National and Kapodistrian University of Athens
School of Health Sciences
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Chemical and biological activity of: *Calea* sp. from Panama; *Centaurea* sp. from Algeria; *Hypericum* sp. from Crete; *Euphorbia* sp. from Central Greece.

Ph.D. DISSERTATION

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Prof. George A. Karikas

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“Εν οίδα ότι ουδέν οίδα (I know that I know nothing).”

- Sokrates

“We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained.”

- Marie Curie

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Στον Κωστή και την Άντι

ABSTRACT

Plant-derived drugs play an important role in both traditional and modern medicine due to nature's high potential of yielding therapeutically relevant bioactive compounds, and natural products are emerging as new lead structures for the development of novel drugs for the prevention and treatment of diseases of modern civilization. On the basis of modern bibliographic data, sesquiterpene lactones from *Centaurea* and *Calea*, phloroglucinols and essential oils from *Hypericum*, as well as diterpenoids from *Euphorbia* were studied, emphasizing on anti-inflammatory and cytotoxic activities. Plants from various families with different phylogenetic relationships and phytochemical profiles have been chosen. *Centaurea*, *Hypericum,* and *Euphorbia* have been referred from Hippocrates (5th century B.C.), and up to now, common uses have been reported for these genera in traditional medicine (including wound-healing, anti-inflammatory, diuretics, antipyretics). When selecting plants from regions with high diversity and endemism (Panama, Algeria, and Greece are biodiversity spots), the probability of finding novel (bioactive or not) substances is higher and demonstrates the potential of different plant species that have never been investigated before.

A total of 84 specialized natural products were isolated from endemic plants of cosmopolitan distribution: 26 from *Calea jamaicensis*, 12 from *Centaurea papposa*, 24 from *Hypericum jovis*, and 22 from *Euphorbia deflexa*. NMR metabolomic strategy was used to target the highly bioactive groups of interest e.g. sesquiterpene lactones, phloroglucinols, and diterpenes in the crude plant extracts and sub-fractions. The isolation procedure included a variety of chromatographic steps using column chromatography in different packing materials (e.g. silica gel, diaion, sephadex), preparative TLC, and HPLC equipped with RI or DAD detector using various columns (eg. RP-C18, RP-Biphenyl, NP). Their chemical structures were elucidated through a combination of HRESIMS, 2D NMR, and X-ray data. 28 sesquiterpene lactones, 14 phloroglucinols, 22 diterpenes, and 20 other compounds were elucidated, among them, 14 sesquiterpene lactones, 2 phloroglucinols, 16 diterpenes, and 3 chromenes are novel natural products and have not been described in the literature before; also the X-ray crystallographic data of 2 diterpenes were reported for the first time. In parallel, more than 120 volatile compounds were identified using GC-MS from the essential oils from *Hypericum* spp. (*H. jovis*, *H. empetrifolium*, *H. amblyocalyx*, *H. triquetrifolium* and *H. perforatum*), belonging mainly to the groups of monoterpene and sesquiterpene hydrocarbons.

Several sesquiterpene lactones were evaluated for their cytotoxic effects *in vitro* reporting $IC_{50} \leq 10$ μM, thus considered to be active and could be potential anti-cancer drugs according to the National Cancer Institute. Carbetolide C afforded IC_{50} values for HeLa, SK-MEL-28 and HePG2 cells similar to that of parthenolide (2.9, 7.5, 12.6 μM and 2.1, 4.9, 10.0 μM, respectively), a well-known cytotoxic sesquiterpene lactone used as a positive control. It is noteworthy that the tested SLs revealed no toxicity in the non-cancerous endothelial HMEC-1 cell line, although Michael reactions of the lactone ring usually also involve non-specific toxicity. In the same cell line, sesquiterpene lactones showed strong inhibition of the TNF-α induced ICAM-1 expression, in a dose-dependent manner. Similar results were obtained from phloroglucinols in the ICAM-1 assay, as well as in the *ex vivo* COX/LOX assay, although only a few studies in the literature demonstrate anti-inflammatory effects from this group of compounds, suggesting the need for further research to explore their anti-inflammatory potential. Regarding diterpenes from *Euphorbia* plants, although abundant and extremely intriguing in their complicated structure elucidation, most of them presented very weak biological activities. Athough the wound healing activities of *Hypericum perforatum* infused oil is known, so far, no studies have been conducted on *Hypericum* essential oils. Besides several non polar ingredients of the essential oil are extracted to the infused oil and contribute to activity. To fulfill this gap, this study describes the investigation of five essential oils *in vivo*, and their significant wound healing effects gave a new insight into the healing properties of genus *Hypericum*. The metabolization of natural products is much less studied than their biosynthetic pathways, thus a final goal was the investigation of the *in vitro* metabolism of the drug-like group of sesquiterpene lactones using human liver microsomes, identified by LC-MS/MS. Sesquiterpene lactones resulted in changeable metabolism due to their structural diversity, though the highly active lactone ring remained the most common metabolic site.

As a concluding mark, high cytotoxic, anti-inflammatory, and wound healing effects were reported from natural products derived from *Calea jamaicensis*, *Centaurea papposa*, *Hypericum jovis*, *H. empetrifolium*, and *Euphorbia deflexa*. For these taxa, almost no records existed in the literature using modern techniques and investigating the biological activities of their constituents. Nature will still be a source of new drugs in the future, and the results of this study contribute to the natural product-based drug discovery, further supporting the strategic use of natural products as a starting point to investigate novel potential effective agents against a variety of human ailments.

ΠΕΡΙΛΗΨΗ

Τα φάρμακα φυσικής προέλευσης παίζουν καίριο ρόλο τόσο στην παραδοσιακή όσο και στην σύγχρονη ιατρική, καθώς η φύση παρέχει μια ανεξάντλητη πηγή βιοδραστικών συστατικών, και ο ρόλος των φυσικών προϊόντων στην εύρεση καινούργιων μορίων-οδηγών για την ανακάλυψη αποτελεσματικών φαρμάκων για μία πληθώρα ασθενειών καθημερινώς ενισχύεται. Με βάση σύγχρονα βιβλιογραφικά δεδομένα, η μελέτη είχε ως στόχο την αναζήτηση και τον εντοπισμό σεσκιτερπενικών λακτονών (από τα ενδημικά είδη *Centaurea* και *Calea*), φαινολικών παραγώγων και αιθερίων ελαίων (από ενδημικά είδη *Hypericum*), καθώς και διτερπενίων (από ενδημική *Euphorbia*), με έμφαση στην αντιφλεγμονώδη και κυτταροτοξική δράση. Αναφορικά με τα τέσσερα υπό εξέταση είδη, αν και διαφέρουν τόσο στην βοτανική κατάταξη, όσο και στο χημικό προφίλ, εμφανίζουν ομοιότητες, που αφορούν στη βιολογική δράση. Το Κενταύριο, το Υπερικό και η Ευφορβία αναφέρονται απο τον Ιπποκράτη (5^{ος} αιώνας π.Χ.) και παρατηρούμε επίσης ότι έχουν καταγραφεί κοινές χρήσεις και για τα τέσσερα γένη στην παραδοσιακή λαϊκή θεραπευτική μέχρι σήμερα, και συγκεκριμένα αναφέρονται ως επουλωτικά, αντιπυρετικά, αντιφλεγμονώδη, διουρητικά, εμμηναγωγά, για γαστρεντερικές διαταραχές. Είναι σημαντικό ότι τα ενδημικά είδη με στενή εξάπλωση αποτελούν δυνητικά σημαντικές πηγές νέων βιοδραστικών δευτερογενών μεταβολιτών, δεδομένου ότι τα αντίστοιχα φυτικά είδη από τα ίδια γένη ευρείας όμως εξάπλωσης εμφανίζουν πληθώρα εθνοφαρμακολογικών αναφορών.

Συνολικά 84 δευτερογενείς μεταβολίτες απομονώθηκαν από ενδημικά φυτά που φύονται ανά τον κόσμο, και συγκεκριμένα 26 από την *Calea jamaicensis*, 12 από την *Centaurea papposa*, 24 από το *Hypericum jovis*, και 22 από την *Euphorbia deflexa*. Η ανίχνευση και απομόνωση μεταβολιτών, που ανήκουν στις κατηγορίες των σεσκιτερπενικών λακτονών, φλορογλουκινών και διτερπενίων ήταν κατευθυνόμενη μέσω ^{1}H NMR (Φασματοσκοπία Πυρηνικού Μαγνητικού Συντονισμού). Οι πορείες απομόνωσης περιελάμβαναν μία πληθώρα χρωματογραφικών τεχνικών, όπως στήλης με διαφορετικά πληρωτικά υλικά (πχ. silica, diaion, sephadex), παρασκευαστική χρωματογραφία λεπτής στιβάδας, καθώς και υγρή χρωματογραφία υψηλής απόδοσης (HPLC) με ανιχνευτές RI και DAD και κατάλληλες στήλες (RP-C18, RP-Biphenyl, NP). Οι χημικές δομές αποδώθηκαν με συνδυασμό από δεδομένα NMR, φασματομετρία μάζης (HRESIMS) και κρυσταλλογραφίας (X-ray). Απομονώθηκαν και ταυτοποιήθηκαν 28 σεσκιτερπενικές λακτόνες, 14 φλορογλουκινόλες, 22 διτερπένια και 20 άλλα συστατικά, μεταξύ αυτών 14 σεσκιτερπενικές λακτόνες, 2 φλορογλουκινόλες, 16 διτερπένια και 3 χρωμένια αποτελούν νέα φυσικά προϊόντα, που περιγράφονται για πρώτη φορά στη παρούσα εργασία, όπως και τα κρυσταλλογραφικά δεδομένα από

δύο διτερπένια. Παράλληλα, περισσότερα από 120 πτητικά συστατικά ταυτοποιήθηκαν με την τεχνική της αεριοχρωματογραφίας συζευγμένης με φασματομετρία μάζης (GC-MS) από φυτά του γένους *Hypericum* (*H. jovis*, *H. empetrifolium*, *H. amblyocalyx*, *H. triquetrifolium* και *H. perforatum*), κυρίως μονοτερπενικοί και σεσκιτερπενικοί υδρογονάνθρακες.

Οι απομονωμένες σεσκιτερπενικές λακτόνες αξιολογήθηκαν *in vitro* σε καρκινικές σειρές, παρουσιάζοντας $IC_{50} \leq 10$ μM, τιμές που κατατάσσουν τα μόρια σε δραστικά και πιθανά αντικαρκινικά φάρμακα με βάση τις οδηγίες από το Εθνικό Ινστιτούτο Καρκίνου (National Cancer Institute). Συγκεκριμένα, ο carbetolide C απέδωσε τιμές IC_{50} για τις καρκινικές σειρές HeLa, SK-MEL-28 και HePG2 παρόμοιες με αυτές του παρθενολιδίου (2.9, 7.5, 12.6 μM και 2.1, 4.9, 10.0 μM, αντίστοιχα), που αποτελεί ισχυρή αντικαρκινική σεσκιτερπενική λακτόνη και η οποία χρησιμοποιήθηκε ως πρότυπη ουσία-μάρτυρας (positive control). Είναι σημαντικό πως στο σύνολό τους οι σεσκιτερπενικές λακτόνες δεν παρουσίασαν τοξικότητα στα φυσιολογικά/μη καρκινικά κύτταρα HMEC-1, παρότι οι αντιδράσεις τύπου Michael του λακτονικού δακτυλίου συνήθως περιλαμβάνουν και φαινόμενα μη ειδικής τοξικότητας. Στην κυτταρρική σειρά HMEC-1, οι σεσκιτερπενικές λακτόνες οδήγησαν σε δοσοεξαρτώμενη αντιφλεγμονώδη δράση αναστέλλοντας την επαγόμενη μέσω TNF-α έκφραση του μορίου ICAM-1. Ισχυρή αντιφλεγμονώδη δράση παρουσίασαν επίσης και οι φλορογλουκινόλες τόσο στην *in vitro* βιοδοκιμασία του ICAM-1, όσο και στην *ex vivo* βιοδοκιμασία COX/LOX. Πολύ λίγες μελέτες περιγράφουν τις αντιφλεγμονώδεις ιδιότητες αυτών των ουσιών, καταδεικνύοντας την ανάγκη για περαιτέρω διερεύνηση της δυναμικής τους ως αντιφλεγμονώδεις παράγοντες. Όσον αφορά στα διτερπένια από την *Euphorbia*, αν και άφθονα με εξαιρετικό ενδιαφέρον στην περίπλοκη απόδοση δομών, τα περισσότερα από αυτά δεν παρουσίασαν αξιόλογες βιολογικές δράσεις. Περαιτέρω, αν και είναι γνωστή η επουλωτική δράση του βαλσαμέλαιου (Oleum *Hyperici*), δεν είχαν μελετηθεί προηγουμένως οι επουλωτικές ιδιότητες των αιθερίων ελαίων από φυτά του γένους *Hypericum*, παρότι πτητικά συστατικά συνεκχυλίζονται κατά την παρασκευή του βαλσαμέλαιου. Για να καλυφθεί αυτό το κενό, διερευνήθηκαν *in vivo* πέντε αιθέρια έλαια και έδειξαν σημαντική επουλωτική δράση, ενισχύοντας τη γνώση για τις θεραπευτικές ιδιότητες του γένους *Hypericum*. Επιπλέον, ο μεταβολισμός των φυσικών προϊόντων είναι πολύ λιγότερο μελετημένος σε σχέση με τα βιοσυνθετικά τους μονοπάτια, οπότε ένας τελικός στόχος της παρούσας διατριβής αποτέλεσε η διερεύνηση του *in vitro* μεταβολισμού των σεσκιτερπενικών λακτονών χρησιμοποιώντας μικροσώματα ανθρώπινου ήπατος και οι μεταβολίτες ανιχθεύθηκαν με LC-MS/MS. Οι σεσκιτερπενικές λακτόνες οδήγησαν σε ποικίλους μεταβολίτες λόγω της δομικής τους ποικιλομορφίας, αν και ο εξαιρετικά ενεργός δακτύλιος λακτόνης παρέμεινε η πιο κοινή μεταβολική θέση.

Συμπερασματικά, αναφέρθηκε υψηλή κυτταροτοξική, αντιφλεγμονώδης και επουλωτική δράση από φυσικά προϊόντα που προέρχονται από τα φυτά που μελετήθηκαν, i.e. *Calea jamaicensis, Centaurea papposa, Hypericum jovis, H. empetrifolium και Euphorbia deflexa*. Για αυτά τα είδη, υπήρχαν ελάχιστες αναφορές στη βιβλιογραφία. Στη παρούσα διδακτορική διατριβή μελετήθηκαν αυτά τα είδη χρησιμοποιώντας σύγχρονες τεχνικές και διερευνώντας τις βιολογικές επιδράσεις των συστατικών τους. Η φύση θα συνεχίσει να αποτελεί μια πηγή νέων φαρμάκων στο μέλλον και τα αποτελέσματα αυτής της μελέτης συμβάλλουν στην ανακάλυψη δραστικών ουσιών βασισμένων στα φυσικά προϊόντα, υποστηρίζοντας περαιτέρω τη στρατηγική χρήση φυσικών προϊόντων ως αφετηρία για τη διερεύνηση νέων δομών ως δυνητικών παραγόντων για ποικίλες ανθρώπινες παθήσεις.

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12-HETE: 12(S)-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenoic acid

12-HHT: 12(S)-hydroxy(5Z,8E,10E)-heptadecatrienoic acid

ADME: Absorption, Distribution, Metabolism, Excretion properties

Ala: alamethicin

CD: Circular dichroism

COX: cyclooxygenase

DAD: Diode-Array Detection

DMAPP: dimethylallyl diphosphate

DNCB: 2,4-dinitrochlorobenzene

DTs: Diterpenes

EMA: European Medicine Agency

EOs: essential oils

FACS: fluorescence-activated cell sorting analysis

FDA: Food and Drug Administration

FPP: farnesyl diphosphate

G6P: glucose-6-phosphate sodium salt

G6PDH: glucose-6-phosphate dehydrogenase

GC-MS: Gas Chromatography-Mass Spectrometry

HA: Hypericum amblyocalyx

HE: Hypericum empetrifolium

HJ: Hypericum jovis

HLM: Human Liver Microsomes

HP: Hypericum perforatum

HPLC: High Performance Liquid Chromatography

HRESIMS: High resolution electrospray ionization mass spectroscopy

HT: Hypericum triquertifolium

IC50: half maximal inhibitory concentration

ICAM-1: Intercellular Adhesion Molecule 1

IPP: isopentenyl diphosphate

LC-MS/MS: Liquid Chromatography-Mass Spectrometry

LOX: lipoxygenase

MEP: 2-C-methyl-D-erythritol 4-phosphate pathway

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

MVA mevalonate pathway

NADP: nicotinamide adenine dinucleotide phosphate sodium salt hydrate

NMR: Nuclear Magnetic Resonance

NP: normal phase

NSAIDS: nonsteroidal anti-inflammatory drugs

p.c.: positive control

PAPs: prenylated acylphloroglucinols

PBS: phosphate-buffered saline

PGE2: prostaglandin E2

PPAPs: polycyclic polyprenylated acylphloroglucinols

RI: Refractive index detector

RP: reversed phase

SLs: sesquiterpene lactones

SPS: sesquiterpene synthase

TEWL: Transepidermal water loss

TNF-α: tumor necrosis factor α

TXB2: thromboxane B2

UDPGA: uridine 5’-diphosphoglucuronic acid trisodium salt

UGT: uridine 5'-diphospho-glucuronosyltransferase

1. INTRODUCTION

1.1. Natural Products

1.1.1. The role of Natural Products

Despite its impressive progress, modern Western medicine is incapable of dealing with chronic degenerative disorders associated with the environment, lifestyle, and stress that plague modern society. Thus, an important, complementary role for traditional herbal medicines emerges in the prevention and cure of diseases of modern civilization and natural products provide a variety of lead structures for the development of new drugs (Mushtaq et al., 2018). The rapid development of sciences and pharmaceutical industry, after the industrial revolution, overlooked much of the past knowledge and the use of some plant products in medicines for the prevention and treatment of diseases. Nowadays, we observe the increasing popularity of plant preparations for a range of therapeutic applications. Individuals are taking greater responsibility for their own health and quality of life, taking confidence in herbal and alternative therapies. WHO reported that 80% of the world's population uses medicinal plants for primary health needs (Khan & Ahmad, 2019).

Bioactive compounds relevant to human/animal health that are derived from natural sources, e.g., plants, animals and microorganisms, are defined as natural products. Nature is an important source for the discovery of new bioactive compounds and natural products have a significant potential to expand the field of disease prevention and treatment. Natural products constitute a strategic starting point for the development of novel drugs (drug leads) in comparison to synthetic compounds, since they exhibit a wide range of pharmacophores (chemical diversity) and a large number of chiral centers that allow the interaction with proteins and biological targets. Research in the field of natural products has gained great attention in the past few decades, a trend expected to continue in the coming years. Considering the current decrease of new drugs introduced to the market, as well as nature's high potential of yielding therapeutically relevant bioactive compounds, plant metabolites are emerging as new lead structures for the development of novel drugs for treating various diseases (Mushtaq et al., 2018, Shin et al., 2018). According to Newman & Cragg (2020): "natural products still hold out the best options for finding novel agents/active templates, which when worked on in conjunction with synthetic chemists and biologists, offer the potential to discover novel structures that can lead to effective agents in a variety of human diseases".

1.1.2. Cancer and Inflammation

Across the globe, cancer is the leading cause of mortality and morbidity (Fridlender et al., 2015). The number of cancer patients is continuously increasing every year, with a speculation of 15.5 million people worldwide becoming cancer patients by 2030, with 11.5 million are expected to be proven fatal (Amin et al., 2009). Regardless of the plethora of efforts to treat cancer with various methods (surgery, radiotherapy, chemotherapy, and immunotherapy), the severe side effects have a negative impact on patients, impeding the process of curing cancer (Morrissey et al., 2016). In the area of cancer, according to Newman and Cragg (2020), over the time frame from around the 1940s to date, of the 259 small antitumor molecules, 206, or 79%, are other than synthetic, including all the well-known anticancer plant derived agents such as paclitaxel and vinblastine/vincristine, as well as the more recently approved ingenol mebutate (see 1.2.4.1).

Moreover, the role of natural products in the discovery of new drugs with anti-inflammatory and immunomodulatory potential is progressively enhanced (Aswad et al., 2018). Inflammation is an important mechanism to fight infections and injuries in order to restore tissue homeostasis, however chronic inflammation plays a crucial role in the pathogenesis of various diseases such as asthma, allergies, rheumatoid arthritis, multiple sclerosis, psoriasis, chronic inflammatory bowel disease, type II diabetes, neurodegenerative diseases and higher risk of cancer development (Scrivo et al., 2011). They place a significant burden on patients suffering and economic cost in Western society (Wylezinski et al., 2019) and the estimated prevalence is 5 to 7% (El-Gabalawy et al., 2010). In Europe, asthma affects one child in seven, while allergies notably have continued to increase dramatically over the past 30 years (Sánchez-Borges et al., 2018). Rheumatoid arthritis affects approximately 17.6 million people worldwide (Centers for Disease Control and Prevention, 2018). Glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDS) are the most common agents used, however, they are often proved to be ineffective, and they present several adverse effects. Thus, researchers around the world are interested in novel substances as safe and effective therapies for chronic inflammatory diseases (Atanasov et al., 2015). Based on the ancient use of salicylic acid, the well-known aspirin was synthesized, while other examples of plant-derived drugs used in such diseases include galantamine for Alzheimer's, semaglutide for diabetes, and siponomod fumarate for multiple sclerosis (Newman & Cragg, 2020).

1.1.3. Why selecting plants from regions with high biodiversity

When selecting plants from regions with high biodiversity and endemism, the probability of finding novel bioactive or not substances is certainly higher and demonstrates the potential of different plant species that have never been investigated before. This type of approach provides an unlimited source of previously undescribed compounds, since nature is a vast chemical laboratory, and when combined with chemotaxonomic and ethnomedicinal data it could lead to the selection of propitious plant taxa with therapeutically relevant bioactive metabolites (Ramos Barbosa et al., 2012). Based on these criteria, plants from various families with different phylogenetic relationships and phytochemical profiles have been chosen, namely, *Calea jamaicensis* from Panama, *Centaurea papposa* from Algeria, *Hypericum jovis* from Crete and *Euphorbia deflexa* from Central Greece. Panama, Algeria and Greece are biodiversity spots (Myers et al., 2000, Figure 1), and the under-investigation taxa have some further common features, except for being narrow endemic in regions of high biodiversity. For example, *Centaurea*, *Hypericum* and *Euphorbia* have been indicated as medicinal plants being referred from Hippocrates (5th century B.C.), while up to now some common uses are being reported for these four genera in folkmedicine (including wound healing, inflammation, diuretics, antipyretics) (see 1.2).

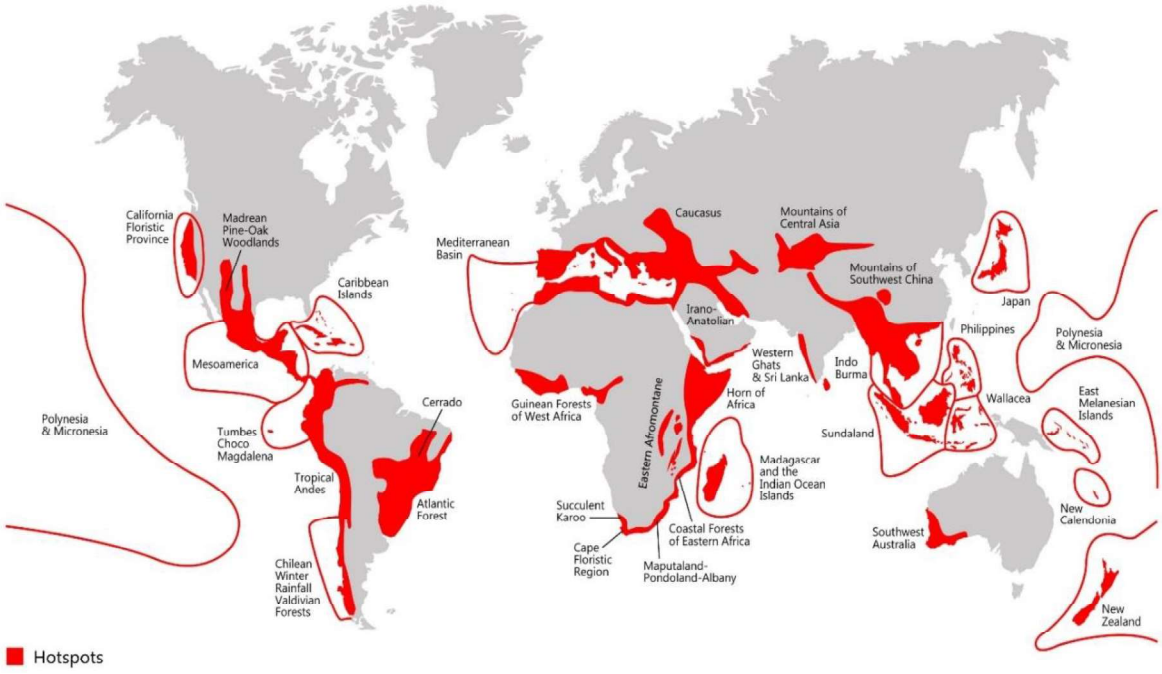

Figure 1. Biodiversity hotspots (taken from Myers et al., 2000).

1.2. The under-investigation plant taxa

1.2.1. The genus *Calea* L.

Calea L. is a genus of the family Asteraceae (tribe Helianthae) with more than 120 species growing in tropical and subtropical regions of the Americas (Kadereit & Jeffrey, 2007). The etymology of the name *Calea* originates from the Ancient Greek word kalos ("κάλλος" meaning beautiful) in reference to the showy inflorescence (Quattrocchi, 2012). Members of the genus are used throughout their geographic distribution for gastrointestinal and skin disorders, hypertension, diabetes, infections, and even cancer (Amaral et al., 2017). *C. zacatechichi* (*C. tenuifolia*) is well-known *Calea* taxa, which is being used from Mexico to Costa Rica for fever, diarrhea, malaria and to enhance dreams (Köhler et al., 2002). Sesquiterpene lactones (SLs) are the principal bioactive constituents of the genus, while chromenes and p-hydroxyacetophenone derivatives are also frequently isolated (Lima et al., 2018).

1.2.1.1. Sesquiterpene Lactones

In recent years, the interest of the scientific community around SLs, their isolation, semi-synthesis, and biological activities, reports a constant growth, to a great extent because of the Nobel Prize in Physiology and Medicine, for the evaluation of artemisinin and its use in the treatment of malaria (Guo, 2016). Numerous plants rich in SLs, such as *Arnica montana, Tanacetum parthenium, Matricaria chamomilla*, are used in folk medicine around the world for the treatment of a variety of ailments including fever, respiratory and gastrointestinal disorders, infections, wound healing and rheumatism (Merfort, 2011). SLs are mostly produced by plants belonging to Asteraceae family, but they can also be isolated in lower amounts from other families, such as Acanthaceae, Amaranthaceae, Anacardiaceae, Apiaceae, Aristolochiaceae, Burseraceae, Cortiariaceae, Cannellaceae, Euphorbiaceae, Hepaticae, Illiciaceae, Lauraceae, Lamiaceae, Menispermaceae, Magnoliaceae, Rutaceae, Winteraceae, as well as in Marchantiophyta, gymnosperms and fungi (Chadwick et al., 2013, Sokovic et al., 2017, Canales et al., 2005, Maries et al., 1995, Rodriguez et al., 1976). Besides artemisinin, other well-known SLs are thapsigargin, parthenolide, alantolactone and costunolide (Figure 2.A), which possess important cytotoxic and anti-inflammatory effects on various targets and they are in cancer clinical or pre-clinical studies (Merfort, 2011, Moujir et al., 2020). SLs are terpenoids with a basic skeleton of 15 carbon atoms with a fused α-methylene-γ-lactone ring, resulting in different skeletal types, eg. guaianolides, eudesmanolides, elemanolides, germacranolides, xantholides etc, which have been reported to exhibit important pharmacological activities (Figure 2.B). Structure-activity

relationships suggest that the fused α-methylene-γ-lactone ring plays a crucial role in the high bioactivity of this group of compounds, by acting as an alkylating center on sulphidric groups from biological systems through Michael additions (Figure 2.C) (Schmidt, 2018). The non-specific toxicity, as a result of the high reactivity of the SL ring, and the unstable absorption/extensive metabolism do not hinder the investigation or exploitation of SLs as therapeutic agents, mainly because of their dominant medicinal potential and drug-like physicochemical properties (Lipinski's rule of 5), suggesting them as promising drug leads.

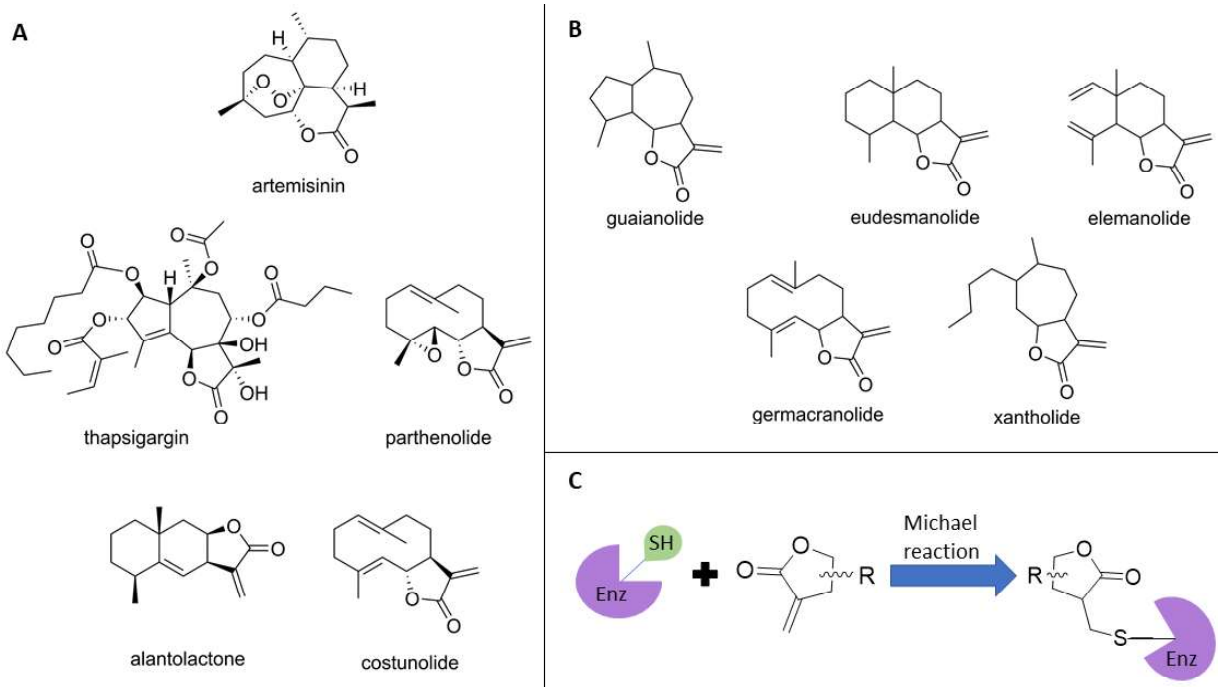

Figure 2. A. Structures of the well-known SLs artemisinin, thapsigargin, parthenolide, alantolactone and costunolide. B. Types of SLs. C. Michael type addition of SL ring to SH groups in biological systems.

As terpenoids, SLs derive from the universal isoprenoid precursors isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Padilla-Gonzalez et al., 2016). IPP and DMAPP are synthesized through two independent metabolic routes, the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, which are localized in the cytosol and the chloroplast, respectively. It has been generally assumed that the MVA pathway supplies isoprenoid precursors for the synthesis of sesquiterpenes, as well as sterols and triterpenes, while the MEP pathway is responsible for monoterpenes, diterpenes and carotenoids (Figure 3). The biosynthesis of SLs starts with the cyclization of farnesyl diphosphate (FPP) by a sesquiterpene synthase (SPS). The reaction is followed by a series of oxidations and

hydroxylations. The biosynthesis of SLs is presented in Figure 4, together with the proposed biosynthesis of the previously undescribed SLs from *Calea Jamaicensis*.

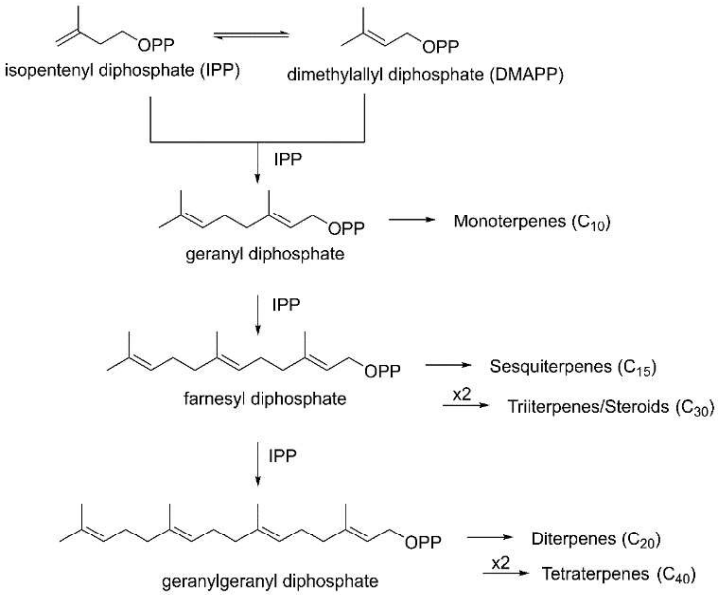

Figure 3. Biosynthesis of terpene categories from activated 5-C units (IPP and DMAPP)

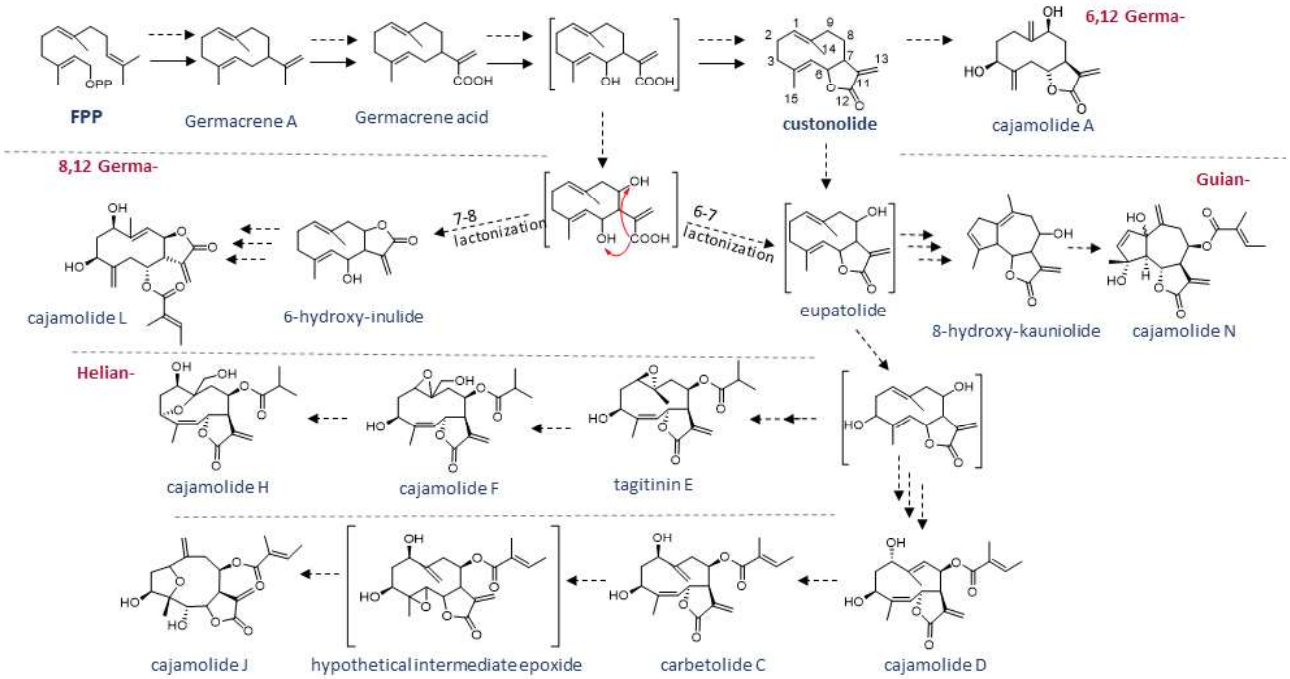

Figure 4. Biosynthetic pathway of SLs: Solid arrows represent confirmed pathway in Cichorium intybus (de Kraker, 2002) and dashed arrows represent proposed pathway for previously undescribed SLs from Calea jamaicensis.

1.2.1.1. *Calea jamaicensis* (L.) L.

The selected plant, *C. jamaicensis* (L.) L., is a narrow endemic species from the region of Panama, more specifically its distribution is from Mexico to Central America and Jamaica, as presented in Figure 5 (Wussow et al., 1985). It is a shrub with opposite, simple leaves of broadly ovate shape with different types of trichomes, capitulescence with numerous, few-flowered heads in cymose cluster, yellow florets with five-lobed, yellow disc corollas. Resin-globules are present on the achenes back, obconic to cylindrical, and somewhat prismatic (Wussow et al., 1985) (Figure 6). *C. jamaicensis* is the type species of the genus *Calea*, and together with a few other Mexican, Central American, and Jamaican species, a fairly close-knit group is formed within *Calea*, as suggested by Wussow et al. (1985). A summary of SLs isolated from this group is presented in Appendix 7.1.

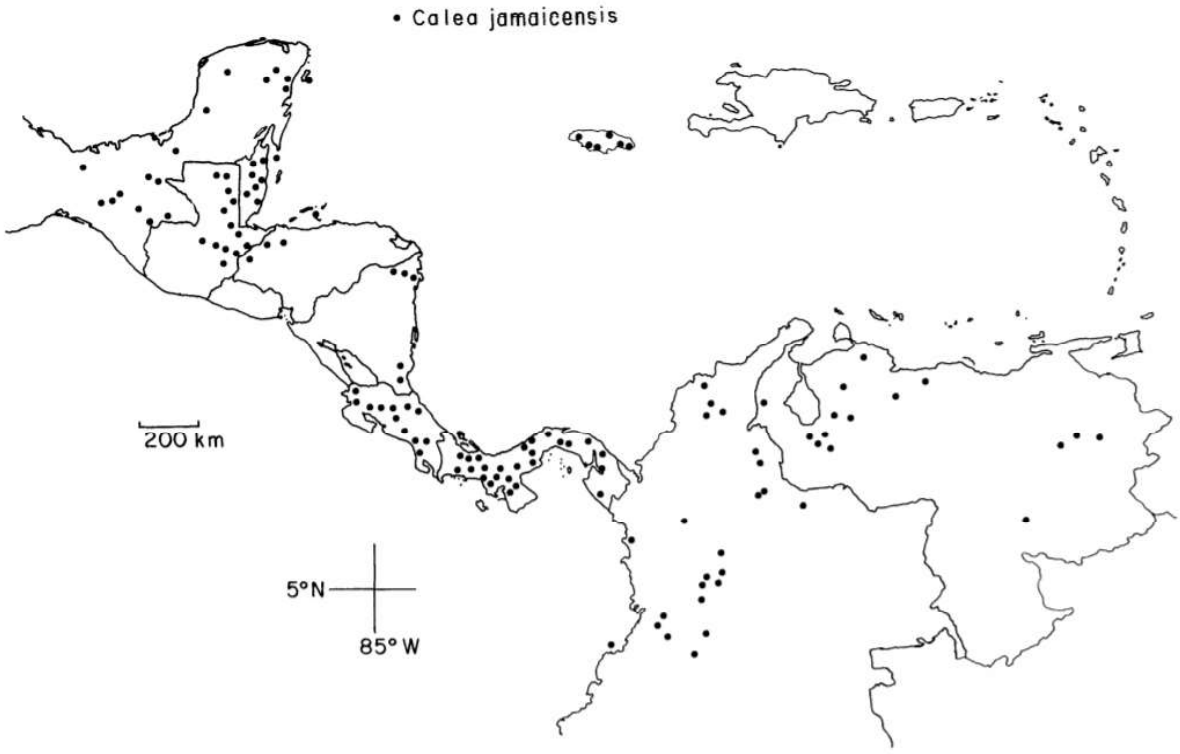

Figure 5. Distribution of Calea jamaicensis in Latin America and Jamaica

Figure 6. Photo of the plant Calea jamaicensis (http://www.inaturalist.org/observations/46113883)

1.2.2. The genus *Centaurea* L.

The genus *Centaurea* L. (Asteraceae, tribe Cynareae) consists of more than 500 taxa, which are mainly distributed in the area of the Mediterranean basin, as well as in Western Asia (Mabberley, 1997). The name *Centaurea* derives from the Greek word kéntauros ("κένταυρος" meaning centaur) due to the mythological discovery of its medicinal properties by Chiron the Centaur (Carnoy, 1959). Many *Centaurea* species are well-known for the treatment of a variety of ailments, including gynaecological, gastrointestinal and skin disorders, inflammation, fever, diabetes and rheumatism (Sokovic et al., 2017). Dioscorides reports the use of the greater centaury (*Centaurea centaurium*) as a wound healing agent (Berendes, 1970). The genus *Centaurea* is also characterized by the biosynthesis of SLs and the common constituents are by order of their abundance, guaianolides, germacranolides, eudesmanolides and elemanolides. SLs are recognized as chemophenetic markers for the genus (Sokovic et al., 2017), while in parallel their high bioactivity in living systems explains the long-term use of *Centaurea* spp. in traditional medicine. Interestingly, several SLs isolated from *Centaurea* spp. have reported possessing strong cytotoxic and anti-inflammatory activities *in vitro*, such as cnicin (Sen et al., 2017), 8α-O-(3,4-dihydroxy-2-methylene-butanoyloxy)-dehydromelitensin (Koukoulitsa et al., 2002, Saroglou et al., 2005) and salograviolide A (El-Najjar et al., 2008) (Figure 7), suggesting the potential of *Centaurea* plants to offer new drug leads.

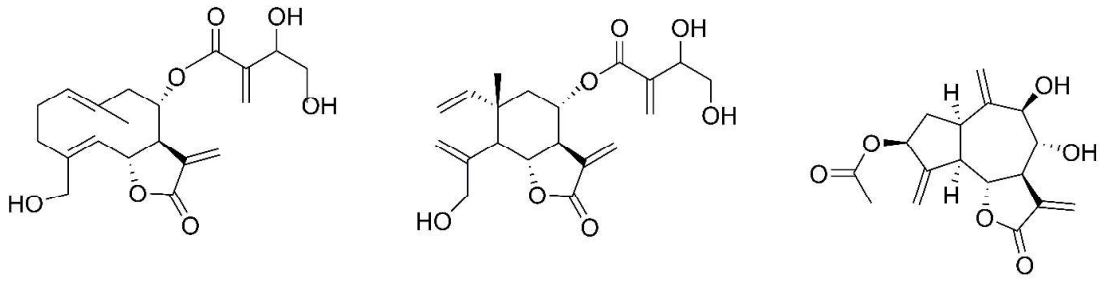

Figure 7. SLs isolated from Centaurea spp. possessing strong cytotoxic and anti-inflammatory activities.

1.2.2.1 *Centaurea papposa* (Coss.) Greuter

About 50 *Centaurea* spp. are located in Algeria (Quezel & Santa, 1963), including the narrow endemic *C. papposa* (Coss.) Greuter. It is a perennial plant covered with dense short hairs, giving a grayish-white appearance. It has tomentose leaves, capitulescence with purple florets homomorphic or dimorphic, involucre oval or spherical with bracts in many rows, bracts either uniform or dimorphic or polymorphic (Figure 8).

The under-investigation species belongs to the subsection Acrolophus (Cass.) DC. of section Centaurea (especially *C. cineraria* group, see Figure 9), which is combining the former sections Acrolophus (sect. Centaurea s. str.), Phalolepis and Willkommia, based on modern classification reports (Hilpold et al., 2011, i.d., 2014). SLs isolated from section Centaurea, with a reference to the three subsections, are presented in Appendix 7.2.

Figure 8. Photo of the plant Centaurea papposa.

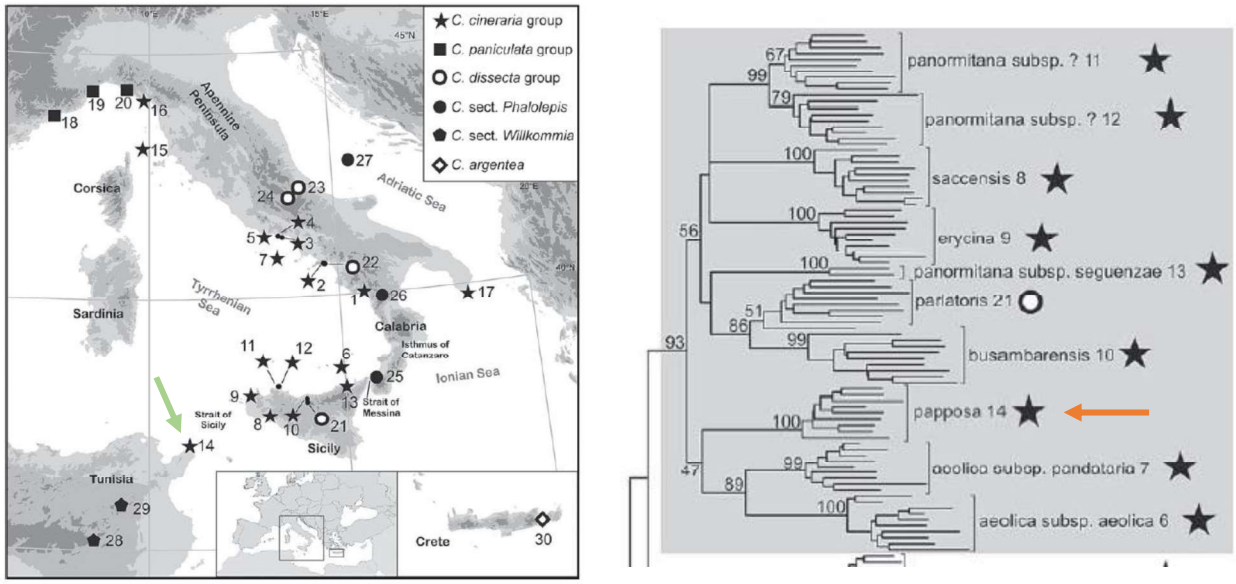

Figure 9. C. papposa belongs to section Centaurea, C. cineraria group (taken from Hilpold et al., 2011).

1.2.3. The genus *Hypericum* L.

Regarding the genus *Hypericum* L. (Hypericaceae), it includes more than 500 taxa with a worldwide distribution (Crockett & Robson, 2012). The botanical name derives from the Greek word hypericon ("υπέρ εικόνα" meaning above the icon), suggesting its use against spirits and demons. The use of *Hypericum* has been reported even during classical Antiquity by Hippocrates (Totelin, 2009), Dioscorides (Berendes, 1970), and later on in the Medieval era by Nikolaos Myrepsos (Valiakos et al., 2015; id. 2017). Several *Hypericum* spp. are used throughout the world in folk medicine, as astringent, febrifuge, diuretic, antiphlogistic agent, analgesic, and antidepressant agents (Zhang et al., 2020). Modern medical research has shown that *H. perforatum* is an effective herbal medicine for the treatment of mild to moderate depression [EMA, herbal medicine with well-established use], while the monograph is also mentioning the wound healing properties [EMA, herbal medicine with traditional use]. The active constituents of the genus belong to the groups of phloroglucinols, napthodianthrones, xanthones, flavonoids (Avato, 2005) and essential oil (Guedes et al., 2012) (Figure 10). These metabolites display a wide range of biological activities. Apart from the well-established antidepressant activity, many substances have become the subject of intense studies concerning their cytotoxic and anti-inflammatory effects.

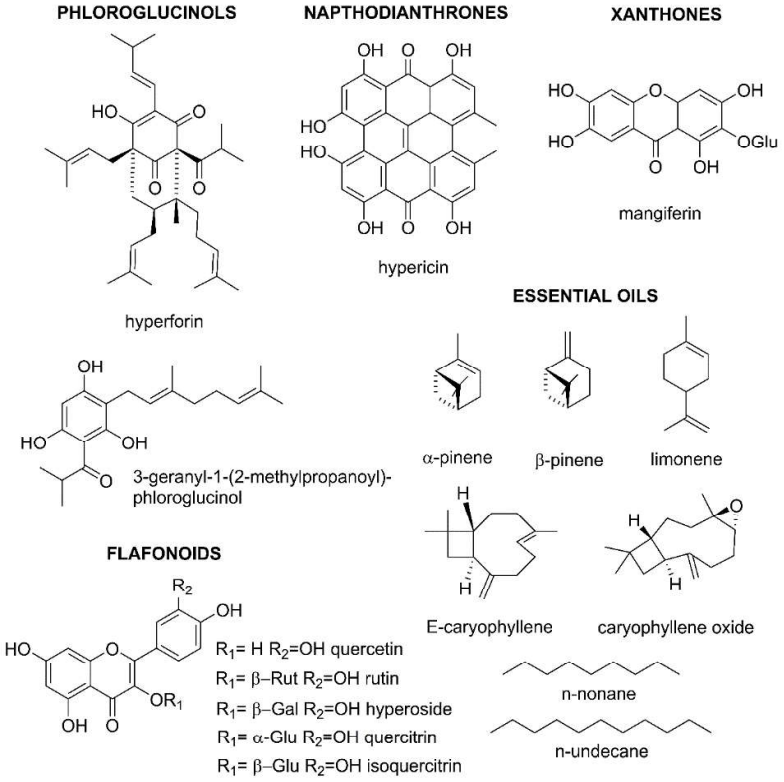

Figure 10. Bioactive specialized metabolites in Hypericum taxa.

1.2.3.1. Phloroglucinols

Phloroglucinols belong to the principal constituents of the genus *Hypericum*, with more than 429 compounds identified from *Hypericum* spp., mainly prenylated acylphloroglucinols (PAPs), chromanes and chromenes, and polycyclic polyprenylated acylphloroglucinols (PPAPs) (Bridi et al., 2018). Phloroglucinol derivatives are phenolic compounds with a wide distribution in different natural sources (Pal Singh & Bharate, 2006). Of the 36 *Hypericum* sections, those presenting phloroglucinols are Adenosepalum, Androsaemum, Ascyreia, Brathys, Campylosporus, Coridium, Crossophyllium, Drosocarpium, Hirtella, Hypericum, Humifusoideum, Myriandra, Oligostema, Olympia, Roscyna, Sampsonia, Takasagoya, Thasia, and Trigynobrathys. Phloroglucinols are polyketides and as such their main skeleton derives from the acetate-malonate pathway, while the prenyl substitutions derive from the universal isoprenoid precursors (Figure 3). Figure 11 presents the proposed phloroglucinol biosynthetic pathway on the example of hyperforin through 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol (Adam 2002, Beerhues 2011).

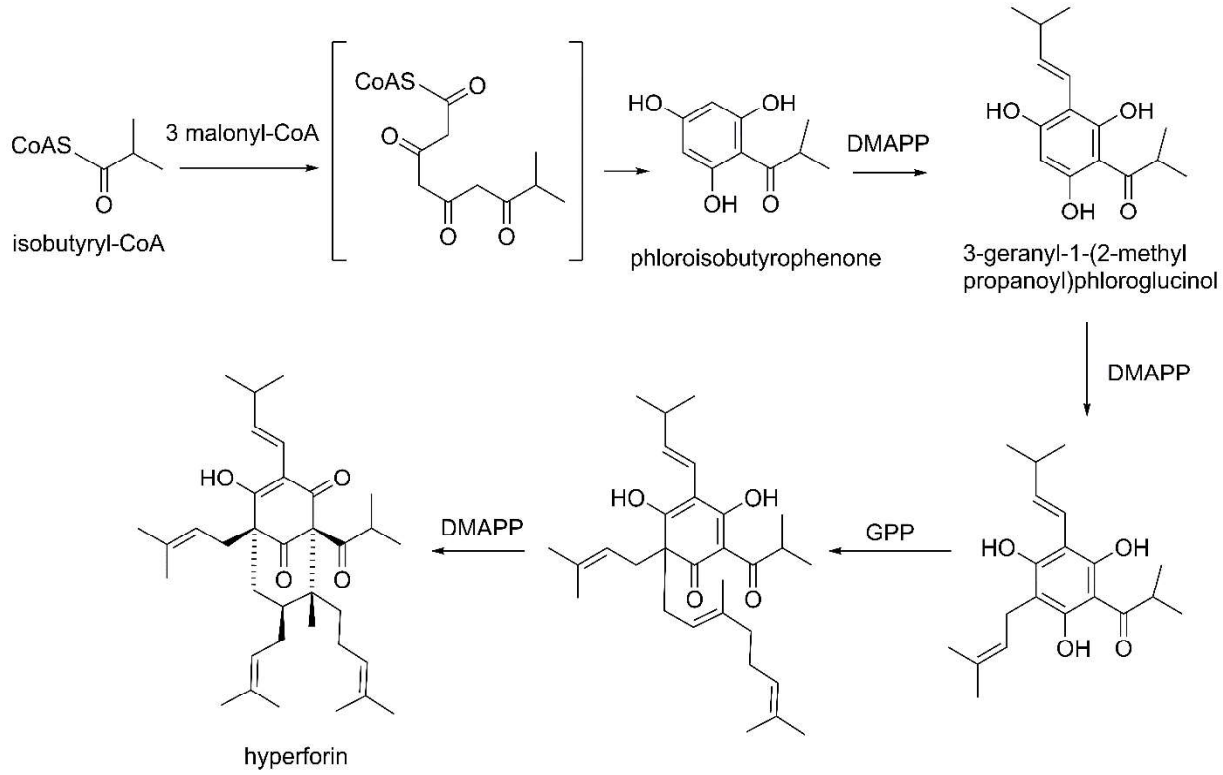

Figure 11. Proposed phloroglucinol biosynthetic pathway on the example of hyperforin through 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol (Adam 2002, Beerhues 2011).

Several studies indicated that phloroglucinol derivatives are responsible for most of the biological activities of *Hypericum* spp. Hyperforin, which is an abundant PPAPs, besides the proven antidepressant, antimicrobial and anti-proliferative activities, is also acting as a strong inhibitor of inflammation- or tumor-triggered angiogenesis, providing new therapeutic approaches to halting pathology-associated angiogenesis, e.g. for wound healing, tumor growth, as well as age-related macular degeneration (Lorusso et al., 2009). Hyperforin was also found to be a dual inhibitor of cyclooxygenase (COX)-1 and 5-lipoxygenase (LOX), both *in vitro* (Albert et al., 2002) and *in vivo* (Koeberle et al., 2011). Other phloroglucinol derivatives have been reported to possess strong anti-inflammatory activity *in vitro*, more specifically two monomeric PAPs (3-geranyl-1-(2-methylpropanoyl)-phloroglucinol and 3-geranyl-1-(2-methylbutanoyl)-phloroglucinol isolated from *H. empetrifolium* also act as strong anti-inflammatory agents by inhibiting COX-1, COX-2 and 5-LOX (Crockett et al., 2008). Furthermore, hyperforin has also been proved to inhibit both VCAM-1 (Vascular cell adhesion protein 1) and ICAM-1 (Intercellular Adhesion Molecule 1) expression (Le Bot et al., 2016). As hyperforin and highly complex phloroglucinols often show instability in aqueous solutions (Wolfender et al., 2003, Ang et al., 2004), the search for more simple and stable templates with the bioactive phloroglucinol skeleton is an intriguing challenge.

1.2.3.2. Essential oils

Another interesting group of compounds is essential oils (EOs), which have been reported to possess strong antimicrobial, antioxidant, antiangiogenetic, gastroprotective activities (Guedes et al., 2012), although *H*ypericum species are generally classified as EO-poor plants (Crockett, 2010). According to the European Pharmacopeia, EOs (Aetherolea) are odorous products, usually of complex composition, obtained mainly by steam distillation. These complex mixtures mainly consist of mono- and sesquiterpenes (Figure 3), in the form of hydrocarbons or oxygenated derivatives. Other substances that could be identified in EOs from different plants are diterpenes, phenols, fats, coumarins, anthraquinones, certain alkaloids and several compounds derived during distillation process (artifacts). EOs from diverse plants have a plethora of uses in the cosmetic, pharmaceutical, and food industry (Ríos, 2016).

EOs and phloroglucinols share partially a common biosynthetic route and, regarding *Hypericum*, they are both bioaccumulated in the translucent glands of the plants (vs the dark glands that bioaccumulate naphodianthrones (Crockett & Robson 2011). The content of the translucent glands (EOs and PAPs) is being extracted in the infused oil (Oleum *Hyperici*) (Crockett, 2010), which is the well-known preparation used since the times of ancient Greeks for the treatment of wounds. A big part of the scientific community is investigating the wound healing properties of *Hypericum*, evaluating extracts of different polarities,

phloroglucinol derivatives and even naphthodianthrones (although only degradation products of this group of compounds can be found in the infused oil), while extensive literature survey showed, that, unlike the rest of the phytochemical groups, the wound healing efficacy of EOs from *Hypericum* spp. has not still been evaluated and compared between species.

1.2.3.3. *Hypericum jovis* Greuter

Among *Hypericum* species, 12 taxa are endemic for Greek territory and 28 have a wider spread (Pylara et al., 2007). Crete is an island with extremely high rates of endemism, having 1825 different plant species in total, of which 19.3% are endemic (Trigas et al. 2013). Taxon, *H. jovis* Greuter, examined in the extensive phytochemical study (3.3.), is a narrow endemic species of Crete, one of the seven taxa growing wild in this island (Table 1). It is a dwarf shrub with erect and caespitose stems of strict branching, simple, linear, hairless, glaucous leaves in whorls of 4, yellow, solitary flowers, sepals stalkless, margins without glands, deciduous petals and stamens, capsule narrowly ellipsoid (Figure 12). It is a close relative of the already studied *H. empetrifolium* (Schmidt et al., 2012a; 2012b) and *H. amblyocalyx* (Winkelmann et al., 2003), all belonging to the section Coridium (Figure 13) (Robson, 2013). The isolated PAPs reported from plants of the section Coridium can be found in Appendix 7.3. Moreover, the previously mentioned three taxa of the section Coridium together with two other *Hypericum* species commonly used throughout Greece, i.e. *H. perforatum* and *H. triquetrifolium* (section Hypericum), were also investigated regarding the EO content and bio-activity. An extensive review of EOs reported from the whole genus *Hypericum* is presented in Appendix 7.4.

Table 1. Endemic Hypericum taxa in the island of Crete, Greece.

***Hypericum* spp.**	**Section**	**Distribution**
Hypericum aciferum N. K. B. Robson	Adenotrias	Western Crete
Hypericum amblyocalyx Coust. & Gand.	Coridium	Eastern Crete
Hypericum empetrifolium ssp. oliganthum (Rech. Fil.) Hagemann	Coridium	Whole Crete
Hypericum empetrifolium ssp. tortuosum (Rech. Fil.) Hagemann	Coridium	Western and Central Crete
Hypericum jovis Greuter	Coridium	Western Crete
Hypericum kelleri Bald.	Oligostema	Western Crete
Hypericum trichocaulon Boiss. & Heldr.	Drosocarpium	Western and Central Crete

Figure 12. Photo of the plant Hypericum jovis.

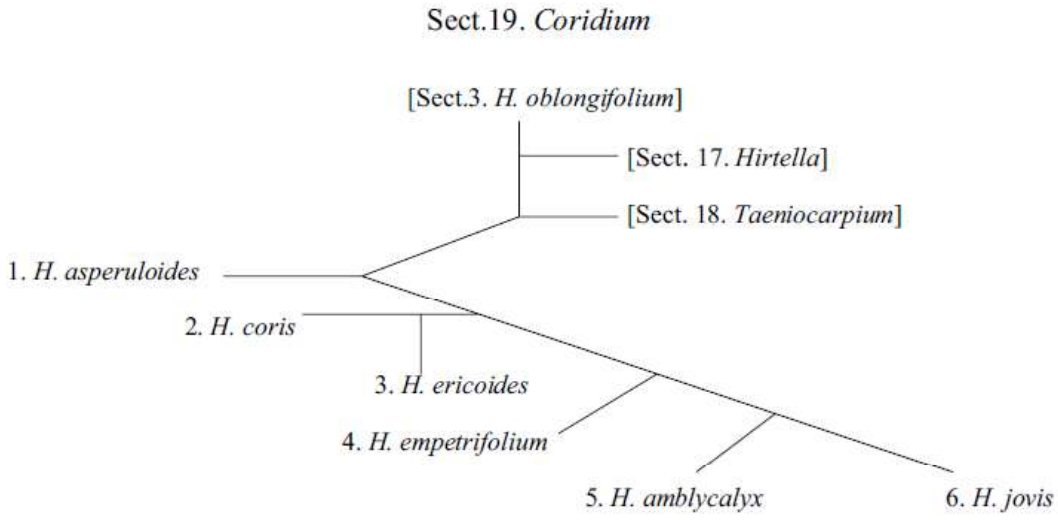

Figure 13. H. jovis, together with H. empetrifolium and H. amblyocalyx, belong to section Coridium (taken from Robson, 2013).

1.2.4. The genus *Euphorbia* L.

The genus *Euphorbia* L. (Euphorbiaceae) has captured the attention of humankind since antiquity or prehistoric era based on the use of *E. lancifolia* by early Mayan Indians (Schultes et al., 1987), while the medicinal effects of "euphorbium" (gum from *E. resinifera*) have been documented by Hippocrates, Galen and Dioscorides (Ernst et al., 2015). The name of the genus derives from the Greek physician Euphorbos of King Juba II of Numidia, who reported the use of a cactus-like *Euphorbia* as a powerful laxative. Recent studies have reported that more than 5% of *Euphorbia* spp. are used in folk medicine for the treatment of skin, digestive, genitourinary and respiratory system disorders, intestal parasites, infections, poisoning, pain and migraines, etc (Ernst et al., 2015). Over 2000 taxa from the genus *Euphorbia* L. have a wide distribution across the globe and are being distinguished by the production of a milky irritant latex, as well as the biosynthesis of diterpenes.

1.2.4.1. Diterpenes

Diterpenes (DTs) found in Euphorbiaceae family are also referred to as "Euphorbiaceae diterpenes" or "lower diterpenoids", in comparison to the "higher diterpenoids". Possessing an additional isoprene unit compared to SLs (see Figure 3 and Figure 14.A), the latter are bearing the classical "concertina-like" polycyclic cyclization of geranylgeranyl diphosphate and are not specific for Euphorbiaceae, while lower diterpenoids follow a different cyclization pattern through the formation of a 14-member macrocyclic ring (Figure 14.A). These DTs are considered important chemotaxonomic markers, because of their restricted distribution in Thymelaeaceae and Euphorbiaceae families. They are the focus of natural product drug discovery, as they are characterized by high therapeutically relevant bioactivity as well as chemical diversity, including the types of casbanes, jatrophanes, lathyranes, myrsinanes, tiglianes, ingenanes, segetanes, palarianes, pepluanes and euphoractines, see Figure 14.B (Shi et al., 2008, Vasas & Hohmann, 2014).

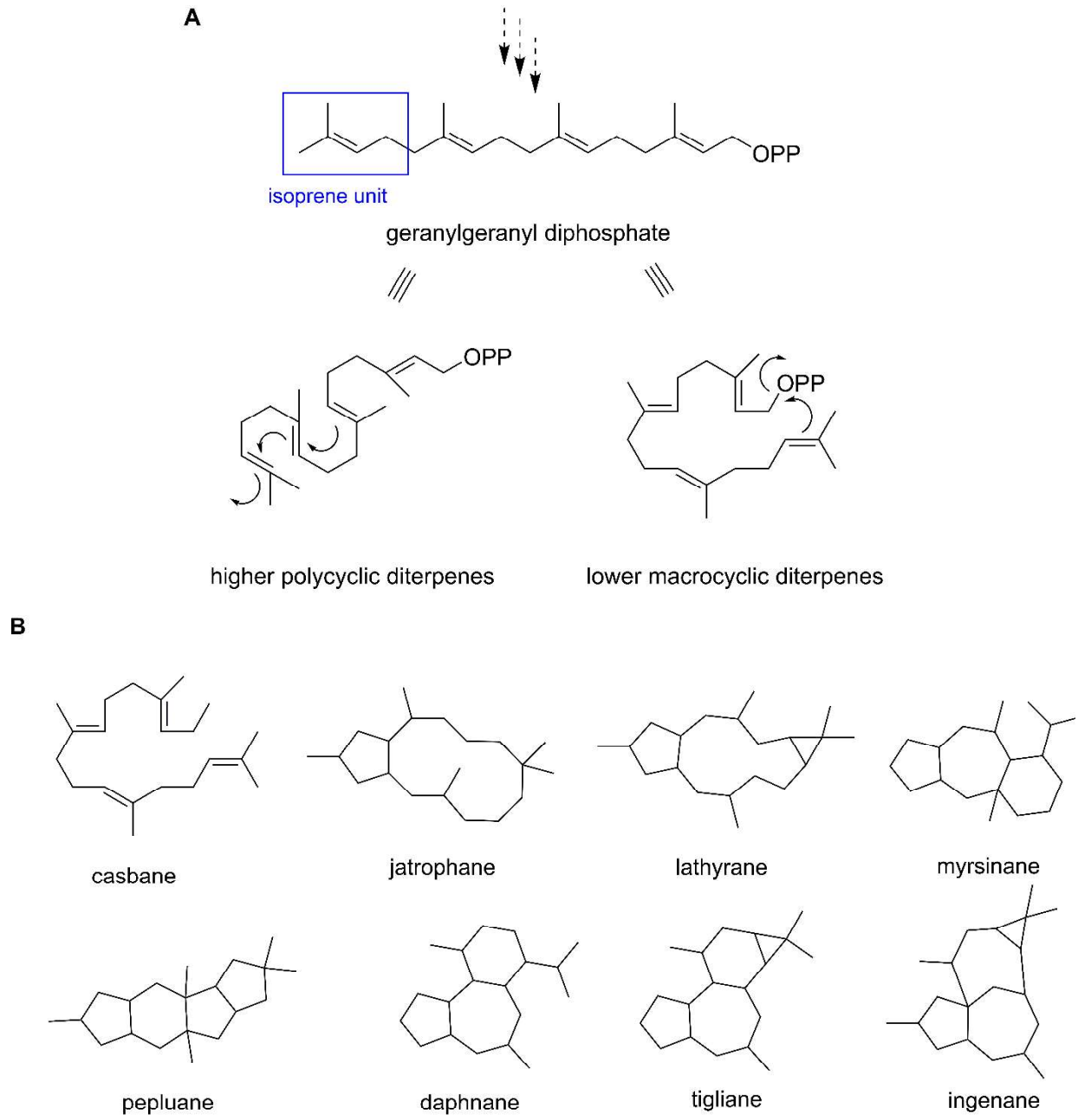

Figure 14. A. "Higher diterpenes" vs "lower diterpenes"; B. skeletal types of "lower diterpenes"

From the genus *Euphorbia* L., more than 650 DTs have been isolated up to date (Shi et al., 2008, Vasas & Hohmann, 2014). Ingenol-3-angelate (ingenol mebutate) has been approved by FDA in 2012 and EMA in 2013 for actinic keratosis, a precancerous skin condition (Saraiva et al., 2018), while some other DTs are in clinical or preclinical stages, such as resiniferatoxin, a capsaicin analog (Kissin et al., 2011) and prostratin, an anti-HIV agent (Sánchez-Duffhues et al., 2011) (Figure 15). Many more DTs have been reported to possess

important antiproliferative activity against different human cancer cell lines, including breast, gastric, ovarian, lung, and colon carcinomas. Taking into consideration the fact that only a few *Euphorbia* species have been investigated (ca. 5%), it is reasonable to expect finding other taxa producing higher amounts of ingenol mebutate, or DTs with similar or other therapeutically relevant biological activities (Ernst et al., 2016).

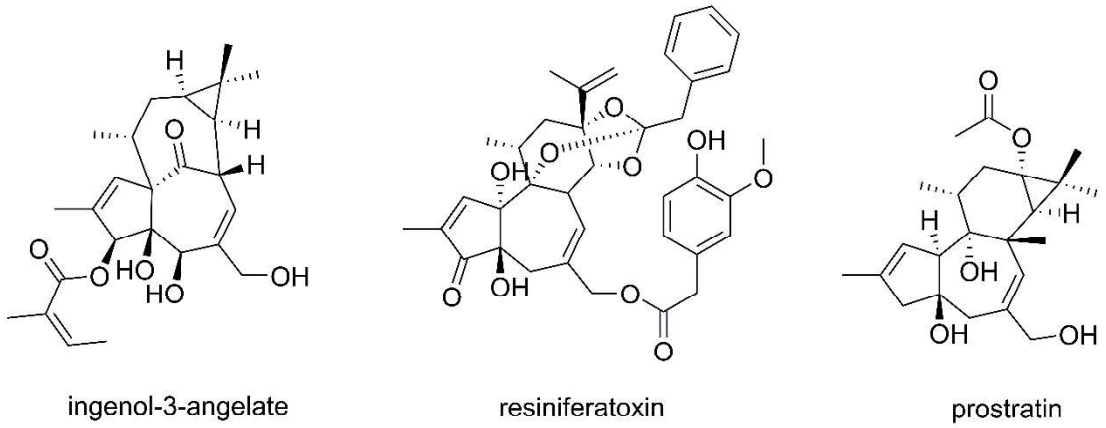

Figure 15. Notable DTs isolated from Euphorbia species.

1.2.4.2. *Euphorbia deflexa* Sibth. & Sm.

The under-investigation *Euphorbia* sp*., E. deflexa* Sibth. & Sm., is a narrow endemic plant from Central Greece. It is a glabrous, glaucous, ascending, caespitose perennial plant, branched at the base with orbicular to obovate oblong petiolate leaves, rays up to 3 times dichotomous, glands with 2 long horns, cyathium inflorescence, and grey ovoid seeds (Figure 16.A). The selected species belongs to section Paralias, subgenus Esula (Figure 16B). Extensive phylogenetic studies have supported the recognition of four major subgeneric clades (Geltman, 2015), with subgenus Esula containing one of the highest number of species with described medicinal uses (Ernst et al., 2015), while another recent study showed that *Euphorbia* species used medicinally are significantly phylogenetically clustered (Ernst et al., 2016). DTs isolated from section Paralias are presented in Appendix 7.5.

A

B

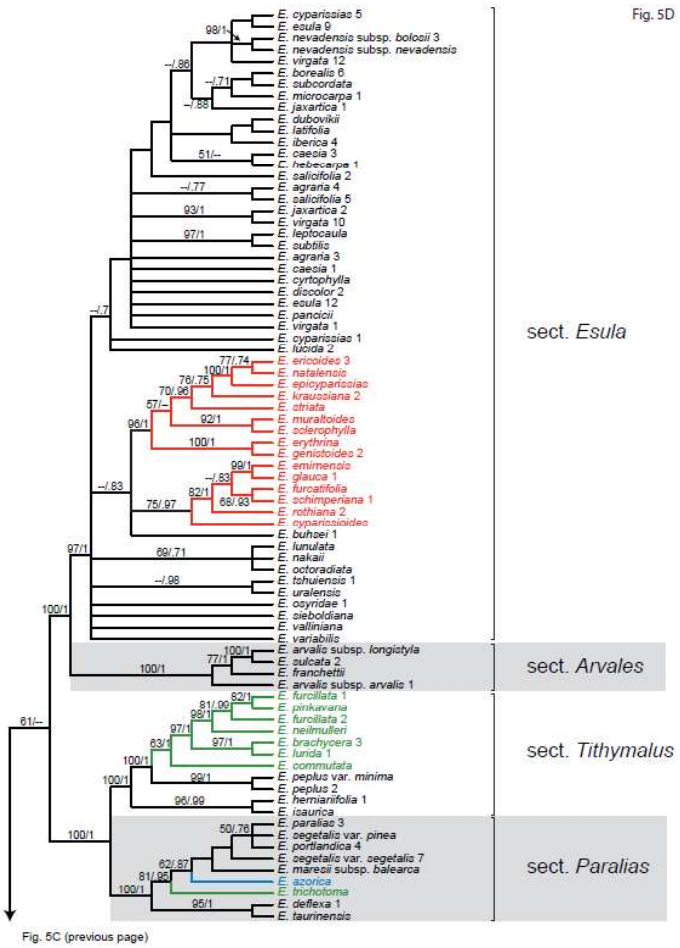

Figure 16. A. Photo of the plant Euphorbia; B. E. deflexa belongs to section Paralias, taken from Riina et al. (2013).

1.3. AIMS

The aim of this study was the investigation of endemic plants of cosmopolitan distribution, with the intention to discover novel drug leads against inflammation and cancer, which are major health problems in modern society. Emphasis was given to the highly bioactive groups of sesquiterpene lactones, acylphloroglucinols and diterpenes, which were targeted using the NMR-metabolomic strategy. Further use of state-of-the-art techniques enabled their isolation (HPLC-RI, HPLC-DAD), elucidation (NMR, MS/MS), and evaluation of pharmacological potential against multiple targets (MTT, ICAM, COX-LOX assays, and *in vivo* models), including *in silico* predictions of pharmacokinetic profiles. A further objective of this study was to broaden the current knowledge about the metabolism of sesquiterpene lactones.

The innovative aspects of the study covered by the topic of this thesis are multiple, considering that there are almost no records in the literature regarding the selected species using modern techniques and investigating the biological effects of their constituents. The project applied a multidisciplinary scientific approach relying on phytochemistry, chemotaxonomy, pharmacology, and drug design, while the evaluation of different phytochemical groups in parallel enabled a better understanding of their pharmacological potential.

2. MATERIALS AND METHODS

The phytochemical methods used (including general experimental procedures and plant material, extraction, and isolation) are described exhaustively in the Results section. Herein, a table (Table 2) including the equipment used will be presented in 2.1.1, as well as a summary of the plant material used in 2.1.2 (Table 3). Furthermore, the state-of-the-art spectrochemical methods and biological assays will be described in 2.1.3 and 2.2, respectively.

2.1. Phytochemical methods

2.1.1. Equipment

The solvents, chemicals and instruments used used for the phytochemical investigations during the course of this thesis are summarized in Table 2.

Table 2. List of solvents, chemicals and instruments used during the phytochemical analysis

Solvents & chemicals	
Acetonitrile	Supelco
Anisaldehyde-sulfuric acid agent (4-methoxybenzaldehyde)	Merck
Cellulose TLC plates	Merck
Chloroform-d	Sigma-Aldrich
Cyclohexane	Fischer Scientific
Diaion HP-20 (250-850 µm)	Supelco
Dichloromethane	Fischer Scientific
Ethyl acetate	Fischer Scientific
Methanol	Fischer Scientific
Methanol-d	Merck
n-Hexane	Sigma-Aldrich
Sephadex LH-20	Sigma-Aldrich
Silica gel (9385 230-400 mesh)	Merck
SVP D40 silica gel Flash column (13 x 4 cm, SI60 15–40 µm, 90 g)	Götec Labortechnik GmbH
TLC silica gel plates 60 F-254	Merck

Vanillin–sulfuric acid reagent (vanillin)	Merck
HPLC columns	
Kinetex Biphenyl (5 µm, 4.6 x 250 mm)	Phenomenex
Kinetex Biphenyl (5 µm, 21.2 x 250 mm)	Phenomenex
Kromasil RP-18 100si Semi-prep (5 µm, 10 x 250 mm)	Sigma-Aldrich
Zorbax RX-SIL normal phase (5 µm, 9.4 x 250 mm)	Agilent
XDB-C18 PrepHT (5 µm, 21.2 x 250 mm)	Agilent
Instruments	
Agilent 1290 Infinity prep. HPLC system, DAD detector	Agilent
Analytical scale Cubis	Sartorius
Astacus LS Ultra-Pure Water Purification System	MembraPure
Avance III 600 5 mm TBI CryoProbe	Agilent
Bruker DRX 400	Bruker BioSpin
Elite LaChrom Hitachi anal. HPLC system, DAD detector	Hitachi
Camag TLC visualizer	Camag AG
Cary 50 Scan UV-spectrophotometer	Varian
Jasco PU-2080 semi-prep HPLC system, RI detector	Jasco, Schimazu
JMS-T200GC AccuTOF GCX	JEOl
J-715 CD spectropolarimeter	JASCO
Melting Point B-545	BÜCHI
MS Q-TOF 6540 spectrometer	Agilent
Spot Flash Liquid Chromatography system	Interchim
Thermoplate S	Desaga Sarstedt-Gruppe
Ultrasonic bath: Ultrasonic cleaner	VWR
UniPol L 1000 polarimeter	Schmidt + Haensch GmbH & Co

2.1.2. Experimental part

A summary of the plants used in this study is presented in Table 3, and Figure 17 depicts the collection sites of the plants. Extraction and isolation procedures are described exhaustively in the Results section.

Table 3. Plant materials: collection sites, dates, and herbarium information of the under-investigation plants.

Plant	**Abb.**	**Collection site**	**Date of collection**	**Voucher specimen number**
Calea jamaicensis (L.) L.	CJ	Parque Nacional, Altos de Campana, Panama	11/07/2010	Herbarium of Panama University, no. Florpan 5458
Centaurea papposa (Coss.) Greuter	CP	"Cape de Garde" Annaba, Algeria	02/09/2016	Herbarium of Department of Biology, University Badji Mokhtar Annaba, (Djeddi & Skaltsa BV 01/2017)
Euphorbia deflexa Sibth. & Sm.	ED	Mountain Parnitha, Central Greece	16/05/2018	Department of Pharmacognosy and Chemistry of Natural Products, NKUA (Skaltsa & Grafakou 02)
***Hypericum* spp.**				
H. ambloycalyx Coustur. & Gand	HA	Avdou Village, Heraklion, Crete	13/05/2018	Herbarium of Natural History Museum, University of Crete, no. 15979
H. empetrifolium Willd.	HE	Potamies Village, Heraklion, Crete	13/05/2018	Herbarium of Natural History Museum, University of Crete, no. 15981
H. jovis Greuter	HJ	Kamaron gorge, Crete	22/06/2017	Herbarium of Natural History Museum, University of Crete, no. 15880
H. perforatum L.	HP	purchased from Florihana (LOT FLEO59-B120917F), France	-	-
H. triquetrifolium Turra	HT	Thessaloniki	27/05/2015	Department of Pharmacognosy and Chemistry of Natural Products, NKUA (Skaltsa & Grafakou 03)

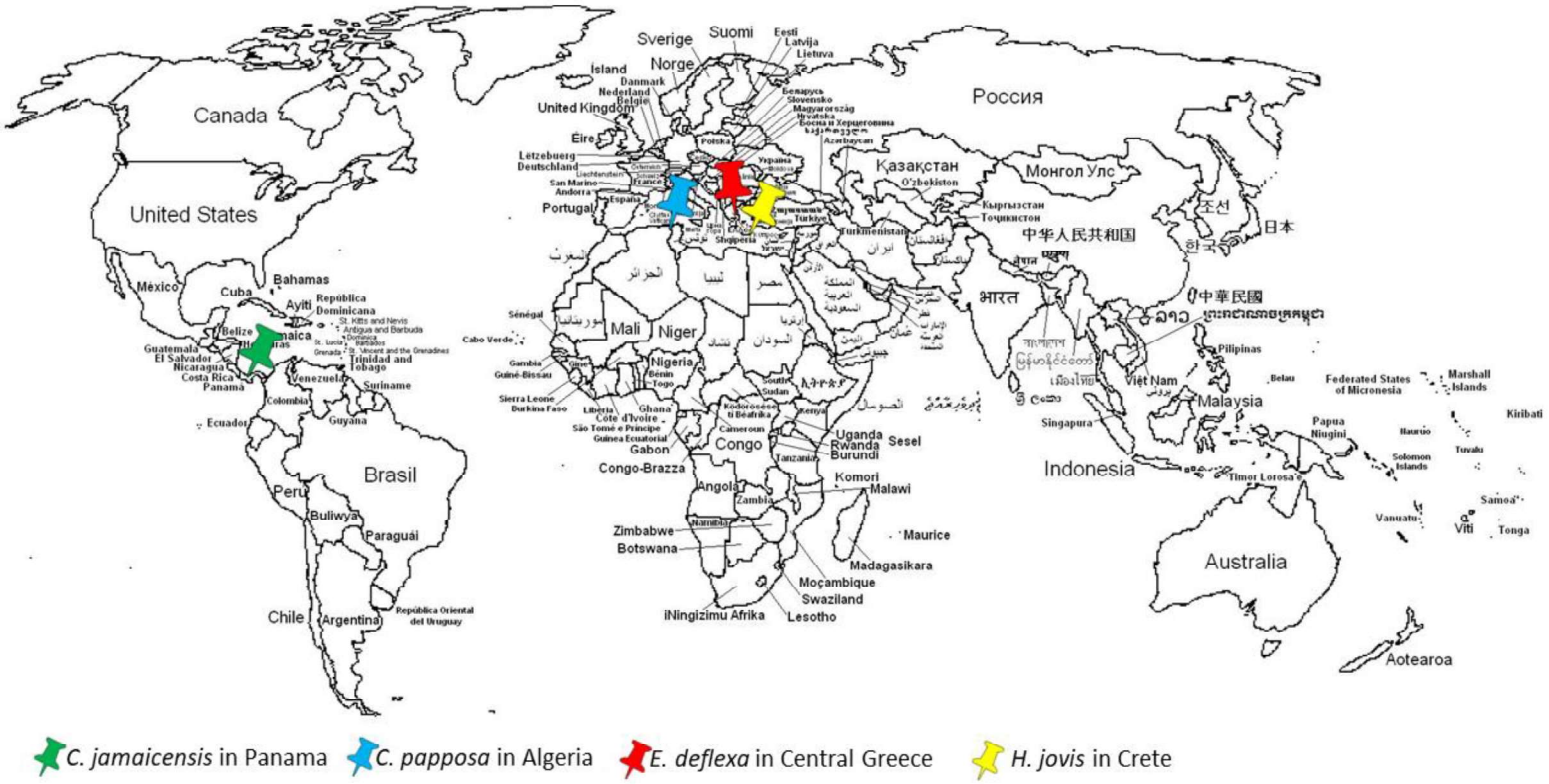

Figure 17. Map of the collection sites of the under-investigation species.

2.1.3. Spectrochemical analysis

2.1.3.1. NMR spectroscopy

NMR spectroscopy is an advanced analytical method, which is being used for the identification and characterization of biological small molecules. Following the NMR-metabolomic strategy, the whole fractionation and final isolation procedure are continuously monitored by NMR. More specifically, the extraction and fractionation are carried out by non-deuterated solvents and the resultant solutions are dried under vacuum and subsequently re-dissolved in a deuterated solvent (chloroform-d or methanol-d), followed by measurements of ^{1}H NMR data in 400 MHz NMR device and further analyzed in each step to identify the compounds of interest in the crude extracts/fractions.

After final purification steps, 2D spectra of the compounds were measured in 600 MHz NMR device (T = 298 K, in almost all measurements) equipped with CryoProbe (Bruker) at the Central Analytical Department, Faculty for Chemistry and Pharmacy, University of Regensburg. HSQC (heteronuclear single quantum coherence), HMBC (heteronuclear multiple bond correlation), COSY (correlation NMR spectroscopy) and NOESY (nuclear Overhauser enhancement spectroscopy) were used for the structure elucidation (processed in Topspin 4.1.1., Bruker).

2.1.3.2. Mass spectrometry

For the previously undescribed compounds, HRESIMS data were measured. A small aliquot of each isolated compound was analyzed in a Q-TOF 6540 spectrometer on the Central Analytical Department for Mass Spectrometry, Faculty for Chemistry and Pharmacy, University of Regensburg. Electrospray ionization was used in both positive and negative modes to ensure optimal ionization of the analyzed compound. The accurate mass and, therefore, the molecular formula, was calculated from the ions present in the spectrum, usually $[M+H]^+$ and $[M+Na]^+$, as well as from $[M–H]^–$ in negative mode.

For the *in vitro* metabolism assay, samples were analyzed with LC-MS/MS using the same spectrometer mentioned above. The separation was performed on a Phenomenex Luna Omega column (C18, 1.6 u, 100 A°, 50 × 2.1 mm) using a gradient of 0.1% formic acid (solvent A) and MeCN supplemented with 0.1% formic acid (solvent B). Gradient: 0.0–8.0 min, 0→30% B; 8.0–8.1 min, 30→98% B; 8.1–9.1 min, 98% B; 9.1–9.2 min, 98→5% B; 9.2–10.0 min, 5% B; flow rate, 0.6 mL/min; injection volume, 1 µL; oven

temperature, 40 °C. Data analysis was performed by MassHunter Workstation Software Qualitative Analysis (B.07.00, Agilent) using automatic mass spectrum integration.

For the essential oils, GC-MS analyses were carried out in Hewlett- Packard 7820A-5977B MSD system at the Department of Pharmacognosy and Chemistry of Natural Products, Faculty of Pharmacy, NKUA, operating in EI mode (70 eV), equipped with an HP- 5MS fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μm), and a split-splitless injector. A gradient from 60 to 300 °C at a rate of 3 °C/min was used, and helium was the carrier gas. A small amount of essential oil was diluted in pentane and the injection volume was 2 μM. Data analysis was performed with MSD ChemStation Data Analysis (Agilent).

2.1.3.3. UV/Vis spectroscopy

For the previously undescribed compounds, UV/Vis spectroscopic data were measured. A quartz cuvette (path length 10 mm) was used, the wavelength range was 200–800 nm, the temperature was 22-25 °C, pure MeOH was used as blank and the concentrations of the samples were chosen to be in the absorption linear range (< 1 AU), between 0.02 and 0.05 mg/mL. The molar absorption coefficient was expressed in the logarithmic form (log ε), based on Lambert–Beer law (Figure 18).

$$\varepsilon = \frac{A}{c \cdot d}$$

ε = molar absorption coefficient [L x mol^{-1} x cm^{-1}]
A = absorbance
c = concentration [mol/L]
d = path length [cm]

Figure 18. Formula for calculation of molar absorption coefficient.

2.1.3.4. Optical rotation

For the previously undescribed compounds, optical rotations were measured. Samples were dissolved in MeOH, in a concrete concentration around 1 mg/mL, and poured into a measuring cylinder (l = 50 mm). The wavelength was set to 589 nm – the wavelength of (sodium D line), the temperature was 22-25 °C and pure MeOH was used as blank. The specific rotation was calculated based on the formula given in Figure 19.

$$[a]_D^T = \frac{100 \cdot a}{c \cdot l}$$

α = optical rotation [°]
c = concentration [g/100 mL]
l = path length [cm]
T = temperature [°C]
D = value of sodium D line [589.44 nm]

Figure 19. Formula for calculation of specific rotation.

2.1.3.5. CD spectropolarimeter

For the previously undescribed compounds, CD-spectroscopic data were measured. Quartz cuvettes (l = 1 mm) were used, MeOH was used prior to measurements, concentrations were 250 µM in MeOH, and the temperature was 22-25 °C. The CD-spectrometric data – molar ellipticity (θ) were graphically plotted vs. wavelengths used during the analysis (190–350 nm). The formula for the calculation of θ is given in Figure 20.

$$[\theta]_M = \frac{\theta \cdot M}{10 \cdot c \cdot l}$$

$[\theta]_M$ = molar ellipticity [° x cm^2 x dmol^{-1}]
θ = ellipticity [°]
M = molar mass [g × mol-1]
c = concentration [g × mL-1]
l = path length [cm]

Figure 20. Formula for calculation of molar ellipticity.

2.1.3.6. X-ray Crystallography Analysis.

Suitable crystals were collected on a XtaLAB Synergy R, DW system, HyPix-Arc 150 diffractometer operating at T = 123.00(10) K, equipped with Cu Kα radiation (λ = 1.54184Å). The structure and absolute configurations were solved with the ShelXT 2018/223 solution program using dual methods and by using Olex2 1.3-alpha24 as the graphical interface (see Results 3.6).

2.2. BIOASSAYS

2.2.1. MTT Assay

The cytotoxicity of the SLs, DTs, and PAPs (Results 3.1, 3.2, 3.3, 3.6 and Discussion), was evaluated using the colorimetric MTT assay. MTT is a yellow tetrazolium dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which is reduced to its insoluble purple formazan in living cells (Figure 21.A). A solution of the detergent sodium dodecyl sulfate solution is usually added to dissolve the formazan product into a colored solution, and the absorbance is being measured at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer (Figure 21.B).

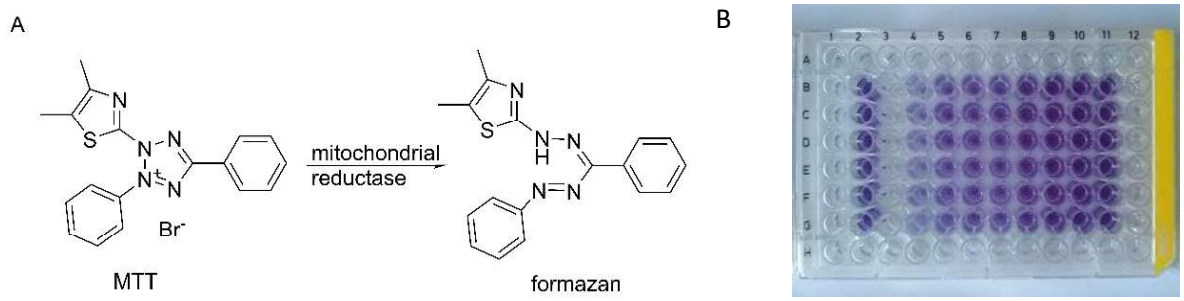

Figure 21. A. Reduction of MTT into formazan. B. Microplate after MTT assay.

The assay was performed as described before (Heilmann et al., 2001). In brief, the cytotoxicity was determined using the human cancer cell lines HeLa (cervical carcinoma, ATTC), SK-MEL-28 (malignant melanoma, DSMZ), HepG2 (hepatocellular carcinoma, DSMZ), and PC-3 (prostate carcinoma, DSMZ), as well as the non-cancerous HMEC-1 cell line (human microvascular endothelial cells (Ades et al., 1992)). Culture medium was MEM (Sigma; supplemented with L-Glutamine, FCS, and NEA, BioChrom), DMEM-HAM'S (50-50%, Invitrogen and BioChrom; supplemented with FCS), RPMI (BioChrom; supplemented with L-Glutamine and heat-activated FCS), and ECGM (EASY Endothelial Cell Growth Medium, supplemented with EASY Growth supplement and Gentamycin-Amphotericin (all PELOBiotech), and heat-activated FCS), respectively. Stock solutions of the samples were prepared in DMSO, and the test concentrations were freshly prepared with medium on the day of the experiment (final concentration of DMSO was 0.33%). Nine different concentrations of the compounds (100.0 to 0.39 µM) together with the cell suspension (final concentration in wells: 6 x 10^4 cells/mL, 3 x 10^4 cells/mL, 12.5 x 10^4 cells/mL, and 7.5 x 104 cells/mL, for HeLa, SK-MEL-28, HePG2, and PC-3, respectively), were added to the 96 well plates. The HMEC-1 cells in a final cell suspension of 90 x 10^4 cells/mL were placed in the 96-wells, and 24 h later they were treated

with 6.25, 12.50, 25.00, 50.00 µM of the tested SLs. After incubation for 68 h (or 24 h for the HMEC-1) (IBS Integra Biosciences, 37 °C, 5% CO_2 and 90% relative humidity), MTT was added (final concentartion in wells: 0.4 mg/mL), and after 4 h the solution was exchanged with 10% SDS. The plates were then placed in the dark and 24 h later, the absorbance at 560 nm was measured (SpectraFluor plus plate reader, Tecan).

2.2.2. ICAM assay

There are multiple mediators of inflammation and the inhibition of adhesion molecules like VCAM-1 (Vascular cell adhesion protein 1) and ICAM-1 (Intercellular Adhesion Molecule 1) is being investigated, aiming to discover novel anti-inflammatory agents. Upon cytokine stimulation, expression of adhesion molecules is increased on the endothelial cells, facilitating the leukocyte endothelial adhesion, in order to generate intracellular signals followed by inflammation processes under active participation of the endothelium, as shown in Figure 22 for ICAM-1 (Cook-Mills & Deem 2005, Harjunpää et al., 2019).

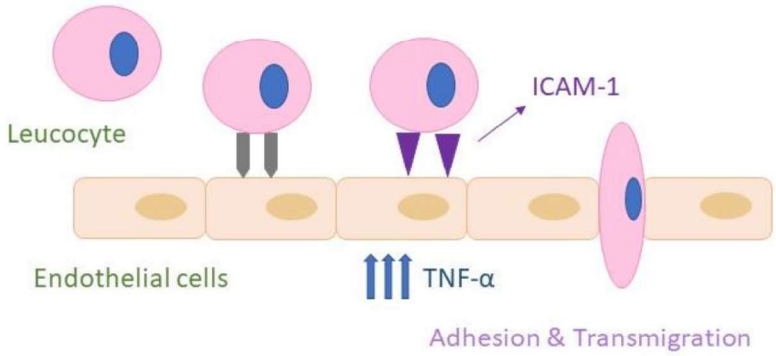

Figure 22. The role of ICAM-1 in the inflammation process.

Several SL and PAP derivatives were tested on the ICAM-1 assay (Results 3.1, 3.2 and Discussion), which was performed as described before (Freischmidt et al., 2012). In brief, confluent grown HMEC-1 cells from a culture flask (150 cm^2) were split (1:3), suspended in 13 mL medium and seeded in a 24 well plate [500 µL/well; plates were treated first with 0.25 % v/v Collagen G in PBS (both Sigma-Aldrich)]. They were cultivated for 48 h at 37 °C, 5% CO_2, and 90% relative humidity until they formed a monolayer. Subsequently, the supernatant was removed and the HMEC-1 cells were treated with the test compounds at not toxic concentrations based on the MTT results (as described above in 2.2.1.), parthenolide (5 µM, positive control, Calbiochem), and medium (ECGM, negative control). After 30 min, TNF-α (10 ng/mL,

Sigma-Aldrich) was added (except for two wells, in which no TNF-α was added in order to evaluate the ICAM-1 production in normal cells), and the plates were incubated at 37 °C, 5% CO_2 and 90% relative humidity, for 24 h. Then, the cells were washed with PBS, removed from the plates with trypsin/EDTA, and fixed with formalin. After centrifugation and removal of the supernatant layer, the cells were incubated with 5 µL of the Mouse Anti-human CD54: FITC (Bio-Rad) for 20 min, followed by fluorescence-activated cell sorting (FACS) analysis (Becton Dickinson FACSCalibur), measuring fluorescence intensity.

2.2.3. COX-1/12-LOX Assay

To further evaluate the anti-inflammatory potential, the tested compounds were subjected to the *ex vivo* cyclooxygenase (COX)-1 and 5-lipoxygenase (LOX) assay using human platelets, obtained from donors. COX and LOX are key enzymes in the production of eicosanoids, including leukotrienes, prostaglandins, and thromboxane, which are potent inflammatory mediators (Dennis & Norris, 2015), and the method is based on the inhibition of the biosynthesis of eicosanoids, such as 12(S)-hydroxy(5Z,8E,10E)-heptadecatrienoic acid (12-HHT), thromboxane B_2 (TXB_2), prostaglandin E_2 (PGE_2) and 12(S)-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenoic acid (12-HETE) (Figure 23), which are measured with LC-MS/MS.

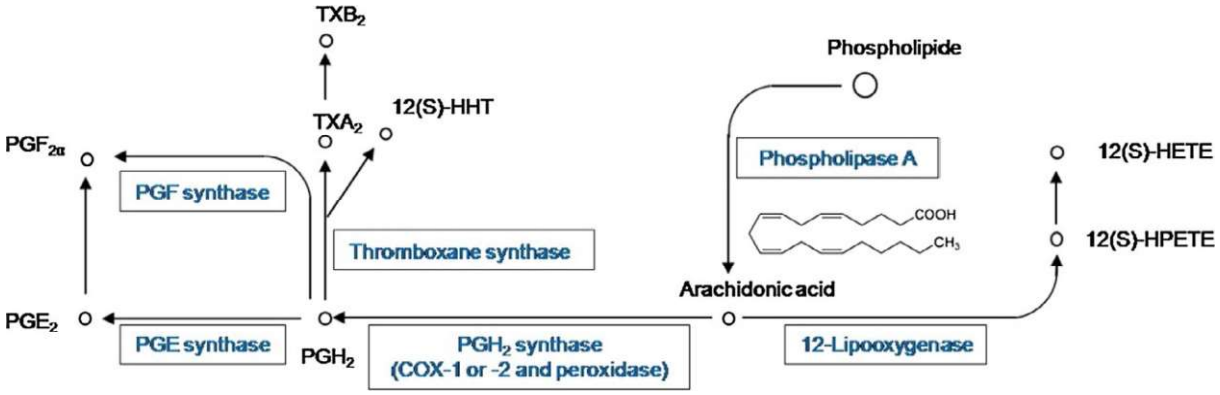

Figure 23. COX-1 and 12-LOX branches of AA metabolic pathway. 12-HPETE–12-hydroperoxyeicosatetraenoic acid; PGH2, PGE2 and PGF2α – prostaglandins; TXA2 and TXB2 –thromboxanes, taken from (Lesjak et al., 2020).

Compounds belonging to PAPs were tested in the assay (Results 3.3 and Discussion), based on the method described by Lesjak et al. (2020). In brief, an aliquot of human platelets (source of COX-1 and 12-LOX enzymes), viable, but outdated for medical treatment, which contained 4×10^8 cells, were suspended in buffer (0.137 mol/L NaCl, 2.7 mmol/L KCl, 2.0 mmol/L KH_2PO_4, 5.0 mmol/L Na_2HPO_4 and 5.0 mmol/L glucose, pH 7.2) to obtain the final volume of 2 mL. The tested concentrations of the samples in the test

tubes ranged from 0.25 to 2 μM. The mixture was slowly mixed at 37 °C for 5 min. Then, 0.1 mL of isolated compounds, or positive controls (aspirin and quercetin,) or DMSO (control and blank probes for control), and 0.1 mL of calcimycin (Calcium Ionophore A23187, 125 μmol/L in DMSO) were added and the mixtures were incubated for 2 min at 37 °C, with moderate shaking. Afterward, 0.3 mL of $CaCl_2$ aqueous solution (16.7 mmol/L), substituted with distilled water in the blank probe and blank probe for the control, were added and the mixture was incubated for another 5 min at 37 °C with shaking. Acidification with cold 1% aqueous formic acid (5.8 mL) to pH 3 terminated the reaction. The internal standard prostaglandin B_2 (PGB_2; 50 μL of 6 μg/mL solution in DMSO) was added to mixtures and extraction of products was done with a solution of chloroform/methanol (1:1 v/v, 8.0 mL) with vigorous vortexing for 15 min. After centrifugation (7012 g, 15 min, 4 °C), the organic layer was separated, evaporated to dryness, dissolved in methanol (0.5 mL), filtered, and used for LC-MS/MS analysis of eicosanoids (12-HHT, TXB_2, PGE_2, and 12-HETE). All samples and controls were analyzed in triplicate. Levels of COX-1 and 12-LOX inhibition achieved by different samples concentrations were calculated using the following equation: $I(\%) = 100 \times (R_0 - R)/R_0$, where R_0 and R were response ratios (metabolite peak area/internal standard peak area) in the control reaction and the samples of examined samples, respectively. Both R and R_0 were corrected for the value of appropriate blank probes.

2.2.4. Disc diffusion and double dilution assays

A screening of PAPs and DTs was performed using the following two techniques. The bacteria used were *Staphylococcus aureus* (DSM20231) and *Streptococcus pyogenes* (DSM20565-0316-001) cultivated in 2% v/v LB Broth, Alfa Aesar and 3.7 w/v Brain Heart Infusion Broth, Sigma-Aldrich, respectively at 37 °C (Incubating Shaker, VWR).

For the disc diffusion method, using aseptic techniques, broth overnight cultures of *S. aureus* and *S. pyogenes* were collected with a sterile swab and seeded into the respective agar plates (2% LB Broth + 1.5% Agar in bi-distillated water or 20 g Sheep Blood Agar Base in 500mL bi-distillated water for *S. aureus* and *S. pyogenes* respectively, autoclave for 15 min in 125 °C, let cool at 50 °C; for *S. pyogenes* add 7% sheep blood before placing in the agar plates). To obtain uniform growth, the agar plate was streaked with the swab in one direction, rotated 120° and streaked again, rotated another 120° and streaked again. Using flame-sterilized forceps the filter paper disks (BD Sesni-Disk) were impregnated into the samples or chloramphenicol (positive control, Sigma-Aldrich) stock solutions (1mg/mL), and then placed on top of the agar surface. Plates were then incubated overnight (37 °C, IFPlus, Memmert).

The double dilution method was carried out as described before by Winkelmann et al. (2003) with slight modifications. The overnight cultures of *S. aureus* and *S. pyogenes* were diluted to 10^8 cells/mL in fresh medium. The required amounts of samples or chloramphenicol (positive control) (1mg/mL) were pipetted into the 96 well plates, left to dry and redissolved with 50 µL DMSO, 50 µL medium and 100 µL diluted bacterial suspension. Incubation at 37 °C (IFPlus, Memmert) for 20 h was followed by the addition of 20 µL aqueous 0.25 % MTT, re-incubation for 4 h and detection of the living bacteria as violet turbid solutions.

2.2.5. In vivo assays

The Small Animal Laboratory of the Faculty of Pharmacy (EL 25 BIO 07) is operating following the guidelines established by the European Communities Council Directive (Directive 2010/63/EU of 22 September 2010). The protocols used were approved by the National Peripheral Agricultural & Veterinary Authority, Region of Attica. The SKH-hr1 mice, originated from the breeding stock of the Small Animal Laboratory, were living in special cages (Tecniplast) of dimensions in accordance with current legislation, in which there was unrestricted continuous access to fresh water and standard chow diet (Nuevo SA-Farma-Efyra Industrial and Commercial SA, Greece) 24 hours a day. The lighting (day-light cycles of 12 hours), temperature (22-25 °C) and humidity (35-55%) conditions were selected and maintained according to the respective Greek and European legislation.

2.2.5.1. Wound healing

Wound healing is a complex process of four phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution. Failure in one of these phases, related to infection, ischemia, metabolic conditions, immunosuppression, age, poor nutritional status, etc., leads to chronic non-healing wounds, whose management is still in need of new treatments (Mahmoudi & Gould, 2020). As a multifactorial phenomenon, wound healing cannot be satisfactorily simulated by *in vitro* models, thus *in vivo* models are selected. As mentioned in 1.2.3.2., *Hypericum* has long-term use in wound-healing, however, the EOs which are important constituents have not been tested, so far. For this reason, EOs from three *Hypericum* spp. were obtained in amounts high enough to conduct *in vivo* testing and the experimental procedures are exhaustively described in Results (3.4).

The experimental procedure was approved by the National Peripheral Agricultural & Veterinary Authority, Region of Attica (Protocol Number: 1064/20-02-2019) after the affirmative opinion of the Animal Protocols Evaluation Committee.

In brief, female SKH-hr1 (age 3 weeks-3 months) were subjected to full-thickness (i.e., epidermis, dermis, and subcutis) wounds of 1 cm^2 (1.0 cm x 1.0 cm) on their dorsal skin under anesthesia using intra-peritoneal administration of a cocktail of ketamine (100 mg/kg) and xylazine (7 mg/kg). The EOs were formulated into ointments using petrolatum jelly as excipient at two doses (0.05 and 0.5%) (Table 4). adecassol cream (*Centella asiatica* 1%) was used as a positive control. The topical formulations were applied once daily for 14 days, until healing was clinically observed in one of the groups. The evaluation of the wounds was based on measurements of skin parameters (TEWL, hydration, erythema, skin thickness and elasticity), photodocumentation, clinical and histopathological assessment.

Table 4. Treatments/ Topical formulations used in wound healing protocol.

Control	**Control group, w/o treatment**
Petrolatum	Petrolatum only (excipient)
Madecassol	Madecassol cream (positive control)
HP 0.05%	*H. perforatum* 0.05% ointment
HP 0.5%	*H. perforatum* 0.5% ointment
HT 0.05%	*H. triquetrifolium* 0.05% ointment
HT 0.5%	*H. triquetrifolium* 0.5% ointment
HE 0.05%	*H. empetrifolium* 0.05% ointment
HE 0.5%	*H. empetrifolium* 0.5% ointment

2.2.5.2. Atopic-Dermatitis

Atopic dermatitis is the most common inflammatory skin disease worldwide. It is a multifactorial phenomenon, involving environmental and genetic factors, especially immune system dysfunction and difficulties with the permeability of the skin (Tollefson et al., 2014). No cure is known, and the most common treatments are topical corticosteroids and calcineurin inhibitors, which cause a variety of side effects, thus there is an emerging need for new drug leads against atopic dermatitis. One compound

isolated from *Hypericum jovis* in high amounts and with proven anti-inflammatory effects (Results 3.3), namely 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol, was tested *in vivo* on atopic dermatitis (Results 3.7.2), based on the protocol described by Kesente (2018) and Drakopoulou (2019).

The experimental procedure was approved by the National Peripheral Agricultural & Veterinary Authority, Region of Attica (Protocol Number: 163329/26-02-2020) after the affirmative opinion of the Animal Protocols Evaluation Committee.

In brief, male hairless SKH-hr1 mice (age 1.5-4 months) were treated with hapten 2,4-dinitrochlorobenzene (DNCB) in a period of 43 days (Figure 24). This is a satisfactory model that mimics the human condition, experimentally and physiologically (Loddé et al., 2020, Oiso et al., 2005). Topical formulations (0.15% w/w) of 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol and rosmarinic acid (positive control) were developed using PEG400/PEF3000 (8/2) (excipient) (Table 5) and applied on the dorsal skin of the mice daily for 20 days (Figure 24). Similar to the wound healing protocol (Results 3.4), the skin condition was evaluated based on measurements of skin parameters (TEWL, hydration, erythema, skin thickness), photodocumentation, clinical and histopathological assessment, as well as behavioral monitoring using a video camera (distance, rearing, freezing and scratching analyzed with Kinovea).

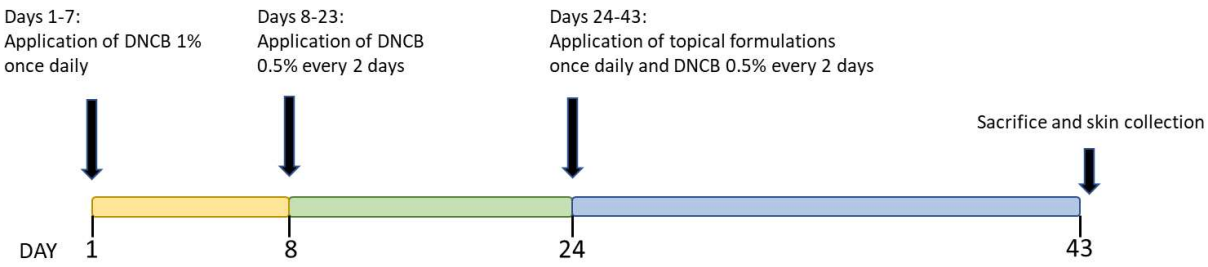

Figure 24. Workframe of the experimental procedure for atopic dermatitis protocol.

Table 5. Treatments/ Topical formulations used for atopic dermatitis protocol.

Control	**Control group, w/o treatment, only DNCB**
PEG	DNCB and excipient only (PEG400/PEF3000 8:2)
Rosmarinic acid	DNCB and topical formulation of rosmarinic acid 0.15% in PEG400/PEF3000 8:2 (positive control)
3-geranyl-1-(2-methylpropanoyl)-phloroglucinol	DNCB and topical formulation of 3-gernayl-1-(2-methylpropanoyl)-phloroglucinol 0.15% in PEG400/PEF3000 8:2 (test compound)

2.2.6. *In vitro* metabolism using Human Liver Microsomes

Drug metabolism is carried out in two general phases: in phase I polar groups are either introduced by oxidation, reduction, and hydrolysis, or uncovered by dealkylation, and in phase II polar groups are conjugated with glucuronic acid, sulfate, glycine, glutamine, glutathione, acetate, or methyl groups (Gillette, 1971), to de-activate and facilitate the excretion of the parent drug. In some cases, more active forms of the parent drug (prodrug) or toxic metabolites can arise from the biotransformation reactions, which occur mainly in the liver, but also in other tissues, including the microbiota in the GI tract.). *In vitro* drug metabolism studies account for a useful tool in basic research, as well as preclinical screening of drug-like properties. There is a number of different *in vitro* systems, and among them, human liver microsomes (HLM) are the model of choice for drug metabolic profiling, providing an affordable way to give a good indication of the CYP and UDP-glucuronyltransferases (UGT) involved in the metabolism of a drug (Parmentier et al., 2007, Sowjanya et al., 2019). Microsomes are artificial subcellular particles derived from the hepatocyte endoplasmic reticulum of hepatic cells, formed during tissue homogenization (Jagow et al., 1965). They are prepared by differential centrifugation at 10,000 and 100,000 × g and contain membrane phase I enzymes namely CYPs, flavine-containing monooxygenases (FMO), esterases, amidases, and epoxide hydrolases, and also the phase II enzymes such as UGTs, but they lack soluble enzymes. For the catalytic activity of both phase I and II enzymes, the addition of exogenous cofactors such as NADPH for CYPs and FMO, and UDPGA/alamethicin for UGTs is necessary.

Sesquiterpene lactones, as promising drug leads with drug-like physicochemical properties, were selected for further investigation of their *in vitro* metabolites using HLM and LC-MS/MS (Figure 25). Despite increasing data regarding bioactivity, to date, there are only a few reports on the metabolic pathways and

metabolites of some SLs, reporting unstable absorption and extensive metabolism with the identification of phase I and II metabolites using different metabolism systems, mainly through *in vivo* rat models or liver microsomes (Yu et al., 2021).

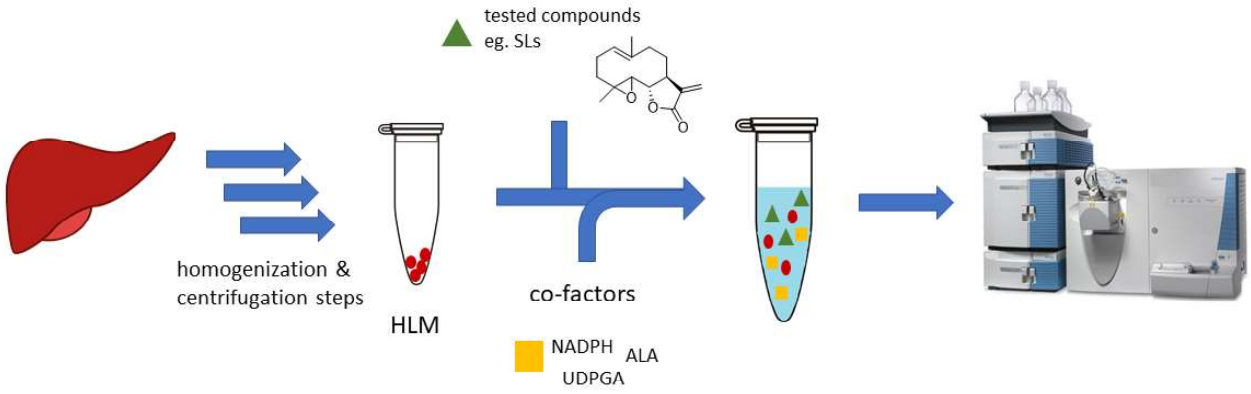

Figure 25. In vitro metabolism study using HLM and LC-MS/MS.

The protocol used was based on the study of Zenger et al. (2015). The reagents and chemicals used and especially: Glucose-6-phosphate sodium salt (G6P), glucose-6-phosphate dehydrogenase (G6PDH), nicotinamide adenine dinucleotide phosphate sodium salt hydrate (NADP), uridine 5'-diphosphoglucuronic acid trisodium salt (UDPGA), and alamethicin (Ala) from Trichoderma viride were obtained from Sigma-Aldrich; frozen human pooled liver microsomes (HMMCPL) and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Gibco (Thermo Fischer Scientific). G6P, G6PDH, NADP, UDPGA, Ala and microsomes were stored prior to use in aliquotes at -20 and -80 °C, respectively, to avoid frequent-thaw cycles. Methanol (MeOH) and ethanol (EtOH) were obtained from Merck. In brief, phase I incubation mixtures (NADPH regeneration system) with a total volume of 1mL in DPBS contained 0.5 mg HLM, 3.3 mM $MgCl_2$, 3.3 mM G6P, 0.4 U/mL G6PDH, 1.29 mM NADP, and 10 µM test compound. Phase II glucuronidation is based on the addition of UPDGA and the mixtures comprised 0.5 mg HLM, 3.3 mM $MgCl_2$, 2 mM UDPGA, 25 µg/mL Ala, and 10 µM test compound. Combined phase I and II metabolism mixtures included all of the above-mentioned reagents of Phase I and II. Mixtures without microsomes, or cofactors (NADP or UDPGA) were used as negative controls, matrix samples without test compound were used as blank controls and mixtures with the addition of only the tested compound in DPBS were used to investigate the stability of the compounds in the given conditions of the assay. The incubation systems are presented in Table X. Incubation was carried out in a stirred water bath at 37 °C for 3 h and was terminated by the addition of the same volume of ice-cold EtOH. Mixtures were vigorously vortexed

for 5 min and centrifuged for another 5 min (14000 rpm, 4 °C). The supertants were dried under N2 and stored at −20 °C until further analysis. All experiments were repeated at least three times. The Liquid Chromatography High-Resolution Quadrupole Time-Of-Flight Mass Spectrometry (LC-Q-TOF-MS/MS) analysis was performed with UHPLC Agilent 1290 infinity system as described in 2.1.3.2.

Table 6. Pipetting scheme for the different incubation systems (total volume 1 mL) in µL.

Reagents*	matrix	stability	w/o microsomes	w/o cofactors	Ph I	Ph II	Ph I+II
DPBS	775	990	790	845	865	835	765
MgCl2	30	-	30	30	30	30	30
G6P	30	-	30	30	30	-	30
G6PDH	10	-	10	10	10	-	10
Ala	50	-	50	50	-	50	50
Micr	25	-	-	25	25	25	25
Stock	-	10	10	10	10	10	10
NADP	30	-	30	-	30	-	30
UDPGA	50	-	50	-	-	50	50

* $MgCl_2$ 110 mM in DPBS; G6P 110 mM in DPBS; G6PDH diluted in 5 mM sodium citrate solution to 40 U/mL; Ala dissolved in MeOH and ultra-pure water (2%, v/v) to a final concentration of 0.5 mg/mL; NADP 43 mM in DPBS; UDPGA 40 mM in DPBS. The reagents were pipetted in descending order.

2.2.7. *In silico* predictions of ADME properties

An important tool to study pharmacodynamic and pharmacokinetic properties of chemical compounds is via application of Molecular Modelling, which is a technique that marked an enormous growth in the last years, providing important information for the development of innovative drugs (drug design) (Aminour et al., 2019). Although molecular modeling still has not found general application in natural product research, several studies bring in light its important contribution in the natural drug discovery and refer to the

combination of Ethnopharmacology and molecular docking, virtual screening of a pure compound library for the discovery of novel bioactive natural compounds, virtual screening of a protein library for target identification, discovering natural lead compounds, study of ADME (Absorption, Distribution, Metabolism, Excretion) properties. Based on the obtained results from the *in vitro* bioassays and, five promising compounds were selected, and using the freely available in silico ADME tool SwissADME the pharmacokinetic profiles were predicted (Daina et al. 2017). It is a web tool that gives access to fast and robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness, and medicinal chemistry friendliness.

2.3. Statistical Analysis

IC_{50} values were calculated from nine different concentrations and tests were performed in sextuplicate for the MTT assay. For ICAM-1 assay each experiment was performed in duplicate and GraphPad Prism 8.4.2 version was used for the creation of the graphical representations, as well as the calculations of the IC_{50} values using non-linear regression. For all the bio-assays, all experiments were repeated at least three times and the results were presented as means ± SD for each sample. For the *in vivo* testing, means of two groups were compared with paired samples t-test or one sample t-test where applicable. Statistical significance (2-tailed) was set at $*p < 0.05$, $**p < 0.01$, or $***p < 0.001$. All continuous variables were checked for normality and statistical analyses were performed using IBM SPSS statistics 26.

3. RESULTS

3.1. Calea jamaicensis (L.) L.

The isolation of the compounds from *C. jamaicensis* is presented in Figure 26. Extraction, isolation, structure elucidation, and biological activities are presented in Grafakou et al. (2021a).

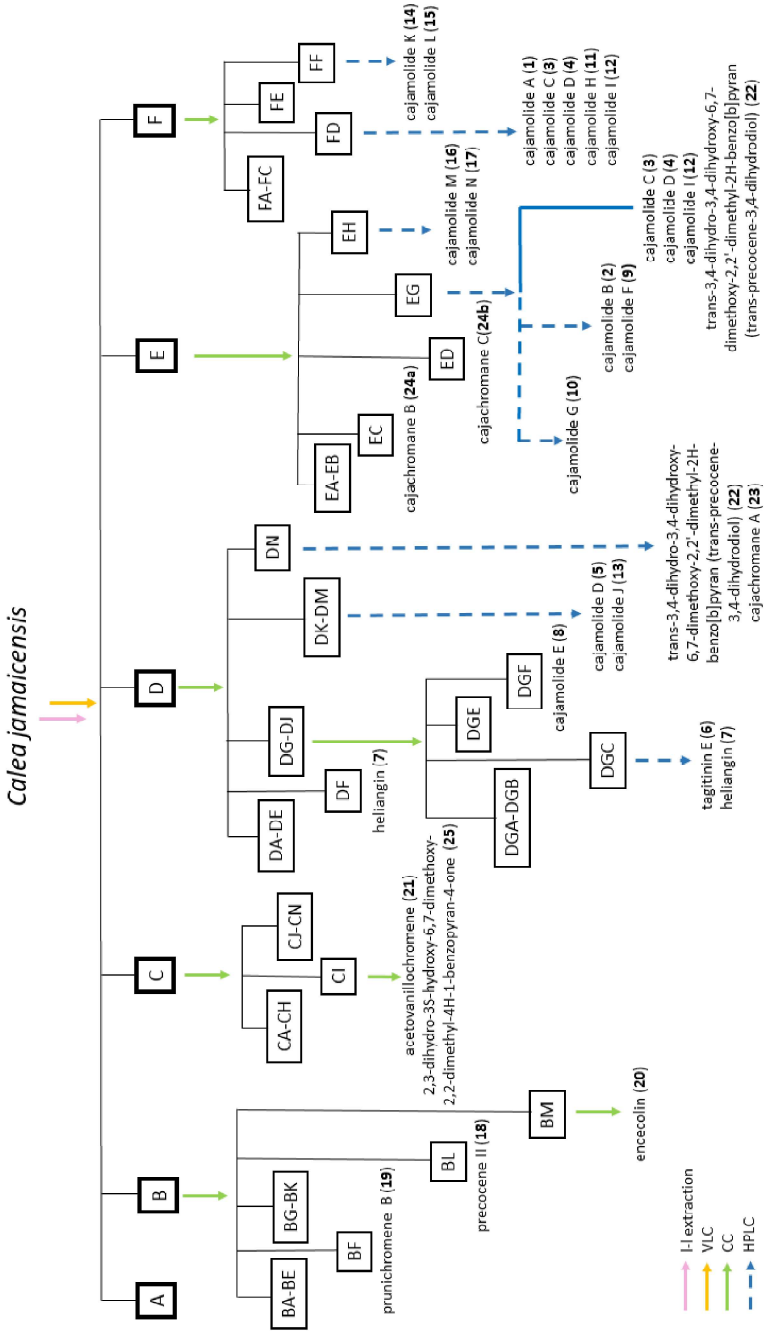

Figure 26. Chromatographic isolation of the compounds from C. jamaicensis.

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Cajamolides A-N: Cytotoxic and anti-inflammatory sesquiterpene lactones from *Calea jamaicensis*☆

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ABSTRACT

In the search for bioactive compounds from natural sources against cancer and inflammation, *Calea jamaicensis* (L.) L., a plant endemic to Panama, was investigated. Using multiple chromatographic steps, seventeen sesquiterpene lactones together with nine chromene derivatives were isolated from the non-polar extract of the plant. Among them, fourteen sesquiterpene lactones and three chromanes are described herein for the first time. Structure elucidation was achieved by extensive spectroscopic analysis and comparison with reported data. The isolated sesquiterpene lactones were tested against HeLa, SK-MEL-28 and HePG2 cancerous cell lines for their cytotoxic effects, as well as in the ICAM-1 assay for their anti-inflammatory potential. This study revealed strong cytotoxic agents on the one hand and strong inhibitors on the other. The compounds inhibited the TNF-α induced ICAM-1 expression on the endothelial HMEC-1 cells in a dose-dependent manner and with no toxicity observed on this non-cancerous cell line. In addition to the well-known cytotoxic activities of sesquiterpene lactones, further exploration may offer a novel therapeutic approach to cope with inflammatory diseases and the genus *Calea* may serve as a biobank for the isolation of potential phytopharmaceuticals, which could be utilized as leads in drug discovery and therapy.

1. Introduction

In modern society, the developed countries are required to cope with chronic degenerative disorders associated with the environment, lifestyle, stress, as well as the increasing life expectancy of the general population. Chronic inflammatory diseases place a significant burden on patients suffering and economic cost in the developed world [1]; overall, the estimated prevalence of inflammatory disorders in Western countries is estimated from 5 to 7% [2]. Chronic inflammation may also increase the risk of certain cancers, as persistent inflammation results in DNA damage, which in turn leads to cancer [3]. Considering the current decrease of new drugs introduced to the market, along with nature's high potential of yielding therapeutically relevant bioactive compounds, plant metabolites are emerging as new lead structures for the development of novel drugs for the prevention and cure of diseases of modern civilization [4–6]. The exploitation of natural products as sources of novel structures is reporting constant growth, a trend expected to continue in the coming years, i.e., in the area of cancer 74.8% of the small molecules are other than synthetic, with 48.6%, actually being either natural products or directly derived therefrom. In other areas, the influence of natural product structures is quite remarkable, with the anti-infective area being dependent on natural products and their structures [7]. Moreover, the role of natural products in the discovery of new drugs with anti-inflammatory and immunomodulatory potential is progressively enhanced [8].

The Asteraceae family is characterized by the ability to biosynthesize sesquiterpene lactones (SLs), which are produced by widely used medicinal plants such as *Arnica montana*, *Tanacetum parthenium*, *Matricaria chamomilla* [9]. SLs are highly active compounds, bearing an α-methylene-γ-lactone ring which is reacting through Michael type additions with sulfhydryl groups in biological systems [10]. These compounds exhibit a broad range of biological activities [11], and they are well

☆ This article is dedicated to the memory of Professor Mahabir P. Gupta (University of Panama) for his immense contribution to the field of Pharmacognosy.

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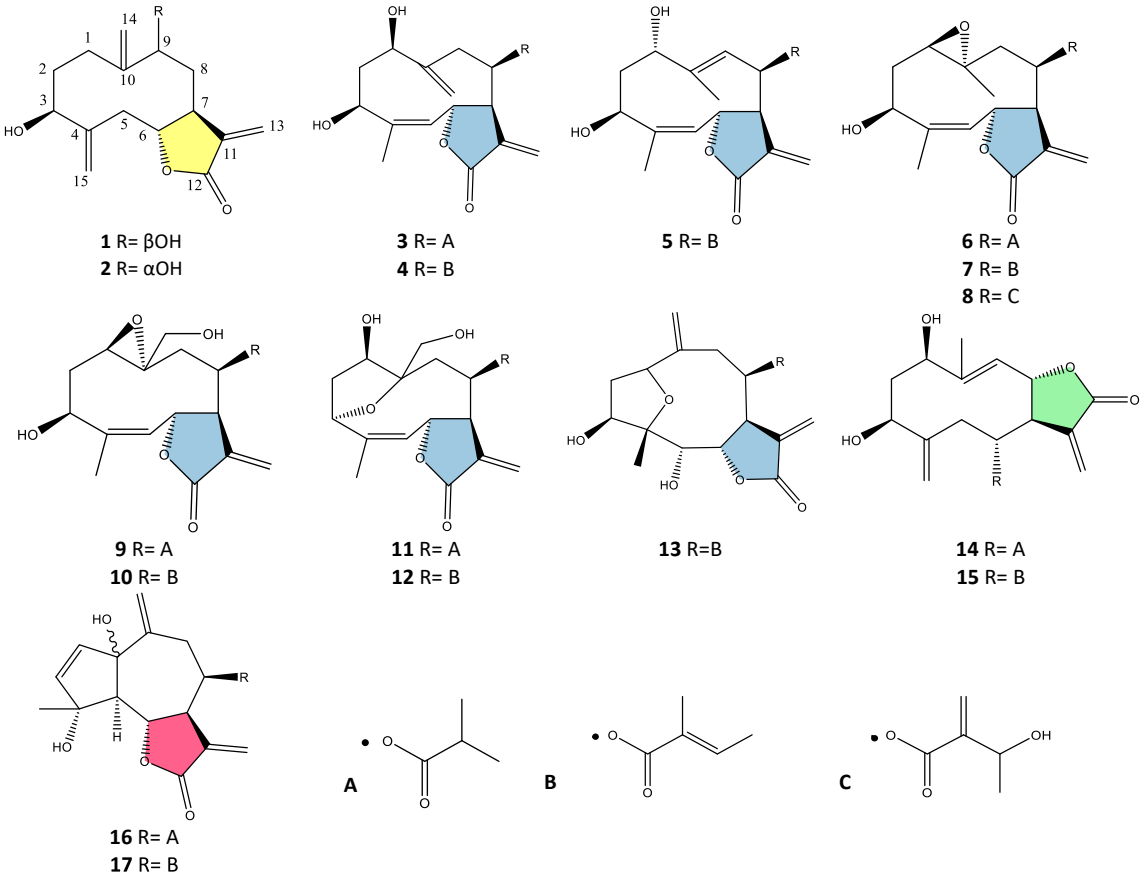

Fig. 1. Structures of compounds **1**–**17**. Different colors indicate germacranolides (yellow), heliangolides (blue), 8–12-germacranolide (green) and guaianolides (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

known for their cytotoxic effects, with some of them being in cancer clinical or preclinical trials (thapsigargin, parthenolide, or their semi-synthetic derivatives) [12], while others could possibly reach clinical phases in the near future (alantolactone, costunolide) [13].

The genus *Calea* L. belongs to the family Asteraceae (tribe Heliantheae Cass., subtribe Melampodiinae Less.), comprises about 125 species and its distribution is mainly in tropical and subtropical areas of the Americas [14]. The name *Calea* is derived from the Ancient Greek word *κάλλος* (kalos, 'beautiful') in allusion to the ostentatious inflorescence [15]. The genus has a long-term use in folk medicine throughout its geographic distribution, including the uses in hypertension, skin infections and dermatitis, gastroenteritis and diarrhoea, rheumatism, diabetes, ulcers, fever, liver and urinary problems [16]. *Calea* species are characterised by the presence of secondary metabolites belonging to SLs, chromenes and p-hydroxyacetophenone derivatives [16].

In case of selecting uninvestigated plants from regions with high diversity and endemism, the probability of finding novel bioactive compounds is higher and more propitious, due to nature's high potential of yielding therapeutically relevant bioactive structures [17]. Based on these criteria, *C. jamaicensis* (L.) L. was selected, which is an endemic plant in Mexico and Central America, as well as Jamaica [18]. This whole region belongs to the biodiversity hotspots [19]. Thus, the aim of

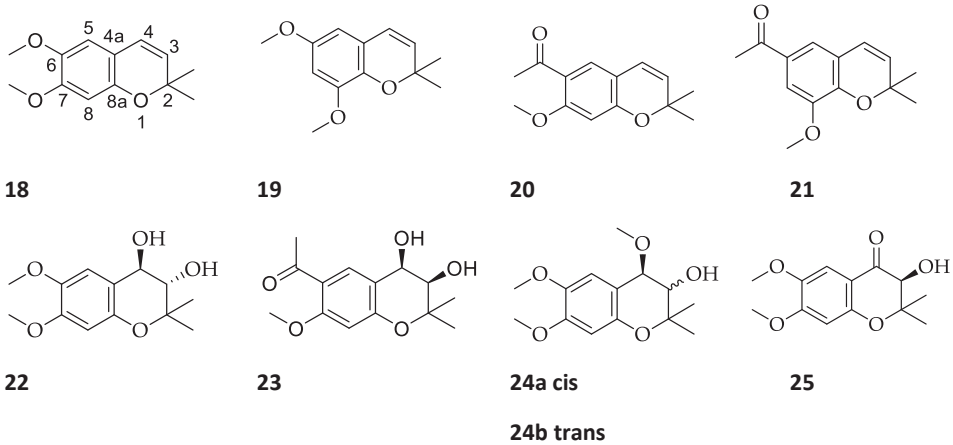

Fig. 2. Structures of compounds **18**–**25**.

Table 1
^{1}H NMR data of compounds **1**–**3**, **5** and **8**; Solvent: $CDCl_3$, Ava 600 MHz (T = 298 K).

	1		2		3		5		8	
Position	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c
1	2.15, *, 2.11, *	22.2	2.28, m, 1.85, m	26.5	4.08, dd (11.5, 3.0)	72.0	4.80, dd (11.5, 4.2)	65.5	2.82, dd (10.2,4.3)	60.5
2	2.18, *, 2.09, *	31.9	2.22, m, 1.97, m	31.6	2.38, m, 2.09, dd (5.2, 3.3)	37.7	2.23, ddd (15.0, 11.5, 3.2)	35.4	2.47, dt (14.9, 4.3)	32.6
							2.12 dt (15.0, 4.2)		1.75, m	
3	4.13, dd (10.4, 4.2)	73.0	4.23, *	73.4	4.52, dd (5.2, 2.5)	72.4	4.55, br t (3.2)	74.0	4.51, br t (2.3)	72.4
4	–	143.4	–	144.5	–	141.6	–	146.2	–	142.1
5	3.11, dd (12.7, 5.7) 2.10, *	40.0	3.10, dd (13.0, 4.7), 2.11, m	39.9	5.33 dd (9.3, 1.5)	126.4	5.33, d (9.6)	120.6	5.34, d (11.0)	126.6
6	4.23, ddd (10.7, 5.7, 3.7)	83.2	4.23, *	83.5	6.30 dd (9.3, 1.5)	73.3	5.96, dd (9.6, 5.9)	67.9	6.67, dt (11.0, 1.6)	74.0
7	2.52, m	39.8	2.82, m	38.9	3.09 *m*	47.1	3.17, m	50.2	2.89, m	48.5
8	1.90, m 1.94, m	38.9	2.02, m	37.9	5.16, m	75.1	5.92, t (9.8)	74.9	5.20, m	76.9
9	4.31, dd (11.6, 4.7)	77.0, *	4.29, m	72.3	2.84 dd (15.1, 6.1), 2.57, dd (15.1, 3.5)	29.4	5.43, d (11.1)	122.9	2.86, dd (15.1, 4.4), 1.36, *	43.6
10	–	148.2	–	149.2	–	144.4	–	144.2	–	59.9
11	–	141.2	–	140.5	–	137.4	–	136.0	–	137.9
12	–	169.3	–	170.3	–	169.6	–	169.7	–	169.7
13	6.24, d (3.3) 5.65, d (2.8)	121.1	6.29 d (3.4) 6.09 d (2.7)	124.5	6.34, d (2.3) 5.73, d (2.1)	124.3	6.28, d (3.2) 6.07 d, (2.9)	121.8	6.38, d (1.6) 5.78, d (1.6)	124.9
14	5.26, s 5.22, s	116.4	5.33, s 5.20, s	111.4	1.78, s	22.9	1.86, s	16.8	1.47 s	19.8
15	5.28, s 5.37, s	116.6	5.34, s 5.24, s	116.9	5.50, s 5.16, s	117.9	1.82, s	23.3	1.82 s	23.0
					OiBu		**OTig**		**OEtac-3-OH**	
1′					–	176.2	–	167.4		165.9
2′					2.52, h (7.1)	34.2	–	128.4		143.6
3′					1.10, d (7.1)	18.7	6.87, m	138.4	4.59, m	66.9
4′					1.12, d (7.1)	18.9	1.83, s	14.5	6.19, s 5.87, s	125.4
5′							1.85, s	12.2	1.36 d (6.6)	22.0

*overlapped signals.

this work was the updated chemical characterization and pharmacological evaluation of SLs isolated from *C. jamaicensis*. Seventeen SLs were isolated, belonging to the sesquiterpene-types of heliangolides, germacranolides and guaianolides; among them, only three have been previously described in the literature (Fig. 1), whilst jamaicolides A-D were formerly reported by Ober et al. [20] from the title species. The isolated SLs were evaluated for their cytotoxic effects in three cancerous cell lines (HeLa, SK-MEL-28 and HePG2 cells), revealing compounds possessing $IC_{50} \leq 10$ μM, which could be potential chemotherapeutic agents for cervical cancer or melanoma according to NCI guidelines [21]. In addition, based on our previous findings reporting strong inhibition of TNF-α induced ICAM-1 expression on the endothelial HMEC-1 cells from SLs isolated from *Centaurea papposa* [22], we further subjected three of the isolated SLs in the ICAM-1 assay, showing strong anti-inflammatory activity in a dose-dependent manner. In parallel, nine benzopyrane derivatives were isolated from *C. jamaicensis*, including four chromanes previously undescribed from natural sources (Fig. 2).

2. Materials and methods

2.1. Plant material

The plant material (leaves) was collected at Parque Nacional, Altos de Campana (Panama) in August 2010 and identified by Dr. Mireya Correa (Panama University). A voucher specimen has been deposited in the Herbarium of Panama University (No. Florpan 5458).

2.2. General experimental procedures

Optical rotations were obtained an UniPol L 1000 polarimeter (Schmidt + Haensch). UV-spectra were recorded on a Cary 50 Scan UV-spectrophotometer (Varian). CD spectra were measured on a J-715 spectropolarimeter (JASCO). NMR spectra were measured in an AVANCE III 600 equipped with a 5 mm TBI CryoProbe (^{1}H NMR 600.25 MHz, ^{13}C NMR 150.95 MHz) or a Bruker DRX 400 (^{1}H NMR 400 MHz, Bruker BioSpin) at 298 K (or 363 K if indicated, see Table 3). Chemical shifts are given in ppm (δ) and were referenced to the solvent signals at 7.26 and 77.0 ppm for ^{1}H-/^{13}C NMR, respectively. HRESIMS spectra were obtained with an Agilent MS Q-TOF 6540 UHD or JEOl AccuTOF GCX spectrometer. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed on a silica gel 60H (Merck). Fractionation was always monitored by TLC silica gel 60F-254 (Merck) with visualization under UV (254 and 365 nm) and spraying with vanillin–sulfuric acid reagent (vanillin, Merck) and anisaldehyde-sulfuric acid agent (4-methoxybenzaldehyde Merck). Semi-prep HPLC was conducted on a Jasco PU-2080 system (flow 1 mL/min) equipped with a RI detector (Shimadzu 10A), using an Kromasil RP-18 100si Semi-prep (10 × 250 mm) or a Hibar Purospher RP-8e (4 × 250 mm) on an Agilent 1290 Infinity system with a DAD detector (flow rate 21 mL/min; 205 nm), using an XDB-C18 PrepHT column (21.2 × 250 mm, Agilent).

2.3. Extraction and isolation

The leaves (240 g) of *C. jamaicensis* were extracted by cyclohexane

Table 2
^{1}H NMR data of compounds **9**–**13**; Solvent: $CDCl_3$, Ava 600 MHz (T = 298 K).

	9[#]		10		11[#]		12		13	
Position	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c
1	2.93, dd (10.0, 5.0)	59.9	2.96, dd (9.9, 4.8)	60.4	4.15, br d (4.3)	79.0	4.14, d (4.0)	79.0	4.57, br d (7.7)	76.6 *
2	2.48, m	31.6	2.51, td (14.5, 4.8)	32.2	2.41, m	41.9	2.38, dd (13.3,6.1)	40.9	2.45, dd (12.3, 6.3)	34.4
	1.97, m		2.01, ddd (14.5, 9.9, 3.4)		1.95, m		1.96 *m*		2.28, m	
3	4.51, br t (3.3)	72.0	4.52, br t (3.4)	72.4	4.88, dd (10.6, 6.1)	83.6	4.89, dd (11.1, 6.1)	82.7	4.69, dd (10.9, 6.3)	72.2
4	–	140.7	–	140.9	–	137.6	–	137.2	–	83.0
5	5.32, dd (11.0, 1.3)	126.3	5.35, d (10.9)	127.8	5.44, d (5.6)	126.	5.45, m	125.9	4.24, br s	78.5
6	6.59, d (11.0, 2.4)	73.2	6.65, dd (10.9, 2.4)	73.7	6.31, t (5.6)	76.1	6.36, br t (5.7)	76.4	5.19, br t (2.6)	78.2
7	2.85, m	47.6	2.88, m	48.5	2.88, m	52.9	2.91, m	53.1	3.40, m	38.5
8	5.21, m	75.0	5.20, m	75.6	5.41, m	73.8	5.46, m	73.0	5.09, m	78.0
9a	3.05, dd (15.3, 4.4)	38.3	3.10, dd (15.2, 4.6)	38.9	2.38, dd (14.0, 2.7)	38.6	2.49, dd (15.4,7.1)	38.5	2.73, dd (12.3, 7.7)	31.9
9b	1.21, *		1.28, *		1.42, dd (14.0, 3.6)		1.45, *		2.49, dd (12.3, 1.5)	
10	–	60.2	–	61.1	–	86.8	–	86.6	–	147.0
11	–	136.4	–	137.0	–	138.1	–	137.8	–	137.4
12	–	169.5	–	169.6	–	169.5	–	169.6	–	170.2
13	6.37, d (2.3)	124.5	6.36, d (2.1)	124.9	6.34, d (3.0)	124.2	6.33, d (3.0)	123.2	6.25, d (2.5)	122.7
	5.76, d (2.3)		5.76, d (1.7)		5.63, d (2.8)		5.64, d (3.0)		5.65, d (2.0)	
14	4.05, d (12.9)	63.6	4.00, d (12.9)	63.7	3.90, d (11.1)	66.0	3.84, d (11.1)	64.8	5.11, s	112.1
	3.94, d (12.9)		3.95, d (12.9)		3.75, d (11.1)		3.69, d (11.1)			
15	1.81, s	22.5	1.82, s	23.0	1.67, s	20.0	1.66, s	20.1	1.33, s	24.7
	OiBu		**OTig**		**OiBu**		**OTig**		**OTig**	
1′	–	174.8	–	164.8	–	176.1	–	167.0		167.0
2′	2.56, h (7.1)	34.1	–	128.7	2.54, h (7.1)	33.7		127.7		128.0
3′	1.14, d (7.1)	18.4	6.84, m	137.9	1.14, d (7.1)	18.6	6.85, m	139.5	6.77, m	138.4
4′	1.13, d (7.1)	19.1	1.79, s	14.6	1.12, d (7.1)	18.6	1.80, s	14.6	1.75, s	14.5
5′			1.81, s	12.0			1.80, s	12.1	1.75, s	11.9

*overlapped signals, [#] assigned by HSQC/HMBC.

(cHex)- ether-methanol (MeOH) 1:1:1 and then partitioned between water and ethyl acetate (EtOAc) (terpene rich residue) according to the Bohlmann method, slightly modified as previously described [23]. The residue was pre fractionated by VLC on silica gel, using cHex-EtOAc-acetone (Me_2CO) mixtures of increasing polarity as eluents to give nine fractions (LEA_{A-I}). The whole fractionation and final isolation procedure are continuously monitored by NMR metabolomic strategy, which enables the characterization of extracts and any derived sub-fraction in detail. Specifically, ^{1}H NMR spectra of the fractions have given evidence of compounds belonging to SLs and chromenes.

Further purification was accomplished by CC on silica gel and RP_{18}-HPLC. More specifically, fraction LEA_B (eluted with cHex-EtOAc 75:25, 124.0 mg) was subjected to CC (8 cm × 2 cm) on silica gel, using cHex: dichloromethane(DCM):EtOAc:MeOH as eluents and afforded 90 fractions (20 mL each) which were combined to 21 groups ($LEA_{B(A-U)}$), according to TLC analysis. Fractions LEA_{BF} (5.1 mg) and LEA_{BL} (5.6 mg) were identified as compounds **19** and **18**, respectively. LEA_{BM} (41.1 mg) was similarly subjected to CC (7 cm × 0.7 cm) affording 27 fractions of about 3 mL, which were combined to 15 groups ($LEA_{BM(A-O)}$) and LEA_{BMI} (1.1 mg) was identified as compound **20**. LEA_C (eluted with cHex-EtOAc 50:50, 179.2 mg) was similarly subjected to CC (7 cm × 2.3 cm) affording 58 fractions (20 mL), which were combined to 14 groups ($LEA_{C(A-N)}$). LEA_{CI} (33.2 mg) was similarly subjected to CC (8.5 cm × 1.3 cm) affording 35 fractions (15 mL), which were combined to 12 groups ($LEA_{CI(A-L)}$). LEA_{CID} (0.7 mg) and LEA_{CIG} (1.1 mg) afforded compounds **21** and **25** respectively. LEA_D (eluted with cHex-EtOAc 25:75, 224.2 mg) was similarly subjected to CC (11 cm × 1.8 cm) affording 53 fractions (20 mL), which were combined to 16 groups ($LEA_{D(A-O)}$). LEA_{DF} (3.5 mg) was identified as compound **7**. Groups $LEA_{D(G-J)}$ (=$LEA_{DG'}$ 39.7 mg) were similarly subjected to CC (9 cm × 1.3 cm) resulting in 46 fractions (15 mL), which were combined to 8 groups ($LEA_{DG'(A-H)}$). $LEA_{DG'F}$ (2.5 mg) was identified as compound **8**. $LEA_{DG'D}$ (14.1 mg) was subjected to RP_{C18}-HPLC (Kromasil RP-18, 1 mL/min, MeOH:H_2O 2:1) and afforded compounds **6** (1.6 mg, t_R 23.2 min) and **7** (1.6 mg, t_R 25.7 min). Groups $LEA_{D(K-L)}$ (=$LEA_{DK'}$ 73.2 mg) were similarly subjected to RP_{C18}-HPLC (Kromasil RP-18, 1 mL/min, MeOH:H_2O 2:1) and afforded compounds **5** (1.2 mg, t_R 39.3 min) and **13** (0.5 mg, t_R 53.0 min). LEA_{DN} (33.2 mg) was subjected to RP_{C18}-HPLC (Kromasil RP-18, 1 mL/min, MeOH:H_2O 2:1) and afforded compounds **22** (1.4 mg, t_R 21.0 min) and **23** (2.0 mg, t_R 29.5 min). LEA_E (eluted with EtOAc 100%, 549.1 mg) was similarly subjected to CC (8 cm × 3.3 cm) resulting in 58 fractions (30 mL), which were combined to 10 groups ($LEA_{E'(A-J)}$). LEA_{EC} (1.8 mg) and LEA_{ED} (10.8 mg) were identified as compounds **24a** and **24b**, respectively. LEA_{EG} (55.2 mg) was subjected to RP_{C18}-HPLC (Kromasil RP-18, 1 mL/min, MeOH:H_2O 4:3) and afforded compounds **3** (2.3 mg, t_R 21.9 min), **22** (4.2 mg, t_R 23.1 min), **4** (7.4 mg, t_R 25.4 min) and **12** (0.7 mg, t_R 35.0 min). LEA_{EG-11} (4,4 mg) was subjected to RP-HPLC (Hibar RP_8, 1 mL/min, 0–15 min ACN 0 → 20%, 15–20 min ACN 20 → 90%, 20–23 min ACN 90%) and afforded compounds **2** (0.5 mg, t_R 10.9 min) and **9** (0.6 mg, t_R 13.3 min), while LEA_{EG-13} (1.9 mg) was subjected to RP-HPLC (Hibar RP_8, 1 mL/min, ACN 30%) and afforded compound **10** (0.5 mg, t_R 3.7 min). LEA_{EH} (80.5 mg) was subjected to RP_{C18}-HPLC (XDB-C18, 21 mL/min, 0–6 min ACN 10%, 6–25 min ACN 10–40%, 25–29 min ACN 40–90%) and afforded compounds **16** (1.1 mg, t_R 20.3 min) and **17** (2.1 mg, t_R 24.6 min). LEA_F (eluted with Me_2CO-EtOAc 10:90, 345.5 mg) was similarly subjected to CC (7 cm × 2.5 cm) affording 25 fractions (20 mL), which were combined to 9 fractions ($LEA_{F(A-I)}$). LEA_{FD} (30.0 mg) was subjected to RP_{C18}-HPLC (Kromasil RP-18, 1 mL/min, MeOH:H_2O 4:3) and afforded compounds **1** (3.8 mg, t_R 14.1 min), **3** (1.2 mg, t_R 22.1 min), **4** (2.0 mg, t_R 25.5 min), **11** (0.5 mg, t_R 31.5 min) and **12** (1.1 mg, t_R 33.9 min). LEA_{FF} (25.5 mg) was subjected to RP_{C18}-HPLC (Kromasil RP-18, 1 mL/min, MeOH:H_2O 4:3) and afforded compounds **14** (2.2 mg, t_R 29.0 min) and **15** (4.6 mg, t_R 33.6 min).

Cajamolide A (**1**): yellow oil; $[\alpha]_D^{25}$ + 8.2 (c 0.18, MeOH); UV λ_{max} (MeOH) log(ε): 279.1 (5.5), 205.0 (5.4); CD: S85.; ^{1}H and ^{13}C NMR data: Table 1; HRESIMS *m/z* 265.1435 $[M + H]^+$ (calcd. for $C_{15}H_{21}O_4^+$, 265.1434).

Cajamolide B (**2**): yellow oil; $[\alpha]_D^{25}$ −50.4 (c 0.05, MeOH); UV λ_{max} (MeOH) log(ε): 292.9 (5.6), 204.0 (5.4); CD: S85.; ^{1}H and ^{13}C NMR data: Table 1; HRESIMS *m/z* 265.1431 $[M + H]^+$ (calcd. for $C_{15}H_{21}O_4^+$, 265.1434).

Cajamolide C (**3**): yellow oil; $[\alpha]_D^{25}$ + 22.9 (c 0.08, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 1;

Table 3
^{1}H NMR data of compounds **14** and **15**; Solvent: DMSO, Bruker 400 (T = 363 K), and of compounds **16** and **17**; Solvent: CDCl3, Ava 600 MHz (T = 298 K).

	14[a]	**15**[a]	**16**[b]		**17**[b]	
position	δ_H	δ_H	δ_H	δ_c	δ_H	δ_c
1	4.04, dd (10.3, 3.2)	4.05, dd (10.3, 3.2)	–	86.2	–	86.2
2	1.91, m 1.83, m	1.91, m 1.84, m	5.64, d (5.1)	134.3	5.64, d (5.5)	134.4
3	3.97, m	3.96, m	6.04, d (5.1)	141.0	6.04, d (5.5)	140.9
4	–	–	–	82.1	–	82.1
5	2.38, dd (16.2, 4.5)2.08, m	2.39, dd (16.2, 4.5)2.16, m	2.47, d (11.3)	67.5	2.49, d (11.1)	67.4
6	5.64, ddd (9.7,4.5, 2.3)	5.69, ddd (9.7, 4.5, 2.3)	4.69, dd (11.3, 9.1)	76.5	4.73, dd (11.1, 9.0)	76.6
7	3.40, m	3.40, m	3.55, m	46.4	3.56, m	46.5
8	4.94, t (10.0)	4.99, t (10.0)	5.56, m	67.2	5.67, m	67.5
9	5.34, d (10.0)	5.36, d (10.0)	3.16 dd (14.5, 3.7),2.44, dd (14.5, 3.2)	35.9	3.17, dd (14.5, 2.7), 2.47, dd (14.5, 6.6)	36.0
10	–	–	–	143.5	–	143.6
11	–	–	–	134.7	–	134.5
12	–	–	–	169.5	–	169.4
13	6.07, d (3.5) 5.43, d (3.2)	6.05, d (3.5) 5.43, d (3.2)	6.33, d (3.6) 5.62, (d 3.1)	122.4	6.31, d (3.6) 5.63, d (3.1)	122.7
14	1.75 s	1.75 s	4.91, s 4.88, s	117.2	4.91, s 4.88, s	117.2
15	5.15, s 4.85, s	5.15, s 4.82, s	1.41, s	24.6	1.40, s	24.5
	OiBu	**OTig**	**OiBu**		**OTig**	
1′	–	–	–	167.5	–	166.9
2′	2.49, *	–	2.52, h (7.1)	34.1	6.76, m	128.2
3′	1.08, d (7.1)	6.79, m	1.10, d (7.1)	19.1	1.77, s	138.2
4′	1.07, d (7.1)	1.76, s	1.12, d (7.1)	18.9	1.76, s	14.5
5′		1.77, s			–	12.0

[a] measured in DMSO; [b] measured in $CDCl_3$; *overlapped signals.

HRESIMS *m/z* 351.1802 $[M + H]^+$ (calcd. for $C_{19}H_{27}O_6^+$, 351.1802).

Cajamolide D (**5**): yellow oil; $[\alpha]_D^{25}$ + 32.7 (c 0.10, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 1; HRESIMS *m/z* 363.1803 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_6^+$, 363.1802).

Cajamolide E (**8**): yellow oil; $[\alpha]_D^{25}$ −8.6 (c 0.10, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 1; HRESIMS *m/z* 379.1748 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_7^+$, 379.1751).

Cajamolide F (**9**): yellow oil; $[\alpha]_D^{25}$ −27.6 (c 0.06, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 2; HRESIMS *m/z* 367.1744 $[M + H]^+$ (calcd. for $C_{19}H_{27}O_7^+$, 367.1751).

Cajamolide G (**10**): yellow oil; $[\alpha]_D^{25}$ + 10.8 (c 0.06, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 2; HRESIMS *m/z* 379.1744 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_7^+$, 379.1751).

Cajamolide H (**11**): colourless oil; $[\alpha]_D^{25}$ −22.1 (c 0.05, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 2; HRESIMS *m/z* 389.1563 $[M + Na]^+$ (calcd. for $C_{19}H_{26}O_7Na^+$, 389.1571).

Cajamolide I (**12**): colourless oil; $[\alpha]_D^{25}$ −15.4 (c 0.13, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 2; HRESIMS *m/z* 379.1754 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_7^+$, 379.1751).

Cajamolide J (**13**): colourless oil; $[\alpha]_D^{25}$ −20.7 (c 0.05, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 2; HRESIMS *m/z* 379.1746 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_7^+$, 379.1751).

Cajamolide K (**14**): yellow oil; $[\alpha]_D^{25}$ + 32.9 (c 0.08, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 3; HRESIMS *m/z* 351.1808 $[M + H]^+$ (calcd. for $C_{19}H_{27}O_6^+$, 351.1802).

Cajamolide L (**15**): yellow oil; $[\alpha]_D^{25}$ −79.5 (c 0.11, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 3; HRESIMS *m/z* 363.1807 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_6^+$, 363.1802).

Cajamolide M (**16**): yellow oil; $[\alpha]_D^{25}$ −13.4 (c 0.08, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 3; HRESIMS *m/z* 349.1650 $[M + H]^+$ (calcd. for $C_{19}H_{25}O_6^+$, 349.1646).

Cajamolide N (**17**): yellow oil; $[\alpha]_D^{25}$ −7.1 (c 0.17, MeOH); UV (MeOH): strong end absorption; CD: S85.; ^{1}H and ^{13}C NMR data: Table 3; HRESIMS *m/z* 361.1648 $[M + H]^+$ (calcd. for $C_{20}H_{25}O_6^+$, 361.1646).

Table 4
^{1}H NMR data of compounds 22, 23, 24a and 24b; Solvent: $CDCl_3$, Ava 600 MHz (T = 298 K).

	22		23		24a		24b	
Position	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c	δ_H	δ_c
2	–	77.8	–	80.0	–	77.5	–	77.0
3	3.60, d (8.0)	77.3	3.59, d (8.0)	76.8	3.85, *	67.2	3.83, *	67.4
4	4.51, d (8.0)	70.1	4.54, d (8.0)	69.1	4.38, d (4.2)	74.4	4.31, d (7.0)	78.8
4a	–	146.5	–	115.0	–	146.6	–	146.6
5	6.93, s	109.6	7.96, s	130.9	6.86, s	110.7	6.79, s	111.46
6	–	143.8	–	121.9	–	143.4	–	143.8
7	–	150.0	–	157.2	–	150.1	–	150.0
8	6.37, s	100.6	6.37, s	99.7	6.38, s	100.6	6.37, s	100.9
8a	–	143.1	–	161.2	–	138.9	–	143.2
9	1.48, s	24.9	1.51, s	26.6	1.50, s	24.7	1.44, s	25.5
10	1.23, s	23.1	1.25, s	19.4	1.25, s	23.3	1.28, s	23.1
4-OCH_3	–	–	–	–	3.59, s	56.9	3.48, s	56.8
6-OCH_3	3.85, s	56.6	–	–	3.83, s	56.4	3.84, s	56.2
6-$COCH_3$	–	–	2.57, s	31.9/197.5				
7-OCH_3	3.83, s	55.8	3.86, s	55.7	3.81, s	55.8	3.82, s	56.0

*overlapped signals.

Cajachromane A (**23**): green amorphous solid; $[\alpha]_D^{25}$ −12 (c 0.13, MeOH); UV λ_{max} (MeOH) log(ε): 296.0 (3.7), 218.0 (3.6); CD: S85.; ^{1}H and ^{13}C NMR data: Table 4; HRESIMS *m/z* 267.1229 $[M + H]^+$ (calcd. for $C_{14}H_{19}O_5^+$, 267.1232).

Cajachromane B (**24a**): green amorphous solid; $[\alpha]_D^{25}$ −6 (c 0.09, MeOH); UV λ_{max} (MeOH) log(ε): 292.0 (3.6), 216.0 (3.2); CD: S85.; ^{1}H and ^{13}C NMR data: Table 4; HREIMS *m/z* 268.1300 $[M]^+$ (calcd. for $C_{14}H_{20}O_5^+$, 268.1305).

Cajachromane C (**24b**): green amorphous solid; $[\alpha]_D^{25}$ + 11 (c 0.10, MeOH); UV λ_{max} (MeOH) log(ε): 293.0 (3.5), 214.0 (3.2); CD: S85.; ^{1}H and ^{13}C NMR data: Table 4; HREIMS *m/z* 268.1309 $[M]^+$ (calcd. for $C_{14}H_{20}O_5^+$, 268.1305).

2.4. MTT assay

The cytotoxicity of the SLs was determined using the human cancer cell lines HeLa (cervical carcinoma, ATTC), SK-MEL-28 (malignant melanoma, DSMZ) and HepG2 (hepatocellular carcinoma, DSMZ), as well as the non-cancerous HMEC-1 cell line (human microvascular endothelial cells [24]). Culture medium was MEM (Sigma; supplemented with L-Glutamine, FCS and NEA, BioChrom), DMEM-HAM'S (50–50%, Invitrogen and BioChrom; supplemented with FCS), RPMI (BioChrom; supplemented with L-Glutamine and heat activated FCS) and ECGM (EASY Endothelial Cell Growth Medium, supplemented with EASY Growth supplement and Gentamycin-Amphotericin (all PELO-Biotech), and heat activated FCS), respectively. Stock solutions of the samples were prepared in DMSO, and the test concentrations were freshly prepared with medium on the day of the experiment (final concentration of DMSO was 0.33%). Cytotoxicity was evaluated using the colorimetric MTT assay as described before [25]. Briefly, nine different concentrations of the compounds (100.0 to 0.39 μM) together with the cell suspension (final concentration in wells: 6×10^4 cells/mL, 3×10^4 cells/mL and 12.5×10^4 cells/mL, for HeLa, SK-MEL-28 and HePG2, respectively), were added to the 96 well-plates. The HMEC-1 cells in a final cell suspension of 90×10^4 cells/mL were placed in the 96-wells, and 24 h later they were treated with 6.25, 12.50, 25.00, 50.00 μM of the tested SLs. After incubation for 68 h (or 24 h for the HMEC-1) (IBS Integra Biosciences, 37 °C, 5% CO_2 and 90% relative humidity), MTT was added (4 mg/mL), and after 4 h the solution was exchanged with 10% SDS. The plates were then placed in dark and 24 h later, the absorbance at 560 nm was measured (SpectraFluor plus plate reader, Tecan).

2.5. ICAM-1 assay

Regarding the ICAM-1 assay, the HMEC-1 cells were treated as described before [26]. Briefly, the compounds were firstly subjected to MTT viability test, as described in 2.4., in order to exclude any false positive results in the reduction of the expression of ICAM-1 due to cytotoxicity. Since no cytotoxicity was observed (mean viability between 90 and 110%, Supplementary information S88) the HMEC-1 cells were treated with the compounds **1**, **4** and **7** at 6.25, 12.50, 25.00, 50.00 μM, or parthenolide (5 μM, positive control, Calbiochem), or medium (ECGM, negative control, set as 100%) in 24 well-plates (plates were treated firstly with 0.25 % v/v Collagen G in PBS (both Sigma-Aldrich). After 30 min, TNF-α (10 ng/mL, Sigma Aldrich) was added (except from two wells, in which no TNF-α was added in order to evaluate the normal cells ICAM-1 production (untreated control), and the plates were incubated at 37 °C, 5% CO_2 and 90% relative humidity for 24 h. Then, the cells were washed with PBS, removed from the plates with trypsin/EDTA and fixed with formalin. Incubation with the Mouse Anti-human CD54: FITC (Bio-Rad) for 20 min, was followed by fluorescence-activated cell sorting (FACS) analysis (Becton Dickinson FacscaliburTM) measuring fluorescence intensity.

2.6. Statistical analysis

IC_{50} values were calculated from nine different concentrations and tests were performed in sextuplicate for the MTT assay. For ICAM-1 assay each experiment was performed in duplicate. All experiments are repeated at least three times and the results are presented as means ± SD for each sample. Statistical analysis of all continuous variables was checked for normality. Means of two groups were compared with paired samples *t*-test or one sample *t*-test where applicable. Statistical significance (2-tailed) was set at $^*p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$. Statistical analyses were performed using IBM SPSS statistics 26. GraphPad Prism 8.4.2 version was used for the creation of the graphical representations, as well as the calculations of the IC_{50} values using non-linear regression.

3. Results and discussion

3.1. Phytochemical investigation

The terpene rich residue from *C. jamaicensis* was repeatedly purified by VLC, CCs, and semi-preparative HPLC (see section *3.3*) to yield fourteen previously undescribed SLs, cajamolides A – N (**1**–**3**, **5**, **8**–**17**), together with three known SLs, carbetolide C (**4**) [27], tagitinin E (**6**) [28] and heliangin (**7**) [29]. All structures are presented in Fig. 1, and the proposed biosynthetic pathway is illustarted as part of the Supporting Information (S87). Moreover, nine chromene derivatives (Fig. 2) were reported, including precocene II (**18**) [30], prunichromene B (**19**) [31], encecolin (**20**) [32], acetovanillochromene (**21**) [33], 2,3-dihydro-3S-hydroxy-6,7-dimethoxy-2,2-dimethyl-4H-1-benzopyran-4-one (**25**) [34] and *trans*-3,4-dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2′-dimethyl-2H-benzo[*b*]pyran (*trans*-precocene-3,4-dihydrodiol) (**22**) [30], with the latter one reported for the first time from natural source, as well as three new analogues cajachromane A (**23**), cajachromane B (**24a**) and cajachromane C (**24b**).

Cajamolide A (**1**) was isolated as yellow oil and its molecular formula of $C_{15}H_{20}O_4$ was determined by the HRESIMS. The ^{1}H and ^{13}C NMR data (Table 1), with the aid of COSY, HSQC and HMBC analysis revealed the presence of 15 carbons, three non-protonated and three protonated olefinic, one carbonyl, three oxygen bearing and four aliphatic carbons, indicating the presence of a SL backbone (NMR data Table 1). An α-methylene-γ-lactone moiety was confirmed by the diagnostic signals in the ^{1}H NMR spectrum of two doublets at δ_H 6.24 (3.3 Hz) and δ_H 5.65 (2.8 Hz) attributed to position 13, following by their characteristic COSY correlation signal with H-7 and the HMBC cross-peak to C-12 of the lactone ring. In addition, two pairs of singlets at δ_H 5.26, 5.22 and δ_H 5.28, 5.37 were observed in the ^{1}H NMR spectrum, supporting a SL structure with three exomethylenic double bonds in total, on positions 11, 4 and 10, respectively. All signals could be assigned by spin decoupling systems of C$\underline{H_2}$-13/ C$\underline{H}$-7/ C$\underline{H_2}$-8/ C$\underline{H}$-9, C$\underline{H}$-7/ C$\underline{H}$-6/ C$\underline{H_2}$-5 and C$\underline{H}$-3/ C$\underline{H_2}$-2/ C$\underline{H_2}$-1, suggesting a germacrene type SL. The relative stereochemistry was deduced by NOESY difference spectroscopy, which showed clear effects between H-3 (δ_H 4.13), H-7 (δ_H 2.52) and H-9 (δ_H 4.31) indicating the α- orientation of these protons (Figure S89, SI).

Cajamolide B (**2**) was isolated as yellow oil and its molecular formula of $C_{15}H_{20}O_4$ was determined by the HRESIMS. The NMR data of **2** (Table 1) were similar to that of **1**. As expected, all protonated carbons were assigned by the COSY correlation signals of C$\underline{H_2}$-13/ C$\underline{H}$-7/ C$\underline{H_2}$-8/ C$\underline{H}$-9, C$\underline{H}$-7/ C$\underline{H}$-6/ C$\underline{H_2}$-5 and C$\underline{H_2}$-3/ C$\underline{H_2}$-2/ C$\underline{H}$-1, with the aid of the HSQC spectrum. Contrarily to **1**, the presence of an α-OH on C-9 was deduced from the lack of the NOE signal between H-9 (δ_H 4.29) and H-7 (δ_H 2.82). Proton H-3, similarly to compound **1**, also gave a NOE signal with H-7 and this proton was up shielded (δ_H 4.23 in **2** and δ_H 4.13 in **1**) (Figure S89, SI).

Furthermore, analysis of the NMR data showed that **3** and **4** are

closely related structures to other heliangolide-type SLs with a side chain located at C-8 that have been previously described in the literature from *Calea* species [27]. In fact, compound **4** was identified as carbedolide C. This compound has been previously found in *C. berteriana* [27,31], *C. prunifolia* and *C. solidaginea* [31]. Heliangolides are considered the main SLs type in the genus *Calea* [16]. Compound **3**, named cajamolide C, was obtained as a yellow oil. Its molecular formula was determined to be $C_{19}H_{26}O_6$ by the HRESIMS. A comparative analysis of the NMR data of **3** (Table 1) with those reported in the literature [23,27], suggested a SL backbone with a structure related to the abovementioned compound **4**, indicating a different substitution at C-8. In comparison with **4**, the chemical shifts of the proton and carbon signals at positions 8, 9 and 14 of **3** were slightly different from those of carberolide C (**4**) due to the tigloyl-side chain. In fact, the diagnostic differences were the signal at δ_C 176.2, corresponding to the carbonyl group (C-1′), as well as the methine septet signal (CH-2′) at δ_H 2.52 (7.1 Hz) and the vicinal methyls at δ_H 1.10 and 1.12 (d 7.1 Hz), which supported the presence of an isobutyl group [24]. The location of the side chain group at C-8 (δ_C 75.1) was supported by the HMBC correlation signals between H-8 (δ_H 5.16) and the carbonyl carbon C-1′. The relative configuration was assigned by the NOE signals H-1/ H-3/ H-7/ H-8, indicating the α-orientation of these protons, similarly to carberolide C (**4**) [27].

Compound **5**, named cajamolide D, was isolated as a yellow oil and its molecular formula was determined to be $C_{20}H_{26}O_6$ from the ^{13}C NMR (Table 1) and HRESIMS. The NMR data of **5** were similar to those of **3** and **4** [27], but with a distinct absence of the exomethylenic double bond at C-10, followed by the appearance of a signal of an angular methyl at δ_H 1.82 (singlet) and an olefinic proton at δ_H 5.43 ($\Delta_{(9)10}$, d 11.1 Hz). Its COSY spin-coupling systems C$\underline{H}_2$-13/ C$\underline{H}$-7/ C$\underline{H}_2$-8/ C$\underline{H}$-9, C$\underline{H}$-7/ C$\underline{H}$-6/ C$\underline{H}$-5 and C$\underline{H}$-3/ C$\underline{H}_2$-2/ C$\underline{H}$-1 constructed the planar structure of **5**. Moreover, an olefinic proton at δ_H 6.87 and two characteristic secondary methyl signals at δ_H 1.83 and 1.85 observed at the ^1H NMR spectrum indicated the presence of a tigloyl group (C_5H_7O) located again at C-8 (Table 1), which was further verified by the significant fragment peak at *m/z* 83.0487 in the MS spectrum. The relative configuration was assigned by the NOE signals H-3/ H-7/ H-8, indicating the α-orientation of these protons and the diaxial orientation of H-1 (δ_H 4.80).

Compound **8**, named cajamolide E, was obtained as a yellow oil and its molecular formula was determined to be $C_{20}H_{26}O_7$ from the HRESIMS. The NMR data of this compound unveiled high similarity with the basic structure of the known compounds tagitinin E (**6**) and heliangin (**7**), featuring an 1,10-epoxide. Its planar structure was supported by the COSY spin-coupling systems C$\underline{H}_2$-13/ C$\underline{H}$-7/ C$\underline{H}$-8/ C$\underline{H}_2$-9, C$\underline{H}$-7/ C$\underline{H}$-6/ C$\underline{H}$-5 and C$\underline{H}$-3/ C$\underline{H}_2$-2/ C$\underline{H}$-1. Furthermore, instead of the typical signals of the substitution of the tigloyl or isobutyl group, compound **8** showed in ^1H NMR a pattern of signals (two singlets at δ_H 5.87 and δ_H 6.19, one multiplet at δ_H 4.59 and a douplet at δ_H 1.36) which together with the characteristic fragment on the mass spectrum at *m/z* 115.0395 $[C_5H_7O_3]^+$, clearly indicated the presence of 3-hydroxy-2-ethylacrylate moiety (Table 1). Such a side-chain substitution has been previously reported from *C. berteriana* [35]. The relative configuration, based on NEOSY correlation signals and coupling constants, was in accordance with the known compounds tagitinin E (**6**) and heliangin (**7**) [28,29].

Compounds **9** and **10** were nearly identical regarding their ^1H NMR data, differing only in the signals due to the lateral chain, with the first bearing an isobutyl and the other bearing a tigloyl group. Comparably to compounds **6**, **7**, and **8**, these compounds are also characterized by the presence of an 1,10 epoxide, however position C-14 is further substituted by a hydroxyl residue (δ_H 4.05, 3.94 and 4.00, 3.95, δ_C 63.6 and 63.7, for 9 and 10, respectively), which is in full accordance with the obtained mass spectra. More specifically, regarding compound **9**, which was named cajamolide F, its molecular formula was determined to be $C_{19}H_{26}O_7$ from the HRESIMS, while accordingly, compound **10**, was named cajamolide G, with the molecular formula $C_{20}H_{26}O_7$ by the HRESIMS. Both compounds were isolated as yellow oils. The direct comparison of their ^1H and ^{13}C NMR spectra (Table 2) with 14-hydroxyleptocarpin reported by Spring et al. [36], shows that **9** and **10** are analogs of this compound, with the only difference in the lateral chain at C-8 (angeloyl-group in 14-hydroxyleptocarpin, vs. isobutyl and tigloyl in **9** and **10**, respectively). The relative configuration proved to be the same with the pattern observed in compounds **6**–**8** [28,29] (Figure S89, SI).

The occurrence of identical basic skeleton differentiated only by the substitution of a tigloyl or an isobutyl group was also observed with compounds **11** and **12**, named cajamolide H and cajamolide I, respectively**.** Comparably to **9** and **10**, these compounds are also characterized by the presence of an epoxide and the same substitution on position C-14 (hydroxyl group), which is in full accordance with the obtained mass spectra. More specifically, the molecular formula of compound **11** was determined to be $C_{19}H_{26}O_7$ from the HRESIMS and for compound **12** $C_{20}H_{26}O_7$ from the HRESIMS. In this case, the downfielded shift of C-3 (δ_C 83.6 and 82.7 of compounds **11** and **12**, respectively) suggests this position as the bridgehead for the epoxide. Precisely, they proved to be analogues of furanonobilin quoted by De Mieri et al. [37], distinguished by the presence of the C-8 substitution (Table 2). The NOESY correlations of H-7/H-1 and H-1/H-8, along with the lack of cross-peaks with H-3, suggested the relative configurations of compounds **11** and **12**, as shown in Figure S89 (SI).

Compound **13**, named cajamolide J, was assigned a molecular formula of $C_{20}H_{26}O_7$ by the HRESIMS and was obtained as a colourless oil. Its structure is based on the presence of two characteristic exomethylenic double bonds, one methyl and five oxygen bearing methines, in combination with 2D NMR experiments. Moreover, an epoxy bridge between C-1 (δ_C 76.6) and C-4 (δ_C 83.0) was supported by the HMBC correlation from C-4 to H-1 (δ_H 4.57), in parallel with the chemical shifts of H-1. It is noteworthy that in comparison with the formerly described 1,10-epoxides (**6**–**10**), position 1 resonates at higher fields (δ_H 4.57 and δ_C 76.6 vs ca. δ_H 2.60 and δ_C 60, see Table 2), due the different location of the epoxy group. The mass spectrum supported the absence of a hydroxy group. Moreover, oxygen bearing tertiary C-4 was downfielded at 83.0 ppm, which is characteristic for epoxide bridges situated at non-protonated carbons [33]. Moreover, the cross-peaks from H-3 (δ_H 4.69) to C-15 (δ_C 24.7) and C$\underline{H}_3$-15 (δ_H 1.33) to C-5 (δ_C 78.5), as well as H-6 (δ_H 5.19) to C-5 in the HMBC spectrum supported the location of the hydroxy groups at C-3 and C-5, respectively, consistent with the correlations of C$\underline{H}_2$-13/ C$\underline{H}$-7/ C$\underline{H}$-6/ C$\underline{H}$-5, C$\underline{H}$-7/ C$\underline{H}$-8/ C$\underline{H}_2$-9 and C$\underline{H}$-3/ C$\underline{H}_2$-2/ C$\underline{H}$-1 in the $^1H-^1H$ COSY spectrum. The NOESY correlations of H-3/H-7 (δ_H 3.40) and H-5 (δ_H 4.24)/ C$\underline{H}_3$-15 confirmed the diaxial orientation of hydroxyl groups at the abovementioned positions (3 and 5) (Figure S89, SI).

The ^1H NMR spectra of compounds **14** and **15** indicated similar structures to jamaicolide D [20], as it is known that analogous lactones featuring a germacran-8,12-olide with such medium-sized rings exist as mixtures of conformers, which interconvert slowly at room temperature with the appearance of broad ^1H NMR signals. The signals are drastically sharpened at higher temperature (363 K), which gives the possibility for better interpretation, thus the experiments were performed at higher temperature in deuterated DMSO. Unfortunately, the small amount available of **14** and **15** and the intrinsic width of several signals in the NMR spectra of such germacrane derivatives, prevented the recording of ^{13}C NMR spectra at 150 MHz (Table 3). However, the resolution of individual signals of ^1H NMR and the results of the HRESIMS experiments permitted a structural assignment. More specifically, compound **14**, named cajamolide K, was obtained as a yellow oil. Its molecular formula was determined to be $C_{19}H_{26}O_6$ from the HRESIMS. More specifically, this germacran-12,6-olide is bearing three double bonds $\Delta_{11(13)}$, $\Delta_{4(15)}$ and $\Delta_{10(9)}$ and two hydroxy groups, as well as an isobutyl substitution on position 6, and is presenting in $^1H-^1H$ COSY the spin systems C$\underline{H}_2$-13/ C$\underline{H}$-7/C$\underline{H}$-6/C$\underline{H}_2$-5, C$\underline{H}$-7/C$\underline{H}$-8/C$\underline{H}$-9 and C$\underline{H}$-1/C$\underline{H}_2$-2/C$\underline{H}$-3, which permitted the structure elucidation of **14.** Accordingly**,** compound **15**,

named cajamolide L, was isolated as a yellow oil. Its molecular formula was determined to be $C_{20}H_{26}O_6$ from the HRESIMS. Structurally, **15** was identical to the previously described compound, differing only at the substitution on position 6. In fact, a tigloyl-side chain was observed in contrast to the isobutyl group of **14**. The relative configuration of **14** and **15** was deduced by NOESY correlations (Figure S89, SI), as well as coupling constants of H-6, H-7 and H-8 ($J_{7,8}$ and $J_{6,7} \approx 10$ Hz, indicated antiperiplanar arrangement) and in comparison with jamaicolide D the structures differentiated only in the chiral center of position 6 [20].

In addition to cajamolides A-L, two highly oxygenated guaianolides were isolated (compounds **16** and **17**). Compound **16**, named cajamolide M, was obtained as a yellow oil. The HRESIMS supported its molecular formula to be $C_{19}H_{24}O_6$. The 1D and 2D NMR spectra of **16** (Table 3) showed 19 carbons, 15 assignable to a SL moiety and 4 to a lateral chain, displaying 3 methyl groups (one oxygenated and two from the side chain), 3 methylenes (including two olefinic), 7 methines (including 2 oxygenated and 2 olefinic), and 6 non-protonated carbons (including 2 carbonyl, 2 oxygenated, and 2 olefinic). An α-methylene-γ-lactone moiety was evident by the two doublets at δ_H 6.33 ($J = 3.6$ Hz) and δ_H 5.62 ($J = 3.1$ Hz) in the ^{1}H NMR spectrum. Moreover, two singlets at δ_H 4.91 and δ_H 4.88 confirmed the presence of a second exo-methylenic double bond. Moreover, a pair of doublets at δ_H 6.04 and δ_H 5.64 with a 5.1 Hz coupling clearly showed that a 2,3-double bond was present. In addition, a singlet at δ_H 1.41 suggested an oxygenated methyl group. Further analysis of COSY and HMBC data permitted the complete assignment of the protons and carbons of a guaiane-type skeleton. More specifically, COSY correlations between C$\underline{H}_2$-13/ C$\underline{H}$-7/ C$\underline{H}$-6/ C$\underline{H}$-5, C$\underline{H}$-7/ C$\underline{H}$-8/ C$\underline{H}_2$-9 and C$\underline{H}$-2/ C$\underline{H}$-3, as well as HMBC correlations from H-6 (δ_H 4.69) to C-4 (δ_C 82.1), C-5 (δ_C 67.5) and C-7 (δ_C 46.4), from H-13 (δ_H 6.04) to C-7 (δ_C 46.4), C-11 (δ_C 134.7) and C-12 (δ_C 169.5), from C$\underline{H}_3$-15 (δ_H 1.41) to C-1 (δ_C 86.2), C-5 (δ_C 67.5), C-9 (δ_C 35.9), and from H-2 (δ_H 134.3) and H-3 (δ_H 6.04) to C-4 (δ_C 82.1) and C-5 (δ_C 67.5) confirmed the guaiane-moiety. The NOESY spectra of compound **16** showed the correlations of H-7 (δ_H 3.55) with H-9α (δ_H 2.44), H-5 (δ_H 2.47) and of H-8 (δ_H 5.56) with H-5 (δ_H 2.47), indicating that these protons are in the same face of the molecule. Moreover, H-6 and the protons of the methyl group at C-15 (δ_H 1.41) are in the other face of the molecule in β-orientation (Figure S89, SI). This result is in accordance with literature data for analogue compounds with the same guaianolide skeleton, such as subcordatolide A [35]. The location of the side chain group at the carbon resonating at δ_C 67.2 (C-8) was supported by the HMBC correlations signals between H-8 (δ_H 5.56) and the carbonyl carbon at δ_C 167.5. Further evidence for this assumption was provided by the downfield shift of H-8 at δ_H 5.56. ^{1}H NMR indicated the presence of an isobutyl- side chain with the characteristic signals of δ_H 1.10 and 1.12 (d, 7.1 Hz), together with a heptet methine proton at δ_H 2.52.

Similarly, compound **17** was also isolated as a yellow oil and named cajamolide N. The positive HRESI-MS of **17** suggested the molecular formula of $C_{20}H_{24}O_6$. The ^{1}H and ^{13}C NMR data of **17** showed very similar structural resemblance to those for **16**, apart from the absence of the isobutyl- side chain signals, which were replaced by the characteristic features of a tigloyl group (an olefinic proton at δ_H 6.76 and two methyl groups at δ_H 1.76 and 1.77).

Compounds **18**–**25** showed closely related NMR data (Table 4, NMR data of **22**, **23**, **24a** and **24b**). Further study of the 1D and 2D NMR experiments of these compounds are consistent with those of characteristic chromene type moieties. The NMR spectra showed the indicative peaks of 2,2-dimethylchromene system bearing acetyl or methoxy substitutions. More specifically apart from the former known chromenes, three analogues are reported herein for the first time, along with *trans*-3,4-dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2′-dimethyl-2H-benzo[*b*] pyran (*trans*-precocene-3,4-dihydrodiol) (**22**) which was previously described only as a synthetic derivative [30] (for comparison reasons the NMR data of **22** are presented in Table 4). Compound **23** was obtained as a green oil and named cajachromane A. The positive HRESIMS of **23** suggested a molecular formula of $C_{14}H_{18}O_5$ (calcd. 267.1232). The ^{1}H NMR showed the presence of two meta coupled aromatic protons at δ_H 7.96 and δ_H 6.37, four singlets due to a methoxy and an acetyl group at δ_H 3.86 and δ_H 2.57, respectively, as well as the diagnostic methyl groups at δ_H 1.51 and δ_H 1.25. Also, two oxygen bearing methine protons resonated at δ_H 3.59, (d, 8.0 Hz) and δ_H 4.54, (d, 8.0 Hz) indicated the high similarity of this isolate with compound **22.** The configurations of 3,4 diols were assigned from their ^{1}H NMR spectra. In these compounds, and in all related structures, $J_{3,4}$-trans was greater than 7 Hz and $J_{3,4}$-cis was<5 Hz [38]. Thus, compound **23** is bearing the cis configuration in accordance with the literature and the reported one of **22**.

Compound **24a** was obtained as an amorphous solid and named cajachromane B. The positive HREIMS of **24a** suggested a molecular formula of $C_{14}H_{20}O_5$. Structurally, this compound was also analogue of **22** with an additional methoxy group located at C-4 (δ_H 4.38, δ_C 74.4). Of note, **24a** proved to be in cis configuration with a $J_{3,4} = 4.2$ Hz. In addition, compound **24b** proved to be the trans analogue with a $J_{3,4} = 7.0$ Hz [38]. The positive HREIMS of **24b** indicated a molecular formula of $C_{14}H_{20}O_5$. Their NMR data were almost identical with the H-4 slightly shifted at δ_H 4.31 (from δ_H 4.38 for compound **24a**) and compound **24b** was named cajachromane C, accordingly. The epoxide of precocene II could be the precursor of compounds **22**, **24a**, **24b** and **25**, as epoxide of compound **20** for **23**; similar events reported the formation of chromene metabolites by a non-enzymatic pathway in *Streptoyces griseus* [30]. Apart from the natural biosynthesis, isolates such as **24a** and **24b** could be originated by the reaction of precocene II epoxide with the methanol used during the extraction of the plant [30].

Table 5
IC_{50} values calculated from the MTT assay against HeLa, SK-MEL-28 and HePG2 cell lines.

Compounds	HeLa	SK-MEL-28	HePG2
1	10.8 ± 0.2	23.8 ± 0.7	26.3 ± 1.6
3	7.5 ± 0.3	18.3 ± 1.5	51.9 ± 6.1
4	2.9 ± 0.1	7.5 ± 0.6	12.6 ± 0.6
6	5.8 ± 0.1	n.t.	n.t.
7	6.2 ± 0.3	12.6 ± 0.4	24.0 ± 3.2
8	13.4 ± 0.3	n.t.	n.t.
14	6.4 ± 0.7	n.t.	n.t.
15	4.9 ± 0.5	6.5 ± 0.7	19.3 ± 3.1
16	7.2 ± 0.2	n.t.	n.t.
17	5.8 ± 0.2	5.2 ± 0.8	17.4 ± 2.2
P	2.1 ± 0.5	4.9 ± 1.1	10.0 ± 0.7

P: parthenolide (positive control); n.t.: not tested.

3.2. Cytotoxic and anti-inflammatory activities

Taking into consideration the amount of the compounds, ten SLs were subjected to cytotoxic screening (Table 5) and parthenolide was used as positive control. Interestingly, all tested compounds, except from compound **8**, showed strong activity against HeLa cells, with IC_{50} values ranging from 2.9 to 10.8 μM. SK-MEL-28 and HepG2 cells were generally more resilient than HeLa series, resulting in lower cytotoxic activities for all the tested compounds. However, compounds **4**, **15** and **17** were highly active against SK-MEL-28 cell line with IC_{50} values of 7.5, 6.5 and 5.2 μM, respectively. It is noteworthy that carbetolide C (**4**), exerted IC_50 values of 2.9, 7.5 and 12.6 μM for the HeLa, SK-MEL-28 and HePG2 cells, which are similar to that of the positive control parthenolide. To conclude, the majority of the tested compounds could be potential chemotherapeutic agents for cervical cancer or even melanoma, since compounds having an $IC_{50} \leq 10$ μM are considered active and could be potential anti-cancer drugs according to NCI guidelines [21].

In addition, our previous study on SLs isolated from *C. papposa* [22], revealed the strong inhibition of TNF-α induced ICAM-1 expression on the endothelial HMEC-1 cells, thus we further subjected three of the isolated SLs in the ICAM-1 assay, in order to evaluate the anti-inflammatory potential. As it is known, the expression of surface

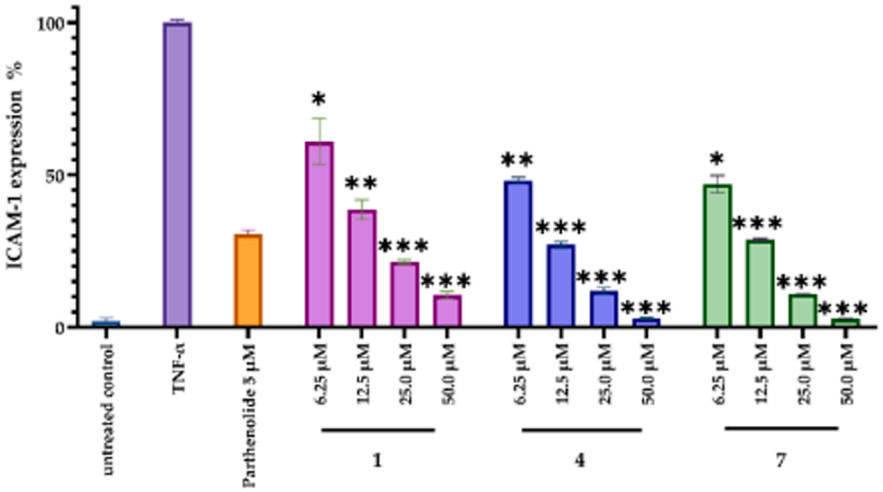

Fig. 3. ICAM-1 expression (%) in HMEC-1 cell line is expressed as means ± SD. Data are representative of 3 independent experiments with similar results. Significance levels were set at $p < 0.5$*, $p < 0.01$**, $p < 0.001$*** vs. TNF-α, as determined by paired sample *t*-test. Untreated control (pure medium, without addition of TNF-α), TNF-α (10 ng/mL, negative control), parthenolide (5 μM, positive control) and different concentrations (6.25, 12.5, 25.0 and 50.0 μM) of compounds **1**, **4**, and **7**.

molecules like ICAM-1 (Intercellular Adhesion Molecule 1) and VCAM-1 (Vascular cell adhesion protein 1) at the endothelial cells, triggered by cytokines such as IFNγ, IL-1b, and TNF-α, leads to adhesion and transmigration of leukocytes in the inflammation site [39,40]. As for the ICAM-1 assay, the samples were firstly subjected to MTT viability test on the HMEC-1 cells, in order to exclude any false positive results in the reduction of the expression of the adhesion molecule due to cytotoxicity, reporting no toxicity (mean viability 90–110%, Figure S88). The determination of natural ICAM-1 expression of the HMEC-1 cells in the control group was about 2% (untreated control), and parthenolide was used as positive control (5.0 μM), reducing its expression to 30% of the control (TNF-α) (see Fig. 3).

Interestingly, compounds **1**, **4** and **7** revealed remarkable inhibiting effects in a dose-dependent manner (IC_{50} values 8.5, 5.5 and 5.4, respectively, Figure S86.). More specifically, compounds **1**, **4** and **7** reduced the ICAM-1 expression by 89.5, 97.1 and 97.2 % respectively at 50.0 μM. Even at this high concentration, no cytotoxic effects were observed in the HMEC-1 cells, while it is worth mentioning that even the lowest concentration tested (6.25 μM) proved to be active, reaching a reduction ca. 50 %. These results are in accordance with our previous study, revealing the strong anti-inflammatory effects of SLs (cnicin and 8α-O-(3′, 4′-dihydroxy-methylenebutanoyloxy)-dehydromelitensin with IC_{50} values of 21.9 and 5.7, respectively) through the ICAM-1 pathway [22]. The latter is supported also by other researchers, reporting that parthenolide (8 μM) strongly inhibits the TNF-α induced ICAM-1 expression by 93 % in human synovial fibroblasts [41]. The lactone ring is present in all the strong inhibitors like parthenolide, cnicin and 8α-O-(3′, 4′-dihydroxy-methylenebutanoyloxy)-dehydromelitensin, as well as in compounds **1**, **4** and **7**, in contrast to compounds lacking the α,β-unsaturated lactone moiety, which showed the lower inhibitory potential. More specifically, in the same test system described in this study, matricine (which lacks the α-methylene-γ-lactone group) and 6α,8α,15-trihydroxyelema-1,3,11(13)-trien-12-olide methyl ester (an homologous sesquiterpene) only reduced the ICAM-1 expression to 52 % and 65 % of the control at the higher concetrations tested (75 μM and 50.0 μM, respectively [42,22]. Thus, it is suggested that the the α-methylene-γ-lactone ring is essential for the anti-inflammatory activity through the ICAM-1 pathway. Although Michael reactions of the lactone ring with -SH groups of biological systems usually also involve non-specific toxicity [43], it is worth mentioning that the tested SLs revealed no toxicity in the endothelial HMEC-1 cells in all the range of the tested concentrations (6.25–50.0 μM).

4. Conclusions

This study showed that the specialized metabolites isolated from *Calea* spp. possess strong cytotoxic and anti-inflammatory activities, reconfirming thus, the traditional use of the genus in folk medicine for the treatment of inflammation and various ailments. Further exploration of the sesquiterpene lactones would certainly offer a novel therapeutic approach to cope with inflammatory diseases. In conclusion, the genus *Calea* could serve as a biobank of potential phytopharmaceuticals, which could be utilized as leads in drug discovery and therapy.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.105351.

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Supporting information

Cajamolides A-N: cytotoxic and anti-inflammatory sesquiterpene lactones from *Calea jamaicensis*.

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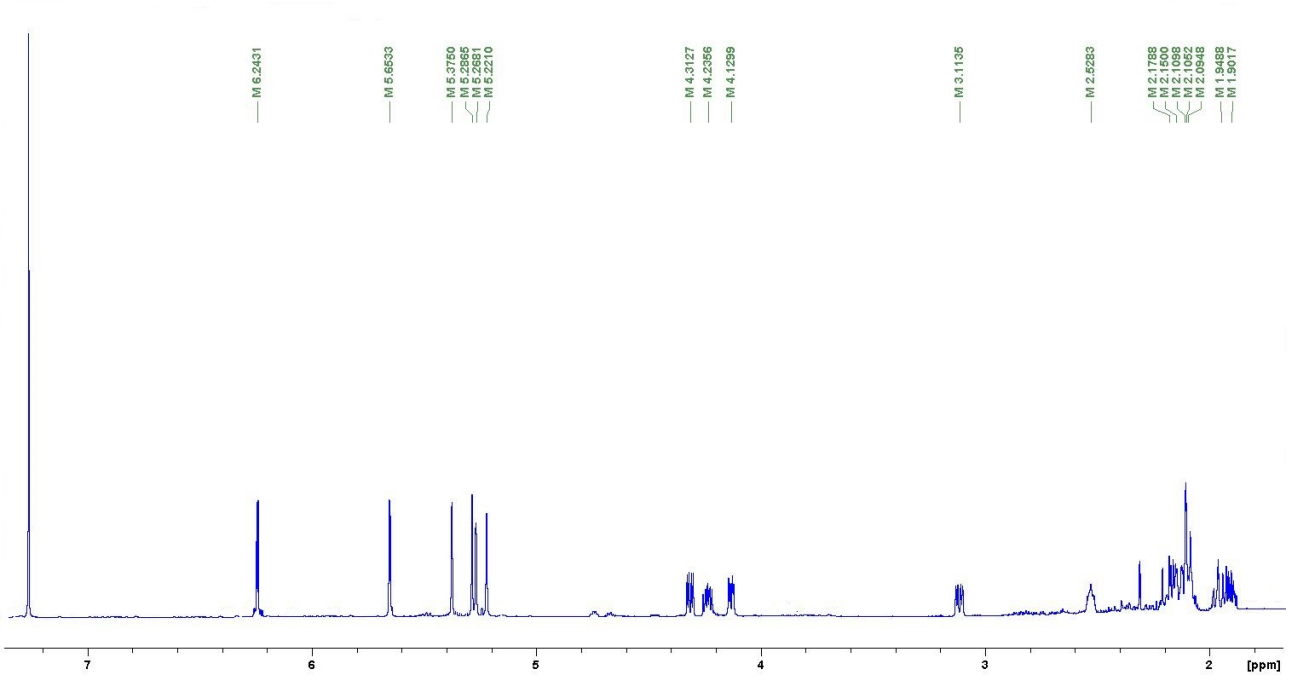

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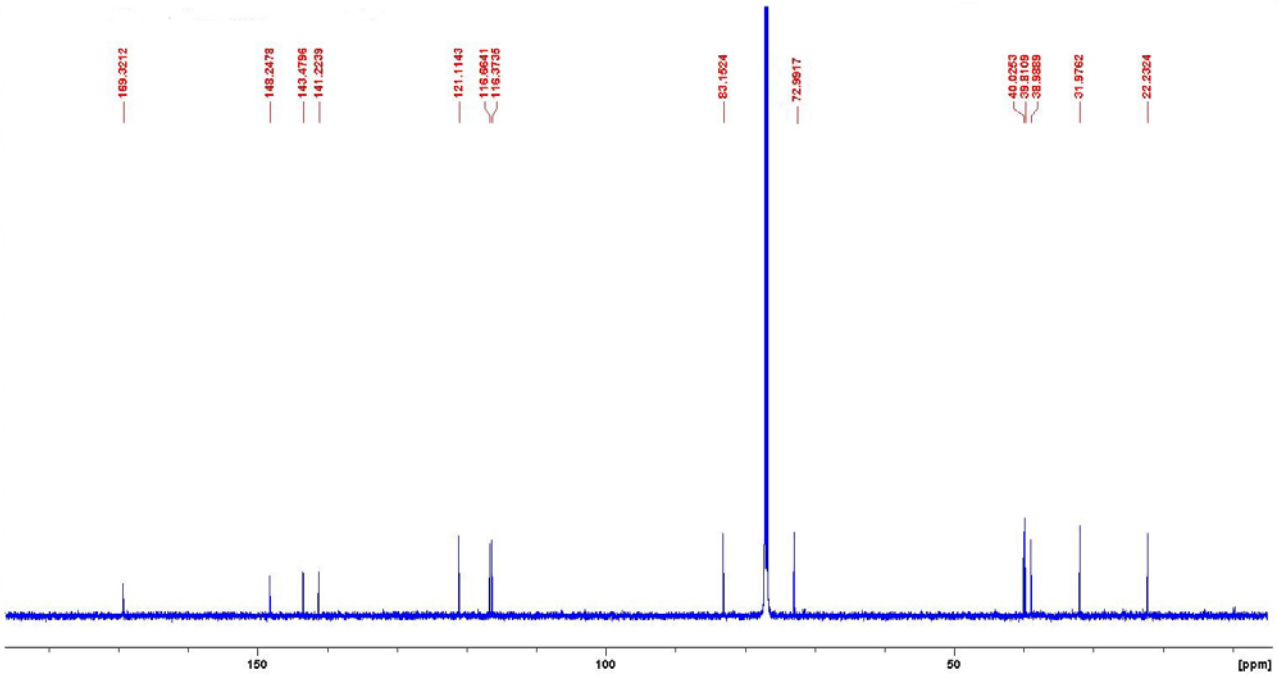

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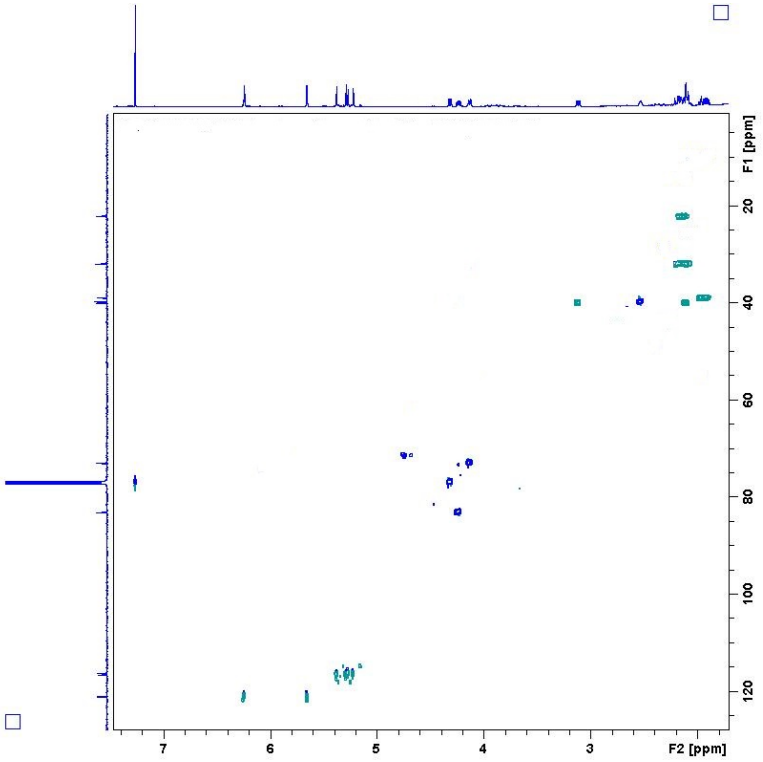

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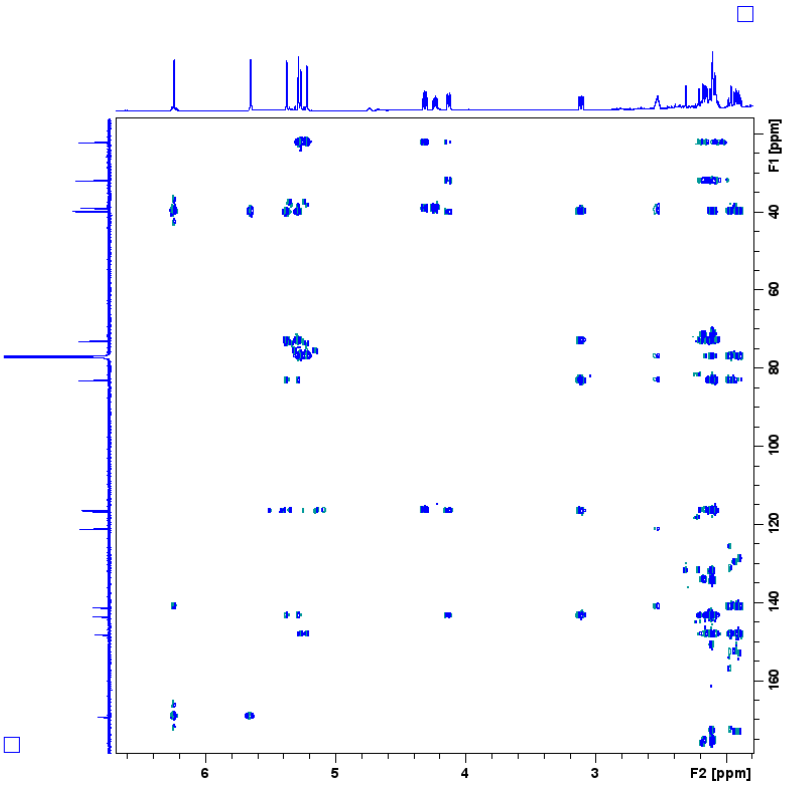

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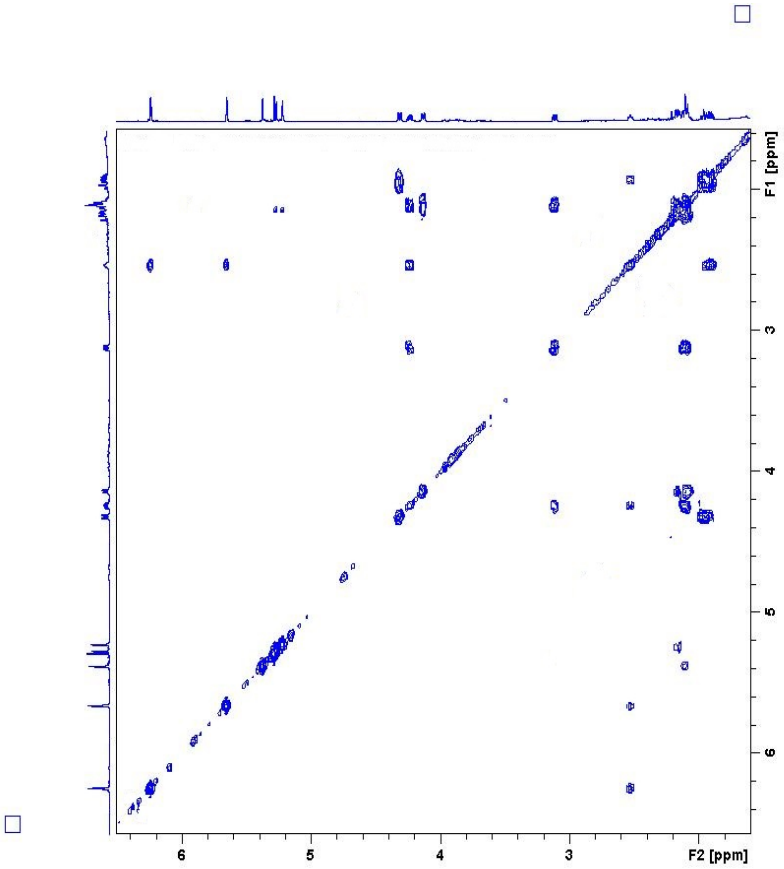

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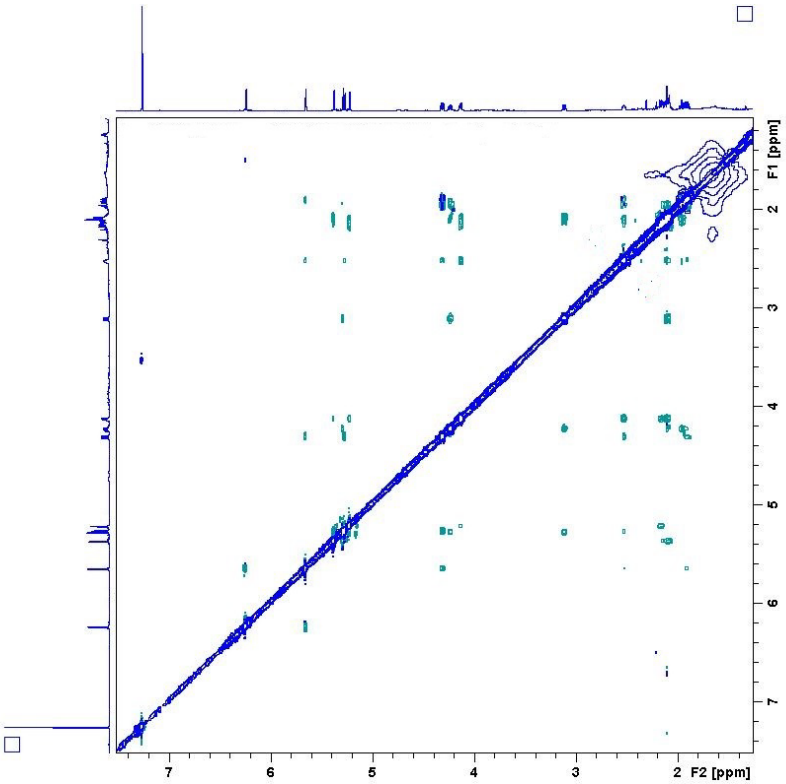

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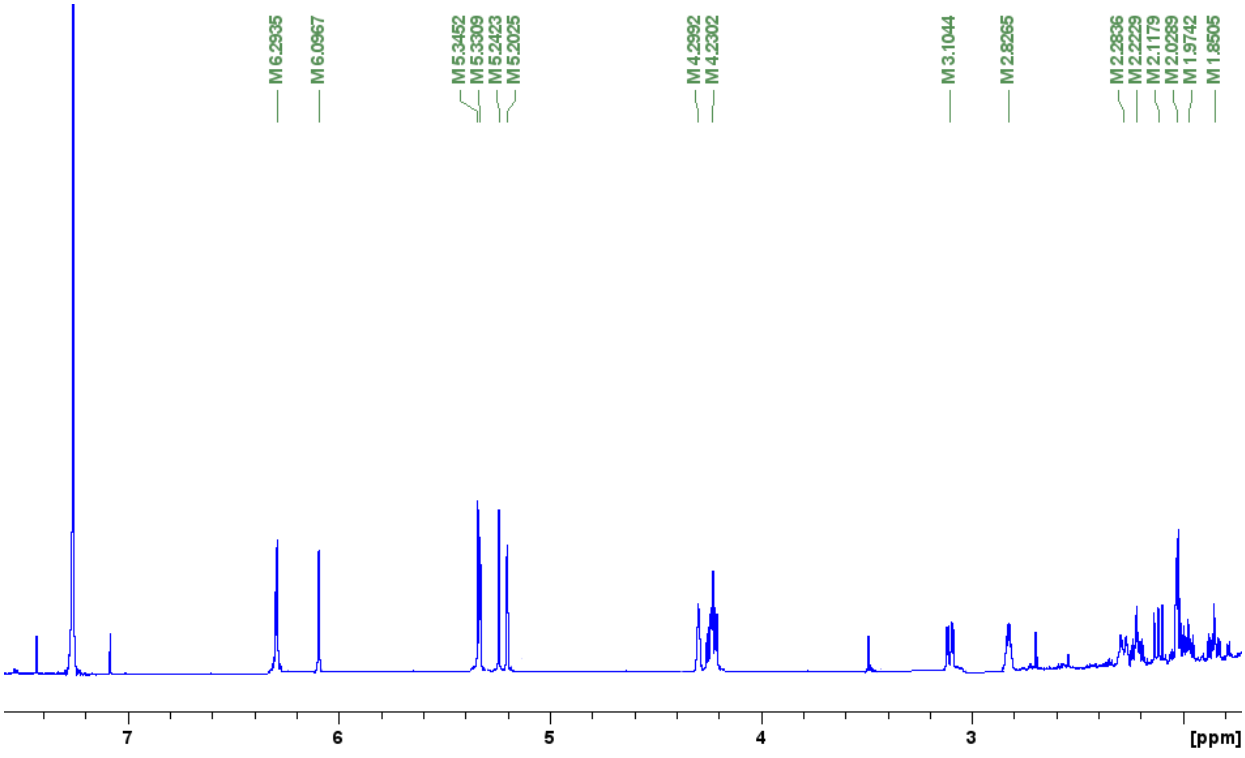

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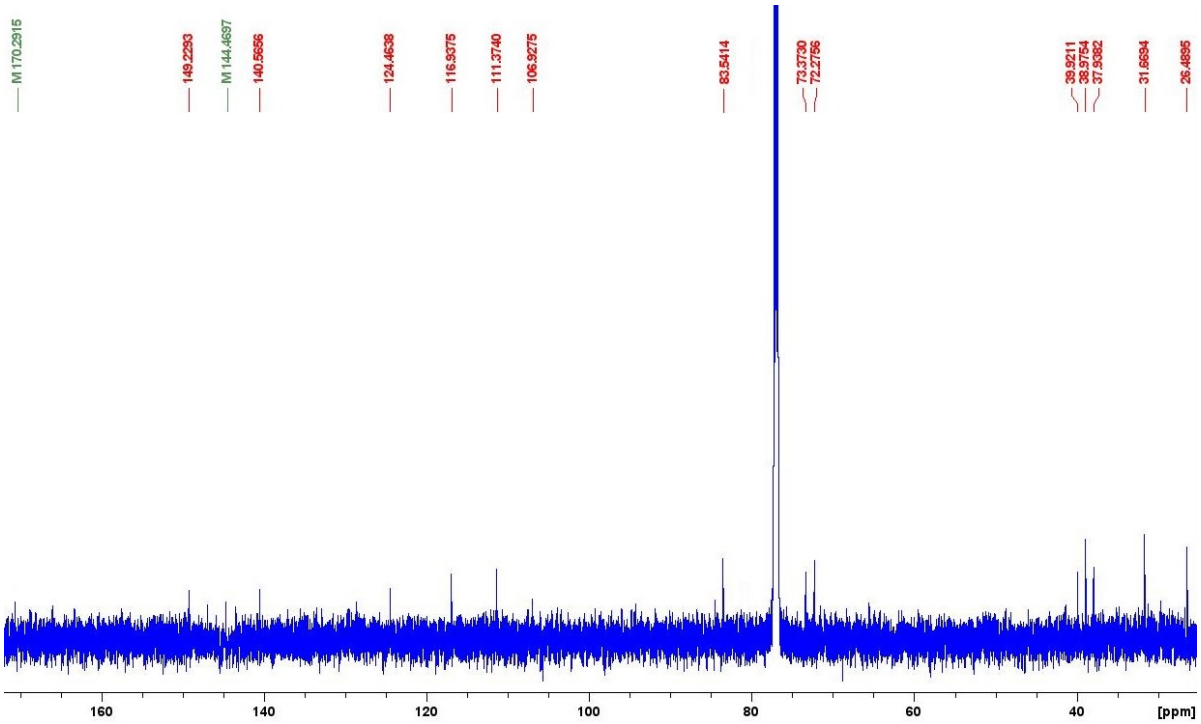

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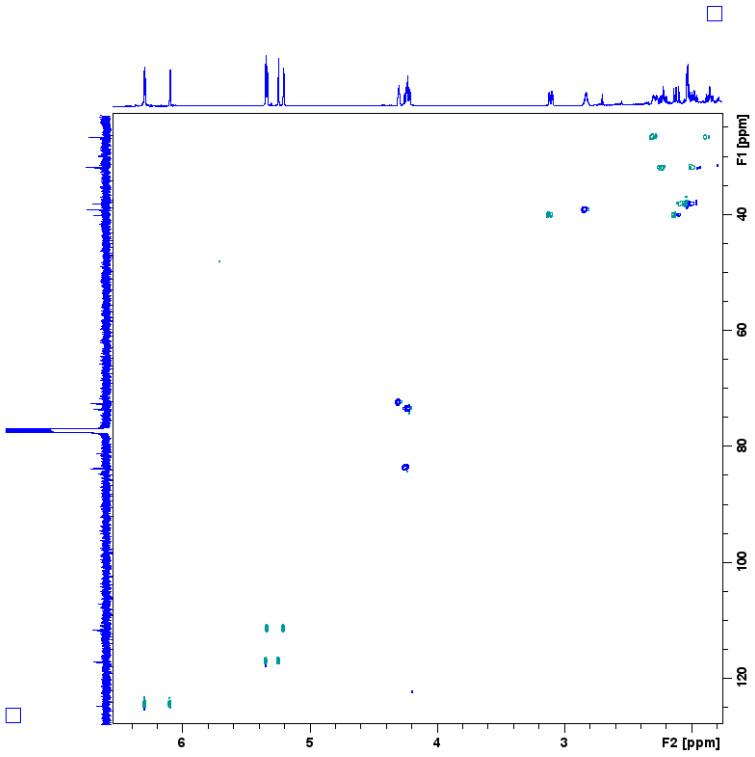

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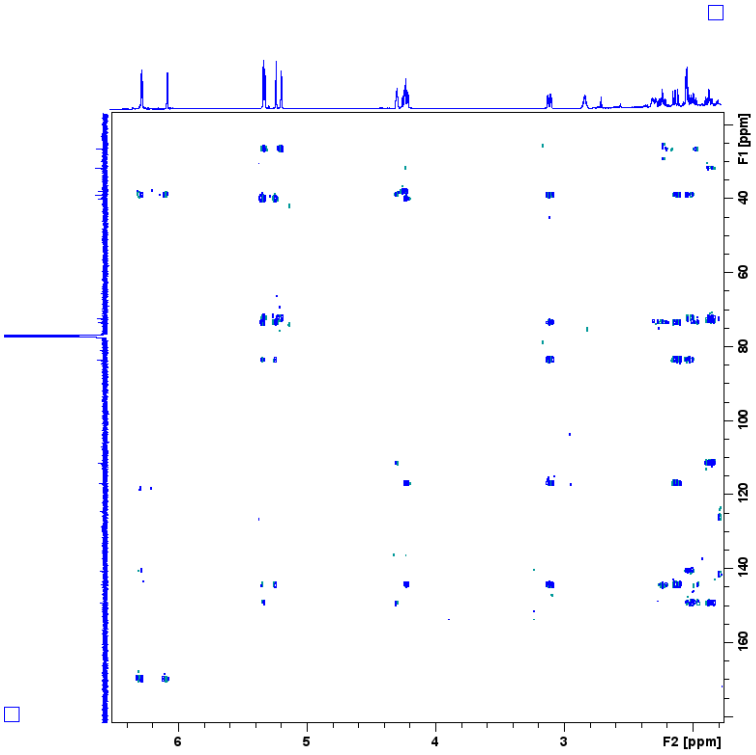

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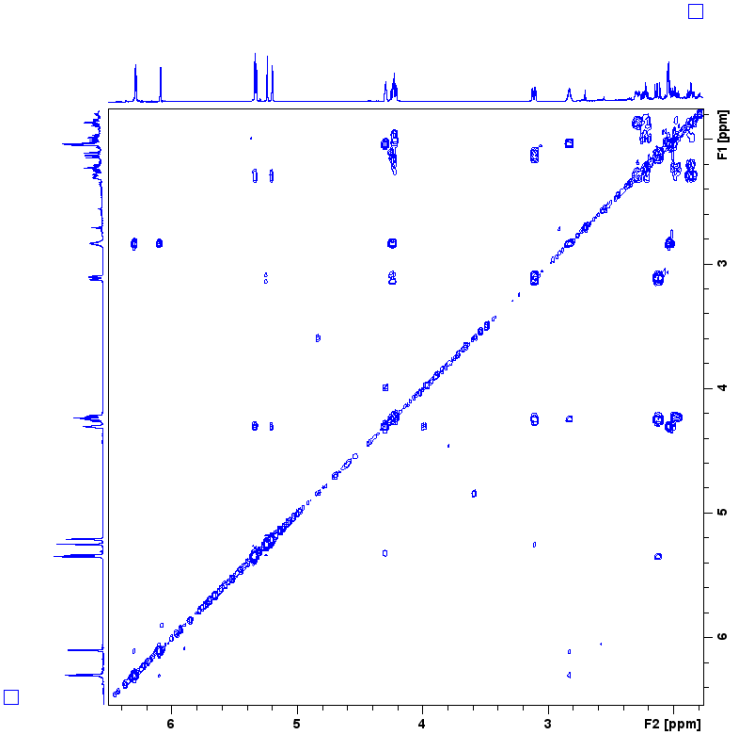

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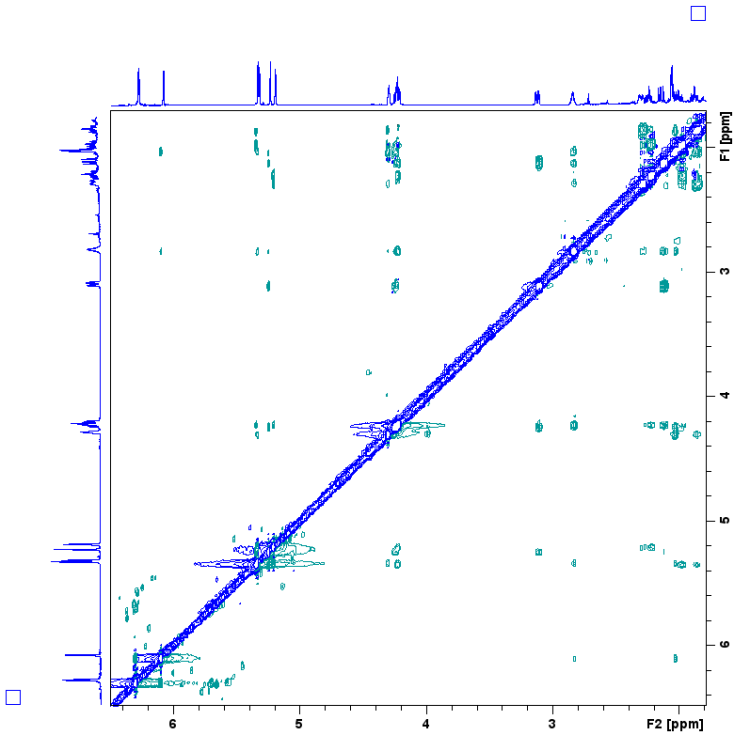

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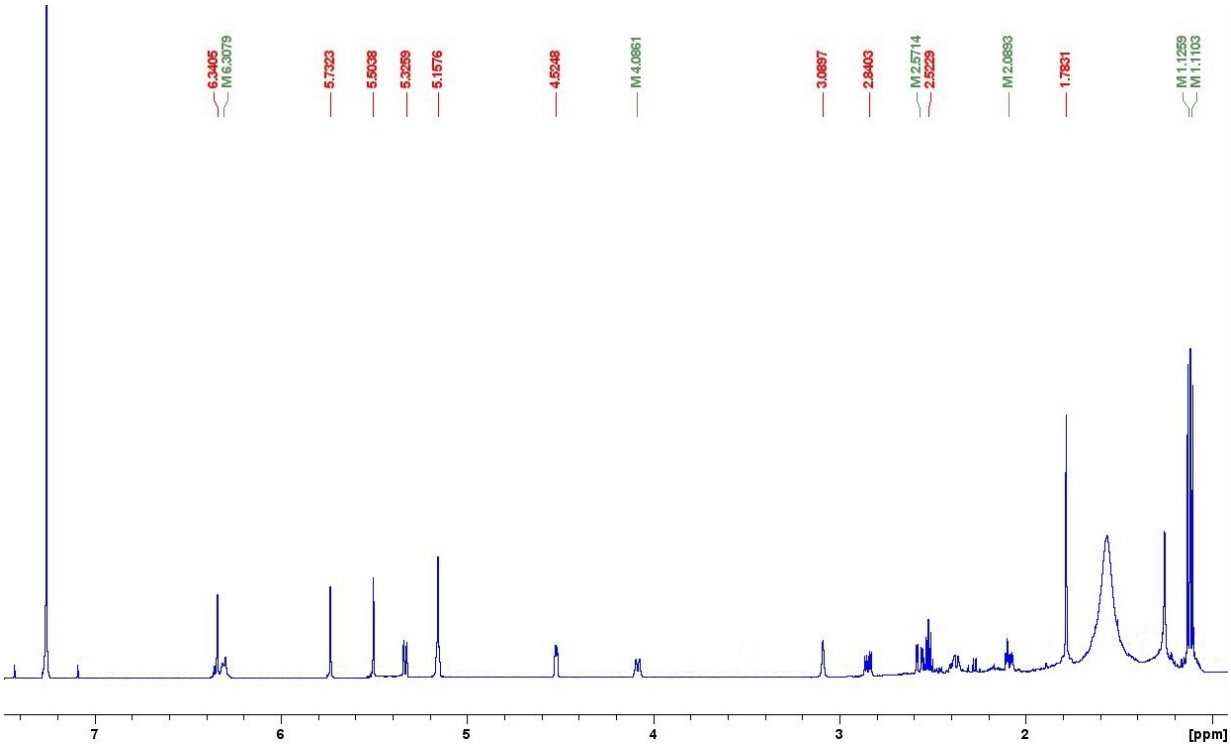

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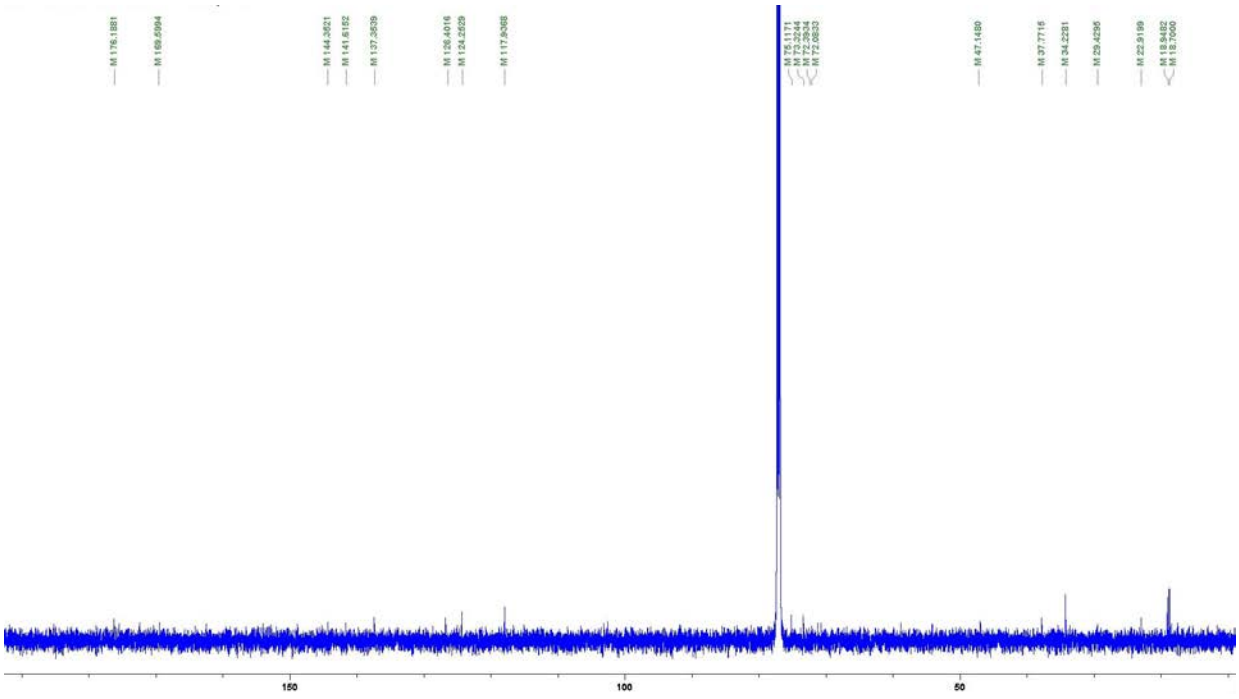

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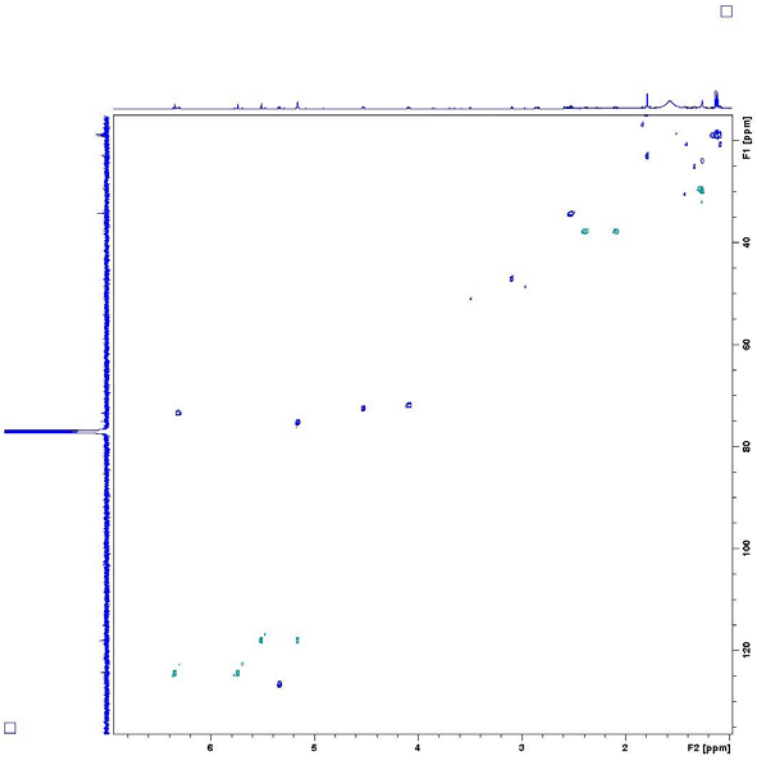

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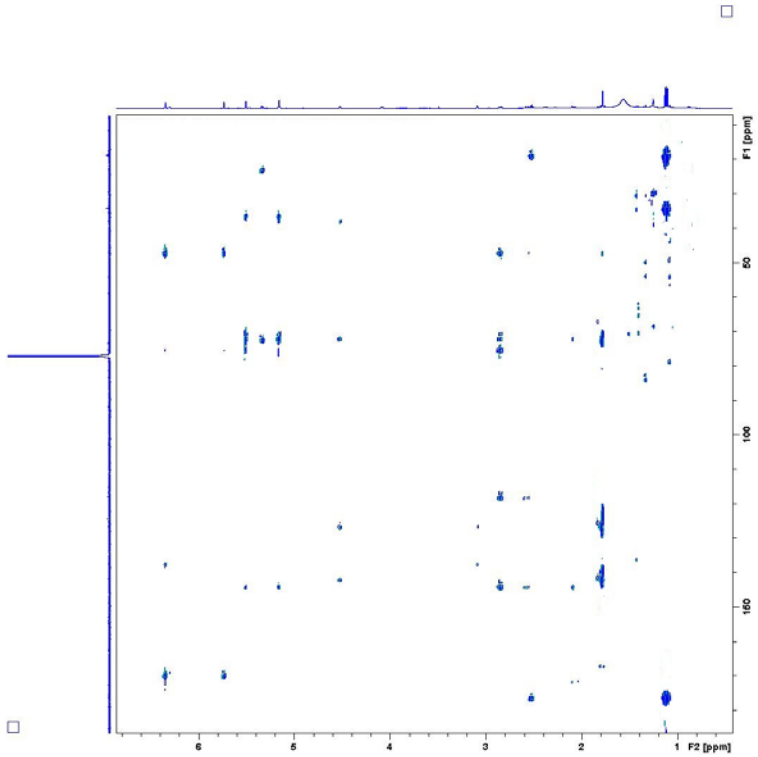

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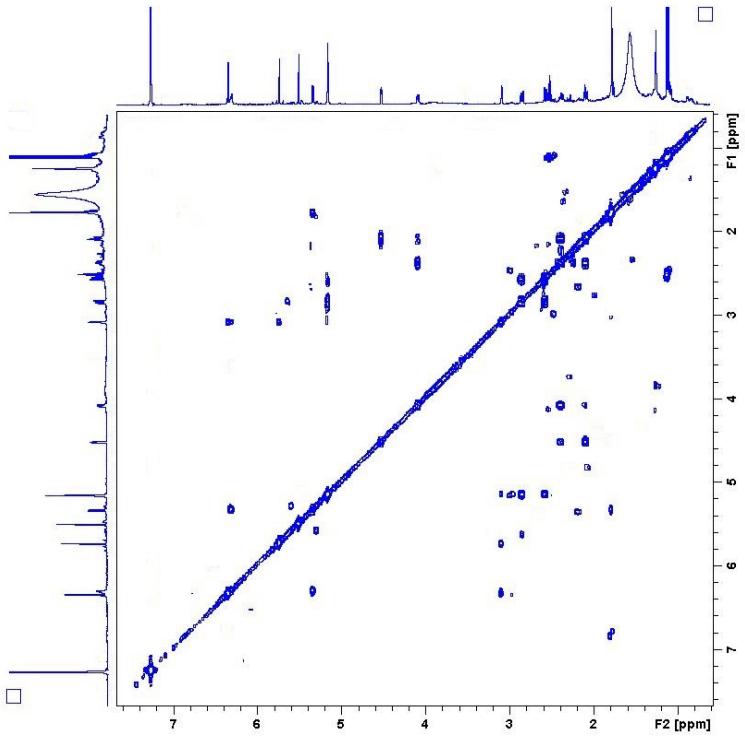

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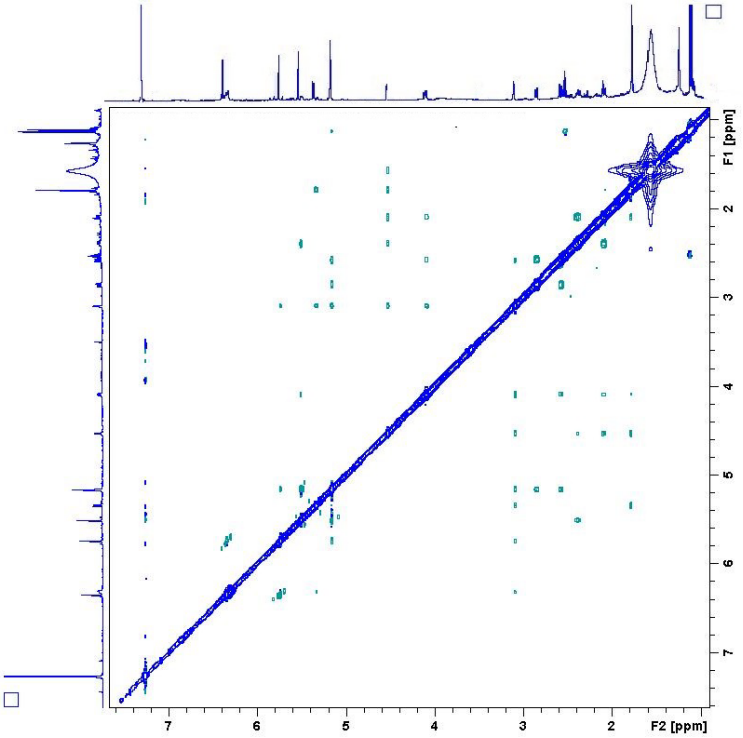

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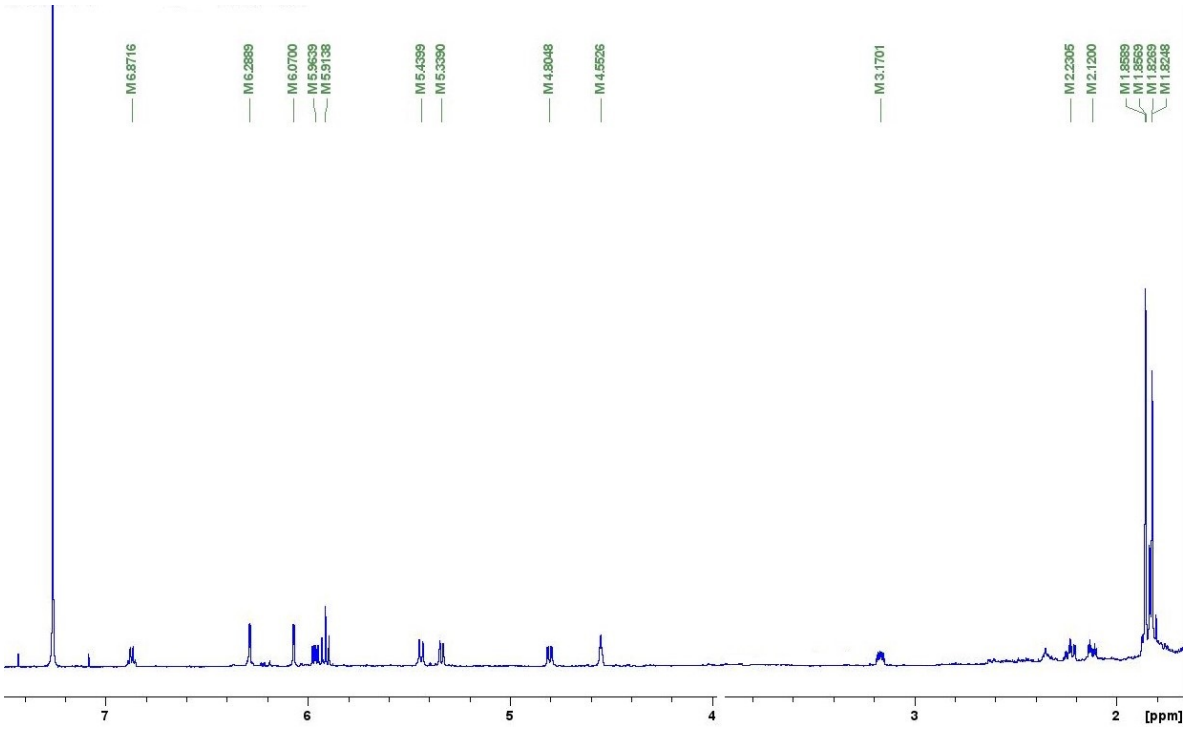

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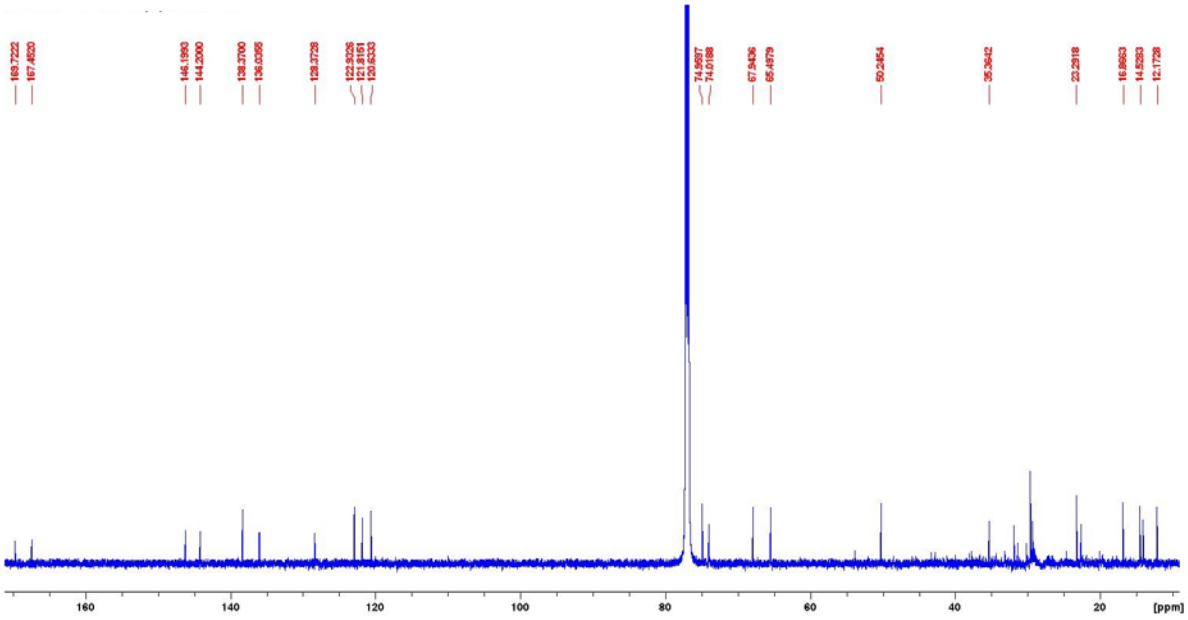

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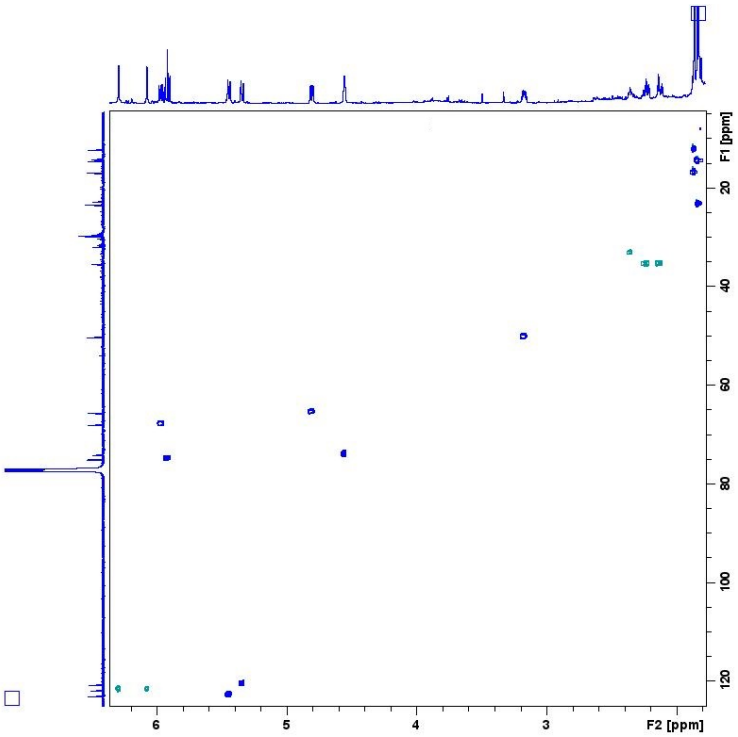

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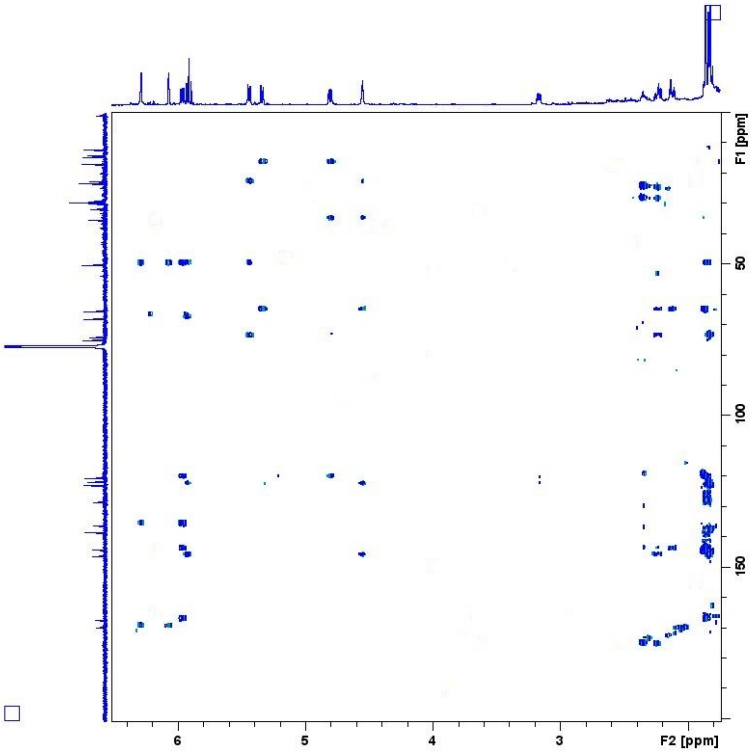

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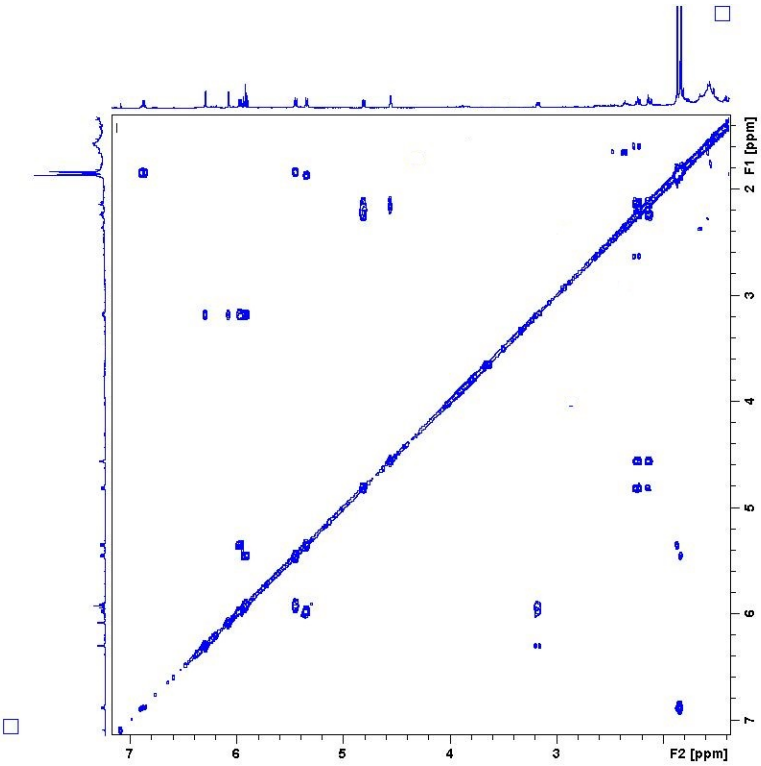

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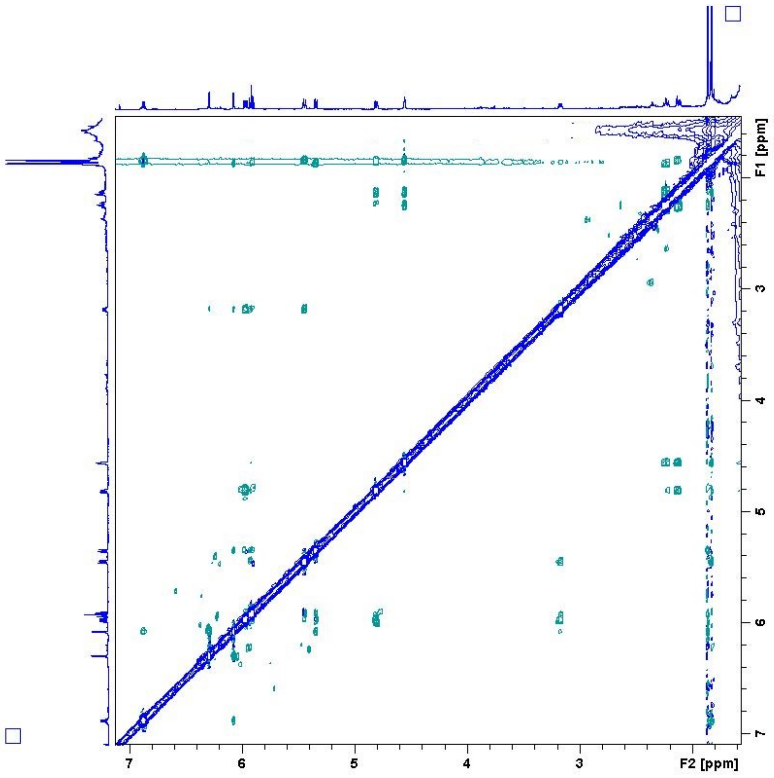
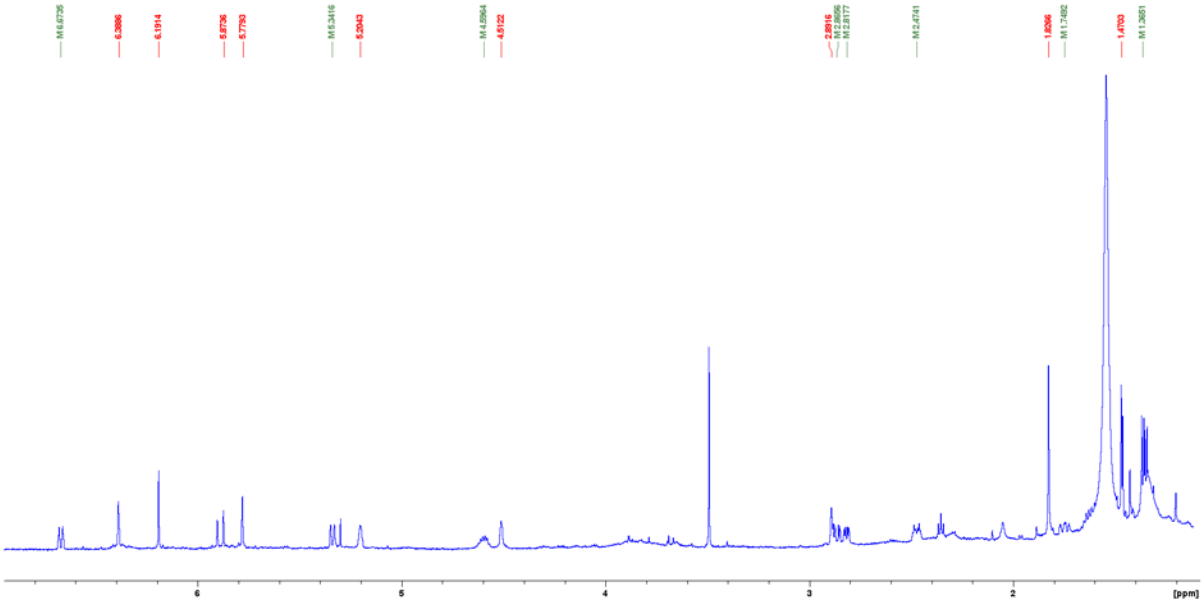
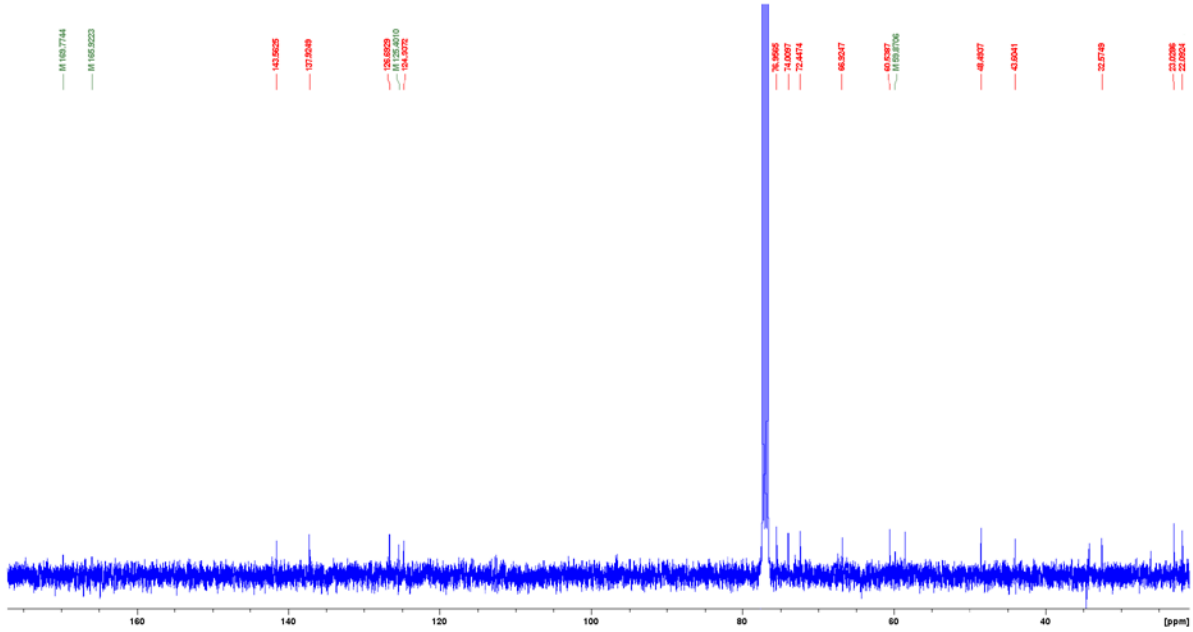


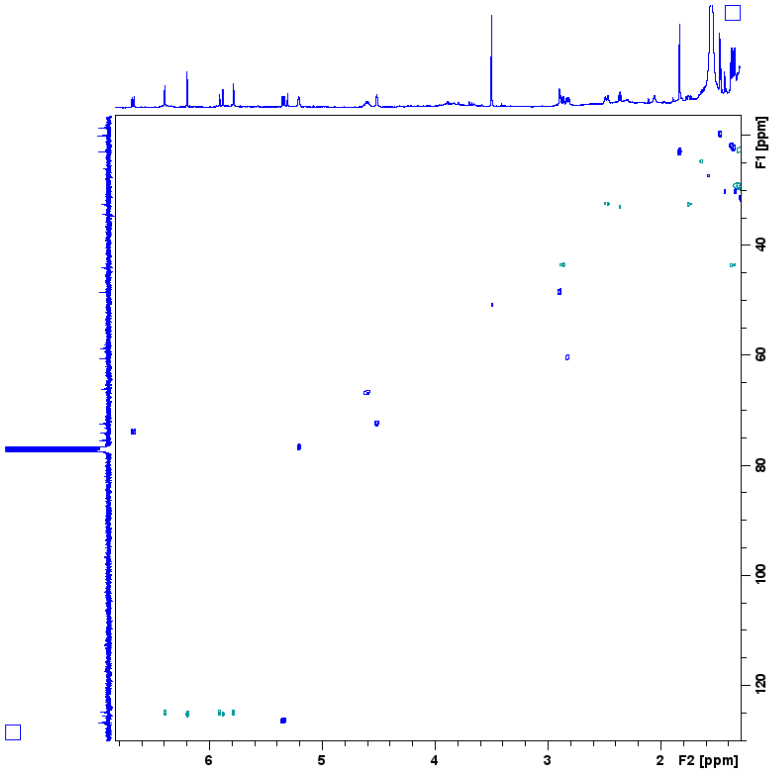
S24. NOESY spectrum of 5



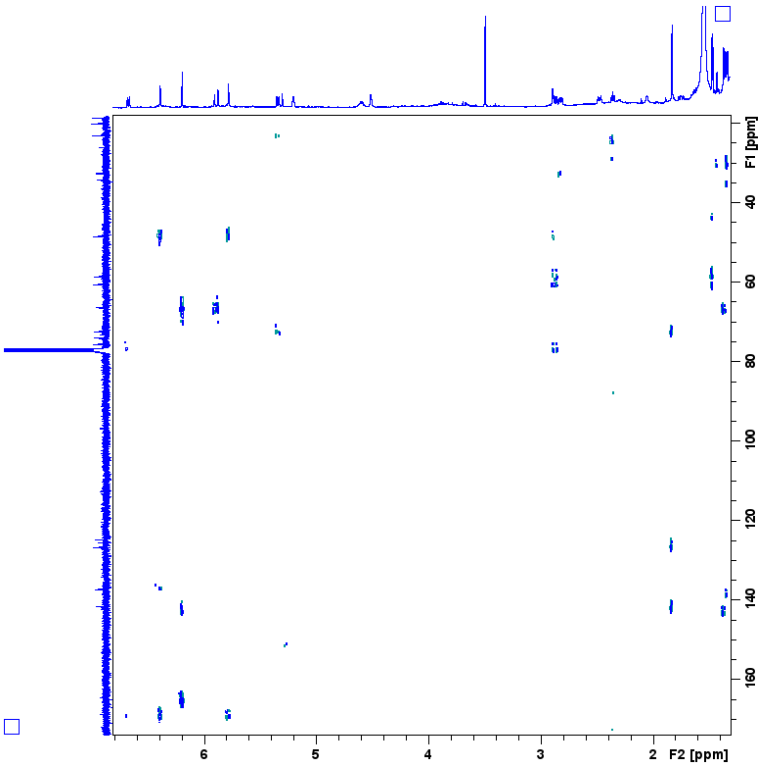
S25. ^{1}H NMR spectrum of 8



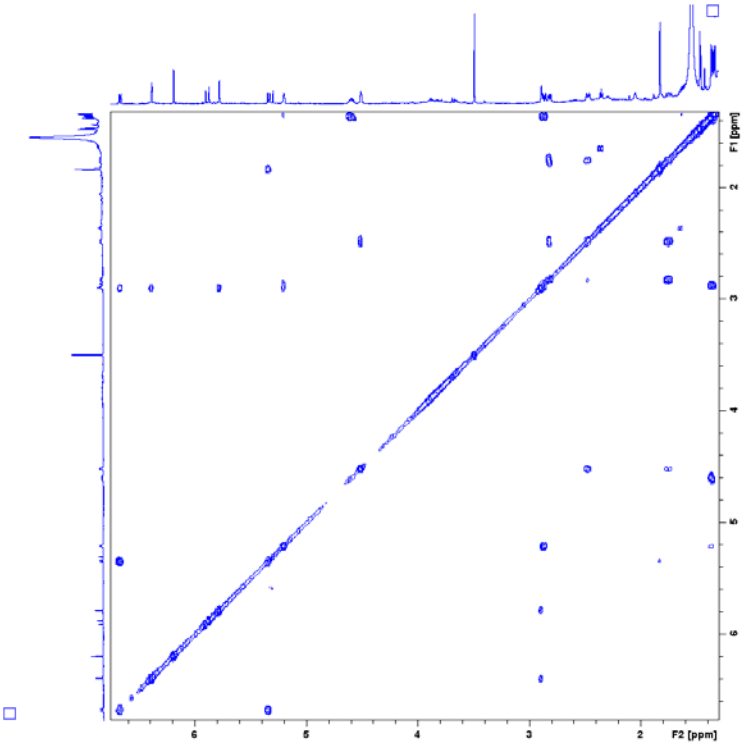
S26. ^{13}**C NMR spectrum of 8**



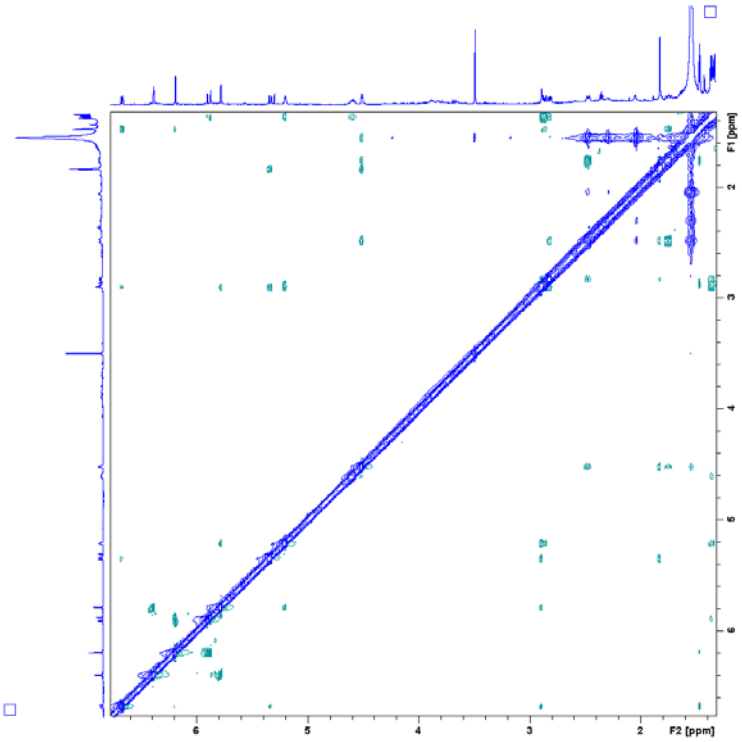
S27. HSQC spectrum of 8



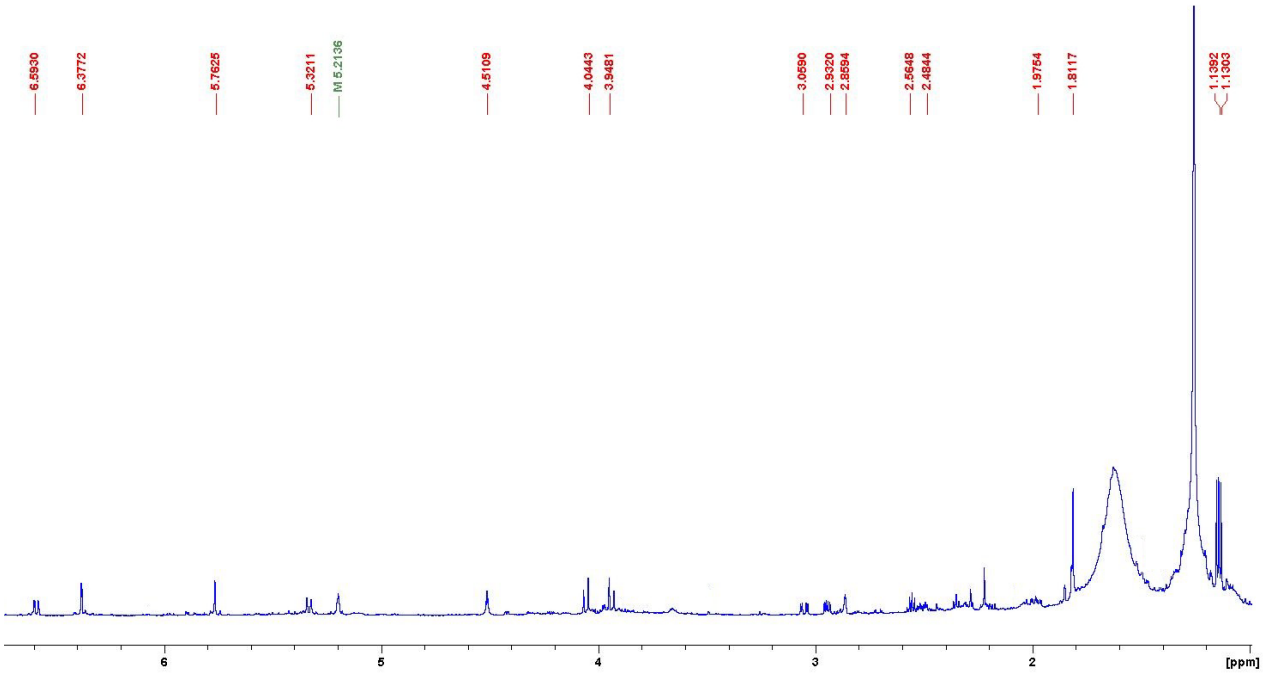
S28. HMBC spectrum of 8



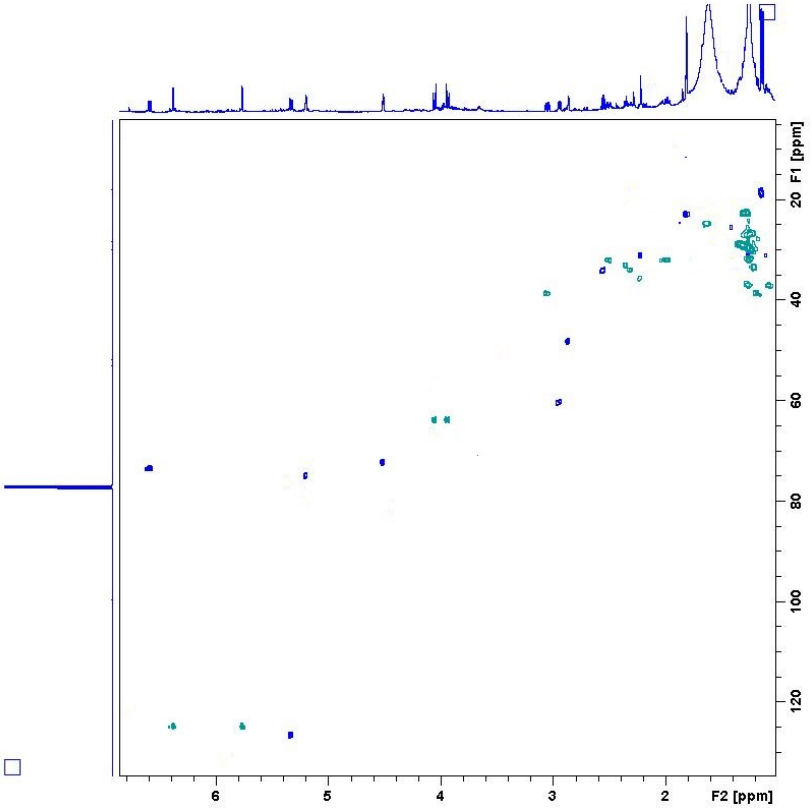
S29. COSY spectrum of 8



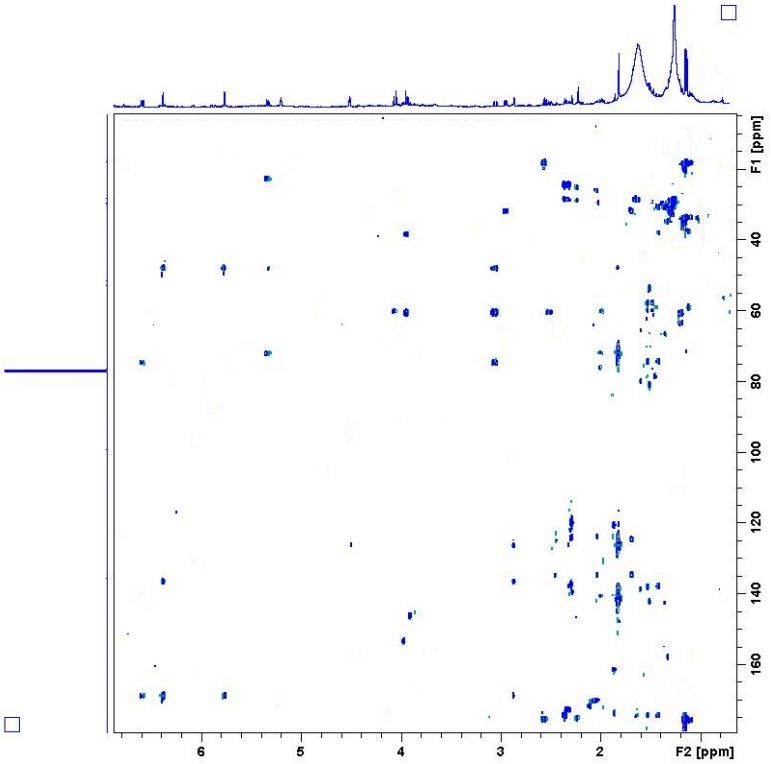
S30. NOESY spectrum of 8



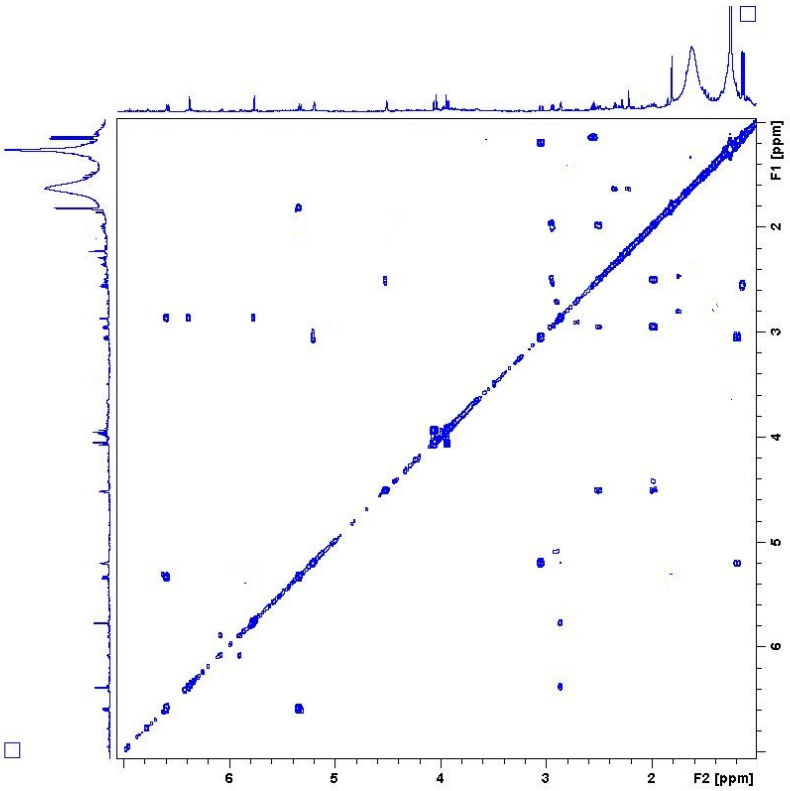
S31. ^{1}H NMR spectrum of 9



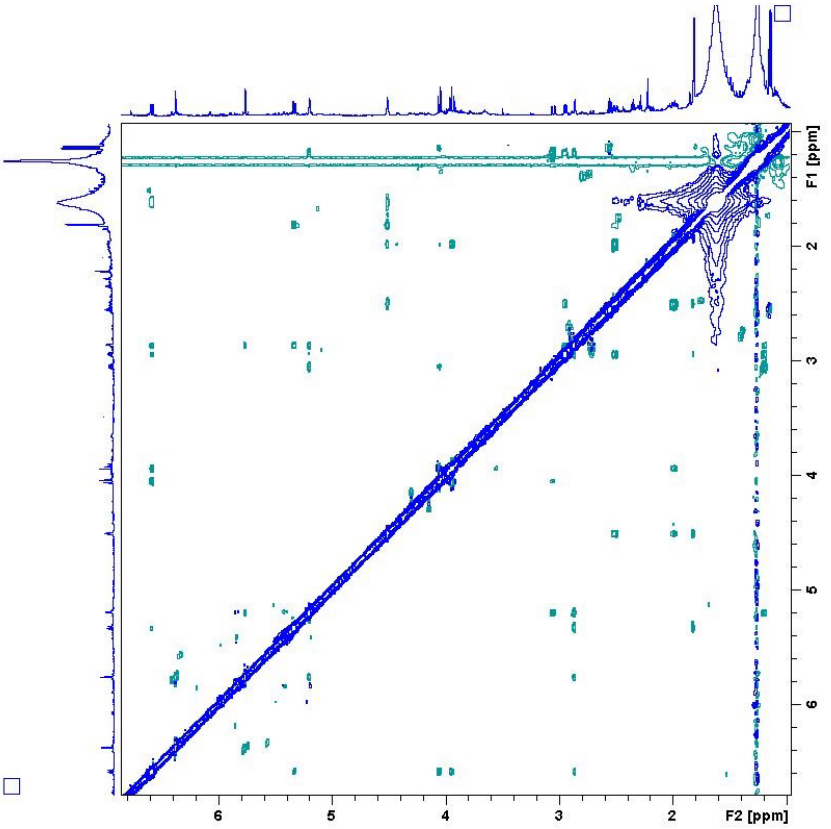
S32. HSQC spectrum of 9



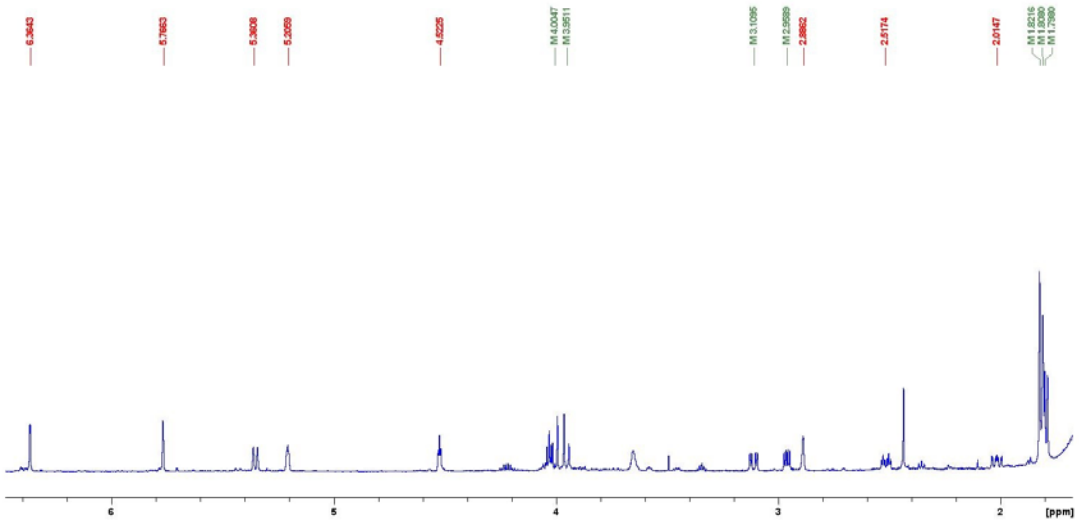
S33. HMBC spectrum of 9



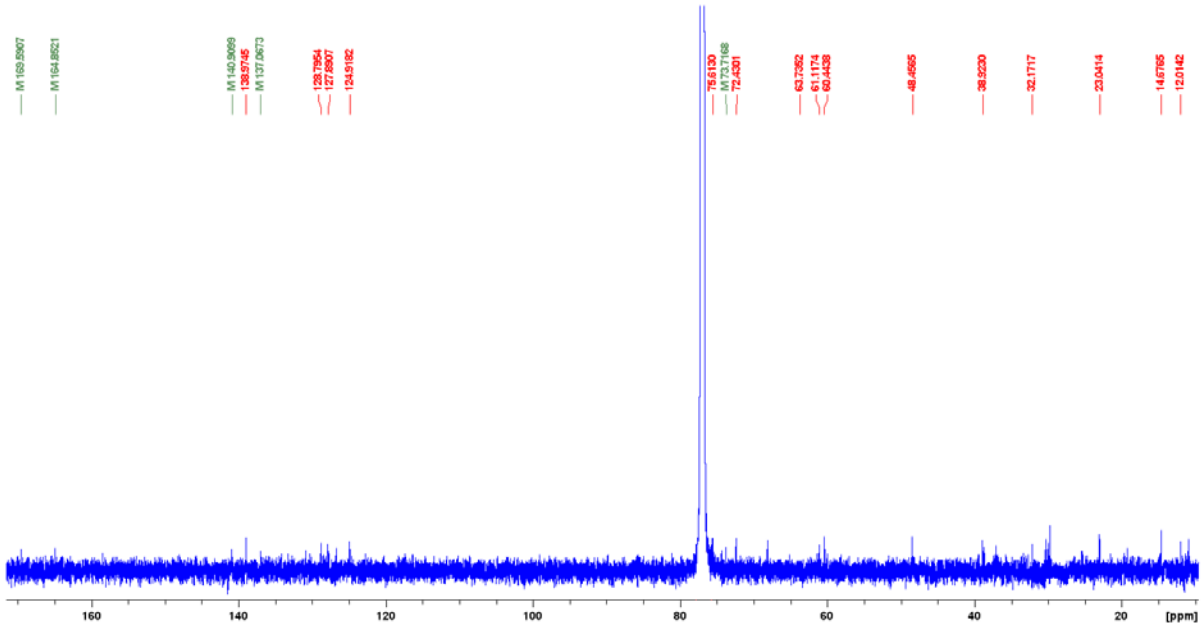
S34. COSY spectrum of 9



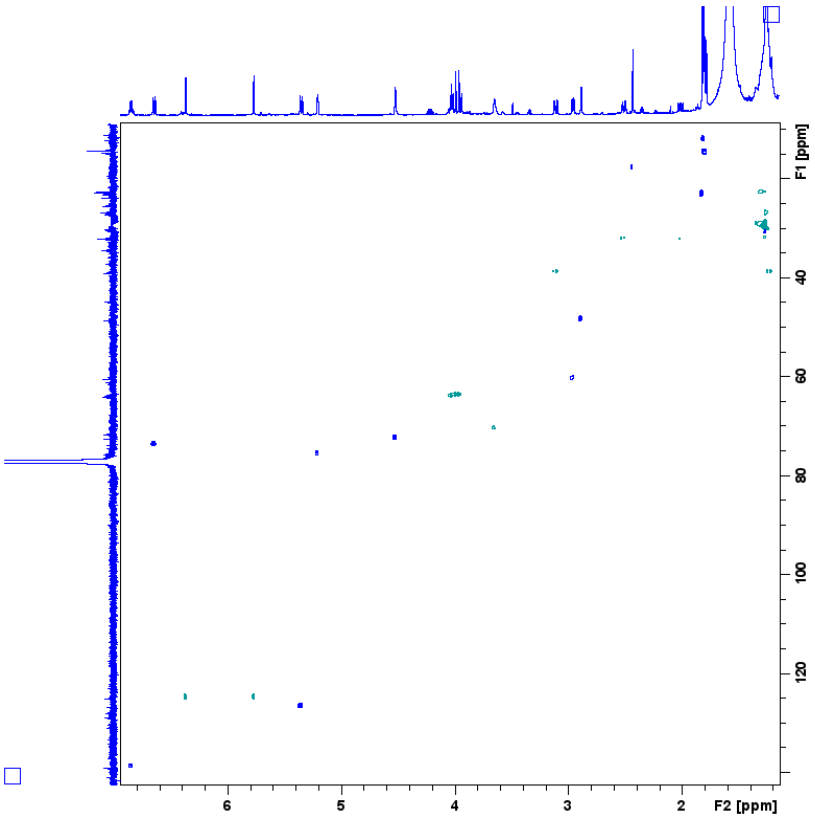
S35. NOESY spectrum of 9



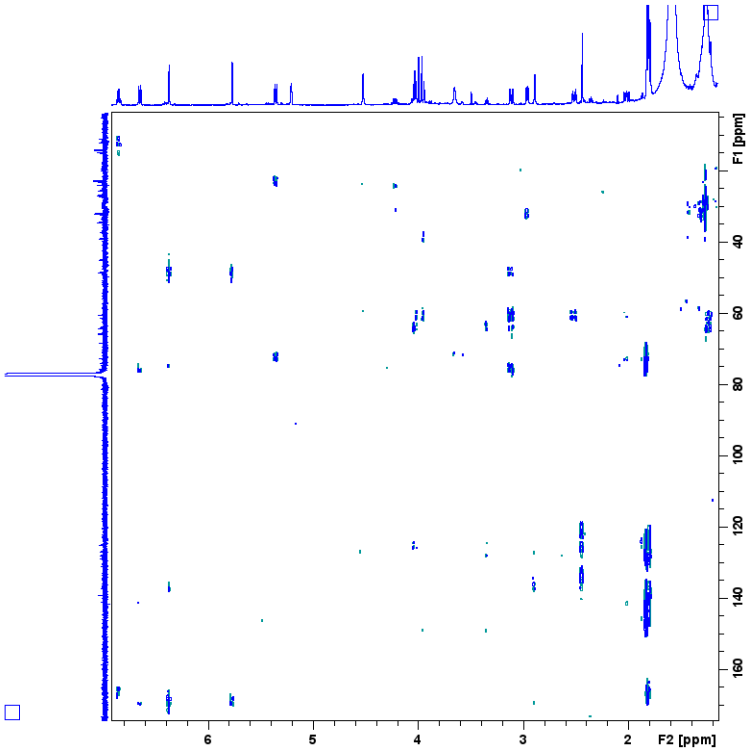
S36. ^{1}H NMR spectrum of 10



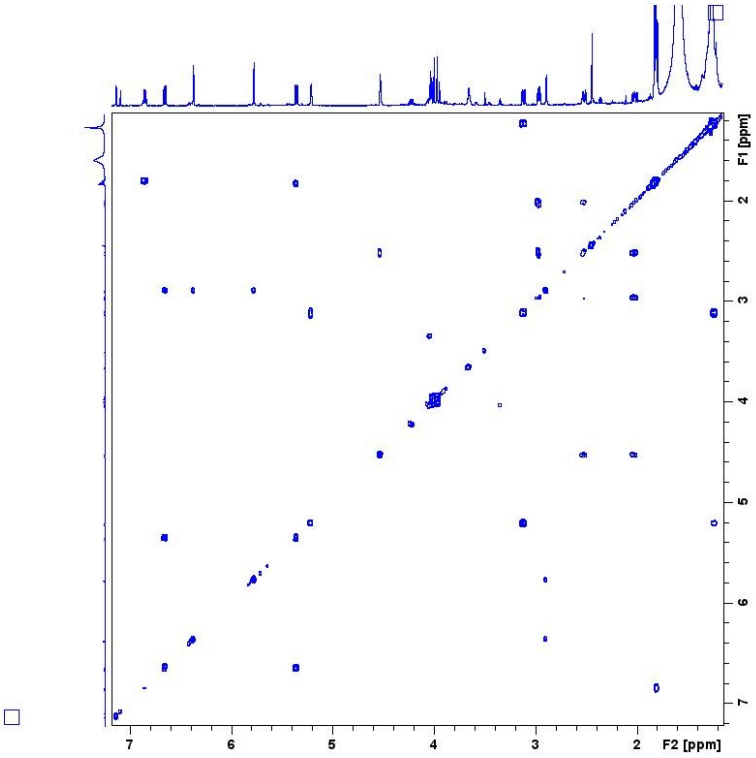
S37. ^{13}C NMR spectrum of 10



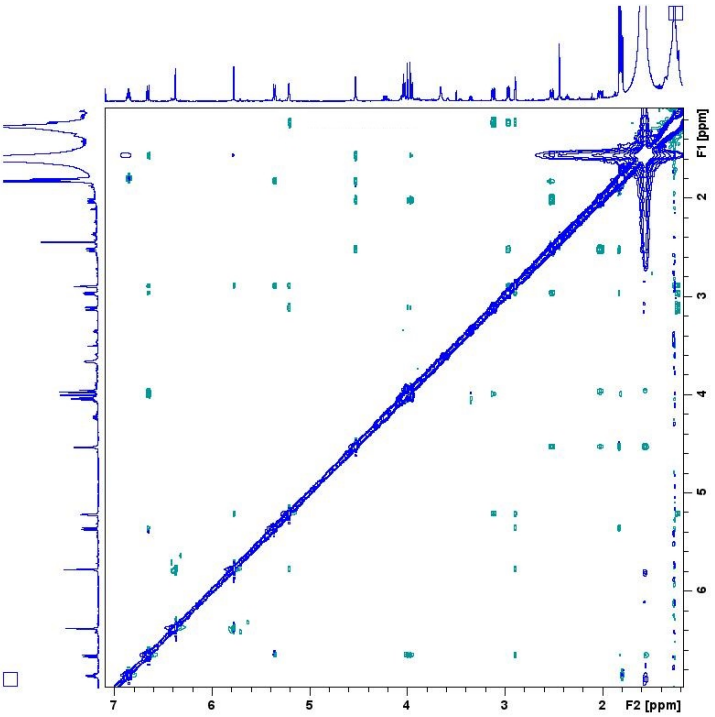
S38. HSQC spectrum of 10



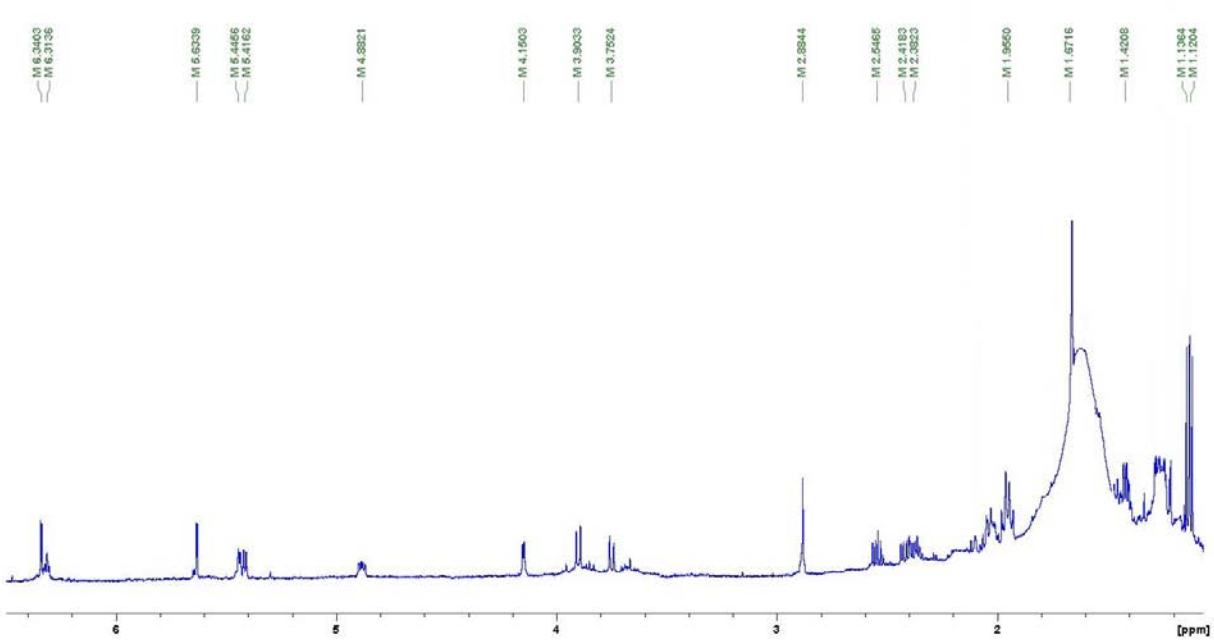
S39. **HMBC spectrum of 10**



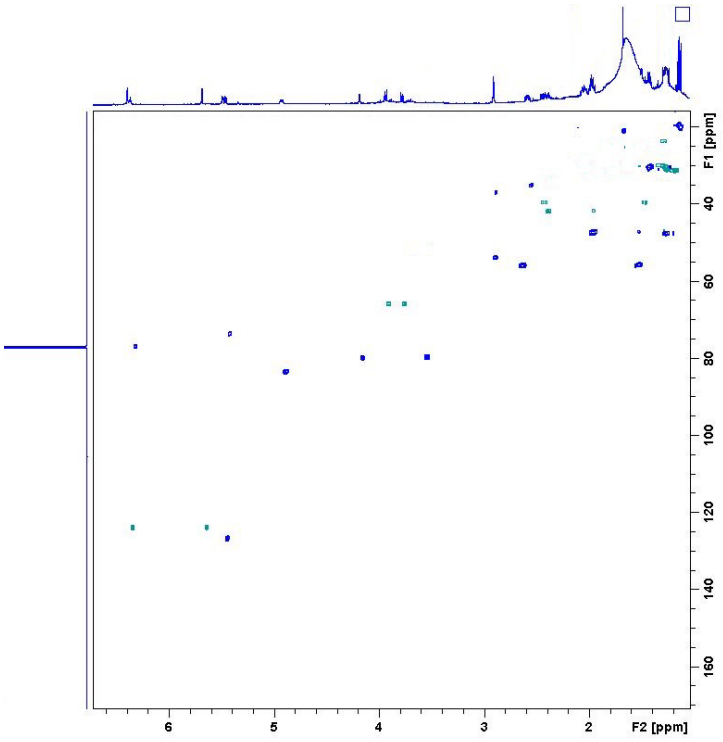
S40. **COSY spectrum of 10**



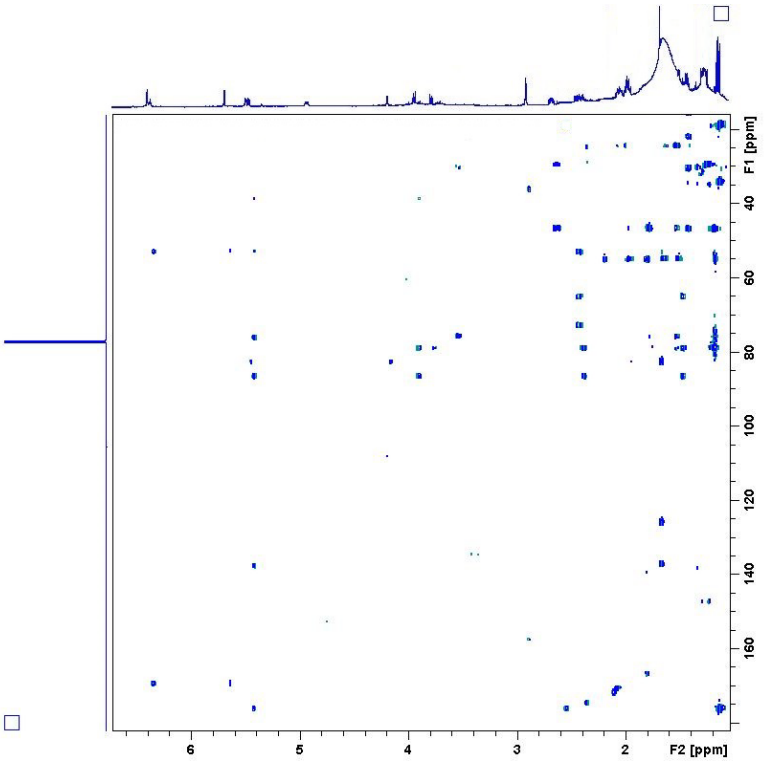
S41. **NOESY spectrum of 10**



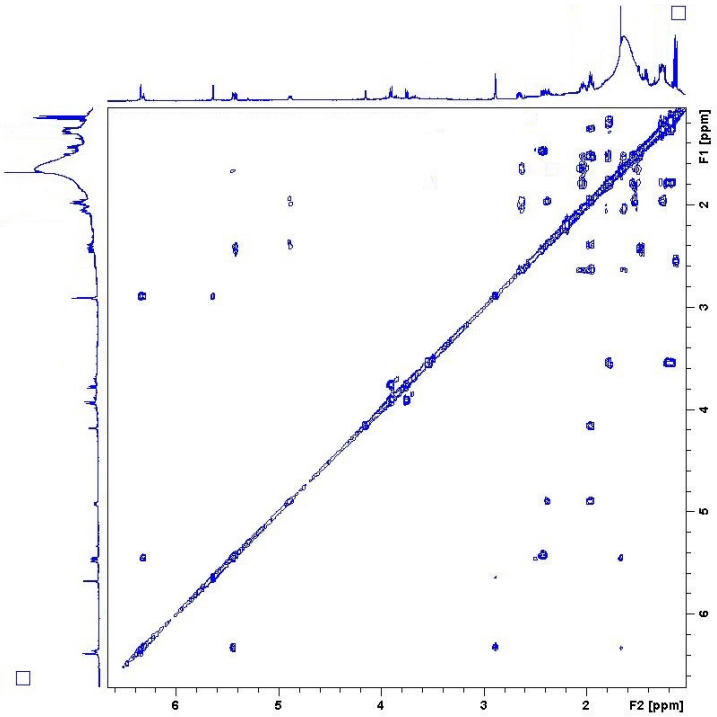
S42. ^{1}**H NMR spectrum of 11**



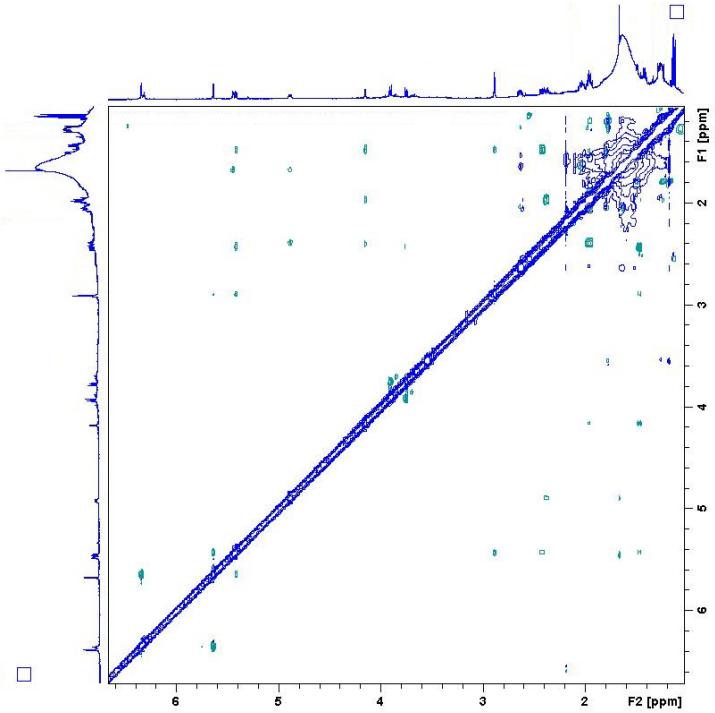
S43. **HSQC spectrum of 11**



S44. **HMBC spectrum of 11**

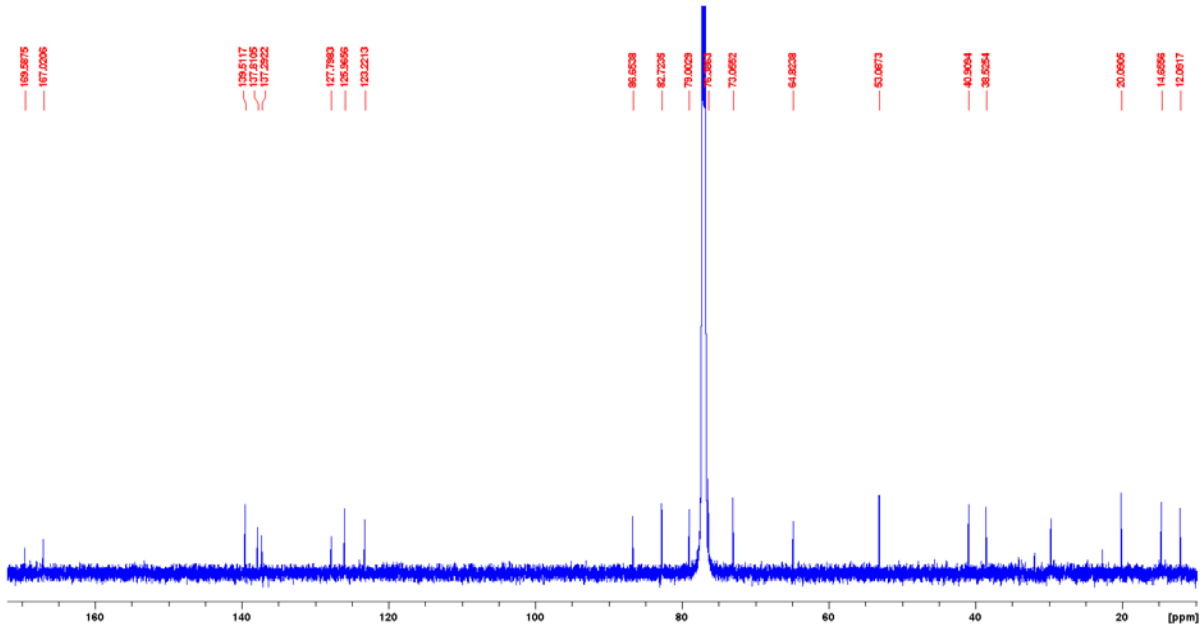


S45. **COSY spectrum of 11**

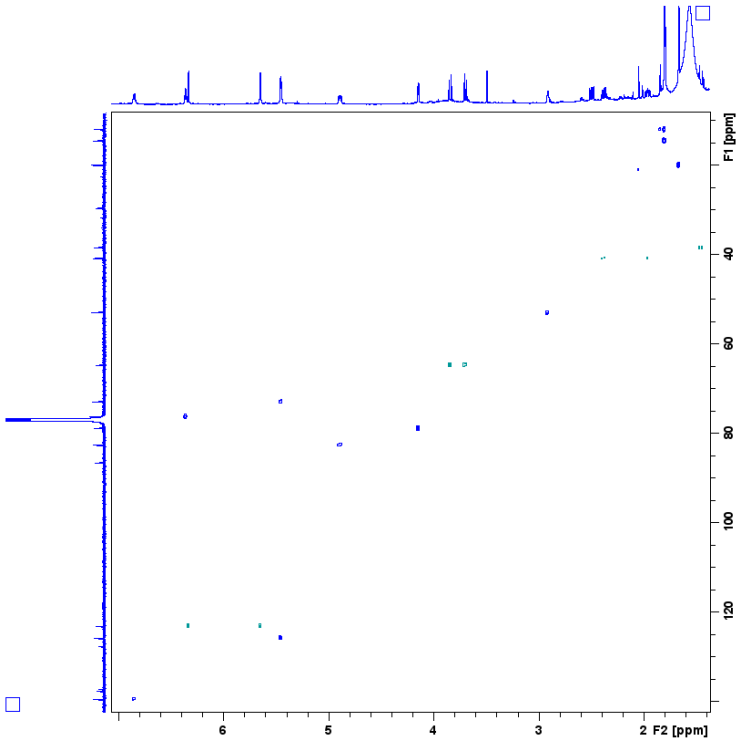


S46. **NOESY spectrum of 11**

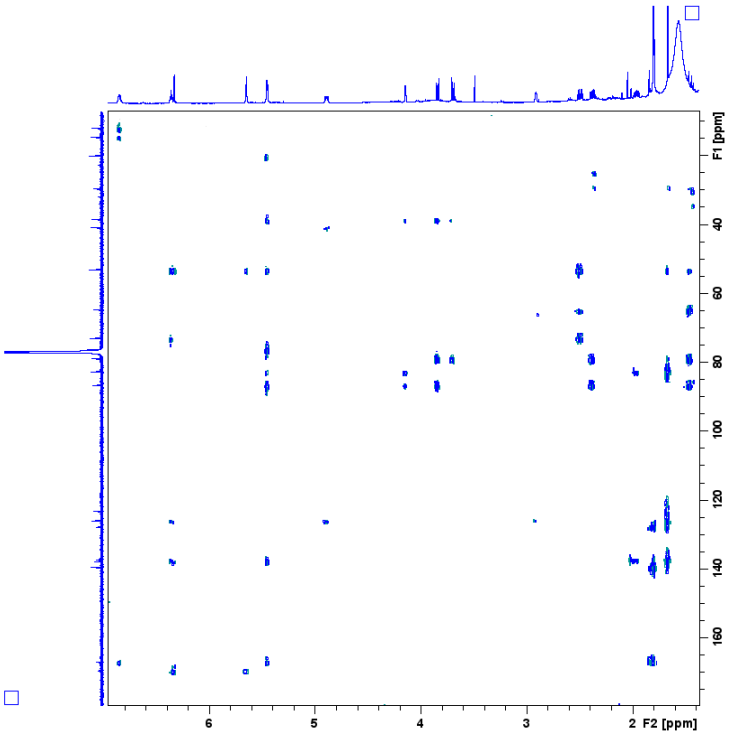
S47. **^{1}H NMR spectrum of 12**



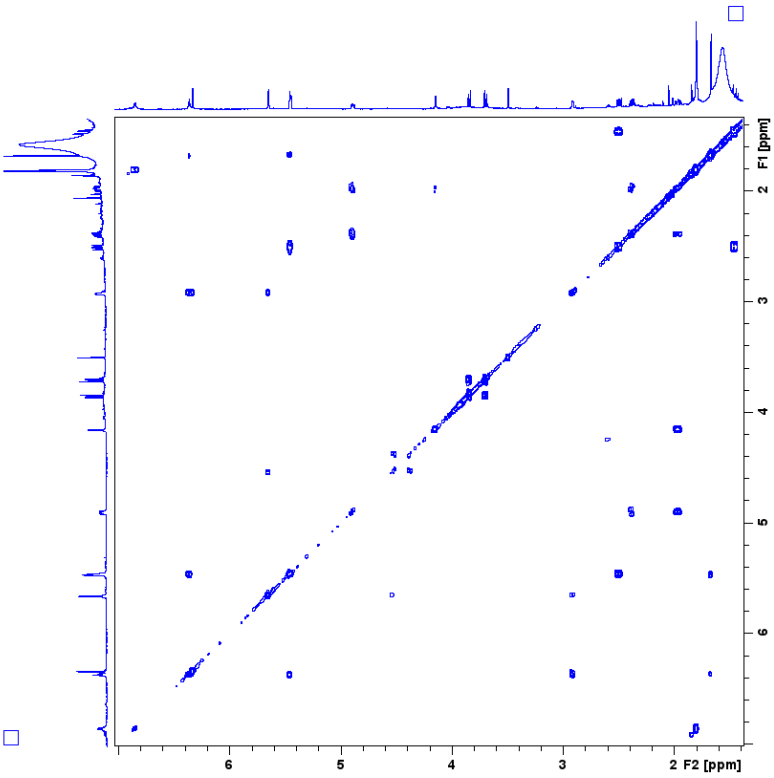
S48. ^{13}**C NMR spectrum of 12**



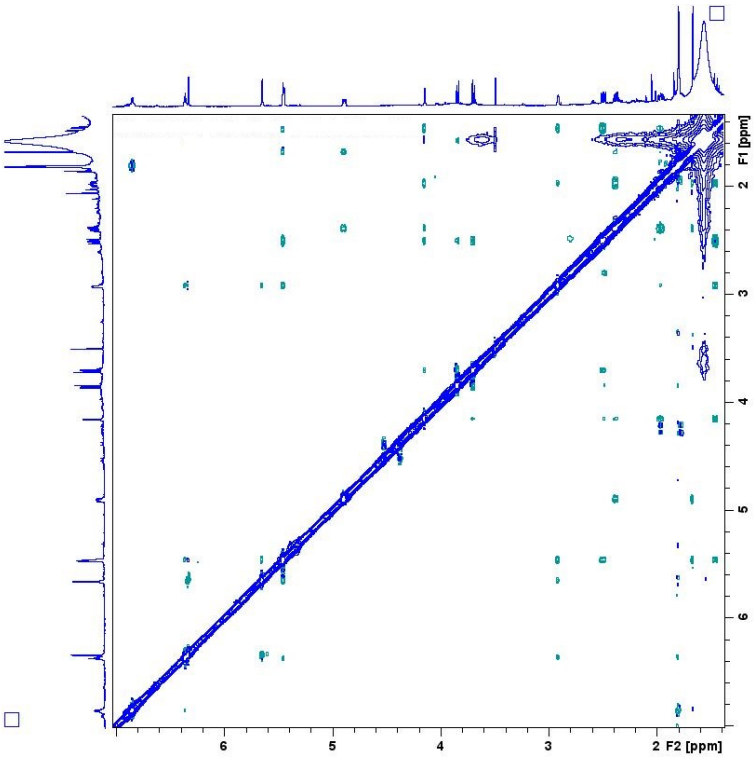
S49. HSQC spectrum of 12



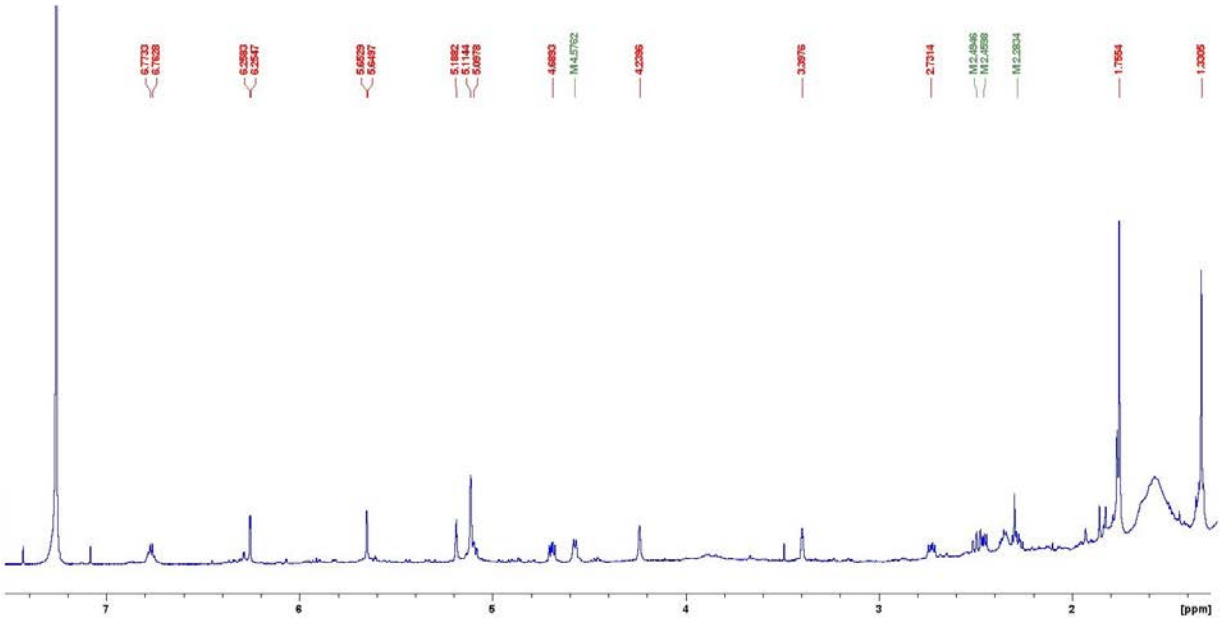
S50. HMBC spectrum of 12



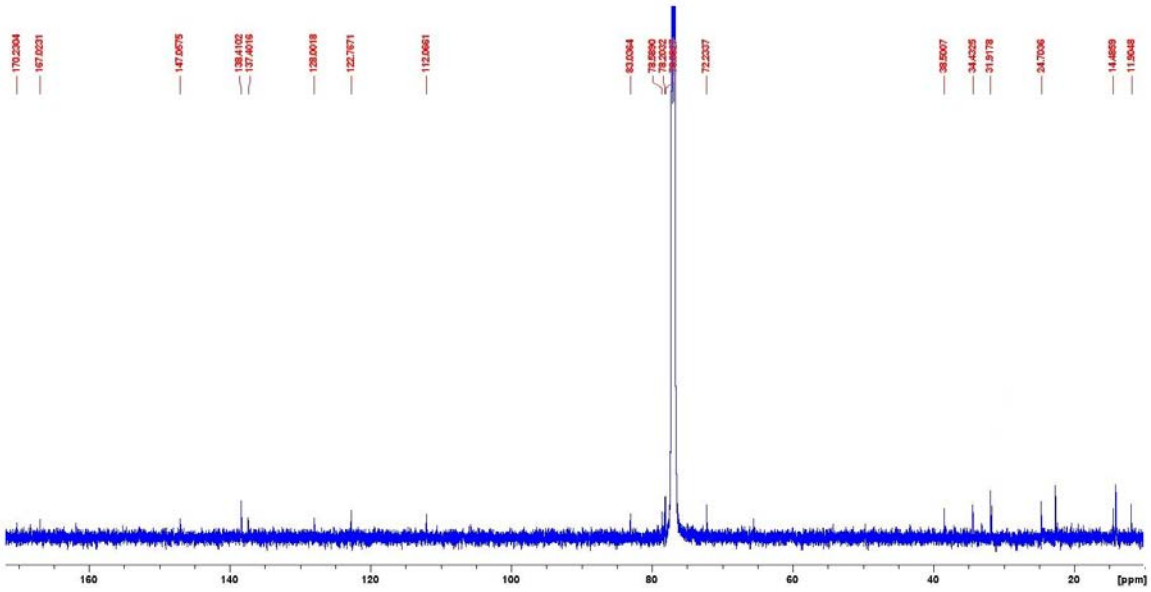
S51. COSY spectrum of 12



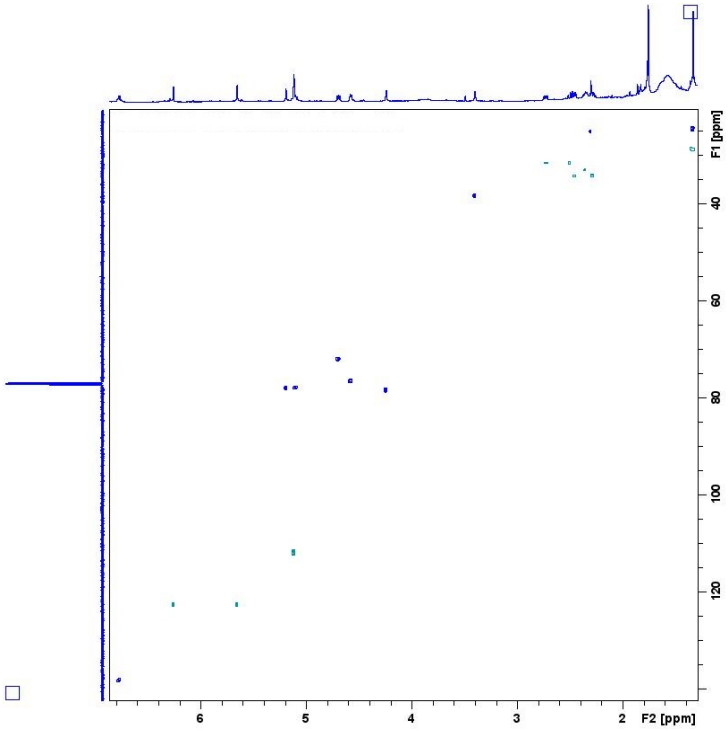
S52. NOESY spectrum of 12



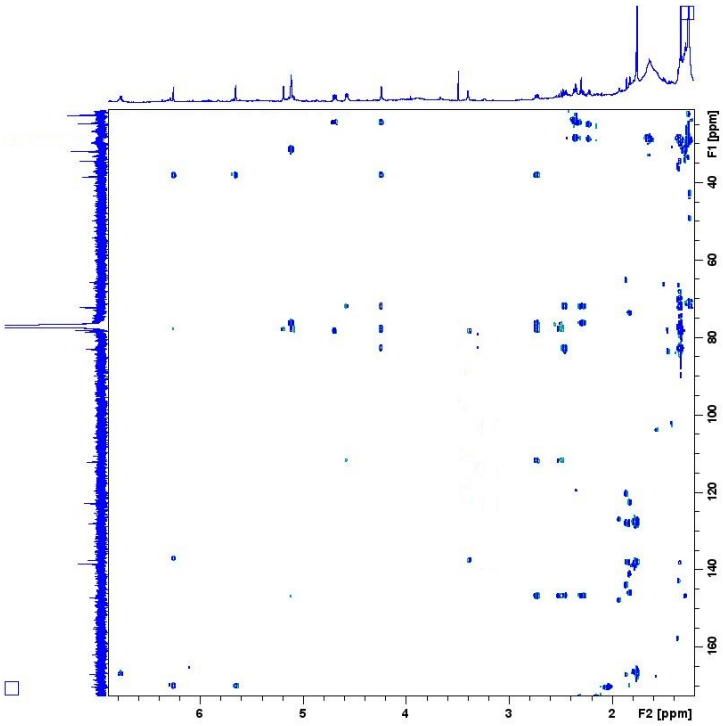
S53. ^{1}H NMR spectrum of 13



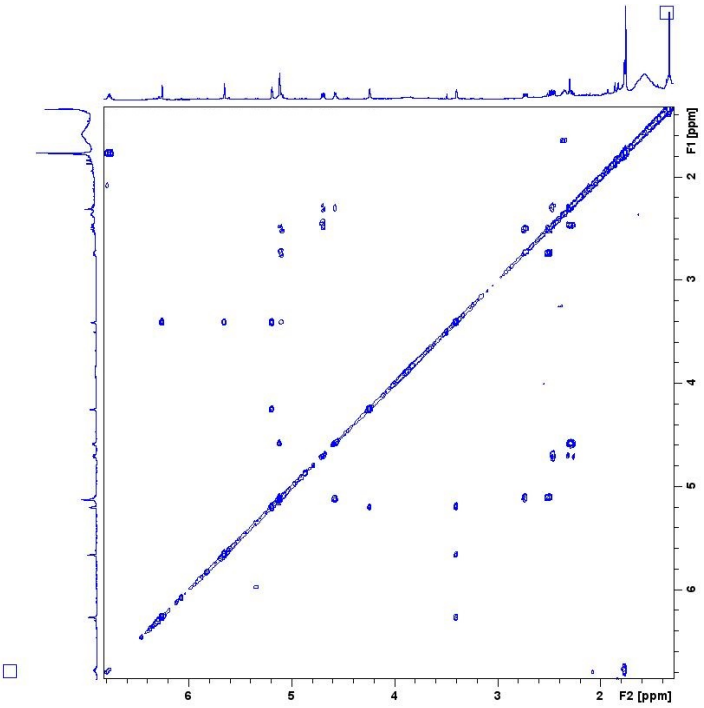
S54. ^{13}**C NMR spectrum of 13**



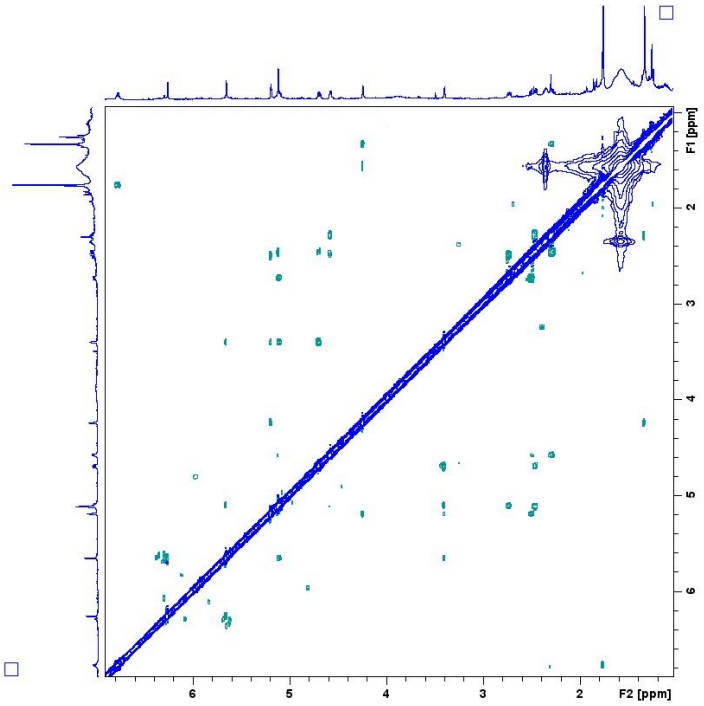
S55. **HSQC spectrum of 13**



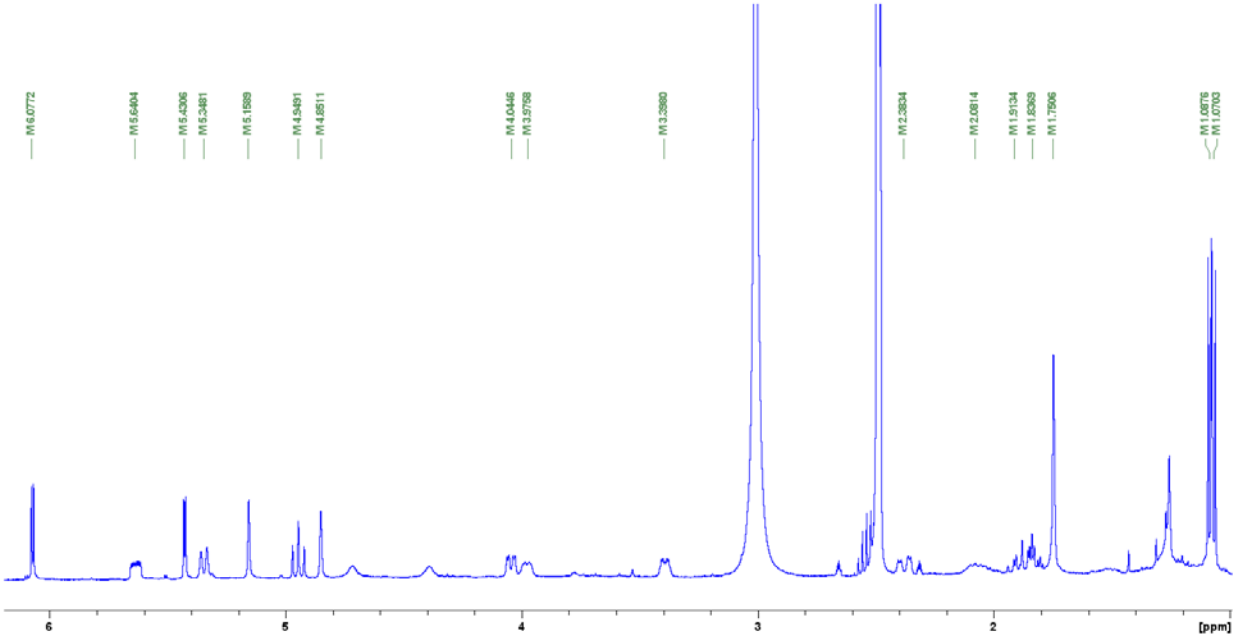
S56. **HMBC spectrum of 13**



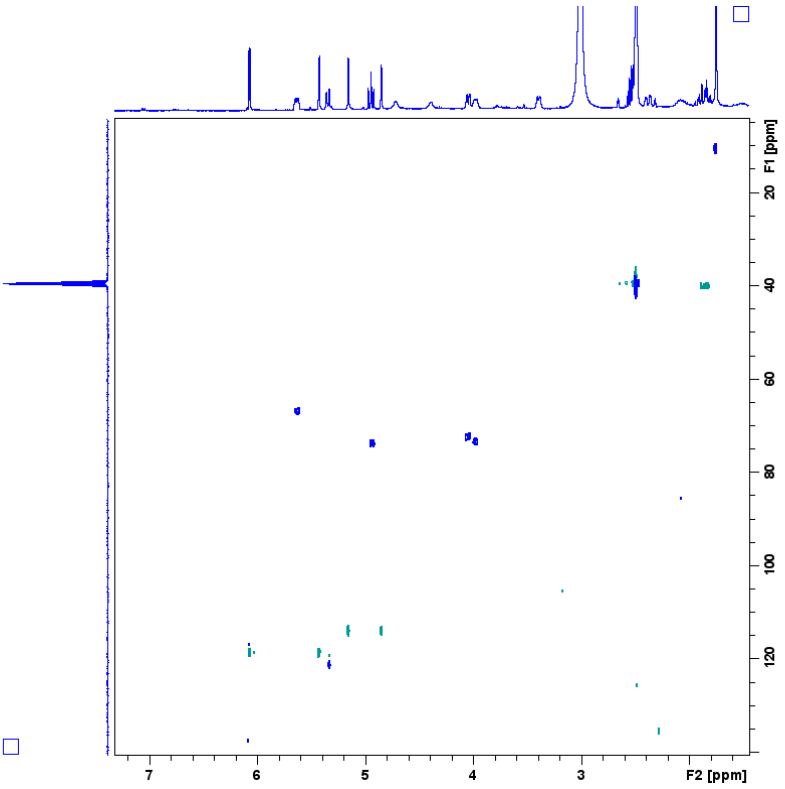
S57. COSY spectrum of 13



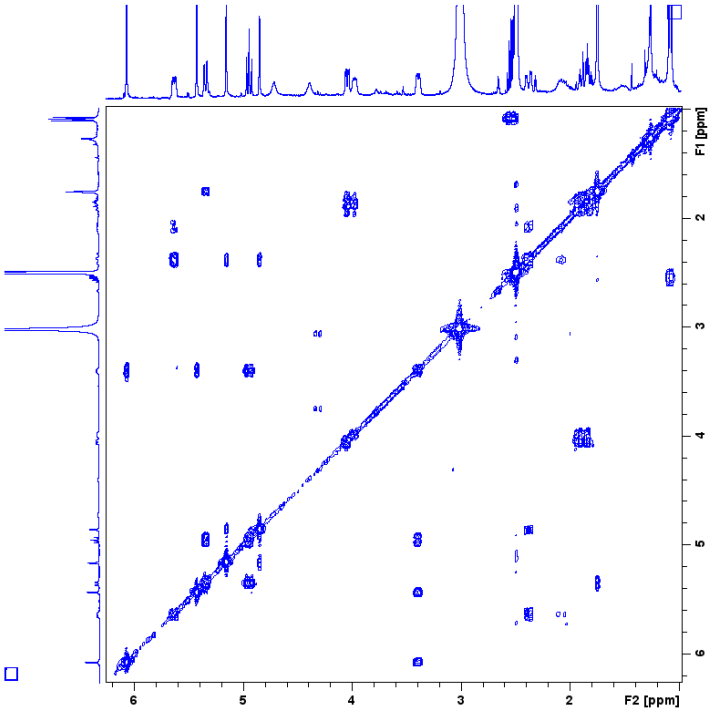
S58. NOESY spectrum of 13



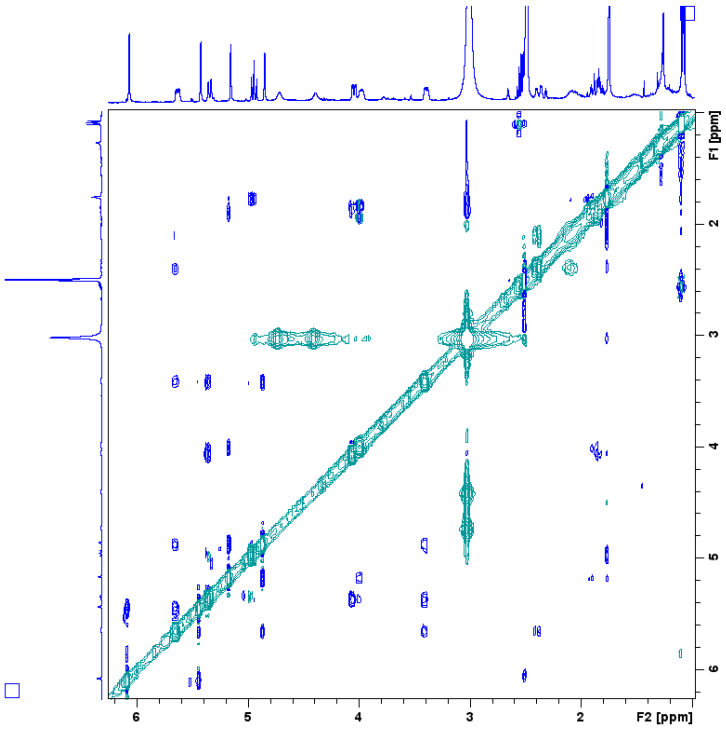
S59. ^{1}H NMR spectrum of 14



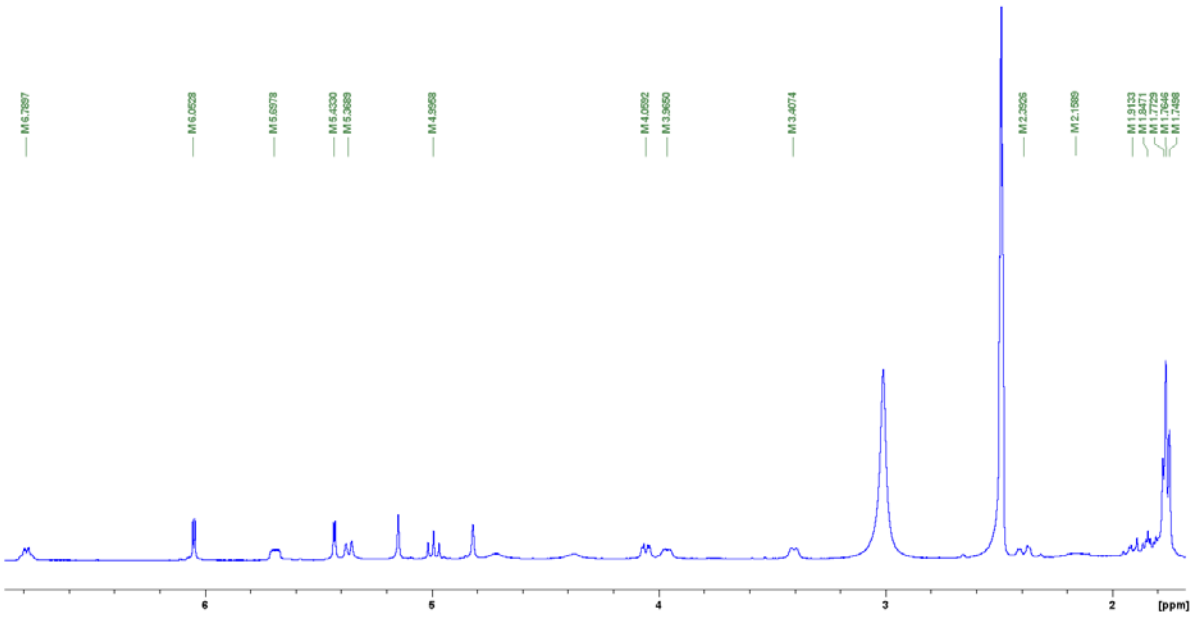
S60. HSQC spectrum of 14



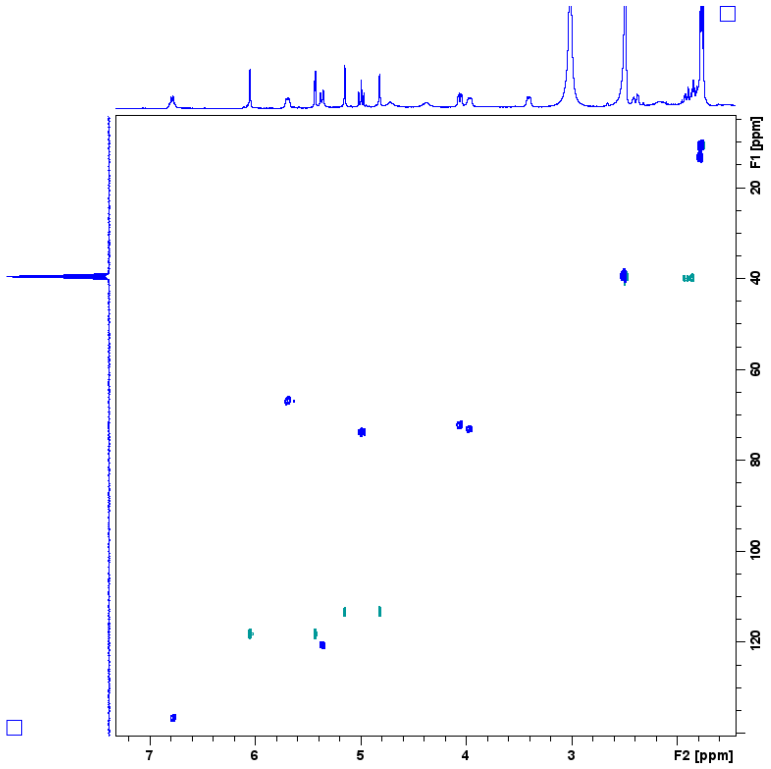
S61. **COSY spectrum of 14**



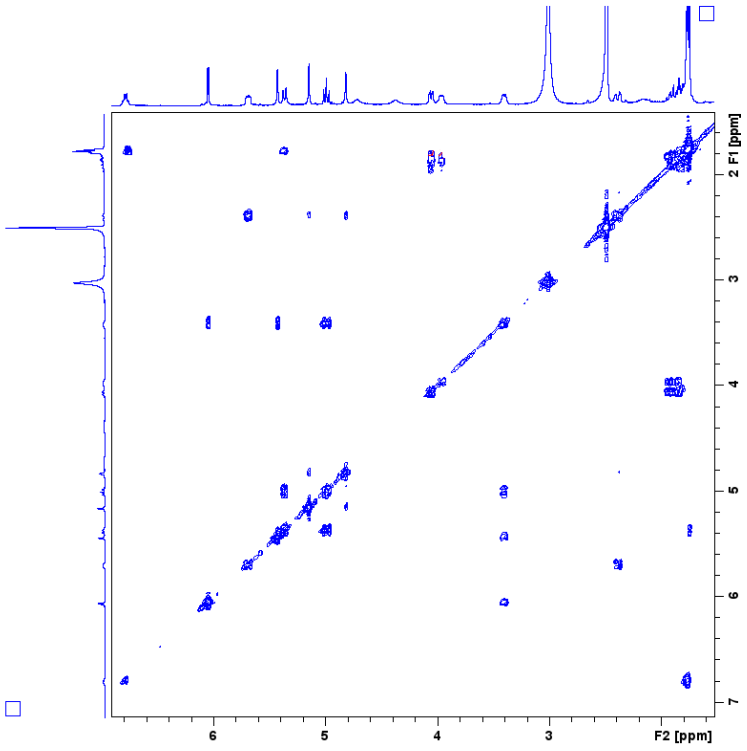
S62. **NOESY spectrum of 14**



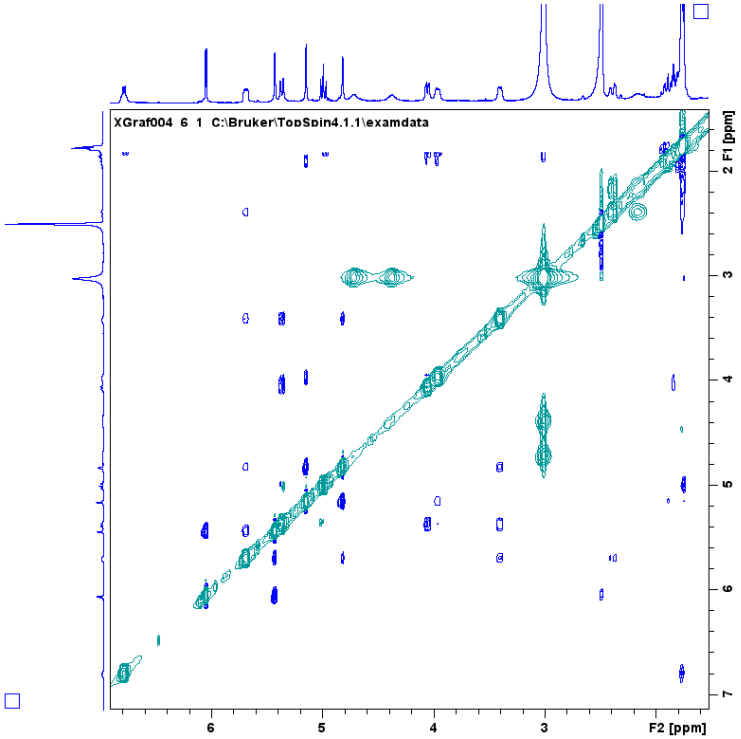
S63. ^{1}H NMR spectrum of 15



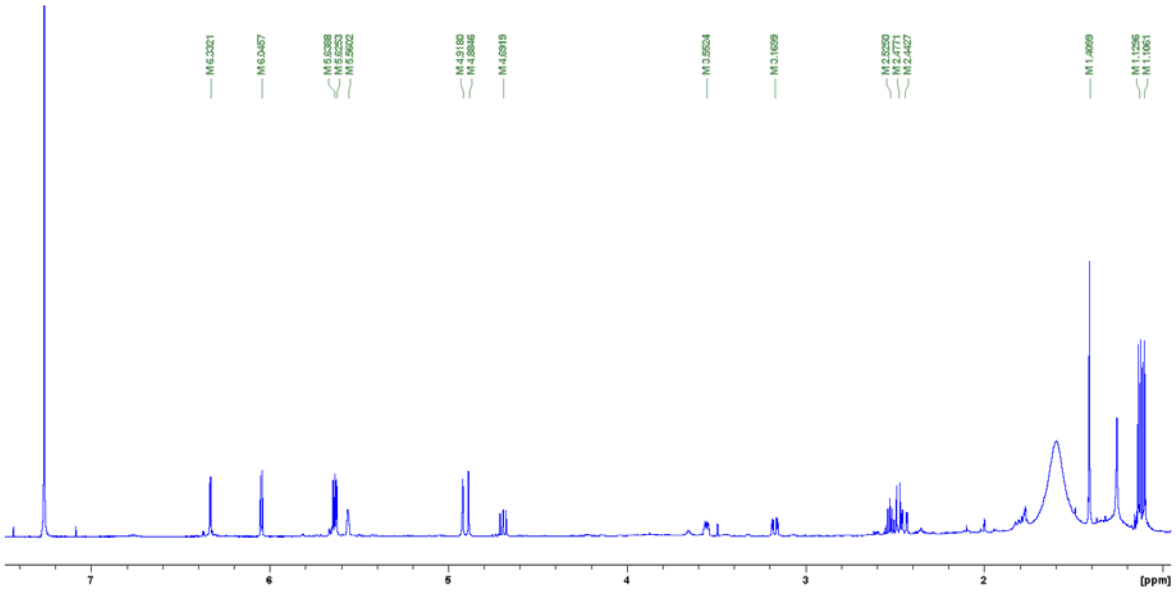
S64. HSQC spectrum of 15



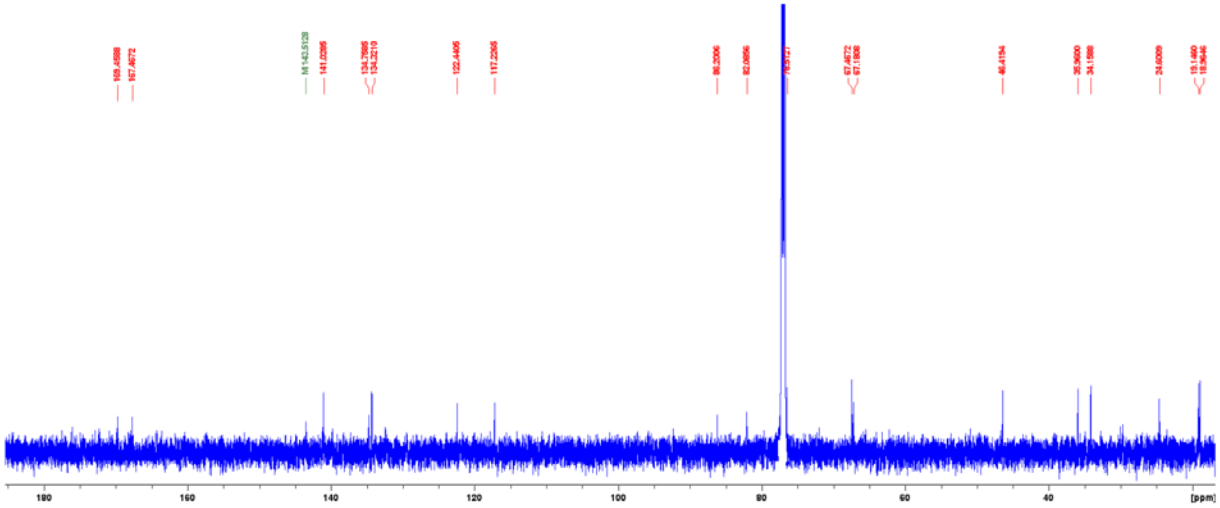
S65. COSY spectrum of 15



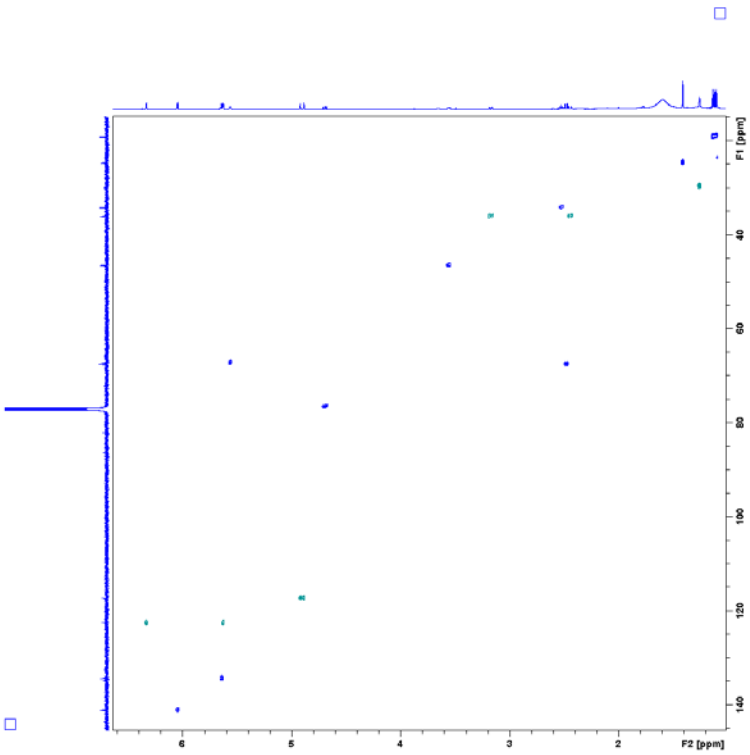
S66. NOESY spectrum of 15



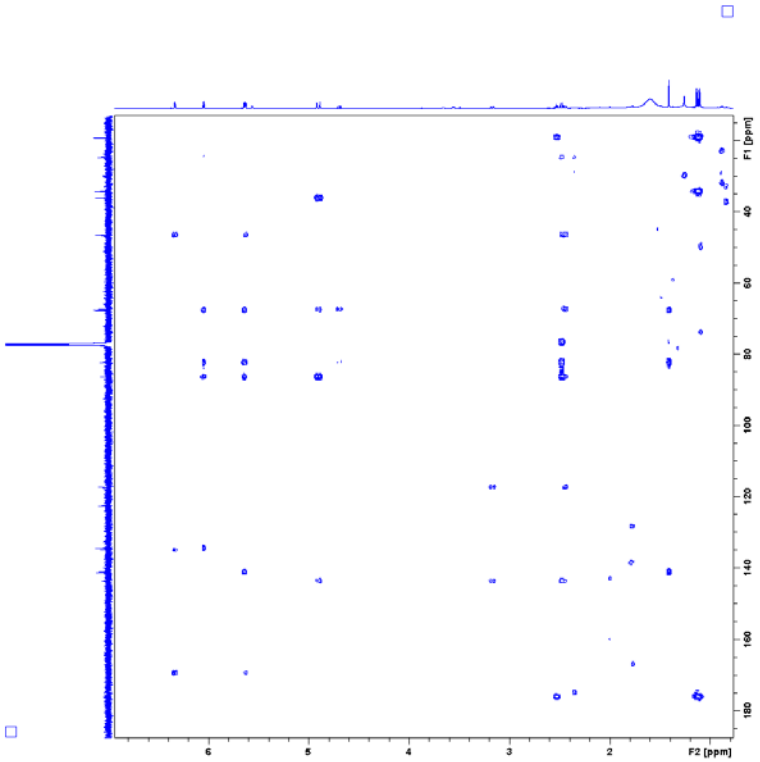
S67. ^{1}H NMR spectrum of 16



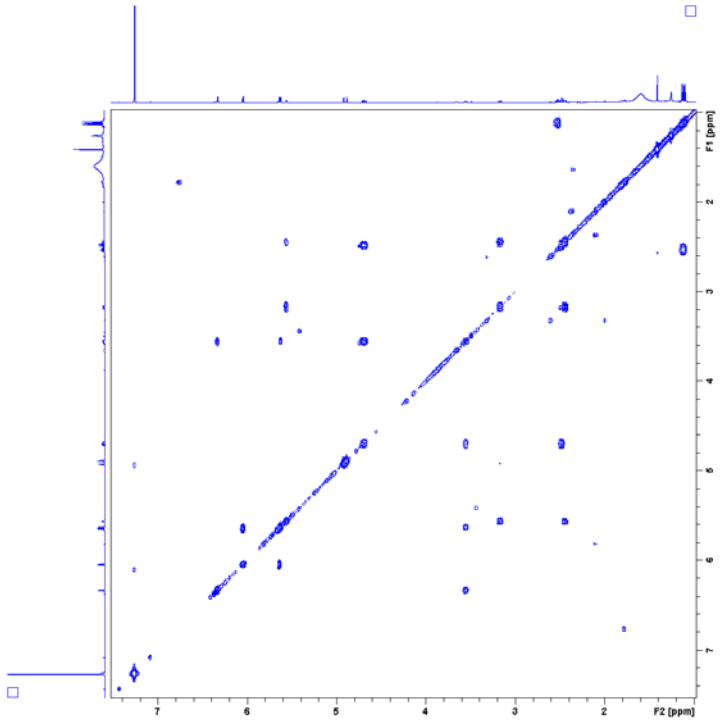
S68. ^{13}**C NMR spectrum of 16**



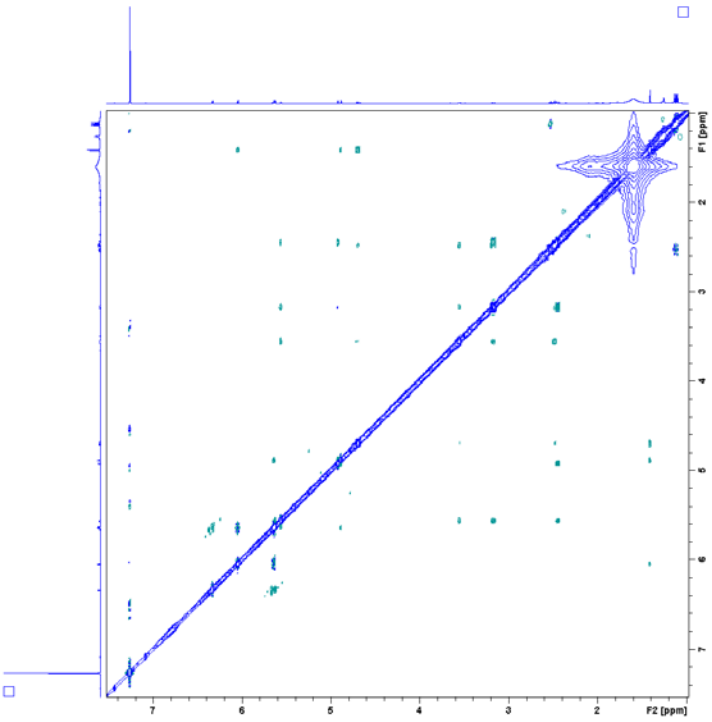
S69. HSQC spectrum of 16



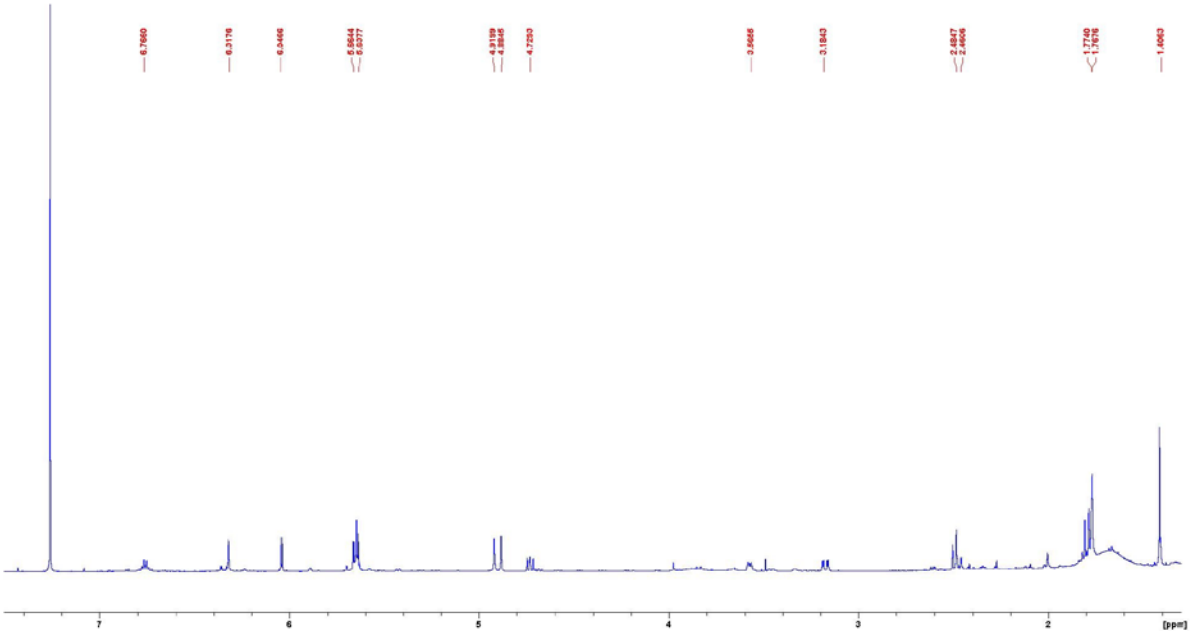
S70. HMBC spectrum of 16



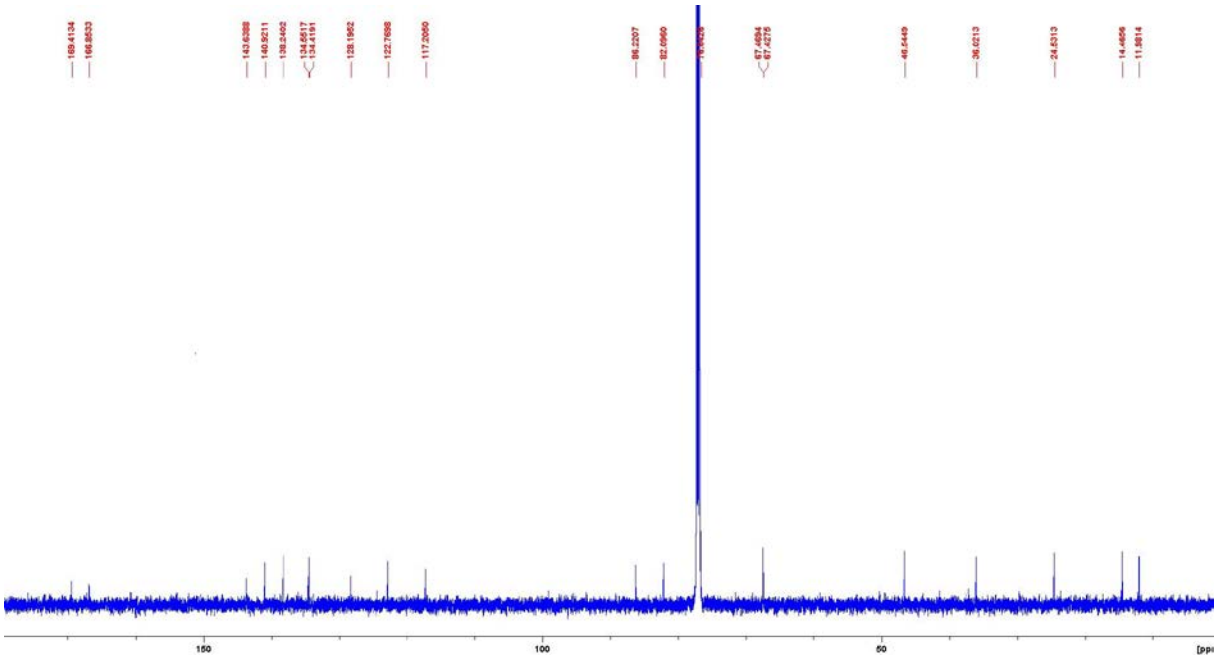
S71. COSY spectrum of 16



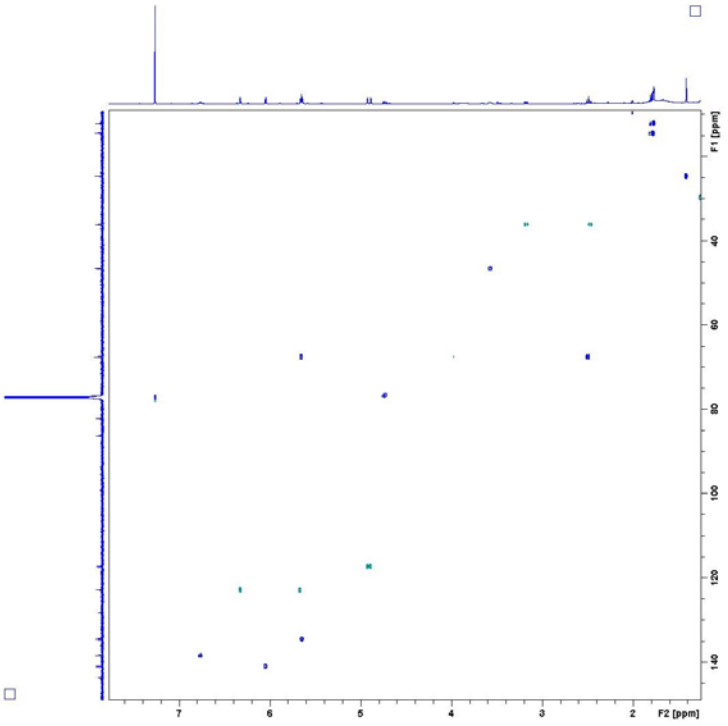
S72. **NOESY spectrum of 16**



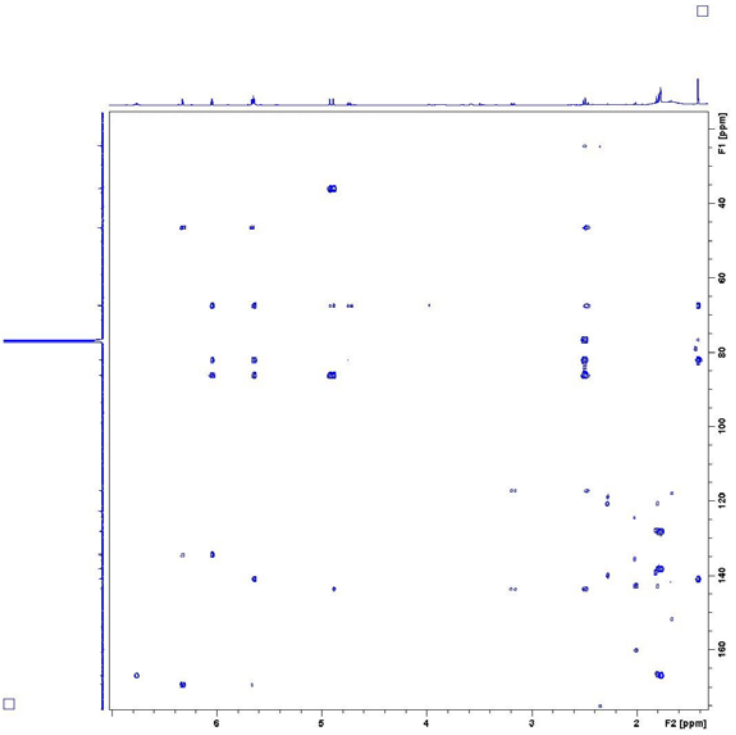
S73. **^{1}H NMR spectrum of 17**



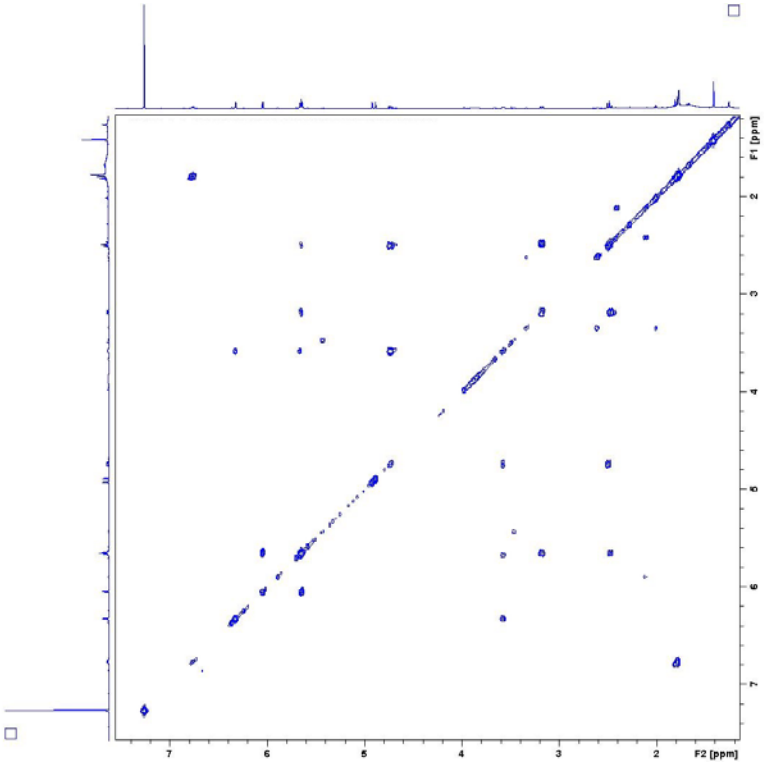
S74. ^{13}**C NMR spectrum of 17**



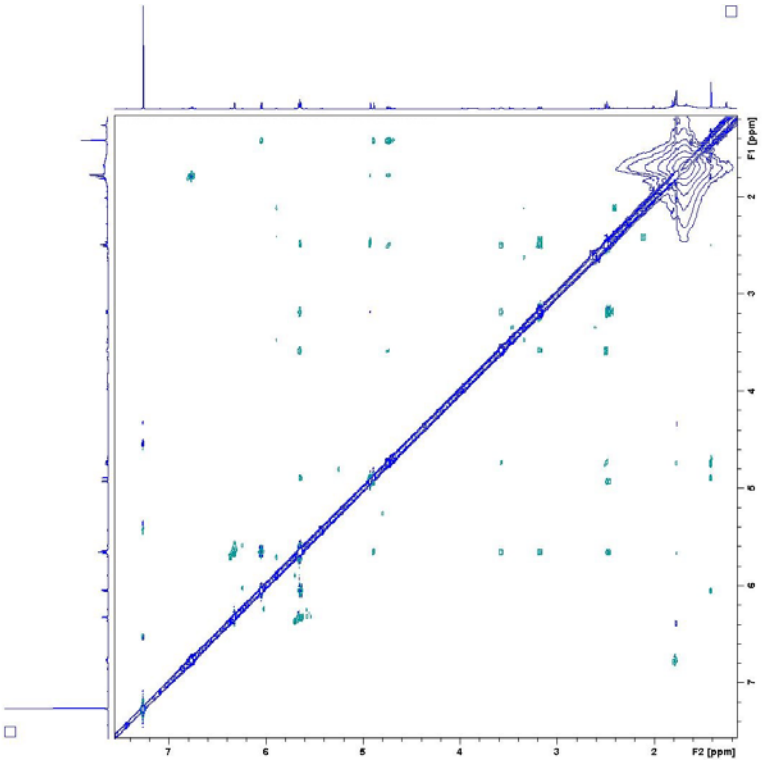
S75. HSQC spectrum of 17



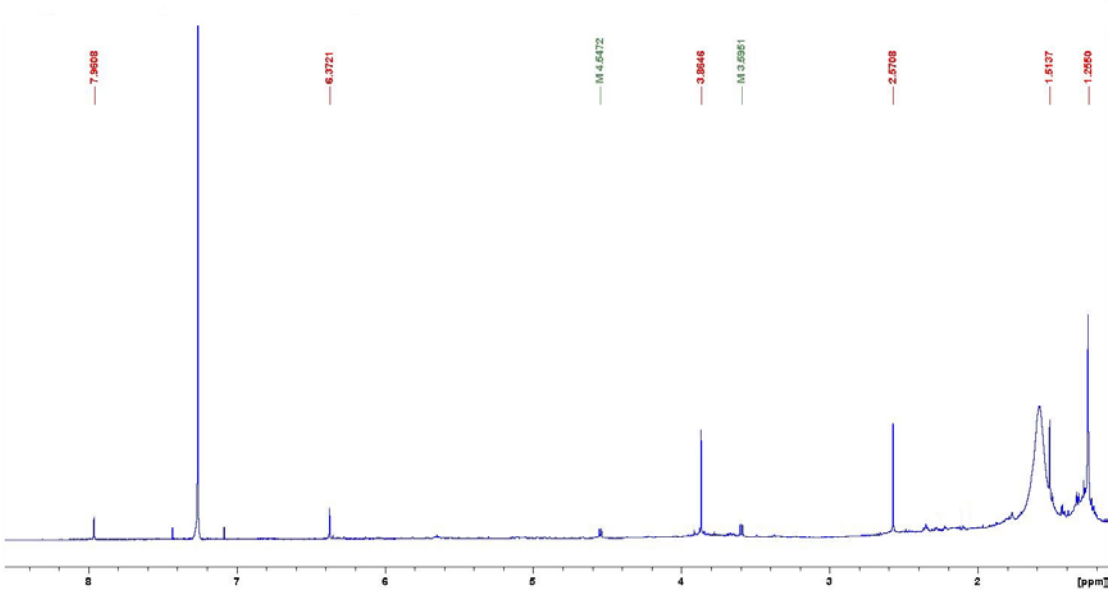
S76. HMBC spectrum of 17



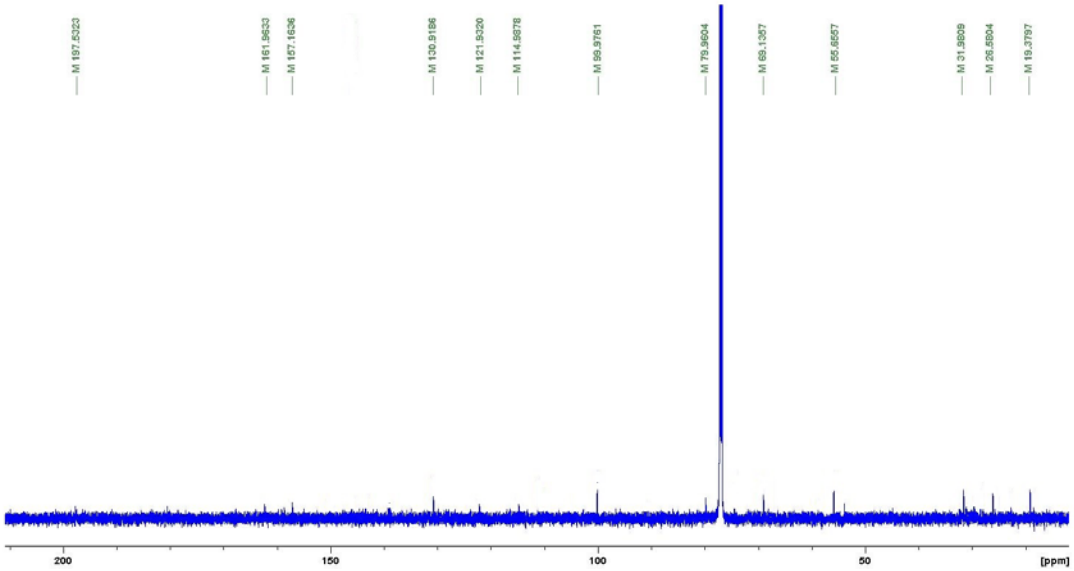
S77. COSY spectrum of 17



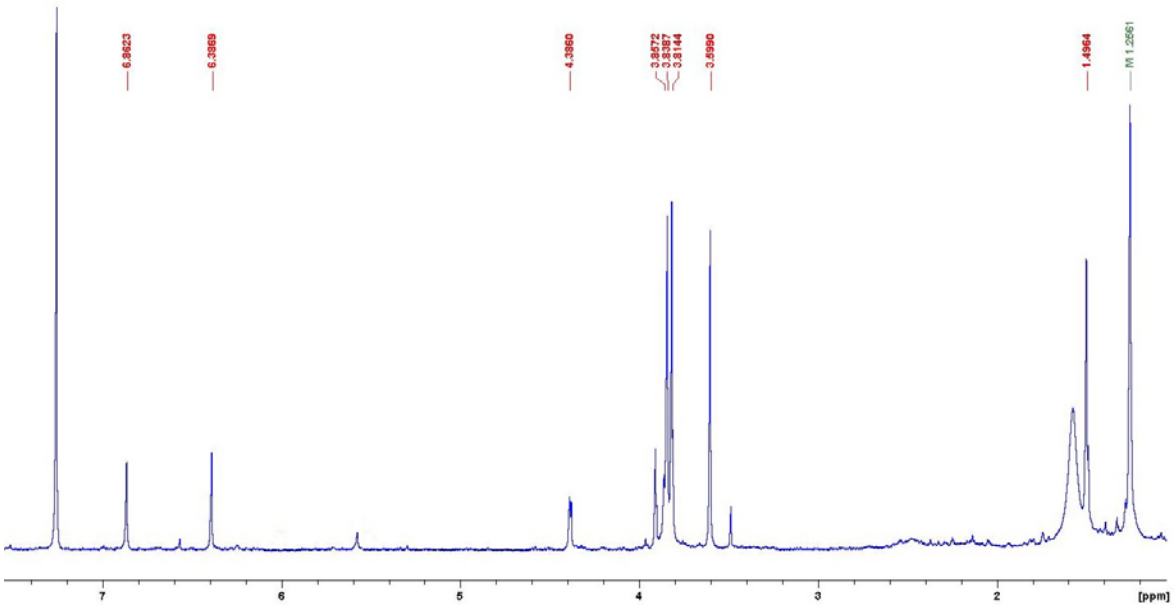
S78. NOESY spectrum of 17



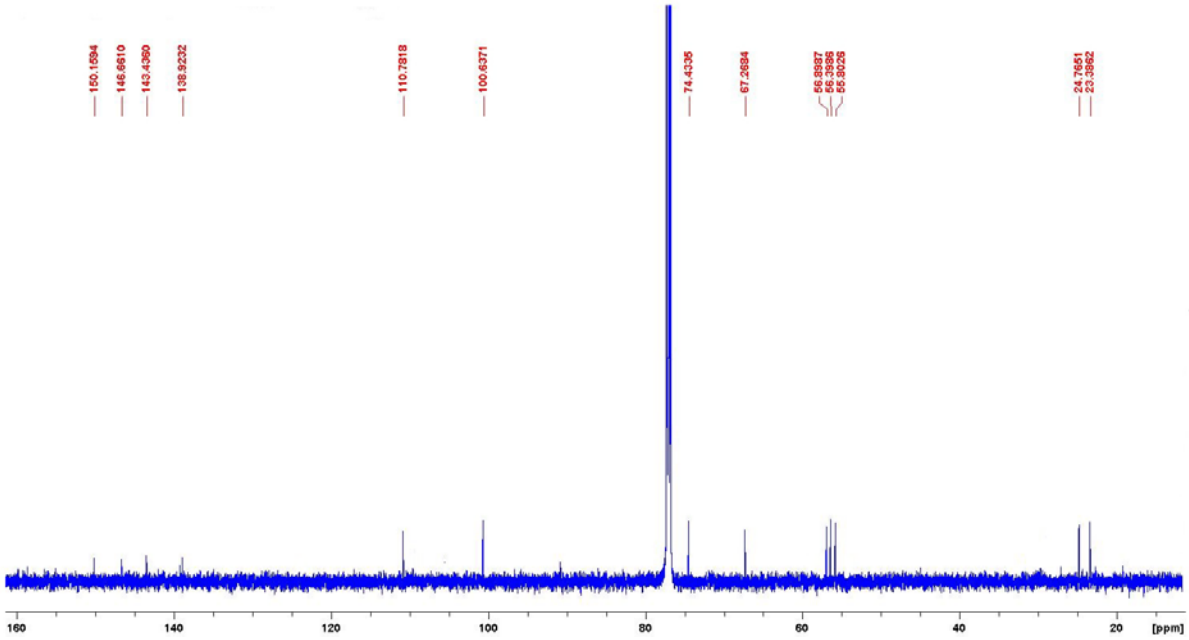
S79. ^{1}H NMR spectrum of 23



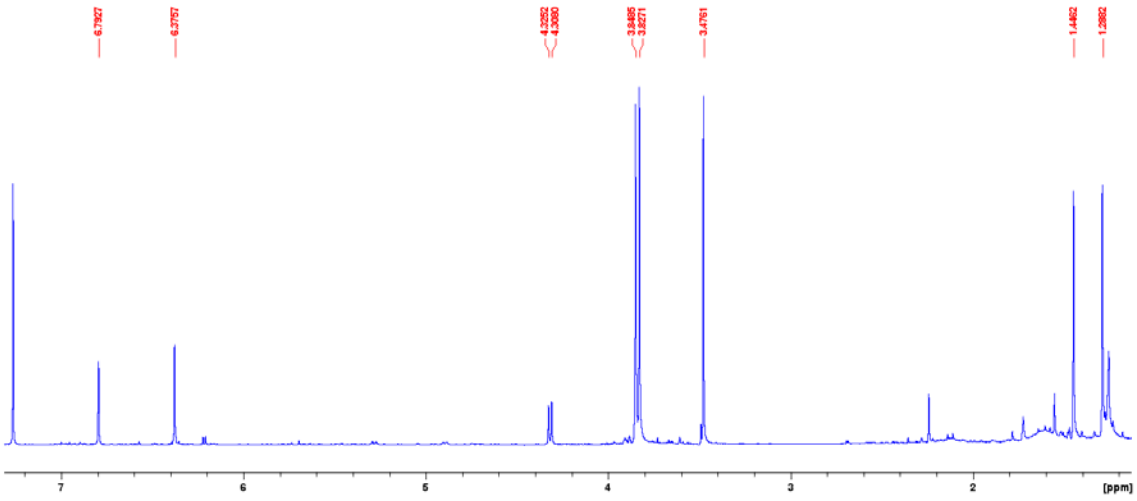
S80. ^{13}C NMR spectrum of 23



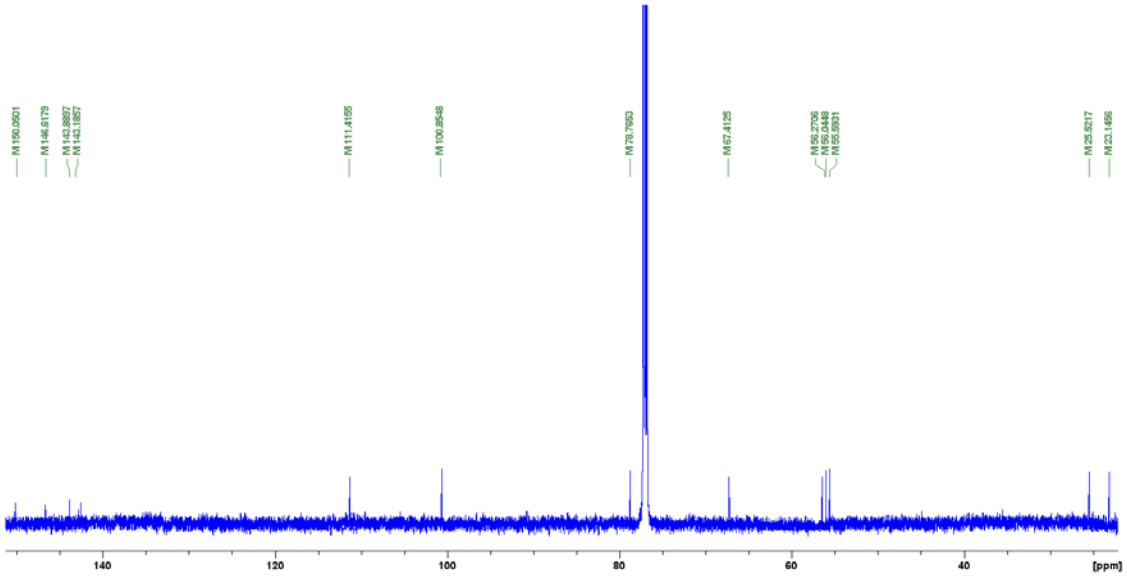
S81. ^{1}H NMR spectrum of 24a



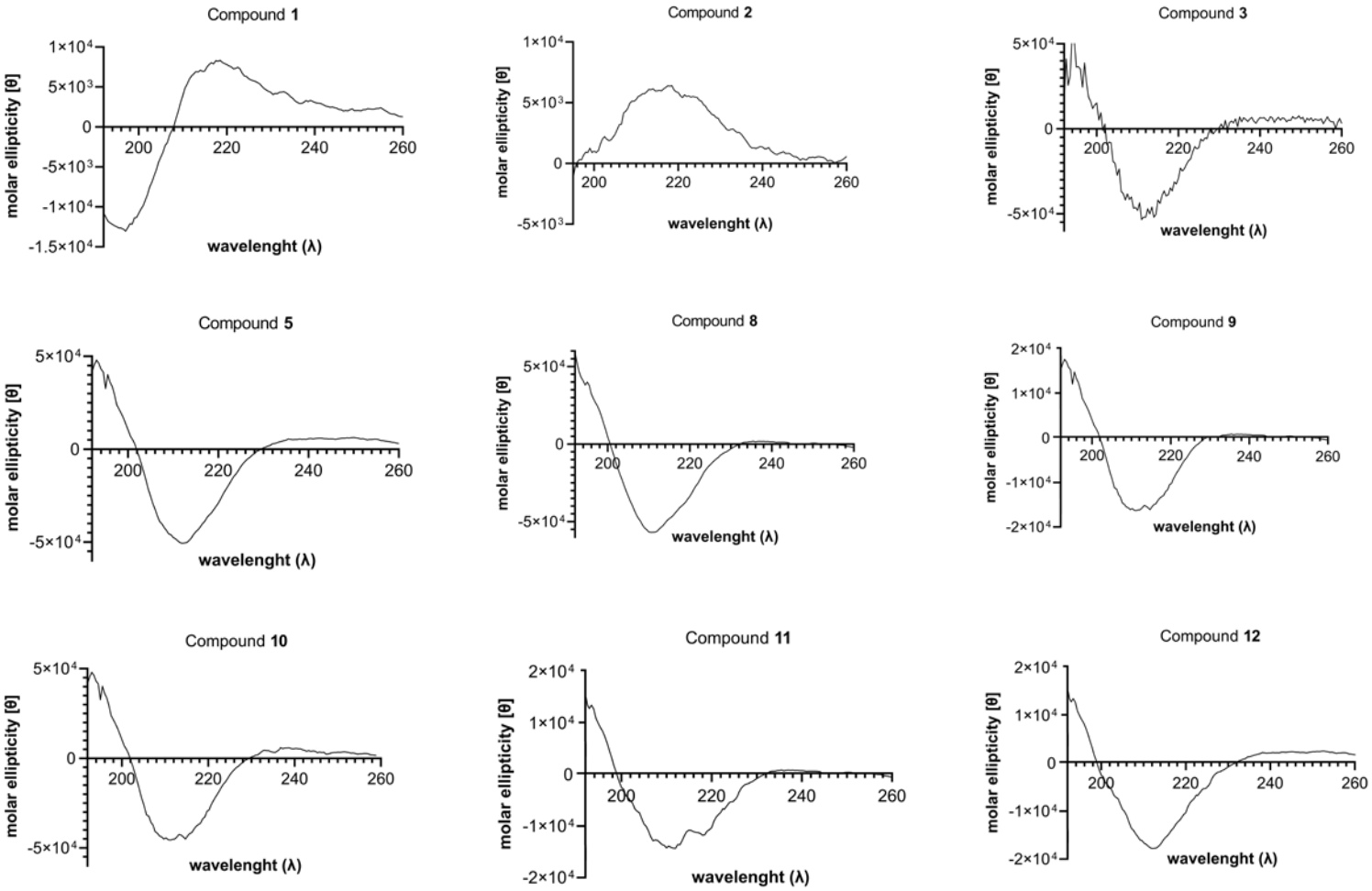
S82. ^{13}C NMR spectrum of 24a

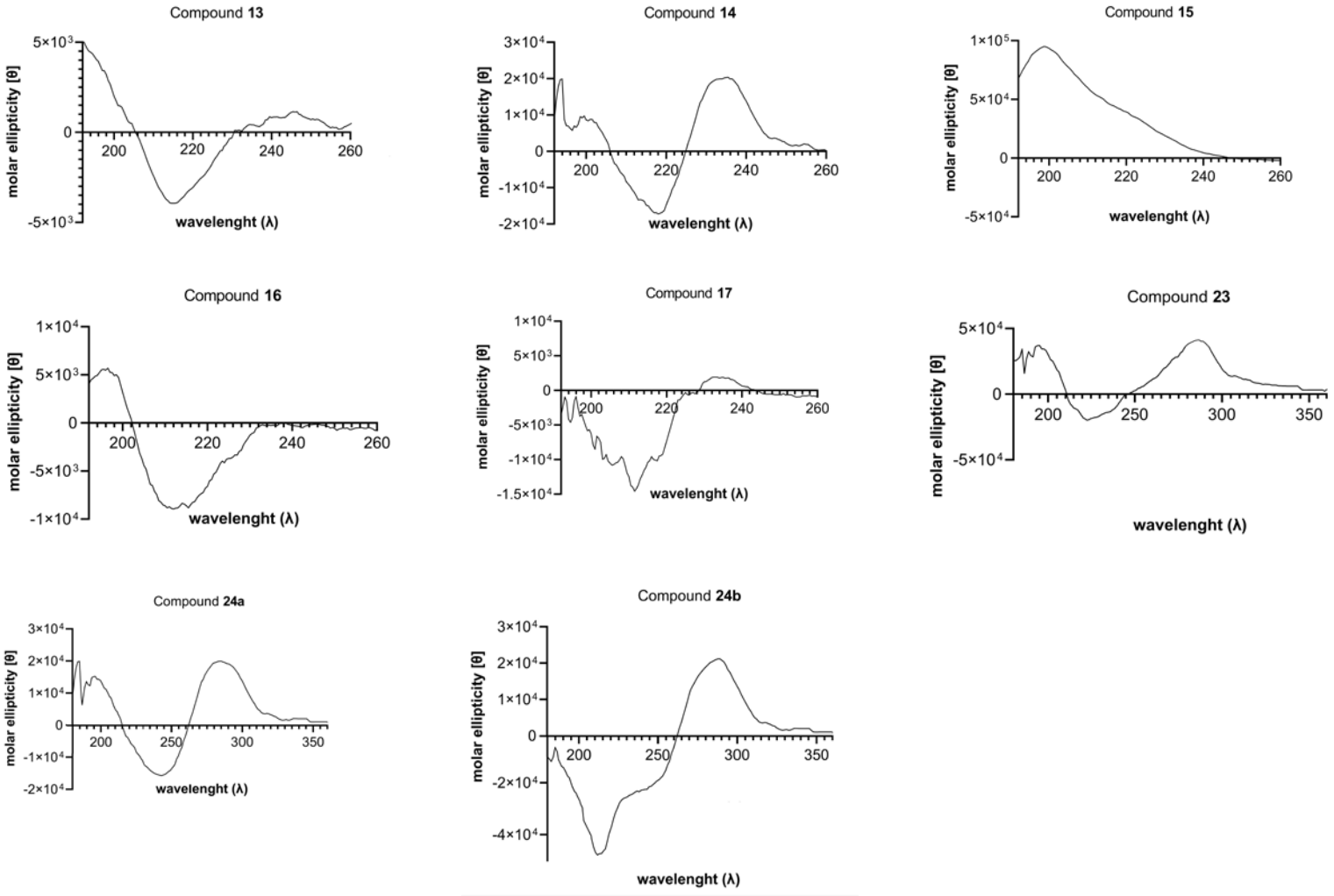


S83. ^{1}H NMR spectrum of 24b

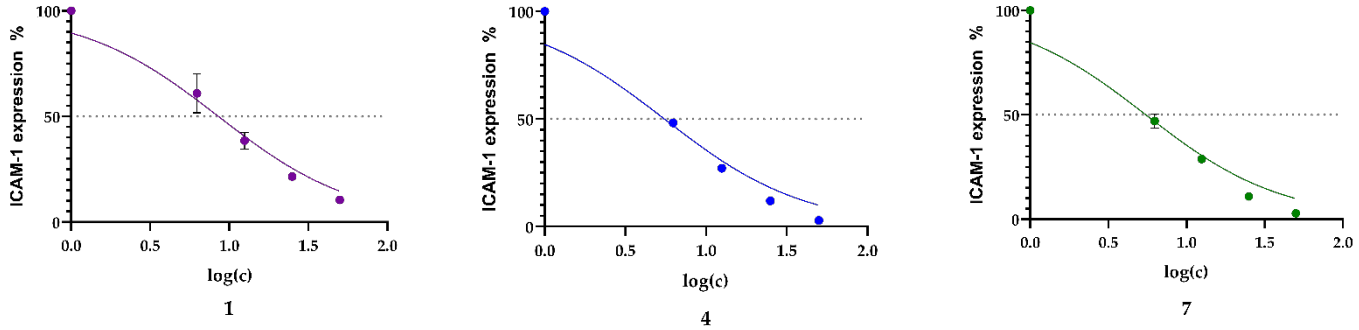


S84. ^{13}**C NMR spectrum of 24b**

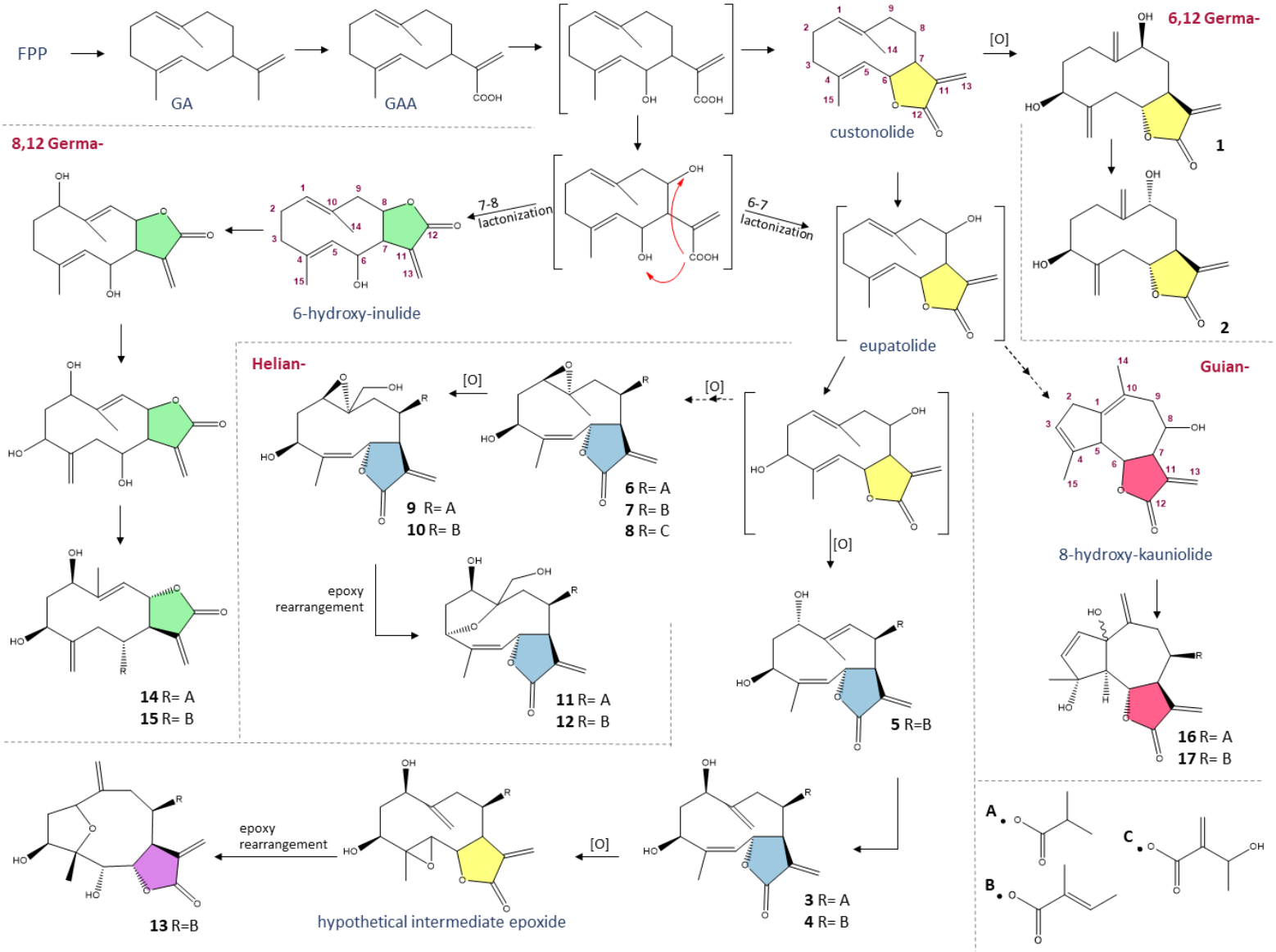




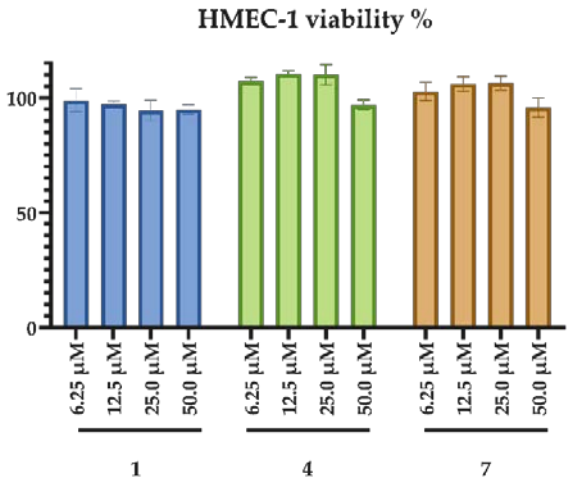
S85. CD of new compounds 1-3, 5, 8-17, 23-24b.



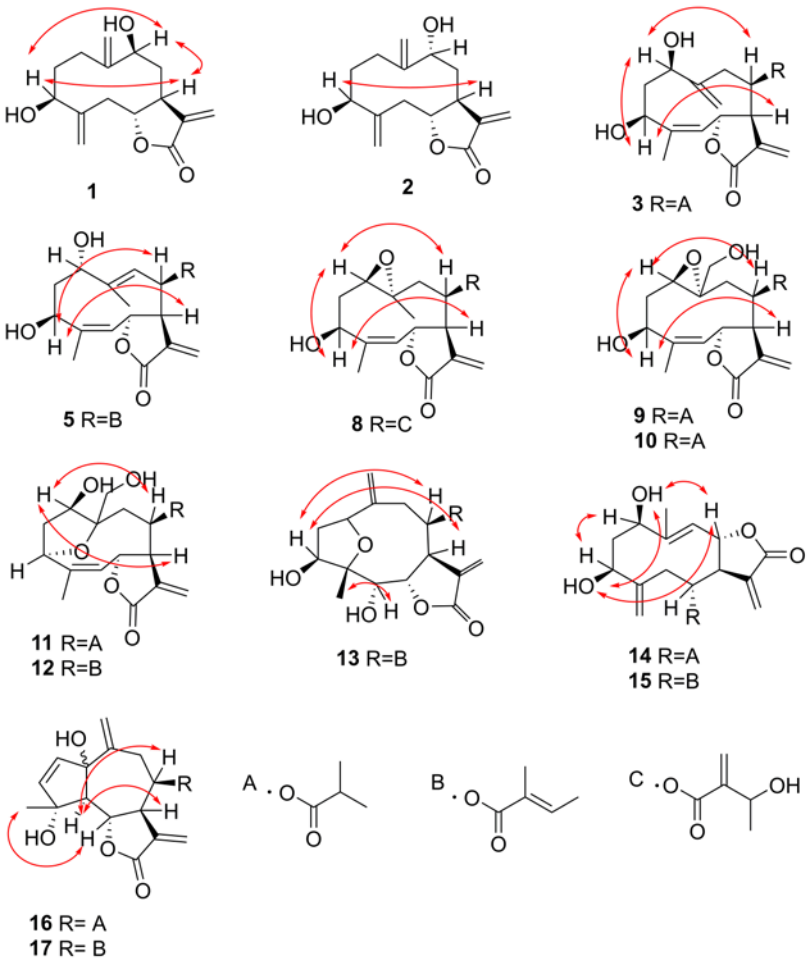
S86. **Non-linear regression for the calculations of the IC_{50} values of 1, 4 and 7 using GraphPad Prism 8.4.2**



S87. **Structures of compounds 1-17 as part of the proposed biosynthetic pathway.**



S88. MTT viability test on the non-cancerous cell line HMEC-1 for compounds 1, 4 and 7, expressed as % mean ± SD. Data are representative of 3 independent experiments with similar results. No toxicity was observed in the cells (viability 90-110%).



S89. Key NOESY correlations for the new SLs 1-3, 5, 8-17.

3.2. *Centaurea papposa* (Coss.) Greuter

The isolation of the compounds from *C. papposa* is presented in Figure 27. The numbering of the compounds are in accordance with Grafakou et al. (2021b). Extraction and isolation are presented in Grafakou et al. (2018) and (2021b); structure elucidation and biological activities are presented in Grafakou et al. (2021b).

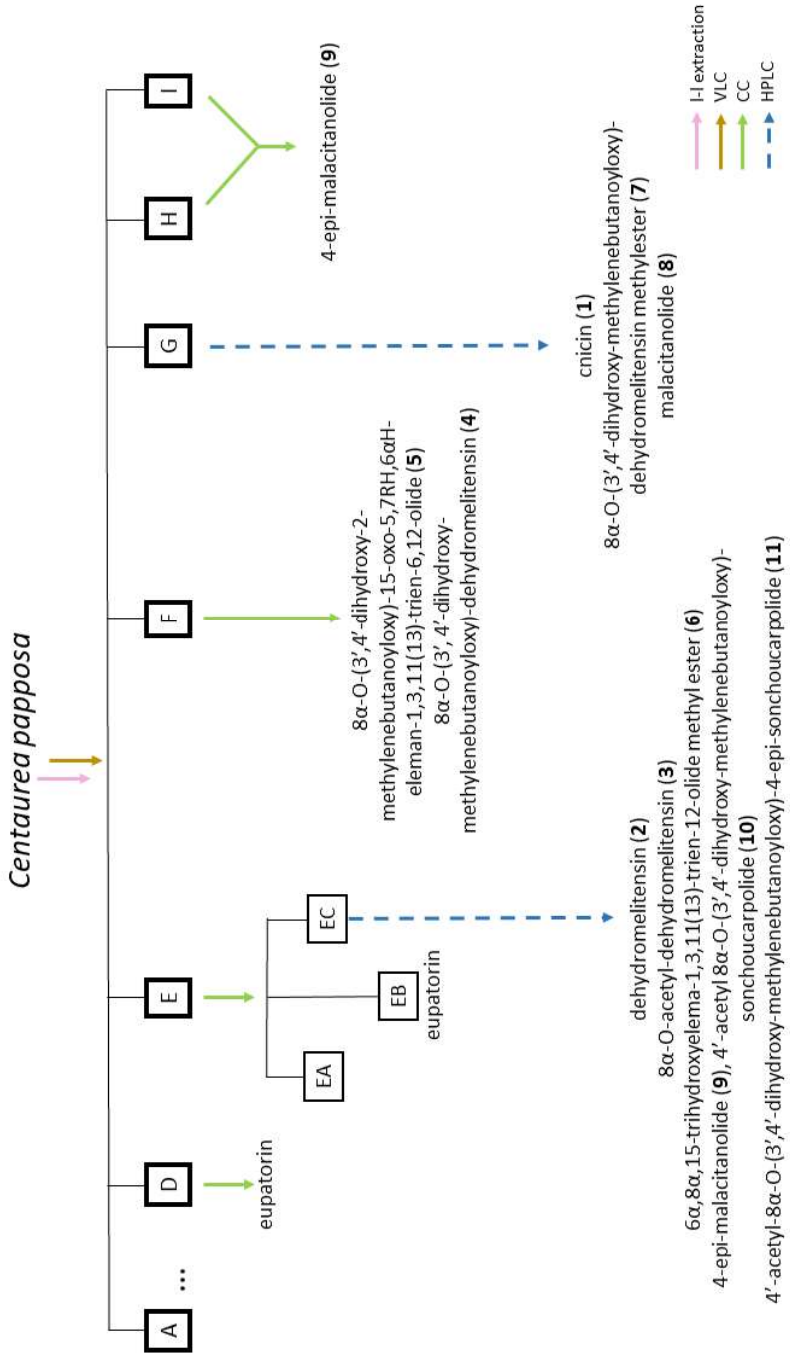


Figure 27. Chromatographic isolation of the compounds from C. papposa.

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Secondary metabolites from the aerial parts of *Centaurea papposa* (Coss.) Greuter

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ABSTRACT

The aerial parts of *Centaurea papposa*, a species growing wild in Algeria and Tunisia, were investigated for the occurrence of sesquiterpene lactones. The germacranolide cnicin is the main compound. In addition one eudesmanolide, two elemanolides, one elemane derivative and the flavonoid eupatorin were isolated.

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1. Introduction

The genus *Centaurea* L. (Asteraceae, Carduae) comprises about 500 species and its distribution is mainly in Europe and Asia (Mabberley, 1997). The genus is characterized by its ability to biosynthesize sesquiterpene lactones (Seaman, 1982) and flavonoids (Formisano et al., 2012), which are important chemotaxonomic markers. In addition, they exhibit high activity in living systems, thus have a strong pharmacological interest, as well as explaining the long-term use of the genus in folk medicine (Khammar and Djeddi, 2012; Boulos, 1983; Perrot and Paris, 1971). *C. papposa* is a species growing wild in Algeria and Tunisia (Quezel and Santa, 1963), belonging to the Centaurea section, subsection Acrolophus (Cass.) DC. (Hilpold et al., 2011). Many previous chemical studies have been recorded on species belonging to the same section and subsection (Tables 1 and 2).

2. Materials and methods

2.1. Plant material

The aerial parts of the plant were collected at "Cape de Garde" Lighthouse at the northwest of the port of Annaba city (Algeria) on September 2016; altitude 58 m; Coordinates (WGS84): 36°57′28″N; 007°46′10″E. The plant was identified by Dr. Hamel Tarek and voucher specimens were deposited to the Herbarium of Department of Biology, University Badji Mokhtar Annaba, Algeria, under the name Djeddi & Skaltsa BV 01/2017.

2.2. Isolation and identification of the compounds from C. papposa

The air-dried and finely ground aerial parts of the plant (0.5 kg) were extracted at room temperature with cyclohexane:Et_2O:MeOH (1:1:1) and the isolation of the secondary metabolites was carried out according to the Bohlmann method (Bohlmann et al., 1984), slightly modified. The extract was washed with brine and the aqueous layer re-extracted with EtOAc. The residue (1.26 g) was pre fractionated by VLC on silica gel using DM-EtOAc-MeOH mixtures of increasing polarity as eluents to give several fractions (A-I). The fractions D (57.2 mg; eluted with cHex-EtOAc 25:75) and E (149.9 mg; eluted with EtOAc 100%) subjected to CC over silica gel using DM:EtOAc:MeOH mixtures, yielded eupatorin (**6**; 22.7 mg,

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Table 1
Sesquiterpene lactones isolated from *Centaurea* L. species, belonging to section Centaurea, subsections Acrolophus (Cass.) DC., Phalolepis (Cass.) and Willkommia (Blanca). A:Acrolophus P:Phalolepis W:Willkommia.

Centaurea species*	Sub-section	Sesquiterpene lactones	Literature
C. affinis Friv.	A	Cnicin, salonitenolide	Janaković et al., 2004
C. affinis subsp. *pallidior* (Halácsy) Hayek (= *C. pallidior* Halácsy subsp. *pallidior*)	A	Cnicin	Nowak et al., 1984
C. aggregata Fisch. & C.A.Mey. ex DC.	A	Cnicin	Nowak et al., 1984
C. alba L.	P	Cnicin, cnicin 4′-*O*-acetate, salonitenolide, 11β,13-dihydrosalonitenolide, salonitenolide 8α-*O*-(4-acetoxy-5-hydroxy)-angelate	Fernandez et al., 1995
C. aplolepa Moretti (= *C. aplolepa* subsp. *aplolepa*)	A	Cnicin	Nowak et al., 1984
C. aplolepa Moretti subsp. *lunensis* (Fiori) Dostál	A	Cnicin	Nowak et al., 1984
C. arenaria M.Bieb. ex Willd. (= *C. arenaria* Bieb. Aex Willd. subsp. *arenaria*)	A	Cnicin Cnicin	Nowak et al., 1984 Csapi et al., 2010
C. attica Nyman (= *C. attica* Nyman subsp. *attica*)	A	Cnicin, cnicin 4′-*O*-acetate, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, methyl 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-6α,15-dihydroxyelema-1,3,11(13)-trien-12-oate Malacitanolide, atticin, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-4-*epi*-sonchucarpolide	Skaltsa et al., 1999 Skaltsa et al., 2000
C. bombycina Boiss. ex DC.	W	Cnicin, salonitenolide, 8α-*O*-(4-acetoxy-5-hydroxyangelate)-salonitenolide	Barrero et al., 2000
C. bovina Velen (= *C. diffusa* Lam. var. *brevispina* Boiss.)	A	Cnicin	Nowak et al., 1984
C. busambarensis Guss. (= *C. cineraria* subsp. *busambarensis* (Guss.) Dostál)	A	Cnicin, dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin	Bruno et al., 1998
C. cadmea Boiss.	P	2α-hydroxy-5αH-eudesman-4(15),11(13)-dien-12,8β-olide (ivalin)	Karamenderes et al., 2007
C. caliacrae Prodan [= *C. alba* subsp. *caliacrae* (Prodan) Dostál]	P	Salonitenolide	Geppert et al., 1983
C. calolepis Boiss.	A	Cnicin	Erel et al., 2011
C. castellana Boiss. [= *C. paniculata* subsp. *castellana* (Boiss. & Reut.) Dostál]	A	Artemissifolin, Dehydromelitensin, 11β,15-dihydroxysaussurea lactone	Gonzalez et al., 1984
C. cineraria L. (= C. *cineraria* subsp. *cineraria)*	A	Cnicin	Nowak et al., 1984
C. cineraria subsp. *circae* (Sommier) Cela Renz. & Viegi	A	Cnicin	Nowak et al., 1984
C. crithmifolia Vis.	A	Salonitenolide	Geppert et al., 1983
C. cuneifolia Sibth. & SM. (= *C. cuneifolia* subsp. *cuneifolia*)	A	Cnicin, dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin	Aslan and Öksüz, 1999
C. cuneifolia Sibth. & SM. subsp. *pallida* (Friv.) Hayek	A	Cnicin	Nowak et al., 1984
C. derventana Vis. & Pančić	A	Cnicin, cnicin-4′-*O*-acetate, salonitenolide, 8α-*O*-(4-acetoxy-5-hydroxyangelate) salonitenolide	Tešević et al., 1998
C. deusta Ten.	P	Salonitenolide Cnicin, cnicin-4′-*O*-acetate, cnicin-3′-acetate 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)	Geppert et al., 1983 Karioti et al., 2002

Table 1 (*continued*)

Centaurea species*	Sub-section	Sesquiterpene lactones	Literature
		dehydromelitensin, salonitenolide, methyl 8α-*O*-(3,4-dihydroxy-2-meth-ylene-butanoyloxy)-6α,15-dihydroxy-elema-1,3,11(13)-trien-12-oate, methyl 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-6α, 15-dihydroxyelema-1,3, 11(13)-trien-12-oate, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-4-*epi*-sonchucarpolide, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-sonchucarpolide	
C. diffusa Lam.	A	Cnicin	Milkova et al., 1993
		Cnicin, cnicin-4′-*O*-acetate, 8α-*O*-(3-hydroxy-4-acetoxy-2-methylenebutanoyloxy) dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7RH,6αH-eleman-1,3,11(13)-trien-6,12-olide	Fortuna et al., 2002
C. bovina Velen. (= *C. diffusa* Lam. var. *brevispina* Boiss.)	A	Cnicin	Nowak et al., 1984
C. friderici Vis.	A	Salonitenolide	Nowak et al., 1984
C. galicicae Micevski	A	Cnicin	Tešević et al., 2014
C. grisebachii (Nyman) Heldr. [= *C. grisebachii* subsp. *grisebachii* = *C. grisebachii* (Nyman) Formánek)]	A	Cnicin	Nowak et al., 1984
		Cnicin, dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, salonitenolide, 8α-*O*-(4-acetoxy-5-hydroxyangelate)-salonitenolide, 8α-*O*-hydroxy-4-*epi*-sonchucarpolide, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-4-*epi*-sonchucarpolide, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-sonchucarpolide, malacitanolide, 4-*epi*-malacitanolide	Djeddi et al., 2008
C. grisebachii (Nyman) Heldr. subsp. *confusa* (Halácsy) Dostál	A	Cnicin	Nowak et al., 1984
C. grisebachii subsp. *transiens* (Halácsy) T.Georgiadis (= *C. transiens* Halácsy)	A	Cnicin	Nowak et al., 1984
C. kartschiana Scop.	A	Cnicin	Nowak et al., 1984
C. kilaea Boiss.	A	Cnicin, dehydromelitensin	Sen et al., 2017
C. laureotica Heldr. ex Halácsy (= *C. mantoudii* Georg.)	A	Cnicin	Nowak et al., 1984
C. leucophaea Jord. (= *C. leucophaea* Jordan subsp. *leucophaea*)	A	Cnicin	Nowak et al., 1984
C. majorrovii Dumbadze (= *C. arenaria* subsp. *majorowii* (Dumb.) Dostál)	A	Cnicin	Nowak et al., 1984
C. odessana Prodan [= *C. arenaria* subsp. *odessana* (Prodan) Dostál]	A	Cnicin	Nowak et al., 1984
C. orphanidea Heldr. & Sart. ex Boiss. (=*C. orphanidea* Heldr. & Sart. ex Boiss. subsp. *orphanidea*)	A	Cnicin	Nowak et al., 1984
		Cnicin, cnicin-4′-*O*-acetate, salonitenolide, malacitanolide, 8α-hydroxy-4-*epi*-sonchucarpolide, 8α-*O*-(3-hydroxy-4-acetoxy-2-methylenebutanoyloxy)-4-*epi*-sonchucarpolide, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin	Gousiadou and Skaltsa, 2003
C. ossaea Halácsy (= *C. attica* Nyman subsp. ossaea)	A	Cnicin	Nowak et al., 1984
C. paniculata L.	A	Salonitenolide	Geppert et al., 1983
C. panormitana subsp. *umbrosa* (Fiori)	A		Bruno and Herz, 1988

(*continued on next page*)

Table 1 (*continued*)

Centaurea species*	Sub-section	Sesquiterpene lactones	Literature
Greuter [= *C. cineraria* L. subsp. *umbrosa* (Lacaita)]		Cnicin, cnicin-4′-*O*-acetate, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin	
C. paui Willk.	W	8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7αH,6,11βH-elema-1,3,11(13)-trien-12-olide, (3R)-15-acetoxy-8α-*O*-(3,4-dihydro-2-methylenebutanoyloxy)-6βH,7αH-germacra-1E,4Z,11(13)-trien-6,12-olide, (3R)-15-acetoxy-8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-6βH,7αH-germacra-1E,4Z,11(13)-trien-6,12-olide,	Cardona et al., 1994
		Cnicin, cnicin-4′-*O*-acetate, salonitenolide, stoebenolide, dehydromelitensin, 11β,13-dihydrosalonitenolide, 8α-*O*-(4-acetoxy-5-hydroxyangeloyl)-salonitenolide, 8α-*O*-hydroxy-15-oxo-5,7αH,6,11βH-elema-1,3-dien-6,12-olide, 8α-*O*-hydroxy-15-oxo-5,7αH,6,11βH -elema-1,3,11(13)-trien-6,12-olide, methyl 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-6,16-dihydroxy-5,7αH,6βH-elema-1, 3, 11 (13)-trien-6, 12-olide, 15-acetoxy-8α-*O*-hydroxy-6βH,7αH -germacra-1E,4Z,11(13)-trien-6,12-olide, (3R)-15-acetoxy-8α-(3,4-dihydroxy-2-methylenebutanoyloxy)-1β-hydroperoxy-10-methylene, 6βH,7αH -germacra-4Z,10(14),11(13)-trien-6,12-olide, (3R)-15-acetoxy-8α-(3,4-dihydroxy-2-methylenebutanoyloxy)-1β-hydroxy-10-methylene, 6βH,7αH-germacra-4Z,10(14),11(13)-trien-6,12-olide, 15-acetoxy-1β,8α-dihydroxy-6βH,7αH -germacra-4Z,10(14),11(13)-trien-6,12-olide, 15-acetoxy-1β,8α-dihydroperoxy-6βH,7αH-germacra-4Z,10(14),11(13)-trien-6,12-olide	Cardona et al., 1997
C. pelia DC.	A	Cnicin	Nowak et al., 1984
C. pseudomaculosa Dobrocz.	A	Cnicin	Al-Easa and Rizk, 1992
C. reichenbachii DC. (= *C. calvescens* Pancić)	A	Cnicin	Nowak et al., 1984
C. savranica Klokov [= *C. rhenana* subsp. *savranica* (Klokov) Dostál]	A	Cnicin	Nowak et al., 1984
C. soskae Hayek	A	Cnicin	Tešević et al., 2014
C. spinosa L. (= *C. spinosa* var. *spinosa*)	A	Cnicin	Nowak et al., 1984
		Cnicin, cnicin-4′-*O*-acetate, 4-*epi*-malacitanolide, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-4-*epi*-sonchucarpolide, malacitanolide, 4′-acetyl-malacitanolide, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7βH,6αH-eleman-1,3,11(13)-trien-6,12-olide, 8R-*O*-(4-acetoxy-2-hydroxymethyl-buten-2-oyloxy) salonitenolide, methyl 8R-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-6α,15- dihydroxy-eleman-1,3,11(13)-trien-12-oate	Saroglou et al., 2005
C. stoebe L. (= *C. maculosa* Lam.)	A	Cnicin	Suchy and Herout, 1962
		Cnicin, salonitenolide, 8α-O-(4-acetoxy-5-hydroxyangelate)-salonitenolide, stoebenolide	Huneck et al., 1986
C. thessala subsp. *drakiensis* (Freyn & Sint.) T. Georgiadis [= *C. attica* Nyman subsp. *drakiensis* (Freyn & Sint.) Dostál]	A	Cnicin	Nowak et al., 1984
		Cnicin, cnicin-4′-*O*-acetate, 8α-*O*-(4-acetoxy-5-hydroxyangelate)-salonitenolide	Skaltsa et al., 1999
		8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-*O*-(3-hydroxy-4-acetoxy-2-methylenebutanoyloxy)-	Skaltsa et al., 2000

Table 1 (*continued*)

Centaurea species*	Sub-section	Sesquiterpene lactones	Literature
		dehydromelitensin, 8α-hydroxy-4-*epi*-sonchucarpolide	
C. tomorosii Micevski	A	Cnicin	Tešević et al., 2014
C. tougourensis Boiss. & Reut.	P	Cnicin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, (6R, 7R, 8S, 30R) 8α-*O*-(3, 4-dihydroxy-2-methylene-butanoyloxy)-15-oxo-helianga 1(10), 4(5), 11(13) trien-6-olide, (6R, 7R, 8S, 30R) 8α-*O*-(3, 4-dihydroxy-2-methylene-butanoyloxy)-15-acetoxy-helianga 1(10), 4(5), 11(13) trien-6-olide	Nacer et al., 2012
C. tymphaea Hausskn. (= *C. tymphaea* Hausskn. subsp. *tymphaea*)	A	Cnicin	Nowak et al., 1984
C. tymphaea subsp. *brevispina* (Hausskn.) Dostál	A	Cnicin	Nowak et al., 1984
C. vallesiaca (DC.) Jord.	A	Cnicin	Geppert et al., 1983
C. virgata Lam.	A	Cnicin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-hydroxy-sonchucarpolide	Tuzun et al., 2017
C. zuccariniana DC.	A	Cnicin	Nowak et al., 1984
		Cnicin, cnicin-4′-*O*-acetate, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-*O*-hydroxy-sonchucarpolide	Koukoulitsa et al., 2002
		Cnicin, cnicin-4′-*O*-acetate, dehydromelitensin, 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-*O*-(3,4-dihydro-2-methylenebutanoyloxy)-15-oxo-5,7αH,6βH-eleman-1,3,11(13)-trien-6, 12-olide, (1E,4Z)-15-hydroxy-8α-*O*-(4-acetoxy-3-hydroxy-2-methylidenebutanoyl)-6βH,7αH-germacra-1,4,11(13)-trien-6,12-olide, methyl 8α-*O*-[(4-acetoxy-3-hydroxy-2-methylenebutanoyl)oxy]-6α,15-dihydroxyelema-1,3,11(13)-trien- 12-oate, 8α-*O*-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)-sonchucarpolide and its 4-epimer, malacitanolide, 8α-*O*-(4,5-diacetoxyangeloyl) sonchucarpolide, zuccarinin	Cirić et al., 2012

*The species have been classified to subsections according to Hilpold et al. (2014); the current accepted botanical names of the species are presented according to Plant List Database and when missing according to Euro+Med PlantBase.

5.4 mg, respectively) (Yam et al., 2010). CC of the fraction F (414.6 mg; eluted with EtOAc: Me_2CO 9:1) on silica gel using DM:MeOH:H_2O mixtures afforded eupatorin (**6**; 2.6 mg), 8α-*O*-(3,4-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7RH, 6αH-eleman-1,3,11(13)-trien-6,12-olide (**4**; 7.2 mg) (Cardona et al., 1994), 8α-*O*-(3,4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin (**3**; 11.8 mg) (Bruno and Herz, 1988) and cnicin (**1**, 20.0 mg) (Sen et al., 2017). The fraction G (85.5 mg; eluted with EtOAc:Me_2CO 75:25) subjected to RP_{18}-HPLC (MeOH-H_2O 2:1), yielded malacitanolide (**2**; 9.1 mg) (Barrero et al., 1997), 8α-*O*-(3, 4-dihydroxy-methyl-enebutanoyloxy)-dehydromelitensin methylester (**5**; 3.8 mg) (Cardona et al., 1997) and cnicin (**1**; 7.0 mg). The combined fractions H and I (581.5 mg; eluted with Me_2CO and MeOH, respectively) subjected to repeated CC over silica gel using DM:EtOAc:MeOH mixtures, and afforded, as well, eupatorin (**6**; 3.5 mg) (Fig. 1).

The structures of all compounds were assigned by spectroscopic methods. NMR spectra were recorded on a Bruker DRX 400 spectrometer at 295 K. Chemical shifts are reported in ppm (δ) using the residual solvent signal (δ_H 7.27 in ^1H and δ_C 77.0 in ^{13}C, $CDCl_3$) or (δ_H 3.31 in ^1H and δ_C 49.0 in ^{13}C, CD_3OD) as reference. COSY, HSQC, HMBC, and NOESY experiments were performed using standard Bruker microprograms. MS: Hewlett-Packard mod. 5973; 70eV. UV spectra were recorded using Shimadzu UV-160 A spectrophotometer, according to Mabry et al. (1970). Vacuum liquid chromatography (VLC) was performed on a silica gel (Merck: 43–63 μm) (Coll and Bowden, 1986), column chromatography (CC) on silica gel 60H (SDS: 40–63 μm). Gradient elution with the solvents mixtures indicated in each case. Semi-preparative RP_{18}-HPLC was performed on a HPLC system: PU-2080 pump Plus (JASCO); refractive index detector RID-10A (Shimazdu); software: Clarity (JASCO), with Kromasil RP-18 column (i.d:10 mm, length: 250 mm, 10 μm) and flow 1.5–1.8 ml/min. Fractions monitoring to follow separation was performed by TLC on silica gel 60 F254 (Merck, Art. 5554) and cellulose (Merck Art. 5685). Compounds were detected

Table 2
Flavonoids isolated from *Centaurea* L. species, belonging to section Centaurea, subsections Acrolophus (Cass.) DC., Phalolepis (Cass.) and Willkommia (Blanca). A:Acrolophus P:Phalolepis W:Willkommia.

Centaurea species[a]	Subsection	Flavonoids	References
C. affinis Friv.	A	Apigenin, eupatorin, salvigenin, 3′-methyl-eupatorin	Janacković et al., 2004
C. aggregata Fisch. & C.A.Mey. ex DC.	A	Eupatorin, 3′-methyl-eupatorin	Zapesochnaya et al., 1978
C. arenaria Bieb. ex Wild (= *C. arenaria* Bieb. ex Wild. subsp. *arenaria*)	A	Eupatilin, eupatorin, 3′-methyl-eupatorin, apigenin, isokaempferide	Csapi et al., 2010
C. cadmea Boiss.	P	Eupatorin, 5-hydroxy-3′,4′,6,7- tetramethoxyflavone	Karamenderes et al., 2007
		Scutellarein	Astari et al., 2013
C. calolepis Boiss.	A	Luteolin-6,8-di-C-β-D-glucoside, schaftoside, apigenin-6,8-di-C-β-D-glucoside, vitexin, isovitexin, isorientin, rutin, orientin, luteolin-7-*O*-glucoside	Erel et al., 2011
C. cariensis Boiss.	A	Pectolinarigenin, eupatorin, 3,5,4′-trihydroxy-6,7-dimethoxyflavone, 5,7,3′-trihydroxy-3,6,4′-trimethoxyflavone	Halfon et al., 1989
C. cineraria L.	A	5,7,4′-trihydroxy-8-methoxyflavone	El-Emary et al., 1983
C. cuneifolia Sibth. & SM. (= *C. cuneifolia* subsp. *cuneifolia*)	A	4′,7-dimethyl-scutellarein, acacetin, eupatilin, eupatorin, jaceosidin, salvigenin	Öksüz et al., 1988
		Salvigenin, eupatilin, jaceosidin, acacetin, kaempferol, 3-methyl-kaempferol, 3′-methyl-eupatorin, 5-hydroxy-3′,4′,7,8- tetramethoxyflavone	Aslan and Öksüz, 1999
C. derventana Vis. & Pančić	A	Apigenin, eupatilin	Tešević et al., 1998
C. deusta Ten.	P	Cirsimaritin, salvigenin	Karioti et al., 2002
C. diffusa Lam.	A	5-hydroxy-6,7,3′,4′-tetramethoxyflavone, cirsimaritin, cirsilineol	Fortuna et al., 2002
C. galicicae Micevski	A	Apigenin, isokaempferide, hispidulin, eupatorin, santoflavone, salvigenin	Tešević et al., 2014
C. grisebachii (Nyman) Heldr. [= *C. grisebachii subsp. grisebachii*, = *C. grisebachii* (Nyman) Formánek]	A	Eupatorin, 3′-methyl-eupatorin, jaseosin, cirsilineol, salvigenin	Djeddi et al., 2008
C. horrida Badarò	A	Horridin	Flamini et al., 2000
		Apigenin, rutin, apigenin-3-*O*-glucuronide, kaempferol-3-*O*-glucuronide, apigenin-8-C-α-L-arabinoside, apigenin-6-C-α-L- arabinoside, apigenin-7-*O*-β-D-glucoside, apigenin-6,8-di-C-β-D-glucoside, scutelarein-7-*O*-β-D-glucoside, quercetin-3-*O*-α-L-rhamnoside,, kaempferol-3-*O*-β-D-glucoside, kaempferol-3-*O*-α-L- ramnoside, vitexin, isovitexin, orientin, saftoside	Flamini et al., 2002
		Hispidulin, quercetin, fisetin, quercetin-3-*O*-β-D-galactoside	Boukhary et al., 2017
C. kilaea Boiss.	A	3′-*O*-methyl-eupatorin, apigenin, cirsimaritin, eupatorin, jaceosidin, pectolinarigenin, salvigenin	Sen et al., 2017
C. orphanidea Heldr. & Sart. ex Boiss.	A	Apigenin, 3-*O*-methyl-kaempferol, luteolin, cirsimaritin	Gousiadou and Skaltsa, 2003
C. panormitana subsp. *umbrosa* (Fiori) Greuter [= *C. cineraria* L. subsp. *umbrosa* (Lacaita)]	A	Salvigenin, 3′-*O*-methyl-eupatorin, eupatilin, jaceosidin	Bruno and Herz, 1988
C. pseudomaculosa Dobrocz.	A	6-methoxyflavone	Al-Easa and Rizk, 1992
C. soskae Hayek	A	Apigenin, isokaempferide, hispidulin, eupatorin, santoflavone, salvigenin	Tešević et al., 2014
C. spinosa L. (= *C. spinosa* var. *spinosa*)	A	Cirsimaritin, salvigenin, desmethoxysudachtin, desmethoxycentauridin, 6,8-dihydroxy-7,4′-dimethyl-luteolin, 6-hydroxy-7,3′-dimethyl-luteolin, nepetin, 5,6,8,3′,4′-pentahydroxy-7-methoxy-flavone, retusin	Saroglou et al., 2005
C. thessala Hausskn. subsp. *drakiensis* (Freyn & Sint.) Georg.	A	Hispidulin, jaseosidin, cirsisineol, eupatorin, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone	Georgiadou, 1999
C. tomorosii Micevski	A	Apigenin, isokaempferide, hispidulin, eupatorin, cirsimaritin, santoflavone, salvigenin	Tešević et al., 2014
C. virgata Lam.	A	Apigenin, hispidulin, salvigenin, eupatorin, 3′-methyleupatorin, isokaempferide	Tuzun et al., 2017
C. zuccariniana DC.	A	Apigenin, genkwanin, hispindulin, cirsimaritin,salvigenin, 7-methylkaempferol, cosmoside, scuttelarein-7-*O*-β-D-glucoside, 6-methoxy-apigenin-7-*O*-β-D-glucoside, luteolin-7-*O*-β-D-glucoside, luteolin-4′-*O*-β-D-glucoside, kaempferol-3-*O*-β-D-glucoside, 6-methoxy-kaempferol-3-*O*-β-D-glucoside	Cirić et al., 2012

[a] The species have been classified to subsections according to Hilpold et al. (2014); the current accepted botanical names of the species are presented according to Plant List Database and when missing according to Euro + Med PlantBase.

using UV absorbance (λ 254 and λ 365 nm). Anisaldehyde/sulphuric acid reagent (anisaldehyde 5% in H_2SO_4/AcOH 1:50) was used for detection of SLs and lignans at TLC chromatography, while Neu's reagent (Neu, 1957) was used for flavonoids. Analytical solvents were obtained from Panreac Quimica SA (Barcelone, Spain, Italy), while deuterated solvents were purchased from Merck, KGaA (Darmstadt, Germany).

3. Results & discussion

C. papposa was previously classified to section Acrolophus (Cass.) DC., which is now a subsection, as combined with the

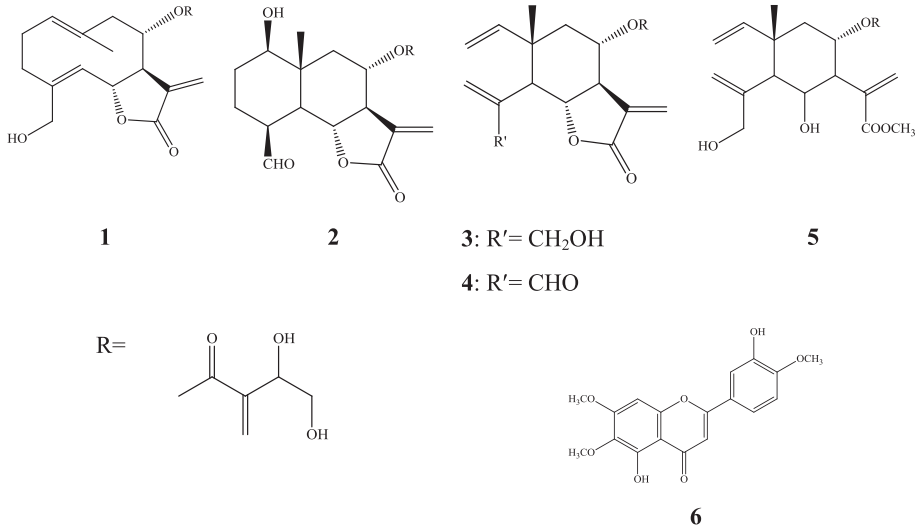

Fig. 1. Chemical structures of the isolated compounds.

sections Phalolepis (Cass.) and Willkommia (Blanca) in section Centaurea (Hilpold et al., 2011, 2014). The present study revealed that the chemical profile of *C. papposa* is similar to that of previous investigated *Centaurea* taxa belonging to the same section (Sokovic et al., 2017).

Regarding the sesquiterpene lactones, common constituents of *Centaurea* species are by order of their abundance, guaianolides, germacranolides, eudesmanolides and elemanolides. Although guaianolides are very common metabolites in the genus, the subsections Acrolophus and Phalolepis are characterized by the absence of those secondary metabolites (Bruno et al., 2013). Cnicin (**1**) is a germacranolide isolated from most *Centaurea* species (Table 1) and is considered as a marker of subsection Acrolophus (Cass.) DC. (Nowak et al., 1984). This generalization is true, since it has been found in the 44 of the 47 studied taxa, and it also applies to the combined *Centaurea* section, as cnicin appears in 48 of the 54 species (Table 1). Some previously studied species (Nowak et al., 1984) belong now to other sections and this replacement seems to be chemotaxonomically correct, as cnicin was absent, while guaianolides were found.

Although eudesmanolides are considered to be rare constituents of the genus *Centaurea* (Skaltsa et al., 2000), concerning the subsection Acrolophus they are markers; specifically the 6,12-eudesmanolides bearing a *trans*-decalin skeleton), such as malacitanolide (**2**) (Djeddi et al., 2008). Regarding the isolated elemanolides, 8α-*O*-(3, 4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin (**3**) has been found in many taxa, while 8α-*O*-(3, 4-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7RH, 6αH-eleman-1,3,11(13)-trien-6,12-olide (**4**) has been isolated only from 5 *Centaurea* species (Table 1). In addition, the elemane 8α-*O*-(3, 4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin methylester (**5**) has been found in 4 *Centaurea* species (Table 1).

It is noteworthy that all isolated sesquiterpene lactones in the present study contain the same side chain, which is common throughout the *Centaurea* species (Table 1).

Finally, the isolated flavonoid, eupatorin (**6**), belongs to the methylated flavones, which are common metabolites of the genus (Table 2).

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SHORT COMMUNICATION

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In vitro cytotoxic and anti-inflammatory activities of sesquiterpene lactones from *Centaurea papposa* (Coss.) Greuter

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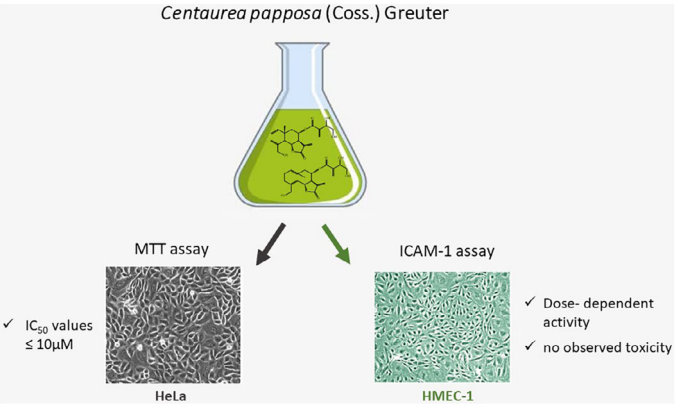
ABSTRACT

In the pursuit of highly active specialized metabolites from endemic plants, *Centaurea papposa* (Coss.) Greuter, an endemic plant in Algeria and Tunisia, was investigated and afforded eleven sesquiterpene lactones (**1**-**11**). Cytotoxic evaluation of these compounds using the *in vitro* MTT assay on three human cancerous cell lines (HeLa, SK-MEL-28 and HepG2), revealed that isolates **4**, **8** and **9** ($IC_{50} \leq 10\,\mu M$) could be potential anti-cancer drugs for cervical cancer according to the National Cancer Institute. Further evaluation of the *in vitro* anti-inflammatory activity showed that compounds **1** and **4** inhibited the TNF-α induced ICAM-1 expression in HMEC-1 endothelial cells at a maximum of 21% and 2% of the control (IC_{50} values 21.9 and 5.7 μM, respectively). The Michael reactions of the α-methylene-γ-lactone ring seem to be responsible for the strong activity, while no toxicity was observed in the HMEC-1 cells in all the range of tested concentrations (6.25-50.0 μM).



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1. Introduction

The Asteraceae family is characterised by the ability to biosynthesize sesquiterpene lactones (SLs), which are produced by widely used medicinal plants, such as *Arnica montana*, *Tanacetum parthenium*, *Matricaria chamomilla* (Merfort 2011). Some SLs and their semi-synthetic derivatives underwent clinical trials (thapsigargin, parthenolide) (Ghantous et al. 2010). Interestingly, cnicin, a SL which characterizes the genus *Centaurea* L., could be a potential chemotherapeutic agent for breast cancer, since it showed strong activity against the MCF-7 series with IC_{50} of 3.25 μg/mL (Sen et al. 2017). Compounds possessing IC_{50} equal or less than 4-5 μg/mL could be potential anti-cancer drugs according to the National Cancer Institute (NCI) (Suffness and Pezzuto 1990). In this project, aiming to investigate the cytotoxicity of the SLs isolated from the Algerian endemic plant *Centaurea papposa* (Coss). Greuter, we used the *in vitro* MTT assay on three human cancerous cell lines (HeLa, SK-MEL-28 and HepG2) and one normal non-cancerous cell line (HMEC-1), and further evaluated the anti-inflammatory potential *in vitro* for the reduction of the ICAM-1 expression induced by TNF-α in the endothelial HMEC-1 cells.

2. Results and discussion

A total of eleven SLs have been isolated from *C. papposa* (Figure S1), namely cnicin (**1**), 8α-O-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7RH,6αH-eleman-1,3,11(13)-trien-6,12-olide (**5**), 8α-O-(3′, 4′-dihydroxy-methylenebutanoyloxy)-dehydromelitensin (**4**), malacitanolide (**8**), 8α-O-(3′,4′-dihydroxy-methylenebutanoyloxy)-dehydromelitensin methylester (**7**) (Grafakou et al. 2018), together with dehydromelitensin (**2**), 8α-O-acetyl-dehydromelitensin (**3**), 6α,8α,15-trihydroxyelema-1,3,11(13)-trien-12-olide methyl ester (**6**), 4-epi-malacitanolide (**9**), 4′-acetyl 8α-O-(3′,4′-dihydroxy-methylenebutanoyloxy)-sonchoucarpolide (**10**) and 4′-acetyl-8α-O-(3′, 4′-dihydroxy-methylenebutanoyloxy)-4-epi-sonchoucarpolide (**11**) (present study). To the best of our knowledge, compound **3**, was isolated for the first time in the genus *Centaurea*. Regarding the methyl ester **6** (Cardona et al. 1992; Demir et al. 2017), its high field ^{1}H and ^{13}C data in $CDCl_3$ have not been reported previously, thus they are presented here (Table S1), together with the spectroscopic data of **2** for comparison purposes, since compound **6** is considered the biosynthetic precursor of **2** (de Kraker et al. 2002).

Taking in consideration the amount of the compounds, seven SLs have been subjected to cytotoxic screening (Table S2). Compound **10** showed great variance in the IC_{50} values at the MTT assay, which could be attributed to instability in the DMSO stock solution, thus it is not presented in Table S2. SK-MEL-28 and HepG2 cells were generally more resilient than HeLa series, resulting in lower cytotoxic activities from all the tested compounds. Compound **1,** previously isolated from *C. arenaria* and *C. calolepis*, has also been tested in HeLa and SK-MEL-28 cells and the reported IC_{50} values are in accordance to our results regarding the HeLa cell line (34.5 μM; Csapi et al. 2010), while the differences in SK-MEL-28 values could be attributed in different incubation times in the MTT assays (14.0 μM; Erel et al. 2011). Compounds **4** and **9** showed strong activity against HeLa cells, with IC_{50} 9.2 and 8.5 μM respectively, and could be

potential chemotherapeutic agents for cervical cancer, since compounds having an $IC_{50} \leq 10\,\mu M$ are considered active and could be potential anti-cancer drugs according to NCI guidelines (Suffness and Pezzuto 1990). The epimers **8** and **9** showed similar cytotoxic activity (IC_{50} 10.6 and 8.5 μM respectively), thus the stereochemistry of C-4 is not important for the activity. Compound **2** showed reduced cytotoxic potential in comparison to its 8α-O-(3′,4′-dihydroxy-methylenebutanoyloxy) derivative **4**, which could be attributed to the loss of the active centers of the C-8 lateral chain. These observations are in accordance with previous results (Koukoulitsa et al. 2002; Saroglou et al. 2005).

Moreover, homologous sesquiterpenes (methyl esters, such as compound **6**) are not active cytotoxic agents as SLs due to the loss of the Michael center (Koukoulitsa et al. 2002). Thus, compounds **1**, **4** together with **6** were tested for their anti-inflammatory potential using the ICAM-1 assay. The expression of ICAM-1 (Intercellular Adhesion Molecule 1) and of other surface molecules, like VCAM-1 (Vascular cell adhesion protein 1), is triggered by cytokines and leads to endothelia adhesion and generation of intracellular signals on the endothelial cells during the inflammation process (Cook-Mills et al. 2011; Harjunpää et al. 2019).

For the ICAM-1 assay, the samples were firstly subjected to MTT viability test on the HMEC-1 cells in order to exclude any false positive results in the reduction of the expression of the adhesion molecule due to cytotoxicity. No toxicity was observed in the cells (viability 95-105%, Figure S2A), and the compounds were further evaluated for the reduction of the TNF-α induced ICAM-1 expression (Figure S2B). The natural ICAM-1 expression of the HMEC-1 cells was also determined in the control group and was about 2%. Parthenolide, used as positive control (5.0 μM), was able to reduce the total expression of the surface molecule by around two-thirds to 30% of the control. It is noteworthy that although compound **6** resulted in moderate reduction of the TNF-α induced ICAM-1 expression, compounds **1** and **4** revealed remarkable inhibiting potential in a dose-dependent manner (IC_{50} values 21.9 and 5.7, respectively, Figure S3). More specifically, compound **1** reduced the ICAM-1 expression down to 21% of control, but in comparison to parthenolide, it needed a ten-fold higher concentration to achieve this reduction (50.0 μM). Interestingly, at 50.0 μM compound **4** reduced the ICAM-1 expression by 98% (at 2% of control), showing no cytotoxic effects in HMEC-1 cells, while it is worth mentioning that even the lowest concentration tested (6.25 μM) proved to be active, reaching a reduction of 49%. Similarly, as reported from Piela-Smith and Liu (2001), parthenolide (8 μM) strongly inhibits the TNF-α induced ICAM-1 expression by 93% in human synovial fibroblasts. However, matricine, an SL which lacks the α,β-unsaturated lactone moiety, only achieves an inhibition of 52% in HMEC-1 with a concentration of 75 μM (Flemming et al. 2015). Taken together, the moderate activities of matricine and compound **6**, in contrast to the strong inhibitors **1**, **4** and parthenolide and their structures, it is suggest that the α-methylene-γ-lactone ring is essential for Michael-type additions resulting to this type of anti-inflammatory events. Moreover, Michael reactions with functional biological nucleophiles that contain sulfhydryl groups usually also involve non-specific toxicity (Amorim et al. 2013), however it is noteworthy that for the tested SLs no toxicity was observed in the endothelial HMEC-1 cells in all the range of the tested concentrations (6.25-50.0 μM). These

findings further support that SLs are the specialized compounds responsible for the medicinal features of *Centaurea* species (Sokovic et al. 2017).

3. Experimental

See Supporting Information.

4. Conclusions

This study provides the *in vitro* cytotoxic and anti-inflammatory activities of seven SLs isolated from *C. papposa*. The MTT assay on the three human cancerous cell lines (HeLa, SK-MEL-28 and HepG2) suggested once more the cytotoxic potential of the SLs, revealing compounds **4**, **8** and **9** as potential chemotherapeutic agents for cervical cancer. Moreover, the SLs showed a remarkable inhibiting activity in a dose-dependent manner for the reduction of the ICAM-1 expression induced by TNF-α in the endothelial HMEC-1 cells, with no observed cytotoxicity in all the range of the tested concentrations. Further exploration of their mechanism of action may offer a novel therapeutic approach to cope with inflammatory diseases.

Disclosure statement

No potential conflict of interest was reported by the authors.

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SUPPLEMENTARY MATERIAL

In vitro cytotoxic and anti-inflammatory activities of Sesquiterpene Lactones from *Centaurea papposa* (Coss.) Greuter.

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ABSTRACT

In the pursuit of highly active specialized metabolites from endemic plants, *Centaurea papposa* (Coss.) Greuter, an endemic plant in Algeria and Tunisia, was investigated and afforded eleven sesquiterpene lactones (**1**-**11**). Cytotoxic evaluation of these compounds using the *in vitro* MTT assay on three human cancerous cell lines (HeLa, SK-MEL-28 and HepG2), revealed that isolates **4**, **8** and **9** (IC_{50} ≤ 10 μM) could be potential anti-cancer drugs for cervical cancer according to the National Cancer Institute. Further evaluation of the *in vitro* anti-inflammatory activity showed that compounds **1** and **4** inhibited the TNF-α induced ICAM-1 expression in HMEC-1 endothelial cells at a maximum of 21% and 2% of the control (IC_{50} values 21.9 and 5.7 μM, respectively). The Michael reactions of the α-methylene-γ-lactone ring seem to be responsible for the strong activity, while no toxicity was observed in the HMEC-1 cells in all the range of tested concentrations (6.25-50.0 μM).

Keywords: *Centaurea papposa*, Asteraceae, Sesquiterpene lactones, MTT, I-CAM-1, cytotoxic, anti-inflammatory

Experimental Section

Plant material

Aerial parts of the *C. papposa* were collected (Cape de Garde Lighthouse, Annaba, Algeria, September 2016) and identified by Dr. Hamel Tarek (Department of Biology, University Badji, Mokhtar Annaba, Algeria). A voucher specimen (no. Djeddi & Skaltsa BV 01/2017) is deposited to the Herbarium of Department of Biology (University Badji Mokhtar Annaba, Algeria).

Isolation and identification of the sesquiterpene lactones from *C. papposa*

Following the initial extraction (cyclohexane:ether:methanol 1:1:1), fractionation and isolation of secondary metabolites from *C. papposa* (Grafakou et al. 2018), the plant was re-investigated and afforded six further SLs. Cnicin (**1**)**,** as the main compound, was previously isolated from the plant together with 8α-O-(3',4'-dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7RH,6αH-eleman-1,3,11(13)-trien-6,12-olide (**5**), 8α-O-(3', 4'-dihydroxy-methylenebutanoyloxy)-dehydromelitensin (**4**), malacitanolide (**8**), 8α-O-(3',4'-dihydroxy-methylenebutanoyloxy)-dehydromelitensin methylester (**7**) (Grafakou et al. 2018). The re-investigation of the plant in the present study afforded for the first time from *C. papposa* dehydromelitensin (**2**) (Cardona et al. 1989)**,** 8α-O-acetyl-dehydromelitensin (**3**) (Del R. Cuenca et al. 1993), 6α,8α,15-trihydroxyelema-1,3,11(13)-trien-12-olide methyl ester (**6**) (Cardona et al. 1992), 4-epi-malacitanolide (**9**), 4'-acetyl 8α-O-(3',4'-dihydroxy-methylenebutanoyloxy)-sonchoucarpolide (**10**) and 4'-acetyl-8α-O-(3',4'-dihydroxy-methylenebutanoyloxy)-4-epi-sonchoucarpolide (**11**) (Saroglou et al. 2005) (Figure S1). More specifically, fraction CPA-E (149.9 mg; eluted with EtOAc 100%) subjected to CC over silica gel (DM:EtOAc:MeOH mixtures) and RP_{18}-HPLC (MeOH-H_2O 3:2) and yielded compounds **11** (0.5 mg; t_R: 15.2 min), **10** (1.4 mg; t_R: 19.0 min), **6** (2.1 mg; t_R: 21.3 min), **2** (1.5 mg; t_R: 20.1 min) and **3** (0.5 mg; t_R: 39.4 min). Furthermore, the combined fractions CPA-H and CPA-I (581.5 mg; eluted with Me_2CO and MeOH, respectively) were subjected to repeated CC over silica gel using DM:MeOH:H_2O mixtures and yielded 4-epi-malacitanolide **9** (3.2 mg). The structures of all compounds were assigned by spectroscopic methods. 1D and 2D NMR spectra were recorded on an Avance 600 Kryo or a Bruker DRX 400 spectrometer at 298 K. Chemical shifts are reported in ppm (δ) using the residual solvent signal (δ_H 7.26 in ^{1}H and δ_C 77.0 in ^{13}C, $CDCl_3$) as reference. CC and HPLC were performed as described before (Grafakou et al. 2018).

MTT assay

The cytotoxicity of the compounds was determined using the human cancer cell lines HeLa (cervical carcinoma, ATTC), SK-MEL-28 (malignant melanoma, DSMZ) and HepG2 (hepatocellular carcinoma, DSMZ), as well as the non-cancerous HMEC-1 cell line (human microvascular endothelial cells). Culture medium was MEM (Sigma; supplemented with L-Glutamine, FCS and NEA, BioChrom), DMEM-HAM'S (50-50%, Invitrogen and BioChrom; supplemented with FCS), RPMI (BioChrom; supplemented with L-Glutamine and heat activated FCS) and ECGM (EASY Endothelial Cell Growth Medium, supplemented with EASY Growth supplement and Gentamycin-Amphotericin (all PELOBiotech), and heat activated FCS), respectively. Stock solutions of the samples were prepared in DMSO, and the test concentrations were freshly prepared with medium at the day of the experiment (final concentration of DMSO was 0.33%). Cytotoxicity was evaluated using the colorimetric MTT assay as described before (Heilmann et al. 2001). Briefly, nine different concentrations of the compounds (100.0 to 0.39 μM) together with the cell suspension (final concentration in wells: $6x10^4$ cells/mL, $3x10^4$ cells/mL and $12.5x10^4$ cells/mL, for HeLa, SK-MEL-28 and HePG2 respectively), were added to the 96 well-plates. The HMEC-1 cells in a final cell suspension of $90x10^4$ cells/mL were placed in the 96-wells, and 24 h later they were treated with 6.25, 12.50, 25.00, 50.00 μM of the tested SLs. After incubation for 68 h (or 24 h for the HMEC-1) (IBS Integra Biosciences, 37 °C, 5% CO_2), MTT was added (4 mg/mL), and after 4 h the solution was exchanged with 10% SDS. The plates were then placed in dark and 24 h later, the absorbance at 560 nm was measured (SpectraFluor plus plate reader, Tecan, Cralsheim).

I-CAM-1 assay

Regarding the ICAM-1 assay, the HMEC-1 cells were treated as described before (Freischmidt et al. 2012). Briefly, the compounds were firstly subjected to MTT viability test, as described in 2.2., in order to exclude any false positive results in the reduction of the expression of ICAM-1 due to cytotoxicity. Since no cytotoxicity was observed (mean viability between 95-15%, Figure 2), the HMEC-1 cells were treated with the compounds **1**, **4** and **6** at 6.25, 12.50, 25.00, 50.00 μM, or parthenolide (5 μM, positive control, Calbiochem), or medium (ECGM, negative control, set as 100%) in 24 well-plates (plates were treated firstly with 0.25% v/v Collagen G in PBS (both Sigma-Aldlich). After 30 min, TNF-α (10 ng/mL, Sigma Aldrich) was added (except from two wells, in which no TNF-α was added in order to evaluate the normal cells ICAM-1 production (untreated control), and the plates were incubated for 24 h. Then, the cells were washed with PBS, removed from the plates with trypsin/EDTA and fixed with formalin. Incubation with the Mouse Anti-human CD54: FITC (Bio-Rad) for 20 min, was followed by FACS analysis (Becton Dickinson FacscaliburTM) measuring fluorescence intensity.

Statistical analysis

IC_{50} values were calculated from nine different concentrations and tests were performed in sextuplicate for the MTT assay. For ICAM-1 assay each experiment was performed in duplicate. All experiments repeated at least three times and the results are presented as means ± SD for each sample. Statistical analysis of all continuous variables was checked for normality. Means of two groups were compared with paired samples t-test or one sample t-test where applicable. Statistical significance (2-tailed) was set at *p < 0.05, **p < 0.01, or ***p < 0.001. Statistical analyses were performed using IBM SPSS statistics 26. GraphPad Prism 8.4.2 version was used for the creation of the graphical representations, as well as the calculations of the IC_{50} values using non-linear regression.

Table S1. ^{1}H-NMR data of compounds **2**, **6**; Solvent: $CDCl_3$, Ava 600 MHz (T=298 K).

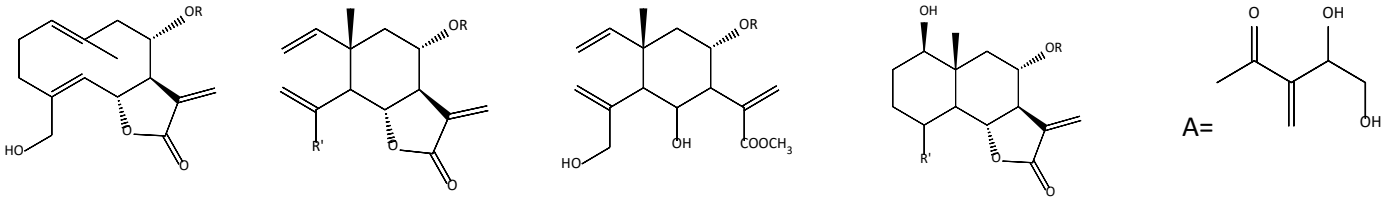
	2		**6**	
Position	δ_H	δ_c	δ_H	δ_c
1	5.78, dd (10.6, 17.4)	146.3	5.71, dd (10.7, 17.5)	147.4
2a	5.05, d (10.6)	112.8	4.96, d (10.7)	112.1
2a	4.98, d (17.4)		4.93 d (17.5)	
3a	5.40, s	115.0	5.40, s	114.7
3b	4.94, s		5.03, s	
4	-	145.8	-	
5	2.51, d (11.3)	50.6	2.07, d (10.6)	55.5
6	4.14, t (11.3)	78.8	4.15, t (10.6)	70.8
7	2.63, tt (3.0, 11.3)	55.1	2.43, t (10.6)	58.9
8	4.12, m	67.6	4.15, m	67.9
9a	1.88, dd (4.1, 13.1)	49.8	1.87, dd (4.3, 12.8)	47.5
9b	1.66, dd (10.9, 13.1)		1.53*	
10	-	41.6	-	40.9
11	-	137.1	-	138.7
12	-	170.1	-	167.7
13a	6.18, d (3.1)	120.6	6.41, s	128.9
13b	5.98, d (2.9)		5.82, s	
14a	4.08, d (13.9)	67.6	4.08, d (13.5)	67.9
14b	4.00, d (13.9)		3.96, d (13.5)	
15	1.11, s	18.9	1.12, s	18.6
-OMe			3.49, s	51.7

*overlapped

Table S2. IC_{50} (µM) values of the tested compounds for the three cancerous cell lines

Compounds	HeLa	SK-MEL-28	HePG2
1	22.9 ± 0.6	40.7 ± 1.5	80.7 ± 5.4
2	34.2 ± 1.7	64.6 ± 3.0	n.t.
4	9.2 ± 0.9	20.3 ± 1.9	28.7 ± 3.1
5	34.2 ± 2.3	64.6 ± 2.4	n.t.
8	10.6 ± 0.4	28.1 ± 1.2	33.1 ± 2.5
9	8.5 ± 0.5	22.4 ± 1.5	24.3 ± 1.8
P	2.1 ± 0.5	4.9 ± 1.1	10.0 ± 0.7

P: Parthenolide (positive control, Calbiochem), n.t.: not tested



1: R= A

2: R= H, R'= CH_2OH

3: R= Ac, R'= CH_2OH

4: R= A, R'= CH_2OH

5: R= A, R'= CHO

6: R= H

7: R= A

8: R= A, R'= βCHO

9: R= A, R'= αCHO

10: R= B, R'= βCHO

11: R= B, R'= αCHO

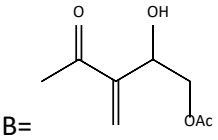


Figure S1. Structures of isolated compounds **1**-**11** from *Centaurea papposa*

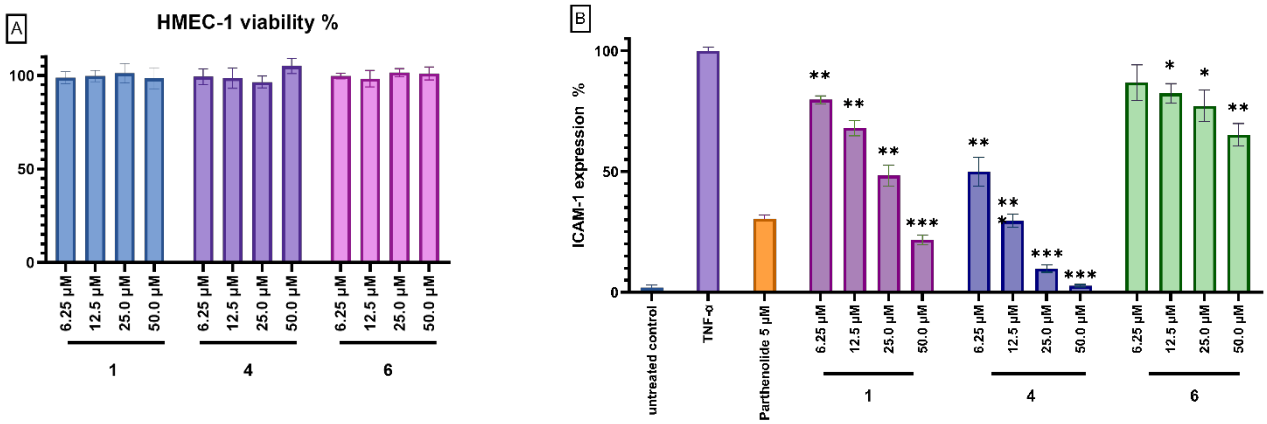


Figure S2. **A.** MTT viability test on the non-cancerous cell line HMEC-1 for compounds **1**, **4** and **6**, expressed as % mean ± SD. Data are representative of 3 independent experiments with similar results. No toxicity was observed in the cells (viability 95-105%). **B.** ICAM-1 expression (%) in HMEC-1 cell line is expressed as means ± SD. Data are representative of 3 independent experiments with similar results. Significance levels were set at p<0.5*, p<0.01**, p<0.001*** vs. TNF-α, as determined by paired sample t-test. Untreated control (pure medium, without addition of TNF-α), TNF-α (10 ng/mL, negative control), parthenolide (5 µM, positive control) and different concentrations (6.25, 12.5, 25.0 and 50.0 µM) of compounds **1**, **4**, and **6**.

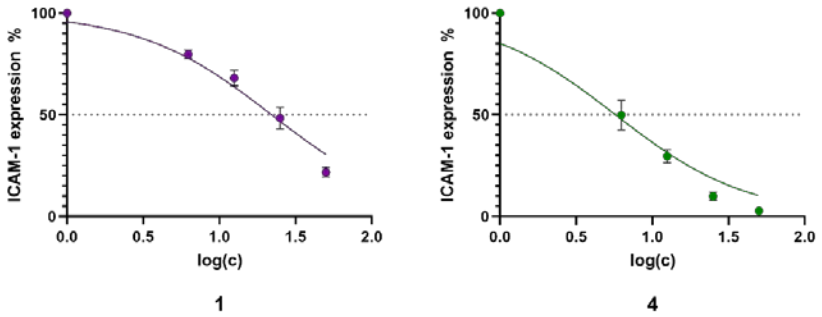


Figure S3. Non-linear regression for the calculations of the IC_{50} value of **1** and **4** using GraphPad.

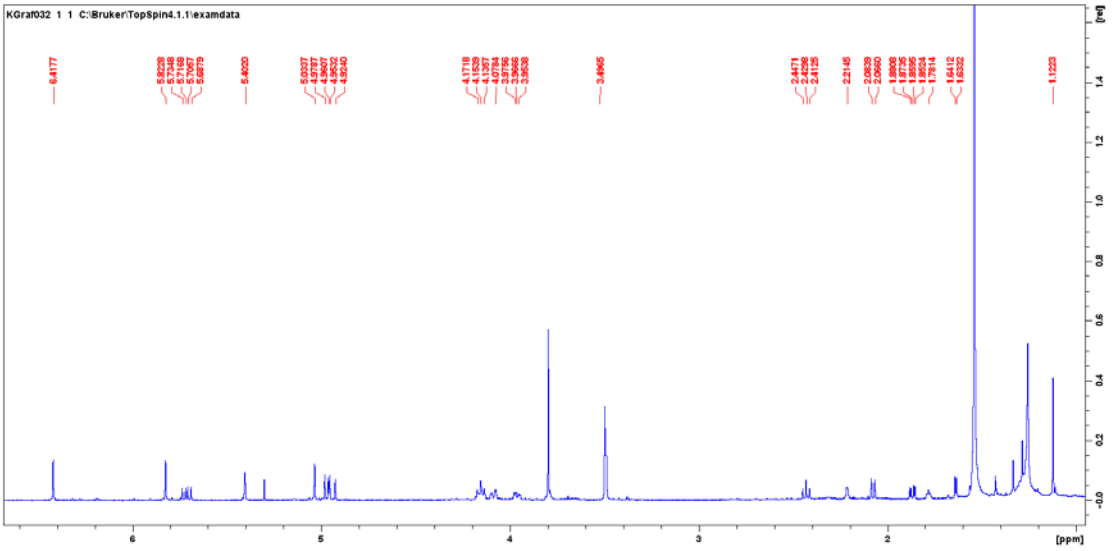


Figure S4. ^{1}H-NMR spectrum of compound **6**

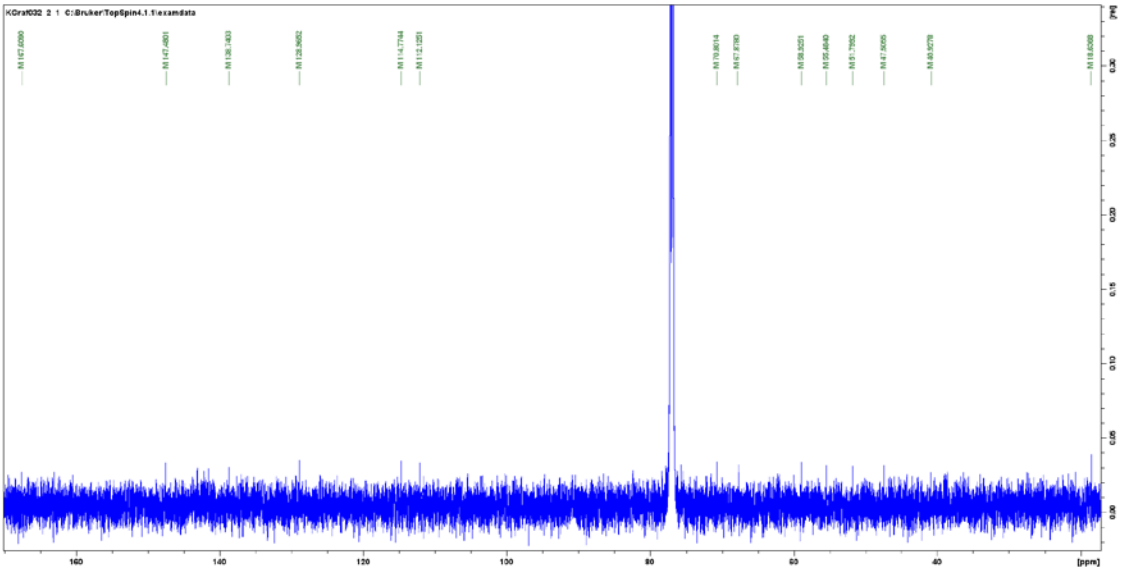


Figure S5. C^{13}-NMR spectrum of compound **6**

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3.3. *Hypericum jovis* Greuter

The isolation of the compounds from *H. jovis* is presented in Figure 28. Extraction, isolation, structure elucidation, and biological activities are presented in Grafakou et al. (2021c).

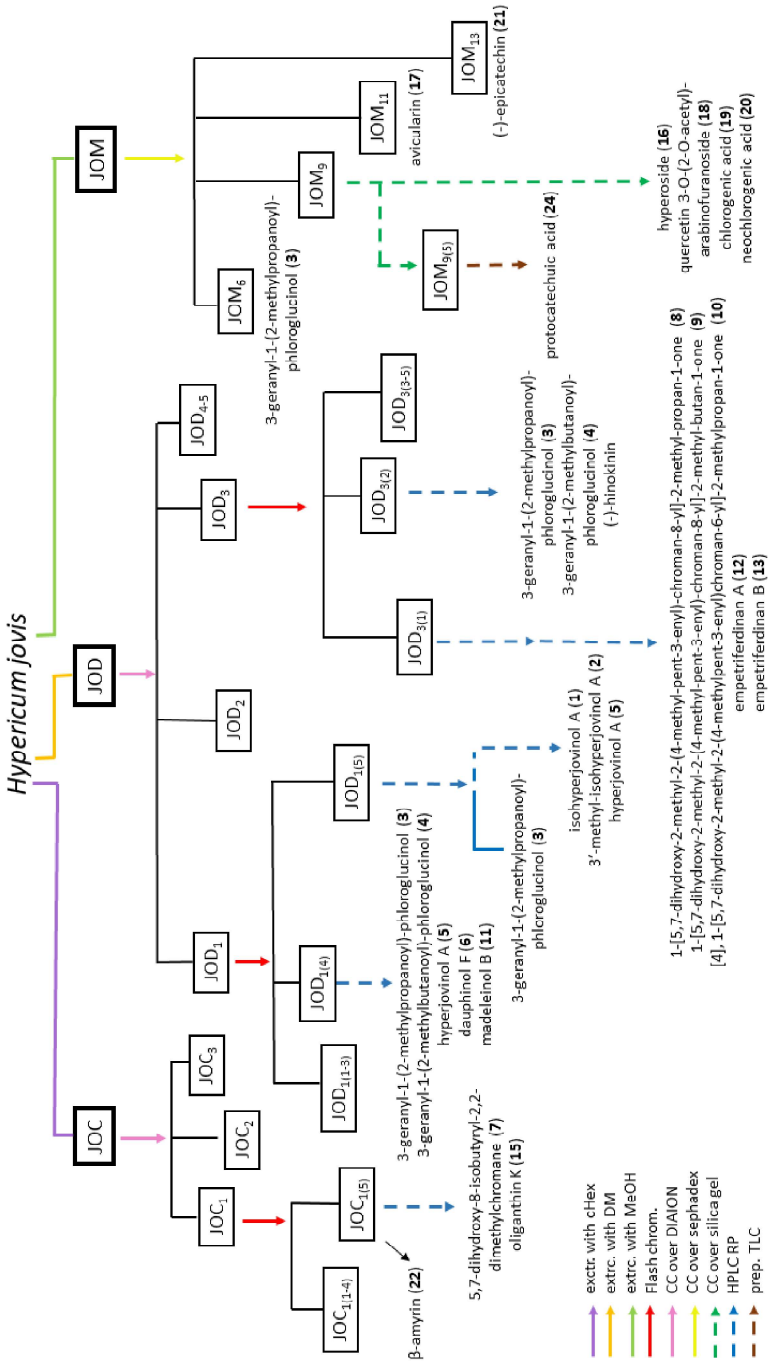

Figure 28. Chromatographic isolation of the compounds from H. jovis.

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Prenylated Acylphloroglucinols from *Hypericum jovis* with Anti-inflammatory Potential#

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Key words
Hypericum jovis, Hypericaceae, prenylated acylphloroglucinols, inflammation, ICAM-1, COX-LOX

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ABSTRACT

Thirteen prenylated acylphloroglucinols (**1–13**), including 2 previously undescribed compounds (**1**) and (**2**), were isolated from *Hypericum jovis*. Their structures were elucidated by high-field NMR spectroscopy. The isolated prenylated acylphloroglucinols were evaluated for their anti-inflammatory effects *in vitro* through the reduction of the intercellular adhesion molecule 1 expression induced by TNF-α in the human microvascular endothelial cells 1 cell line. Compounds **3, 5, 6, 8,** and **12** significantly reduced intercellular adhesion molecule 1 expression in a concentration-dependent manner with IC_{50} values of 16.9, 34.4, 4.0, 3.2, and 7.7 µM, respectively. In addition, compound **12** showed notable inhibitory activity on the formation of cyclooxygenase-1- and 12-lipoxygenase-derived inflammatory mediators in an *ex vivo* cyclooxygenase-lipoxygenase assay. Eleven further constituents were isolated (**14–24**), including the rare quercetin 3-O-(2-O-acetyl)-arabinofuranoside (**18**).

ABBREVIATIONS

COX	cyclooxygenase
ECGM	EASY endothelial cell growth medium
HMEC-1	human microvascular endothelial cells 1
ICAM-1	intercellular adhesion molecule 1
LOX	lipoxygenase
PAP	prenylated acylphloroglucinol derivatives
VCAM-1	vascular cell adhesion protein 1

Introduction

The genus *Hypericum* L. (Hypericaceae) comprises more than 500 taxa (species and subspecies) worldwide [1], among which 40 taxa (including 12 endemic taxa) grow in Greece. Crete is an island with extremely high rates of endemism, having 1825 different plant species in total, of which 19.3 % are endemic [2]. *H. jovis*

Dedicated to Prof. Dr. Otto Sticher on the occasion of his 85th birthday.

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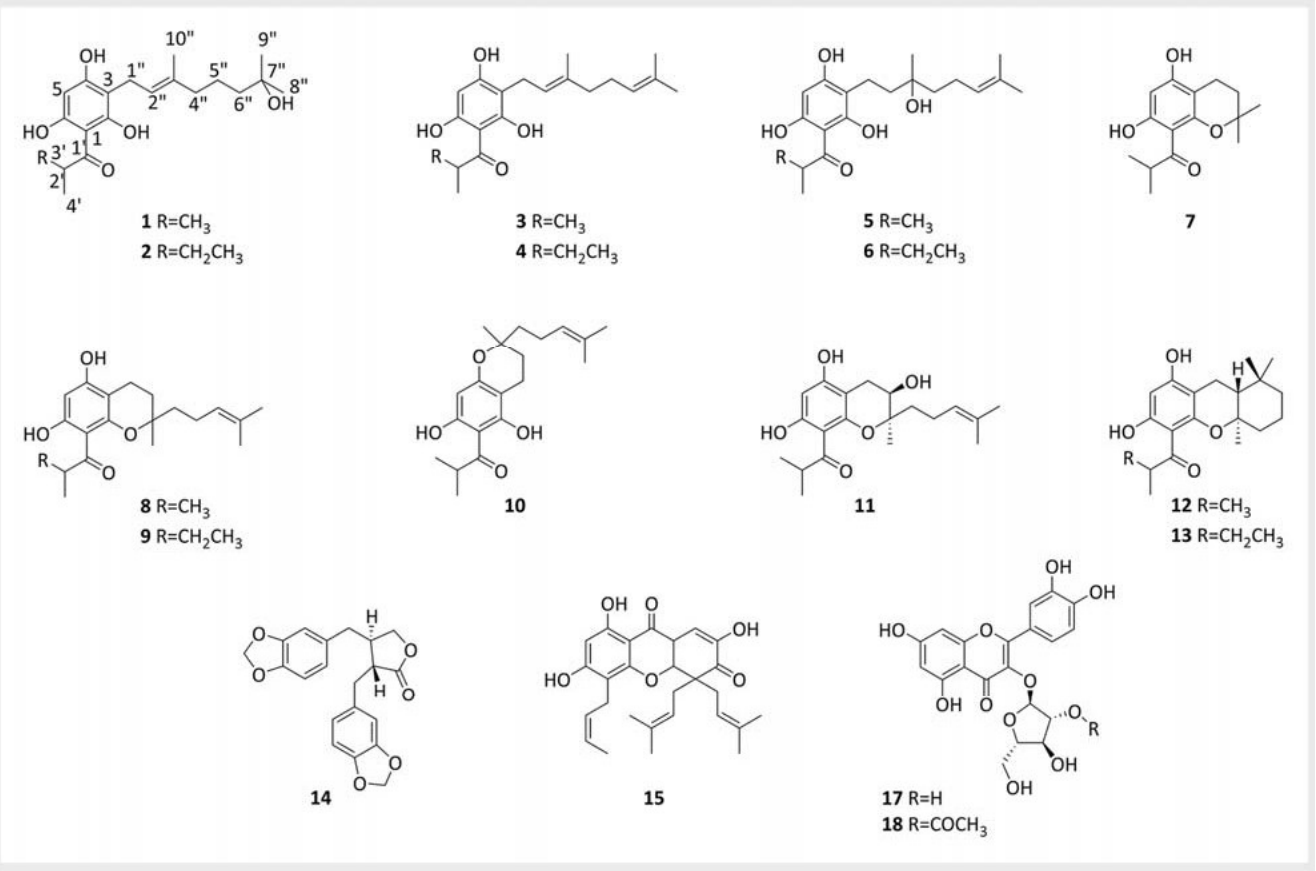

▶ **Fig. 1** Compounds **1**–**15** and **17**, **18**.

Greuter examined in this study is a narrow endemic species of West Crete and is closely related to the previously studied *H. empetrifolium* Willd. [3, 4] and *H. amblyocalyx* Coust. & Gand [5], both belonging to the section *Coridium* [6].

Several studies have investigated the anti-inflammatory potential of PAPs isolated from the genus *Hypericum*. For example, hyperforin, a poly-prenylated acylphloroglucinol from *H. perforatum* was found, besides its proven antidepressant, anti-microbial and anti-proliferative activities, to be a dual inhibitor of COX-1 and 5-LOX, both *in vitro* [7] and *in vivo* [8]. COX and LOX are key enzymes in the production of eicosanoids, including leukotrienes, prostaglandins, and thromboxane, which are potent inflammatory mediators [9]. Interestingly, 2 monomeric PAPs isolated from *H. empetrifolium* also act as strong anti-inflammatory agents by inhibiting COX-1, COX-2, and 5-LOX [10]. Furthermore, hyperforin has also been shown to inhibit both VCAM-1 and ICAM-1 expression [11]. Upon cytokine stimulation, the expression of adhesion molecules like VCAM-1 and ICAM-1 is increased on the endothelial cells, facilitating the leukocyte-endothelial adhesion, thereby triggering intracellular signals followed by inflammation processes [12, 13].

The aim of this study was to identify PAPs from *H. jovis* with potential anti-inflammatory activity and to analyze the acyl-phloroglucinol profile from a chemotaxonomical point of view. Since hyperforin and other highly complex PAPs often show instability in aqueous solutions [14, 15], the search for simpler and more stable analogues is warranted. In comparison to the study of Athanasas et al. [16], the present study revealed a variety of PAPs from *H. jovis*, including the previously undescribed iso-hyperjovinol-A (**1**) and 3′-methyl-isohyperjovinol A (**2**). The known PAPs comprised 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol (**3**) [17], 3-geranyl-1-(2-methylbutanoyl)-phloroglucinol (**4**) [3], hyperjovinol A (**5**) [16], dauphinol F (**6**) [18], 5,7-dihydroxy-8-isobutyryl-2,2-dimethylchromane (**7**) [19], 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one (**8**) [5], 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-butan-1-one (**9**) [4], 1-[5,7-dihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-6-yl]-2-methylpropan-1-one (**10**) [4], madeleinol B (**11**) [20], empetriferdinan A (**12**) [4], and empetriferdinan B (**13**) [4].

In addition, structurally diverse compounds were isolated including the lignan (−) hinokinin (**14**) [21], the xanthone oliganthin K (**15**) [22], the flavonoids hyperoside (**16**) [23], avicularin (**17**) [24] and quercetin 3-O-(2-O-acetyl)-arabinofuranoside (**18**) [24] (▶ **Fig. 1**), chlorogenic acid (**19**) and neochlorogenic acid (**20**) [25], (−)-epicatechin (**21**) [26], *β*-amyrin (**22**) [27], lupeol (**23**) [28], and protocatechuic acid (**24**) [29]. The isolated PAPs were evaluated for their anti-inflammatory effects *in vitro* for the reduction of the ICAM-1 expression induced by TNF-*α* in the HMEC-1 cell line. The most potent compounds were further evaluated *ex*

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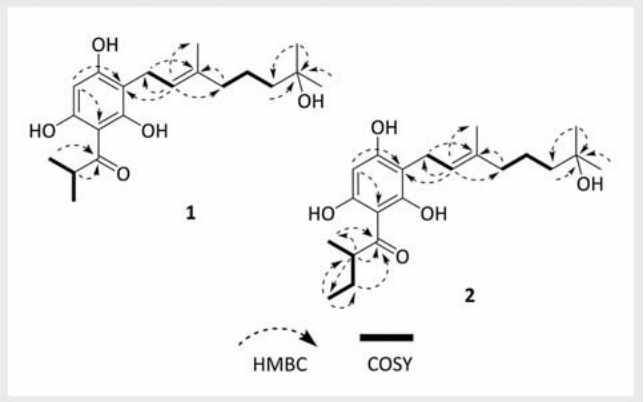
vivo for the inhibition of COX-1 and 12-LOX activity to investigate their anti-inflammatory effects through the arachidonic acid pathway.

Results & Discussion

Compound **1** was isolated as a yellow oil with the molecular formula $C_{20}H_{30}O_5$ established by the quasi-molecular $[M + H]^+$ ion at *m/z* 351.2172 (calc. 351.2166) in HRESIMS. The ^{13}C NMR spectrum showed 20 carbon signals with diagnostic resonances at δ_C 162.8 (C-2), δ_C 160.9 (C-4), and δ_C 160.9 (C-6), which indicated the presence of a phloroglucinol scaffold as in the known congeners **3–6**. The carbons were sorted by HSQC into 5 methyls, 4 methylenes, and 3 methines, which indicated the presence of 8 nonprotonated carbons in combination with the ^{13}C NMR data. Substitution at C-1 and C-3 in *meta* positions on the phloroglucinol moiety was indicated by the chemical shifts at δ_C 104.0 and δ_C 105.8, respectively. The cross-peaks between these carbons and the singlet at δ_H 5.85 in the HMBC spectrum confirmed the presence of methine hydrogen located at C-5. The substituent at C-1 proved to be an isobutyryl group (C-1′–C-4′). The most downfield signal at δ_C 210.8 corresponded to the carbonyl group (C-1′), and the diagnostic methine signal at δ_H 3.88 appeared as a septet due to the coupling to the vicinal methyls at δ_H 1.16 (d 6.4 Hz). A characteristic triplet at 5.26 ppm suggested the presence of an isoprenyl moiety [3,4]. Indeed, the ^{13}C and 2D NMR data (COSY, HSQC, and HMBC) displayed diagnostic signals and correlations of a substituted geranyl side chain (10 carbons) linked to C-3. The geranyl moiety was substituted on the second prenyl group by a hydroxyl group at C-7″ (δc 71.6), which resulted in a downfield shift of the methyl groups CH_3-9″ and CH_3-8″ observed at 29.1 ppm in the ^{13}C-NMR spectrum. This was further supported by the HMBC cross-peaks from CH_2-6″ (δ_H 1.43), CH_3-8″ (δ_H 1.20), and CH_3-9″ (δ_H 1.20) to C-7″ (▶ **Fig. 2**). Based on these data, compound **1** was identified as 3-(7-hydroxy-3,7-dimethyloct-2-en-1-yl)-1-(2-methylpropanoyl)-phloroglucinol and named isohyperjovinol A.

Compound **2** was isolated as a yellow oil with the molecular formula $C_{21}H_{32}O_5$, established by HRESIMS ($[M + H]^+$ *m/z* 365.2328, calcd. 325.2323). The ^1H and ^{13}C NMR data were similar to those of compound **1** but showed signals of additional methylene (δ_H 1.84, 1.40, δc 26.9), which was correlated with the methine at δ_H 3.73 (*pseudo* sext, H-2′) and the methyl at δ_H 0.91 (t, H-5′) in the ^1H-^1H COSY spectrum, thereby revealing the presence of a different side-chain at C-1. The HMBC correlations (▶ **Fig. 2**) were in good agreement with the presence of a 2-methylbutanoyl side chain. Compound **2**, named 3′-methyl-isohyperjovinol A, was thus assigned the structure 3-(7-hydroxy-3,7-dimethyloct-2-en-1-yl)-1-(2-methylbutanoyl)-phloroglucinol.

PAPs are chemotaxonomic markers of the genus *Hypericum* [30]. The majority of the isolated PAPs (compounds **3, 4, 6, 8, 9, 10, 12,** and **13**) have been previously described in 2 further species of the section *Coridium, H. empetrifolium,* and *H. amblyocalyx* [3–5], while compound **11** has been found in *H. roeperianum* G. W. Schimp. ex A. Rich [20]. Compound **6** has been only reported from *Garcinia dauphinensis* P. W. Sweeney & Z. S. Rogers [18] and is described herein for the first time in the genus *Hypericum*. When



▶ **Fig. 2** Key ^1H–^1H COSY and HMBC correlations of compounds **1** and **2**.

considering the investigation of *H. jovis* by Athanasas et al. [16], only compounds **3, 5, 8,** and **10** were previously reported, while the rest of the PAPs are described herein for the first time in *H. jovis*. In addition, the common lignan (−)-hinokinin (**14**) isolated from several plant species, has never been described before from the genus *Hypericum* [21]. The xanthone oliganthin K (**15**) has been described from *Garcinia oligantha* Merr. [22], while similar structures have been isolated from some *Hypericum* spp. [31]. It is noteworthy, that the genus *Garcinia* L. belongs to the Clusiaceae family (*sensu stricto*), which according to a former classification system was also including *Hypericum* spp. (Clusiaceae *sensu lato*, [32]). The isolated flavonoid derivatives, such as compounds **16** and **17**, are characteristic metabolites of the genus *Hypericum*, except the rare quercetin 3-O-(2-O-acetyl)-arabinofuranoside (**18**), which has been isolated twice in the plant kingdom, from *Tibouchina semidecandra* Cogn. (Melastomataceae) [24] and *Berchemia floribunda* (Wall.) Brongn. (Rhamnaceae) [33]. Its NMR data in CD_3OD are reported here for the first time for future comparison (**Table 20S**, Supporting Information).

Twelve PAPs were tested *in vitro* for the reduction of TNF-α induced ICAM-1 expression. Based on the results of the cytotoxicity assay on the HMEC-1 cells (viability 90–110%, see **Fig. 16S**, Supporting Information), the PAPs were tested in a range of concentrations from 0.5 to 50.0 µM (▶ **Fig. 3**). Hyperforin dicyclohexylammonium salt was also tested for comparison reasons. The natural ICAM-1 expression of the HMEC-1 cells was determined in the control group and was about 2%. The sesquiterpene lactone parthenolide (positive control, 5 µM) was able to reduce the total expression of ICAM-1 by around ⅔. Compounds **1, 3, 4**, and **8** were tested at concentrations of 6.25, 12.5, 25.0, and 50.0 µM and compound **10** at concentrations of 5.00, 6.25, 12.5, and 25.0 µM (▶ **Fig. 3**). The ICAM-1 expression was significantly reduced in a concentration-dependent manner by compounds **3, 5,** and **8** (IC_{50} values 16.9, 34.4, and 3.2 µM, respectively). Compounds **3** and **4** have been previously reported to possess important anti-inflammatory activities [10]. Compounds **1** and **10** also significantly reduced ICAM-1 expression, however not in a dose-dependent manner (▶ **Fig. 3**, Supporting Information). Compounds **2, 9**, and **11** were found to possess moderate ICAM-1 inhibitory potential

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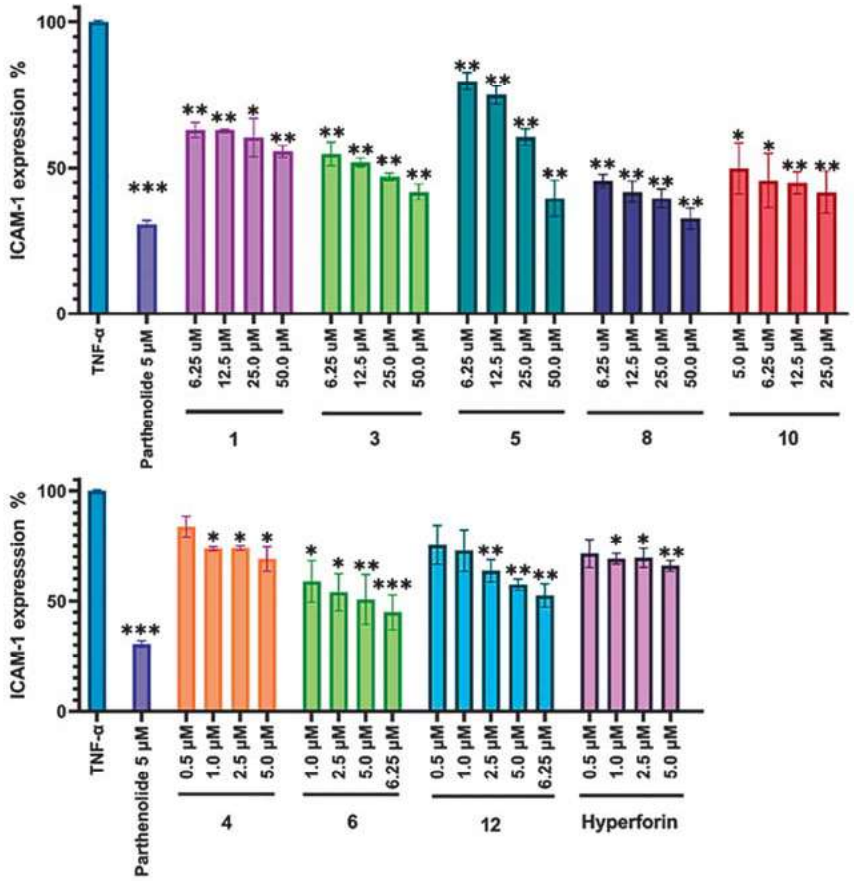

▶ **Fig. 3** ICAM-1 expression (%) in HMEC-1 cell line is expressed as means ± SD. Data are representative of 3 independent experiments with similar results. Significance levels were set at $p < 0.5^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$ vs. TNF-α, as determined by paired sample t-test. TNF-α (10 ng/mL), parthenolide (5 μM, positive control) and different concentrations of compounds **1, 3, 5, 8** (6.25, 12.5, 25.0 and 50.0 μM) and compound **10** (5.0, 6.25, 12.5 and 25.0 μM), as well as compound **4** and hyperforin (0.5, 1.0, 2.5, 5.0 μM) and compounds **6, 12** (0.5, 1.0, 2.5, 5.0 and 6.25 μM), based on the viability assay on the HMEC-1 cells.

(see **Fig. 17S**, Supporting Information). Compounds **4, 6, 12,** and hyperforin were tested at concentrations of 0.50, 1.00, 2.50, 5.00 μM (and additionally 6.25 μM for **6** and **12**) (▶ **Fig. 3**), as higher concentrations proved to have toxic effects or increase the metabolic activity of HMEC-1 cells (see **Fig. 16S**, Supporting Information). Interestingly, **6** and **12** were particularly effective in the reduction of ICAM-1 expression with IC_{50} values of 4.0 and 7.7 μM, respectively. In comparison to hyperforin, which was able to reduce the ICAM-1 expression to around 60 % at all tested concentrations, **6** and **12** showed a concentration-dependent reduction, with 4.9 ± 7.9 and 52.5 ± 5.3 % expression, respectively, at 6.25 μM, the highest concentration tested.

Based on these results, 4 PAPs, **3, 5, 8**, and **12,** were selected to be further tested for their anti-inflammatory potential in an *ex vivo* COX-1/12-LOX assay. Selected PAPs were tested in a concentration range from 0.25 to 2 μM. IC_{50} values are listed in ▶ **Table 1**. All investigated PAPs showed inhibition activity regarding the formation of at least 1 COX-1 or 12-LOX-derived product (see **Fig. S19**, Supporting Information). Compounds **5, 8,** and **12** inhibited the activity of 12-LOX with similar potency and were also active regarding the inhibition of the production of different COX-1-derived products. Compound **3** efficiently inhibited the formation of TXB_2, while showing no activity regarding the inhibition of production of other COX-1 or 12-LOX-derived products. Taken together, compound **12** was the most active in the applied COX-1/12-LOX assay.

Overall, the isolated PAPs from *H. jovis* showed promising results as anti-inflammatory agents through pathways involving the ICAM-1 production and/or COX-1/12-LOX. Further *in vitro* as well as *in vivo* studies are warranted to unveil the anti-inflammatory potential of acylphloroglucinols from the genus *Hypericum*.

Material and Methods

General experimental procedures

Optical rotations were obtained on a Unipol L 1000 polarimeter (Schmidt + Haensch GmbH & Co.). UV-spectra were recorded on a Cary 50 Scan UV-spectrophotometer (Varian Deutschland GmbH). CD spectra were measured on a J-715 spectropolarimeter

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▶ **Table 1** Anti-COX-1/LOX-12 activities of examined and standard compounds.

IC_{50} values (μM)	Test compounds				Positive controls	
	3	5	8	12	aspirin	quercetin
COX-1 pathway						
12-HHT	na.	na.	< 0.25	na.	27.64 ± 2.43[a]	74.28 ± 7.01[b]
TXB_2	0.84 ± 0.056[b]	> 2	na.	0.36 ± 0.013[a]	27.64 ± 0.33[c]	117.64 ± 8.17[d]
PGE_2	na.	> 2	> 2	1.56 ± 0.11[a]	30.97 ± 2.94[b]	42.18 ± 0.86[c]
12-LOX pathway						
12-HETE	na.	< 0.25	0.27 ± 0.009[a]	< 0.25	na.	24.61 ± 2.15[b]

Values are means ± SD of 3 measurements. Means within each row with different letters ([a–d]) differ significantly ($p < 0.05$); na. 50% inhibition not achieved

(JASCO). NMR spectra were measured in an AVANCE III 600 instrument equipped with a 5 mm TBI CryoProbe (^{1}H-NMR 600.25 MHz, ^{13}C-NMR 150.95 MHz) or a Bruker DRX 400 (^{1}H-NMR 400 MHz, Bruker BioSpin GmbH) at 298 K. HRESIMS spectra were obtained with an Agilent MS Q-TOF 6540 spectrometer. Flash chromatography was performed using a Spot Flash Liquid Chromatography system (Interchim), with an SVP D40 silica gel column (13 × 4 cm, SI60 15–40 μm, 90 g, Götec Labortechnik GmbH). Semi-prep HPLC was conducted on an Agilent 1290 Infinity system (flow rate 21 mL/min; detection at 210 and 290 nm), using an XDB-C_{18} PrepHT column (21.2 × 250 mm, 5 μm, Agilent), or a Jasco PU-2080 system (flow rate 1 mL/min) equipped with a RI detector (Shimadzu 10A), using a Kromasil 100si Semi-prep C_{18} column (10 × 250 mm, 5 μm, Nouryon). Column chromatography (CC) was performed on Diaion HP-20 (250–850 μm; Supelco), silica gel (230–400 mesh, Merck), and Sephadex LH-20 (Sigma-Aldrich). Thin-layer chromatography (pTLC) was performed on silica gel (Merck) and cellulose (Merck).

Plant material

Aerial parts from *H. jovis* were collected from natural populations in the island of Crete (Greece) during the flowering stage at Kamaron gorge, Crete, on 22 June 2017. The collected plant materials were authenticated by Prof. Z. Kypriotakis (Department of Agriculture, Hellenic Mediterranean University). A voucher specimen was deposited at the Herbarium of Natural History Museum, University of Crete (voucher no. 15880).

Extraction and isolation

Air-dried and comminuted aerial parts (0.5 kg) were extracted by maceration at room temperature with cyclohexane, dichloromethane, and MeOH (each solvent 2 L 3 times), successively. The cyclohexane extract (JOC, 47.2 g) was washed with MeOH at 0 °C. The supernatant layer was collected giving, after removal of the solvent under reduced pressure, a residue (3.1 g), which was further defatted by CC on Diaion HP-20 eluted with MeOH 90%, MeOH 100%, and *n*-hexane 100% to yield 3 fractions ($JOC_{1–3}$). Fraction JOC_1 (0.9 g) was further fractionated in 4 portions by flash chromatography on an SVP D40 silica gel column with a gradient of *n*-hexane-EtOAc (0–5 min 100% *n*-hexane, 5–55 min 100–0% *n*-hexane, 55–75 min 100% EtOAc) at a flow rate 30 mL/min. Fractions (20 mL) were combined based on TLC analysis to give a total of 7 fractions ($JOC_{1(1–7)}$). The nonsoluble residue (MeCN) of fraction 5 ($JOC_{1(5)}$) was identified as **22** (12.9 mg). The soluble part of fraction 5 ($JOC_{1(5)}$) (159.5 mg) was subjected to RP_{18}-HPLC with a H_2O-MeCN gradient (40% MeCN 0–2 min; 40% → 70% MeCN 2–15 min; 70% → 90% MeCN 15–25 min; 90% → 125% MeCN 25–36 min) to yield compounds **7** (3.5 mg; t_R = 15.9 min) and **15** (5.0 mg; t_R = 29.4 min). Fraction 7 ($JOC_{1(7)}$) was identified as **23** (10.3 mg).

The dichloromethane extract (JOD, 27.3 g) was subjected to open CC on Diaion HP-20 (eluted with MeOH 90%, MeOH 100%, and *n*-hexane 100% to yield 5 fractions ($JOD_{1–5}$). Fraction JOD_1 (6.3 g; eluted with MeOH 90%) was separated in 4 portions by flash chromatography using the system and the method described for JOC_1. Fractions (20 mL) were combined based on TLC analysis to give a total of 6 fractions ($JOD_{1(1–6)}$). Part of $JOD_{1(4)}$ (350.6 mg) subjected to further purification by RP_{18}-HPLC using a H_2O-MeCN gradient (40% MeCN 0–2 min; 40% → 70% MeCN 2–15 min; 70% → 90% MeCN 15–25 min; 90% → 100% MeCN 25–36 min) yielded compounds **5** (7.8 mg; t_R 11.9 min), **6** (2.3 mg; t_R 15.0 min), **11** (3.9 mg; t_R 18.1 min), **3** (37.0 mg; t_R 22.3 min), and **4** (4.9 mg; t_R 25.5). Fraction $JOD_{1(5)}$ (130.0 mg) was subjected to RP_{18}-HPLC using H_2O-MeCN gradient (10% MeCN 0–2 min; 10% → 90% MeCN 2–28 min; 90% MeCN 28–33) to yield an additional amount of **3** (3.3 mg; t_R 30.1 min). Fraction 4 ($JOD_{1(5(4))}$) (t_R 24–17 min; 70.2 mg) was subjected to further purification by RP_{18}-HPLC using a H_2O-MeCN gradient (50% MeCN 0–22 min; 50–100% MeCN 22–25 min) to give compounds **1** (10.4 mg; t_R 14.6 min), **2** (1.5 mg; t_R 20.7 min), and **5** (1.6 mg; t_R 23.2). Fraction JOD_3 was subjected to flash chromatography as described above to give 5 fractions ($JOD_{3(1–5)}$). Part of $JOD_{3(1)}$ (205.7 mg) was further purified by 2 successive steps of RP_{18}-HPLC using a H_2O-MeCN gradient (40% → 80% MeCN 0–9 min; 80% MeCN [isocratic] 9–18 min; 80% → 100% MeCN 18–20 min) to yield compounds **8** (25.5 mg; t_R 19.6 min), **10** (16.2 mg; t_R 20.8 min), **12** (11.5 mg; 21.5 min), **9** (9.5 mg; t_R 22.6 min), and **13** (4.0 mg; t_R 24.8 min). $JOD_{3(2)}$ was subjected to RP_{18}-HPLC using a H_2O-MeCN gradient (40% → 90% MeCN 0–21 min; 90–100% MeCN 22–26 min) to afford compounds **14** (1.5 mg; t_R 4.3 min), **3** (4.5 mg; t_R 22.1 min), and **4** (2.5 mg; t_R 23.6 min).

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The MeOH extract (JOM, 11.5 g) was subjected to CC (30 cm × 4 cm) on Sephadex LH-20 (MeOH). A total of 148 fractions were collected which were combined into 20 fractions ($JOM_{1–20}$). Fraction, JOM_6 yielded a further amount of **3** (15.6 mg), JOM_{11} gave **17** (1.3 mg), and JOM_{13} afforded **21** (5.2 mg). JOM_9 (2.8 g) was subjected to CC (10.5 × 3.5 cm) on silica gel. A total of 85 fractions were collected which were combined into 18 fractions ($JOM_{9\ [1–18]}$) to yield compounds **18** ($JOD_{9(6)}$, 1.9 mg), **16** ($JOD_{9(12)}$, 2.1 mg), **19,** and **20** ($JOD_{9(13)}$, 2.1 mg). Separation of $JOD_{9(5)}$ (11.9 mg) by pTLC (cellulose, eluted with 30% AcOH) yielded compound **24** (1.0 mg).

Isohyperjovinol A (**1**): yellow oil; UV (MeOH) λ_{max} (log ε): 292 (6.71), 228 (6.60); ^1H and ^{13}C-NMR data ($CDCl_3$, 600 and 150 MHz, respectively) see ▶ **Table 2**; HRESIMS *m/z* 351.2172 $[M + H]^+$ (calcd. for $C_{20}H_{31}O_5^+$, 351.2166).

3′-Methyl-isohyperjovinol A (**2**): yellow oil; UV (MeOH) λ_{max} (log ε): 292 (6.73), 228 (6.62); $[\alpha]_D^{25}$ + 10 (c 0.07, MeOH); CD: See **Fig. 18S** (Supporting Information); ^1H and ^{13}C-NMR data ($CDCl_3$, 600 and 150 MHz, respectively) see ▶ **Table 1**; HRESIMS *m/z* 365.2328 $[M + H]^+$ (calcd. for $C_{21}H_{33}O_5^+$, 325.2323).

▶ **Table 2** ^1H and ^{13}C NMR Data of compounds **1** and **2** in $CDCl_3$.

position	1[a] δ_H	1[b] δ_C	2[a] δ_H	2[b] δ_C
1	–	104.0	–	104.6
2	–	162.8	–	162.8*
3	–	105.8	–	105.8
4	–	160.9	–	160.7
5	5.85, s	95.2	5.84, s	95.3
6	–	160.9	–	160.7
1′	–	210.8	–	210.3
2′	3.88, sept (6.4)	39.8	3.73, *pseudo* sext (6.6)	45.9
3′a	1.16, d (6.4)	19.2	1.84, m	26.9
3′b	–		1.40, m	
4′	1.16, d (6.4)	19.2	1.15, d (6.6)	16.7
5′			0.91, t (7.7)	11.9
1″a	3.35, d (6.9)	21.4	3.37, d (6.9)	21.5
1″b				
2″	5.24, t (6.9)	122.1	5.26, t (6.9)	121.7
3″	–	138.8	–	139.4
4″	2.05, m	39.2	2.05, m	39.9
5″	1.49, m	22.3	1.49, m	22.3
6″	1.43, m	43.1	1.41, m	43.2
7″	–	71.6	–	71.1
8″	1.20, s	29.1	1.20, s	29.2
9″	1.20, s	29.1	1.20, s	29.2
10″	1.80, s	16.1	1.81, s	16.2

[a] Recorded at 600 MHz′; [b] Recorded at 150 MHz; * extracted from the HMBC spectrum.

MTT viability test

HMEC-1 [34] passage 3 were subcultured in 40 mL (25 cm^2) flasks using EASY Endothelial Cell Growth Medium (ECGM, PELOBiotech), supplemented with EASY Growth supplement and Gentamycin-Amphotericin (PELOBiotech) and 10% FCS Superior (Merck), in an incubator (IBS Integra Biosciences) at 37 °C, 5% CO_2 and 90% relative humidity. The cells were used between passage 2–11 in a final cell suspension of 1.8×10^6 cells/mL and placed in the 96-wells (50 µL), and 24 h later they were treated with 6.25, 12.5, 25.0, 50.0 µM (50 µL) of the test PAPs (compounds **1–6, 8–12,** and hyperforin). After further incubation for 24 h, MTT (10 µL, 4 mg/mL) was added and after 3 h the solution was exchanged with 10% SDS. The plates were then placed in the dark, and after 24 h the absorbance at 560 nm was measured with a SpectraFluor plus plate reader (Tecan). Cell viability was calculated as a percentage compared to the average absorbance of the negative control group (only ECGM).

ICAM-1 assay

The ICAM-1 assay was performed as described before [35]. Confluent grown HMEC-1 cells from a culture flask (150 cm^2) were split (1 : 3), suspended in 13 mL medium, and seeded in a 24 well plate (500 µL/well; plates were treated first with 0.25% v/v Collagen G in PBS [both Sigma-Aldrich]). They were cultivated for 48 h at 37 °C, 5% CO_2, and 90% relative humidity until they formed a monolayer. Subsequently, the supernatant was removed and the HMEC-1 cells were treated with the compounds (**1–6, 8–12,** and hyperforin) at not toxic concentrations based on the MTT results (see **Fig. 16S**, Supporting Information), parthenolide (5 µM, positive control, Calbiochem), and medium (ECGM, negative control). After 30 min, TNF-α (10 ng/mL, Sigma Aldrich) was added (except for 2 wells, in which no TNF-α was added to evaluate the ICAM-1 production in normal cells), and the plates were incubated at 37 °C, 5% CO_2, and 90% relative humidity for 24 h. Then, the cells were washed with PBS, removed from the plates with trypsin/EDTA, and fixed with formalin. After centrifugation and removal of the supernatant layer, the cells were incubated with 5 µL of the Mouse Anti-human CD54: FITC (Bio-Rad) for 20 min, followed by fluorescence-activated cell sorting (FACS) analysis (Becton Dickinson FACSCalibur).

COX-1/12-LOX assay

Compounds **3, 5, 8,** and **12** were tested in a COX-1/12-LOX assay using human platelets, according to [36]. The method is based on the inhibition of the biosynthesis of eicosanoids, such as 12(S)-hydroxy(5Z,8E,10E)-heptadecatrienoic acid (12-HHT), thromboxane B_2 (TXB_2), prostaglandin E_2 (PGE_2), and 12(S)-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenoic acid (12-HETE). The tested concentrations of the samples in the test tubes ranged from 0.25 to 2 µM.

In brief, an aliquot of human platelets (source of COX-1 and 12-LOX enzymes), viable but outdated for medical treatment, which contained 4×10^8 cells, was suspended in buffer (0.137 mol/L NaCl, 2.7 mmol/L KCl, 2.0 mmol/L KH_2PO_4, 5.0 mmol/L Na_2HPO_4,

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and 5.0 mmol/L glucose, pH 7.2) to obtain the final volume of 2 mL. The mixture was slowly mixed at 37 °C for 5 min. Then, 0.1 mL of isolated compounds, or positive controls (aspirin and quercetin,) or DMSO (control and blank probes for control), and 0.1 mL of calcimycin (Calcium Ionophore A23187, 125 µmol/L in DMSO) were added and the mixtures were incubated for 2 min at 37 °C, with moderate shaking. Afterward, 0.3 mL of $CaCl_2$ aqueous solution (16.7 mmol/L), substituted with distilled water in the blank probe and blank probe for the control, were added, and the mixture was incubated for another 5 min at 37 °C with shaking. Acidification with cold 1 % aqueous formic acid (5.8 mL) to pH 3 terminated the reaction. The internal standard prostaglandin B_2 (PGB_2; 50 µL of 6 µg/mL solution in DMSO) was added to mixtures and extraction of products was done with a solution of chloroform/methanol (1 : 1 v/v, 8.0 mL) with vigorous vortexing for 15 min. After centrifugation (7012 g, 15 min, 4 °C), the organic layer was separated, evaporated to dryness, dissolved in methanol (0.5 mL), filtered, and used for LC-MS/MS analysis of eicosanoids (12-HHT, TXB_2, PGE_2, and 12-HETE). All samples and controls were analyzed in triplicate. Levels of COX-1 and 12-LOX inhibition achieved by different samples concentrations were calculated by using the following equation: $I(\%) = 100 \times (R_0 - R)/R_0$, where R_0 and R were response ratios (metabolite peak area/internal standard peak area) in the control reaction and the samples of examined samples, respectively. Both R and R_0 were corrected for the value of appropriate blank probes.

Statistical analysis

Each experiment was performed in sextuplicate for the MTT viability assay, in duplicate for the ICAM-1 assay, and in triplicate for the COX-1/12-LOX assay. All experiments were repeated at least 3 times and the results are presented as means ± SD for each sample. Statistical analysis of all continuous variables was checked for normality. Means of 2 groups were compared with paired samples t-test or 1 sample t-test where applicable. Statistical significance (2-tailed) was set at $p < 0.05$, $p < 0.01$, or $p < 0.001$. Statistical analyses were performed using IBM SPSS statistics 26. GraphPad Prism 8.4.2 version was used for the creation of the graphical representations, as well as the calculations of the IC_{50} values using nonlinear regression.

Supporting Information

NMR spectra for compounds **1, 2**, and **18** (**Fig. 1S–15S**); % viability on HMEC-1 cell line (MTT assay) (**Fig. 16S**); ICAM-1 expression % for compounds **2, 9,** and **11** (**Fig. 17S**); CD spectra of **2** (**Fig. 18S**); Scheme of COX-1 and 12-LOX branches of the arachidonic acid metabolic pathway (**Fig. 19S**); and NMR data of **18** in CD_3OD (**Table 1S**) are provided as Supporting Information.

Contributors' Statement

Concept and design of the work: M. E. G., H. S. and J. H.; data collection (Isolation, NMR, ICAM-1): M. E. G.; statistical analysis: C. B.; COX-1/12-LOX data: D. P. and M. L.; analysis and interpretation of the data: M. E. G., C. B., J. H. and M. L.; supervision: H. S. and H. J.; drafting the manuscript: M. E. G. and C. B.; critical revision of the manuscript: H. S., M. L. and J. H. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Supplementary material

Prenylated Acylphloroglucinols from *Hypericum jovis* with Anti-inflammatory Potential

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* Dedicated to Prof. Dr. Otto Sticher on the occasion of his 85th birthday.

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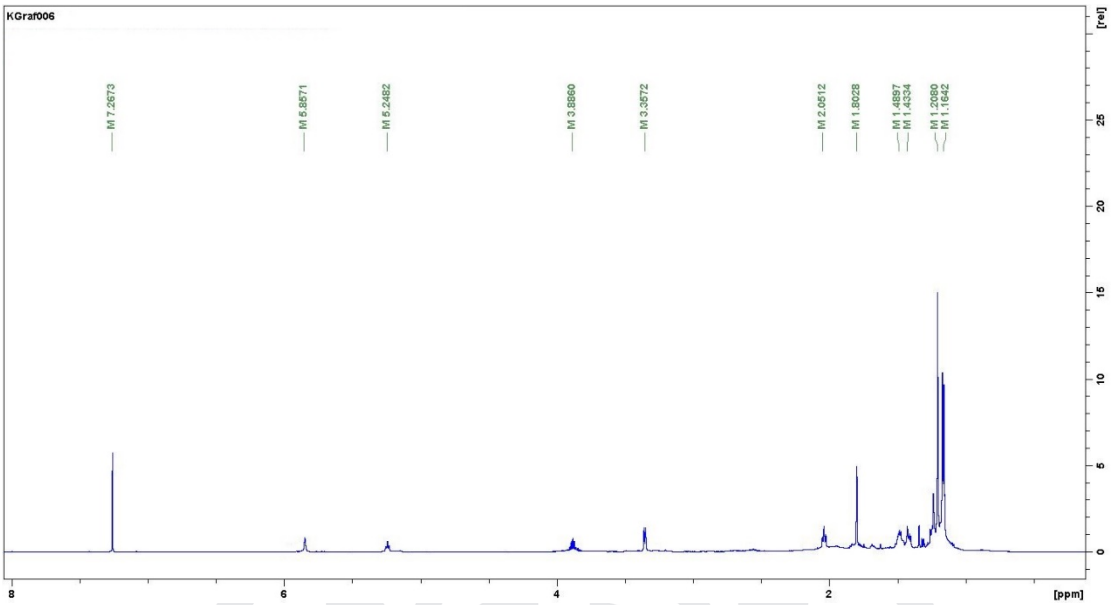
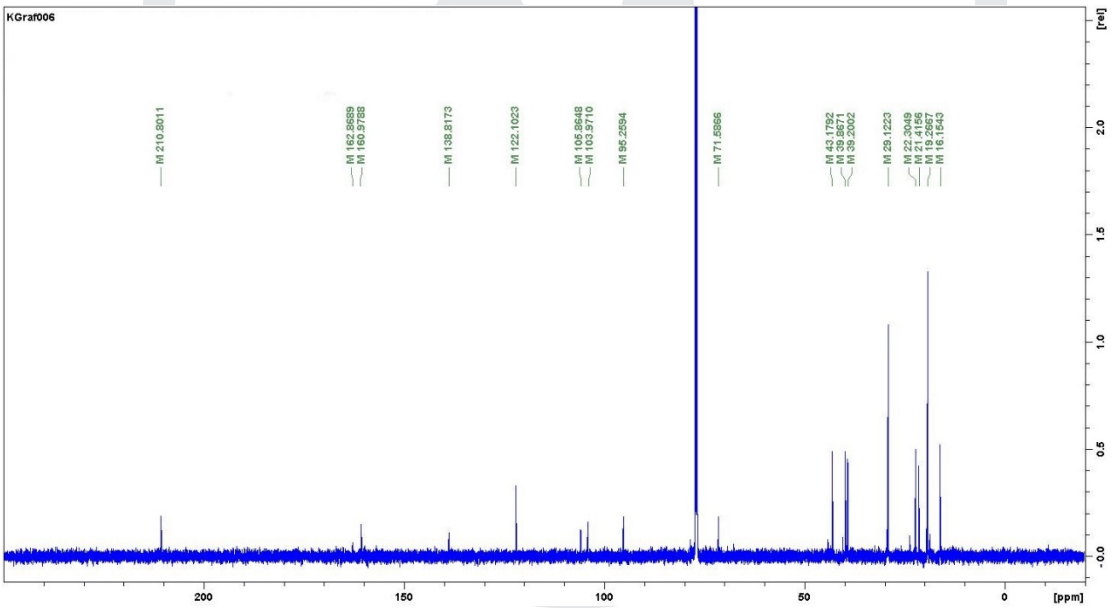


Fig. 1S. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **1.**



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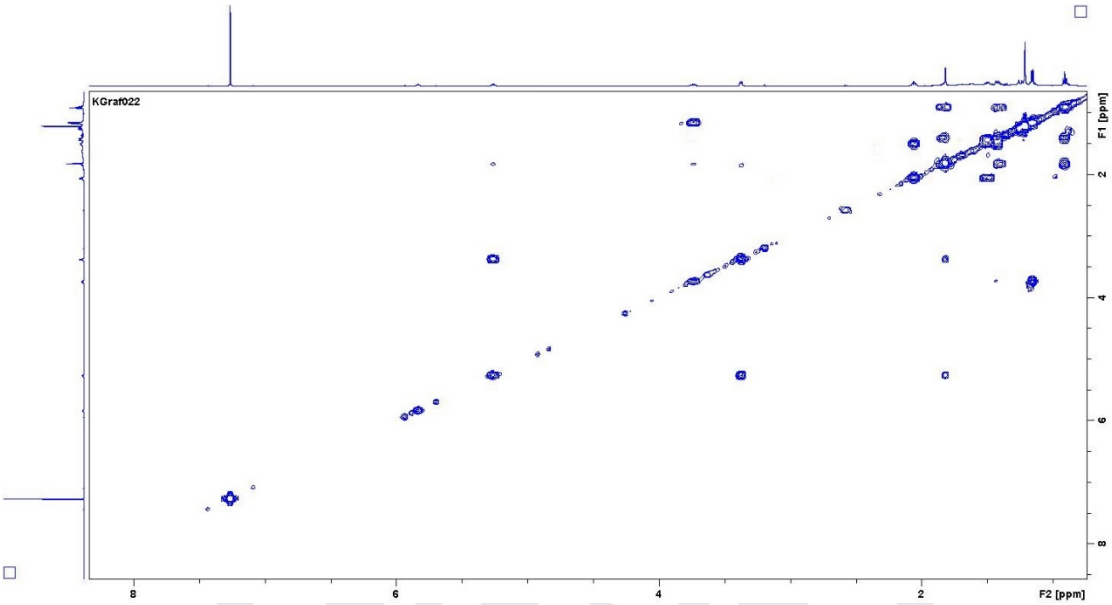

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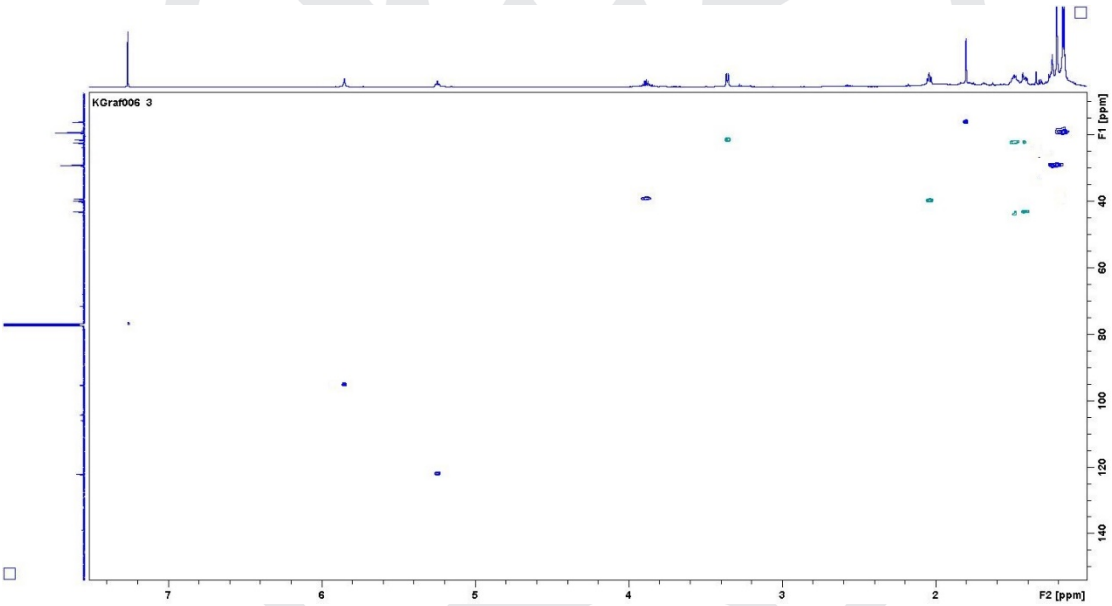

Fig. 4S. HSQC-DEPT spectrum of **1.**

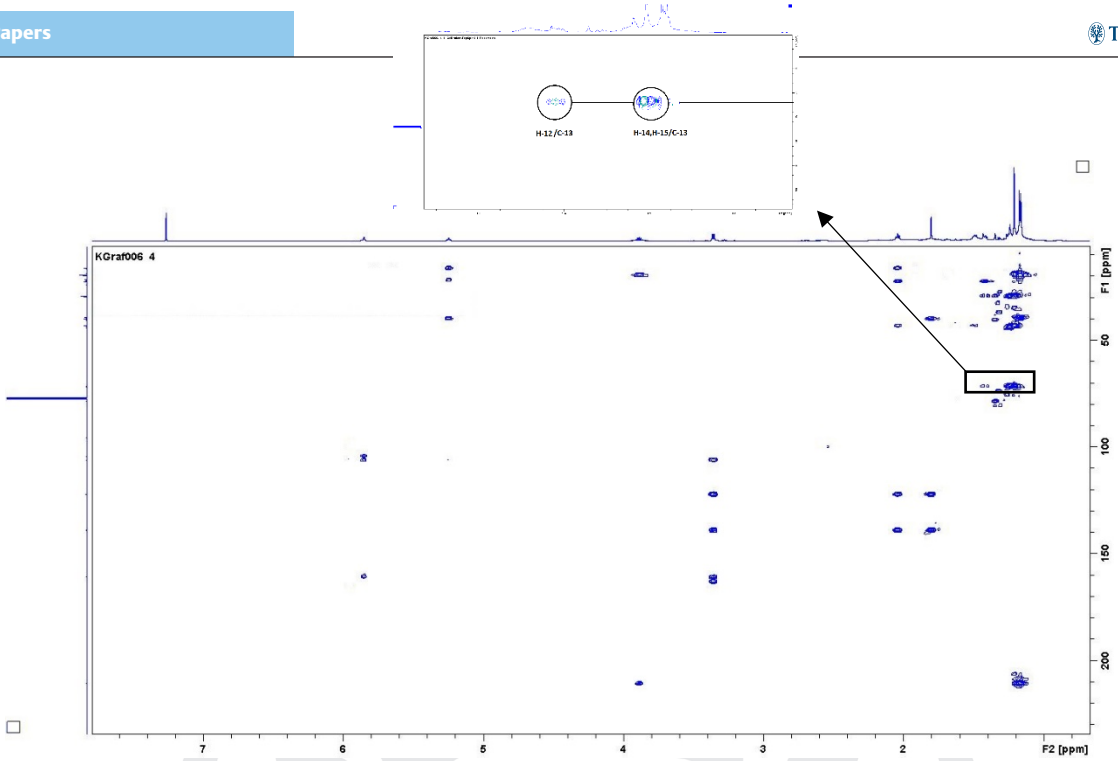

Fig. 5S. HMBC spectrum of **1.**

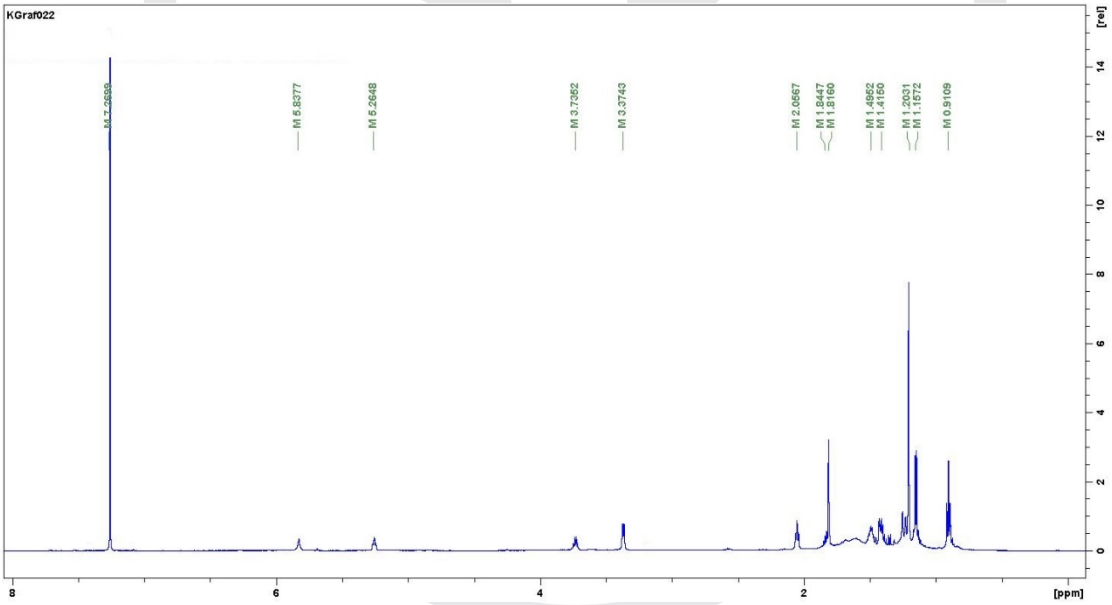

Fig. 6S. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2.**

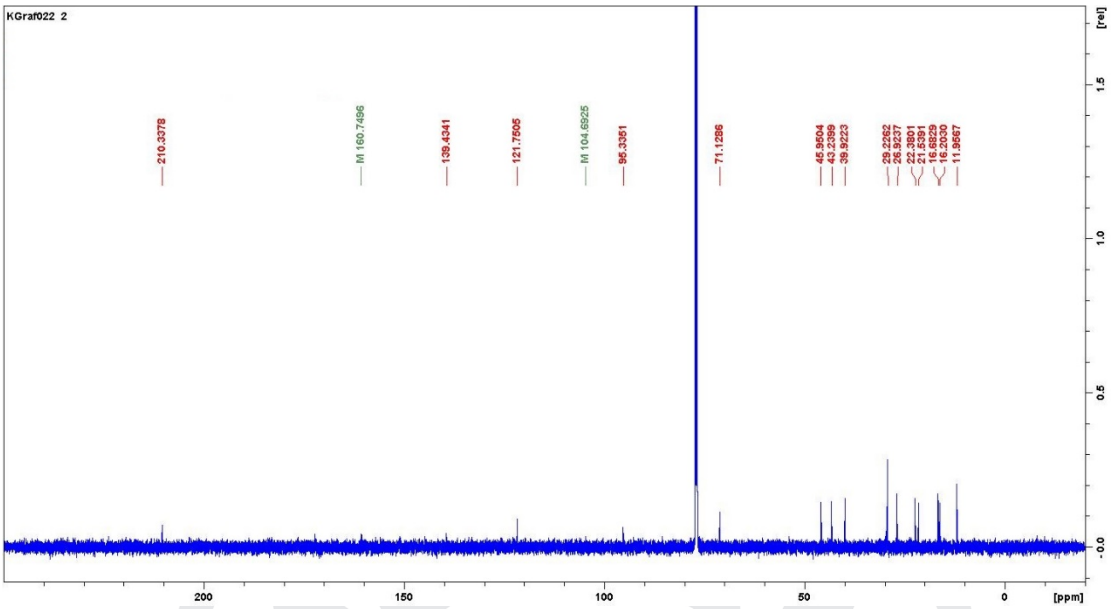

Fig. 7S. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of **2.**

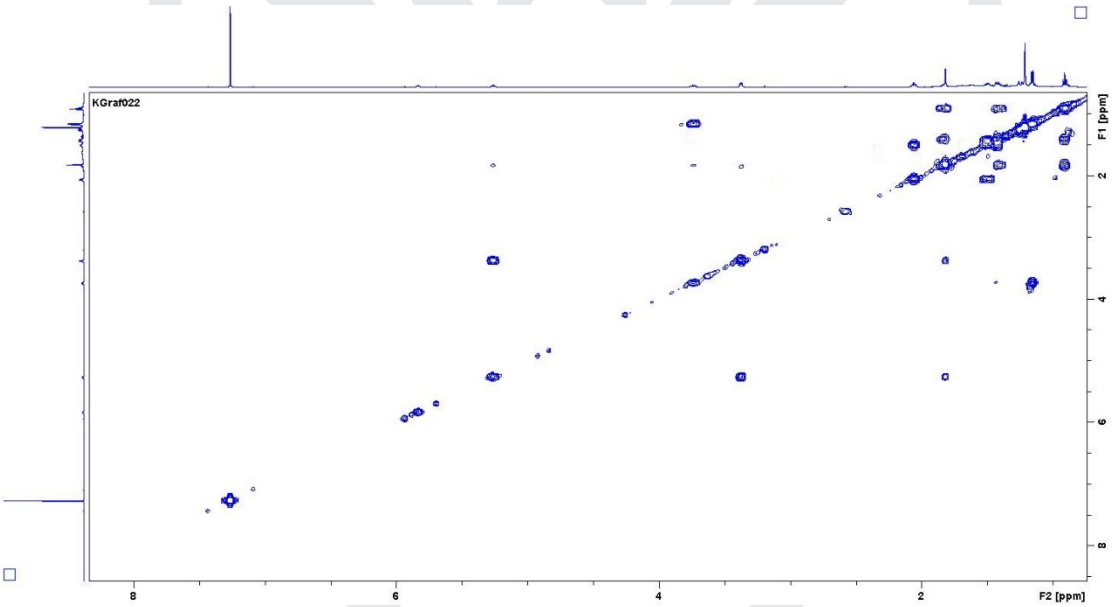

Fig. 8S. ^{1}H-^{1}H COSY NMR (600 MHz, $CDCl_3$) spectrum of **2.**

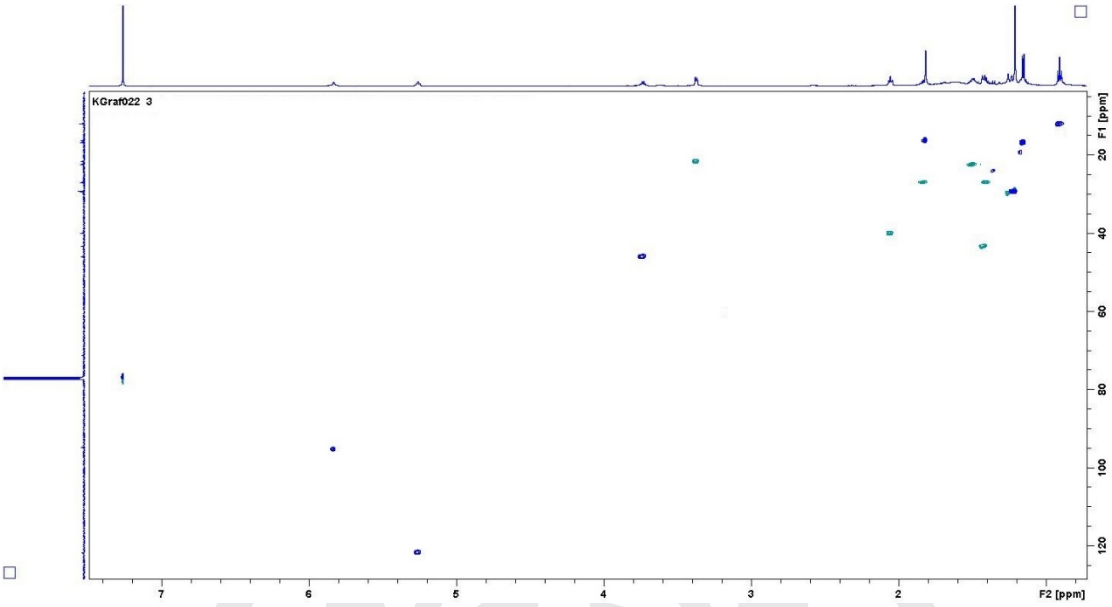

Fig. 9S. HSQC-DEPT spectrum of **2.**

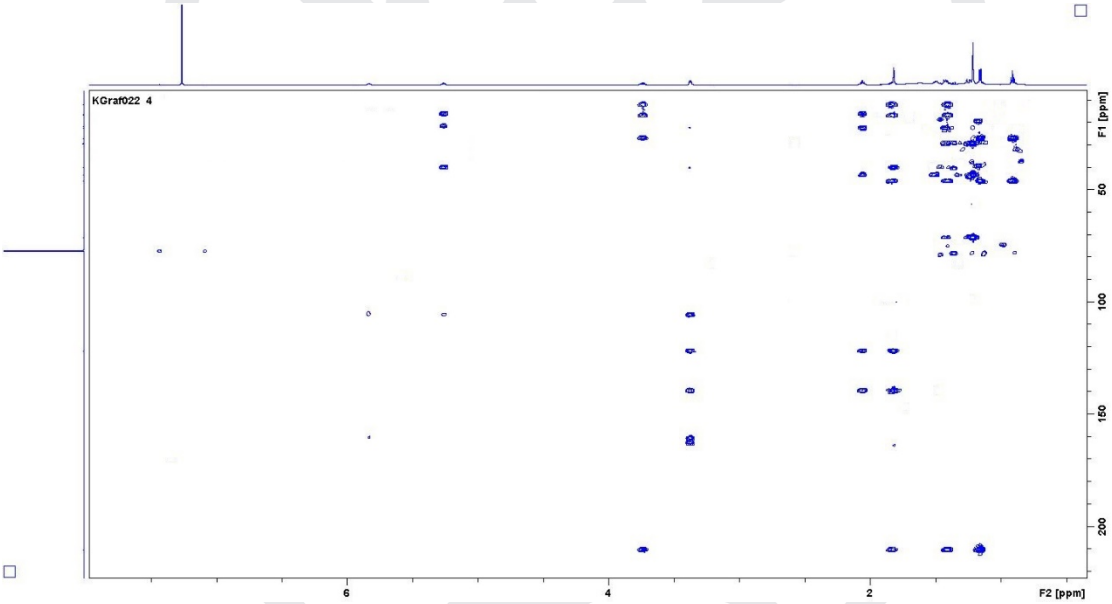

Fig. 10S. HMBC spectrum of **2.**

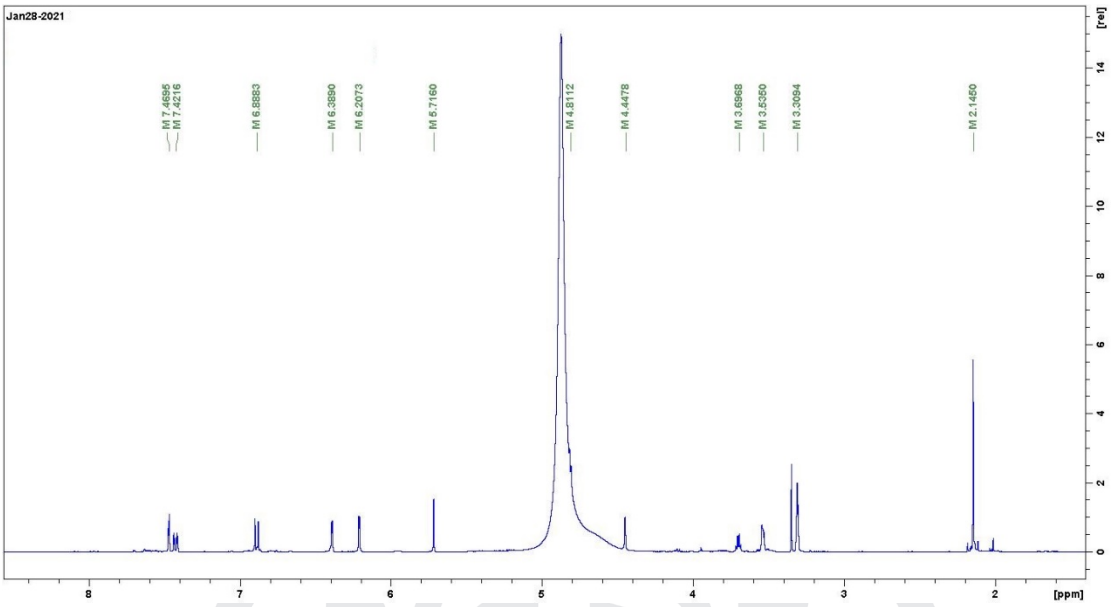

Fig. 11S. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **18.**

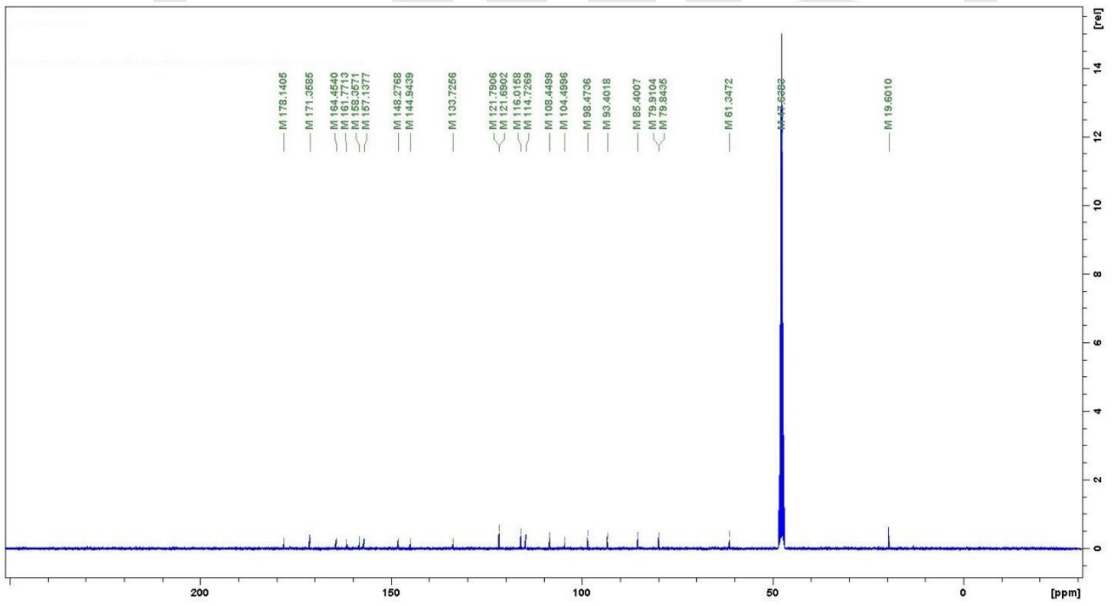

Fig. 12S. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of **18.**

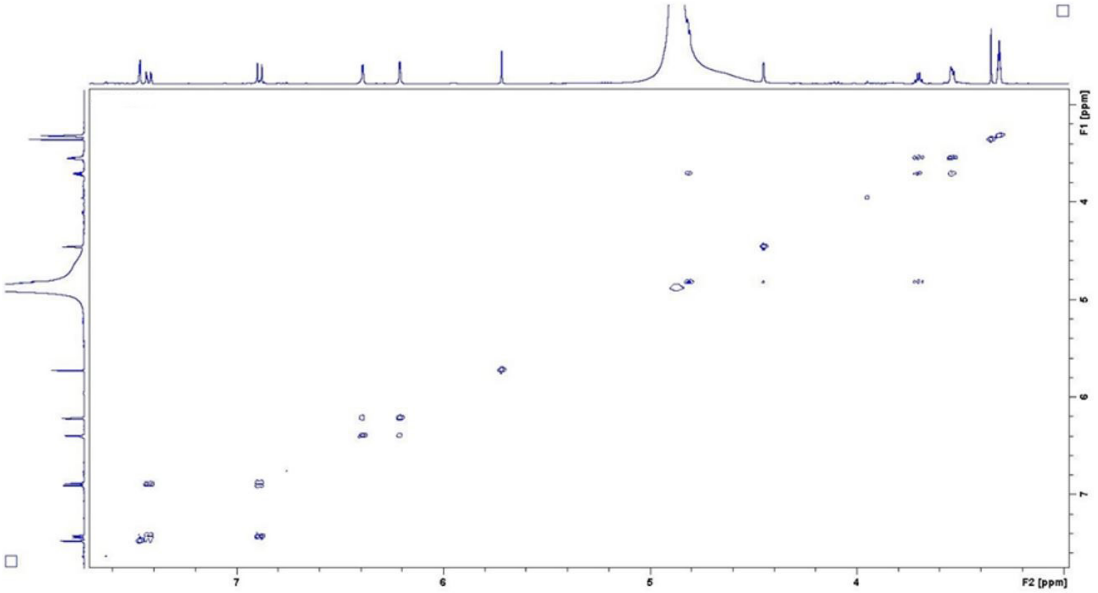

Fig. 13S. ^{1}H-^{1}H COSY NMR (600 MHz, $CDCl_3$) spectrum of **18.**

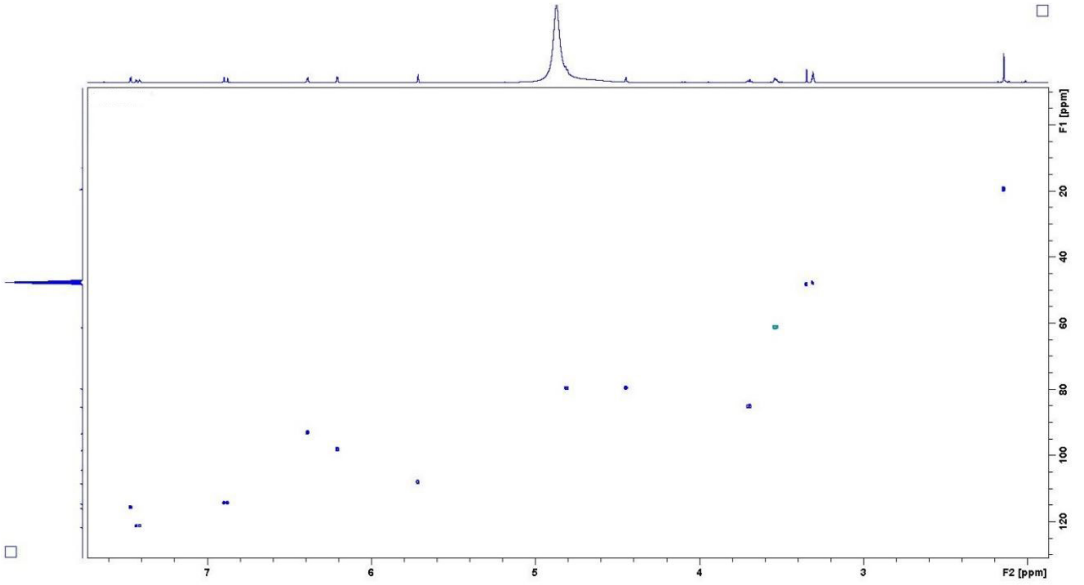

Fig. 14S. HSQC-DEPT spectrum of **18.**

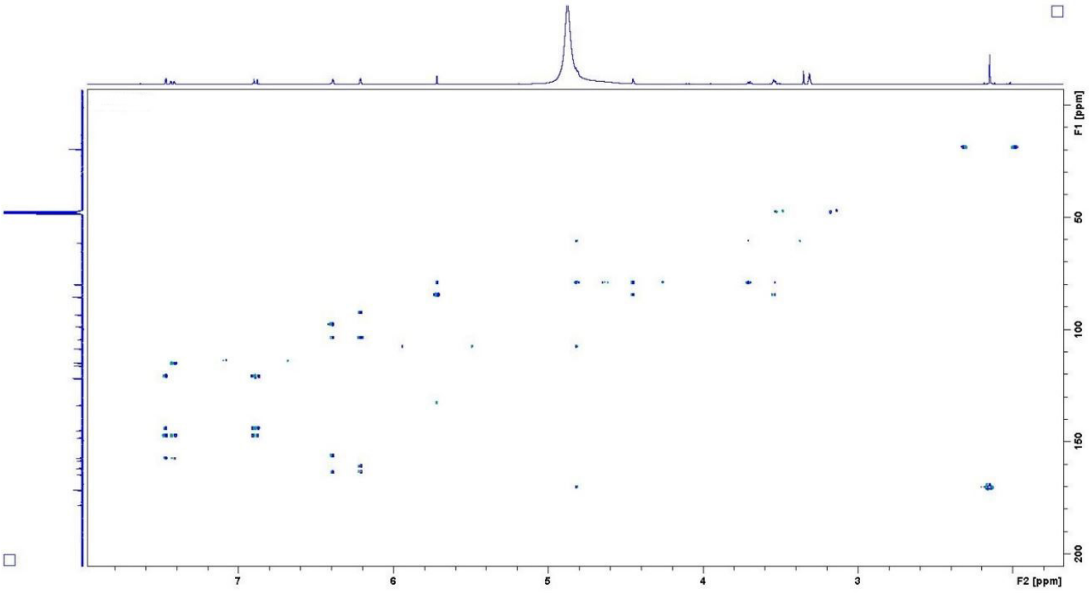

Fig. 15S. HMBC spectrum of **18.**

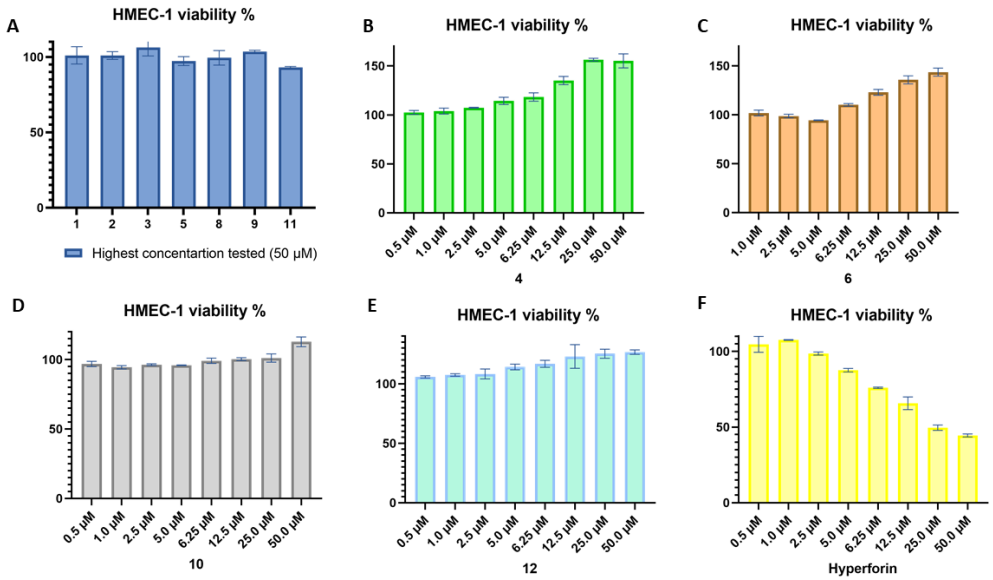

Fig. 16S. % viability on HMEC-1 cell line (MTT assay).

A: No cytotoxicity observed for **1**, **2**, **3**, **5**, **8**, **9**, **11** (presented in the highest concentration tested 50.0 µM); **B–E**: increase the metabolic activity of the HMEC-1 cells for 4, 6, 10, 12; **F**: toxic effects from hyperforin.

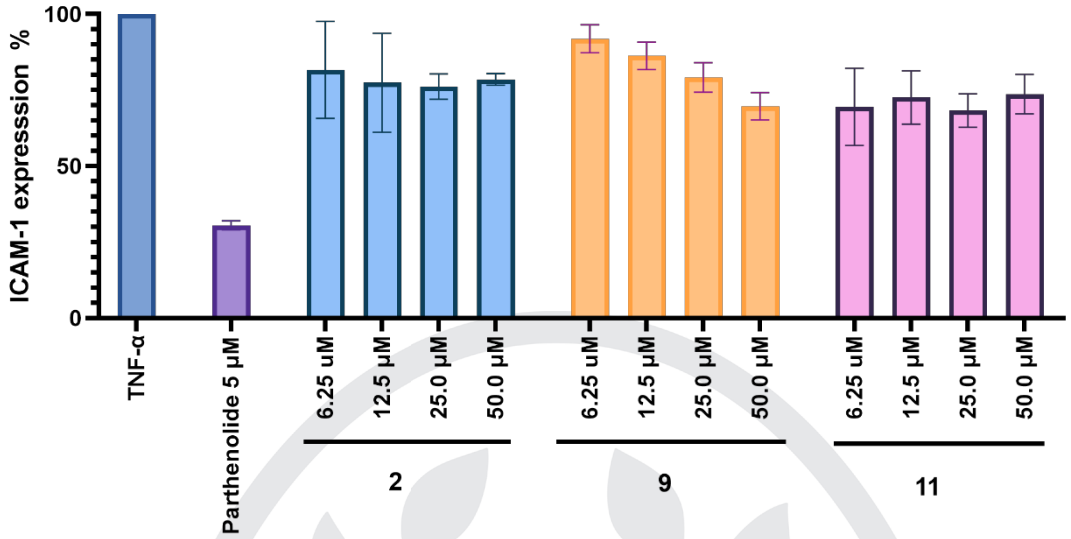

Fig. 17S. ICAM-1 expression % compounds **2**, **9,** and **11.**

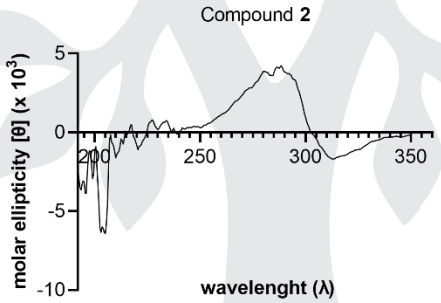

Fig. 18S. CD spectrum of **2.**

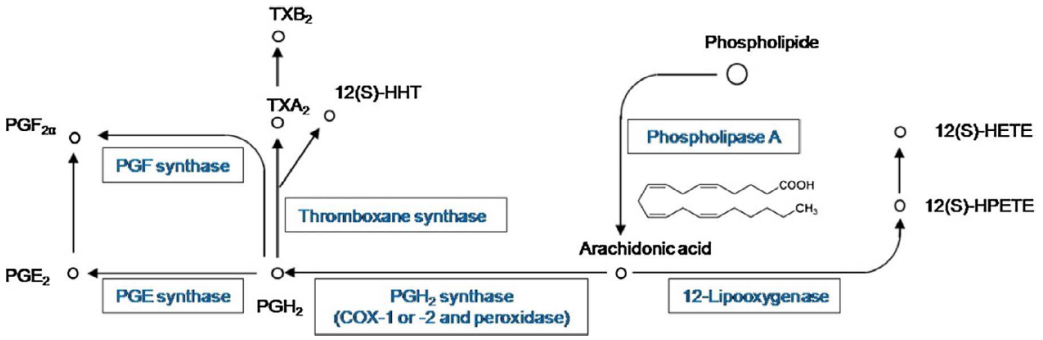

Fig. 19S. COX-1 and 12-LOX branches of the arachidonic acid metabolic pathway. 12-HPETE: 12-hydroperoxyeicosatetraenoic acid; PGH_2, PGE_2, and $PGF_2\alpha$: prostaglandins; TXA_2 and TXB_2: thromboxanes, are inflammation mediators derived from arachidonic acid metabolism, which is catalyzed by enzymes of the inflammatory response, COX-1 and 12-LOX (taken from [36]).

Table 1S. ^{1}H and ^{13}C NMR Data of 18 in CD_3OD

position	δ_{Ha}	δ_{Cb}
2	-	158.3
3	-	133.7
4	-	178.1
5	-	161.7
6	6.20 d (2.0)	98.5
7	-	164.4
8	6.38 d (2.0)	93.4
9	-	157.1
10	-	104.5
1’	-	121.8
2’	7.47 d (2.1)	116.0
3’	-	144.9
4’	-	148.2
5’	6.88 d (8.4)	114.7
6’	7.42 dd (8.4, 2.1)	121.7
arabinoside		
1”	5.71 brs	108.4
2”	4.81 dd (0.6, 4.2)	79.9
3”	4.44 brs	79.8
4	3.69 m	85.4
5”	3.53 m	61.3
Acetyl		
	2.14	19.6
C=0	-	171.3

3.4. *Hypericum* Essential Oils

Figure 29 below presents the GC-MS chromatograms of the essential oils from *H. jovis*, *H. amblyocalyx*, *H. empetrifolium* (Grafakou et al., 2020), followed by the results for wound healing by essential oils from *H. empetrifolium*, *H. triquertifolium* and *H. perforatum* (Grafakou et al., 2021d).

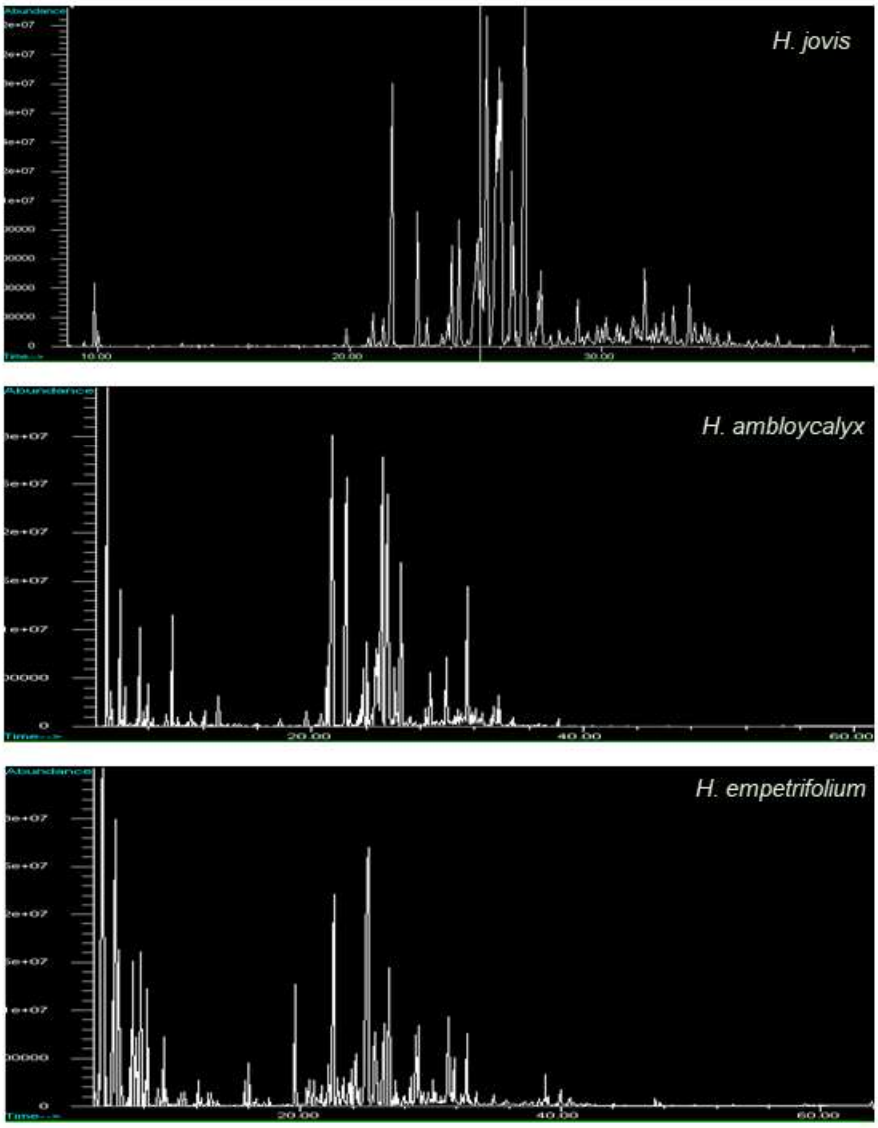

Figure 29. GC-MS chromatograms of the essential oils from H. jovis, H. amblyocalyx, H. empetrifolium.

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Article

Chemical Composition and Antimicrobial Activity of the Essential Oils of Three Closely Related *Hypericum* Species Growing Wild on the Island of Crete, Greece

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Abstract: The volatile compositions of three closely related *Hypericum* species growing wild on the island of Crete were studied, all belonging to the section Coridium. Hydro-distillation in a modified Clevenger-type apparatus was performed according to the Hellenic Pharmacopoeia in order to obtain the essential oils, which were analyzed by GC-MS. Identification of the compounds was carried out by comparison of MS spectra and retention indices with literature data, as well as by co-chromatography with authentic samples. In total, 123 different compounds were identified and the main compounds were by order of their abundance as follows: *H. empetrifolium*: α-pinene, germacrene D, β-pinene, E-caryophyllene; *H. amblycalyx*: β-elemene, β-selinene, α-pinene, E-caryophyllene, α-selinene; *H. jovis*: trans-calamenene, α-selinene, β-elemene. The chemical results revealed the differences and similarities (qualitative and quantitative) between the studied oils, supporting the hypothesis that essential oils from *Hypericum* spp. do not serve as chemotaxonomic markers. Moreover, the essential oils were subjected to antimicrobial screening. According to the given results, the essential oils possessed better antifungal and anticandidal activities than antibacterial activities.

Keywords: *Hypericum*; section Coridium; essential oils; GC-MS; antifungal; anticandidal

1. Introduction

The genus *Hypericum* L. of the family Hypericaceae consists of more than 460 species divided in 36 sections [1] and has a long-term use in traditional medicine. It has been described by Hippocrates [2], Dioscorides [3], and later on in the Medieval era by Nikolaos Myrepsos [4,5]. *H. perforatum* (St. John's Wort) has been used as an ancient folk cure for diseases including wounds, kidney and lung ailments, and depression, while in modern times, its monograph was included in 2009 in the European Pharmacopoeia [6]. *Hypericum* plants are also used in foodstuff, after the evaluation of the concentration of hypericin and xanthone derivatives (Council of Europe, 2000) [7]. Externally, *Hypericum* is a valuable healing and anti-inflammatory remedy for burns, wounds, sores, bruises, and other skin disorders [6].

The genus produces a plethora of bioactive secondary metabolites, mainly phenols, flavonoids, tannins, xanthones, phloroglucinols, naphtodianthrones and essential oil (EO) [8]. Apart from the well-established antidepressant activity, many extracts and substances have become subject of intense studies concerning a variety of biological activities [8]. Although volatile constituents have been reported to contribute to these activities, only few *Hypericum* species have been investigated regarding their essential oil content [9–11]. *Hypericum* is considered to be an essential oil-poor genus [9], and so far studies have revealed that its volatile oil demonstrates antimicrobial, antioxidant, anti-angiogenic, gastroprotective, larvicidal, insecticidal activities [10–14].

In continuation to our work on *Hypericum* spp., we investigated the volatile constituents of three closely related taxa [15], *H. empetrifolium* Willd., *H. amblycalyx* Coustur. and Gand, and *H. jovis* Greuter, which were collected across a relatively restricted geographic range, in the island of Crete (Greece). All three belong to the section Coridium; *H. empetrifolium* is considered the ancestor of *H. amblycalyx* and *H. jovis*, the eastern and western derivatives, respectively [15]. The section Coridium comprises six species. It is worth mentioning that all three species under investigation grow on the island of Crete, while the rest are totally absent from Greece. Therefore, we have undertaken the present study, in order to compare the EOs of the Greek taxa belonging to the section Coridium, and based on the results revealed from those closely related *Hypericum* taxa, examine the hypothesis that essential oils from *Hypericum* taxa serve as chemotaxonomic markers. Regarding the chemotaxonomy, many studies describe volatile compounds as chemotaxonomic markers for the genus *Hypericum*, while Crockett [9] mentioned that statistical analysis of essential oils should not be used to infer phylogeny. The present results corroborate with the latter study, since significant differences (qualitative and quantitative) have been observed. In parallel, a second aim of the present study was the investigation of the antimicrobial activity from the obtained EOs, against a variety of fungi and bacteria. This is the first report of the chemical composition of the essential oils obtained from *H. amblycalyx* and *H. jovis*, as well as the first report of the antimicrobial activity of all three *Hypericum* taxa.

2. Materials and Methods

2.1. Plant Material

Aerial parts from the three *Hypericum* taxa under-investigation were collected from natural populations in the island of Crete (Greece), during the flowering stage. The plants were collected and identified by Dr. Z. Kypriotakis and voucher specimens of each population were deposited in the Personal Herbarium of Dr. Kypriotakis (Heraklion-Crete). In Table 1, collection sites on the island of Crete, dates of collection and essential oil yield are summarized, while the collection sites are also presented in Figure 1.

Table 1. Collection sites, dates and essential oil yield of the three under-investigation *Hypericum* spp.

***Hypericum* spp.**	**Abbreviations**	**Collection Sites**	**Date of Collection**	**EO Yield (% v/dry Weight)**
H. empetrifolium Willd.	HE	Potamies Village, Heraklion, Crete; altitude: 215 m	13/05/2018	0.9
H. amblycalyx Coustur. and Gand	HA	Avdou Village, Heraklion, Crete; altitude: 520 m	13/05/2018	0.4
H. jovis Greuter	HJ	Kamaron gorge, Crete; altitude: 680 m	22/06/2017	0.5

Figure 1. Collection sites of the three under-investigation *Hypericum* spp.; Map of Crete, Greece; ○ *H. jovis*, □ *H. empetrifolium*, Δ *H. amblycalyx*.

Figure 1. Collection sites of the three under-investigation *Hypericum* spp.; Map of Crete, Greece; ○ *H. jovis*, □ *H. empetrifolium*, Δ *H. amblycalyx*.

2.2. Hydro-Distillation of Essential Oils

In order to obtain the EOs, the air-dried plant material from each sample was finely comminuted and separately subjected to hydro-distillation for 3 h. About 45 g from each plant in 500 mL of distilled water were used and the distillation was performed in a modified Clevenger-type apparatus with a water-cooled oil receiver to reduce artifacts produced during hydro-distillation by over-heating, according to the Hellenic Pharmacopoeia [16]. The EO yield (% *v/w*; Table 1) was calculated based on dry weight. GC (Gas Chromatography) grade n-pentane was used for the collection of the EOs, with the addition of anhydrous sodium sulphate to reduce any moisture. The EOs were subsequently analyzed by GC-MS (Gas chromatography-mass spectrometry analysis) and finally stored at −20 °C.

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2.3. Gas Chromatography-Mass Spectrometry Analysis

GC–MS analyses were carried out using a Hewlett- Packard 7820A-5977B MSD system operating in EI mode (70 eV), equipped with a HP- 5MS fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm), and a split-splitless injector. The temperature program was from 60 °C at the time of the injection, raised to 300 °C at a rate of 3 °C/min and subsequently held at 300 °C for 10 min. Helium was used as a carrier gas at a flow rate of 2.0 mL/min. The injected volume of the samples was 2 μL. The analyses were carried out twice, resulting in reproducible results.

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2.4. Identification of Compounds

Retention indices for all compounds were determined according to the Van der Dool approach [17], with reference to a homologous series of n-alkanes from C_9 to C_{25}. The identification of the chemical components was based on comparison of both relative retention times and mass spectra with those reported in the NIST/NBS and Wiley libraries, as well as those described by Adams [18] and other literature data. In many cases, the essential oils were subjected to co-chromatography with authentic compounds (Fluka, Sigma). Component relative percentages were calculated based on GC peak areas without using correction factors. Optical rotation values of the Eos $[\alpha]_D^{20}$ were determined at 20 °C at 589 nm in cyclohexane (cHex), Polarimeter: Perkin Elmer 341.

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2.5. Microbial Cultures

The Gram-positive bacteria *Bacillus cereus* (human isolate), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (ATCC 11632), and the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 13311) were used in order to determine the potential antimicrobial activity of EOs of the three *Hypericum* species. For the determination of the antifungal activity three yeast species *Candida albicans* (clinical isolate), *Candida tropicalis* (ATCC 750), and *Candida krusei* (clinical isolate) and four micromycetes *Aspergillus fumigatus* (ATCC 9197), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum* var. *cyclopium* (food isolate) were tested for their susceptibility. The bacterial strains were cultured on solid Tryptic Soy agar (TSA), while micromycetes were cultured on solid malt agar (MA) and yeasts were sustained on Sabouraud dextrose agar (SDA) medium. The cultures were sub-cultured once a month and stored at 4 °C for further utilization. All the tested microorganisms

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are deposited at the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stankovic", University of Belgrade.

2.6. In Vitro Antibacterial and Antifungal Assays

The minimum inhibitory and bactericidal/fungicidal concentrations (MICs, MBCs and MFCs) were determined using 96-well microdilution plates with a flat bottom [19,20]. The oils were dissolved in 5% dimethylsulfoxide (DMSO) solution that contained 0.10% Tween 80 (*v/v*) and added appropriate medium with bacterial/fungal inoculum.

For antibacterial assay, bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. Afterward, the bacterial inoculum was added to this mixture in order to achieve the appropriate concentrations. The microplates were incubated for 24 h at 37 °C. The MIC/MBC values for bacteria were detected following the addition of 40 µL of p-iodonitrotetrazolium violet (INT) 0.2 mg/mL (Sigma I8377) and incubation at 37 °C for 30 min [21]. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. Streptomycin (Sigma-Aldrich S6501, St Louis, MO, USA) and ampicillin (Sigma-Aldrich A9393, Germany) were used as positive controls for bacteria (1 mg mL^{-1} in sterile physiological saline). Sterilized distilled water containing 0.1% Tween 80 and 5% DMSO was used as negative control.

The assay for antifungal activity was performed in the following manner: fungal spores/yeast cells were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (*v/v*). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. MIC/MFC determination was performed by a serial dilution technique using 96-well plates. Microplates were incubated for 24 h at 37 °C for yeasts and for other fungi 72 h at 28 °C. Commercially available antifungal agent—ketoconazole (Zorkapharma, Šabac, Serbia) was used as a positive control. Sterilized distilled water containing 0.1% Tween 80 and 5% DMSO was used as negative control.

3. Results

3.1. Chemical Analysis

As presented in Table 2, the EOs of the under-investigation *Hypericum* spp., namely *H. empetrifolium* (HE), *H. amblycalyx* (HA) and *H. jovis* (HJ), were complex mixtures; in total 123 individual compounds were identified representing more than 94% of the total EOs, while even the main constituents never exceeded 19% (α-pinene in HE). More specifically, 94.2%, 94.5% and 95.2% of the total EOs were identified regarding HE, HA and HJ, respectively. The main compounds have been revealed as follows: HE: α-pinene (19.0%), germacrene D (12.5%), β-pinene (8.7%), E-caryophyllene (5.3%); HA: β-elemene (17.4%), β-selinene (10.5%), α-pinene (10.2%), E-caryophyllene (8.8%), α-selinene (8.7%); HJ: *trans*-calamenene (13.5%), α-selinene (8.3%), β-elemene (7.6%). Chemical structures of the most abundant compounds are presented in Figure 2. The under investigation *Hypericum* spp. yielded EO 0.9%, 0.4% and 0.5 *v/w*% for HE, HA and HJ respectively, which was calculated in dry weight (Table 1).

Table 2. Qualitative and quantitative composition (% *v/v*) of EOs.

	Compounds	RI	HE	HA	HJ
1	(3E)-2.3-dimethylhepta-1.3-diene	902	0.2	-	-
2	α-thujene	913	1.0	-	-
3	α-pinene	928	19.0	10.2	-
4	α-fenchene	938	0.5	0.1	-
5	camphene	940	0.3	0.3	-
6	3-methyl-nonane	962	3.5	-	-
7	β-pinene	972	8.7	1.5	-
8	6-methyl-5-hepten-2-one	979	-	0.1	0.1
9	myrcene	984	1.8	0.5	
10	hexenyl acetate	985	-	-	0.1
11	n-decane	994	0.2	-	-
12	α-phellandrene	1001	tr	-	-
13	α-terpinene	1011	0.1	-	-
14	p-cymene	1018	0.8	0.2	-
15	limonene	1022	1.6	1.2	0.1
16	*cis*-ocimene	1030	0.7	0.2	-
17	*trans*-ocimene	1040	1.9	0.5	-
18	γ-terpinene	1050	0.3	0.1	tr
19	2-methyl-decane	1056	1.8	-	-
20	terpinolene	1080	0.2	0.2	-
21	n-undecane	1094	1.0	2.0	1
22	n-nonanal	1097	-	0.2	0.2
23	endo-fenchol	1108	0.2	0.1	
24	α-campholenal	1119	0.1	-	-
25	allo-ocimene	1128	0.1	-	-
26	*trans*-pinocarveol	1129	0.2	0.2	-
27	neo-allo-ocimene	1133	-	0.1	-
28	*trans*-verbenol	1135	0.2	-	-
29	camphor	1137	tr	-	-
30	camphene hydrate	1141	tr	-	-
31	isoborneol	1152	0.1	-	-
32	pinocarvone	1153	-	0.2	-
33	borneol	1158	0.3	0.3	-
34	3-methyl-undecane	1162	-	-	-
35	terpinen-4-ol	1167	0.1	-	-
36	α-terpineol	1181	0.2	0.5	-
37	myrtenol	1186	0.2	-	-
38	verbenone	1198	0.1	-	-
39	citronellol	1219	tr	-	-
40	linalool acetate	1246	0.4	-	-
41	2-undecanone	1285	0.1	-	-
42	tridecane	1290	0.1	-	-
43	carvacrol	1292	-	0.2	-
44	α-longipinene	1337	2.1	0.3	-
45	α-cubebene	1338	-	-	0.4
46	cyclosativene	1359	-	-	0.2
47	α-ylangene	1360	0.3	-	0.6
48	α-copaene	1363	0.4	0.2	0.6
49	β-bourbonene	1370	0.4	-	-
50	geranyl acetate	1373	0.1	-	-
51	β-cubebene	1376	0.1	-	-
52	β-elemene	1380	0.2	17.4	7.6
53	β-longipinene	1384	0.3	-	-
54	α-cedrene	1389	0.1	-	-
55	E-caryophyllene	1406	5.3	8.8	2.9
56	β-cedrene	1409	0.9	0.2	0.5
57	β-gurjunene	1424	0.5	0.3	0.2

Table 2. *Cont.*

	Compounds	RI	HE	HA	HJ
58	aromadendrene	1430	0.6	0.7	0.7
59	α-himachalene	1433	0.4	-	-
60	α-humulene	1437	0.6	1.2	2.1
61	E-β-farnesene	1445	1.6	-	2.8
62	allo-aromadendrene	1450	0.4	-	-
63	*cis*-cadina-1(6).4-diene	1453	-	-	0.1
64	ishwarane	1453	2.0	2.3	-
65	4,5 di-epi-aristolochene	1453	-	0.3	-
66	γ-muurolene	1461	0.7	3.5	6.2
67	germacrene D	1466	12.5	2.9	3.7
68	β-selinene	1473	1.0	10.5	10
69	valencene	1476	-	0.6	-
70	α-selinene	1480	1.0	8.7	8.3
71	α-muurolene	1483	0.8	2.1	5.6
72	β-himachalene	1485	-	2.0	-
73	epizonarene	1488	-	-	6.5
74	trans-β-guaiene	1492	-	-	0.3
75	E.E-alpha-farnesene	1492	0.8	-	-
76	δ-amorphene	1494	-	0.1	1.6
77	γ-cadinene	1496	1.5	0.5	4.5
78	7-epi-α-selinene	1499	-	0.4	0.2
79	δ-cadinene	1506	3.1	-	-
80	*trans*-calamenene	1508	-	4.4	13.5
81	γ-dehydro-ar-himachelene	1514	-	-	1.2
82	cadina-1.4-diene	1514	0.2	-	0.3
83	α-cadinene	1519	0.4	0.1	0.3
84	α-calacorene	1524	0.1	0.2	1.5
85	β-calacorene	1544	tr	-	0.3
86	E-nerolidol	1547	0.5	-	-
87	3Z-hexenyl-benzoate	1553	0.1	0.3	0.2
88	himachalene epoxide	1556	-	-	-
89	spathulenol	1558	1.5	-	-
90	caryophyllene oxide	1563	1.8	1.2	1.1
91	cubeban-11-ol	1573	0.1	-	-
92	salvial-4(14)-en-1-one	1574	-	-	0.3
93	viridiflorol	1578	0.2	-	0.4
94	rosifoliol	1581	0.3	-	-
95	humulene epoxide II	1589	tr	-	0.3
96	junenol	1597	0.3	0.2	0.2
97	cubenol 1.10-di-epi	1608	-	0.2	-
98	1-epi-cubenol	1608	0.1	-	-
99	epi-α-cadinol	1609	-	-	0.5
100	caryophylla-4(12).8(13)-dien-5-alpha-ol	1619	-	0.4	-
101	cubenol	1620	0.5	-	0.3
102	τ-muurolol	1626	2.3	0.5	1.3
103	Torreyol = α-muurolol	1632	0.5	0.2	0.4
104	β-eudesmol	1635	-	-	0.2
105	epoxide-allo-aromadendrene	1637	-	-	-
106	α-cadinol	1640	0.8	-	1.9
107	selin-11-en-4-α-ol	1643	-	3.8	-
108	cis-calamenen-10-ol	1647	-	-	0.2
109	intermedeol	1652	-	0.4	-
110	calamenen-10-ol trans	1656	0.2	-	-
111	14-hydroxy-9-epi-E-caryophyllene	1661	-	-	0.3
112	cadalene	1664	tr	-	0.6
113	germacra-4(15).5.10(14)-trien-1-α-ol	1665	-	0.1	0.2
114	eudesma-4(15).7-dien-1β-ol	1676	-	-	0.9

Table 2. *Cont.*

	Compounds	RI	HE	HA	HJ
115	amorpha-4.9-dien-2-ol	1703	-	-	0.5
116	nootkatol	1710	-	-	0.2
117	γ-costol	1714	-	0.6	0.5
118	cyclocolorenone	1743	-	0.2	0.3
119	benzyl-benzoate	1757	0.1	-	-
120	6.10.14-trimethyl-pentadec-2-one	1843	-	-	0.2
121	n-hexadecanol	1877	0.3	-	-
122	nonadecane	1898	0.1	-	-
123	heneicosane	2097	0.1	-	-
	Total identification		94.2	94.5	95.2
	$[\alpha]_D^{20}$		−14.89 (c 0.10)	1.13 (c 1.06)	−1.66 (c 0.48)
	Grouped Components		**HE**	**HA**	**HJ**
	Monoterpene hydrocarbons		36.9	15.1	0.1
	Oxygenated monoterpenes		2.1	1.4	0
	Sesquiterpene hydrocarbons		38.3	67.9	83.3
	Oxygenated sesquiterpenes		9.2	7.6	10.0
	Others		7.6	2.5	1.8

Components listed in order of elution from a HP 5MS column. RI: Retention indices calculated against C_9–C_{25} n-alkanes on the HP 5MS column; tr: traces; concentrations below 0.01% are marked as -.

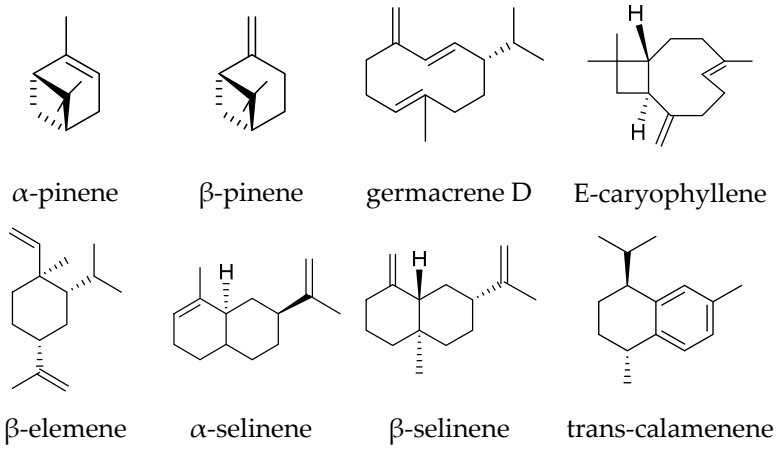

Figure 2. Chemical structures of the most abundant compounds.

3.2. Antimicrobial Activity

The antibacterial and antifungal activities of the three *Hypericum* EOs are presented as MIC and MBC/MFC in Tables 3 and 4.

Table 3. Antibacterial activity of *Hypericum* EOs mg/mL.

Bacteria	HJ MIC MBC	HE MIC MBC	HA MIC MBC	Streptomycin MIC MBC	Ampicilin MIC MBC
Staphylococcus aureus	0.015 0.030	0.030 0.060	0.015 0.030	0.05 0.10	0.25 0.40
Bacillus cereus	0.0075 0.015	0.015 0.030	0.0025 0.0050	0.10 0.20	0.25 0.40
Listeria monocytogenes	0.020 0.040	-	0.010 0.020	0.20 0.40	0.40 0.50
Pseudomonas aeruginosa	0.0015 0.0030	0.005 0.010	0.0025 0.0050	0.20 0.40	0.75 1.25
Salmonella typhimurium	-	-	-	0.20 0.40	0.40 0.50
Escherichia coli	0.0015 0.0030	0.010 0.015	0.0025 0.0050	0.20 0.40	0.25 0.50

-: the determination of the MIC and MBC values was not possible since expected MIC and MBC values were not in the tested range of concentrations (0.16 mg/mL).

Table 4. Antifungal activity of *Hypericum* EOs mg/mL.

Fungi	HJ MIC MFC	HE MIC MFC	HA MIC MFC	Ketoconazole MIC MFC
Aspergillus fumigatus	0.015 0.030	0.030 0.060	0.010 0.020	0.20 0.50
Aspergillus niger	-	-	-	0.20 0.50
Penicillium funiculosum	0.015 0.030	0.030 0.060	0.010 0.020	1.50 2.00
Penicillium verucosum	0.025 0.05	0.030 0.060	0.010 0.020	0.20 0.50
Candida albicans	-	0.005 0.010	-	0.50 1.00
Candida tropicalis	0.010 0.030	0.001 0.002	0.005 0.010	0.30 0.50
Candida krusei	0.010 0.030	0.001 0.002	0.005 0.010	0.50 1.00

-: the determination of the MIC and MFC values was not possible since expected MIC and MFC values were not in the tested range of concentrations (0.16 mg/mL).

4. Discussion

The three under investigation species contained EOs in low amounts, consistent with previous studies reporting *Hypericum* as an EO-poor genus [9]. Despite this fact, many studies have been conducted on *Hypericum* spp. regarding the volatile constituents [9–11]; in many cases, the plant material has been collected in Greece [22–26]. Regarding chemotaxonomy, some controversial hypotheses exist; some researchers accept volatile constituents of the genus *Hypericum* as possible chemotaxonomic markers [27,28] and make use of statistical analysis to divide *Hypericum* spp. into taxonomic sections [24,29,30], while Crockett [9] mentioned the variability of the EOs from this genus concerning a broader distribution, taxonomic rank or phenological state. Our results are in accordance with the latter author, as both differences and similarities (qualitative and quantitative) were observed between the studied oils. The investigated *Hypericum* species are closely related taxa, as all belong

to the section Coridium; HA and HJ are very close to HE, referred as recent derivatives of the latter and they are considered as the eastern and western member of a species pair, respectively [15]. The *Hypericum* taxa of the section Coridium (six species in total) are distributed in disjunct geographic areas, except from the derivative taxa of HE in Crete, while the rest of them are totally absent from Greece [15]. HA and HJ are distributed in restricted areas of Crete only, thus being narrow endemic, and this is the first report of the chemical composition of their EOs, although studies have been conducted concerning their content in phloroglucinols derivatives [31,32]. Regarding HE, its populations occupy a wider geographical range: Crete and other Greek Islands, Central Greece, Albania, W. Turkey, Cyrenaica [15,33]. Intraspecific-variability has been observed, in comparison to some previous studies using plant material collected from Mount Parnitha, Attica [21] and Kos Island, Aegean Sea [26]. In those studies, the plant material has also been subjected to hydro-distillation using a Clevenger apparatus [24,26], allowing the comparison of the chemical composition of the EOs. The main compound of the EO from HE was α-pinene (19.0%), a result that is consistent with previous studies for the dominant compound. However, both Petrakis et al. [24] and Fanouriou et al. [26] reported similarly higher percentages (approx. 35%), while the latter study also describes a high amount of iswarane (30.5%), a compound totally absent in the analysis of Petrakis et al. [21], which was revealed in small amounts (2.0%) in our study. The same compound was also identified in HA (2.3%). Generally, the most abundant group of compounds was found to be sesquiterpene hydrocarbons; however, some frequently reported constituents to other *Hypericum* spp., such as germacrene-D and β-caryophyllene [9,10], were either absent or revealed only in small amounts regarding HA and HJ. Such differences have also been found in the composition of *H. perforatum* growing wild in Greece [11]. It is noteworthy that HJ was found to be deficient in monoterpene hydrocarbons (0.1%), while this group of compounds was present in HE (36.9%) and HA (15.1%). HA and HJ were also found to be deficient in aliphatic hydrocarbons, like n-nonane, n-decane and n-undecane, which are common compounds in other *Hypericum* spp. [9,10]; e.g., *H. caprifolium* and *H. myrianthum* are characterized by the production of n-nonane and n-undecane, 55% and 20%, respectively [34]. Similar results have been reported for *H. coris*, the type species of the section Coridium [35]. Finally, *H. ericoides*, also belonging to the section Coridium, has been studied regarding its EO; however, these studies report quite diverse results, which could be explained by the different extraction methods that have been applied: isolation of the EO from the hexane extract of the plant [36], hydro-distillation [37], and headspace solid phase microextraction [38].

Regarding their anti-microbial potential, the obtained EOs were further subjected to the evaluation of antimicrobial activity in a variety of bacteria and fungi. The selected bacteria were some clinical and food isolates. More specifically, most food-borne illness is caused by infection of *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*, while *S. aureus*, *Pseudomonas aeruginosa*, *E. coli* give rise to some hospital-acquired infections. The tested fungi of *Aspergillus* and *Penicillium* genera are plant, animal and human pathogens, food contaminators and producers of potentially carcinogenic mycotoxins. *Penicillium* species can produce nephrotoxic, carcinogenic, teratogenic and immunotoxic metabolites, such as ochratoxin A, and contaminate cereals and cereal-based products [39]. Thus, in European countries, *P. verrucosum*, which produces this mycotoxin, is a major cause of cereal contamination [40]. Therefore, the presence of toxigenic fungi in cereals and food poses a potential risk to human and animal health. Moreover, *Aspergillus fumigatus* can cause invasive aspergillosis in immunocompromised patients [41]. Regarding the selected *Candida* species, they are responsible for the fungal infections called candidiasis. *C. albicans* is the cause for oral and genital infections, while *C. krusei* is the pathogen for fungemia (bloodstream infection).

According to given results of the antibacterial tests, EOs showed good activity against tested strains, but in variable degree. Minimal inhibitory concentrations ranged between 0.0025–0.030 mg/mL, and minimal bactericidal concentration was 0.005–0.060 mg/mL. All EOs showed higher antibacterial activity in comparison to commercial drugs Ampicillin and Streptomycin. The Gram-negative *E. coli* and *P. aeruginosa* were the most sensitive bacteria, since they were inhibited by all tested oils (MIC

values in the range of 0.015–0.010 mg/mL). In contrast, the tested oils did not exhibit antibacterial activity against *S. typhimurium*.

The examined oils were more effective compared to ketoconazole (MIC 0.200–1.500 mg/mL and MFC 0.500–2.000 mg/mL), a commercially available fungicide used as positive control. The most sensitive fungi were the yeasts *C. tropicalis* and *C. krusei* with MIC 0.001–0.010 mg/mL and MFC 0.002–0.030 mg/mL. No remarkable activity was detected against *A. niger* and *C. albicans*.

Generally, among all tested essential oils, antimicrobial potential could be presented as following: HA > HJ > HE. The *Hypericum* EOs possessed better antifungal and anticandidal activities than antibacterial activities, with MIC values ranging between 0.001 mg/mL to 0.030 mg/mL, whereas fungicidal activates were within the 0.002 mg/mL−0.060 mg/mL range. Their inhibition and bactericidal/fungicidal concentrations (<100 mg/mL) indicate a good potency level as antibiotics [42], and support their use in the traditional medicine against diseases caused by different species of microorganisms. This is in accordance with previous reports on the antimicrobial activities of EOs from the genus *Hypericum*, and could be explained due to the presence of α-pinene, β-pinene and (E)-caryophyllene, which are known for their antimicrobial effects [11].

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Wound Healing Effects from 3 *Hypericum* spp. Essential Oils

OPEN ACCESS

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ABSTRACT

Hypericum species have a long-term use as wound healing agents, with the most common preparation being the infused oil from the aerial parts. It contains naphthodianthrones, phloroglucinols, and essential oil. An extensive literature survey shows that, unlike napthodianthrones and phloroglucinols, essential oils from *Hypericum* spp. have not yet been evaluated for their wound healing efficacy. The present study aims to assess the wound healing efficacy of essential oils from *H. perforatum*, a plant recognized in European Pharmacopoeia for having wound healing properties, as well from 2 other *Hypericum* species commonly used in Greece as wound healing agents since classical antiquity, namely, *H. empetrifolium* and *H. triquetrifolium*. So far, only the wound healing effects of Hypericum oil are known, which is a different herbal preparation containing nonvolatile compounds, while the essential oils under investigation contain only volatile constituents. The essential oils were subjected to GC-MS analyses. Wounds were created on the upper back of hairless SKH-hr1 mice. Healing was evaluated by clinical, histopathological, and biophysical assessment. The essential oils showed a significantly faster wound healing rate in comparison to the controls and the vehicle-treated groups. *H. empetrifolium* possessed the most significant healing properties while for *H. perforatum* and *H. triquetrifolium* skin inflammation persisted. The essential oils from *Hypericum* spp. showed promising results as wound healing agents and are likely to contribute to the wound healing efficacy of the *Hypericum* preparations. *H. empetrifolium*, being the most potent anti-inflammatory and wound healing agent, confirms the traditional use of this plant in Greece for wounds and skin inflammations.

Introduction

The genus *Hypericum* L. (Hypericaceae) comprises more than 450 taxa worldwide [1] and 51 taxa in Greece, of which 15 are endemic [2]. *Hypericum* species have been used as wound healing agents since classical antiquity, and they have been described by Hippocrates [3], Dioscorides [4], and later on in the Medieval era by Nikolaos Myrepsos [5, 6].

In modern times, a monograph of *H. perforatum* L. was included in 2009 in the European Pharmacopoeia, mentioning wound healing properties (www.ema.eu) [7]. The translucent glands on the leaves of the plant look like perforations, and its preparations are red resembling blood. The use of the plant in wound treatment was suggested since ancient times [8].

The infused oil (Oleum *Hyperici*) is the most common preparation for the treatment of wounds and skin inflammation. It is obtained by macerating the fresh aerial parts under sunlight, usually in olive or sunflower oil for a period of 2 – 3 weeks. Oleum *Hyperici* has a red color, resulting from the degradation of naphthodianthrones, which are not extracted in the infused oil. The dark glands contain this group of compounds, which are important bioactive secondary metabolites of other preparations of the *Hyperici* herba, such as tincture, also used for wound healing [7]. The content of the translucent glands (phloroglucinols and essential oil) is extracted in the infused oil [9].

Phloroglucinols are reported to be sensitive metabolites that are quickly degraded in the presence of air, heat, and light [10], and there is much controversy in the scientific community regarding the composition and stability of the formulations containing this group of compounds [11].

The translucent glands also contain essential oils (EOs), which are partially derived from the same biosynthetic pathway [12]. According to the European Pharmacopeia, EO (Aetherolea) are odorous products, usually of complex composition, obtained mainly by steam distillation. Although *Hypericum* spp. are classified as EO-poor plants [9], studies have shown that their volatile oils possess antimicrobial, antioxidant, antiangiogenetic, and gastroprotective activities [13]. An extensive literature survey shows, that, unlike napthodianthrones and phloroglucinols, the wound healing efficacy of EOs from *Hypericum* spp. was not still evaluated and compared between species. Thus, the aim of the present study is the investigation of the wound healing efficacy of the EOs specifically used for this reason: *H. perforatum* L. (HP) and 2 other *Hypericum* species commonly used in Greece since classical antiquity, namely *H. empetrifolium* Willd. (HE) and *H. triquetrifolium* Turra (HT).

Results and Discussion

As presented in ▶ **Table 1**, the EOs of the under-investigation *Hypericum* spp., namely HE, HP, and HT, were complex mixtures. In total, 122 individual constituents were identified, representing 88.1–96.8 % of the EOs. Even the main constituents never exceeded 19.0 % (α-pinene in HE). Regarding HE, its chemical analysis has been recently completed [14] and is presented in ▶ **Table 1** to facilitate the comparison. The *Hypericum* spp. Under investigation yielded EOs 0.9 and 0.6 v/w % for HE and HT respectively, which were calculated in dry weight (▶ **Table 1**). It is noteworthy that when only inflorescences and leaves were carefully selected instead of total aerial parts of HE, the yielded EO reached 0.6 mL (13 v/w %). The main compounds have been identified as follows: HE: α-pinene, germacrene D, β-pinene, E-caryophyllene; HP: ishwarane, α-himachalene, α-pinene, β-pinene; and HT: α-pinene, 3-methyl-nonane, caryophyllene oxide, germacrene D. In comparison to different previous studies [9, 13, 15, 16], intraspecific-variability has been observed for all the under-investigation species, which could be explained by the different extraction methods that have been applied, as well as different collection times and sites.

Regarding the skin parameters, the most sensitive measurement is transepidermal water loss (TEWL), when it is cautiously measured in areas fully healed. TEWL has been recovered in several treatment groups, especially in HE 0.5 % and HP 0.05 % ointments, while the other remains at relatively higher levels (highest levels in the control groups, petrolatum, and the HT 0.5 %, ▶ **Fig. 1a**). Moreover, skin hydration has been recovered. Despite this, the healed skin generally shows higher hydration; however, this does not reflect the reality, as cysts or edema are frequently formed in wounds (▶ **Fig. 1b**). An overall increase of the erythema factor was observed for all treatments. This observation is inconsistent with previous observations of our laboratory, where the high sensitivity of the detector often leads to the evaluation of healed skin as inflamed. Furthermore, skin thickness was increased in all treatments, while it generally takes more than 2 years to recover completely. Regarding treatments HT 0.05 % and HT 0.5 %, as well as treatments HE 0.05 % and HE 0.5 %, the elasticity increased, probably as a result of neovascularization.

Based on the clinical evaluation of the mice, it became apparent that the treatment with the low dose of HP 0.05 % ointment led to almost complete wound healing (99.9 %) with expected scarring. This was also the criterion for terminating the experiment. Compared to the treatment with the same EO at the highest dose (0.5 %), a similar degree of healing was observed by day 8, but at the end of the experiment, for HP 0.5 %, the degree decreased and reached a healing rate of 96.6 %. This leads to the suspicion of possible dose-dependent toxicity, which is characteristic of EOs. Also, HP 0.05 % showed positive results compared to the control and the petrolatum, while HP 0.5 % resulted in less healing in comparison to the control and the petrolatum.

HT ointment showed 99.2 % healing at a low dose and 97.9 % at the highest (HT 0.05 % and HT 0.05 %, respectively). Therefore, it is evident, again, that the low dose has better results compared to the control group and the treatment with petrolatum, but the high dose showed a similar clinical impact with them. Treatments with 0.05 % and 0.05 % of HE ointment showed a very good effect in both doses (99.4 % and 98.9 % degree of healing, respectively). Moreover, both the high and the low doses showed a positive effect compared to the control group (97.1 %), the petrolatum (98.1 %), and Madecassol (99.6 %) treatments but also to the treatments with HP and HT ointments.

▶ **Fig. 2** shows the overall degree of healing of each group. In treatment with 0.05 % HP ointment, there were wounds of greater initial area, but this ointment resulted in 99.9 % healing on day 15. It is noteworthy that HE ointments had the best healing rates, due to the faster reduction in wound area compared to other treatments. The photo documentation in ▶ **Fig. 3** shows representative images of the wound areas of the various mice groups.

▶ **Table 1** Qualitative and quantitative composition (% v/v) of EOs.

	Compounds	RI aver.	HE	HP	HT
1.	(3E)-2,3-dimethylhepta-1,3-diene	902	0.2	–	–
2.	α-thujene	913	1.0	0.1	0.4
3.	α-pinene	928	**19.0**	6.4	**13.9**
4.	α-fenchene	938	0.5	0.2	tr
5.	camphene	940	0.3	0.6	tr
6.	dehydrosabinene = thuja-2,4(10)-diene	943	–	tr	–
8.	3-methyl-nonane	962	3.5	–	**10.2**
8.	β-pinene	972	**8.7**	6.1	1.5
9.	6-methyl-5-hepten-2-one	979	–	tr	–
10.	myrcene	984	1.8	0.9	1.4
11.	hexenyl acetate	985	–	–	–
12.	n-decane	1000	0.2	–	0.4
13.	α-phellandrene	1001	tr	0.1	tr
14.	α-terpinene	1011	0.1	0.3	1.2
15.	p-cymene	1018	0.8	0.2	0.9
16.	limonene	1022	1.6	2.2	0.6
17.	cis-ocimene	1030	0.7	0.3	tr
18.	trans-ocimene	1040	1.9	0.2	tr
19.	γ-terpinene	1050	0.3	0.5	2
20.	2-methyl-decane	1056	1.8	0.8	4
21.	terpinolene	1080	0.2	0.8	0.5
22.	n-undecane	1094	1.0	0.7	1.8
23.	n-nonanal	1097	–	tr	tr
24.	endo-fenchol	1108	0.2	0.2	–
25.	α-campholenal	1119	0.1	0.1	tr
26.	allo-ocimene	1128	0.1	–	–
27.	trans-pinocarveol	1129	0.2	0.2	–
28.	trans-verbenol	1135	0.2	–	–
29.	camphor	1137	tr	0.1	–
30.	camphene hydrate	1141	tr	0.1	–
31.	isoborneol	1152	0.1	0.6	–
32.	pinocarvone	1153	-	–	tr
33.	borneol	1158	0.3	0.3	–
34.	3-methyl-undecane	1162	–	1.0	–
35.	terpinen-4-ol	1167	0.1	0.2	tr
36.	α-terpineol	1181	0.2	0.9	–
37.	myrtenol	1186	0.2	0.2	–
38.	verbenone	1198	0.1	-	–
39.	citronellol	1219	tr	0.1	–
40.	geraniol	1245	–	tr	–
41.	linalool acetate	1246	0.4	–	–
42.	2-undecanone	1285	0.1	0.1	–
43.	tridecane	1300	0.1	–	–
44.	α-longipinene	1337	2.1	0.2	–
45.	α-cubebene	1338	–	–	tr
46.	α-ylangene	1360	0.3	0.5	tr
47.	α-copaene	1365	0.4	0.2	1.2
48.	α-duprezianene	1367	–	0.1	–
49.	β-bourbonene	1370	0.4	–	tr
50.	geranyl acetate	1373	0.1	–	–
51.	β-cubebene	1376	0.1	–	–

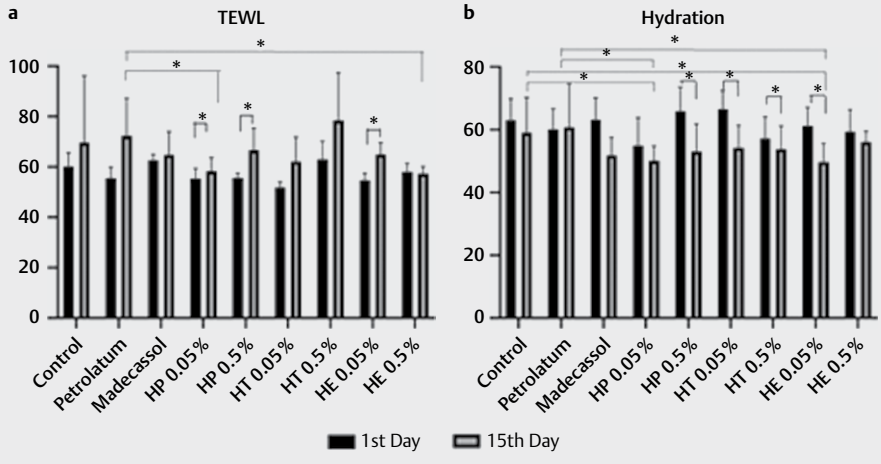
▶ **Table 1** Continued.

	Compounds	RI aver.	HE	HP	HT
52.	italicene	1379	–	0.1	–
53.	β-elemene	1380	0.2	–	–
54.	β-longipinene	1384	0.3	–	–
55.	longifolene	1389	–	0.4	–
56.	α-cedrene	1389	0.1	–	–
57.	2-epi-β- funebrene	1397	–	0.1	tr
58.	E-caryophyllene	1406	**5.3**	2.6	**14.0**
59.	β-cedrene	1409	0.9	–	–
60.	β-duprezianene	1413	–	0.6	–
61.	β-copaene	1413	–	–	0.5
62.	β-gurjunene	1424	0.5	–	–
63.	aromadendrene	1430	0.6	–	–
64.	α-himachalene	1433	0.4	**6.9**	–
65.	α-humulene	1437	0.6	–	1.8
66.	E-β-farnesene	1445	1.6	–	–
67.	allo-aromadendrene	1450	0.4	–	–
68.	ishwarane	1453	2.0	**22.0**	–
69.	γ-muurolene	1461	0.7	3.4	3.3
70.	germacrene D	1466	**12.5**	1.0	**8.2**
71.	γ-himachalene	1467	–	1.2	1.2
72.	β-selinene	1473	1.0	2.2	–
73.	valencene	1476	–	1.4	1.3
74.	α-selinene	1480	1.0	1.9	1.2
75.	4-epi-cis-dihydroagaro-furan	1482	–	0.6	–
76.	α-muurolene	1483	0.8	–	0.9
77.	β-himachalene	1485	–	3.5	–
78.	epizonarene	1488	–	0.2	–
79.	trans-β-guaiene	1492	–	0.2	–
80.	E, E-α-farnesene	1492	0.8	–	–
81.	δ-amorphene	1494	–	–	tr
82.	γ-cadinene	1496	1.5	0.8	2.2
83.	7-epi-α-selinene	1499	–	0.2	–
84.	δ-cadinene	1506	3.1	1.4	4
85.	γ-dehydro-ar-himachelene	1514	–	0.4	–
86.	cadina-1,4-diene	1514	0.2	–	tr
87.	γ-vetivenene	1518	–	0.4	–
88.	α-cadinene	1519	0.4	–	tr
89.	α-calacorene	1524	0.1	0.1	0.5
90.	β-calacorene	1544	tr	0.2	–
91.	E-nerolidol	1547	0.5	–	–
92.	caryophyllenol	1550	–	0.6	–
93.	3Z-hexenyl-benzoate	1553	0.1	–	–
94.	himachalene epoxide	1556	–	0.2	–
95.	spathulenol	1558	1.5	–	0.9
96.	caryophyllene oxide	1563	1.8	0.9	**9.7**
97.	cubeban-11-ol	1573	0.1	–	–
98.	salvial-4(14)-en-1-one	1574	–	–	1.1
99.	viridiflorol	1578	0.2	–	–
100.	rosifoliol	1581	0.3	–	–
101.	humulene epoxide II	1589	tr	–	0.5
102.	junenol	1597	0.3	–	–
103.	1-epi-cubenol	1608	0.1	–	tr
104.	epi-α-cadinol	1609	–	tr	–
105.	cubenol	1620	0.5	–	–

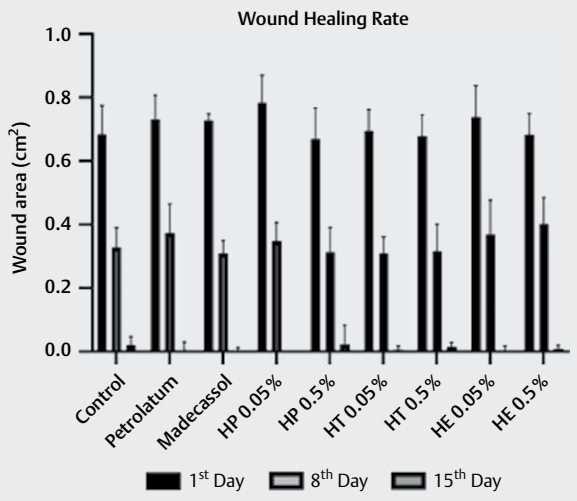
▶ **Table 1** Continued.

	Compounds	RI aver.	HE	HP	HT
106.	τ-muurolol	1626	2.3	–	0.9
107.	torreyol = α-muurolol	1632	0.5	–	–
108.	allo-aromadendrene epoxide	1637	–	–	0.9
109.	α-cadinol	1640	0.8	–	0.9
110.	himachalol	1640	–	3.1	–
111.	selin-11-en-4-α-ol	1643	–	2.6	–
112.	allohimachalol	1650	–	0.5	–
113.	intermedeol	1652	–	1.7	–
114.	trans-calamenen-10-ol	1656	0.2	–	–
115.	cadalene	1664	tr	0.9	–
116.	germacra-4(15), 10(14)-trien-1-α-ol	1665	–	–	1.0
117.	eudesma-4(15),7-dien-1β-ol	1676	–	–	1.8
118.	cyclocolorenone	1743	–	0.1	–
119.	benzyl benzoate	1757	0.1	0.1	–
120.	n-hexadecanol	1877	0.3	–	–
121.	nonadecane	1898	0.1	–	–
122.	heneicosane		2097	0.1	0.1
Total identification			94.2	88.1	96.8
[α]D20			- 14.89	− 0.25	− 12.58
			(c 0.10)	(c 1.61)	(c 0.103)
EO yield (% v/dry weight)			0.9	–	0.6
Grouped components			**HE**	**HP**	**HT**
Monoterpene hydrocarbons			37.1	18.9	22.4
Oxygenated monoterpenes			2.1	3.0	0.0
Sesquiterpene hydrocarbons			38.3	53.1	40.3
Oxygenated sesquiterpenes			9.1	10.3	17.7
Others			7.6	2.8	16.4

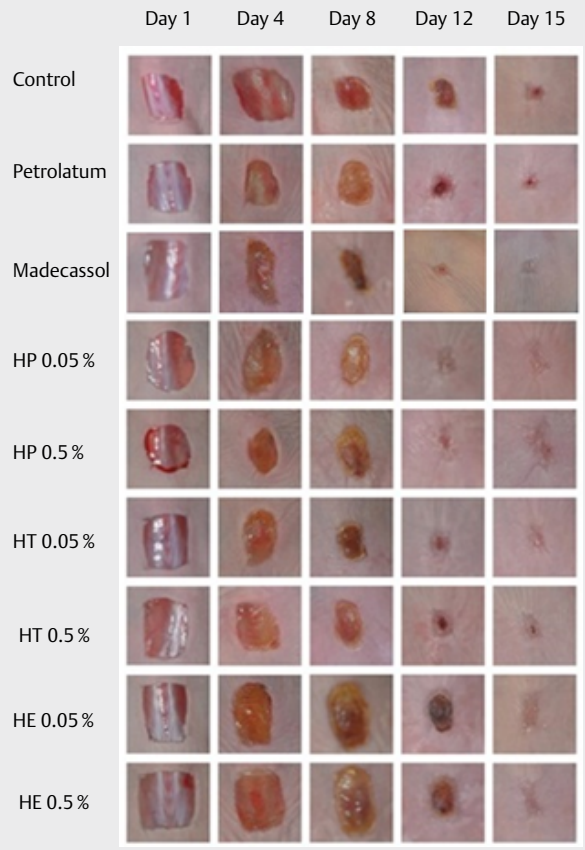
Components listed in order of elution from a HP 5MS column. RI aver. Retention indices calculated against C9-C25 n-alkanes on the HP 5MS column; average value from three samples. tr: traces. Concentrations below 0.01 % are marked as -; **main compounds** >5 %.



▶ **Fig. 1** (**a**) Transepidermal water loss (TEWL) values for the various mice groups (control; petrolatum; Madecassol; HP 0.05 %; HP 0.5 %; HT 0.05 %; HT 0.5 %; HE 0.05 %; HE 0.5 %) on Day 1 and Day 15 of the experiment. (**b**) Hydration values for the various mice groups on Day 1 and Day 15 of the experiment. Values are presented as mean ± SD of 3-–4 independent experiments (n = 6 mice per group). Statistical analysis was performed using Student's t-test or One-way ANOVA (in comparison to the control group and the group treated with petrolatum); * $p < 0.05$.



▶ **Fig. 2** The effect of the different treatments (control; petrolatum; Madecassol; HP 0.05 %; HP 0.5 %; HT 0.05 %; HT 0.5 %; HE 0.05 %; HE 0.5 %) on the wound healing process on young mice. The wounds from each group were photographed at time 0 and on Day 1, Day 8, and Day 15. A Nikon Nikkor AF-S Micro 60 mm f/2.8 G ED, SWMED IF camera was used, located at a distance of 30 cm from the animals. The photographs were digitized, and the wound area (cm2) was measured using Adobe Photoshop C5.



▶ **Fig. 3** Representative images of the wound areas of the various mice groups (control; petrolatum; Madecassol; HP 0.05 %; HP 0.5 %; HT 0.05 %; HT 0.5 %; HE 0.05 %; HE 0.5 %), as recorded over a 15-days period.

After histopathological examination of skins from each group, no total healing was observed in any treatments, and their degree of inflammation was significantly different.

The lowest inflammation was observed in the group treated with the low dose of HE ointment, 0.05 %. This is illustrated by the following images (▶ **Fig. 4**), which belong to the mice treated with HE 0.05 %. Interestingly, the skin's normal structure was maintained, and it is also worth noting that some elements of regenerated hair follicles existed in the wounded skins. HE showed significant healing properties also at the highest dose (0.5 %), similar to that of the lower dose (0.05 %).

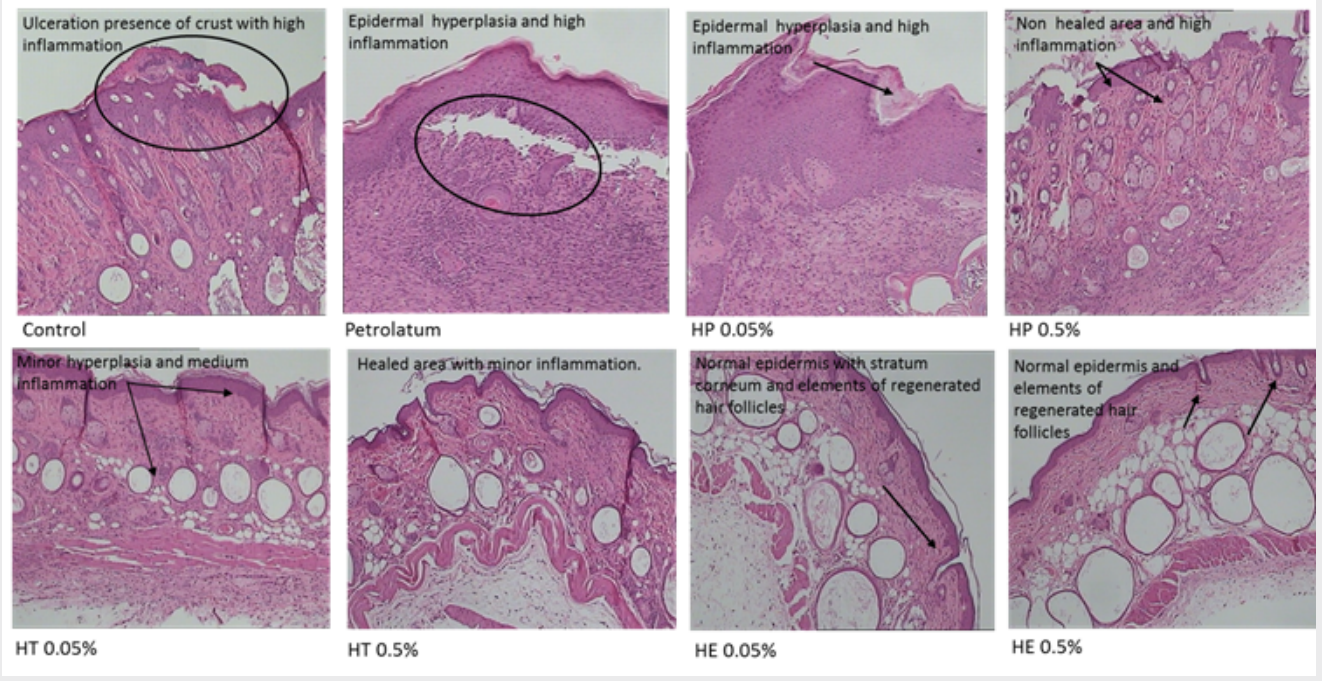
On the contrary, we observed that there was still acute inflammation in treatment with the control that had not been administered any formulation. More specifically, the image even shows ulceration in the presence of "inflammatory overgrowth." But also, treatment with petrolatum had strong inflammation, as the nuclei of the polymorphonuclear cells are visible, characteristic of acute inflammation, shown as black dots under microscope observation. The structure of the skin with the characteristic layers has changed.

Treatment with a 0.5 % high dose of HT ointment was also effective, presenting a healed area with minor inflammation, whereas treatment with the lower concentration of 0.05 % ointment of the same plant had weaker results, with minor hyperplasia and with medium inflammation. These are evident in some photos from the microscope (▶ **Fig. 4**).

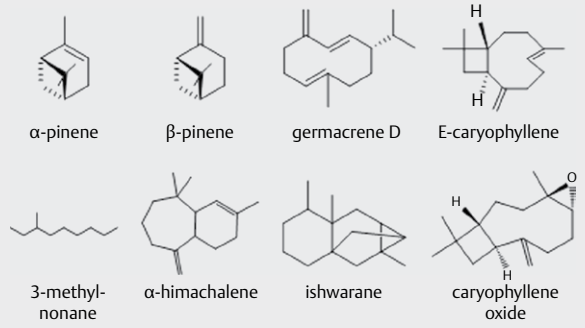
The effect of HP ointment at either low (HP 0.05 %) or high concentration (HP 0.5 %) was not shown to be effective during the experiment. Especially in the mice with HP 0.05 % treatment, the inflammation (in the presence of polymorphonuclear cells) is predominant, mainly in the dermis, similar to that of treatment with petrolatum.

To the best of our knowledge, the present work is the first study revealing the wound healing properties of the EOs from *Hypericum* spp., which are likely to contribute to the wound healing efficacy of the *Hypericum* preparations. Taking everything into account, it appeared that the control group and the group treated with petrolatum had normal healing progress due to the skin's ability to self-heal but also had most of the elements of inflammation and alteration of the skin structure. Low-dose HP and HT had a good clinical picture, but it was not the same for the degree of inflammation. Moreover, higher concentrations resulted in wound healing delay, indicating dose-dependent toxicity related to the EOs, except for *H. empetrifolium*, which showed significant wound healing and anti-inflammatory effects in both EO doses. In comparison with the other 2 species under investigation, HE EO yields a high concentration of monoterpenes hydrocarbons (37.1 %, *vs.* 18.9 % and 22.4 % for HP and HT, respectively). Furthermore, α-pinene (19.0 % in HE) (▶ **Fig. 5**) has been previously reported to possess anti-inflammatory activities [17].

In conclusion, the significant wound healing properties of HE confirm the traditional use of this plant in Greece for wounds and skin inflammations [18]. It is worth mentioning that in the quote by Dioscorides, *hypericon* could be attributed to HE since the pre-



▶ **Fig. 4** Representative histopathological images of the back skin of SKH-hr1 hairless mice (magnification 100×) on Day 15 of treatment with petrolatum, HP 0.05%, HP 0.5%, HT 0.05%, HT 0.5%, HE 0.05%, HE 0.5%, and without treatment (control) (magnification 100×). Samples were stained with hematoxylin and eosin. Arrows and circles pointing the events of wound healing (*i. e.* ulceration presence of crust with high inflammation [control]; epidermal hyperplasia and high inflammation [petrolatum and HP 0.05%]; non-healed area and high inflammation [HP 0.5%]; minor hyperplasia and medium inflammation [HT 0.05%]; normal epidermis with stratum corneum and elements of regenerated hair follicles [HE 0.05%]; normal epidermis with elements of regenerated hair follicles [HE 0.05%].



▶ **Fig. 5** Chemical structures of the most abundant compounds.

viously reported *H. coris*[4] is not growing wild in Greece, in contrast to its closely related species, HE [19].

Material and Methods

Plant material

Aerial parts from HE and HT were collected from natural populations in Greece (in Crete and Thessaloniki, GPS position 35.25463477968957, 25.37766337130051 and 40.636971, 22.976209, respectively), during the flowering stage. The collected plant materials were recognized and authenticated by Prof. Z. Kypriotakis and Dr. E. Antaloudaki for HE (Department of Agriculture, TEI of Crete, and Department of Biology, University of Crete) and by Assoc. Prof. Th. Constantinidis for HT (Biology Department, NKUA). Voucher specimens were deposited in the Herbarium of Natural History Museum, University of Crete (15981) and in the Laboratory of Pharmacognosy and Chemistry of Natural Products (Skaltsa & Grafakou 03), respectively. The EO of HP was purchased from Florihana (France, LOT FLEO59-B120917F) and further subjected to GC-MS analysis. The plant names have been checked according to http://www.theplantlist.org [20].

Hydro-distillation of essential oils, GC-MS spectrometry analysis, identification of compounds

To obtain the EOs from HE and HT, the air-dried plant materials were subjected to hydro-distillation, according to the procedure described before [14]. The 3 EOs were subsequently analyzed by GC-MS and finally stored at −20 °C before being used for the *in vivo* experiments. GC-MS analyses and identification of the chemical compounds were carried out as described previously [14].

Animals

Animal care was performed according to the guidelines established by the European Council Directive 2010/63/EU. Fifty-four female SKH-hr1 hairless mice (3–12 weeks old, 17–40 g, n = 6) were used in this study. All mice originated from the breeding stock of

the Small Animal Laboratory of the Section of Pharmaceutical Technology, Department of Pharmacy (EL 25 BIO 07). The animal room was kept at 23 ± 1 °C and 25–55 % humidity and was illuminated by yellow fluorescent tubes in a 12 h light and dark cycle. The mice had unrestricted continuous access to standard chow diet (Nuevo SA-Farma-Efyra Industrial and Commercial SA, Greece) and fresh water. The experimental procedure was approved by the National Peripheral Veterinary Authority (Protocol Number: 1064/20-02-2019) after the affirmative opinion of the Animal Protocols Evaluation Committee.

Experimental design for in vivo wound healing effect in a mouse model

The experimental protocol for the evaluation of wound healing has been used for years from the Laboratory of Dermatopharmacology, and has been recently described by Sofrona and colleagues [21]. Briefly, full-thickness (*i. e.*, epidermis, dermis, and subcutis) wounds of 1 cm^2 (1.0 cm × 1.0 cm) were induced on the dorsal skin of anesthetized mice by intraperitoneal administration of a cocktail of ketamine (100 mg/kg) and xylazine (7 mg/kg).

Mice (n = 54) were randomly divided into 9 treatment groups of 6 animals per group. The first group was the control (untreated mice); the second group received the petrolatum vehicle (100 % petroleum jelly); and the third group received the Madecassol cream (*Centella asiatica* extract used as positive control), the rest of the groups received 0.05 and 0.5 % w/w petrolatum ointment of each EO (▶ **Table 2**). The different treatments were applied once per day for 14 days, where complete (99.9 %) healing was clinically observed in one of the groups (HP 0.05 %).

Evaluation of TEWL, hydration, erythema, thickness, and elasticity

Skin parameters, including TEWL, hydration, erythema, skin thickness, and elasticity were evaluated with noninvasive biophysical methods. TEWL and skin hydration were measured by using the Tewameter TM 210 (Courage + Khazaka Electronic GmbH) and the Corneometer CM820 (Courage + Khazaka Electronic GmbH), respectively. Erythema was measured by using the spectrophotometer Mexameter MX 18 (Courage-Khazaka). Skin thickness was scored in mm using an electronic digital caliper (Powerfix Prof Milomex Ltd), 3 cm above the tail. Elasticity was measured by using CUTOMETER MPA 580 (Courage-Khazaka). All of the above-mentioned measurements were conducted on the first (just before the induction of the wounds) and last day in healed areas, as described before [19]. This is particularly important, as they are sensitive measurements, which evaluate the skin barrier restoration; as a result, they must be cautiously measured the last day only in areas fully healed to provide reliable results.

▶ **Table 2** Treatments.

Control	Control group
Petrolatum	Petrolatum
Madecassol	Madecassol cream
HP 0.05 %	*H. perforatum* 0.05 % ointment
HP 0.5 %	*H. perforatum* 0.5 % ointment
HT 0.05 %	*H. triquetrifolium* 0.05 % ointment
HT 0.5 %	*H. triquetrifolium* 0.5 % ointment
HE 0.05 %	*H. empetrifolium* 0.05 % ointment
HE 0.5 %	*H. empetrifolium* 0.5 % ointment

Photodocumentation, percentage of wound healing, and histological analysis

The wounds from each group were photographed at time zero and then on the 4th, 8th, 12^{th,} and 15th day. A Nikon Nikkor AF-S Micro 60 mm f/2.8 G ED, SWMED IF camera was used, located at a distance of 30 cm from the animals. The photographs were digitized, and the wound area was measured using Adobe Photoshop C5. Wound closure was defined as a reduction in the wound area and the results were expressed as a percentage (%) of the original wound area. After the mice were sacrificed, the back skin was removed, fixed in formaldehyde, and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin (H&E). The extent of inflammatory cell infiltration, parakeratosis, and hyperkeratosis, epidermal thickness, and Munro abscess were blindly evaluated by an experienced anatomopathologist.

Statistical analysis

All results are presented as means ± SEM of 3 different experiments for each sample. The data were tested for normality and distribution. Data were evaluated by Student's t-test and 1-way analysis of variance (ANOVA) using the SPSS v 18.0 statistical analysis software (IBM SPSS software package, Inc.). The p-value of ≤ 0.05 was set as a significance level for all data. Graphs were generated using GraphPad Prism 8.4.2 (GraphPad Software, Inc.).

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Conflict of Interest

The authors declare no conflicts of interest regarding the current research work.

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Supplementary Material

Wound Healing Effects from 3 *Hypericum* spp. Essential Oils

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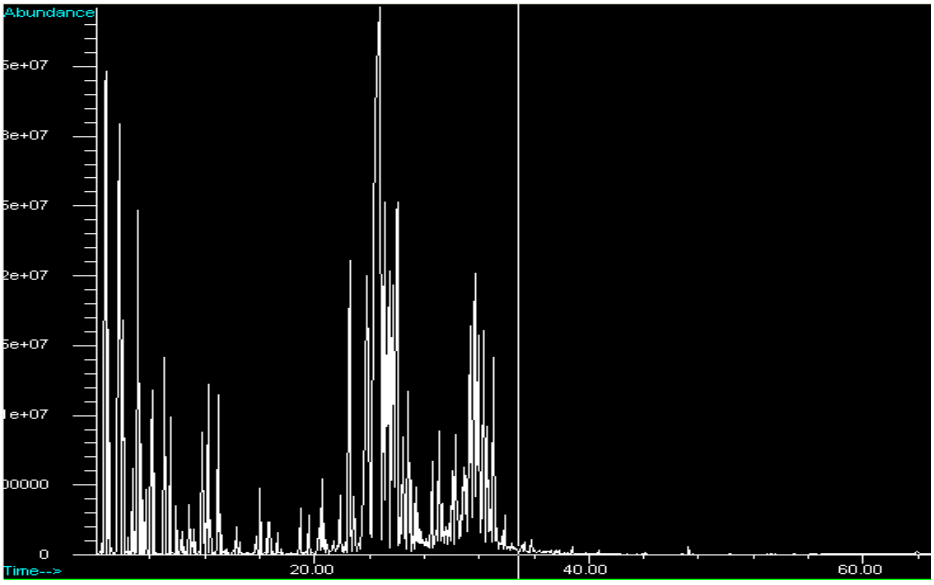

Fig. S1. HP-EO.

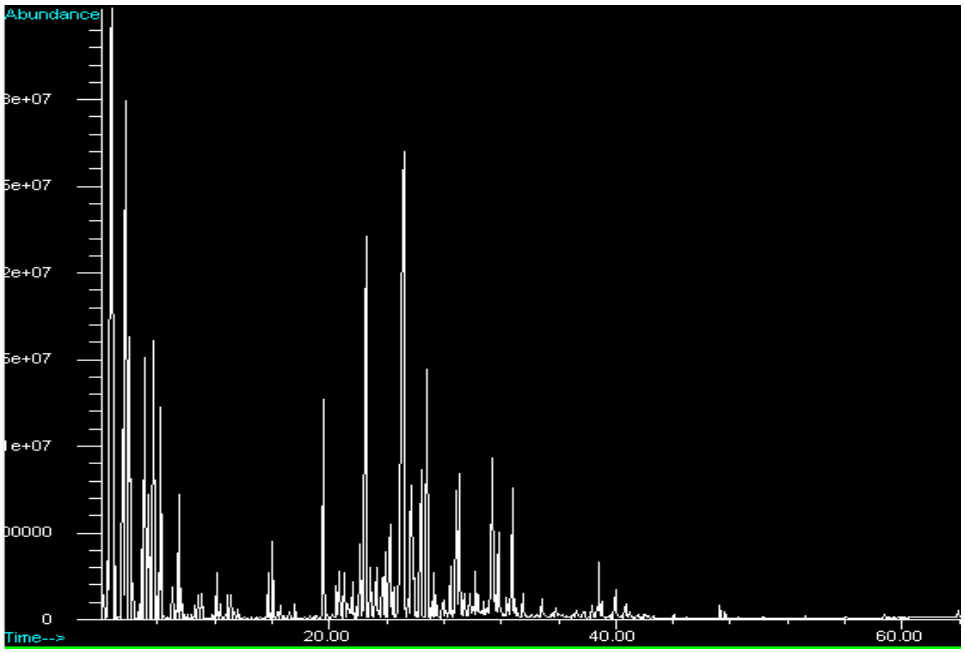

Fig. S2. HE-EO.

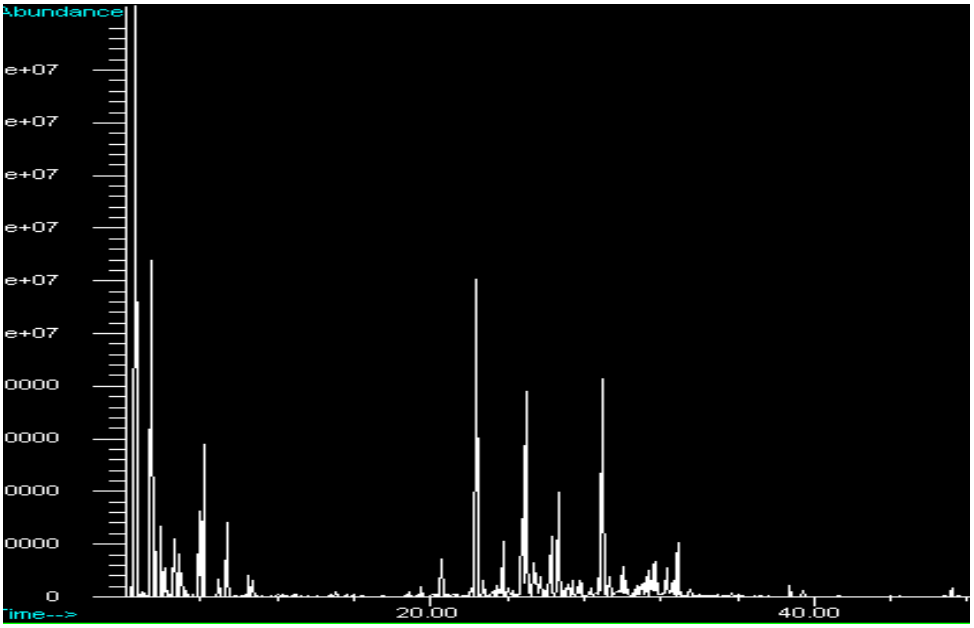

Fig. S3. HT-EO.

3.6. *Euphorbia deflexa* Sibth. & Sm.

The isolation of the compounds from *E. deflexa* is presented in Figure 30. Extraction, isolation, structure elucidation, and biological activities are presented in Grafakou et al. (2021e, accepted).

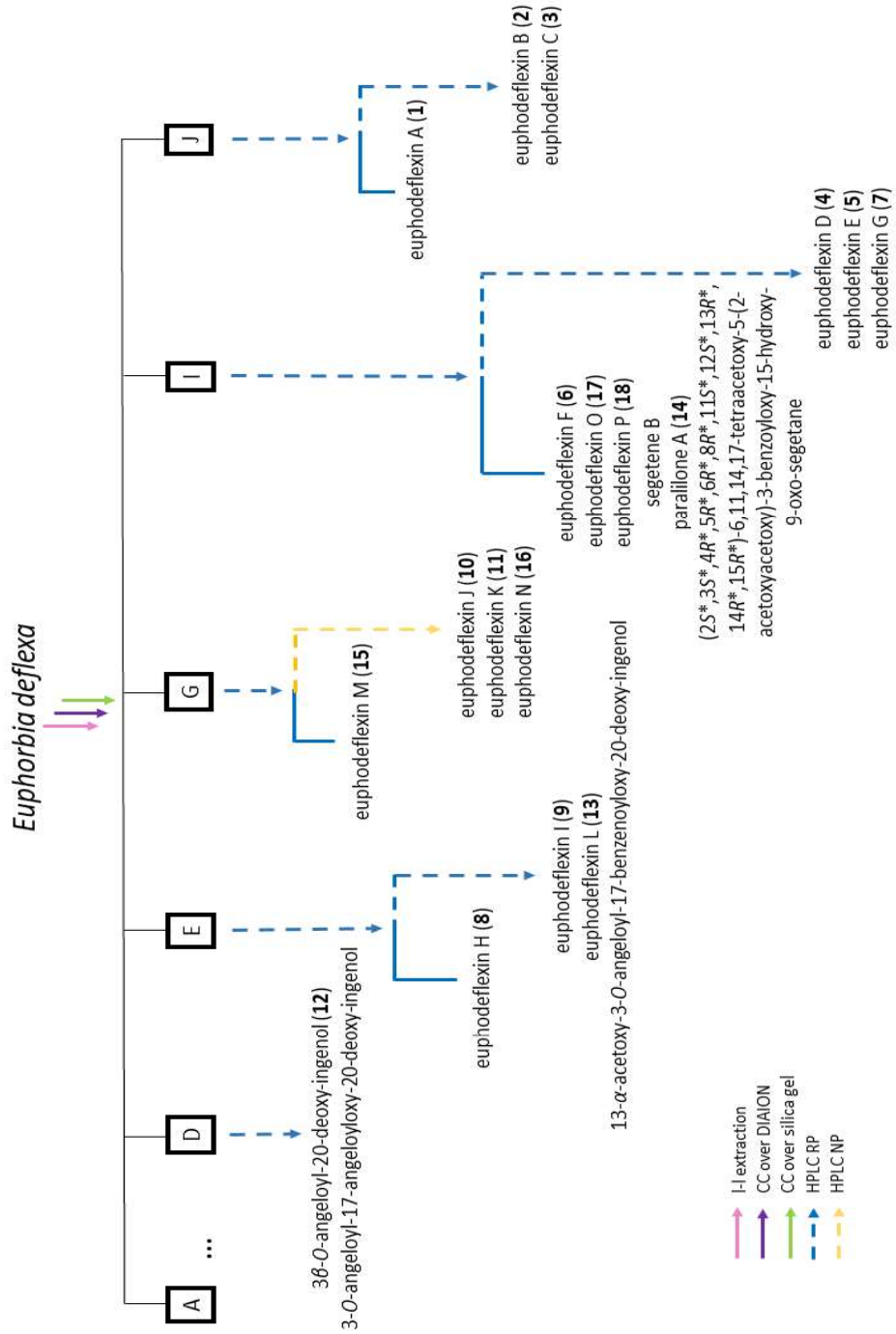

Figure 30. Chromatographic isolation of the compounds from E. deflexa.

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Article

Macrocyclic Diterpenoid Constituents of *Euphorbia deflexa*, an Endemic Spurge from Greece

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ABSTRACT: *Euphorbia deflexa*, an endemic spurge from Greece, was investigated for the occurrence of its diterpene constituents. Through continuous monitoring by ^{1}H NMR, 22 diterpenoids were isolated, including 16 previously undescribed compounds (euphodeflexins A–P), which belong to the jatrophane, ingenane, segetane, and pepluane diterpene types. Their chemical structures were elucidated through a combination of HRESIMS, NMR spectroscopy, and X-ray data. The isolated diterpenoids were tested against a panel of human cancer cell lines, as well as against two bacterial strains. Compounds **1**, **13**, and **17** were active against the HeLa cell line with IC_{50} values 9.9, 9.8, and 5.8 μM, respectively.

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Euphorbia L. (Euphorbiaceae) is one of the largest genera of flowering plants, with more than 2000 species distributed worldwide. Members of the genus, although diverse in morphology, are all readily distinguishable by the production of a milky skin-irritant latex.[1] The use of *Euphorbia* species has been reported in different human societies, and the genus includes taxa of economic importance, such as *E. tetragona*, *E. triangularis* (inferior rubber), *E. antisyphylitica* (candellila wax), and *E. resinifera* ("euphorbium").[2] Recent studies have reported that more than 5% of *Euphorbia* spp. are being used as herbal remedies.[3]

Diterpenes found in the genus *Euphorbia* have been the focus of natural product drug discovery, because of their structural diversity (e.g., jatrophane, ingenane, daphnane, tigliane skeletons) and their wide range of pharmacological activities, including antitumor and antimicrobial properties, anti-inflammatory, cardiovascular, and neuroprotective effects.[4−6] These specialized metabolites are considered important chemophenetic markers due to their restricted distribution, existing only in the families Thymelaeaceae and Euphorbiaceae.[4] Ingenol-3-angelate (ingenol mebutate), isolated from *Euphorbia peplus*, has attracted the interest of the scientific community and has been approved by the U.S. FDA in 2012 and the EMA in 2013 for actinic keratosis, a precancerous skin condition.[7,8] Despite being released to the market, obtaining ingenol mebutate by cultivation from *E. peplus* has been proven inefficient; thus alternative sources, such as synthetic or biosynthetic approaches, are being investigated.[8,9]

Taking into consideration the low percentage of *Euphorbia* species that have been investigated phytochemically so far (ca. 5%), it is likely that other taxa will be found producing higher amounts of ingenol mebutate or compounds with similar or other therapeutically relevant biological activities.[10] Thus, the aim of this project was the isolation, structure elucidation, and evaluation of the cytotoxic and antibacterial effects of diterpenoids from *E. deflexa* Sibth. & Sm., an endemic species of Greece. Through continuous monitoring by ^{1}H NMR spectroscopy, 22 diterpenoids were isolated, including 16 previously undescribed compounds (**1**−**11**, **13**, **15**−**18**, Figure 1).

RESULTS AND DISCUSSION

Eleven previously undescribed jatrophane-type diterpenes were isolated and characterized in the present study (compounds **1**−**11**, Figure 1). Compound **1**, named euphodeflexin A, was purified as colorless crystals, and its molecular formula was deduced as $C_{40}H_{48}O_{14}$ based on the obtained HRESIMS ion at m/z 753.3122 ($[M + H]^+$, calcd for $C_{40}H_{49}O_{14}^+$, 753.3117). The MS data, in combination with 1D and 2D NMR spectra, as well as literature records on isolates from the genus *Euphorbia*, suggested its polyesterified diterpenoid nature. The presence of three acetyl and two benzoyl groups was deduced from the typical ^{1}H and ^{13}C NMR signals (Table 1), which showed 20 carbon atoms in total. The remaining 20 carbon

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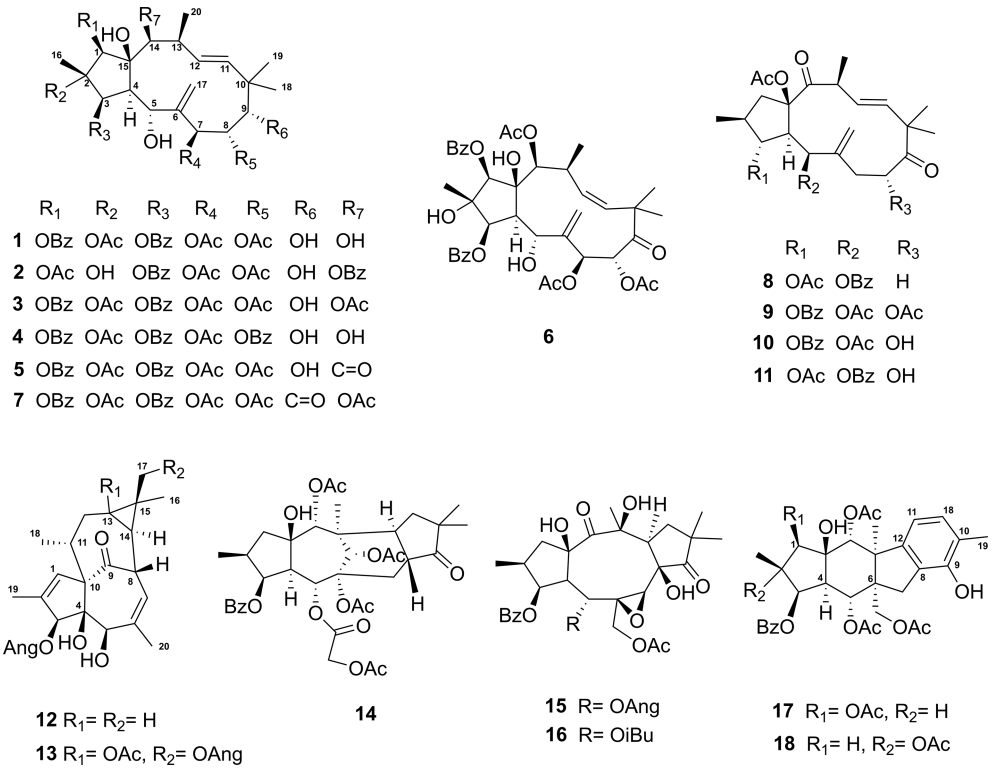

Figure 1. Structures of compounds **1**−**18**.

atoms comprised the diterpene skeleton with the characteristic pattern of four methyl groups and one exomethylene double bond. Eleven methines (two aliphatic, two olefinic and seven oxygenated) and one oxygenated tertiary carbon accounted for the remaining signals. The structural fragments A, B, and C as shown by bold bonds (Figure 2) were established from the ^{1}H−^{1}H COSY spectrum: H-3 (δ_H 6.10)/H-4 (δ_H 3.47)/H-5 (δ_H 5.19) (fragment A), H-7 (δ_H 5.44)/H-8 (δ_H 5.16)/H-9 (δ_H 3.47) (fragment B), H-11 (δ_H 5.90)/H-12 (δ_H 5.87)/H-13 (δ_H 2.78)/H-14 (δ_H 3.65) and H-13/H$_3$-20 (δ_H 1.20) (fragment C). The five-membered ring was elucidated by the HMBC cross-peaks from H-3 to C-1 (δ_C 77.2), C-2 (δ_C 90.7), C-4 (δ_C 41.5), and C-15 (δ_C 84.7). The connection of fragments A and B was suggested due to the long-range ^{1}H−^{1}H COSY signals of the H$_2$-17 (δ_H 4.80/4.82) to H-5 and H-7. This was supported further by the HMBC correlations (Figure 2) of H-5 and H-7 with C-6 (δ_C 146.9) and C-17 (δ_C 109.1), as well as of H$_2$-17 with C-5 (δ_C 70.9) and C-7 (δ_C 68.8). Concerning fragments B and C, H$_3$-18 (δ_H 1.08) and H$_3$-19 (δ_H 1.13) gave HMBC cross-peaks to C-9 (δ_C 81.1), C-10 (δ_C 41.4), and C-11 (δ_C 132.4). In turn, H-14 gave HMBC signals with C-1, C-4, and C-15, while H-7 and H-8 exhibited cross-peaks to carbonyl groups (δ_C 168.9 and 170.5), permitting the allocation of two acetoxy moieties at C-7 and C-8. The correlation between AcO-2 (δ_H 2.16) and C-2 was used to place the remaining acetoxy group at this position. Furthermore, the correlations of H-1 and H-3 with the related carbonyl carbons (δ_C 165.7, 165.3) endorsed the location of the two benzoyl groups at C-1 and C-3 (δ_C 79.4), respectively. The key NOE correlations (Figure 2) were consistent with the α-orientation of H-1, H-3, H-4, H-7, H-13, and H-14 and the β-orientation of H-5, H-8, H-9, and H$_3$-20, indicating a relative configuration in accordance with the pattern of jatrophanes reported from other *Euphorbia* spp.[4,5] The structure of **1** was confirmed by X-ray crystallographic analysis of a crystal, and its absolute configuration was assigned as (1*R*,2*S*,3*R*,4*S*,5*R*,-7*S*,8*S*,9*S*,13*S*,14*S*,15*R*)-1,3-dibenzoyloxy-2,7,8-triacetoxy-jatropha-6(17),11*E*-diene-14,15-diol (Figure 3).

Euphodeflexin B (**2**) was found to possess the same molecular formula ($C_{40}H_{48}O_{14}$) as compound **1**, as indicated by the sodium adduct ion observed at *m*/*z* 775.2943 (calcd for $C_{40}H_{48}O_{14}Na^+$, 775.2936). Spectroscopic data analysis indicated a similar structure to that of **1**, differing only in the placement of the substituents. Based on the HMBC spectrum, one acetyl and one benzoyl group were attached to C-1 (δ_C 82.7) and C-14 (δ_C 79.0), respectively, with C-2 (δ_C 81.5) having a free hydroxy group. The relative stereochemistry of **2** was identical to **1**, based on the coupling constants (Table 1) and the interpretation of the NOE signals.

The molecular formula of euphodeflexin C (**3**) was determined as $C_{42}H_{50}O_{15}$, according to its HREIMS ion at *m*/*z* 793.3074 [M − H]$^-$ (calcd for $C_{42}H_{49}O_{15}^-$, 793.3077). Structurally, compound **3** was shown to be related to compound **1** closely, but the observation of an additional acetyl group in the ^{1}H NMR spectrum was confirmed by the MS data. The former oxygenated H-14 was deshielded at 5.11 ppm (Table 1), and the HMBC cross-peak from this proton to the extra carbonyl carbon (δ_C 171.7) was used to place the remaining acetyl group (δ_H 2.27) at C-14 (δ_C 78.9). The relative configuration of **3** was assigned as being identical to that of **1**, on the basis of NOESY signals observed.

Euphodeflexin D (**4**) was deduced to have a molecular formula of $C_{45}H_{50}O_{14}$, based on the HREIMS ion at *m*/*z* 813.3136 [M − H]$^-$ (calcd for $C_{45}H_{49}O_{14}^-$, 813.3128). Analysis of the NMR data (Table 1) revealed **4** to be also structurally related to the above-mentioned isolates **1**−**3**, although an extra set of benzoyl group signals was observed. The position of the additional benzoyl moiety (δ_C 73.0) was

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data (600 and 150 MHz, $CDCl_3$) for Compounds 1−4

	euphodeflexin A (1)		euphodeflexin B (2)		euphodeflexin C (3)		euphodeflexin D (4)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C[b], type	δ_H (J in Hz)
1	77.2[a] CH	6.14, s	82.7 CH	5.30,[a] s	76.9[a] CH	6.26, s	77.2 CH	6.16, s
2	90.7 C		81.5 C		90.7 C		90.7 C	
3	79.4 CH	6.10, d (6.8)	81.4 CH	5.72, d (5.2)	79.4 CH	6.17, d (6.8)	79.5 CH	6.14, d (6.6)
4	41.5[a] CH	3.47[a]	41.3 CH	3.16, dd (5.2, 2.1)	42.1 CH	3.57, dd (6.8, 3.1)	41.5 CH	3.53, dd (6.6, 3.4)
5	70.9 CH	5.19, br s	70.2 CH	5.03, br s	70.8 CH	5.20, br s	71.2 CH	5.28, br s
6	146.9 C		146.3 C		146.0 C		146.8 C	
7	68.8 CH	5.44, s	69.2 CH	5.48, s	69.1 CH	5.47, s	69.0 CH	5.56, s
8	72.3 CH	5.16, s	72.1 CH	5.12, s	72.2 CH	5.23, d (2.1)	73.0 CH	5.38, s
9	81.1 CH	3.47,[a]	81.1 CH	3.55, s	80.1 CH	3.49, d (2.1)	81.1 CH	3.63, s
10	41.4[a] C		42.4 C		41.6 C		41.7 C	
11	132.4 CH	5.90, d (16.0)	134.3 CH	5.85, d (16.0)	134.6 CH	5.94, d (16.3)	132.5 CH	5.93, d (16.0)
12	132.9 CH	5.87, dd (16.0, 9.4)	130.8 CH	5.65, dd (16.0, 9.6)	131.8 CH	5.67, dd (16.3, 9.6)	133.2 CH	5.98, dd (16.0, 8.6)
13	37.9 CH	2.78, m	37.0 CH	2.89, m	37.6 CH	2.92, m	37.9 CH	2.81, m
14	77.6 CH	3.65, s	79.0 CH	5.30,[a] s	78.9 CH	5.11, s	77.7 CH	3.67, s
15	84.7 C		84.9 C		85.0 C		84.9 C	
16	18.1 CH_3	1.44, s	20.0 CH_3	1.26, s	18.3 CH_3	1.39, s	18.2 CH_3	1.45, s
17a	109.1 CH_2	4.80, s	110.1 CH_2	4.97, br s	109.0 CH_2	4.82, br s	109.7 CH_2	4.72, s
17b		4.82, s		4.98, br s		4.84, br s		4.80, s
18	23.5 CH_3	1.08, s	23.5 CH_3	1.10, s	23.2 CH_3	1.05, s	23.5 CH_3	1.15, s
19	26.6 CH_3	1.13, s	26.5 CH_3	1.13, s	26.6 CH_3	1.16, s	26.6 CH_3	1.16, s
20	24.2 CH_3	1.20, d (6.9)	23.5 CH_3	1.17, d (7.0)	23.1 CH_3	1.10, d (6.9)	24.2 CH_3	1.23, d (6.9)
OAc-1			21.0 CH_3	2.26, s				
C=O			171.9 C					
OAc-2	22.1 CH_3	2.16, s			21.9 CH_3	2.17, s	21.0 CH_3	2.18, s
C=O	171.2 C				171.8 C		171.2 C	
OAc-7	20.9 CH_3	2.13, s	21.1 CH_3	2.18, s	20.9 CH_3	2.14, s	22.2 CH_3	2.17, s
C=O	168.9 C		169.9 C		168.8 C		168.8 C	
OAc-8	20.7 CH_3	2.00, s	20.7 CH_3	2.04, s	20.7 CH_3	2.03, s		
C=O	170.5 C		170.2 C		170.3 C			
OAc-14					21.2 CH_3	2.27, s		
C=O					171.7 C			
OBz	*1/3-*		*1/3-*		*1/3-*		*1/3/8-*	
C=O	165.7 C/ 165.3 C		166.1 C/ 165.7 C		165.7 C/ 165.1 C		166.0 C/ 165.7 C/ 165.6 C	
1	129.5 C/ 129.9 C		130.0 C/ 129.9 C		130.1 C/ 130.0 C		133.0 C/ 129.9 C	
2, 6	129.9 CH	8.11, d (7.6)/ 8.12, d (7.6)	129.9 CH/ 129.8 CH	8.08, d (7.4)/ 8.06, d (7.6)	130.0 CH/ 129.8 CH	8.13, d (7.8)/ 8.06, d (7.8)	130.0 CH/ 129.9 CH	8.12, d (7.6)/8.17, d (7.6)/8.0, d (7.6)
3, 5	128.5 CH/ 128.4 CH	7.44, d (7.6)/ 7.43, d (7.6)	128.6 CH/ 128.5 CH	7.44, d (7.4)/ 7.42, d (7.4)	128.6 CH/ 128.5 CH	7.48, d (7.8)/ 7.43, d (7.8)	128.6 CH/ 128.5 CH	7.46, d (7.6)/7.40[a]
4	133.4 CH/ 133.0 CH	7.56, t (7.6)/7.57 t (7.6)	133.7 CH/ 133.1 CH	7.59, t (7.4)/7.56 t (7.4)	133.7 CH/ 133.1 CH	7.60, t (7.8)/ 7.56, t (7.8)	133.4 CH/ 133.2 CH	7.58, t (7.6)/7.54,[a] 7.52,[a]

[a]Overlapped signals. [b]Assigned from the HSQC/HMBC spectra.

determined at C-8 by the HMBC cross-peak between H-8 (δ_H 5.38) and the carbonyl carbon (δ_C 165.6) of the benzoyl group. Following similar biosynthetic reasoning, the relative configuration of **4** was proposed as being the same as that of compounds **1**−**3**.

The (−)-HREIMS data of euphodeflexin E (**5**) showed an $[M + Cl]^-$ ion at m/z 785.2584 (calcd for $C_{40}H_{46}O_{14}Cl^-$, 785.2582), corresponding to the molecular formula $C_{40}H_{46}O_{14}$. The NMR data (Table 2) revealed that **5** is also a jatrophane-type diterpene that exhibited some similarities to the above-mentioned compounds, with a key difference being the lack of an oxygenated H-14, with also a downfield-shifted H-13 (δ_H 4.04) and the appearance of a carbonyl group signal (δ_C 210.6) at ^{13}C NMR spectrum. The HMBC signals within the C-13−C-15 sequence demonstrated the carbonyl group to be located at C-14, similar to previously isolated jatrophanes.[11,12]

Euphodeflexin F (**6**) afforded the same molecular formula, $C_{40}H_{46}O_{14}$, as was determined for compound **5** by the HREIMS ion at m/z 749.2816 $[M - H]^-$ (calcd for $C_{40}H_{45}O_{14}^-$, 749.2815), while euphodeflexin G (**7**) gave the molecular formula $C_{42}H_{48}O_{15}$, based on the HREIMS ion at m/z 827.2695 $[M + Cl]^-$ (calcd for $C_{42}H_{48}O_{15}Cl^-$, 827.2687). The ^{1}H NMR spectrum and the MS data of **7** suggested the presence of an additional acetyl group in comparison to compound **6**. Similar to **5**, compounds **6** and **7** were shown to be characterized by the absence of H-9, followed by the downfield-shifted H-8 (δ_H ~5.80), as well as the appearance of a carbonyl group resonance (δ_H ~205) in the ^{13}C NMR

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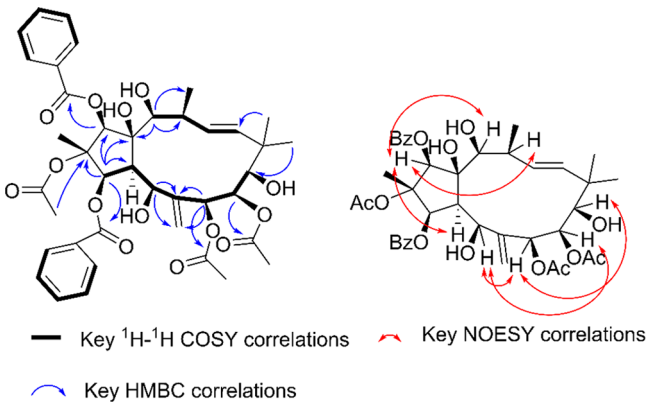

Figure 2. Key HMBC, ^{1}H−^{1}H COSY, and NOESY correlations of euphodeflexin A (**1**).

spectrum (Table 2). In this case, the carbonyl group could be located at C-9, based on the HMBC cross-peaks within the C-7−C-11 sequence. A noticeable difference of compound **6** reflected the lack of coupling constant between protons H-11 and H-12, which appeared as singlets in the ^{1}H NMR spectrum (vs doublets J 16.0 Hz for the remainder of the isolated compounds), allowing the *cis*-configuration of the Δ_{11-12} double bond to be proposed. Compound **7** displayed the typical configuration of the jatrophanes, and the above-mentioned additional acetyl group was placed at position C-2 based on the NMR data obtained.

The molecular formula of euphodeflexin H (**8**) was determined as $C_{31}H_{38}O_8$, based on the HRESIMS ion at m/z 539.2643 ([M + H]$^+$, calcd for $C_{31}H_{39}O_8^+$, 539.2639). Comparison of its 1D and 2D NMR data with those of the above-mentioned compounds suggested that it also possessed a jatrophane scaffold, but with a different substitution pattern, and it was identified as a 9,14-dione with two aliphatic methylenes at C-7 and C-8 (Table 3). This was supported by the chemical shifts of C-9 (δ_C 212.0) and C-10 (δ_C 50.5), as well as C-7 (δ_C 34.2), C-8 (δ_C 25.6), H_2-7 (δ_H 2.19, 2.65), and H_2-8 (δ_H 1.88, 2.16), together with the HMBC correlations of H-12 (δ_H 5.77), H_3-20 (δ_H 1.37), and H_2-1 (δ_H 2.13, 2.86) to C-14 (δ_C 203.6), of H_2-7, H_2-8, H-11 (δ_H 5.62), H_3-18 (δ_H 1.20), and H_3-19 (δ_H 1.22) to C-9, and of H_2-8 to C-7. The cross-peaks between H-3 (δ_H 5.66) and the carbonyl carbon at δ_C 170.0, H-5 (δ_H 5.99) and the carbonyl carbon at δ_C 165.0, and the methyl group of the AcO-15 (δ_H 2.12) and C-15 (δ_C 90.7) supported placing the benzoyl and acetyl groups at positions C-3, C-5, and C-15, respectively. The relative configuration of **8** was deduced by the NOE signals between H-2/H-4, which were assigned as α-orientated, while the correlations between H-3/H_3-16, H-3/H_3-20, and H_3-16/AcO-15 indicated the β-orientation. This was supported by the values of $J_{2,3}$ and $J_{3,4}$ (~3 Hz) and $J_{4,5}$ (~10 Hz).[13]

Compound **9**, euphodeflexin I, gave a molecular formula of $C_{33}H_{40}O_{10}$, based on the HRESIMS ion observed at m/z 597.2692 ([M + H]$^+$, calcd for $C_{33}H_{41}O_{10}^+$, 597.2694). The planar structure of **9** proved to be comparable to compound **8**. On the basis of the ^{1}H NMR data (Table 3), an additional acetyl group signal was observed, as well as the exchange of one methylene with an oxygenated methine. In comparison to the majority of the isolated jatrophanes, the ^{1}H−^{1}H COSY experiment showed the extra spin system H_2-7/H-8, with H-8 being shifted downfield to 5.22 ppm, suggesting the location of the additional acetyl group at C-8. These inferences were supported by the HMBC correlations from H_2-7 (δ_H 2.07) to C-6 (δ_C 137.9) and C-17 (δ_C 116.39), together with the cross-peaks from H-8 (δ_H 5.22) to C-6 and the carbonyl carbon of AcO-8 (δ_C 170.1).

Compounds **10** and **11**, euphodeflexins J and K, were characterized as positional isomers with the molecular formula of $C_{31}H_{38}O_9$, based on the HRESIMS ions obtained observed at m/z 555.2591 and 555.2592, respectively ([M + H]$^+$, calcd for $C_{31}H_{39}O_9^+$, 555.2589). The NMR data (Table 3) permitted the structure elucidation of these compounds. Featuring the same carbon skeleton as compound **8**, isolates **10** and **11** were differentiated by their respective substitution at position 8. Both **10** and **11** were found to be similar to compound **9**, but C-8 of both compounds **10** and **11** (δ_C 71.0 and δ_C 71.2, respectively) was substituted by −OH. In the HMBC experiment, a correlation signal of C-8 with the carbonyl carbon at δ_C 213.8 (C-9) supported the given structures. However, **10** and **11** differed in their respective esterification pattern in the jatrophane skeleton. A benzoyl and an acetyl group were linked at C-3 (δ_C 77.0) and C-5 (δ_C 72.2), respectively, for compound **10**, while these substitutions were reversed for compound **11** (C-3 at δ_C 76.5; C-5 at δ_C 73.5), based on the HMBC correlations from the corresponding protons to the carbonyl carbons of the substitution, i.e., compound **10**: H-3 (δ_H 5.84)/OBz-3 (δ_C 165.7) and H-5 (δ_H 5.80)/OAc-5 (δ_C 168.6); compound **11**: H-3 (δ_H 5.63)/OAc-3 (δ_C 170.0) and H-5 (δ_H 6.08)/OBz-5 (δ_C 164.7).

In addition, four diterpenoids possessing an ingenol-type scaffold were isolated, namely, 3β-*O*-angeloyl-20-deoxyingenol (**12**),[14] 3-*O*-angeloyl-17-angeloyloxy-20-deoxyingenol,[14] and 13α-acetoxy-3-*O*-angeloyl-17-benzenoyloxy-20-deoxyinge-

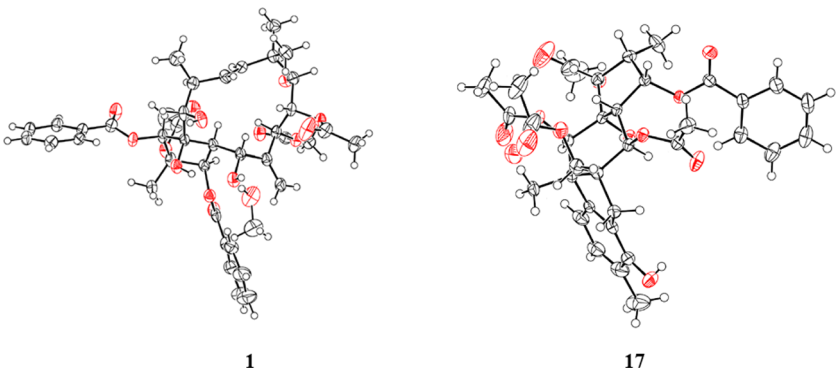

Figure 3. ORTEP diagrams of compounds **1** and **17**.

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Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data (600 and 150 MHz, $CDCl_3$) for Compounds 5−7

	euphodeflexin E (5)		euphodeflexin F (6)		euphodeflexin G (7)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	77.8 CH	6.14, s	82.3[b] CH	5.25, s	77.6,[a] CH	6.25, s
2	90.8 C		81.5[b] C		90.3 C	
3	77.6 CH	6.17, d (5.8)	80.9 CH	5.53, d (4.7)	78.5 CH	5.97, d (7.3)
4	41.1 CH	3.71, dd (5.8, 2.0)	43.6[b] CH	3.28, dd (4.7, 2.3)	42.2 CH	3.60, dd (7.3, 2.4)
5	69.0 CH	4.26, br s	69.5 CH	5.05, br s	69.8 CH	5.09, br s
6	144.9 C		143.2[b] C		143.5 C	
7	69.1 CH	5.38,[a]	67.1 CH	5.82, br s	66.9 CH	5.75 br s
8	71.6 CH	4.89, br s	71.1 CH	5.76, br s	70.7 CH	5.81, br s
9	81.6 CH	3.53, br s	205.1 C		204.8 C	
10	41.3 C		49.9 C		50.2 C	
11	137.5 CH	6.05, d (16.0)	134.8[b] CH	5.88, s	135.3 CH	6.07, d (16.0)
12	129.1 CH	5.39, dd (16.0, 9.5)	134.8[b] CH	5.88, s	134.0 CH	5.85, d (16.0, 9.7)
13	44.6 CH	4.04, m	36.9[b] CH	3.08, m	37.5 CH	2.99, m
14	210.6 C		80.1 CH	5.45, br s	79.0 C	5.13, br s
15	89.3 C		85.0 C		85.2 C	
16	16.7 CH_3	1.56, s	19.7 CH_3	1.30, s	18.2 CH_3	1.40, s
17a	112.0 CH_2	5.02, br s	112.0 CH_2	5.17, br s	111.3 CH_2	4.99, br s
17b		5.11, br s		5.22, br s		5.12, br s
18	23.2 CH_3	1.11, s	24.4 CH_3	1.20, s	24.2 CH_3	1.24, s
19	26.3 CH_3	1.11. s	25.6 CH_3	1.33, s	25.5 CH_3	1.31. s
20	19.9 CH_3	1.40, d (6.6)	18.9 CH_3	1.28, d (6.6)	23.4 CH_3	1.18, d (6.9)
OAc-2	22.1 CH_3	2.22, s			21.9	2.16
C═O	170.6 C				170.4	
OAc-7	21.1 CH_3	2.16, s	20.8 CH_3	2.18, s	20.8 CH_3	2.13, s
C═O	169.2 C		170.3 C		169.6 C	
OAc-8	20.6 CH_3	1.99, s	20.4 CH_3	2.08, s	20.4 CH_3	2.06, s
C═O	169.8 C		170.6 C		169.9 C	
OAc-14			20.9	2.25, s	20.9	2.27, s
C═O			170.6 C		172.0 C	
OBz	*1/3-*		*1/3-*		*1/3-*	
C═O	165.1 C/165.3 C		165.3 C/165.9 C		165.0/167.0	
1	129.7 C/129.8 C		129.9 C		129.2/129.4	
2,6	129.9 CH/130.1 CH	8.03, d (7.6)/8.14, d (7.6)	129.7 CH/129.8 CH	8.07, d (7.4)/8.09, d (7.4)	129.9/130.0	8.10, d (7.4)/8.12, d (7.4)
3,5	128.5 CH/128.6 CH	7.42, d (7.6)/7.44, d (7.6)	128.6 CH/128.7 CH	7.44,[a]	128.6/128.7	7.47, d (7.4)/7.49, d (7.4)
4	133.1 CH/133.7 CH	7.56, t (7.6)/7.59 t (7.6)	133.2 CH	7.59,[a]	133.4	7.59, t (7.4)/7.62, t (7.4)

[a]Overlapped signals. [b]Assigned from the HSQC/HMBC spectra.

nol,[11] together with the previously undescribed euphodeflexin L (**13**). Compound **13** was isolated as a white amorphous solid and was assigned a molecular formula of $C_{32}H_{42}O_9$ based on the HRESIMS ion observed at m/z 571.2914 $[M + H]^+$ (calcd for $C_{32}H_{43}O_9^+$, 571.2902). A direct comparison of the NMR spectra (Table 4) of compound **13** with the co-occurring 13α-acetoxy-3-*O*-angeloyl-17-benzenoyloxy-20-deoxyingenol[11] revealed that they were almost identical and different only at the substitution on C-17. In the ^{1}H NMR spectrum, the signals for the benzoyl group were missing and were replaced by a second set of angelate signals. The additional angelate group was placed at this position (C-17, δ_C 64.4) as proposed by the HMBC signals from H_2-17 (δ_H 4.25, 4.35) to the carbonyl carbon (δ_C 167.9) (Figure 4).

Moreover, three segetane-type diterpenes were isolated, namely, segetene B,[15] paralinone A (**14**),[16] and (2*S**,3*S**,4*R**,5*R**,6*R**,8*R**,11*S**,12*S**,13*R**,14*R**,15*R**)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxy-9-oxo-segetane,[11] together with two previously undescribed presegetanes, euphodeflexins M (**15**) and N (**16**). The latter compounds were isolated as white amorphous solids, and their molecular formulas were deduced, in turn, as $C_{34}H_{42}O_{12}$ and $C_{33}H_{42}O_{12}$ (m/z 643.2753 $[M + H]^+$, calcd for $C_{34}H_{43}O_{12}^+$, 643.2749 and m/z 631.2760 $[M + H]^+$, calcd for $C_{33}H_{43}O_{12}^+$, 631.2749, respectively). In comparison to the closely related structure of presegatanin,[16] an upfield shift of the oxygenated carbon C-6 (~δ_C 60) in the ^{13}C NMR spectrum and the absence of two −OH signals in the ^{1}H NMR spectrum were observed (Table 5). These resonances provided evidence for the presence of an epoxy group, which was in full accordance with the respective molecular formulas of **15** and **16** obtained from the HRESIMS. Based on the 1D and 2D NMR data of compound **15**, one acetyl, one benzoyl, and one angeloyl group each were detected as substituents of the main diterpene skeleton, encompassing the remaining 20 carbons. In detail, the planar structure consisted of four methyl groups, two carbonyl groups, eight oxygenated carbons (three protonated, five tertiary), three methylenes, two methines, and one quaternary carbon. The $^{1}H-^{1}H$ COSY experiment (Figure 5) revealed two spin systems: H_2-1 (δ_H 2.53, 1.83)/H-2 (δ_H 2.46)/H-3 (δ_H 5.57)/H-4 (δ_H 2.99)/H-5 (δ_H 5.46), H-2/H_3-16 (δ_H 1.00) and H_2-11 (δ_H 2.00, 2.33)/H-12 (δ_H 3.08). Furthermore, apart from these signals in the ^{1}H NMR spectrum, one isolated proton (H-7, δ_H 3.25) was detected.

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Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data (600 and 150 MHz, $CDCl_3$) for Compounds 8−11

	euphodeflexin H (8)		euphodeflexin I (9)		euphodeflexin J (10)		euphodeflexin K (11)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1a	44.8 CH_2	2.13,[a]	45.1 CH_2	2.27,[a]	45.1 CH_2	2.26,[a]	45.0 CH_2	2.15, dd (15.6, 12.9)
1b		2.86, dd (15.7, 7.5)		2.93, dd (15.9, 8.3)		2.94, dd (15.7, 8.2)		2.87, dd (15.6, 7.8)
2	39.0 CH	2.34, m	39.0 CH	2.42, m	39.1 CH	2.40, m	39.0 CH	2.34, m
3	76.6 CH	5.66, t (3.9)	77.4[a] CH	5.86,[a]	77.0[a] CH	5.84, t (3.4)	76.5 CH	5.63, t (3.8)
4	49.5 CH	2.80, dd (9.5, 3.9)	49.2 CH	2.76, dd (10.1, 3.5)	49.5 CH	2.73, dd (10.0, 3.4)	49.2 CH	2.77, dd (9.9, 3.8)
5	72.9 CH	5.99, d (9.5)	72.3 CH	5.80, d (10.1)	72.2 CH	5.80,[a]	73.1 CH	6.08, d (9.9)
6	141.5 C		137.9 C		138.6 C		138.7 C	
7a	34.2 CH_2	2.19, m	29.0 CH_2	2.07, m	33.4 CH_2	1.65,[a]	33.3 CH_2	1.17,[a]
7b		2.65, m				2.18, d (17.7)		2.24, d (17.9)
8(a)	25.6 CH_2	1.88, m	73.0 CH_2	5.22, dd (7.4, 1.9)	71.0 CH_2	4.48, d (7.9)	71.2 CH_2	4.49, d (7.9)
8b		2.16, m						
9	212.0 C		207.6 C		213.7 C		213.8 C	
10	50.5 C		49.5 C		49.5 C		48.2 C	
11	132.5 CH	5.62, d (16.0)	134.9 CH	5.62, d (16.0)	134.8 CH	5.66, d (16.1)	132.6 CH	5.68, d (16.0)
12	134.9 CH	5.77, dd (16.0, 9.2)	132.9 CH	5.86,[a]	132.7 CH	5.78,[a]	134.8 CH	5.80, dd (16.0, 9.2)
13	44.0 CH	3.37, m	43.9 CH	3.37, m	43.5 CH	3.39, m	43.3 CH	3.38, m
14	203.6 C		203.2 C		203.2 C		203.3 C	
15	90.7 C		90.6 C		90.6 C		90.5 C	
16	13.9 CH_3	1.00, d (6.4)	13.8 CH_3	1.07, d (6.7)	13.8 CH_3	1.05, d (6.5)	13.8 CH_3	1.00, d (6.6)
17a	116.5 CH_2	4.94, br s	116.9 CH_2	4.96, br s	117.7 CH_2	5.32, br s	117.8 CH_2	5.40, br s
17b		5.26, br s		5.27, br s		5.39, br s		5.45, d (2.1)
18	23.2 CH_3	1.20, s	23.9 CH_3	1.25, s	25.2 CH_3	1.25, s	25.2 CH_3	1.26, s
19	23.3 CH_3	1.22, s	25.3 CH_3	1.39, s	24.0 CH_3	1.31, s	23.5 CH_3	1.32, s
20	20.1 CH_3	1.37, d (6.5)	20.2 CH_3	1.40,[a]	20.1 CH_3	1.40,[a]		20.1 CH_3
OAc-3	20.6 CH_3	1.98, s					20.6 CH_3	2.01, s
C═O	170.0 C						170.0 C	
OAc-5			20.8 CH_3	1.89, s	20.7 CH_3	1.84, s		
C═O			168.9 C		168.6 C			
OAc-8			20.5 CH_3	2.06, s				
C═O			170.1 C					
OAc-15	21.3 CH_3	2.12, s	21.3 CH_3	2.28, s	21.3 CH_3	2.25, s	21.3 CH_3	2.18, s
C═O	169.2 C		169.0 C		169.1 C		169.3 C	
OBz	5-		3-		3-		5-	
C═O	165.0 C		165.7 C		165.7 C		164.7 C	
1	129.8 C		129.6 C		129.7 C		129.8 C	
2,6	129.6 CH	7.95, d (7.5)	129.5 CH	7.99, d (7.6)	129.5 CH	8.00, d (7.5)	129.5 CH	7.91, d (7.5)
3,5	128.4 CH	7.42, d (7.5)	128.5 CH	7.45, d (7.6)	128.4 CH	7.45, d (7.5)	128.4 CH	7.42, d (7.5)
4	133.2 CH	7.56, t (7.5)	133.24 CH	7.59, t (7.6)	133.3 CH	7.58, t (7.5)	133.1 CH	7.55, t (7.5)

[a]Overlapped signals.

A five-membered ring could be located at C-4/C-15 based on the HMBC correlations (Figure 5) from H_2-1, H-2, H-3, and H-4 to C-15 (δ_C 87.9). The carbonyl group at δ_C 220.0 was assigned at C-14 due to the observed HMBC correlations from H_2-1, H-4, and H_3-20 (δ_H 1.61). The location of a second five-membered ring was assigned based on the HMBC signals between H_3-18 (δ_H 1.11)/H_3-19 (δ_H 1.27) and C-11 (δ_C 34.7), C-12 (δ_C 46.8), and C-9 (the second carbonyl group δ_C 212.9), H-12 and C-7 (δ_C 64.1), C-8 (δ_C 76.2), and C-13 (δ_C 82.1), and OH-8 (δ_H 5.23) and C-8, C-9, and C-12. Further data from the HMBC spectrum, and particularly the correlations from H-4, H-5, and H_2-17 (δ_H 3.23, 5.28) to the oxygenated tertiary C-6 (δ_C 59.6) and from H_2-17 to C-7, allowed the structure elucidation of the basic diterpene skeleton. The cross-peaks from H-3, H-5, and H_2-17 to the carbonyl groups at δ_C 165.7, 165.4, and 172.7 were used to place the benzoyl, angeloyl, and acetyl groups at these positions, respectively. Compound **16** was found to be identical to compound **15**, differing only at the substitution of position C-5. This was assigned with an isobutyl group, since instead of the typical angeloyl signals, a septet and two doublets appeared in the ^{1}H NMR spectrum. The relative configurations of compounds **15** and **16** were identical to that of other members of the segetane diterpenoid class isolated to date,[11,17,18] based on the coupling constants (Table 5) and interpretation of NOE signals (Figure 5).

Two diterpenoids (**17** and **18**) belonging to the pepluane class were isolated. Euphodeflexin O (**17**), isolated as colorless crystals, was found to possess a molecular formula of $C_{35}H_{40}O_{12}$, as indicated by the sodium adduct ion observed at m/z 675.2419 (calcd for $C_{35}H_{40}O_{12}Na^+$, 675.2412). The characteristic geminal methyl signals were absent, and the ^{13}C NMR data indicated the presence of a tetrasubstituted aromatic ring. The ^{1}H NMR data of **17** (Table 6) were almost identical to that of (1*S**,2*R**,3*S**,4*R**,5*R**,6*R**,13*S**,14*R**,15*R**)-1,5,14,17-tetraacetoxy-3-benzoyloxy-8,10,(18)11-hexadehydropepluane-11,15-diol, isolated from *E. segetalis*,[11] possessing the same pattern of protonated and quaternary carbons of the diterpene scaffold. These corresponded to three methyl groups (including one aromatic), two

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Table 4. ^{1}H and ^{13}C NMR Spectroscopic Data (600 and 150 MHz, $CDCl_3$) for Euphodeflexin L (13)

position	δ_C, type	δ_H (J in Hz)
1	131.5 CH	6.05, d (1.0)
2	136.2 C	
3	82.7 CH	5.46, s
4	84.7 C	
5	77.4 CH	3.70, s
6	138.0 C	
7	122.2 C	5.67, dd (4.4, 1.0)
8	42.5 CH	4.06, br d
9	205.9 C	
10	72.0 C	
11	37.9 CH	2.59, m
12	35.2 CH_2	2.35, (16.9, 5.0)
		2.76, (16.9, 3.5)
13	68.7 C	
14	28.6 CH_3	1.38, d (12.5)
15	33.9 C	
16	18.5 CH_3	1.17, s
17	64.4 CH_2	4.25, d (11.8)
		4.35, d (11.8
18	17.9 CH_3	0.99, d (7.1)
19	15.6 CH_3	1.81, d (1.0)
20	21.9 CH_3	1.76, s
OH-4		3.53, s
OH-5		3.08, br
OAng-3		
C=O	168.6 C	
	15.9 CH_3	1.99, br s
	127.8 C	
	140.2 CH	6.17, dq (7.3, 1.3)
	20.7 CH_3	1.91, br s
OAng-17		
C=O	167.9 C	
	15.8 CH_3	2.0, br s
	126.9 C	
	138.3 CH	6.09, dq (7.3, 1.3)
	20.6 CH_3	1.92, brs
OAc-13	21.1 CH_3	1.98, s
C=O	170.8 C	

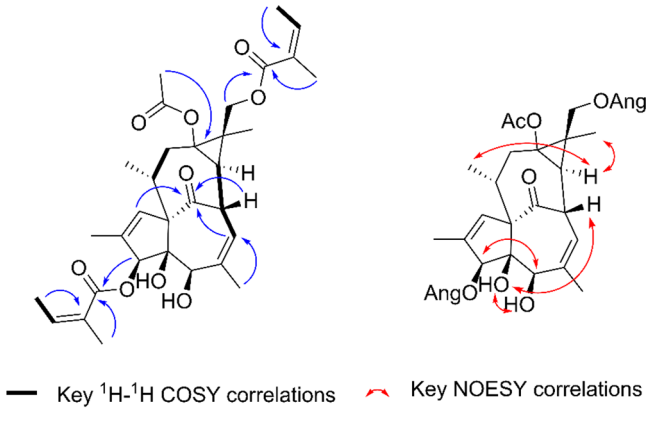

Figure 4. Key HMBC, ^{1}H−^{1}H COSY, and NOESY correlations of euphodeflexin L (**13**).

methylenes (one oxygenated), and eight methines (two aliphatic, four oxygenated, and two aromatic), as well as seven nonprotonated carbons (including two quaternary, one oxygenated tertiary, and four aromatic). Indeed, the 7.8 Hz vicinal coupling of the aromatic signals required their *ortho* positioning. A close observation of the HMBC spectrum revealed a different arrangement of the six-membered ring, with correlations of signals from H-14 (δ_H 5.59) and H_3-20 (δ_H 1.07) to C-12 (δ_C 148.3), as well as from H_2-7 (δ_H 2.60, 2.85) to C-8 (δ_C 123.2), C-9 (δ_C 151.2), and C-12 (δ_C 148.3). The HMBC experiment was used also to infer the locations of the substituents, establishing the linkage of four acetyl groups and one benzoyl group at C-1, C-5, C-13, C-17, and C-3, respectively. The relative configuration of compound **17** was deduced from the NOE correlated signals of H-4 (δ_H 2.72) with H-1 (δ_H 5.02) and H-2 (δ_H 2.83), respectively, indicating that they are α-oriented. The NOEs of H-7a (δ_H 2.60) with H-2 and H_3-20 (δ_H 1.07) suggested these all to be cofacial. However, H-5 (δ_H 5.52) and H-14 gave a NOE signal indicating them as being β-oriented (Figure 6). The structure of compound **17** was confirmed by X-ray crystallographic analysis as (1*S*,2*R*,3*S*,4*R*,5*R*,6*R*,13*S*,14*R*,15*R*)-1,5,14,17-tetraacetoxy-3-benzoyloxy-8,9,10,18,11,12-hexadehydropepluane-9,15-diol (Figure 3). The proposed biogenesis of compound **17** is presented in Figure S1, Supporting Information.

Compound **18** gave the same molecular formula as **17**, based on its HRESIMS at m/z 675.2413 (calcd for $C_{35}H_{40}O_{12}Na^+$, 675.2412). The ^{1}H NMR spectrum of **18** was similar to that of **17**, with a replacement of the doublet at 5.0 ppm with two doublets due to a methylene at position C-1 (H_2-1, δ_H 2.07, 2.47), the absence of a H-2 signal, and a downfield shift of H_3-16 (from 0.77 to 1.44 ppm) appearing as a singlet (Table 6). Moreover, the downfield-shifted signal of H-4 at δ_H 3.43 was a result of the cofacial orientation with the acetyl group at C-2 (δ_C 90.3). The relative stereochemistry of **18** was the same as **17**, as well as other pepluanes, based on the NOESY spectrum.[11,19]

Seven isolated diterpenoids based on different skeletons (compounds **1**, **12**−**15**, **17**, **18**) that were available in significant quantities were subjected to an MTT cytotoxicity evaluation[20] against four human cancer cell lines, i.e., HeLa, SK-MEL-28, HePG2, and PC-3 cells. Parthenolide was used a positive control. Compounds **1**, **13**, and **17** exhibited cytotoxic effects against HeLa cells (Table 7), while the remaining cell lines were not affected. With IC_{50} values (for HeLa cells) of 9.9, 9.8, and 5.8 μM, respectively, compounds **1**, **13**, and **17** are considered active according to NCI guidelines.[21] Compounds **12**, **14**, **15**, **17**, and **18** did not show significant cytotoxic activity against any of the cell lines. Moreover, compounds **1**, **13**, and **15** were further assessed against *Staphylococcus aureus* and *Streptococcus pyogenes* and showed no discernible antibacterial effects using the double dilution method[22] and chloramphenicol as a positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. See S1, Supporting Information.

Plant Material. The aerial parts from *E. deflexa* were collected from natural populations in Mount Parnitha, Central Greece (coordinates (WGS84): latitude: 38°08′58.4″ N; longtitude: 23°43′18.0″ E) during the flowering stage in May 2018. The collected plant material was identified and authenticated by Prof. Th. Contsantinidis (Faculty of Biology, NKUA). A voucher specimen has been deposited in the Department of Pharmacognosy and Chemistry

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Table 5. ^{1}H and ^{13}C NMR Spectroscopic Data (600 and 150 MHz, $CDCl_3$) for Compounds 15 and 16

	euphodeflexin M (15)		euphodeflexin N (16)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1a	53.6 CH_2	1.83,[a]	53.8 CH_2	1.82, d (7.5)
1b		2.53, dd (13.4, 7.6)		2.56, dd (13.4, 7.5)
2	38.9 CH	2.46, m	38.9 CH	2.47, m
3	78.0 CH	5.57, t (3.5)	77.9 CH	5.57, t (3.5)
4	61.7 CH	2.99, dd (10.4, 3.5)	61.6 CH	2.99, dd (10.4, 3.5)
5	64.2[a] CH	5.46, d (10.4)	64.0 CH	5.32, d (10.4)
6	59.6 C		59.5 C	
7	64.1[a] CH	3.25, s	64.0 CH	3.23, s
8	76.2 C		76.1 C	
9	212.9 C		212.6 C	
10	44.1 C		44.1 C	
11a	34.7 CH_2	2.00, dd (12.3, 6.3), 2.33, t (12.3)	34.6 CH_2	2.00, dd (12.6, 5.8), 2.33, t (12.6)
11b				
12	46.8 CH	3.08, dd (12.3, 6.3)	46.8 CH	3.07 dd (12.6, 5.8)
13	82.1 C		82.1 C	
14	220.0 C		219.9 C	
15	87.9 C		87.9 C	
16	13.6 CH_3	1.00, d (6.5)	13.6 CH_3	1.01, d (6.3)
17	65.3 CH_2	3.23, d (11.5)	65.2 CH_2	3.23, d (11.4)
		5.28, d (11.5)		5.24, d (11.4)
18	26.3 CH_3	1.11, s	26.2 CH_3	1.11, s
19	27.2 CH_3	1.27, s	27.2 CH_3	1.28, s
20	23.6 CH_3	1.61, s	22.6 CH_3	1.61, s
OAc-17	20.7 CH_3	1.81, s	20.6 CH_3	1.79, s
C═O	172.7 C		172.6 C	
OAng-5				
C═O	165.4 C			
	15.7 CH_3	1.86, dd (7.2, 1.5)		
	127.4 C			
	137.9 CH	5.99, dq (7.2, 1.5)		
	20.4 CH_3	1.84, d (1.5)		
OiBu-5				
C═O			174.5 C	
			33.8 CH	2.53, m
			19.8 CH_3	1.12, d (2.6)
			19.9 CH_3	1.14, d (2.6)
OBz-3				
C═O	165.7 C		165.6 C	
	129.8 C		129.7 C	
	129.7 CH	8.02, d (7.7)	129.7 CH	8.02, d (7.4)
	128.4 CH	7.49, t (7.7)	128.4 CH	7.50, t (7.4)
	133.1 CH	7.59, t (7.7)	133.1 CH	7.61, t (7.4)
OH-8		5.23, s		5.14, s
OH-13		4.49, s		4.48, s
OH-15		3.25, s		3.23, s

[a]Overlapped signals.

of Natural Products (Faculty of Pharmacy, NKUA) under the code Skaltsa & Grafakou 02.

Extraction and Isolation. The plant material (air-dried and powdered, 220 g) was extracted by maceration at room temperature with methanol (MeOH), and the extracts were evaporated at reduced pressure below 45 °C. The residue (dissolved in MeOH−H_2O, 1:1) was then subjected to liquid−liquid extraction [hexane, dichloromethane (CH_2Cl_2)]. Further fractionation was carried out by successive column chromatography (CC) and HPLC-DAD. The CH_2Cl_2 extract was purified on CC over Diaion (using MeOH−H_2O, 9:1, MeOH, hexane) in order to remove the remaining chlorophyll and waxes. Fraction DED-A (MeOH−H_2O, 9:1, 3 g) was subjected to CC (5 cm × 10 cm) over silica gel using mixtures of hexane, ethyl acetate, and MeOH of increasing polarity as eluents, to give 79 fractions, which were combined based on TLC similarities into 12 groups (DED-A-(A−L)).

Fraction DED-A-E (280.7 mg) was subjected to RP-HPLC (XDB-C_{18}, 21 mL/min, 0−30 min, acetonitrile (CH_3CN) in H_2O from 30% to 50%). Peaks collected at t_R 22.1 min (3.4 mg) and t_R 29.1 min (16.5 mg) were further subjected to RP-HPLC (Kinetex Biphenyl 4.6 × 250 mm, 1 mL/min, 0−35 min, CH_3CN in H_2O 50%) and RP-HPLC (Kinetex Biphenyl 21.2 × 250 mm, 21 mL/min, 0−35 min, CH_3CN in H_2O 50%) to yield compounds **8** (0.6 mg, t_R 18.2 min), **9** (0.7 mg, t_R 20.3 min), and **13** (2.2 mg, t_R 30.6 min), respectively.

Fraction DED-A-G (300.8 mg) was subjected to RP-HPLC (Kinetex Biphenyl 21.2 × 250 mm, 21 mL/min, 0−35 min,

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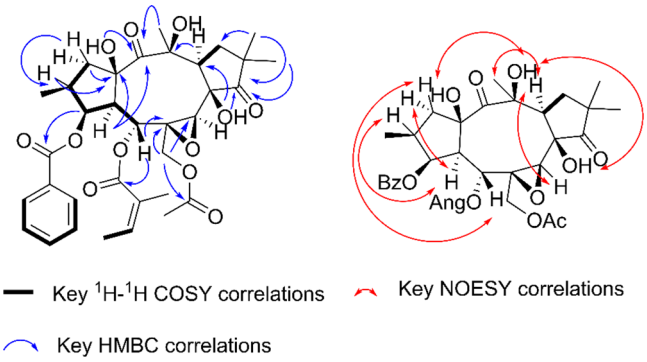

Figure 5. Key HMBC, ^{1}H−^{1}H COSY, and NOESY correlations of euphodeflexin M (**15**).

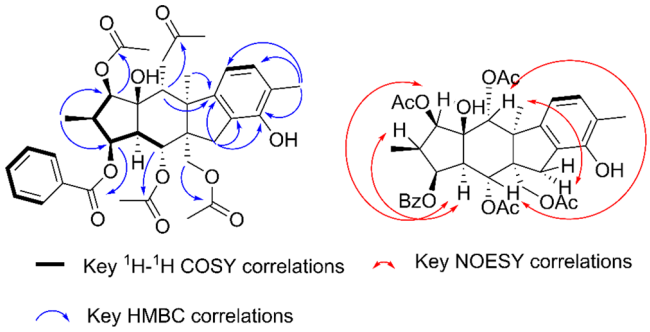

Figure 6. Key HMBC, ^{1}H−^{1}H COSY, and NOESY correlations of euphodeflexin O (**17**).

CH_3CN in H_2O 50%) and afforded compound **15** (10.6 mg, t_R 14.6 min). Peaks collected at t_R 8.05−10.5 min (21.3 mg) were further subjected to NP-HPLC (Zorbax RX-SIL, 5 mL/min, 0−20 min, 95:5 hexane−isopropanol) to yield compound **16** (1.0 mg, t_R 8.7 min), as well as **10** (1.1 mg, t_R 11.1 min) and **11** (1.0 mg, t_R 13.2 min) in addition to several fatty acids.

Table 6. ^{1}H and ^{13}C NMR Spectroscopic Data (600 and 150 MHz, $CDCl_3$) for Compounds 17 and 18

	euphodeflexin O (**17**)		euphodeflexin P (**18**)	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	74.1 CH_2	5.02, d (10.2)	48.6 CH_2	2.07, d (14.3)
				2.47, d (14.3)
2	38.0 CH	2.83,[a]	90.3 C	
3	72.7 CH	5.74 dd (6.8, 5.8)	77.9 CH	5.84, d (6.6)
4	43.1 CH	2.72 dd (12.1, 5.8)	44.2 CH	3.43, dd (12.6, 6.6)
5	69.6 CH	5.52 d (12.1)	69.7 CH	5.31, d (12.6)
6	54.8 C		54.7 CH	
7a	33.3 CH_2	2.60 d (15.9)	33.5 CH_2	2.58, d (15.7)
7b		2.85 d (15.9)		2.85, d (15.7)
8	123.2 C		123.8 C	
9	151.2 C		151.3 C	
10	122.1 C		123.5 C	
11	114.7 CH	6.82 d (7.8)	114.8 CH	6.85, d (7.8)
12	148.3 C		146.9 C	
13	54.8 C		54.6 C	
14	71.9 CH	5.59, s	73.0 CH	5.62, s
15	81.7 C		82.6 C	
16	9.9 CH_3	0.77 d (7.6)	22.7	1.44, s
17	63.6 CH_2	4.42 d (12.0)	63.4 CH_2	4.49, s
		4.51 d (12.0)		
18	129.9 CH	7.04 d (7.8)	130.5 CH	7.06, d (7.8)
19	15.4 CH_3	2.24, s	15.1 CH_3	2.23, s
20	23.8 CH_3	1.07, s	24.0 CH_3	1.08, s
OAc-1	20.7 CH_3	2.15, s		
C=O	170.2			
OAc-2			21.0[a] CH_3	2.18, s
C=O			170.1 C	
OAc-5	20.9 CH_3	1.95	21.2[a] CH_3	2.18, s
C=O	170.5 C		170.1 C	
OAc-14	20.9 CH_3	2.18, s	22.2 CH_3	2.04, s
C=O	169.7		169.9 C	
OAc-17	21.3 CH_3	2.13, s	20.9 CH_3	1.96, s
C=O	170.5		170.8 C	
OBz-3	129.4 C	1.98, s	129.7 C	
	128.3 CH	7.34, 2H t (7.2)	128.3 CH	7.35, 2H t (7.6)
	129.7 CH	7.49, 1H t (7.2)	133.2 CH	7.50, 1H t (7.6)
	129.7 CH	7.83, 2H d (7.2)	129.7 CH	7.85, 2H d (7.6)
C=O	166.2 C		165.6 C	

[a]Overlapped signals.

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Table 7. IC_{50} Values (μM) Calculated from the MTT Assay against HeLa, SK-MEL-28, HePG2, and PC-3 Cell Lines

	1	12	13	14	15	17	18	parthenolide
HeLa	9.9 ± 1.0	>10	9.8 ± 1.1	>10	>10	5.8 ± 0.7	>10	2.1 ± 1.3
SK-MEL-28	>10	>10	>10	>10	>10	>10	>10	4.9 ± 0.9
HePG2	>10	>10	n.t.[a]	>10	>10	n.t.[a]	n.t.[a]	5.6 ± 1.2
PC-3	>10	>10	n.t.[a]	>10	>10	n.t.[a]	n.t.[a]	>10

[a]n.t.: not tested.

Fraction DED-A-I (222.4 mg) was subjected to RP-HPLC (Kinetex Biphenyl 21.2 × 250 mm, 21 mL/min, 0−35 min, CH_3CN in H_2O 50%) and afforded compounds **17** (5.1 mg, t_R 11.9 min), **18** (3.2 mg, t_R 12.5 min), and **6** (1.4 mg, t_R 21.1 min). Peaks collected at t_R 22.0−26.0 min (26.7 mg) were further subjected to RP-HPLC, as described above, and afforded compound **5** (1.4 mg, t_R 27.8 min). Peaks collected at t_R 33.2−35.0 min (3.9 mg) were also subjected to RP-HPLC (Kinetex Biphenyl 4.6 × 250 mm, 1 mL/min, 0−15 min, CH_3CN in H_2O 64.5%) to yield compounds **4** (0.9 mg, t_R 7.8 min) and **7** (0.7 mg, t_R 8.4 min).

Fraction DED-A-J (345.1 mg) was purified using RP-HPLC (XDB-C_{18}, 21 mL/min, 0−2 min CH_3CN in H_2O 10%, 2−28 min CH_3CN from 10% to 90%, 28−31 min 90%, 31−33 min from 90% to 10%, 33−35 min 10%) and afforded compound **1** (18.5 mg, t_R 23.5 min). Peaks collected at t_R 21.0−27.0 min (96.9 mg) were further purified using RP-HPLC (Kinetex Biphenyl 21.2 × 250 mm, 21 mL/min, 0−35 min, CH_3CN in H_2O 50%) and afforded compounds **2** (1.4 mg, t_R 13.5 min) and **3** (1.3 mg, t_R 22.9 min).

For full details of the extraction and isolation process followed for the known compounds reported in this study, see S2, Supporting Information.

*Euphodeflexin A (**1**):* colorless crystals (MeOH); mp 131 °C; $[\alpha]^{25}_D$ −14 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.27), nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1; HREIMS *m*/*z* 753.3122 $[M + H]^+$ (calcd for $C_{40}H_{49}O_{14}$, 753.3117).

*Euphodeflexin B (**2**):* white amorphous solid; $[\alpha]^{25}_D$ +47 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.18) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1; HREIMS *m*/*z* 775.2943 $[M + Na]^+$ (calcd for $C_{40}H_{48}O_{14}Na$, 775.2936).

*Euphodeflexin C (**3**):* white amorphous solid; $[\alpha]^{25}_D$ −19 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.89) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1; HREIMS *m*/*z* 793.3074 $[M - H]^-$ (calcd for $C_{42}H_{49}O_{15}$, 793.3077).

*Euphodeflexin D (**4**):* white amorphous solid; $[\alpha]^{25}_D$ −22 (*c* 0.2, MeOH UV (MeOH) λ_{max} (log ε) 227 (4.31), nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1; HREIMS *m*/*z* 813.3136 $[M - H]^-$ (calcd for $C_{45}H_{49}O_{14}$, 813.3128).

*Euphodeflexin E (**5**):* white amorphous solid; $[\alpha]^{25}_D$ +18 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.05) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 2; HREIMS *m*/*z* 785.2584 $[M + Cl]^-$ (calcd for $C_{40}H_{46}O_{14}Cl$, 785.2582).

*Euphodeflexin F (**6**):* white amorphous solid; $[\alpha]^{25}_D$ +32 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.27) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 2; HREIMS *m*/*z* 749.2816 $[M - H]^-$ (calcd for $C_{40}H_{45}O_{14}$, 749.2815).

*Euphodeflexin G (**7**):* white amorphous solid; $[\alpha]^{25}_D$ +14 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.99) nm; CD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 2; HREIMS *m*/*z* 827.2695 $[M + Cl]^-$ (calcd for $C_{42}H_{48}O_{15}Cl$, 827.2687).

*Euphodeflexin H (**8**):* white amorphous solid; $[\alpha]^{25}_D$ +121 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (4.08) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 3; HREIMS *m*/*z* 539.2643 ($[M + H]^+$, calcd for $C_{31}H_{39}O_8$, 539.2639).

*Euphodeflexin I (**9**):* white amorphous solid; $[\alpha]^{25}_D$ +111 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.08) nm; CD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 3; HREIMS *m*/*z* 597.2692 ($[M + H]^+$, calcd for $C_{33}H_{41}O_{10}$, 597.2694).

*Euphodeflexin J (**10**):* white amorphous solid; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 3; HREIMS *m*/*z* 555.2591 ($[M + H]^+$, calcd for $C_{31}H_{39}O_9$, 555.2589).

*Euphodeflexin K (**11**):* white amorphous solid; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 3; HREIMS *m*/*z* 555.2590 ($[M + H]^+$, calcd for $C_{31}H_{39}O_9$, 555.2589).

*Euphodeflexin L (**13**):* white amorphous solid; $[\alpha]^{25}_D$ +32 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.20) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 4; HREIMS *m*/*z* 571.2914 $[M + H]^+$ (calcd for $C_{32}H_{43}O_9$, 571.2902).

*Euphodeflexin M (**15**):* white amorphous solid; $[\alpha]^{25}_D$ +95 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.28) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 5; HREIMS *m*/*z* 643.2753 $[M + H]^+$ (calcd for $C_{34}H_{43}O_{12}$, 643.2749).

*Euphodeflexin N (**16**):* white amorphous solid; $[\alpha]^{25}_D$ +117 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.57) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 5; HREIMS *m*/*z* 631.2760 $[M + H]^+$ (calcd for $C_{33}H_{43}O_{12}$, 631.2749).

*Euphodeflexin O (**17**):* colorless crystals (MeOH); mp 186 °C; $[\alpha]^{25}_D$ +41 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.70) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 6; HREIMS *m*/*z* 675.2419 $[M + Na]^+$ (calcd for $C_{35}H_{40}O_{12}Na$, 675.2412).

*Euphodeflexin P (**18**):* white amorphous solid; $[\alpha]^{25}_D$ +21 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.91) nm; ECD see S96, Supporting Information; ^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 6; HREIMS *m*/*z* 675.2413 $[M + Na]^+$ (calcd for $C_{35}H_{40}O_{12}Na$, 675.2412).

X-ray Crystallography Analysis. Suitable crystals of **1** and **17** were collected on an XtaLAB Synergy R, DW system, HyPix-Arc 150 diffractometer operating at *T* = 123.00(10) K, equipped with Cu Kα radiation (λ = 1.541 84 Å). The structures and absolute configurations were solved with the ShelXT 2018/2[23] solution program using dual methods and by using Olex2 1.3-alpha[24] as the graphical interface. The crystallographic data of these compounds have been deposited at the Cambridge Crystallographic Data Center (deposition numbers 2094702 and 2096413).

*Crystallographic data for compound **1**.* $C_{40}H_{48}O_{14}{\cdot}CH_4O$, M = 775.37 g/mol, space group $P2_12_12_1$, *a* = 13.2073(4) Å, *b* = 16.4790(3) Å, *c* = 18.5699(4) Å, $\alpha = \beta = \gamma = 90°$, *V* = 4041.61(17) Å^3, *T* = 123.00(10) K, *Z* = 4, D_x = 1.274 g/cm^3, μ(Cu Kα) = 0.808 mm^{-1}, 42 456 reflections measured (6.802° ≤ 2θ ≤ 147.376°), 6454 unique (R_{int} = 0.0851, R_{sigma} = 0.0541). The final R_1 was 0.0434 ($I \geq 2\sigma(I)$) and wR_2 was 0.1010 (all data). The goodness of fit was GooF = 1.037 and Flack parameter = 0.02(12).

*Crystallographic data for compound **17**.* $C_{35}H_{40}O_{12}$, M = 652.67 g/mol, space group $P2_12_12_1$, *a* = 11.30706(5) Å, *b* = 13.99129(6) Å, *c* = 20.65257(8) Å, $\alpha = \beta = \gamma = 90°$, *V* = 3267.25(2) Å^3, *T* = 123.00(10) K, *Z* = 4, D_x = 1.327 g/cm^3, μ(Cu Kα) = 0.835 mm^{-1}, 145 025 reflections measured (6.802° ≤ θ ≤ 147.376°), 6740 unique

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(R_{int} = 0.0371, R_{sigma} = 0.0083). The final R_1 was 0.0426 ($I \geq 2\sigma(I)$) and wR_2 was 0.1125 (all data). The goodness of fit was G*oo*F = 1.072 and Flack parameter = 0.01(3).

MTT Assay. For experimental details of the cytotoxic assay, see S3, Supporting Information.

Antibacterial Assay. For experimental details of the antibacterial assay, see S4, Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.1c00654.

Crystallographic data of **1** (CIF)
Crystallographic data of **17** (CIF)
Experimental procedures, extraction and isolation, MTT assay, antibacterial assay, proposed biogenesis of pepluane **17**, 1D and 2D NMR spectra, and ECD of compounds **1**−**10**, **13**, and **15**−**18** (PDF)

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Notes

The authors declare no competing financial interest.

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Macrocyclic Diterpenoid Constituents of *Euphorbia deflexa*, an Endemic Spurge from Greece

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Supporting information

1. Experimental

S1. General Experimental Procedures.

Melting points were measured in a Melting Point B-545 (BÜCHI). Optical rotations were obtained an UniPol L 1000 polarimeter (Schmidt + Haensch GmbH & Co.). UV-spectra were recorded on a Cary 50 Scan UV-spectrophotometer (Varian). CD spectra were measured on a J-715 spectropolarimeter (JASCO). NMR spectra were measured in an AVANCE III 600 instrument equipped with a 5 mm TBI CryoProbe (^{1}H-NMR 600.25 MHz, ^{13}C-NMR 150.95 MHz) at 298 K. Chemical shifts are given in ppm (δ) and were referenced to the solvent signals at 7.26 and 77.0 ppm for ^{1}H-/^{13}C-NMR, respectively. HRESIMS spectra were obtained with an Agilent MS Q-TOF 6540 spectrometer. Column chromatography was performed on Diaion HP-20 (250-850 μm; Supelco) and silica gel (9385 230-400 mesh, Merck). Fractionation was always monitored by TLC silica gel 60 F-254 (Merck) with visualization under UV (254 and 365 nm) and spraying with vanillin–sulfuric acid reagent (vanillin, Merck) and anisaldehyde-sulfuric acid agent (4-methoxybenzaldehyde, Merck). Semi-prep HPLC was conducted on an Agilent 1290 Infinity system (flow

rate 5 or 21 mL/min; DAD detector: 205 and 230 nm), or an Elite LaChrom Hitachi system (flow rate 1 mL/min; DAD detector: 205 and 230 nm), using the reverse-phase columns XDB-C18 PrepHT (5 μm, 21.2 x 250 mm, Agilent), Kinetex Biphenyl (5 μm, 21.2 x 250 mm, Phenomenex) and Kinetex Biphenyl (5 μm, 4.6 x 250 mm, Phenomenex), or the normal-phase ZORBAX RX-SIL (5 μm, 9.4 x 250 mm, Agilent).

S2. Extraction and Isolation.

Fraction DED-A-D (40.3 mg) was subjected to RP-HPLC (XDB-C18, 21 mL/min, 0-2 min ACN in H_2O 10 %, 2-28 min from 10 to 90%, 28-31 min 90%, 31-33 from 90 to 10%, 33-35 min 10%) and afforded 3*β*-*O*-angeloyl-20-deoxy-ingenol (**12**)[1] (3.9 mg, t_R 30.3 min) and 3-*O*-angeloyl-17-angeloyloxy-20-deoxy-ingenol[1] (1.6 mg, t_R 32.2 min). Fraction DED-A-E (280.7 mg) was subjected to RP-HPLC (XDB-C18, 21 mL/min, 0-30 min, ACN in H_2O from 30 to 50%). Peak collected at t_R 29.1 min (16.5 mg) was further subjected to RP-HPLC (Kinetex Biphenyl 21.2 x 250 mm, 21 mL/min, 0-35 min, ACN in H_2O 50%) to yield 13-*α*-acetoxy-3-*O*-angeloyl-17-benzenoyloxy-20-deoxy-ingenol[1] (0.8 mg, t_R 26.6 min). Fraction DED-A-I (222.4 mg) was subjected to RP-HPLC (Kinetex Biphenyl 21.2 x 250 mm, 21 mL/min, 0-35 min, ACN in H_2O 50%) and afforded segetene B[2] (0.6 mg, t_R 14.1 min), paralilone A (**14**)[3] (9.8 mg, t_R 18.3 min) and (2*S**,3*S**,4*R**,5*R**,6*R**,8*R**,11*S**,12*S**,13*R**,14*R**,15*R**)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxy-9-oxo-segetane[4] (5.4 mg, t_R 19.5 min).

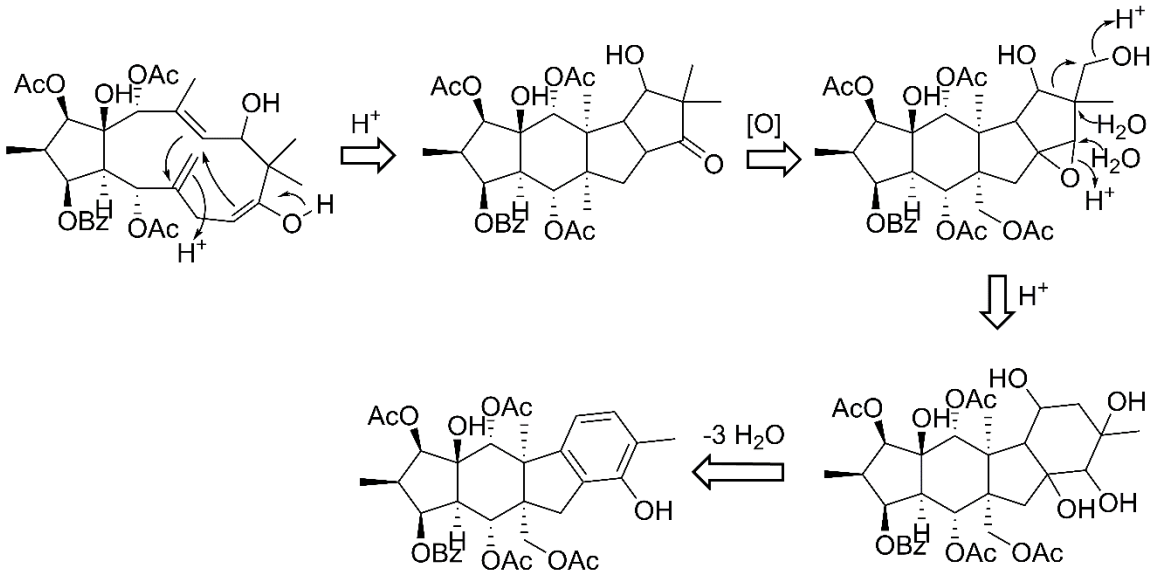
S3. MTT Assay.

The cytotoxicity of the diterpenes was determined using the human cancer cell lines HeLa (cervical carcinoma, ATTC), SK-MEL-28 (malignant melanoma, DSMZ) and HepG2 (hepatocellular carcinoma, DSMZ), and PC-3 (prostate carcinoma, DSMZ). Culture medium was MEM (Sigma; supplemented with L-Glutamine, FCS and NEA, BioChrom), DMEM-HAM'S (50-50%, Invitrogen and BioChrom; supplemented with FCS), RPMI (BioChrom; supplemented with L-Glutamine and heat activated FCS) and HAM'S (Biochrom; supplemented with FCS) respectively. Stock solutions of the samples were prepared in DMSO, and the test concentrations were freshly prepared with medium at the day of the experiment (final concentration of DMSO was 0.33%). Cytotoxicity was evaluated using the colorimetric MTT assay as described before.[5] Briefly, nine different concentrations (100.0 to 0.39 μM) of the tested compounds (**1**, **12-14**, **15**, **17-18**) together with the cell suspension (final concentration in wells: 6 x 10^4 cells/mL, 3 x 10^4 cells/mL, 12.5 x 10^4 cells/mL and 7.5 x 10^4 cells/mL, for HeLa, SK-MEL-28, HePG2 and PC-3 respectively), were added to the 96 well-plates. After incubation for 68 h (IBS Integra Biosciences, 37 °C, 5% CO2 and 90% relative humidity), MTT (4 mg/mL) was added, and after 4 h the solution was exchanged with 10% SDS. The plates were then placed in dark and 24 h later, the absorbance at 560 nm was measured (SpectraFluor plus plate reader, Tecan).

S4. Antibacterial Assay

The double dilution method was carried out as described before,[6] slightly modified. The bacteria used were *Staphylococcus aureus* (DSM20231) and *Streptococcus pyogenes* (DSM20565-0316-001), and their overnight cultures (2% v/v LB Broth, Alfa Aesar and 3.7 w/v Brain Heart Infusion Broth, Sigma Aldrich, 37 °C, Incubating Shaker, VWR) were diluted to 10^8 cells/mL in fresh medium. The required amounts of samples (**1**, **13** and **15**) or chloramphenicol (positive control, Sigma Aldrich) (1mg/mL) were pipetted in the 96-well plates, left to dry and redissolved with 50μL DMSO, 50 μL medium and 100 μL diluted bacteria suspension. Incubation at 37 °C (IFPlus, Memmert) for 20 h was followed by addition of 20 μL aqueous 0.25 % MTT, re-incubation for 4 h and detection of the living bacteria as violet turbid solutions.

2. Figure S1. Proposed biogenesis of pepluane 17, starting from a suitable substituted jatrophane



3. Spectra of the new compounds 1-11,13, 15-18.

Figure S2. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **1**

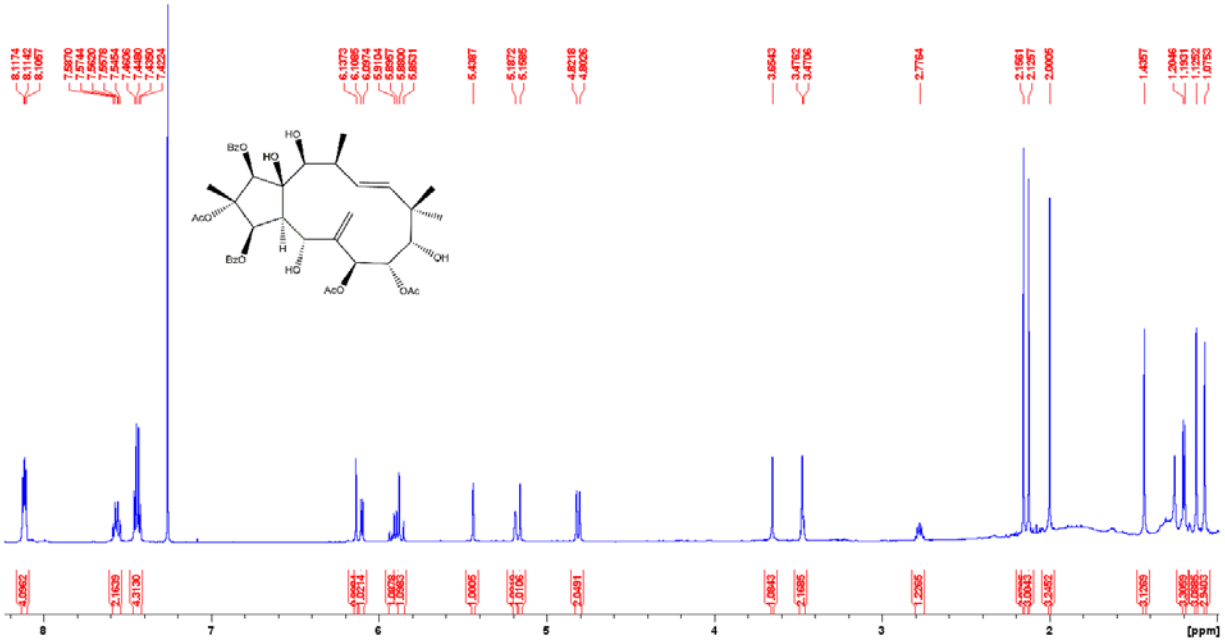


Figure S3. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **1**

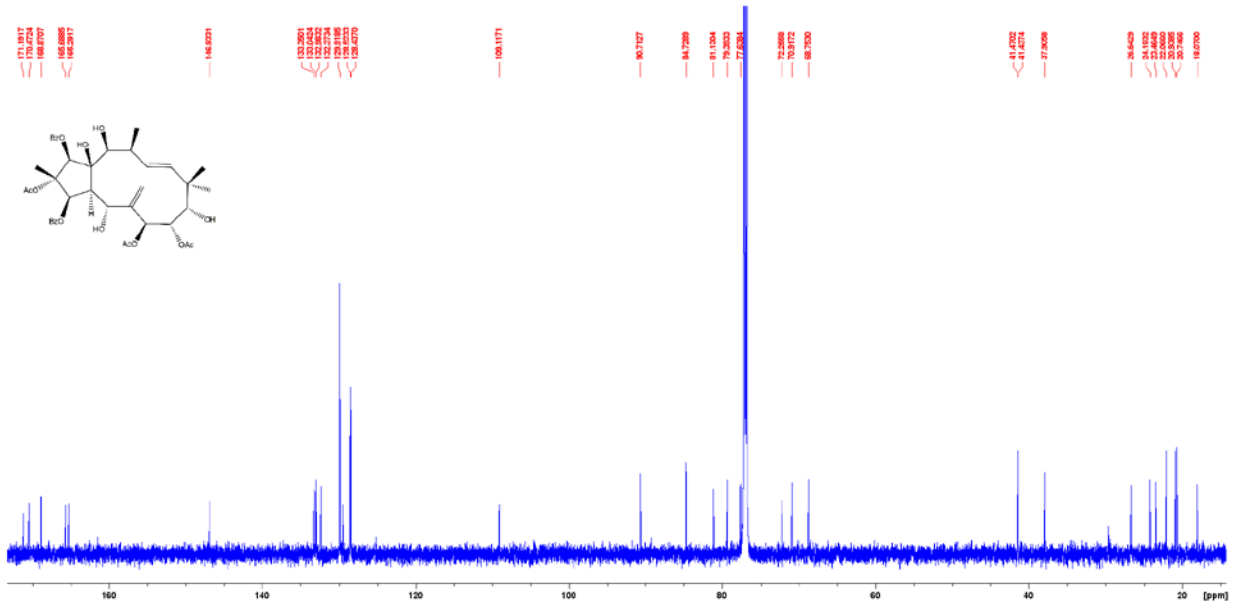


Figure S4. HSQC ($CDCl_3$) spectrum of the new compound **1**.

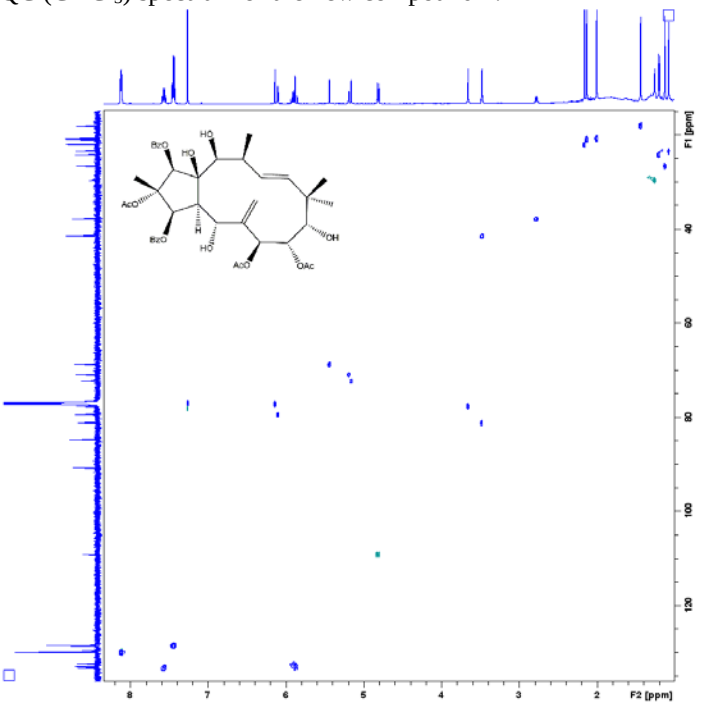


Figure S5. HMBC ($CDCl_3$) spectrum of the new compound **1.**

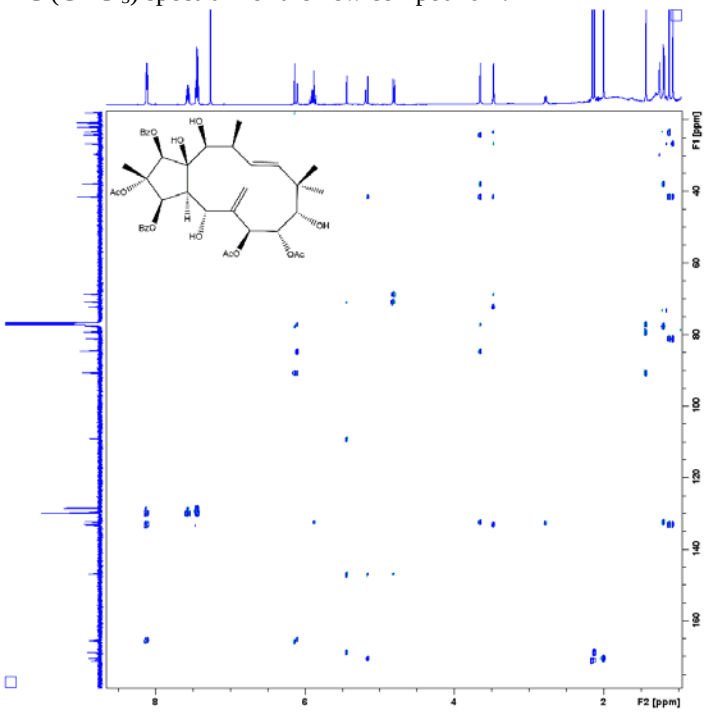


Figure S6. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **1.**

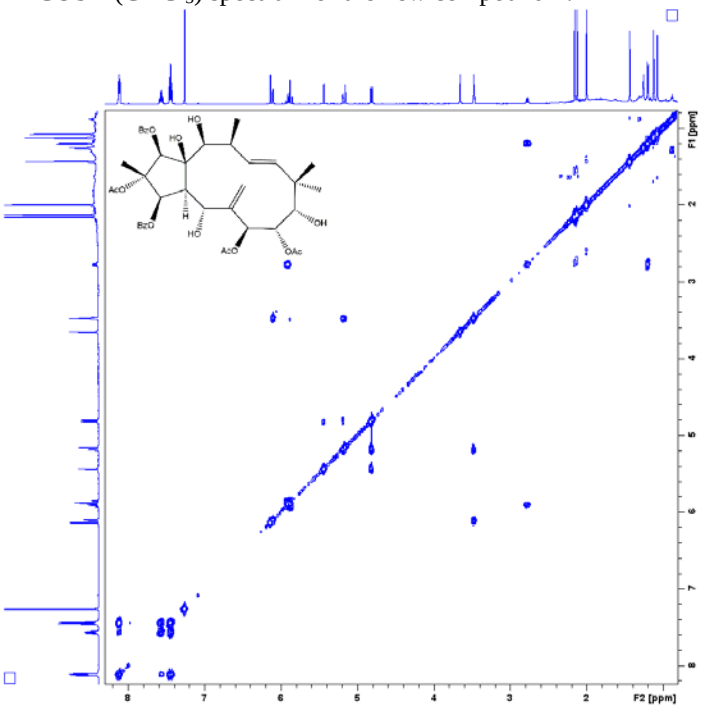


Figure S7. NOESY ($CDCl_3$) spectrum of the new compound **1.**

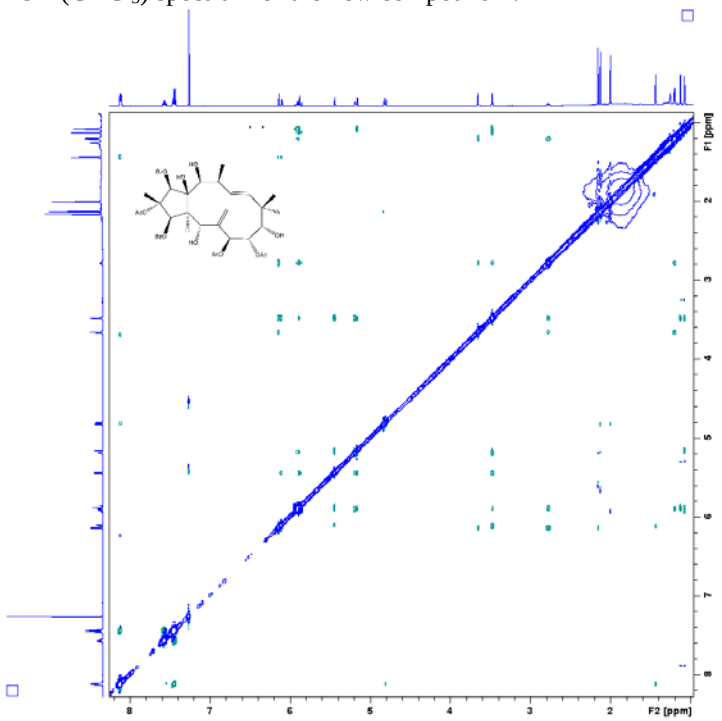


Figure S8. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **2**.

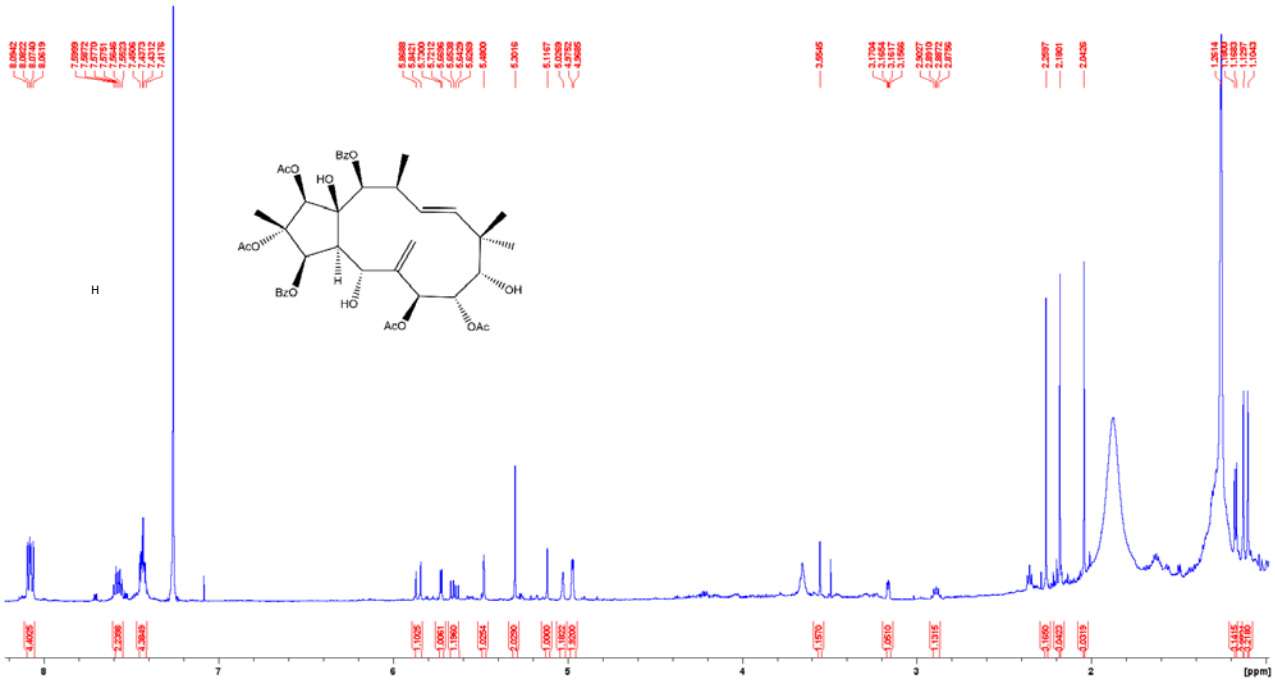


Figure S9. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **2**.

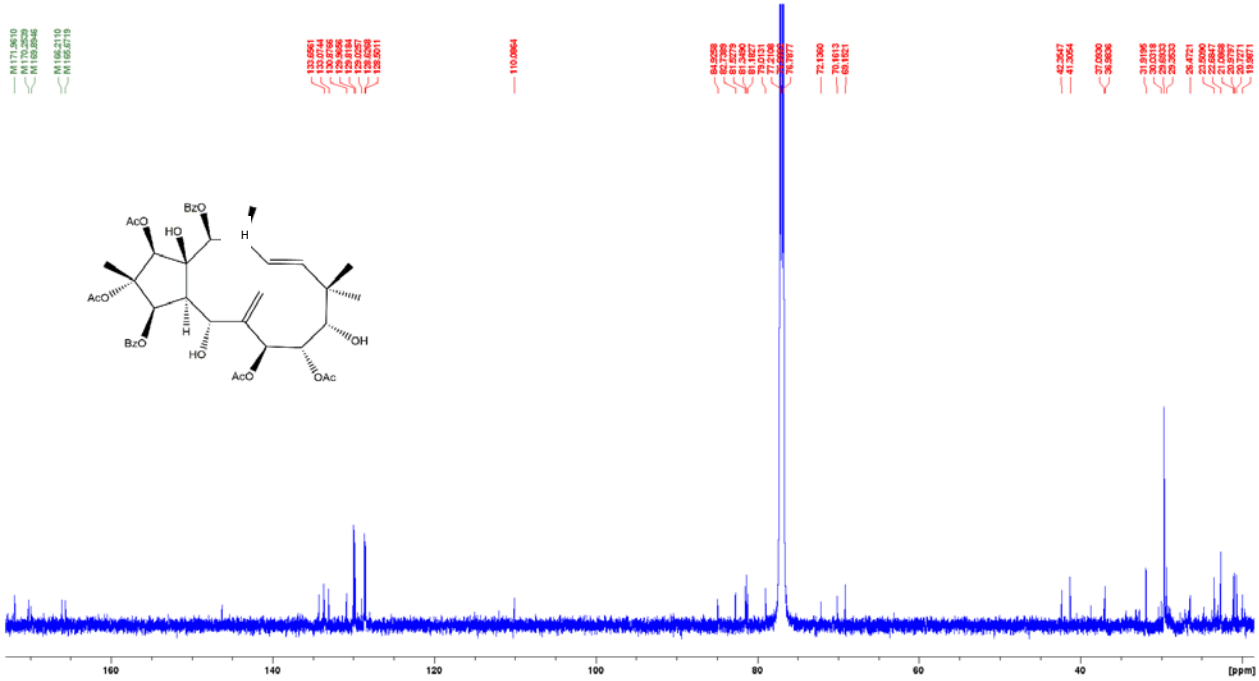


Figure S10. HSQC ($CDCl_3$) spectrum of the new compound **2**.

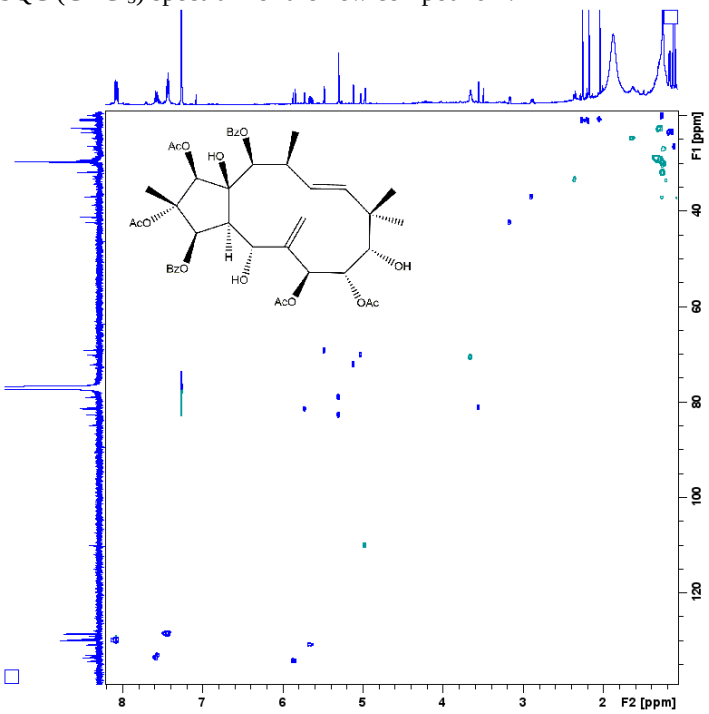


Figure S11. HMBC ($CDCl_3$) spectrum of the new compound **2**.

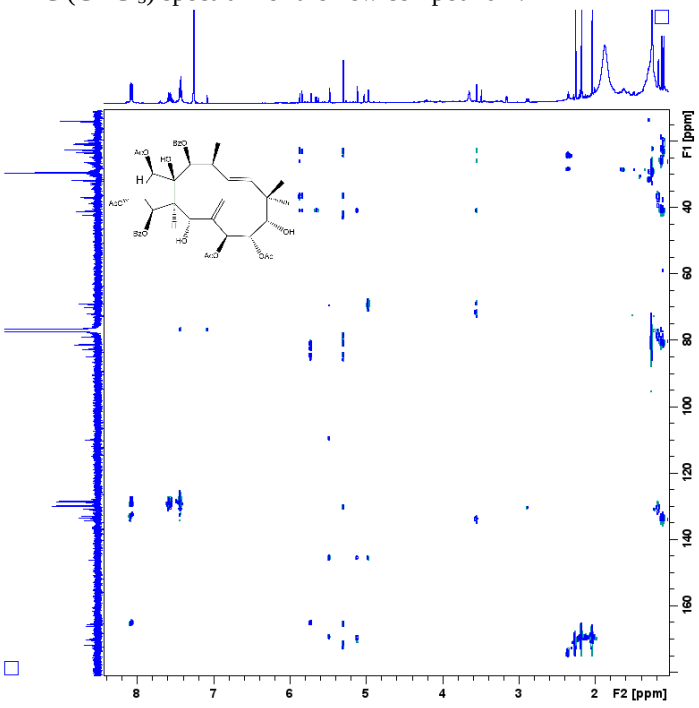


Figure S12. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **2**.

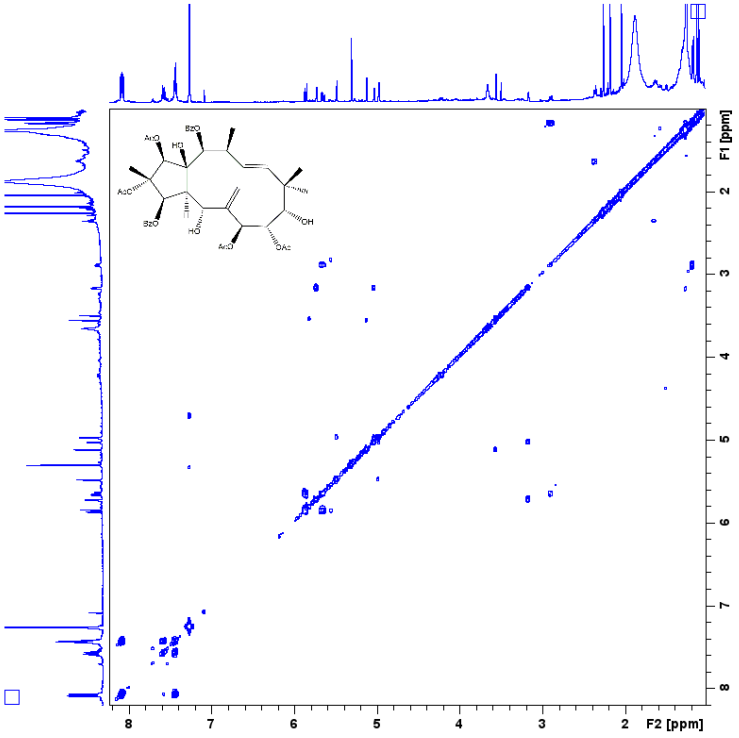


Figure S13. NOESY ($CDCl_3$) spectrum of the new compound **2**.

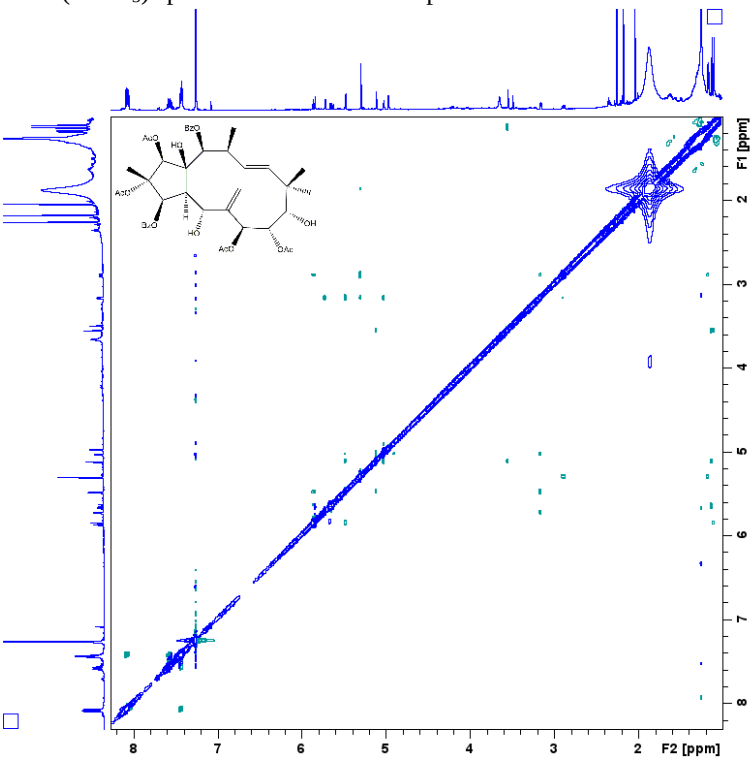


Figure S14. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **3**.

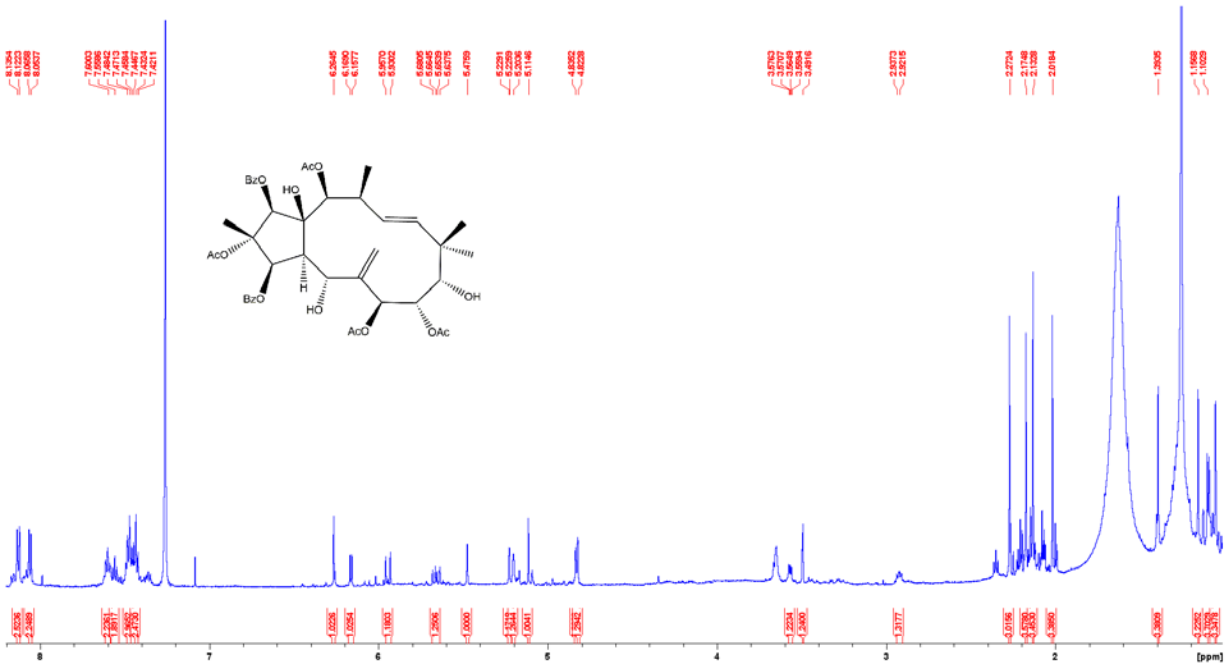


Figure S15. ^{13}C (150 MHz, $CDCl_3$) spectrum of the new compound **3**.

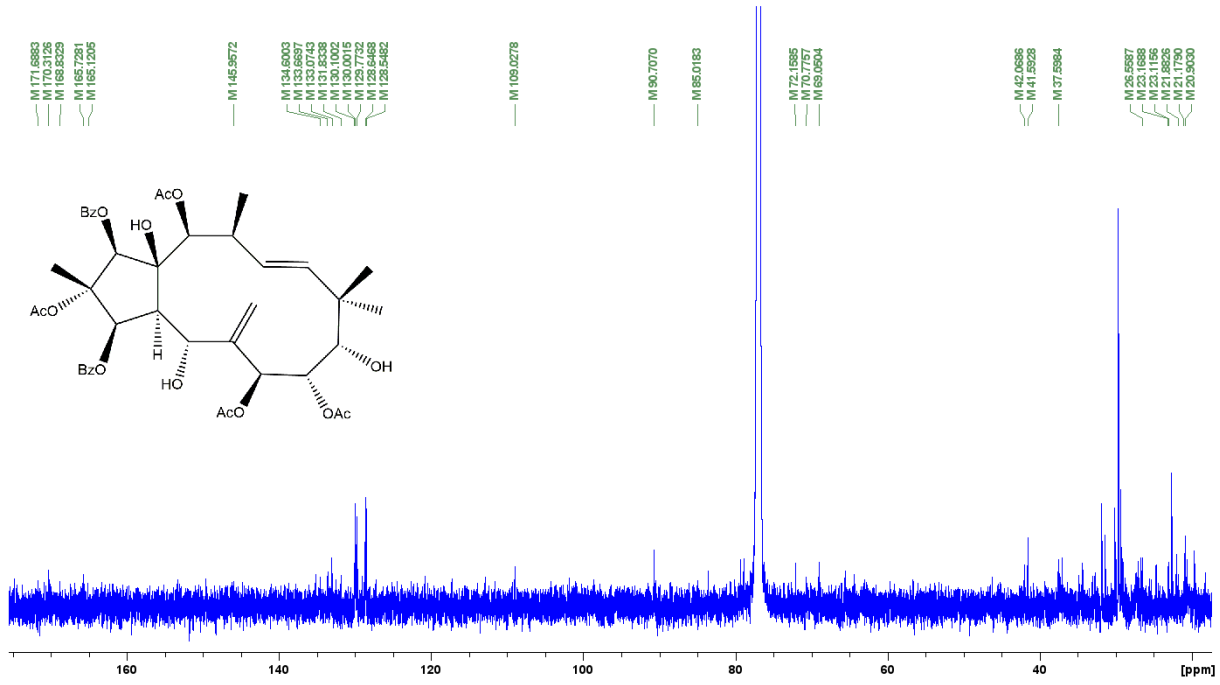


Figure S16. HSQC ($CDCl_3$) spectrum of the new compound **3**.

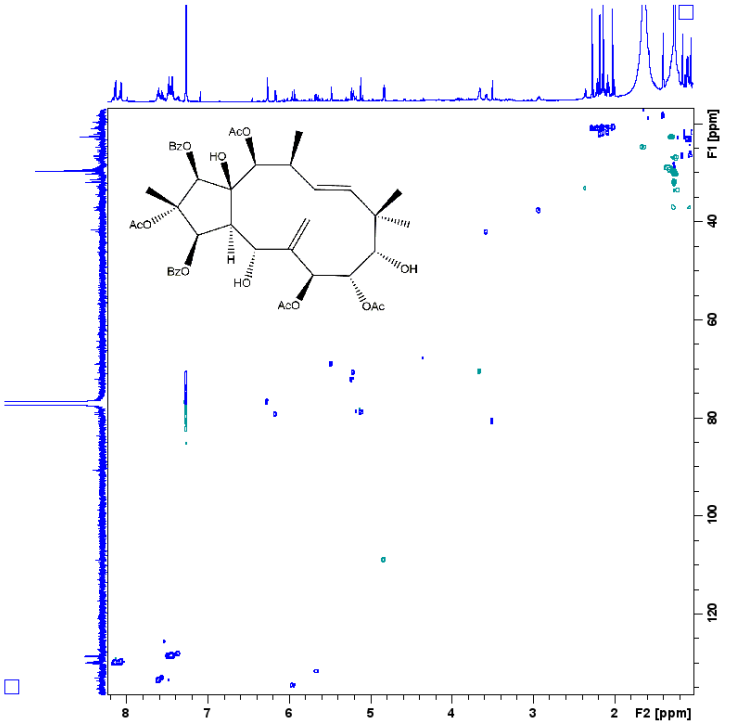


Figure S17. HMBC ($CDCl_3$) spectrum of the new compound **3**.

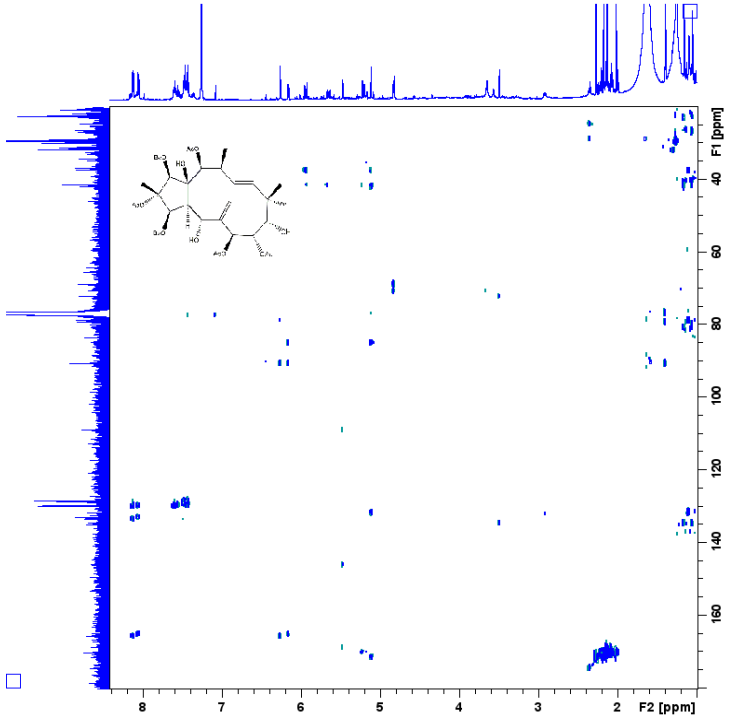


Figure S18. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **3**.

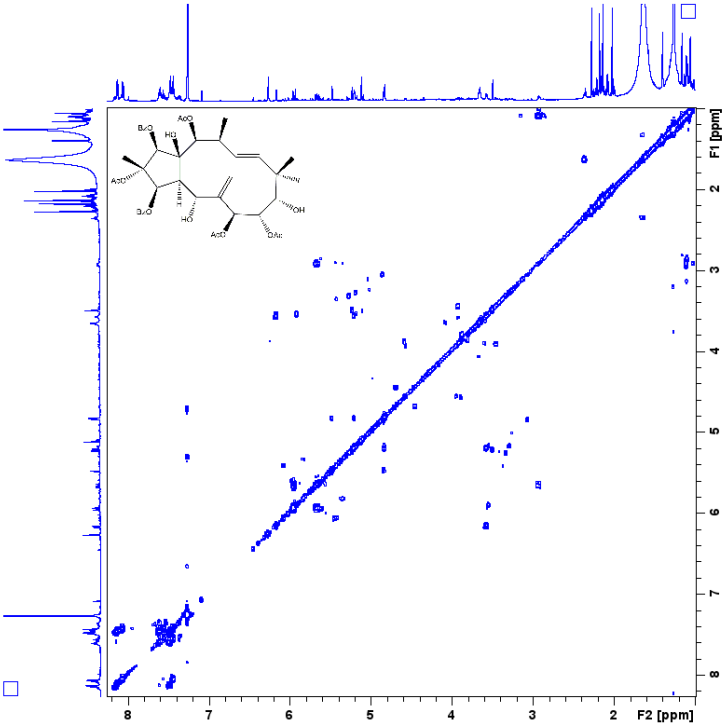


Figure S19. NOESY ($CDCl_3$) spectrum of the new compound **3**.

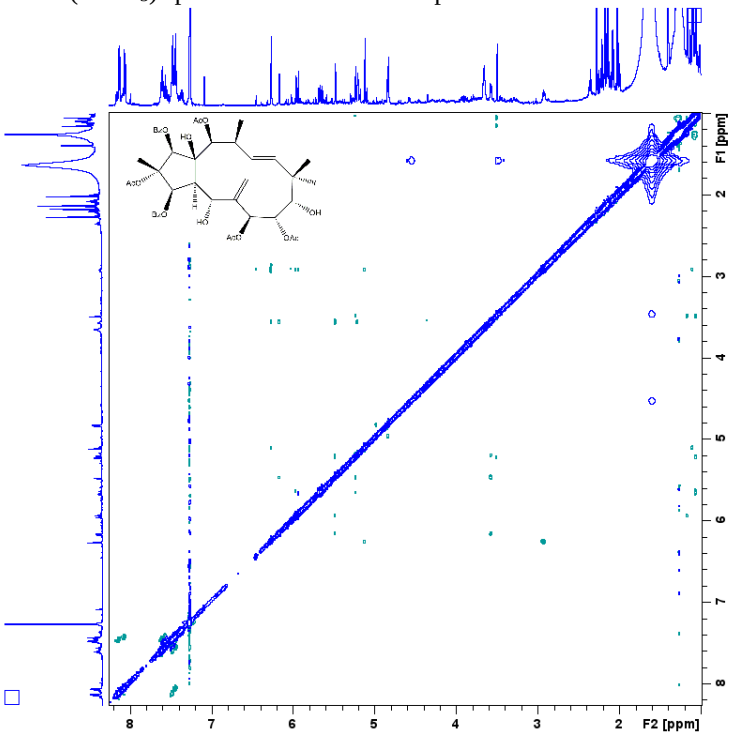


Figure S20. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **4**.

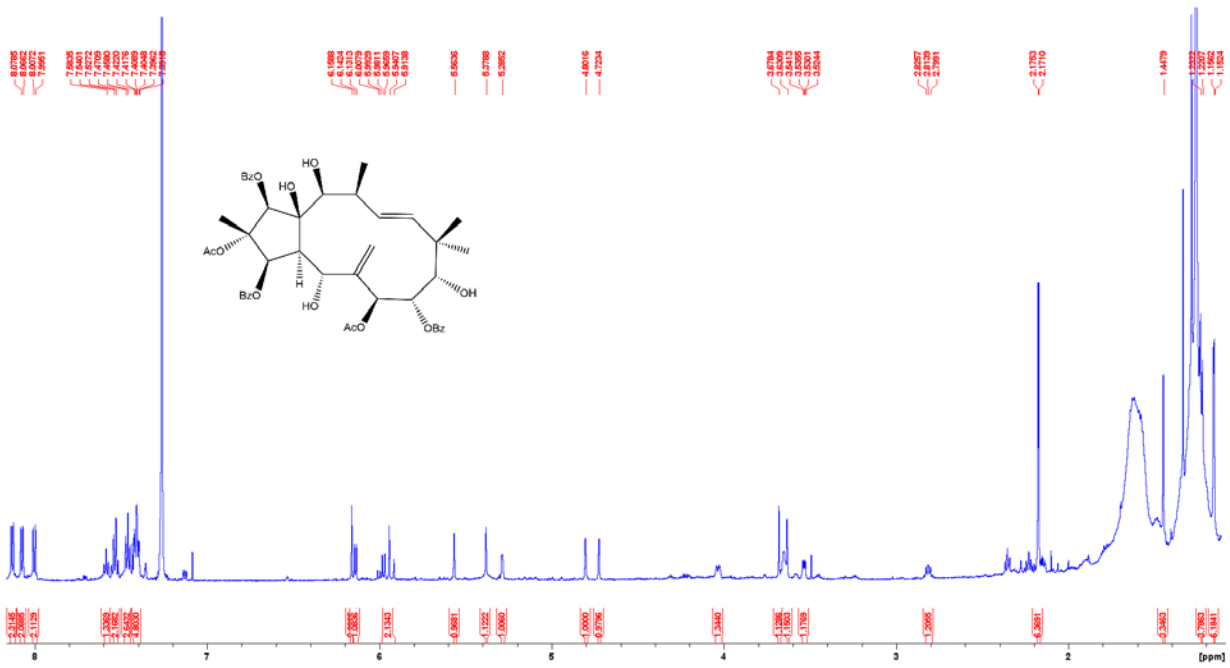


Figure S21. HSQC ($CDCl_3$) spectrum of the new compound **4**.

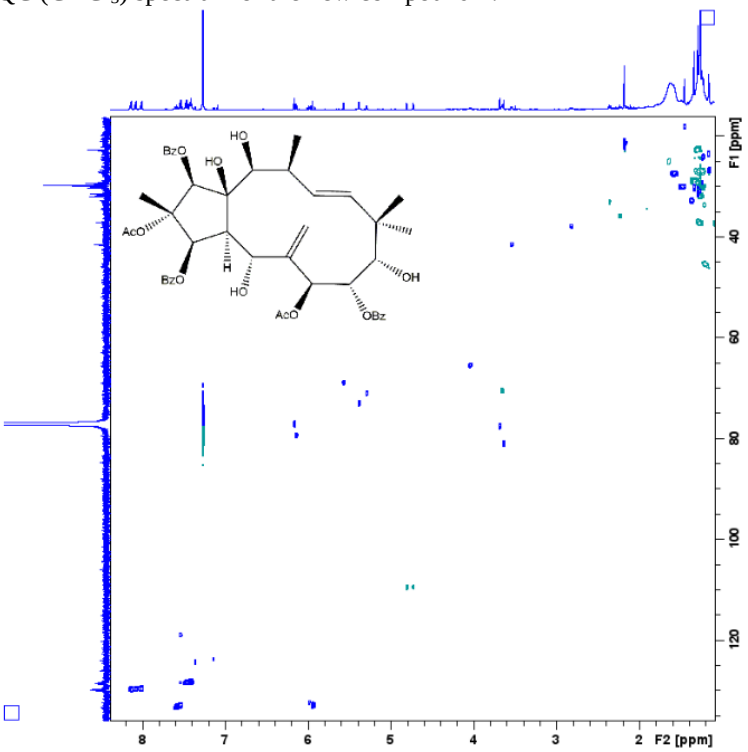


Figure S22. HMBC ($CDCl_3$) spectrum of the new compound **4**.

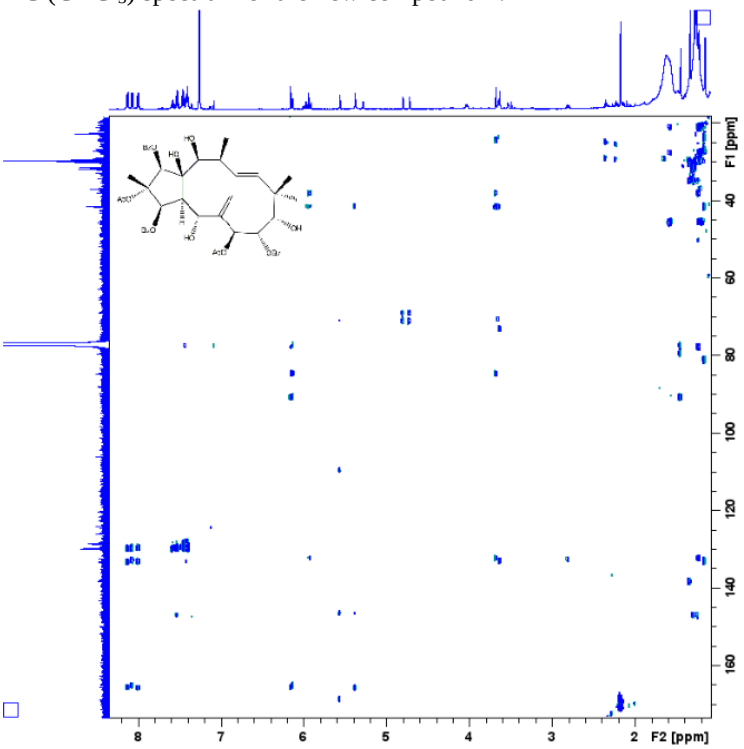


Figure S23. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **4**.

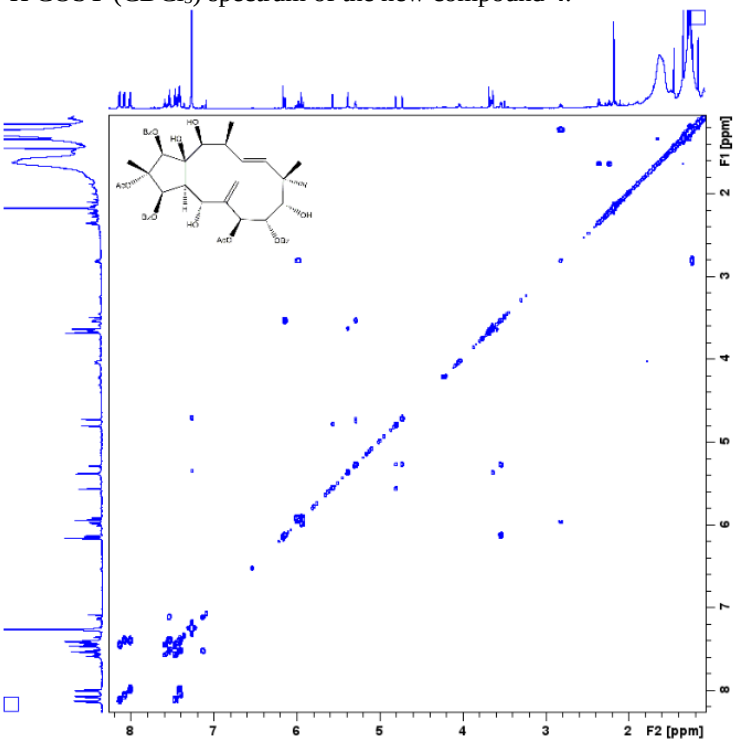


Figure S24. NOESY ($CDCl_3$) spectrum of the new compound **4**.

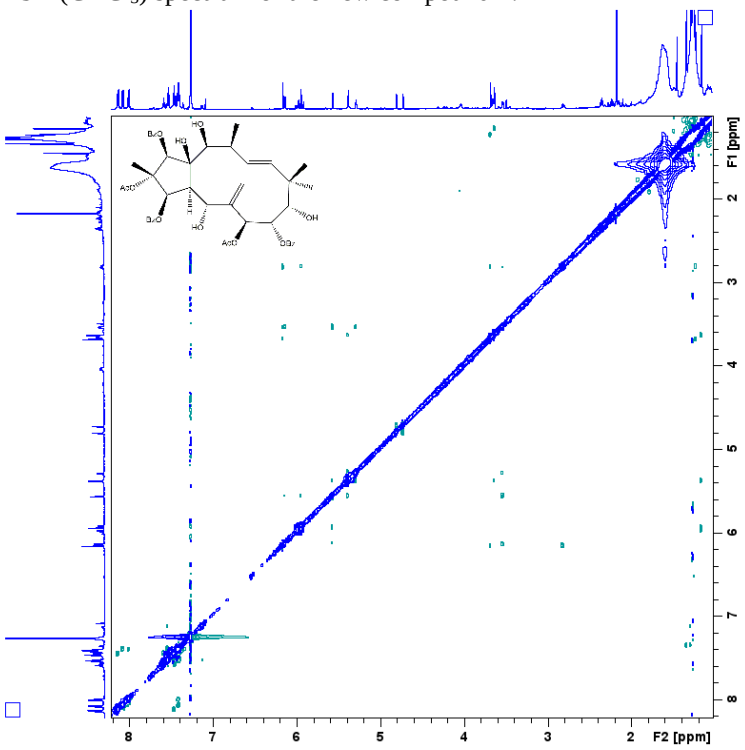


Figure S25. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **5**.

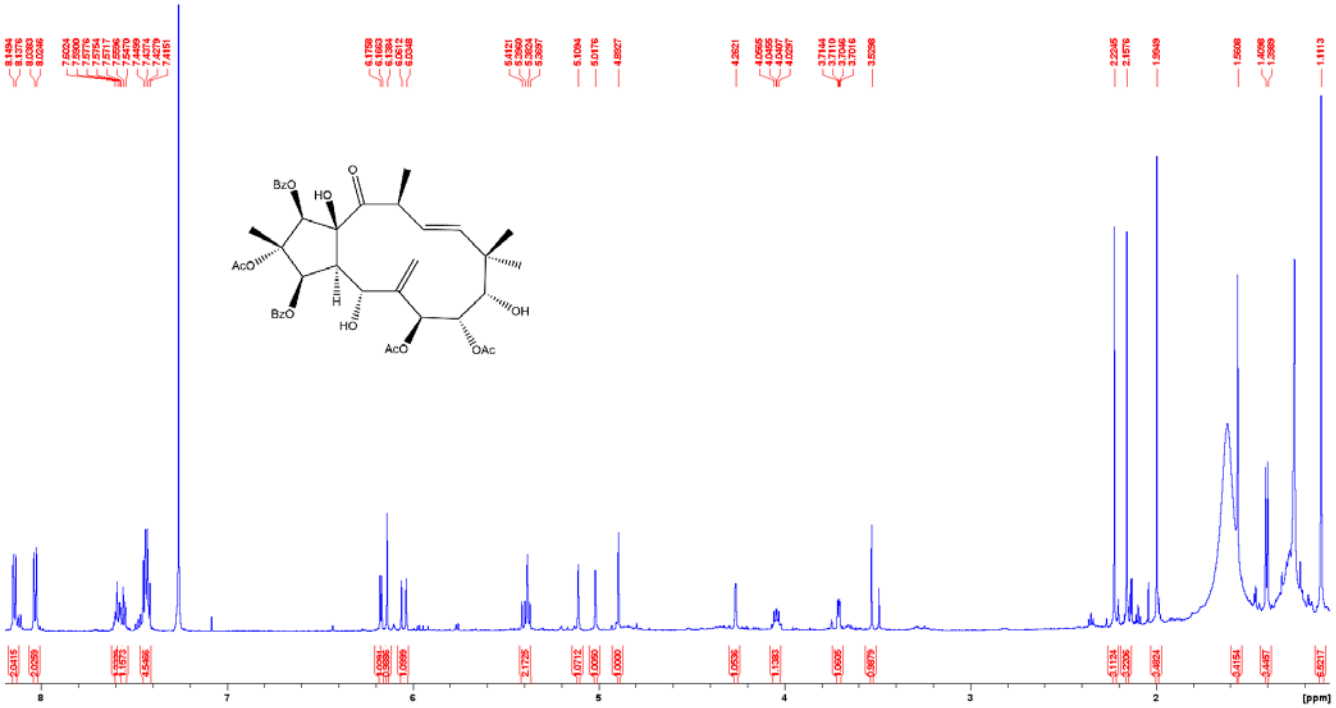


Figure S26. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **5**.

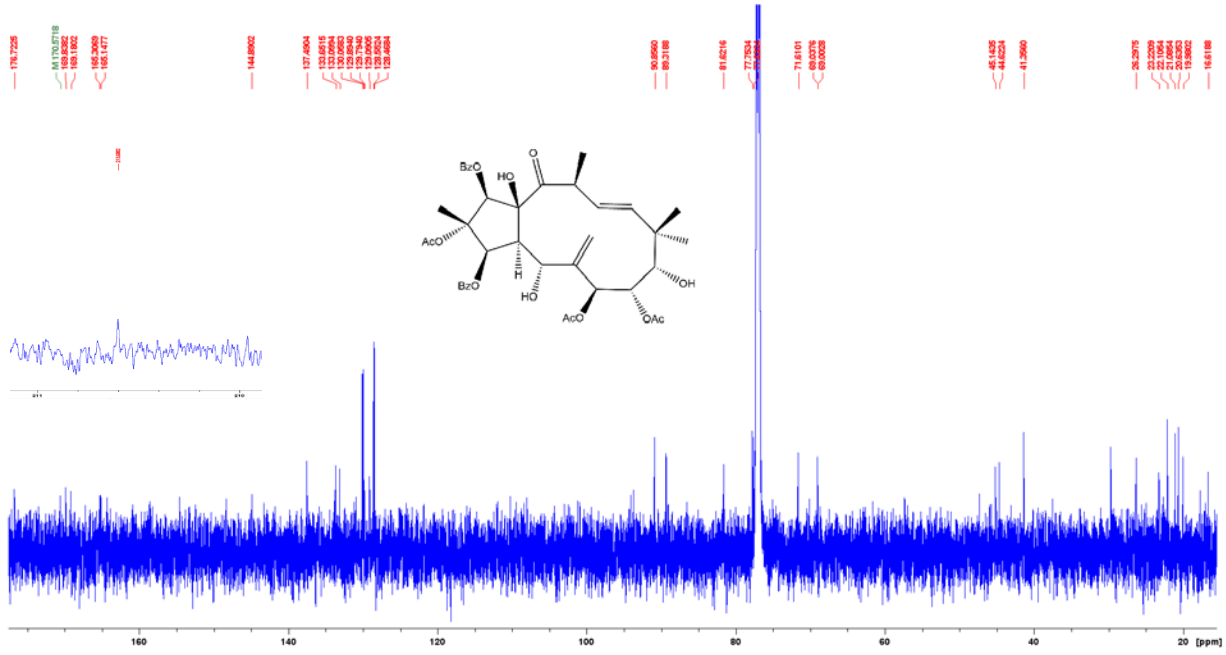


Figure S27. HSQC ($CDCl_3$) spectrum of the new compound **5**.

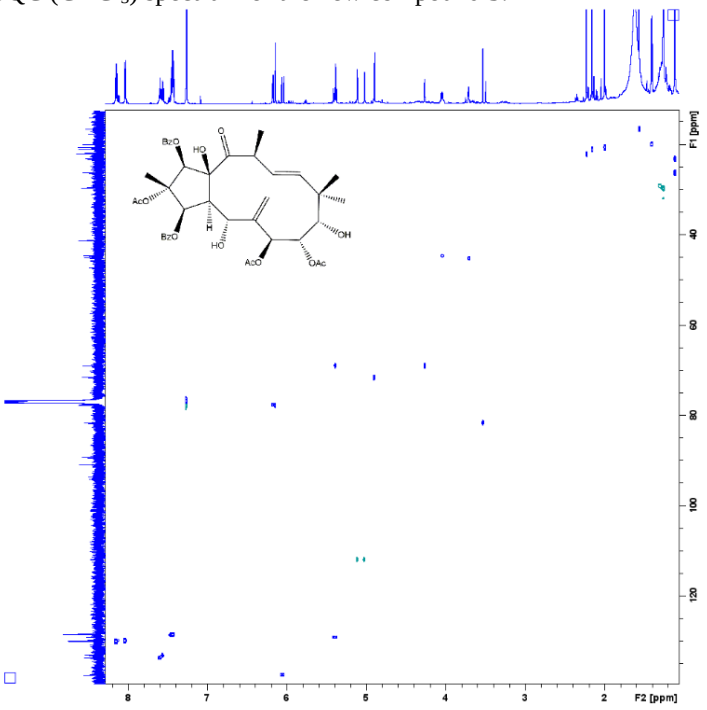


Figure S28. HMBC ($CDCl_3$) spectrum of the new compound **5**.

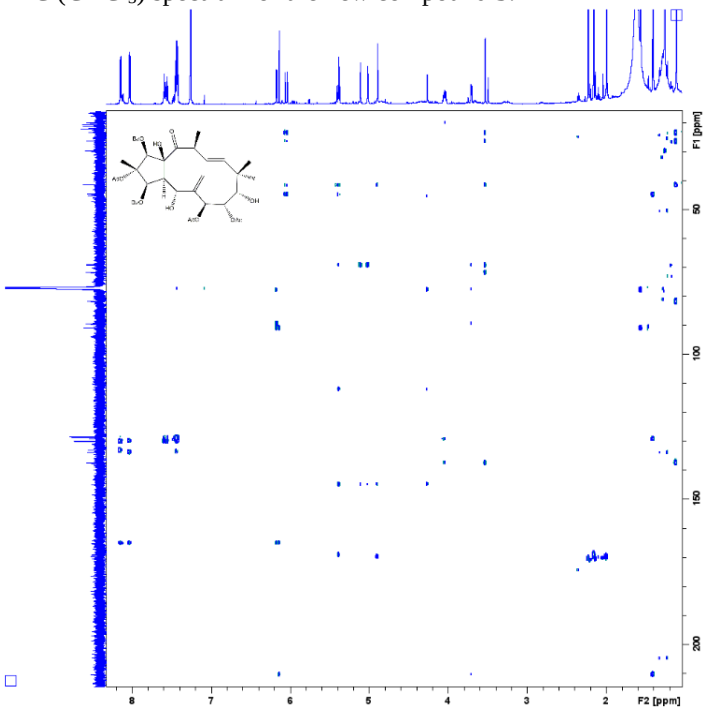


Figure S29. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **5**.

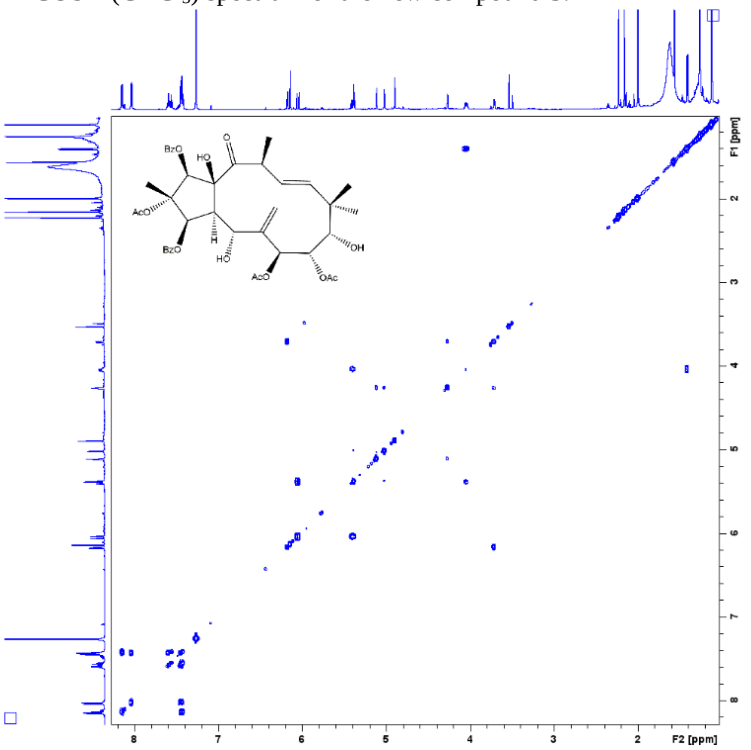


Figure S30. NOESY ($CDCl_3$) spectrum of the new compound **5**.

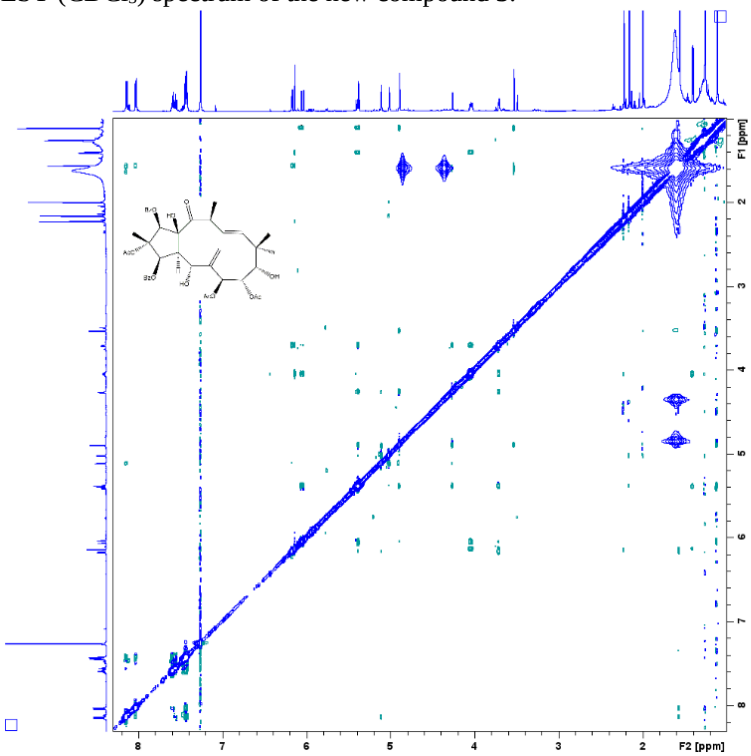


Figure S31. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **6**.

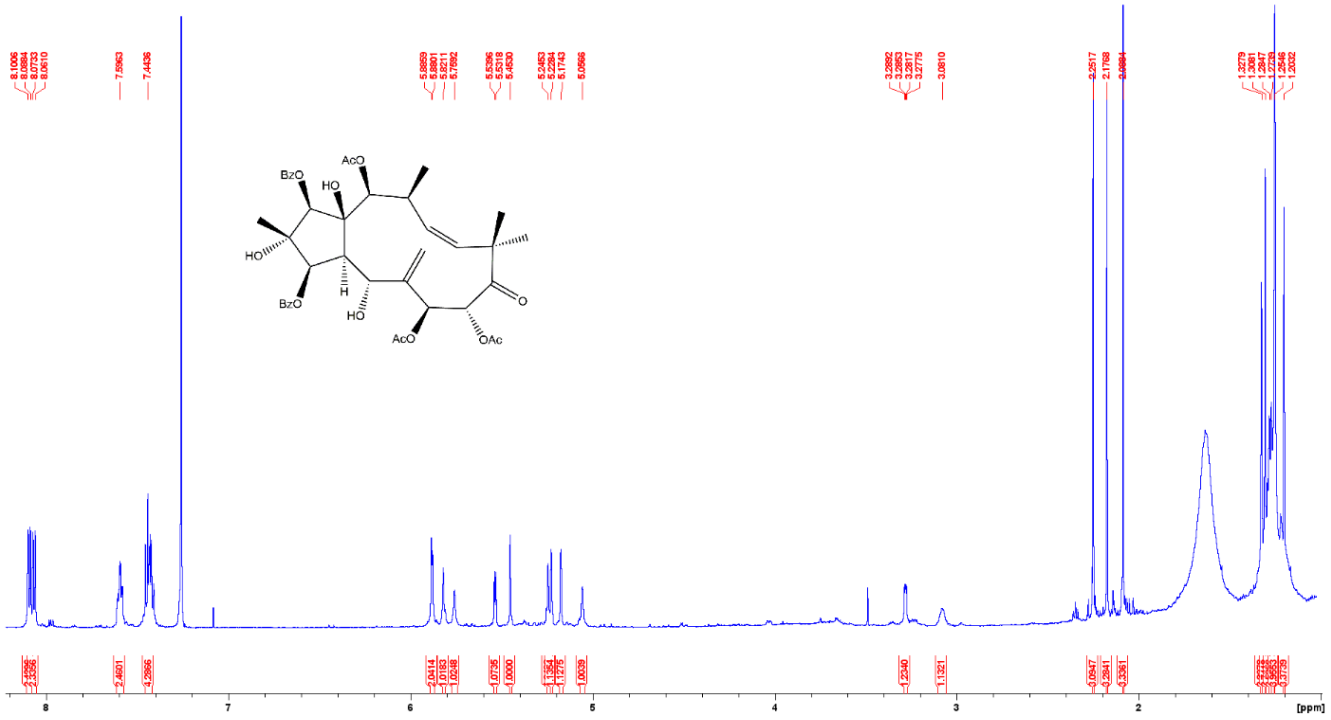


Figure S32. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **6**.

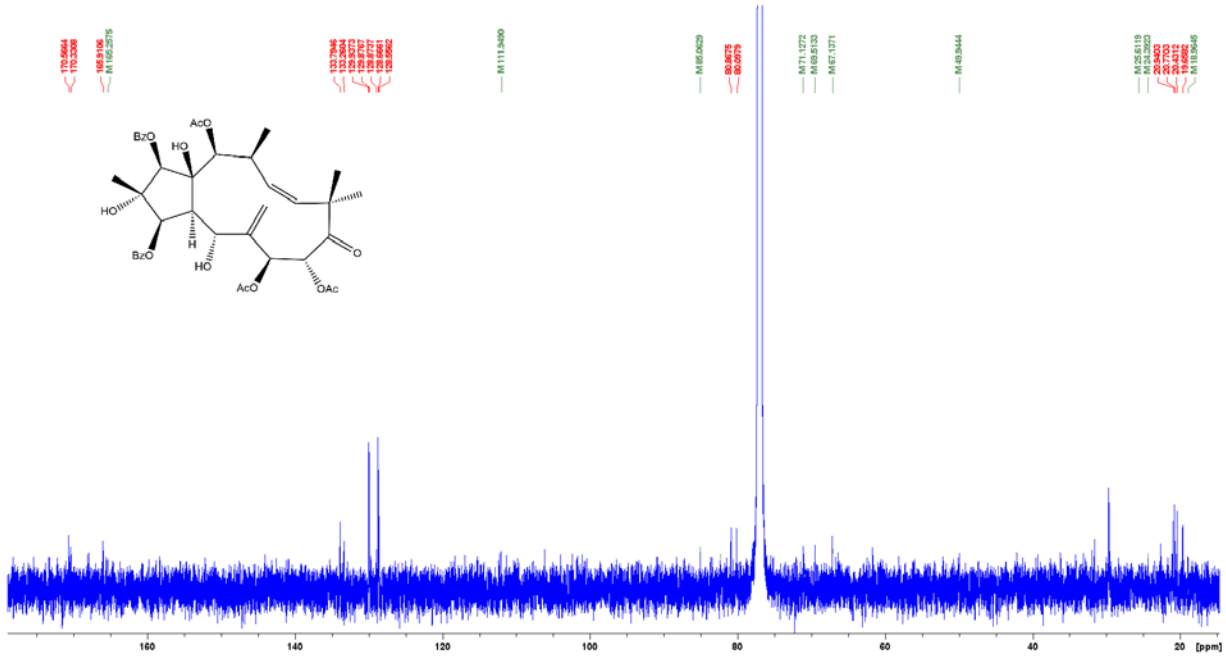


Figure S33. HSQC ($CDCl_3$) spectrum of the new compound **6**.

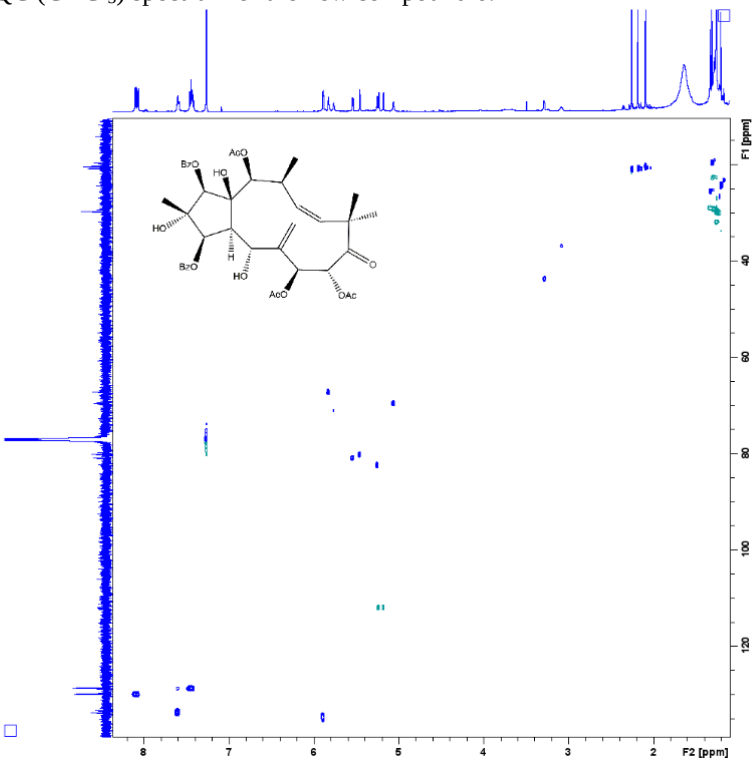


Figure S34. HMBC ($CDCl_3$) spectrum of the new compound **6**.

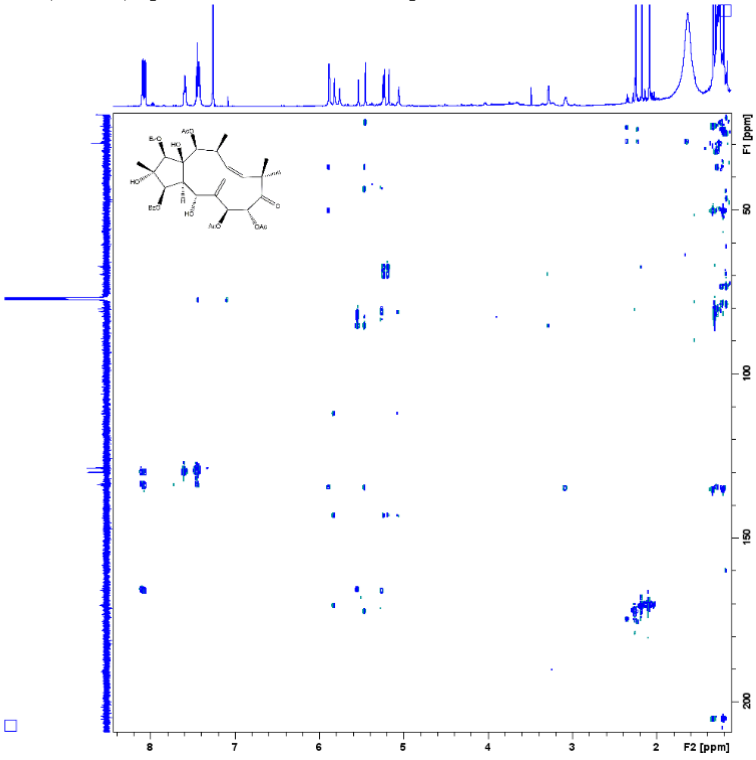


Figure S35. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **6**.

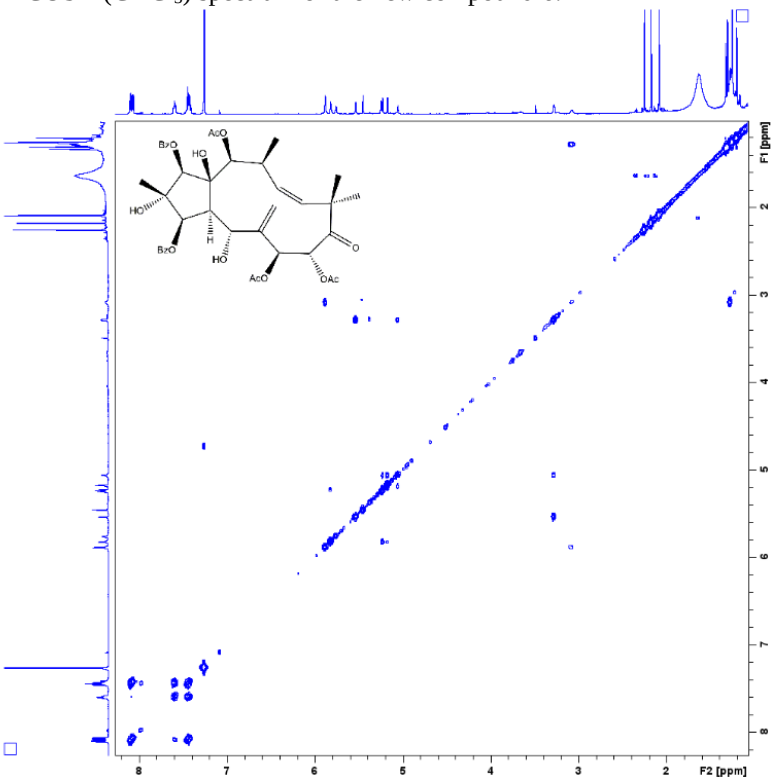


Figure S36. NOESY ($CDCl_3$) spectrum of the new compound **6**.

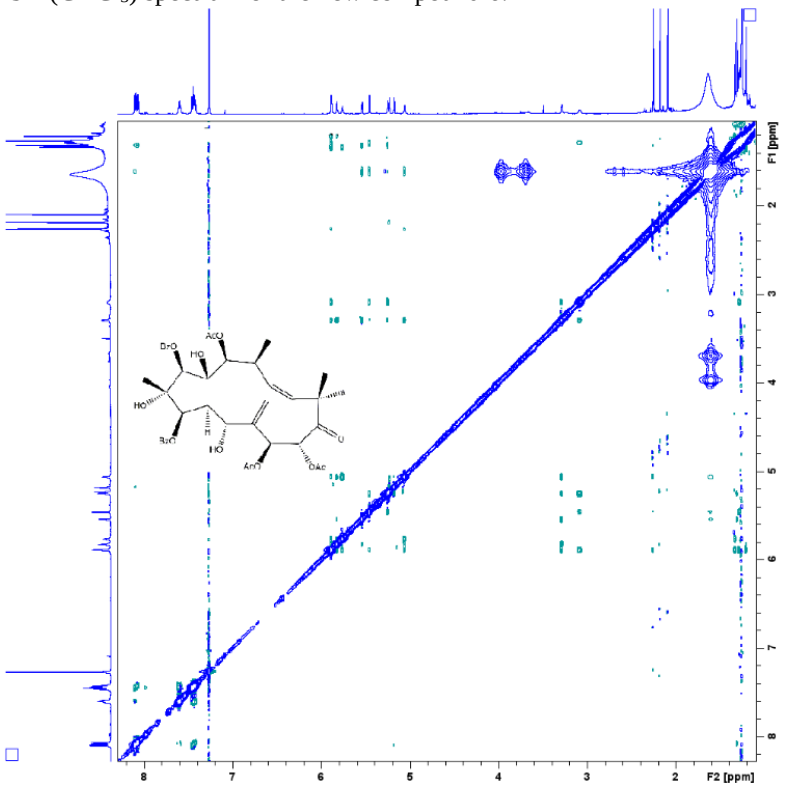


Figure S37. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **7**.

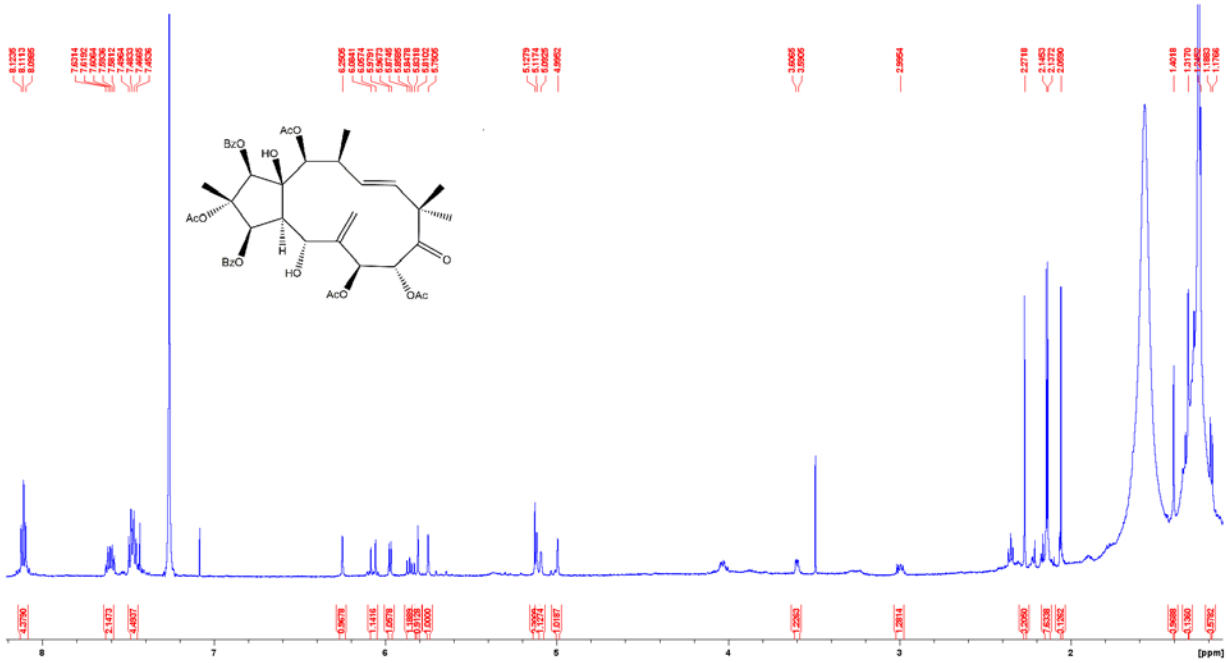


Figure S38. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **7**.

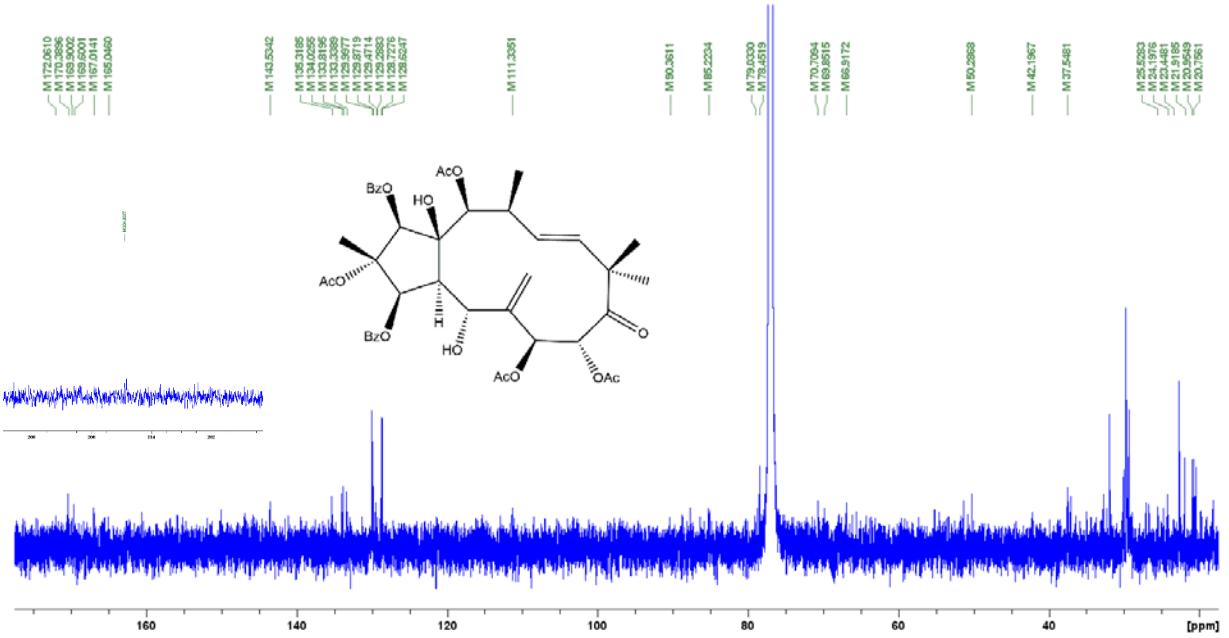


Figure S39. HSQC ($CDCl_3$) spectrum of the new compound **7**.

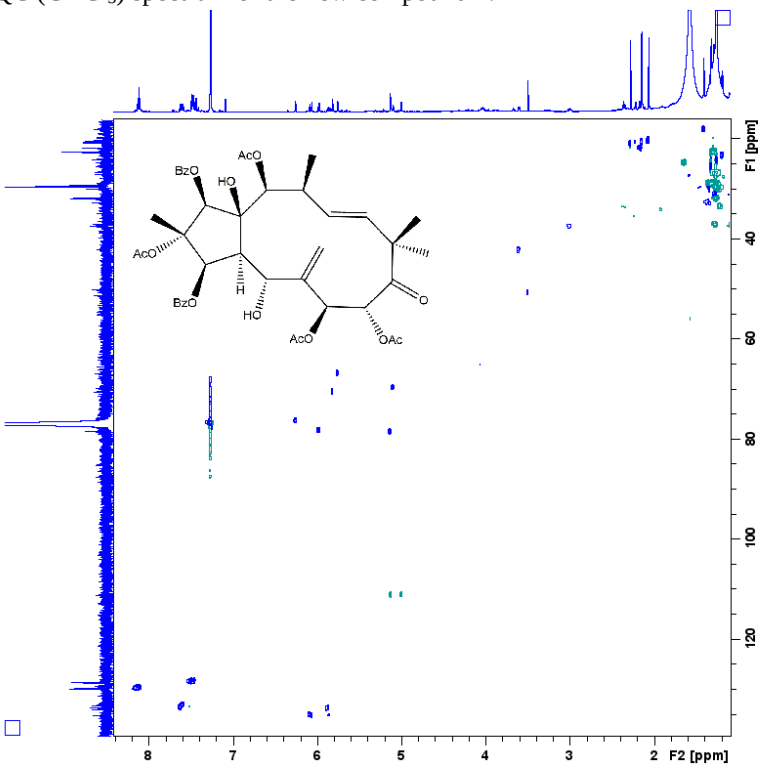


Figure S40. HMBC ($CDCl_3$) spectrum of the new compound **7**.

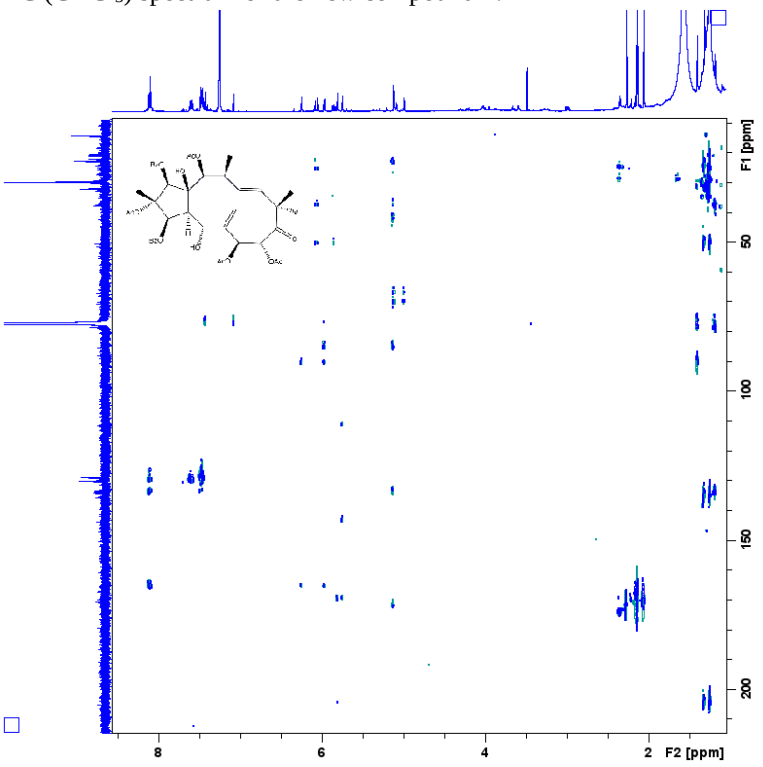


Figure S41. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **7**.

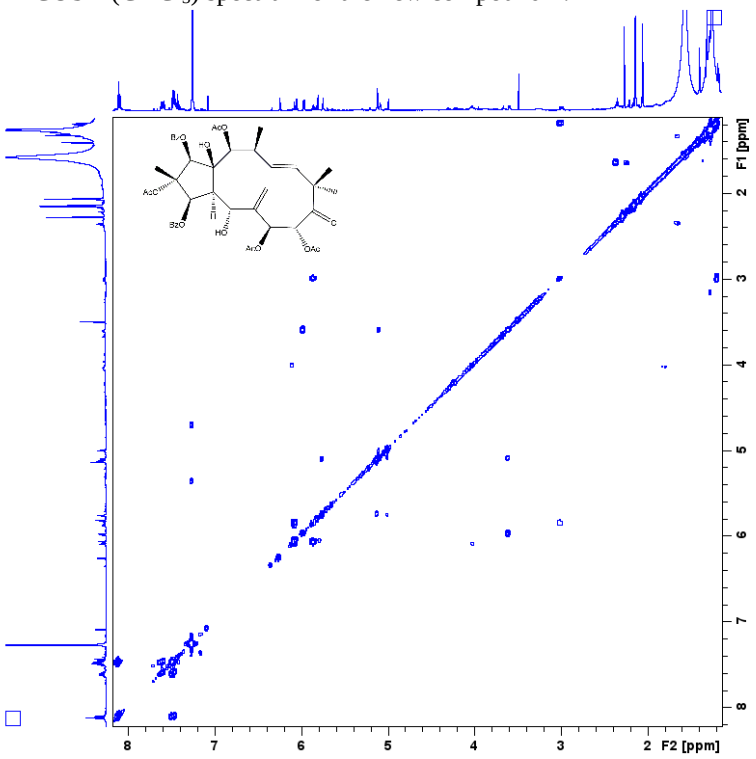


Figure S42. NOESY ($CDCl_3$) spectrum of the new compound **7**.

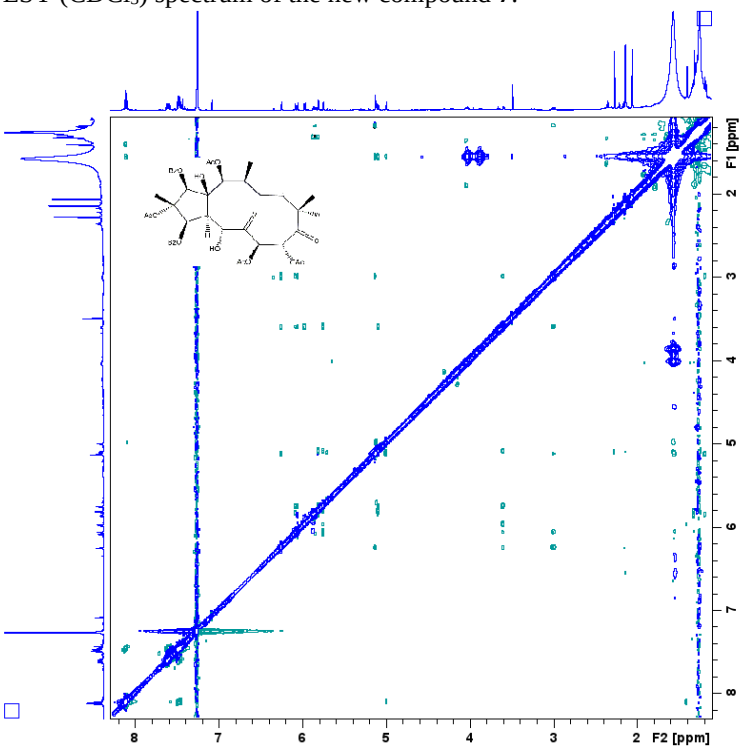


Figure S43. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **8**.

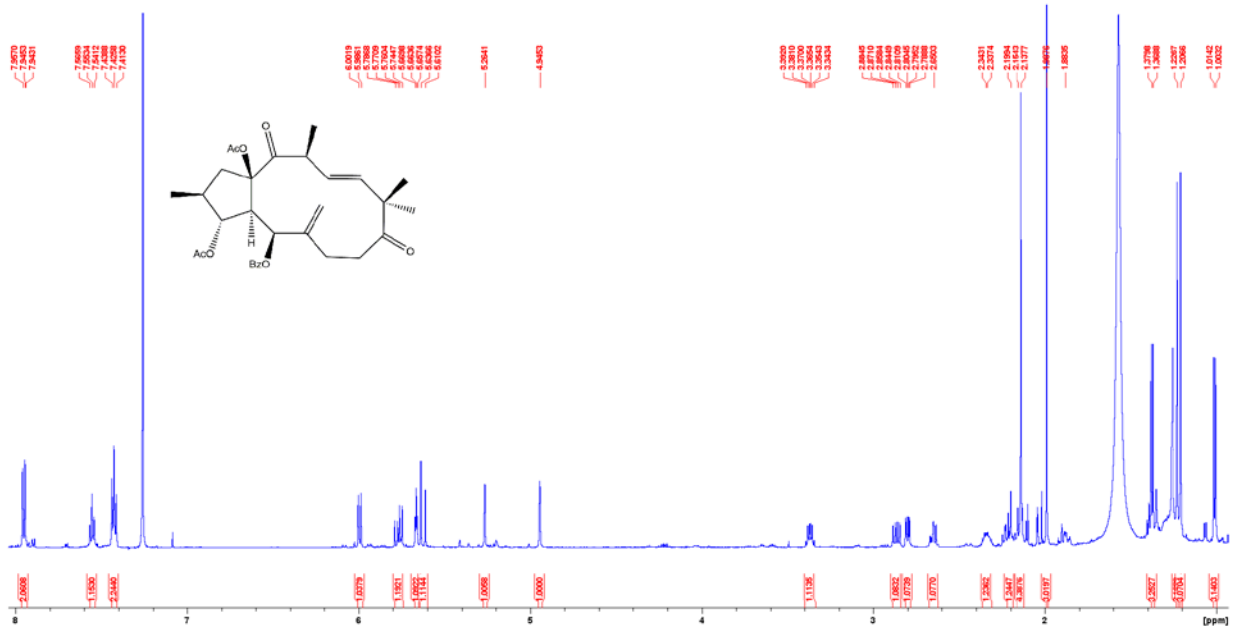


Figure S44. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **8**.

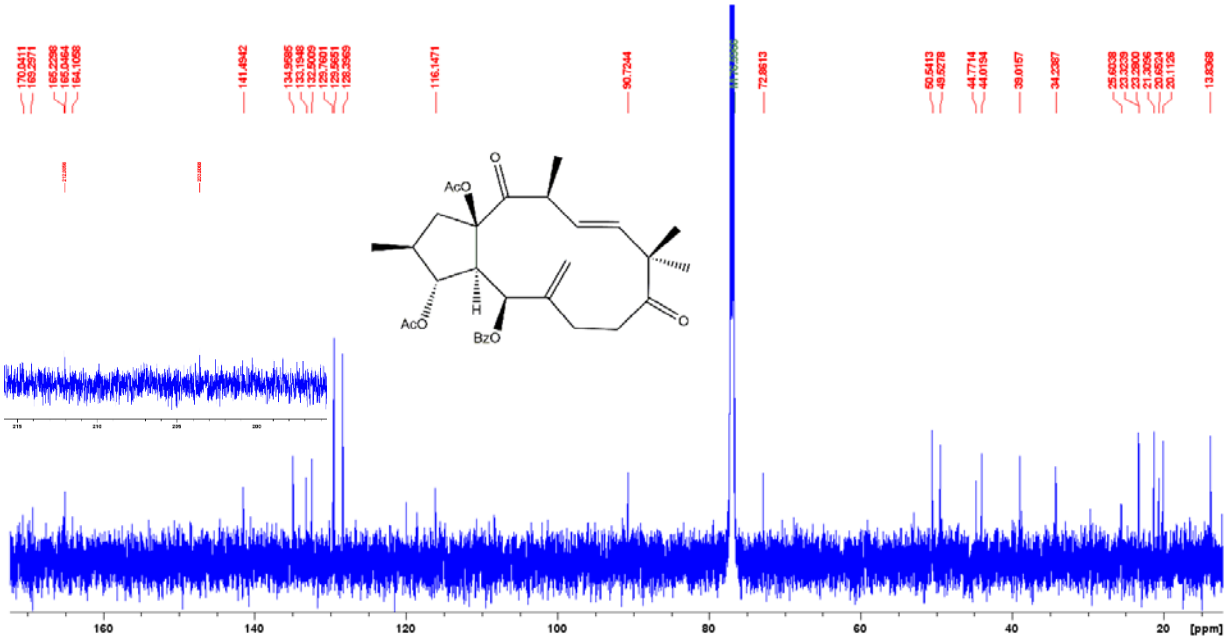


Figure S45. HSQC ($CDCl_3$) spectrum of the new compound **8**.

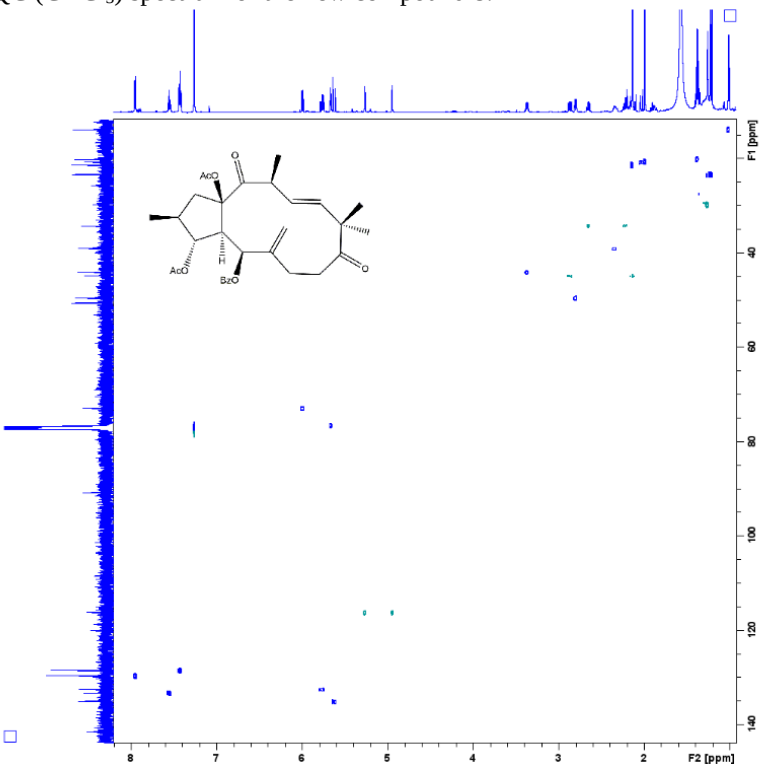


Figure S46. HMBC ($CDCl_3$) spectrum of the new compound **8**.

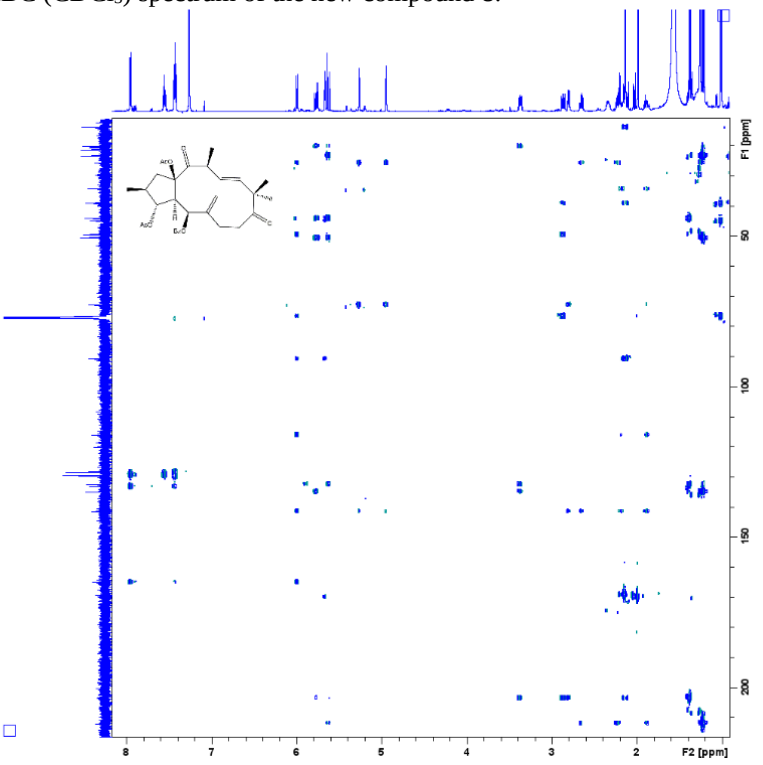


Figure S47. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **8**.

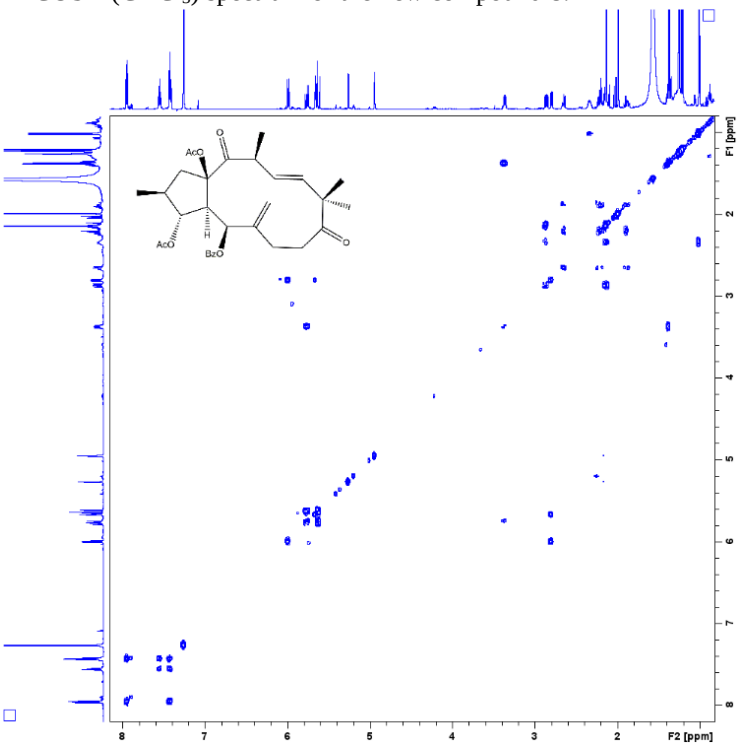


Figure S48. NOESY ($CDCl_3$) spectrum of the new compound **8**.

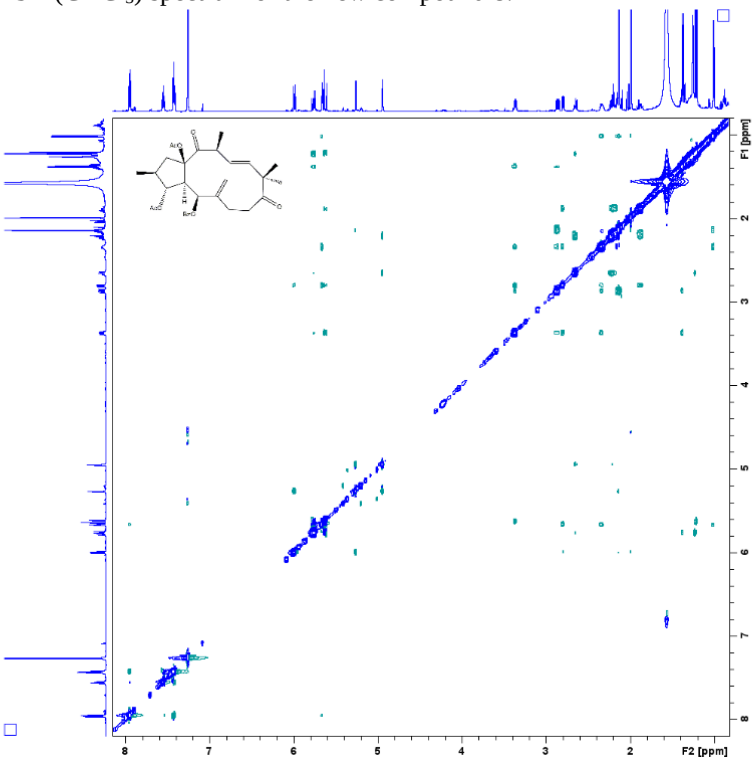


Figure S49. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **9**.

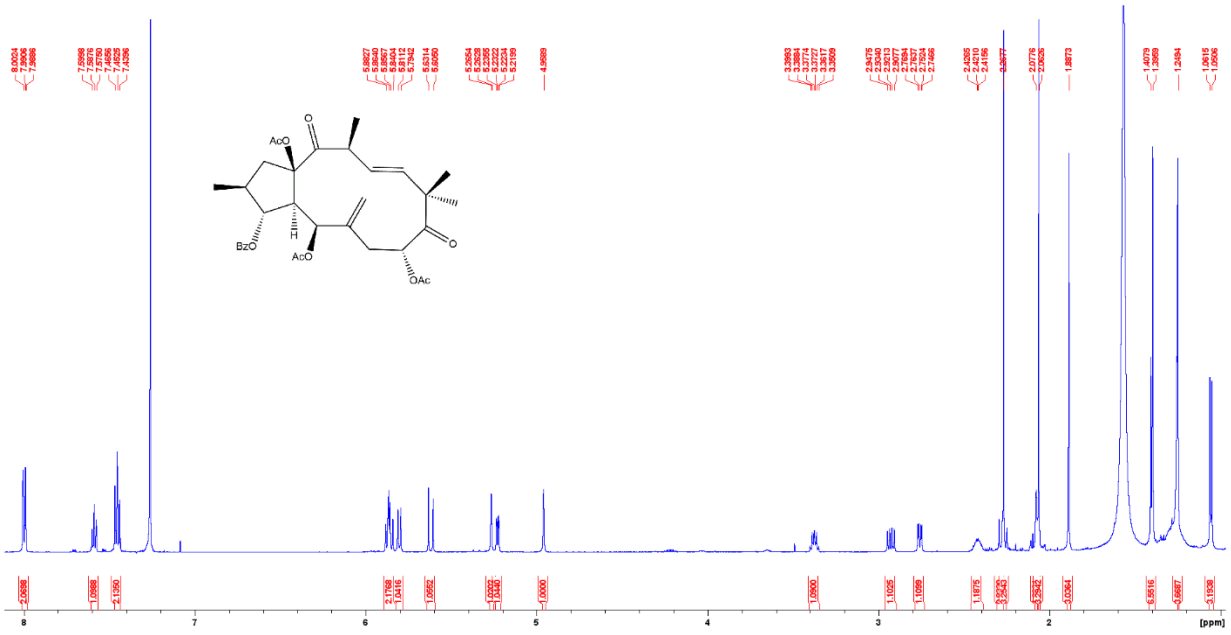


Figure S50. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **9**.

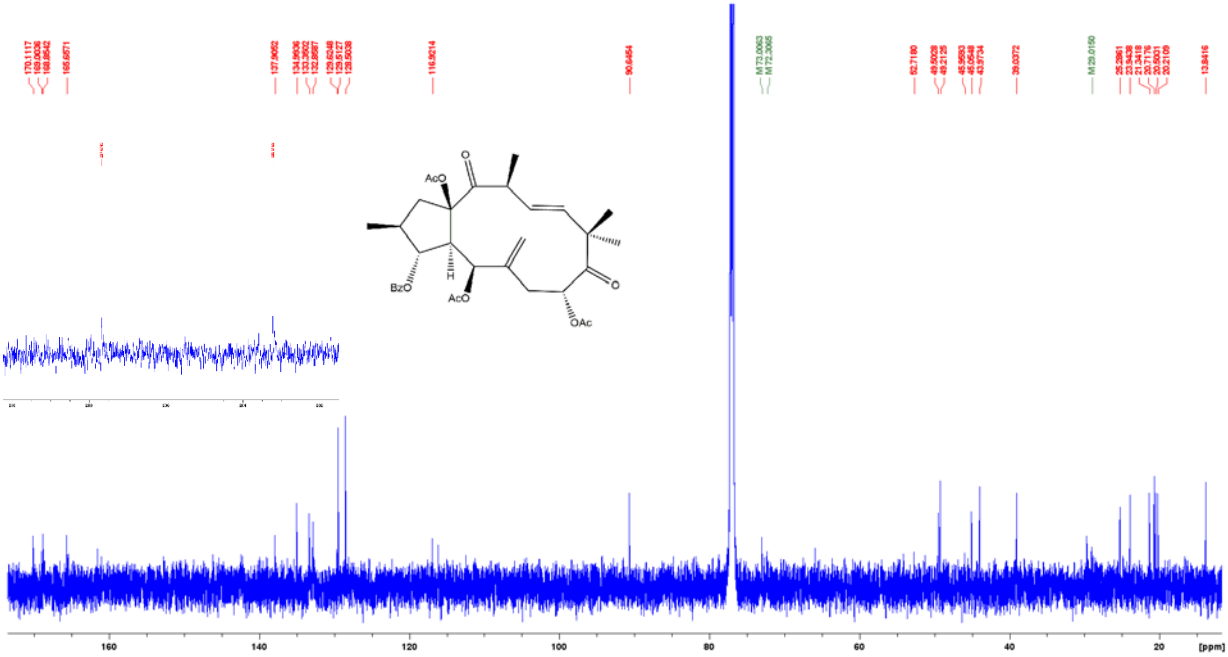


Figure S51. HSQC ($CDCl_3$) spectrum of the new compound **9**.

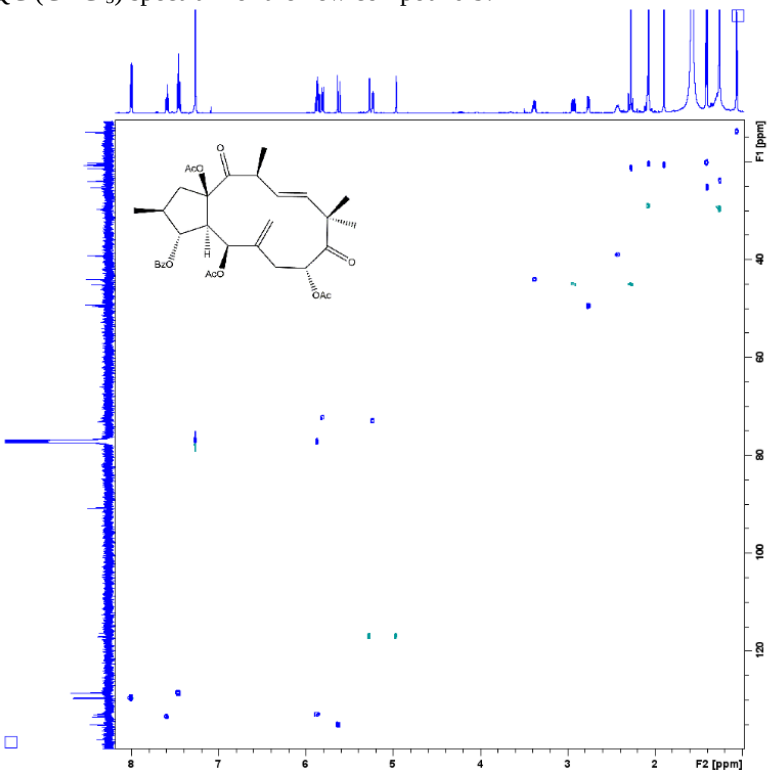


Figure S52. HMBC ($CDCl_3$) spectrum of the new compound **9**.

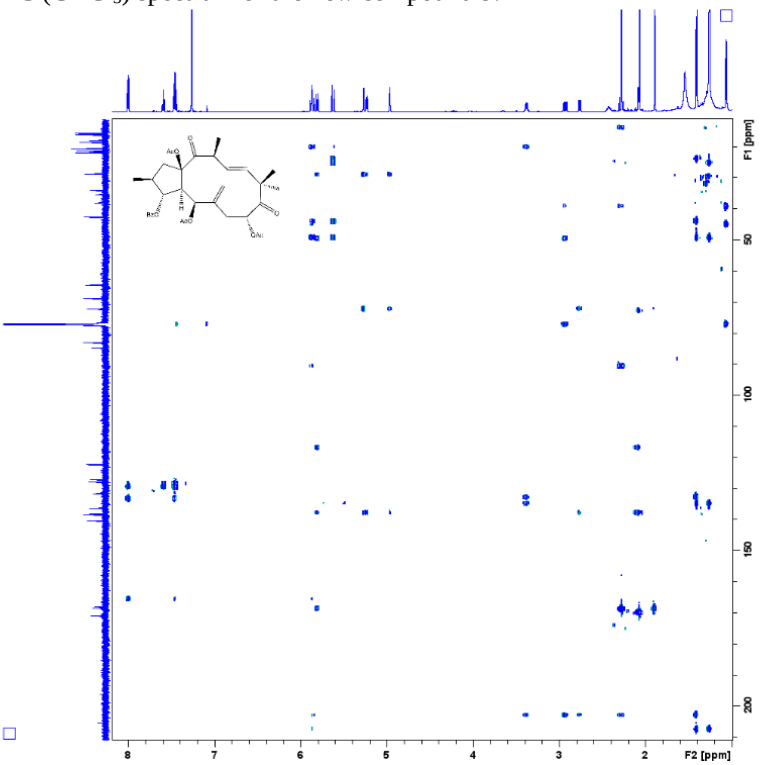


Figure S53. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **9**.

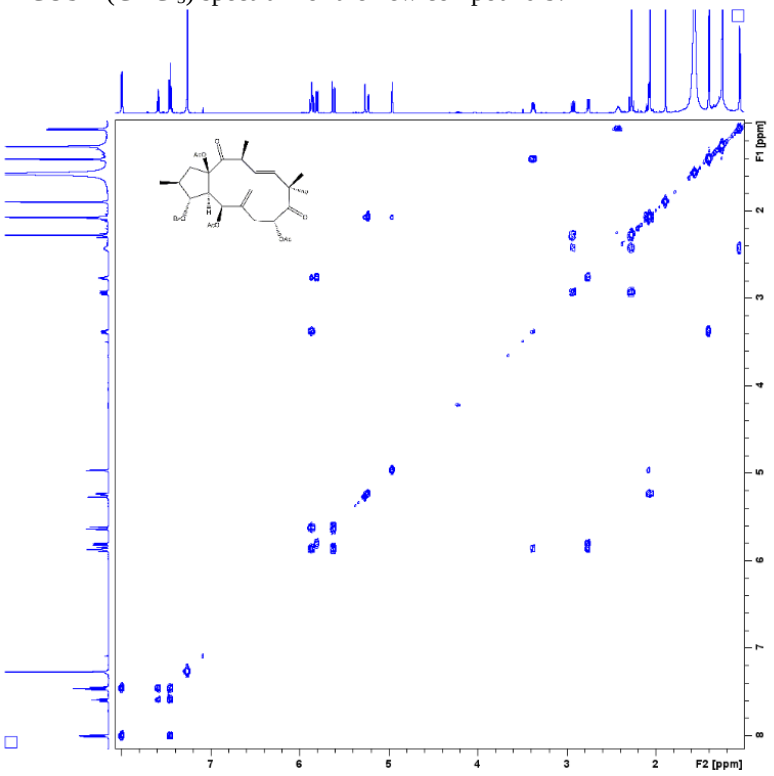


Figure S54. NOESY ($CDCl_3$) spectrum of the new compound **9**.

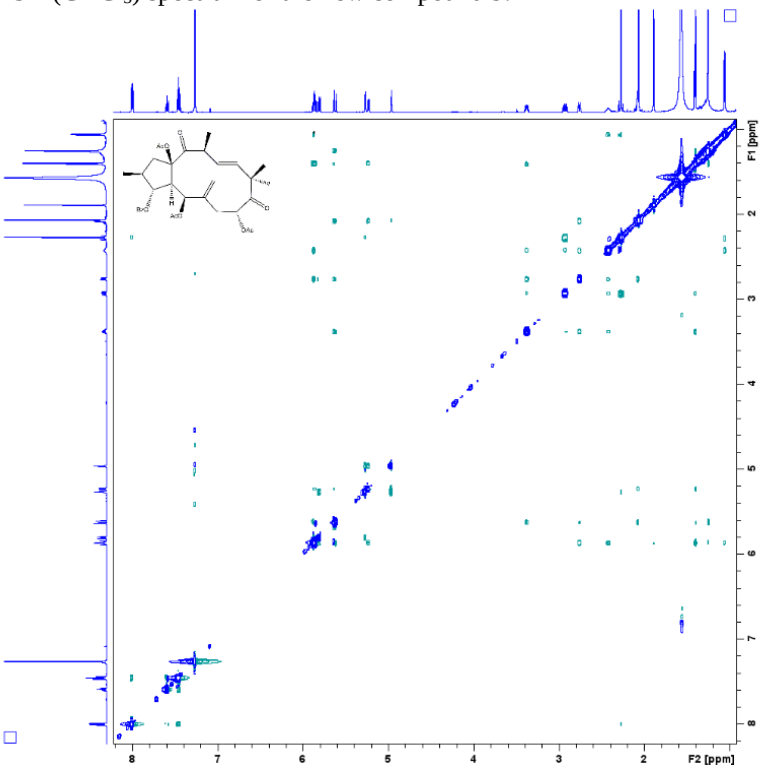


Figure S55. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **10**.

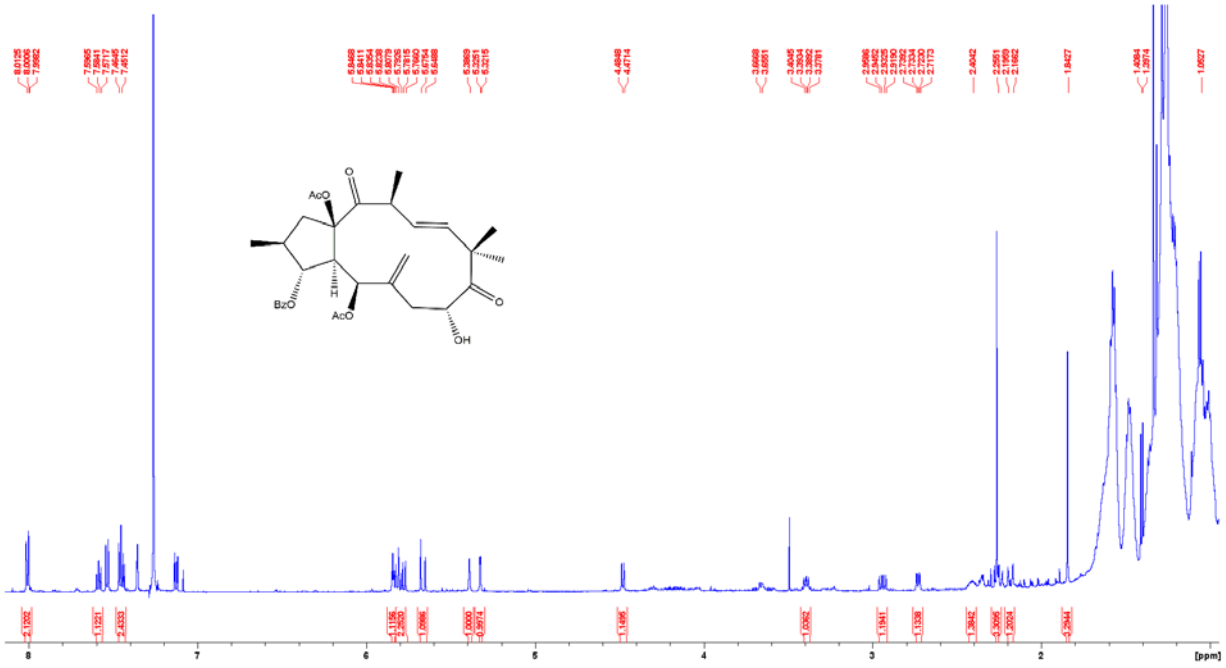


Figure S56. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **10**.

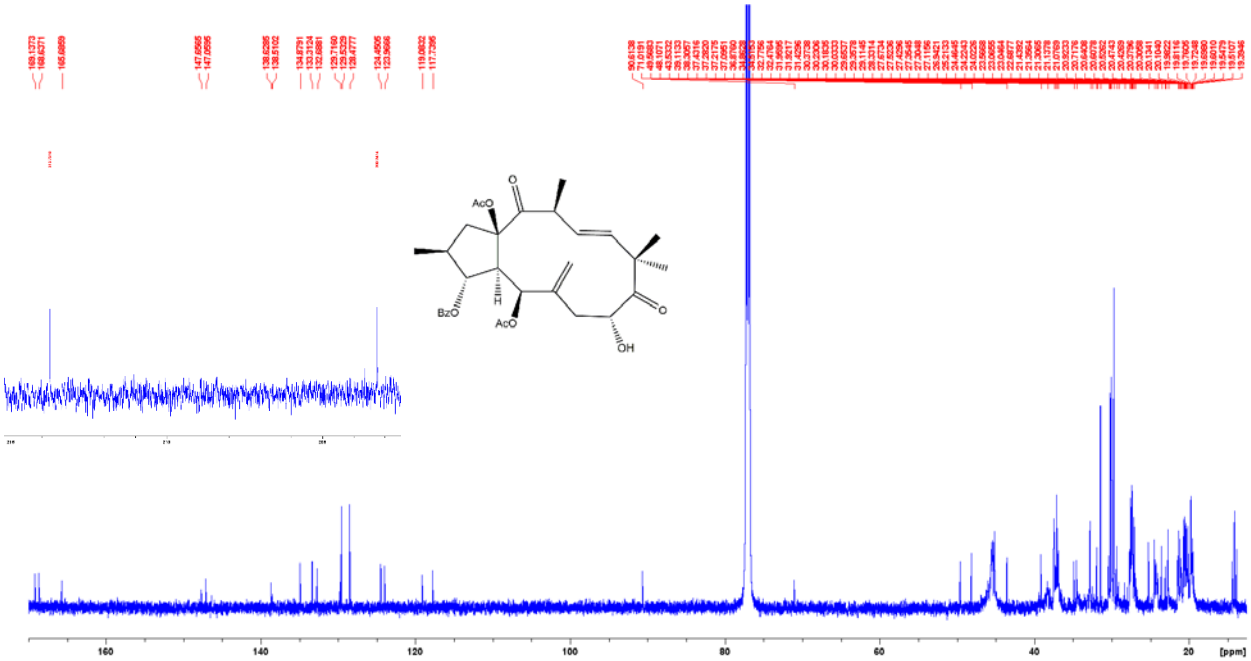


Figure S57. HSQC ($CDCl_3$) spectrum of the new compound **10**.

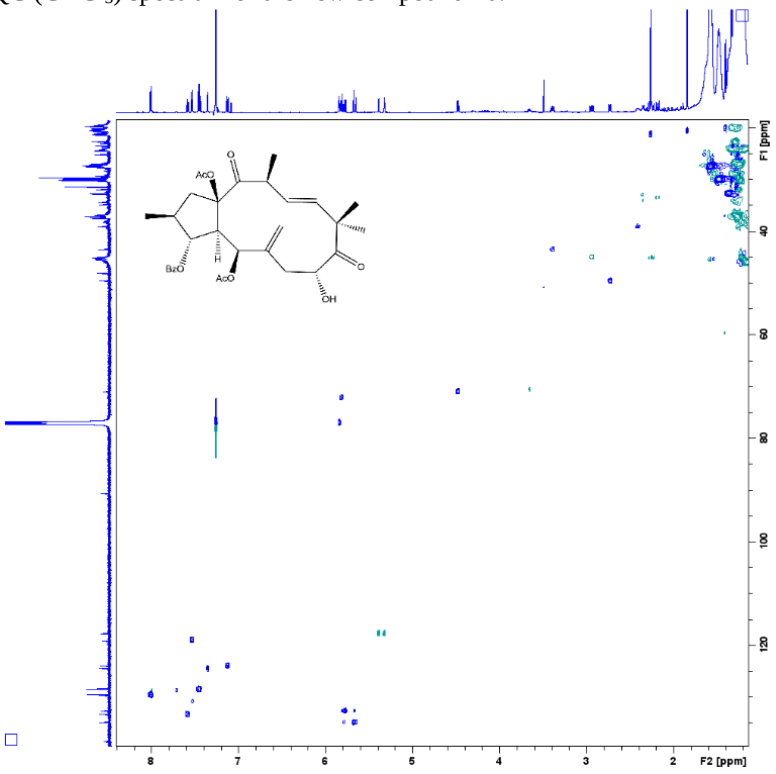


Figure S58. HMBC ($CDCl_3$) spectrum of the new compound **10**.

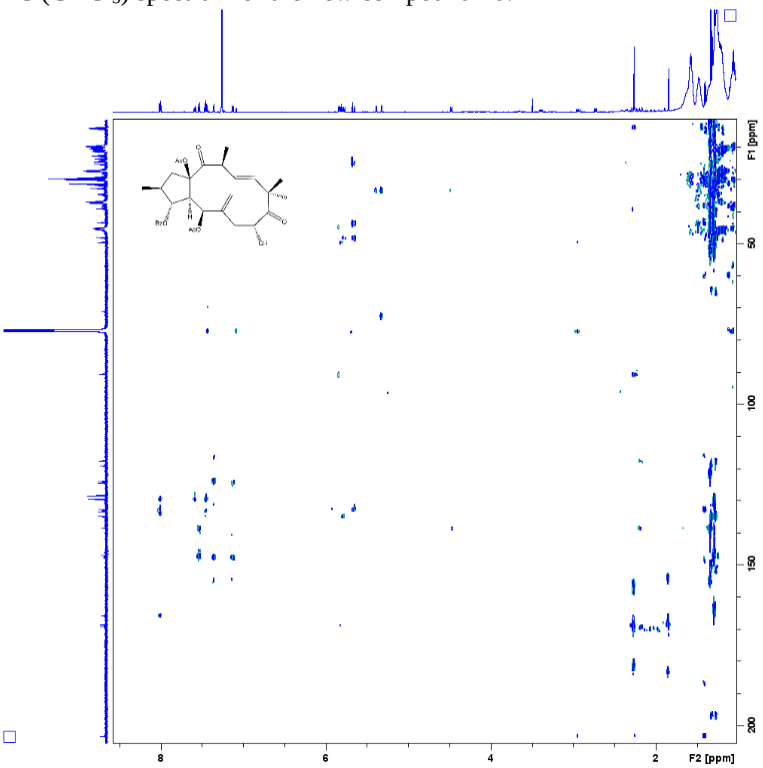


Figure S59. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **10**.

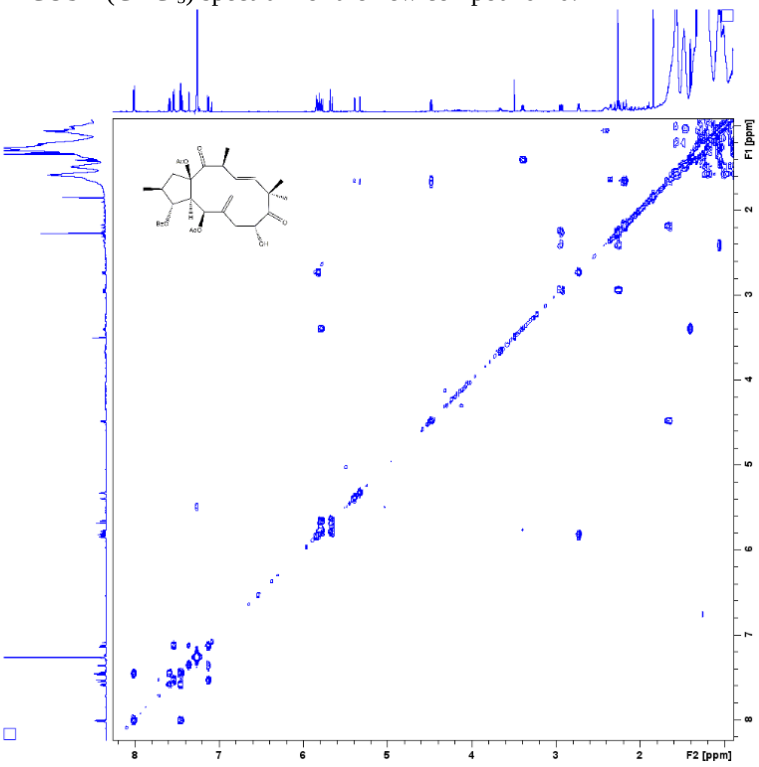


Figure S60. NOESY ($CDCl_3$) spectrum of the new compound **10**.

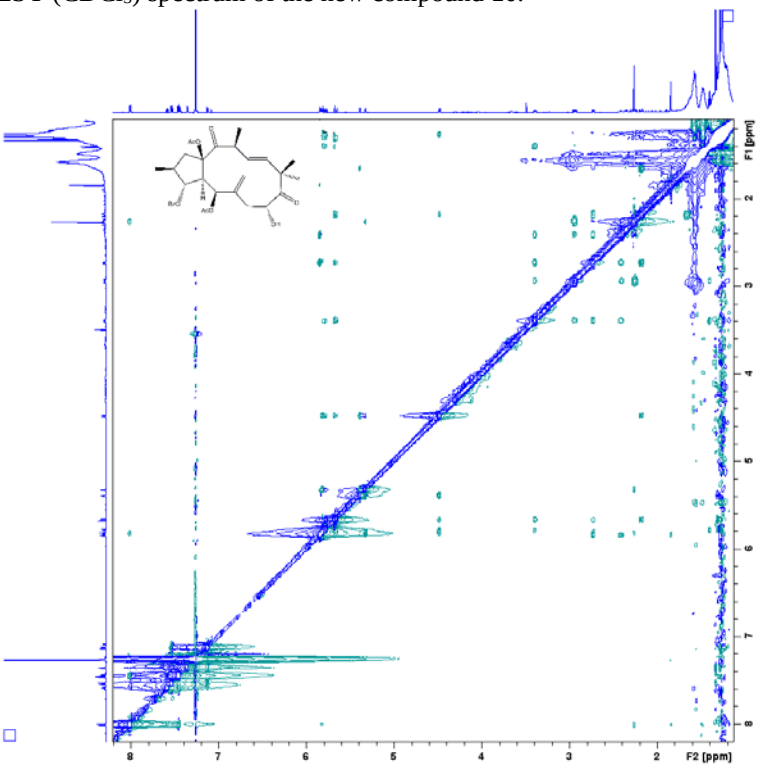


Figure S61. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **11**.

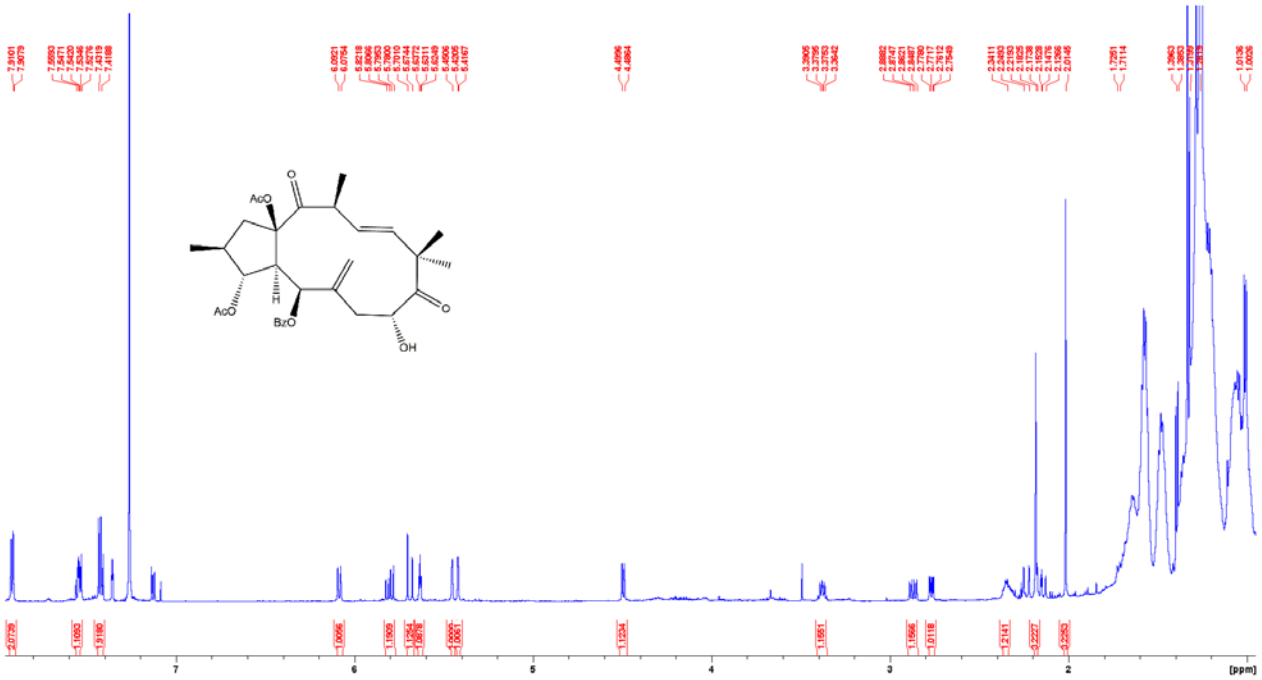


Figure S62. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **11**.

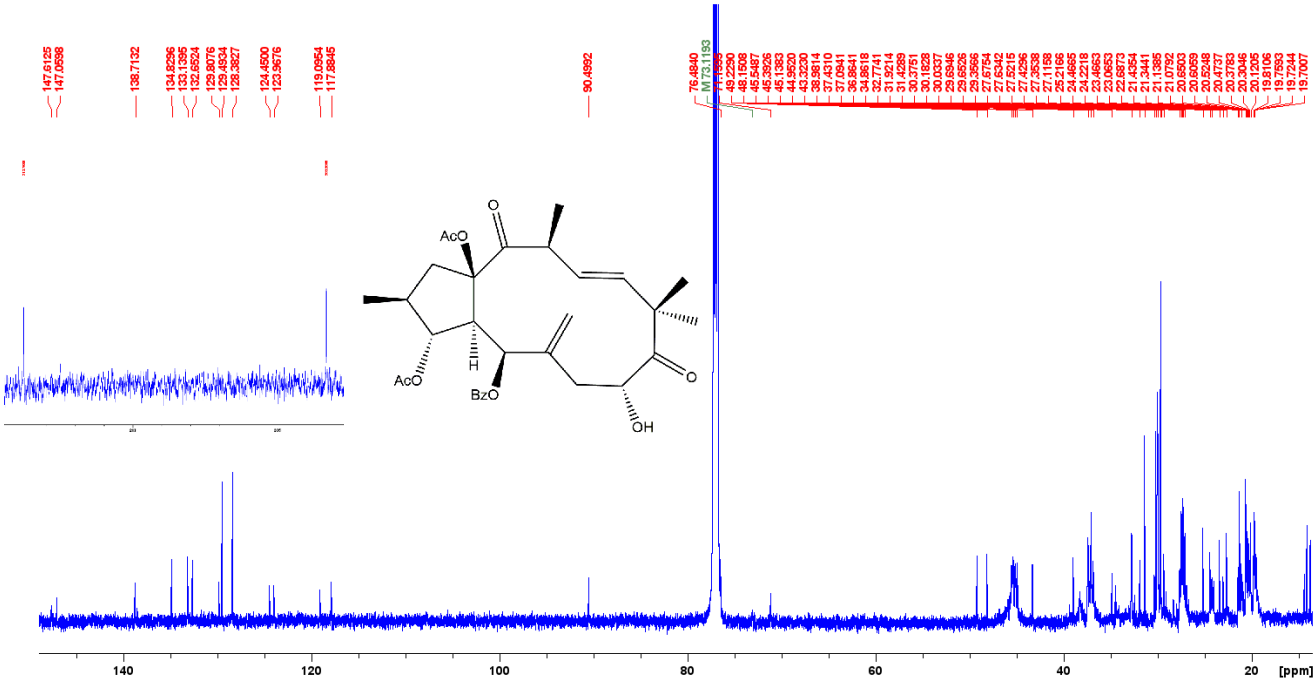


Figure S63. HSQC ($CDCl_3$) spectrum of the new compound **11**.

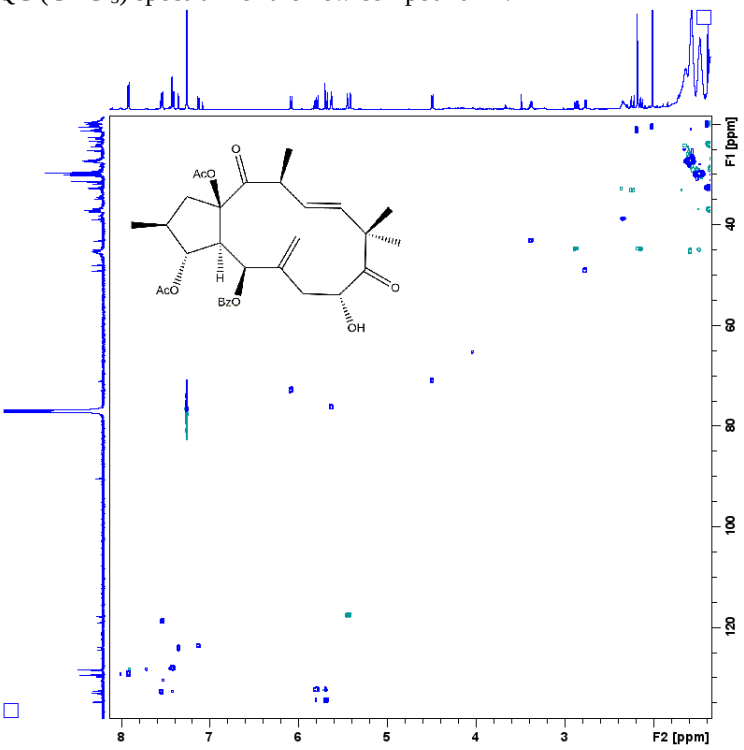


Figure S64. HMBC ($CDCl_3$) spectrum of the new compound **11**.

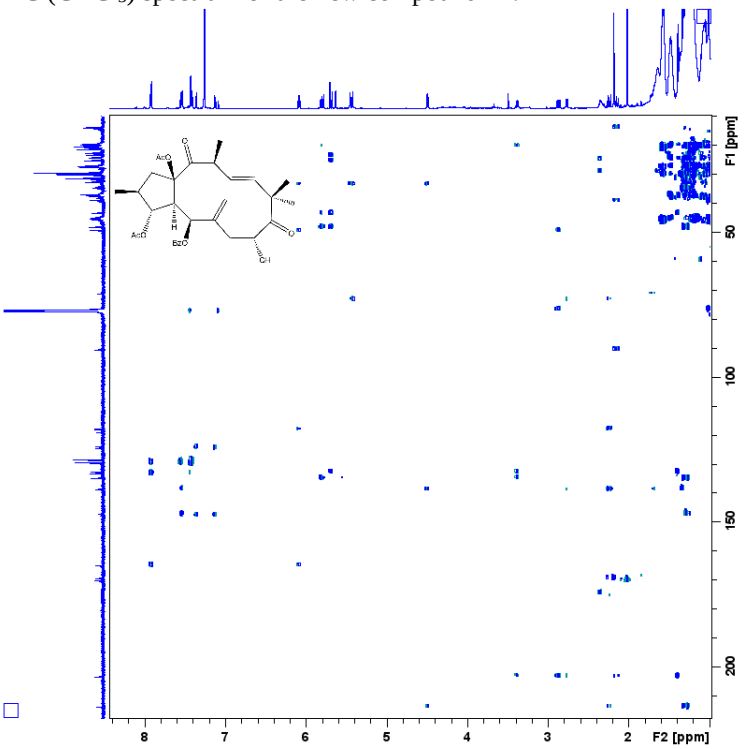


Figure S65. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **11**.

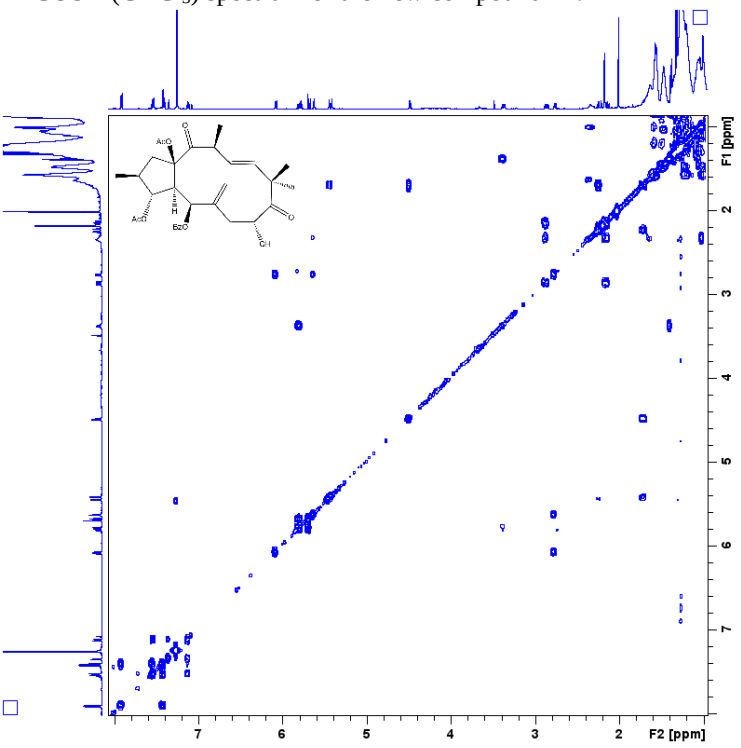


Figure S66. NOESY ($CDCl_3$) spectrum of the new compound **11**.

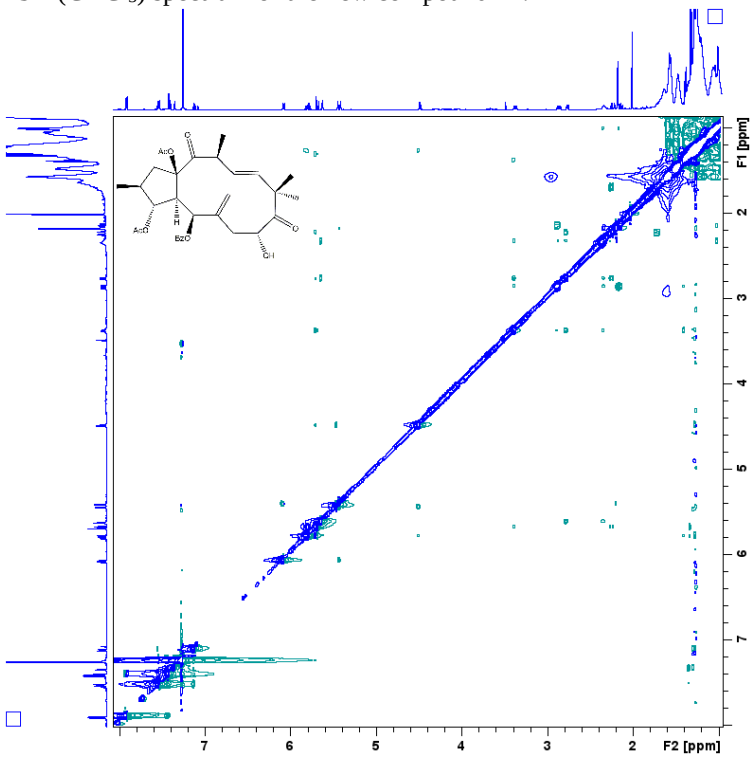


Figure S67. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **13**.

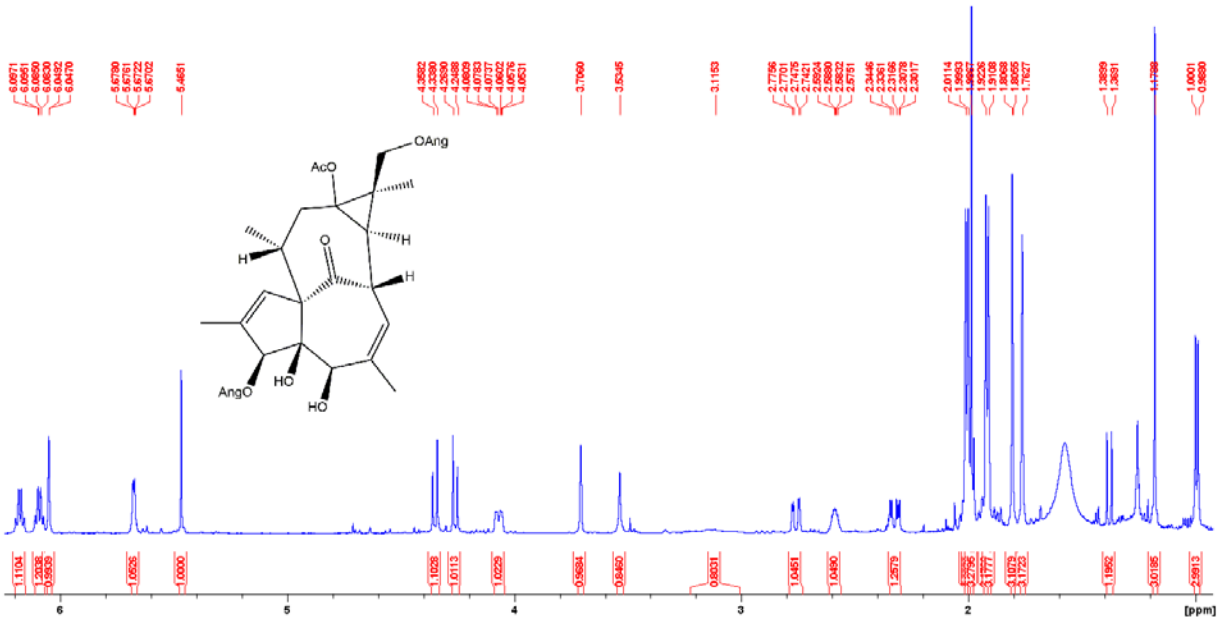


Figure S68. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **13**.

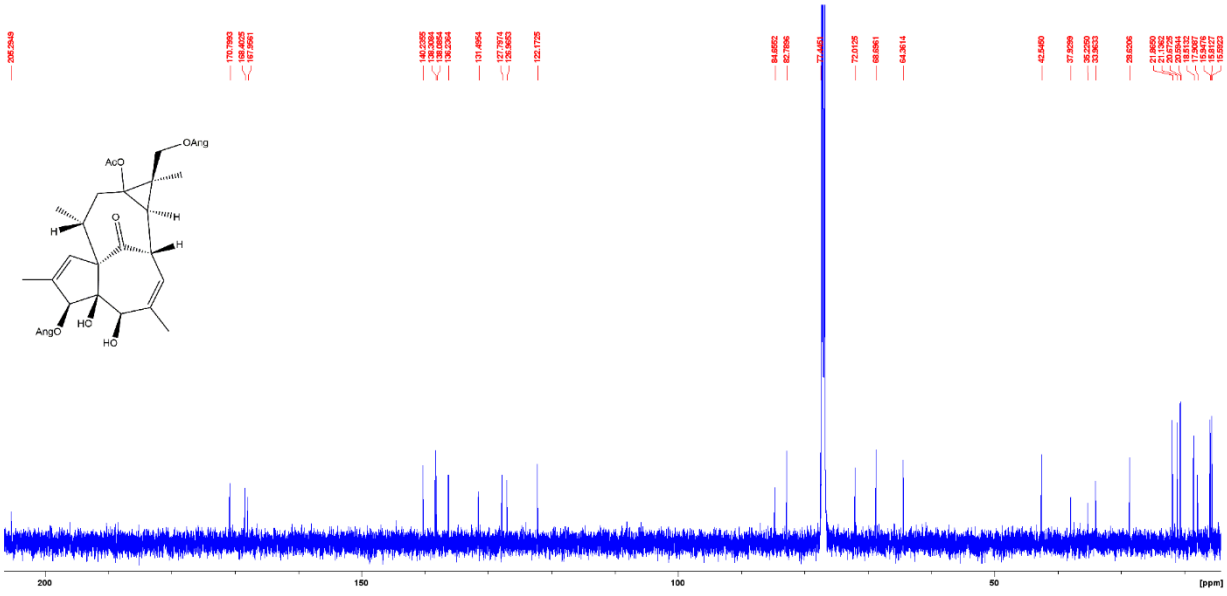


Figure S69. HSQC ($CDCl_3$) spectrum of the new compound **13**.

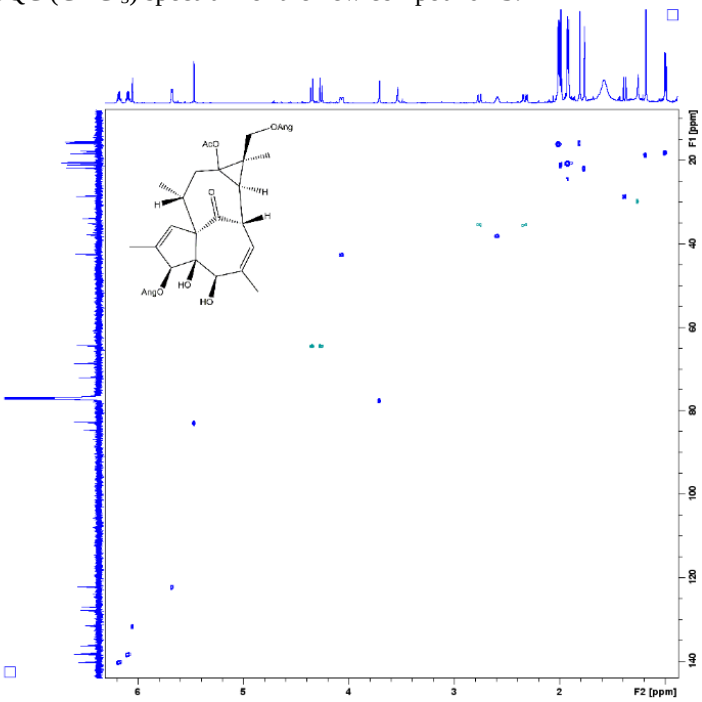


Figure S70. HMBC ($CDCl_3$) spectrum of the new compound **13**.

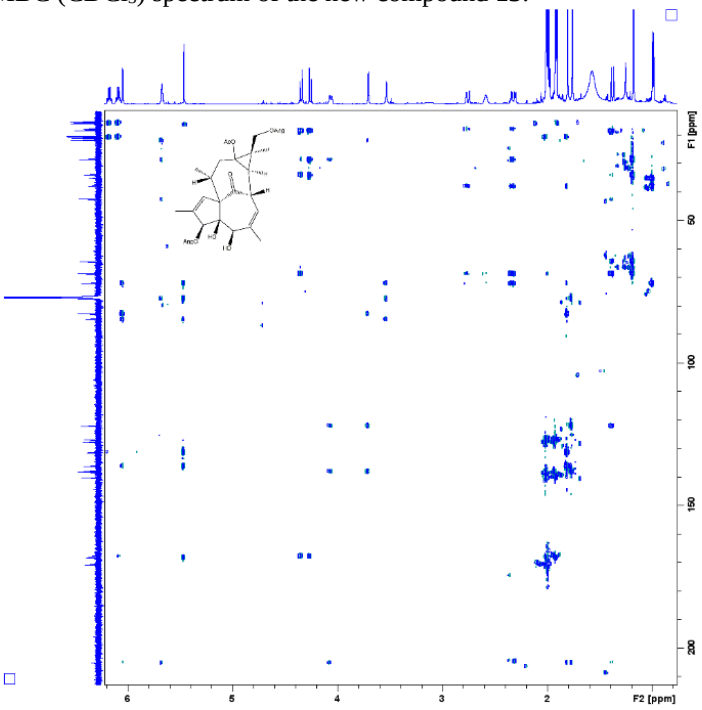


Figure S71. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **13**.

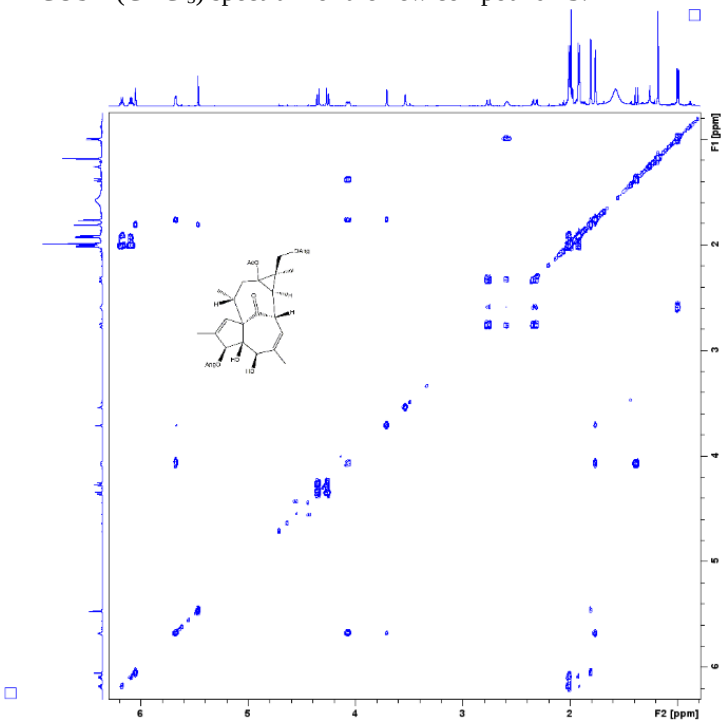


Figure S72. NOESY ($CDCl_3$) spectrum of the new compound **13**.

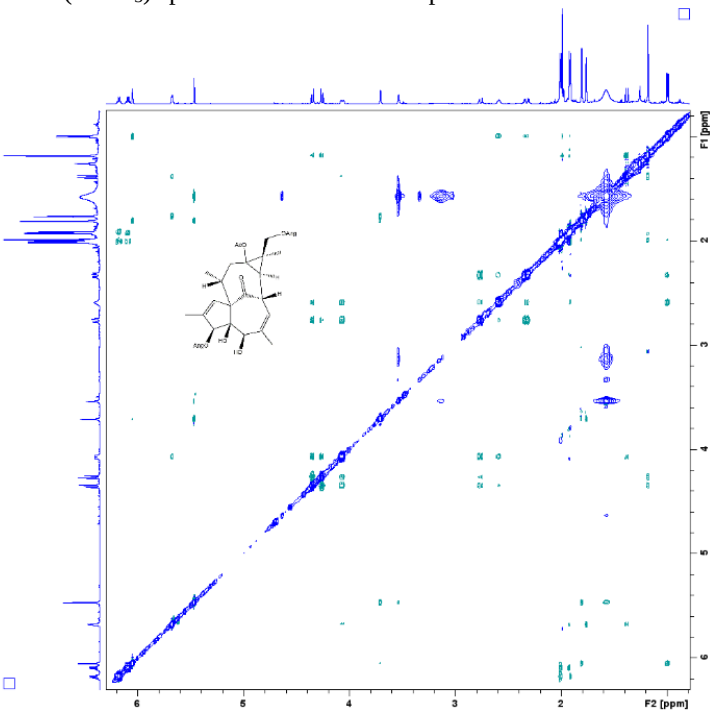


Figure S73. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **15**.

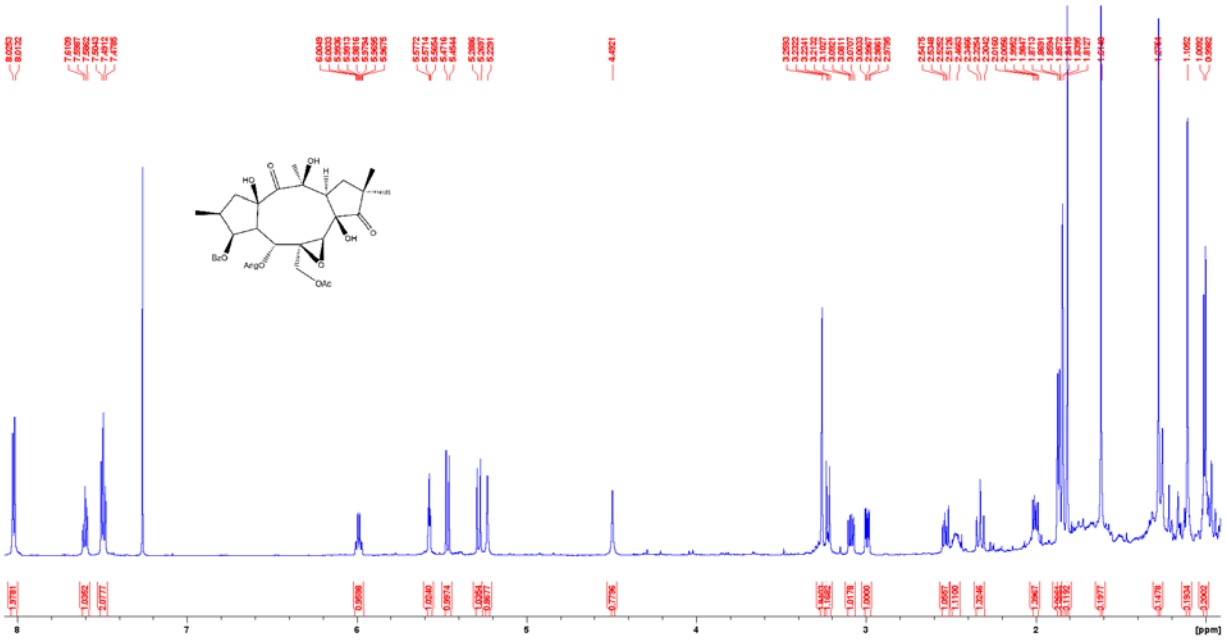


Figure S74. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **15**.

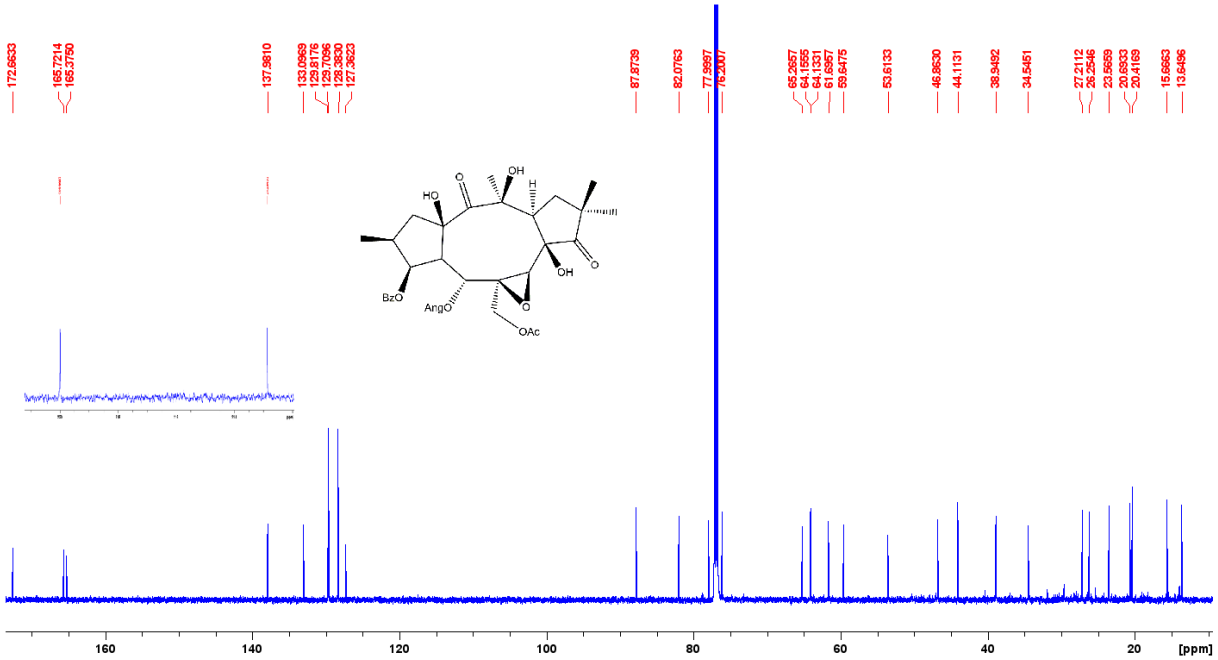


Figure S75. HSQC ($CDCl_3$) spectrum of the new compound **15**.

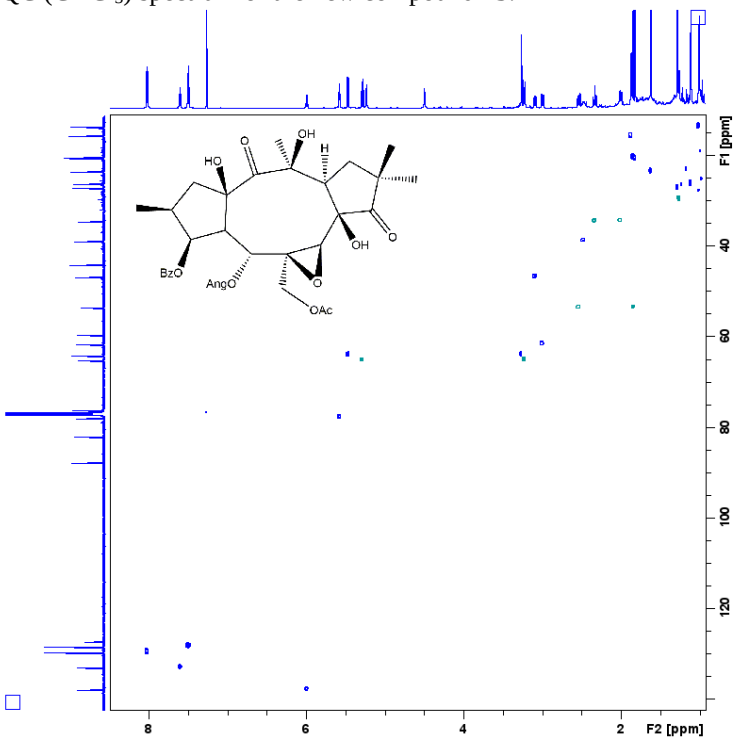


Figure S76. HMBC ($CDCl_3$) spectrum of the new compound **15**.

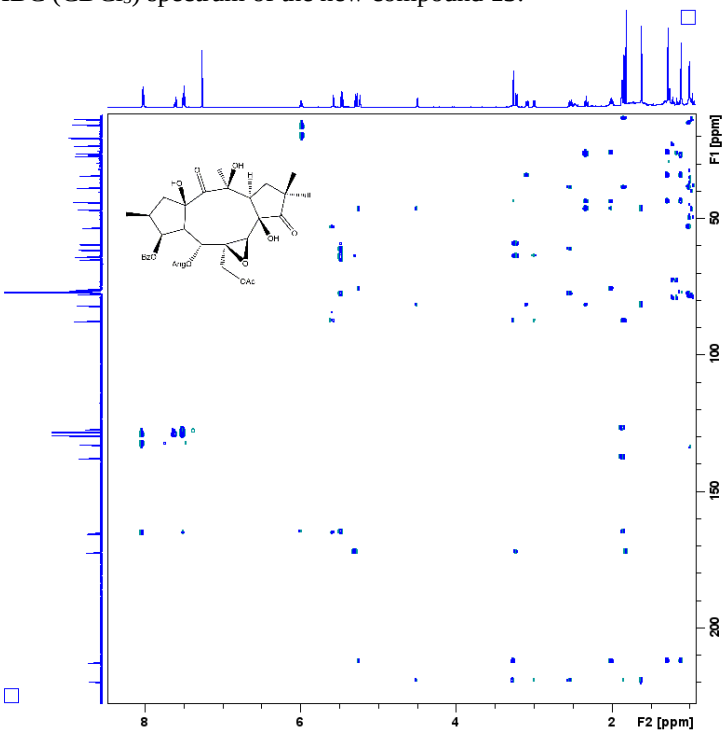


Figure S77. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **15**.

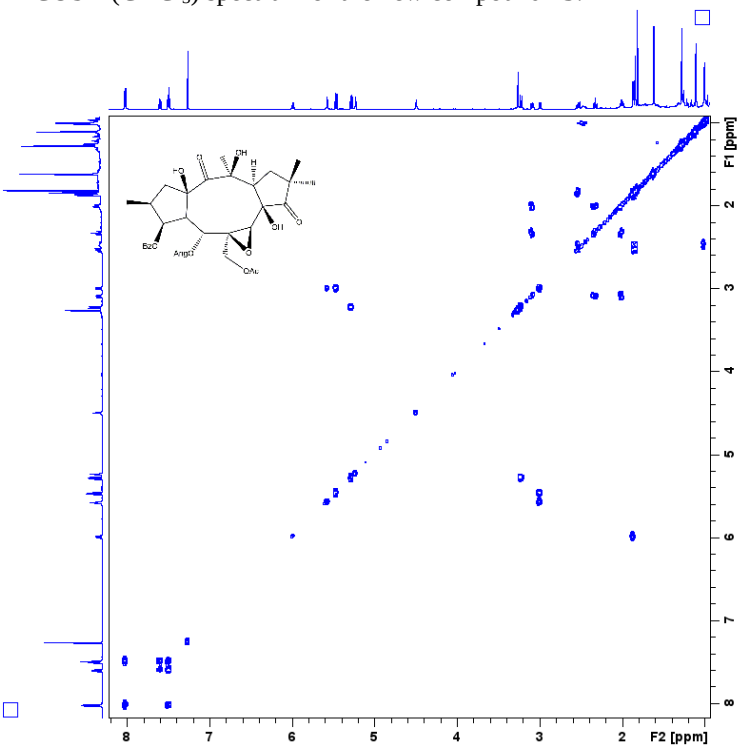


Figure S78. NOESY ($CDCl_3$) spectrum of the new compound **15**.

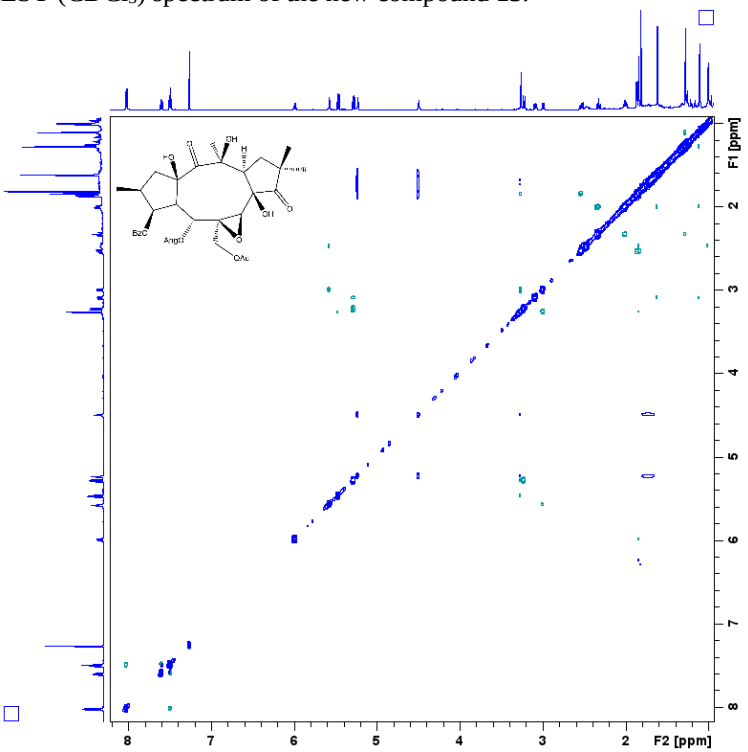


Figure S79. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **16**.

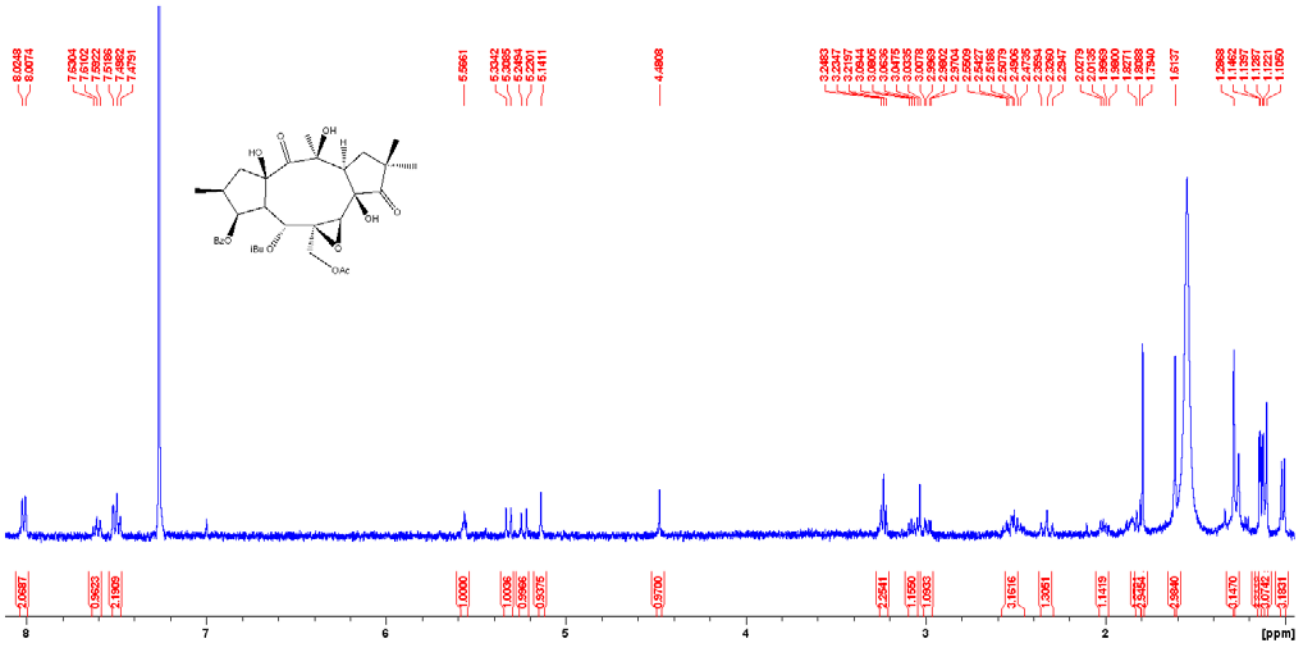


Figure S80. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **16**.

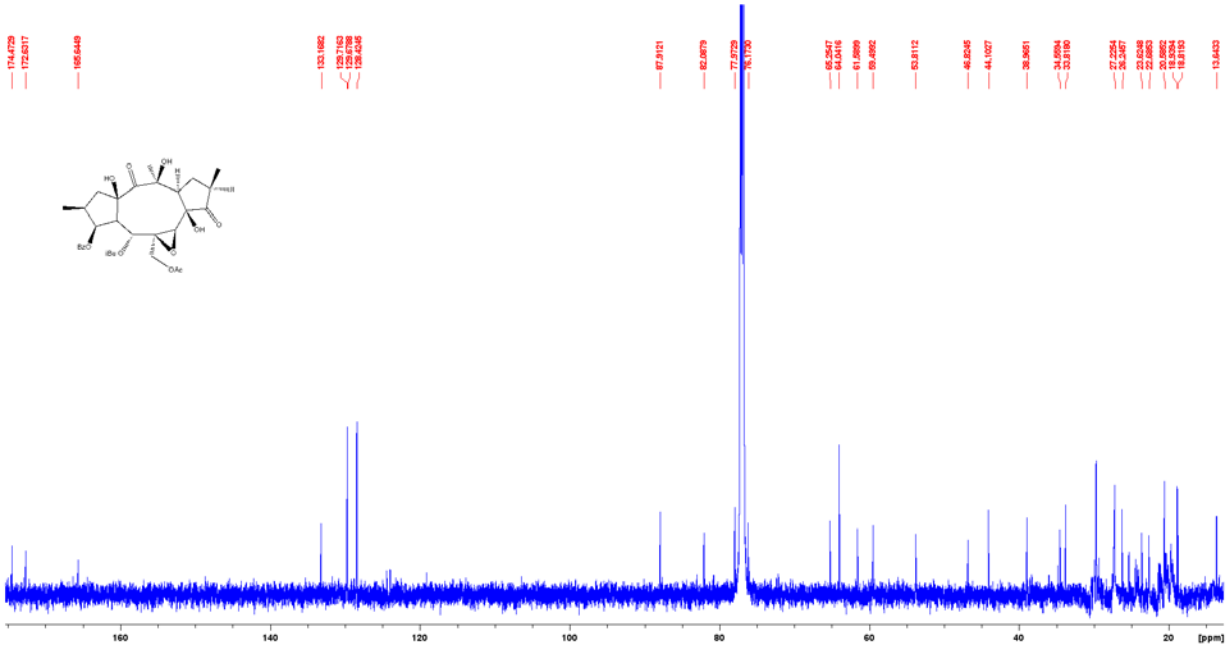


Figure S81. HSQC ($CDCl_3$) spectrum of the new compound **16**.

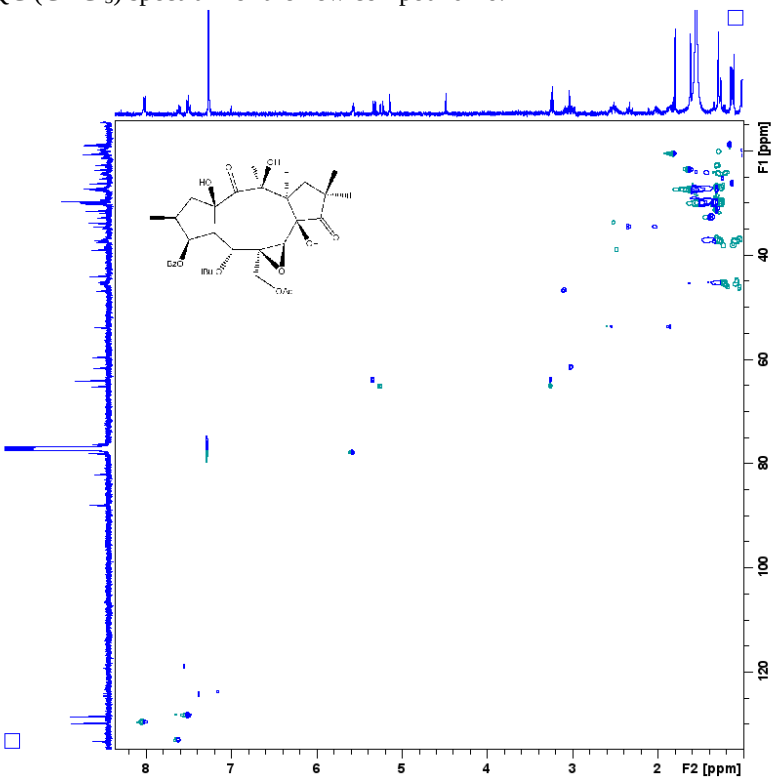


Figure S82. HMBC ($CDCl_3$) spectrum of the new compound **16**.

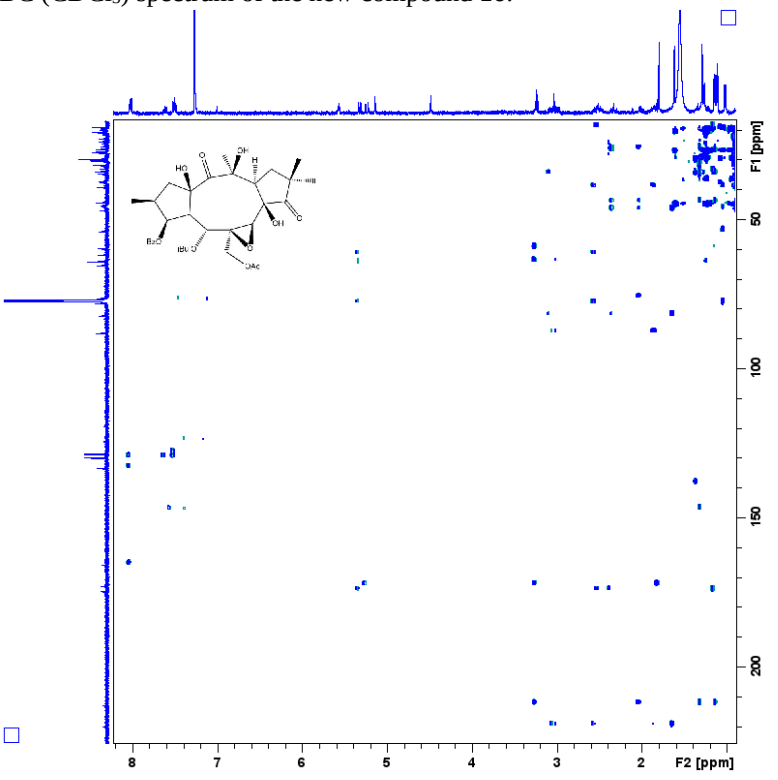


Figure S83. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **16**.

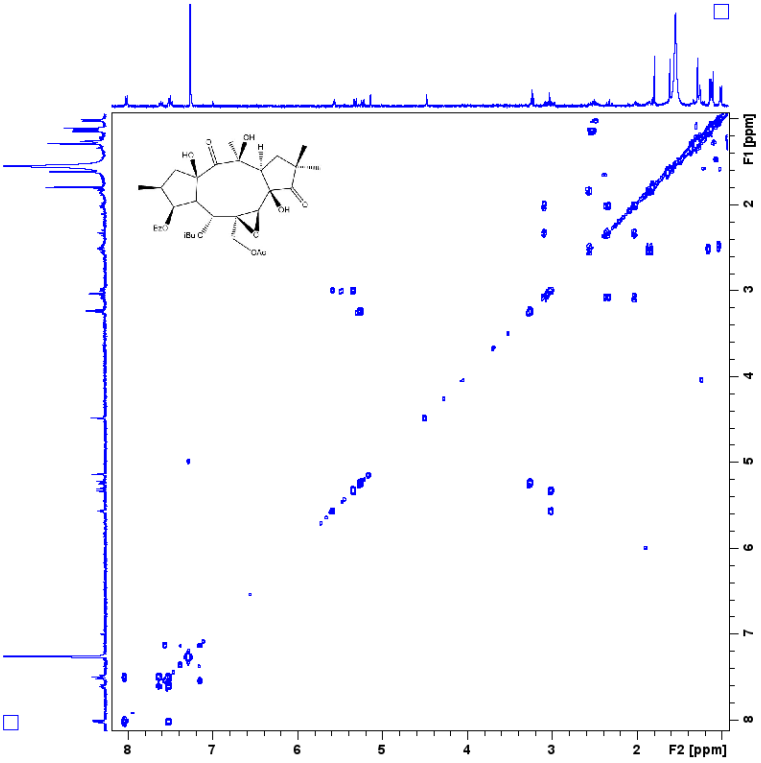


Figure S84. NOESY ($CDCl_3$) spectrum of the new compound **16**.

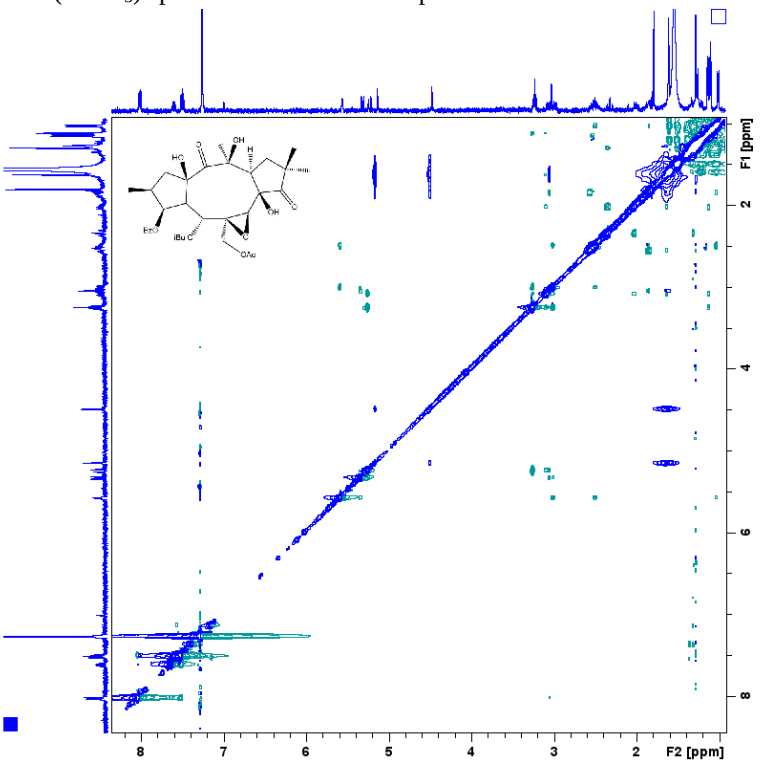


Figure S85. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **17**.

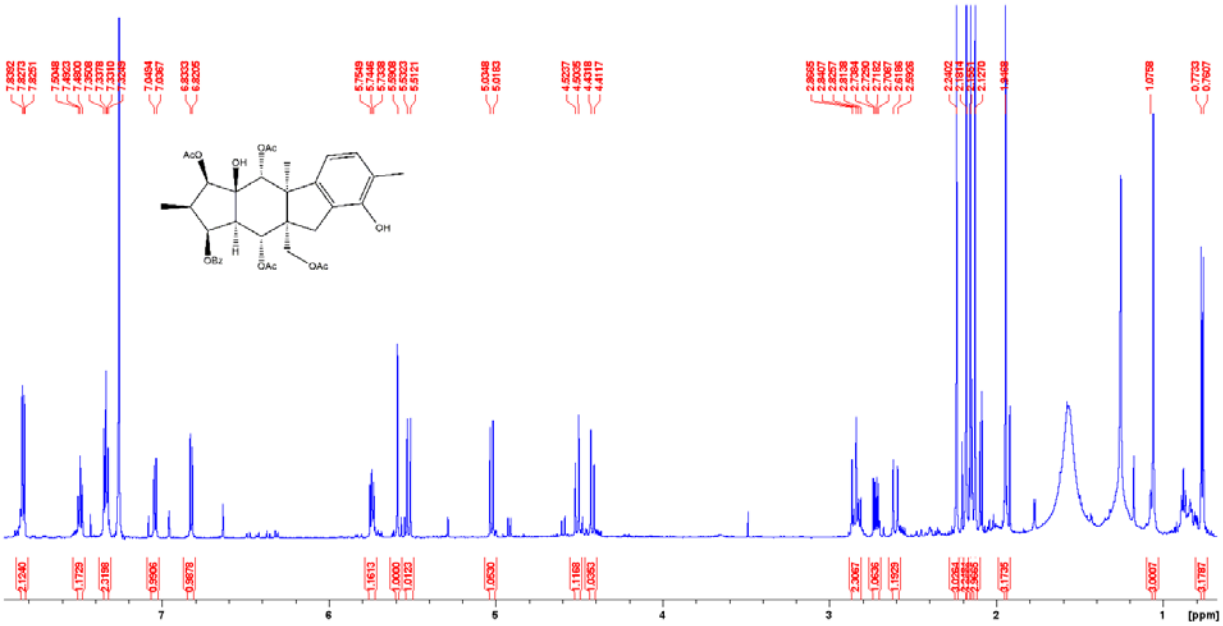


Figure S86. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **17**.

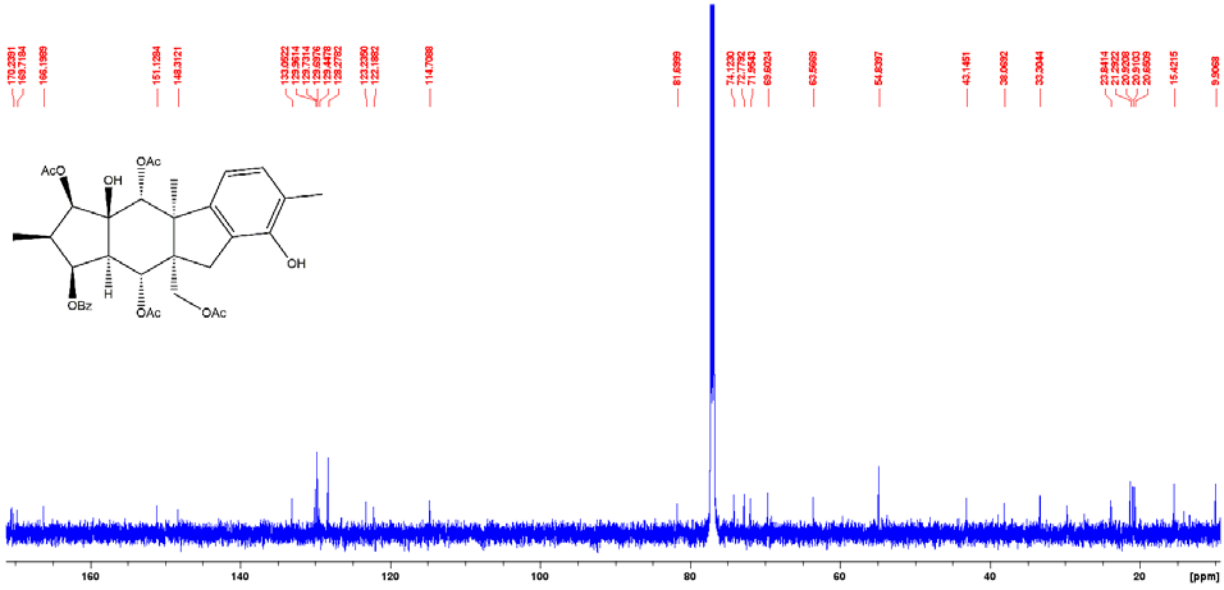


Figure S87. HSQC ($CDCl_3$) spectrum of the new compound **10**.

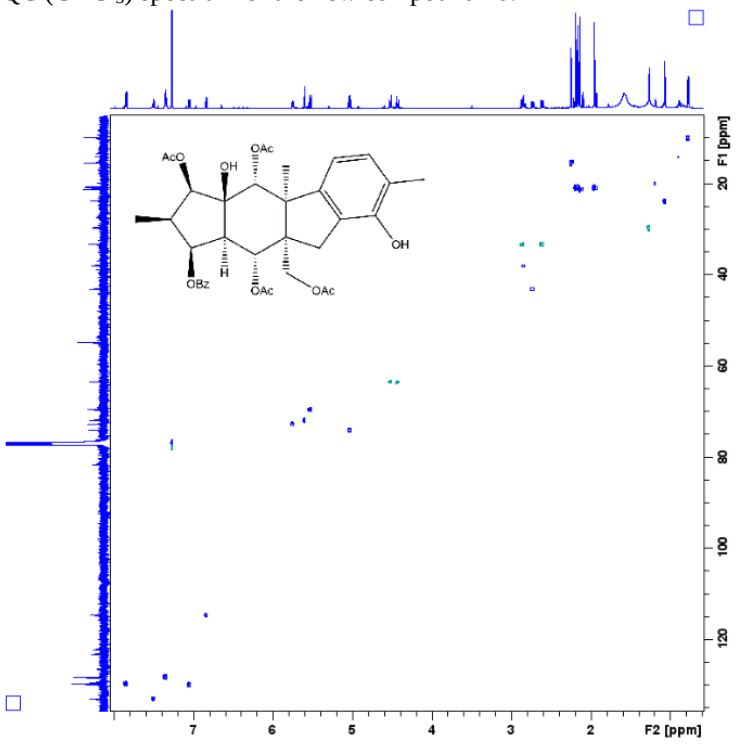


Figure S88. HMBC ($CDCl_3$) spectrum of the new compound **17**.

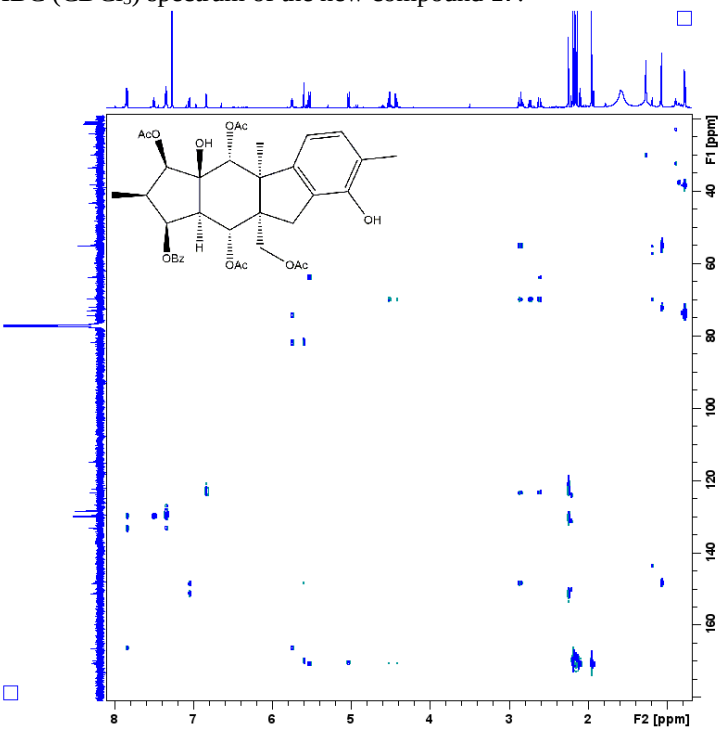


Figure S89. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **17**.

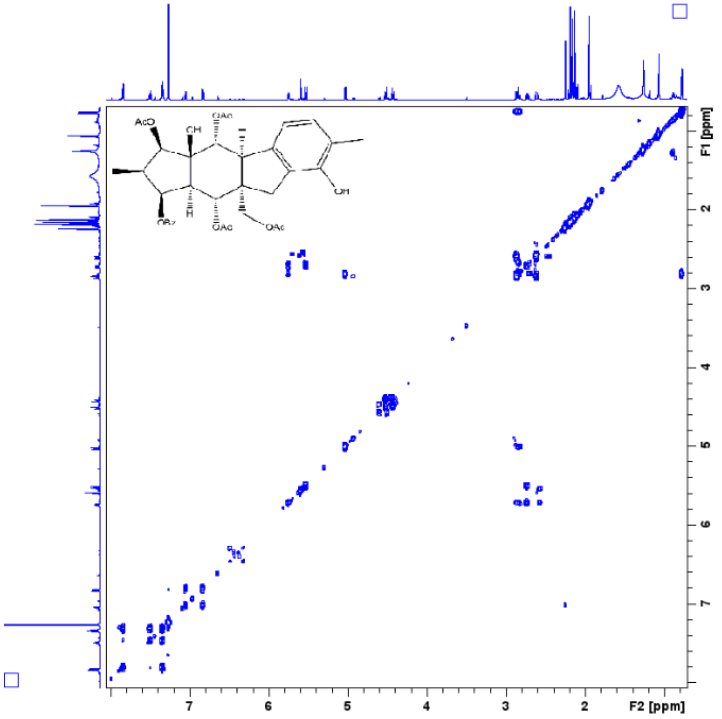


Figure S90. NOESY ($CDCl_3$) spectrum of the new compound **17**.

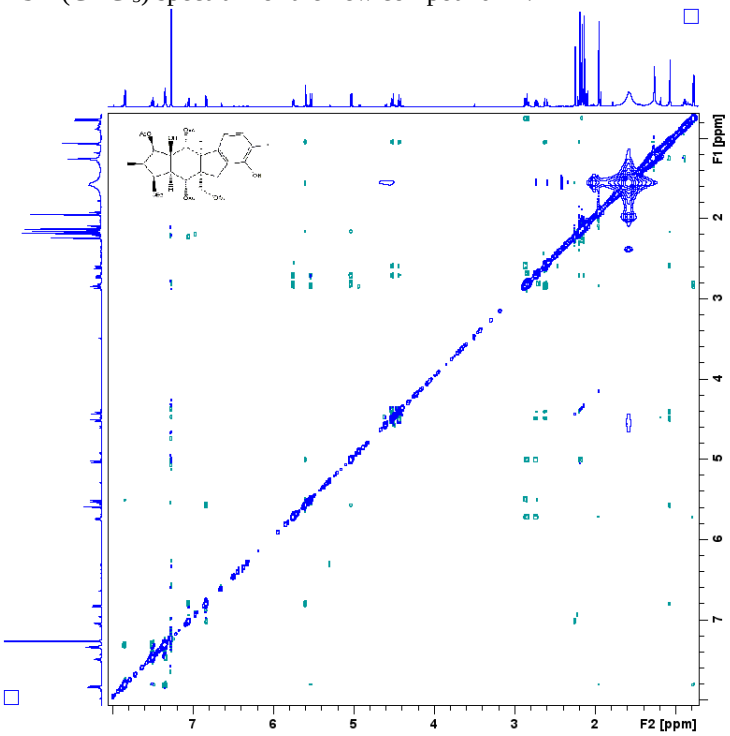


Figure S91. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of the new compound **18**.

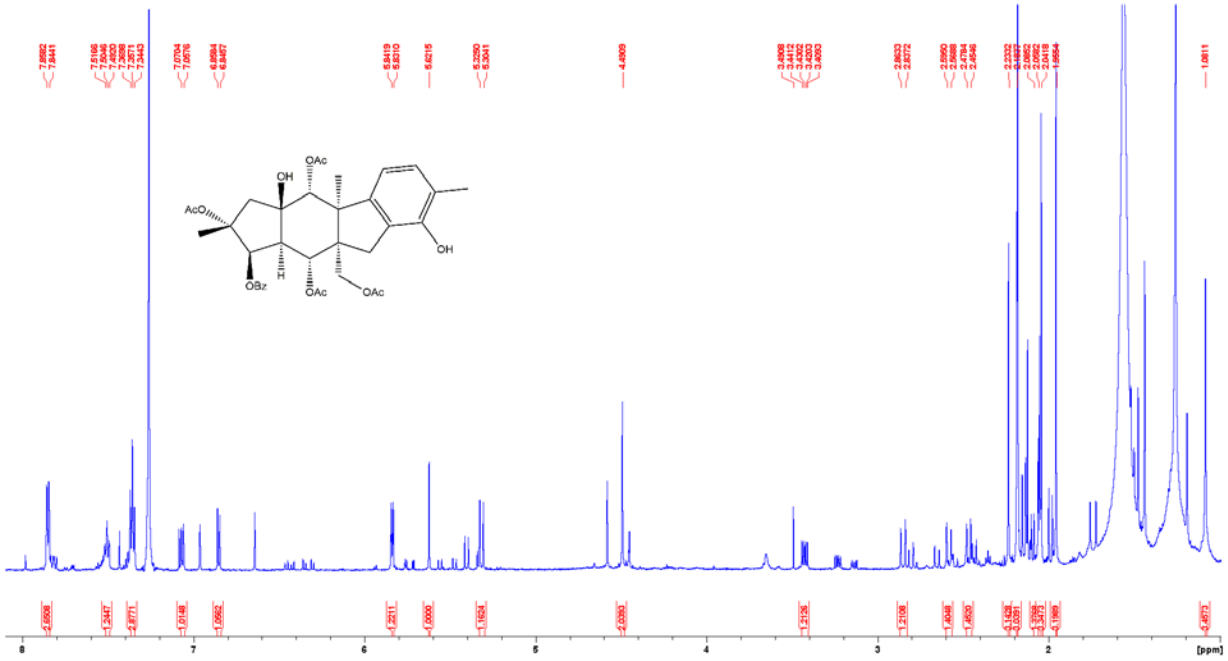


Figure S92. ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of the new compound **18**.

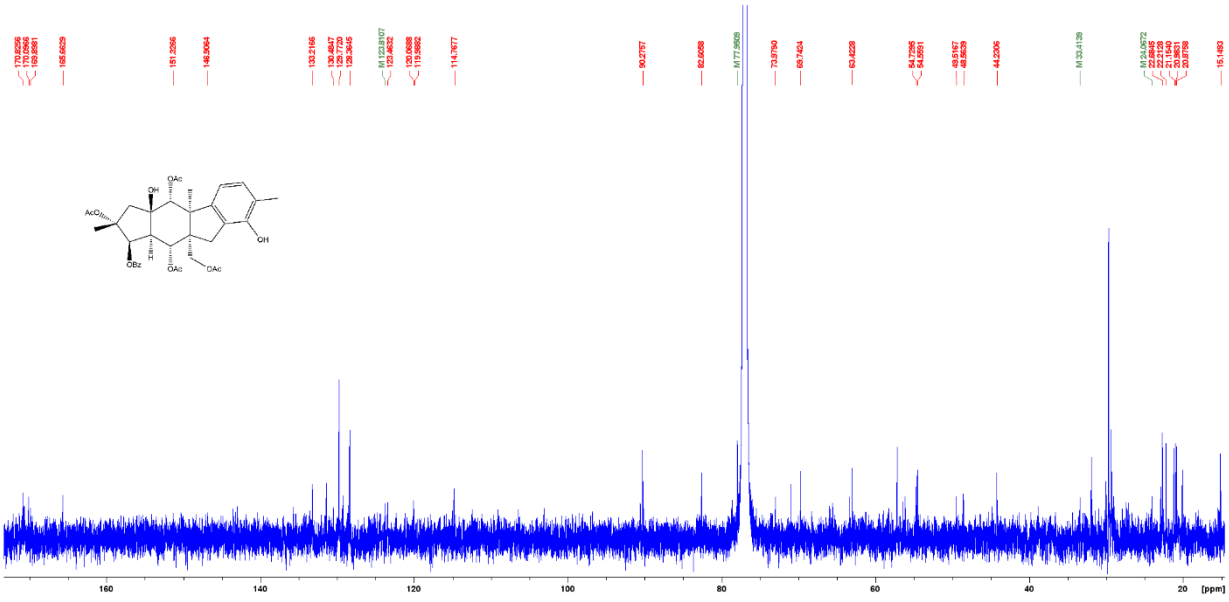


Figure S93. HSQC ($CDCl_3$) spectrum of the new compound **18**.

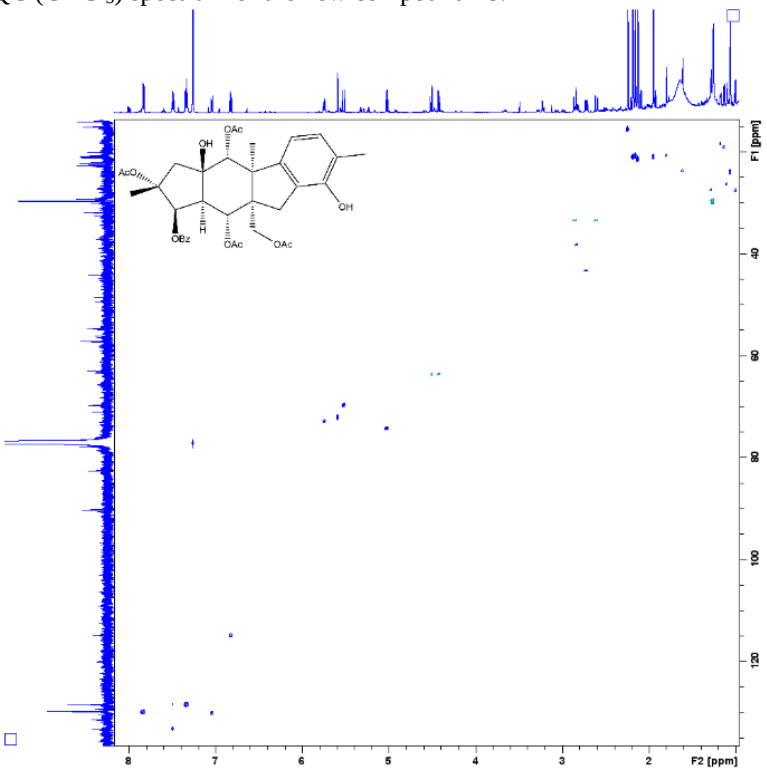


Figure S94. HMBC ($CDCl_3$) spectrum of the new compound **18**.

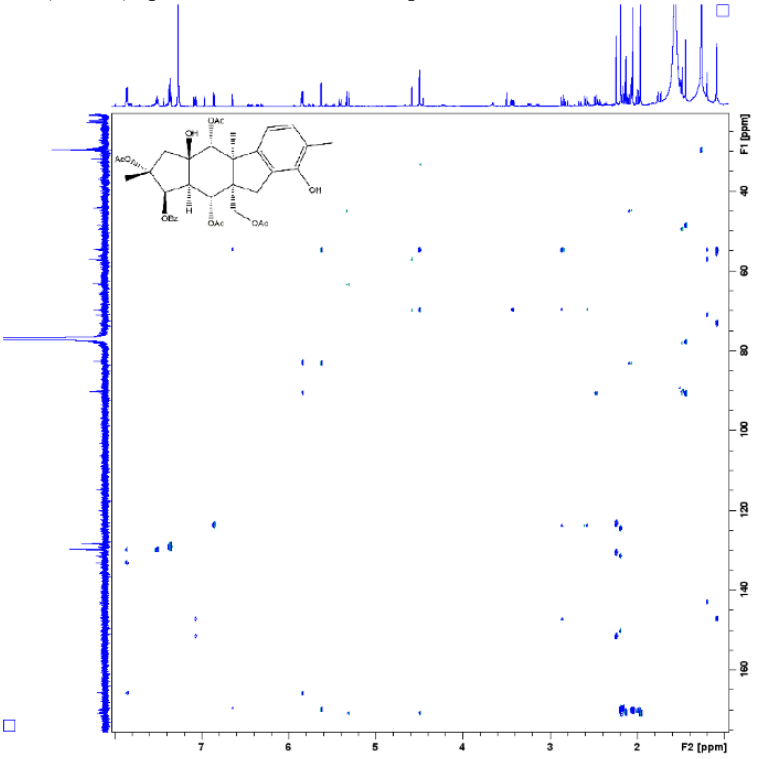


Figure S95. ^{1}H-^{1}H COSY ($CDCl_3$) spectrum of the new compound **18**.

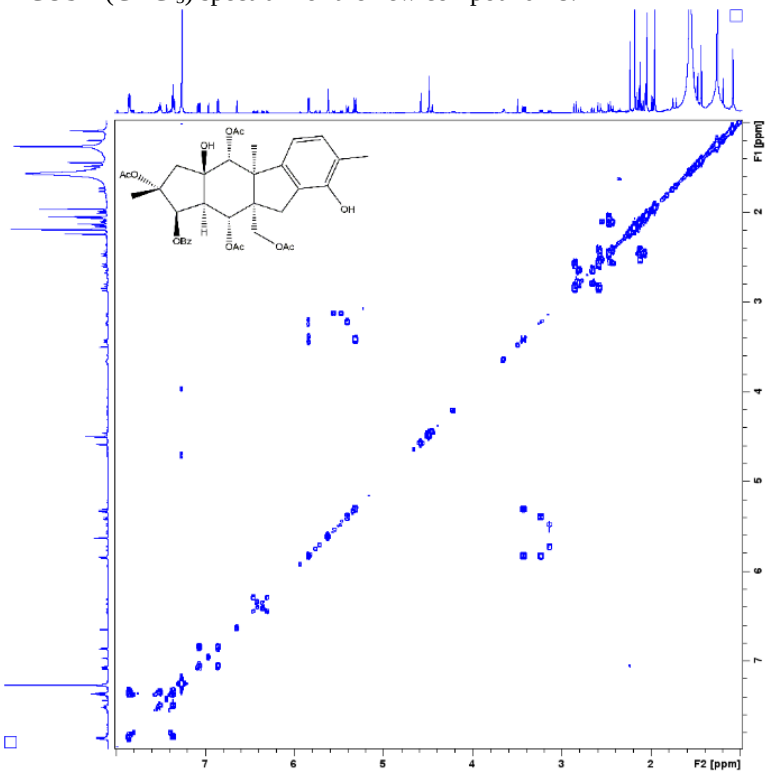
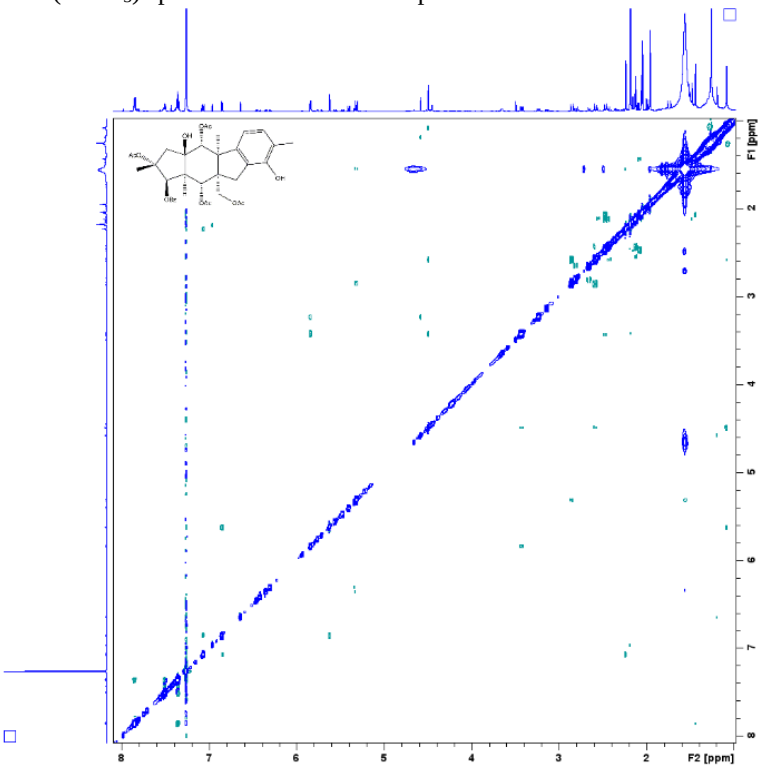


Figure S96. NOESY ($CDCl_3$) spectrum of the new compound **18**.



4. ^{1}H NMR spectra of the known compounds.

Figure S97. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of 3*β*-*O*-angeloyl-20-deoxy-ingenol (**12**).

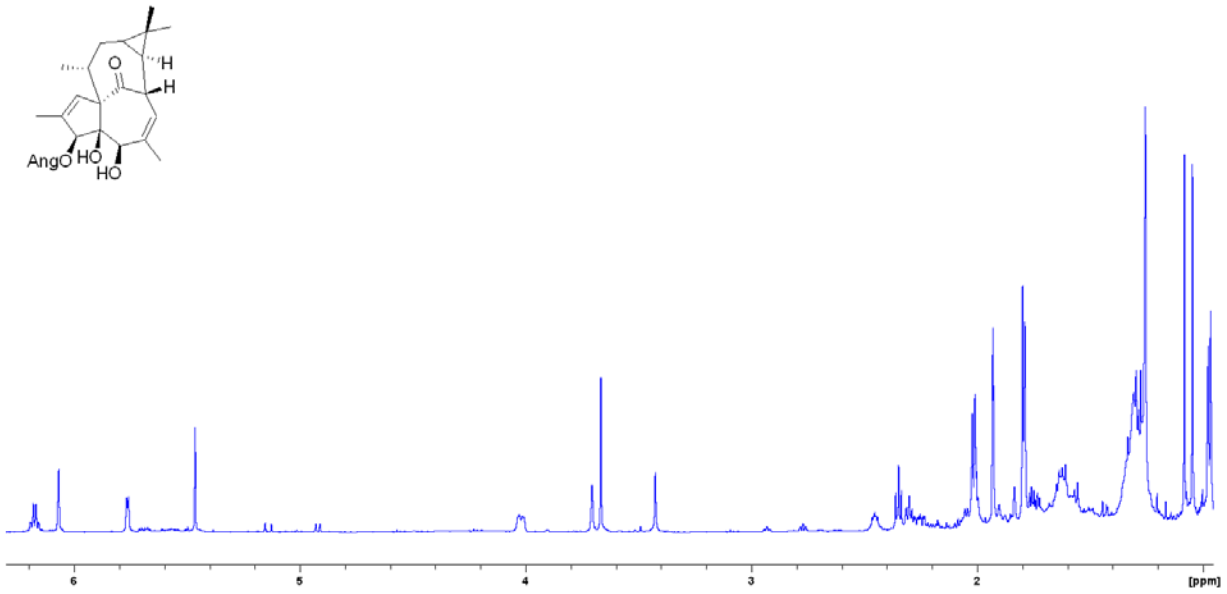


Figure S98. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of 3-*O*-angeloyl-17-angeloyloxy-20-deoxy-ingenol.

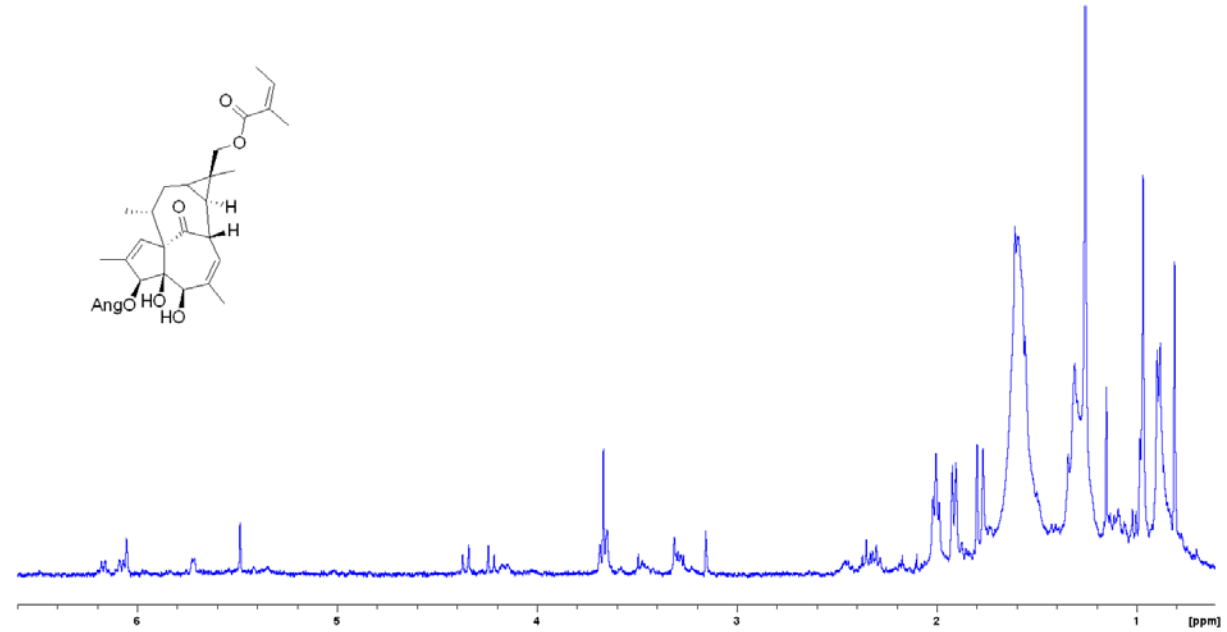


Figure S99. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of 13*α*-acetoxy-3-*O*-angeloyl-17-benzenoyloxy-20-deoxy-ingenol.

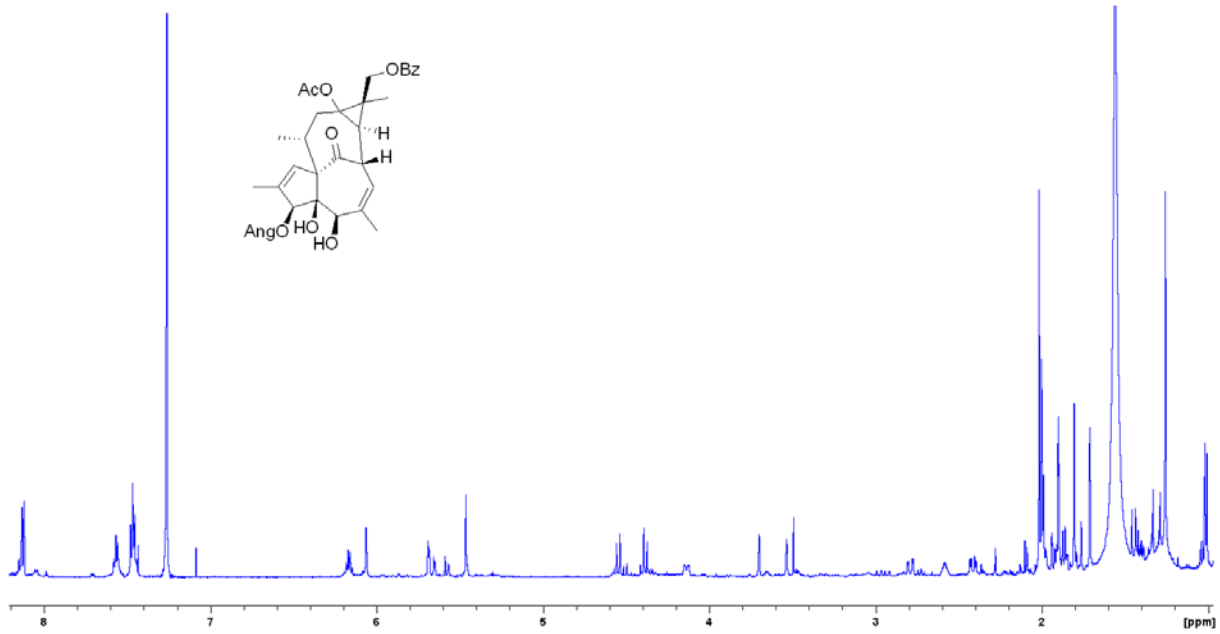


Figure S100. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of segetene B.

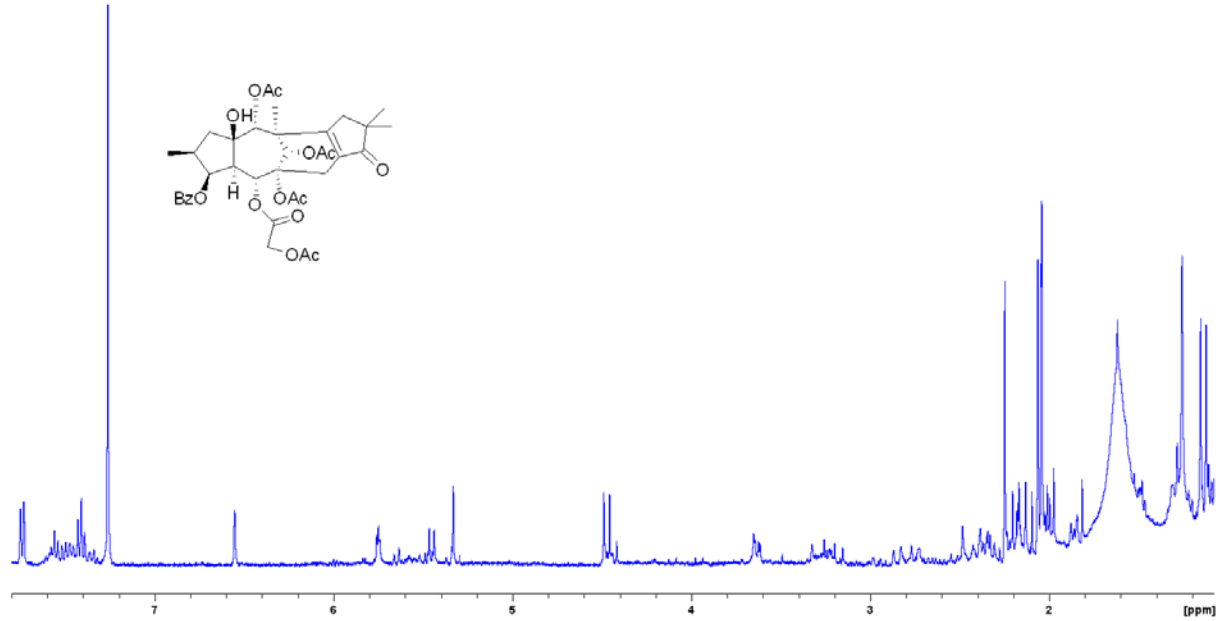


Figure S101. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of paralinone A (**14**).

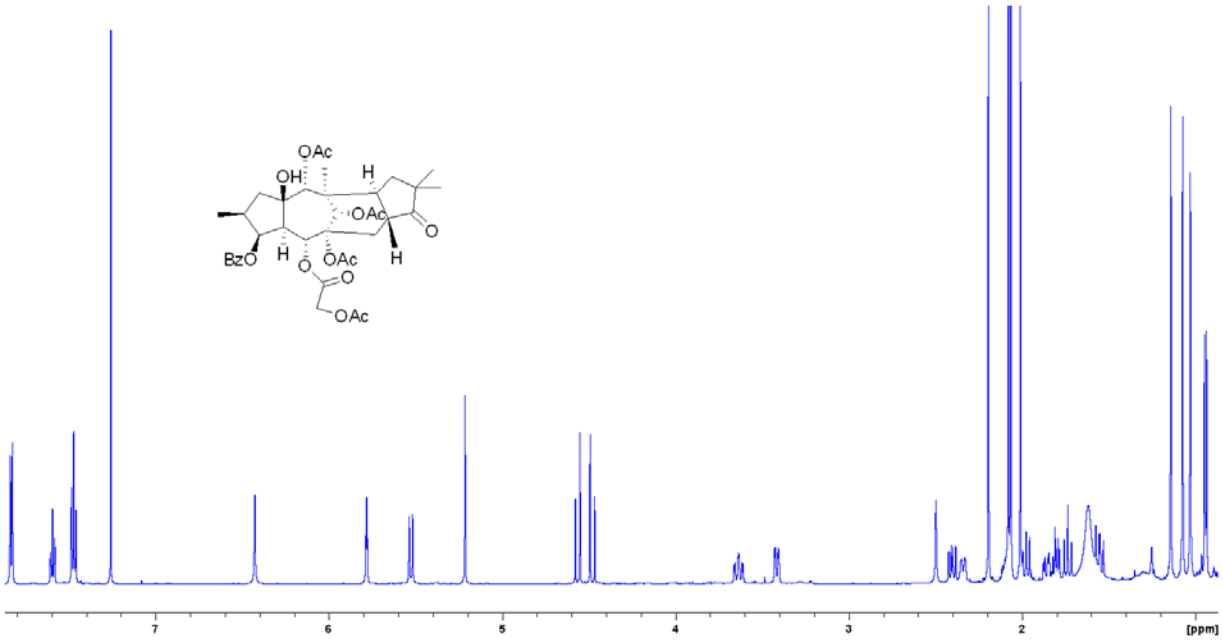
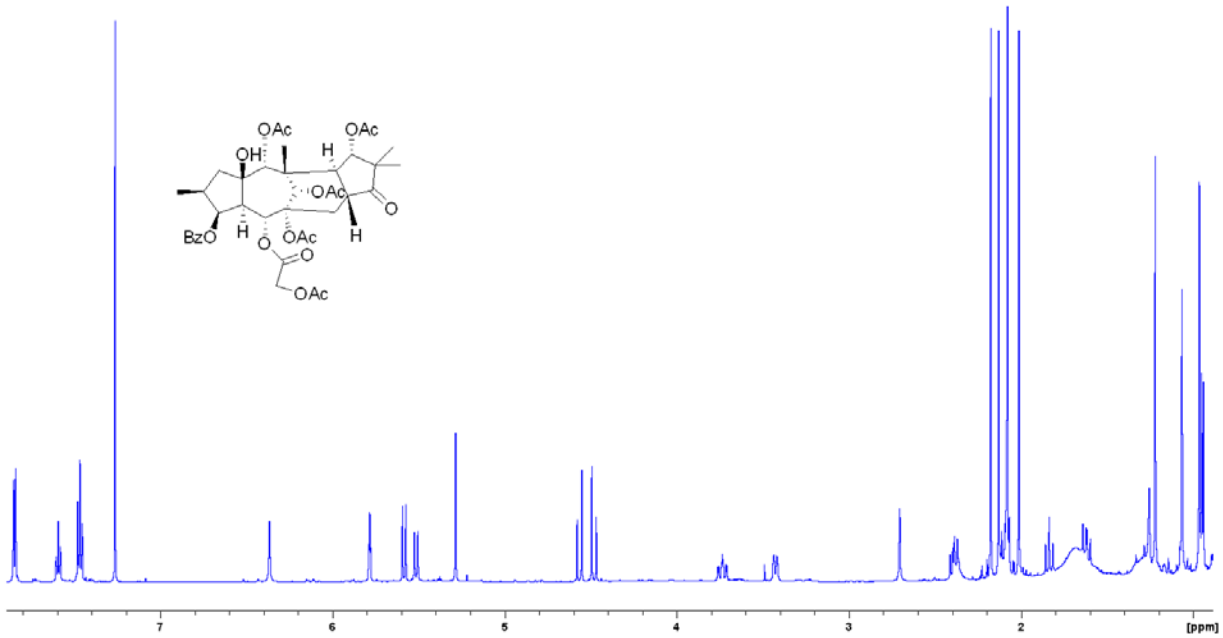
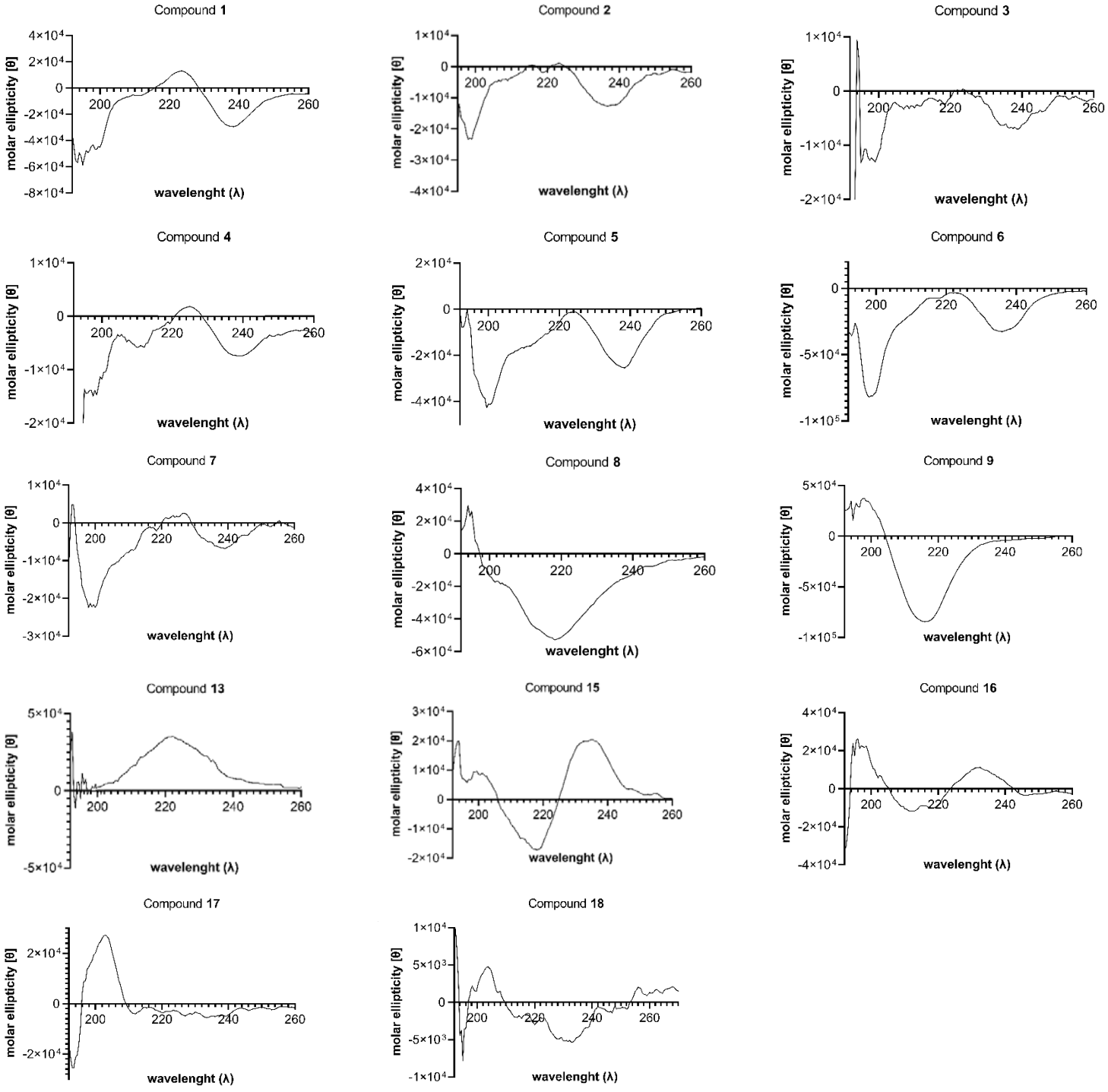


Figure S102. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of (2*S**,3*S**,4*R**,5*R**,6*R**,8*R**,11*S**,12*S**,13*R**,14*R**,15*R**)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxy-9-oxo-segetane.



5. Figure S103. CD spectra of compounds **1-9**, **13**, **15-18**.



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(6) Winkelmann, K.; Heilmann, J.; Zerbe, O.; Rali, T.; Sticher, O. *J. Nat. Prod.* **2000**, *63*, 104–108.

3.7. UNPUBLISHED DATA

3.7.1. Antimicrobial results for Phloroglucinols and Diterpenes

Two PAPs and three DTs were subjected to antimicrobial screening against *Staphylococcus aureus* and *Streptococcus pyogenes* using the disc diffusion and double dilution methods (2.2.4.), with chloramphenicol as a positive control. Photos of the agar plates from the disc diffusion assay are presented in Figure 31. Similar to the results of the MTT assay, the tested DTs (euphodeflexins A, L and M, see 3.6) showed no antibacterial effects. The two tested PAPs (3-geranyl-1-(2-methylpropanoyl)-phloroglucinol and 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one) (see 3.3) were active as expected (Wilkenmann et al., 2003), and the MICs against *S. aureus* were calculated using the double dilution method in 96 well plates as 16 and 32 µg/mL, respectively.

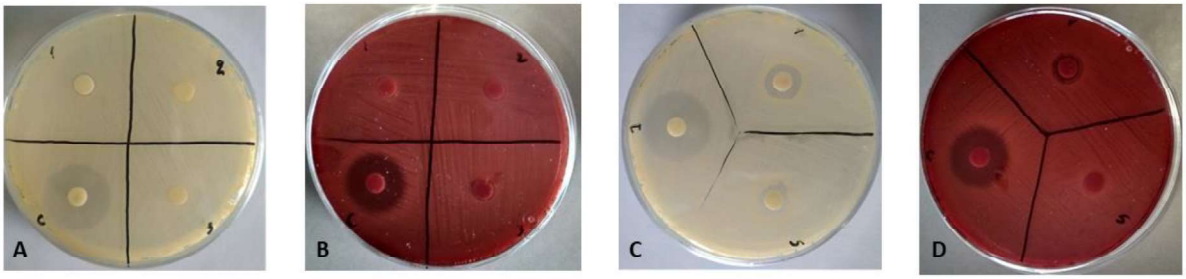


Figure 31. Disc diffusion agar plates for A. DTs against S. aureus; B. DTs against S. pyogenes; C. PAPs against S. aureus; D. PAPs against S. pyogenes. 1,2,3 = euphodeflexins A, L and M, respectively and 4,5= 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol and 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one, respectively, C= chloramphenicol.

3.7.2. 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol in Atopic Dermatitis

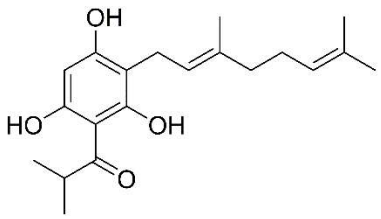
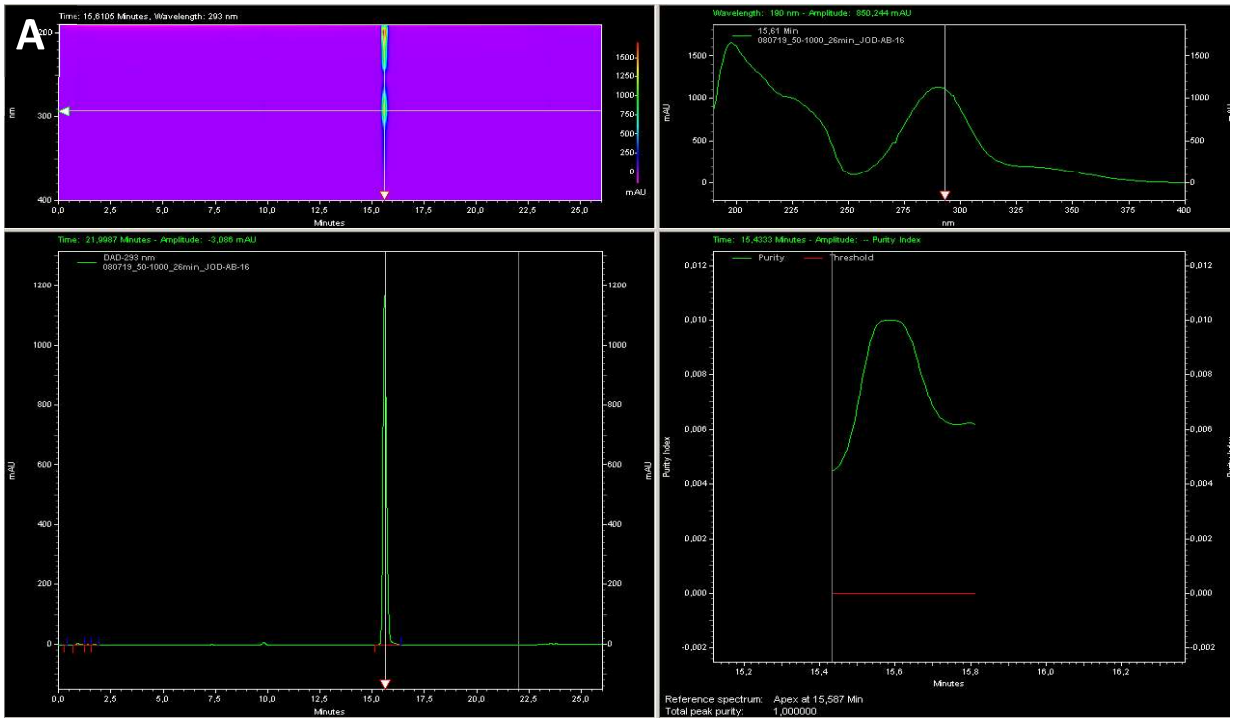


Figure 32. Structure of 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol.

3-geranyl-1-(2-methylpropanoyl)-phloroglucinol (Figure 32) is a PAP isolated from *Hypericum jovis* (Results 3.3). In fact, it is the main compound of this plant as suggested by the HPLC analysis of the crude extract (Figure 33). This compound was obtained in high amounts (yield ~ 2%), which gives the opportunity for *in vivo* testing.



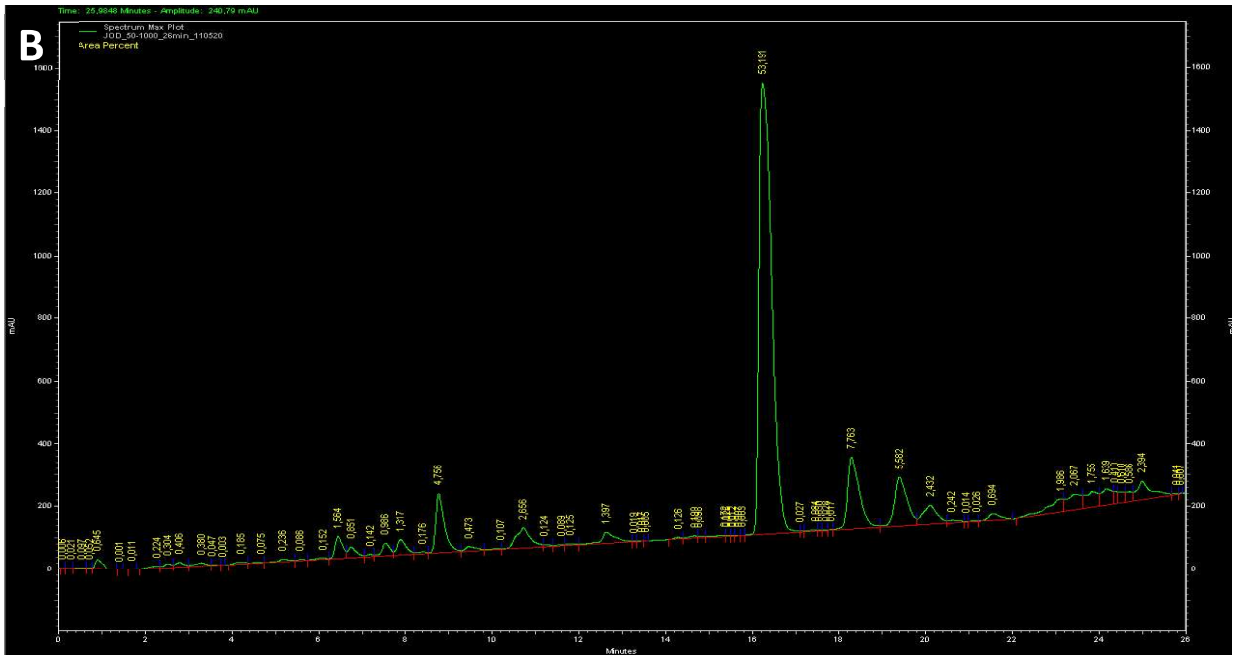


Figure 33. Analytical HPLC chromatogram under the same conditions of: A. purified 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol, B. crude extract of Hypericum jovis containing 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol as the main compound.

3-geranyl-1-(2-methylpropanoyl)-phloroglucinol has been previously reported to possess strong antioxidant activity at cellular level comparable to Trolox, the water-soluble form of vitamin C (Athanasas et al., 2004), as well as strong anti-inflammatory activity (inhibition of COX-1, COX-2 and 5-LOX enzymes, with IC_{50} values of 6.0, 29.9, 2.2 μM) (Crockett et al., 2008). During the course of the present study, this compound was further investigated for its anti-inflammatory potential and significantly reduced the ICAM-1 expression in a dose-depended manner with IC_{50} values of 16.9 μM (Results 3.3). These properties advocate the investigation of this compound in atopic dermatitis. A recent study in an *ex vivo* skin model by Takada et al. (2017), suggested that hyperforin (the only PAP that has been tested in atopic dermatitis), could serve as a possible therapeutic agent for atopic dermatitis. Unfortunately, in the present study, 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol showed only moderate activity.

Clinical evaluation of the mice showed that the treatment with the under-investigation compound led to xeroderma, scaling, and wounds, similar to the control group and the group treated only with the excipient (PEG) (Figure 34). The histopathological assessment showed that the tested compound presented significantly lower inflammation levels in the epidermis in comparison with the other treatments (Figure 35). However, in the photo from the microscope, there is evidence of a slight thickening of the skin as well

as deep inflammation over the muscle fibers. This observation could be explained by the low permeability of the tested compound (in association with the excipient or the dose used), which is active in the upper layers of the skin, but fails to penetrate in the lower levels of the dermis. Taken together, 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol seemed not to be promising for the treatment of atopic dermatitis under the given conditions.

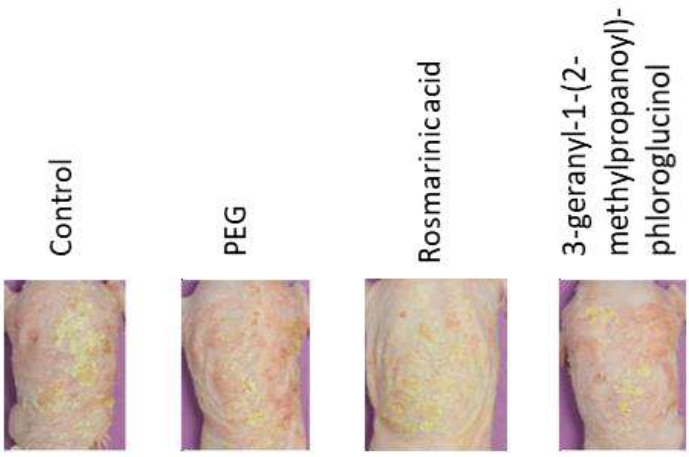


Figure 34. Representative images of the treatment groups on day 44.

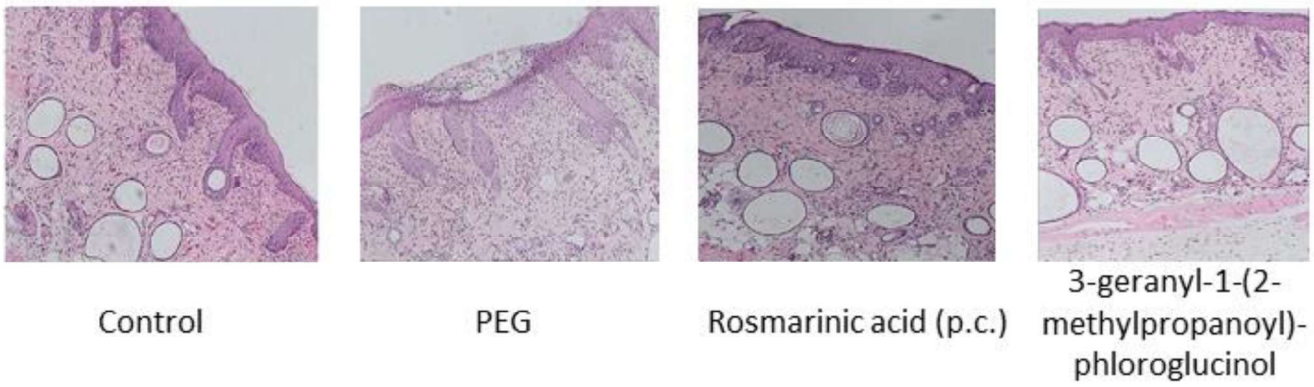
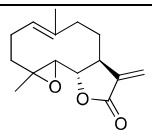
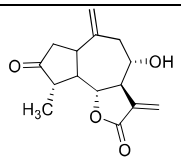
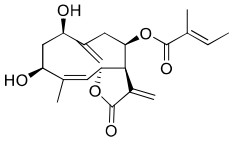
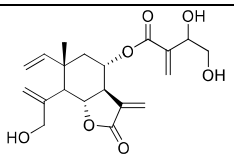
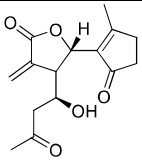


Figure 35. Representative histopathological images of the back skin of the mice (x100).

3.7.3. Metabolism of natural sesquiterpene lactones in human liver microsomes

In this part of the Ph.D. thesis, the *in vitro* metabolism of six naturally occurring sesquiterpene lactones (SLs) (Table 7) using HLM was analyzed. SLs were selected as promising drug leads with drug-like physicochemical properties, whose unstable absorption and extensive metabolism need further investigation (Yu et al., 2021).

Table 7. Tested SLs on human liver microsomes assay and plant origin.

	Compound	Genus	Structure	Source/Reference
1	Parthenolide	*Tanacetum parthenium*		Sigma-Aldrich Sztiller-Sikorska & Czyz, 2020
2	Grosheimin	*Cynara* spp., *Crepis* spp.		Barda et al., 2018
3	Carbetolide C	*Calea* spp.		Grafakou et al., 2021a
4	8α-O-(3,4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin	*Centaurea* spp.		Grafakou et al., 2021b
5	Arteludovicinolide A	*Artemisia dubia*, *Achillea coarctata*		Papakosta et al., 2020, Kreuzer et al., 2013

By screening reactive compound metabolites of phase I, as well as phase II acyl-glucuronide conjugate reactivities we can achieve some understanding of such complex procedures that are occuring in metabolic systems. On this basis, we set under-investigation structurally diverse plant-derived SLs to be characterized in detailed level by HPLC-MS/MS after *in vitro* metabolism on HLM. Step by step, we underwent different microsomal incubation systems, starting with phase I reactions and phase II glucuronidation by the addition

of UDPGA, to a combined system that encompasses both activations of phase I enzymes and UDP-glucuronosyl transferases. In parallel, all compounds were subjected to stability test in DPBS, while control incubation systems without cofactors or microsomes were examined (Table 6). The proposed metabolites are presented in Figure 36 and the corresponding MS data are reported in Tables 8-12.

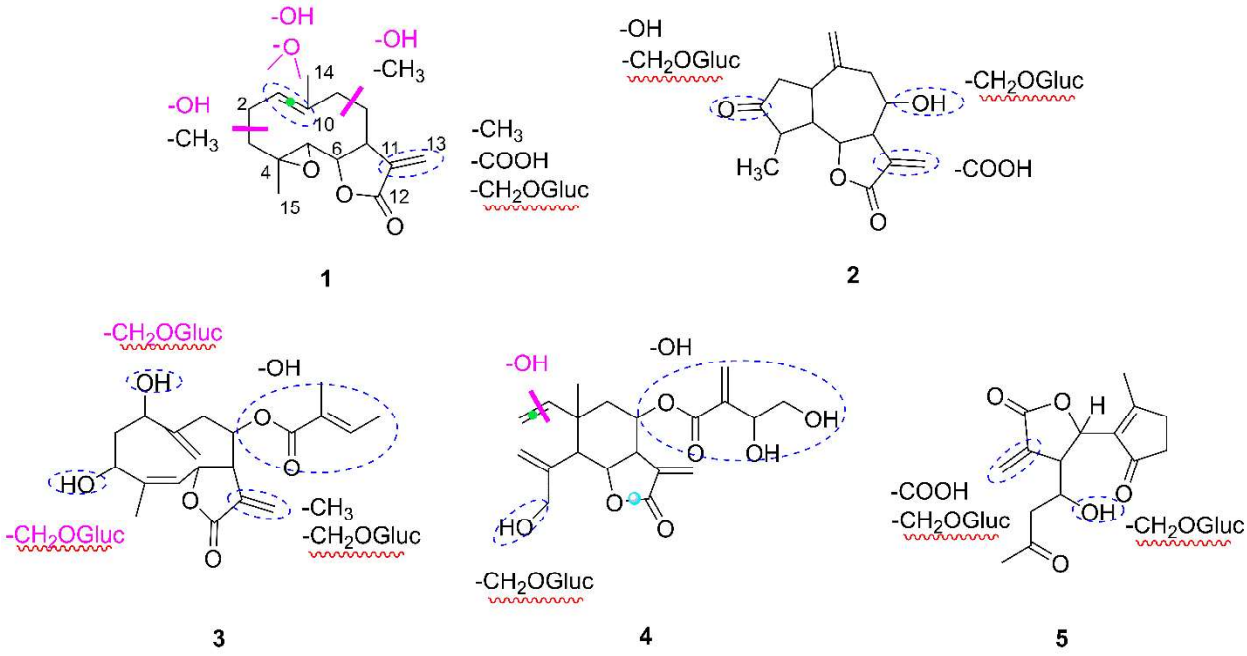


Figure 36. Metabolic sites of tested SLs. A formation of one of these metabolites; • conversion between single and double bond; ○ opening of the lactone ring; ∿∿ Ph.II metabolite formed under given conditions (glucuronidation).

More specifically, regarding parthenolide (**1**), five phase I metabolites were found. According to retention times (t_R) and LC-MS data (Table 8), they were identified as one hydroxylated (t_R = 3.12 min; *m/z* $[M + H]^+$ 265.1444) and three methylated derivatives (one lost the double bond at $\Delta_{10\text{-}1}$) (t_R = 4.60 min; *m/z* $[M + H]^+$ 263.1652; t_R = 5.13 min; *m/z* $[M + H]^+$ 263.1649; t_R = 6.23 min; *m/z* $[M + H]^+$ 265.1801). Methylation of the main skeleton has been described before in SL metabolism (Cai et al., 2015, Jiang et al., 2019) and the additional carbon atom is supposed to be attached enzymatically, similar to biosynthetic pathways mediated by methyltransferases (Liscombe et al., 2012). Moreover, no metabolites with an opened lactone ring could be identified, however the exomethylenic double bond at $\Delta_{11\text{-}13}$ was subject of reduction or carboxylation (t_R = 5.45 min; *m/z* $[M + H]^+$ 251.1652, t_R = 6.75 min; *m/z* $[M + H]^+$ 281.1385). Combined phase I and II metabolism led to the formation of a glucuronide on the lactone moiety (t_R = 5.17 min, *m/z* $[M - H]^-$ 443.1892). In accordance with the literature data, the lactone ring, which acts as a strong Michael acceptor (Schmidt, 2018), is the main location for metabolism and conjugation (Yu et al., 2021).

Table 8. HRESIMS data in positive and negative ion modes from parthenolide (1).

		m/z $[M+H]^+$		*m/z* $[M-H]^-$		Molecular formula
	t_R (min)	found	calcd	found	calcd	
1	5.31	249.1485	249.1585			$C_{15}H_{20}O_3$
Phase I	3.12	265.1444	265.1434			$C_{15}H_{20}O_4$
	4.60	263.1652	263.1642			$C_{16}H_{22}O_3$
	5.13	263.1649	263.1642			$C_{16}H_{22}O_3$
	5.45	251.1652	251.1642			$C_{15}H_{22}O_3$
	6.23	265.1801	265.1798			$C_{16}H_{24}O_3$
	6.75	281.1387	281.1384			$C_{15}H_{20}O_5$
Phase I+II	5.17			443.1892	443.1912	$C_{21}H_{32}O_{10}$

Regarding grosheimin (**2**), four metabolites were detected (Table 9). In phase I similar events were conducted on the lactone ring (carboxylation, t_R = 1.21 min, *m/z* $[M + H]^+$ 295.1191), while in that case another metabolic site proved to be the carbonyl group of C-3 (t_R = 1.94 min, *m/z* $[M + H]^+$ 265.144). Carbonyl groups in other SLs are also found to be subject to metabolism (Yu et el., 2021). The latter position, as well as C-8 hydroxyl group were further conjugated by glucuronides on phase II and I + II (t_R = 4.30 min, *m/z* $[M - H]^-$ 437.1449; t_R = 4.01 min, *m/z* $[M - H]^-$ 439.1604), while it is worth mentioning that no glucuronide conjugates could be detected on $\Delta_{11\text{-}13}$, contrary to compound **1**.

Table 9. HRESIMS data in positive and negative ion modes from grosheimin (2).

		m/z $[M+H]^+$		*m/z* $[M-H]^-$		Molecular formula
	t_R (min)	found	calcd	found	calcd	
2	5.66	263.1274	263.1278	261.1124	261.1132	$C_{15}H_{18}O_4$
Phase I	1.21	295.1191	295.1175			$C_{15}H_{18}O_6$
	1.94	265.1444	265.1434			$C_{15}H_{20}O_4$
Phase II	4.30	439.1595	439.1595	437.1449	437.1453	$C_{21}H_{26}O_{10}$
Phase I+II	4.01			439.1604	439.1610	$C_{21}H_{28}O_{10}$

Furthermore, two metabolites of carbetolide C (**3**) were found after phase I metabolism (t_R = 1.70 min, *m/z* $[M + H]^+$ 281.1385; t_R = 3.16 min, m/z $[M - H]^-$ 363.1803). The first was observed as a result of the replacement of 8-substitution with a hydroxyl group, while the second metabolite was formed by saturation on $\Delta_{11\text{-}13}$. Glucuronide conjugations were observed on one of the free hydroxyl groups, as well as the lactone ring, based on phase II and phase I + II reactions (t_R = 4.83 min, *m/z* $[M - H]^-$ 537.1986; t_R = 6.92 min, *m/z* $[M - H]^-$ 555.2182) (Table 10).

Table 10. HRESIMS data in positive and negative ion modes from carbetolide C (3).

		m/z $[M+H]^+$		*m/z* $[M-H]^-$		Molecular formula
	t_R (min)	found	calcd	found	calcd	
3	6.15	363.1804	363.1802	361.1641	361.1657	$C_{20}H_{26}O_6$
Phase I	1.70	281.1385	281.1384	279.1228	279.1238	$C_{15}H_{20}O_5$
	3.16			363.1803	363.1813	$C_{20}H_{28}O_6$
Phase II	4.83	539.2130	539.2123	537.1986	537.1978	$C_{26}H_{34}O_{12}$
Phase I+II	6.92			555.2182	555.2083	$C_{26}H_{36}O_{13}$

Regarding 8α-O-(3,4-dihydroxymethylenebutanoyloxy)-dehydromelitensin (**4**), four metabolites were detected (Table 11). More specifically, in phase I, a derivative with a free hydroxyl group was present due to the replacement of 8-substitution (t_R = 3.86 min, *m/z* $[M + H]^+$ 265.1456). Similarly, a metabolite detected from Ixerin Z was formed by the loss of a glucose substitution on the main SL skeleton (Cai et al., 2015). Two further metabolites were formed by hydrolysis events, supposing that the addition of H_2O is taking place at the double bond 1-2 or the lactone ring (t_R = 4.16 min, *m/z* $[M + H]^+$ 397.1882; t_R = 4.76 min, *m/z* $[M - H]^-$ 393.1561). Thus, contrary to the previous metabolites (**1**-**3**), in this case, the opening of the lactone ring was observed, similar to the reported metabolite of the SL 11α,13-dihydrohelenaline acetate formed in pig liver microsomes (Jürgens et al., 2020). Moreover, in phase II the glucuronidation is taking place on position 15 (t_R = 3.79 min, *m/z* $[M - H]^-$ 553.1933).

Table 11. HRESIMS data in positive and negative ion modes from 8α-O-(3,4-dihydroxymethylenebutanoyloxy)-dehydromelitensin (4).

		m/z $[M+H]^+$		*m/z* $[M-H]^-$		Molecular formula
	t_R (min)	found	calcd	found	calcd	
4	1.69	379.1756	379.1751	377.1611	377.1606	$C_{20}H_{26}O_7$
Phase I	3.86	265.1456	265.1436			$C_{15}H_{20}O_4$
	4.16	397.1882	397.1857			$C_{20}H_{28}O_8$
	4.76			393.1561	393.1555	$C_{20}H_{26}O_8$
Phase II	3.79	555.2083	555.2072	553.1933	553.1927	$C_{26}H_{34}O_{13}$

Concerning arteludovicinolide A (**5**), we detected one metabolite in each phase I, II and I + II (t_R = 2.37 min, *m/z* $[M + H]^+$ 311.1099; t_R = 3.53 min, *m/z* $[M - H]^-$ 453.1415; t_R = 3.71 min, *m/z* $[M - H]^-$ 471.1499), respectively. In accordance with the above-mentioned compounds, a carboxylate formation is taking place on the lactone ring at position $\Delta_{11\text{-}13}$, which further formed a glucuronide conjugate during phase I + II. Additionally to the glucuronidation of the combined metabolism, in phase II, the former free hydroxyl group led to another glucuronide conjugation. These data are depicted in Table 12.

Table 12. HRESIMS data in positive and negative ion modes from arteludovicinolide A (5).

		m/z $[M+H]^+$		*m/z* $[M-H]^-$		Molecular formula
	t_R (min)	found	calcd	found	calcd	
5	4.0	279.1224	279.1227	277.1078	277.1081	$C_{15}H_{18}O_5$
Phase I	2.37	311.1099	311.1125	309.0994	309.0980	$C_{15}H_{18}O_7$
Phase II	3.53			453.1415	453.1402	$C_{21}H_{26}O_{11}$
Phase I+II	3.71			471.1499	471.1508	$C_{21}H_{28}O_{12}$

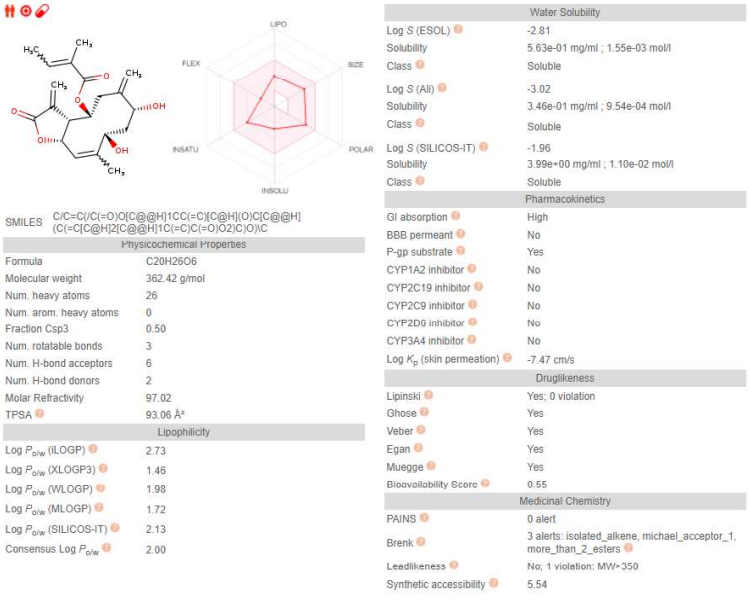
Due to structural polymorphism, metabolic sites and pathways for SLs are warranted to be changeable. Such metabolic processes may occur simultaneously or sequentially and undergo phase I to convert lipophilic drugs into more polar commonly by CYP450s (or reduction and hydrolysis). In phase I, metabolites are often still active and further proceed to phase II to yield larger polar metabolites by enzymatic reactions such as glucuronidation (most common), methylation, acetylation, sulfation, or conjugation with glutathione and amino acids. Outlining our results, we detected monohydroxylated, hydrated, carboxylated, methylated derivatives, as well as corresponding monoglucuronides of phase II and combined metabolism. Under the given condition of our *in vitro* system, we can observe the formation of glucuronides that are likely also occurring *in vivo*, however, these metabolites represent just a part of the total metabolization throughout the body's cells. Sulfate, cysteine, acetylcysteine, and glucuronide conjugates have been described from SLs using different metabolism systems (Yu et al., 2021). For an overall view and to cover all possible phase II bioproducts, further studies using liver cells and *in vivo* systems should be utilized. It is also worth mentioning that the reactivity of the basic SL backbone is relatively weak, while the lactone ring is a Michael center, which, similar to bioactivity, is the main site of metabolic reactions. The exomethylenic double bond is subject to reduction, oxidation, or conjugation, while some cases are reporting the opening of the lactone ring. The inherent structures play an integral role in the metabolism outcome, thus even similar compounds result in different pathways.

3.7.4. Pharmacokinetic profiles using SwissADME

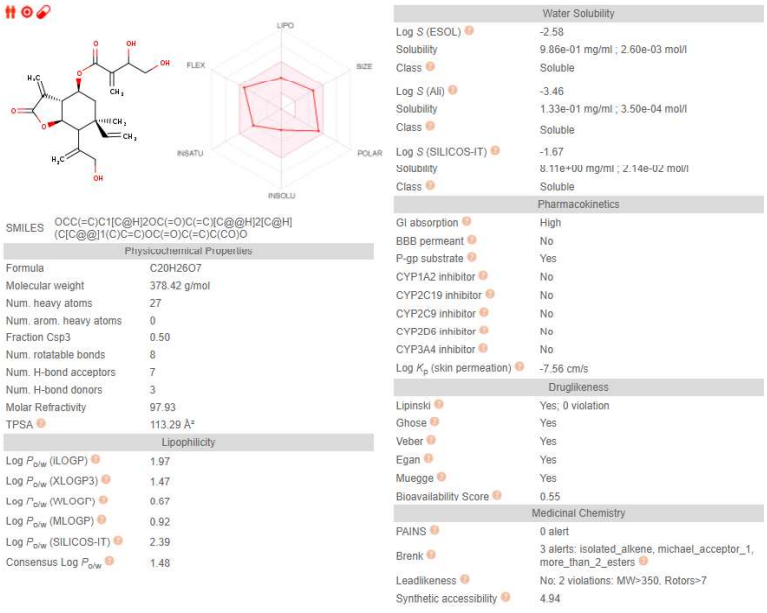
During drug discovery and development, early investigation of pharmacokinetic profiles is important to identify compounds, which have the best chance to become effective drug leads. Some of the most promising compounds were submitted in the web-tool SwissADME, that gives access to a fast and robust alternative to experimental procedures for the prediction of ADME. Physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug-likeness, and medicinal chemistry properties are calculated, as presented in Figure 37.

According to obtained values and the Bioavailability Radar (on the left top of each scheme) which is a rapid appraisal of drug-likeness, taking into account lipophilicity, size, polarity, solubility, flexibility, and saturation, the sesquiterpene lactones carbetolide C (Figure 37A) and 8α-O-(3,4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin (Figure 37B), as well as the phloroglucinols 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol (Figure 37C) and 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one (Figure 37D) are drug-like compounds, following the Lipinski's rule, with high solubility and GI absorption, while the diterpene euphodeflexin O (Figure 37E) does not follow Lipinski's rule, is poorly soluble and with low GI absorption.

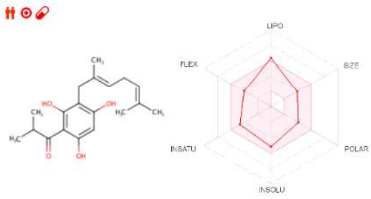
A



B



C

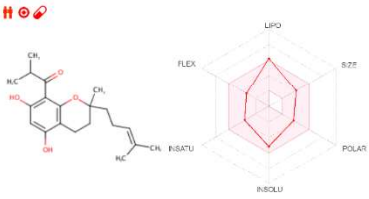


SMILES CC(=CC/C=C(/Cc1c(O)cc(c(c1O)C(=O)C(C)C)O)/C)C

Physicochemical Properties	
Formula	C19H26O4
Molecular weight	318.41 g/mol
Num. heavy atoms	23
Num. arom. heavy atoms	6
Fraction Csp3	0.42
Num. rotatable bonds	6
Num. H-bond acceptors	4
Num. H-bond donors	3
Molar Refractivity	94.79
TPSA	77.76 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.16
Log $P_{o/w}$ (XLOGP3)	5.62
Log $P_{o/w}$ (WLOGP)	4.49
Log $P_{o/w}$ (MLOGP)	2.70
Log $P_{o/w}$ (SILICOS-IT)	4.31
Consensus Log $P_{o/w}$	4.06

Water Solubility	
Log *S* (ESOL)	-5.15
Solubility	2.24e-03 mg/ml ; 7.05e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-7.02
Solubility	3.07e-05 mg/ml ; 9.64e-08 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-3.58
Solubility	8.47e-02 mg/ml ; 2.66e-04 mol/l
Class	Soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-4.25 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: isolated_alkene
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	2.87

D



SMILES CC(=CCCC1(C)CCc2c(O1)c(c(cc2O)O)C(=O)C(C)C)C

Physicochemical Properties	
Formula	C20H28O4
Molecular weight	332.43 g/mol
Num. heavy atoms	24
Num. arom. heavy atoms	6
Fraction Csp3	0.55
Num. rotatable bonds	5
Num. H-bond acceptors	4
Num. H-bond donors	2
Molar Refractivity	97.66
TPSA	66.76 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.54
Log $P_{o/w}$ (XLOGP3)	5.42
Log $P_{o/w}$ (WLOGP)	4.77
Log $P_{o/w}$ (MLOGP)	2.62
Log $P_{o/w}$ (SILICOS-IT)	4.85
Consensus Log $P_{o/w}$	4.04

Water Solubility	
Log *S* (ESOL)	-5.17
Solubility	2.24e-03 mg/ml ; 6.75e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-6.58
Solubility	8.79e-05 mg/ml ; 2.65e-07 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-4.61
Solubility	8.14e-03 mg/ml ; 2.45e-05 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-4.48 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: isolated_alkene
Leadlikeness	No; 1 violation: XLOGP3>3.5
Synthetic accessibility	3.76

E

SMILES CC(=O)OC[C@]12Cc3c[C@]2(C)[C@H]([C@@]2([C@@H]([C@H]1O[C@H]1OC(=O)C)[C@@H](OC(=O)c1ccccc1)[C@H]([C@H]2OC(=O)C)C)O)OC(=O)Ccc(c3O)C

Physicochemical Properties	
Formula	C35H40O12
Molecular weight	652.68 g/mol
Num. heavy atoms	47
Num. arom. heavy atoms	12
Fraction Csp3	0.51
Num. rotatable bonds	12
Num. H-bond acceptors	12
Num. H-bond donors	2
Molar Refractivity	165.12
TPSA	171.96 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	4.19
Log $P_{o/w}$ (XLOGP3)	3.85
Log $P_{o/w}$ (WLOGP)	3.10
Log $P_{o/w}$ (MLOGP)	2.65
Log $P_{o/w}$ (SILICOS-IT)	4.01
Consensus Log $P_{o/w}$	3.56

Water Solubility	
Log *S* (ESOL)	-5.71
Solubility	1.28e-03 mg/ml ; 1.95e-06 mol/l
Class	Moderately soluble
Log *S* (Ali)	-7.16
Solubility	4.54e-05 mg/ml ; 6.96e-08 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-6.30
Solubility	3.25e-04 mg/ml ; 4.98e-07 mol/l
Class	Poorly soluble
Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-7.55 cm/s
Druglikeness	
Lipinski	No; 2 violations: MW>500, NorO>10
Ghose	No; 3 violations: MW>480, MR>130, #atoms>70
Veber	No; 2 violations: Rotors>10, TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	No; 3 violations: MW>600, TPSA>150, H-acc>10
Bioavailability Score	0.17
Medicinal Chemistry	
PAINS	0 alert
Brenk	1 alert: more_than_2_esters
Leadlikeness	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Synthetic accessibility	6.21

Figure 37. SwissADME results for: A. carbetolide C; B. 8α-O-(3,4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin; C. 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol; D. 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one; E. euphodeflexin O.

4. DISCUSSION & CONCLUSIONS

In the present study and with the aid of NMR metabolomic strategy, a total of 84 specialized metabolites were isolated from endemic plants of cosmopolitan distribution: 26 from *Calea jamaicensis,* 12 from *Centaurea papposa*, 24 from *Hypericum jovis*, and 22 from *Euphorbia deflexa*. In parallel, more than 120 volatile compounds were reported using GC-MS from the essential oils from *Hypericum* spp. (*H. jovis*, *H. empetrifolium*, *H. amblyocalyx*, *H. triquetrifolium* and *H. perforatum*). Most of the isolated compounds belong to the groups of SLs, PAPs, DTs, chromenes, and flavonoids. Among them, 14 SLs, 2 PAPs, 16 DTs, and 3 chromenes are new natural products and have not been described in the literature before. With the intention to discover novel drug leads against cancer and inflammation, the isolated compounds, based on the obtained amounts, were subjected to evaluation of pharmacological potential against multiple targets (MTT, ICAM, COX-LOX assays, and *in vivo* models). SLs proved to be the most drug-like compounds, followed by PAPs. Although DTs are extremely interesting in their complicated structure elucidation, most of them presented very low biological activities.

Regarding cytotoxicity, taking into consideration the obtained amounts, out of 28 isolated SLs, 17 were subjected to cytotoxic screening using the *in vitro* MTT assay on three human cancer cell lines (HeLa, SK-MEL-28, and HepG2) with parthenolide as a positive control (Results 3.1 and 3.2). From *C. papposa*, only 8α-O-(3,4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin, malacitanolide and its 4-epimere exerted IC_{50} values ≤ 10 μM against HeLa cells, while SK-MEL-28 and HepG2 cells were more resilient. All the tested SLs from *C. jamaicensis* showed strong activity against HeLa cells, with IC_{50} values ranging from 2.9 to 13.4 μM. Carbetolide C, together with cajamolides L and N were also highly active against SK-MEL-28 cell line (IC_{50} values 7.5, 6.5, and 5.2 μM, respectively). It is important to note that carbetolide C afforded IC_{50} values of 2.9, 7.5, and 12.6 μM for the HeLa, SK-MEL-28, and HePG2 cells, which are similar to that of parthenolide, a SL with strong anti-cancer potential (Sztiller-Sikorska & Czyz, 2020) and whose semi-synthetic derivative was assayed in phase I trials against acute myeloid and lymphoblastic leukemia, and other blood and lymph node cancers (Moujir et al., 2020). In accordance with the guidelines by National Cancer Institute, stating that compounds with IC_{50} ≤ 10 μM are considered active and could be potential anti-cancer drugs, most of the tested SLs could be potential chemotherapeutic agents for cervical cancer or even melanoma. On the other hand, out of the 7 DTs tested (Results 3.6), 3 were active against only HeLa cells (euphodeflexins A, L, O with IC_{50} values of 9.9, 9.8 and 5.5 μM), and in parallel low activity was observed against two bacterial strains.

In addition to cytotoxicity, there is a growing interest on the anti-inflammatory mechanisms of SLs, and numerous compounds from different taxa have been investigated using *in silico*, *in vitro,* and *in vivo* assays

(Matos et al., 2021). In the present study, out of the total SLs isolated from *C. jamaicensis* and *C. papposa*, six were tested using the *in vitro* ICAM-1 assay in order to investigate their anti-inflammatory potential (Results 3.1 and 3.2). The samples were firstly subjected to MTT viability test on the endothelial (non-cancerous) HMEC-1 cells, in order to exclude any false-positive results. Interestingly, cajamolide A, carbetolide C and heliangin from *C. jamaicensis*, together with cnicin and 8α-O-(3,4-dihydroxy-methylenebutanoyloxy)-dehydromelitensin from *C. papposa* strongly inhibited the TNF-α induced ICAM-1 expression in HMEC-1 cells, in a dose-dependent manner (with IC_{50} values 8.5, 5.5, 5.4, 21.9 and 5.7, respectively). These results are in accordance with other researchers reporting parthenolide's strong inhibition of the TNF-α induced ICAM-1 expression in human synovial fibroblasts (Piela-Smith & Liu, 2001). These strong inhibitors all have in common the lactone ring, in contrast to compounds lacking the α,β-unsaturated lactone moiety. 6α,8α,15-trihydroxyelema-1,3,11(13)-trien-12-olide methyl ester, a homologous sesquiterpene from *C. papposa*, showed lower inhibitory potential, similar to matricine, which lacks the α-methylene-γ-lactone group and was evaluated in the same test system described in this study by Flemming et al. (2015). Thus, it is suggested that the moiety of lactone ring is essential for the anti-inflammatory activity through the ICAM-1 pathway. It is noteworthy that the tested SLs revealed no toxicity in the endothelial HMEC-1 cells in all the range of the tested concentrations (6.25-50.0 μM), though Michael reactions of SLs usually also involve non-specific toxicity (Matos et al., 2021).

Of note, several of the isolated PAPs showed similar results in the ICAM-1 assay (Results 3.3). Although the majority of the PAP derivatives are investigated for their antidepressant, cytotoxic and anti-angiogenetic, or antimicrobial effects, the present work was driven by the observations of Crockett et al. (2008), mentioning the strong anti-inflammatory effects of 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol and 3-geranyl-1-(2-methylboutanoyl)-phloroglucinol isolated from *Hypericum empetrifolium*. In fact, *H. empetrifolium* and *H. jovis* taximomically are close relative plants, and many of the isolated compounds from *H. jovis* were previously reported from *H. empetrifolium*, including 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol and 3-geranyl-1-(2-methylboutanoyl)-phloroglucinol. Following this idea, the isolated PAPs from *H. jovis* were tested for their anti-inflammatory potential in the ICAM-1 assay, and compounds 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol, hyperjovinol A, dauphinol F, 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one and empetriferdinan A significantly reduced ICAM-1 expression in a concentration-dependent manner (IC_{50} values of 16.9, 34.4, 4.0, 3.2 and 7.7 μM). The most potent compounds (based also on the obtained amounts) were further subjected to the *ex-vivo* COX/LOX assay and empetriferdinan A showed notable inhibitory activity on the formation of COX-1 and 12-LOX derived inflammatory mediators. In comparison

to the SLs which showed no toxicity in the HMEC-1 cells, some of the tested PAPs showed either toxicity in higher concentrations (eg. empetriferdinan A and hyperforin) or increased metabolic activity, thus tested in lower concentrations. Moreover, 3-geranyl-1-(2-methylpropanoyl)-phloroglucinol showed, in addition to the anti-inflammatory effects described above, antioxidant (Athanasas et al., 2004) and antimicrobial activities (Results 3.7.1), and in parallel it was not toxic to the normal cells and was isolated in huge amounts, which gave the opportunity for *in vivo* testing (Results 3.7.2). Unfortunately, this compound showed only moderate effects for the treatment of atopic dermatitis under the given conditions.

The possibility for *in vivo* testing was also given by obtaining high amounts from some *Hypericum* EOs (Results 3.4). More specifically, as mentioned above *H. jovis* is a close relative of *H. empetrifolium* and together with *H. amblyocalyx* they constitute the three taxa of section Coridium growing wild in the island of Crete. The volatile oils of the three species were investigated for their chemical profiles and subjected to antimicrobial screening, displaying good activity against fungi and bacteria. It is noticeable that *H. empetrifolium* yielded high amounts of EO (up to 13% v/w). For this reason, this plant, together with *H. triquetrifolium* (both are used in folk medicine in Greece) and *H. perforatum* (the officially used by EMA) were evaluated for wound healing *in vivo*. EOs are bioaccumulated in the translucent glands of *Hypericum* plants, together with phloroglucinols, and these non-polar constituents are highly likely to be extracted in the infused oil (Oleum *Hyperici*), which is the most common preparation used in wound treatments (Crockett, 2010). Up to this point, the EOs from *Hypericum* had never been investigated for wound healing, most probably because of the possibility of skin irritation/toxicity and low yields. Indeed, *H. perforatum* and *H. triquetrifolium* showed a lower degree of healing at the last days of the experiment which could suggest a dose-depended toxicity. Interestingly, this was not observed with *H. empetrifolium*, which showed significant wound healing effects and confirmed its traditional use in Greece for wounds and skin inflammations (Vokou et al., 1993). It is worth mentioning that in the quote by Dioscorides, *Hypericon* could be attributed to *H. empetrifolium* since the previously reported *H. coris* (Berendes, 1970) is not growing wild in Greece, in contrast to its closely related species, *H. empetrifolium* (Robson, 2010).

A final goal was the investigation of the metabolization of natural products, which is much less studied than their biosynthetic pathways. Structural diverse SLs were selected as a case study, because of their strong biological effects and drug-like properties. Metabolites were produced *in vitro* through phase I and phase II (glucuronidation) or combined I and II using human liver microsomes. Such bioproducts are structurally unreported and their biological existence-effects are fully unidentified. SLs proved to be

subject of changeable metabolism although the highly active lactone ring is the most common metabolic site. Further detailed studies are warranted to address the metabolic pathways of natural products and to define actions or interactions that result in the final formations of the component in a living system.

As a concluding mark, the present study applied an interdisciplinary approach to natural product-based drug discovery, utilizing state-of-the-art techniques and bioassays, *in vivo* models, *in silico* pharmacokinetic and *in vitro* metabolism studies. High cytotoxic, anti-inflammatory, and wound-healing potential were reported from natural products derived from *Calea jamaicensis*, *Centaurea papposa*, *Hypericum jovis* and *Euphorbia deflexa*. For these taxa, almost no records existed in the literature using modern techniques and investigating the biological effects of their constituents. Out of more than 310.000 plant species, only 5-15% have been investigated so far phytochemically and/or pharmacologically (Atanasov et al., 2015), indicating a horizon of further plants-involving studies. The results of this study further supported the strategic use of natural products as a starting point to investigate novel structures as potential effective agents against a variety of human ailments.

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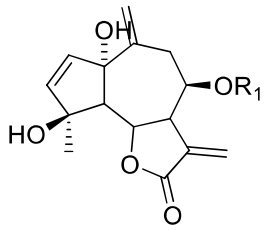
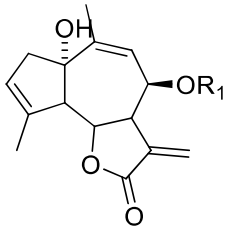
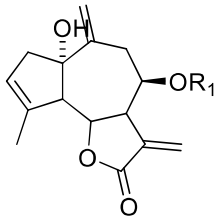
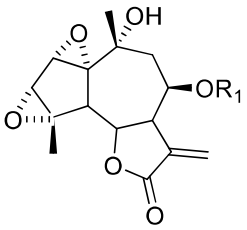
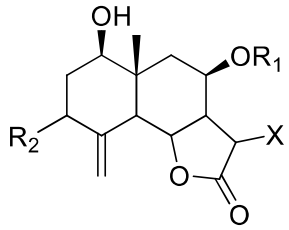
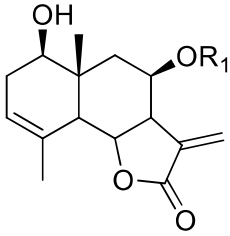
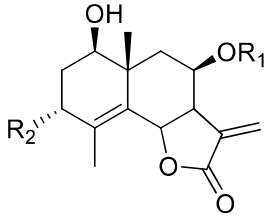
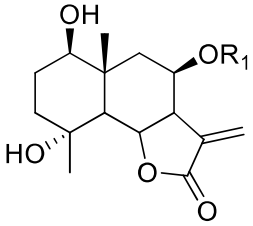
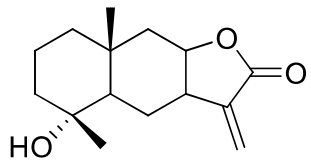
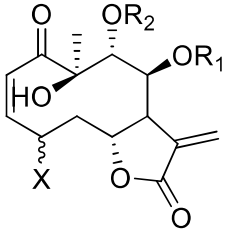
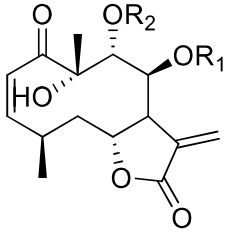
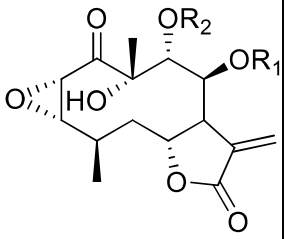
7. APPENDIX

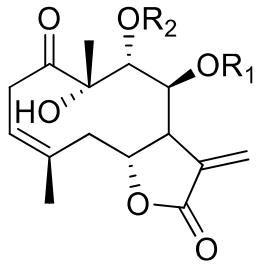
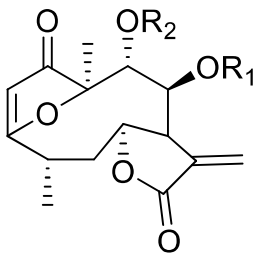
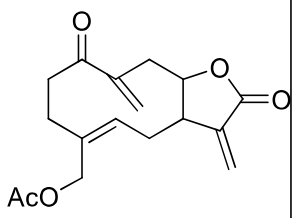
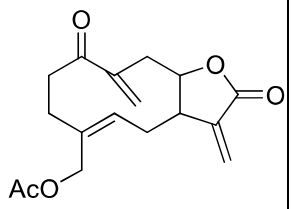
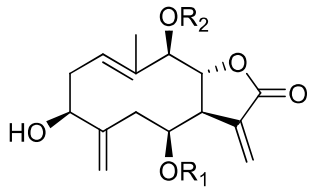
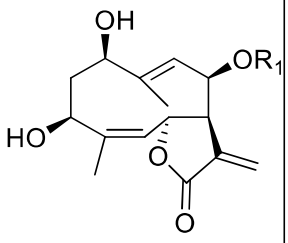
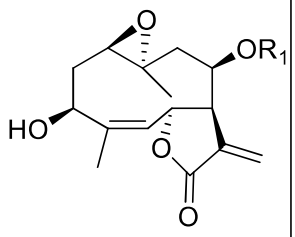
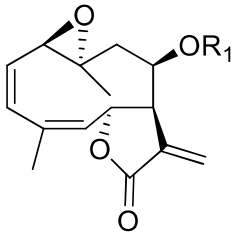
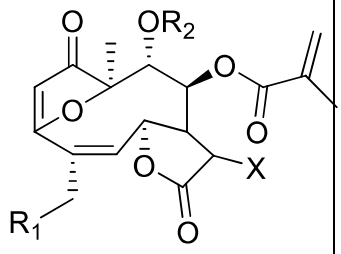
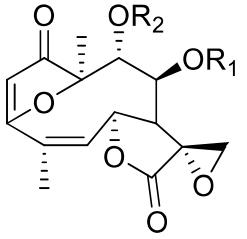
7.1. Summary of the isolated Sesquiterpene Lactones from the genus *Calea*

Sesquiterpene lactones isolated from Mexican, Central American, and Jamaican *Calea* spp. grouped by Wussow et al. (1985).		
***Calea* spp.**	**Sesquiterpene lactones**	**References**
C. berteriana DC.	calbertolides A-C, heliangin	Ober et al., 1985a
	calbertolide C, desacyl-8-tiglylsubcordatolide A, 8-epi-stiglylrupicolins A-B	Ober et al., 1985b
C. crocinervosa Wussow, Urbatsch & G.A.Sullivan	9α-hydroxyatripliciolide-8-O-methylacrilate, septuplinolide	Ortega et al., 1989
C. jamaicensis (L.) L.	jamaicolides A-D	Ober et al. 1986
C. ternifolia var. var. *calyculata* (B.L.Rob.) "Wussow, Urbatsch & G.A.Sullivan	9α-acetoxyzexbrevin, 9α-Hydroxy-11,13-dihydro-11α,13-epoxyatripliciolide-8β-O[2-methylacrylate], 9α-Hydroxy-11,13-dihydro-atripliciolide-8β-O-[2-methylacrylate]	Lee et al., 1982a
	8β-angeloyloxy-9α-hydroxycalycultolide, 8β-methylacryloyloxy-9α-hydroxycalyculatolide, 15-hydroxy- 11.13-dihydro- 11α, 13-epoxyatripliciolide-8β-O-angelate	Fischer et al., 1984
	calein A, 8β-angeloyloxy-9α-acetoxyternifolin, 8β-angeloyloxy-9α-[2-methylbutanoyloxy] -ternifolin	Lee et al., 1982b

C. ternifolia Oliv.	calein A calein C	Escandón-Rivera et. al., 2017
C. trichotoma Donn.Sm.	trichomatolide A, 8β-tiglinoyloxyreynosin, 1β-hydroxy-8β-tiglinoyloxyarbusculin B, 8β-tiglinoyloxybalchanin, 1β,4α-dihydroxy-8β-tigloxy-11(13)-eudesmen-6α,12-olide	Ober et al., 1984a
	3-deoxy-2,3-dehydroheliangin, trichomatolides C-E	Ober et al., 1984b
C. urticifolia (Mill.) DC.	4-epi-caleurticolide-angelicate, 4-epi-caleurticolide-[2-methylacrylate] , 4-epi-caleurticolide-isoburitate	Herz & Kumar 1980
	calealactone B, arucanolide, 2,3-epoxy-juanislamin, 2,3-epoxy-calealactone A, calealactone A	Ohguchi et al., 2009
	calealactones A-C, 2,3-epoxy-calealactone A, calein D, juanislamin, 2,3-epoxy- juanislamin	Yamada et al., 2004
	calealactone A, 2,3-epoxy-calealactone A, calealactone B, calealactone C, arucanolide, juanislamin,	Nakagawa et al., 2005

	2,3-epoxy- juanislamin	
	9α-Hydroxy-atripliciolid-8-O-[2-methylacrylate] 9α-[senecioyloxy]-15-hydroxy-atripliciolid-8-O-[2-methylacrylate], 9α-[angeloyloxy]-15-hydroxy-atripliciolid-8-O-[2-methylacrylate, 9α-[isovaleryloxy]-15-hydroxy-atripliciolid-8-O-[2-methylacrylate, caleurticolidacetate, caleurticolide-[2-methylacrylate], caleurticolide-angelicate	Bohlmann and Jakupovic (1979)

 **A**	 **B**	 **C**	 **D**
 **E**	 **F**	 **G**	 **H**
 **I**	 **G**	 **K**	 L

 **M**	 **N**	 **O**	 **P**
 **Q**	 **R**	 **S**	 **T**
 **U**	 **V**		

		R_1	R_2	X
desacyl-8-tiglylsubcordatolide A	**A**	Tig		
jamaicolide A	**A**	Ang-4-OAc		
8-epi-stiglylrupicolin A	**B**	Tig		
8-epi-Stiglylrupicolin B	**C**	Tig		
jamaicolide B	**D**	Ang-4-OAc		
trichomatolide A	**E**	Tig	H	CH_2OH
8β-tiglinoyloxyreynosin	**E**	Tig	H	$=CH_2$
trichomatolide B	**E**	Tig	OH	$=CH_2$
trichomatolide D	**E**	Etacr-3-OH	H	$=CH_2$
trichomatolide E	**E**	1-Metoxy-sen	H	$=CH_2$
8β-tiglinoyloxybalchanin	**F**	Tig		
trichomatolide C	**G**	Tig	OH	

1β-hydroxy-8β-tiglinoyloxyarbusculin B	**G**	Tig	H	
1β,4α-dihydroxy-8β-tigloxy-11(13) eudesmen-6α,12-olide	**H**	Tig		
septuplinolide	**I**			
calein A	**G**	Ang	Ac	αCH_3
calein C	**G**	Tig	Ac	βCH_3
caleurticolidacetate	**G**	Meacr	Ac	αCH_3
caleurticolide-[2-methylacrylate]	**G**	Meacr	Meacr	αCH_3
caleurticolide-angelicate	**G**	Meacr	Ang	αCH_3
4-epi-caleurticolide-angelicate	**G**	Ang	Meacr (or reverse)	βCH_3
4-epi-caleurticolide-[2-methylacrylate]	**G**	Meacr	Meacr	βCH_3
4-epi-caleurticolide-isoburitate	**G**	iBu	Meacr (or reverse)	βCH_3
calein D	**K**	Ac	Meacr	
calealactone A	**K**	Meacr	i-Val	
calealactone B	**K**	Meacr	Ac	
juanislamin	**K**	Meacr	Etacr	
arucanolide	**K**	Ac	Meacr	
2,3-epoxy-calealactone A	**L**	Meacr	i-Val	
2,3-epoxy-juanislamin	**L**	Etacr	Acryl	
calelactone C	**M**	Meacr	Ac	
9α-acetoxyzexbrevin	**N**	Meacr	Ac	
8α-angeloyloxy-9α-hydroxycalyculatolide	**N**	Ang	H	
8β-methylacryloyloxy-9α hydroxycalyculatolide	**N**	Meacr	H	
calbertolide A	**O**			
calbertolide B	**P**			
jamaicolide D	**Q**	Ang	Ac (or reverse)	
calbertolide C	**R**	Tig		
jamaicolide C	**R**	Ang		
heliangin	**S**	Tig		
3-deoxy-2,3-dehydroheliangin	**T**	Tig		

9α-hydroxyatripliciolide 8-O-methylacrilate	**U**	H	H	$=CH_2$
9α-Hydroxy-11,13-dihydro-atripliciolide-8β-O-[2-methylacrylate]	**U**	H	H	CH_3
9α-[senecioyloxy]-15-hydroxy-atripliciolid-8-O-[2- methylacrylate]	**U**	OH	iVal	
9α-[angeloyloxy]-15-hydroxy-atripliciolid-8-O-[2- methylacrylate	**U**	OH	Sen	
9α-[isovaleryloxy]-15-hydroxy-atripliciolid-8-O-[2- methylacrylate	**U**	OH	Ang	
9α-Hydroxy-11,13-dihydro-11α,13 epoxyatripliciolide-8β-*O*[2-methylacrylate]	**V**	Meacr	H	
15-hydroxy- 11.13-dihydro-11α, 13-epoxyatripliciolide-8β-O-angelate	**V**	Ang	H	

7.2. Summary of the isolated Sesquiterpene Lactones from the genus *Centaurea, section Centaurea*

Sesquiterpene lactones isolated from *Centaurea* L. species, belonging to section Centaurea, subsections Acrolophus (A), Phalolepis (P) and Willkommia (W).			
***Centaurea* spp.**	**Sub-section**	**Sesquiterpene lactones**	**References**
C. affinis Friv.	A	cnicin	Janackovic et al., 2004
C. affinis subsp. *pallidior* (Halácsy) Hayek (= *C. pallidior* Halácsy subsp. *pallidior*)	A	cnicin	Nowak et al., 1984
C. aggregata Fisch. & C.A.Mey. ex DC	A	cnicin	Nowak et al., 1984
C. alba L.	P	cnicin, 4′-acetylcnicin, salonitenolide, 11β,13-dihydrosalonitenolide, salonitenolide-8-O-(4'-acetoxy-5'-hydroxy)-angelate	Fernandez et al., 1995
C. aplolepa Moretti (= *C. aplolepa* subsp. *aplolepa*)	A	cnicin	Nowak et al., 1984
C. aplolepa Moretti subsp. *lunensis* (Fiori) Dostál	A	cnicin	Nowak et al., 1984
C. arenaria M.Bieb. ex Willd.	A	cnicin cnicin	Nowak et al., 1984

(= *C. arenaria* Bieb. Aex Willd. subsp. *arenaria*)			Csapi et al., 2010
C. attica Nyman (= *C. attica* Nyman subsp. *attica*)	A	cnicin, cnicin 4′-O-acetate, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, methyl 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-6a,1 5-dihydroxyelema-1,3,1 1(13)-trien-12-oate malacitanolide, atticin, 8α-(4′-acetoxy-3′-hydroxy-2′-methylenebutanoyloxy)-4-epi-sonchucarpolide	Skaltsa et al., 1999 Skaltsa et al., 2000
C. bombycina DC.	W	cnicin, salonitenolide, 8-O-(4′-acetoxy-5′-hydroxyangelate)salonitenolide	Barrero et al., 2000
C. bovina Velen (= *C. diffusa* Lam. var. *brevispina* Boiss.)	A	cnicin	Nowak et al., 1984
C. busambarensis Guss. (= *C. cineraria* subsp. *busambarensis* (Guss.) Dostál)	A	cnicin, dehydromelitensin, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin	Bruno et al., 1998
C. cadmea Boiss.	P	2α-hydroxy-5αH-eudesman-4(15),11(13)-dien-12,8β-olide (ivalin)	Karamenderes et al., 2007
C. caliacrae Prodan	P	salonitenolide	Geppert et al., 1983

[= *C. alba* subsp. *caliacrae* (Prodan) Dostál]			
C. calolepis Boiss.	A	cnicin	Erel et al., 2011
C. castellana Boiss. [= *C. paniculata* subsp. *castellana* (Boiss. & Reut.) Dostál]	A	artemissifolin, dehydromelitensin 11β,15-dehydroxysaussurea	Gonzalez et al., 1984
C. cineraria L. (= C. *cineraria* subsp. *cineraria)*	A	cnicin	Nowak et al., 1984
C. cineraria subsp. *circae* (Sommier) Cela Renz. & Viegi	A	cnicin	Nowak et al., 1984
C. crithmifolia Vis.	A	calonitenolide	Geppert et al., 1983
C. cuneifolia Sibth. & SM. (= *C. cuneifolia* subsp. *cuneifolia*)	A	cnicin, dehydromelitensin, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin	Aslan & Öksüz, 1999
C. cuneifolia Sibth. & SM. subsp. *pallida* (Friv.) Hayek	A	cnicin	Nowak et al., 1984
C. derventana Vis. & Pančić	A	cnicin, cnicin-4′-O-acetate, salonitenolide, 8-O-(4′-acetoxy-5′-hydroxyangelate) salonitenolide	Tešević et al., 1998

C. deusta Ten.	P	salonitenolide cnicin, cnicin-4'-O-acetate, 3'-acetylcnicin, 8α-(3',4'-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)dehydromelitensin, salonitenolide, methyl 8α-(3,4-dihydroxy-2-meth-ylene-butanoyloxy)-6α,15-dihydroxy-elema-1,3,11(13)-trien-12-oate, methyl 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-6α,15-dihydroxyelema-1, 3, 11(13)-trien-12-oate, 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-4-epi-sonchucarpolide, 8α-(4-acetoxy-3-hydroxy-2-methylene-butanoyloxy)-sonchucarpolide	Geppert et al., 1983 Karioti et al., 2002
C. diffusa Lam.	A	cnicin cnicin, 4'-acetylcnicin, 8α-(3'-hydroxy-4'-acetoxy-2-methylene-butanoyloxy) dehydromelitensin,	Milkova et al., 1993 Fortuna et al., 2002

		methyl 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-6α,1 5-dihydroxyelema-1,3,1 1(13)-trien-12-olide	
C. diffusa Lam. var. *brevispina* Boiss. (= *C. bovina* Velen.)	A	cnicin	Nowak et al., 1984
Centaurea friderici Vis.	A	cnicin	Nowak et al., 1984
C. galicicae Micevski	A	cnicin	Tešević et al., 2014
C. grisebachii (Nyman) Heldr. [= *C. grisebachii* subsp. *grisebachii* = *C. grisebachii* (Nyman) Formánek)]	A	cnicin cnicin, dehydromelitensin, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, salonitenolide, 8-O-(4′-acetoxy-5′-hydroxyangelate)salonitenolide, 8α-hydroxy-4-epi- sonchucarpolide, 8α-(4′-acetoxy-3′-hydroxy-2′-methylenebutanoyloxy)-4-epi-sonchucarpolide, 8α-(4′-acetoxy-3′-hydroxy-2′-methylenebutanoyloxy)-sonchucarpolide, malacitanolide, 4-epi-malacitanolide	Nowak et al., 1984 Djeddi et al., 2008

C. grisebachii (Nyman) Heldr. subsp. *confusa* (Halácsy) Dostál	A	cnicin	Nowak et al., 1984
C. grisebachii subsp. *transiens* (Halácsy) T.Georgiadis (= *C. transiens* Halácsy)	A	cnicin	Nowak et al., 1984
C. kartschiana Kcop.	A	cnicin	Nowak et al., 1984
C. kilaea Boiss.	A	cnicin, dehydromelitensin	Sen et al., 2017
C. laureotica Heldr. ex Halácsy (= *C. mantoudii* Georg.)	A	cnicin	Nowak et al., 1984
C. leucophaea Jord. (= *C. leucophaea* Jordan subsp. *leucophaea*)	A	cnicin	Nowak et al., 1984
C. majorrovii Dumbadze (= *C. arenaria* subsp. *majorowii* (Dumb.) Dostál)	A	cnicin	Nowak et al., 1984

C. odessana Prodan [= *C. arenaria* subsp. *odessana* (Prodan) Dostál]	A	cnicin	Nowak et al., 1984
C. orphanidea Heldr. & Sart. ex Boiss.	A	cnicin cnicin, 4′-O-acetylcnicin, salonitenolide, malacitanolide, 8α- hydroxy-4-epi-sonchucarpolide, 8α-(3 ′-hydroxy,4′-acetoxy-2-methylenebutanoyloxy)-4-epi-sonchucarpolide, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin,	Nowak et al., 1984 Gousiadou & Skaltsa, 2003
C. ossaea Halácsy (= *C. attica* Nyman subsp. ossaea)	A	cnicin	Nowak et al., 1984
C. paniculata L.	A	salonitenolide	Geppert et al., 1983
C. panormitana subsp. *umbrosa* (Fiori) Greuter [= *C. cineraria* L. subsp. *umbrosa* (Lacaita)]	A	cnicin, 4′-O-acetylcnicin, 8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-dehydromelitensin	Bruno & Herz, 1988
C. paui Loscos ex Willk.	W	8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-15-oxo-5,7αH,6,11 β H-elema-1,3,1 1(13)-trien-12-olide,	Cardona et al., 1994

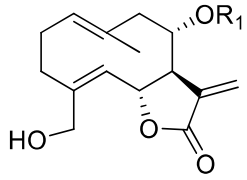
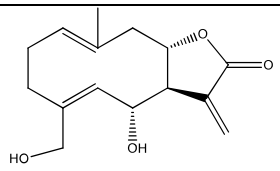
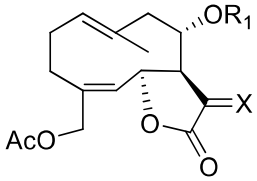
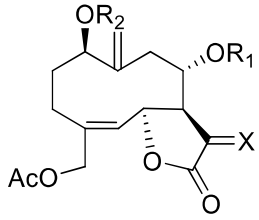
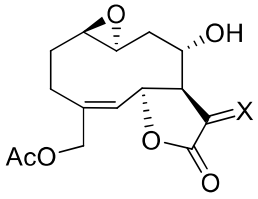
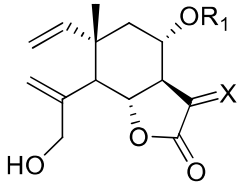
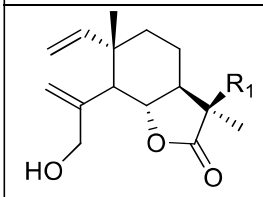
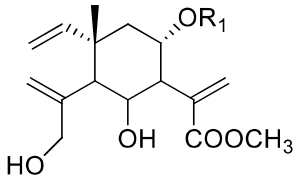
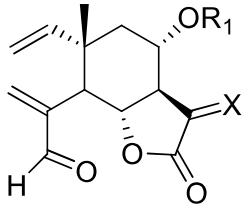
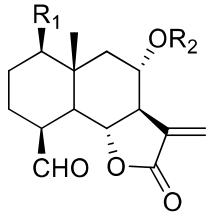
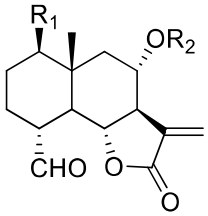
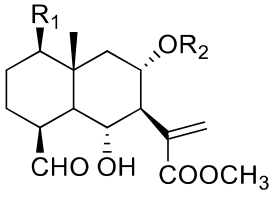
		(3′R)-15-acetoxy-8α-(3′,4′-dihydro-2′-methylenebutanoyloxy)-6βH,7αH-germacra-1E,4Z,11(13)-trien-6,12-olide, (3′R)-15-acetoxy-8α-O-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)- 6βH,7αH-germacra-1E,4Z,11(13)-trien-6,12-olide, cnicin, 4′-acetylcnicin, salonitenolide, stoebenolide, dehydromelitensin, 11β,13-dihydrosalonitenolide, 8α-O-(4′-acetoxy-5′-hydroxyangeloyl)salonitenolide, 8α -hydroxy-15-oxo-5,7αH,6,11βH-elema-1,3-dien-6,12-olide, 8α -hydroxy-15-oxo-5,7αH,6,11βH -elema-1,3,11(13)-trien-6,12-olide, methyl 8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-6,16-dihydroxy-5,7αH,6βH-elema-1, 3, 11 (13)-trien-6, 12-olide, 15-acetoxy-8α-hydroxy-6βH,7αH -germacra-1E,4Z,11(13)-trien-6,12-olide, (3′R)-15-acetoxy-8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-1β-hydroperoxy-10-methylene, 6βH,7αH -germacra-4Z,10(14),11(13)-trien-6,12-olide,	Cardona et al., 1997

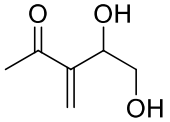
		(3′R)-15-acetoxy-8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-1β-hydroxy-10-methylene, 6βH,7αH-germacra-4Z,10(14),11(13)-trien-6,12-olide, 15-acetoxy-1β,8α-dihydroxy-6βH,7αH -germacra-4Z,10(14),11(13)-trien-6,12-olide, 15-acetoxy-1β,8α-dihydroperoxy-6βH,7αH-germacra-4Z,10(14),11(13)-trien-6,12-olide	
C. pelia DC.	A	cnicin	Nowak et al., 1984
C. pseudomaculosa Dobrocz.	A	cnicin	Al-Easa & Rizk, 1992
C. reichenbachii DC. (= *C. calvescens* Pancić)	A	cnicin	Nowak et al., 1984
C. savranica Klokov [= *C. rhenana* Boreau subsp. *savranica* (Klokow) Dostál]	A	cnicin	Nowak et al., 1984
C. soskae Hayek	A	cnicin	Tešević et al., 2014
C. spinosa L. (= C. *spinosa* var. *spinosa*)	A	cnicin cnicin, 4′-acetyl-cnicin, 4-epi-malacitanolide, 8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-4-epi-sonchucarpolide,	Nowak et al., 1984 Saroglou et al., 2005

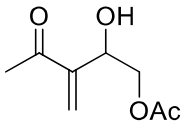
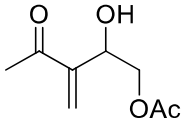
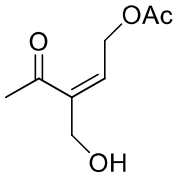
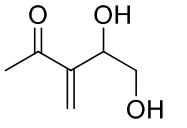
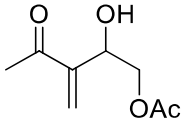
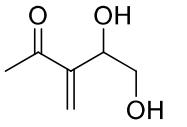
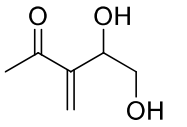
		malacitanolide, 4′-acetyl-malacitanolide, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-O-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-15-oxo-5,7βH,6αH-eleman-1,3,11(13)-trien-6,12-olide, 8R-O-(4-acetoxy-2-hydroxymethyl-buten-2-oyloxy) salonitenolide, methyl 8R-O-(3,4-dihydroxy-2-methylenebutanoyloxy)-6α,15- dihydroxy-eleman-1,3,11(13)-trien-12-oate	
C. stoebe L. (= *C. maculosa*)	A	cnicin cnicin, salonitenolide, 8-O-(4′-acetoxy-5′-hydroxyangelate)-salonitenolide, stoebenolide	Nowak et al., 1984 Huneck et al., 1986
C. thessala Hausskn. subsp.*drakiensis* (Freyn & Sint.) T. Georgiadis [= *C. attica* Nyman subsp. *drakiensis* (Freyn, Sint.) Dostál]	A	cnicin cnicin, 4′-acetylcnicin, 8-O-(4′-acetoxy-5′-hydroxyangelate)-salonitenolide 8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-dehydromelitensin, 8α-(3′-hydroxy,4′-acetoxy-2′-methylenebutanoyloxy)-dehydromelitensin, 8α-hydroxy-4-epi-sonchucarpolide	Nowak et al., 1984 Skaltsa et al., 1999 Skaltsa et al., 2000

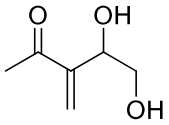
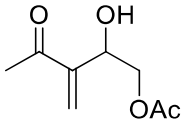
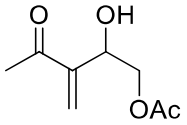
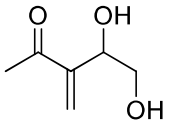
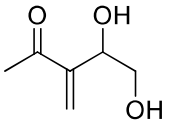
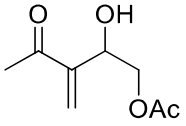
C. tomorosii Micevski	A	cnicin	Tešević et al., 2014
C. tougourensis Boiss. & Reut.	P	cnicin, 8α-(3',4'-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, (6R, 7R, 8S, 30R) 8α-(3, 4-dihydroxy-2-methylene-butanoyloxy)-15-oxo-helianga 1(10), 4(5), 11(13) trien-6-olide, (6R, 7R, 8S, 30R) 8α-(3, 4-dihydroxy-2-methylene-butanoyloxy)-15-acetoxy-helianga 1(10), 4(5), 11(13) trien-6-olide	Nacer et al., 2012
C. tymphaea Hausskn. (= *C. tymphaea* Hausskn. subsp. *tymphaea*)	A	cnicin	Nowak et al., 1984
C. tymphaea subsp. *brevispina* (Hausskn.) Dostál	A	cnicin	Nowak et al., 1984
C. vallesiaca (DC..) Jordan	A	cnicin cnicin	Nowak et al., 1984, Geppert et al., 1983
C. virgata Lam.	A	cnicin, 8α-(3',4'-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-O-hydroxy-sonchucarpolide	Tuzun et al., 2017

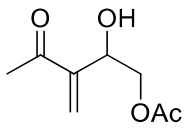
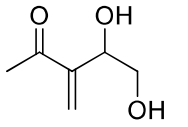
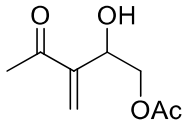
C. zuccariniana DC..	A	cnicin	Nowak et al., 1984
		cnicin, 4′-acetylcnicin, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-O-hydroxy-sonchucarpolide	Koukoulitsa et al., 2002
		cnicin, 4′-acetylcnicin, dehydromelitensin, 8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin, 8α-O-(4′-acetoxy-3′-hydroxy-2′-methylenebutanoyloxy)-dehydromelitensin, 8α-(3′,4′-dihydro-2′-methylenebutanoyloxy)-15-oxo-5,7αH,6βH-eleman-1, 3, 11 (13)-trien-6, 12-olide, (1E,4Z)-15-hydroxy-8α-O-(4′-acetoxy-3′-hydroxy-2′-methylidenebutanoyl)-6βH,7αH-germacra-1,4,11(13)-trien-6,12-olide, methyl 8α-O-[(4-acetoxy-3-hydroxy-2-methylenebutanoyl)oxy]-6α,15-dihydroxyelema-1,3,11(13)-trien-12-oate, 8α-O-(4′-acetoxy-3′-hydroxy-2′-methylenebutanoyloxy)-sonchucarpolide and its 4-epimer, malacitanolide, 8α-(4′,5′-diacetoxyangeloyl)sonchucarpolide, zuccarinin	Cirić et al., 2012

 **I**	 **Ia**	 **II**
 **III**	 **IV**	 **V**
 **Va**	 **Vb**	 **VI**
 **VII**	 **VIII**	 **IX**

		R_1	R_2	X
cnicin	**I**			CH_2

4′-acetylcnicin	**I**			CH_2
salonitenolide	**I**	H		CH_2
11β,13-dihydrosalonitenolide	**I**	H		βH, αMe
8a-O-(3-hydroxy-4-aketoxy-2-metheleno-butanoyloxy)salonitenolide	**I**			CH_2
8-O-(4′-acetoxy-5′-hydroxyangelate) salonitenolide	**I**			CH_2
artemissifolin	**Iα**			
(3′R)-15-acetoxy-8α-(3′,4′-dihydro-2′-methylenebutanoyloxy)-6βH,7αH-germacra-1E,4Z,11(13)-trien-6,12-olide	**II**			CH_2
15-acetoxy-8α-hydroxy-6βH,7αH -germacra-1E,4Z,11(13)-trien-6,12-olide	**II**	H		CH_2
(3′R)-15-acetoxy-8α-O-(4-acetoxy-3-hydroxy-2-methylenebutanoyloxy)- 6βH,7αH-germacra-1E,4Z,11(13)-trien-6,12-olide	**II**			CH_2
(3′R)-15-acetoxy-8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-1β-hydroxy-10-methylene, 6βH,7αH-germacra-4Z,10(14),11(13)-trien-6,12-olide,	**III**		OH	CH_2
15-acetoxy-1β,8α-dihydroperoxy-6βH,7αH-germacra-4Z,10(14),11(13)-trien-6,12-olide	**III**	OH	OH	CH_2
(3′R)-15-acetoxy-8α-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-1β-hydroperoxy-10-methylene,6βH,7αH -germacra-4Z,10(14),11(13)-trien-6,12-olide	**III**		H	CH_2

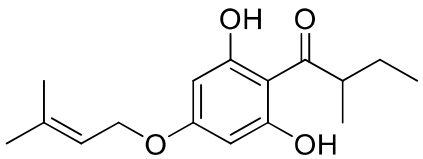
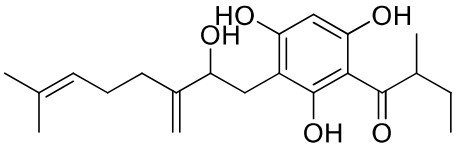
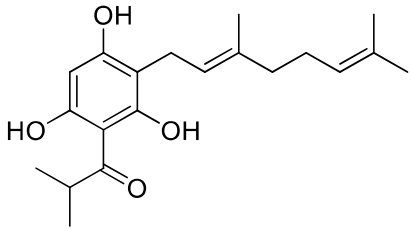
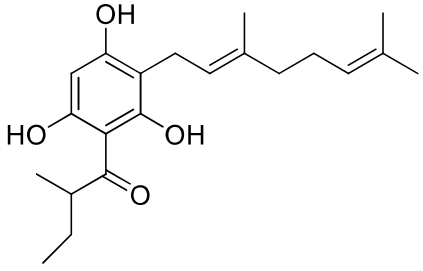
15-acetoxy-1β,8-dihydroxy-6βH,7H -germacra-4Z,10(14),11(13)-trien-6,12-olide,	**III**	H	H	CH_2
15-acetoxy-1β,10α-epoxy-6βH,7aH -germacra-4Z,11(13)-dien-6,12-olide	**IV**			CH_2
8α-(3′,4′-dihydroxy-2-methylenebutanoyloxy)-dehydromelitensin,	**V**			CH_2
8α-O-(4′-acetoxy-3′-hydroxy-2′-methylenebutanoyloxy)-dehydromelitensin	**V**			CH_2
Saussurea (11β,15-dehydromelitensin)	**Va**	OH		CH_2
8α-O-(4′ acetoxy -3-dihydroxy-2methylenobutanoyloxy)-6α,15-dihydroxy-eleman-1,3,11(13)-trien-12-olide methylester	**Vb**			CH_2
11,13- dehydromelitensin	**V**	H		CH_2
8α -hydroxy-15-oxo-5,7αH,6,11βH-elema-1,3-dien-6,12-olide	**VI**	H		βH, αMe
8α -hydroxy-15-oxo-5,7αH,6,11βH -elema-1,3,11(13)-trien-6,12-olide	**VI**	H		CH_2
8α-O-(3′,4′-dihydroxy-2′-methylenebutanoyloxy)-15-oxo-5,7βH,6αH-eleman-1,3,11(13)-trien-6,12-olide	**VI**			CH_2
stoebenolide	**VII**	H	H	
8α-hydroxy-4-epi- sonchucarpolide	**VII**	OH	H	
malacitenolide	**VII**	OH		
4′-acetyl-malacitanolide	**VII**	OH		

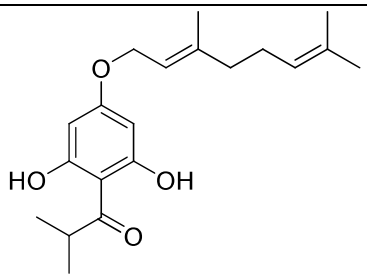
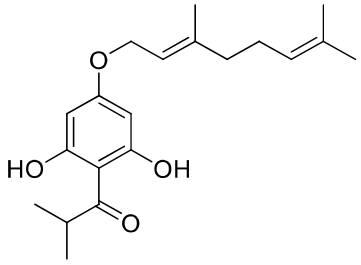
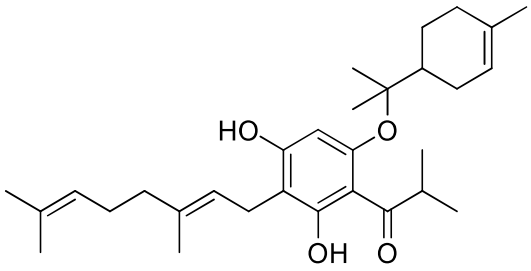
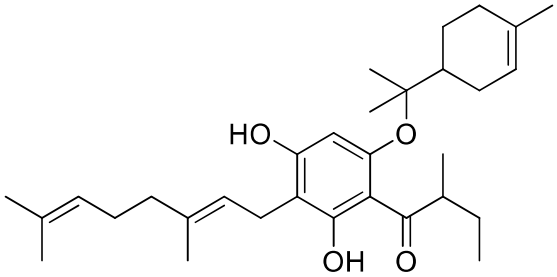
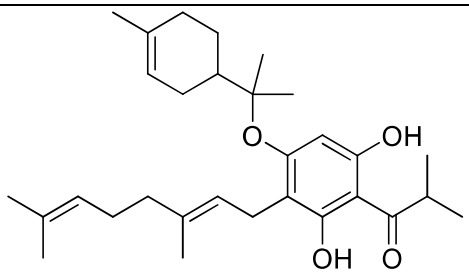
8α-(4'-acetoxy-3'-hydroxy-2'-methylene-butanoyloxy)-sonchucarpolide	**VIII**	OH		
8α-hydroxy-sonchucarpolide	**VIII**	OH		
atticin	**IX**	OH		

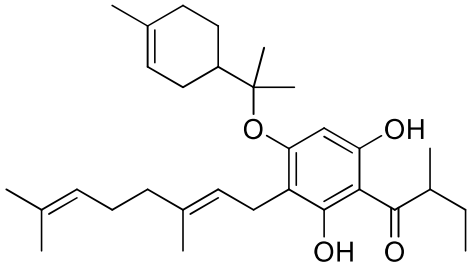
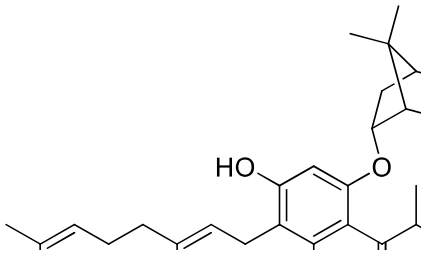
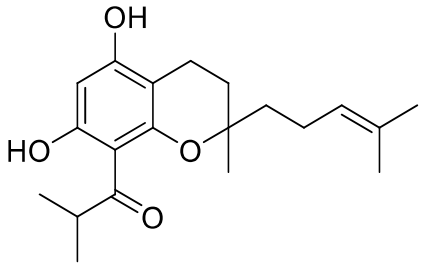
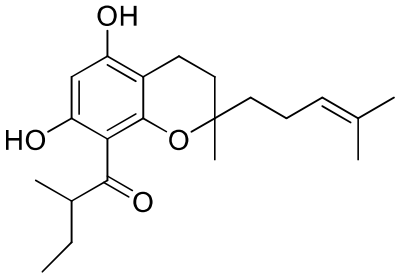
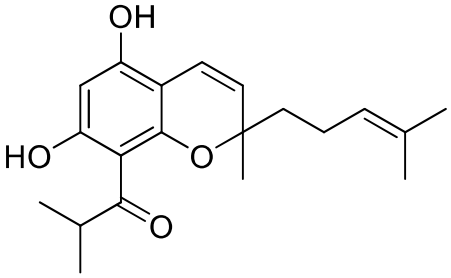
7.3. Summary of the isolated Phloroglucinols from the genus *Hypericum, section Coridium*

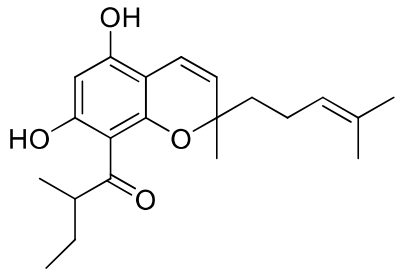
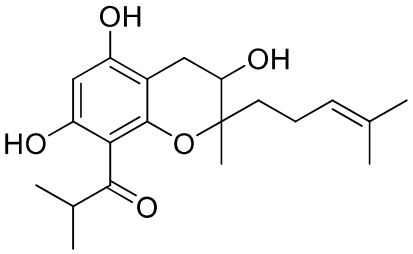
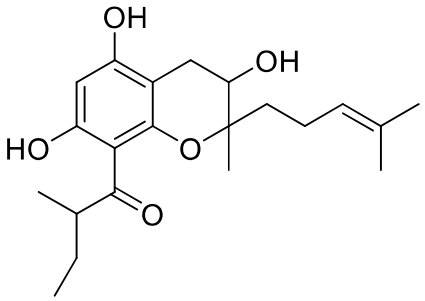
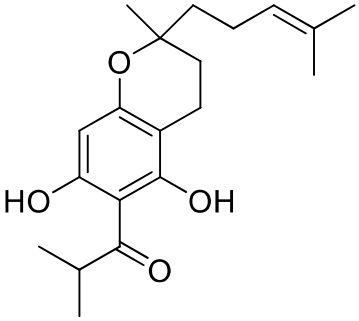
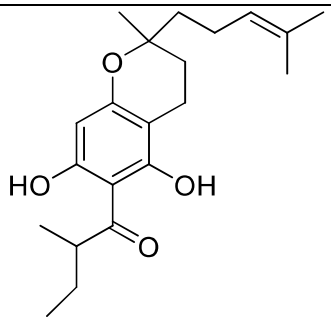
Phloroglucinols isolated from *Hypericum* spp., section Coridium		
***Hypericum* spp.**	**Compounds**	**References**
H. ambloycalyx Coust. & Gand.	hypercalyxones A/B, 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one, 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-butan-1-one	Winkelmann et al., 2003
	4-geranyl-2-(2'-methylbutyryl)-phloroglucinol, 4-geranyl-4-(2'-isobutyryl)-phloroglucinol, 1-[(2R*, 3S*)-3,5,7-trihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-8-yl]-2-methylbutan-1-one, 1-[(2R*, 3S*)-3,5,7-trihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-8-yl]-2-methyl-propan-1-on	Mathioudaki et al., 2013
H. empetrifolium Willd.	empetrifelixins A–D, empetrikajaforin {(1-O-(bornan-200-yl)-4-geranyl-2-(20-methylbutyryl)-phloroglucinol}, 1-(2-methylbutanone)-4-O-prenylphloroglucinol, 3-geranyl-1-(2'-methylpropanoyl)phloroglucinol, 3-geranyl-1-(2'-methylbutanoyl)phloroglucinol, empetrikathiforin {(3-(2-hydroxy-7-methyl-3-methyleneoct-6-enyl)-1-(20-methylbutyryl)-phloroglucinol}	Schmidt et al., 2012a
	empetrikarinen, A/B, empetrikarinols A/B, 1-[5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-6-yl]-2-methylpropan-1-one, 1-[5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-6-yl]-2-methylbutan-1-one, 1-[5,7-dihydroxy-2-methyl-3-(3-methylbut-2-enyl)-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one	Schmidt et al., 2012b

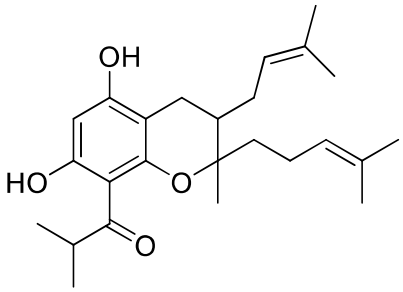
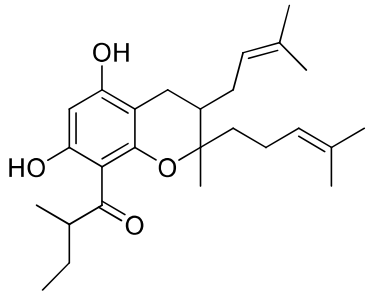
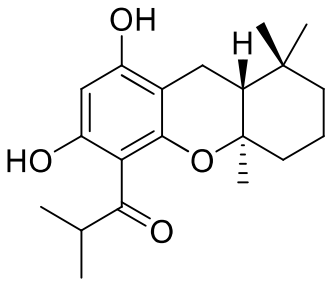
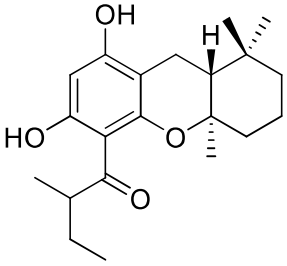
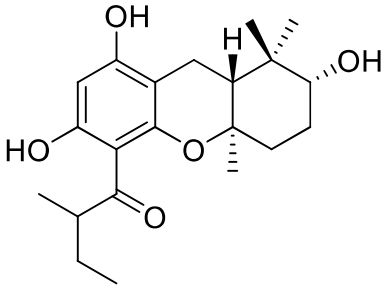
	1-[5,7-dihydroxy-2-methyl-2-(4-methylpent-3-enyl)-chroman-8-yl]-2-methylpropan-1-one, empetriferdinans A/B, empetriferdinol, empetrifranzinans A/B/C	
	3-geranyl-1-(2′-methylpropanoyl)phloroglucinol, 3-geranyl-1-(2′-methylbutanoyl)phloroglucinol	Crokett et al., 2008
H. jovis Greuter	hyperjovinols A/B, 3-geranyl-1-(3-methylbutanoyl) phloroglucinol, 1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one, 1-[5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-6-yl]-2-methylpropan-1-one, 4-geranyloxy-1-(2-methylpropanoyl)-phloroglucinol	Athanasas et al., 2004

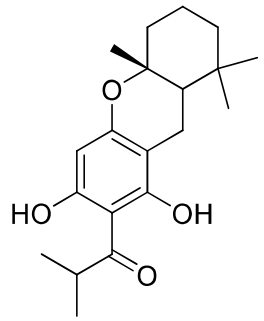
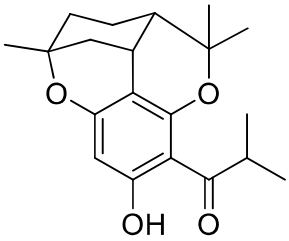
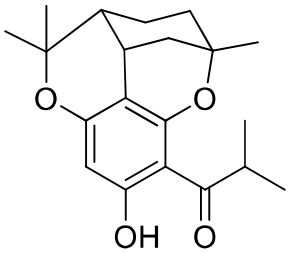
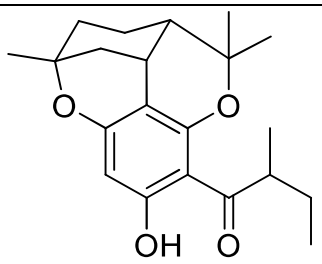
1-(2-methylbutanone)-4-O-prenylphloroglucinol	
Empetrikathiforin {(3-(2-hydroxy-7-methyl-3-methyleneoct-6-enyl)-1-(2′-methylbutyryl)-phloroglucinol}	
3-geranyl-1-(2′-methylpropanoyl)phloroglucinol	
3-geranyl-1-(2′-methylbutanoyl)phloroglucinol	

hyperjovinol A	
4-geranyloxy-1-(2-methylpropanoyl)-phloroglucinol	
empetrifelixin A (4-geranyl- 1-O-(p-menthen-8’’-yl)-2-(20-methylpropionyl)- phloroglucinol)	
empetrifelixin B	
empetrifelixin C	

empetrifelixin D	
empetrikajaforin {(1-O-(bornan-2’’-yl)-4-geranyl-2-(2’-methylbutyryl)-phloroglucinol}	
(1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-propan-1-one	
1-[5,7-dihydroxy-2-methyl-2-(4-methyl-pent-3-enyl)-chroman-8-yl]-2-methyl-butan-1-one	
empetrikarinen A	

empetrikarinen B	
empetrikarinol A	
empetrikarinol B	
1-[5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-6-yl]-2-methylpropan-1-one	
1-[5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-6-yl]-2-methylbutan-1-one	

hypercalyxone A	
hypercalyxone B	
empetriferdinan A	
empetriferdinan B	
empetriferdinol	

hyperjovinol B	
empetrifranzinan A	
empetrifranzinan B	
empetrifranzinan C	

7.4. Summary of the Essential Oils isolated from the genus *Hypericum*

***Hypericum* spp.**	**Constituents from the EO**	**References**
H. acmosepalum N. Robson	α-pinene, undecane, β-pinene, myrcene, limonene, p-cymene, terpinolene, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid), α-copaene, linalool, β-caryophyllene, (Z)-β-farnesene, γ-muurolene, β-selinene, δ-cadinene, γ-cadinene, ar-curcumene, calamenene, p-cymen-8-ol, α-calacorene, caryophyllene oxide, ar-turmerol, cadalene, selina-11-en-4-α-ol, δ-cuparenol	Demirci et al., 2005
H. adenotrichum Spach	α-Pinene, Hexanal, Undecane, β-Pinene, Myrcene, Limonene, (Z)-3-Hexenal, Amylfuran (2-Pentylfuran), Tridecane, Nonanal, Bicycloelemene, α-Copaene, β-Bourbonene, β-Cubebene, β-Funebrene, β-Ylangene, β-Caryophyllene, Alloaromadendrene, (Z)-β-Farnesene, α-Humulene, γ -Muurolene, α-Terpineol, Germacrene D, α-Muurolene, Bicyclogermacrene, (E,E)-α-Farnesene, δ-Cadinene, γ -Cadinene, Cadina-1,4-diene (=Cubenene), Myrtenol, 3,7-Guaiadiene, (E,E)-2,4-Decadienal, Nonadecane, Dodecanol, Globulol, Heneicosane, Hexahydrofarnesyl acetone, Spathulenol, (Z)-3-Hexen-1-yl benzoate, T-Cadinol, T-Muurolol, δ-Cadinol, Carvacrol, α-Cadinol, Dodecanoic acid	Erken et al., 2001
H. aegypticum subsp. *aegypticum* (SPACH) N. ROBSON	α-pinene, undecane, β-pinene, limonene, β-phellandrene, p-cymene, α-copaene, isocaryophyllene, β-caryophyllene, guaia-6,9-diene, ishwarane, eudesma-4,11-diene, δ-selinene, valencene, selina-4(15),7(11)-diene, γ-cadinene, selina-3,7(11)-diene, guaia-3,7-diene, eudesma-5,7(11)-diene, calamenene (correct isomer not identified), α-calacorene, caryophyllene oxide, methyl eugenol, humulene epoxide-II, cubenol, 1-epi-cubenol, spathulenol, T-cadinol, T-muurolol, α-eudesmol, selina-11-en-4α-ol, eudesm-11-en-4-ol stereoisomer (correct isomer not identified), caryophylladienol-II, eudesm-11-en-4-ol stereoisomer (correct isomer not identified), caryophyllenol-I, caryophyllenol-II	Crockett et al., 2007

	α-Pinene, β-Pinene, p-Cymene, Limonene, β-Phellandrene, n-Undecane, α-Copaene, Methyl eugenol, (E)-Caryophyllene, Guaia-6,9-diene, Guaia-3,7-diene, Ishwarane, Valencene, Eudesma-4,11-diene, Eudesma-5,7(11)-diene, δ-Selinene, γ-Cadinene, Calamenene, Selina-4(15),7(11)-diene, Selina-3,7(11)-diene, α-Calacorene, Spathulenol, Caryophyllene oxide, Isocaryophyllene, Humulene epoxide, 1-epi-Cubenol, Eudesm-11-en-4-ol, Caryophylladienol, Caryophyllenol, τ-Muurolol, epi-α-Cadinol (τ-Cadinol), Cubenol, α-Eudesmol, Selina-11-en-4α-ol	Marcetic et al., 2016
H. aegypticum L. ssp. *marrocanum* (Pau) N. Robson	β-caryophyllene, γ-himachalene, β-curcumene, caryophyllene oxide, methyl eugenol, spathulenol, δ-cadinol, caryophylladienol-II, caryophyllenol-I, caryophyllenol-II	Crockett et al., 2007
	Methyl eugenol, (E)-Caryophyllene, γ-Himachalene, β-Curcumene, Spathulenol, Caryophyllene oxide, δ-Cadinol, Caryophylladienol, Caryophyllenol	Marcetic et al., 2016
H. aegypticum subsp. *webbii* (SPACH) N. ROBSON	n-Nonane, α-Thujene, α-Pinene, α-Fenchene, Camphene, 3-Methylnonane, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, β-Phellandrene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, Linalool, n-Undecane, endo-Fenchol, exo-Fenchol, trans-Pinocarveol, trans-Verbenol, Isoborneol, Borneol, α-Terpineol, α-Copaene, (E)-Caryophyllene, β-Gurjunene, (E)-β-Farnesene, γ-Himachalene, δ-Selinene, γ-Cadinene, δ-Cadinene, α-Cadinene, α-Calacorene, Spathulenol, Caryophyllene oxide, epi-α-Cadinol (τ-Cadinol), Selina-3,11-dien-6α-ol, Benzyl benzoate (Porto Vromi, Zakynthos, Greece)	Marcetic et al., 2016
	n-Nonane, α-Thujene, α-Pinene, α-Fenchene, Camphene, 3-Methylnonane, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, γ-Terpinene, Terpinolene, Linalool, n-Undecane, endo-Fenchol, exo-Fenchol, trans-Pinocarveol, Camphene hydrate, Isoborneol, Borneol, α-Terpineol, (E)-Caryophyllene, Valencene, γ-Himachalene, δ-Selinene, γ-Cadinene, δ-Cadinene, α-Cadinene, α-Calacorene, Spathulenol, Caryophyllene oxide, epi-α-Cadinol (τ-Cadinol), Selina-3,11-dien-6α-ol (Porto Vromi distilled after 4 years of storage)	

	α-Pinene, Camphene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Sylvestrene, (Z)-β-Ocimene, γ-Terpinene, Terpinolene, Linalool, endo-Fenchol, Isoborneol, Borneol, Terpinen-4-ol, α-Terpineol, Bicycloelemene, α-Copaene, (E)-Caryophyllene, β-Gurjunene, Aromadendrene, β-Copaene, γ-Himachalene, δ-Selinene, δ-Cadinene, α-Cadinene, Occidentalol, epi-α-Cadinol (τ-Cadinol) (Kefalonia, Greece)	
	n-Nonane, α-Thujene, α-Pinene, α-Fenchene, Camphene, 3-Methylnonane, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, β-Phellandrene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, Linalool, n-Undecane, endo-Fenchol, trans-Pinocarveol, trans-Verbenol, Camphene hydrate, Borneol, α-Terpineol, α-Copaene, (E)-Caryophyllene, β-Gurjunene, (E)-β-Farnesene, Valencene, γ-Himachalene, δ-Selinene, γ-Cadinene, δ-Cadinene, α-Cadinene, α-Calacorene, Spathulenol, Caryophyllene oxide, epi-α-Cadinol (τ-Cadinol), Selina-3,11-dien-6α-ol, Benzyl benzoate (Volimes, Zakynthos, Greece)	
H. alpinum Waldst. & Kit.	Nonane, α-Thujene, (-)-β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, (-)-Limonene, cis-Ocimene, trans-Ocimene, γ-Terpinene, α-Terpinolene, Undecane, n-Nonanal, α-Campholenal, (+)-α-Terpineol, Myrtenol, Decanal, n-Decanol, α-Cubebene, Undecanol, (-) α-Copaene, β-Elemene, Dodecanal, α-Gurjunene, (-)-(E)-Caryophyllene, (Z)-β-Farnesene, α-Humulene, Dodecanol, α-Amorphene, Germacrene D, epi-Bicyclosesquiphellandrene, (E)-β-Ionone, β-Selinene, α-Selinene, α-Muurolene, γ-Cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, (E)-Nerolidol, Globulol, α-Copaen-8-ol, Salvial-4(14)-en-1-one, (-)-Caryophyllene oxide, Torreyol, β-Eudesmol, α-Cadinol	Saroglou et al., 2007
H. androsaemum L.	nonane, α-pinene, β-pinene, β-myrcene, limonene, cineole, linalool, n-undecane, α-copaene, β-caryophyllene, α-humulene, trans-β-farnesene, germacrene D, bicyclogermacrene, δ-cadinene, $C_{15}H_{24}$, caryophyllene oxide, 3-octadecene	Nogueira et al., 1998
	β-Pinene, Limonene, n-Undecane, α–Copaene, α-Santalene, α-Guaiene, cis-Muurola-3,5-diene, β-Selinene, Valencene, γ-Cadinene, γ-Selinene, Italicene ether, Caryophyllene oxide, Humulene epoxide II, epi-α-cadinol, Selin-11-en-4α-ol, 5-	

H. androsaemum L.	Isocedranol, Khusinol, Eudesma-4(15),7-dien-1β-ol, Eudesm-7(11)-en-4-ol, Cedroxyde, 2E,6E-Farnesol, Palustrol, α–Cyperone, 14-Hydroxy-α-muurolene, α-Bisabolol acetate, n-Nonadecane (flowers)	Morteza-Semnani & Saeedi, 2005
	n-Undecane, α–Copaene, β–Bourbonene, β-Caryophyllene, β-Gurjunene, (E)-β-Farnesene, Ishwarane, β-Acoradiene, γ-Muurolene, β-Selinene, cis-β-Guaiene, Valencene, γ-Cadinene, γ-Selinene, Italicene ether, Dendrolasin, Caryophyllene oxide, Humulene epoxide II, Alloaromadendrene epoxide, Himachalol, Khusinol, Cedroxyde, Cedr-8(15)-en-9-α-ol,acetate, 8α-11-Elemodiol, β-Bisabolenol, β-Eudesmol acetate (leaves)	
H. annulatum Moris	3-methyl-2-buten-1-ol (syn. prenol), 3-methyl-2-butenal (syn. prenal), octane, furfural, (Z)-3-hexenol, (Z)-2-hexenol, hexanol, nonane, (E,E)-2,4-hexadienal (syn. sorbaldehyde), α-thujene, α-pinene, camphene, (E)-2-heptenal, Thuja-2,4(10)-diene, benzaldehyde, sabinene, β-pinene, 6-methyl-5-hepten-2-one, myrcene, 2-pentylfuran, decane, octanal, α-phellandrene, (E,E)-2,4-heptadienal, α-terpinene, p-cymene, limonene, 1,8-cineole, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, acetophenone, p-mentha-3,8-diene, terpinolene, p-cymenene, Undecane, linalool, Nonanal, (E)-6-methyl-3,5-heptadien-2-one, endo-fenchol, dehydrosabinaketone, α-campholenal, trans-pinocarveol, camphene hydrate, (E)-2-nonenal, pinocarvone, terpinen-4-ol, α-terpineol, safranal, decanal, (E,E)-2,4-nonadienal, trans-carveol, β-cyclocitral, pulegone, (E)-2-decenal, 2-undecanone, tridecane, (E,E)-2,4-decadienal, α-cubebene, (E)-2-undecenal, α-copaene, β-bourbonene, β-elemene, dodecanal, α-gurjunene, β-caryophyllene, β-copaene, aromadendrene, (E)-β-farnesene, α-humulene, homofarnesane (correct stereoisomer not determined) (syn. 2,6,8-trimethyltridecane), allo-aromadendrene, 4,5-di-epi-aristolochene, selina-4,11-diene (correct stereoisomer not determined), γ-muurolene, germacrene D, α-amorphene, β-selinene, 2-tridecanone, valencene, α-selinene, bicyclogermacrene, germacrene A, γ-cadinene, methyl dodecanoate, δ-cadinene, (E)-γ-bisabolene, α-cadinene, dodecanoic acid, (Z)-3-hexenyl benzoate, spathulenol, caryophyllene oxide, viridiflorol, cubeban-11-ol, rosifoliol, eudesma-6-en-1a-ol, humulene epoxide II, caryophyll-5-en-12-al, selin-11-en-4-ol, 7-epi-α-eudesmol, tetradecanol, 2-	Radulović et al., 2010b

	pentadecanone, methyl tetradecanoate, tetradecanoic acid, benzyl benzoate, hexahydrofarnesyl acetone, nonadecane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, hexacosanal, nonacosane	
H. apricum Kar. and Kir.	Nonane, α-Pinene, β-Pinene, β-Myrcene, Decane, p-Cymene, Limonene, Pinocarvone, α-Terpineol, Cyclohexasiloxane, α-Cubebene, α-Longipinene, α-Ylangene, α-Copaene, β-Bourbonene, β-Caryophyllene, β-Cubebene, β-Farnesene, α-Humulene, Octadecane, Cycloheptasiloxane, α-Amorphen, Germacrene D, δ-cadinene, Epibicyclosesquiphllendrene, Spathulenol, Caryophyllene oxide, Silane, Benzoic acid, α-Cadinol, Dehydroaromadendrene, Tetradecanoic acid, Benzilbenzoate, 2-Pentadecanol, Cyclotetradecane, Nonadecane, Cyclodecasiloxane, n-Decanoic acid, Heneicosane, Cyclononasiloxane, Tricosane, Nonacosane	Bagci & Yuce, 2011c
H. asperulum Jaub. & Spach	n-Nonane, -Pinene, Camphene, β-Pinene, Myrcene, n-Decane, -Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, n-Undecane, Linalool, -Terpineol, -Cubebene, -Copaene, -Gurjunene, β-Caryophyllene, β-Copaene, -Guaiene, -Humulene, (E)-β-Farnesene, n-Dodecanol, γ-Muurolene, -Amorphene, Germacrene D, β-Selinene, -Selinene, Bicyclogermacrene, -Muurolene, (Z)- -Bisabolene, Germacrene A, γ-Cadinene, -Cadinene, -Cadinene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide, Tetradecanal, β-Eudesmol, -Cadinol, Tetradecanol, n-Heptadecane, n-Octadecane	Azadi, 2013
H. atomarium Boiss	2,4-diisopropenyl-1-methyl-1-vinyl-cyclohexane, dodecanol, isocariophyllene, γ-cadinene, β-selinene, δ-guaiene, δ-cadinene	Gudzic et al., 2004
H. aviculariifolium Jaub. et Spach subsp. *depilatum* var.	Nonane, α-Pinene, Camphene, Verbenene, β-Pinene, β-Myrcene, α-Terpinene, p-Cymene, Limonene, γ-Terpinene, α-Terpinolene, Undecane, Nonanal, Chrysantenone, Borneol, α-Terpineol, Citronellol, Myrtenol, α-Longipinene, β-Bourbonene, β-Elemene, β-Caryophyllene, Trans-β-Farnesene, α-Amorphene, Germacrene D, Bicyclogermacrene, Naphthalene, δ-Cadinene, Cis-Calemenene, Spathulenol, Caryophyllene oxide, α-Cadinol, Caryophyllene-II, Allo	Yuce & Bagci, 2012

depilatum (Freyn et Bornm.) Robson	aromadendrene, Cyercene, Benzilbenzoate, 2-Pentadecanone, 2-Heptadecanone, n-Hexadecanoic acid, Tricosane, Nonacosane	
H. balearicum L.	α-pinene, α-fenchene, camphene, β-pinene, myrcene, limonene, γ-terpinene, p-cymene, terpinolene, 6-methyl-5-hepten-2-one, γ-campholene aldehyde, camphor, linalool, isopinocamphone, pinocarvone, β-gurjunene, myrtenal, acetophenone, trans-verbenol, α-terpineol, borneol, verbenone, α-muurolene, carvone, δ-cadinene, γ-cadinene, myrtenol, trans-carveol, calamenene (correct isomer not identified), p-cymen-8-ol, perillyl alcohol, eremoligenol, α-eudesmol, β-eudesmol	Crockett et al., 2007
H. barbatum Jacq.	Nonane, α-Thujene, (-)-α-Pinene, Camphene, (-)-β-Pinene, Myrcene, Decane, α-Phellandrene, α-Terpinene, p-Cymene, (-)-Limonene, cis-Ocimene, trans-Ocimene, γ-Terpinene, α-Terpinolene, Undecane, (-)α-Copaene, (-)-(E)-Caryophyllene, (Z)-β-Farnesene, α-Amorphene, β-Selinene, α-Selinene, δ-Cadinene, α-Calacorene, Spathulenol, Globulol, (-)-Caryophyllene oxide	Saroglou et al., 2007
H. beanii N.Robson	α-pinene, undecane, β-pinene, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid), cis-linalool oxide (furanoid), α-copaene, β-caryophyllene, allo-aromadendrene, (Z)-β-farnesene, acetophenone, α-humulene, γ-muurolene, germacrene D, β-selinene, (E,E)-α-farnesene, δ-cadinene, γ-cadinene, calamenene, α-calacorene, caryophyllene oxide, humulene epoxide-II, spathulenol, neointermedeol, α-bisabolol, β-eudesmol, selina-11-en-4-α-ol, caryophylladienol-II, caryophyllenol-II	Demirci et al., 2005
H. brasiliense Choisy	cis-β-Ocimene, trans-β-Ocimene, α-Copaene, α-Gurjunene, β-Caryophyllene, Aromadendrene, α-Humulene, γ-Muurolene, Acoradiene, Ledene, α-Selinene, γ-Cadinene, Δ-Cadinene, α-calacorene, Ledol, Spathulenol, Caryophyllene oxide, Humulene epoxide II, Cubenol, epi-α-Muurolol	Abreu et al., 2004

H. bupleuroides Griseb.	α-pinene, undecane, δ-3-carene, limonene, 1,8-cineole, (Z)-3-hexenal, amyl furan (2-pentyl furan), p-cymene, tridecane, 6-methyl-5-hepten-2-one, nonanal, α-copaene, decanal, β-caryophyllene, γ-elemene, (Z)-β-farnesene, α-humulene, γ-muurolene, dodecanal, zingiberene, β-selinene, α-selinene, δ-cadinene, γ-cadinene, β-sesquiphellandrene, selina-3,7(11)-diene, selina-5,7(11)-diene, germacrene-B, caryophyllene oxide, selin-7(11)-en-4α-ol	Demirci & Baser, 2006
H. calycinum L.	α-pinene, camphene, hexanal, undecane, β-pinene, myrcene, limonene, (Z)-3-hexenal, (E)-β-ocimene, terpinolene, α-cubebene, α-ylangene, α-copaene, β-bourbonene, β-ylangene, β-copaene, β-caryophyllene, aromadendrene, allo-aromadendrene, α-humulene, α-terpineol, germacrene D, α-muurolene, δ-cadinene, γ-cadinene, guaia-3,7-diene, calamenene, α-calacorene, caryophyllene oxide, salvial-4(14)-en-1-one, humulene epoxide-II, spathulenol, cadina-4,10(14)-dien-1-α-ol, T-cadinol, valerianol, α-eudesmol, α-cadinol	Demirci et al., 2005
H. calycinum L.	4-Methyl-2-pentanone, α-Pinene, α-Fenchene, Camphene, Hexanal, Undecane, β-Pinene, Limonene, (Z)-3-Hexenal, p-Cymene, Terpinolene, trans-Linalool oxide (Furanoid), α,p-Dimethylstyrene, cis-Linalool oxide (Furanoid), α-Campholene aldehyde, Camphor, Pinocarvone, Fenchyl alcohol, Myrtenal, trans-Pinocarveol, p-Mentha-1,5-dien-8-ol, α-Humulene, α-Terpineol, Borneol, Verbenone, Carvone, Myrtenol, trans-Carveol, p-Cymen-8-ol, o-Cresol, Humulene epoxide-II, Spathulenol	Erken et al., 2001
H. canariense L.	(2E)-hexenal, 2-methyl-octane, n-hexanol, n-nonane, α-thujene, α-pinene, α-fenchene, camphene, β-pinene, myrcene, α-phellandrene, n-decane, dehydroxy-cis-linalool oxide, α-terpinene, p-cymene, limonene, 1,8-cineole, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, terpinolene, n-undecane, n-nonanal, endo-fenchol, α-campholenal, trans-pinocarveol, camphene hydrate, pinocarvone, borneol, cis-pinocamphone, terpinen-4-ol, n-nonanol, α-terpineol, myrtenal, safranal, n-decanal, β-cyclocitral, 2-undecanone, δ-elemene, α-longipinene, α-ylangene, α-copaene, β-bourbonene, β-elemene, (Z)-caryophyllene, β-longipinene, italicene, (E)-caryophyllene, α-trans-bergamotene, β-copaene, γ-elemene, α-humulene, 6,9-guaiadiene, geranyl acetone, β-acoradiene, (E)-β-farnesene, γ-muurolene, γ-himachalene, ar-	Zorzetto et al., 2015

	curcumene, δ-selinene, amorpha-4,7(11)-diene, α-selinene, valencene, γ-curcumene, β-himachalene, α-muurolene, (Z,E)-alpha-farnesene, γ-cadinene, δ-cadinene, δ-amorphene, α-alaskene, β-curcumene, selina-3,7(11)-diene, germacrene B, α-calacorene, caryophyllenyl alcohol, (E)-nerolidol, spathulenol, caryophyllene oxide, salvial-4(14)-en-1-one, β-oplopenone, muurola-4,10(14)-dien-1-β-ol, α-acorenol, caryophylla-4(12),8(13)-dien-5-ol, α-cadinol, eudesm-7(11)-en-4-ol, eudesma-4(15),7-dien-1β-ol, 2-pentadecanone,6,10,14-trimethyl-, hexadecanoic acid, phytol, n-tricosane, n-tetracosane, n-pentacosane, n-heptacosane, n-nonacosane	
H. capitatum Choisy var. *luteum* Robson	α-Pinene, Camphene, β-Pinene, α-Myrcene, Benzene-1-methyl 2, Limonene, cis-Ocimene, 1,3,6-octatriene, γ-Terpinene, α-Terpinolene, Undecane, Neoalloocimene, trans-Pinocarveol, Camphor, Borneol, α-Terpineol, α-Ylangene, α-Copaene, α-Elemene, α-Caryophyllene, α-Cubebene, α-Muurolene, α-Amorphene, α-Farnesene, Naphthalene, Germacrene D, Bicyclogermacrene, Hotrienol, Spathulenol, Caryophyllene oxide, trans-β-Caryophyllene, α-Cadinol, Vulgarol B, 2-Pentadecanone, 1,2-Benzenedicarboxylic acid, Cyclotetradecane, Pentacosane, Cyclodecasiloaxane	Bagci & Yuce, 2011b
H. capitatum Choisy var. *capitatum botn*	Isononane, α-Pinene, Camphene, 3-Methyl nonane, β-Pinene, Cineole, 2-Methyl decane, Linalool, Anisaldehyde, Dihydrocarvyl acetate, α-Terpinyl acetate, α-Copaene, Iso-Longifolene, β-Cubebene, Caryophyllene, β-Gurjunene, τ-Elemene, β-Farnesene, α-Himachalane, α-Humulene, Alloaromadendrene, Guaiene, Valencene, β-Selinene, α-Selinene, β-Himachalene, τ-Cadinene, β-Cadinene, Elemol, Spathulenol, Caryophyllene oxide, Globulol, Longiborneol, α-Cadinol, Bulnesol, Cadalene, 2-Methyl heptadecane, Arachidic acid, Cholic acid	Boga et al., 2016
	Nonane, α-Pinene, β-Pinene, α-Myrcene, Benzene-1-methyl 2, Limonene, 1,8-Cineole, cis-Ocimene, 1,3,6-octatriene, α-Campholene aldehyde, γ-Terpinene, Fencholenic aldehyde, Undecane, trans-Verbenol, Camphor, α-Terpineol, trans-Carveole, Cyclohexasiloxane, Hexadecane, α-Longipinene, α-Copaene, α-Bourbonene, Tridecanal, α-Caryophyllene, Cycloheptasiloxane, α-Cubebene, α-Muurolene, α-Amorphene, α-Humulene, Octadecane, Naphthalene, δ-Cadinene, Dodecanoic acid, cis-3-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, Cyclododecane, Benzoic acid, α-Cadinol,	Bagci & Yuce, 2011b

	Caryophyllenol II, 12-Norcyercene-B, Cyclononasiloxane, 2-Pentadecanone, 1,2-Benzenedicarboxylic acid, Cyclohexadecane, Pentacosane, Hexadecanoic acid, Tricosane	
H. caprifoliatum Cham. & Schlecht. (flowering)	trans-2-Hexanal, 2-Methyloctane, Nonane, α–Pinene, 3-Methylnonane, β–Pinene, β–Myrcene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, Nonanal, Undecane, Decanal, Decanol, β–Elemene, β–Caryophyllene, Aromadendrene, α–Humulene, allo-Aromadendrene, γ–Muurolene, Germacrene D, Bicyclogermacrene, Germacrene A, γ–Cadinene, β–Cadinene, Caryophyllene oxide, Globulol, α –Cadinol, Heneicosane, Nonadecanal	Ferraz et al., 2005
H. caprifoliatum Cham. & Schlecht. (vegetative)	trans-2-Hexanal, 2-Methyloctane, Nonane, α-Pinene, 3-Methylnonane, β–Pinene, β–Myrcene, (Z)-β-Ocimene, (E)-β-Ocimene, Nonanal, Undecane, Decanal, β–Elemene, β-Caryophyllene, Aromadendrene, α-Humulene, allo-Aromadendrene, γ-Muurolene, Germacrene D, Bicyclogermacrene, Germacrene A, γ–Cadinene, β–Cadinene, Spathulenol, Globulol, α-Cadinol, Octanodecanol, Nonadecanal	Ferraz et al., 2005
H. carinatum Griseb. (flowering)	2-Methyloctane, Nonane, β-Pinene, Limonene, Undecane, α-Copaene, β-Elemene, Isocaryophyllene, β-Caryophyllene, γ-Elemene, α-trans-Bergamoptene, α-Humulene, (E)-β-Farnesene, allo-Aromadendrene, Germacrene D, Curcumene, Viridiflorene, Bicyclogermacrene, α-Muurolene, Germacrene A, β-Bisabolene, γ-Cadinene, cis-Calamenene, β-Cadinene, α-Calacorene, Nerolidol, Spathulenol, Caryophyllene oxide, Globulol, 5-epi-7-epi-α-Eudesmol, Humulene oxide II, γ-Eudesmol, τ-Cadinol, τ-Muurolol, α-Muurolol, α-Cadinol, Nonadecane, Heneicosane	Ferraz et al., 2005
H. cerastoides (Spach) Robson	α-Pinene, Hexanal, Undecane, β-Pinene, Myrcene, Limonene, (Z)-3-Hexenal, Amylfuran (2-Pentylfuran), (Z)-β-Ocimene, γ -Terpinene, (E)-β-Ocimene, p-Cymene, Terpinolene, Nonanal, 1-Octen-3-ol, α-Cubebene, α-Copaene, β-Bourbonene, α-Gurjunene, β-Cubebene, β-Ylangene, β-Elemene, β-Caryophyllene, Aromadendrene, Myrtenal, Alloaromadendrene, trans-Pinocarveol, (Z)-β-Farnesene, α-Humulene, γ-Muurolene, α-Terpineol, Dodecanal, Germacrene D, Valencene, α-Selinene, α-Muurolene, Bicyclogermacrene, δ-Cadinene, γ-Cadinene, Cadina-1,4-diene(=Cubenene), 3,7-Guaiadiene,	Erken et al., 2001

	trans-Carveol, cis-Calamenene, (E)-Geranyl acetone, Nonadecane, α-Calacorene, 1,5-Epoxy-salvial(4)14-ene, Caryophyllene oxide, Salvial-4(14)-en-1-one, Ledol, Humulene epoxide-II, Heneicosane, Hexahydrofarnesyl acetone, Spathulenol, (Z)-3-Hexen-1-yl benzoate, 3,4-Dimethyl-5-pentylidene-2(5H)-furanone, T-Cadinol, T-Muurolol, α-Cadinol, Decanoic acid, Tricosane	
H. choisyanum Wall. ex N.Robson	α-ylangene, α-copaene, β-bourbonene, cis-eudesma-6,11-diene, β-ylangene, β-copaene, allo-aromadendrene, selina-4,11-diene, γ-muurolene, germacrene D, valencene, δ-cadinene, γ-cadinene, selina-3,7(11)-diene, guaia-3,7-diene, cuparene, calamenene, α-calacorene, (E)-β-ionone, γ-calacorene, caryophyllene oxide, ledol, humulene epoxide-II, spathulenol, T-cadinol, copaborneol, isospathulenol, eudesma-4(15),7-dien-1-β-ol, diphenylamine	Demirci et al., 2005
H. confertum Choisy	Decane, α-Pinene, Camphene, Undecane, β-Pinene, Myrcene, α-Phellandrene, Limonene, 2-Pentyl furane, (E)-β-Ocimene, Terpinolene, Nonanal, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, β-Ylangene, β-Copaene, β-Elemene, β-Caryophyllene, Cadina-3,5-diene, Alloaromadendrene, epi-Zonarene, α-Humulene, γ-Muurolene, Germacrene D, α-Muurolene, Bicyclogermacrene, (E,E)- α-Farnesene, δ-Cadinene, γ-Cadinene, Cadina-1,4-diene, α-Cadinene, 1,5-Epoxy-salvial-4(14)-ene, Caryophyllene oxide, Ledol, Humulene epoxide-II, Cubenol, Octanoic acid, 1-epi-Cubenol, Globulol, Viridifurolol, Hexahydrofarnesyl acetone, 6-epi-Cubenol, t-Cadinol, t-Muurolol, α-Muurolol, α-Cadinol, Tricosane, Hexadecanol, Pentacosane, Dodecanoic acid, Phytol, Benzyl benzoate, Tetradecanoic acid, Nonacosane, Hexadecanoic acid	Kiyan et al., 2014
	2-E-Hexenal, α-Pinene, Sabinene, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, 2-Penthylfuran, p-Cimene, Benzene acetaldeide, Acetophenone, cis-Linalool oxide, Undecane, Linalool, Nonanal, 4-Terpineol, α-Terpineol, p-Cymen-8-ol, α-Terpineol, Safranal, Verbenone, Carvacrol, α-Cubebene, Cyclosativene, α-Copaene, β-Bourbunene, β-Elemene, α-Gurjunene, β-Caryophyllene, B-Gurjunene, Aromadendrene, E-β-Farnesene, α-Humulene, Alloaromadendrene, Drima-7,9(11)-diene, cis-Muurola-4-(14),5-diene, γ-Muurolene, Germacrene D, β-Selinene, trans-Muurola-4-(14),5-diene, α-	Bertoli et al., 2018

	Selinene, α-Muurolene, cis-γ-Cadinene, δ-Cadinene, trans-Calamenene, trans-Cadina-1(2), 4-diene, α-Cadinene, α-Calacorene, trans-Nerolidol, Spathulenol, Caryophyllene oxide, Globulol, Humulene epoxide II, 1,10-Diepi-cubenol, 1-Epicubenol, cis-cadin-4-en-7-ol, τ-Cadinol (epi-a), τ-muurolol (epi-a), α-Muurolol, α-Cadinol, n-Heptadecane, 6,10,14-Trimethylpentadecanone, n-Nonadecane, n-Eicosane, n-Heneicosane, n-Tricosane, n-Pentacosane	
H. connatum Lam. (flowering)	Nonane, α-Pinene, β-Pinene, Limonene, Undecane, α-Terpineol, β-Elemene, Isocaryophyllene, β-Caryophyllene, Aromadendrene, α-Humulene, allo-Aromadendrene, Germacrene D, β-Selinene, δ-Selinene, Valencene, α-Selinene, Bicyclogermacrene, α-Muurolene, Germacrene A, β-Bisabolene, γ-Cadinene, 7-epi-α-Selinene, cis-Calamenene, Caryophyllene oxide, Humulene oxide I, Humulene oxide II, 1-epi-Cubenol, Caryophylladienol II, τ-Cadinol, τ-Muurolol, α-Muurolol, α-Cadinol, Heneicosane	Ferraz et al., 2005
H. cordatum (Vell.) N.Robson (leaf oil)	α-pinene, camphene, β-pinene, myrcene, decane, α-terpinene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, terpinolene, undecane, allo-ocimene (correct isomer not identified) , borneol, terpinen-4-ol, α-terpineol, bornyl acetate, β-elemene, β-caryophyllene, α-humulene, β-selinene, trans-β-guaiene, δ-cadinene, globulol, benzyl benzoate	Ladeira et al., 2009
H. coris L.	linalyloxide cis, linalyloxide trans, linalool, geraniol, α-copaene, β-caryophyllene, α-humulene, γ-muurolene, β-himachalene, α-curcumene, β-selinene, α-muurolene, (E,E)-α-farnesene, γ-cadinene, calamenene, δ-cadinene, calacorene, nerolidol, spathulenol, caryophyllene oxide, humulene oxide, T-cadinol, α-cadinol, bisabol, benzyl benzoate	Schwob et al., 2002a
H. davisii Robson	Nonane, α-Pinene, β-Pinene, β-Myrcene, p-Cymene, Limonene, cis-Ocimene, δ-3-Carene, Undecane, trans-Pinocarvone, Camphor, α-Terpineol, Chrysanthenone, α-Copaene, β-Caryophyllene, β-Farnesene, Cycloheptasiloxane, α-Amorphen, Germacrene D, Bicyclogermacrene, Naphtalene, δ-Cadinene, Spathulenol, Caryophyllene oxide, Cyclosativene, Benzoic acid, α-Cadinol, Cyclodecasiloxane, Cyclononasiloxane, Nonacosane	Bagci & Yuce, 2011b

H. delphicum Boiss. & Heldr.	α-pinene, undecane, β-pinene, p-cymene, tridecane, α-ylangene, β-bourbonene, benzaldehyde, β-ylangene, β-copaene, β-caryophyllene, β-cyclocitral, phenylacetaldehyde, γ-muurolene, γ-cadinene, ar-curcumene, caryophyllene oxide, humulene epoxide-II, spathulenol, 3,4-dimethyl-5-pentylidene-2(5H)-furanone, caryophylladienol-I, caryophylladienol-II, caryophyllenol-I, caryophyllenol-II, phytol	Crockett et al., 2007
H. dogonbadanicum Assadi	nonane, tricyclene, α-pinene, camphene, β-pinene, myrcene, α-phellandrene, α-terpinene, p-cymene, limonene, γ-terpinene, linalool oxide, terpinolene, linalool, α-fenchol, α-carnpholenal, trans-pinocarveol, borneol, α-terpineol, myrtenol, β-bisabolenol, cedren-13-01-acetate	Sajjadi et al., 2001
	Hexanal, Nonane, Santolinatriene, Tricyclene, α-Pinene, Camphene, Thuja-2,4(10)-diene-2, Benzaldehyde, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, Dehydroxy-trans-linalool oxide, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, (E)-β-Ocimene, γ-Terpinene, Acetophenone, trans-Linalool oxide (furanoid), Terpinolene, cis-Linalool oxide (furanoid), Undecane, Linalool, Nonanal, β-Fenchol, α-Campholenal, Nopinone, trans-Pinocarveol, Camphor, Camphene hydrate, trans-Pinocamphone, Pinocarvone, Borneol, Terpinen-4-ol, α-Terpineol, Methyl chavicol, Decanal, Verbenone, Neral, Carvone, Geraniol, Geranial, Isobornyl acetate, 2-Undecanone, Tridecane, 2E,4E-Decadienal, α-Terpinyl acetate, α-Ylangene, α-Copaene, Dodecanal, β-Caryophyllene, Aromadendrene, 2-(E)-Dodecenal, Germacrene-D, (E)-β-Ionone, γ-Amorphene, α-Muurolene, γ-Cadinene, δ-Cadinene, α-Cadinene, α-Calacorene, 3-(Z)-Hexenyl benzoate, Dodecanoic acid, Heptadecane, Bicyclovetivenol, Octadecane, 6,10,14-Trimethyl-2-pentadecanone, Nonadecane, Farnesyl acetone, Hexadecanoic acid, Eicosane, Heneicosane, Docosane	Javidnia et al., 2008
H. elegans Stephan ex Willd.	Pentan-1-ol, (Z)-Pent-2-en-1-ol, 3-Methylbut-2-en-1-ol, Pentane-2,4-dione, 3-Methylbut-2-enal, Octane, 2-Methylbutanoic acid, Furfural, (E)-Hex-3-en-1-ol, (E)-Hex-2-enal, (Z)-Hex-3-en-1-ol, 2-Methyloctane, 3-Methyloctane, 2-Butylfuran, Nonane, (2E,4E)-Hexa-2,4-dienal, α-Thujene, α-Pinene, α-Fenchene, Camphene, (E)-Hept-2-enal, 2-Methylnonane, Benzaldehyde, 5-Methylfurfural, 3-Methylnonane, Sabinene, β-Pinene, 6-Methylhept-5-en-2-one,	Radulović et al., 2010a

	Myrcene, 2-Pentylfuran, trans-Dehydroxylinalool oxide, Decane, (E)-Hex-3-en-1-yl acetate, α-Phellandrene, cis-Dehydroxylinalool oxide, (2E,4E)-Hepta-2,4-dienal, α-Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, (E)-Oct-2-enal, γ-Terpinene, 2-Methyldecane, 3-Methyldecane, Acetophenone, cis-Linalool oxide (furanoid), Terpinolene, Undecane, Linalool, Nonanal, (E)-6-Methylhepta-3,5-dien-2-one, endo-Fenchol, α-Campholenal, (Z)-Myroxide, (E)-Non-2-enal, Octanoic acid, 3-Methylundecane, Terpinen-4-ol, α-Terpineol, Dodecane, Safranal, Decanal, (2E,4E)-Nona-2,4-dienal, β-Cyclocitral, (Z)-Hex-3-en-1-yl 2-methylbutanoate, (Z)-Hex-3-en-1-yl 3-methylbutanoate, Neral, Geraniol, Hexylbenzene, 2-Methyldodecane, Nonanoic acid, Geranial, (E)-Dec-2-en-1-ol, Undecan-2-one, Tridecane, Undecanal, Hex-3-en-1-yl 2-methylbut-2-enoate, p-Vinylguaiacol, (2E,4E)-Deca-2,4-dienal, Isobutyl benzoate, Bicycloelemene, α-Cubebene, 1,2-Dihydro-1,1,6-trimethylnaphthalene (dehydro-ar-ionene), (E)-Undec-2-enal, Decanoic acid, α-Ylangene, α-Copaene, (Z)-Hex-3-en-1-yl hexanoate, β-Bourbonene, 1,5-Di-epi-α-Bourbonene, β-Elemene, Tetradecane, 1,5-Di-epi-β-bourbonene, Dodecanal, α-Gurjunene, β-Ylangene, β-Caryophyllene, β-Copaene, Isoamyl benzoate, Aromadendrene, cis-Muurola-3,5-diene, Geranyl acetone, trans-Muurola-3,5-diene, Humulene, Homofarnesane (2,6,8-trimethyltridecane), Octylbenzene, allo-Aromadendrene, cis-Cadina-1(6),4-diene, cis-Muurola-4(14),5-diene, γ-Muurolene, Germacrene D, (E)-β-Ionone, γ-Himachalene, γ-Amorphene, Pentadecane, Bicyclogermacrene, (E,E)-α-Farnesene, δ-Amorphene, γ-Cadinene, Methyl dodecanoate, δ-Cadinene, Zonarene, trans-Cadina-1,4-diene, cis-Calamenene, α-Cadinene, α-Calacorene, exo-1,5-Epoxysalvial-4(14)-ene, Dodecanoic acid, (Z)-Hex-3-en-1-yl benzoate, Maaliol, Spathulenol, Caryophyllene oxide, Viridiflorol, Cubeban-11-ol, Muurola-4,10(14)-dien-1-ol, Rosifoliol, Ledol, β-Oplopenone, 1,10-Di-epi-cubenol, Junenol, 5-Guaiene-11-ol, 1-epi-Cubenol, epi-α-Cadinol (syn. τ-cadinol), epi-α-Murrolol (syn. τ-muurolol), α-Muurolol (syn. torreyol), cis-Guaia-3,9-dien-11-ol, α-Cadinol, Heptadecane, Tetradecanoic acid, Benzyl benzoate, Octadecane, Hexahydrofarnesyl acetone, Nonadecane, Hexadecanoic acid, Eicosane, Heneicosane, Docosane, Tricosane, Tetracosane, Pentacosane, 3-Methylhexacosane	

H. elongatum C.A.Mey.	Heptane, Nonane, α-Pinene, Camphene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, o-Cymene, 3-Δ-Carene, β-Z-Ocimene, β-E-Ocimene, γ-Terpinene, Terpinolene, Undecane, Nonanal, α-Campholenal, Terpinen-4-Ol, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, α-Gurjunene, E-Caryophyllene, β-Copaene, Aromadendrene, α-Humulene, Allo-aromadendrene, γ-Muurolene, Germacrene D, γ-Cadinen, Δ-Cadinene, α-Cadinene, β-Eudesmol	Ghasemi et al., 2007
H. empetrifolium Willd.	α-Pinene, β-Pinene, Myrcene, Limonene, γ-Terpinene, 2-Methyldecane, Terpinolene, Undecane, 3-Methylundecane, α-Terpineol, β-Caryophyllene, (E)-β-Farnesene, α-Himachalene, Iswarane, γ-Muurolene, γ-Himachalene, β-Selinene, α-Selinene, β-Himachalene, Trans-calamenene	Fanouriou et al., 2018
	n-Nonane, α-Pinene, Camphene, Verbenene, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, n-Decane, α-Phellandrene, α-Terpinene, o-Cymene, p-Cymene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, 2-Methyl-decane, Terpinolene, Linalool, n-Undecane, n-Nonanal, endo-Fenchol, exo-Fenchol, α-Campholenal, trans-Pinocarveol, Camphor, Camphene hydrate, Isoborneol, Borneol, Terpinen-4-ol, Naphtalene, α-Terpineol, Myrtenol, trans-Carveol, Citronellol, Geraniol, α-Terpinen-7-al, 2-Undecanone, α-Cubebene, α-Longipinene, Cyclosativene, α-Ylangene, α-Copaene, β-Patchulene, β-Elemene, α-Cedrene, β-Cedrene, (E)-Caryophyllene, Aromadendrene, α-Humulene, (E)-β-Farnesene, allo-Aromadendrene, γ-Gurjunene, γ-Muurolene, γ-Curcumene, Germacrene-D, (E)-β-Ionone, β-Selinene, Valencene, Viridiflorene, α-Selinene, Bicyclogermacrene, α-Muurolene, α-Chamigrene, trans-β-Guaiene, α-Bulnesene, (E,E)-α-Farnesene, γ-cadinene, 7-epi-α-Selinene, cis-Calamene, δ-Cadinene, trans-Calamene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, Caryophyllene alcohol, (Z)-3-Hexenyl-benzoate, Spathulenol, Caryophyllene oxide, 1-epi-Cubenol, Cubenol, α-Muurolol, α-Cadinol, Valerianol, Bulnesol, β-Bisabolol, Cadalene, Khusinol, Acorenone, Benzyl benzoate	Petrakis et al., 2005
H. ericoides L.	n-hexanal, (E)-2-hexanal, 2-heptanone, n-nonane, 2-methyl-4-heptanone, α-pinene, α-fenchene, (Z)-Hept-4-en-1-ol, β-pinene, 6-methyl-5-hepten-2-one, myrcene, n-decane, n-octanal, α-phellandrene, α-terpinene, -cymene, limonene,	Rouis et al, 2011

	benzyl alcohol, lavender lactone, (E)β-ocimene, bergamal, -terpinene, cis-linalool oxide, cis-sabinene hydrate, trans-linalool oxide, 2-nonanone, n-undecane, cis-thujone, exo-fenchol, cis- -menth-2-en-1-ol, methyl octanoate, α-campholenal, octyl formate, trans-pinocarveol, camphor, isopolegol<neo>, (E,Z)-2,6-nonadienal, iso-borneol, trans-pinocamphone, pinocarvone, -cymen-8-ol, α-terpineol, methyl salicylate, myrtenol, n-dodecane, safranal, n-decanal, verbenone, trans-carveol, carvone, butyrophenone, geranial, nonanoic acid, n-undecanone, n-tridecane, α-cubebene, n-undecanol, cyclosativene, α-ylangene, α-copaene, β-patchoulene, β-maaliene, α-duprezianane, β-bourbonene, β-cubebene, β-elemene, β-longipinene, longifolene, iso-caryophyllene, 1,7 di-epi-β-cedrene, α-cedrene, β-caryophyllene, β-copaene, β-gurjunene, cis-thujopsene, -elemene, α-guaiene, cis-muurola-3,5-diene, α-himachalene, khusimene, geranyl acetone, α-humulene, β-farnesene, allo-aromadendrene, cis-muurola-4(14),5-diene, dehydro-aromadendrene, β-acoradiene, -muurolene, germacrene-D, AR-curcumene, β-selinene, (E)-β-ionone, cis-β-gaiene, valencene, α-muurolene, n-pentadecane, trans- -cadinene, δ-cadinene, zonarene, cis-calamenene, α-cadinene, α-calacorene, β-calacorene, spathulenol, caryophellen oxide, 1-hexadecene, n-hexadecane, tetradecanal, 1-epi-cubenol, hinesol, epi-α-cadinol, α-cadinol, cadalene, khusinol, apiol, n-heptadecane, n-octadecane, musk xylol, n-nonadecane, cembrene, beyerene, phytol, heneicosane, n-decosane, n-tricosane, n-tetracosane	
H. ericoides ssp. *roberti* (Coss.) Maire & Wilczek	n-Octane, 2-Methyloctane, n-Nonane, α-Pinene, β-Pinene, 3-Methylnonane, Limonene, Acetophenone, 2-Methyldecane, Undecane, α-Terpineol, Pulegone, α-Gurjunene, Tetradecane, Methyl eugenol, 1-Dodecanol, Germacrene-D, δ-Cadinene, Lauric acide, Caryophyllene oxide, Hexadecane, T-Muurolol, Phytol, 1,3-Dimethyl-2(2,4,6-trimethyl-phenyl)-1,2-benzene-dicarboxylic acide, Cyclododecane, 3,5-Heptanedione	Hosni et al., 2011b
H. forrestii (Chitt.) N.Robson	3-methylnonane, α-pinene, hexanal, undecane, β-pinene, myrcene, limonene, (Z)-3-hexenal, 2-pentylfuran, p-cymene, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid),cis-linalool oxide (furanoid), α-copaene, β-bourbonene, linalool, cis-eudesma-6,11-diene, β-copaene, β-caryophyllene, allo-aromadendrene, (Z)-β-farnesene, acetophenone, α-humulene, γ-muurolene, germacrene D, α-muurolene, (E,E)-α-farnesene, δ-cadinene, γ-cadinene, calamenene, α-	Demirci et al., 2005

	calacorene, caryophyllene oxide, salvial-4(14)-en-1-one, humulene epoxide-II, spathulenol, 3,4-dimethyl-5-pentylidene-2(5H)-furanone, T-cadinol, α-eudesmol, caryophyllenol-II	
H. grandifolium Choisy	2-methyl-octane, n-nonane, α-pinene, camphene, β-pinene, myrcene, 2-pentyl furan, n-decane, p-cymene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, terpinolene, 3-nonanone, n-undecane, 2-nonanol, n-nonanal, trans-pinocamphone, pinocarvone, cis-pinocamphone, terpinen-4-ol, α-terpineol, safranal, n-dodecane, n-decanal, β-cyclocitral, n-tridecane, δ-elemene, α-cubebene, α-ylangene, α-copaene, β-bourbonene, β-elemene, (Z)-caryophyllene, (E)-caryophyllene, β-ylangene, β-copaene, γ-elemene, α-humulene, 6,9-guaiadiene, geranyl acetone, (E)-β-farnesene, γ-muurolene, α-amorphene, β-selinene, 4,5-di-epi-aristolochene, eremophilene, (E)-β-ionone, δ-selinene, amorpha-4,7(11)-diene, α-selinene, epizonarene, α-muurolene, γ-cadinene, trans-calamenene, δ-cadinene, δ-amorphene, selina-3,7(11)-diene, selina-4(15),7(11)-diene, germacrene B, α-calacorene, caryophyllenyl alcohol, (E)-nerolidol, caryophyllene oxide, gleenol, muurola-4,10(14)-dien-1-β-ol, caryophylla-4(12),8(13)-dien-5-ol, α-cadinol, eudesm-7(11)-en-4-ol, 2-pentadecanone,6,10,14-trimethyl-, 2-pentadecanone,6,10,14-trimethyl-, methyl hexadecanoate, hexadecanoic acid, ethyl hexadecanoate, phyllocladene, phytol, n-heneicosane, n-tricosane, n-tetracosane, n-pentacosane, n-heptacosane, n-nonacosane	Zorzetto et al., 2015
H. helianthemoides (Spach) Boiss.	Hexanal, 2-(E)-Hexenal, α-Pinene, Camphene, Thuja-2,4(10)-diene-2, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, 3-(Z)-Hexenyl acetate, α-Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Acetophenone, Terpinolene, p-Cymenene, Undecane, Linalool, Nonanal, α-Fenchol, α-Campholenal, trans-Pinocarveol, Camphor, trans-Chrysanthenol, Pinocarvone, Borneol, p-Cymen-8-ol, α-Terpineol, Myrtenol, Verbenone, Carvone, α-Longipinene, α-Ylangene, α-Copaene, β-Bourbonene, Isocaryophyllene, β-Caryophyllene, 6-epi-α-Cubebene, α-Guaiene, α-Humulene, E-(β)-Farnesene, trans-Cadina-1(6)-4-diene, γ-Muurolene, β-Selinene, Bicyclogermacrene, α-Muurolene, γ-Cadinene, δ-Cadinene, α-Cadinene, α-Calacorene, Caryophyllenyl alcohol, Spathulenol, Caryophyllene oxide, Salvia-4(14)-en-1-one, Humulene epoxide II, Caryophylla-4(14),8(15)-dien-5-β-ol, β-Eudesmol, 14-Hydroxy-9-epi-(E)-	Javidnia et al., 2008

	caryophyllene, Eudesma-4(15),7-dien-1-β-ol, Benzyl benzoate, Tetradecanoic acid, 6,10,14-Trimethyl-2-pentadecanone, Farnesyl acetone, Hexadecanoic acid, Phytol, Docosane, Tricosane, Tetracosane, Pentacosane	
	α-Thujan, α-Pinene, α-Fenchene, Camphene, β-Pinene, β-Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, β-Phellandrene, (Z) β-Ocimene, (E) β-Ocimene, γ-Terpinene, Terpinolene, Fenchol, endo, Borneol, Terpin 4 ol, Thymol, Carvacrol, α-Cubebene, α-Copaene, β-Bourbonene, β-Elemene, β-Caryophyllene, Aromadendrene, α-Humulene, Germacrene-D, Bicyclogermacrene, γ-Cadinene, Δ-Cadinene, Spathulenol, Hinesol, β-Eudesmol, α-Eudesmol	Pirbalouti et al., 2014
H. hemsleyanum H.Lév. & Vaniot	Phenol,3-methyl, Palmitic acid, Hexadecanoic acid,ethyl ester, 2H-1-benzopyran, 9,12-Octadecadienoic acid (Z,Z), Linoleic acid ethyl ester, Osthole, Lomatin acetate, 1,2-dihydrocyclobuta[b]anthracen-1-one	Wagan et al., 2016
	Phenol, 3-methyl, Palmitic acid, Hexadecanoic acid, ethyl ester, 2H-1-benzopyran, 9,12-Octadecadienoic acid (Z,Z), Linoleic acid ethyl ester, Osthole, Lomatin acetate, 1,2-dihydrocyclobuta[b]anthracen-1-one	Wagan et al., 2016
H. heterophyllum Vent.	α-Pinene, β-Pinene, β-Myrcene, n-Decane, α-Phellandrene, p-Cymene, Limonene, n-Undecane, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, Methyl eugenol, Decyl acetate, Isocaryophyllene, α-Gurjunene, β-Caryophyllene, α-Humulene, γ-Muurolene, Germacrene D, β-Selinene, Valencene, Germacrene B, α-Longipinene, γ-Cadinene, δ-Cadinene, γ-Elemene, β-Calacorene, Germacrenol, Spathulenol, β-Caryophyllene oxide, 1-Hexadecene, Farnesol, n-Heptadecane, Hexahydrofarnesyl acetone	Cakir et al., 2004
H. hircinum L.	N-Nonane, α-Pinene, β-Pinene, Limonene, Undecane, α-Copaene, (E)-Caryophyllene, β-Copaene, Aromadendrene, (E)-β-Farnesene, γ-Muurolene, γ-Amorphene, α-Muurolene, γ-Cadinene, Methyl dodecanoate, δ-Cadinene, Caryophyllene oxide	Fanouriou et al., 2018
H. hircinum L.	n-Octane, n-Nonane, α-Pinene, Camphene, 3-Methyl-nonane, β-Pinene, β-Myrcene, p-Cymene, Limonene, (E)-β-Ocimene, Benzeneacetaldehyde, γ-Terpinene, Terpinolene, 2-Nonanone, Linalool, β-Fenchol, n-Undecane, n-Nonanale,	Marandino et al., 2011

	α-Campholene aldehyde, trans-Pinocarveol, trans-Limonene oxide, Camphor, Pinocarvone, Borneol, Terpinen-4-ol, α-Terpineol, Myrtenal, Myrtenol, n-Decanale, trans-Carveol, Verbenone, cis-Carveol, Carvone, Geraniol, α-Copaene, Isoledene, β-Bourbonene, β-Maaliene, α-Gurjunene, β-Caryophyllene, β-Gurjunene, Aromadendrene, (E)-β-Farnesene, α-Humulene, allo-Aromadendrene, trans-Cadina-1(6),4-diene, γ-Gurjunene, β-Selinene, γ-Muurolene, δ-Selinene, cis-β-Guajene, α-Selinene, (E,E)-α-Farnesene, δ-Cadinene, β-Calacorene, Caryophyllene oxide, Viridiflorol	
H. hircinum L.	α-Pinene, Camphene, Undecane, β-Pinene, Myrcene, α-Terpinene, Limonene, (Z)-β-Ocimene, γ-Terpinene, (E)-β-Ocimene, Terpinolene, β-Caryophyllene, (E)-2-Decenal, α-Humulene, α-Terpinol, (E)-2-Undecenal, Caryophyllene oxide, Hexahydrofarnesyl acetone	Kyan et al., 2014
H. hircinum L. (fruits)	Octane, nonane, α-pinene, camphene, β-pinene, myrcene, p-cymene, limonene, terpinolene, 2-nonanone, linalool, undecane, β-fenchol, α-campholene aldehyde, trans-pinocarveol, camphor, pinocarvone, borneol, terpinen-4-ol, α-terpineol, myrtenal, verbenone, trans-carveol, carvone, geraniol, α-gurjunene, β-bourbonene, β-caryophyllene, β-gurjunene, caryophyllene oxide	Bertoli et al., 2000
H. hircinum L. (leaves)	Octane, nonane, α-pinene, camphene, 3-methylnonane, β-pinene, myrcene, p-cymene, limonene, undecane, camphor, terpinen-4-ol, myrtenal, geraniol, α-gurjunene, β- bourbonene, β-caryophyllene, β-gurjunene, allo-aromadendrene, (E,E)-α-farnesene, caryophyllene oxide	Bertoli et al., 2000
H. hircinum L. subsp. *majus* (Aiton) N. Robson (leaves)	Nonane, α-Pinene, 3-Methylnonane, β-Pinene, p-Cymene, Limonene, Benzene acetaldehyde, (E)-β-Ocimene, Undecane, Nonanal, Dodecane, Decanal, α-Copaene, Isoledene, β-Maaliene, α-Gurjunene, (E)-Caryophyllene, α-Humulene, Allo-aromadendrene, (E)-β-Farnesene, Trans-cadina-1(6),4-diene, β-Selinene, γ-Gurjunene, γ-Muurolene, α-Selinene, δ-Selinene, cis-β-guaiene, (E,E)-α-Farnesene, δ-Cadinene, β-Calacorene, Caryophyllene oxide, Isolongifolan-7-α-ol, β-Eudesmol, Benzyl benzoate, Guaiazulene, Hexadecanoic acid, Phytol, Linoleic acid, Docosane, Tricosane, Tetracosane, Pentacosane, Hexacosane, Heptacosane, Nonacosane	Quassinti et al., 2013

H. hirsutum L.	Nonane, Nonane,3-methyl, Decane, (-)-Limonene, cis-Ocimene, trans-Ocimene, Undecane, n-Nonanal, Nonanol, Decanal, 4-Undecanone, Thymyl methyl ether, 3-Undecanone, n-Decanol, 2-Undecanone, α-Cubebene, α-Ylangene, Undecanol, (-)α-Copaene, β-Bourbonene, β-Elemene, Dodecanal, α-Gurjunene, (-)-(E)-Caryophyllene, (+)-Aromadendrene, (Z)-β-Farnesene, α-Humulene, Dodecanol, α-Amorphene, Germacrene D, epi-Bicyclosesquiphellandrene, (E)-β-Ionone, β-Selinene, α-Selinene, Viridiflorene, α-Muurolene, γ-Cadinene, δ-Cadinene, Salvial-4(14)-en-1-one, (-)-Caryophyllene oxide, α-Cadinol	Saroglou et al., 2007
	α-Thujene, n-Undecane, n-Tridecane, α-Longipinene, β-Bourbonene, β-Elemene, Dodecanal, (Z)-α-Bergamotene, Patchoulene, Germacrene D, α-Selinene, Valencene, γ-Cadinene, Spathulenol, Caryophyllene oxide, τ-Cadinol, n-Tetradecanol, n-Heptadecane, 1-Hexadecanol, n-Nonadecane, n-Eicosane, n-Heneicosane, n-Tricosane, n-Tetracosane, n-Pentacosane, n-Heptacosane, n-Nonacosane	Gudzic et al., 2007
H. hirtellum (Spach) Boiss.	Hexanal, 2-(E)-Hexenal, 3-(Z)-Hexenol, Nonane, α-Thujene, α-Pinene, Camphene, Thuja-2,4(10)-diene-2, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, 3-(Z)-Hexenyl acetate, 2E,4E-Heptadienal, α-Terpinene, p-Cymene, Limonene, 1,8-Cineol, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, Undecane, Linalool, cis-p-Menth-2-en-1-ol, α-Campholenal, Camphor, Pinocarvone, p-Mentha-1,5-dien-8-ol, Terpinen-4-ol, α-Terpineol, Isobornyl acetate, Lavandulyl acetate, Bicycloelemene, α-Cubene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, β-Elemene, (Z)-Jasmone, Longifolene, Methyl eugenol, β-Caryophyllene, β-Copaene, α-Guaiene, α-Humulene, E-(β)-Farnesene, Germacrene-D, (E)-β-Ionone, Bicyclogermacrene, α-Muurolene, Eudesma-2,4(15),11-triene, γ-Cadinene, δ-Cadinene, trans-Cadina-1,4-diene, α-Cadinene, α-Calacorene, β-Calacorene, Caryolan-1-ol, Spathulenol, Salvia-4(14)-en-1-one, Humulene epoxide II, Caryophylla-4(14),8(15)-dien-5-β-ol, Eudesma-4(15),7-dien-1-β-ol, Benzyl benzoate, Tetradecanoic acid, 6,10,14-Trimethyl-2-pentadecanone, Farnesyl acetone, Hexadecanoic acid, Hexadecyl acetate, Geranyl linalool, Heneicosane	Javidnia et al., 2008

	n-Octane, trans-2-Hexenal, cis-3-Hexen-1-ol, 2-Methyloctane, n-Nonane, α-Thujene, α-Pinene, Camphene, β-Pinene, 3-Methylnonane, 2-Penthyl furan, β-Myrcene, n-Decane, Benzene acetaldehyde, p-Cymene, 1,8-Cineole, Limonene, Acetophenone, cis-β-Ocymene, trans-β-Ocymene, γ-Terpinene, 2-Methyldecane, α-Terpinolene, Phenyl ethyl alcohol, n-Nonanal, n-Undecane, trans-Pinocarveol, Pinocarvone, Terpinen-4-ol, α-Terpineol, Myrtenol, n-Decanal, Undecanal, γ-Elemene, α-Cubebene, α-Copaene, β-Elemene, Dodecanal, β-Cedrene, β-Funebrene, β-Caryophyllene, β-Copaene, γ-Elemene, α-Himachalene, α-Humulene, Kushimene, trans-β-Farnesene, β-Acoradiene, γ-Muurolene, Dodecanol, Germacrene D, β-Selinene, δ-Selinene, Viridiflorene, α-Muurolene, trans,trans-α-Farnesene, β-Curcumene, γ-Cadinene, δ-Cadinene, α-Cadinene, β-Sesquiphellandrene, Germacrene B, trans-Nerolidol, Dodecanoic acid, β-Caryophyllene oxide, δ-Cadinol	Nogueira et al., 2008
H. humifusum L.	n-hexanal, (E)-2-hexanal, 2-heptanone, n-nonane, 2-methyl-4-heptanone, α-pinene, α-fenchene, (Z)-Hept-4-en-1-ol, β-pinene, 6-methyl-5-hepten-2-one, myrcene, n-decane, n-octanal, α-phellandrene, α-terpinene, -cymene, limonene, benzyl alcohol, lavender lactone, (E)β-ocimene, bergamal, -terpinene, cis-linalool oxide, cis-sabinene hydrate, trans-linalool oxide, 2-nonanone, n-undecane, cis-thujone, exo-fenchol, cis- -menth-2-en-1-ol, methyl octanoate, α-campholenal, octyl formate, trans-pinocarveol, camphor, isopolegol<neo>, (E,Z)-2,6-nonadienal, iso-borneol, trans-pinocamphone, pinocarvone, -cymen-8-ol, α-terpineol, methyl salicylate, myrtenol, n-dodecane, safranal, n-decanal, verbenone, trans-carveol, carvone, butyrophenone, geranial, nonanoic acid, n-undecanone, n-tridecane, α-cubebene, n-undecanol, cyclosativene, α-ylangene, α-copaene, β-patchoulene, β-maaliene, α-duprezianane, β-bourbonene, β-cubebene, β-elemene, β-longipinene, longifolene, iso-caryophyllene, 1,7 di-epi-β-cedrene, α-cedrene, β-caryophyllene, β-copaene, β-gurjunene, cis-thujopsene, -elemene, α-guaiene, cis-muurola-3,5-diene, α-himachalene, khusimene, geranyl acetone, α-humulene, β-farnesene, allo-aromadendrene, cis-muurola-4(14),5-diene, dehydro-aromadendrene, β-acoradiene, -muurolene, germacrene-D, AR-curcumene, β-selinene, (E)-β-ionone, cis-β-gaiene, valencene, α-muurolene, n-pentadecane, trans- -cadinene, δ-cadinene, zonarene, cis-calamenene, α-cadinene, α-calacorene, β-	Rouis et al, 2011

	calacorene, spathulenol, caryophellen oxide, 1-hexadecene, n-hexadecane, tetradecanal, 1-epi-cubenol, hinesol, epi-α-cadinol, α-cadinol, cadalene, khusinol, apiol, n-heptadecane, n-octadecane, musk xylol, n-nonadecane, cembrene, beyerene, phytol, heneicosane, n-decosane, n-tricosane, n-tetracosane	
	α-pinene, β-pinene, β-myrcene, limonene, cineole, n-undecane, dodecanal, β-caryophyllene, trans-β-farnesene, germacrene D, bicyclogermacrene, γ-cadinene, δ-cadinene, $C_{15}H_{24}$, nerolidol	Nogueira et al., 1998
	n-Nonane, α-Pinene, β-Pinene, Myrcene, Limonene, n-Undecane, Carvacrol, α-Cubebene, α-Copaene, iso-Caryophyllene, β-Caryophyllene, γ-Muurolene, Germacrene D, α-Selinene, trans- -Cadinene, δ-Cadinene, Methyl dodecanoate, Spathulenol, Caryophyllene oxide, Humulene epoxide II, epi-10-γ-Eudesmol, 1-epi-Cubenol, Caryophylla-4(14),8(15)-dien-5-ol, epi-α-Cadinol, Cubenol, α-Cadinol, Cadalene, Hexahydrofarnesylacetone, n-Heneicosane, n-Tricosane, n-Pentacosane	Rouis et al., 2013
H. hyssopifolium Chaix subsp. *elongatum* (Ledeb.) Woron var. *microcalycinum.* (Boiss. & Heldr.) Boiss.	3-Methyl nonane, Decane, α-Pinene, α-Thujene, Camphene, Undecane, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, Limonene, β-Phellandrene, (Z)-β-Ocimene, γ-Terpinene, (E)-β-Ocimene, p-Cymene, Terpinolene, Longipinene, α-Copaene, β-Caryophyllene, Aromadendrene, Alloaromadendrene, (Z)-β-Farnesene, γ-Muurolene, Germacrene D, Bicyclogermacrene, (E,E)-α-Farnesene, δ-Cadinene, γ-Cadinene, α-Cadinene, Dodecyl acetate, Dodecanol, Cubenol, Globulol, Heneicosane, Viridifurolol, t-Cadinol, t-Muurolol, α-Cadinol, Tricosane, Pentacosane, Dodecanoic acid, Phytol, Benzyl benzoate, Heptacosane, Nonacosane	Kiyan et al., 2014
H. hyssopifolium subsp.	α-Pinene, β-Pinene, β-Myrcene, n-Decane, α-Phellandrene, p-Cymene, Limonene, (Z)- and (E)-Ocimene, γ-Terpinene, Acetophenone, Terpinolene, p-Cymenene, n-Undecane, α-Thujone, Fenchol, α-Campholenal, neo-allo-ocimene, (E)-Pinocarveol, Camphene hydrate, Camphor, Menthol, Terpinen-4-ol, p-Cymen-8-ol, α-Terpineol, Myrtenol, Verbenone,	Cakir et al., 2004

elongatum var. *elongatum*	(E)-Carveol, Nerol, Myrtenyl acetate, Pulegone, Carvone, Piperitone, Geraniol, (E)-Myrtanol, Geranial, Piperitenone, α-Cubebene, α-Copaene, β-Bourbonene, Methyl eugenol, Isocaryophyllene, β-Caryophyllene, α-Humulene, n-Dodecanol, γ-Muurolene, Germacrene D, α-Longipinene, Butylated hydroxytoluene, γ-Cadinene, δ-Cadinene, γ-Elemene, β-Calacorene, Germacrenol, Spathulenol, β-Caryophyllene oxide, 1-Hexadecene, n-Hexadecane, Farnesol, n-Heptadecane	
H. hyssopifolium Chaix ssp. *hyssopifolium*	nonane, α-pinene, linalool, undecane, terpinen-4-ol, α-terpineol, 2-methyldodecane, tridecane, α-longipinene, α-ylangene, α-copaene, 2-phenylethyl propionate, β-caryophyllene, α-himachalene, (E)-β-farnesene, 2-methyltetradecane, dodecanol, γ-muurolene, γ-himachalene, ar-curcumene, 2-phenylethyl 2-methylbutyrate, 2-phenylethyl 3-isovalerate, β-selinene, γ-amorphene, α-selinene, α-muurolene, α-cuprenene, γ-cadinene, cis-calamenene, δ-cadinene, α-cadinene, α-calacorene, (E)-nerolidol, spathulenol, caryophyllene oxide, epi-α-cadinol, α-cadinol, tetradecanol	Schwob et al., 2006
H. hyssopifolium var. *microcalycinum* (Boiss. & Heldr.) Boiss.	α-Pinene, β-Pinene, Linalol, Terpinen-4-ol, α-Terpineol, α-Ylangene, β-Elemene, α-Humulene, α-Amorphene, Valencene, cis-Calamenene, Spathulenol, Caryophyllene oxide, Caryophyllene alcohol, Dodecanol, 1-Tetradecanolo	Toker et al., 2006
H. japonicum Thunb. ex Murray	(E)-Hex-2-enal, 2-Methyl octane, n-Nonane, α-Pinene, Camphene, 3-Methyl nonane, β-Pinene, Myrcene, Decane, α-Phellandrene, p-Cymene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, 2-Methyl decane, (E)-2-Nonen-1-ol, 2-Nonanone, n-Undecane, Nonanal, (2Z)-Nonenol, n-Nonanol, α-Terpineol, (4Z)-Decenal, n-Decanal, (3Z)-Hexenyl-2-methylbutanoate, (3Z)-Hexenyl-3-methylbutanoate, 3-Undecanone, n-Decanol, 2-Undecanone, n-Tridecane, 1,8-Octanediol, Decanoic acid, (2E)-Undecenol, β-Cubebene, Sibirene, Dodecanal, α-Cedrene, (E)-Caryophyllene, α-Aromadendrene, α-Humulene, (E)-β-Farnesene, allo-Aromadendrene, n-Dodecanol, 10-epi-β-Acoradiene, Germacrene	Verma et al., 2012

	D, (Z)-Cadina-1,4-diene, 10-Undecenol acetate, β-Himachalene, (E,E)-α-Farnesene, δ-Amorphene, γ-Cadinene, δ-Cadinene, α-Calacorene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide, Globulol, Humulene epoxide II, 10-epi-γ-Eudesmol, 1-epi-Cubebol, γ-Eudesmol, allo-Aromadendrene epoxide, β-Eudesmol, α-Cadinol, (Z)-α-Santalol, α-Bisabolol, (2Z,6E)-Farnesol, Octadecane	
H. kouytchense H.Lév.	α-copaene, β-caryophyllene, guaia-6,9-diene, allo-aromadendrene, cis-β-guaiene, γ-muurolene, β-selinene, α-muurolene, eremophillene, δ-cadinene, γ-cadinene, selina-3,7(11)-diene, calamenene, α-calacorene, caryophyllene oxide, humulene epoxide-II, T-cadinol, α-cadinol, porosadienol, tricosane, caryophylladienol-II, pentacosane, heptacosane	Demirci et al., 2005
H. lancasteri N.Robson	hexanal, undecane, longipinene, α-ylangene, α-copaene, β-ylangene, β-copaene, β-caryophyllene, α-himachalene, acetophenone, selina-4,11-diene, γ-muurolene, γ-himachalene, germacrene D, β-selinene, α-muurolene, γ-cadinene, selina-3,7(11)-diene, guaia-3,7-diene, calamenene, β-calacorene, α-calacorene, γ-calacorene, caryophyllene oxide, humulene epoxide-II, cubenol, 1-epi-cubenol, spathulenol, T-muurolol, isospathulenol, α-eudesmol, β-eudesmol, porosadienol, α-cyperone, juniper camphor, eudesmadienone (correct isomer not identified)	Demirci et al., 2005
H. leschenaultia Choisy	undecane, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid), α-cubebene, α-ylangene, β-bourbonene, linalool, β-ylangene, β-copaene, β-caryophyllene, allo-aromadendrene, (Z)-β-farnesene, γ-muurolene, β-selinene, (E,E)-α-farnesene, α-cuprenene, γ-cadinene, ar-curcumene, guaia-3,7-diene, cuparene, α-calacorene, γ-calacorene, caryophyllene oxide, ledol, humulene epoxide-II, cubenol, globulol, spathulenol, T-cadinol, T-muurolol, α-bisabolol, cadalene, heptacosane	Demirci et al., 2005
H. linarifolium Vahl.	n-Octane, trans-2-Hexenal, cis-3-Hexen-1-ol, 2-Methyloctane, n-Nonane, α-Thujene, α-Pinene, Camphene, β-Pinene, 3-Methylnonane, β-Myrcene, Hexanoic acid, n-Decane, Benzene acetaldehyde, α-Terpinene, p-Cymene, 1,8-Cineole, Limonene, Acetophenone, cis-β-Ocymene, trans-β-Ocymene, γ-Terpinene, 2-Methyldecane, α-Terpinolene, Phenyl	Nogueira et al., 2008

	ethyl alcohol, n-Nonanal, Linalol, n-Undecane, trans-Verbenol, Pinocarvone, Terpinen-4-ol, Myrtenal, α-Terpineol, Myrtenol, n-Decanal, Geraniol, γ-Elemene, α-Cubebene, α-Copaene, β-Elemene, Dodecanal, β-Funebrene, β-Caryophyllene, β-Copaene, γ-Elemene, α-Himachalene, α-Humulene, Kushimene, trans-β-Farnesene, β-Acoradiene, γ-Muurolene, Germacrene D, β-Selinene, cis-β-Guaiene, δ-Selinene, α-Selinene, α-Muurolene, trans, trans-α-Farnesene, γ-Cadinene, trans-Calamenene, δ-Cadinene, α-Calacorene, Hexenyl benzoate, Germacrene B, trans-Nerolidol, Dodecanoic acid, β-Caryophyllene oxide, α-Cadinol, Benzyl benzoate, Guaiazulene	
	nonane, α-pinene, β-pinene, β-myrcene, limonene, cineole, 2-methyl-decane, linalool, n-undecane, α-copaene, β-elemene, dodecanal, β-caryophyllene, α-humulene, trans-β-farnesene, germacrene D, γ-cadinene, δ-cadinene, $C_{15}H_{24}$, nerolidol, caryophyllene oxide, 1-hexadecene, 3-octadecene	Nogueira et al., 1998
H. linarioides Bosse	2-Methyl-2-cyclohexenol, n-Undecane, n-Nonanal, Borneol, Terpinen-4-ol, p-Cymen-8-ol, α-Terpineol, (E)-Carveol, Neral, (Z)-Verbenyl acetate, Nonanoic acid, n-Tridecene, Thymol, Carvacrol, α-Cubebene, Piperitenone, α-Ylangene, Decanoic acid, α-Copaene, β-Bourbonene, β-Elemene, Italicene, Methyl eugenol, β-Caryophyllene, Aromadendrene, α-Cadinene, (Z)-β-Farnesene, allo-Aromadendrene, γ-Gurjunene, γ-Muurolene, γ-Curcumene, Germacrene-D, β-Selinene, Viridiflorene, α-Selinene, α-Longipinene, α-Muurolene, α-Farnesene, β-Bisabolene, γ-Cadinene, δ-Cadinene, (1S)-(E)-Calamenene, β-Muurolene, (E)-γ-Bisabolene, α-Calacorene, γ-Elemene, β-Calacorene, Dodecanoic acid, (Z)-3-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, Globulol, Viridiflorol, Carotol, Dodecyl acetate, α-Guaiol, Cedrol, Cubenol, γ-Eudesmol, Gossonorol, T-Cadinol, T-Muurolol, δ-Cadinol, α-Cadinol, (E)-α-Bergamotol, Myristic acid, Benzyl benzoate, (Z,E)-Farnesyl acetate, Hexahydrofarnesyl acetone, Dibutylphthalate, 2-Phenylethylbenzoate, Farnesyl propionate, Hexadecanoic acid, Phytol	Cakir et al., 2005
H. lydium Boiss.	3-Methyl nonane, Decane, α-Pinene, Undecane, β-Pinene, Thuja-2,4(10)-diene, Myrcene, Limonene, p-Cymene, Longipinene, α-Campholene aldehyde, α-Copaene, β-Caryophyllene, Myrtenal, trans-Pinocarveol, (Z)-β-Farnesene,	Serbetci et al., 2012

	trans-Verbenol, γ-Muurolene, 2-Undecanol, Valencene, β-Selinene, δ-Cadinene, γ-Cadinene, 7-epi-α-Selinene, Myrtenol, Caryophyllene oxide, Humulene epoxide-II, Hexahydrofarnesyl acetone, Spathulenol, Copaborneol, Clovenol, Cadalene, Decanoic acid, Tricosane, Caryophylla-2(12),6(13)-dien-5β-ol (=Caryophylladienol I), Caryophylla-2(12),6-dien-5α-ol (=Caryophyllenol I), Caryophylla-2(12),6-dien-5β-ol (=Caryophyllenol II), Pentacosane, Dodecanoic acid, Tetradecanoic acid, Heptacosane, Nonacosane, Hexadecanoic acid	
	Myrcene, p-Cimene, Limonene, Benzene acetaldeide, cis-Linalool oxide, trans-Linalool oxide, Linalool, Nonanal, Dehydrosabinaketone, p-Cymen-8-ol, α-Terpineol, Verbenone, trans-Carveol, β-Bourbunene, B-Gurjunene, Alloaromadendrene, γ-Muurolene, β-Selinene, α-Selinene, cis-γ-Cadinene, δ-Cadinene, α-Calacorene, Spathulenol, Caryophyllene oxide, Humulene epoxide II, γ-Eudesmol, Caryophyla-4(14), 8(15)-dien-5-ol, α-Eudesmol, Santalol-7-a, n-Nonadecane, n-Heneicosane	Bertoli et al., 2018
H. lysimachioides var. *lysimachioides* Boiss. & Noë	α-Pinene, β-Pinene, Limonene, Terpinen-4-ol, α-Terpineol, α-Ylangene, β-Elemene, α-Longifolene, α-Humulene, α-Amorphene, β-Selinene, α-Selinene, cis-Calamenene, Spathulenol, Caryophyllene oxide, Dodecanol	Toker et al., 2006
H. maculatum Crantz	Nonane, (-)-α-Pinene, Camphene, Verbenene, Sabinene, (-)-β-Pinene, α-Terpinene, p-Cymene, (-)-Limonene, cis-Ocimene, trans-Ocimene, γ-Terpinene, cis-Sabinene hydrate, α-Terpinolene, Undecane, n-Nonanal, 1-Terpineol, α-Campholenal, trans-Pinocarveol, trans-Verbenol, Pinocarvone, Nonanol, (+)-Terpinen-4-ol, (+)-α-Terpineol, (-)-Myrtenal, Decanal, Verbenone, Pulegone, Cuminal, Tridecane, α-Cubebene, Cyclosativene, α-Ylangene, (-)α-Copaene, β-Bourbonene, β-Cubebene, α-Gurjunene, α-Cedrene, (+)-β-Funebrene, (-)-(E)-Caryophyllene, (+)-Aromadendrene, (Z)-β-Farnesene, α-Humulene, α-Patchoulene, α-Acoradiene, α-Amorphene, Germacrene D, epi-Bicyclosesquiphellandrene, (E)-β-Ionone, α-Muurolene, γ-Cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-	Saroglou et al., 2007

	Calacorene, (E)-Nerolidol, Spathulenol, Globulol, Salvial-4(14)-en-1-one, T-cadinol, T-muurolol, Torreyol, α-Cadinol, 6,10,14-Trimethyl pentadecan-2-one	
	n-Nonane, Tricyclene, β-Pinene, p-Cymene, Limonene, 1,8-Cineole, γ-Terpinene, Terpinolene, n-Undecane, α-Thujone, trans-Pinocarveol, Pinocarvone, Terpinen-4-ol, α-Terpineol, Myrtenal, Myrtenol, Cuminyl aldehyde, (E)-Anethole, n-Tridecane, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, α-Gurjunene, β-Caryophyllene, β-Gurjunene, Aromadendrene, α-Himachalene, α-Caryophyllene, β-Farnesene, α-Acoradiene, γ-Muurolene, Eremophyllene, α-Muurolene, γ-Cadinene, Calamenene, δ-Cadinene, Cadina-1,4-diene, α-Calacorene, β-Calacorene, Nerolidol, Spathulenol, Caryophyllene oxide, Globulol, Viridiflorol, Humulene epoxide, epi-α-Cadinol, α-Cadinol	Gudzic et al., 2002
	2-Methyloctane, 2-Butylfuran, Nonane, α-Thujene, α-Pinene, Camphene, Thuja-2,4(10)-diene, 3-Methylnonane, Sabinene, β-Pinene, β-Myrcene, Decane, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, β-Phellandrene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, 2-Methyldecane, cis-Sabinene hydrate, Terpinolene, Undecane, trans-Sabinene hydrate, cis-p-Menth-2-en-1-ol, α-Campholenal, trans-Pinocarveol, trans-Verbenol, Sabina ketone, Pinocarvone, Borneol, p-Mentha-1,5-dien-8-ol, Terpinen-4-ol, Myrtenol/Myrtenal, Verbenone, β-Cyclocitral, Tridecane, α-Cubebene, α-Longipinene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, β-Elemene, Sativene, α-Gurjunene, β-Funebrene, β-Ylangene, (E)-Caryophyllene, β-Cedrene, β-Copaene, Aromadendrene, trans-Muurola-3,5-diene, α-Humulene, (E)-β-Farnesene, Alloaromadendrene, β-Acoradiene, cis-Muurola-4(14),5-diene, trans-Cadina-1(6),4-diene, γ-Muurolene, Germacrene D, (Z,E)-α-Farnesene, γ-Amorphene, trans-Muurola-4(14),5-diene, Bicyclogermacrene, α-Muurolene, (E,E)-α-Farnesene, δ-Amorphene, Germacrene A, γ-Cadinene, δ-Cadinene, β-Sesquiphellandrene, trans-Cadina-1,4-diene, α-Cadinene, α-Calacorene, Salviadienol, (E)-Nerolidol, β-Calacorene, Palustrol, Spathulenol, Caryophyllene oxide, Salvial-4(14)-en-1-one, Cubeben-11-ol, Viridiflorol, Ledol, 1,10-Diepicubenol, Humulene epoxide II, Junenol, Epicubenol, Muurola-4,10(14)-dien-1-ol, epi-α-Cadinol (syn. t-Cadinol), epi-α-Muurolol (syn. t-Muurolol), α-Muurolol (syn.	Dordevic et al., 2014

	Torreyol), α-Cadinol, Cadalene, Mustakone, Amorpha-4,9-dien-2-ol, Hexahydrofarnesyl acetone, Hexadecan-1-ol, Nonadecane, Isophytol, Eicosane, Heneicosane, Tricosane, Tetracosane, Pentacosane, Heptacosane, Nonacosane	
H. monogynum L.	α-pinene, β-pinene, myrcene, (Z)-β-ocimene, 6-methyl-5-hepten-2-one, cis-eudesma-6,11-diene, β-caryophyllene, guaia-6,9-diene, γ-elemene, allo-aromadendrene, (Z)-β-farnesene, acetophenone, α-humulene, γ-muurolene, germacrene D, α-muurolene, (E,E)-α-farnesene, δ-cadinene, γ-cadinene, selina-3,7(11)-diene, germacrene B,α-calacorene, γ-calacorene, caryophyllene oxide, salvial-4(14)-en-1-one, ledol, humulene epoxide-II, spathulenol, T-cadinol, α-bisabolol, α-eudesmol, porosadienol, tricosane, tetracosane, pentacosane	Demirci et al., 2005
H. montbretii Spach	α-Pinene, Undecane, β-Pinene, Myrcene, α-Terpinene, Limonene, γ-Terpinene, Terpinolene, α-Copaene, Fenchyl alcohol, β-Caryophyllene, γ-Muurolene, α-Terpineol, Borneol, Germacrene D, Valencene, α-Cadinene, Bicyclogermacrene, (E,E)-α-Farnesene, δ-Cadinene, γ-Cadinene, Cadina-1,4-diene(=Cubenene), 3,7-Guaiadiene, (E)-Nerolidol, 1-epi-Cubenol, Spathulenol, T-Cadinol, T-Muurolol, α-Cadinol, Hexadecanoic acid	Erken et al., 2001
H. myrianthum Cham. & Schlecht. (flowering)	2-Methyloctane, Nonane, α-Pinene, 3-Methylnonane, β-Pinene, β-Myrcene, Decane, Limonene, (E)-β-Ocimene, 2-Methyldecane, Undecane, α-Copaene, β-Caryophyllene, Aromadendrene, α-Himachalene, α-Humulene, Dehydro-aromadendrane, allo-Aromadendrene, γ-Himachalene, γ-Curcumene, β-Selinene, δ-Selinene, Valencene, α-Selinene, Viridiflorene, β-Himachalene, β-Bisabolene, γ-Cadinene, 7-epi-α-Selinene, cis-Calamenene, β-Cadinene, α-Calacorene, Nerolidol, Caryophyllene oxide, Himachalol	Ferraz et al., 2005
H. olympicum L.	2-Methyl-octane, n-Nonane, α–Pinene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, 2-Methyl-decane, Terpinolene, Linalool, Camphor, Terpin-4-ol, α-Terpineol, Pulegone, Geraniol, Geranial, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, (E)-Caryophyllene, α-Humulene, allo-Aromadendrene, Germacrene D, Bicyclogermacrene, α-Muurolene, γ-Cadinene, δ-Cadinene, α-Cadinene, α-Calacorene, (Z)-3-Hexenyl benzoate, Spathulenol, α-Muurolol, α-Cadinol, Cadalene, Benzyl benzoate	Pavlovic et al., 2006

	Tricyclene, 2,5,6-trimethyl-hepta-1,3,6-triene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, Ocimene, Fenchone, Undecane, γ-Terpineol, Estragole, Carvone, (E)-Anethole, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, α-Gurjunene, β-Caryophyllene, β-Gurjunene, Patchoulene, Bicyclosesquiphellandrene, α-Caryophyllene, β-Farnesene, γ-Muurolene, Germacrene D, Eremophyllene, α-Muurolene, α-Farnesene, γ-Cadinene, γ-Bisabolene, Calamenene, δ-Cadinene, Cadina-3,9-diene, Cadina-1,4-diene, Muurola-4,9-diene, α-Calacorene, Germacrene B, β-Calacorene, Spathulenol, (1-epi)-Cubenol, α-Elemene, (epi-α)-Cadinol, (epi-α-Muurolol, α-Cadinol, α-Bisabolol	Gudzic et al., 2001
H. orientale L.	α-Pinene, Myrcene, p-Cimene, Benzene acetaldeide, cis-Linalool oxide, trans-Linalool oxide, Linalool, α-Terpineol, α-Cubebene, α-Longipinene, Cyclosativene, α-Copaene, β-Bourbunene, β-Elemene, β-Caryophyllene, B-Gurjunene, Aromadendrene, α-Humulene, Alloaromadendrene, Drima-7,9(11)-diene, γ-Muurolene, α-Amorphene, β-Selinene, Valencene, α-Selinene, α-Muurolene, δ-Cadinene, trans-Calamenene, α-Cadinene, α-Calacorene, Spathulenol, Caryophyllene oxide, Globulol, β-Oplopenone, Humulene epoxide II, γ-Eudesmol, τ-Cadinol (epi-a), α-Cadinol, Selina-11-en-6-a-ol, Santalol-7-a, 6,10,14-Trimethylpentadecanone, n-Heneicosane	Bertoli et al., 2018
H. patulum Thunb	α-pinene, hexanal, undecane, β-pinene, myrcene, limonene, 2-pentylfuran, (Z)-β-ocimene, γ-terpinene, p-cymene, terpinolene, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid), α-cubebene, cis-linalool oxide (furanoid), α-ylangene, α-copaene, β-bourbonene, linalool, italicene, 6-methyl-3.5-heptadien-2-one, β-caryophyllene, allo-aromadendrene, (Z)-β-farnesene, acetophenone, γ-muurolene, β-selinene, α-muurolene, (E,E)-α-farnesene, δ-cadinene, γ-cadinene, ar-curcumene, selina-3,7(11)-diene, calamenene, p-cymen-8-ol, epi-cubebol, β-calacorene, α-calacorene, cubebol, γ-calacorene, caryophyllene oxide, ledol, humulene epoxide-II, 1-epi-cubenol, spathulenol, T-cadinol, ar-turmerol, α-bisabolol, cadalene, porosadienol, cyperenone, tricosane, caryophyllenol-II, pentacosane, heptacosane	Demirci et al., 2005

	Nonane, α-Thujune, α-Pinene, Camphene, β-Pinene, Myrcene, α-Terpinene, p-Cymene, Limonene, β-Ocimene (Z), β-Ocimene (E), γ-Terpinene, α- Terpinolene, Linalool, Terpinene-4-ol, α-Termineol, trans-Anethol, Thymol, α-Copaene, β-Burbonene, β-Elemene, trans-Caryophyllene, γ-Elemene, α-Humulene, trance-β-Farnesene, allo-Aromadenarene, Germacrene-d, β-Selinene, α-Selinene, β-Dihydroagarofuran, E,E-alpha-farnesene, γ-Cadinene, Δ-Cadinene, Zonarene, Germacrene B, Caryophylleneoxide, Guaiol, 10-epi-γ-Eudesmol, 1-epi-Cubenol, γ-Eudesmol, Cubenol, Agarospirol, β-Eudesmol, α-Eudesmol, Selin-11-en-4-α-ol, Phytol	Morshedloo et al., 2014
H. perfoliatum L. .	n-Octane, 2-Methyloctane, n-Nonane, α-Thujene, α-Pinene, Camphene, β-Pinene, 3-Methylnonane, β-Myrcene, n-Decane, α-Terpinene, p-Cymene, Limonene, γ-Terpinene, 2-Methyldecane, p-Mentha-2,4(8)-diene, α-Terpinolene, n-Nonanal, Linalol, n-Undecane, Terpinen-4-ol, Myrtenal, α-Terpineol, n-Decanal, α-Longipinene, α-Copaene, β-Bourbonene, β-Elemene, α-Gurjunene, β-Caryophyllene, Aromadendrene, α-Humulene, allo-Aromadendrene, Germacrene D, Viridiflorene, α-Muurolene, γ-Cadinene, δ-Cadinene, α-Calacorene, trans-Nerolidol, Spathulenol, Ledol, trans-Cadinol, δ-Cadinol, α-Cadinol	Nogueira et al., 2008
	n-hexanal, (E)-2-hexanal, 2-heptanone, n-nonane, 2-methyl-4-heptanone, α-pinene, α-fenchene, (Z)-Hept-4-en-1-ol, β-pinene, 6-methyl-5-hepten-2-one, myrcene, n-decane, n-octanal, α-phellandrene, α-terpinene, -cymene, limonene, benzyl alcohol, lavender lactone, (E)β-ocimene, bergamal, -terpinene, cis-linalool oxide, cis-sabinene hydrate, trans-linalool oxide, 2-nonanone, n-undecane, cis-thujone, exo-fenchol, cis- -menth-2-en-1-ol, methyl octanoate, α-campholenal, octyl formate, trans-pinocarveol, camphor, isopolegol<neo>, (E,Z)-2,6-nonadienal, iso-borneol, trans-pinocamphone, pinocarvone, -cymen-8-ol, α-terpineol, methyl salicylate, myrtenol, n-dodecane, safranal, n-decanal, verbenone, trans-carveol, carvone, butyrophenone, geranial, nonanoic acid, n-undecanone, n-tridecane, α-cubebene, n-undecanol, cyclosativene, α-ylangene, α-copaene, β-patchoulene, β-maaliene, α-duprezianane, β-bourbonene, β-cubebene, β-elemene, β-longipinene, longifolene, iso-caryophyllene, 1,7 di-epi-β-cedrene, α-cedrene, β-caryophyllene, β-copaene, β-gurjunene, cis-thujopsene, -elemene, α-guaiene, cis-muurola-3,5-diene, α-himachalene, khusimene,	Rouis et al, 2011

	geranyl acetone, α-humulene, β-farnesene, allo-aromadendrene, cis-muurola-4(14),5-diene, dehydro-aromadendrene, β-acoradiene, -muurolene, germacrene-D, AR-curcumene, β-selinene, (E)-β-ionone, cis-β-gaiene, valencene, α-muurolene, n-pentadecane, trans- -cadinene, δ-cadinene, zonarene, cis-calamenene, α-cadinene, α-calacorene, β-calacorene, spathulenol, caryophellen oxide, 1-hexadecene, n-hexadecane, tetradecanal, 1-epi-cubenol, hinesol, epi-α-cadinol, α-cadinol, cadalene, khusinol, apiol, n-heptadecane, n-octadecane, musk xylol, n-nonadecane, cembrene, beyerene, phytol, heneicosane, n-decosane, n-tricosane, n-tetracosane	
	n-Octane, trans-2-Hexenal, 2-Methyl octane, n-Nonane, α-Pinene, 3-Methyl nonane, Sabinene, β-Pinene, β-myrcene, Limonene, 1-8-Cineol, (E)-β-Ocimene, 2-Methyl decane, Undecane, Terpinene-4-ol, α-Terpineol, α-Longipinene, β-Elemene, β-Caryophyllene, 1-Decene, Allo-Aromadendrene, γ-Muurolene, Germacrene-D, β-Selinene, α-Selinene, β-Himachalene, α-Calacorene, δ-Cadinene, Caryophyllene oxide, Spathulenol, Ledene, α-Cadinol	Hosni et al., 2008
	n-Nonane, α-Pinene, Camphene, Verbenene, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, n-Decane, α-Terpinene, p-Cymene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, n-Undecane, n-Nonanal, exo-Fenchol, α-Campholenal, trans-Pinocarveol, Borneol, Naphtalene, α-Longipinene, Cyclosativene, α-Ylangene, α-Copaene, β-Patchulene, (E)-Caryophyllene, β-Gurjunene, Aromadendrene, α-Humulene, allo-Aromadendrene, γ-Muurolene, Germacrene-D, (E)-β-Ionone, Viridiflorene, Bicyclogermacrene, α-Muurolene, γ-cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, (Z)-3-Hexenyl-benzoate, Spathulenol, Caryophyllene oxide, 1-epi-Cubenol, Cubenol, α-Muurolol, α-Cadinol, Cadalene	Petrakis et al., 2005
	α-Pinene, p-Cymene, Eucalyptol, γ-Terpinene, Camphor, Propanediamide, Thymol, 4,5-Dimethyl-2-ethylphenol, α-Farnesene, (–)-Spathulenol, Caryophyllene oxide, Diethyl phthalate, 4-(2,6,6-Trimethyl)-2-cyclohexen-1-yl-3-buten-2-ol, Isoaromadendrene epoxide, Alloaromadendrene oxide, T-Cadinol, α-Cadinol, trans-(Z)-α-Bergamotol, α-Bisabolol, 8-Cedren-13-ol, Lilac alcohol, Lilac alcohol formate, trans-(Z)-α-Bisabolene epoxide, 6,10,14-Trimethyl-2-pentadecanone,	Touafek et al., 2005

	Acetyldamantane, Nonadecane, n-Hexadecanoic acid, Eicosane, 2-Nonadecanone, Phytol, Dibutyl phthalate, Dioctyl + diisooctyl phthalates, Docosane, Tricosane, Tetracosane	
	2-methyl-octane, nonane, α-pinene, β-pinene, β-myrcene, limonene, cineole, 2-methyl-decane, linalool, n-undecane, α-copaene, β-elemene, β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, γ-cadinene, δ-cadinene, $C_{15}H_{24}$, spathulenol, 1-hexadecene	Nogueira et al., 1998
	n-Octane, 2-Methyloctane, n-Nonane, α-Pinene, Verbenene, Camphene, 3-Methyl-nonane, β-Pinene, 6-Methyl-5-hepten-2-one, β-Myrcene, n-Decane, α-Terpinene, p-Cymene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, Linalool, β-Fenchol, n-Undecane, n-Nonanale, trans-Pinocarveol, Camphor, Borneol, α-Terpineol, n-Decanale, Thymol, α-Longipinene, Cyclosativene, α-Ylangene, β-Patchoulene, α-Copaene, β-Bourbonene, β-Elemene, α-Gurjunene, β-Caryophyllene, β-Gurjunene, Aromadendrene, α-Humulene, allo-Aromadendrene, Germacrene D, γ-Muurolene, E-(β)-Ionone, Viridiflorene, Bicyclogermacrene, α-Muurolene, (E,E)-α-Farnesene, γ-Cadinene, δ-Cadinene, α-Cadinene, Cadina-1,4-diene, α-Calacorene, Ledol, trans-Nerolidol, Spathulenol, Caryophyllene oxide, iso-Aromadendrene epoxide, allo-Aromadendrene oxide, δ-Cadinol, τ-Cadinol, α-Muurolol, Cubenol, α-Cadinol, α-Bisabolol, 8-Cedren-13-ol, epi-Cubebol	Marandino et al., 2011
H. perforatum L.	Octane,2-methyl, Nonane, (-)-α-Pinene, Camphene, Verbenene, Nonane,3-methyl, (-)-β-Pinene, Myrcene, Decane, α-Phellandrene, α-Terpinene, p-Cymene, cis-Ocimene, trans-Ocimene, γ-Terpinene, Decane,2-methyl, α-Terpinolene, Undecane, n-Nonanal, trans-Thujone, α-Campholenal, Pinocarvone, (+)-Terpinen-4-ol, p-Cymen-8-ol, (+)-α-Terpineol, (-)-Myrtenal, Pulegone, Cuminal, Dodecane,2-methyl, Tridecane, α-Cubebene, α-Ylangene, (-) α-Copaene, β-Bourbonene, β-Cubebene, (+)-β-Funebrene, (-)-(E)-Caryophyllene, Calarene, (+)-Aromadendrene, (Z)-β-Farnesene, α-Humulene, α-Patchoulene, Germacrene D, (E)-β-Ionone, trans-β-Guaiene, α-Muurolene, (E,E)-α-Farnesene, γ-Cadinene,	Saroglou et al., 2007

	δ-Cadinene, (E)-Nerolidol, Spathulenol, Globulol, Salvial-4(14)-en-1-one, Viridiflorol, β-Eudesmol, Tetradecanol, Pentadecanol, Guaiazulene, 6,10,14-Trimethyl pentadecan-2-one, Hexadecanol	
	nonane, 2-methyloctane, tricyclene, 2-methylcyclopentane, 3-methylnonane, β-pinene, decanal, p-cymene, p-mentha-2-4 (8)-diene, 2,3-diethylpyrazine, p-cymenene, α-terpineol, α-longipinene, α-ylangene, α-copaene, isoledene, β-bourbonene, β-cubebene, β-gurjunene, patchoulene (correct isomer not identified), α-guaiene, α-patchoulene, seychellene, allo-aromadendrene, β-selinene, bicyclosesquiphellandrene, α-farnesene (correct isomer not identified), calamenene (correct isomer not identified), δ-cadinene, α-calacorene, nerolidol (correct isomer not identified), (Z)-3-hexenyl benzoate, viridiflorol, β-acorenol, tetradecanol	Rancic et al., 2005
	Octane,2-Methyl, Nonane, α-Pinene, Nonane,2-Methyl, Myrcene, Decane, p-Cymene, Limonene, 1,8 Cineole, 2-Octanol,2-Methyl, cis-Ocimene, Artemisia ketone, Decane,2-Methyl, Undecane, cis-Thujone, trans-Thujone, α-Campholenal, trans-Pinocarveol, α-Terpineol, Verbenone, Linalyl acetate, Dodecane,2-Methyl, Tridecane, Caryophyllene, β-Copaene, Caryophyllene oxide, 1-Octadecene	Tognolini et al., 2006
	n-Octane, trans-2-Hexenal, 2-Methyloctane, n-Nonane, α-Pinene, Sabinene, β-Pinene, 3-Methylnonane, β-Myrcene, α-Phellandrene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, 2-Methyldecane, Undecane, Terpinene-4-ol, α-Terpineol, Dodecane, α-Longipinene, α-Copaene, α-Ylangene, β-Elemene, trans-β-Farnesene, allo-Aromadendrene, α-Amorphene, Germacrene-D, β-Selinene, α-Selinene, Zingiberene, β-Himachalene, δ-Cadinene, (E)-Nerolidol, Caryophyllene oxide, Spathulenol, Viridiflorol, Ledene, α -Cadinol, 2-Pentadecanone, Phytol, (+-)-5-epi-Neointermedol, Cyclododecane	Hosni et al., 2011b
	3-Methylnonane, α-Pinene, 2-Methyldecane, Undecane, β-Pinene, Myrcene, α-Terpinene, Limonene, 1,8-Cineole, β-Phellandrene, Amylfuran(2-pentylfuran), (Z)-β-Ocimene, γ-Terpinene, (E)-β-Ocimene, p-Cymene, Terpinolene, (Z)-3-Hexenol, Nonanal, γ-Campholene aldehyde, (E)-2-Octenal, α-Cubebene, α-Ylangene, α-Copaene, α-Campholene aldehyde, Isomenthone, Camphor, β-Bourbonene, (E)-2-Nonenal, Linalool, Octanol, Linalyl acetate, trans-p-Menth-2-	Baser et al., 2002

	en-1-ol, Pinocarvone, β-Funebrene, Terpinen-4-ol, β-Caryophyllene, Aromadendrene, Myrtenal, (Z)-β-Farnesene, p-Mentha-1,5-dien-8-ol, trans-Verbenol, α-Humulene, γ-Muurolene, γ-Himachalene, Germacrene D, β-Silenene, α-Silenene, Bicyclogermacrene, (E,E)-α-Farnesene, (E)-2-undecenal, δ-Cadinene, γ-Cadinene, Methyl salicylate, Myrtenol, 3,7-Guaiadiene, (E,E)-2,4-Decadienal, trans-Carveol, cis-Calamenene, Geraniol, (E)-Geranyl acetone, Benzyl isovalerate, 2-methyl butyl benzoate, α-Calacorene, 1,5-Epoxy-salvial(4)14-ene, (E)-β-Ionone, cis-Jasmone, Dodecanol, γ-Calacorene, 2-Phenylethyl-2-methyl-butyrate, Caryophyllene oxide, Methyl eugenol, Salvial-4(14)-en-1-one, (E)-Nerolidol, Ledol, Humulene epoxide-II, Tridecanol, Cubenol, 1-epi-Cubenol, Globulol, Viridiflorol, Spathulenol, (Z)-3-Hexen-1-yl benzoate, 3,4-Dimethyl-5-pentylidene-2(5H)-furanone, Tetradecanol, T-Cadinol, Nonanoic acid, Thymol, T-Muurolol, δ-Cadinol, α-Bisabolol, Carvacrol, trans-α-Bergamotol, α-Cadinol, Selin-11-en-4α-ol, Decanoic acid, Caryophylla-2(12),6(13)-dien-5α-ol (caryophylladienol II), (2Z,6E)-Farnesol, Caryophylla-2(12),6-dien-5α-ol (caryophyllenol I), Dodecanoic acid, Phytol, Benzyl benzoate, Tetradecanoic acid, Hexadecanoic acid	
	2-Methyl-octane, n-Nonane, β-Pinene, β-Myrcene, n-Decane, p-Cymene, (E)-β-Ocimene, 2-Methyl-decane, p-Cymenene, n-Undecane, Campholenal, iso-Nonanol, (E)-Pinocarveol, Pinocarvone, 4-Terpineol, p-Cumen-8-ol, α-Terpineol, Myrtenol, Carvone, Geraniol, 2-Methyl-dodecane, (E)-Anethole, n-Tridecane, α-Longipinene, α-Copaene, β-Bourbonene, β-Caryophylene, β-Gurjunene, α-Himachalene, α-Caryophyllene, β-Farnesene, γ-Muurolene, α-Farnesene, γ-Cadinene, δ-Cadinene, Nerolidol, 3-(Z)-Hexenyl-benzoate, Spathulenol, Humulene-epoxide, 1-epi-Cubenol, n-Tetradecanol, Benzyl benzoate, n-Hexadecanol, n-Nonadecane	Gudzic et al., 2001
	2-Methyl-octane, n-Nonane, α-Thujene, α-Pinene, 3-Methyl-nonane, β-Pinene, Myrcene, n-Decane, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, 2-Methyl-decane, Terpinolene, Linalool, n-Nonanal, Terpin-4-ol, α-Terpineol, Pulegone, Linalyl acetate, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, β-Elemene, (Z)-Jasmone, 1,7-di-epi-β-Cedrene, (E)-Caryophyllene, Aromadendrene, α-Humulene, (E)-β-Farnesene, allo-Aromadendrene, β-Acoradiene, γ-Muurolene, Germacrene D, ar-Curcumene, β-Selinene, Valencene, α-	Pavlovic et al., 2006

	Muurolene, (E,E)-α-Farnesene, γ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, (E)-Nerolidol, (Z)-3-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, α-Cadinol	
	α - pinene, β - pinene, 2-methyl-decane, undecane, 2-methyl-dodecane, tridecane, longipinene, α - copaene, cis-caryophyllene, 3,7-guaiadiene, β - farnesene, naphtalene 1,2,3,4,4a,5,6,8a-octahydro-7-methylene-1-(1-methylethyl), β - chimachalene, BHT (from diethyl ether used as solvent), cadinene, 1-hexyl-2-propyl-cyclopropane, cyclododecane, 6,10,14-trimethyl-2-pentadecanon, ciclotetradecane, nonadecane, eicosane	Gudzic et al., 1997
	3-Methylnonane, α-Pinene, 2-Methyldecane, Undecane, β-Pinene, Limonene, p-Cymene, Carvacrol methyl ether (=Methyl carvacrol), (Z)-β-Farnesene, β-Selinene, α-Selinene, Tetradecanol, Thymol, Carvacrol	Erken et al., 2001
	2-methyl-octane, nonane, α-thujene, α-pinene, sabinene, β-pinene, myrcene, p-cymene, limonene, cis-β-ocimene, trans-β-ocimene, undecane, terpinen-4-ol, α-terpineol, decanal, α-cubebene, α-ylangene, α-copaene, β-bourbonene, β-elemene, longifolene, β-caryophyllene, β-copaene, α-humulene, E-β-farnesene, germacrene D, β-selinene, bicyclogermacrene, α-muurolene, E,E-α-farnesene, γ-cadinene, δ-cadinene, E-nerolidol, spathulenol, caryophyllene oxide, guaiol, 1-epi-cubenol, muurola-4,10-en-l-b-ol, α-muurolol, α-cadinol	Chauhan et al., 2011
	2-Methyl-octane, n-Nonane, α-Thujene, α-Pinene, 3-Methyl-nonane, β-Pinene, Myrcene, n-Decane, α-Phellandrene, α-Terpinene, o-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, 2-Methyl-decane, Terpinolene, Linalool, n-Nonanal, Terpinen-4-ol, α-Terpineol, trans-Sabinene hydrate acetate, n-Undecane, α-Cubebene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, β-Elemene, (Z)-Jasmone, 1,7-di-epi-β-Cedrene, (E)-Caryophyllene, Aromadendrene, α-Humulene, (E)-β-Farnesene, β-Acoradiene, γ-Muurolene, Germacrene-D, ar-Curcumene, β-Selinene, Valencene, α-Muurolene, (E,E)-α-Farnesene, γ-cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide	Petrakis et al., 2005

	α-Pinene, β-Pinene, 2-Carene, Sabinene, 3-Carene, Myrcene, α-Phellandrene, Limonene, γ-Terpinene, p-Cymene, α-Longipinene, α-Copaene, Longifolene, Isocaryophyllene, β-Caryophyllene, Aromadendrene, allo-Aromadendrene, γ-Gurjunene, Viridiflorene, Germacrene-D, α-Muurolene, γ-Cadinene, Calamenene, Myrtanol, Cadalene, Cedrol, Eugenol	Cakir et al., 1997
	n-Nonane, α-Pinene, Limonene, α-Campholene aldehyde, Isopinocarveol, Verbenol, p-Mentha-1,8-diene-8-ol, p-Cymen-8-ol, Myrtenal, (-)-α-Terpineol, trans-(+)-Carveol, Nopol, 4-Isopropyl-1,6-dimethyldecahydronaphthalene, Thymol, Tridecane, α-Cubebene, α-Copaene, Tetradecane, β-Caryophylene, Aromadendrene, Germacrene-D, α-Amorphene, β-Selinene, Pentadecane, Calarene, α-Muurolene, δ-Cadinene	Akhbari et al., 2012
	3-Methyl nonane, α-Pinene, α-Thujene, 2-Methyl decane, Undecane, β-Pinene, Sabinene, Myrcene, α-Phellandrene, α-Terpinene, Limonene, β-Phellandrene, (Z)-β-Ocimene, γ-Terpinene, (E)-β-Ocimene, p-Cymene, Terpinolene, Tridecane, Nonanal, (Z)-3-Hexenyl-2-methyl butyrate, (Z)-3-Hexenyl 3-methylbutyrate, Bicycloelemene, α-Copaene, Decanal, β-Bourbonene, α-Gurjunene, β-Cubebene, Linalool, β-Ylangene, β-Elemene, Terpinen-4-ol, β-Caryophyllene, Aromadendrene, Cadina-3,5-diene, Alloaromadendrene, (Z)-β-Farnesene, α-Humulene, γ-Muurolene, Ledene, Bicyclosesquiphellandrene, Germacrene D, (Z,E)-α-Farnesene, α-Muurolene, Bicyclogermacrene, (E,E)-α-Farnesene, δ-Cadinene, γ-Cadinene, Cadina-1,4-diene (=Cubenene), α-Cadinene, Calamenene, Dodecanol, Caryophyllene oxide, (E)-Nerolidol, Ledol, Humulene epoxide-II, Tridecanol, Cubenol, 1-epi-Cubenol, Globulol, Viridiflorol, Hexahydrofarnesyl acetone, Rosifoliol, Spathulenol, 6-epi-Cubenol, Nor-Copaonone, Tetradecanol, T-Cadinol, T-Muurolol, δ-Cadinol (=alpha-muurolol), trans-α-Bergamotol, α-Cadinol, Eudesma-4(15),7-dien-4 ß-ol, Hexadecanol, Pentacosane, Dodecanoic acid, Phytol, Benzyl benzoate, Heptacosane, Nonacosane, Hexadecanoic acid	Alan et al., 2010
	α-Thujene, α-Pinene, Camphene, β-Pinene, Decane,2-Methyl, δ-2-Carene, D-Limonene, β-Phellandrene, Ocimene, γ-Terpinene, Terpinolene, Caryophyllene, β-Farnesene, α-Himachalene, α-Muurolene, D-Germacrene, 10s-11s-Himachala-3(12),4-diene, γ-Elemene, γ-Cadinene, Caryophyllene oxide	Jianu et al., 2016

	n-hexanol, (Z)-3-methyl-4-nonene, 5-methylnonane, 3-methylnonane, Ethylcyclohexane, Decane, 1,2,3-trimethylcyclohexane, sec-butylcyclohexane, 2,6-dimethyldecane, 1-hexyl-3-methylcyclopentane, Dodecane, L(-)-carvone, Tetradecane, Hexadecane	Parchin et al., 2016
	α-Thujene, α-Pinene, Camphene, Sabinene, β-Pinene, β-Phellandrene, Decane,2-methyl, a-Carene, D-Limonene, p-Menth-3-ene, Undecane, β-cis-Ocimene, γ-Terpinene, Dodecane,2-methyl, Caryophyllene, 1,4,7-Cycloundecatriene,1,5,9,9-tetramethyl, α-Muurolene, Germacrene D, β-Bisabolene, γ-Elemene, γ-Cadinene, γ-Muurolene	Moleriu et al., 2017
	n-octane, Norbornene, Nonane, α-pinene, 5-methyl-3-heptanone, β- pinene, Myrcene, n- decane, p-cymene, Limonene, Allylhexanoate, Undecane, Tetrahydrolavandulol, Tridecane, α-longipinene, Ylangene, α-copaene, Nepetalactone, β-elemene, Dodecanal, β- funebrene, E-caryophyllene, γ-Elemene, α- humulene, E-β-farnesene, α-acroadiene, N- dodecanol, γ-Himachalene, β-selinene, δ- selinene, α-selinene, E,E-α-farnesene, γ-cadinene, δ-cadinene, E-Nerolidol, Caryophyllene oxide, B-Eudesmol, Selin-11-en-4-α-ol, β- bisabolol, N-tetradlecanol, 5-iso cedranol, Epi-α-bisabolol, Ethyl tetradecanoate, Isophytol, Nonacosane	Morshedloo et al., 2015
	2-methyl-octane, nonane, α-pinene, β-pinene, β-myrcene, limonene, cineole, 2-methyl-decane, linalool, n-undecane, α-copaene, β-caryophyllene, α-humulene, trans-β-farnesene, germacrene D, bicyclogermacrene, γ-cadinene, δ-cadinene, $C_{15}H_{24}$, spathulenol, 1-hexadecene, 3-octadecene	Nogueira et al., 1998
	Undecane, 5-methyl, Nonane, α-Thujan, α-Pinene, Nonane, 3-methyl, β-Pinene, β-Myrcene, Decane, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, 1,8-Cineole, (Z) β-Ocimene, (E) β-Ocimene, γ-Terpinene, Decane, 2-methyl, Terpinolene, Undecane, n-Nonanal, 1-Octanol, 2-methyl, Pulegone, Tridecane, Carvacrol, α-Cubebene, Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, α-Cedrene, β-Caryophyllene, Aromadendrene, α-Humulene, β-Farnesene <Z>,	Pirbalouti et al., 2014

	Bicyclosesquiphellandrene <Epi->, 1-Dodecanol, α-Amorphene, Germacrene-D, β-Selinene, α-Selinene, α-Farnesene, γ-Cadinene, Δ-Cadinene, 1,4-Cadinadiene, Spathulenol, Caryophyllene oxide, Germacrene B, Phytol	
	(E)-3-Hexanal, n-Nonane, α-Pinene, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, p-Cymene, Limonene, cis-Linalool oxide (furanoid), n-Undecane, α-Campholenal, trans-Verbenol, Pinocarvone, α-Terpineol, Myrtenol, trans-Carveol, α-Cubebene, α-Ylangene, α-Copaene, Sativene, β-Caryophyllene, α-Himachalene, Geranyl acetone, β-Selinene, α-Selinene, trans- -Cadinene, δ-Cadinene, α-Cadinene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide, Humulene epoxide II, β-Oplopenone, 1-epi-Cubenol, epi-α-Cadinol, epi-α-Muurolol, α-Cadinol, Cadalene, 1-Tetradecanol, Eudesma-4(15),7-dien-1-β-ol, (E)-Sesquilavandulyl acetate, Aristolone, n-Eicosane, n-Heneicosane, n-Tricosane, n-Pentacosane	Rouis et al., 2013
	2-Methyloctane, n-Nonane, α-Pinene, β-Pinene, β-Myrcene, n-Decane, p-Cymene, Limonene, (E)-β-Ocimene, p-Cymenene, Linalool, n-Undecane, Campholenal, trans-Pinocarveol, Pinocarvone, iso-Nonanol, Terpinen-4-ol, p-Cimen-8-ol, α-Terpineol, Myrtenol, cis-Carveol, Carvone, Geraniol, (E)-Anethole, 2-Methyldodecane, n-Tridecane, α-Cubebene, α-Longipinene, α-Copaene, β-Bourbonene, β-Elemene, α-Caryophyllene, β-Caryophyllene, β-Copaene, β-Gurjunene, Aromadendrene, α-Himachalene, (E)-β-Farnesene, α-Humulene, Dodecanol, β-Selinene, Germacrene D, γ-Muurolene, Bicyclogermacrene, α-Selinene, α-Muurolene, (E,E)-α-Farnesene, γ-Cadinene, δ-Cadinene, (Z)-3-Hexenyl-benzoate, trans-Nerolidol, Spathulenol, Caryophyllene oxide, Humulene epoxide, 1-epi-Cubenol, n-Tetradecanol, Benzyl benzoate, n-Hexadecanol, n-Nonadecane	Marandino et al., 2011
	3-Methyl nonane, α-Pinene, 2-Methyl decane, Undecane, β-Pinene, Myrcene, Limonene, (Z)-β-Ocimene, γ-Terpinene, (E)-β-Ocimene, p-Cymene, Terpinolene, Tridecane, 6-Methyl-5-hepten-2-one, Nonanal, Tetradecane, Longipinene, α-Ylangene, α-Copaene, α-Campholene aldehyde, Pentadecane, β-Bourbonene, Linalool, Hexadecane, β-Caryophyllene, (Z)-β-Farnesene, α-Humulene, Selina-4,11-diene, Heptadecane, γ-Muurolene, β-Selinene, α-Selinene, δ-Cadinene, γ-	Kiyan et al., 2014

	Cadinene, Calamenene, Benzyl 2-methylbutyrate, Nonadecane, 2-Methyl butyl benzoate, α-Calacorene, Dodecanol, Caryophyllene oxide, (E)-Nerolidol, Humulene epoxide-II, Hexahydrofarnesyl acetone, Spathulenol, Tetradecanol, Clovenol, Cadalene, Selin-11-en-4 a-ol , Tricosane, Caryophylla-2(12),6(13)-dien-5β-ol (=Caryophylladienol I), Hexadecanol, Caryophylla-2(12),6-dien-5α-ol (=Caryophyllenol I), Pentacosane, Dodecanoic acid, Benzyl benzoate, Heptacosane, Nonacosane, Hexadecanoic acid	
	Carvacrol, β-Caryophyllene, Caryophyllene 9-epi (E), n-Dodecanol, Germacrene D, β-Selinene, δ-Selinene, γ-Cadinene, δ-Cadinene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide, Humulene epoxide II, Caryophylla-4(12),8(13)dien-5α-ol, α-Bisabolol oxide B, Selin-11-en-4α-ol, (Z)-α-Santolol, n-Tetradecanol, Eudesma-4(15),7-dien-1β-ol	Hodaj-Çelikua et al., 2017
	α-Pinene, β-Pinene, Myrcene, α-Phellandrene, p-Cymene, Limonene, 1,8-Cineole, Santolina alcohol, (E)-β-Ocimene, γ-Terpinene, Linalool, cis-Thujone (= α-Thujone), trans-Thujone (= β-Thujone), Octyl formate, Menthone, Borneol, (2E)-Nonenol, Terpinen-4-ol, α-Terpineol, Pulegone, Carvone, cis-Piperitone epoxide, Linalool acetate, cis-Chrysanthenyl acetate, Citronellyl formate, Thymol, Carvacrol, α-Cubebene, Piperitenone oxide, (2E)-Undecenol, β-Bourbonene, β-Elemene, 2-epi-β-Funebrene, (E)-Caryophyllene, β-Copaene, α-Humulene, (E)-β-Farnesene, Sesquisabinene, (2E)-Dodecenal, n-Dodecanol, γ-Muurolene, Germacrene D, β-Selinene, trans-Muurola-4(14),5-diene, Bicyclogermacrene, α-Muurolene, Germacrene A, (E,E)-α-Farnesene, γ-Cadinene, δ-Cadinene, (E)-Nerolidol, (3Z)-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, Globulol, Ledol, Junenol, 1-epi-Cubenol, α-Muurolol (= Torreyol), β-Eudesmol, α-Cadinol, 14-Hydroxy-9-epi-(E)-caryophyllene, n-Tetradecanol, Germacra-4(15),5,10(14)-trien-1α-ol, Phytone, (E)-Phytol	Sharopov et al., 2010
	Nonane, α-Pinene, Camphene, β-Pinene, cis-3-Hexenyl acetate, α-Terpinene, Limonene, trans-Ocimene, γ-Terpinene, Terpinolene, Linalool, endo-Fenchol, α-Terpineol, α-Cubebene, α-Longipinene, α-Copaene, β-Cubebene, β-Elemene, α-Gurjunene, β-Caryophyllene, α-Guaiene, β-Farnesene, α-Humulene, Germacrene-D, β-Selinene, α-Muurolene, δ-	Ghiasvand et al., 2016

	Cadinene, Nerolidol, Germacrene-B, 3-Hexenyl-benzoate, Spathulenol, Caryophyllene oxide, Viridiflorol, Globulol, Guaiol, allo-Aromadendrene, α-Eudesmol, 1-Hexadecanol, n-Heptacosane	
	2-methylheptane, octane, (E)-2-hexenal, 2-methyloctane, nonane, 2,4-dimethyl-2,4-heptadiene, α-thujene, α-pinene, camphene, 2-methylnonane, heptanol, β-pinene, myrcene, α-terpinene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, 2-methyldecane, terpinolene, linalool, nonanol, nonanal, terpinen-4-ol, α-terpineol, decanal, undecanal, β-bourbonene, β-elemene, β-cedrene, β-caryophyllene, β-gurjunene, aromadendrene, α-himachalene, α-humulene, (E)-β-farnesene, γ-muurolene, germacrene D, β-selinene, α-selinene, bicyclogermacrene, α-farnesene, γ-cadinene, (Z)-γ-bisabolene, δ-cadinene, cadina-1,4-diene, α-cadinene, (E)-nerolidol, (Z)-3-hexenylbenzoate, spathulenol, caryophyllene oxide, globulol, cedrol, 1-epi-cubenol, β-acorenol, T-muurolol, α-muurolol, α-cadinol, tetradecanol, α-bisabolol, heptadecane, benzyl benzoate, 6,10,14-trimethyl-2 pentadecanone, n-pentadecanol, tetradecanoic acid, octadecane, nonadecane, phytol, docosane	Chatzopoulou et al., 2006
H. perforatum L. (cultivated)	2-methylheptane, octane, 2-methyloctane, nonane, 2,4-dimethyl-2,4-heptadiene, α-thujene, α-pinene, 2-methylnonane, heptanol, β-pinene, myrcene, α-terpinene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, 2-methyldecane, nonanol, nonanal, terpinen-4-ol, β-bourbonene, β-caryophyllene, β-gurjunene, aromadendrene, α-humulene, (E)-β-farnesene, γ-muurolene, germacrene D, bicyclogermacrene, α-farnesene, γ-cadinene, δ-cadinene, α-cadinene, (E)-nerolidol, (Z)-3-hexenylbenzoate, spathulenol, caryophyllene oxide, cedrol, 1-epi-cubenol, β-acorenol, T-muurolol, α-muurolol, α-cadinol, tetradecanol, α-bisabolol, heptadecane, benzyl benzoate, 6,10,14-trimethyl-2 pentadecanone, n-pentadecanol, tetradecanoic acid, octadecane, nonadecane, phytol, docosane	Chatzopoulou et al., 2006
H. perforatum L. (flowers and fruits)	α-pinene, α-campholene Aldehyde, isopinocarveol, Verbenol, P-Menth-1,8-diene-8-ol, P-Cymen-8-Ol, (-)-α-Terpineol, Myrtenol, NOPOL, 4-Isopropyl-1,6-dimethyldecahydronaphthalene, Trans-(+)-Carveol, Thymol, Tridecane, α-Cubebene,	Akhbari & Batooli, 2009

	α-Copaene, Tetradecane, β-Caryophylene, Germacrene-D, Aromadendrene, α-Amorphene, β-Selinene, Calarene, α-Muurolene, Pentadecane, δ-Cadinene, Spathulenol	
H. perforatum L. (flowers)	α-Pinene, β-Pinene, Myrcene, p-Cymene, Limonene, (E)-β-Ocimene, α-Copaene, β-Elemene, α-Gurjunene, β-Caryophyllene, (E)-β-Farnesene, β-Acoradiene, γ-Gurjunene, γ-Muurolene, β-Selinene, α-Selinene, γ-Cadinene, δ-Cadinene, Spathulenol, Caryophyllene oxide	Kakhky et al., 2008
H. perforatum L. (leaf)	Oct-1-ene, Nonane, α-pinene, Camphene, 6-Methylhept-5-en-2-one, β-Pinene, Myrcene, α-Phellandrene, 5-Methylheptan-2,4-dione, 6-Methylheptan-2,4-dione, α-Terpinene, p-Cymene, Limonene, (Z)-Ocimene, (E)-Ocimene, Acetophenone, γ-Terpinene, Pinol (4,7,7-Trimethyl-6-oxabicyclo[3.2.1]oct-3-ene), Terpinolene, Linalol, Undecane, Fenchol, Neo-allo-ocimene, Borneol, Terpinen-4-ol, α-Terpineol, α-Campholenol, Nerol, Neral, Geraniol, Geranial, Bornyl acetate, Methyl geranate, α-Terpinyl acetate, Bicycloelemene, Neryl acetate, Geranyl acetate, β-Bourbonene, β-Elemene, Caryophyllene, (E)-β-Farnesene, α-Humulene, Ishwarane, α-Amorphene, Selina-4,11-diene, γ- and ar-Curcumene, β-Selinene, α-Selinene, α-Cuprenene, δ-Cadinene, Elemol, Caryophyllene epoxide, Guaiol, γ-Eudesmol, β-Eudesmol, α-Eudesmol, 2,2-Dimethyl-7-sec-butyl-2H,5H-pyrano[4,3-b]pyran-5-one, 2,2-Dimethyl-7-isobutyl-2H,5H-pyrano[4,3-b]pyran-5-one	Weyerstahl et al., 1995
H. perforatum L. (leaves)	Cyclopentane, methyl-, camphene, α-pinene, verbenene, β-pinene, Myrcene, p-Cymene, Limonene, Trans-β-Ocimene, gamma-terpinene, Ethanone 1-phenyl, Linalool Oxide, o-Isopropenyltoluene, Undecane, α-campholene Aldehyde, trans-Pinocarveol, Verbenol, Trans-P-Menth-2-ene-1,8-diol, Octanoic acid, Terpinene-4-ol, p-cymene-8-ol, 1-.alpha.-Terpineol, Myrtenal, Trans-(+)-Carveol, Carvone, Thymol, Carvacrol, α-Cubene, α-Longipinene, α-Ylangene, α-Copaene, β-Caryophyllene, (+)-Aromadendrene, Germacrene-D, α-Humulene, β-Selinene, α-Amorphene, α-gurjunene, δ-Cadinene, α-Cadinene, (-)-Caryophyllene Oxide, α-Calacorene, (+)-Spathulenol, Eremophilene, Cadina-1,4-diene,	Akhbari & Batooli, 2009

	glubulol, β-oplopinone, Calarene, 4-Isopropyl-1,6-dimethyldecahydronaphthalene, guaiazolene, Valerenal, Octadecane, Nonadecane, Hexadecanoic acid, Heneicosane	
H. perforatum L. (leaves)	α-Pinene, sabinene, β-Caryophyllene, (E)-β-Farnesene, Germacrene D, β-Selinene, α-Selinene, 6,10,14-trimetyl-2-pentadecanone, Heneicosane	Kakhky et al., 2008
H. perforatum L. (roots)	α-Pinene, β-Pinene, β-Phellandrene, (E)-β-Ocimene, α-Copaene, β-Elemene, β-Caryophyllene, β-Gurjunene, (Z)-β-Farnesene, α-humulene, γ-Muurolene, Germacrene D, β-Selinene, α-Selinene, (Z)-α-Bisabolene, γ-Cadinene, δ-Cadinene, Spathulenol, Caryophyllene oxide, Dillapiole	Kakhky et al., 2008
H. perforatum L. (stems)	α-Pinene, Terpinolene, Undecane, Camphor, α-Copaene, β-Elemene, β-Caryophyllene, (E)-β-Farnesene, γ-Muurolene, Germacrene D, Myristicin, δ-Cadinene, α-Calacorene, Elemicin, Spathulenol, Caryophyllene oxide	Kakhky et al., 2008
H. perforatum L. ssp. *angustifolium*	Nonane, α-Pinene, 3-Methylnonane, β-Pinene, p-Cymene, α-Terpineol, 2-Methyldecane, trans-Linalool oxide, cis-Linalool oxide, β-Thujene, α-Thujene, Pinocarveol, Terpinene-4-ol, (-)-α-Terpineol, Myrtenol, 2-Methyl-6-propyl-dodecane, trans-Anethole, Tridecane, α-Cubebene, Tridecanal, α-Copaene, β-Bourbonene, trans-Caryophyllene, Germacrene-D, trans-β-Farnesene, Ciklopropane, γ-Muurolene, α-Muurolene, δ-Cadinene, α-Cadinene, α-Calacorene, trans-Nerolidol, Caryophyllene oxide, 3-Tetradecane, 6,10,14-Trimethyl-pentadecane-2-on, 1-Heptadecanol, n-Nonadecane	Šmelcerović et al., 2004
H. perforatum L. subsp. *perforatum* (flower oils)	2,6-dimethylheptane, 2-methyloctane, nonane, 2-methyl-4-heptanone, α-pinene, α-fenchene, camphene, 2-methyldecane, β-pinene, sabinene, myrcene, limonene, 1,8-cineole, (Z)-β-ocimene, γ-terpinene, (E)-β-ocimene, p-cymene, 2,3,5-trimethyldecane, linalool, β-caryophyllene, terpinen-4-ol, ethyl benzoate, myrtenal, α-himachalene, trans-pinocarveol, allo-aromadendrene, α-humulene, β-bisabolene, α-terpineol, γ-muurolene, α-muurolene, γ-cadinene, cis-carveol, α-calacorene, (Z)-nerolidol, caryophyllene oxide, humulene epoxide-I, (Z)-3-hexenyl benzoate,	Maggi & Ferretti, 2008

	viridiflorol, globulol, spathulenol, T-cadinol, epi-α-muurulol, thymol, α-cadinol, (Z,E)-farnesol, decanoic acid, hexadecanol, dodecanoic acid, hexacosane, (E)-phytol, tetradecanoic acid, hexadecanoic acid	
H. perforatum L. var. *angustifolium* DC.	2-methyloctane, n-nonane, α-pinene, β-pinene, myrcene, α-terpinene, limonene, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, terpinolene, linalool, undecane, (Z)-3-hexenyl isobutyrate, terpinen-4-ol, α-terpineol, decanal, tridecane, δ-elemene, α-cubebene, α-longipinene, isoledene, α-copaene, β-cubebene, β-elemene, β-funebrene, β-caryophyllene, β-copaene, β-gurjunene, aromadendrene, α-humulene, (E)-β-farnesene, allo-aromadendrene, germacrene D, γ-muurolene, bicyclogermacrene, trans-β-guaiene, γ-cadinene, δ-cadinene, α-cadinene, (E)-nerolidol, caryophyllene alcohol, α-cedrene epoxide, longiborneol, 1,10-di-epi-cubenol, cis-cadin-4-en-7-ol, epi-α-cadinol, α-muurolol, α-cadinol, heptadecane	Pintore et al., 2005
H. perforatum subsp. *veronense* (Schrank) H.Lindb. (flower oils)	octane, 2,6-dimethylheptane, 2-methyloctane, nonane, 2-methyl-4-heptanone, α-pinene, α-fenchene, camphene, 2-methyldecane, β-pinene, sabinene, myrcene, limonene, 1,8-cineole, γ-terpinene, p-cymene, 2,3,5-trimethyldecane, α-longipinene, α-ylangene, α-campholenal, α-copaene, camphor, β-bourbonene, linalool, pinocarvone, β-funebrene, β-caryophyllene, terpinen-4-ol, ethyl benzoate, myrtenal, α-himachalene, trans-pinocarveol, (E)-β-farnesene, allo-aromadendrene, α-humulene, β-bisabolene, α-terpineol, γ-muurolene, verbenone, valencene, α-muurolene, γ-cadinene, myrtenol, cis-calamenene, p-cymen-8-ol, cis-carveol, (Z)-nerolidol, caryophyllene oxide, aristolene epoxide, humulene epoxide-I, (Z)-3-hexenyl benzoate, (E)-nerolidol, viridiflorol, globulol, spathulenol, T-cadinol, carvacrol, epi-α-muurulol, thymol, α-cadinol, (Z,E)-farnesol, decanoic acid, hexahydrofarnesyl acetone, hexadecanol, dodecanoic acid, hexacosane, hexadecanoic acid	Maggi & Ferretti, 2008
H. philonotis Cham. & Schlecht.	n-Octane, 2-Methyl-octane, n-Nonane, 2,3,5-Trimethyl-1,3-hexadiene, Camphene, 3-Methyl-nonane, β-Pinene, Myrcene, n-Decane, 2-Methyldecane, n-Decanal, 1-Decanol, Limonene dioxide, Patchoulene, (E)-β-Caryophyllene, (E)-β-Farnesene, (E)-Nerolidol, β-Caryophyllene oxide	García-de la Cruz et al., 2013

H. polyanthemum Klotzsch ex Reichardt. (flowering)	Nonane, α-Pinene, β-Pinene, 2-Methyldecane, Undecane, α-Copaene, β-Elemene, β-Caryophyllene, α-Humulene, allo-Aromadendrene, γ-Muurolene, Germacrene D, β-Selinene, δ-Selinene, Valencene, cis-β-Guaiene, α-Selinene, Viridiflorene, Bicyclogermacrene, β-Himachalene, Germacrene A, γ-Cadinene, δ-Cadinene, Z-γ-Bisabolene, β-Cadinene, trans-Calamenene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, 1-epi-Cubenol, τ-Cadinol, β-Eudesmol, α-Cadinol, 6-isobutyryl-5,7-dimethoxy-2,2-dimethyl-benzopyran, 6-isobutyryl-5-methoxy-2,2-dimethyl-benzopyran, 6-isobutyryl-7-methoxy-2,2-dimethyl-benzopyran, Eicosane, Heneicosane	Ferraz et al., 2005
H. pseudohenryi N.Robson	3-methylnonane, α-pinene, α-thujene, undecane, β-pinene, myrcene, limonene, 2-pentylfuran, (Z)-β-ocimene, γ-terpinene, p-cymene, terpinolene, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid), α-cubebene, cis-linalool oxide (furanoid), α-copaene, β-bourbonene, linalool, β-elemene, 6-methyl-3.5-heptadien-2-one, β-caryophyllene, γ-elemene, allo-aromadendrene, (Z)-β-farnesene, acetophenone, α-humulene, limonen-4-ol, γ-muurolene, germacrene D, β-selinene, (E,E)-α-farnesene, δ-cadinene, γ-cadinene, ar-curcumene, selina-3,7(11)-diene, guaia-3,7-diene, calamenene, p-cymen-8-ol, β-calacorene, α-calacorene, γ-calacorene, caryophyllene oxide, humulene epoxide-II, cubenol, 1-epi-cubenol, spathulenol, T-cadinol, ar-turmerol, α-bisabolol, cadalene, porosadienol, tricosane, cis-nuciferyl acetate, pentacosane, heptacosane	Demirci et al., 2005
H. pseudolaeve N.Robson	Nonane, α-Pinene, Camphene, β-Pinene, β-Myrcene, 1-Phellandrene, α-Terpinene, Benzene-1-methyl-2, δ-Limonene, cis-Ocimene, 1,3,6-Octatriene, γ-Terpinene, α-Terpinolene, Undecane, Nonanal, allo-Ocimene, 3-Cyclohexen-1-ol, α-Terpineol, Decanal, 1-Decanol, α-Cubebene, α-Longipinene, α-Ylangene, α-Copaene, β-Elemene, allo-Aromadendrene, trans-Caryophyllene, trans-β-Farnesene, α-Humulene, Naphthalene, Germacrene D, β-Selinene, α-Selinene, Spathulenol, Caryophyllene oxide, trans-β-Caryophyllene, Azulene, α-Cadinol, Caryophyllenol-II, Benzilbenzoate, 2-Pentadecanone, n-Hexadecanoic acid, Ethyllinoleolate, Tricosane, Tetracosane	Bagci & Yuce, 2011c

H. pulchrum L.	trans-2-Hexenal, n-Heptanal, n-Nonane, α-Thujene, α-Pinene, Camphene, β-Pinene, β-Myrcene, n-Decane, Benzene acetaldehyde, p-Cymene, 1,8-Cineole, Limonene, Acetophenone, cis-β-Ocymene, trans-β-Ocymene, γ-Terpinene, α-Terpinolene, n-Nonanal, Linalol, n-Undecane, trans-Pinocarveol, Pinocarvone, δ-Terpineol, Terpinen-4-ol, Myrtenal, α-Terpineol, Myrtenol, n-Decanal, α-Cubebene, α-Copaene, β-Elemene, Dodecanal, β-Caryophyllene, α-Humulene, Kushimene, allo-Aromadendrene, Germacrene D, β-Selinene, α-Selinene, α-Muurolene, trans,trans-α-Farnesene, γ-Cadinene, trans-Calamenene, δ-Cadinene, α-Cadinene, α-Calacorene, Hexenyl benzoate, Spathulenol, Dodecanoic acid, β-Caryophyllene oxide, Savial-4(14)-en-1-one, δ-Cadinol, Intermediol, α-Cadinol, Eudesma-1,4(15),11-triene	Nogueira et al., 2008
	nonane, α-pinene, β-pinene, β-myrcene, limonene, cineole, linalool, n-undecane, α-copaene, β-elemene, β-caryophyllene, α-humulene, trans-β-farnesene, germacrene D, bicyclogermacrene, γ-cadinene, δ-cadinene, spathulenol, 1-hexadecene	Nogueira et al., 1998
H. reflexum L.f. (La Esperanza)	(3Z)-hexenol, n-hexanol, n-nonane, α-thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, dehydroxy-trans-linalool oxide, α-phellandrene, n-decane, dehydroxy-cis-linalool oxide, α-terpinene, p-cymene, limonene, 1,8-cineole, 2,2,6-trimethyl-cyclohexanone, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, terpinolene, n-undecane, n-nonanal, endo-fenchol, α-campholenal, borneol, menthol, terpinen-4-ol, α-terpineol, methyl salicylate, safranal, n-dodecane, β-cyclocitral, geraniol, geranial, 2-undecanone, n-tridecane, δ-elemene, α-cubebene, α-ylangene, cyclosativene, α-copaene, β-bourbonene, β-cubebene, β-elemene, isoledene, (Z)-caryophyllene, α-gurjunene, (E)-caryophyllene, aromadendrene, β-copaene, α-humulene, 6,9-guaiadiene, cis-muurola-3,5-diene, allo-aromadendrene, cis-cadina-1(6),4-diene, γ-muurolene, germacrene D, trans-cadina-1(6),4-diene, β-selinene, (E)-β-ionone, α-selinene, epizonarene, α-muurolene, cis-cadina-1,4-diene, γ-cadinene, trans-calamenene, δ-cadinene, δ-amorphene, α-cadinene, trans-cadina-1,4-diene, (E)-α-bisabolene, α-calacorene, caryophyllenyl alcohol, spathulenol, β-calacorene, 1α,10α-epoxy-amorph-4-ene, caryophyllene oxide, salvial-4(14)-en-1-one, gleenol, copaborneol, β-copaen-4-α-ol, β-oplopenone, junenol, rosifoliol, 1-epi-cubenol, muurola-4,10(14)-dien-1-β-ol, 1,10-di-epi-cubenol, α-muurolol, epi-α-	Zorzetto et al., 2015

	muurolol, caryophylla-4(12),8(13)-dien-5-ol, α-cadinol, cadalene, eudesma-4(15),7-dien-1β-ol, n-heptadecane, mint sulfide, 2-pentadecanone,6,10,14-trimethyl-, n-nonadecane, hexadecanoic acid, phytol, n-heneicosane, n-tricosane, n-pentacosane, n-hexacosane, n-heptacosane, n-octacosane, n-nonacosane	
H. reflexum L.f. (Ifonche)	(3Z)-hexenol, n-hexanol, n-nonane, α-thujene, α-pinene, α-fenchene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, n-decane, dehydroxy-cis-linalool oxide, α-terpinene, p-cymene, limonene, 1,8-cineole, 2,2,6-trimethyl-cyclohexanone, (Z)-β-ocimene, (E)-β-ocimene, γ-terpinene, terpinolene, n-undecane, n-nonanal, endo-fenchol, α-campholenal, borneol, terpinen-4-ol, α-terpineol, safranal, n-dodecane, β-cyclocitral, geraniol, 2-undecanone, n-tridecane, α-longipinene, α-cubebene, α-ylangene, cyclosativene, α-copaene, β-bourbonene, sativene, β-cubebene, β-elemene, isoledene, (Z)-caryophyllene, α-gurjunene, (E)-caryophyllene, aromadendrene, β-copaene, α-himachalene, α-humulene, allo-aromadendrene, (E)-β-farnesene, γ-muurolene, germacrene D, γ-himachalene, trans-cadina-1(6),4-diene, β-selinene, (E)-β-ionone, α-selinene, β-himachalene, α-muurolene, cis-cadina-1,4-diene, γ-cadinene, (E,E)-α-farnesene, trans-calamenene, δ-cadinene, δ-amorphene, α-cadinene, trans-cadina-1,4-diene, (E)-α-bisabolene, α-calacorene, palustrol, caryophyllenyl alcohol, spathulenol, β-calacorene, caryophyllene oxide, salvial-4(14)-en-1-one, gleenol, copaborneol, β-copaen-4-α-ol, β-oplopenone, junenol, rosifoliol, 1-epi-cubenol, muurola-4,10(14)-dien-1-β-ol, 1,10-di-epi-cubenol, α-corocalene, α-acorenol, α-muurolol, epi-α-cadinol, caryophylla-4(12),8(13)-dien-5-ol, α-cadinol, cadalene, eudesma-4(15),7-dien-1β-ol, n-heptadecane, 2-pentadecanone,6,10,14-trimethyl-, n-nonadecane, hexadecanoic acid, phytol, n-heneicosane, n-tricosane, n-pentacosane, n-hexacosane, n-heptacosane, n-octacosane, n-nonacosane	Zorzetto et al., 2015
H. retusum Aucher	Nonane, α-pinene, Camphene, Verbenene, β-pinene, β-myrcene, p-cymene, Limonene, 1,8-cineole, δ3-carene, γ-terpinene, α-terpinolene, Benzene-1-methyl-4, Undecane, β-thujene, Chrysantenone, allo-ocimene, Trans-pinocarveole, Trans-verbenol, Pinocarvone, Borneol, Terpinene-4-ol, Thymol, α-terpineol, Trans-carveol, Sabinyl acetate, 2-cyclohexene-1-one, Bicycloelemene, α-cubebene, α-longipinene, α-ylangene, α-copaene, β-bourbonene, β-	Bagci & Yuce, 2011a

	elemene, β-cubebene, Aromadendrene, Neo alloocimene, Naphthalene, Germacrene D, Bicyclogermacrene, α-amorphene, δ-cadinene, Calacorene, 3-hexene-1-ol, Spathulenol, Copaene, α-cadinol, Benzilbenzoate, n-hexadecanoic acid, Aromadendrene epoxide, Phytol, Tricosane, Tetracosane, Eicosane	
H. richeri Vill.	2,6-Dimethyl heptane, 2-Methyl octane, n-Nonane, 2-Methyl 4-heptanone, α-Pinene, β-Pinene, Sabinene, β-Myrcene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, p-Cymene, 2,3,5-Trimethyl decane, Pentyl isovalerate, (E)-Thujone, α-Ylangene, α-Copaene, Linalool, β-Funebrene, β-Caryophylene, Terpinen-4-ol, α-Himachalene, β-Guaiene, trans-Pinocarveol, allo-Aromadendrene, β-Bisabolene, Germacrene D, α-Terpineol, Verbenone, Valencene, α-Muurolene, γ-Cadinene, Myrtenol, α-Calacorene, (Z)-Nerolidol, Caryophyllene oxide, Humulene epoxide-I, (E)-Nerolidol, Viridiflorol, Globulol, epi-α-Cadinol, Spathulenol, epi-α-Muurulol, Thymol, α-Cadinol, Decanoic acid, Dodecanoic acid, n-Hexacosane, trans-Phytol, Hexadecanoic acid	Ferretti et al., 2005
H. richeri Vill. subsp. *grisebachii* (Boiss.) Nyman	Nonane, α-Thujene, α-Pinene, Camphene, Thuja-2,4(10)-diene, Sabinene, β-Pinene, Myrcene, 2-Pentylfuran, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, Undecane, Fenchol, α-Campholenal, trans-Pinocarveol, trans-Verbenol, Camphene hydrate, Pinocarvone, p-Mentha-1,5-dien-8-ol, Terpinen-4-ol, α-Terpineol, cis-Dihydro carvone, trans-Dihydro carvone, Decanal, (Z)-3-Hexenyl-3-methylbutanoate, Tridecane, Undecanal, α-Cubebene, α-Ylangene, α-Copaene, 1-Undecanol, β-Bourbonene, β-Cubebene, β-Elemene, Dodecanal, β-Caryophyllene, β-Copaene, trans-Muurola-3,5-diene, (E)-β-Farnesene, α-Humulene, allo-Aromadendrene, cis-Muurola-4(14),5-diene, trans-Cadina-1(6),4-diene, γ-Muurolene, α-Amorphene, Germacrene D, β-Selinene, trans-Muurola-4(14),5-diene, Bicyclogermacrene, α-Muurolene, (E,E)-α-Farnesene, Germacrene A, γ-Cadinene, δ-Cadinene, Zonarene, Methyl dodecanoate, trans-Cadina-1,4-diene, α-Cadinene, α-Calacorene, Salviadienol, trans-Nerolidol, 1,5-epoxysalvial-4(14)-ene, Spathulenol, Caryophyllene oxide, Globulol, salvial-4(14)-en-1-one, Viridiflorol, Rosifoliol, Ledol, Humulene epoxide II, 1,10-di-epi-Cubenol, Junenol, 1-epi-Cubenol, Acorenol, epi-α-Cadinol (syn. τ-cadinol), epi-α-Murrolol (syn. τ-muurolol), α-Muurolol (syn. torreyol), cis-Guaia-3,9-dien-11-ol, α-Cadinol, Selin-11-en-4-ol, Germacra-	Đorđević et al., 2011

	4(15),5,10(14)-trien-1-α-ol, Acorenone, Heptadecane, Hexahydrofarnesyl acetone, Nonadecane, Isophytol, Eicosane, Octadecanal, Heneicosane, Phytol, 1-Docosene, Tricosane, Tetracosane, Pentacosane, Tetracosanal, Heptacosane, Hexacosanal, Nonacosane, Hentriacontane	
H. roeperianum G.W.Schimp. ex A.Rich.	α-pinene, undecane, 2-pentylfuran, 6-methyl-5-hepten-2-one, α-copaene, β-bourbonene, β-caryophyllene, aromadendrene, α-himachalene, (Z)-β-farnesene, acetophenone, α-humulene, γ-curcumene, γ-himachalene, α-muurolene, β-curcumene, δ-cadinene, γ-cadinene, ar-curcumene, calamenene (correct isomer not identified), α-calacorene, caryophyllene oxide, humulene epoxide-II, spathulenol, α-eudesmol, β-eudesmol, caryophylladienol-II, (2E,6E)-farnesol	Crockett et al., 2007
H. rumeliacum Boiss.	Nonane, α-Thujene, (-)-α-Pinene, (-)-β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, (-)-Limonene, cis-Ocimene, γ-Terpinene, α-Terpinolene, Undecane, (+)-Terpinen-4-ol, (+)-α-Terpineol, Undecanal, β-Elemene, Dodecanal, (-)-(E)-Caryophyllene, (Z)-β-Farnesene, α-Amorphene, ar-Curcumene, Germacrene D, α-Selinene, γ-Cadinene, δ-Cadinene, Spathulenol, (-)-Caryophyllene oxide	Saroglou et al., 2007
H. rumeliacum Boiss. subsp. *apollinis* Robson & Strid	α-Pinene, Camphene, β-Pinene, Myrcene, n-Decane, α-Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, n-Undecane, n-Nonanal, α-Campholenal, allo-Ocimene, Camphor, Isoborneol, α-Longipinene, α-Ylangene, α-Copaene, β-Elemene, β-Longipinene, (E)-Caryophyllene, β-Gurjunene, α-Himachalene, α-Humulene, Dehydro-Aromadendrene, γ-Muurolene, Germacrene-D, β-Selinene, α-Selinene, α-Muurolene, γ-cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, Caryophyllene oxide	Petrakis et al., 2005
H. rumeliacum subsp. *apollinis* (Boiss. & Heldr.) N.Robson & Strid	α-Pinene, Camphene, β-Pinene, Myrcene, n-Decane, α-Terpinene, p-Cymene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, n-Undecane, n-Nonanal, α-Campholenal, allo-Ocimene, Camphor, Isoborneol, α-Longipinene, α-Ylangene, α-Copaene, β-Elemene, β-Longipinene, (E)-Caryophyllene, β-Gurjunene, α-Himachalene, α-	Couladis et al., 2003

	Humulene, dehydro-Aromadendrane, γ-Muurolene, Germacrene D, β-Selinene, α-Selinene, α-Muurolene, γ-Cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, Caryophyllene oxide, Vulgarone B	
H. salsolifolium Hand. - Mazz.	Nonane, α-pinene, Camphene, Verbenene, β-pinene, β-myrcene, p-cymene, Limonene, 1,8-cineole, δ3-carene, γ-terpinene, α-terpinolene, Benzene-1-methyl-4, Undecane, β-thujene, Chrysantenone, allo-ocimene, Trans-pinocarveole, Trans-verbenol, Pinocarvone, Borneol, Terpinene-4-ol, Thymol, α-terpineol, D-verbonene, Trans-carveol, Sabinyl acetate, 2-cyclohexene-1-one, 2,6-octadiene-1-ol, Bicycloelemene, α-cubebene, α-longipinene, α-ylangene, α-copaene, β-bourbonene, β-elemene, β-cubebene, Aromadendrene, Naphthalene, Germacrene D, Adamantane, Bicyclogermacrene, α-amorphene, δ-cadinene, Calacorene, 3-hexene-1-ol, Spathulenol, Globulol, Copaene, α-cadinol, β-humulene, Benzilbenzoate, Benzoicacide, n-hexadecanoic acid, Tricosane, Tetracosane	Bagci & Yuce, 2011a
H. scabrum L.	α-Pinene, Camphene, Hexanal, Undecane, β-Pinene, Myrcene, α-Terpinene, Limonene, 1,8-Cineole, o-Mentha-1(7),5,8-triene, (Z)-3-Hexenal, Amylfuran(2-pentylfuran), γ-Terpinene, p-Cymene, Terpinolene, Octanal, 2-Nonanone, Nonanal, γ-Campholene aldehyde, β-Thujone, 6-Methyl-5-hepten-2-ol, cis-Linalool oxide (furanoid), (E,Z)-2,4-Heptadienal, 3-Nonanol, α-Campholene aldehyde, 2-Nonanol, Camphor, β-Bourbonene, Linalool, Isopinocamphone, trans-p-Menth-2-en-1-ol, Pinocarvone, Fenchyl alcohol, (E,Z)-2,6-Nonadienal, 6-Methyl-3,5-heptadien-2-one, Terpinen-4-ol, β-Caryophyllene, Aromadendrene, cis-p-Menth-2-en-1-ol, Myrtenal, Acetophenone, p-Mentha-1,5-dien-8-ol, trans-Verbenol, p-Mentha-1,8-dien-4-ol (limonen-4-ol), γ-Muurolene, α-Terpineol, Borneol, Verbenone, Germacrene D, α-Muurolene, Carvone, Decanol, δ-Cadinene, γ-Cadinene, p-Methyl acetophenone, Myrtenol, 2-Tridecanone, (E,E)-2,4-Decadienal, trans-Carveol, p-Cymen-8-ol, (E)-Geranyl acetone, cis-Carveol, cis-p-Mentha-1(7),8-diene-2-ol, α-Calacorene, 1,5-Epoxy-salvial(4)14-ene, Dodecanol, Heptanoic acid, γ-Calacorene, Isocaryophyllene oxide, Caryophyllene oxide, Salvial-4(14)-en-1-one, Ledol, Humulene epoxide-II, Cubenol, Octanoic acid, Globulol, Heneicosane, Cumin alcohol, Spathulenol, 3,4-Dimethyl-5-pentylidene-2(5H)-furanone, Isothymol(2-isopropyl-4-methylphenol), Nonanoic acid, Thymol, T-Muurolol, δ-Cadinol, Isocarvacrol (4-isopropyl-2-methylphenol), α-Bisabolol,	Baser et al., 2002

	Carvacrol, Elimicine, trans-α-Bergamotol, Cadalene, Oxo-α-Ylangene, Myristicine, Decanoic acid, Tricosane, Caryophylla-2(12),6(13)-dien-5β-ol (caryophylladienol I), Caryophylla-2(12),6-dien-5α-ol (caryophyllenol I), Caryophylla-2(12),6-dien-5β-ol (caryophyllenol II), 4-Isopropyl-6-methyl-1,2,3,4-tetrahydronaphthalen-1-one, Dodecanoic acid, Benzyl benzoate, Tetradecanoic acid, Hexadecanoic acid	
	Hexanal, 2-(E)-Hexenal, Nonane, α-Pinene, Camphene, Thuja-2,4(10)-diene-2, β-Pinene, Myrcene, α-Phellandrene, 3-(Z)-Hexenyl acetate, α-Terpinene, p-Cymene, Limonene, 1,8-Cineol, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Acetophenone, Terpinolene, Undecane, Linalool, α-Campholenal, trans-Pinocarveol, Camphene hydrate, trans-Pinocamphone, Pinocarvone, p-Mentha-1,5-dien-8-ol, Borneol, α-Terpineol, Myrtenol, Myrtenal, Verbenone, cis-Carveol, Bicycloelemene, α-Terpinyl acetate, α-Ylangene, α-Copaene, β-Cubebene, (Z)-Jasmone, Methyl eugenol, Dodecanal, 6-epi-α-Cubebene, α-Guaiene, Aromadendrene, α-Humulene, E-(β)-Farnesene, trans-Cadina-1(6)-4-diene, γ-Muurolene, Germacrene-D, γ-Amorphene, Valencene, Bicyclogermacrene, Eudesma-2,4(15),11-triene, γ-Cadinene, δ-Cadinene, trans-Cadina-1,4-diene, α-Cadinene, α-Calacorene, β-Calacorene, 3-(Z)-Hexenyl benzoate, Spathulenol, Salvia-4(14)-en-1-one, α-Muurolol, α-Cadinol, Montsulfide, Benzyl benzoate, 6,10,14-Trimethyl-2-pentadecanone, Nonadecane	Javidnia et al., 2008
	Methylcyclopentane, Cyclohexane, 2-Methylhexane, n-Heptane, n-Octane, Hexanal, 2(E)-Hexenal, n-Nonane, α-Pinene, Camphene, Thuja-2,4(10)-diene, β-Pinene, 6-Methyl-5-hepten-2-one, 2-Pentylfuran, Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, (E)-β-Ocimene, γ-Terpinene, Acetophenone, trans-Linalool oxide, m-Cymenene, Terpinolene, Linalool, n-Undecane, 6-Camphenol, α-Campholenal, trans-Pinocarveol, cis-Verbenol, Camphor, Pinocarvone, Borneol, p-Mentha-1,5-dien-8-ol, Terpinen-4-ol, p-Cymen-8-ol, α-Terpineol, Myrtenal, n-Decanal, Verbenone, trans-Carveol, Carvone, Geraniol, Thymol, Carvacrol, α-Cubebene, α-Ylangene, Isoledene, α-Copaene, β-Elemene, β-Caryophyllene, β-Copaene, Aromadendrene, α-Humulene, allo-Aromadendrene, α-Amorphene, Germacrene D, trans-Muurola-4(14),5-diene, γ-Amorphene, Bicyclogermacrene, δ-Amorphene, γ-Cadinene, δ-	Morteza-Semnani et al., 2006

	Cadinene, trans-Calamenene, trans-Cadina-1(2),4-diene, α-Calacorene, (E)-Nerolidol, 3(Z)-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, Viridiflorol, n-Hexadecane, 1,10-di-epi-Cubenol, epi-α-Cadinol, Caryophylla-4(14),8(15)-dien-5-β-ol, epi-α-Muurolol, α-Muurolol, α-Eudesmol, α-Cadinol, Cadalene, Eudesma-4(15),7-dien-1-β-ol, n-Heptadecane, n-Octadecane, n-Nonadecane	
	Tricyclene, α-Pinene, β-Pinene, 2-Carene, Sabinene, 3-Carene, Myrcene, α-Phellandrene, Limonene, cis-Ocimene, trans-Ocimene, γ-Terpinene, p-Cymene, α-Longipinene, α-Copaene, Isocaryophyllene, β-Caryophyllene, allo-Aromadendrene, α-Humulene, γ-Gurjunene, Viridiflorene, Germacrene-D, γ-Cadinene, Calamenene, Cuparene, Cadalene, Cedrol, Eugenol	Cakir et al., 1997
	n-Nonane, α-Pinene, β-Pinene, Limonene, Undecane, α-Campholene aldehyde, cis-Pinocarveol, p-Mentha-1,8-diene-8-ol, p-Cymen-8-ol, (+)-Isomenthol, Myrtenal, Verbenone, trans-(+)-Carveol, Carvone, Thymol, Carvacrol, α-Cubebene, Piperitenone oxide, α-Ylangene, α-Copaene, β-Caryophylene, Aromadendrene, Germacrene-D, Alloaromadendrene, α-Amorphene, β-Selinene, α-Elemene, α-Muurolene, γ-Cadinene, δ-Cadinene, α-Cadinene, Calacorene, Spathulenol, α-Cadinol, β-Eudesmol	Akhbari et al., 2012
	α-Pinene, Camphene, Undecane, β-Pinene, Sabinene, Myrcene, α-Phellandrene, α-Terpinene, Limonene, β-Phellandrene, (Z)-β-Ocimene, γ-Terpinene, (E)-β-Ocimene, p-Cymene, Terpinolene, (Z)-3-Hexenyl acetate, α-Campholene aldehyde, β-Bourbonene, Linalool, Terpinen-4-ol, Acetophenone, (Z)-3-Hexenyl tiglate, α-Humulene, γ-Muurolene, α-Terpineol, Borneol, Germacrene D, Valencene, α-Selinene, Bicyclogermacrene, δ-Cadinene, γ-Cadinene, Calamenene, α-Calacorene, Caryophyllene oxide, Spathulenol, (Z)-3-Hexen-1-yl benzoate, T-Cadinol, Eremoligenol, T-Muurolol, α-Eudesmol, α-Cadinol, Pentacosane	Tabanca et al., 2015
	n-Nonane, α-Thujene, α-Pinene, Camphene, Sabinene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, 1,8-Cineole, γ-Terpinene, Terpinolene, n-Undecane, Linalool, Nonanal, β-Thujone, cis-p-Menth-2-en-1-ol, α-Campholenal, trans-	Tozlu et al., 2011

	Pinocarveol, trans-p-Menth-2-en-1-ol, trans-Verbenol, Camphor, Menthone, trans-Pinocamphone, Pinocarvone, Terpinen-4-ol, p-Cymen-8-ol, α-Terpineol, Myrtenal, Verbenone, Pulegone, Carvone, Piperitone, Bornyl acetate, Thymol, Carvacrol, α-Cubebene, Piperitenone, Piperitenone oxide, α-Copaene, β-Bourbonene, β-Elemene, 4aα,7α,7aβ-Nepetalactone, 4aα,7β,7aα-Nepetalactone, β-Caryophyllene, β-Gurjunene, Aromadendrene, (Z)-β-Farnesene, α-Humulene, Alloaromadendrene, cis-Muurola-4(14),5-diene, γ-Gurjunene, γ-Muurolene, Germacrene D, β-Selinene, Valencene, δ-Selinene, Viridiflorene, α-Muurolene, β-Bisabolene, γ-Cadinene, δ-Cadinene, cis-Calamenene, α-Cadinene, α-Calacorene, β-Calacorene, cis-3-Hexenyl benzoate, Spathulenol, Caryophyllene epoxide, Viridiflorol, 1-epi-Cubenol, Cubenol, α-Cadinol, (Z,Z)-Farnesyl acetone	
	α-Thujan, α-Pinene, α-Fenchene, Camphene, β-Pinene, β-Myrcene, α-Phellandrene, α-Terpinene, p-Cymene, Limonene, (Z) β-Ocimene, (E) β-Ocimene, γ-Terpinene, Terpinolene, α-Campholene aldehyde, Borneol, Terpin 4 ol, Thymol, Carvacrol, α-Cubebene, α-Copaene, β-Elemene, β-Caryophyllene, α-Humulene, Germacrene-D, Bicyclogermacrene, γ-Cadinene, Δ-Cadinene, Spathulenol, α-Cadinol <Epi->, Phytol	Pirbalouti et al., 2014
	p-Cymene, Thymol, Carvacrol, Camphor, Methylcyclopentane, n-Nonane, α-Pinene, β- pinene, Myrcene, Limonene, Acetophenone, α-Campholenal, p-Cymen-8-ol, Verbenone, α-Amorphene, Bicyclogermacrene, Γ-Cadinene	Cetin et al., 2011
	α-Pinene, Thuja-2,4(10)-diene, β-Pinene, α-Campholenal, trans-Pinocarveol, trans-Verbenol, Pinocarvone, p-Cymen-8-ol, α-Terpineol, Myrtenol, Verbenone, trans-Carveol, cis-Tetrahydrojasmine, cis-Verbenyl acetate, α-Campholenic acid, trans-Soberol, α-Guaiene, γ-Muurolene, β-Selinene, α-Muurolene, γ-Cadinene, trans-Calamenene, (3Z)-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, α-Cadinol	Sharopov et al., 2010
	n-Nonane, α-Thujene, α-Pinene, Camphene, Thuja-2,4-diene, β-Pinene, β-Myrcene, p-Cymene, Limonene, n-Undecane, α-Pinene oxide, Eucarvone, Camphor, Pinocarvone, 4-Terpineol, α-Terpineol, Myrtenal, Berbenone, α-Cubebene, α-Copaene, β-Bourbonene, β-Elemene, E-Caryophyllene, Aromadendrene, α-Humulene, 9-epi-E-Caryophyllene, γ-	Dadkhah et al., 2014

	Muurolene, α-Amorphene, Germacrene D, β-Selinene, α-Muurolene, γ-Cadinene, cis-Calamenene, α-Calacorene, cis-3-Hexenyl benzoate, Spathulenol, Caryophyllene oxide, Salvia-4(14)-en-1-one, Humulene epoxide II, epi-α-Muurolol, α-Cadinol, Cadalene, Tricosane	
	2-Methyl-octane, Nonane, α-Pinene, Camphene, β-Pinene, cis-3-Hexenyl acetate, α-Phellandrene, α-Terpinene, Limonene, trans-Ocimene, γ-Terpinene, 2-Methyl-decane, α-Campholene aldehyde, α-Terpineol, α-Cubebene, α-Yalangene, α-Copaene, β-Elemene, α-Gurjunene, β-Caryophyllene, β-Farnesene, α-Humulene, allo-Aromadrene, α-Amorphene, Germacrene-D, Valencene, γ-Cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, cis-Calamenene, cis-3-Hexenyl-benzoate, Spathulenol, Caryophyllene oxide, tau-Cadinol, Torreyol, α-Cadinol, Pentacecanone trimethyl(cas), n-Tetracosane	Ghiasvand et al., 2016
H. scabrum L. (flower)	Carvacrol, α-Copaene, β-Caryophyllene, Germacrene-D, Aromadendrene, β-Selinene, α-Amorphene, α-Selinene, γ-Cadinene, Dodecanoic acid,methyl ester (lauric acid), Caryophyllene oxide, Tetradecanoic acid,methyl ester (myristic acid), Pentadecanoic acid,methyl ester (pentadecanoic acid), 2-Pentadecanone,6,10,14-trimethyl, 9-Hexadecenoic acid,methyl ester (9-hexadecenoic acid), Hexadecanoic acid,methyl ester (palmitic acid), 9,12-Octadecadienoic acid,methyl ester (linoleic acid) or ω-6, 9,12,15-Octadecatrienoic acid,methyl ester (linolenic acid) or ω-3, Phytol, Octadecanoic acid,methyl ester (stearic acid), Eicosanoic acid,methyl ester (arachidic acid), Tetracosane, Pentacosane, Docosanoic acid, methyl ester (behenic acid), Bis (2-ethylhexyl) phthalate, Hexatriacontane, Tetracosanoic acid, methyl ester (lignoceric acid)	Shafaghat, 2011
H. scabrum L. (leaf)	Carvacrol, Camphene, α-Cubebene, β-Elemene, β-Caryophyllene, Germacrene-D, Aromadendrene, β-Selinene, α-Amorphene, α-Selinene, E,E-α-Farnesene, γ-Cadinene, Dodecanoic acid,methyl ester (lauric acid), Spathulenol, Tetradecanoic acid,methyl ester (myristic acid), Pentadecanoic acid,methyl ester (pentadecanoic acid), 2-Pentadecanone,6,10,14-trimethyl, 1-Hexadecanol, Nonadecane, 9-Hexadecenoic acid,methyl ester (9-hexadecenoic	Shafaghat, 2011

	acid), Hexadecanoic acid,methyl ester (palmitic acid), Heptadecanoic acid,methyl ester (margaric acid), 9,12-Octadecadienoic acid,methyl ester (linoleic acid) or ω-6, 9,12,15-Octadecatrienoic acid,methyl ester (linolenic acid) or ω-3, Phytol, Octadecanoic acid,methyl ester (stearic acid), Tricosane, Eicosanoic acid,methyl ester (arachidic acid), Tetracosane, Pentacosane, Docosanoic acid,methyl ester (behenic acid), Bis (2-ethylhexyl) phthalate, Oxirane,heptadecyl, Hexatriacontane, Tetracosanoic acid,methyl ester (lignoceric acid), 2,6,10,14,18,22-Tetracosahexaene, Tetracosanal, Nonacosane, Hexacosanoic acid,methyl ester (cerotic acid), 22,23-dihydrostigmasterol	
H. scabrum L. (seed)	Thymol, Caryophyllene oxide, Dibutyl phthalate, 9,12-Octadecadienoic acid,methyl ester (linoleic acid) or ω-6, 9,12,15-Octadecatrienoic acid,methyl ester (linolenic acid) or ω-3, Phytol, Octadecanoic acid,methyl ester (stearic acid), Eicosanoic acid, methyl ester (arachidic acid), Docosanoic acid,methyl ester (behenic acid), Bis (2-ethylhexyl) phthalate, Tetracosanoic acid,methyl ester (lignoceric acid), Squalene, Nonacosane, 22,23-dihydrostigmasterol	Shafaghat, 2011
H. scabrum L. (stem)	β-Caryophyllene, Germacrene-D, β-Selinene, α-Amorphene, α-Selinene, γ-Cadinene, Dodecanoic acid,methyl ester (lauric acid), Caryophyllene oxide, Hexadecane,Tetradecanoic acid,methyl ester (myristic acid), Pentadecanoic acid,methyl ester (pentadecanoic acid), 7-Hexadecenoic acid,methyl ester (7-hexadecenoic acid), Hexadecanoic acid,methyl ester (palmitic acid), Heptadecanoic acid,methyl ester (margaric acid), 9,12-Octadecadienoic acid,methyl ester (linoleic acid) or ω-6, 9,12,15-Octadecatrienoic acid,methyl ester (linolenic acid) or ω-3, Phytol, Octadecanoic acid,methyl ester (stearic acid), Nonadecanoic acid,methyl ester (nonadecanoic acid), 9-Tricosane, Eicosanoic acid,methyl ester (arachidic acid), Heneicosanoic acid,methyl ester (heneicosanoic acid), Pentacosane, Docosanoic acid,methyl ester (behenic acid), Bis (2-ethylhexyl) phthalate, Oxirane,heptadecyl, Hexatriacontane, Tetracosanoic acid,methyl ester (lignoceric acid), Squalene, Nonacosane, Hexacosanoic acid,methyl ester (cerotic acid), 1-Dotriacontanol, γ-Sitosterol, 22,23-dihydrostigmasterol	Shafaghat, 2011

H. silenoides Juss.	n-Octane, trans-Hex-2-enal, 3-Hexen-1-ol, 2-Methyl-octane, n-Nonane, 2,3,5-Trimethyl-1,3-hexadiene, α-Thujene, α-Pinene, Camphene, β-Pinene, Myrcene, n-Decane, α-Phellandrene, p-Cymene, Terpinolene, Linalool, n-Undecane, n-Nonanal, Camphor, α-Terpineol, n-Decanal, 4-Octyl, propanoate, 1-Decanol, 1-Undecanol, Patchoulene, n-Dodecanal, (E)-β-Caryophyllene, trans-α-Bergamotene, β-Caryophyllene oxide, α-Santalol	García-de la Cruz et al., 2013
H. ternum A. St. Hil. (flowering)	Nonane, α-Pinene, β-Pinene, Limonene, (Z)-β-Ocimene, (E)-β-Ocimene, Undecane, α-Copaene, β-Elemene, Isocaryophyllene, β-Caryophyllene, Aromadendrene, α-Humulene, Dehydro-aromadendrane, allo-Aromadendrene, γ-Muurolene, Germacrene D, β-Selinene, δ-Selinene, α-Selinene, Bicyclogermacrene, α-Muurolene, Germacrene A, γ-Cadinene, 7-epi-α-Selinene, cis-Calamenene, β-Cadinene, trans-Calamenene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, Selina-3,7(11)-diene, Germacrene B, Nerolidol, Spathulenol, Caryophyllene oxide, Globulol, Epiglobulol, 5-epi-7-epi-α-Eudesmol, Humulene oxide II, 10-epi-γ-Eudesmol, 1-epi-Cubenol, γ-Eudesmol, τ-Cadinol, τ-Muurolol, α-Muurolol, β-Eudesmol, α-Eudesmol, α-Cadinol, 6-isobutyryl-5,7-dimethoxy-2,2-dimethyl-benzopyran, 6-isobutyryl-5-methoxy-2,2-dimethyl-benzopyran, 6-isobutyryl-7-methoxy-2,2-dimethyl-benzopyran	Ferraz et al., 2005
H. tetrapterum Fries	2-Methyl-octane, n-Nonane, α-Pinene, 3-Methyl-nonane, α-Terpinene, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, Terpinolene, n-Undecane, (Z)-4-Decenal, Nerol, Geraniol, Geranial, α-Longipinene, α-Copaene, β-Bourbonene, (E)-Caryophyllene, β-Gurjunene, allo-Aromadendrene, γ-Muurolene, ar-Curcumene, (E)-β-Ionone, β-Selinene, β-Himachalene, δ-Cadinene, α-Calacorene, (Z)-3-Hexenyl benzoate, Caryophyllene oxide, 1,10-di-epi-Cubenol, Himachalol, α-Cadinol	Pavlovic et al., 2006
H. thymbrifolium Boiss. & Noë	Nonane, α-Thujene, α-Pinene, β-Pinene, β-Myrcene, Benzene-1-methyl-2, δ-Limonene, cis-Ocimene, 1,3,6-Octatriene, γ-Terpinene, α-Terpinolene, Undecane, Chrysanthenone, allo-Ocimene, trans-Verbenol, Borneol, 3-Cyclohexen-1-ol, α-Terpineol, trans-Carveol, Geraniol, Cyclohexasiloxane, α-Cubebene, α-Longipinene, α-Ylangene, α-Copaene, β-Bourbonene, allo-Aromadendrene, β-cubebene, Aromadendrene, trans-β-Farnesene, Naphthalene, Germacrene D,	Bagci & Yuce, 2011c

	Bicyclogermacrene, α-Amorphene, δ-Cadinene, 3-Hexen-1-ol, Spathulenol, Cyclododecane, trans-β-Caryophyllene, α-Cadinol, 12-Norcyercene-B, Benzilbenzoate, 2-Pentadecanone, Cyclotetradecane, Nonadecane, n-Hexadecanoic acid, Eicosane, Heneicosane, Ethyllinoleolate, Docosane, Pentatriacontane, Tricosane, Nonacosane, Tetracosane	
H. thymopsis Boiss	α-pinene, undecane, tridecane, α-cubebene, longipinene, α-ylangene, α-copaene, pentadecane, β-bourbonene, β-cubebene, β-ylangene, bornyl acetate, β-copaene, hexadecane, β-elemene, β-caryophyllene, aromadendrene, allo-aromadendrene, epi-zonarene, heptadecane, γ-muurolene, germacrene D, α-muurolene, bicyclogermacrene, δ-cadinene, γ-cadinene, cadina-1,4-diene (= cubenene), α-cadinene, (E,E)-2,4-decadienal, cuparene, calamenene (correct isomer not identified), (E)-geranyl acetone, epi-cubebol, nonadecene, β-calacorene, 2,3,4-trimethylbenzaldehyde, α-calacorene, 1,5-epoxy-salvial(4)14-ene, cubebol, (E)-β-ionone, dodecanol, γ-calacorene, eicosane, 2,3,6-trimethylbenzaldehyde, salvial-4(14)-en-1-one, muurola-4,10(14)-dien-1-ol, cubenol, heneicosane, rosifoliol, spathulenol, (Z)-3-hexenyl benzoate, salviadienol, 6-epi-cubenol, 3,4-dimethyl-5-pentylidene-2(5H)-furanone, T-cadinol, docosane, T-muurolol, copaborneol, α-muurolol, carvacrol, trans-α-bergamotol, α-cadinol, torilenol, eudesma-4(15),7-dien-1β-ol, hexadecanol, pentacosane, octadecanol, heptacosane, eicosanol, octacosane, nonacosane, hexadecanoic acid	Ozkan et al., 2009
H. tomentosum L.	n-Octane, trans-2-Hexenal, 2-Methyl octane, n-Nonane, Heptanal, α-Pinene, Benzaldehyde, 3-Methyl nonane, Sabinene, β-Pinene, 6-Methyl-5-hepten-2-one, β-myrcene, (E,E)-2-4-Heptadienal, p-Cymene, Limonene, 1-8-Cineol, 2-Methyl decane, cis-Linalool oxyde (furanoid), Octanol, trans-Linalool oxyde (furanoid), Undecane, Linalool, β-Thujone, cis-p-Menth-2-en-1-ol, Menthone, Terpinene-4-ol, Neomenthol, α-Terpineol, Myrtenol, Verbenone, trans-Carveol, β-Cyclocitral, Cuminal, Geranial, cis-Chrysantenyl acetate, Tridecane, (E,E)-2-4-Decadienal, α-Terpinyl acetate, α-Cubebene, α-Copaene, α-Gurjunene, Tetradecane, Methyl eugenol, β-Caryophyllene, α-Humulene, ar-Curcumene, cis- β-Guaiene, Allo-Aromadendrene, γ-Muurolene, β-Ionone, Germacrene-D, α-Calacorene, δ-Cadinene, (E)-Nerolidol,	Hosni et al., 2008

	Lauric acid, Caryophyllene oxide, Hexadecane, Spathulenol, β-Oplopenone, T-Cadinol, α-Cadinol, α-Muurolol, Cadalene, Myristic acid, Octadecane, Nonadecane, Eicosane	
	α-pinene, limonene, cineole, cis-linalool oxide, 2-methyl-3-nonanone, 2-methyl-decane, linalool, n-undecane, pulegone, α-copaene, β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, γ-cadinene, δ-cadinene, $C_{15}H_{24}$, spathulenol, caryophyllene oxide, 1-hexadecene	Nogueira et al., 1998
H. tomentosum L. (Fernana)	(E)-3-Hexanal, n-Nonane, α-Pinene, 4-(Z)-Hepten-1-ol, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, p-Cymene, Limonene, trans-Linalool oxide (furanoid), n-Undecane, α-Campholenal, trans-Verbenol, neo-Isopulegol, Pinocarvone, α-Terpineol, Myrtenol, trans-Carveol, α-Copaene, β-Caryophyllene, alloaromadendrene, γ-Muurolene, β-Selinene, trans- -Cadinene, δ-Cadinene, Methyl dodecanoate, α-Cadinene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide, Humulene epoxide II, β-Oplopenone, 1-epi-Cubenol, Caryophylla-4(14),8(15)-dien-5-ol, epi-α-Cadinol, α-Cadinol, Cadalene, Eudesma-4(15),7-dien-1-β-ol, n-Eicosane, 1-Heneicosene, n-Heneicosane, n-Pentacosane	Rouis et al., 2013
H. tomentosum L. (Tbarka)	(E)-3-Hexanal, n-Nonane, α-Pinene, 4-(Z)-Hepten-1-ol, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, p-Cymene, Limonene, cis-Linalool oxide (furanoid), trans-Linalool oxide (furanoid), n-Undecane, α-Campholenal, Camphor, Pinocarvone, 1-Nonanol, α-Terpineol, Myrtenol, trans-Carveol, α-Cubebene, α-Ylangene, α-Copaene, Sativene, β-Caryophyllene, Geranyl acetone, alloaromadendrene, γ-Muurolene, β-Selinene, trans- -Cadinene, δ-Cadinene, α-Cadinene, (E)-Nerolidol, Spathulenol, Caryophyllene oxide, Humulene epoxide II, β-Oplopenone, 1-epi-Cubenol, Caryophylla-4(14),8(15)-dien-5-ol, epi-α-Cadinol, epi-α-Muurolol, α-Cadinol, Eudesma-4(15),7-dien-1-β-ol, Hexahydrofarnesylacetone, n-Eicosane, n-Heneicosane, n-Tricosane, n-Pentacosane	Rouis et al., 2013
H. triquetrifolium Turra	1-Hexanal, Nonane, α-Pinene, 3-methylnonane, β-Pinene, β-Myrcene, Decane, p-Cymene, Limonene, γ-Terpinene, 2-methyldecane, α-Terpinolene, Undecane, Nonanal, Borneol, Decanal, 1,1,2-trimethylcyclopentane, 1-Decanol, Cyclohexane, α-Cubebene, α-Longipinene, β-Caryophyllene, β-Cubebene, Aromadendrene, Nerylacetone, α-	Yuce & Bagci, 2012

	Humulene, α-Amorphene, Valencene, α-Muurolene, Naphthalene, δ-Cadinene, Cis-Calemenene, α-Cadinene, Calacorene, Caryophyllene oxide, Salvial-4(14)-en-1-one, Ylangene, α-Cadinol, Caryophyllene-II, 2-Pentadecanone, Heneicosane, Phytol, 9,12-Octadecadienoic acid, 9,12,15-Octadecatrien-1-ol, Tricosane	
	2-Methyl-octane, n-Nonane, α-Pinene, Camphene, 3-Methyl-nonane, Sabinene, β-Pinene, 6-Methyl-5-hepten-2-one, Myrcene, n-Decane, α-Phellandrene, α-Terpinene, o-Cymene, p-Cymene, Limonene, 1,8-Cineole, (Z)-β-Ocimene, (E)-β-Ocimene, γ-Terpinene, 2-Methyl-decane, Terpinolene, n-Undecane, n-Nonanal, endo-Fenchol, exo-Fenchol, α-Campholenal, trans-Pinocarveol, Borneol, Terpinen-4-ol, Naphtalene, α-Terpineol, Myrtenal, Verbenone, trans-Carveol, Pulegone, Carvone, Geraniol, Geranial, 3'-Methoxy-acetophenone, (E,E)-2,4-Decadienal, δ-Elemene, α-Cubebene, Cyclosativene, α-Ylangene, α-Copaene, β-Bourbonene, β-Cubebene, β-Elemene, β-Longipinene, Longifolene, β-Cedrene, (E)-Caryophyllene, β-Gurjunene, Aromadendrene, α-neo-Glovene, α-Humulene, (E)-β-Farnesene, Dehydro-Aromadendrene, allo-Aromadendrene, Drima-7,9(11)-diene, γ-Muurolene, Germacrene-D, (E)-β-Ionone, β-Selinene, Valencene, Viridiflorene, epi-Cubebol, Bicyclogermacrene, α-Muurolene, trans-β-Guaiene, (Z)-α-Bisabolene, β-Bisabolene, γ-cadinene, δ-Cadinene, Cadina-1,4-diene, α-Cadinene, α-Calacorene, α-Agarofuran, (E)-Nerolidol, (Z)-3-Hexenyl-benzoate, Spathulenol, Caryophyllene oxide, Humulene epoxide II, β-Oplopenone, 1-epi-Cubenol, γ-Eudesmol, Cubenol, Cedr-8(15)-en-9-a-ol, α-Muurolol, Vulgarone B, α-Cadinol, 14-Hydroxy-9-epi-(E)-caryophyllene, Bulnesol, Cadalene, Khusinol, α-Bisabolol, Phytol	Petrakis et al., 2005
	trans-2-hexenal, nonane, α-pinene, 3-methylnonane, β-pinene, myrcene, p-cymene, γ-terpinene, undecane, nonanal, α-terpineol, thymol, tridecane, α-cubebene, cyclosativene, α-ylangene, α-copaene, β-bourbonene, β-elemene, β-caryophyllene, β-copaene, aromadendrene, cis-muurola-3,5-diene, α-humulene, trans-β-farnesene, germacrene-D, β-selinene, δ-selinene, E-E-α-farnesene, γ-cadinene, δ-cadinene, trans-cadina-1(2),4-diene, α-cadinene, α-calacorene, germacrene-B, cis-3-hexenyl benzoate, caryophyllene oxide, salvial-4(14)-en-1-one, β-copaen-4-α-ol, juniperol, 1,10-di-	Sajjadi et al., 2015

	epi-cubenol, epi-α-cadinol, α-cadinol, khusinol, hexadecanoic acid, trans-phytol, ethyl linoleolate, n-tricosane, n-tetracosane, n-pentacosane	
	Hexene-3-ol, n-Nonane, α-Thujene, α-Pinene, Camphene, 1-Octen-3-ol, 6-Methyl-5-hepten-2-one, Sabinene, β-Pinene, Myrcene, α-Phellandrene, δ-3-Carene, α-Terpinene, 1.8-Cineole, (E)-β-Ocimene, Nonan-2-one, cis-Linalool oxide (furanoïde), trans-Linalool oxide (furanoïde), Terpinolene, α-Thujone, n-Undecane, β-Thujone, Linalool, allo-Ocimene, Fenchol, Isopentyl isovalerate, cis-Verbenol, p-Cymen-8-ol, α-Terpineol, p-Cymen-7-ol, Carvacrol methyl ether, Geraniol, (Z)-Methyl cinnamate, Carvacrol, trans-Carvyl acetate, Piperitenone, δ-Elemene, α-Cubebenez, Thymyl acetate, α-Copaene, α-Bourbonene, β-Damascenone, β-Bourbonene, Methyl eugenol, α-Cedrene, α-Gurjunene, β-Caryophyllene, β-Cubebene, Aromadendrene, Cadina-3.5-diene, α-Humulene, allo-Aromadendrene, 1-Dodecanol, γ-Muurolene, γ-Gurjunene, Germacrene D, ß-Ionone, Eremophilene, Bicyclogermacrene, 4-epi-Cubebol, α-Muurolene, α-Selinene, β-Bisabolene, β-Sesquiphellandrene, γ-Cadinene, β-Curcumene, δ-Cadinene, 7-epi-α-Selinene, cis-Calamenene, trans-Calamenene, Elemicine, Globulol, (E)-γ-Bisabolene, α-Calacorene, Germacrene-B, cis-Davanone, (E)-Nerolidol, Ledol, Palustrol, (Z)-Nerolidol, Spathulenol, Caryophyllene oxide, (Z)-Asarone, Salvial-4(14)-en-1-one, Humulene epoxide I, Rosifoliol, β-Oplopenone, Humulene epoxide II, 10-epi-γ-Eudesmol, Guaia-6. 10(14)-dien-4ß-ol, Cubenol, 12-epi-Cedrol, Caryophylla-4(14).8(15)-dien-5 á-ol, allo-Aromadendrene epoxide, α-Acorenol, γ-Eudesmol, T-Cadinol, ‘I-Muurolol, α-Selin-11-en-4-ol, α-Cadinol, β-Eudesmol, Eudesma-4(15).7-dien-4β-ol, α-Bisabolol, trans-α-Bergamotol, Germacrone, (2E.6E)-Farnesol, α-Cyperone, Betulenol, α-Eudesmol acetate	Hosni et al., 2007
H. triquetrifolium Turra (flowering)	Oil yield, n-Octane, 2-Methyloctane, n-Nonane, α-Pinene, 3-Methylnonane, β-Pinene, (E,E)-2-4-Heptadienal, Limonene, cis-Ocimene, 2-Methyldecane, α-Terpinolene, cis-Linalol oxide (furanoid), trans Linalol oxide (furanoid), Undecane, Linalool, α-Terpineol, (E,E)-2-4-Decadienal, α-Cubebene, α-Longipinene, Longicyclene, β-Elemene, β-Caryophyllene, α-	Hosni et al., 2011a

	Humulene, allo-Aromadendrene, ar-Curcumene, α-Amorphene, γ-Muurolene, Germacrene-D, β-Selinene, α-Selinene, γ-Cadinene, δ-Cadinene, (E)-Nerolidol, Caryophyllene oxide, Spathulenol, T-Cadinol, α-Muurolol, α-Cadinol	
H. triquetrifolium Turra (fruiting)	Oil yield, n-Octane, trans-2-Hexenal, 2-Methyloctane, n-Nonane, Heptanal, α-Pinene, 3-Methylnonane, β-Pinene, β-Myrcene, α-Phellandrene, (E,E)-2-4-Heptadienal, Limonene, 1,8-Cineole, cis-Ocimene, 2-Methyldecane, α-Terpinolene, cis-Linalol oxide (furanoid), Octanol, trans Linalol oxide (furanoid), Undecane, Linalool, Fenchol, cis-p-Menth-2-en-1-ol, Terpinène-4-ol, α-Terpineol, Dodecane, Geraniol, cis-Chrysantenyl acetate, Geranial, Tridecane, (E,E)-2-4-Decadienal, α-Cubebene, α-Longipinene, α-Copaene, α-Ylangene, Longicyclene, α-Gurjunene, Tetradecane, β-Elemene, Methyl eugenol, β-Caryophyllene, trans-β-Farnesene, α-Humulene, allo-Aromadendrene, 1-Dodecanol, ar-Curcumene, α-Amorphene, γ-Muurolene, cis-β-Guaiene, Germacrene-D, β-Selinene, α-Selinene, Zingiberene, β-Himachalene, δ-Cadinene, α-Calacorene, (E)-Nerolidol, Lauric acid, Caryophyllene oxide, Spathulenol, Ledene, Hexadecane, β-Oplopenone, T-Cadinol, α-Muurolol, α-Cadinol, 2-Pentadecanone, Myristic acid, Cyclododecane	Hosni et al., 2011a
H. triquetrifolium Turra (vegetative)	Oil yield, n-Octane, 2-Methyloctane, n-Nonane, α-Pinene, 3-Methylnonane, β-Pinene, Limonene, cis-Ocimene, 2-Methyldecane, α-Terpinolene, Undecane, Fenchol, Terpinène-4-ol, α-Terpineol, Geraniol, (E,E)-2-4-Decadienal, α-Cubebene, α-Longipinene, α-Copaene, Longicyclene, α-Gurjunene, β-Elemene, Methyl eugenol, β-Caryophyllene, trans-β-Farnesene, α-Humulene, allo-Aromadendrene, α-Amorphene, Germacrene-D, β-Selinene, α-Selinene, δ-Cadinene, Caryophyllene oxide, Spathulenol, T-Cadinol, α-Cadinol, Myristic acid	Hosni et al., 2011a
H. X moserianum auct.	α-pinene, hexanal, undecane, β-pinene, myrcene, limonene, 2-pentylfuran, 6-methyl-5-hepten-2-one, trans-linalool oxide (furanoid), cis-linalool oxide (furanoid), α-ylangene, α-copaene, β-bourbonene, β-cubebene, β-copaene, β-caryophyllene, aromadendrene, allo-aromadendrene, (Z)-β-farnesene, acetophenone, α-humulene, γ-muurolene, α-muurolene, (E,E)-α-farnesene, δ-cadinene, γ-cadinene, selina-3,7(11)-diene, calamenene, α-calacorene, γ-calacorene,	Demirci et al., 2005

	caryophyllene oxide, humulene epoxide-II, spathulenol, muurola-4,10(14)-dien-1-ol, T-cadinol, α-cadinol, caryophylladienol-II, caryophyllenol-II, pentacosane, heptacosane	

7.5. Summary of the isolated Diterpenes from the genus *Euphorbia, section Paralias*

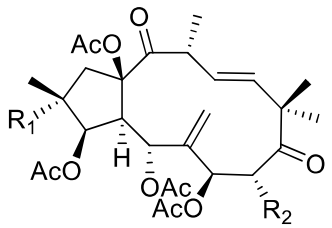
Diterpenes isolated from *Euphorbia* spp., section Paralias		
***Euphorbia* spp.**	**Compounds**	**References**
E. paralias L.	(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-isobutyryloxyjatropha-6(17),11 E-diene- 9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-angeloyloxyjatropha-6(17),11 E-diene-9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-3,5,7,15-tetraacetoxy-8-isobutyryloxyjatropha-6(17),11 E-diene-9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-(2-methylbutanoyloxy)jatropha-6(17),11E-diene-9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-benzoyloxyjatropha-6(17),11 E-diene-9,14-dione (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,8,15-hexaacetoxyjatropha-6(17),11 E-diene-9,14-dione, 3β-O-angeloyl-17-angeloyloxy-20-deoxyingenol, 3β-O-angeloyl-20-deoxyingenol, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-3-benzoyloxy-15-hydroxy-5-(2-hydroxyacetoxy)segetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,11 S*,12 S*,13 R*,14 R*,15 R*)-5,6,11,14,17-pentaacetoxy-3-benzoyloxy-15-hydroxysegetan-9-one (2 S*,3 S*,4 R*,5 R*,6 S*,8 R*,11 S*,12 S*,13 R*,14 R*,15 R*)-6,11,14-triacetoxy-3-benzoyloxy-15-hydroxy-5-(3-oxobutanoyl)-9-segetanone,	Jakupovic et al., 1998a

	(2 S*,3 S*,4 R*,5 R*,6 R*,8 S*,12 S*,13 S*,14 R*,15 R*)-5,8,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one	
	paralinone A, paralinone B	Oksuz et al., 1997
	presegetanin, segetatanin A, segetatanin B	Barile & Lanzotti, 2007
	segetene A, segetene B, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,11 S*,12 S*,13 R*,14 R*,15 R*)-5,6,11,14,17-pentaacetoxy-3-benzoyloxy-15-hydroxysegetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-3-benzoyloxy-15-hydroxy-5-(2-hydroxyacetoxy)segetan-9-one, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-isobutyryloxyjatropha-6(17),11 E-diene- 9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-angeloyloxyjatropha-6(17),11 E-diene-9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-3,5,7,15-tetraacetoxy-8-isobutyryloxyjatropha-6(17),11 E-diene-9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-(2-methylbutanoyloxy)jatropha-6(17),11E-diene-9,14-dione, (2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-benzoyloxyjatropha-6(17),11 E-diene-9,14-dione,	Abdelgaleil, 2001

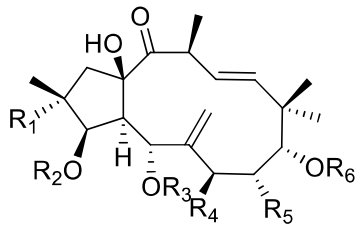
	(1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-1,5,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 S*,12 S*,13 S*,14 R*,15 R*)-5,8,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one	
E. portlandica L.	euphoportlandol A, euphoportlandol B	Madureira et al., 2006
E. segetalis L.	(2 S*,3 S*,4 R*,5 R*,7 S*,8 S*,9 S*,l3 S*,15 R*)-3,9,15-trihydroxy-5,7,8-triisobutyryloxyjatropha-6(17),11 E-dien-14-one, (2 S*,3 S*,4 R*,5 R*,7 S*,8 S*,9 S*,l3 S*,15 R*)-3,9,15-trihydroxy-7-isobutyryloxy-5,8-bis(2-methylbutanoyloxy)jatropha-6(17),11 E-dien-14-one, (2 S*,3 S*,4 R*,5 R*,7 S*,8 S*,9 S*,l3 S*,15 R*)-3,9,15-trihydroxy-5,7-diisobutyryloxy-8-(2-methylbutanoyloxy)jatropha-6(17),11 E-dien-14-one, 1,5,8,9-tetraacetoxy-2-benzoyloxyacetoxy-7-isobutyryloxyjatropha-6(17),11E-dien-14-one, 5-acetoxy-3-benzoyloxy-9-cinnamoyloxy-15-hydroxyjatropha-6(17),11E-dien-14-one, 5-acetoxy-3,9-dicinnamoyloxy-15-hydroxyjatropha-6(17),11E-dien-14-one, (2 S,3 S,4 S,5 R,7 S,8 S,9 S,11 E,13 S,15 R)-5,7,8-tris(2-methylbutanoyloxy)-3,9,15-trihydroxyjatropha-6(17),11-dien-14-one, terracinolides A/B/C/E/H/I, 13R-hydroxyterracinolide B/I, 3-dehydro-7,12-di-O-acetyl-8-O-angeloyl-2-epi-ingenol, 14-hydroxy-7,12-di-O-acetyl-8-O-angeloyl-2-epi-ingenol, 14-hydroxy-12-O-acetyl-8-O-angeloyl-2-epi-ingenol, 20-O-acetylingenol, 20-deoxyingenol, 13α-acetoxy-3β-O-benzoyl-17-benzoyloxyingenol, 20-acetoxy-3β-O-angeloyl-17-angeloyloxyingenol,	Jakupovic et al., 1998b

	13α-acetoxy-3β-O-angeloyl-17-benzoyloxyingenol, 3β-O-angeloyl-17-angeloyloxy-20-deoxyingenol, 3β-O-angeloyl-20-deoxyingenol, 20-O-acetyl-3β-O-angeloylingenol, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-3-benzoyloxy-15-hydroxy-5-(2-hydroxyacetoxy)segetan-9-one, (2 S*,3 S*,4 R*,5 R*,6 R*,7 S*,8 R*,12 R*,13 S*,14 R*,15 R*)-5-angeloyloxy-3-benzoyloxy-6,14:8,14-diepoxy-7,13,15,17-tetrahydroxy-15-epi-presegetan-9-one, (1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 S*,12 S*,13 S*,14 R*,15 R*)-1,5,8,14-tetraacetoxy-3-benzyloxy-15-hydroxyparalian-9-one, (1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-1,5,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one, (2 R*,3 R*,4 S*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-2,5,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one, (1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-1,5,14,17-tetraacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one, (1 S*,2 R*,3 S*,4 R*,5 R*,6 R*,13 S*,14 R*,15 R*)-1,5,14,17-tetraacetoxy-3-benzoyloxy-8,10(18)11-hexadehydropepluane-11,15-diol	
	(1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-1,5,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one (= segetalol-1,5,14-triacetate-3-benzoate)	Ferreira et al., 1998
E. taurinensis All.	6,14-Diacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxy-9-oxo-segetane,	Redei et al., 2018

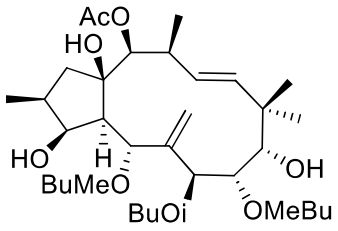
	paralinone A, 5,9-Diacetoxy-3-cinnamoyloxy-15-hydroxy-14-oxo-jatropha-6 (17),11E-diene, 5-acetoxy-3,9-dicinnamoyloxy-15-hydroxyjatropha-6(17),11E-dien-14-one, 3-O-angeloyl-20-deoxyingenol, 3-O-angeloyl-17-angeloyloxy-20-deoxyingenol, 20-O-acetyl-3-O-angeloyl-17-angeloyloxyingenol	

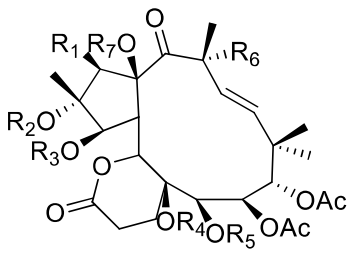


	R_1	R_2
(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-isobutyryloxyjatropha-6(17),11 E-diene- 9,14-dione	OAc	OiBu
(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-angeloyloxyjatropha-6(17),11 E-diene-9,14-dione	OAc	OAng
(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-3,5,7,15-tetraacetoxy-8-isobutyryloxyjatropha-6(17),11 E-diene-9,14-dione	H	OiBu
(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-(2-methylbutanoyloxy)jatropha-6(17),11E-diene-9,14-dione	OAc	OMeBu
(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,15-pentaacetoxy-8-benzoyloxyjatropha-6(17),11 E-diene-9,14-dione	OAc	OBz
(2 R*,3 R*,4 S*,5 R*,7 S*,8 R*,13 R*,15 R*)-2,3,5,7,8,15-hexaacetoxyjatropha-6(17),11 E-diene-9,14-dione	OAc	OAc

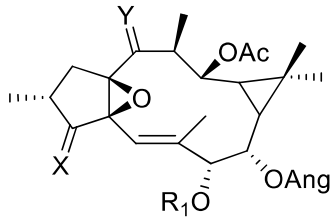


	R_1	R_2	R_3	R_4	R_5	R_6
(2 S*,3 S*,4 R*,5 R*,7 S*,8 S*,9 S*,l3 S*,15 R*)-3,9,15-trihydroxy-5,7,8-triisobutyryloxyjatropha-6(17),11 E-dien-14-one	H	H	iBu	OiBu	OiBu	H
(2 S*,3 S*,4 R*,5 R*,7 S*,8 S*,9 S*,l3 S*,15 R*)-3,9,15-trihydroxy-7-isobutyryloxy-5,8-bis(2-methylbutanoyloxy)jatropha-6(17),11E-dien-14-one	H	H	MeBu	OiBu	OMeBu	H
(2 S*,3 S*,4 R*,5 R*,7 S*,8 S*,9 S*,l3 S*,15 R*)-3,9,15-trihydroxy-5,7-diisobutyryloxy-8-(2-methylbutanoyloxy)jatropha-6(17),11E-dien-14-one	H	H	iBu	OiBu	OMeBu	H
1,5,8,9-tetraacetoxy-2-benzoyloxyacetoxy-7-isobutyryloxyjatropha-6(17),11E-dien-14-one	OAc	BzOAc	Ac	OiBu	OAc	Ac
5-acetoxy-3-benzoyloxy-9-cinnamoyloxy-15-hydroxyjatropha-6(17),11E-dien-14-one	H		Ac	H	H	Cinn
5-acetoxy-3,9-dicinnamoyloxy-15-hydroxyjatropha-6(17),11E-dien-14-one	H	Cinn	Ac	H	H	Cinn
5,9-Diacetoxy-3-cinnamoyloxy-15-hydroxy-14-oxo-jatropha-6(17),11E-diene	H	Cinn	Ac	H	H	Ac

(2 S,3 S,4 S,5 R,7 S,8 S,9 S,11 E,13 S,15 R)-5,7,8-tris(2-methylbutanoyloxy)-3,9,15-trihydroxyjatropha-6(17),11-dien-14-one	

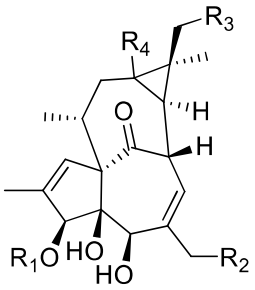


	R_1	R_2	R_3	R_4	R_5	R_6	R_7
terracinolide A	H	Ac	Ac	Bz	iBu	H	Ac
terracinolide B	H	Ac	Ac	Ac	iBu	H	Ac
terracinolide C	H	Ac	H	Ac	iBu	H	Ac
terracinolide E	H	Ac	Ac	Bz	Pr	H	Ac
terracinolide H	H	Ac	H	iBu	iBu	H	Ac
13R-hydroxyterracinolide B	H	Ac	Ac	Ac	iBu	OH	Ac
13R-hydroxyterracinolide I	OAc	Ac	Ac	Ac	iBu	OH	Ac
terracinolide I	OAc	Ac	Ac	Ac	iBu	H	Ac

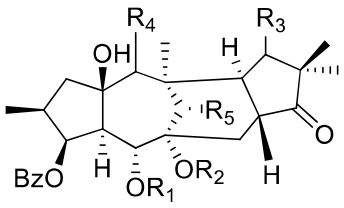


	R_1	X	Y
3-dehydro-7,12-di-O-acetyl-8-O-angeloyl-2-epi-ingenol	OAc	O	O

14-hydroxy-7,12-di-O-acetyl-8-O-angeloyl-2-epi-ingenol	Ac	β-OH, H	α-OH, H
14-hydroxy-12-O-acetyl-8-O-angeloyl-2-epi-ingenol	H	β-OH, H	α-OH, H

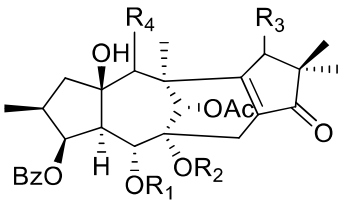


	R_1	R_2	R_3	R_4
20-O-acetylingenol	H	OAc	H	H
20-deoxyingenol	H	H	H	H
13α-acetoxy-3β-O-benzoyl-17-benzoyloxyingenol	Bz	H	OBz	OAc
20-acetoxy-3β-O-angeloyl-17-angeloyloxyingenol	Ang	OAc	OAng	H
13α-acetoxy-3β-O-angeloyl-17-benzoyloxyingenol	Ang	H	OBz	OAc
3β-O-angeloyl-17-angeloyloxy-20-deoxyingenol	Ang	H	OAng	H
3β-O-angeloyl-20-deoxyingenol	Ang	H	H	H
20-O-acetyl-3β-O-angeloylingenol	Ang	OAc	H	H

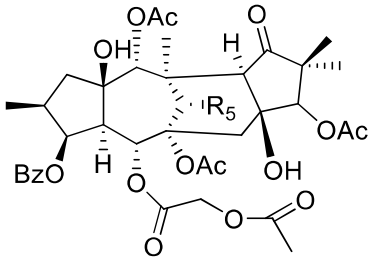
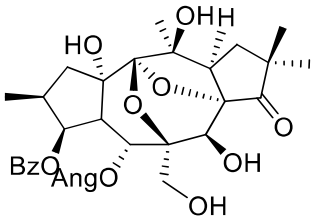
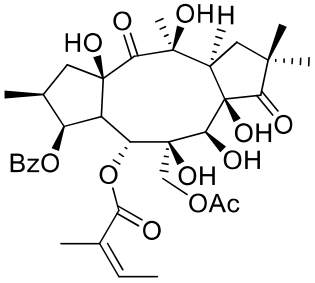


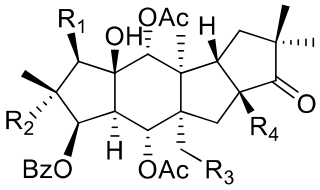
	R_1	R_2	R_3	R_4	R_5
(2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one	$COCH_2OAc$	Ac	H	αOAc	OAc

(2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,11,14,17-tetraacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxysegetan-9-one	$COCH_2OAc$	Ac	OAc	αOAc	OAc
(2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,12 S*,13 R*,14 R*,15 R*)-6,14,17-triacetoxy-3-benzoyloxy-15-hydroxy-5-(2-hydroxyacetoxy)segetan-9-one	$COCH_2OAc$	Ac	H	αOAc	OAc
euphoportlandol B	Ac	H	OAc	αOAc	OAc
(2 S*,3 S*,4 R*,5 R*,6 R*,8 R*,11 S*,12 S*,13 R*,14 R*,15 R*)-5,6,11,14,17-pentaacetoxy-3-benzoyloxy-15-hydroxysegetan-9-one	Ac	Ac	OAc	αOAc	OAc
paralinone B	$COCH_2OAc$	Ac	OAc	αOAc	OAc
paralinone A	$COCH_2OAc$	Ac	H	αOAc	OAc
segetanin A	Ac	Ac	OH	αOAc	OAc
6,14-Diacetoxy-5-(2-acetoxyacetoxy)-3-benzoyloxy-15-hydroxy-9-oxo-segetane	$COCH_2OAc$	Ac	H	βOAc	H
(2 S*,3 S*,4 R*,5 R*,6 S*,8 R*,11 S*,12 S*,13 R*,14 R*,15 R*)-6,11,14-triacetoxy-3-benzoyloxy-15-hydroxy-5-(3-oxobutanoyl)-9-segetanone	$COCH_2OAc$	Ac	OAc	αOAc	H



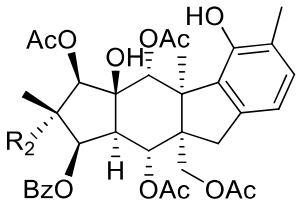
	R_1	R_2	R_3	R_4
euphoportlandol A	Ac	H	OAc	αOAc
segetene A	Ac	Ac	Ac	βOAc
segetene B	$COCH_2OAc$	Ac	H	αOAc

segetanin B	
(2 S*,3 S*,4 R*,5 R*,6 R*,7 S*,8 R*,12 R*,13 S*,14 R*,15 R*)-5-angeloyloxy-3-benzoyloxy-6,14:8,14-diepoxy-7,13,15,17-tetrahydroxy-15-epi-presegetan-9-one	
presegetanin	



	R_1	R_2	R_3	R_4
(1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 S*,12 S*,13 S*,14 R*,15 R*)-1,5,8,14-tetraacetoxy-3-benzyloxy-15-hydroxyparalian-9-one	OAc	H	H	OAc
(1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-1,5,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one	OAc	H	H	H
(2 R*,3 R*,4 S*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-2,5,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one	H	OAc	H	H

(1 R*,2 R*,3 S*,4 R*,5 R*,6 R*,8 R*,12 R*,13 S*,14 R*,15 R*)-1,5,14,17-tetraacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one	OAc	H	OAc	H
(2 S*,3 S*,4 R*,5 R*,6 R*,8 S*,12 S*,13 S*,14 R*,15 R*)-5,8,14-triacetoxy-3-benzoyloxy-15-hydroxyparalian-9-one	H	H	H	OAc

(1 S*,2 R*,3 S*,4 R*,5 R*,6 R*,13 S*,14 R*,15 R*)-1,5,14,17-tetraacetoxy-3-benzoyloxy-8,10(18)11-hexadehydropepluane-11,15-diol	

7.6. CV Grafakou Maria-Eleni

Birth: 22/06/1994, Sparta, Greece
Address: Agias Elenis 28, Zografos, 15772, Attica, Greece
Tel: +30 6939003938
Email: megrafakou@pharm.uoa.gr
Google Scholar Profile: Maria Eleni Grafakou

Education

Ph.D. candidate, Department of Pharmacognosy and Chemistry of Natural Products, Faculty of Pharmacy, National and Kapodistrian University of Athens (NKUA) *10/2017-10/2021*

- Thesis title: Chemical and biological activity of: *Calea* sp. from Panama; *Centaurea* sp. from Algeria; *Hypericum* sp. from Crete; Euphorbia sp. from Central Greece, supervisor Prof. H. Skaltsa

- Funded by European Social Fund- ESF, implemented by the State Scholarships Foundation-IKY

- 4 years program (2017-2021); special entrance Doctorate students - Bachelor's Degree with honors*

*During my Ph.D I had taken 10 ECTS from Master's Level Curriculum «Improvement, Production and Evaluation of Bioactive Natural Products» as specified from the regulations of the Program of Postgraduate Studies (special entrance Doctorate students on account of Bachelor's Degree with honors (9.15/10); without attending Master's level, as it is generally required) *10/2017-04/2018*

- Chemistry of Natural Products *(Grade 10/10)*
- Spectroscopy II *(Grade 10/10)*

- During my Ph.D. I had also taken the following course (internet based, Uppsala University) *01-03/2019*

- Pharmaceutical Bioinformatics (7.5 ECTS), *Pass with distinction (Grade 17/20)*

Guest Researcher: Chair of Pharmaceutical Biology, Universität Regensburg *10/2020-04/2021*

Erasmus+ Program Intern: Chair of Pharmaceutical Biology, Universität Regensburg *03-09/2020 & 03-08/2019*

- Part of my PhD was conducted under the supervision of Prof. Dr. Joerg Heilmann

Diploma of Pharmacy, NKUA *2012-2017*
excellent marks (9.15/10)

High Faculty Gythio, Lakonia, Greece *2009-2012*
excellent marks (19.6/20)

Experience

Teaching Assistant: *05-09/2021*
Undergraduate program, Department of Pharmacognosy and Chemistry of Natural Products, Faculty of Pharmacy, NKUA (Introduction to Laboratory Practices, Laboratory Practical Courses of Pharmacognosy 1 and 2)

Academic Service: *03/2021-ongoing*
Reviewed for Journals: Natural Product Research (Taylor & Francis), Phytochemistry (Elsevier)

Research fellow *03-08/2019 &*
Laboratory of Pharmaceutical Biology, Universität Regensburg, Germany *03/2020-04/2021*

Teaching Assistant in 1st years' course "Introduction to Laboratory Practices", Faculty of Pharmacy, NKUA *10/2017-02/2018*

Pharmacy Intern Community Open Pharmacy Kraniotis Xaralampos (Zografos, Attica, Greece) *01-07/2017*

Hospital Pharmacy Intern 'Andreas Syggros' Hospital of Cutaneous & Venereal Diseases, Athens, Greece *10/2016-01/2017*

Skills

Instrumentation experience NMR, HPLC-DAD, HPLC-RI, Flash-LC, hydro-distillation, GC-MS, LC-MS, UV/Vis, a_D, CD

Bio-assays experience cultivation of normal/cancerous cell lines and gram+ bacteria, MTT assay, ICAM-1 assay, disc-diffusion assay, double-dilution assay, in vitro metabolism assay with human liver microsomes, animal handling

Digital skills Topspin, DataAnalysis, MassHunter, Graphpad, SwissADME, Reaxys, Chemdraw, Microsoft Office

Languages English Cambridge Certificate of Proficiency (C2); German (Goethe-Zertifikat B2); Greek (mother tongue)

Teaching Skills Assistance in undergraduate student courses; Supervision of undergraduate research

Honors, Awards, Grants

Compensatory Scholarship Teaching Assistant, Faculty of Pharmacy, NKUA *05-09/2021*

Young Researcher Workshop, eSymposium, Society for Medicinal Plant and Natural Product Research (GA) Selected (1/12 young researchers) to present a 10min talk: Another proof of wound healing activity from the genus *Hypericum*? *07/11/2020*

Best Poster Presentation Award *10/2019*
Poster: Phytochemical investigation and cytotoxic evaluation of *A. coarctata* Poir. on MCF-7 and HeLa cell lines. 1st Panhellenic Congress of Ethnopharmacology, 11-13/10/2019, Athens, Greece

Young Investigator Fellowship Award *05/2019*
19th International Congress of the Society of Ethnopharmacology, June12-14/2019, Dresden, Germany

Doctorate Scholarship *2018-2021*
State Scholarships Foundation (IKY) «IKY Fellowships of Excellence for Postgraduate Studies in Greece» through the Operational Programme (Greece-EU) «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432)

Graduation with honors *08/11/2017*
third place in the graduation ceremony

Undergraduate Scholarship *2014-2017*
"Legacy Thomas Sourlis"

Excellence Award *03/10/2014*
Laboratory of Experimental Physiology, Faculty of Medicine, NKUA

Excellence Award *2013*
Association of the Lakonians "Lykourgos"
Excellence Student Award *2013*
"The Great Moment for Education" by Eurobank in collaboration with the Greek Ministry of Education, Research and Religious Affairs

Publications in peer-reviewed journals

- **Maria-Eleni Grafakou**, Christina Barda, Helen Skaltsa, Joerg Heilmann. Study on the metabolism of natural sesquiterpene lactones in human liver microsomes using LC-Q-TOF-MS/MS, under submission.
- **Maria-Eleni Grafakou**, Christina Barda, Joerg Heilmann, Helen Skaltsa. Macrocyclic diterpenes from *Euphorbia deflexa*, an endemic spurge from Greece. Journal of Natural Products, 84, 11, 2893–2903, 2021, DOI 10.1021/acs.jnatprod.1c00654.
- **Maria-Eleni Grafakou**, Christina Barda, George Karikas, Joerg Heilmann, Helen Skaltsa. Cajamolides A-N: cytotoxic and anti-inflammatory sesquiterpene lactones from the leaves of *Calea jamaicensis* (L.) L. Biorganic Chemistry, 116, 105351, 2021, DOI 10.1016/j.bioorg.2021.105351.
- **Maria-Eleni Grafakou**, Christina Barda, Diandra Pintać, Marija Lesjak, Joerg Heilmann, Helen Skaltsa. Prenylated acylphloroglucinols from *Hypericum jovis* with anti-inflammatory potential. Planta Medica, 87(14):1184-1191, 2021, DOI 10.1055/a-1556-9721.
- **Maria-Eleni Grafakou**, Christina Barda, Joerg Heilmann, Helen Skaltsa. *In vitro* cytotoxic and anti-inflammatory activities of sesquiterpene lactones from *Centaurea papposa*. Natural Products Research, 2021, eprint, DOI 10.1080/14786419.2021.1955882.
- Artemis Daskalakis, **Maria-Eleni Grafakou**, Barda Christina, Zacharias Kypriotakis, Jörg Heilmann, Helen Skaltsa. Secondary metabolites from *Hypericum trichocaulon* Boiss. & Heldr., growing wild in the island of Crete. Biochemical Systematics and Ecology, 97, 104294, 2021. DOI 10.1016/j.bse.2021.104294
- Elias Valiakos, Marios Marselos, **Maria-Eleni Grafakou**, Eleni Skaltsa, Nikolaos Sakellaridis. Animal medicines and treated diseases in Nikolaos Myrepsos' Dynameron. Journal of Ethnopharmacology, 276, 11419, 2021, DOI 10.1016/j.jep.2021.114191
- **Maria-Eleni Grafakou**, Aggeliki Diamanti, Eleytheria Simirioti, Asimina Terezaki, Christina Barda, Ioannis Sfiniadakis, Michail Rallis, Helen Skaltsa. Essential oils from *Hypericum* spp.: wound healing effects in mice. Planta Medica International Open, 8(02), e69-e77, 2021. DOI: 10.1055/a-1492-3634
- Christina Barda, **Maria-Eleni Grafakou**, Eleutherios Kalpoutzakis, Joerg Heilmann, Helen Skaltsa. Chemical composition of *Crepis foetida* L. and *C. rubra* L. volatile constituents and evaluation of the in vitro anti-inflammatory activity of salicylaldehyde rich volatile fraction. Biochemical Systematics and Ecology, 96, 104256, 2021, DOI 10.1016/j.bse.2021.104256
- **Maria-Eleni Grafakou,** Christina Barda, Ekaterina-Michaela Tomou, Helen Skaltsa. The genus *Genista* L.: A rich source of bio-active flavonoids. Phytochemistry, 181, 112574, 2021, DOI 10.1016/j.phytochem.2020.112574
- Konstantina Papakosta, **Maria-Eleni Grafakou**, Christina Barda, Ioannis V. Kostopoulos, Ourania Tsitsilonis, Helen Skaltsa. Anti-cancer potential of the genus *Achillea* L. and evaluation of cytotoxic activity of *A. coarctata* Poir. on MCF-7 and HeLa cell lines. Current Medicinal Chemistry, 2020, DOI 10.2174/0929867327666200505092514
- **Maria-Eleni Grafakou**, Aggeliki Diamanti, Eleftheria Antaloudaki, Zacharias Kypriotakis, Ana Ćirić, Marina Soković, Helen Skaltsa. Chemical composition and antimicrobial activity of the essential oils of three *Hypericum* species growing wild in the island of Crete, Greece. Applied Sciences, Section Chemistry, 2020, 10(8), 2823. DOI 10.3390/app10082823

- **Maria-Eleni Grafakou**, Helen Skaltsa. Flavonol arabinofuranosides from the methanolic extract of *Hypericum jovis*. Planta Medica, 85, 1484, 2019. DOI 10.1055/s-0039-3399884
- **Maria-Eleni Grafakou**, Aggeliki Diamanti, Elefteria Simirioti, Asimina Terezaki, Christina Barda, Michail Rallis, Helen Skaltsa. Preliminary results: Essential oils from *Hypericum* spp. growing wild in Greece and their wound healing effects. Planta Medica, 85, 1543, 2019. DOI 10.1055/s-0039-3400074
- Eirini Christodoulou, **Maria-Eleni Grafakou**, Helen Skaltsa, Nikolaos Kadoglou, Nikolaos Kostomitsopoulos, Georgia Valsami. Preparation, chemical characterization and determination of serum and tissue pharmacokinetics of crocetin after oral and intravenous administration of Saffron (*Crocus sativus* L.) aqueous extract to C57/BL6J mice. Journal of Pharmacy and Pharmacology, 71, 753–764, 2019. DOI 10.1111/jphp.13055
- **Maria-Eleni Grafakou**, Samah Djeddi, Hamel Tarek, Helen Skaltsa. Secondary metabolites from the aerial parts of *Centaurea papposa* (Coss.) Greuter. Biochemical Systematics and Ecology, 76, 15-2, 2018. DOI 10.1016/j.bse.2017.11.005

Book Chapter

Maria-Eleni Grafakou, Christina Barda Helen Skaltsa. *Teucrium* species: Biology and Applications, Chapter VIII: Secondary metabolites of *Teucrium* species with toxic effects. Springer Nature, 2020. DOI 10.1007/978-3-030-52159-2

Poster Presentations in conferences

- **Maria-Eleni Grafakou**, Christina Barda, Joerg Heilmann, Helen Skaltsa. ICAM-1 bioassay for the evaluation of sesquiterpene lactones from *Calea* and *Centaurea* spp., 69th Annual Conference of the Society for Medicinal Plant and Natural Product Research (GA), Bonn, Germany, Virtual Conference, 05-08/09/2021, published in the book of abstracts.
- **Maria-Eleni Grafakou**, Christina Barda, Helen Skaltsa, Joerg Heilmann. Identification of parthenolide metabolites in human liver microsomes by LC-Q-TOF-MS/MS, 69th Annual Conference of the Society for Medicinal Plant and Natural Product Research (GA), Bonn, Germany, Virtual Conference, 05-08/09/2021, published in the book of abstracts.
- **Maria-Eleni Grafakou**, Aggeliki Diamanti, Elefteria Simirioti, Asimina Terezaki, Christina Barda, Ioannis Sfiniadakis, Michail Rallis, Helen Skaltsa. Wound healing efficacy of EO from *Hypericum* spp. growing wild in Greece. 1st Panhellenic Congress of Ethnopharmacology, Athens, Greece, 11-13/10/2019, published in the book of abstracts.
- Konstantina Papakosta, **Maria-Eleni Grafakou**, Christina Barda, Ioannis V. Kostopoulos, Ourania Tsitsilonis, Helen Skaltsa. Phytochemical investigation and cytotoxic evaluation of *A. coarctata* Poir. on MCF-7 and HeLa cell lines. 1st Panhellenic Congress of Ethnopharmacology, Athens, Greece, 11-13/10/2019, published in the book of abstracts.
- **Maria-Eleni Grafakou**, Helen Skaltsa. Flavonol arabinofuranosides from the methanolic extract of *Hypericum jovis*. 67th Annual Conference of the Society for Medicinal Plant and Natural Product Research (GA), Innsbruck, Austria, 1-5/09/2019, published in the book of abstracts
- **Maria-Eleni Grafakou**, Aggeliki Diamanti, Elefteria Simirioti, Asimina Terezaki, Christina Barda, Michail Rallis, Helen Skaltsa. Preliminary results: Essential oils from *Hypericum* spp. growing wild in Greece and their wound healing effects. 67th Annual Conference of the Society for Medicinal Plant and Natural Product Research (GA), Innsbruck, Austria, 1-5/09/2019, published in the book of abstracts

- **Maria-Eleni Grafakou**, Aggeliki Diamanti, Eleftheria Antaloudaki, Helen Skaltsa. Chemical analysis of the essential oils of five *Hypericum* spp. growing wild in Greece. 19th International Congress of the Society of Ethnopharmacology, June12-14/2019, Dresden, Germany, published in the book of abstracts
- Eirini Christodoulou, **Maria-Eleni Grafakou**, Helen Skaltsa, Nikolaos Kadoglou, Nikolaos Kostomitsopoulos, Georgia Valsami. Saffron aqueous extract: phytochemistry & determination of crocetin's serum and tissue pharmacokinetics after oral and intravenous administration to C57/BL6J mice. 24-29/11/2018: 30th International Symposium on the Chemistry of Natural Products and 10th International Congress on Biodiversity, Athens, Greece, published in the book of abstracts
- **Maria-Eleni Grafakou**, George A. Karikas, Mahabir P. Gupta, Helen Skaltsa. NMR metabolomic strategy: secondary metabolites from Calea jamaicensis. Athens Conference on Advances in Chemistry (ACAC2018), 30 Oct-2 Nov, 2018, Athens, Greece. *Oral* presentation published in the Book of Abstracts
- **Maria-Eleni Grafakou**, George A. Karikas, Mahabir P. Gupta, Helen Skaltsa. Ten metabolites isolated from *Calea jamaicensis* (L.) L. 8th International Conference on Oxidative Stress in Skin Medicine and Biology. 6-9 Sep 2018, Andros, Greece (poster presentation published in the Book of Abstracts)
- **Maria-Eleni Grafakou**, Zacharias Kypriotakis, Helen Skaltsa. Chemical analysis of the essential oil of *Hypericum jovis*, a narrow endemic species of Crete (Greece). 8th International Conference on Oxidative Stress in Skin Medicine and Biology. 6-9 Sep 2018, Andros, Greece (poster presentation published in the Book of Abstracts)
- **Maria-Eleni Grafakou**, George A. Karikas, Mahabir P. Gupta, Helen Skaltsa. Preliminary study: Secondary metabolites from *Calea jamaicensis* (L.) L., First Austrian Summit on Natural Products Science and Technology behind Phytopharmacy, Phytocosmetics and Phytonutrition, Seefeld, Austria, 14-16/01, 2018
- **Maria-Eleni Grafakou**, Samah Djeddi, Hamel Tarek, Helen Skaltsa. Cnicin as chemotaxonomic marker in *Centaurea papposa* (Coss.) Greuter, First Austrian Summit on Natural Products Science and Technology behind Phytopharmacy, Phytocosmetics and Phytonutrition, Seefeld, Austria, 14-16/01, 2018.
- **Maria-Eleni Grafakou**, Samah Djeddi, Helen Skaltsa. Secondary metabolites from the aerial parts of *Centaurea papposa* (Coss.) Greuter. 3rd International Conference on Natural Products Utilization: From Plants to Pharmacy Shelf, Bansko, Bulgaria 18-21/11, 2017, PP85.

ORAL Presentations in conferences

- **Grafakou M.E.** what is the potential of *Hypericum* plants in drug discovery and therapy?, 9th International Conference on Oxidative Stress in Skin Medicine and Biology, eCongress, 12/09/2021 (15 min).
- **Grafakou M.E** Wound healing effects from Essential Oils from Hypericum spp. growing wild in Greece. 20th International Congress of the International Society of Ethnopharmacology, Virtual Congress, 18-20/04/21 (12 min).
- **Grafakou M.E** Another proof of wound healing activity from the genus *Hypericum*? (10 min), 14th Young Researcher Workshop (selected as 1 of 12 young researchers to present their results), eSymposium, Society for Medicinal Plant and Natural Product Research (GA), 07/11/2020.
- **Grafakou M.E**. Wound healing efficacy of EO from *Hypericum* spp. growing wild in Greece (selected from posters to be orally presented -in Greek) 1st Panhellenic Congress of Ethnopharmacology, 11-13/10/2019, Athens, Greece
- **Grafakou M.E.** NMR strategy in plant metabolomics (presented in Greek, 20 min). 3rd Panhellenic Scientific Conference of Pharmacy Students, 08-09/12/2018, Thessaloniki, Greece
- **Grafakou M.E.** NMR metabolomic strategy: Secondary metabolites from *Calea jamaicensis*. Athens Conference on Advances in Chemistry (ACAC2018), 30 Oct-2 Nov, 2018, Athens, Greece (10 min)
- **Grafakou M.E.** Chemical analysis of the essential oil of *Hypericum jovis*, a narrow endemic species of Crete (Greece). Poster presentation presented (3 min) orally in 8th International Conference on Oxidative Stress in Skin Medicine and Biology. 6-9 Sep 2018, Andros, Greece

- **Grafakou M.E.** Ten metabolites isolated from *Calea jamaicensis* (L.) L. Poster presentation presented (3 min) orally in 8th International Conference on Oxidative Stress in Skin Medicine and Biology. 6-9 Sep 2018, Andros, Greece

Conference attendance – Seminars

08-12/09/2021: 9th International Conference on Oxidative Stress in Skin Medicine and Biology, Andros Greece, eCongress

05-08/09/2021: 69th Annual Conference of the Society for Medicinal Plant and Natural Product Research (GA), Bonn, Germany, Virtual Conference

05/08/2021: How to green your lab, Nature Research webcasts

28/07/21: How to write an impressive thesis using an AI language assistant. Webinar by Trinka & Enago Academy

27-31/07/21: Cutting Edge Science in Cellular and Molecular Biomedicine, 5-Day online Faculty Enrichment Programme, Amity University of Molecular Medicine and Stem Cell Research, Amity University Uttar Pradesh India

14/06/2021: Online seminar for ERC & MSCA from NKUA Funding Attraction Office.

07/11/2020: eSymposium: 14th Young Researcher Workshop, Society for Medicinal Plant & Natural Product Research GA

8,15,22/10/20: Webinar "Real Skills for Scientists" National Documentation Center (EKT)

29/09/2020: Webinar "Funding and career opportunities for young researchers" National Documentation Center (EKT)

23/09/2020: Webinar "Developing Patents in the fields of Chemistry and Health Sciences". Presented by Dr. Dimitrios Roukounas, European Patent Attorney. Archimides Center for Research, Innovation and Business.

21/04/2020: Webinar "Best Practices in Submitting Research for Highly Selective Journals". Researcher Academy on Campus. Presented by Dr. Robert D. Eagling, Editor-in-Chief Chem.

11-13/10/2019: 1st Panhellenic Congress of Ethnopharmacology, Athens, Greece

01-05/09/2019: 67th Annual Conference of the Society for Medicinal Plant and Natural Product Research (GA), Innsbruck, Austria

12-14/06/2019: 19th International Congress of the Society of Ethnopharmacology, Dresden, Germany

14/12/2018: One day Conference: Aspects of Medicine in Byzantine Times, Faculty of History and Archaeology, NKUA

08-09/12/2018: 3rd Panhellenic Scientific Conference of Pharmacy Students, Thessaloniki, Greece

24-29/11/2018: 30th International Symposium on the Chemistry of Natural Products and 10th International Congress on Biodiversity, Athens, Greece

30/10/2018-2/11/2018, Athens Conference on Advances in Chemistry (ACAC2018), Athens, Greece

22/09/2018: Scientific event: Women in Science Classes, Year1, L'oreal Hellas- Unesco, Athens

13/09/2018: Merck Chromatography Seminar, Merck Hellas, Athens

06-09/09/2018 8th International Conference on Oxidative Stress in Skin Medicine and Biology. Andros, Greece

11-12/5/2018-: One day conference «The Herbal plants in our lives», Center of Makrynitsa Environmental Education, Institute of Agriculture Sciences, Kifisias Avenue 182, Athens

4-5/05/2018 Rails girls Athens vol. 3, programming workshop

22/03/2018: Educational seminar for the electronic database /*REAXYS */ Elsevier from Mr. Carlos Rodriguez del Rio, Sciences Library, NKUA

15/03/2018: European Competitive Programs and their connection with Education and Training, State Scholarships Foundation (IKY)

14-16/01/2018: First Austrian Summit on Natural Products Science and Technology behind Phytopharmacy, Phytocosmetics and Phytonutrition, Seefeld, Austria

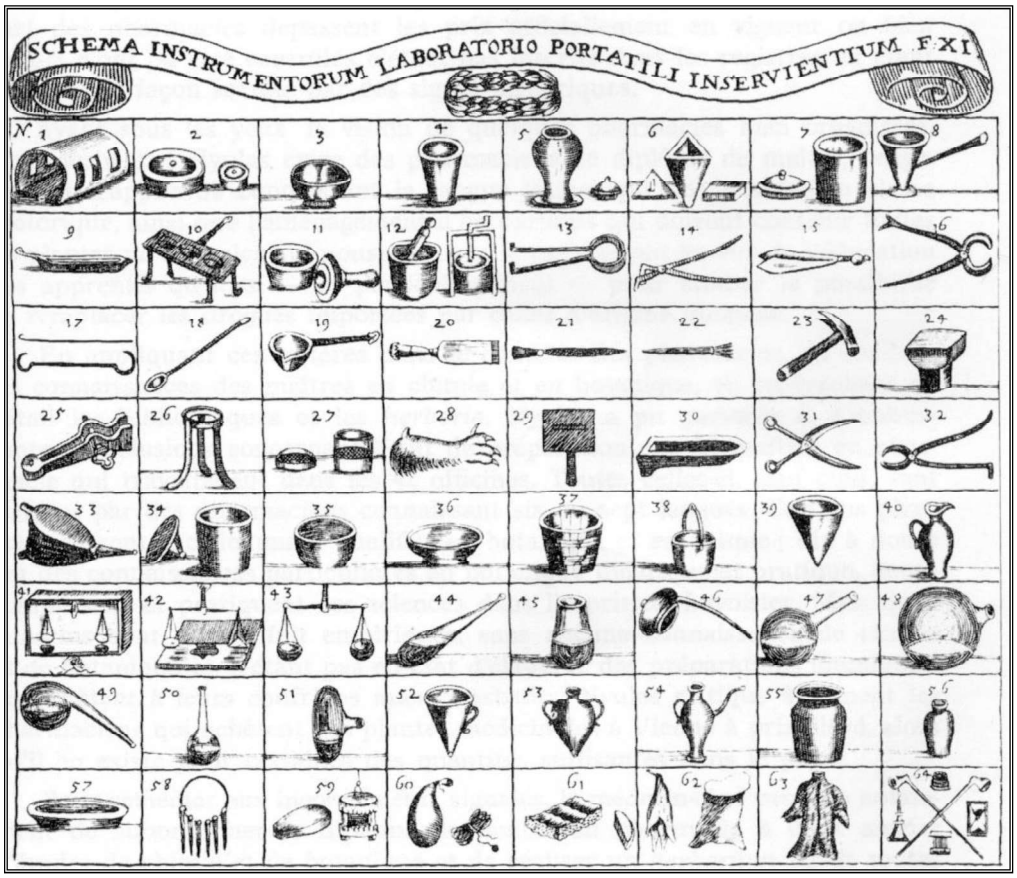
18-21/10/2017: 3rd International Conference on Natural Products Utilization: From Plants to Pharmacy Shelf, Bansko, Bulgaria
22/09/2017: Scientific event: The Importance of Vaccines (Prevention, Myths and Truths). NKUA, Center for Disease Control and Prevention
22/05/2017: International Conference Modern Greek Enlightenment: Views, Intersections, Investigations, Foundation for Pedagogical Studies and Applications
11-14/05/2017: 1st Hellenic-Chinese Congress on Health, Eugenides Foundation, Athens, Greece.
08/10/2015–11/10/2015: 17ο Panhellenic Pharmaceutical Congress
18/04/2015: Certificate of attendance in one day conference: Phytotherapy and its Development in the field of Pharmacy. Faculty of Pharmacy NKUA, Hellenic Pharmaceutical Society, Athens (Greece)
13-15/10/2014: 1st International Congress: From drug discovery to drug delivery, Royal Olympic Hotel, Athens, Greece
03/10/2014–05/10/2014: Days of Physiology in Mani. NKUA, Faculty of Medicine, Department of Physiology, Vathi, Municipality of Mani (Greece)
17/05/2013–18/05/2013: Certificate of attendance in 1st Student Conference in Anatomy NKUA, Faculty of Medicine, Anatomy Department, Athens (Greece)

Volunteer Experience

04/2018 - ongoing: Member of European Pharmaceutical Students Association-EPSA
03-04/2018: Athens Science Festival 2018
10/2017 - ongoing: Greek Pharmaceutical Students' Federation - GPSF
07/2017 - ongoing: Melios Animal protection
04/04/2017: Let's do it Greece 2017, Reforestation, Ymittos, Volunteer Group of Agricultural University of Athens

Interests

Sailing (participation in several National Championships, as well as in 2009 Balkan Sailing Championship, Servia), sewing, ornithology, fishing


SCHEMA INSTRUMENTORUM LABORATORIO PORTATILI INSERVIENTIUM F.XI