Investigation of Anti-infective Compounds within the Flowers of Myrtaceae

Sarath Parakumge Dayani Senadeera
BSc (Hons), MSc

Griffith School of Environment

Griffith Sciences

Griffith University, Australia

A thesis submitted in fulfilment of the requirements of the degree of

Doctor of Philosophy

March 2017

ABSTRACT

This thesis reports on the chemistry and anti-infective activity of the flowering plant family Myrtaceae (a family whose members dominate the Australian landscape). To understand the significance and importance of the Myrtaceae family from a phytochemical and medical perspective, a systematic literature review was carried out. The conclusion from this review was that the family is a major source of β-triketone, phloroglucinol, and volatile terpene natural products with the β-triketones in particular being a major focus of recent research. Over hundred new compounds from this class, many of which possess novel ring systems, have been reported in the last ten years. Furthermore their chemical diversity is matched with their high biological activity, most notably anti-infective activities including antiplasmodial, antibacterial and antiviral being reported for these compounds. All the findings of the literature review are documented in **Chapter 2** of this thesis.

As a result of this review the experimental research carried out in this dissertation focused on the antiplasmodial and antibacterial activity of natural product constituents isolated from the flowers of three Australian Myrtaceae plant species, *Corymbia intermedia*, *C. torelliana*, and *Angophora woodsiana* collected from South East Queensland. These plants were chosen because of the literature precedent for *Corymbia* species to be a major source of bioactive β-triketones and although *Angophora* species have not previously been investigated their close taxonomic relationship to the *Corymbia* suggested that they would likely be an additional source of β-triketone constituents.

A total of 24 β-triketone compounds, 14 of which are new, were isolated from methanol extracts of flowers of these three species. Structures of all the new and known compounds were elucidated using one-dimensional and two-dimensional NMR spectroscopy and mass spectrometry. The relative configurations of the stereogenic centres in the new compounds were assigned from detailed analysis of coupling constants in the ^{1}H NMR spectra and correlations observed in ROESY spectra.

Angophora woodsiana, a plant endemic to Australia yielded two new β-triketones, woodsianone A and woodsianone B and nine known β-triketones. (**Chapter 5**). The new compound, woodsianone A is only the third example of a β-triketone adduct containing a cadinene sesquiterpene. Woodsianone B is the first β-triketone epoxide derivative to be

isolated from a natural source. Woodsianone B is an oxidation product of an olefin which has previously been proposed to be an intermediate in the biosynthesis of β-triketone-terpene adducts. This study was the first chemical investigation of any species from the genus *Angophora* and revealed that it is another Myrtaceae genus to contain β-triketones. Some of the known β-triketones discovered in the flowers of *Angophora woodsiana* have previously been reported from other *Corymbia* species including *C. ficifolia,* and *C. watsoniana,* thus providing chemical evidence to support the morphological and molecular evidence for the close taxonomic relationship between the genus *Angophora* and *Corymbia*.

Five new β-triketone/pinene adducts, intermediones A-E (**Chapter 6**) were isolated from *Corymbia intermedia*. Intermediones A-C, are C-6 phenyl analogs of 4*S*/4*R*-ficifolidione reported previously from *C. ficifolia,* while intermedione E is a structural isomer of intermedione A. The three compounds intermedione A-C are diastereomeric and detailed analysis of ROESY data was used to resolve their relative configurations. Intermedione D, an oxidized analogue of intermedione A posesses a novel oxadispiro[bicyclo[4.1.1]octane-2,2'-furan-5',1"-cyclohexane tetracyclic ring system. The β-triketone chemistry of *Corymbia intermedia* was in agreement with previous chemical investigations of the genus and provided further evidence to substantiate the taxonomic differences between *Corymbia* and the genus *Eucalytpus* to which these species were previously assigned.

Chemical investigation of the flowers of *Corymbia torelliana* yielded six new β-triketones, torellianones A-F, torellianol A and the four known β-triketones 4*S*/4*R* ficifolidiones, kunzeanone A and kunzeanone B (**Chapter 7**). Six of the new compounds, torellianones A-F, which are β-triketone/flavanone adducts, were isolated as inseparable diastereomeric mixtures. To further complicate the interpretation of 1D and 2D NMR data for torellianones E and F, each diastereomer was present as rotating mixture of conformations and this resulted in NMR spectra in which signals for four isomers were present. Despite this complexity the structures and relative configuration of the two diastereomers as well as the structures of each rotamer were determined from NMR analysis. A search of the literature revealed regioisomers of torellianones E and F had been reported earlier; however, the ^{1}H NMR data previously reported was identical with that of the torellianones E and F. It was therefore concluded that the structures formerly reported was incorrect and has now been corrected. In addition, the published ^{1}H and ^{13}C NMR data for one of the known compounds, kunzeanone B, isolated previously from two *Kunzea* species was inconsistent with 1D and 2D NMR obtained in this study of *C. torelliana* and as a result the ^{1}H and ^{13}C NMR data for this

compound have now been corrected. The fourth new compound, torellianol A is a 1,2,3,5-tetrahydroxycyclohexane and is likely to be reduced analog of a simple β-triketone. This structure class is rare and to date torrelianol A is the only fully reduced β-triketone to be reported in the literature.

The isolated compounds were tested for antiplasmodial activity against the chloroquine-sensitive 3D7 strains of *Plasmodium falciparum*. Compounds showing 5 μM potency were also tested against the chloroquine resistant Dd2 strain of *P. falciparum*. Most of the compounds had IC_{50} values ranging from 1.5 μM - 15 μM. The most potent compounds were rhodomyrtone (IC_{50} 1.8 ± 1.0 μM against the 3D7 strain) and woodsianone B (2.53 ± 0.11 μM against the Dd2 strain). The antibacterial activity of the isolated compounds was tested against *Staphylococcus aureus* ATCC 157293 and the most potent activity (MIC 0.02 mM) was reported from woodsianone B. The structure-activity relationship of the isolated β-triketones was assessed for antiplasmodial and antibacterial activities. It was identified that the β-triketone part of the molecules was essential for both activities. The poor water solubility demonstrated by some of the compounds suggests that synthetic modifications to increase their water solubility could lead to improved bioactivity.

DECLARATION

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed) _____ (Date) 14/03/2017

Sarath Parakumge Dayani Senadeera

ACKNOWLEDGEMENTS

First, I am sincerely and heartily grateful to my principal supervisor Tony (Anthony) Carroll for the invaluable support, guidance, encouragement and patience he showed to me throughout my PhD study. Without his supervision, kind advice, unsurpassed knowledge and sheer enthusiasm in science, this thesis would not have been possible. I am truly indebted to him for directing me towards exciting research in natural products chemistry. Also I am sincerely thankful to my associate supervisor Vicky Avery for the support and guidance always provided to me during the course of my study. My knowledge and skills have definitely enriched under the supervision of Tony and Vicky, and for that I am most grateful.

I would also like to thank Griffith University for providing Griffith University Postgraduate Research Scholarship (GUPRS) and Griffith University International Postgraduate Research Scholarship (GUIPRS) and Griffith School of Environment for providing additional funding, which allowed me to pursue my research full-time.

I would like to thank Wendy Lao, Dongdong Wang, Mario Wibowo and Chao Wang for help with NMR and other experimental activities at Eskitis Institute, Sandra Duffy and Leonardo Lucantoni at Discovery Biology, Eskitis Institute for antiplasmodial activity testing and Joshua Hayton, Griffith School of Environment for antibacterial activity testing. Also, my thanks go to staff at Griffith School of Environment (ENV), Environmental Futures Research Institute (EFRI), Smart Water Research Centre (SWRC), Medical Research Institute (MRI), Sri Lanka. Especially, I would like to thank Catherine Pickering for great advice and support as HDR convenor, and Belinda Hachem, Christina Perry, Jennifer Dickson and Hee Lee for their great help with administration.

I would like to extend sincere thanks to my research group: Tanja Grkovic, Leesa Habener, Fan Yang, Joshua Hayton, Larissa Buedenbender, Laurence Jennings, Luke Robertson, Guy Kleks, Marion Pactat, Shimony Mohanty, Casey Hall and Parichat Thepthong for their ever willing help, friendship, advice and kind cooperation given throughout my study.

At last but not least, I would like to thank my beloved husband Niroshana, two sons Ishara and Tharusha, parents, my two sisters Chandi and Shyamanthi, relatives and friends for their love, encouragement, inspiration and commitments.

TABLE OF CONTENTS

Glossary

1D	one dimensional
2D	two dimensional
Da.	Daltons
br	broad
C_{18}	octadecyl bonded silica
C	degree Celsius
^{13}C NMR	carbon nuclear magnetic resonance
cm^{-1}	wave number unit
$CDCl_3$	deuterated chloroform
CH_2Cl_2	dichloromethane
COSY	correlation spectroscopy
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
d	doublet
dd	doublet of doublet
d_6-DMSO	deuterated dimethylsulfoxide
g	gram
HMBC	heteronuclear multiple bond correlation spectroscopy
HMQC	heteronuclear multiple quantum correlation spectroscopy
^{1}H NMR	proton nuclear magnetic resonance
ed-HSQC	*edited*-heteronuclear single quantum correlation spectroscopy
HPLC	high performance liquid chromatography
Hz	hertz
HRESIMS	high resolution electron spray ionization mass spectrometry
IC_{50}	50% inhibitory concentration
IR	infrared spectroscopy

J	coupling constant
$^{n}J_{CH}$	n bond hydrogen to carbon correlation (n = 2, 3, or 4)
MHz	megahertz
m	multiplet
μg	microgram
mg	milligram
mL	milliliters
m/z	mass-to-charge ratio
MeOH	methanol
MW	molecular weight
NMR	nuclear magnetic resonance spectroscopy
me	methyl
MS	mass spectrometry
q	quartet
ROESY	rotating-frame overhauser effect spectroscopy
s	singlet
t	triplet
UV	ultraviolet
ν_{max}	maximum absorption frequency
λ_{max}	maximum absorption wave length
δ	chemical shift
$[\alpha]_D$	specific rotation

CHAPTER 1

INTRODUCTION

1.0 Introduction

Natural products, known as primary and secondary metabolites, are chemical substances biosynthesized by living organisms. Primary metabolites found in all living organisms include carbohydrates, lipids, amino acids, nucleosides and proteins. They are essential for the survival of organisms and are involved in basic life functions such as cell division and growth, respiration, storage, and reproduction. Primary metabolites participate in metabolic processes but are also building blocks in the biosynthesis of secondary metabolites.[1] Secondary metabolites, which are not essential for the basic life functions of an organism, are produced by living organisms either to adapt to their environment or to acquire chemical defence mechanism against predators. For instance, it has been observed that organisms deprived of an immune system produce a higher number of secondary metabolites than others that possess an immune system.[2] Consequently, these secondary metabolites with diverse chemical structures often exhibit biological activities such as antimicrobial, antiparasitic, antigermination and antifeeding and insecticidal. As a result, natural products can be used to treat a wide range of biomedical problems, and have been used to treat and prevent particular diseases all over the world for thousands of years. This is evidenced by the clay tablets found from Mesopotamia, which document uses of medicinal oils from cypress and *Commiphora* species (myrrh) from cypress dating back to 2600 B.C., The Elbers Papyrus from Egypt in 2900 B.C., describes medications that originate from 700 plants and the Chinese Materia Medica contains 52 prescriptions written by Wu Shi Er Bing Fang in 1100 B.C.[3] The later development of the pain relieving drug, aspirin (**1.1**) in 1899, from salicin isolated from the willow tree, *Salix alba*, the isolation of morphine (**1.2**) from *Papaver somniferum* L. (opium poppy) in 1803 and the discovery of penicillin F (**1.3**) in 1928 are three examples of historically important natural product derived drugs (Figure 1.1).[4] Moreover, according to the world health organization (WHO), even today more than 50% of the population in developing countries use plants as their primary source of medicine.[5] A recent survey by Newman *et al.* has shown that 25% of today's drugs in the market are of natural product origin.[6]

Today many efforts are being made in the world to discover new bioactive compounds from natural sources that can be developed as anti-infective agents to fight the emergence of new infectious diseases such as HIV/AIDS, dengue, chikungunya, and avian influenza, drug-resistant pathogens: *Plasmodium falciparum* and *Mycobacterium tuberculosis*, and also to develop drugs with fewer side effects than the drugs currently in use. Similarly, new drug

candidates need to be developed imperatively to treat non-infectious diseases such as diabetes mellitus, heart diseases, cancer and Alzheimer's disease since they too have become some of the leading causes of death in the world.

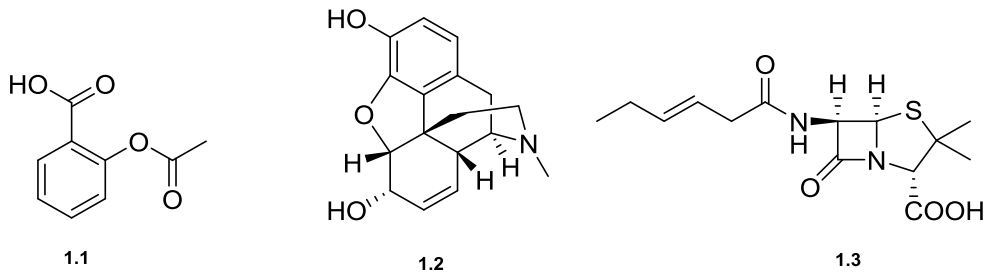


Figure 1.1. Historically important natural product derived drugs

The vast array of known and yet to be discovered plants, microbes, and marine organisms that make natural products is surprising. Only about 200,000 natural products have been identified to date and only 5-15% of higher plants have been exploited for their natural products.[7] The percentage of natural product structure classes that have been identified from different sources found in the Bioactive Natural Products Database (BNPD) is highlighted below (Figure 1.2).

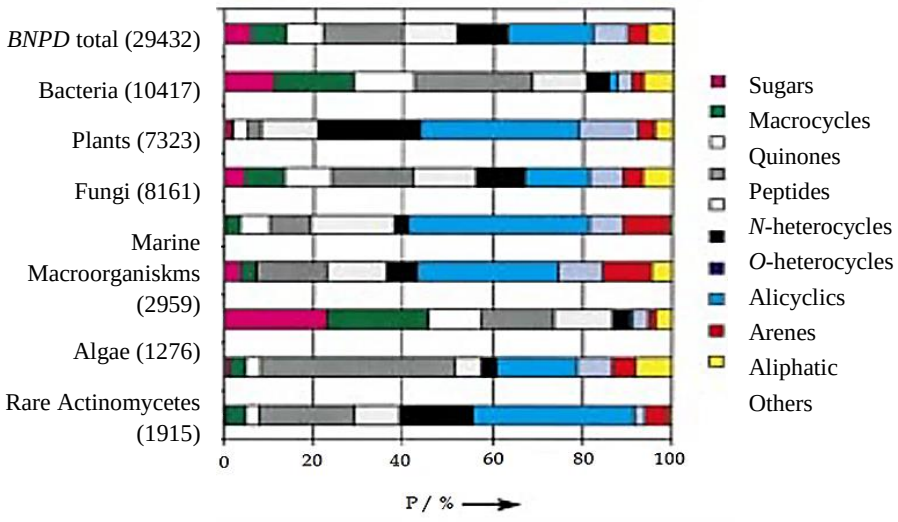


Figure 1.2. Percentage of natural products identified from different sources[8]

Continuing research on natural products to discover new bioactive compounds from terrestrial plants, terrestrial microorganisms, marine organisms, terrestrial invertebrates, and vertebrates is important in modern drug development.

1.1 Plants as a source of natural products

There are estimated to be over 250,000 flowering plant species on the planet and some of these species have proven to be rich sources of natural products with complex chemical structures containing a diversity of different functional groups. These phytochemicals are used as pharmaceuticals, food additives, fragrances and pesticides and can be grouped as alkaloids, phenolics, terpenes, sterols, flavonoids, lignins, tannins etc. according to their biosynthetic pathways as shown in figure1.3.[9]

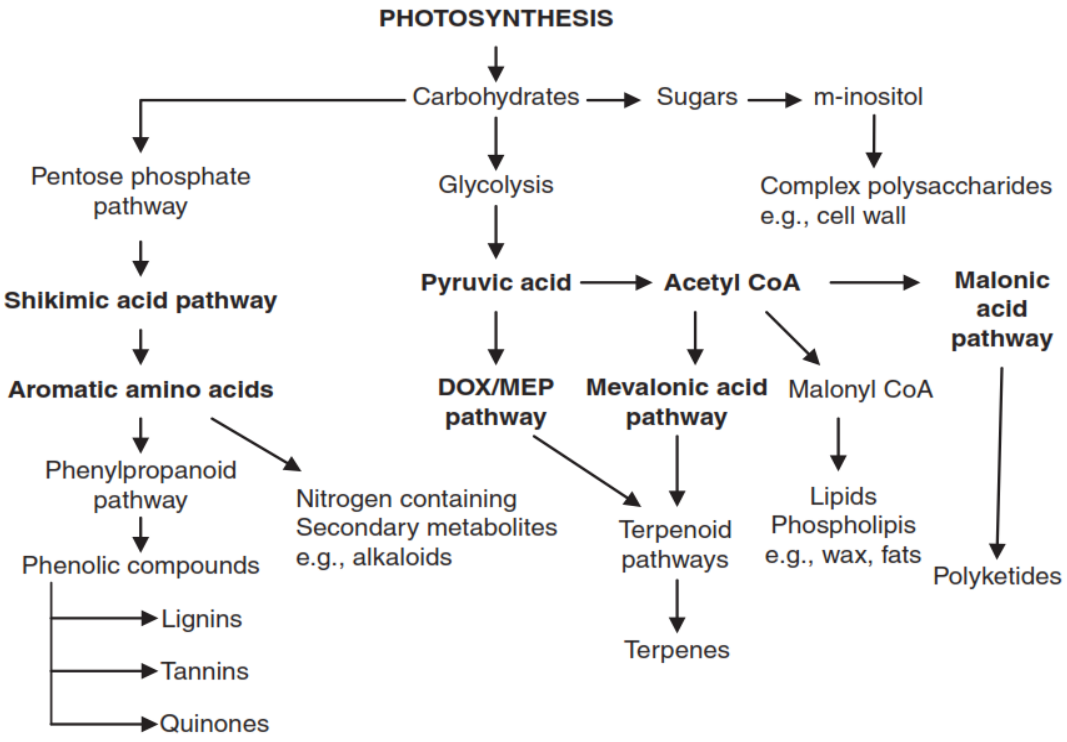


Figure 1.3. Biosynthetic pathways of secondary metabolites[10]

Plant secondary metabolites play important ecological functions within plants. They provide protection against microbial or insect attacks, serve as attractants (odour, colour, taste) for pollinators and seed-dispersing animals, function as mediators of plant-plant competition and plant-microbe symbioses.[11]

1.2 Plant natural products in drug discovery

For more than a thousand years people throughout the world have been using plants as medicines in the form of tinctures, poultices, teas, powders and herbal formulations to treat and prevent various ailments. Evidence dates back to the Middle Palaeolithic age (i.e. 60,000 years ago) according to the fossil records.[12] Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Ayurveda, Unani, Kampo and traditional Chinese medicine are the well-established traditional medical systems of the world while the past and present indigenous people in Africa and the America have used herbs in their healing rituals. [13]

With the isolation of the natural product, morphine (**1.2**) from the opium poppy, *Papaver somniferum*, in 1803, plants as medicines moved into a new era, and scientists started isolating bioactive compounds from medicinal plants to identify lead compounds that could be developed into drugs. Consequently in the 1870s the natural product morphine (**1.2**) was converted into its diacetyl derivative, diacetyl morphine (heroin) (**1.4**), by boiling with acetic anhydride and to codeine (**1.5**), a naturally occurring painkiller found in opium, by methylation. The use of *Digitalis purpurea* (another traditional plant medicine) dates back to the 10th century in Europe and was used in ancient Rome as a herbal remedy, while in Ireland, Germany, and England it was used to treat dropsy and other ailments in the 16th century. The use of digitalis in modern medicine was initiated by William Withering in 1785 when he treated 163 hospitalized cardiac patients with extracts from the plant. Digitoxin (**1.6**), a cardiotonic glycoside extensively used today for the treatment of cardiac conduction enhancing cardiac contractibility in heart patients, was first purified in 1875, while digoxin (**1.7**), the current cardiotonic drug was isolated for the first time in 1957. Digitalin (**1.8**) is another cardiac glycoside that has been isolated from *Digitalis purpurea*, which is also used to treat patients with heart diseases (Figure 1.4).[4]

Quinine (**1.9**), an alkaloid, isolated from the bark of cinchona (quinaquina) tree in 1820 to treat malaria was the first chemical compound that was used to treat an infectious disease.[14]

Even today this compound saves the lives of millions of people suffering from malaria. Artemisinin (**1.10**), a sesquiterpene endoperoxide, isolated from sweet wormwood (*Artemisia annua*) in the 1970s, is also another effective antimalarial drug.[15] (Figure 1.4). Artemisinin is now the drug of choice in areas where drug resistance to quinine and chloroquine is endemic.

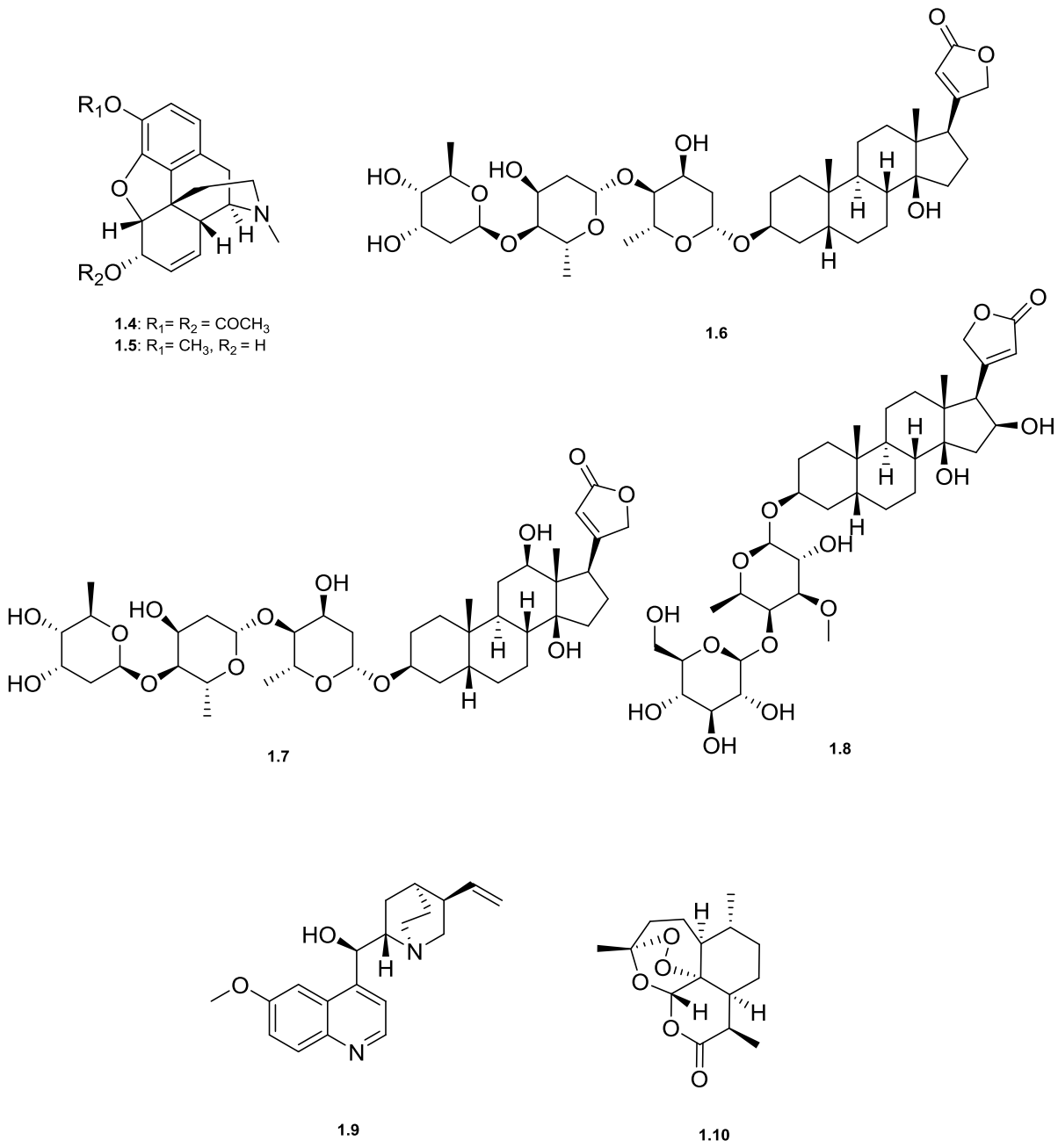


Figure 1.4. Plant derived pain killers, cardiovascular drugs and antimalarial drugs

The *Catharanthus* alkaloids, vincristine (**1.11**) and vinblastine (**1.12**) isolated from *Catharanthus roseus* in the 1950s were the first plant-derived anticancer drugs that were used to treat cancers including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi's sarcoma.[16] Paclitaxel (**1.13**) isolated from the bark of Pacific Yew, *Taxus brevifolia* Nutt. (Taxaceae) was approved as an anticancer drug by the Food and Drug Administration in 1992 and later in 1994 it was recommended for the treatment of metastatic breast cancer.[17] Camptothecin (**1.14**), a quinoline-based alkaloid isolated from the Chinese ornamental plant *Camptotheca acuminate* (Nyssaceae) was advanced to clinical trials by the National Cancer Institute of USA in the 1970s, but was not successfully progressed into the clinic due to severe bladder toxicity. However, semi-synthetic derivatives of camptothecin, topotecan (**1.15**) and irinotecan (**1.16**) are used for the treatment of ovarian and small cell lung cancers, and colorectal cancers, respectively (Figure1.5).[18-20]

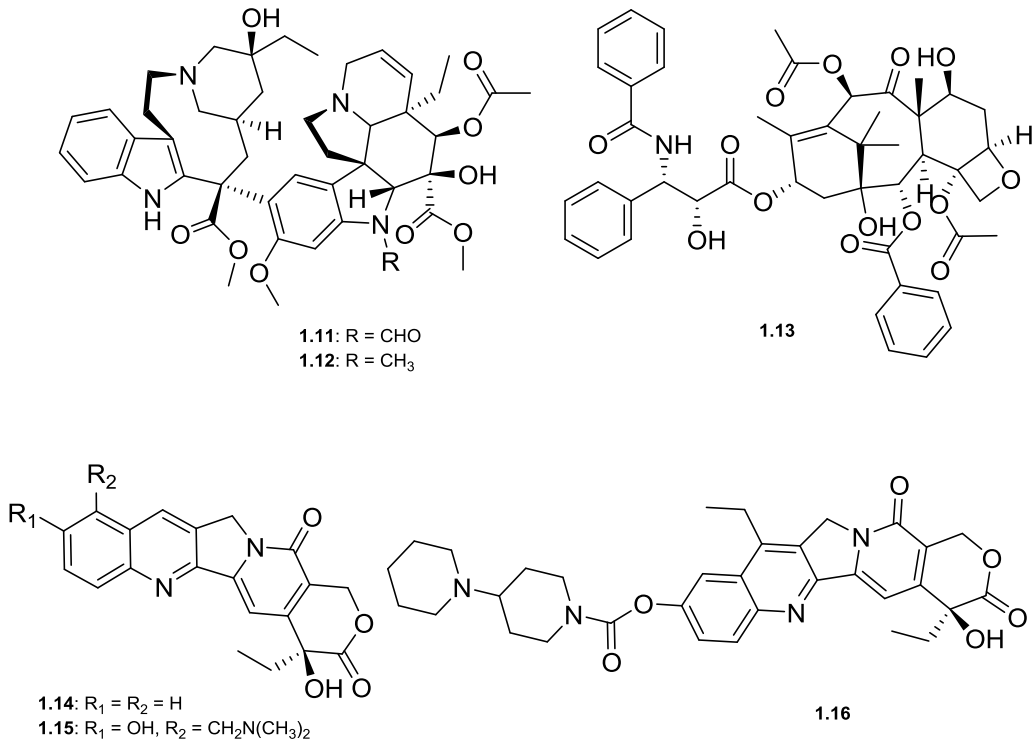


Figure 1.5. Plant derived anticancer drugs

Apart from the natural products mentioned above, there are many other plant-derived drugs that are available on the market today including compounds with anticholinergic and anti-inflammatory activity. As a consequence, global efforts to discover new drugs, new drug

leads and new chemical entities from medicinal plants remains an important area of drug discovery despite the development of other novel approaches such as synthetic chemistry, combinatorial chemistry and molecular modelling that have emerged during the 20th century.

1.3 Australian flora

Australia is an island continent with diverse and exceptional flora. It is one of the 17 countries in the world that a classified as megadiverse in terms of a total number of species and the number of endemic species, genera, and families.[21] Australia possesses about 24,000 native species of plants of which 85% are endemic. The flora native to Australia is unique, because Australia has been geographically isolated from the rest of the world for over 100 million years. Most interestingly, Australia's native plants are diverse because of they survive in a vast array of different habitats, from the mountain ranges, to the rain forests and from the inland deserts, to the white sand dunes of the coast. In tropical regions fruit trees such as figs and green plums are common. In dried climates; i.e. central Australia where water is scarce thinly spread plants can be found. The plant communities of southwest of Western Australia and the rainforests of northern Queensland are very highly diverse with high levels of endemism and these places are internationally known as biodiversity hotspots. Most of the Australian flora has a Gondwanan origin. Three hundred million years ago the supercontinent, Gondwana, consisted of South America, Africa, India, Australia, and Antarctica and it was covered with rain forests and ferns. Around fifty million years ago, Australia separated from Antarctica and drifted away from the Southern polar region.[22] Consequently its climate became warmer and drier and new plant species arrived at the continent from the North. However, there are still many forests that can be found in Australia that originated from Gondwanan flora. Warm temperate rainforests in north-east NSW and southeast Queensland are examples. *Proteacea*e, *Asteraceae, Winteraceae* and *Nothofagaceae* are examples of plant families of Gondwanan origin.[23]

Common species of Australian flora are *Acacia, Eucalyptus, Grevillea, Melaleuca, and Eremophila*. The predominant and largest family of flowering plants found in Australia (with over 1000 species) is the Myrtaceae (myrtles).[24]

1.3.1 Myrtaceae

The Myrtaceae is a large angiosperm family that consists of 140 genera with more than 3,800 species. Species within the family have a Gondwanan origin and are widely distributed in South America, South East Asia, and Australia.[25] Several species are also established in Africa.[26] Traditionally, species in the Myrtaceae have been subdivided into two main classes depending on their fruits the: Leptospermoideae with capsular fruits and the Myrtoideae with fleshy fruits. Floral characteristics, which define plants from the Myrtaceae include oil glands in the entire leaves, the ovary that are half inferior to inferior, flowers that possess numerous stamens, an internal phloem and vestured pits on the xylem.[27, 28] Australian Myrtaceae species are generally shrubs and trees that are found in the tropics, subtropics and temperate Australia. They consist of 70 genera and 1,400 species and most of them are endemic to Australia.[25] The Australian landscape is dominated by plants from the Myrtaceae and Australia can be considered the centre of diversity of the family since over half the genera and nearly half the species in the family are endemic to Australia. In Australia, there are two large clades of Myrtaceae; the eucalypt group and the melaleuca group.[29]

Gum trees, colloquially known as eucalypts, which contains seven closely related genera including *Eucalyptus, Corymbia, Angophora, Stockwellia, Allosyncarpia, Eucalyptopsis* and *Arillastrum* are a dominant part of Australian flora that belongs to Myrtaceae. *Eucalyptus* with more than 600 species is the largest genus of the eucalypt group found in drier open forests, woodlands and semi-arid mallee vegetation, which is primarily distributed in Australia (Figure 1.6). However, some species like *E. deglupta* are found in Timor and New Guinea spreading from Northern New Guinea to New Britain, Sulawesi, Ceram, and Mindanao. The closely related genera to *Eucalyptus* are *Angophora* with thirteen species and *Corymbia* with 113 species distributed in warm temperate climates. Of the other four eucalypt genera, *Stockwellia* from North Queensland, *Allosyncarpia* from the Arnhem Land Plateau (Northern Territory) and northern Australia and *Arillastrum* from the Atherton Tablelands in north-eastern Australia are monotypic genera, while *Eucalyptopsis* found in New Guinea, Moluccan Archipelago, and Woodlark Island consists of two species.[29]

Eucalypts play a vital role in the Australian identity including culture, biodiversity, and socioeconomic activities. Since Aboriginal people first occupied Australia about 40000 years ago, eucalypts have been used as a source of food and medicine as well as for cultural purposes. Utensils and tools such as spears were made from stems, while roots of some plants

were dug to collect water, seeds of certain species were eaten as food and the bark and leaves of some selected species were used to treat cold, influenza, toothaches, snakebites, fevers, diarrhea and other ailments. Kino, an astringent obtained from plants such as *Corymbia ficifolia*, *Corymbia maculata*, *Eucalyptus hemiphloia*, is an example of a medicine obtained from Eucalypts. Eucalypts are also important from an ecological perspective as they provide food and shelter for a significant number of Australian animals. For instance a few varieties of Eucalypt including Swamp Mahogany (*Eucalyptus robusta*), Small Leaf Peppermint (*Eucalyptus nicholii*), Bangalay (*Eucalyptus botryoides*), Scribbly Gum (*Eucalyptus haemastoma*), Willow Gum (*Eucalyptus scoparia*), Grey Gum (*Eucalyptus punctata*), Spotted Gum (*Corymbia maculata*), Drooping Red Gum (*Eucalyptus parramattenis*), Tallowwood (*Eucalyptus microcorys*), Blackbutt (*Eucalyptus pilularis*), Lemon Scented Gum (*Corymbia citriodora*) and Small fruit Grey Gum (*Eucalyptus propinqua*) are the only food eaten by Koalas of Australia. The main uses of Eucalypts today are in forestry, environmental planting, amenity planting, as a source of essential oil, in floriculture, and to make art and crafts. In 2006, *E. globulus* was the main source of Australian hardwood and eucalyptus oil. For instance, *E. globulus* and *E. grandis* are used in many countries for pulp and paper production. Furthermore, useful compounds such as the sesquiterpene aromadendrene, present in leaves and bark of *E. globulus* is being isolated as a by-product of pulp and paper production industries.[30]

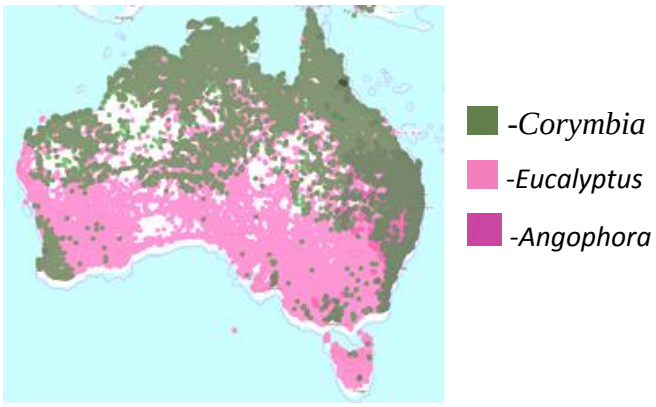


Figure 1.6. Distribution of *Eucalyptus*, *Corymbia* and *Angophora* in Australia[31]

The Melaleuca group consists of nine genera; *Beaufortia*, *Callistemon*, *Calothamnus* Labill, *Conothamnus* Lindl., *Eremaea*, *Lamarchea*, *Melaleuca*, *Phymatocarpus*, *Regelia* with more than 300 species distributed throughout Australia. They are important in southern and eastern heathlands, the monsoon woodlands, and mallee shrublands in the southern arid zone. Species have a potential for forestry, essential oil production, degraded land reclamation, farm tree windbreak plantings, and ornamental horticulture, among other applications. Bottle brushes belong to the genus *Callistemon* and paperbarks belonging to the genus *Melaleuca* are closely related and have 'bottlebrush' shaped flower spikes. There are about 40 species in the genus *Callistemon* and almost all are endemic to Australia. Most of the bottle brushes are distributed in the East and Southeast of Australia (Figure 1.7). Two species occur in the Southwest of Western Australia and four species in New Caledonia. Bottlebrushes can be found growing from Australia's tropical north to the temperate south. They often grow in damp or wet conditions such as along creek beds or in areas which are prone to floods. Some species of bottle brushes are very popular as garden plants. Some common garden plants are *Callistemon brachyandrus* (Prickly Bottlebrush), *Callistemon citrinus* (Crimson Bottlebrush), *Callistemon pallidus* (Lemon Bottlebrush), *Callistemon pityoides* (Alpine Bottlebrush), *Callistemon salignus* (Willow Bottlebrush), *Callistemon subulatus*, and *Callistemon viminalis (*Weeping Bottlebrush).[29, 32]

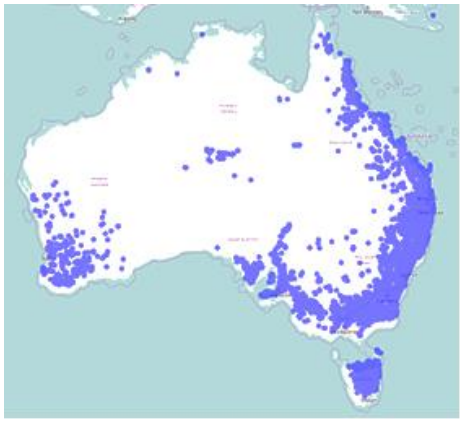
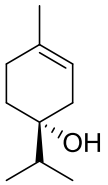


Figure 1.7. Distribution of *Callistemon* in Australia[31]

Paperbarks are aromatic and medicinal plants that belong to the genus of *Melaleuca* and are mainly used to produce medicinal essential oils. Historically, *M. cajuputi, M. leucadendron, M. linariifolia* and *M. quinqueneruia* were used by the Aboriginals for the treatment of

headaches, aches and pains, colds and also as an insect repellent. Mostly leaves and small branches were crushed and the vapour was inhaled to treat coughs and colds. Sometimes bruised leaves were soaked in water and then swallowed or poured over the body to cure sore throats and skin ailments.[33] From the 230 species found in the genus 220 are endemic to Australia. However, they can be found in Indonesia and Papua New Guinea. They are distributed in open forests, woodland or shrub land, along with waterways and the edges of swamps. Essential oils with strong aroma and medicinal application can be extracted from leaves and stem and have anti-cancer activity. Most essential oils are extracted from the broad leaved *M. quinquenervia* (niaouli oil) and *M. cajuputi* (cajuput oil) and the small-leaved *M. alternifolia*, *M. linariifolia* complex.[34] Tea Tree Oil (TTO) extracted from *M. alternifolia* by leaf steam distillation has antimicrobial activity and the main antimicrobial constituent, (+)-terpinen-4-ol (**1.17**) (Figure 1.8) cause structural damages to cell walls and membranes of bacteria and fungi causing disruption of cell integrity.[33]



1.17

Figure 1.8. The main antimicrobial constituent of TTO, (+)-terpinen-4-ol

Upon European settlement in Australia in 1788, the study of the chemistry of plants from the Myrtaceae, particularly *Eucalyptus* species, to isolate and identify biologically active secondary metabolites began. These studies have led to the understanding of the diversity of chemistry found within plants from the Myrtaceae and it is now known that they mainly produce a range of polyphenols, flavonoids, gallo- and ellagitannins, phloroglucinols, and terpenoids.[35] However, to date, only a few species have been chemically exploited and further studies are needed to identify more secondary metabolites of the Myrtaceae family.

1.4 References and Notes

1. Alan Crozier, Michael N. Clifford and H. Ashihara, in *Frontmatter* Blackwell Publishing Ltd, Oxford, UK, 2006, pp. i-xii.
2. R. A. Maplestone, M. J. Stone and D. H. Williams, *Gene*, 1992, **115**, 151-157.
3. D. A. Dias, S. Urban and U. Roessner, *Metabolites*, 2012, **2**, 303-336.
4. D. J. Newman, G. M. Cragg and K. M. Snader, *Nat. Prod. Rep.*, 2000, **17**, 215-234.
5. D. R. A. Mans, *Acad. J. Med. Plants*, 2013, **1**, 101-110.
6. D. J. Newman, G. M. Cragg and K. M. Snader, *J. Nat. Prod.*, 2003, **66**, 1022-1037.
7. M. Fullbeck, E. Michalsky, M. Dunkel and R. Preissner, *Nat. Prod. Rep.*, 2006, **23**, 347-356.
8. T. Henkel, R. M. Brunne, H. Müller and F. Reichel, *Angew. Chem.*, 1999, **38**, 643-647.
9. M. J. Balunas and A. D. Kinghorn, *Life Sci.*, 2005, **78**, 431-441.
10. K. G. Ramawat, S. Dass and M. Mathur, *Herbal Drugs: Ethnomedicine to Modermn Medicine*, Springer, Heidleberg, 2009.
11. M. Wink, *Theor. Appl. Genet.*, 1988, **75**, 225-233.
12. R. S. Solecki, *Science*, 1975, **190**, 880-881.
13. D. A. Dias, S. Urban and U. Roessner, *Metabolites*, 2012, **2**, 303-336.
14. J. Achan, A. O. Talisuna, A. Erhart, A. Yeka, J. K. Tibenderana, F. N. Baliraine, P. J. Rosenthal and U. D'Alessandro, *Malar. J.*, 2011, **10**, 144-144.
15. D. Klayman, *Science*, 1985, **228**, 1049-1055.
16. H. Robert van der, I. J. Denise, S. Wim, H. Didier and V. Robert, *Curr. Med. Chem.*, 2004, **11**, 607-628.
17. N. C. Kampan, M. T. Madondo, O. M. McNally, M. Quinn and M. Plebanski, *BioMed Res. Int.*, 2015, **2015**, 413076.
18. Y. H. Hsiang, R. Hertzberg, S. Hecht and L. F. Liu, *J. Biol. Chem.*, 1985, **260**, 14873-14878.
19. A. Ardizzoni, H. Hansen, P. Dombernowsky, T. Gamucci, S. Kaplan, P. Postmus, G. Giaccone, B. Schaefer, J. Wanders and J. Verweij, *J. Clin. Oncol.*, 1997, **15**, 2090-2096.
20. J. F. Pizzolato and L. B. Saltz, *Lancet*, 2003, **361**, 2235-2242.
21. J. Williams, *Australia State of the Environment Report (Theme Report)*, 2001.

22. R. S. Hill, *History of the Australian Vegetation. Cretaceous to Recent*, Cambridge University Press Great Britain, 1994.
23. J. M. Anderson, H. M. Anderson, S. Archangelsky, M. Bamford, S. Chandra, M. Dettmann, R. Hill, S. McLoughlin and O. Rösler, *J. Afr. Earth Sci.*, 1999, **28**, 145-167.
24. C. E. González-Orozco, S. W. Laffan and J. T. Miller, *Aust. J. Bot.*, 2011, **59**, 601-609.
25. D. Beardsell, S. Obrien, E. Williams, R. Knox and D. Calder, *Aust. J. Bot.*, 1993, **41**, 511-526.
26. K. J. Sytsma, A. Litt, M. L. Zjhra, J. Chris Pires, M. Nepokroeff, E. Conti, J. Walker and P. G. Wilson, *Int. J. Plant Sci.*, 2004, **165**, S85-S105.
27. P. G. Wilson, M. M. O'Brien, P. A. Gadek and C. J. Quinn, *Am. J. Bot.*, 2001, **88**, 2013-2025.
28. E. W. Berry, *Botanical Gazette*, 1915, **59**, 484-490.
29. P. Y. Ladiges, F. Udovicic and G. Nelson, *J. Biogeogr.*, 2003, **30**, 989-998.
30. J. W. Turnbull, *Economic and Social Importance of Eucalypts*, CISIRO, Collingwood, VIC 3066, 2000.
31. Atlas of Living Australia website http://www.ala.org.au, (accessed 15 September 2017).
32. G. K. Brown, F. Udovicic and P. Y. Ladiges, *Aust. Syst. Bot.*, 2001, **14**, 565-585.
33. C. F. Carson and T. V. Riley, *Lett. Appl. Microbiol.*, 1993, **16**, 49-55.
34. B. Luiz Claudio Almeida, S. Cleber José, T. Róbson Ricardo, M. Renata Maria Strozi Alves and P. Antônio Lelis, *Agric. Conspec. Sci.*, 2013, **78**, 11-23.
35. E. L. Ghisalberti, *Phytochemistry*, 1996, **41**, 7-22.

CHAPTER 2

LITERATURE REVIEW

2.0 Literature Review

2.1 Chemistry of Myrtaceae

Plants from the Myrtaceae contain a vast array of secondary metabolites with chemical and functional diversity. These secondary metabolites can be divided into two main groups based on their physical properties: volatile and non-volatile metabolites. A large number of studies have been carried out to investigate the volatile chemistry of the Myrtaceae. However, a limited number of studies have been carried out to explore their non-volatile chemistry. More than 1000 species of Myrtaceae have been studied for their volatile chemistry, while only about 150 species have been studied for non-volatile chemistry. The non-volatile chemistry found within plants from the Myrtaceae includes compounds such as β-triketones, phloroglucinols, flavonoids, polyphenols and their biological activities have been examined.[1-3] These studies indicate that the non-volatile chemistry present within plants from the Myrtaceae is exceedingly bioactive across many targets and since only limited studies have targeted this aspect of Myrtaceae chemistry, further studies are warranted.

2.1.1 Volatile chemistry

Volatile compounds of great economic as well as medicinal importance are found within the species of Myrtaceae. Leaves, fruits, bark and stems of many species of Myrtaceae are a good source of volatile organic compounds.[4, 5] Essential oils, the odorous volatile secondary metabolites, contain complex mixtures of low molecular weight substances including alkanes, alkenes, alcohols, esters, ethers, acids, aromatic phenols, aldehydes, and ketones.[4] The most important characteristic feature of Australian Myrtaceae is their terpenoid dominated essential oils with distinct aromas which are responsible for the odor of Australian flora.[6] Isoprenoids are the most abundant volatile compounds found in plants from the Myrtaceae and they can be subdivided according to the number of isoprene (C5) (**2.1**) units present in the molecule with monoterpenes (C10), sesquiterpenes (C15) being the predominant classes. Most of them are hydrophobic and monoterpenes can be either linear, mono, bi or tricyclic structure.[7] These isoprenoids can be categorized as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and terpene conjugates. The predominant volatile organic compounds of Australian Myrtaceae are

monoterpenes while sesquiterpenes contribute to 10% of the total leaf oil fraction. The major sesquiterpene hydrocarbons of Australian Myrtaceae are aromadendrene (**2.2**), allo aromadendrene (**2.3**) and β-caryophyllene (**2.4**) while the major terpene alcohols are globulol (**2.5**), spathulenol (**2.6**) and eudesmols (**2.7**).[8] Table 2.1 in the following pages shows the main classes of terpenes found within the family of Myrtaceae.[6]

Table 2.1. Terpene hydrocarbons and oxygenated terpenes of Myrtaceae

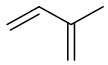
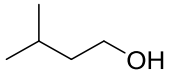
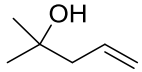
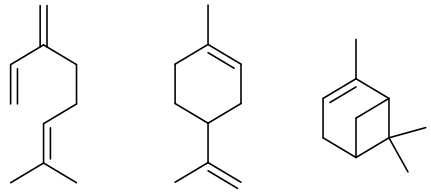
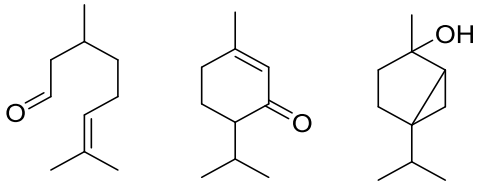
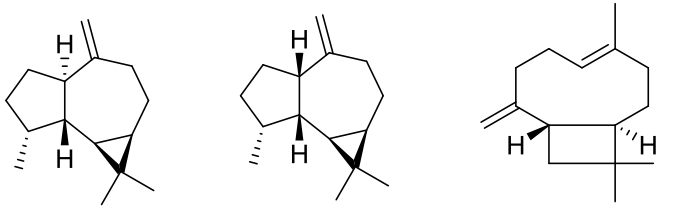
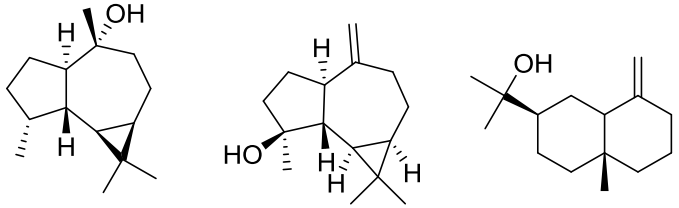
Class	Hydrocarbons	Oxygenated terpenes
Hemiterpenes	 **2.1:** isoprene	 3-methyl-2-butanol  2-methyl-3-buten-2-ol
Monoterpenes	 myrcene limonene pinene	 citronellal peperitone sabinene hydrate
Sesquiterpenes	 **2.2**: aromadendrene **2.3**: allo aromadendrene **2.4**: β -caryophyllene	 **2.5**: globulol **2.6**: spathulenol **2.7**: eudesmol

Table 2.1 (continued)

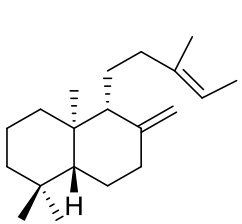
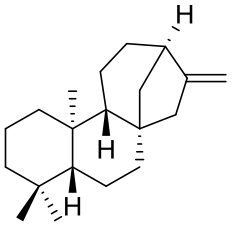
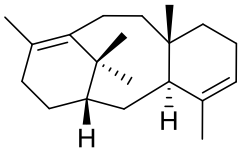
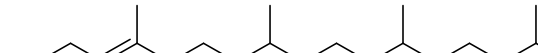
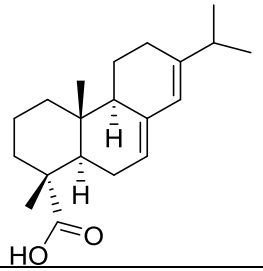
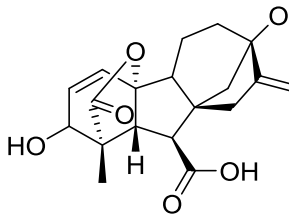
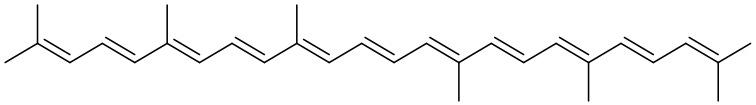
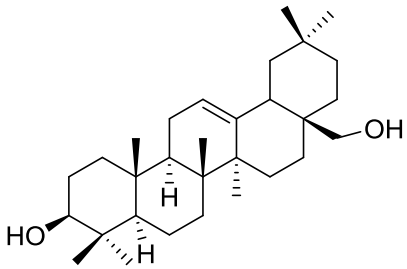
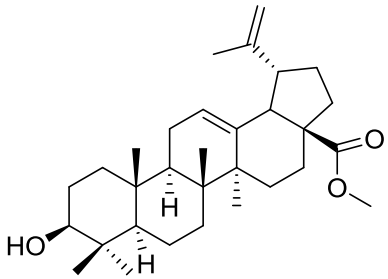
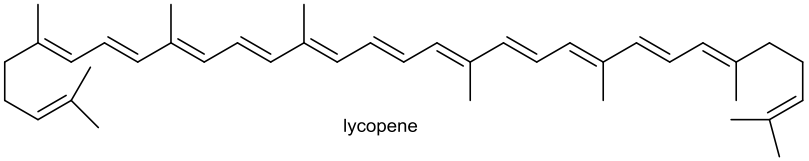
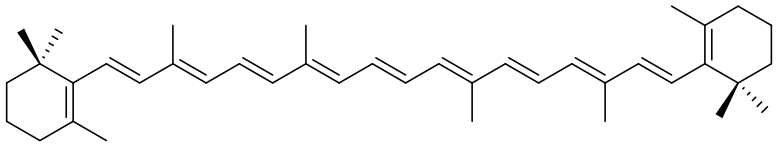
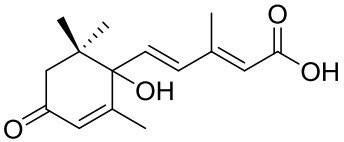
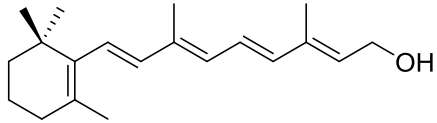
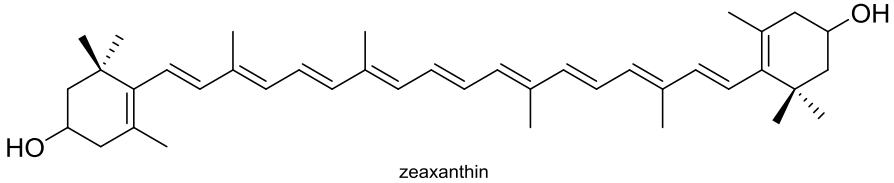
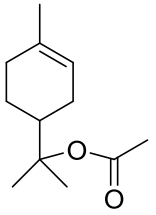
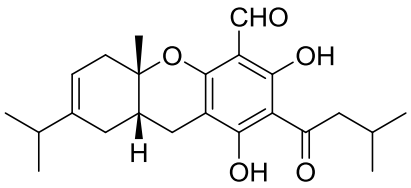
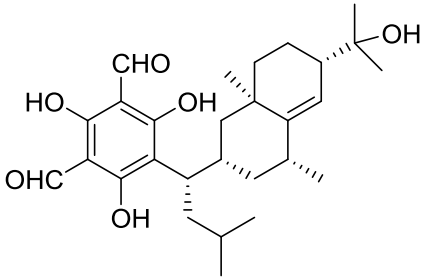
Class	Hydrocarbons	Oxygenated terpenes
Diterpenes	 ent-copaene  ent-kaurene  taxadiene	 phytol  
Triterpenes	 squalene	 erythrodiol  betulinic acid-methyl-ester

Table 2.1 (continued)

Class	Hydrocarbons	Oxygenated terpenes
Tetraterpenes and apocarotenoids	 lycopene  β-carotene	 abscisc acid  retinol/vitamin A  zeaxanthin
Terpene conjugates	 α–terpenyl-acetate  euglobal-G6  macrocarpal-E	

2.1.2 Non-volatile chemistry

Although the Myrtaceae has 140 genera and more than 3800 species; the number of studies carried out to investigate the non-volatile constituents of this family is rather low. A literature survey of the non-volatile chemistry of Myrtaceae species indicates that less than 150 species have been studied for their non-volatile chemistry but these studies have demonstrated that the family is a rich source of non-volatile compounds including β-triketones, phloroglucinols, flavonoids, and polyphenols, while relatively few species contain alkaloids. β-Triketone and phloroglucinol chemistry is distinctive to the family and these compounds have exhibited a wide range of biological activities including antimicrobial, antimalarial, antifeeding, antiinflammatory and antioxidant. A comprehensive systematic literature review carried out on the structural diversity, biological activities, and occurrence of β-triketones and phloroglucinols are discussed in detail in the next section.

STATEMENT OF CONTRIBUTION TO CO-AUTHORED UNPUBLISHED PAPER

This section includes a co-authored article currently in preparation for submission to Natural Product Reports, and has been formatted to that journals style. The bibliographic details (if published or accepted for publication)/status (if prepared or submitted for publication) of this co-authored paper, including all authors are

Sarath P. D. Senadeera, Anthony R. Carroll

The co-author of this manuscript is my principal supervisor Professor Anthony R. Carroll. My contribution to the manuscript involved collection of literature data, analysis, organizing and preparation of manuscript.

(Signed)[redacted] (Date) 14/03/2017

Name of student: Sarath Parakumge Dayani Senadeera

(Countersigned)[redacted] (Date) 14/03/2017

Corresponding author Professor: Anthony R. Carroll

(Countersigned)[redacted] (Date) 14/03/2017

Principle supervisor: Anthony R. Carroll

2.2 The Chemistry and Biological activity of β-Triketones and Phloroglucinols from the Myrtaceae

2.2.1 Abstract

The Myrtaceae is a diverse angiosperm family which contains many species that are important sources of oils and other chemical components with commercial and medicinal value. In this study the non-volatile chemicals reported from the family has been reviewed. β-triketones and phloroglucinols are the predominant constituents present and this article reports their occurrence, various structural classes, biological activity, structure activity relationships and chemotaxonomic importance.

2.2.2 Introduction

The Myrtaceae is a large angiosperm family that consists of 140 genera with more than 3800 species. The family has a Gondwanan origin and distribution, with species widely distributed in South America, South East Asia and Australasia. Only a small number of species are present in Africa.[9] Taxonomically, species in the Myrtaceae are subdivided into two main classes depending on fruit morphology: the Leptospermoideae with capsular fruits and the Myrtoideae with fleshy fruits. Floral characteristics which define plants from the Myrtaceae include oil glands in the entire leaves, ovaries that are half inferior to inferior, flowers that possess numerous stamens, an internal phloem and vestured pits on the xylem.[10, 11] The Australian landscape is especially dominated by plants from the Myrtaceae and Australia can be considered as the centre of diversity of the family since over half the genera and nearly half the species are endemic to this continent.[12]

Plants from the Myrtaceae produce a vast array of secondary metabolites with chemical and functional diversity.[1] Myrtaceae species are a rich source of mono and sesquiterpene dominated essential oils and volatile components from more than 1000 species have been documented.[13] The majority of studies have been carried out by Brophy and his team who have predominantly investigated the volatile chemistry of Australian Myrtaceae species.[14-17] In 2008, Foley et al reviewed the diversity of terpenes of Australian Myrtaceae in terms of molecular perspective[6] and in 2014 they

further discussed the evolution of foliar terpene diversity in the Myrtaceae.[8] Foley has also discussed the genetic basis of foliar terpenes in order to understand the biosynthesis of these terpenes.[18] What has been less well studied are the non-volatile constituents present within Myrtaceae plants since only about 150 species have been studied specifically for these components. Apart from volatile terpenes, the chemistry found within plants from the Myrtaceae includes compounds such as, phloroglucinols, β-triketones, flavonoids, polyphenols, triterpenes and alkaloids.[4, 5, 14, 19] Biological activities of some of these compounds have been examined indicating that many of these chemicals are exceedingly bioactive across many targets. Reported biological activities of these non-volatile natural products can be classified as ecologically significant (such as antifeedant, growth regulators and antifouling) and therapeutically or pharmacologically significant (for example antibacterial, antiprotozoal, Epstein Barr Virus inhibitory, HIV-RT inhibitory, aldose reductase inhibitory).[20] As previous reviews have specifically reported on ellagitannins, and volatile terpenes found within Myrtaceae species, only a brief summary of the tannins from polar extracts, and the mono and sesquiterpene volatile components found in essential oils is presented here. Similarly, numerous reviews have documented the diversity of flavonoids present in a wide array of plant taxa and so Myrtaceae flavonoids will not discuss here.

This review predominantly focuses on the β-triketone and phloroglucinol natural products reported from Myrtaceae species since these compounds represent the second most abundant and diverse class of compounds present in Myrtaceae species after the volatile terpenes. Other non-terpenoid volatile components such as simple acylphlorglucinols and β-triketones are also present in Myrtaceae essential oils and these will be discussed in more detail because they show close similarities to other non-volatile compounds present in Myrtaceae species. Several reviews have discussed the chemistry of specific Myrtaceae genera (eg Eucalyptus) or species (eg Myrtus communis), but there is no comprehensive review of the non-volatile constituents found in Myrtaceae species. The most recent and comprehensive review of the phytochemistry, distribution, biosynthesis and biological activity of phloroglucinols and β-triketone was published in 2006 by Singh and Bharate.[19] In this review structures were grouped according to the number of phloroglucinols (or their β-triketone equivalents) that were present in a molecule and therefore monomeric, dimeric, trimeric, tetrameric or higher phloroglucinols and phlorotannins, were the

major groups presented. This current review however takes a different approach, making a specific distinction between compounds containing β-triketones and those containing only phloroglucinols. This approach was taken because it was evident from a review of the recent literature that clear distinctions in taxonomy and bioactivity relate to the presence and absence of β-triketones or phloroglucinols in the Myrtaceae genera. A significant number of new β-triketone structures reported from Myrtaceae species have appeared in the literature over the last 10 years and this now demonstrates that the family is a major source of this bioactive class of compounds. This review therefore specifically aims to focus on phloroglucinol/β-triketone chemistry of the Myrtaceae to highlight taxonomic trends in their distribution within specific genera and to identify biological activities that are consistent with the structural differences between phloroglucinols and β-triketones.

2.2.3 β-Triketones and Phloroglucinols

β-Triketones and phloroglucinols are secondary metabolites, of polyketide origin and are commonly found in plants from families including, *Apicidaceae, Asteraceae, Cannabinaceae, Clusiaceae, Compositae, Crassukaceae Euphorbiaceae, Fagaceae, Lauraceae, Myrtaceae, Rosaceae,* and *Rutaceae* as well as in marine and microbial sources. Both β-triketones and phloroglucinols share a common 1,3,5-triketocyclohexyl core (**1**), which in phloroglucinols tautomerizes to the more stable 1,3,5 trihydroxybenzene structure, while β-triketones it remains in the keto form because alkyl substituents attached at both C-2 and C-4 prevent tautomerism. It is noteworthy that since they are widespread in nature many plant based traditional medicines which are used to treat various ailments contain either β-triketones or phloroglucinols, and of the β-triketones and phloroglucinols that have been studied for their bioactive properties many have antibacterial activities while others possess anti-HIV, antimalarial, antileishmanial, antituberculosis, antibacterial, antifoulding and antifungal activities.[21]

2.2.3.1 β-Triketones and Related Structures

Within the Myrtaceae, β-triketones and their variants can be categorised into four main groups, simple acyl-β-triketones, β-triketone terpene adducts, mono β-triketone acylphloroglucinol conjugates, and bis β-triketone acylphloroglucinol conjugates. For convenience β-diketones are also discussed in this section.

2.2.3.2 Simple Acyl-β-Triketones

Simple acyl-β-triketones (Figure 1 and Figure 2) are widely distributed secondary metabolites within the family and 30 variants from 43 species and 16 genera have been reported to date. Their structural diversity relates to variations in the acyl side chain, the number of methyl groups directly attached to, and the level of oxygenation of the cyclohexyl group. They have been reported from the genera *Eucalyptus*, *Leptospermum*, *Xanthostemon*, *Darwinia*, *Backhousia*, *Calythrix*, *Corymbia*, *Syncarpia*, *Myrtus*, *Syzygium*, *Rhodomyrtus*, *Baeckea*, *Campomanesia*, *Myrcia* and *Melaleuca*. Syncarpic acid (**2**) or 2,2,4,4-tetramethyl-5-hydroxycyclohexen-1,3-dione, isolated from the leaves of *Syncarpia glomulifera* (previously *Syncarpia laurifolia*) is the simplest β-triketone and is the substructure most commonly found in most β-triketones metabolites present in Myrtaceae species. The first acyl-β-triketone isolated from the Myrtaceae was tasmanone (**3**) which was isolated from *Eucalyptus risdoni* and *Eucalyptus linearis* in 1914 by Robinson and Smith. Initially they thought it was a phenol and named it tasmanol.[22] Later, Trikojus and White suggested a possible molecular formula for it and thought it to be an acid since it reacted with a solution of sodium carbonate as well as giving positive results for other acid tests.[23] However, in 1956 it was identified as a β-triketone by Birch and Elliot and renamed as tasmanone.[24] Tasmanone has also been reported from the leaf oil of *Eucalyptus camfieldii*, *Eucalyptus cloeziana*, *Eucalyptus tasmanica*, and *Eucalyptus oblonga* and as much as 40% of the volatile leaf oil of *Eucalyptus camfieldii* is tasmanone. Tasmanone has been shown to possess potent insecticidal activity, targeting the octopamine receptor in insects and this has led to the compound being currently developed into a new insecticide. Agglomerone (**4**), another β-triketone that has been isolated as a major component from the steam volatile oil of leaves of *Eucalyptus*

agglomerata and *Eucalyptus mckieana* represents, 80% and 50% respectively of their total oil. In solution agglomerone and tasmanone both exist as a mixture of tautomers. Agglomerone has also been isolated from the steam volatile oils of *Eucalyptus oblonga*,[1, 25] *Eucalyptus camerooni*,[1, 25] *Baeckea diosmifolia*,[25] *Xanthostemon oppositifolius*.[25] In 1973, synthesis of agglomerone has also been described by D. R. Baigent and I. R. C. Bick.[26] Leptospermone (**5**) was first isolated in Australia from the leaves and branchlets of *Leptospermum flavescens*[27] in 1921 and was named as leptospermol. Subsequently it was identified from the leaves and branchlets of *Leptospermum ericoids* in New Zealand[28-30] and later from leaves terminal branchlets and seeds of *Leptospermum scoparium*.[31] This has also been reported from leaves and terminal branchlets of *Eucalyptus* species[25] including *E. caliginosa*, *E. phaeotricha*, *E. froggatti*, *E. nitens*, *E. notabilis*, *E. oblonga*, *E. decorticans*, *E. michaeliana*. Additionally, it has also been identified from the leaves and terminal branchlets of *Xanthostemon chrysanthus*,[25] *Melaleuca nodosa*[25] and from the leaves of *Callistemon lanceolatus*.[32] Leptospermone has antimicrobial activity,[33] arcaricidal activity[34] and herbicidal activity.[35] It inhibits the enzyme 4-hydroxyphenylpyruvate dehydrogenase (HPPD). It is important to note that the drug, nitisinone (**6**) (trade name Orfadin®), which is used to treat, a rare inherited disease, tyrosinaemia type I, is derived from leptospermone.[36-38] Initially, a herbicide mesotrione (**7**) was developed from leptospermone in 1977[35] and later in 2002, nitisinone was developed.[38] Nitisinone and mesitrione both inhibit the enzyme HPPD in both humans and maize respectively. Inhibition of the HPPD enzyme by mesotrione in maize causes reduction of plastoquinone and tocopherol biosynthesis, acting as an herbicide. In humans the HPPD enzyme inhibition by nitisinone prevents tyrosine catabolism and the accumulation of toxic bioproducts in the liver and kidneys.[36] Leptospermone co-exists with its lower homologue, flavesone (**8**) in the *Leptospermum flavescens, L. flavescens var grandifiora, L. Lanigerum, Eucalyptus decorticons, E. oblonga* and *Callistemon lanceolatus*.[32, 39] Isoleptospermone (**9**), another β-triketone which differs from leptospermone by replacement of the isopentoyl side chain with a 2-methylbutoyl group has been isolated from the essential oil of *Eucalyptus grandis*,[40, 41] *Leptospermum scoparium*,[42] *Myrciaria dubia*.[43] It also co-occurs with flavesone. The related, β-diketones, angustione (**10**) and dehydroangustione (**11**) have been isolated from the essential oil of leaves and branchlets of *Backhousia angustifolia*,[44, 45] and

dehydroangustione has also been isolated from the leaves and branchlets of *Backhousia myrtifolia*[25] and *Darwinia procera.*[25] Platyphyllol (**12**) isolated from the essential oil of *Melaleuca cajuputi*[46] is a 4-O methyl analogue of dehydroangustione. Xanthostemone (**13**), a homologue of dehydroanguistione, was isolated from the essential oil of the air-dried leaves of *Xanthostemon oppositifolius*.[47] Grandiflorone (**14**), from the leaves of *Leptospermum flavescens* var. *grandiflora*, *L. scoparium*,[48] two distinct morphological forms of *L. lanigerum*,[48] and *Xanthostemon pubescens,*[25] papuanone (**15**), from the leaves of *Corymbia dallachiana*,[42] (**16**) and (**17**) from the leaves of *Leptospermum scoparium*[49] represent additional acyl chain variants of the simple acyl-β-triketones. Syzygiol (**18**), from the seeds of *Syzygium polycephalum*[50] (incorrectly named *S. polycephaloides*) collected in Indonesia occurs as multiple conformations in solution and shows significant inhibition of skin cancer promotion induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). A compound (**19**), champanone D, isolated from the fruit of *Campomanesia lineatifolia*[51] is closely related to syzygiol. A third compound that is closely related to these compounds is myrigalone A (**20**) isolated from *Myrcia gale*.[52] The alkyl β-diketone-glycoside citriodorin (**21**) was isolated from the leaves of *Corymbia citriodora*[53] (previously *Eucalyptus citriodora*). Its structure was confirmed by X-ray analysis. Endoperoxides G1 (**22**) and G2 (**23**) from *Eucalyptus grandis* and G3 (**24**) from both *E. grandis*[54] and *Rhodomyrtus tomentosa*[55] are β-triketones which incorporate a cyclic endoperoxide and have been shown to inhibit root formation in cuttings. Another endoperoxide (**25**) has been isolated from the aerial parts of *Baeckea frutescens*.[56] Gallomyrtucommulones A-D (**26**-**29**), isolated from the leaves of *Myrtus communis*[57] are biogenetically related to endoperoxide G3 (which was co-isolated) but differ since the β-diketones are each conjugated to a 6-galloyl glucose moiety. On testing these compounds against drug resistant strains of *Staphylococcus aureus*, only gallomyrtucommulone A and B showed minimum inhibitory concentrations between 64 – 256 μg/mL. Calythrone (**30**), isolated from the essential oil of *Calythrix tetragona* exhibits keto enol tautomerism and it is a pentacyclic β-triketone. Endoperoxides, baeckenones D, E (**31**, **32**) and baeckenone F (**33**) isolated from *B. frutescens* had cytotoxic activities against human pancreatic (PSN-1), lung (A549), and breast (MDA-MB-231) cancer cell lines with IC_{50}s of 33.3 μM, 34 μM, and 39.3 μM accordingly.

Gallomyrtucommulones D and F (**34**, **35**) isolated from *Myrtus communis*[57] are glycosidated simple β-triketones.

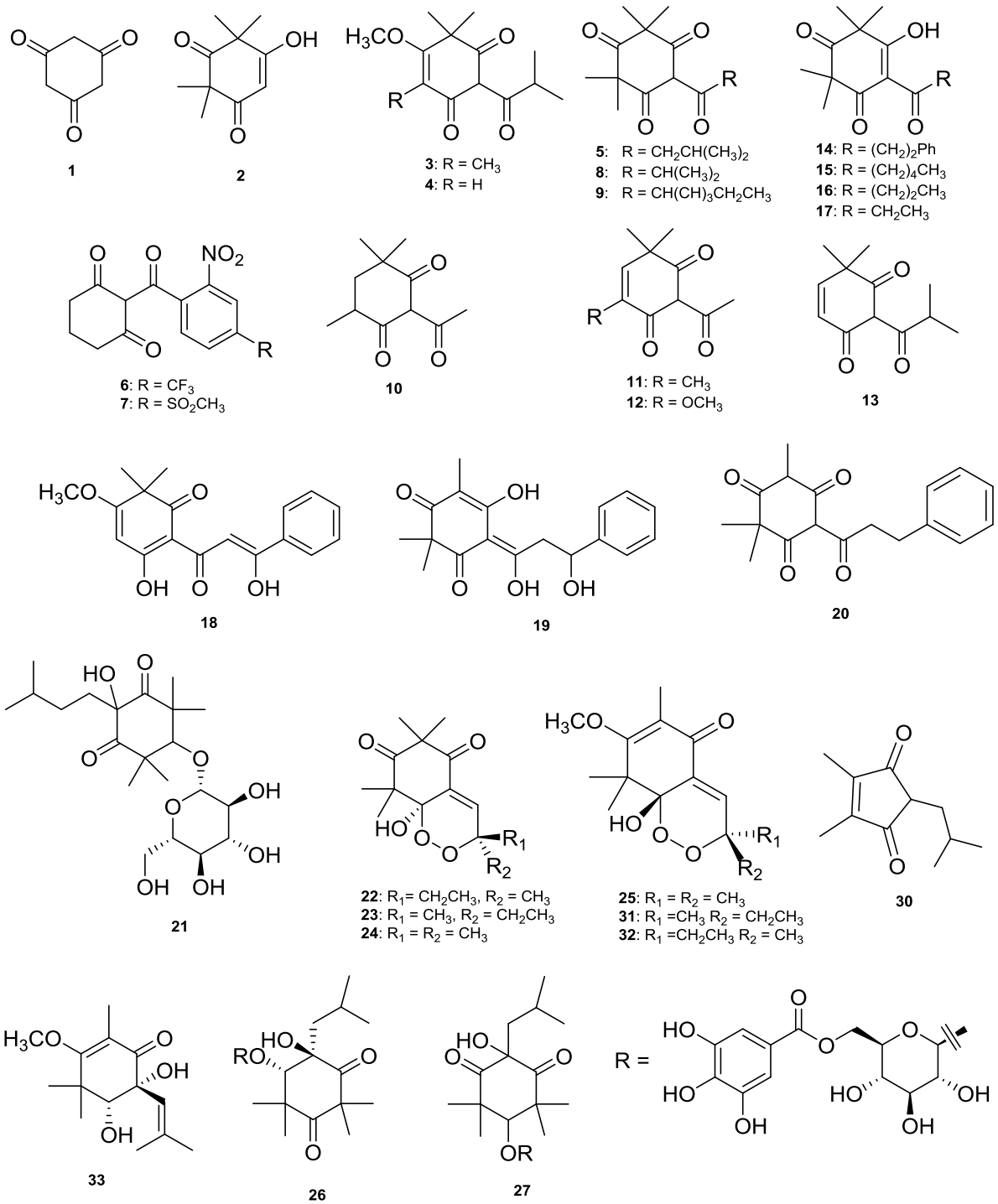


Figure 1. Simple β-triketones and related structures

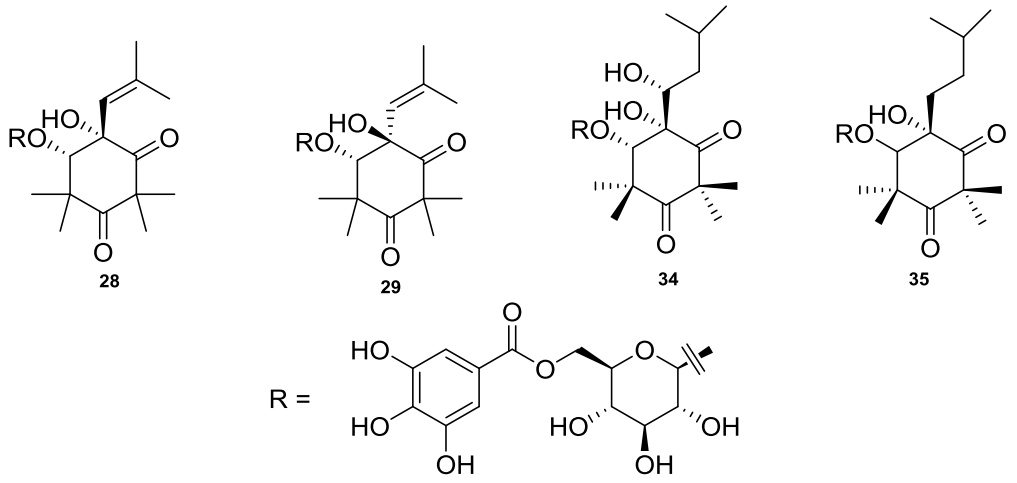


Figure 2. Simple β-triketones and related structures (continued)

2.2.3.3 β-Triketone Terpene Adducts

Twenty five β-triketone terpene adducts (Figure 3 and Figure 4) have been reported from six Myrtaceae genera *Baeckea, Callistemon, Corymbia, Kunzea, Myrtus* and *Rhodomyrtus*. Seven of these compounds incorporate either a mono or sesquiterpene moiety into a pyran ring directly attached to an alkyl β-triketone. BF-2 (**36**) isolated from *Baeckea frutescens*[58] and 4*S*-ficifolidione (**37**) isolated from *Corymbia ficifolia*[59] (previously named *Eucalyptus ficifolia*) and *Kunzea ericoides*[59] incorporate a pinene monoterpene moiety conjugated to either tasmanone or leptospermone. The configuration at C-4 in **37** was been revised based on X-ray crystal analysis of the compound made through total synthesis. Both epimers 4*S*-ficifolidione (**37**) and 4*R*-ficifolidione (**38**) were isolated from leaves of *Rhodomyrtus tomentosa*.[60] BF-2 shows strong cytotoxic activity (IC_{50} 5.0 μg/mL) against L 1210 leukaemia cells,[61] 4*S*-ficifolidione (**37**) was reported to possess insecticidal activity towards *Aedes aegypti*, *Culex quinquefasciatus* and *Frankliniella occidentalis* at a dose of 0.2 μg/insect causing mortality of 48%, 30% and 26% respectively after 48 h. The moderate insecticidal activities of 4*R*-ficifolidione include, LD_{50} for topical application to the aphid *Aphis fabae*, at 12 μg/insect, and to the thrips, *Thrips tabaci*, at 5.9 μg/insect. In addition, 45% of mortality was reported on the third stadium larvae of the cabbage white butterfly, *Pieris brassica* at a dose of 10 μg/insect.[59] However, reinvestigation of

insecticidal activity of ficifolidione and its C-4 epimer against adult house flies (*Musca domestica*), mosquito larvae (*Culex pipiens*) and cutworms (*Spodoptera litura*) the similar activity has not been observed.[62] Ficifolidione had cell cytotoxicity against insect (Sf9) (IC_{50} 32 μM), mouse carcinoma (Colon26) (IC_{50} 9 μM), human promyelocytic leukemia (HL60) (IC_{50} 3 μM), and African green monkey epithelial (Vero) (IC_{50} 12 μM) cell lines.[62]

Kunzeanone C (**39**) isolated from *Kunzea ambigua*[63] is an adduct between an epoxide of the sesquiterpene bicyclogermacrene and leptospermone. Kunzeanone C (**39**) isolated from *Kunzea ambigua*[63] is an adduct between an epoxide of the sesquiterpene bicyclogermacrene and leptospermone.

Viminadione A and B (**40**, **41**) from *Callistemon viminalis*[64] are both adducts of terpene and leptospermone and diastereomeric at C-7. Viminadione A and B showed moderate insecticidal activities in comparison with natural pyrethrum extract. Insecticidal activity of viminadione A, to houseflies, *Musca domestica*, 1.9 μg/insect; to the aphid, *Aphis fabae*, 5.9 μg/insect; and to the thrips, *Thrips tabaci*, 4.2 μg/insect was observed. In comparison, the corresponding figures for pyrethrum extract, an established botanical insecticide, were 0.01, 3.8, and 7.9 μg/insect.[64] Viminadione B was less active, killing only 60% of houseflies at a dose of 10 μg.

Myrtucommulones K and L (**42**, **43**) are terpene adducts of flavesone and were isolated from the leaves of *Myrtus communis*.[65] The structure of myrtucommulone K was revised to **42** recently based on X-ray crystallographic analysis.[66] Neither compound was cytotoxic to the human haematological tumor cell line MT-4.

Two additional β-triketone terpene adducts which don't contain a pyran ring are calliviminone A and B (**44**, **45**) which were isolated from the fruits of *Callistemon viminalis*,[67] a cultivated Australian native plant, collected from the Guangdong province of China. The compounds were proposed to be adducts formed by the Diels-Alder cyclization of the monoterpene myrcene and an isobutyl syncarpic acid moiety. A biomimetic synthesis using these precursors successfully produced both isomers. Both compounds moderately inhibited the production of NO in lipopolysaccharide-induced RAW264.7 macrophages. Rhodomyrtal D (**46**) has an unusual coupling of a terpene and β-triketone since it incorporates a eudesmane sesquiterpene linked to the

C-2 methyl carbon of 2-demethylleptospermone. It was isolated from the Australian rainforest plant *Rhodomyrtus psidioides.*[68]

Calliviminones C-H (**47**-**52**), the polymethylated β-triketone derivatives were isolated from the fruits of *Callistemon viminalis* and they moderately inhibited nitric oxide production in lipopolysaccharide-induced RAW264.7 macrophages. Tomentosenol A (**53**) isolated from *Rhodomyrtus tomentosa*[60] is a pyran ring opened form of 4*R*-ficifolidione (**38**) with moderate antitumor activities towards the four human cancer cell lines MCF-7 (human breast adeno carcinoma cell line), NCI-H460 (human non-small cell lung cancer cell line), SF-268 (human glioma cell line) and HepG-2 (human hepatoma carcinoma cell line) with IC_{50} values of 8.66 ± 0.24 mM, 8.62 ± 0.31 mM, 10.01 ± 0.41 mM, and 9.44 ± 0.36 mM. It exhibited antibacterial activity and MIC values were 4.74 µM against *S. aureus,* comparable to the positive control vancomycin (MIC = 1.23 µM). Seven other meroterpenoid epimers, tomentodiones A-D (**54**, **55, 56, 57**), β-triketone conjugated with caryophyllene moiety, rhodomyrtials A and B, rhodomentone A (**58, 59, 60**) were also isolated from the leaves of *Rhodomyrtus tomentosa*.[60, 69, 70]

2.2.3.4 Mono-β-Triketone Phloroglucinol Conjugates

Compounds which contain a bond linking C-1 of the side chain of a simple acyl-β-triketone to an aromatic carbon of an acylphloroglucinol have been increasingly reported in Myrtaceae species (Figure 5 and Figure 6). Since the last comprehensive phloroglucinol review in 2006, 36 additional mono β-triketone phloroglucinol conjugates have been reported increasing the total known compounds to 40. These compounds have been reported from 15 species and ten genera within the Myrtaceae, including *Baeckea*, *Callistemon*, *Callythranthes*, *Corymbia*, *Kunzea*, *Lophomyrtus*, *Luma*, *Melaleuca*, *Myrtus*, and *Rhodomyrtus.* The least complex mono β-triketone phloroglucinol adducts contain an alkyl syncarpic acid moiety connected to the phloroglucinol through only one bond. Hydrogen bonds between the phloroglucinol hydroxyls ortho to the point of attachment of the β-triketone with the carbonyl and enol oxygens of the syncarpic acid moiety in $CDCl_3$ leads to stable rotamers about the C-6/C-1' bond being observed in solution. This generally produces a doubling of all

NMR signals in compounds from this general structure class which can make their structure determination challenging.

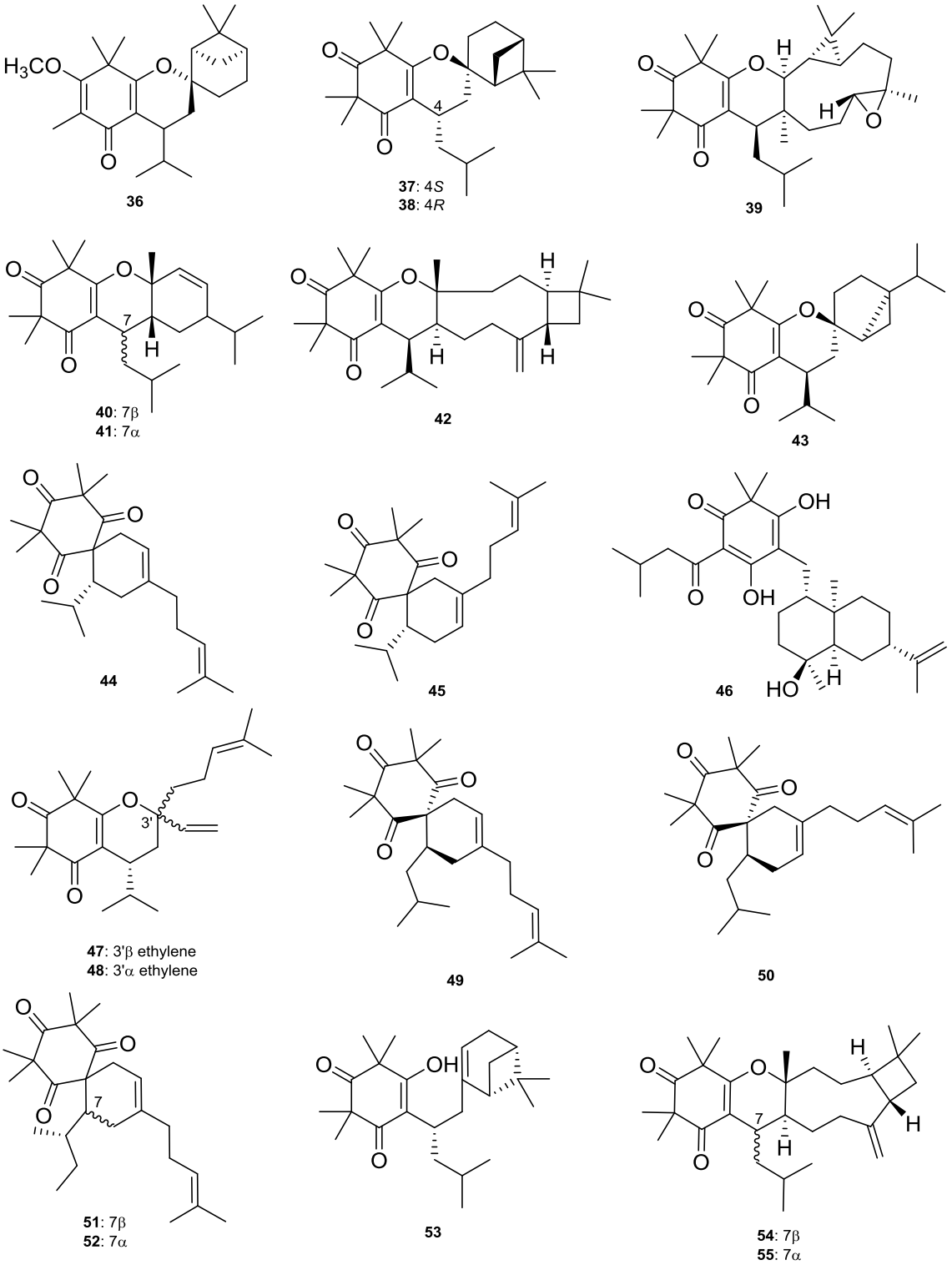


Figure 3. β-Triketone terpene adducts

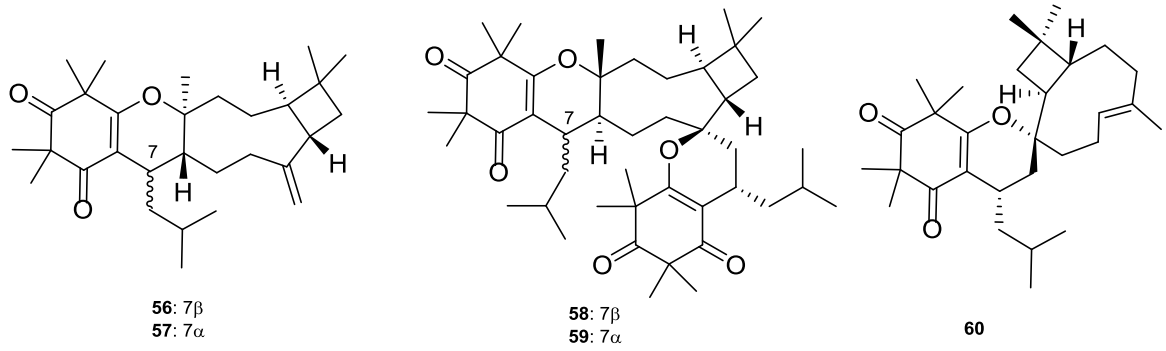


Figure 4. β-Triketone terpene adducts (continued)

Semimyrtucommulone (**61**) from the leaves of Mediterranean tree *Myrtus communis*[71, 72] was the first compound in this series to be characterised and it can be considered to be a conjugate of the acyl-β-triketone flavesone and an isobutanoylphloroglucinol. It possesses antibacterial activity against multi-drug resistant *Staphylococcus aureus* at MIC 32-64 μg/mL.

Two compounds isolated from *Kunzea ericoides*[30] (**62** and **63**) both contain an *O*-methyl-acylphlorogucinol. They showed whole-well (17 mm) inhibition of cytopathic effects of herpes simplex type 1 and polio type 1 viruses at 40 μg per disk.

Callistenone C (**64**), a homologue of semimyrtucommulone (**61**) from the leaves of *Callistemon lanceolatus*[32] showed equally potent antibacterial activity against *Staphylococcus aureus* ATCC 25923 and methicillin resistant *Staphylococcus aureus* SK1 at 8 μg/mL.

Corymbones A and B (**65**, **66**) which are also closely related to semimyrtucommulone have been isolated from the flowers of the tree *Corymbia peltata*[73] from Queensland, Australia. They possess thyrotropin releasing hormone receptor 2 binding affinity with IC_{50} values of 23 and 19 μM respectively. Biogenetically, corymbones are proposed to be derived from the condensation of 2′,4′,6′-trihydroxy-3′-methyldihydrochalcone with the acyl-β-triketones, grandiflorone and leptospermone respectively.

The tetramethylcyclohexenedione (**67**) which was isolated as an inseparable mixture of two diastereomers from aerial parts of *Kunzea ambigua*[74] and *Kunzea baxterii*,[74] is closely related to corymbone B and could potentially be an artefact obtained after non stereo selective cyclization of the chalcone derivative of corymbone B to form a

flavonoid. It possesses moderate insecticidal activity compared to natural pyrethrum extract.

A related compound (**68**) also isolated as a diastereomeric mixture in a ratio of 3:2 has been reported from the aerial parts of *Baeckea frutecens*[56] collected in Hong Kong. The compound lacks a methyl substituent at C-2 of the β-triketone moiety, but this is replaced with methyl enol ether at C-3. Bullataketals A (**69**) and B (**70**) diastereomeric compounds isolated as a mixture from the leaves of the New Zealand shrub *Lophomyrtus bullata*[75] are more complex derivatives of semimyrtucommulone since each contains an unusual bicyclic ketal moiety attached to the phloroglucinol. *L. bullata* also contains the unique lactone bullatenone (**71**) which the authors considered to be a plausible biogenetic building block which could undergo an aldol like condensation with a demethyl-semimyrtucommulone derivative to form **69** and **70**. Interestingly, the compounds were isolated as racemic mixtures. The compounds were active against the P388 mouse leukaemia cell line (IC_{50} 1 μg/ml), and also showed antimicrobial activity against *Bacillus subtilis* (MIC 30 μg/disk).

In a second series of mono β-triketone-phloroglucinol conjugates, the β-triketone moiety and phloroglucinol form a chroman ring. Simple mono β-triketone phloroglucinol adducts are the most likely precursors since hemiketal cyclization and subsequent dehydration has been shown to generate these chroman derivatives. Since there are generally two hydroxyl groups ortho to the attachment point of the alkyl-β-triketone, two possible chroman regioisomers can result from cyclization. It is therefore no surprise that pairs of regioisomers are commonly co-isolated from the same plant source. There is also a possibility that some of these compounds could be artefacts that result from the extraction and purification process since these chromans can be easily made by treating uncyclised precursors with acid.

Myrtucommulone B (**72**) was the first compound in the class to be reported. It was isolated from the leaves *Myrtus communis*[71] and reported to possess significant antimicrobial activity against gram positive bacteria. Two papers have since suggested that myrtucommulone B may actually be the regioisomer with the acyl side chain attached at C-7. Shaheen et al[76] proposed that myrtucommulone B was the C-7 keto regioisomer based on single crystal X-ray analysis of a compound they isolated from *M. communis*, however, no NMR data was presented for their compound. The NMR data originally presented by Kasham et al[71] provides convincing evidence that the acyl

side chain is attached to C-5 in myrtucommulone B since only one of the phenolic protons is intramolecular hydrogen bonded, resonating downfield at δ_H 14.30 while the other phenol proton resonates at δ_H 6.10. Appendino et al[72] reported NMR data for a compound isomeric with myrtucommulone B which they named isomyrtocommulone B (**73**).

The phenolic protons for this compound resonated at δ_H 11.2 and 13.9 and this was consistent with both phenolic protons forming hydrogen bonds to the adjacent ketone carbonyl carbon. Based on this evidence myrtucommulone B is the C-5 acyl isomer (**72**) and isomyrtucommulone B (**73**) is the C-7 acyl isomer. Isomyrtucommulone B shows moderate α-glucosidase activity (IC_{50} 40 μM).

Rhodomyrtone (**74**), and rhodomyrtosone B (**75**) isolated from the leaves of *Rhodomyrtus tomentosa*,[55, 77, 78] an evergreen shrub, which is distributed throughout Southeast Asia, are a pair of regioisomers containing an isobutyl ketone attached to either C-5 or C-7 of the phloroglucinol. Rhodomyrtone is closely related to myrtucommulone B since both isopropyl groups in **72** are replaced with isobutyl groups in **74**. Antibacterial activity for rhodomyrtone has been studied extensively and it is active against epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA), vancomycin- intermediate *Staphylococcus aureus* and vancomycin-resistant enterococcal strains. Interestingly, the MIC and minimum bactericidal concentration values for rhodomyrtone against MRSA ranged from 0.39 to 0.78 μg/mL, which is very close to those of vancomycin. Protein profiling studies on rhodomyrtone treated MRSA have demonstrated that it changes the expression of several major functional classes of bacterial proteins. Rhodomyrtone treated *Staphylococcus aureus* exhibited reduced pigmentation and rhodomyrtone treatment led to a dose-dependent increase in the susceptibility of the pathogen to H_2O_2 and exposure to singlet oxygen. Consequently, the survival of treated organisms decreased in freshly isolated human whole blood. It has been assumed that rhodomyrtone may be acting via effects on the enzymes DnaK and/or σ^B, resulting in many additional effects on bacterial virulence. Rhodomyrtone has also been reported from the bark and twigs of cultivated *Eucalyptus globulus*[79] growing on the banks of the Nile in Egypt. However since none of the typical *E. globulus* metabolites were reported in this study a question arises about the true identity of this plant material studied.

Rhodomyrtosone I (**76**) has been isolated from the fruits of *R. tomentosa.* It differs from rhodomyrtone by the replacement of the isobutyl group attached to C-9 with a phenyl group.

Callistenone A and B (**77**, **78**) isolated from the leaves of *Callistemon lanceolatus* are another pair of chroman regioisomers. Callistenone A is closely related to rhodomyrtone since the only difference between the two compounds is the replacement of the C-9 isobutyl group in **74** with an isopropyl group in **77**. Likewise, callistenone B (**78**) is the C-9 isoproyl analogue of rhodomyrtosone B (**75**). They exhibited antibacterial activity against *S. aureus* ATCC25923 and methicillin-resistant *S. aureus* SK1 at concentrations of 0.5, 1 μg/mL and 8 μg/mL respectively. The antibacterial activity observed within these series suggests that the position of the ketone side chain affects the antimicrobial activity. Compounds with acyl groups attached at C-5 displayed more potent activity than ones in which the acyl group is attached at C-7. Seven additional chroman derivatives which each contain an ethylphenyl ketone attached to the phloroglucinol have been reported in the literature.

Rhodomyrtosone E (**79**), containing a β-triketone–dihydrochalcone skeleton, has been isolated from the leaves of *Corymbia citriodora* (previously named *Eucalyptus citriodora*).[80] It showed weak GLUT4 translocation effects with a 0.49 fold enhancement after 10 min. Rhodomyrtosone F (**80**) with strong antiplasmodial activity (IC_{50} of 0.10 ± 0.02 μM).has been isolated from the *Syncarpia glomulifera*.[81] Watsonianone C (**81**) isolated from the flowers of *Corymbia watsoniana*[82] is closely related to **58** since it differs from **58** only because of the phloroglucinol group is both methylated at C-5 and more highly oxidised. Watsonianone C showed antiplasmodial activity against chloroquine sensitive and resistant strains of *P. falciparum* at 1.18 μM and 1.07 μM respectively.

Kunzeanones A (**82**) and B (**83**) from *Kunzea ambigua*[63] are also closely related to rhodomyrtosone E since cyclization of the ethylphenyl side chain form a flavanone and the addition of a methyl substituent at C-5 are the only difference observed. Single crystal X-ray analysis of a 1:1 mixture **82** and **83** indicated that they were both present in the unit cell and demonstrated that they were epimeric at C-9. Kunzeanone A (**60**) was tested for its ichthyotoxicity towards medaka fish, but showed no toxicity at 30 μg/mL.

BF-4 (**84**), BF-5 (**85**) and BF-6 (**86**) isolated from the leaves of *Baeckea frutescens*[58] were each obtained as racemic mixtures. All three compounds contain an ethylphenyl ketone attached at C-7, however, unlike the kunzeanones, this moiety forms a flavanone through cyclization with oxygen attached at C-8. BF-4 and BF-5 are epimers at C-9 and exhibited equally strong cytotoxic activity against leukaemia cells (L1210 IC_{50} 0.25 μg/mL). BF-6 was only weakly cytotoxic in the same assay (IC_{50} 10 μg/mL) suggesting that the substituent attached at C-9 is important for cytotoxicity. Four diasteromeric compounds, leucadenones

A-D (**87**-**90**) that are C-11 hemiketal derivatives of BF-6 have been isolated from the fresh leaves of *Melaleuca leucadendron.*[83]

Rhodomyrtosone A (**91**) and watsonianone B (**92**) each containing a fused bisfurano-β-triketone-phloroglucinol structure, have been isolated from the leaves of *Rhodomyrtus tomentosa*[55] and the flowers of *Corymbia watsoniana*[82] respectively. While **91** contains an isobutyl ketone, **92** contains an ethylphenyl ketone. Watsonianone B is a potent anti-plasmodial compound with IC_{50} of 0.44 μM and 0.29 μM against chloroquine resistant (Dd2) and sensitive strains (3D7) respectively of the parasite, *Plasmodium falciparum*. Stage specificity studies revealed that watsonianone B is active against young ring stages of *P. falciparum.*

Usnone A (**93**) and its regioisomer isousnic acid (**94**) isolated from *Myrtus communis*[84, 85] is also a β-triketone phloroglucinol conjugate, however, the phloroglucinol was directly bonded to the cyclohexanone, forming a benzofuran. Pallenic acid (**95**) isolated from the fruits of the rare plant *Calyptranthes pallens*[86] from Florida, USA is a phloroglucinol compound with a new carbon skeleton. Its structure suggests that it is likely to be an oxidation product of a β-triketone phloroglucinol conjugate.

Lumiaflavone A, lumiaflavone B (**96**, **97**) and lumiaflavone C (**98**), have been isolated from *Luma chequen*.[87] Lumiaflavones A and B, lumiaflavone C are C-9 isopropyl derivatives of BF-6 and leucadenones A-D respectively. A mixture of **96** and **97**, **98** was tested for anifeedant activity, brine shrimp toxicity and antifungal activity against *Spodoptera littoralis*, *Artemia salina* and *Botrytis cinerea* respectively. LC_{50} value against *Artemia salina* for a mixture of **96** and **98** was > 100 ppm while it was 1.97 ppm for lumiaflavone C. The antifeedant activity (feeding ratio (FR_{50})) for the mixture of lumiaflavone A and lumiaflavone B, lumiaflavone C was 0.68 ± 0.11, 0.11 ± 0.02 respectively. Strong anti-fungal activity was also reported for mixture of lumiaflavone A and lumiaflavone B.

Callistrilone A (**99**) and Callistrilone B (**100**) were isolated from *Callistemon rigidus*[88]. They are composed of pentacyclic ring system with an isobutyl syncarpic acid unit, an isobutyryl phloroglucinol moiety and α-phellandrene monoterpene unit. Callistrilone A is moderately active towards Gram-positive bacteria *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Acinetobacter baumanni* ATCC 19606, *Klebsiella pneumoniae* ATCC BAA- 2146 and *Pseudomonas aeruginosa* ATCC 27853 with MIC values ranging from 16 μg/mL - 32 μg/mL. It has stronger activity

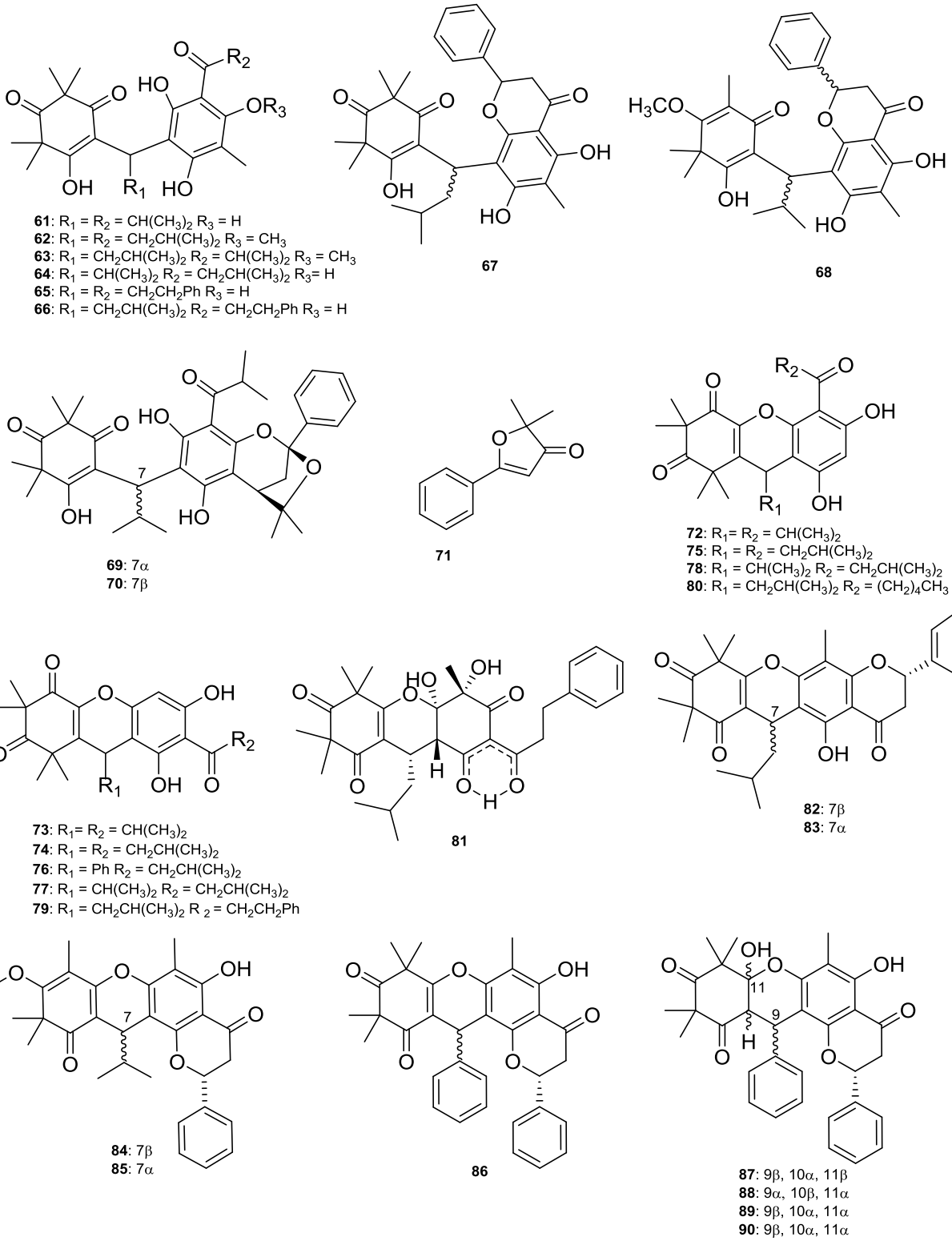


Figure 5. Mono-β-triketone phloroglucinol conjugates

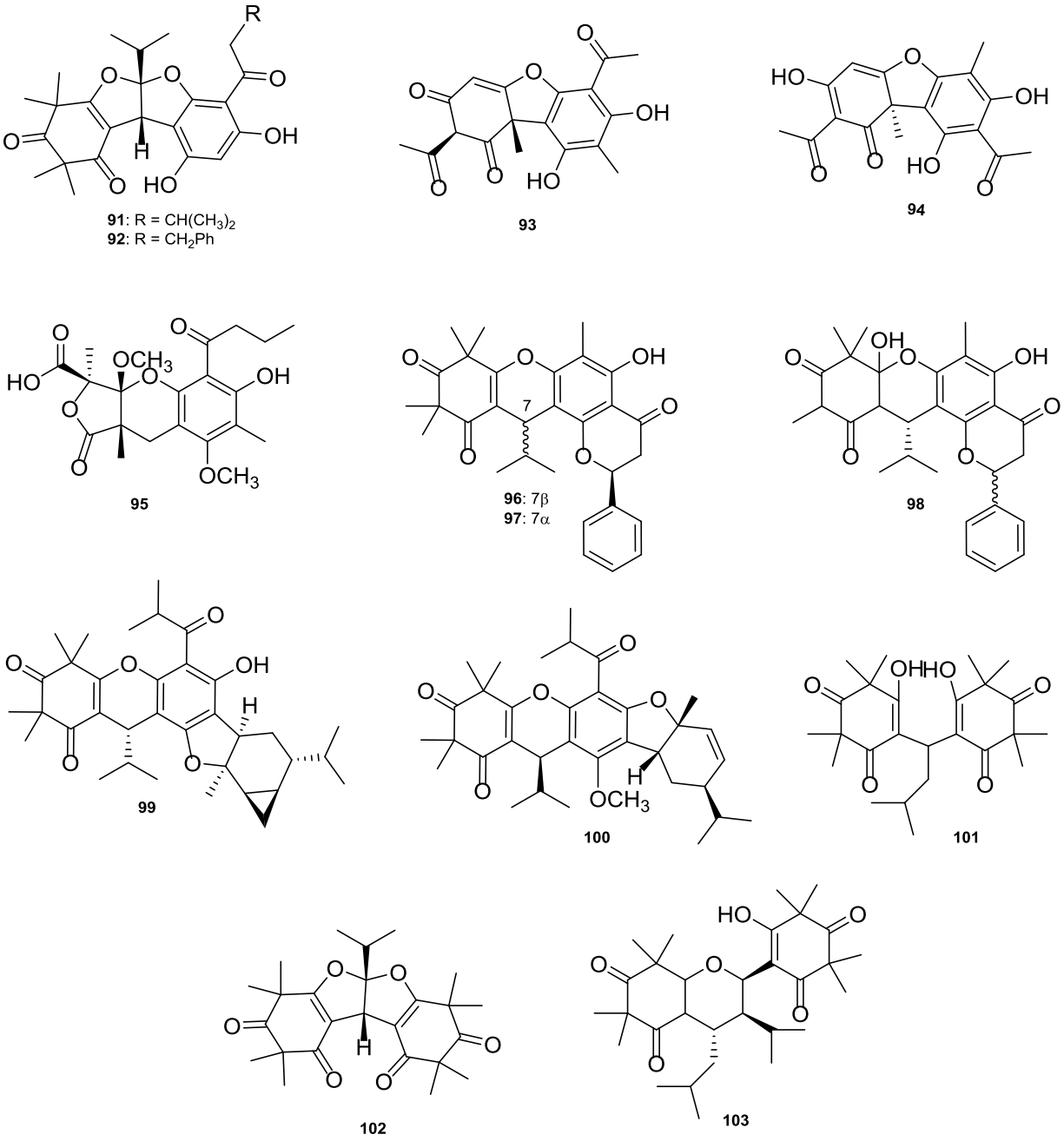


Figure 6. Mono-β-triketone phloroglucinol conjugates (continued) and β-triketone dimers

towards multi drug resistant strains *Staphylococcus aureus* ATCC 33591, *S. aureus* Mu50, and *Enterococcus faecium* 13-01 than the positive control oxacillin (MIC 256 - 512 μg/mL).

2.2.3.5 β-Triketone Dimers

β-Triketone dimers (Figure 6) are rarely found in nature and watsonianone A (**101**) isolated from the flowers of *Corymbia watsoniana*[82] from Queensland, Australia is reported to be the first naturally occurring methylene bridged bis-tetramethylcyclohexatrione in the literature. Watsonianone A possesses antiplasmodial activity against chloroquine sensitive 3D7 *Plasmodium falciparum* (IC_{50} 5.3 μM) and chloroquine resistant Dd2 (IC_{50} 8.8 μM) *Plasmodium falciparum*.

The symmetrical metabolite, rhodomyrtosone D (**102**) isolated from the leaves of *Rhodomyrtus tomentosa*[55] collected in southern Thailand, also contains a fused bisfurano moiety but in this case two β-triketone moieties are linked to form the two furan rings. This compound was proposed to be derived from **101,** a compound (watsonianone A) which has subsequently been isolated from *Corymbia watsoniana.*

Ericifolione (**103**) isolated from the leaves of the New Zealand native shrub *Kunzea ericifolia*[89]cultivated at Kew Gardens in the UK is an insecticidal compound containing two β-triketone moieties linked by a pyran ring. The structure of the compound shows remarkable similarities to the sideroxylonals (see below). In a series of whole insect assays ericifolione showed LD_{50} values of 1.4 μg/insect (houseflies, *Musca domestica*), 5.2 μg/insect (aphid, *Aphis fabae*) and 5.3 μg /insect (thrips, *Thrips tabaci*). The vast majority (97%) of mustard beetles, *Phaedon cochleariae* were killed at a dose of 10 μg.

2.2.3.6 Bis-β-Triketone Phloroglucinol Conjugates

The majority of bis-β-triketone phloroglucinol conjugates (Figure 7 and Figure 8) have been reported from *Callistemon, Corymbia, Myrtus, Pilidiostigma* and *Rhodomyrtus* species. They generally contain an additional alkyl substituted syncarpic acid moiety attached at C-5 or C-7 of the phloroglucinol of a mono β-triketone phloroglucinol conjugate forming either a methylene linkage or a chroman ring. The methylene linked bis β-triketone phloroglucinol conjugates can form multiple stable rotamers in solution which leads them to possess highly complex NMR spectra which has significantly hampered their structure determination.

Myrtucommulone A (**104**) isolated from *Myrtus communis*,[65, 71, 72] *Callistemon lanceolatus*[90] and the seeds of *Corymbia scabria*[91] from Australia is a flavesone conjugate of semimyrtucommulone. It has demonstrated antibacterial activity against multidrug resistant clinically important bacteria *Staphylococcus aureus* at MICs 0.5 - 2 μg/mL and anti-inflammatory activity. Myrtucommulone A, also inhibits microsomal prostaglandin E2 synthase-1 and induces apoptosis in cancer cells via the mitochondrial pathway involving caspase-9. Myrtucommulone A also showed cytotoxicity against human haematological MT-4 cells with IC_{50} values ranging from 4.7 to 14.0 μM.

Myrtucommulone F (**105**) and H (**106**) which are pentylketone analogues of **104** have also been isolated from the seeds *Corymbia scabria.*[91] Under mildly acidic conditions Myrtucommulones A, F and H cyclize to give myrtucommulones D (**107**), G (**108**) and I (**109**) respectively. Myrtucommulones A, D and F-I showed rat TRH receptor -2 binding affinity with IC_{50} values 39, 11, 16, 24, 31 and 16 μM respectively. Myrtucommulones C-E (**110**, **107**, **111**) also isolated from *Myrtus communis*[76] are dehydrated and cyclized chroman derivatives of **104**. Myrtucommulones C, D and E showed α-glucosidase inhibitory activity (IC_{50} 35.4, 84.3 and 46.6 μM respectively).

Another two compounds that are analogues of myrtucommulone G and myrtucommulone E respectively are callistenones D (**112**) and E (**113**) which have also been isolated from the leaves of *Callistemon lanceolatus*[32] collected in southern Thailand. Rhodomyrtosone C (**114**) isolated from the leaves of the *Rhodomyrtus tomentosa*[55] is also closely related to myrtucommulone E.

Myrtucommuacetalone (**115**), a compound with a unique carbon skeleton has been isolated from *Myrtus communis.*[85] The structure of this compound was elucidated using NMR techniques and X-ray diffraction studies. These studies indicated that this compound contains an unprecedented bridged furochromene moiety. This unusual side chain is most likely derived from flavesone and therefore potentially could be a rearranged derivative of myrtucommulone A. It has exhibited inhibitory effect against nitric oxide production and substantial antiproliferative activity of IC_{50}< 0.5 μg/mL against T-cell proliferation. These findings suggest that the compound has a highly significant inhibitory activity on cellular immune response and might be useful in suppressing various allergic, inflammatory, and autoimmune disorders.

Myrtucommulone J (**116**) also isolated from *Myrtus. communis*[65] exists as a pair of isomers in solution in a ratio of 9:1. The authors suggest that these isomers are the result of keto-enol tautomerism of the syncarpic acid moiety; however a more likely explanation is that the NMR signal doubling is associated with rotamers about the phloroglucinol-isobutenyl syncarpic acid bond. It is likely that myrtucommuacetalone and myrtucommulone J are biosynthetically related, with **115** being a potential precursor to **116**. It showed cytotoxic activity against human haematological MT-4 cells with IC_{50} values ranging from 2.1 to 3.0 μM.

Tomentosones A (**117**) and B (**118**) are novel hexacyclic compounds that have also been isolated from the leaves of *Rhodomyrtus tomentosa*.[92] Both compounds incorporate a rhodomyrtone substructure to which an additional alkyl syncarpic acid moiety is attached forming a fused bisfurano moiety identical with that present in rhodomyrtosone A. Tomentosone A inhibited the growth of chloroquine resistant and sensitive strains of the malaria parasite *Plasmodium falciparum*, with IC_{50} values of 1.49 μM and 1.0 μM, respectively, while tomentosone B was significantly less active.

Eucalyptone G (**119**) is a bis-β-triketone phloroglucinol conjugate isolated from the bark of *Eucalyptus globulus*[79] cultivated in Egypt. As mentioned above, based on the extensive list of compounds isolated in the study, the species studied may not be *E. globulus*. The proposed structure of eucalyptone G incorporates a rhodomyrtone directly attached to an acylcyclohexadione. The structure of the pendent acylcyclohexadione is atypical of simple acyl-β-triketones reported from Myrtaceae species since the acyl side chain is vicinal to the germinal dimethyl carbon and this, along with ambiguous 2D NMR correlations and dubious ^{13}C chemical shift assignments suggest that the proposed structure may be incorrect. The compound inhibits the growth of gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*.

Myrtucommulone M (**120**) isolated from *Myrtus communis*[85] is a symmetrical dimer of myrtucommulone B generated by the insertion of a methylene bridge between the two phloroglucinols. It was inactive in nitric oxide production and antiproliferative assays in T-cells.

Pilidiostigmin (**121**) isolated from the leaves of the Australian rainforest shrub *Pilidiostigma glabrum*[93] was orginially proposed to contains two β-triketone phloroglucinol moieties ether linked to form a dimer. The fact that the authors claim that the compound was isolated as a stable sodium alkoxide salt even after purification

under aqueous conditions, called into question the true identity of this compound and the structure was revised in 2016 to **121**. Pilidiostigmin was cyctotoxic to RAW 2647 mouse monocyte macrophage cells with an EC_{50} 8.65 μM and showed 60% inhibition of nitrous oxide production in the same cells stimulated with LPS at 7.8 μM.

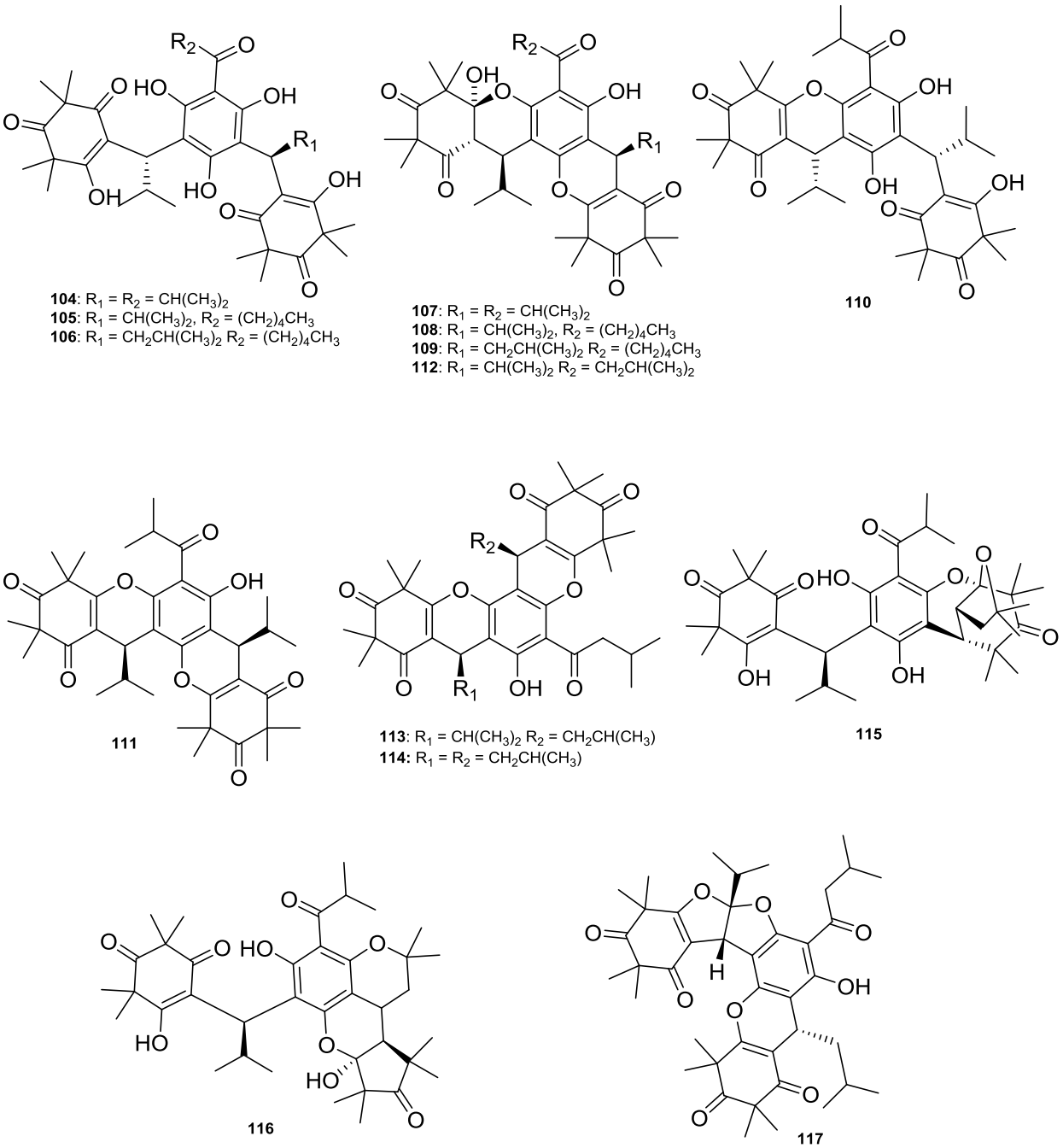


Figure 7. Bis β-triketone phloroglucinol conjugates

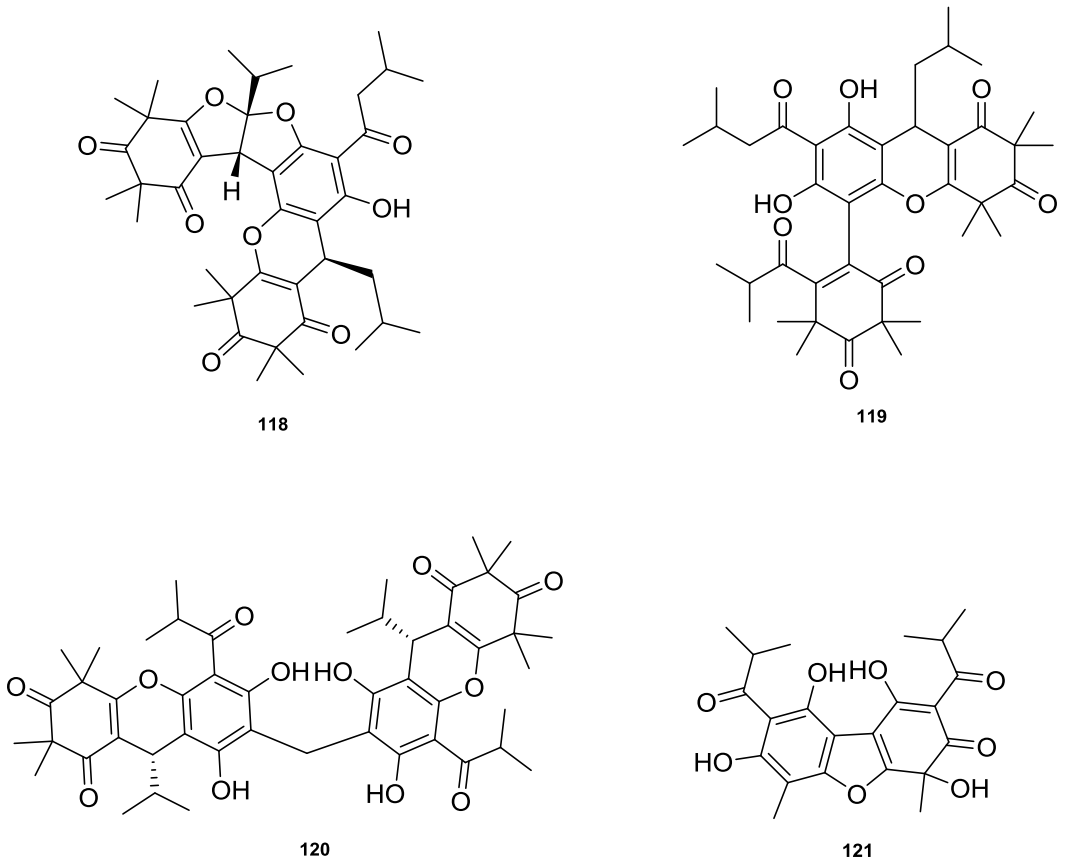


Figure 8. Bis β-triketone phloroglucinol conjugates (Continued)

2.2.4 Phloroglucinols

2.2.4.1 Acyl Phloroglucinols

Acyl phloroglucinols (Figure 9 - Figure 12) are characterized by the presence of at least one acyl chain, generally a -COR group, attached directly to the phloroglucinol.[94] The R group can either be isovaleryl, methylbutyryl, isobutyryl, acetyl, ethylphenyl or benzoyl. To date 84 acylphloroglucinol compounds have been isolated from 36 species and 13 genera within the Myrtaceae. Members of this class of compounds exhibit a variety of biological activities such as antimicrobial, antioxidant, anti-inflammation and antimalarial making them of interest for drug development.[19, 95] The presence of an acyl side chain is vital for their biological activity. Compounds containing a long acyl side chain and with more than one acyl group are particularly bioactive against methicillin resistant *Staphylococcus aureus* (MRSA),

vancomycin-resistant *Enterococcus faecalis* (VRE) and multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB).[96] The benzoyl oxygen can predominantly form intramolecular hydrogen bonds with ortho-hydroxyl groups and this often determines the conformation of the molecule, contributing to antioxidant and antiradical activities of certain acylphloroglucinol molecules.[94]

Species from the Myrtaceae produce acylphloroglucinols containing various combinations and proportions of aryl *C* methylation or formylation and/or *O* methylation which contributes to their varied biological activities such as antibacterial activity, HIV-RTase inhibitory activity, aldose reductase inhibitory activity, antiplasmodial activity, inhibition of Epstein-Barr Virus activation and cell cytotoxicity.[1, 19] The vast majority of acylphloroglucinols reported from Mytraceae are *C*-methylated and all but one of the seven formylated phloroglucinols reported have been isolated exclusively from *Eucalyptus* species suggesting that the formyl substituent could be a taxonomic marker for the genus.

Grandinol (**122**) and homograndinol (**123**) are the most widely studied Myrtaceae acylphloroglucinols. These two compounds (**122**, **123**) with one formyl substituent have been isolated from the mature leaves of *E. grandis*, *E. perriniana*, *E. globulus* and *E. pulverulenta*.[97-99] Both grandinol and homograndinol have exhibited germination inhibitory activity, Epstein Barr Virus inhibitory activity and photosynthetic electron transport inhibitory activity. Grandinol inhibits germination[97], transpiration and stomatal opening. On the cress germination test, grandinol showed an IC_{50} of 10 ppm.[97] It also showed antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* at 5 μg/mL and 25 μg/mL respectively.[97-99] The phloroglucinol, jensenone (**124**), isolated from *E. jensenii*,[100, 101] a small iron bark tree of northern Australia is substituted by two formyl groups in addition to the isovaleryl group suggesting that it is the precursor of more complex secondary metabolites isolated from Myrtaceae species including the euglobals, macrocarpals and sideroxylonals (Figures 13-19). Initially, jensenone was isolated by acid base extraction of a crude extract of *E. jensenii*[100] prepared by Soxhlet extraction of dried and powdered leaves with 20% acetone in petroleum ether. It was later isolated from the leaf essential oils of *E. apodophylla*[102] and *Choriocarpia subargentea.*[103] Jensonone is a potent mammalian antifeedant.[104] It is effective against *Candida albicans* with a half-maximal inhibition concentration (IC_{50}) of 5.5 mg/mL and exhibited moderate anti-leishmanial activity in vitro (IC_{50}) at 19 mg/mL against *Leishmania donovanii* promastigotes.[105] Furthermore, jensenone inhibits photosynthetic electron transport although it is somewhat less active compared to grandinol.[100]

The analysis of oil samples obtained by hydrodistillation of leaves of *E. apodophylla*[102] led to the isolation of two diformyl acylphlorglucinols (**125**) and (**126**). The most wide spread acylphloroglucinol compound found in the Myrtaceae is torquatone (**127**) isolated from the leaves of various *Eucalyptus* species including *E. angulosa, E. brachycalyx, E. caesia, E. canaliculata, E. calygona, E. celastroides, E. ceratocorys, E. celandi, E. flocktoniae, E. spathulata var grandiflora, E. incerata, E. miniata, E. pumila, E. recta, E. suggrandis, E. tenera, E. torquata, E. vegrandis*.[14, 106, 107]

Miniatone (**128**) from *E. miniata* and *E. jensenii* and 4-*O*-demethyl miniatone (**129**) reported from *E. jensenii* are structurally related to torquatone.[101, 108] The leaves of *E. apodophylla* also contain two methyl phloroglucinols, apodophyllone (**130**) and isotorquatone (**131**). These compounds have also been isolated from the leaves of *Eucalyptus chartaboma.*[108] Robustaol B (**132**), isolated from the Australian native tree, *E. robusta*, (a plant used to prepare the Chinese anti-malarial traditional medicine "Da Ye An"), *Kunzea sinclairii*[30] and *Kunzea ericoides*[30] is another isobutyryl analogue of phloroglucinol which has shown whole-well (17 mm) inhibition of the cytopathic effect on *Herpes simplex* Type 1 and Polio Type 1 viruses at 5 μg/disk. Other than robastaol B compounds **133** and **134** with phosphodiesterase activity (ED_{50} 100 μg/mL and 125 μg/mL respectively) have also been reported from the leaves of *E. robusta*.[109]

Conglomerone (**135**) isolated from the essential oil of *E. conglomerata*[19] is an isobutyryl analogue of phloroglucinol. Another alkylated and isobutyryl analogue, **136** has been isolated from *K. sinclairii* and it exhibits antiviral activity against against *Herpes simplex* Type 1 and Polio Type 1 viruses at 5 μg/disk.[30] A dimethoxy compound, baeckeol (**137**) has been identified from the leaves of *Baeckea crenulata,*[19] *Baeckea frutescens*[61] and *Calythrix angulata*[106]. From the leaves of *Baeckea frutescens,*[61] a medicinal plant which has been used as an antifebrile in southeast Asia and China, two acylphloroglucinol compounds including BF-1 (**138**) and the phloroisovalerophenone analogue homoisobaeckeol (**139**) were reported. Homoisobaeckeol has also been isolated from *Eucalyptus chartaboma.*[108]

Eucalyptus pulverulenta,[97] a rare eucalypt that grows naturally in only a few localities of the central and southern tablelands of New South Wales (Australia), but is widely cultivated in California and to a lesser extent in Japan produces pulverulentone A and B^1 (**140**, **141**) in its fresh leaves and stems. These two compounds have exhibited weak cress seed germination activity. Another dimethoxy acylphloroglucinol compound bancroftinone (**142**) has been isolated from the essential oil of *Syzygium aromaticum* (previously named *Eugenia*

caryophyllus)[110] a plant that naturally grows in the Moluku Islands of Indonesia and *Backhousia bancroftii*.[111]

Two more dimethoxy compounds, 6-hydroxy-2,4-dimethoxy-3-methylbenzophenone (**143**) and 2-hydroxy-4,6-dimethoxy-3-methy1benzophenone (**144**) have been reported from the leaves of *Leptospermum luehmannii*[112] which is endemic to the rocky peaks and slopes of the Glasshouse Mountains of the Sunshine Coast, Queensland, Australia. The leaves and twigs of the Florida tree *Calyptranthes pallens*,[86] contains two acylphloroglucinols, 2,6-dihdroxy-3-methyl-4-methoxyacetophenone (**145**) and aspidinol (**146**).

2,4,6-Trimethoxytoluene (**147**) has been isolated from the primitive eucalypt *Stockwellia* sp.[113] *Leptospermum recurvum*,[114] a plant endemic to Mt Kinabalu in Malaysia contains 2,4,6-trihyroxy-3-methyldihydrochalcone (**148**) as an inseparable mixture with two related flavonoids. It has also been found in flower extracts of *Corymbia peltata*. Synthetic (**149**) was mildly antibacterial towards *Bacillus subtillis* and *Tricophyton mentagrophytes* at 60 µg/disk.

Two additional chalcones, 2'-hydroxy-4',6'-dimethoxy-3'-methyldihydro-chalcodione (**150**) and 2'-hydroxy-4',6'-dimethoxy-3'-methyl-3-hydroxydihydrochalcone (**151**) have been isolated from the leaves of *Leptospermum scoparium*. From the leaves of the *Eucalyptus loxophleba*[20] a cyclised formylated phloroglucinol compound loxophlebene (**152**) have been isolated and it is noncytotoxic to peripheral blood mononuclear cells. A structurally related compound (**153**), in which the *C*-8 formyl group is replaced with a methyl, and which was glycosylated at *O*-7 has been isolated from *Eucalyptus cypellocarpa*.[115] It showed potent short term in vitro and in vivo antitumour promoting activity. Several simple chromans have been isolated from a number of Myrtaceae species.

Noreugenin, eugenitin, melachromonone, 8-methyleugenitol, angustifolionol (**154**-**158**), **159**, and **160**, have been isolated from the leaves of *Melaleuca cajuputi*.[116, 117] Angustifolionol has also been isolated from *Backhousia angustifolia*[118] while eugenitin has also been isolated from *Syzygium aromaticum* (previously named *Eugenia caryophyllata*).[119] Two additional chromenes (**161**, **162**) have been isolated from *Calyptranthes tricona.*[120]A series of *C*- and *O*-glucosylated and acylphloroglucinols kunzeachromone A-F (**163**-**168**) and kunzeaphlogins A-F (**169**-**174**) have been isolated from the leaves of the Australian native species *Kunzea ambigua*[121, 122] collected from the Royal Botanic Gardens in Japan. The kunzeachromones contain a chromone moiety, while the kunzeaphlogons contain either an isobutyl ketone or an isopropyl ketone. The majority of these compounds are gallic acid esters. Two of the kunzeachromonones C and E were also isolated from *Baeckea frutescens*[123] from

Indonesia. An additional six (**175-180**) related compounds (all of which were previously reported as hydrolysis products of the kunzeachromononones) were also isolated. Four of these compounds kunzeachromononones C, E and 2'-degallykunzeacheomonone A and 2'-degallykunzeacheomonone B inhibited copper induced LDL oxidation in the range 3.5 - 3.9 µM. Biflorin and isobiflorin (**176**, **180**) have also been isolated from *Syzygium aromaticum.* (previously named *Eugenia caryophyllus*)[124] while isobiflorin has also been isolated from the leaves of *Eucalyptus cypellocarpa.*[115] An additional two *C*-glucosylated chromenes (**181**) and (**182**) have been isolated from the leaves of *Eucalyptus maiden*,[125-127] while the 6'-gallic acid ester of isobiflorin (**183**) has been isolated from the clove buds of *Syzygium aromaticum*.[128] Two additional *O*-7 glucosylate chromones named cypellocarpins B and C (**184**, **185**) both of which are esterified at C-6' of the glucose moiety by an oleurpeic acid have been isolated from the leaves of *E. cypellocarpa*[115] as potent in vitro and in vivo antitumor promoters. Three phloroglucinol compounds eucamaldusides A-C (**186-188**), which are chromenone glucosides acylated with monoterpene acids, have been isolated *from Eucalyptus camaldulensis var. obtusa.*[129] Four acylphloroglucinols (**189-192**) containing 5 (a), 7 (b, c) or 9 (d) carbon ketones have been isolated from the whole plant of *Syzygium levinei.*[130] Three of the compounds (a, b, d) inhibited the growth of leukaemia K562 and HL-60 cells while also inhibiting differentiation of K562 cells. Six additional long chain acyl-polyhydroxmethylbenzene compounds (**193-198**) have been isolated from the leaves of *Syzygium polyanthium.*[131] In one paper Saifudin et al[132] reported four related compounds each with an unexpected 2,3,5-trihydroxy-3-methylphenyl moiety. Three of the compounds (**193-195**) showed protein tyrosine phosphatase 1B (PTP-1B) inhibitory activity. (IC_{50} 13.1, 5.77, 4.01 µM respectively). In a separate paper Har et al[131] reported two additional long chain acylphloroglucinols, anthuminoate and anthuminone (**199**, **200**) from the same species. The structures of **193-196** have recently been revised to **197-200** after reinterpretation of the NMR data originally published.[133] An additional five phloroglucinol *O*-glycosides, eucalmainosides A- E (**201-205**) were isolated from the fresh fruits of *Eucalyptus maideini*.[134] Eight additional glucosylated acetophenones (**206-213**) have been isolated from the dried leaves of *Syzygium aromaticum*[135] collected in Indonesia. Two glucosylated phloroacetophenones myrciaphenone A and B (**214**, **215**) have been isolated from *Myrcia multiflora*.[136] Compounds isolated from *Syzygium aquem* (**216-218**) are related to 2,4,6-trihydroxy-3-methyldihydrochalcone (**148**).

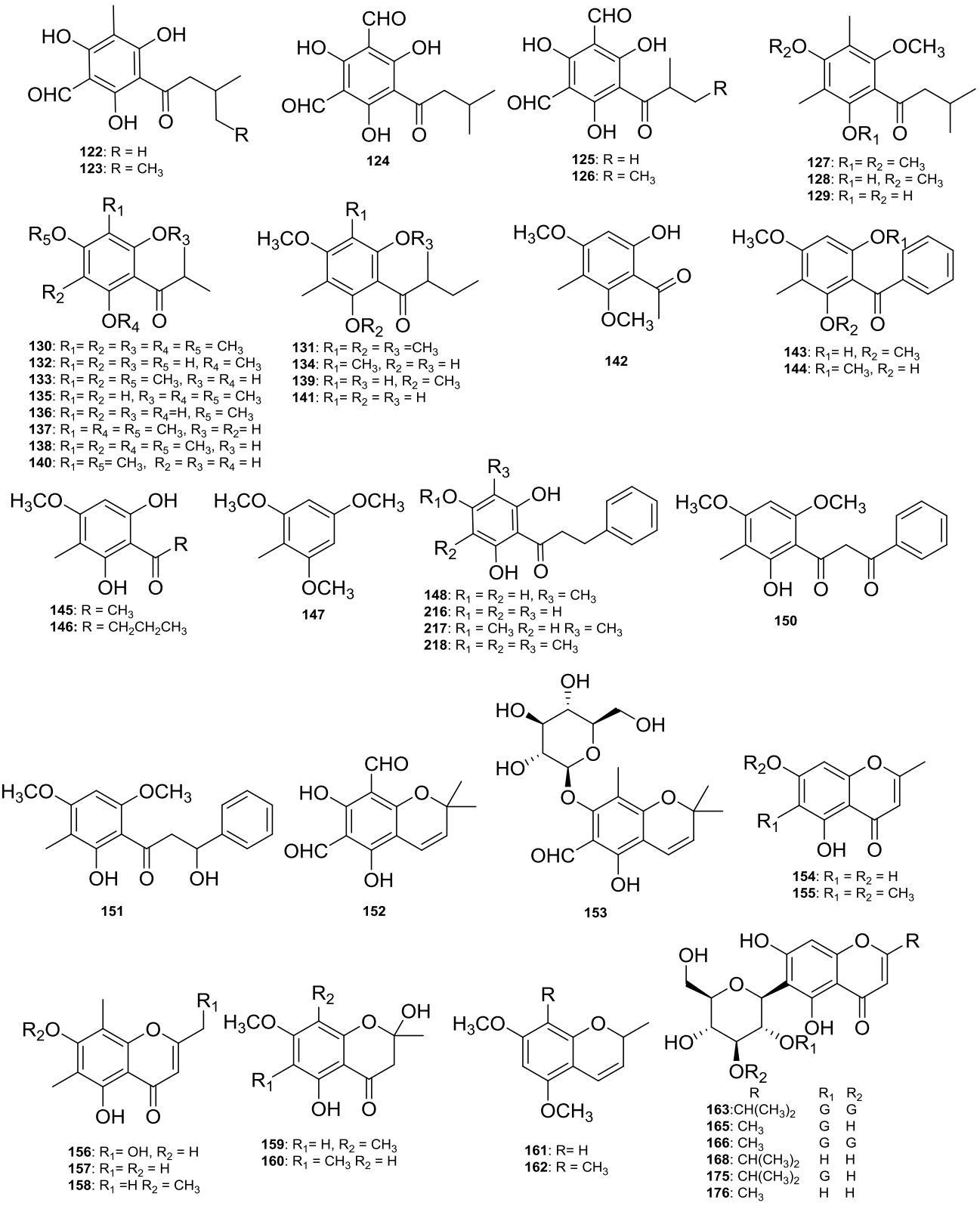


Figure 9. Acyl phloroglucinols

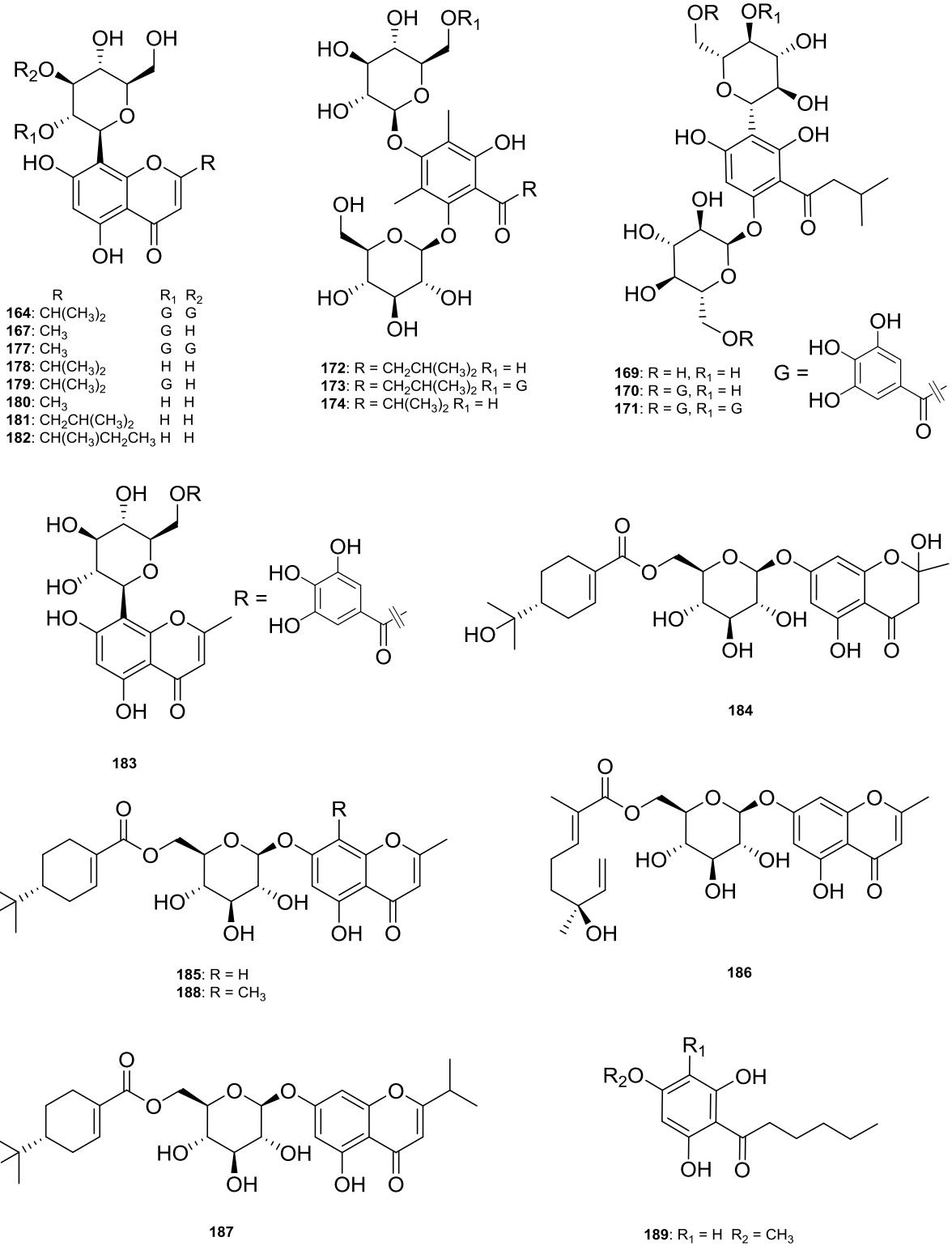


Figure 10. Acyl phloroglucinols (continued)

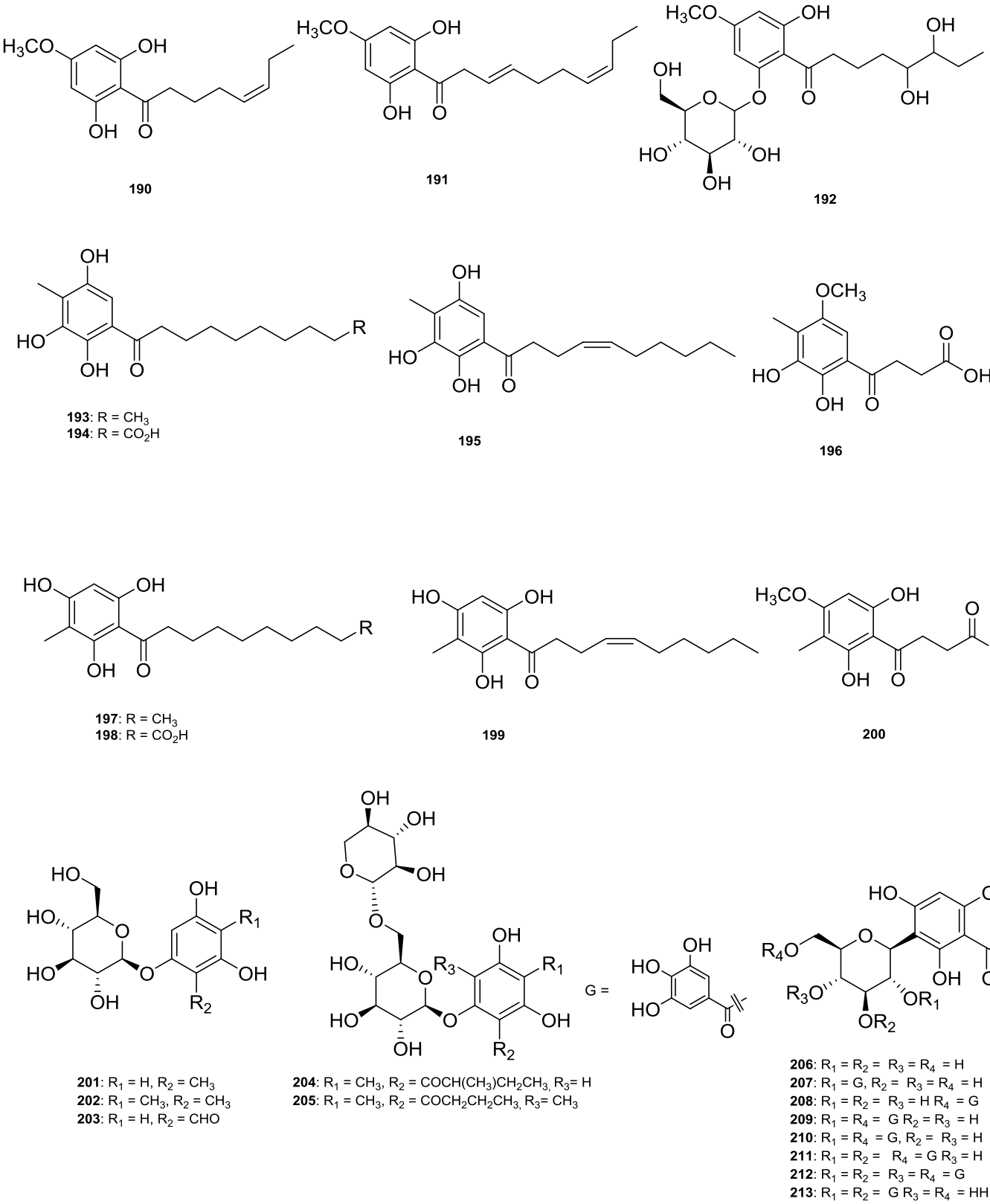


Figure 11. Acyl phloroglucinols (continued)

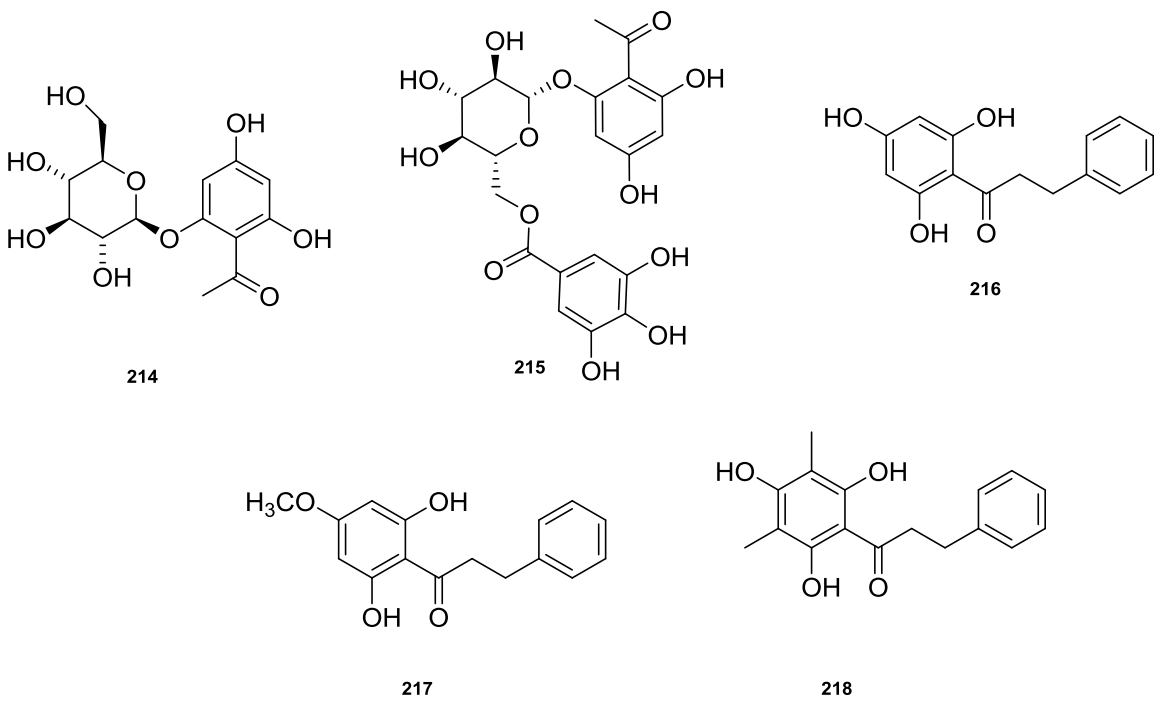


Figure 12. Acyl phloroglucinols (continued)

2.2.4.2 Meroterpenes

A large number of natural products have been reported that contain both a phloroglucinol and a prenyl or terpene unit. Compounds which contain isoprenoid substituents directly attached to the phloroglucinol core have been reported from a number of families, predominantly the Asteraceae, Clusiaceae and Rutaceae. These compounds are prenylated, farnasylated or geranylated and can often contain multiple isoprenoid substituents in one molecule. Meroterpenes containing a phloroglucinol moiety in comparison are a class of compound that are exclusive to the Myrtaceae. Even within the family the vast majority of these compounds have been reported from *Eucalyptus* species. From a biogenetic perspective, the terpene unit is likely to conjugate to the acyl carbon of a phloroglucinol precursor. The genera *Eucalyptus*, *Myrcia*, *Psidium,* and *Rhodomyrtus* have yielded a total of 87 meroterpenes. Over 73% of the compounds isolated come from the 10 *Eucalyptus* species and a further 20% have been isolated from one *Psidium* species. Among the meroterpenes reported to date, chemical diversity results from variation in the structure of the terpenoid moitey. The terpenoid unit can either be a monoterpene or a sesquiterpene and the majority of

compounds that fall within this class have been named as euglobals (Figure 13 and Figure 14) and macrocarpals (Figure 17 and Figure18). Another key feature of the euglobals and macrocarpals is the presence of one or more formyl groups attached to aromatic carbons ortho to the hydroxyls of the phloroglucinol. A distinguishing feature that separates the euglobals from the macrocarpals is that in the euglobals one of the phloroglucinol phenolic hydroxyl groups is ether linked to the terpene unit, with the vast majority forming a chroman ring. All macrocarpals contain an uncyclized phloroglucinol.

2.2.4.2.1 Euglobals

The euglobals have been reported from several Eucalyptus, species as well as *Myrcia multiflora*, and the leaves of the plant that produces the edible fruit, guava, *Psidium guajava*. Euglobals can be broadly subdivided into two main groups depending on the subtype of tepenoid moiety (either monoterpene or sesquiterpene). The terpene group binds to the phloroglucinol moiety either directly to the acyl side chain or to the formyl side chain. Some of the euglobals are strong Epstein Barr Virus activation inhibitors induced by the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate and also possess cancer chemoprevention activity, antileishmanial activity and antimalarial activity.

2.2.4.2.1.1 Euglobals with Monoterpene Adducts

Out of 59 isolated euglobals 31 were monoterpene adducts where majority of them have been isolated from the leaves and buds of *Eucalyptus*, *Psidium* and *Myrcia* genera. The monoterpene groups included α-pinene, β-pinene, sabinene, α-phellandrene, β-phellandrene, terpinolene, α-terpinene, γ-terpinene. In sixteen of the compounds (**219**-**235**), the terpene group bound to the phloroglucinol moiety via formyl side chain (Figure 13) while in others (**236**-**249**) through acyl side (Figure 14) chain.

Monoterpene group binding via formyl group

Euglobal IIc (**219**) in which the terpene group binds via a formyl group was isolated from the buds of *Eucalyptus globulus*.[137] and from the leaves of *Eucalyptus grandis*[138] and *Eucalyptus tereticornis*.[139] It weakly inhibited the Epstein Barr Virus activation.[139] Similarly, euglobal T1 (**220**) which belongs to the same class of compounds was isolated from the leaves of Eucalyptus tereticornis[139] and weakly inhibited the Epstein Barr virus activation. Euglobals G1 to G4 (**221**-**224**) and G6 to G12 (**225**-**231**) identified from the juvenile leaves of *Eucalyptus grandis*[138, 140] were also phloroglucinol-monoterepene adducts and euglobals G1, G2, G4 and G5 exhibited strong Epstein Barr Virus activation induced by (TPA).[138, 141] (100% inhibition of activation at 1×10^3 mol ratio/TPA, more than 70% inhibition of activation at 5×10^2 mol/TPA and 25-55% inhibition of activation at 1×10^2 mol ratio/TPA). They also preserved the high viability of Raji cells even at a high concentration. Euglobal G1 also exhibited potent antitumour promoting activity on a two stage carcinogenesis test of mouse pulmonary tumours using 4-nitroquinoline-*N*-oxide (4-NQO) as an initiator and glycerol as a promoter. Euglobals G1 - G4 (**221**-**224**), synthesised by a biomimetic approach showed antileishmanial activity with IC_{50} values against promatigotes of *Leishmania denovani* of 3.6, 7.1, 3.9 and 12 μg/mL respectively.[142] Moreover, euglobals Ia1, Ia2 and IIb had strong cytotoxicity on Raji cells exhibiting less than 40% viability of Raji cells at $1\times10^3$and 5×10^2 mol ratio of compound/TPA. Euglobal III has also been tested for activation of Epstein Barr Virus early antigen inhibition including 100% and 70% of inhibition of activation at 1×10^3 and 5×10^2 mol ratio/TPA, respectively and has preserved the high viability of Raji cells. Euglobal R1 (**232**) with a skeleton formed from formyl-isovaleryl phloroglucinol coupled to β-phellandrene via formyl group attached to the phloroglucinol and euglobal R2 (**233**) have also been isolated from the leaves of *Eucalyptus robusta*.[143] Eugenial A (**234**) and B (**235**), isolated from the fruits of the Brazilian plant *Myrcia multiflora*[144] (previously named *Eugenia multiflora*) are regioisomeric compounds that are closely related to euglobal G6 and G7.

Monoterpene group binding via acyl group

Six granulation inhibiting agents in which the monoterpene group binds via an acyl side chain to the phloroglucinol moiety, euglobals Ia1, Ia2, Ib, Ic, IIa, IIb (**236**-**241**) have been isolated from the buds of *Eucalyptus globulus*.[137] Euglobals Ib, Ic and IIb have also been isolated from the juvenile leaves of *Eucalyptus blakelyi*.[145] Euglobal-Ib showed strong Epstein Barr Virus early antigen activation (EBV-EA) (more than 70-80% inhibition at 1×10^3 mol ratio of compound/TPA and 50% inhibition at 1×10^2 mol ratio) whilst euglobals Ic and IIa showed inhibitory effects on EBV-EA (more than 80% inhibition at 1×10^3 mol ratio of compound/TPA and 20-30% inhibition at 1×10^2 mol ratio).[145] Euglobals Am-1 and Am-2 (**242, 243**), were isolated from *Eucalyptus amplifolia* and among them euglobal Am-2 had EBV-EA activity and euglobal IVb had strong cytotoxic activity on Raji cells.

Robustadials A and B (**244, 245**) isolated from *Eucalyptus robusta* leaves exhibited strong in vivo antimalarial activity against *Plasmodium berghei*.[146, 147] However, in vitro antimalarial activity of synthetic robustadials A and B against *Plasmodium falciparum* strains including chloroquine sensitive D6 clone (IC_{50} of 4.76 μg/mL) and chloroquine resistant W2 clone (IC_{50} of 2.80 μg/mL) as weak. The antileishmanial activity of synthetic robustadials A and B, determined by the Alamar Blue assay against *L. donovani* promastigotes was moderate with IC_{50} values of 20 and 16 μg/mL, respectively. Robustadial B (**245**) together with euglobals III, IVb and Ia2 were isolated from the leaves of *Eucalyptus globulus*.[148]

Also from the juvenile leaves of *Eucalyptus blakelyi*, euglobal BI-1 (**246**) has been isolated along with euglobals Ib, Ic and IIa and it has also exhibited strong EBV-EA activation of more than 70-80% inhibition at 1×10^3 mol ratio of compound/TPA.

Guadial A (**247**) which was co-isolated with psiguadial C and D (**275**, **276**) was the first monoterpene euglobal to be isolated from *P. guajava*.[149] It is the C-7 phenyl analogue of euglobal IIa. Its absolute configuration was determined by ECD.

Guadial B and C (**248, 249**) are also monoterpene adducts. Guadial B is structurally related to euglobals G1 and G2, while guadial C is related to the robustadials. Neither compounds inhibited human hepatoma cell growth. Guapsidial was co-isolated with guadial B and C. It is closely related to guajadial except that the condensation of the diformylbenzophenone with caryophyllene is via one of the formyl groups rather than

the benzoyl group. This results in a compound containing a methylene bridge leading to a phenyl ketone being present in the molecule.

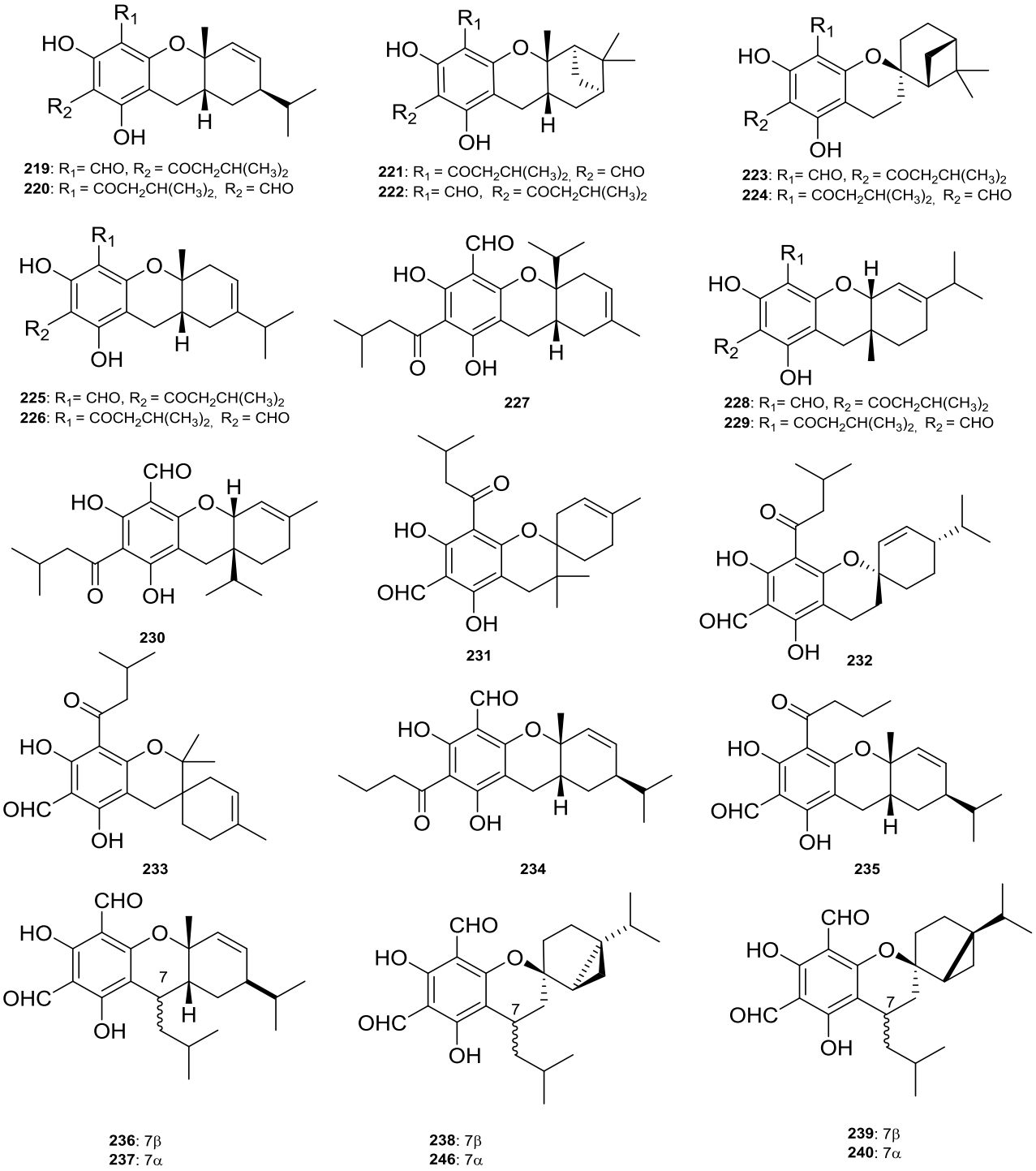


Figure 13. Euglobals with monoterpene group binding via formyl group

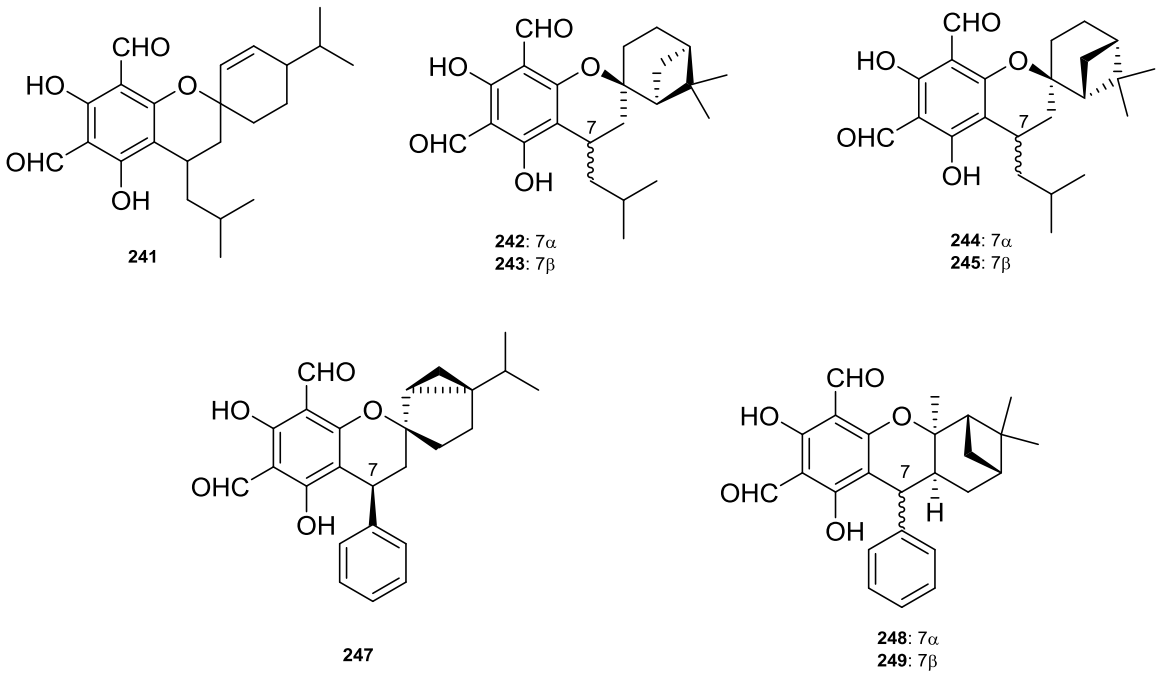


Figure 14. Euglobals with monoterpene group binding via acyl and benzoyl side chains

2.2.4.2.1.2 Euglobals with Sesquiterpene Adducts

To date 30 sesquiterpene adducts of euglobals (Figure 15) have been isolated from Eucalyptus and Psidium species. The terpene groups bound to phloroglucinol moiety are bicyclogermacrene and caryophyllene. In a search for compounds with granulation inhibiting activity, the crude extract of the buds of *Eucalyptus globulus*[150] showed activity and euglobal III (**250**) was isolated as the bioactive component by Sawada et al. Euglobal III also exhibited potent antitumour promoting activity on a two stage carcinogenesis test of mouse pulmonary tumours using 4-nitroquinoline-*N*-oxide (4-NQO) as an initiator and glycerol as a promoter.

Euglobals IVb (**251**) and VII (**252**) isolated from *Eucalyptus amplifolia* are another two sesquiterpene adduct euglobals. More recently euglobal III a (**253**) has been isolated from the leaves of *Eucalyptus incrassate*.[140] Euglobal IIIa (**253**), an acylphloroglucinol sesquiterpene derivative with cytotoxic activities against five human cancer cell lines including breast cancer MCF-7, hepatocellular carcinoma and 17.6 μM respectively) has been reported from *Eucalyptus robusta*.[151] Euglobal IVa (**254**) isolated from the leaves of *Eucalyptus globulus*.[148] Euglobal VII (**252**), was

isolated from *Eucalyptus amplifolia* and it had strong cytotoxic activity on Raji cells. Euglobal V (**255**) isolated from *Eucalyptus globulus* and *Eucalyptus incrassata* is a granulation inhibiting agent. Euglobal IX (**256**) together with euglobals III IVb Ia2 and robustadial B were isolated from the leaves of *Eucalyptus globulus*.[148] Euglobal IX inhibited the catalytic activity of CYP3A4 (IC_{50} = 38.8 μM).

Eucalyptals A-E (**257**-**261**) 3,5-diformyl-isopentyl phloroglucinol coupled to different sesqueterpenoid moieties including cadinane and aromadendrene have been isolated from the fruits of *Eucalyptus globulus*[152, 153] and they have exhibited cytotoxic activities. Eucalyptals A-C had selective toxicity against human leukaemia (HL-60) cell line with IC_{50} values of 1.7, 6.8 and 17 μM. Eucalyptals D and E also showed cytotoxicity against human BGC-823, KE-97 gastric, Huh-7 hepato and Jurkat T lymphoma cancer cell lines.

Euglobals In-1, In-2 and In-3 (**262**-**264**) have also been isolated from the *Eucalyptus incrassata.* Euglobal-In-1 exhibited Epstein Barr Virus activation (more than 80% inhibition at 1×10^3 mol ratio of compound/TPA and more than 40% inhibition at 5×10^2 mol ratio of compound/TPA). Up until 2007 euglobals had been reported from only *Eucalyptus* species. However, since then, 16 related compounds have been isolated from the leaves of *Psidium guajava*. The most notable difference between the Eucalyptus and Psidium euglobals is that the Psidium compounds (Figure 16) are all derived from a 3,5-diformyl-2,4,6-trihydroxybenzophenone precursor, whereas the *Eucalyptus* compounds are derived from jensenone.

Guajadial (**265**) contains a caryophyllene terpene conjugate and is the first euglobal to contain a caryophyllene terpene.[154] Guajadial shows potent cytotoxity towards doxorubicin sensitive and resistant human hepatoma cells (HepG2 and HepG2/ADM) IC_{50} 158 nM. Psidial A (**266**), a C-1' epimer of guajadial also isolated from the leaves of *P. guajava*,[155] was inactive against the enzyme, protein tyrosine phosphatase 1B (PTP1B) and several cancer cell lines. The relative configuration of the stereogenic centres proposed for both compounds have been confirmed by a short biomimetic total synthesis, which also allowed their absolute configurations to be established.[156]

Guajadial B (**267**) obtained from the leaves of *P. guajava*[157] collected in Vietnam was isolated as a racemate and contains a humulene terpene unit. Its structure was also confirmed by a short biomimetic synthesis. Guajadials C-F (**268**-**271**) were also isolated from the leaves of *P. guajava*[158] collected in Vietnam and each contains a

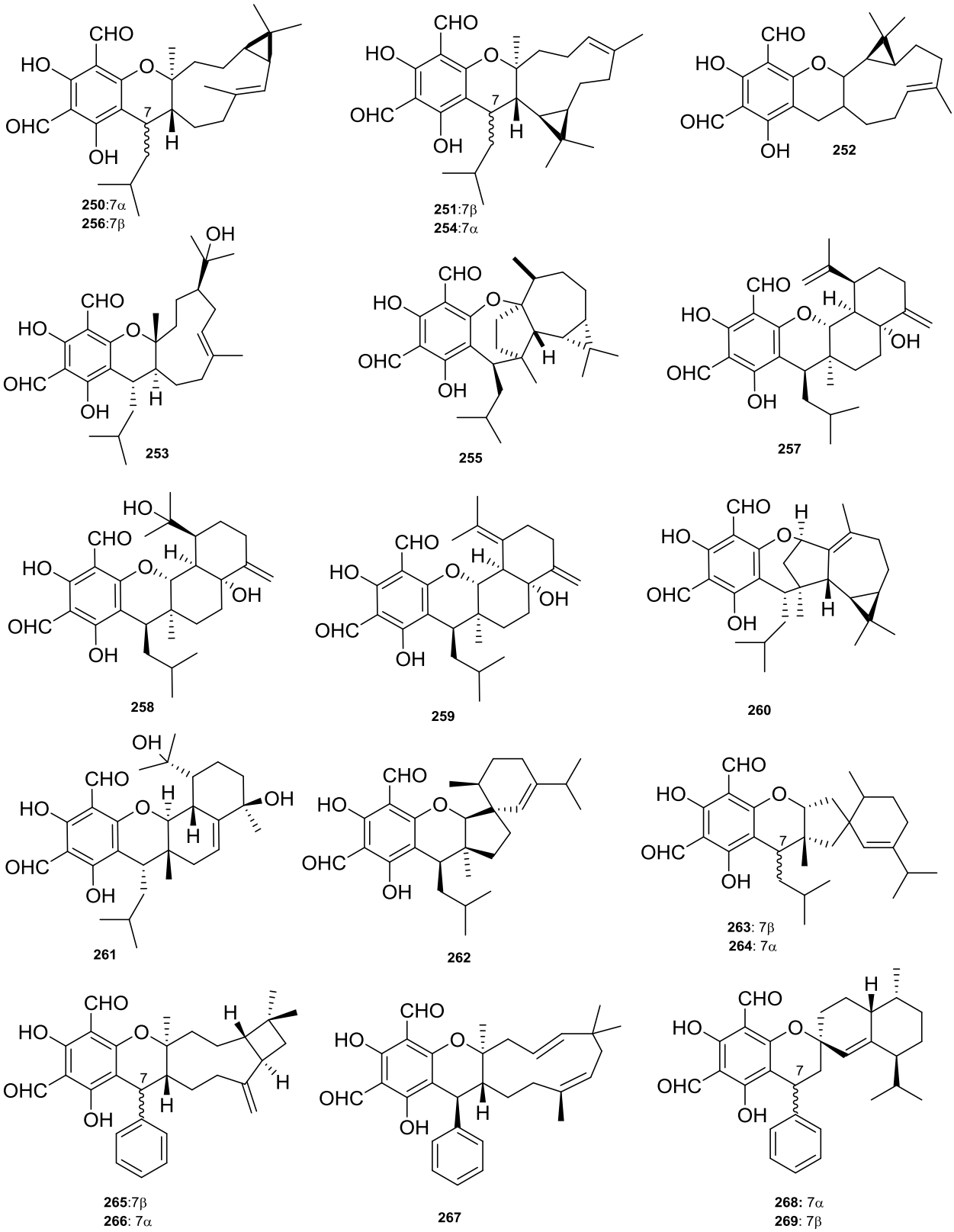


Figure 15. Euglobals with sesquiterpene adducts

cadenine type sesquiterpene adduct with C and D, and E and F being epimeric pairs. The compounds showed *in vitro* growth inhibitory effects against five human cancer cell lines (MCF-7, A-549, SMMC-7721, SW480, and HL-60) with IC_{50}'s mostly in the 5 - 40 μM range. A phenyl ether bridged dimer of guajadial named diguajadial (**272**) has also been reported from the leaves of *P. guajava*.[159]

The structures of Psiguadial A and B (**273**, **274**), which also possess unusual sesquiterpene units have been determined by a combination of NMR and X-ray analysis and their absolute configuration established by comparison of experimental ECD spectra with quantum chemical calculated spectra.[160] Psiguadial A is the C-1' phenyl analogue of euglobal V. The compounds showed potent cytotoxic activity towards doxorubicin sensitive and resistant human hepatoma cells (HepG2 and HepG2/ADM) with IC_{50} values of 61 nM and 46 nM respectively.

Psiguadial C and D (**275, 276**), also isolated from *P. guajava*,[149] both contain a bicyclogermerene sesquiterpene unit. They were potently cytotoxic to doxorubicin sensitive human hepatoma cells (HepG2) with IC_{50} values of 104 nM and 128 nM respectively, but were significantly less toxic towards doxorubicin resistant cells (HepG2/ADM) with IC_{50} values of 21 mM and 24 mM respectively suggesting that they might be P-glycoprotein efflux transporter substrates.

Guapsidial A (**277**) was only weakly toxic to doxorubicin resistant human hepatoma cells (HepG2, IC_{50} 37 μM).[161] Guavadial (**278**) isolated from *Psidium guajava* is another isomer of guajadial (**265**) with the skeleton of 4',6'-diformylbenzyl resorcinol coupled terpenoid. This showed antidiabetic activity on mouse with diabetes induced by streptozotozin and antioxidant activity in vivo.

2.2.4.2.2 Macrocarpals

The first compound belonging to the macrocarpal compound class was macrocarpal A (**279**), isolated from *Eucalyptus macrocarpa*[162] and *Eucalyptus cinerea*.[163] Later, macrocarpals B-G (**280**-**285**), from *Eucalyptus macrocarpa*[164], Macrocarpal H-J (**286**-**288**) from *Eucalyptus globulus*,[165] Macrocarpal am-1 (**289**) together with macrocarpals, A, B, and E from *Eucalyptus amplifolia*,[166] Macrocarpal K (**290**) from *Eucalyptus globulus*,[167] and eucalyptone (**291**) also from the leaves of *E. globulus*[168]

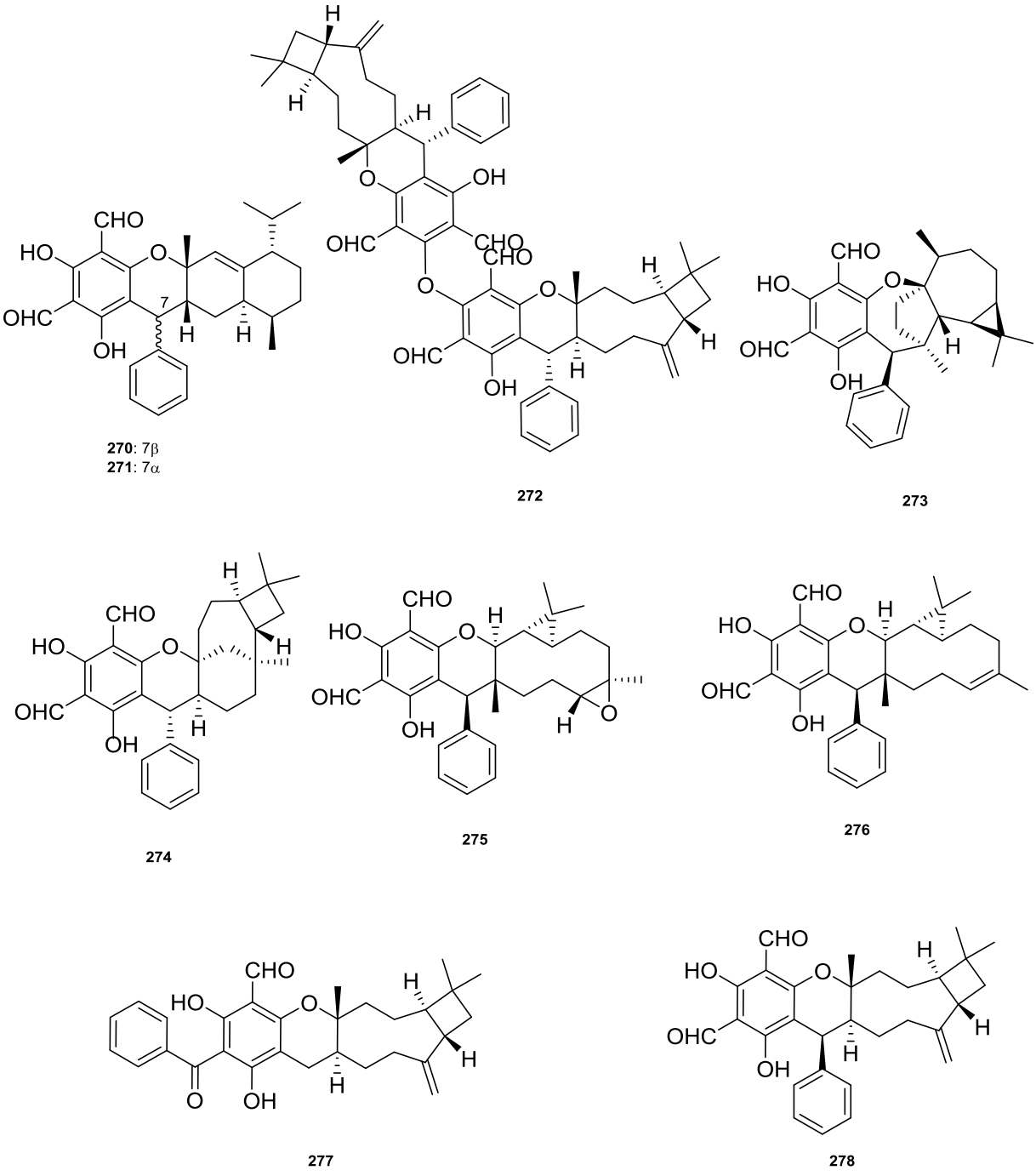


Figure 16: Euglobals with sesquiterpene adducts (continued)

were isolated. Macrocarpal F was proposed to possess structure (**284**), but no detailed spectroscopic analysis has been presented to corroborate this structural assignment. Macrocarpal am-1 and eucalytpone possess the same skeleton, similar optical rotations and the same relative configuration at all of the stereogenic centres that could be determined by NMR analysis (the relative configuration at C-9 was not determined for

either molecule). They could therefore be either the same compound or epimers at C-9. Macrocarpals exhibit a range of biological activities including antibacterial activity against Gram-positive bacteria and periodontopathic bacteria, anti-HIV activity, attachment inhibitory activity against the blue mussels, *Mytilus edulis* and aldose reductase inhibitory activity. Macrocarpal A showed antibacterial activity against Gram-positive bacteria such as *Bacillus subtilis* PCI219 and *Staphylococcus aureus* FDA209P with minimum inhibitory concentration < 0.2 μg/ml and 0.4 μg/ml, respectively but was not active against Gram-negative bacteria such as *Escherichia coli* NIHJ and *Pseudomonas aeruginosa* P-3, yeast and fungi such as *Saccharomyces cerevisiae* and *Aspergillus niger*.[162] In addition, this compound showed cytotoxic activity of 14.8 ± 0.55 μg/mL, 11.4 ± 0.45 μg/mL and 7.8 ± 0.3 μg/mL against MCF7 (breast carcinoma cell line), HEP2 (laryngeal carcinoma), CaCo (colonic adenocarcinoma) respectively and 50.1 ± 1.1 μg/mL against normal human cell line.[163] Macrocarpals A, B, E, am-1, H and K showed potent attachment-inhibiting activity against the blue mussel, which was 1-3 times higher than the standard antifoulant $CuSO_4$.[167] Furthermore, macrocarpals A, B and C showed activity against periodontopathic bacteria *Porphyromonas gingivalis* ATCC 33277. Macrocarpals D and E showed HIV-RTase inhibitory activity with IC_{50} values at 12 μM and 8.1 μM respectively.[169] Eucalyptone showed antibacterial activity towards several strains of cariogenic bacteria including *Streptococcus mutans, S. sobrinus* with MIC of 12.5 mg/mL.[168] Macrocarpals A (**279**), B (**280**), C (**281**), D (**282**), H (**286**) and eucalyptone (**291**) exhibited 100% inhibition of adherent water insoluble glucan synthesis by GTase at 100 μg/mL and more than 44.0% inhibition at 10 μg/mL.[165, 168]

Macrocarpals L-O (**292**-**295**) together with the macrocarpals A, B, D and E were isolated from the leaves of *E. globulus*.[170] Macrocarpal L and M are epimers at C-9, while O is the C-9 epimer of D. Macrocarpals P and Q (**296**, **297**) have also been reported from the leaves of *E. globulus*. Macrocarpal Q is the C-9 epimer of macrocarpal E and its isolation has allowed the configurational assignment of all stereogenic centres in both compounds.[171]

Eucalmaidial A and B (**298**, **299**) are the first macrocarpals to contain an iphionane sesquiterpene skeleton. They were isolated from the young leaves of the Australian tree, *E. maideni*[172] growing in southern China. The eucalmaidials are C-9' epimers. Macrocarpals A, B, E, G, H, K, I, J and eucalyptone were also isolated from *E.*

maideni. Eucalmaidial A showed potent antifungal activity against *Candida glabrata* (IC_{50} 0.75 μg/mL).

Eucalyptin A (**300**) isolated from the fruits of *E. globulus* along with macrocarpals B and C was shown to possess potent inhibition on HGF/c-Met axis, and thus could be important in inhibiting malignancies. A brief SAR study revealed that the combination of phloroglucinol and sesquiterpene were important for inhibitory activity.[173] The phloroglucinol sesquiterpene linkage in eucalyptin A differs from all previously isolated macrocarpals, since the two subunits are linked by an unsubstituted methylene bridge and the phloroglucinol has an isobutyl ketone replacing one of the formyl groups. Eucalyptin A also shows antibacterial activity against *S. epidermidis* ATCC35984 with MIC and MBC values at 1.7 and 3.5 μg/mL.[68, 173]

Psidial B and C (**301**, **302**) are the only macrocarpals that have been isolated from *Psidium guajava*.[155] Psidial B is the C-7 phenyl analogue of macrocarpal A, while psidial C is an analogue of macrocarpal E. Both psidial B and C moderately inhibited PTP1b at 10 μM, but were inactive against several cancer cell lines.

Rhodomyrtals A-C (**303-305**), incorporating a eudesmane sesquiterpene bonded to a benzylic methylene, together with the known compound eucalyptin A (**300**) have been isolated from the Australian rainforest plant *Rhodomyrtus psidioides.*[68] These compounds together with eucalptin A are the only other macrocarpals to have a ketone moiety attached to the phloroglucinol.

Rhodomyrtals A-C were antibacterial towards *S. epidermidis* ATCC35984 (2.4, 10, 15 μg/mL respectively) and *S. aureus* ATCC 29213 (9.4, 40, 30 μg/mL respectively) but were inactive towards *P. aureginosa* ATCC 27853.

Compound GR95647X (**306**) isolated from *Eucalyptus globulus*[174] is structurally similar to macrocarpal A and it has antibacterial activity (MIC 8μg/mL against methicillin resistant *Staphylococcus aureus*).The structure was reported in a poster abstract but unfortunately no spectroscopic data was presented and since the structure has an unusual methyl substitution of the terpene unit, this suggests that the structure may be incorrect. It was postulated that the compound is derived biosynthetically by condensation of an isovaleryl phloroglucinol dialdehyde unit with α-gurjunene.

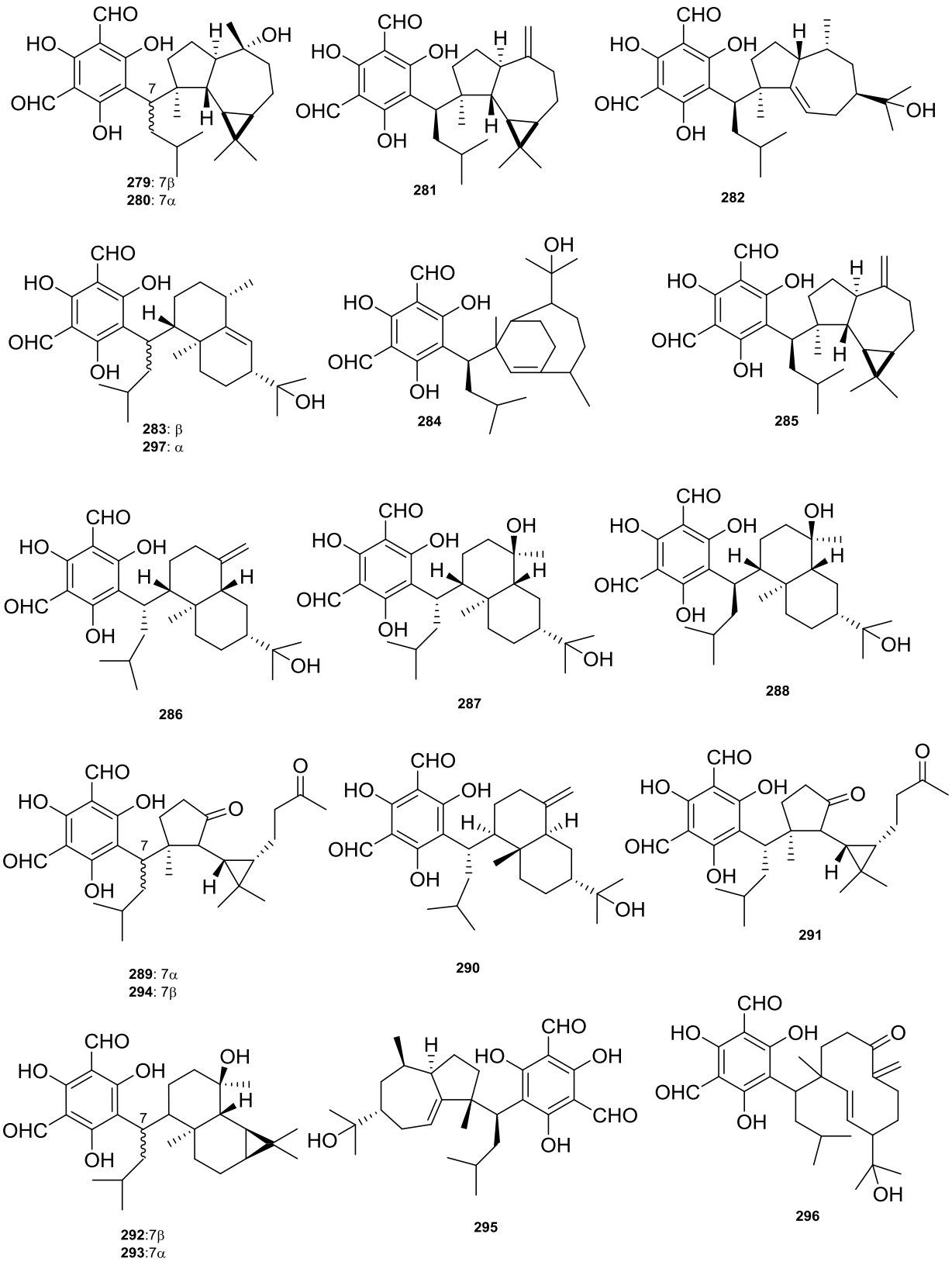


Figure 17. Macrocarpals

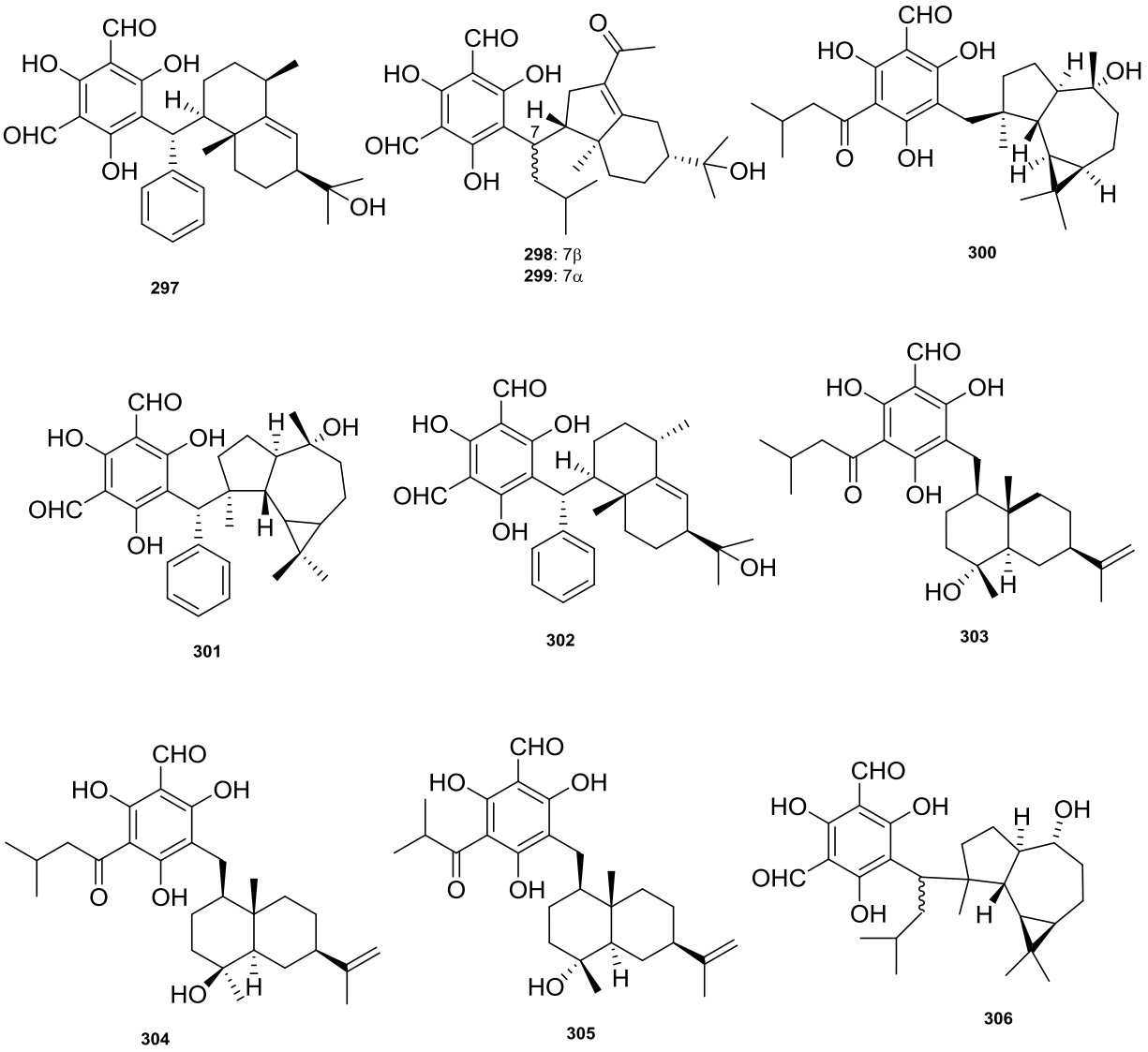


Figure 18. Macrocarpals (continued)

2.2.4.3 Sideroxylonals

Sideroxylonals (Figure 19) are derived from the aldol condensation of two acylphloroglucinol moieties forming either a 3,4-dialkyl or 3 alkyl substituted flavan ring system. All sideroxylonals contain either three or four formyl substituents attached to the two phloroglucinol units. Sideroxylonals have been exclusively reported from the leaves, stems or flower buds of *Eucalyptus* species.[1, 19] Within this genus the sideroxylonals have so far only been reported from the subgenus *Symphyomyrtus*, which include *Eucalyptus globulus*, *Eucalyptus nitens*, and the oil

mallees such as *E. kochii*, *E. loxophleba*. Sideroxylonals vary based on the configuration at C-7, C-8 and C-9 of the molecule (compound **307**). These compounds also exhibit a wide range of biological activities including inhibition of bacterial growth, inhibition of human plasminogen activation and marine antifouling. The presence of these compounds in *Eucalytpus* leaves also limits the consumption of leaves by koalas and other folivorous marsupials and as well as insects.[175] Sideroxylonals A and B (**307**, **308**) were isolated from *Eucalyptus sideroxylon*[176] in a yield of 0.0012 and 0.0009% respectively. They showed antibacterial activity against Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* at 3.9 and 7.8 μg/disc respectively. Correspondingly, sideroxylonals A and B inhibit aldose reductase at IC_{50} of 1.25 and 2.47 μg/mL respectively and inhibit the growth of HeLa cells. Furthermore, sideroxylonal A isolated from leaves of *Eucalyptus grandis*[177] showed a potent attachment inhibiting activity against the marine fouling organism, the blue mussel, *Mytilus edulis*. Sideroxylonal B had also been isolated from *Eucalyptus cinerea.*[163] Sideroxylonal B was tested against three human cancer cell lines; MCF7 (breast carcinoma cell line), HEP2 (laryngeal carcinoma), CaCo (colonic adenocarcinoma) and one type of normal human cell line; 10 FS (fibroblast cells) and it exhibited cytotoxic activity with IC_{50} 7.2 ± 0.5 μg/mL against HEP2, 4 ± 0.36 μg/mL against CaCo, 4.4 ± 0.25 μg/mL against MCF7 respectively. Interestingly, sideroxylonal B showed low cytotoxicity against normal cell line 10 FS, with IC_{50} of 43 ± 0.8 μg/mL.

Sideroxylonal C (**309**) isolated from leaves of *Eucalyptus melliodora*[178] and flowers of *Eucalyptus albens*[179] showed human plasminogen activation at 4.7 μM without any significant effect on human tissue.

Moreover, two sideroxylonals, loxophlebal A (**310**) and B (**311**) together with sideroxylonals A, B and C were reported by Sidana et al. from the leaves of *Eucalyptus loxophleba*[180, 181] and shown to have potent antibacterial activity

Grandinal (**312**) is also another dimeric phloroglucinol possessing a chroman ring and isolated from the leaves of *Eucalyptus grandis*[182] which showed attachment inhibiting activity against the blue mussel, antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* at MIC of 50 and 100 μg/mL respectively. Biogenetically, it is assumed that grandinal is formed from intermediates derived from grandinol (**95**) and jensenone (**97**).

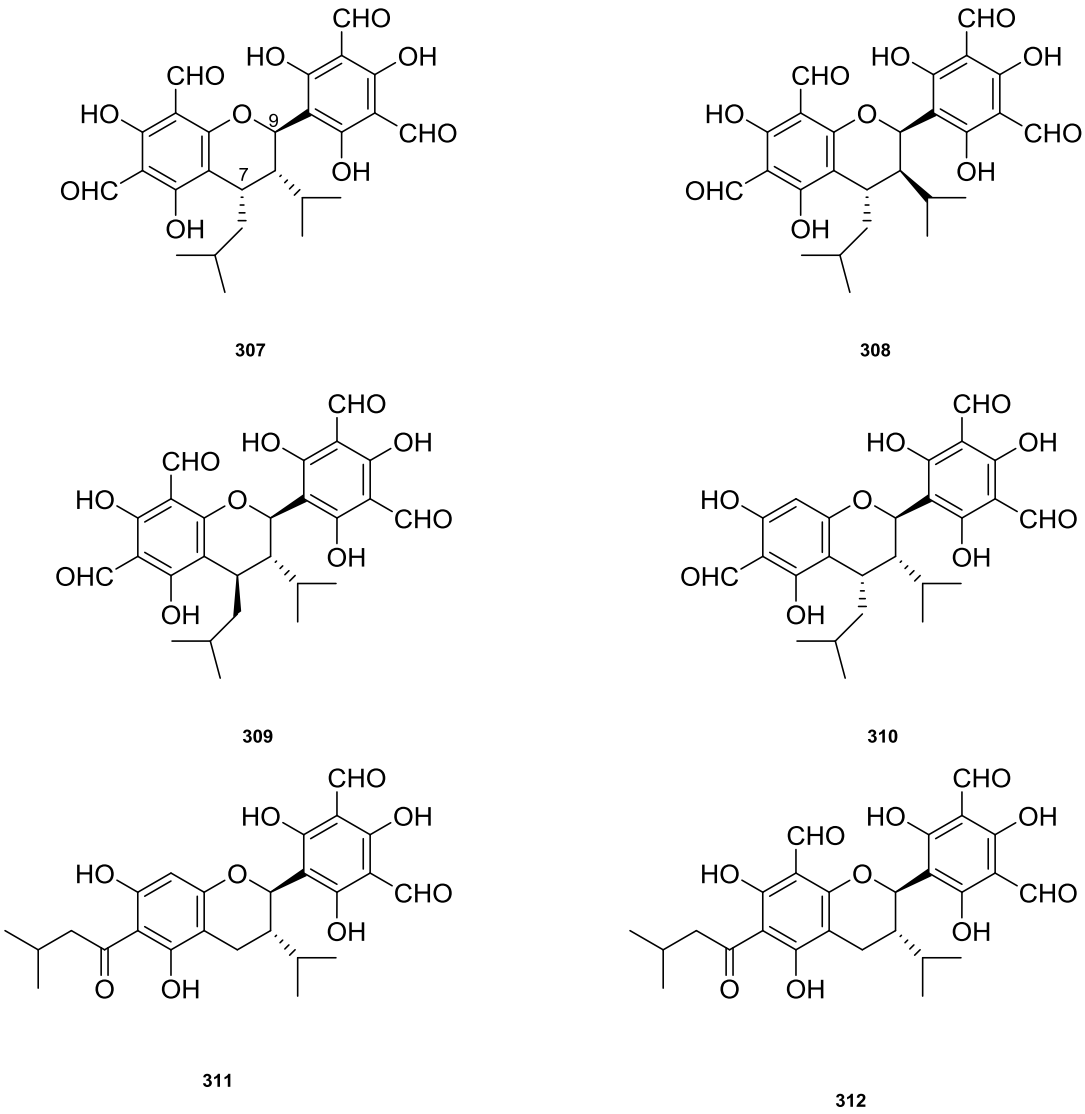


Figure 19. Sideroxylonals,

2.2.4.4 Methylene Bridged Bis-Phloroglucinols

Although widely distributed in other plant families, methylene bridged bis-acylphloroglucinols are represented by only five compounds (Figure 20) in the Myrtaceae, Three are formylated derivatives isolated from Australian *Eucalyptus* species Robustaol A (**313**): isolated from the leaves of *Eucalyptus robusta*,[183] jensenone dimer (**314**) isolated from the leaves of of *E. saligna* and *E. jensenii*, jensenal (**315**) isolated from the leaves of *E. jensenii.* Robustaol exhibits antimalarial activity. Melanervin (**316**) isolated from the flowers of the Australian species *Melaleuca quinquenervia*,[184] cultivated in Florida, USA contains a

triphenyl methane moiety. Methylene-bis-aspidinol (**317**) isolated from *Calyptranthes pallens*[86] was selectively active against the human oral epidermoid carcinoma (KB) cell line (ED_{50} 2.7 μg/mL).[19]

2.2.4.5 Dibenzofurans

A fourth subset of compounds containing two phloroglucinol groups that are present in Myrtaceae species are the dibenzofurans (Figure 20). Rhodomyrtoxin (**318**), rhodomyrtoxin B (**319**) rhodomyrtoxin C (**320**) and ψ- rhodomyrtoxin (**321**) were isolated from the unripe fruits of *Rhodomyrtus macrocarpa*,[185, 186] which if ingested can reportedly cause permanent blindness. Five new dibenzofurans (**322**-**326**) and rhodomyrtoxin C (**320**) were isolated from the Australian rainforest shrub *Pilidiostigma glabrum.*[187] The *P. glabrum* dibenzofurans which contained 2,8-acyl substituents had low micromolar antibacterial activity against Gram-positive bacteria, while the 4,6-acyldibenzofurans were less active. All but **326** showed low micromolar inhibition of nitric oxide synthesis in RAW264 macrophages and only the 2,8-acyldibenzofurans inhibited the synthesis of PGE2 in 3T3 cells. Rhodomyrtoxin B (**319**) has also been isolated from the bark of *Pilidiostigma tropicum*.[188] All of these compounds lack formyl groups attached to the phenyl ring with methyl substituents replacing them and shown to be a potent antibacterial agent against *Bacillus cereus* and *S. aureus* (MIC 0.14 μM and 0.28 μM respectively) and showed cytotoxicity towards Hep-G2 and MDA-MB-231 cells (IC_{50} 19 μM and 2.5 μM respectively).

2.2.5 Conclusions

In reviewing and analysing the literature on the chemistry of the Myrtaceae it can be concluded that only a tiny fraction of the family has been chemically investigated and the chemistry typical to the family can be divided as volatile and non-volatile. Volatile chemistry has been studied broadly while only minute fraction of the species has been studied for non-volatile chemistry. Among non-volatiles, phloroglucunols and β-triketones are unique to the family and formylated phloroglucinol chemistry is almost exclusively found within the genus *Eucalyptus* whilst β-triketone chemistry is from other genera such as *Corymbia*, *Kunzea*, *Rhodomyrtus*, *Myrtus*, *Leptospermum* and *Callistemon*. This more widespread distribution of

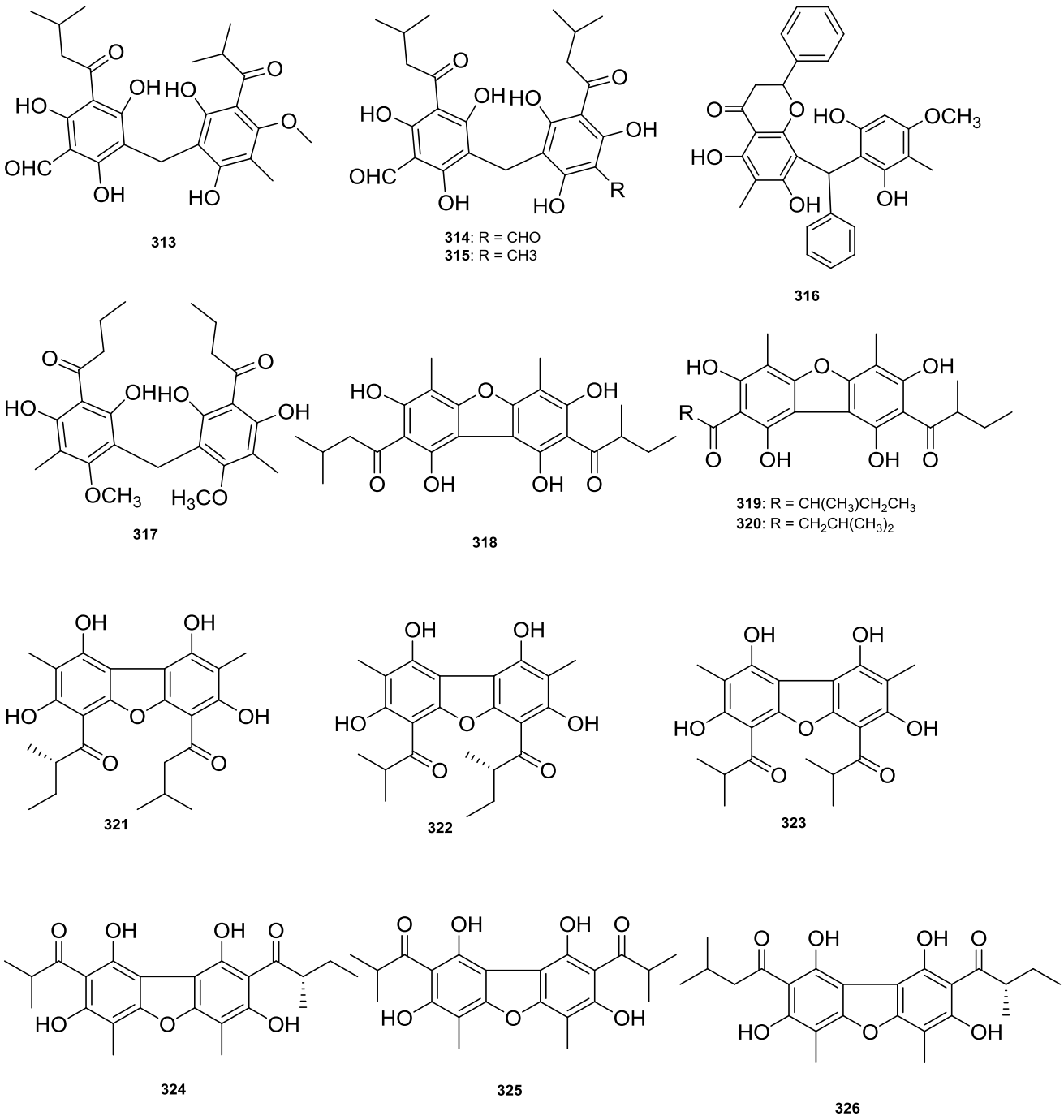


Figure 20. Methylene bridged bis-phloroglucinols and benzofurans

β-triketones within the family is interesting from a chemotaxonomic perspective and more studies on the β-triketone chemistry of other genera within the family would be fruitful to establish the breadth of β-triketone distribution within the family. Both phloroglucinols and β-triketones have exhibited biological activities including anti-infective and non-anti-infective. Interestingly, many β-triketones have drug like properties with less cytotoxicity and higher activity

against drug resistant pathogens. In future, more studies focussing on the non-volatile chemistry of the family are recommended and this is likely to reveal interesting and unique chemistry. A focus on plant parts such as stems, bark, seeds and flowers which have been less frequently studied compared to leaves is likely to yield novel biological activities of the known and newly isolated compounds that could be developed into novel drug leads. Similarly, the study of the non-volatile chemistry of more Myrtaceae species will help to address taxonomic issues and ambiguities within the family.

2.2.6 References and Notes

1. E. L. Ghisalberti, *Phytochemistry*, 1996, **41**, 7-22.
2. E. Wollenweber, R. Wehde, M. Dörr, G. Lang and J. F. Stevens, *Phytochemistry*, 2000, **55**, 965-970.
3. T. Barboni, M. Cannac, L. Massi, Y. Perez-Ramirez and N. Chiaramonti, *Molecules*, 2010, **15**, 7849.
4. A. A. A. El-Mageed, A. K. Osman, A. Q. Tawfik and H. A. Mohammed, *Res. J. Phytochem.*, 2011, 1-8.
5. A. Ebadollahi, *Annu. Res. Rev. Biol.*, 2013, **3**, 148-175.
6. A. Keszei, C. L. Brubaker and W. J. Foley, *Aust. J. Bot.*, 2008, **56**, 197-213.
7. A. Padovan, A. Keszei, C. Külheim and W. J. Foley, *Phytochem Rev*, 2014, **13**, 695-716.
8. A. Padovan, A. Keszei, C. Külheim and W. Foley, *Phytochem Rev*, 2014, **13**, 695-716.
9. K. J. Sytsma, A. Litt, M. L. Zjhra, J. C. Pires, M. Nepokroeff, E. Conti, J. Walker and P. G. Wilson, *Int. J. Plant Sci.*, 2004, **165**, S85-S105.
10. P. G. Wilson, M. M. O'Brien, P. A. Gadek and C. J. Quinn, *Am. J. Bot.*, 2001, **88**, 2013-2025.
11. E. W. Berry, *Bot. Gaz.*, 1915, **59**, 484-490.
12. D. Beardsell, S. Obrien, E. Williams, R. Knox and D. Calder, *Aust. J. Bot.*, 1993, **41**, 511-526.
13. M. É. A. Stefanello, A. C. R. F. Pascoal and M. J. Salvador, *Chem. Biodivers.*, 2011, **8**, 73-94.

14. C. M. Bignell, P. J. Dunlop and J. J. Brophy, *Flavour Frag. J.*, 1998, **13**, 131-139.
15. C. M. Bignell, P. J. Dunlop and J. J. Brophy, *Flavour Frag. J.*, 1997, **12**, 277-284.
16. D. J. Boland and J. J. Brophy, in *Bioactive volatile compounds from plants*, ed. R. Teranishi, et al., American Chemical Society, Washington, DC, 1993.
17. J. J. Brophy and D. J. Boland, *J. Essent. Oil Res.*, 1990, **2**, 87-90.
18. H. Webb, W. J. Foley, uuml and C. lheim, *Plant Biotechnol.*, 2015, **31**, 363-376.
19. I. Pal Singh and S. B. Bharate, *Nat. Prod. Rep.*, 2006, **23**, 558-591.
20. J. Sidana, S. Singh, S. K. Arora, W. J. Foley and I. P. Singh, *Fitoterapia*, 2011, **82**, 1118-1122.
21. M. M. Kabanda and L. Mammino, *Int. J. Quantum Chem*, 2012, **112**, 3691-3702.
22. H. G. S. Robert Robinson, *J. Proc. R. Soc. N.S.W.*, 1914, **48**, 518-519.
23. D. P. V. M. Trikojus, D. E. White, *J. Proc. R. Soc. N.S.W.*, 1932, **66**, 279-283.
24. A. Birch and P. Elliott, *Aust. J. Chem.*, 1956, **9**, 95-104.
25. R. Hellyer, *Aust. J. Chem.*, 1968, **21**, 2825-2828.
26. D. Baigent and I. R. C. Bick, *Aust. J. Chem.*,1973, **26**, 2065-2066.
27. A. R. Penfold, *J. Proc. R. Soc. N.S.W.*, 1921, **49**, 170.
28. R. Gardner, *J. S. C. I.*, 1925, 528T-530 T.
29. R. Penfold, *J. S. C. I.*, 1924, **34T-35T**.
30. S. J. Bloor, *J. Nat. Prod.*, 1992, **55**, 43-47.
31. L. H. Briggs, C. H. Hassall and W. F. Short, *J. Chem. Soc.*, 1945, 706-709.
32. S. Rattanaburi, W. Mahabusarakam, S. Phongpaichit and A. R. Carroll, *Tetrahedron*, 2013, **69**, 6070-6075.
33. E.-Y. Jeong, J.-H. Jeon, H.-W. Kim, M.-G. Kim and H.-S. Lee, *Food Chem.*, 2009, **115**, 1401-1404.
34. E.-Y. Jeong, M.-G. Kim and H.-S. Lee, *Pest Manag. Sci.*, 2009, **65**, 327-331.
35. G. Mitchell, D. W. Bartlett, T. E. M. Fraser, T. R. Hawkes, D. C. Holt, J. K. Townson and R. A. Wichert, *Pest Manag. Sci.*, 2001, **57**, 120-128.
36. M. J. Balunas and A. D. Kinghorn, *Life Sci.*, 2005, **78**, 431-441.
37. M. S. Butler, *J. Nat. Prod.*, 2004, **67**, 2141-2153.
38. S. Frantz and A. Smith, *Nat. Rev. Drug Discov.*, 2003, **2**, 95-96.
39. I. R. C. Bick, A. J. Blackman, R. O. Hellyer and D. H. S. Horn, *J. Chem. Soc.*, 1965, 3690-3693.

40. F. N. M. de Oliveira, P. H. Ferri, J. R. Paula, J. C. Seraphin and E. P. Filho, *J. Essent. Oil Res.*, 2008, **20**, 555-560.
41. J. Boland, J. J. Brophy and A. P. N. House, *Eucalyptus Leaf Oils: Use, Chemistry, Distillation and Marketing.* , ACIAR/CSIRO, Australian Central for International Agricultural Research, Inkata Press, Melbourne, 1991.
42. J. W. van Klink, J. J. Brophy, N. B. Perry and R. T. Weavers, *J. Nat. Prod.*, 1999, **62**, 487-489.
43. C. E. Quijano and J. A. Pino, *J. Essent. Oil Res.*, 2007, **19**, 527-533.
44. J. J. Brophy, J. R. Clarkson and C. J.R. Fookes, *Phytochemistry*, 1989, **28**, 1259-1261.
45. A. R. Penfold, *J. Proc. R. Soc. N.S.W.*, 1923, **57**, 300-312.
46. J. J. Brophy, R. J. Goldsack, P. I. Forster, J. R. Clarkson and C. J. R. Fookes, *J. Essent. Oil Res.*, 1996, **8**, 465-470.
47. A. Birch and P. Elliott, *Aust. J. Chem.*, 1956, **9**, 238-240.
48. R. O. Hellyer and J. T. Pinhey, *J. Chem. Soc. C*, 1966, 1496-1498.
49. N. G. Porter and A. L. Wilkins, *Phytochemistry*, 1999, **50**, 407-415.
50. M. Nishizawa, H. Yamada, J. Sano, S. Itô, Y. Hayashi, H. Ikeda, Chairul, M. Shiro and H. Tokuda, *Tetrahedron Lett.*, 1991, **32**, 211-212.
51. F. Echeverri, J. Gil, W. Quiñones and E. Correa, *Molbank*, 2015, **2015**, M853.
52. K. Oracz, A. Voegele, D. Tarkowská, D. Jacquemoud, V. Turečková, T. Urbanová, M. Strnad, E. Sliwinska and G. Leubner-Metzger, *Plant Cell Physiol.*, 2012, **53**, 81-95.
53. Z. Shen, Q. Yu, Y. Wang and G. Lin, *Chem. Ind. For. Prod.*, 1987, **2**, 35-43.
54. W. D. Crow, W. Nicholls and M. Sterns, *Tetrahedron Lett.*, 1971, **12**, 1353-1356.
55. A. Hiranrat and W. Mahabusarakam, *Tetrahedron*, 2008, **64**, 11193-11197.
56. W.-Y. Tsui and G. D. Brown, *Tetrahedron*, 1996, **52**, 9735-9742.
57. G. Appendino, L. Maxia, P. Bettoni, M. Locatelli, C. Valdivia, M. Ballero, M. Stavri, S. Gibbons and O. Sterner, *J. Nat. Prod.*, 2006, **69**, 251-254.
58. M. Makino and Y. Fujimoto, *Phytochemistry*, 1999, **50**, 273-277.
59. B. P. S. Khambay, D. G. Beddie, A. M. Hooper and M. S. J. Simmonds, *Tetrahedron*, 2003, **59**, 7131-7133.
60. H.-X. Liu, K. Chen, G.-H. Tang, Y.-F. Yuan, H.-B. Tan and S.-X. Qiu, *RSC Adv.*, 2016, **6**, 48231-48236.

61. Y. Fujimoto, S. Usui, M. Makino and M. Sumatra, *Phytochemistry*, 1996, **41**, 923-925.
62. H. Nishiwaki, S. Fujiwara, T. Wukirsari, H. Iwamoto, S. Mori, K. Nishi, T. Sugahara, S. Yamauchi and Y. Shuto, *J. Nat. Prod.*, 2015, **78**, 43-49.
63. H. Ito, H. Iwamori, N. Kasajima, M. Kaneda and T. Yoshida, *Tetrahedron*, 2004, **60**, 9971-9976.
64. B. P. S. Khambay, D. G. Beddie, A. M. Hooper, M. S. J. Simmonds and P. W. C. Green, *J. Nat. Prod.*, 1999, **62**, 1666-1667.
65. F. Cottiglia, L. Casu, M. Leonti, P. Caboni, C. Floris, B. Busonera, P. Farci, A. Ouhtit and G. Sanna, *J. Nat. Prod.*, 2012, **75**, 225-229.
66. L. Lv, Y. Li, Y. Zhang and Z. Xie, *Tetrahedron*, 2017, **73**, 3691-3695.
67. L. Wu, J. Luo, Y. Zhang, M. Zhu, X. Wang, J. Luo, M. Yang, B. Yu, H. Yao, Y. Dai, Q. Guo, Y. Chen, H. Sun and L. Kong, *Tetrahedron Lett.*, 2015, **56**, 229-232.
68. Q. Shou, J. E. Smith, H. Mon, Z. Brkljaca, A.-S. Smith, D. M. Smith, H. J. Griesser and H. Wohlmuth, *RSC Adv.*, 2014, **4**, 13514-13517.
69. Y.-L. Zhang, C. Chen, X.-B. Wang, L. Wu, M.-H. Yang, J. Luo, C. Zhang, H.-B. Sun, J.-G. Luo and L.-Y. Kong, *Organic Lett.*, 2016, **18**, 4068-4071.
70. H.-X. Liu, K. Chen, Y. Yuan, Z.-F. Xu, H.-B. Tan and S.-X. Qiu, *Org. Biomol. Chem.*, 2016, **14**, 7354-7360.
71. Y. Kashman, A. Rotstein and A. Lifshitz, *Tetrahedron*, 1974, **30**, 991-997.
72. G. Appendino, F. Bianchi, A. Minassi, O. Sterner, M. Ballero and S. Gibbons, *J. Nat. Prod.*, 2002, **65**, 334-338.
73. A. R. Carroll, S. Urban, J. Lamb, R. Moni, G. P. Guymer, P. I. Forster and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 881-883.
74. Khambay, Bhupinder P.S, Beddie and D. G, *Phytochemistry*, 2002, **59**, 69-71.
75. L. Larsen, M. H. Benn, M. Parvez and N. B. Perry, *Org. Biomol. Chem.*, 2005, **3**, 3236-3241.
76. F. Shaheen, M. Ahmad, S. Nahar Khan, S. Samreen Hussain, S. Anjum, B. Tashkhodjaev, K. Turgunov, M. N. Sultankhodzhaev, M. I. Choudhary and R. Atta ur, *Eur. J. Org. Chem.*, 2006, **2006**, 2371-2377.
77. E. N. T. S. Limsuwan, T. R. Kouwen, S. Piersma, A. Hiranrat, W. Mahabusarakam, S. P. Voravuthikunchai, J. M. Van Dijl, O. Kayser, *Phytomedicine*, 2009, **16**, 645-651.

78. A. Hiranrat, W. Chitbankluoi, W. Mahabusarakam, S. Limsuwan and S. P. Voravuthikunchai, *Nat. Prod. Res.*, 2011, **26**, 1904-1909.
79. G. A. Mohamed and S. R. M. Ibrahim, *Arkivoc*, 2007, **XV**, 281-291.
80. C. Wang, J. Yang, P. Zhao, Q. Zhou, Z. Mei, G. Yang, X. Yang and Y. Feng, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3096-3099.
81. Q. Su, S. Dalal, M. Goetz, M. B. Cassera and D. G. I. Kingston, *Bioorg. Med. Chem. Lett.*, 2016, **24**, 2544-2548.
82. A. R. Carroll, V. M. Avery, S. Duffy, P. I. Forster and G. P. Guymer, *Org. Biomol. Chem.*, 2013, **11**, 453-458.
83. C.-K. Lee, *Tetrahedron Lett.*, 1999, **40**, 7255-7259.
84. F. Shaheen, M. Ahmad, S. Nahar Khan, S. Samreen Hussain, S. Anjum, B. Tashkhodjaev, K. Turgunov, M. N. Sultankhodzhaev, M. I. Choudhary and R. Atta ur, *Eur. J. Org. Chem.*, 2006, **2006**, 2371-2377.
85. M. I. Choudhary, N. Khan, M. Ahmad, S. Yousuf, H.-K. Fun, S. Soomro, M. Asif, M. A. Mesaik and F. Shaheen, *Organic Lett.*, 2013, **15**, 1862-1865.
86. T. Lobo-Echeverri, J. F. Rivero-Cruz, B.-N. Su, H.-B. Chai, G. A. Cordell, J. M. Pezzuto, S. M. Swanson, D. D. Soejarto and A. D. Kinghorn, *J. Nat. Prod.*, 2005, **68**, 577-580.
87. J. C. Cecilia Labbé, Joseph D. Connolly, Francesca Faini, Louis J. Farrugia, David S. Rycroftc *Collect. Czech. Chem. Commun.*, 2002, **67**, 115-123.
88. J.-Q. Cao, X.-J. Huang, Y.-T. Li, Y. Wang, L. Wang, R.-W. Jiang and W.-C. Ye, *Organic Lett.*, 2016, **18**, 120-123.
89. B. P. S. Khambay, D. G. Beddie, M. S. J. Simmonds and P. W. C. Green, *J. Nat. Prod.*, 1999, **62**, 1423-1424.
90. M. Lounasmaa, H. S. Puri and C.-J. Widén, *Phytochemistry*, 1977, **16**, 1851-1852.
91. A. R. Carroll, J. Lamb, R. Moni, G. P. Guymer, P. I. Forster and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 1564-1568.
92. A. Hiranrat, W. Mahabusarakam, A. R. Carroll, S. Duffy and V. M. Avery, *J. Org. Chem.*, 2012, **77**, 680-683.
93. Q. Shou, H. Sun, L. K. Banbury, E. H. Lambley, D. E. Renshaw, C. Griesinger, H. J. Griesser and H. Wohlmuth, *Tetrahedron Lett.*, 2013, **54**, 1853-1856.
94. L. Mammino and M. M. Kabanda, *Mol. Simul.*, 2012, **39**, 1-13.
95. R. Ciochina and R. B. Grossman, *Chem. Rev.*, 2006, **106**, 3963-3986.

96. J. W. van Klink, L. Larsen, N. B. Perry, R. T. Weavers, G. M. Cook, P. J. Bremer, A. D. MacKenzie and T. Kirikae, *Bioorg. Med. Chem.*, 2005, **13**, 6651-6662.
97. M. L. Bolte, J. Bowers, W. D. Crow, D. M. Paton, A. Sakurai, N. Takahashi, M. Ujiie and S. Yoshida, *Agric. Biol. Chem.*, 1984, **48**, 373-376.
98. W. D. Crow, T. Osawa, D. M. Paton and R. R. Willing, *Tetrahedron Lett.*, 1977, **18**, 1073-1074.
99. R. Nakayama, M. Murata, S. Homma and K. Aida, *Agric. Biol. Chem.*, 1990, **54**, 231-232.
100. D. J. Boland, J. J. Brophy and C. J. R. Fookest, *Phytochemistry*, 1992, **31**, 2178-2179.
101. J. Sidana, W. J. Foley and I. P. Singh, *Phytochem. Anal*, 2012, **23**, 483-491.
102. C. Menut, J. M. Bessiere, A. D. Samate, J. Millogo-Rasolodimby and M. Nacro, *Phytochemistry*, 1999, **51**, 975-978.
103. J. J. Brophy, R. J. Goldsack and P. I. Forster, *Flavour Frag. J.*, 1994, **9**, 7-10.
104. I. R. Lawler, W. J. Foley, G. J. Pass and B. M. Eschler, *J. Comp. Physiol. B*, 1998, **168**, 611-618.
105. S. B. Bharate, S. I. Khan, N. A. M. Yunus, S. K. Chauthe, M. R. Jacob, B. L. Tekwani, I. A. Khan and I. P. Singh, *Bioorganic Med. Chem. Lett.*, 2007, **15**, 87-96.
106. R. Bowyer and P. Jefferies, *Aust. J. Chem.*, 1962, **15**, 145-149.
107. R. Bowyer and P. Jefferies, *Aust. J. Chem.*, 1959, **12**, 442-446.
108. B. F. Ireland, R. J. Goldsack, J. J. Brophy, C. J. R. Fookes and J. R. Clarkson, *J. Essent. Oil Res.*, 2004, **16**, 89-94.
109. Q. Cheng and J. K. Snyder, *Z. Naturforsch. B Chem. Sci.*, 1991, **46**, 1275-1277.
110. S. Huneck, *Phytochemistry*, 1972, **11**, 3311-3312.
111. J. J. Brophy, R. J. Goldsack, C. J. R. Fookes and P. I. Forster, *J. Essent. Oil Res.*, 1995, **7**, 237-254.
112. V. Powell and M. Sutherland, *Aust. J. Chem.*, 1963, **16**, 282-284.
113. J. J. Brophy, C. J. R. Fookes and A. P. N. Houset, *Phytochemistry*, 1992, **31**, 324-325.
114. K. A. Mustafa, H. G. Kjaergaard, N. B. Perry and R. T. Weavers, *Tetrahedron*, 2003, **59**, 6113-6120.
115. H. Ito, M. Koreishi, H. Tokuda, H. Nishino and T. Yoshida, *J. Nat. Prod.*, 2000, **63**, 1253-1257.
116. S. Rattanaburi, W. Mahabusarakam, S. Phongpaichit and A. R. Carroll, *Nat. Prod. Res.*, 2012, **27**, 221-225.

117. Fun, H.-K.; Chantrapromma, K.; Pullaput, Y.; Boonnak, N.; Chantrapromma, S., *Acta Cryst. E*, 2007, **63** o2017-o2019.

118. A. Birch, P. Elliott and A. Penfold, *Aust. J. Chem.*,1954, **7**, 169-172.

119. H. Sehmid, *Helv. Chim. Acta*, 1949, **32**, 813-820.

120. C. Menut, J. M. Bessiere, H. Ntalani, P. Verin, A. T. Henriques and R. Limberger, *Phytochemistry*, 2000, **53**, 975-979.

121. N. Kasajima, H. Ito, T. Hatano and T. Yoshida, *Phytochemistry*, 2008, **69**, 3080-3086.

122. H. Ito, N. Kasajima, H. Tokuda, H. Nishino and T. Yoshida, *J. Nat. Prod.*, 2004, **67**, 411-415.

123. T. Satake, K. Kamiya, Y. Saiki, T. Hama, F. Yasuo, H. Endang and M. Umar, *Phytochemistry*, 1999, **50**, 303-306.

124. H. J. Kim, J. S. Lee, E.-R. Woo, M. K. Kim, B. S. Yang, Y. G. Yu, Y. Hokoon, Park and S. Lee, *Planta Med.*, 2001, **67**, 277-279.

125. L.-W. Tian, Y.-J. Zhang, Y.-F. Wang, C.-C. Lai and C.-R. Yang, *J. Nat. Prod.*, 2009, **72**, 1608-1611.

126. L.-W. Tian, M. Xu, Y. Li, X.-Y. Li, D. Wang, H.-T. Zhu, C.-R. Yang and Y.-J. Zhang, *Chem. Biodivers.*, 2012, **9**, 123-130.

127. S. A. O. Santos, J. J. Villaverde, C. S. R. Freire, M. R. M. Domingues, C. P. Neto and A. J. D. Silvestre, *Ind Crops Prod*, 2012, **39**, 120-127.

128. A.-R. Han and Y.-S. Paik, *J. Korean Soc. Appl. Biol. Chem.*, 2010, **53**, 847-851.

129. S. Begum, Farhat and B. S. Siddiqui, *Helv. Chim. Acta*, 2011, **94**, 238-247.

130. J. Zou, Y. Mi, W. Chen, Q. Liu, J. Wang, L. Lou and W. Zhao, *Planta Med.*, 2006, **72**, 533-538.

131. K. S. Lee Wei Har, Lee Hong Boon, Fadzly A. Kamarulzaman and Intan S. Ismail, *Nat Prod Commun*, 2012, **7**, 1033-1036.

132. A. Saifudin, K. Tanaka, S. Kadota and Y. Tezuka, *Planta Med.*, 2012, **78**, 1378-1381.

133. A. R. Carroll, *Planta Med Lett*, 2016, **3**, e8-e9.

134. L.-W. Tian, Y.-J. Zhang, C. Qu, Y.-F. Wang and C.-R. Yang, *J. Nat. Prod.*, 2010, **73**, 160-163.

135. B. Ryu, H. M. Kim, J.-H. Woo, J.-H. Choi and D. S. Jang, *Fitoterapia*, 2016, **115**, 46-51.

136. S. H. Yoshikawa M1, Nishida N, Li Y, Toguchida I, Yamahara J, Matsuda H., *Chem. Pharm. Bull.*, 1998, **46**, 113-119.
137. M. Kozuka, T. Sawada, F. Kasahara, E. Mizuta, T. Amano, T. Komiya and M. Goto, *Chem. Pharm. Bull.*, 1982, **30**, 1952-1963.
138. K. Umehara, I. P. Singh, H. Etoh, M. Takasaki and T. Konoshima, *Phytochemistry*, 1998, **49**, 1699-1704.
139. M. Kokumai, T. Konoshima, M. Kozuka, M. Haruna and K. Ito, *J. Nat. Prod.*, 1991, **54**, 1082-1086.
140. M. Takasaki, T. Konoshima, M. Kozuka, M. Haruna, K. Ito and T. Shingu, *Chem. Pharm. Bull.*, 1994, **42**, 2591-2597.
141. M. Takasaki, T. Konoshima, M. Kozuka and H. Tokuda, *Biol. Pharm. Bull.*, 1995, **18**, 435-438.
142. S. B. Bharate, K. K. Bhutani, S. I. Khan, B. L. Tekwani, M. R. Jacob, I. A. Khan and I. P. Singh, *Bioorganic Med. Chem.*, 2006, **14**, 1750-1760.
143. Y.-Q. Jian, Y. Wang, X.-J. Huang, G.-Q. Li, B.-X. Zhao, Q.-Y. Guo and W.-C. Ye, *J Asian Nat Prod Res*, 2012, **14**, 831-837.
144. L. G. Faqueti, C. M. Petry, C. Meyre-Silva, K. E. Machado, A. B. Cruz, P. A. Garcia, V. Cechinel-Filho, A. San Feliciano and F. D. Monache, *Nat. Prod. Res.*, 2012, **27**, 28-31.
145. M. Takasaki, T. Konoshima, M. Kozuka, M. Haruna, K. Ito and S. Yoshida, *Chem. Pharm. Bull.*, 1994, **42**, 2177-2179.
146. R. Xu, J. K. Snyder and K. Nakanishi, *J. Am. Chem. Soc.*, 1984, **106**, 734-736.
147. I. Pal Singh and S. B. Bharate, *Nat. Prod. Rep.*, 2006, **23**, 558-591.
148. T. H. Tetsuro Kawabata, Yuka Nojiri, Chiaki Uchida, Tomoyuki Tsubata, Hikaru Kato, Fumihide Takano, and Tomihisa Ohta, *Heterocycles*, 2011, **83**, 631-636.
149. M. Shao, Y. Wang, Y.-Q. Jian, X.-J. Huang, D.-M. Zhang, Q.-F. Tang, R.-W. Jiang, X.-G. Sun, Z.-P. Lv, X.-Q. Zhang and W.-C. Ye, *Organic Lett.*, 2012, **14**, 5262-5265.
150. T. Sawada, M. Kozuka, T. Komiya, T. Amano and M. Goto, *Chem. Pharm. Bull.*, 1980, **28**, 2546-2548.
151. L.-Y. Peng, J. He, G. Xu, X.-D. Wu, L.-B. Dong, X. Gao, X. Cheng, J. Su, Y. Li and Q.-S. Zhao, *Nat. Prod. Bioprospect.*, 2011, **1**, 101-103.
152. S. Yin, J.-J. Xue, C.-Q. Fan, Z.-H. Miao, J. Ding and J.-M. Yue, *Organic Lett.*, 2007, **9**, 5549-5552.

153. J. Wang, W.-Z. Zhai, Y. Zou, J.-J. Zhu, J. Xiong, Y. Zhao, G.-X. Yang, H. Fan, M. T. Hamann, G. Xia and J.-F. Hu, *Tetrahedron Lett.*, 2012, **53**, 2654-2658.
154. X.-L. Yang, K.-L. Hsieh and J.-K. Liu, *Organic Lett.*, 2007, **9**, 5135-5138.
155. H.-Z. Fu, Y.-M. Luo, C.-J. Li, J.-Z. Yang and D.-M. Zhang, *Organic Lett.*, 2010, **12**, 656-659.
156. A. L. Lawrence, R. M. Adlington, J. E. Baldwin, V. Lee, J. A. Kershaw and A. L. Thompson, *Organic Lett.*, 2010, **12**, 1676-1679.
157. Y. Gao, G.-Q. Wang, K. Wei, P. Hai, F. Wang and J.-K. Liu, *Organic Lett.*, 2012, **14**, 5936-5939.
158. Y. Gao, G.-T. Li, Y. Li, P. Hai, F. Wang and J.-K. Liu, *Nat. Prod. Bioprospect.*, 2013, **3**, 14-19.
159. Z. T. Yaowu, *Chin J Nat Med*, 2008, **6**, 333-335.
160. M. Shao, Y. Wang, Z. Liu, D.-M. Zhang, H.-H. Cao, R.-W. Jiang, C.-L. Fan, X.-Q. Zhang, H.-R. Chen, X.-S. Yao and W.-C. Ye, *Organic Lett.*, 2010, **12**, 5040-5043.
161. Y.-Q. Jian, X.-J. Huang, D.-M. Zhang, R.-W. Jiang, M.-F. Chen, B.-X. Zhao, Y. Wang and W.-C. Ye, *Chem. Eur. J.*, 2015, **21**, 9022-9027.
162. M. Murata, Y. Yamakoshi, S. Homma, K. Aida, K. Hori and Y. Ohashi, *Agric. Biol. Chem.*, 1990, **54**, 3221-3226.
163. F. M. Soliman, M. M. Fathy, M. M. Salama, A. M. Al-Abd, F. R. Saber and A. M. El-Halawany, *Sci. Rep.*, 2014, **4**, 5410.
164. Y. Yamakoshi, M. Murata, A. Shimizu and S. Homma, *Biosci. Biotechnol. Biochem.*, 1992, **56**, 1570-1576.
165. K. Osawa, H. Yasuda, H. Morita, K. Takeya and H. Itokawa, *J. Nat. Prod.*, 1996, **59**, 823-827.
166. I. Pal Singh and H. Etoh, *Biosci. Biotechnol. Biochem.*, 1995, **59**, 2330-2332.
167. I. P. Singh, K. Umehara and H. Etoh, *Nat Prod Lett*, 1999, **14**, 11-15.
168. K. Osawa, H. Yasuda, H. Morita, K. Takeya and H. Itokawa, *Phytochemistry*, 1995, **40**, 183-184.
169. M. Nishizawa, M. Emura, Y. Kan, H. Yamada, K. Ogawa and N. Hamanaka, *Tetrahedron Lett.*, 1992, **33**, 2983-2986.
170. Y. K. Shibuya, Hiroshi; Murphy, Graham K.; Nishizawa, Yoshinori, *J Nat Med*, 2001 **55**, 28-31.

171. S. Chenavas, C. Fiorini-Puybaret, P. Joulia, C. Larrouquet, H. Waton, A. Martinez, H. Casabianca and B. Fabre, *Phytochem Lett*, 2015, **11**, 69-73.
172. L.-W. Tian, M. Xu, X.-C. Li, C.-R. Yang, H.-J. Zhu and Y.-J. Zhang, *RSC Adv.*, 2014, **4**, 21373-21378.
173. S.-P. Yang, X.-W. Zhang, J. Ai, L.-S. Gan, J.-B. Xu, Y. Wang, Z.-S. Su, L. Wang, J. Ding, M.-Y. Geng and J.-M. Yue, *J. Med. Chem.*, 2012, **55**, 8183-8187.
174. H. M. Noble, P. J. Sidebottom, S. J. Lane and M. J. O'Neill, *Planta Med.*, 1990, **56**, 647.
175. I. R. Wallis, A. J. Herlt, B. M. Eschler, M. Takasaki and W. J. Foley, *Phytochem. Anal.*, 2003, **14**, 360-365.
176. H. Satoh, H. Etoh, N. Watanabe, H. Kawagishi, K. Arai and K. Ina, *Chem. Lett.*, 1992, **21**, 1917-1920.
177. K. T. Inder Pal Singh, Hideo Etoh, *Biosci. Biotechnol. Biochem*, 1996, **60**, 1522-1523.
178. B. M. Eschler and W. J. Foley, *Aust. J. Chem.*, 1999, **52**, 157-158.
179. J. Neve, P. de Almeida Leone, A. R. Carroll, R. W. Moni, N. J. Paczkowski, G. Pierens, P. Björquist, J. Deinum, J. Ehnebom, T. Inghardt, G. Guymer, P. Grimshaw and R. J. Quinn, *J. Nat. Prod.*, 1999, **62**, 324-326.
180. J. Sidana, Singh, Sukhvinder, Arora, Sunil Kumar,Foley, William J., R. K. Rohilla, Roy, Nilanjan, R. A. Barrow, Foley, William J. and I. P. Singh, *Fitoterapia*, 2010, **81**, 878-883.
181. J. Sidana, R. K. Rohilla, N. Roy, R. A. Barrow, W. J. Foley and I. P. Singh, *Fitoterapia*, 2010, **81**, 878-883.
182. H. E. Inder Pal Singh, *Nat. Prod. Sci.*, 1997, **3**, 1-7.
183. C. Z.-X. Qin Guo-Wei, Wang Hong-Cheng, Qian Ming-Kun, *Chin. J. Chem.*, 1981, **39**, 83-89.
184. O. Seligmann and H. Wagner, *Tetrahedron*, 1981, **37**, 2601-2606.
185. N. H. Anderson, Ollis, W. D., Underwood, J. G., Scrowston, R. M., *J. Chem. Soc. C*, 1969, 2403-2408.
186. Igboechi, C. A.; Parfitt, R. T.; Rowan, M. G., Phytochemistry, 1984, 23, (5), 1139-1141.
187. Q. Shou, L. K. Banbury, D. E. Renshaw, E. H. Lambley, H. Mon, G. A. Macfarlane, H. J. Griesser, M. M. Heinrich and H. Wohlmuth, *J. Nat. Prod.*, 2012, **75**, 1612-1617.

188. W. Setzer, G. Rozmus, M. Setzer, J. Schmidt, B. Vogler, S. Reeb, B. Jackes and A. Irvine, *J Mol Model*, 2006, **12**, 703-711.

CHAPTER 3

SCREENING TARGETS

3.0 Screening Targets

Based upon the literature review presented above it is clear that the natural products present within Myrtaceae species show significant promise as agents to treat infections. Therefore the proposed research to be carried out in this doctoral study will focus on the discovery of new secondary metabolites from various Myrtaceae species and these compounds will be tested for antimalarial and antibacterial activity. The emergence of drug-resistant pathogens in the areas of malaria and bacterial infections threatens the lives of humans around the world and as a result, the increased morbidity and mortality due to these diseases have led to a renewed need to find new leads to treat these infections.[1, 2] The following sections will further discuss the importance of drug discovery to find leads to treating malaria and bacterial infections.

3.1 Malaria

Malaria is one of the most widespread and life-threatening infectious diseases in the world. It is commonly found in Africa, Asia, and America. Malaria causes millions of deaths each year and according to the WHO, there were about 207 million cases of malaria in 2010 and estimated deaths during that year were 627000. Ninety percent of these deaths were reported from the African continent. Pregnant women and children are the most vulnerable groups to the disease and it has been reported that 85% of the deaths are from children under the age of five.[3-5]

Malaria is a vector-borne disease, which is caused by a protozoan parasite from the genus *Plasmodium.* There are over 200 species of *Plasmodium*. *Plasmodium* species is known to infect mammals, birds and reptiles and *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* are the four main parasites responsible for causing malaria in humans. The parasite uses both a human and female *Anopheles* mosquito host. *P. knowlesi* is also a rare cause of malaria in humans. Humans are infected by the malaria parasite when a female *Anopheles* mosquito injects the parasite into the blood stream in the form of sporozoites while taking a blood meal. Sporozoites are then carried to the liver by the blood circulatory system where they infect hepatocytes. Intracellular sporozoites undergo asexual multiplication known as exoerythrocytic schizogony and develop into schizonts. Schizonts within hepatic cells burst and release thousands of merozoites into the blood stream. Merozoites invade erythrocytes (red blood cells) and within the erythrocyte, merozoites develop into a ring or early

trophozoite form of the parasite. The early trophozoite stage is referred to as the ring form based on its morphological features. Then the early trophozoite advances into a mature trophozoite and undergoes asexual multiplication to form a schizont containing merozoites. Then the erythrocytic schizont ruptures releasing merozoites into the blood stream that attack erythrocytes thus completing the erythrocytic cycle. Some fraction of merozoites differentiates into male and female gametocytes within erythrocyte cells which in turn are taken up by anopheline mosquitoes during a blood meal. Gametocytes that entered the body of the mosquito develop into male and female gametes and fertilized to form a motile zygote or ookinete. This occurs within the lumen of the mosquito gut and the process is known as sporogony. Upon ookinete penetrates the gut wall becoming a visible oocyst within which another phase of multiplication occurs resulting in the formation of sporozoites. Sporozoites then transfer to the salivary glands of a mosquito and transmit to a new human host during a blood meal spreading the disease.[6] Figure 3.1 shows the life cycle of the malaria parasite.

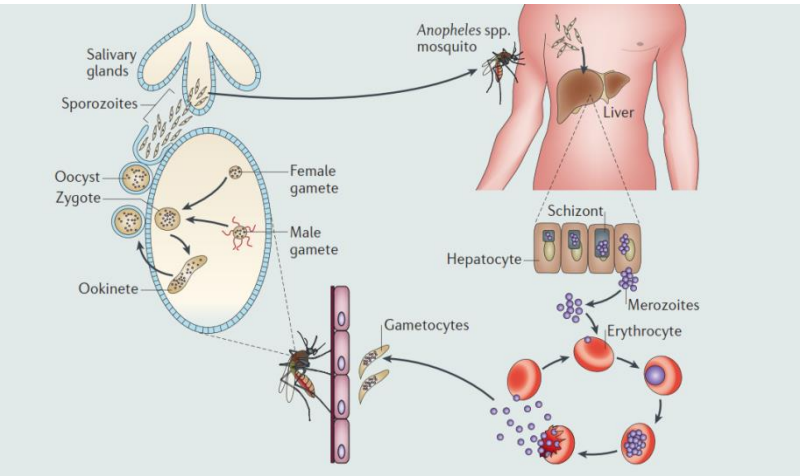


Figure 3.1. Life cycle of Malaria parasite[7]

There was an era where malaria was widespread in regions such as North America, Asia, Africa and Europe. A campaign to eradicate malaria in the 1950s and 1960s resulted in its elimination from North America, Europe and some parts of Asia. The insecticide, dichlorodiphenyltrichloroethane (DDT) (**3.1**) was used to control mosquitoes and drugs such as chloroquine and sulphadoxine-pyrimethamine were used to treat malaria patients. However, since the mosquitos have become resistant to DDT in many regions and the parasite became resistant to chloroquine and sulphadoxine-pyrimethamine drugs, their use

was limited and consequently, malaria became predominant in many regions of the world. In 2004 reported deaths due to malaria were 1.8 million and later on with the introduction of new drugs such as artemisinin, vector control, increased funding and public awareness the mortality rate due to malaria dropped by 30%.[7]

There is limited number of drugs available to treat malaria. Most widely used drugs are quinine (**1.9**) and its derivatives, doxycycline (**3.2**), antifolate combination drugs and artemisinin (**1.10**) and its derivatives.

Quinine (**1.9**), an alkaloid isolated from the bark of cinchona (quinaquina) tree, which is native to South America, was the first chemotherapeutic agent developed in the world to treat malaria. Use of cinchona bark, which is also referred as "Jesuits' bark," "cardinal's bark," or "sacred bark" to treat malaria dates back to the 1600s and these names indicate its use by Jesuit missionaries of South America in 1630. However, historical evidence suggests that native people of South America used to use hot infusions of the bark of cinchona tree to treat shivering in colds and damp conditions long before the Jesuit missionaries. Quinine was first isolated in France in 1820 and its structure was established in early 20th century. Other alkaloids isolated from the cinchona tree include quinidine (**3.3**), cinchonine (**3.4**), and cinchonidine (**3.5**) and all are also active against the malaria parasite. Quinine is schizonticidal against intraerythrocytic malaria parasites and also gametocytocidal for *Plasmodium vivax* and *Plasmodium malariae*, but not for *Plasmodium falciparum*. Nevertheless, antimalarial mechanism of action of quinine is still unknown. It is used to treat severe malaria, multidrug resistant *P. falciparum* infections, and malaria during early stage pregnancy.[8]

Primaquine (**3.6**), an 8-aminoquinoline, is a synthetic malaria drug, which is used to treat *P. vivax* or *P. ovale* malaria. Malaria caused by *P. vivax* or *P. ovale* can return (relapse) parasitemia originating from hypnozoites in the liver. It can relapse from 16 days to several years after the primary infection. Only *P. vivax* and *P. ovale* form hypnozoites. To provide a radical cure from the disease, drug combination therapy was introduced employing chloroquine (**3.7**) and primaquine, which kill parasites present in every tissue of the body. [9] Chloroquine, 4-aminoquinoline is now only used to treat *P. vivax* since the other three species of *Plasmodium* that cause malaria in humans have developed resistance to it.

Antifolate combination drugs are another class of antimalarial drugs (Figure 3.2). They are combinations of dihydrofolate reductase inhibitors and sulfa drugs. When these drugs are used alone parasites become resistance rapidly. Due to synergistic effects, drug combinations

are more active. Combinations are sulfadoxine (**3.8**)/pyrimethamine (**3.9**), sulfalene (**3.10**)/ pyrimethamine (**3.9**) and sulfamethoxazole (**3.11**) /trimethoprim (**3.12**).[10]

In the mid-1970s Chinese scientists isolated a remarkable antimalarial drug artemisinin (**1.10**), a sesquiterpene endoperoxide, from sweet wormwood (*Artemisia annua*).[11] The antimalarial properties of artemisinin (**1.10**) are unquestionable and semisynthetic derivatives of artemisinin, artesunate (**3.13**), dihydroartemisinin (**3.14**), arteether (**3.15**) and artemether (**3.16**) (Figure 3.2) are playing a significant role in the treatment of drug-resistant malaria using artemisinin combination therapy (ACT) (Figure 3.2). In ACT partner drugs include lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine and chlorproguanil/dapsone.[12] There are many advantages of using artemisinin to treat malaria over other antimalarial drugs. Artemisinins can be administered through many routes including oral, intramuscular, intravenous and intrarectal. This allows treating patients individually depending on the severity of the disease and patient age group. For example, children who face difficulties having oral administration can get the drug intra-rectally. The other important characteristic of artemisinin is that of its quick action. It can kill parasites quickly and also inhibit their glycolysis, nucleic acid, and protein synthesis pathways immediately. Moreover, artemisinins can attack various blood stages of parasites starting from merozoites to trophozoites and schizonts. This leads to the delayed formation of gametocytes and as a result transmission of malaria can be reduced. Similarly, artemisinins prevents the cytoadherence to endothelial cells. Antimalarial activity of artemisinins is associated with the peroxide moiety within the 1,2,4-trioxane system and they act through inhibition of the hemoglobin degradation pathway where the parasite normally gets amino acids during blood stage infection.[13]

Although effective drugs are available to treat malaria there are two major issues which make eradication of malaria from the world difficult. The first obstacle is the ability of the malaria parasite to develop resistance to antimalarial drugs currently available to treat the disease.[14] Chloroquine was the first drug to which the malaria parasite, *P. falciparum* became resistant. *P. falciparum* is the causative agent of life-threatening malaria and it was initially reported to show resistance to chloroquine in the 1950s in South East Asia and South America.[15] Resistance was further extended to Thai-Cambodia border and in 1960 resistance was reported from Africa. Chloroquine resistance was due to the point mutation of the transporter gene Pf*crt* and multi-drug resistant gene Pf*mdr*1.[16] Consequently, sulfadoxine–pyrimethamin drug combination therapies were introduced to these regions but parasites developed resistance to these drugs in less than five years.[17] Once, chloroquine and sulfadoxine-

pyrimethamine were no longer effective treatments the WHO recommended artemisinin combination therapy (ACT) and monotherapy to treat *P. falciparum* malaria infections. In 2005, WHO reported that artemisinin resistance and recommended the withdrawal of artemisinin monotherapy and for the first time artemisinin resistance was identified from the Thailand-Cambodia border region. Furthermore, the failure of ACT has been reported from Thailand and if ACT, the front-line antimalarial drug combination, fails there are no other anti-malarial drugs in the world to replace them and this would have a devastating effect on global malaria control and millions of deaths due to malaria would become unavoidable.[18]

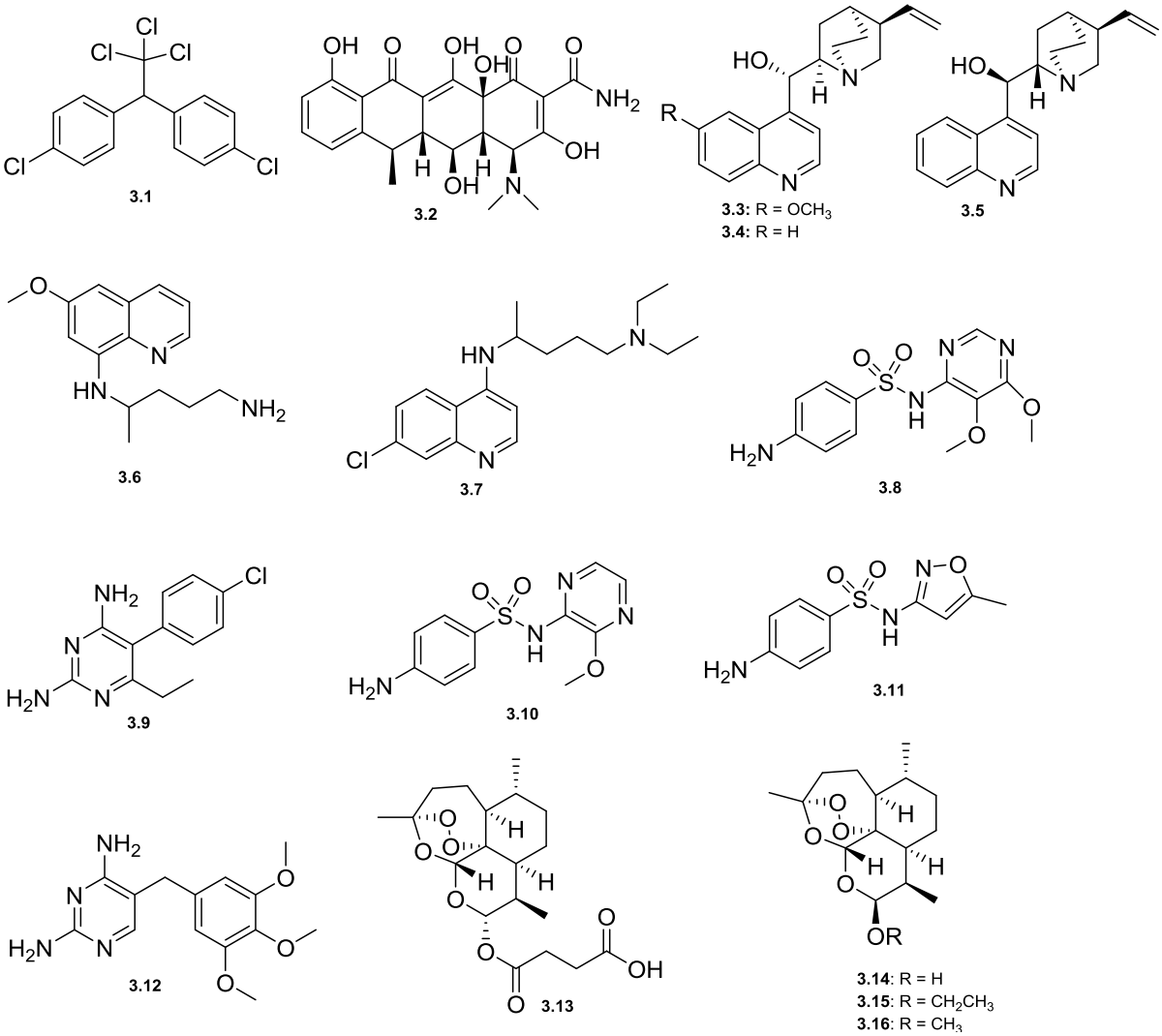


Figure 3.2. Antimalarial drugs

The second problem is that there are no other of drugs to treat *P. vivax* and *P. ovale* infections other than primaquine. *P. vivax* and *P. ovale* form hypnozoites, which are dormant liver stages and if *P. vivax* and *P. ovale* are not eradicated from the liver the disease relapses. Patients are required to take primaquine over 15 days repeatedly to totally cure the disease. On the other hand, primaquine cannot be administered to patients with a glucoce-6-phosphate dehydrogenase deficiency as it is toxic to them. Hence, many people in malaria endemic regions are at risk.[14]

Therefore, to eradicate the malaria epidemic from the world, new drugs that block all stages of malaria parasite life cycle with a new mechanism of action are needed. However, the discovery of new anti-malarial drugs is not an easy process. One important feature that new anti-malarial drugs should possess is minimum side effects, they must be well tolerated, safe since most of the malaria endemic regions occur in the world's poorest countries and these people are unable to have follow-up health care. Similarly, they should be orally bioavailable and be able to be administered orally with minimum health care facilities. Drugs need to have a minimum therapeutic period with 2-3 times day dosing and they need to be used in combination to avoid development of drug resistance and compliance. Furthermore, these drugs should be cheap and affordable for people with low incomes.[19] This current scenario for malaria eradication has led universities, research institutes, funding agencies, governments, non-governmental organizations and pharmaceutical companies to work together to find new anti-malarial drugs that can replace currently available drugs.

3.2 Bacterial infections

Bacteria are unicellular living organisms that look like balls, rods or spirals. They can be found in air, water, soil and food. They live on plants, insects, animals, pets, and even in the human digestive system and upper respiratory tract. There are thousands of various kinds of bacteria. Some bacteria are useful to humans, but a few, about 1%, are harmful. They are classified as Gram-negative and Gram-positive depending on the color they appear after Gram staining. Gram-negative bacteria stain pink and Gram positive bacteria stain blue. Most bacteria help humans to digest food, destroy disease-causing cells and provide vitamins to the body. However, infectious bacteria are harmful to humans and make them ill. Once infected, they reproduce rapidly within the body and produce toxins which are detrimental to the body. Some of such bacteria are *Streptococcus*, *Staphylococcus*, and *Escherichia coli*.

Although humans are exposed to a large number of bacteria in the environment, not everyone is prone to infection. Some are at higher risk of infection. This may be due to low immunity; age, nutritional factors, and genetic predisposition. Neonates and elderly people are more susceptible and most of the time neonates are infected by bacteria like *E. coli*. Lower respiratory tract infections caused by *Streptococcus pneumonia* are common among elderly people.

Bacteria can cause diverse infections including respiratory infections, gastrointestinal infections, skin infections, and healthcare associated infections. Most common respiratory infections are upper respiratory tract infections, otitis media, lower respiratory tract infections and tuberculosis.

Today bacterial infections are one of the major causes of morbidity and mortality. Nearly 50% of deaths in the tropical region and 20% deaths in the United State are due to infectious diseases including bacterial infections.[20] Antibiotics are the main class of drugs that are used to treat bacterial infections (Figure 3.3). The world's first antibiotic agent was salvarsan (**3.17**), which was synthesized by Ehrlich for the treatment of syphilis in 1910. Thereafter discovery of penicillin (**1.3**) from the fungus *Penicillium notatum* by Fleming in 1928 was a dramatic event in antibiotic drug discovery since it paved the pathway to the discovery of antibiotics from other microorganisms. Penicillin came into clinical use in the 1940s and saved the lives of a large number of soldiers during World War II. In 1935, synthetic antibiotic sulphonamides drugs (Figure 3.3) were developed by a research group headed by Domagk and this initiated the discovery of antimycobacterial drug Isoniazid (**3.18**). Discovery of the antibiotic, gramicidin, from soil microbes, was first reported by Rene Dubos in 1939. This drug is still in use to treat skin infections. Later in 1944, streptomycin (**3.19**), an aminoglycoside, was obtained from the soil bacterium *Streptomyces griseus*. After that chloramphenicol (**3.20**), tetracycline (**3.21**) and macrolides were also discovered from soil bacteria. Synthetic compound, nalidixic acid (**3.22**), a quinolone antimicrobial agent was discovered in 1962.[21]

Antibiotics used to treat bacteria are called antibacterial agents and the important characteristic feature of those antibacterial agents is their selective toxicity, which is an ability to attack invading bacteria without harming the host cells. Antibacterial agents can be bactericidal or bacteriostatic. Bactericidal agents kill bacteria while bacteriostatic agents inhibit the growth of bacteria. Bacteriostatic drugs cannot be used to treat immunocompromised patients since they are less effective. Antibiotics can be classified depending on the site of their action and include molecules that inhibit cell wall synthesis,

protein synthesis, nucleic acid synthesis, metabolic pathways and cell membrane function. The following table summarizes the classes of antibiotics based on their site of action.[22]

Table 3.1. Classes of antibiotics based on their site of action.

Class of drug	Drug group	Examples
Cell wall synthesis inhibitors	Beta –lactams	Penicillins, cephamycins
		Cephalosporins, Carbapenems
		Monobactams
	Glycopeptides	Vancomycin, Teicoplanin, Avoparcin
Protein synthesis inhibitors	Aminoglycosides	Gentamicin, Neomycin, Streptomycin
	Tetracyclines	Tetracycline, Oxytetracyclines
	Chloramphenicol	Minocycline, Tigecycline
	Lincosamides	Lincomycin, Clindamycin
	Macrolides	Erythromycin, Clarithromycin
	Streptogramins	Pristinamycin, Virginiamycin
	Oxazolidinones	Linezolid, Posozolid, Tedizolid
	Fusidic Acid	Fusidic Acid
Nucleic acid synthesis inhibitors	Quinolones	Nalidixic acid, Ciprofloxacin, Levofloxacin
	Rifamycins	Rifamycin B, Rifamycin SV
	Sulfonamides	Hydrochlorothiazide, Furosemide
	Trimethoprim	Trimethoprim
Cell membrane function inhibitors	Polymixins	Colistin, Polymyxin B
Metabolic pathway inhibitors	Polymixin	
	Daptomycin	

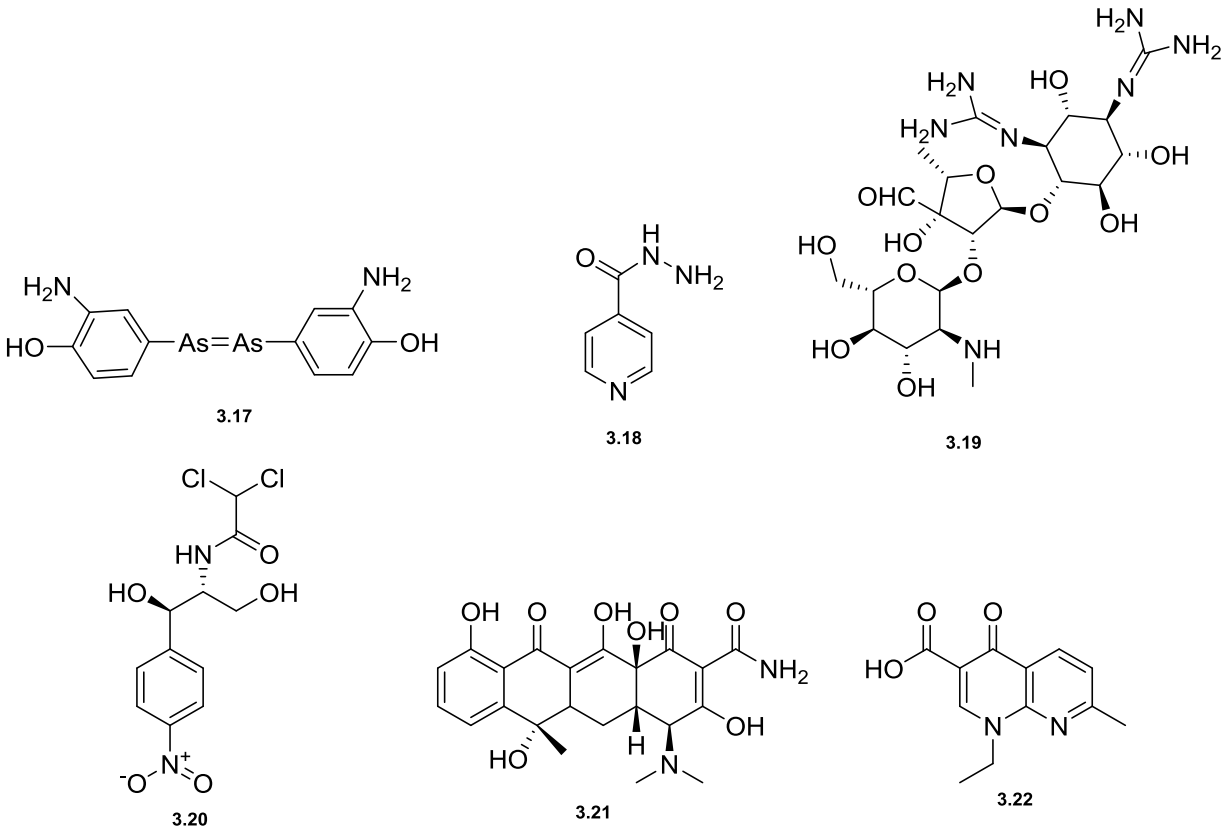
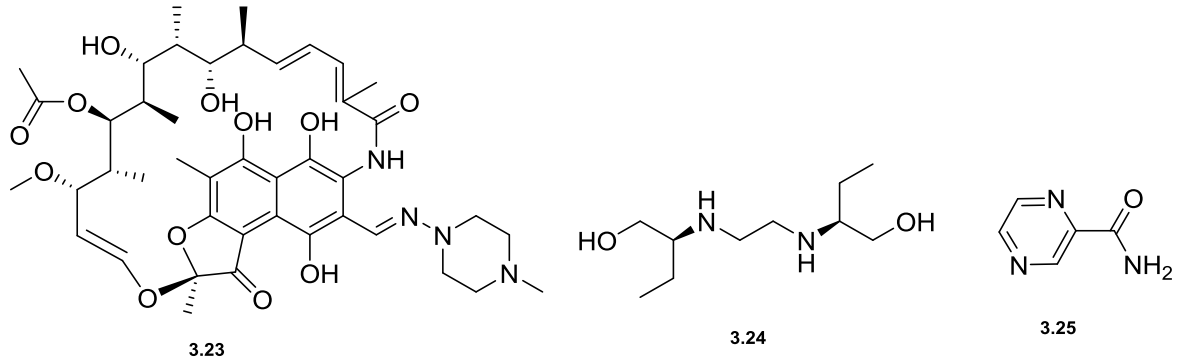


Figure 3.3. Antibiotics used to treat bacterial infections

Despite the availability of a huge number of antibiotics to treat antibacterial infections, emergence and spread of drug-resistant bacteria is a worldwide health problem today. Treatment failure associated with drug-resistant bacteria is one of the most serious issues faced by physicians today. Infections caused by bacteria such as staphylococci, enterococci, *Klebsiella pneumonia*, and *Pseudomonas* species are very common today and lives of those critically ill are at risk. Furthermore, mortality rates of blood stream infections caused by resistant *Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Enterobacter* species, coagulase-negative staphylococci, and enterococci have increased. Spread of drug-resistant bacteria within the community too is a serious health issue as these epidemics are difficult to control and expensive to treat. Methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum-lactamase (ESBL)–producing *E. coli* are commonly found species in the community. Community-associated MRSA strains are less drug resistant than healthcare-associated MRSA though they produce toxins, such as Panton–Valentine leukocidin. The more prone groups of such infections are military groups, children and sports teams.[23] Another devastating health hazard in the world

today is that treatment and control of drug-resistant *Mycobacterium tuberculosis* (TB). The emergence of multidrug resistant (MDR) and extremely drug resistant (XDR) forms of (TB) bacilli are the main reason for this situation. According to the WHO in 2012, 1.3 million deaths occurred due to TB. Currently available drugs such as rifampicin (**3.23**), isoniazid (**3.18**), ethambutol (**3.24**), and pyrazinamide (**3.25**) (Figure 3.4) take a long time (six to twelve months) to treat and cure patients. Consequently, control of TB is very difficult as it can spread to other people during this treatment period.[24]



3.23 3.24 3.25

Figure 3.4. Currently available drugs to treat *Mycobacterium tuberculosis*

Intensive and prolonged use and misuse of antibiotics to treat bacterial infections in humans as well as animals and in the field of agriculture are the major causes for the emergence of drug-resistant bacterial pathogens. Scientists believe that development of drug resistance in bacterial pathogens is an evolutionary process as they confront diverse challenges in their day to day lives and need to adapt to these changing environmental conditions. One of the fascinating features of bacteria is that they can familiarize to the new environments acquiring genetic changes and developing resistance to life-threatening causative agents. Bacteria develop resistance to antibiotics in several ways including mutating drug target sites, changing the cell permeability and efflux, and horizontal transfer of resistance genes.[25] The resistance of *Escherichia coli* to third generation cephalosporins and aminopenicillins; *Staphylococcus aureus* to vancomycin, *Pseudomonas aeruginosa* to piperacillin, ceftazidime, cefotaxime, ciprofloxacin, gentamicin, and tobramycin, Mycobacterium tuberculosis to isoniazid and rifampin are some examples.[26]

3.3 References and Notes

1. J. E. Hyde, *Trends Parasitol.*, 2005, **21**, 494-498.
2. S. B. Levy and B. Marshall, *Nat. Med.*, 2004, **10**, S122-S129.
3. C. J. L. Murray, L. C. Rosenfeld, S. S. Lim, K. G. Andrews, K. J. Foreman, D. Haring, N. Fullman, M. Naghavi, R. Lozano and A. D. Lopez, *Lancet*, 2012, **379**, 413-431.
4. R. F. Schumacher and E. Spinelli, *Mediterr. J. Hematol. Infect. Dis.*, 2012, **4**, e2012073.
5. E. N. Takem and U. D'Alessandro, *Mediterr. J. Hematol. Infect. Dis.*, 2013, **5**, e2013010.
6. I. Sherman, *Malaria: Parasite Biology, Pathogenesis, and Protection*, 1998, 3-10.
7. E. L. Flannery, A. K. Chatterjee and E. A. Winzeler, *Nat. Rev. Microbiol.*, 2013, **11**, 849-862.
8. W. E. Sneader, *Drug Discovery (The History)*, Wiley Online Library, 2005.
9. J. K. Baird and S. L. Hoffman, *Clin. Infect. Dis.*, 2004, **39**, 1336-1345.
10. S. R. Meshnick and M. J. Dobson, in *Antimalarial Chemotherapy*, Springer, 2001, pp. 15-25.
11. D. Klayman, *Science*, 1985, **228**, 1049-1055.
12. J. T. Lin, J. J. Juliano and C. Wongsrichanalai, *Curr. Infect. Dis. Rep.*, 2010, **12**, 165-173.
13. S. Krishna, A.-C. Uhlemann and R. K. Haynes, *Drug Resist. Update*, 2004, **7**, 233-244.
14. E. L. Flannery, A. K. Chatterjee and E. A. Winzeler, *Nat. Rev. Microbiol.*, 2013, **11**, 849-862.
15. D. Payne, *Parasitol. Today*, 1987, **3**, 241-246.
16. A. B. S. Sidhu, D. Verdier-Pinard and D. A. Fidock, *Science*, 2002, **298**, 210-213.
17. C. H. Sibley, J. E. Hyde, P. F. G. Sims, C. V. Plowe, J. G. Kublin, E. K. Mberu, A. F. Cowman, P. A. Winstanley, W. M. Watkins and A. M. Nzila, *Trends Parasitol.*, 2001, **17**, 570-571.
18. T. E. Wellems and C. V. Plowe, *J. Infect. Dis.*, 2001, **184**, 770-776.
19. J. C. Craft, *Curr. Opin. Microbiol.*, 2008, **11**, 428-433.

20. G. B. Mahady, *Medicinal Plants for the Prevention and Treatment of Bacterial Infections*, Bentham Science Publishers, 2005.
21. C. G S, H. V. K R, F. Mirzaei and S. M. Gopinath, *JBSO*, 2013, **1**, 32-37.
22. C. Mims, H. M. Dockrell, R. V. Georing, I. Roitt, D. Wakelin and M. Zuckerman, *Medical Microbiology*, Elsevier Limited, 2004.
23. L. B. Rice, *Am. J. Med.*, 2006, **119**, S11-S19.
24. E. D. Chan and M. D. Iseman, *BMJ*, 2002, **325**, 1282.
25. A. Rodríguez-Rojas, J. Rodríguez-Beltrán, A. Couce and J. Blázquez, *Int. J. Med. Microbiol.*, 2013, **303**, 293-297.
26. F. C. Tenover, *Am. J. Med.*, 2006, **119**, S3-S10.

CHAPTER 4

AIMS OF THE PROJECT

4.0 Aims of the Project

The studied genera of Myrtaceae include *Syzygium, Rhodomyrtus, Pilidiostigma, Callistemon, Leptospermum, Corymbia, Baeckea, Backhousia, Kunzea, Melaleuca, Psidium and Eucalyptus*. The number of species studied from each genus is very low; however, several bioactive and novel secondary metabolites of phloroglucinols and β-triketones have been discovered. The phloroglucinol group, however, showed several adverse properties (including toxicity), whereas many of the β-triketones have shown excellent biological activities and drug-like properties against malaria parasites and drug-resistant bacterial strains like MRSA with limited or no human cell toxicity.[1-4] β-Triketones have predominantly been isolated from genera such as *Corymbia, Leptospermum, Rhodomyrtus and Kunzea*. Hence, exploring diverse β-triketone molecules as anti-infective agents from Myrtaceous species in which β-triketones are dominant was expected to be productive. This approach was further supported by the long history of use of β-triketones as drugs.[5]

Corymbia is a genus of 113 species and limited research work has been carried out on this genus. From the previous studies, it has been identified that flowers from species in the genus *Corymbia* are a promising source of β-triketone molecules. *Angophora* is a genus of Eucalypts that is more closely related to *Corymbia* than *Eucalyptus* and therefore it is anticipated that they too may contain β-triketones even though no previous chemical investigations have been undertaken on plants from this genus. *Angophora* species are endemic to Australia and are distributed in Queensland, New South Wales, and Victoria. There are about 13 species in the genus. Since the chemistry of this genus has not previously been studied, studies into its chemistry may yield new secondary metabolites as anti-infective agents and their study may also help to understand taxonomic relationships between the genera *Eucalyptus* and *Angophora*.[6, 7]

The flush of flowers produced by a majority of *Corymbia* and *Angophora* species are prominent, rich in both nectar and pollen and produced in abundance. The high nectar production should make the flowers susceptible to bacterial and fungal infection, but this is rarely observed in the wild. In addition, honey produced from *Corymbia* species have been reported to possess some of the highest antibacterial properties of any Australian bush honey tested. This provides circumstantial evidence to suggest that the flowers of species in these

genera may possess anti-infective natural products. The hypothesis is further supported by the chemical analyses reported by Carroll et al[2, 8, 9] for *Corymbia watsonianana*, *C. scabria*, and *C. peltata*, which reported novel β-triketone molecules with excellent biological activities. Interestingly, only 6% of chemical investigations targeting non-volatile secondary metabolites from Myrtaceae plants have been directed at flowers compared to the leaves, aerial parts, and bark. There is, therefore, a major gap in the current knowledge of the chemistry of the Myrtaceae flowers. Especially nectar and pollen of flowers are a source of food for insects, bats, birds and some other animals of our environment.[10] The relationship between plants and flowers is mutualistic and beneficial to both groups. As a result reproduction of higher plants occurs through pollination.[11] Secondary metabolites of flowers play a major role in attracting animals providing colours and scent to flowers. Colours are provided to the flowers by compounds such as flavonoids, carotenoids, chlorophylls and betalain alkaloids. These compounds are responsible for colours such as red, blue, yellow, white, purple and green.[12] Volatile chemicals such as terpenoids are responsible for the scent of the flowers and especially bees, night flying insects, bats and moths are attracted to trees by a scent of flowers.[13] Other than chemicals responsible for colour and scent there are other chemical compounds within nectar and pollen which provide nutrient to animals fed on them. Usually, 15-75% of the weight of nectar is sugar and most of the time glucose, fructose and sucrose are present. The amount of each sugar or combination of sugars present within angiosperms differs according to the species. Other nutritious compounds present in pollen are amino acids and proteins. They provide nitrogen to the pollinators and presence of all twenty common amino acids has been reported.[14] They also vary qualitatively as well as quantitatively from species to species. Lipids are also present in nectar and highly abundant in bee-pollinated plants since bees feed oil to their young. Toxins too are present in nectars and especially they provide defence against herbivores as well as harmful animal visitors.[15] The presence of iridoids such as catalpol in the nectar of *Catalpa speciosa* to keep ants away is an example. Though sometimes these toxins are similar to the compounds present in other parts of the plant, this cannot be true always as the mechanism of defence may change with the part of the plant. Pollen usually contains 16-30% proteins, 1-7% starch, 0-15% free sugar and 3-10% fat, carotenoids and flavonoids as colouring agents. Odours of pollens differ from species to species. The responsible constituents of the odour are volatile compounds.[16-18]

The characteristic features of Myrtaceae flowers are that they have five sepals and petals and many stamens. The stamens are usually long and conspicuous. The flowers have various

colours including pink, red, white and yellow.[18] (Figure 4.1). The *Corymbia*, *Angophora,* and *Eucalyptus* are closely related morphologically and difficult to distinguish. To date, genetic studies[6] on these genera have shown differences, but some ambiguity still remains; it is proposed that chemical investigations could aid taxonomic classification of these genera.

Figure 4.1. Some examples of Myrtaceae flower species

This doctoral research aims to:

1.0 Isolate β-triketone molecules from the flowers of species from the Myrtaceae genera *Corymbia* and *Angophora*.

2.0 Elucidate the molecular structures of isolated β-triketones using multidimensional NMR techniques and mass spectrometry. (^{1}H NMR, ^{13}C NMR, HSQC, COSY, HMBC and ROESY).

3.0 Determine the antimalarial and antibacterial activities of the isolated compounds.

4.0 Disseminate the outcome of the research to the scientific community through research publications, conference presentations and a doctoral thesis.

4.1 References and Notes

1. F. Cottiglia, L. Casu, M. Leonti, P. Caboni, C. Floris, B. Busonera, P. Farci, A. Ouhtit and G. Sanna, *J. Nat. Prod.*, 2012, **75**, 225-229.
2. A. R. Carroll, V. M. Avery, S. Duffy, P. I. Forster and G. P. Guymer, *Org. Biomol. Chem.*, 2013, **11**, 453-458.
3. S. Limsuwan, E. N. Trip, T. R. H. M. Kouwen, S. Piersma, A. Hiranrat, W. Mahabusarakam, S. P. Voravuthikunchai, J. M. van Dijl and O. Kayser, *Phytomedicine*, 2009, **16**, 645-651.
4. K. Nisa, T. Ito, T. Kodama, M. Tanaka, Y. Okamoto, Y. Asakawa, H. Imagawa and H. Morita, *Fitoterapia*, 2016, **109**, 236-240.
5. M. J. Balunas and A. D. Kinghorn, *Life Sci.*, 2005, **78**, 431-441.
6. K. D. Hill and L. A. S. Johnson, *Telopea*, 1995, **6**, 185-504.
7. L. A. Johnson and K. D. Hill, *Telopea*, 1990, **4**, 37-108.
8. A. R. Carroll, J. Lamb, R. Moni, G. P. Guymer, P. I. Forster and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 1564-1568.
9. A. R. Carroll, S. Urban, J. Lamb, R. Moni, G. P. Guymer, P. I. Forster and R. J. Quinn, *J. Nat. Prod.*, 2008, **71**, 881-883.
10. H. G. Baker, I. Baker and S. A. Hodges, *Biotropica*, 1998, **30**, 559-586.
11. P. A. Fryxell, *Bot. Rev.*, 1957, **23**, 135-233.
12. T. Seshadri, *Curr. Sci.*, 1964, **33**, 69-72.
13. C. Y. Lin, Y. H. Chen, T. C. Chang, Y. J. Chen, S. S. Cheng and S. T. Chang, *J. Agric. Food Chem.*, 2013, **61**, 12531-12538.
14. M. Nepi, C. Soligo, D. Nocentini, M. Abate, M. Guarnieri, G. Cai, L. Bini, M. Puglia, L. Bianchi and E. Pacini, *Flora - Morphology, Distribution, Functional Ecology of Plants*, 2012, **207**, 475-481.
15. T. Swain, *Annu. Rev. Plant Physiol.*, 1977, **28**, 479-501.
16. J. B. Harborne, *Secondary Metabolites: Attracting Pollinators*, John Wiley & Sons, Ltd, 2001.
17. M. S. Percival, *New Phyto.*, 1961, **60**, 235-281.
18. H. G. Baker, *Apidologie*, 1977, **8**, 349-356.

CHAPTER 5

ANTIPLASMODIAL β-TRIKETONES FROM THE FLOWERS OF THE AUSTRALIAN TREE *Angophora woodsiana*

ANTIPLASMODIAL β-TRIKETONES FROM THE FLOWERS OF THE AUSTRALIAN TREE *Angophora woodsiana*

Flowers of *Angophora woodsiana* was investigated for β-triketone chemistry and extraction, isolation, and structure elucidation of two new and nine known β-triketones and their antiplasmodial and antibacterial activity testing are reported in this chapter.

STATEMENT OF CONTRIBUTION CO-AUTHORED PUBLISHED PAPER. THE BIBLIOGRAPHIC DETAILS OF THE CO-AUTHORED PUBLISHED PAPER, INCLUDING ALL AUTHORS, ARE

Senadeera, S. P. D.; Sandra Duffy; Vicky Avery; Anthony R. Carroll. Antiplasmodial β-triketones from Australian tree *Angophora woodsiana*. Bioorg. Med. Chem. Lett. 2017, 27(11), 2602-2607.

My contribution to the paper involved:

Collection and organization of information, data and references; Preparation of manuscript.

(Signed)[redacted] (Date) 14.03.2017

Name of student: Sarath Parakumge Dayani Senadeera

(Countersigned)[redacted] (Date) 14.03.2017

*Corresponding author: Anthony R. Carroll

(Countersigned)[redacted] (Date) 14.03.2017

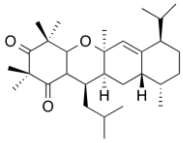
Principle supervisor: Anthony R. Carroll

Graphical Abstract

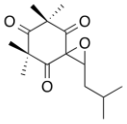
Antiplasmodial β-triketones from the flowers of the Australian tree *Angophora woodsiana*

Leave this area blank for abstract info.

Sarath P. D. Senadeera,[a,b,c] Sandra Duffy,[d] Vicky M. Avery[d] and Anthony R. Carroll[a,b,d,*]



woodsianone A
IC_{50} 27.98 μM (3D7)



woodsianone B
IC_{50} 3.38 μM (3D7)

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Antiplasmodial β-triketones from the flowers of the Australian tree *Angophora woodsiana*

Sarath P. D. Senadeera,[a,b,c] Sandra Duffy,[d] Vicky M. Avery[d] and Anthony R. Carroll[a,b,d,*]

[a]*Environmental Future Research Institute, Griffith University, Gold Coast, QLD 4222, Australia.*
[b]*Griffith School of Environment, Griifth University, Gold Coast, QLD 4222, Australia*
[c]*Department of Natural Products, Medical Research Institute, No 527, Dr. Danister De Silva Mawatha, Colombo 08, Sri Lanka*
[d]*Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia.*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Angophora woodsiana
β-triketones
Myrtaceae
Antiplasmodial activity

ABSTRACT

Chemical investigations of the MeOH extract of air dried flowers of the Australian tree *Angophora woodsiana* (Myrtaceae) yielded two new β-triketones, woodsianones A and B (**1**, **2**) and nine known β-triketones (**3**-**11**). Woodsianone A is a β-triketone-sesquiterpene adduct and woodsianone B is a β-triketone epoxide derivative. The structures of the new and known compounds were elucidated from the analysis of 1D/2D NMR and MS data. The relative configurations of the compounds were determined from analysis of ^{1}H-^{1}H coupling constants and ROESY correlations. All compounds (**1**-**11**) had antiplasmodial activity against the chloroquine sensitive strain 3D7. The known compound rhodomyrtone (**5**) and new compound woodsianone B (**2**) showed moderate antiplasmodial activities against the 3D7 strain (1.84 µM and 3.00 µM, respectively) and chloroquine resistant strain Dd2 (4.00 µM and 2.53 µM, respectively).

1. **Introduction**

Malaria is a vector borne disease caused by protozoan parasites from the genus *Plasmodium*. There are five species of *Plasmodium* that are known to cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and the simian *P. knowles*.[1] Malaria is a significant public health problem that mainly affects communities in the developing world including Sub-Saharan Africa, South-East Asia, Central and South America and the Eastern Mediterranean region. The emerging resistance to currently used drugs is now compounding the problem further. According to the World Health Organization (WHO) 438,000 deaths from malaria occurred in 2015, of which 90% occurred in Africa and 10% in South-East Asia and the Eastern Mediterranean.[2]

There are a limited number of drugs available to treat malaria. The most widely used drugs include quinine and its derivatives, antifolate combination drugs and artemisinin and its derivatives.[3, 4] Although there are currently effective drugs available to treat malaria, the development of resistance to anti-malarial drugs by the *Plasmodium* parasite is widespread.[5] Resistance to chloroquine developed in Thailand by 1957 and later independently developed in South America. By 1980 chloroquine resistance covered most malaria-endemic regions of Sub-Saharan Africa, leading to a sharp increase in the number of deaths in this region. In 1973 sulfadoxine-pyrimethamine (SP) was introduced as a first-line treatment but by 1980 the parasite had developed resistance in Thailand and subsequently resistance was discovered in Asia and Africa. Subsequently, within the span of only 7 years (1985-1992) resistance also developed against mefloquine.[6] The discovery and development of artemisinin, a potently active sesquiterpene extracted from the Chinese plant *Artemisia annua*[7] led to artemisinin based therapies being the treatment of choice in regions where chloroquine resistance has emerged. Artemisinin and its derivatives including artesunate, artemether and dihydroartemisinin are highly effective and rapidly suppress the asexual stage of parasitemia (t1/2 ~ 1h). However, when artemisinin derivatives and many other drugs are used as monotherapies, they are generally required to be taken repeatedly over seven days for complete elimination of parasite.For many patients however, compliance is problematic and many terminate treatment before seven days , resulting in recrudescence and the propagation of drug resistance.[8, 9] Initially, resistance to artemisinin was found in the Pailin province of Western Cambodia where artemisinins were predominantly used as oral monotherapies. In January 2014, WHO reported artemisinin resistance in Lao People's Republic, Myanmar, Thailand and Vietnam.[10, 11] Today there is a concern that artemisinin resistance may spread to other continents beyond the Greater Mekong sub-region, similar to chloroquine and sulfadoxine-pyrimethamine.[11] Termination of artemisinin monotherapies was recommended by WHO in 2007 and re-confirmed in 2011 and 2013.[12] Today, artemisinin and derivatives are only recommended for use in combination with partner drug (such as amodiaquine, mefloquine, lumefantrine, sulfadoxine-pyrimethamine, piperaquine) with a different mechanism and

speed of action.[13] Artemisinin-based combination therapies (ACTs), result in a rapid reduction in the parasite biomass, and prolonged drug exposure with a partner drug with a longer half-life.[14] The emergence of artemisinin resistance in recent years is of grave concern to global health agendas Emergence of multi drug resistance (MDR) parasites would have a devastating effect on global malaria control and millions of deaths due to malaria would become unavoidable.[15] There is therefore an urgent need to discover new anti-malarial drugs with different mechanisms of action to those currently in use. We have previously shown that natural products containing β-triketone moieties show promising antiplasmodial activity and therefore new compounds from this structure class could provide further insights enabling development of these structures as antimalarial drug leads.

The Australian endemic genus *Angophora* from the angiosperm family Myrtaceae is represented by 16 species of "gum" or Eucalypt trees.[16] The genus has been shown through molecular and morphological evidence to be closely related to two other Eucalypt genera, *Eucalyptu*s and *Corymbia*.[17] We have previously shown that the flowers of *Corymbia* species are a rich source of β-triketones, some of which show moderately potent antiplasmodial activity.[18-20] Since *Angophora* species are very closely related to *Corymbia* species, we anticipated that their flowers may also contain β-triketones although no previous studies have investigated their chemistry. In this study the flowers of *Angophora woodsiana*, were chemically explored. Locally known as the smudgy apple, *Angophora woodsiana* is a medium sized rough barked tree of about 20 m height that flowers profusely between December and January.[21]

Methanol extracts from the flowers were purified by reversed phase and normal phase HPLC to yield two new compounds, woodsianone A (**1**) and B (**2**) and the nine known compounds, rhodomyrtosone A (**3**), rhodomyrtosone D (**4**), rhodomyrtone (**5**), tomentodione A (**6**), tomentodione B (**7**) 4*S*-ficifolidione (**8**)**,** kunzeanone A (**9**), watsonianone A (**10**) and watsonianone B (**11**). The known compounds were identified by 1D and 2D NMR analysis and through comparison with literature data. All of the compounds contain β-triketone moieties, corroborating our hypothesis that the genus should be an additional source of this structure class. Structures of the two new compounds were elucidated from analysis of 1D and 2D NMR data and antiplasmodial activities of all compounds were evaluated. This provided further evidence that β-triketones are new scaffolds for malaria drug discovery.

The MeOH extract (8.0 g) of *A. woodsiana* was partitioned between hexane and MeOH to yield a hexane soluble fraction (1.8 g). The remaining MeOH soluble fraction was further partitioned with dichloromethane (DCM) to yield a DCM soluble fraction (3.44 g). One gram of the hexane soluble fraction was further purified by Diol bonded silica gel HPLC (21 mm x 150 mm) and eluted with a 60 min. gradient from hexane to DCM at 9 mL/min and then further eluted for 10 minutes. with DCM to yield 70 fractions. This was repeated three times and fractions were analyzed by ^{1}H NMR. Fractions containing NMR signals of interest were combined yielding four main fractions. Each of these fractions was further purified by preparative (21 mm x 250 mm) phenyl bonded silica gel HPLC with a 60 min gradient from 100% H_2O to 100% MeOH at 9mL/min and further eluted for 10 min. with MeOH, yielding woodsianone A (**1**), and nine known compounds rhodomyrtosone A (**3**)**,** tomentodione A (**6**), tomentodione B (**7**), 4*R*-ficifolidione (**8**)**,** kunzeanone A (**9**), watsonianone A (**10**) and watsonianone B (**11**) (Fig. 1)**.** The DCM fraction was purified by Diol bonded HPLC (21 mm x 150 mm) eluting with a 60 min. gradient from hexane to DCM at 9

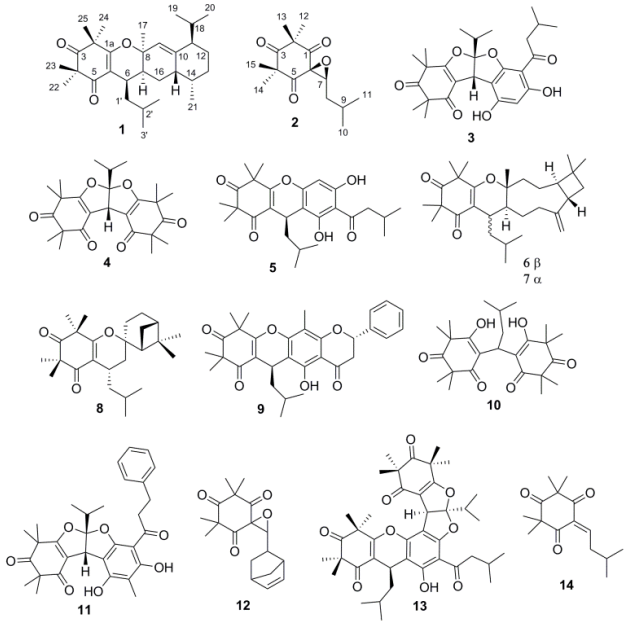


Figure 1. β-triketones isolated from *Angophora woodsiana*

mL/min and then further eluted for 10 minutes. Early eluting fractions were the new compound woodsianone B (**2**). The crude MeOH extract (23g) was purified using medium pressure liquid chromatography (MPLC) with a gradient from 100% H_2O to 100% MeOH separately. Further purification of MPLC fractions 11 and 12 was achieved by Diol bonded HPLC (21 mm x 150 mm) eluting with a 60 min. gradient from hexane to DCM at 9 mL/min and further eluted for10 minutes. yielding rhodomyrtosone D (**4**) and rhodomyrtone (**5**).[22]

Woodsianone A (**1**), was isolated as a colorless amorphous solid and its molecular formula was determined to be $C_{30}H_{46}O_3$ from analysis of (+) HRESIMS data (*m/z* MH^+ 455.3530 calculated 455.3520).[23] The IR spectrum for **1** showed absorption bands at 1610 cm^{-1} and 1380 cm^{-1},indicating carbonyls were present in the molecule. The UV spectrum displayed UV absorption maxima at 267 nm and 204 nm. The ^{1}H NMR spectrum (800 MHz, $CDCl_3$) of **1** (Table 1) had resonances assigned to five secondary (δ_H 0.78, 0.91, 0.94, 0.94, 1.00) and five quaternary aliphatic methyl groups (δ_H 1.26, 1.27, 1.30, 1.31, 1.26), four aliphatic methylene groups (δ_H 1.85/1.29, 1.43/1.37, 1.64/1.68, 1.05/1.94) and eight methine groups (δ_H 1.69, 1.71, 1.86, 1.91, 1.97, 2.40, 2.95, 5.54). The ^{13}C NMR spectrum contained 30 carbon resonances, eight of which were quaternary; two ketone carbonyls (δ_C 213.7, 198.7); one conjugated oxygenated enol carbon (δ_C 167.2), three olefinic carbons (δ_C 109.6, 126.9, 145.8), two aliphatic quaternary carbons (δ_C 55.9, 48.1) and one oxygenated quaternary carbon (δ_C 77.4).

An edited HSQC spectrum revealed that **1** had ten upfield methyl signals, signals for four methylene groups and eight methine groups. HMBC correlations from four of the quaternary methyl resonances (δ_H 1.26, 1.27, 1.30, 1.31) to downfield quaternary carbons at δ_C 167.2, 48.1, 55.9, 213.7, 198.7, 109.6 (C-1a, C-2, C-3, C-4, C-5, C-5a respectively) established the β-diketone moiety. The COSY correlations between δ_H 2.95 (H-6), δ_H 1.91 (H-7) and δ_H 1.05 (H-1'β); δ_H 1.91 (H-7) and δ_H 1.43 (H-16α); δ_H 1.43 (H-16) and δ_H 2.40 (H-15); δ_H 2.40 (H-15) and δ_H 1.97 (H-14), δ_H 1.97 (H-14) and δ_H 0.91 (H_3-21) established the H-1'/H-6/H-7/H-16/H-15/H-14/H-21 partial structure within the molecule. Similarly, COSY correlations from δ_H 1.86 (H-18) to δ_H 0.78 (H_3-19) and δ_H 0.94 (H_3-20) established the isopropyl partial structure. The HMBC correlations from δ_H 0.91 (H_3-21) to

δ_C 29.6 (C-13), δ_C 37.2 (C-14), δ_C 35.6 (C-15); δ_H 0.78 (H_3-19) to δ_C 26.6 (C-18), δ_C 21.4 (C-20) and δ_C 51.2 (C-11); δ_H 0.94 (H_3-20) to δ_C 26.6 (C-18), δ_C 21.4 (C-19), δ_C 21.4 (C-20) and δ_C 51.2 (C-11); and δ_H 1.71 (H-11) to δ_C 26.6 (C-18), δ_C 29.6 (C-13), δ_C 126.9 (C-9), δ_C 145.8 (C-10); δ_H 5.54 (H-9) to δ_C 77.4 (C-8), δ_C 51.2 (C-11), δ_C 35.6 (C-15) and from δ_H 1.26 (H_3-17) to δ_C 126.9 (C-9), δ_C 77.4 (C-8) and δ_C 34.1 (C-7) determined that a cadinene sesquiterpene moiety was present in the molecule. The COSY correlation from the methine proton at δ_H 2.95 (H-6) to δ_H 1.91 (H-7) and HMBC correlations from δ_H 1.26 (H_3-17) to δ_C 34.1 (C-7), δ_C 77.4 (C-8), δ_C 126.9 (C-9) connected the β-diketone moiety to the sequiterpene unit. All these data established the gross structure of the molecule. Correlations observed in a ROESY spectrum were then used to establish the relative configuration of the six stereogenic centers in **1** (Fig. 2). ROESY correlations from δ_H 5.54 (H-9) to δ_H 1.26 (H-17) and δ_H 1.71 (H-11); δ_H 2.95 (H-6) to δ_H 1.00 (H_3-3'), δ_H 1.94 (H-1'α), δ_H 1.26 (H_3-17); δ_H 2.40 (H-15) to δ_H 0.78 (H-19), δ_H 1.43 (H-16), δ_H 1.91 (H-7), δ_H 1.85 (H-13β). These correlations indicated that H-6, H-7 and H_3-17 were on the α face of the molecule, while H-15, H-18, H_3-19 and H-13β were on the β face of the molecule. The ROESY correlations from δ_H 0.91 (H_3-21) to δ_H 1.91 (H-7), δ_H 1.69 (H-2') indicated that these protons were on the α face of the molecule. The relative configuration was therefore established to be 6*R**,7*S**,8*R**,11*R**,14*S**,15*S**.

Table 1.
^{1}H (800 MHz) and ^{13}C NMR (125 MHz) data for **1** and **2** in $CDCl_3$

Position	**1**		**2**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	-	-	201.9	-
1a	167.2	-	-	-
2	48.1	-	59.7	-
3	213.7	-	207.0	-
4	55.9	-	60.5	-
5	198.7	-	202.1	-
5a	109.6	-	-	-
6	28.9	2.95, ddd, 3.4, 6.2, 12.0	63.0	-
7	34.1	1.91, ddd, 4.4, 6.2, 13.7	68.7	3.74, dd, 8.7, 2.9
8	77.4	-	35.8	1.34, m; 1.47, m
9	126.9	5.54, d, 1.7	26.9	1.88, qqt 6.7,6,7,6,7
10	145.8	-	22.7	1.02, d, 6.7
11	51.2	1.71, m	22.6	0.99, d, 6.7
12	23.0	1.64, m; 1.68, m	23.4	1.31, s
13α	29.6	1.29, m	21.4	1.46, s
13β		1.85, m		
14	37.2	1.97, m	19.2	1.40, s
15	35.6	2.40, ddd, 4.5, 6.6, 8.5	24.3	1.37, s
16α	24.5	1.43, m	-	-
16β		1.37, m		
17-Me	24.3	1.26, s	-	-
18	26.6	1.86, m	-	-
19-Me	21.4	0.78, d, 6.6	-	-
20-Me	21.4	0.94, d, 5.6	-	-
21-Me	15.0	0.91, d, 7.1	-	-
22-Me	26.9	1.27, s	-	-
23-Me	21.9	1.31, s	-	-
24-Me	23.0	1.30, s	-	-
25-Me	26.1	1.26, s	-	-
1'α	35.2	1.94, ddd, 3.4, 11.4, 14.5	-	-
1'β		1.05, ddd, 3.0, 12.0, 14.5		
2'	25.3	1.69, m	-	-
3'-Me	20.9	1.00, d, 6.5	-	-
4'-Me	24.6	0.94, d, 5.6		

Woodsianone B (**2**) was isolated as a yellow amorphous solid and based on (+) HRESIMS data at *m/z* 267.1595 (calculated 267.1591) the molecular formula was determined to be $C_{16}H_{22}O_4$.[24] The IR spectrum of **2** showed absorption bands at 1708 and 1512 cm^{-1} indicating carbonyls were present in the molecule. The UV spectrum displayed UV absorption bands at 204 and 274 nm. The ^{1}H NMR spectrum (800 MHz, $CDCl_3$) for **2** (Table 1) indicated the presence of six methyl resonances

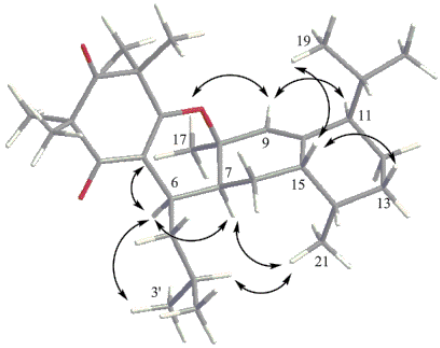


Figure2. ROESY correlations observed for woodsianone A (**1**)

(δ_H 0.99, 1.02, 1.31, 1.37, 1.40, 1.46), two methine resonances (δ_H 3.74, 1.88) and one methylene resonance (δ_H 1.34, 1.47). The ^{13}C NMR spectrum exhibited 15 resonances and edited HSQC data revealed that there are four quaternary methyl groups (δ_C 19.2, 24.3, 21.4, 23.4); two secondary methyls (δ_C 22.7, 22.6), one methylene (δ_C 35.8) and two methines (δ_C 26.9, 68.7). The HMBC correlations from the methyl singlets at δ_H 1.40 (H_3-14) and δ_H 1.37 (H_3-15) to the ketone carbons at δ_C 202.1 (C-5) and 207.0 (C-3) and the aliphatic quaternary carbon at δ_C 60.5 (C-4), and from the other two methyl singlets at δ_H 1.46 (H_3-13) and 1.31 (H_3-12) to the ketone carbons at δ_C 201.9 (C-1) and 207.0 (C-3) and the aliphatic quaternary carbon at δ_C 59.7 (C-2) established the presence of a syncarpic acid moiety in **2**. An isopropyl group was shown to be present in **2** because COSY correlations were observed between both methyl doublets at δ_H 0.99 (H_3-11) and 1.02 (H_3-10) to the methine resonance at δ_H 1.88 (H-9). COSY correlations from the methylene protons at δ_H 1.34 and 1.47 (H_2-8) to the oxygenated methine at δ_H 3.74 (H-7) and the aliphatic methine at δ_H 1.88 (H-9) indicated that the isopropyl group was vicinal to a CH_2CHO group. This was further supported by HMBC correlations from δ_H 1.02 (H_3-10) and δ_H 0.99 (H_3-11) to δ_C 35.8 (C-8) and δ_C 26.9 (C-9) and from δ_H 1.34 (H-8a) to δ_C 68.7 (C-7) establishing the isopentyl side chain within the molecule. No HMBC correlations were observed to the remaining quaternary carbon at δ_C 63.0 (C-6) but the molecular formula derived from MS analysis dictated that this carbon was part of the syncarpic acid moiety and must therefore also be vicinal in C-7. An epoxide oxygen linking C-6 and C-7 provided the final degree of unsaturation to satisfy the molecular formula. The chemical shifts of C-1 to C-7 are consistent with those reported for the related synthetic compound 2-(bicyclo[2.2.1]hept-5-en-2-yl)-5,5,7,7-tetramethyl-1-oxaspiro [2.5]octane-4,6,8-trione (**12**).[25] Woodsianone B was therefore 2-isobutyl-5,5,7,7-tetramethyl-1-oxaspiro[2.5]octane-4,6,8-trione (**2**).

The number of β-triketone terpene adducts reported in the literature has grown substantially over the last five years with compounds being reported from the Myrtaceae genera *Callistemon*, *Kunzea*, *Corymbia*, *Myrtus* and *Rhodomyrtus*.[26-31] Conjugates of the sesquiterpenes, caryophylene, bicyclogermacrene and cadinene have appeared in the literature[22-24] and woodsianone A (**1**) is the third example of a β-triketone adduct containing a cadinene sesquiterpene. It differs from the only other two examples, callistiviminenes A and B in that **1** is likely to be formed by an endocyclic Diels-Alder cycloaddition of cadina-3,5-diene with a β-triketone whereas callistiviminene A and B are likely to be formed from an exocyclic Diels-Alder cycloaddition of bicyclosesqiphellandrene with a β-triketone yielding a spiro product. A variety of simple acylated syncarpic acid derivatives have also been reported from various Myrtaceae plants with the acyl ketone side chain being their point of

difference.[32] Isopropyl, isobutyl, isopentyl, ethyl, propyl, pentyl and ethylphenyl groups attached to the side chain ketone have been reported but no natural epoxide derivatives have been reported. Woodsianone B (**2**) is therefore the first to be isolated from a natural source. The epoxide **2** is likely to be an oxidation product of the olefin **14** which has been used as a precursor for the production of β-triketone-terpene adducts. Several biomimetic syntheses of this type of compounds have been achieved through Diels-Alder cyclization of this olefin intermediate (**14**) with either β-pinene or caryophyllene.[24, 25] The known compounds **3**, **4**, **5**, **6** and **7** have previously been isolated from *Rhodomyrtus tomentosa*,[30,33,25] **8** has been isolated from *Corymbia ficifolia*, *Kunzea ericoides* and *R. tomentosa*,[24, 26] **9** from *Kunzea ambigua*[23] and **10** and **11** from *Corymbia watsoniana*.[18]

The antiplasmodial activities of compounds **1-9** were evaluated using the method described by Duffy and Avery,[27] against the chloroquine sensitive (3D7) strain of *P. falciparum* and human cell cytotoxicity was assessed using the mammalian cell line HEK-293 to determine the selectivity of compounds towards the parasite (Table 2). Compounds showing activity less than 5 μM were further tested against the resistant (Dd2) strain of *P. falciparum*. The antiplasmodial activities of watsonianone B (**11**) and watsonianone A (**10**) have been reported previously to be 0.29 and 5.3 μM, respectively, against the 3D7 strain of *P. falciparum*.[18] During the testing of the β-triketones it was noted that some of the compounds were poorly soluble in aqueous solutions. Measures were taken to reduce handling time enabling the assays to be undertaken as quickly as possible to avoid excessive compound precipitation.

In the current study the most potent antimalarial activity was found for rhodomyrtone (**5**) (IC_{50} against 3D7, 1.8±1.0 μM (n=3)) followed by woodsianone B (**2**) with an IC_{50} against 3D7 of 3.0±0.6 μM (n=3). The least active compound was woodsianone A (**1**) with an IC_{50} of 26.9±2.0 μM (n=3). The two most potent compounds, **5** and **2,** were further tested against the resistant strain, Dd2, yielding IC_{50} values of 4.00±0.30 μM (n=2) and 2.53±0.11 μM (n=2), respectively. Rhodomyrtone (**5**) had an IC_{50} of 11.4±2.1 μM (n=2) against the HEK-293 cells, while woodsianone B (**2**) had an IC_{50} of 15.9±5.7 μM (n=2). This indicated that both compounds were less than tenfold selective towards the parasite compared to HEK-293 cells.

The four β-triketone terpene adducts (**1**, **6**, **7**, **8**) were all only weakly antiplasmodial and this might be associated with their low water solubility. The compounds containing a caryophyllene group (**6**, **7**) show slightly more potent activity and this might be associated with more flexibility in this terpene moiety compared to cadenene and β-pinene in **1** and **8** leading to slightly higher solubility. Similarly a three-fold higher antiplasmodial activity for watsonianone A (**10**) compared to its cyclized analogue, rhodomyyrtosone D (**4**) and six fold higher activity of rhodomyrtone (**5**) compared to kunzeanone A (**9**) may also be associated with higher solubility for the more potent analogues. Comparable antiplasmodial activity for woodsianone B (**2**) and watsonianone A (**10**) suggests that the second syncarpic acid moiety in **10** is not greatly contributing to its activity. A surprising result was the almost 50 fold lower activity observed for rhodomyrtosone A (**3**) compared to watsoniaone B (**11**) and this suggests that the ethyl phenyl side chain in **11** is important for activity. A 20 fold lower activity for **3** compared to another compound that we have previously reported, tomentosone A (**13**) (IC_{50} of 1.0 μM against 3D7),[28] was also observed and since tomentosone A (**13**) is essentially an amalgum of rhodomyrtone (**5**) and rhodomyrtosone A. (**3**) this suggests that the syncarpic acid linked through the bisfurano group in **13** is not contributing to its activity.

Table 2. Antiplasmodial activity for **1**-**11** and **13**

Compound Name	3D7 IC_{50} μM (n=3)	Dd2 IC_{50} μM (n=2)	HEK cytotoxicity IC_{50} μM (n=2)	Ligand Efficiency[d]
Woodsianone A (**1**)	26.9±2.0	-	68%[a]	0.19
Woodsianone B (**2**)	3.0±0.6	2.53±0.11	15.9±5.7	0.40
Rhodomyrtosone A (**3**)	10.5±0.9	-	59% [a]	0.21
Rhodomyrtosone D (**4**)	14.0±6.5	-	IA[a]	0.21
Rhodomyrtone (**5**)	1.8±1.0	4.00±0.30	11.4±2.1	0.24
Tomentodione A (**6**)	8.9[e]	-	-	0.21
Tomentodione B (**7**)	11.3[e]	-	-	0.20
4*S*-Ficifolidione (**8**)	6.7±1.4	-	IA	0.25
Kunzeanone A (**9**)	10.7±1.6	-	IA[a]	0.18
Watsonianone A (**10**)[b]	5.3	8.8	42%	0.23
Watsonianone B (**11**)[b]	0.29	0.44	85%	0.23
Tomentosone A (**13**)[c]	1.0	1.49	IA	0.16
Pyrimethamine[f]	0.008±0.009	62% at 40 μM	62% at 40 μM	
Chloroquine[f]	0.0093±0.0022	0.194±0.038	IA	
Pyronaridine[f]	0.0119±0.0025	0.018±0.003	5.22±1.42	
Puromycin[f]	0.062±0.032	0.061±0.0025	0.399±0.001	
Artesunate[f]	0.001±0.001	0.00067±0.00018	94% at 20 μM	
DHA[f]	0.0004±0.0001	0.00043±0.00007	83% at 20 μM	

[a]% inhibition at the highest concentration tested (100 μM)
[b]activity reported from reference 12, HEK-293 inhibition at 120 μM
[c]activity reported from reference 26 HEK-293 inhibition at 40 μM
[d]Ligand efficiency LE = -1.36*pIC_{50}/(number of heavy atoms)
[e]n=1
[f]reference compounds

A comparison of the ligand efficiency (LE) of the 11 compounds isolated indicates that woodsianone B (**2**) would make a good hit molecule since its LE is 0.40, its molecular weight is low and its structure is relatively simple.[29] The low water solubility observed for some of the compounds suggests that synthetic modifications to increase their water solubility could lead to improved bioactivity.

In conclusion, this is the first chemical investigation of any plant from the genus *Angophora* and it has demonstrated that the genus is also a source of β-triketones. This discovery supports its close taxonomic relationship with the genus *Corymbia*. Antiplasmodial activity for the two new and nine known compounds has added further to our knowledge of structure activity relationships within this class of compound and further indicates that β-triketones can be considered a novel class anti-malarial drug leads.

Acknowledgements

We acknowledge support from Griffith University for a Postgraduate Research Scholarship and a Griffith University International Postgraduate Research Scholarship (awarded to SPDS). The research was also supported by research funding from the Griffith School of Environment and the Australian Research Council (LP120200557 awarded to VMA). The authors wish to thank and acknowledge the Australian Red Cross Blood Bank for the provision of fresh red blood cells, without which this research could not have been performed. The authors thank and acknowledge the contributions of Elspeth Johnson for assistance with parasite and mammalian cell culturing.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at…..

References and notes

1. Biamonte MA, Wanner J, Le Roch KG. *Bioorg Med Chem Lett*. 2013, 23, 2829-2843.
2. World Malaria Report. World Health Organization; 2016.
3. WHO. Guidelines for the treatment of malaria (Third Edition). Geneva, World Health Organization, 2015.
4. White NJ. *N Engl J Med*. 1996, 335, 800-806.
5. Antony HA, Parija SC. *Trop Parasitol*. 2016, 6, 30-41.
6. Bhattacharjee D, Shivaprakash G. *JAPHAC*. 2016, 6, 137-143.
7. Klayman D. *Science*. 1985, 228, 1049-1055.
8. Jackson Y, Chappuis F, Loutan L, Taylor W. *Malaria J*. 2006, 5, 81.
9. Bell D, Winstanley P. *Brit Med Bull*. 2004, 71, 29-43.
10. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. *New Engl J Med*. 2008, 359, 2619-2620.
11. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NPJ, Lindegardh N, Socheat D, White NJ. *N Engl J Med*. 2009, 361, 455-467.
12. Thein ST, Sudhinaraset M, Khin HSS, McFarland W, Aung T. *Malaria J*. 2016, 15, 334.
13. Eastman RT, Fidock DA. *Nat Rev Microbiol*. 2009, 7, 864-874.
14. Maude RJ, Woodrow CJ, White LJ. *Drug Dev Res*. 2010, 71, 12-19.
15. Roberts L. *Science*. 2016, 352, 398-405.
16. Chippendale GM. *Flora of Australia: Vol. 19 Myrtaceae-Eucalyptus, Angophora*. Canberra: Australian Government Publishing Service; 1988.
17. Udovicic F, McFadden G, Ladiges P. *Mol Phylogenet Evol*. 1995, 4, 247-256.
18. Carroll AR, Avery VM, Duffy S, Forster PI, Guymer GP. *Org Biomol Chem.* 2013, 11, 453-458.
19. Carroll AR, Urban S, Lamb J, Moni R, Guymer GP, Forster PI, Quinn RJ. *J Nat Prod.* 2008, 71, 881-883.
20. Carroll AR, Lamb J, Moni R, Guymer GP, Forster PI, Quinn RJ. *J Nat Prod*.. 2008, 71, 1564-1568.
21. Bailey FM. *Proc Linn Soc NSW*. 1882, 6, 143-144.
22. Wu L, Wang X-b, Li R-j, Zhang Y-l, Yang M-h, Luo J, Kong L-Y. *Phytochemistry*. 2016, 131, 140-149.
23. Ito H, Iwamori H, Kasajima N, Kaneda M, Yoshida T. *Tetrahedron*. 2004, 60, 9971-9976.
24. Liu H-X, Zhang W-M, Xu Z-F, Chen Y-C, Tan H-B, Qiu S-X. *RSC Adv*. 2016, 6, 25882-25886.
25. Liu H-X, Chen K, Tang G-H, Yuan Y-F, Tan H-B, Qiu S-X. *RSC Adv*. 2016, 6, 48231-48236.
26. Khambay BPS, Beddie DG, Hooper AM, Simmonds MSJ. *Tetrahedron*. 2003, 59, 7131-7133.
27. Duffy S, Avery VM. *Am J Trop Med Hyg*. 2012, 86, 84-92.
28. Hiranrat A, Mahabusarakam W, Carroll AR, Duffy S, Avery VM. *J Org Chem*. 2012, 77, 680-683.
29. Hopkins AL, Keseru GM, Leeson PD, Rees DC, Reynolds CH. *Nat Rev Drug Discov*. 2014, 13, 105-121.

5.1 Antibacterial Activity of isolated compounds

All the isolated compounds were tested against bacterial strain *Staphylococcus aureus* ATCC 157293. At the initial screening the compounds rhodomyrtone, woodsianone B and watsonianone A inhibited the growth of bacteria at the concentration of 10 mM. The MIC values of these active compounds were evaluated and they were 0.63 mM, 0.02 mM and 10 mM for rhodomyrtone, woodsianone B and watsonainone A respectively.

5.1.1 Antibacterial Assay

The antibacterial activities of the compounds **1-11** were determined using a broth micro dilution method against *S. aureus* ATCC 157293 bacterium. The assay was conducted in 96 well sterilized microtiter plate containing pure compounds at a concentration of 0.5 mM to 0.002 mM. Compounds were dissolved in 100% DMSO. To each well 25 μL of double strength broth was added and then 5 μL of compounds at 10 mM of concentration was added to the well followed by 50 μL of inoculum and 20 μL of sterile water. Plates were incubated at 37°C while being shaken at 100 rpm, for 6 hours. After this period 10 μL of the resazurin stock solution (704 μmol) was added and the plate was incubated for a further hour. After colour has been developed inhibition relative to resazurin was determined using a BMG Labtech FLUO star omega microplate fluorometer (excitation 570 nm; emission 620 nm). All the experiments were carried out in physical containment level 2 (PC2) laboratory facilities.

Antiplasmodial β-triketones from the Australian tree *Angophora woodsiana*

Sarath P. D. Senadeera[a,b, c], Sandra Duffy[d], Vicky Avery[d] and Anthony R.Carroll[*, a,b,d]

[a]*Environmental Future Research Institute, Griffith University, Gold Coast, QLD 4222, Australia.*
[b]*Griffith School of Environment, Griifth University, Gold Coast, QLD 4222, Australia.*
[c]*Department of Natural Products, Medical Research Institute, No 527, Dr. Danister De Silva Mawatha, Colombo 08, Sri Lanka*
[d]*Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia.*

Supplementary Data

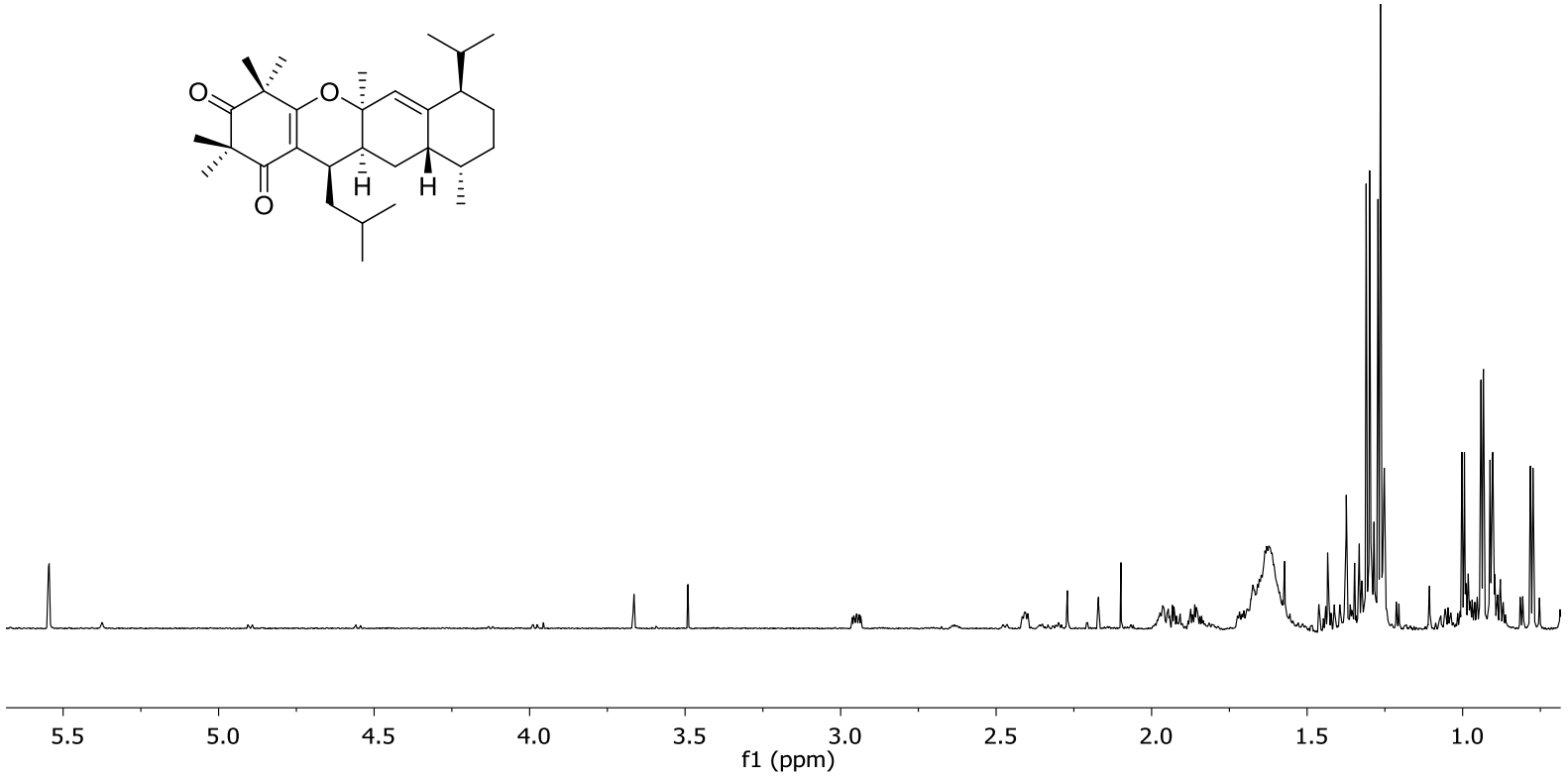


Figure S1.^{1}H NMR spectrum of woodsianone A in $CDCl_3$ (800 MHz)

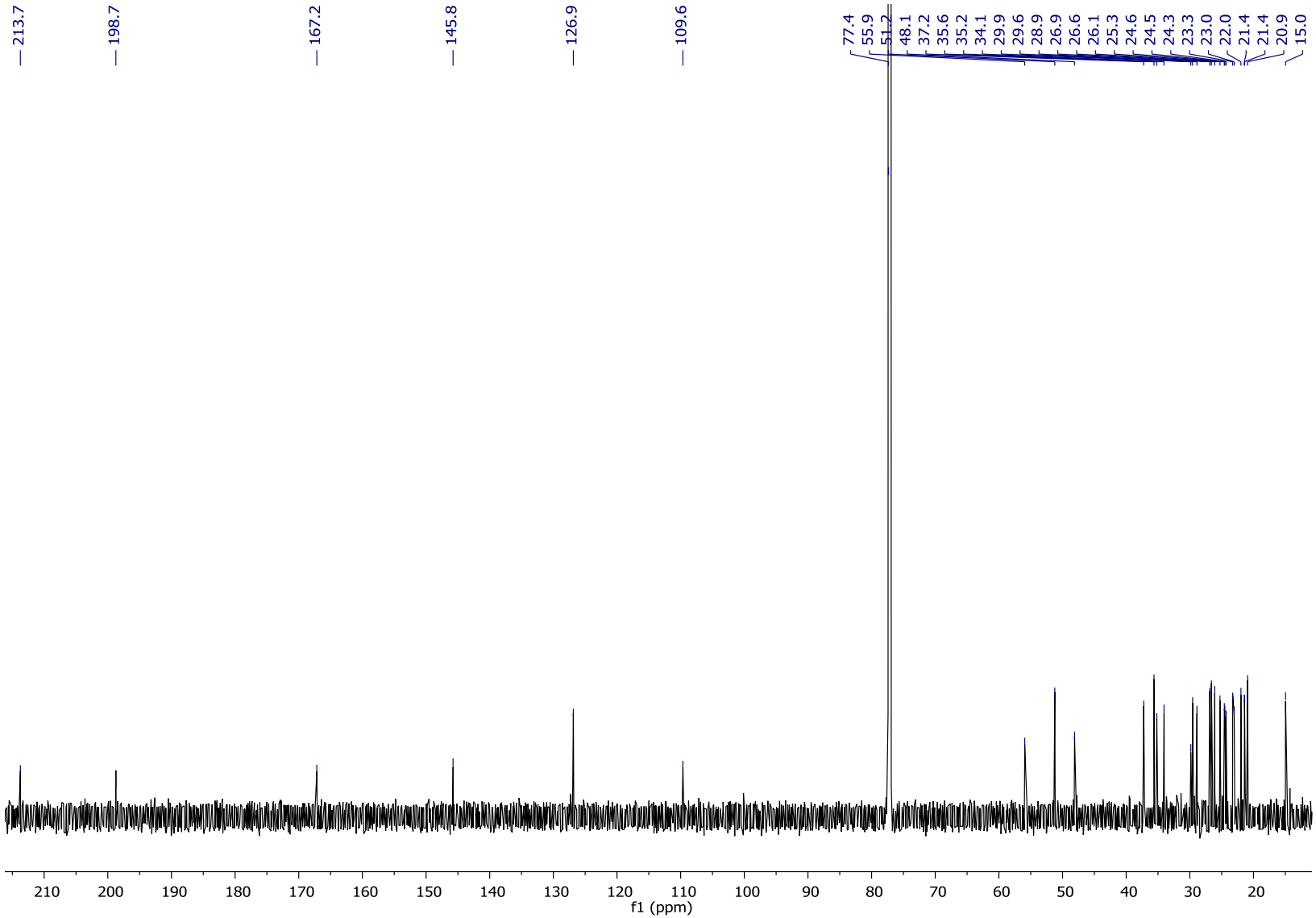


Figure S2. ^{13}C NMR of woodsianone A in $CDCl_3$ (800 MHz)

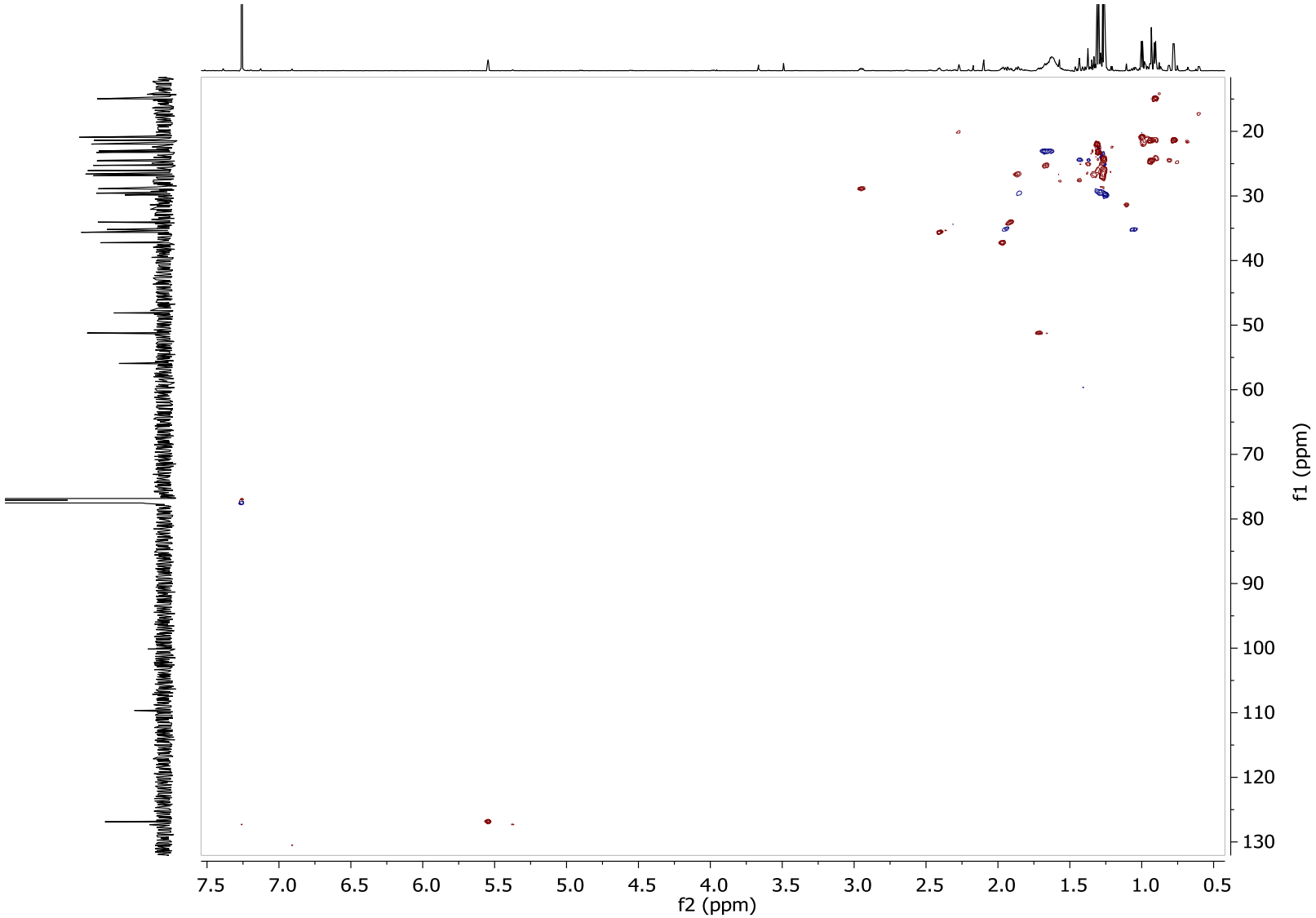


Figure S3. HSQC spectrum of woodsianone A in $CDCl_3$ (800 MHz)

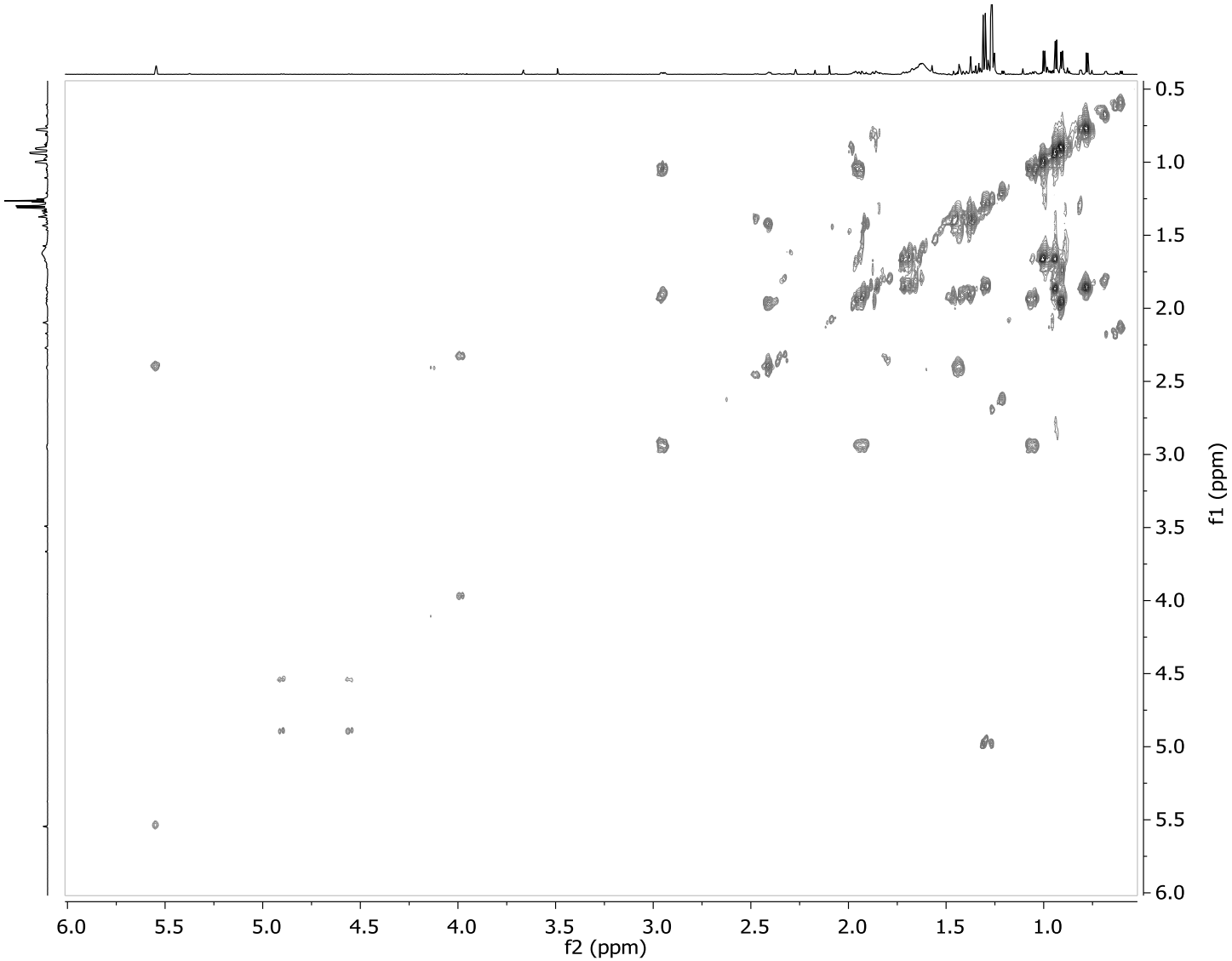


Figure S4. COSY spectrum of woodsianone A in $CDCl_3$ (800 MHz)

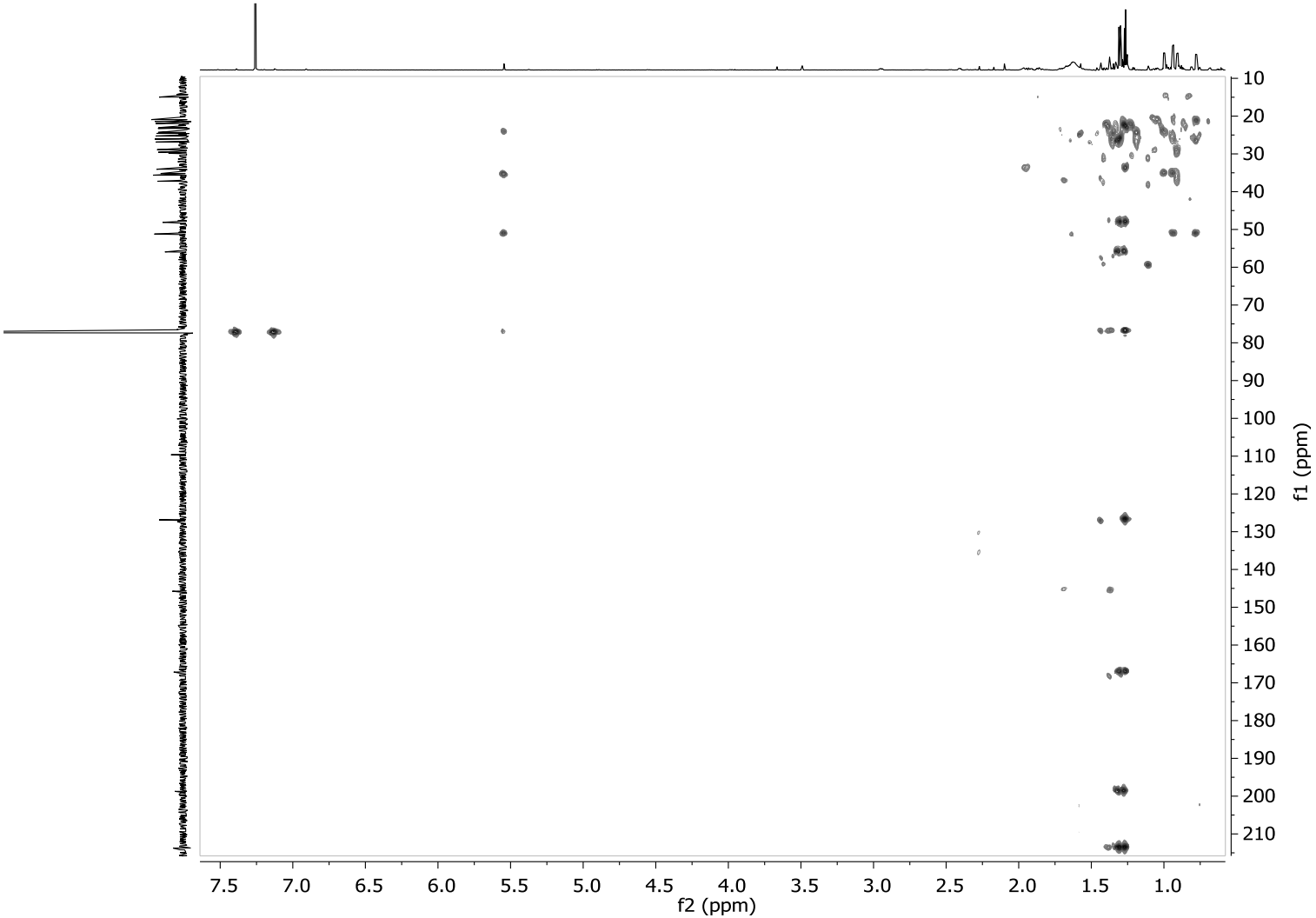


Figure S5. HMBC spectrum of woodsianone A in $CDCl_3$ (800 MHz)

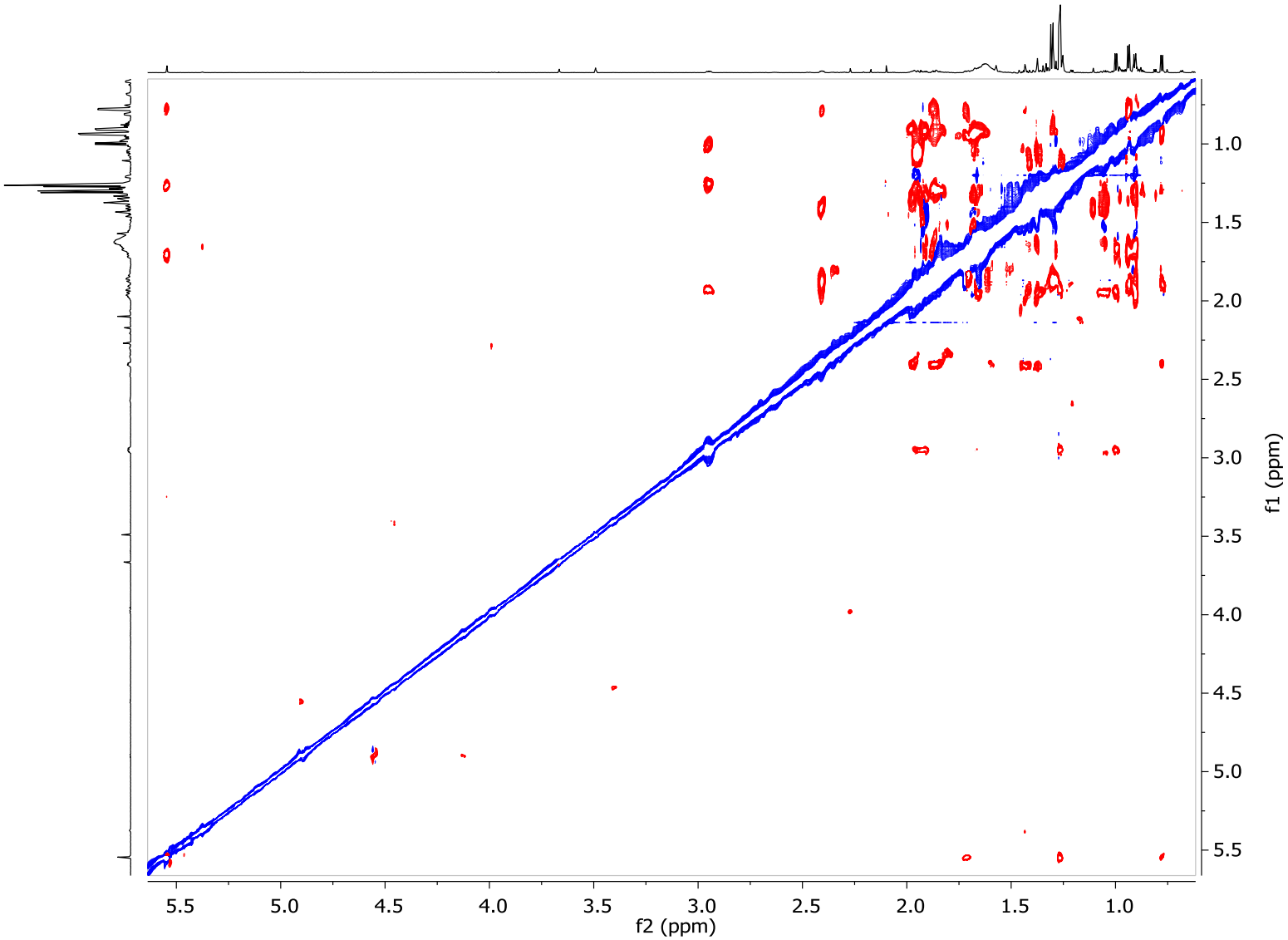


Figure S6. ROESY spectrum of woodsianone A in $CDCl_3$ (800 MHz)

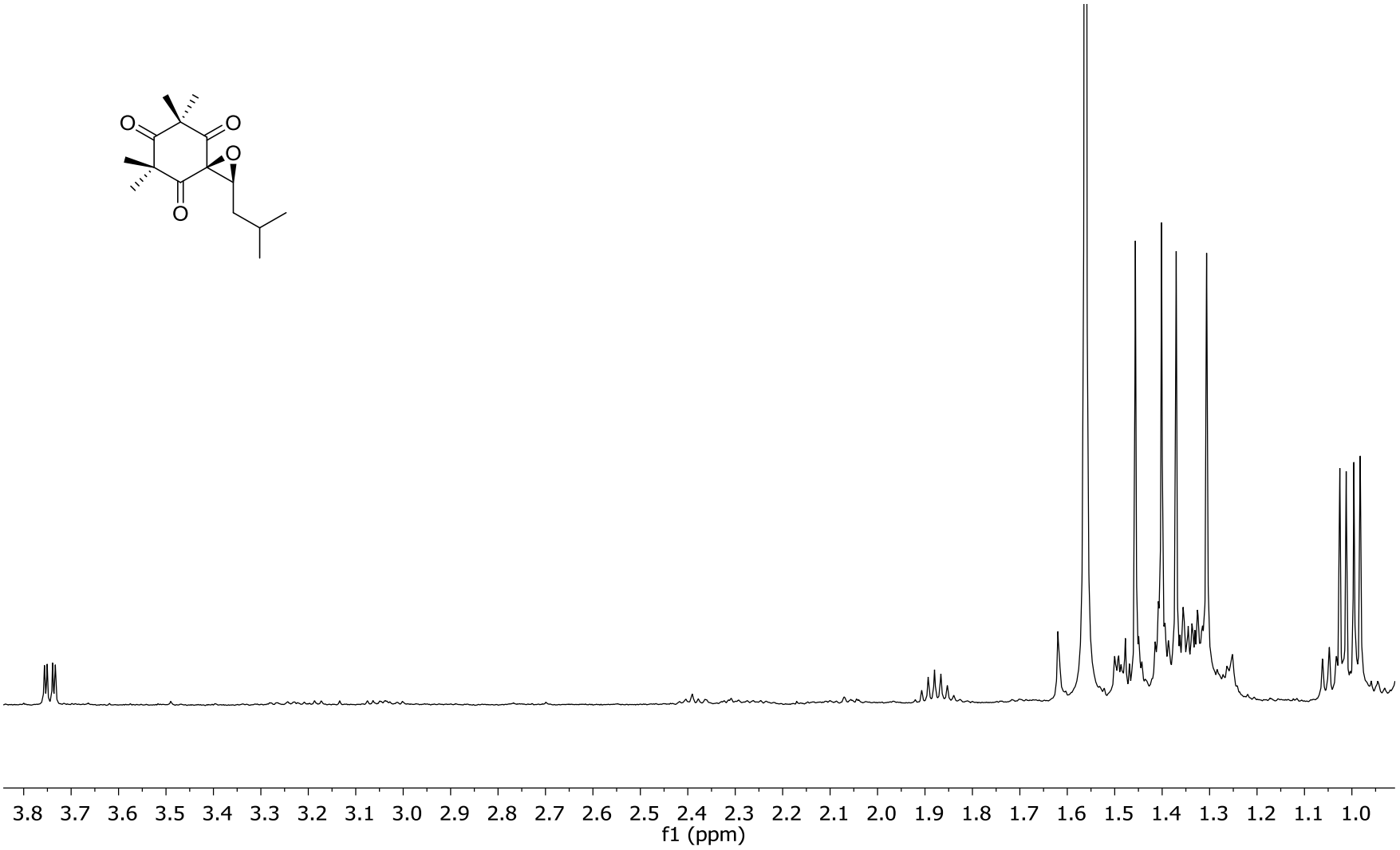


Figure S7 ^{1}H NMR spectrum of woodsianone B in $CDCl_3$ (800 MHz)

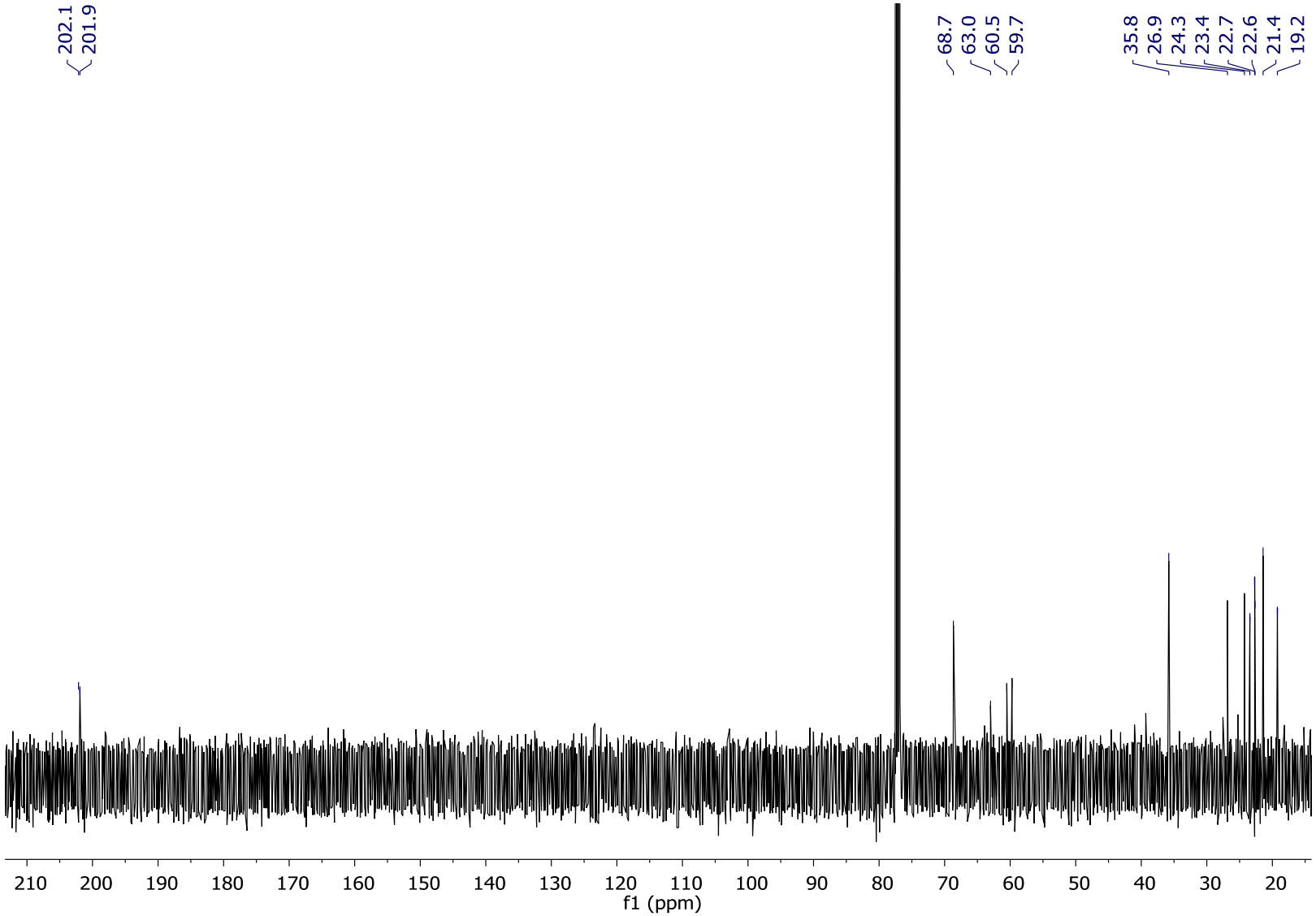


Figure S8 ^{13}C NMR spectrum of woodsianone B in $CDCl_3$ (800 MHz)

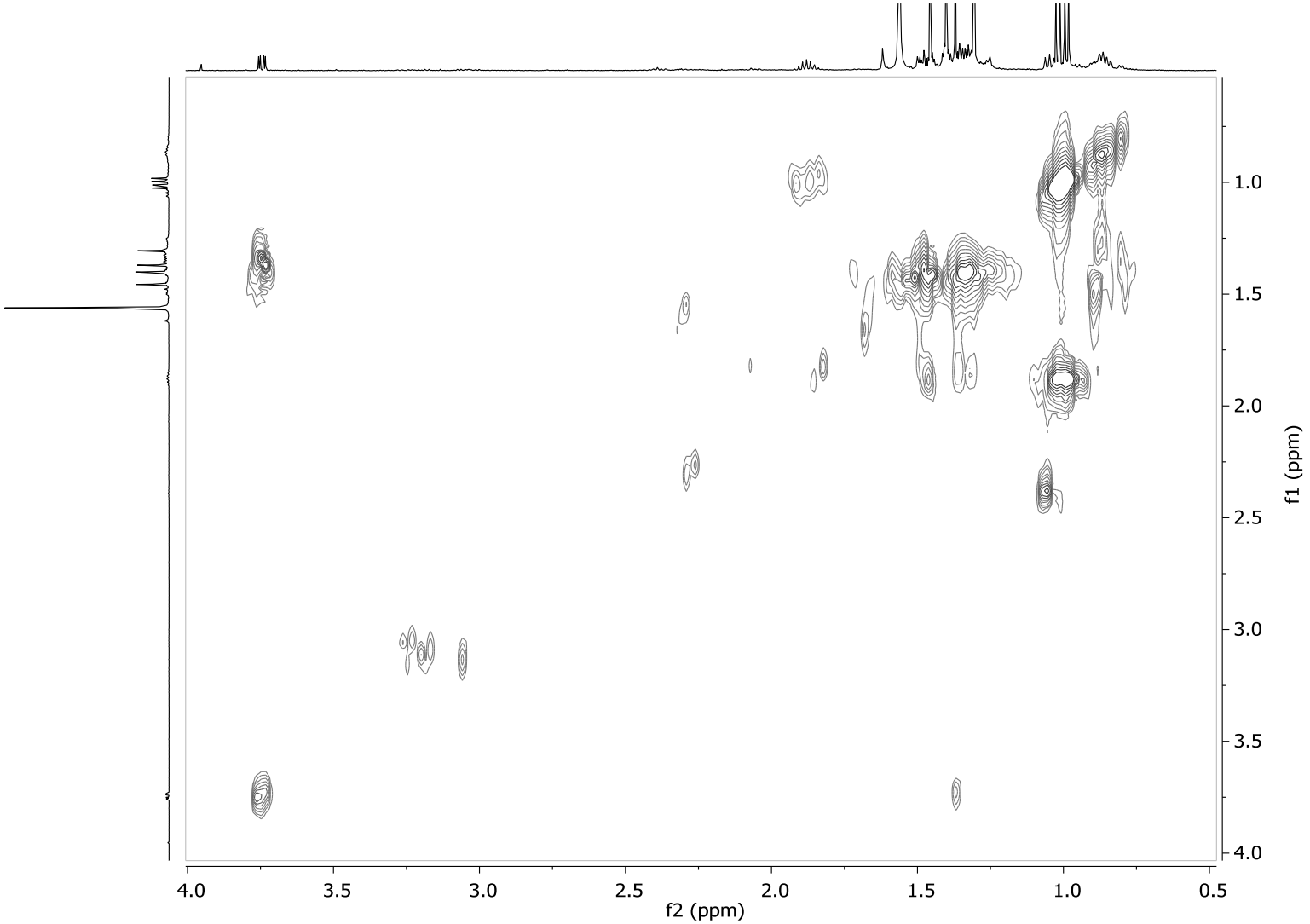


Figure S9. COSY spectrum of woodianone B in $CDCl_3$ (800 MHz)

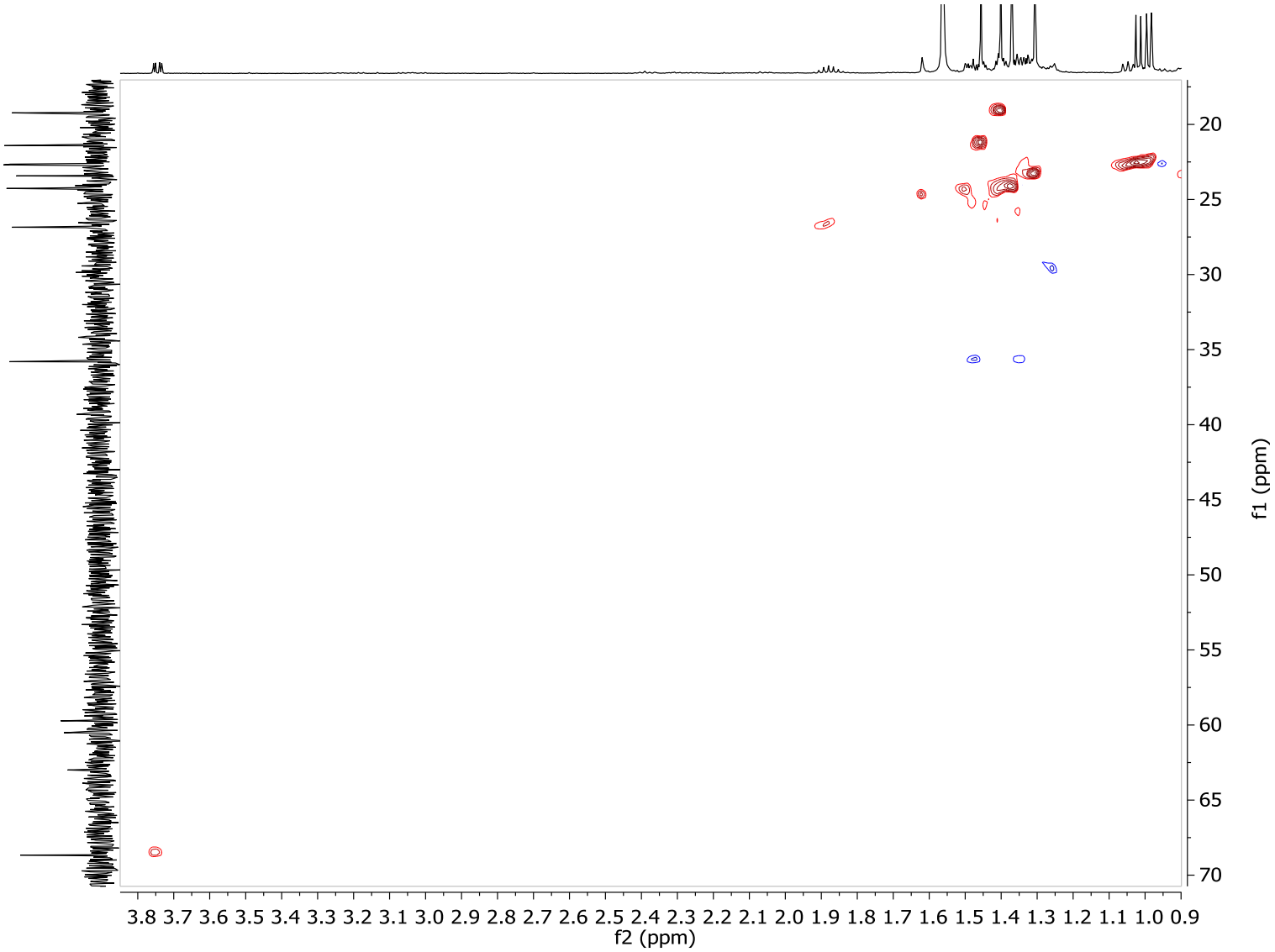


Figure S10. HSQC spectrum of woodianone B in $CDCl_3$ (800 MHz)

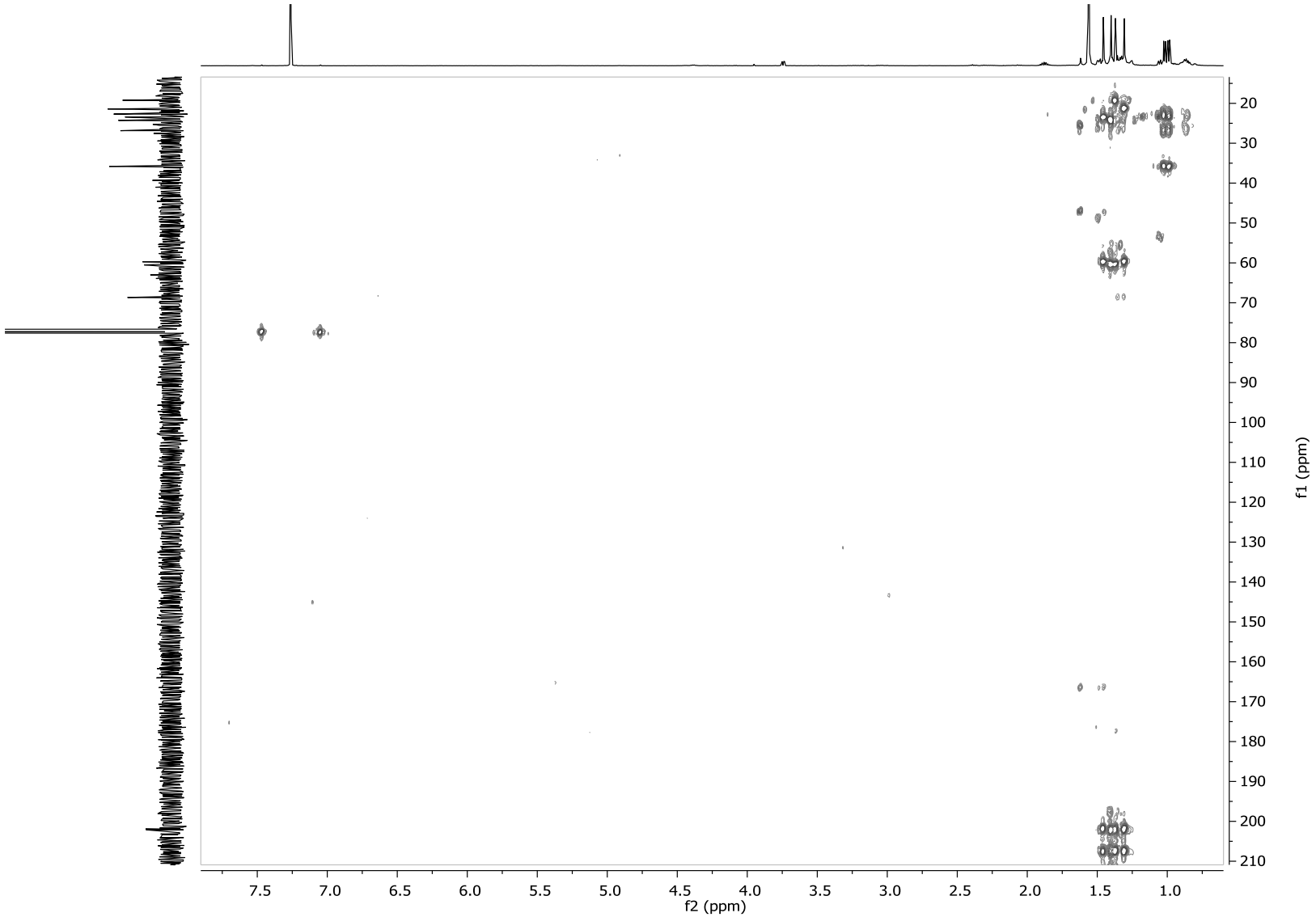


Figure S11. HMBC spectrum of woodianone B in $CDCl_3$ (800 MHz)

CHAPTER 6

FIVE NEW ANTIPLASMODIAL β-TRIKETONES FROM THE FLOWERS OF AUSTRALIAN TREE *Corymbia intermedia*

FIVE NEW ANTIPLASMODIAL β-TRIKETONES FROM THE FLOWERS OF AUSTRALIAN TREE *Corymbia intermedia*

One of the species selected for this study was *Corymbia intermedia.* In this results chapter isolation, structure elucidation, antiplamodial, and antibacterial activity testing of five new β-triketones from the flowers of *C. intermedia* is explained.

STATEMENT OF CONTRIBUTION CO-AUTHORED UNPUBLISHED PAPER

This results chapter includes a co-authored prepared manuscript: 'Five new antiplasmodial β-triketones from the flowers of Australian tree *Corymbia intermedia'* which will be submitted to the Journal of Natural Products. It is formatted to meet the journal requirements.

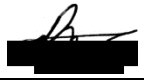
The bibliographic details of the co-authored manuscript, including all authors, are:

Sarath P. D. Senadeera, Sandra Duffy, Joshua Hayton, Vicky M. Avery, Anthony R. Carroll*

My contribution to the paper involved:
Collection and organization of information, data and references; Preparation of manuscript.

(Signed)  (Date) 14/03/2017
Name of student: Sarath Parakumge Dayani Senadeera

(Countersigned)  (Date) 14/03/2017
*Corresponding author: Anthony R. Carroll

(Countersigned)  (Date) 14/03/2017
Principle supervisor: Anthony R. Carroll

6.1 Abstract

Five new β-triketone monoterpene adducts, intermediones A-E (**1**-**5**) have been identified from the flowers of the Australian eucalypt tree *Corymbia intermedia* (Myrtaceae). Intermediones A-C and E are β-triketones that incorporate a pinene moiety attached via a benzyl group to a syncarpic acid, while intermedione D is an oxidized analog of these compounds incorporating a novel ring skeleton. The structures of **1**-**5** were elucidated from the analysis of 1D/2D NMR and MS data. The relative configurations of the compounds were determined from analysis of ^{1}H-^{1}H coupling constants and ROESY correlations. All compounds showed moderate antiplasmodial activity against chloroquine sensitive (3D7) and resistant (Dd2) strains of *Plasmodium falciparum* with IC_{50} values ranging from 12-21 μM (3D7) and 15-26 μM (Dd2) respectively. Intermediones A, B and mixture of intermediones B and C were inactive at 0.5 mM against the bacterium *Staphylococcus aureus* ATCC 157293.

6.2 Introduction

The Myrtaceae is a large angiosperm family that consists of 140 genera with more than 3800 species. Australia can be considered to be the centre of diversity of the family since 17 genera and about 1600 species are endemic to the continent.[1] Non-volatile constituents found in Myrtaceae species are dominated by phloroglucinols and β-triketones and these compounds display a broad range of significant biological activities including mammalian antifeedant, growth regulator, anti-fouling, antibacterial, antiprotozoal, Epstein Barr Virus inhibitory, HIV-RT inhibitory, and aldose reductase inhibitory activities.[2-6] The Myrtaceae genus *Corymbia* contains 113 species of trees distributed in tropical to warm temperate savannah vegetation of northern Australia.[7] The genus was created in 1995, to separate specifically distinct species (based on morphological characteristics of flowers and molecular analysis of sRNA) of bloodwood, spotted gum and ghost gum that were previously classified as *Eucalyptus* species.[7] Previous studies on the chemistry of *Corymbia* species have revealed that they produce a diverse array of β-triketone natural products including simple acyl β-triketones, β-triketone terpene adducts, β-triketone dimers and β-

triketone phloroglucinol conjugates with interesting biological activities such as antiplasmodial activity.[5, 6, 8] This paper reports on the isolation, structure elucidation and biological activity of five new β-triketone monoterpene adducts, intermediones A-E from the flowers of *Corymbia intermedia*.

6.3 Results and Discussion

The ground flowers of *C. intermedia* were extracted with methanol and purified by HPLC on C_{18} silica gel eluting with a linear gradient from H_2O to MeOH. Based on ^1H NMR data, fractions eluting between 50% and 100% MeOH were recombined and purified by HPLC on a diol-bonded silica gel column using a gradient from 100% Hexane to 100% DCM and then further eluted for 10 minutes in DCM. Two fractions from this separation were further purified by HPLC on diol-bonded silica gel eluting with 80% hexane/20% DCM and on phenyl bonded silica gel with a linear gradient from 60% aqueous MeOH to 100% MeOH respectively. This resulted in intermediones A (**1**), B (**2**), D (**4**) and E (**5**) being purified. (Figure 1). A fraction containing a mixture of compounds including more intermedione B (**2**) was further purified using a gradient from 40% H_2O/60% MeOH to 100% MeOH on a phenyl bonded silica gel HPLC column yielding intermedione B (**2**), from this separation. Further attempts to separate **3** from **2** were unsuccessful and so the structure of intermedione C (**3**) was deduced from analysis of 1D and 2D NMR data acquired on the 1:4 mixture of it with intermedione B.

Intermedione A (**1**) was obtained as a yellow amorphous solid (50 mg). The (+) HRESIMS of **1** contained a protonated molecule at *m/z* 407.2605 MH^+ indicating a molecular formula $C_{27}H_{34}O_3$ (calculated *m/z* 407.2581). The IR spectrum of **1** showed absorption bands at 1654 cm^{-1} and 1715 cm^{-1} indicating carbonyls were present in the molecule. The UV spectrum displayed a UV absorption maximum at 266 nm which suggested the molecule contained an aromatic chromophore. The ^1H NMR spectrum (Table 1) for **1** contained resonances for six aliphatic quaternary methyl groups (δ_H 0.97, 1.20, 1.25, 1.30, 1.33, 1.52), four aliphatic methylene groups (δ_H 1.94/2.40, 1.98/1.84, 2.18/1.54, 2.12/1.77) and three methine protons (δ_H 3.78, 2.30, 1.98) while three aromatic proton resonances [δ_H 7.25 (2H), 7.16 (1H), 7.14 (2H)] indicated the presence of a

mono substituted benzene ring in the molecule. The ^{13}C NMR spectrum exhibited 27 carbon signals, nine of which were quaternary; two ketone carbonyls (δ_C 213.7, 197.2); one conjugated oxygenated enol carbon (δ_C 171.6), an olefinic carbon (δ_C 110.6), three aliphatic quaternary carbons (δ_C 55.3, 48.5, 38.5) one aromatic quaternary carbon (δ_C 144.8) and an oxygenated quaternary carbon (δ_C 85.8).

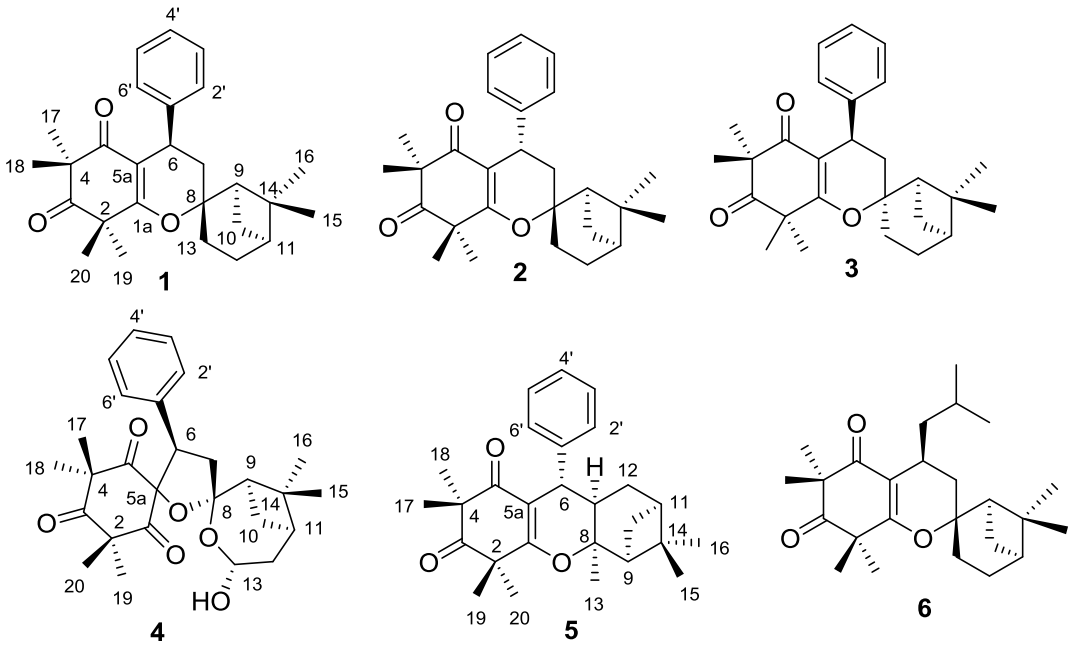


Figure 1. β-triketones isolated from *Corymbia intermedia*

Analysis of edited-HSQC correlations revealed that 13 aliphatic carbon signals in the upfield region of the spectrum could be assigned to six methyls (δ_C 22.7, 23.3, 24.7, 25.8, 26.2, 27.7), four methylenes (δ_C 24.7, 26.2, 30.1 43.3) and three methines (δ_C 36.1, 40.3, 46.6). There were also five aromatic methines (δ_C 128.6 (2C), 126.9 (1C), 126.3(2C)). HMBC correlations from four of the quaternary methyl resonances (δ_H 1.20, 1.25, 1.33, 1.52) to the down field quaternary carbon at δ_C 213.7 (C-3), from δ_H 1.20 and 1.25 to δ_C 55.3 (C-4) and 197.2 (C-5) and from δ_H 1.33 and 1.52 to δ_C 171.6 (C-1a) and 48.5 (C-2) established the syncarpic acid moiety within the molecule. HMBC correlations from δ_H 3.78 to the aromatic carbons at δ_C 144.8 (C-1'), 126.3 (C-2')

and also to the olefinic carbon at δ_C 110.6 (C-5a) and the downfield quaternary carbons at δ_C 171.6 (C-1a) and δ_C 197.2 (C-5) indicated that the phenyl group was attached to the syncarpic acid at C-6. This was further confirmed by HMBC correlations from the aromatic protons, δ_H 7.14 to δ_C 36.1 (C-6). ^{1}H-^{1}H COSY correlations from the methine doublet of doublets at δ_H 3.78 (H-6) to the methylene protons at δ_H 1.94 (dd, J = 14.5 Hz, 11.4 Hz, H-7b) and 2.40 (dd, J = 14.5 Hz, 6.5 Hz, H-7a) established that the phenyl group was vicinal to a $CHCH_2$ group. HMBC correlations from the H_2-7 methylene protons, δ_H 1.94 and 2.40 to δ_C 144.8 (C-1'), also supported the incorporation of this methylene group at position 7. Mutual COSY correlations between the methylene protons at δ_H 1.84, 1.98 1.77 and 2.12 indicated that the molecule contained a CH_2CH_2 group. COSY correlations between the methylene proton at δ_H 2.18 (H-10a) and the methine protons at δ_H 1.98 (H-11) and 2.30 (H-9) were consistent with a $CHCH_2CH$ spin system. The proton geminal to H-10a, (δ_H 1.54 (H-10b)) showed no coupling to H-9 and H-11 suggesting that it was 90° to both H-9 and H-11. A COSY correlation associated with a large w-coupling of 4.8 Hz between 2.30 (H-9) and 1.98 (H-11) suggested that a cyclobutyl ring was present in the molecule. [9] HMBC correlations from the two methyl resonances at δ_H 0.97 and 1.30 to δ_C 38.5, 40.3, 46.6 (C-14, C-11, C-9) further supported the presence of a cyclobutyl moiety. The methine proton resonating at δ_H 2.30 showed HMBC correlations to seven carbons, δ_C 26.2, 27.7, 30.1, 38.5, 40.3, 43.3 and 85.8 (C-10, C-16, C-13, C-14, C-11, C-7 and C-8 respectively) while correlations from the methylene proton δ_H 1.84 to five carbons δ_C 26.2, 30.1, 38.5, 40.3 and 85.8 (C-10, C-13, C-14, C-11 and C-8) established the pinene ring. HMBC correlations from δ_H 1.94/2.40 to δ_C 30.1, 46.6 and 85.8 (C-13, C-9 and C-8 respectively) provided evidence to attach the pinene moiety to the syncarpic acid moiety. The chemical shift of the quaternary carbon resonating at δ_C 85.8 suggested it was oxygenated and an ether linking C-1a and C-8 to form a pyran ring fulfilled the final degree of unsaturation dictated by the molecular formula establishing the 2D structure of **1**.

The relative configuration of the four stereogenic centers in **1** was deduced from analysis of ROESY data (Figure 2) and ^{1}H-^{1}H coupling constants. The proton resonating at δ_H 3.78 (H-6) shows a large (11.4 Hz) coupling to δ_H 1.94 (H-7b) indicating that H-6 and H-7b are diaxial. ROESY correlations from δ_H 3.78 (H-6) to δ_H 0.97 (H_3-15), 2.30 (H-9) and 2.40 (H-7a) provided

evidence to define the configuration of the C-8 spiro center relative to C-6 and this indicated that the cyclobutyl moiety and H-6 were on the α face of the molecule. Furthermore, these correlations indicated that the gem-dimethyl partial structure was oriented towards C-7 and not *O*-1. A ROESY correlation between H-10a and H_3-16 indicated that the methyl group (H_3-16) and the cyclobutyl methylene proton (H-10a) were on the bottom face of the cyclobutyl moiety. ROESY correlations from H-13b and H-12b to H_3-15 further confirmed the position of the methyl H_3-15 and defined the positon of H-12b and H-13b to be on the face as C-15 and orientated towards C-7 (and not *O*-1). A ROESY correlation between H-10b and the syncarpic acid methyl protons, H_3-19 further corroborated the orientation of the methylene protons of the cyclobutyl group to be on the same side as *O*-1 while H_3-19 is on the α face of the cyclohexadione ring. ROESY correlations from the phenyl protons H-2′/H-6′ to H_3-18 and H_3-20 indicated that both of these methyl groups were located on the same face of the cyclohexadione ring. The relative configuration of the stereogenic centers in **1** was therefore assigned as 6*R**, 8*R**, 9*R**, 11*S** as shown in the Figure 2.

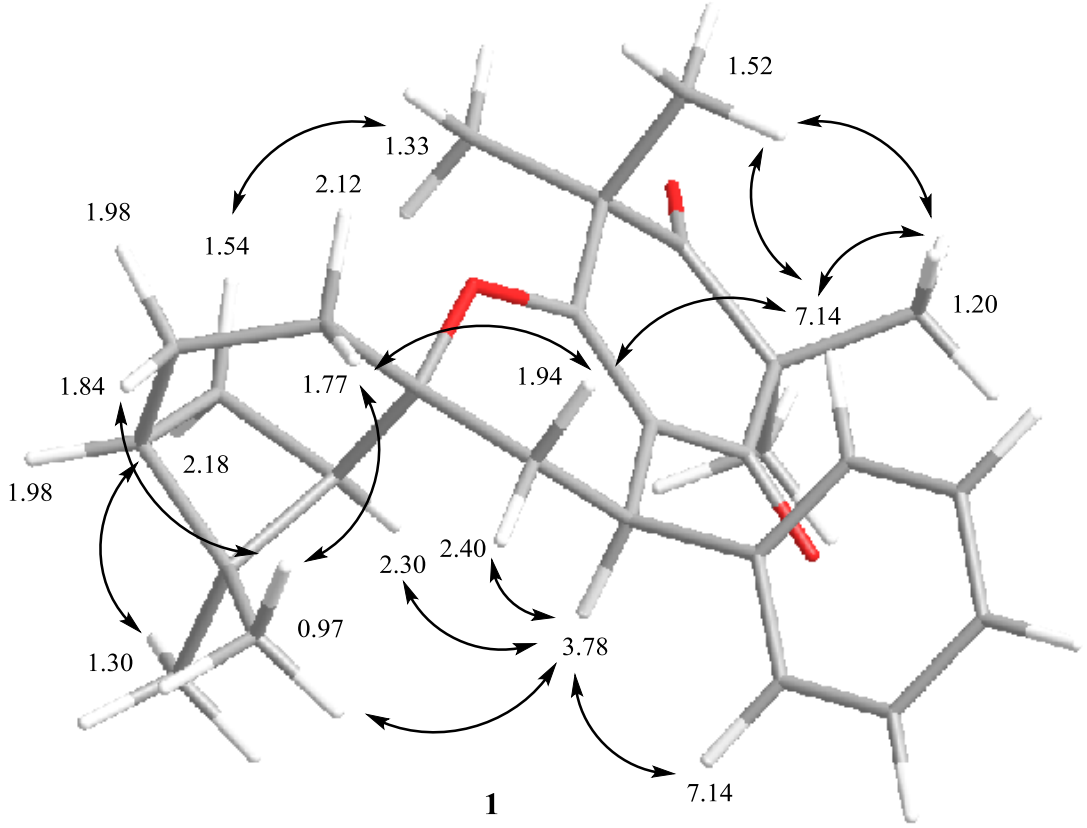


Figure 2. ROESY correlations observed for intermedione A (**1**)

Intermedione B (**2**) was obtained as a colourless amorphous solid (1.8 mg). A molecular formula, $C_{27}H_{34}O_3$, was assigned to **2** based upon analysis of HRESIMS data for the MH^+ ion at *m/z* 407.2608 (calculated 407.2581) and this suggested that **2** was an isomer of **1**. The IR and UV data obtained for **2** closely resembled that of **1**. The ^{13}C NMR data (Table 2) for **2** and **1** were almost identical except for the resonances assigned to C-6, C-9 and C-10, which were shifted from δ_C 36.1 to 34.9, 46.6 to 51.8 and 26.2 to 27.0 respectively, indicating a different configuration at one or more of the stereogenic centers C-6, C-8, C-9, and C-11. In the ^1H NMR spectrum of **2** (Table 1), the change of proton chemical shifts for H-6 and H-9 from δ_H 3.78 to 3.89 and δ_H 2.30 to 2.05 respectively were the most significant differences between the two spectra. Detailed analysis of ^1H NMR, HSQC, ^1H-^1H COSY and HMBC data established the chemical structure of **2** indicating that it possessed the same ring skeleton as **1**. The relative configuration of the stereogenic centers in **2** was assigned from the analysis of ROESY correlations and coupling constants (Figure 3). The proton δ_H 3.89 (H-6) showed a large coupling (10.3 Hz) to δ_H 1.78 (H-7b) and a smaller (6.5 Hz) coupling to δ_H 2.26 (H-7a). This implied that H-6 and H-7b are diaxial. ROESY correlations between δ_H 3.89 (H-6) and δ_H 2.00 (H-13a) and between δ_H 2.26 (H-7a) and δ_H 2.05 (H-9) indicated that H-6 and C-13 were on the β face of the molecule. ROESY correlations between H_3-16 and H-9, H-10b and H-11 indicated that these protons were on the same face of the cyclobutyl group. A ROESY correlation between H-7b and H_3-15 indicated that the gem-dimethyl group was situated on the same side as C-7. These observations suggested that **2** was the C-6 epimer of **1** and thus the relative configuration of **2** was established to be 6*S**, 8*R**, 9*R** and 11*S**. The equatorial position of the phenyl group in both **1** and **2** results from the pinene substructure puckering below the plain of dihydropyran ring in **1** while it puckers above the dihydropyran ring in **2**.

Intermedione C (**3)** was obtained as a mixture with **2 (**80% of **2** and 20% of **3)** and assigned a molecular formula $C_{27}H_{34}O_3$ since no other protonated molecule was observed in the HRESIMS for this mixture. The ^{13}C NMR data for **3** were almost superimposed with that of **2**, indicating that **3** was an isomer of **2**. ^1H-^1H COSY and HMBC correlations for **3** were similar to those observed for **2**. Proton and carbon resonances of **3** were assigned based upon comprehensive analysis of 1D and 2D NMR data (Tables 1 and 2).

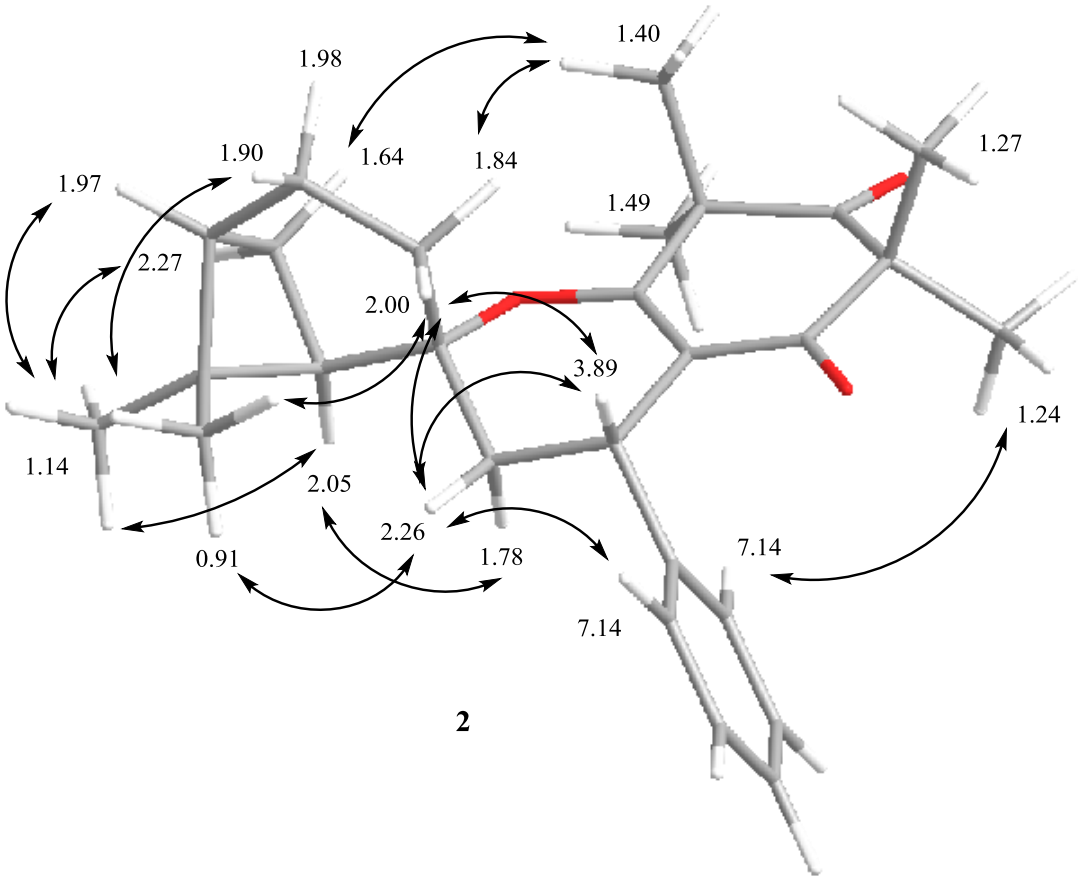


Figure 3: ROESY Correlations observed for intermedione B (**2**)

The relative configuration of the four stereogenic centers in **3** were defined in the same manner as that of **1** and **2** using phase sensitive ROESY data and coupling constants (Figure 4). The proton δ_H 3.86 (H-6) had a large diaxial coupling (11.9 Hz) to δ_H 1.56 (H-7b) and a smaller (6.3 Hz) axial-equatorial coupling to δ_H 2.28 (H-7a). ROESY correlations from δ_H 3.86 (H-6) to δ_H 2.06 (H-13a) and δ_H 2.28 (H-7a) indicated that they are all on the α face. A ROESY correlation between δ_H 1.52 (H-7b) and δ_H 2.04 (H-9) indicated that the cyclobutyl moiety was on the β face of the molecule. The methylene proton H-10a (δ_H 2.30) had a ROESY correlation to δ_H 1.28 (H_3-15) indicating that they were both on the same face of the cyclobutyl moiety. The methylene proton H-10b (δ_H 1.11) also showed a ROESY correlation to H-13a. The methyl protons H_3-16 correlated to both H_3-19 and H_3-20. This data indicated that the gem-dimethyl group attached to the cyclobutyl moiety was on the same side as *O*-1. This combined data indicated that **3** was the C-8 epimer of **1** and thus had a relative configuration of 6*R**, 8*S**, 9*R**, 11*S**.

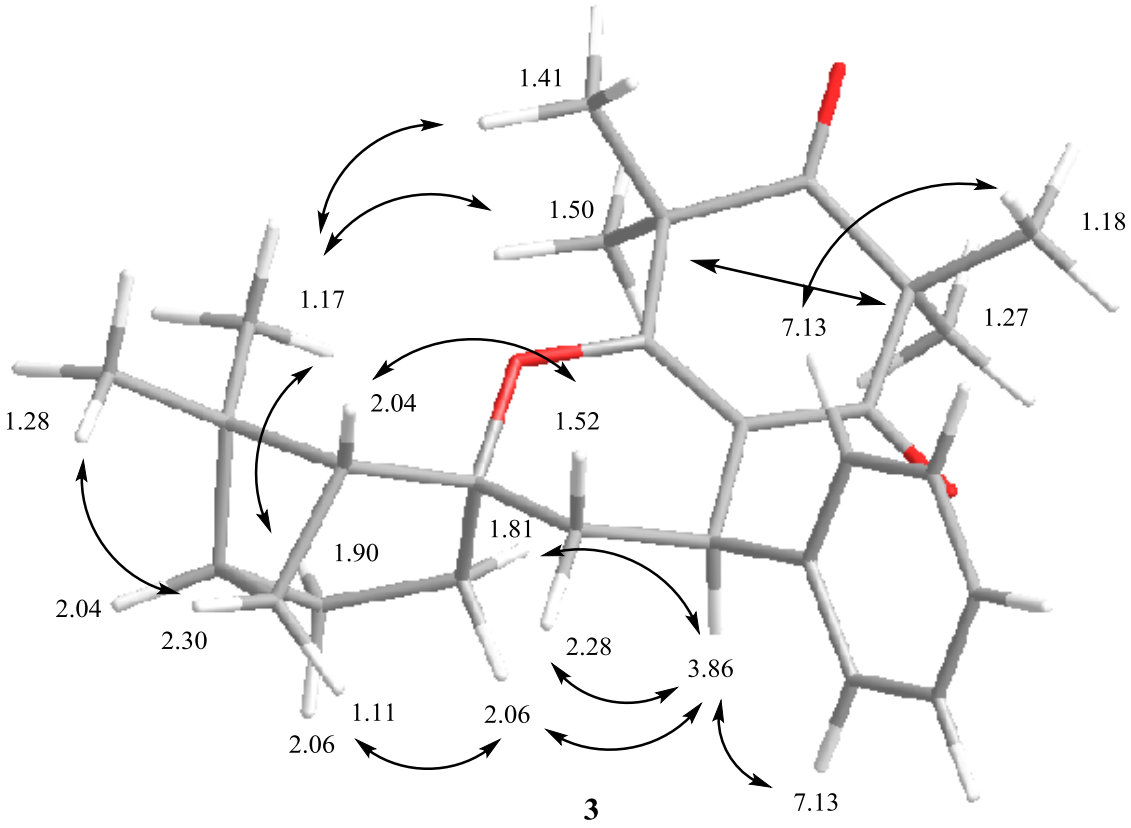


Figure 4: ROESY Correlations observed for intermedione C (**3**)

Intermedione D (**4**) was obtained as a pale yellow amorphous solid (1.3 mg). A molecular formula, $C_{27}H_{34}O_6$, was assigned to **4** based upon analysis of a (+) HRESIMS ion peak at *m/z* 437.2338 (calculated *m/z* 437.2323) which was attributed to dehydrated protonated molecule. This indicated that **4** contained three additional oxygen atoms compared to **1** and **2**. Much of the ^{1}H NMR and ^{13}C NMR data (Tables 1 and 2) obtained for **4** was similar to that of **1,** however, key differences were observed. In particular, the ^{1}H NMR spectrum (Table 1) of **4** exhibited a sharp downfield singlet at δ_H 10.84 and a doublet at δ_H 5.67, replacing signals associated with one of the methylene groups in **1**. The sharp singlet at δ_H 10.84 showed no HSQC correlation and its downfield chemical shift suggested that it could be assigned to a hydrogen bonded hydroxyl group.[10] In the ^{13}C NMR spectrum (Table 2), the carbon chemical shifts for C-1a, C-2, C-4, and C-5 were shifted downfield from 171.6, 48.5, 55.3 and 197.2 in **1** to 205.3, 60.6, 57.8 and 208.4 in **4** while C-3, C-5a were shifted upfield from δ_C 213.7, 110.6 in **1** to 206.2, 92.6 in **4**. These upfield and

downfield shifts for carbons in the cyclohexadione ring could be attributed to the electron delocalization within the β-triketone moiety brought about by opening the pyran ring in **1** to generate a cyclohexa-1,3,5-trione moiety. HMBC correlations (Figure 5) from δ_H 4.30 (H-6) to the ketone carbon at δ_C 205.3 (C-1) and the phenyl carbons at 137.2 (C-1′) and 130.0 (C-2′/C-6′) confirmed delocalization within the β-triketone moiety brought about by opening the pyran ring in **1** to generate a cyclohexa-1,3,5-trione moiety. HMBC correlations (Figure 5) from δ_H 4.30 (H-6) to the ketone carbon at δ_C 205.3 (C-1) and the phenyl carbons at 137.2 (C-1′) and 130.0 (C-2′/C-6′)

Table 1. ^{1}H NMR data for compounds **1-4** ($CDCl_3$, 800MHz)

Position	**1**	**2**	**3**	**4**	**5**
	$\delta_{H,\ mult.}$ (*J* in Hz)	$\delta_{H,\ mult.}$ (*J* in Hz)	$\delta_{H,\ mult.}$ (*J* in Hz)	$\delta_{H,\ mult.}$ (J in Hz)	$\delta_{H,\ mult.}$ (*J* in Hz)
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	
5	-	-	-	-	-
5a	-	-	-	-	-
6	3.78, dd, (11.4, 6.5)	3.89, dd, (10.2, 6.5)	3.86, dd, (11.9, 6.3)	4.30, dd, (8.9, 7.4)	3.99 (s)
7a	2.40, dd, (14.5, 6.5)	2.26, dd, (14.1, 6.5)	2.28, dd, (6.3, 13.2)	2.74, dd, (13.4, 8.9)	2.85, dd, (9.7, 9.7)
7b	1.94, dd, (14.5, 11.4)	1.78, dd, (14.1, 10.2)	1.52, dd, (13.2, 11.9)	2.72, dd, (13.4, 7.4)	-
8	-	-	-	-	-
9	2.30, dd, (6.0, 4.8)	2.05, dd, (5.4, 5.4)	2.04, m	2.27, overlaps with H-10a	2.07,dd, (5.7, 5.7)
10a	2.18, ddd (10.2, 6.0, 2.0)	2.27, m	2.30, m	2.27, overlaps with H-9	2.17, dddd, (10.2, 5.9, 5.7, 2.3)
10b	1.54, d, (10.2)	1.63, d, (10.6)	1.11, d, (9.9)	2.20, m	0.92, d (10.2)
11	1.98, m	1.97, m	2.04, m	1.97, dd, (6.0, 6.2)	1.94, ddd, (5.9, 5.7, 3.7)
12a	1.98, m	1.98, m	2.06, m	2.16, ddd, (15.6, 6.6, 2.3)	2.47, ddd, (13.3, 9.7, 3.7, 2.3)
12b	1.84, dddd, (14.7, 10.7, 4.2, 3.3)	1.90, m	1.90, m	2.03, dd, (15.6, 6.2)	1.49, m
13a	2.12, ddd, (16.2, 11.5, 4.2)	2.00, m	2.06, m	5.67, d, (6.6)	1.01, s
13b	1.77, ddd, (16.2, 10.7, 3.7)	1.84, m	1.81, m	-	-
14	-	-	-	-	-
15-Me	0.97, s	0.91, s	1.28, s	1.03, s	1.03, s
16-Me	1.30, s	1.14, s	1.17, s	1.31, s	1.27, s
17-Me	1.25, s	1.27, s	1.26, s	1.33, s	1.34, s
18-Me	1.20, s	1.24, s	1.18, s	1.40, s	1.42, s
19-Me	1.33, s	1.40, s	1.41, s	1.10, s	1.45, s
20-Me	1.52, s	1.49, s	1.50, s	0.49, s	1.57, s
1'	-	-	-	-	-
2'/6'*	7.14, dd, (7.5, 1.5)	7.14, dd, (7.0, 1.3)	7.13, m	7.40, dd, (8.3, 1.4)	7.13, d, (7.8 Hz)
3'/5'*	7.25, t, (7.5)	7.25, t, (7.5)	7.25, m	7.21-7.28 (m)	7.25, t, (7.5 Hz)
4'*	7.16, t, (7.0)	7.16, t, (7.0)	7.16, m	7.21-7.28 (m)	7.16 t, (7.3 Hz)
13-OH	-	-	-	10.84, s	-

* These signals showed second order couplings. The reported multiplicities and couplings are approximations.

confirmed that a benzyl group was directly bonded to C-5a of the syncarpic acid and COSY correlations from H-6 to δ_H 2.72 and 2.74 (H-7b and H-7a) indicated that H-6 was also vicinal to a methylene group. However, the two protons attached to C-7 resonated at almost identical chemical shifts, δ_H 2.72 and 2.74, and showed 7.4 and 8.9 Hz couplings to H-6 respectively and this was quite different to the couplings and chemical shifts observed for the same protons in **1**. Both H-6 and H_2-7 showed HMBC correlations to quaternary carbons at δ_C 92.6 (C-5a) and 114.9 (C-8) and this suggested that the syncarpic acid moiety contained an oxygen at C-5a and that C-8 was a ketal carbon. The chemical shift of C-5a was ~ 8 ppm further downfield than that observed in another compound containing 2-alkyl-2-hydroxycyclohexan-1,2-dione, gallomyrtucommulone B, suggesting that the oxygen attached to C-5a was likely to be an ether.[11] COSY correlations between δ_H 5.67 (H-13) and the methylene proton δ_H 2.16 (H-12a) on δ_C 31.3 (C-12), from δ_H 2.16 and 2.03 (H-12a and H-12b) to the methine proton δ_H 1.97 (H-11) attached to a carbon at δ_C

Table 2. ^{13}C NMR data for Intermdiones A - E (**1-5)** ($CDCl_3$, 200MHz)

	1	**2**	**3**	**4**	**5**
Position	δ_C	δ_C	δ_C	δ_C	δ_C
1	-	-	-	205.3	-
1a	171.6	170.8	170.7	-	173.8
2	48.5	48.4	48.4	60.6	48.3
3	213.7	213.7	213.7	208.4	213.3
4	55.3	55.5	55.0	57.8	55.7
5	197.2	197.0	197.2	206.2	197.1
5a	110.6	111.3	111.8	92.6	106.8
6	36.1	34.9	35.0	50.0	39.5
7	43.3	44.4	44.4	48.8	41.1
8	85.8	85.2	85.4	114.9	87.1
9	46.6	51.8	52.3	52.6	56.0
10	26.2	27.0	27.4	24.5	28.0
11	40.3	40.5	40.7	40.8	40.0
12	24.7	25.1	24.1	31.3	37.3
13	30.1	27.9	27.7	105.3	28.5
14	38.5	38.3	38.6	38.9	37.7
15-Me	23.3	23.4	27.5	20.3	22.2
16-Me	27.7	27.4	25.5	30.0	27.2
17-Me	22.7	22.4	23.0	25.2	26.5
18-Me	25.8	26.1	24.6	20.9	22.7
19-Me	26.2	25.5	26.6	18.6	23.2
20-Me	24.7	25.1	24.2	20.6	26.2
1'	144.8	144.8	144.8	137.2	144.0
2'/6'	126.3	127.1	126.9	130.0	127.7
3'/5'	128.6	128.5	128.5	128.6	128.3
4'	126.9	126.2	126.3	128.0	127.3

40.8 (C-11), from δ_H 1.97 (H-11) to the methylene protons δ_H 2.27 and δ_H 2.20 (H-10a and H-10b) attached to a carbon at δ_C 24.5 (C-10) and from δ_H 2.27 and 2.20 (H-10a and H-10b) to δ_H 2.27 (H-9) attached to the carbon at δ_C 52.6 indicated that **4** contained a $CHCH_2CHCH_2CH$ spin system. HMBC correlations from the methyl protons δ_H 1.03 (H_3-15) and δ_H 1.31 (H_3-16) to 38.9 (C-14), 40.8 (C-11), 52.6 (C-9) and δ_H 2.27 (H-9) to δ_C 40.8 (C-11) and δ_C 52.6 (C-9) established the presence of a dimethyl cyclobutyl moiety in **4**. A HSQC correlation from δ_H 5.67 (d, J = 6.6 Hz) to a carbon at δ_C 105.3 and a $^1J_{CH}$ = 178 Hz suggested that C-13 was doubly oxygenated.[12, 13] HMBC correlations from δ_H 5.67 (H-13) and 2.27 (H-9) to δ_C 114.9 (C-8) confirmed that an oxygen bridge linked C-8 and C-13. The hydroxyl group was attached to C-13 based on the observation of a ROESY correlation from δ_H 10.84 (13-OH) to δ_H 5.67 (H-13). Finally, an ether linkage between C-5a and C-8 provided the final degree of unsaturation required to fulfill the molecular formula. With the assignment of a tetrahydrofuran ring encompasing C-5a - C-6 - C-7 - C-8, the magnitude of the observed ^{1}H coupling between H-6 and H-7a and H-7b can be rationalized. Five membered rings such as cyclopentanes and tetrahydrofurans have been reported to possess *cis* and *trans* couplings between vicinal protons of almost equal magnitude. This is in contrast to the small equatorial/axial and large axial/axial couplings observed in the chair conformation of cylcohexane systems. The couplings observed between H-6 and H-7a (8.9 Hz) and H-7b (7.4 Hz) are typical for H-3/H-4 *cis* and *trans* couplings in tetrahydrofurans respectively.[14]

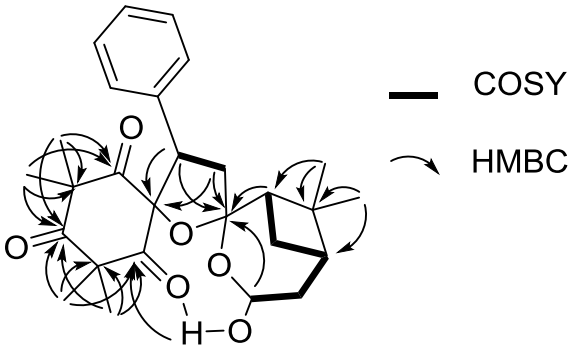


Figure 5: Key COSY and HMBC correlations observed for intermedione D (**4**)

The planar tetracyclic structure of **4** is novel, however, cyclic spiro hemiacetal-ketal structures are common motifs in natural products. Spiro pyranopyrans are commonly encountered in plants, dinoflagelates and actinomycetes[15-19] while furanofurans incorporating acetal/ketal linkages have been reported from sponges.[20] Previously diterpenoid compounds, **7**, aplyroseols-2, 3 and 5 (**8**, **9**, **10**), dendrillol-1 (**11**) and dendrillol-2 (**12**) with acetal/hemiacetal functionality have been isolated from the Australian nudibracnch *Chromodoris reticulate* and New Zealand sponge *Daminella oxeata.* [21-23] (Figure 6). A comparison of the NMR data reported with these molecules provides good agreement with the data we have obtained.

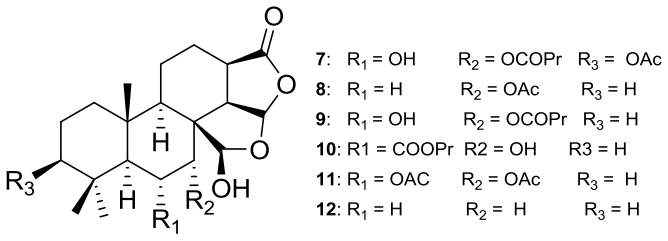


Figure 6: Diterpenoid compounds with acetal hemiacetal functionality

The relative configurations of the six stereogenic centers in **4** were assigned based on the analysis of ROESY correlations (Figure 7). There were ROESY correlations from δ_H 1.03 (H-15) to 5.67 (H-13), δ_H 2.16 (H-12a) and 2.74 (H-7a) indicating that all of these protons were situated on the same face of the cyclobutane ring. ROESY correlations from the methyl protons H-16 to δ_H 2.27 (H-9) and δ_H 2.27 (H-10a) indicated that these protons were all situated on the top face of the cyclobutane ring. A large coupling (8.9 Hz) between H-6 and δ_H 2.72 (H-7b) indicated that these protons were trans to each other. By comparison with the coupling constants of the known compounds with tetrahydrofuran (THF) rings, it was confirmed that this kind of coupling within furan ring is possible.[13, 24, 25] ROESY correlations were observed between H-6, H-9 and the β-triketone methyl protons δ_H 0.49 (H-20) indicating that they were all on the β face of the molecule. H-10a also showed a weak ROESY correlation to the methyl protons, H_3-20 providing fur-

ther evidence that H-10a was situated on the side of the cyclobutyl adjacent to the syncarpic acid moiety. ROESY correlations between δ_H 10.84 (13-OH) and H-10a and H-13. Therefore, **4**

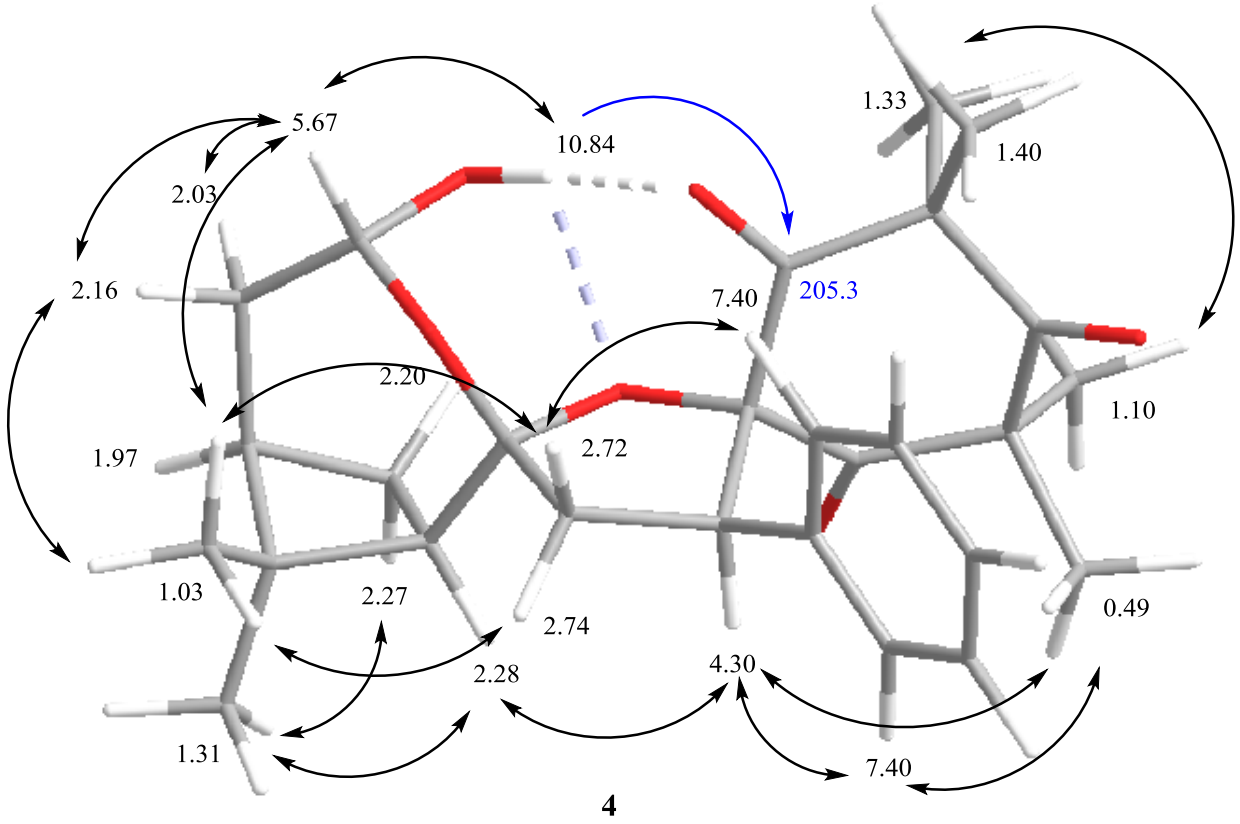


Figure 7: ROESY correlations observed for intermedione D (**4**)

possesses the same relative configuration at C-6, H-9, and C-11 as **1** and a total relative configuration of 6*R**, 8*S**, 9*R**, 11*S** and 13*R**. With the relative configuration and 3D conformation for **4** determined, the unusual downfield chemical shift and sharpness of the hemiacetal hydroxyl proton required some explanation. Molecular modelling was performed using Macromodel with conformational searching using a mixed MCMM/low-mode search and this generated the lowest energy conformation in which the hemiacetal hydroxyl proton shared hydrogen bonds with the C-1 ketone oxygen and the furan oxygen. This hydrogen bonding network led to a 90° angle between H-13 and 13-OH suggesting that no $^1H/^1H$ coupling would be observed between these protons (this was what was observed experimentally). Furthermore on close inspection of the

HMBC spectrum, a weak correlation was observed between 13-OH and C-1. In the β-triketone, watsonianone C, isolated from *Corymbia watsoniana,* a similar phenomenon has been observed where a strongly hydrogen bonded hydroxyl proton showed HMBC correlation to both carbonyl carbons participating in the hydrogen bond.[5] This hydrogen bonding would account for the significant downfield shift of 13-OH since it is sharing its electron density with three oxygens. Furthermore the subtle upfield shift of the carbonyl carbon C-1 (δ_C 205.3) compared to C-5 (δ_C 206.2) can also be rationalized by this hydrogen bonding since C-1 should have a higher electron density as a result and thus resonate slightly upfield of that carbonyl carbon that does not show any hydrogen bonding.

Intermedione **E** (**5**) was obtained as a yellow amorphous solid. A molecular formula, $C_{27}H_{34}O_3$, was established for **5** from analysis of the MH^+ ion in the (+) HRESIMS *m/z* 407.2584 (calculated *m/z* 407.2581). The IR spectrum of **5** had an intense carbonyl stretching band at 1682 cm^{-1}. The ^1H NMR spectrum of **5** (Table 1) was very similar to that of **1-3**, however, seven methyl singlet resonances were observed (instead of the six seen in **1-4**) and this suggested that the conjugation of the β-triketone (**2a)** was with α-pinene instead of β-pinene (Scheme 1). The ^{13}C NMR resonances for **5** (Table 2) were also very similar to those observed in **1**. Analysis of HSQC correlations indicated that **5** contained two additional methines, one methyl, and two less methylene carbons compared to intermediones A-C. The HMBC, HSQC and COSY correlations established the gross chemical structure of **5**. In particular, the benzylic proton H-6 (δ_H 3.99) was a singlet orientated at a 90° angle to the methine proton at δ_H 2.85 (H-7) in **5** instead of a methylene in **1**. H-7 was mutually coupled to a pair of methylene protons at δ_H 1.49 and 2.47 (H-12b and H-12a). A dimethyl cyclobutyl moiety sharing almost identical ^1H and ^{13}C signals as those in **1** were shown to be attached to C-12 because COSY correlations were observed between the methylene protons H_2-12 and H-11. The methyl singlet at δ_H 1.01 (H_3-13) had HMBC correlations to C-7, an oxygenated quaternary carbon δ_C 87.1 (C-8) and the cyclobutyl methine carbon δ_C 56.0 (C-9) providing evidence to establish a cyclohexane ring. NMR signals for a syncarpic acid moiety in **5** were identical with those of **1** and an ether bond linking C-1a and C-8 provided the final structural requirement to fulfill the molecular formula derived from MS analysis. Analysis of ROESY correlations and coupling constants defined the relative configuration of the five

stereogenic centers in **5** (Figure 8). The proton at δ_H 2.85 (H-7) showed a large coupling (10.2 Hz) to δ_H 2.47 (H-12a) indicating that these protons were in a diaxial orientation. ROESY correlations observed from δ_H 2.47 (H-12a) to δ_H 1.03 (H_3-15) indicated that these protons were located on the β face. ROESY correlations from δ_H 2.07 (H-9) to δ_H 1.01 (H_3-13) and similarly from δ_H 1.01 (H_3-13) to the aromatic proton δ_H 7.13 (H-2′/6′) indicated that these protons are on the α face with the phenyl group situated below the plane of the pyran ring. ROESY correlations between the aromatic protons at δ_H 7.13 (H-2′/6′) and the methyl protons at δ_H 1.57 (H_3-20) indicated that H_3-20 was α orientated. . Intermedione E, therefore, has 6*R**,7*R**,8*S**,9*S**,11*R** relative configuration.

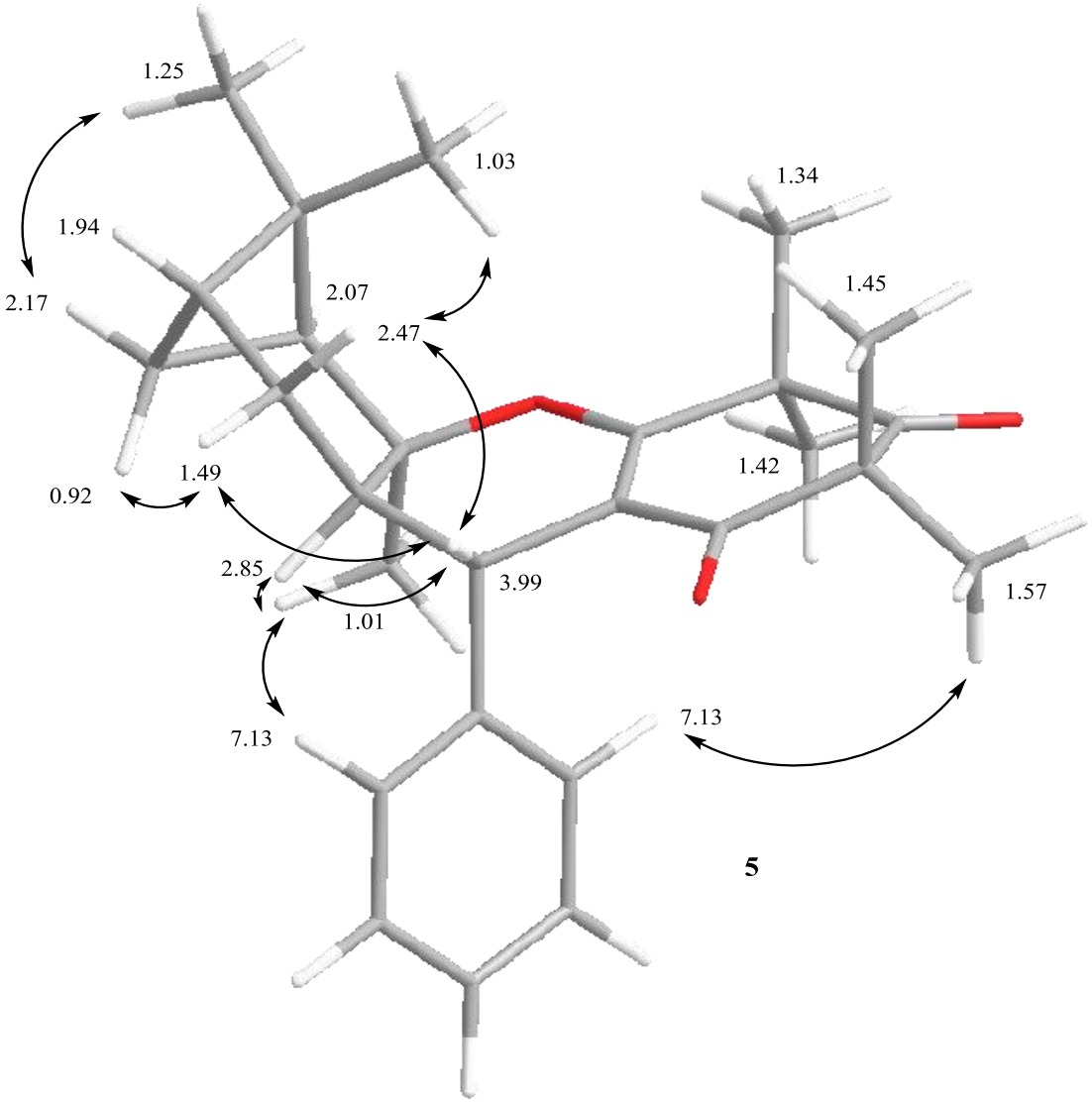


Figure 8. Some important ROESY correlations observed for intermedione E (**5**)

The first β-triketone terpene adduct isolated from the genus *Corymbia* was an insecticidal compound 4*S*-ficifolidione (**6**) from *Corymbia ficifolia* (previously named *Eucalyptus ficifolia*[26]). This compound incorporates a β-pinene monoterpene moiety conjugated to the simple β-triketone, leptospermone. The intermediones A-C (**1**-**3**) are therefore C-6 phenyl analogs of 4*S*-ficifolidione and are likely to be products generated through conjugation of β-pinene with a postulated β-triketone intermediate (**2a**). The proposed biogenetic pathway is shown in scheme **1**. Briefly, reduction of the side chain ketone of the β-triketone (**2a**) to an alcohol and then dehydration to produce the intermediate **3a** could then undergo either exo or endo Diels-Alder cycloaddi tions with β-pinene (**1a)** to generate compounds **1**, **2** and **3** with the different configuration at either C-6 and/or C-8. Similarly, Diels-Alder cycloaddition of (+)-α-pinene (**4a**) with **3a** could generate intermedione E. The structure of intermedione D is intriguing and its biogenesis is likely to result from oxidation of intermedione A (scheme 2). Firstly, oxidation of the pyran double bond could generate the expoxide (**i**) and subsequent hydrolysis would lead to the 2-hydroxy-cyclo-1,3,5-trione (**ii**). Oxidative ring opening of the bicycloheptane would then generate the highly oxygenated pentaketone (**iii**). Nucleophilic attack of the ketone vicinal to the cyclobutane by the hydroxy oxygen and subsequent attach of the aldehyde oxygen by the ketone oxygen would lead to the cyclic spiroketal/hemi acetal. The cyclobutyl stereogenic centres would remain conserved throughout this process.

4*S*-Ficifolidione (**6**) from *Corymbia ficifolia* its C-4 epimer 4*R*-ficifolidione reported from *Rhodomyrtus tomentosa*,[9] BF-2 from *Baeckea frutescens*[27]and callistiviminenes I and J from *Callistemon viminalis*[28] are the most closely related compounds to intermediones A-C since all are β-pinene adducts of syncarpic acid. However, in all of these compounds, the phenyl substituent attached at C-6 in the intermediones is replaced by either an isobutyl or isopropyl moiety.

Intermediones A-E were tested against the chloroquine-sensitive (3D7) strain of the malaria parasite *Plasmodium falciparum.* Intermedione A, the mixture of intermediones B and C and intermedione E were also tested against the chloroquine resistant (Dd2) strain of *P. falciparum*. The cytotoxicity of the compounds was also evaluated using the mammalian cell line HEK-293. Intermediones A, B, D, E and the mixture of intermediones B and C had IC_{50} values of 12.45 μM,

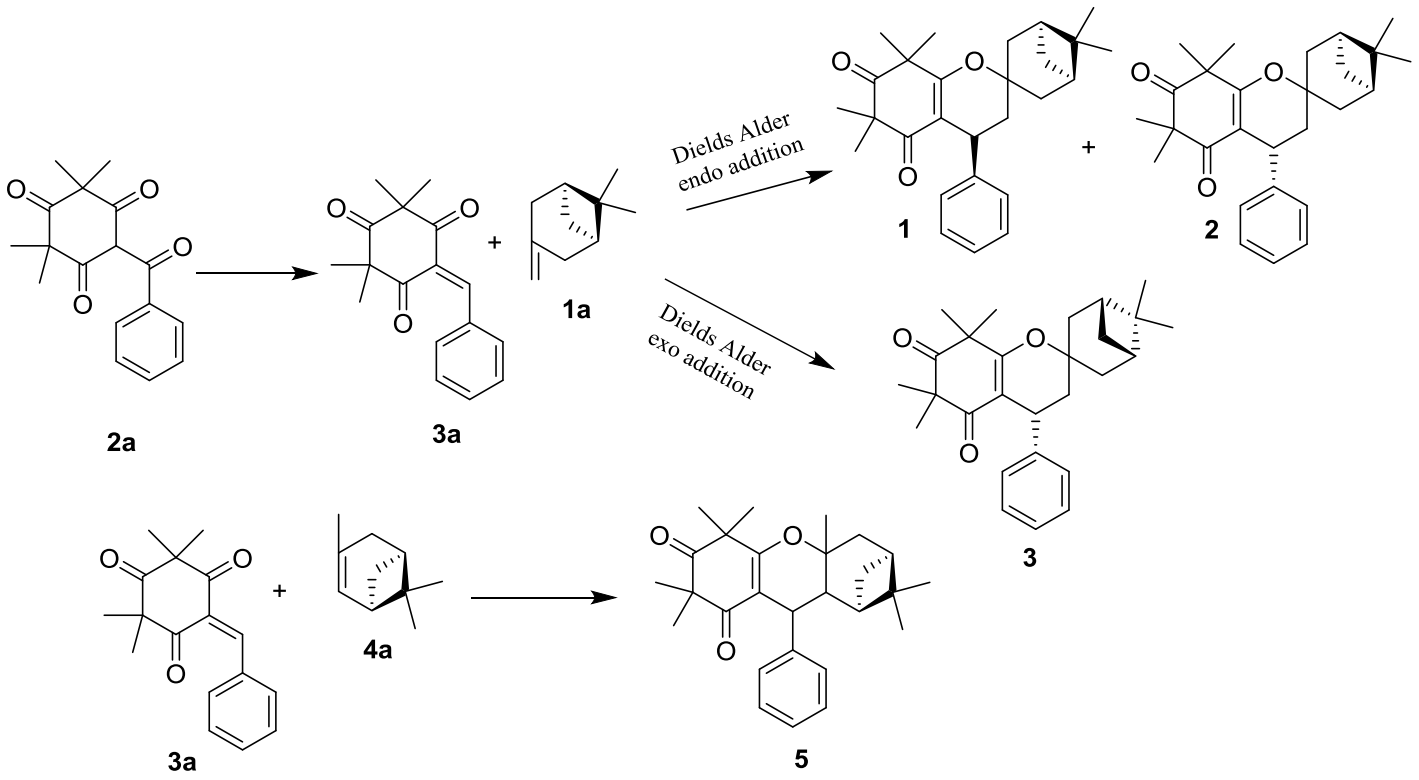
9.93 μM, 13.0 μM, 20.8 μM, 12.6 μM respectively against the 3D7 strain. The antiplasmodial activity of intermedione A, D, the mixture of B and C against Dd2 was 18.5 μM, 26.0 μM, and 15.25 μM respectively. Intermedione B and E were not tested against Dd2 strain due to the insufficient amount of material. In cell cytotoxicity testing intermediones D and E were inactive towards HEK-293 mammalian cell line at 100 μM. Intermedione A and the mixture of intermediones B and C inhibited HEK-293 cells by 64% and 85% at 100μM respectively. The change of configuration at C-6 in intermediones A and B had no significant effect on the antiplasmodial activity.

Intermediones A, B, and mixture of intermediones B and C were tested for antibacterial activity against *Staphylococcus aureus* ATCC 157293 but they were inactive at 0.5mM.

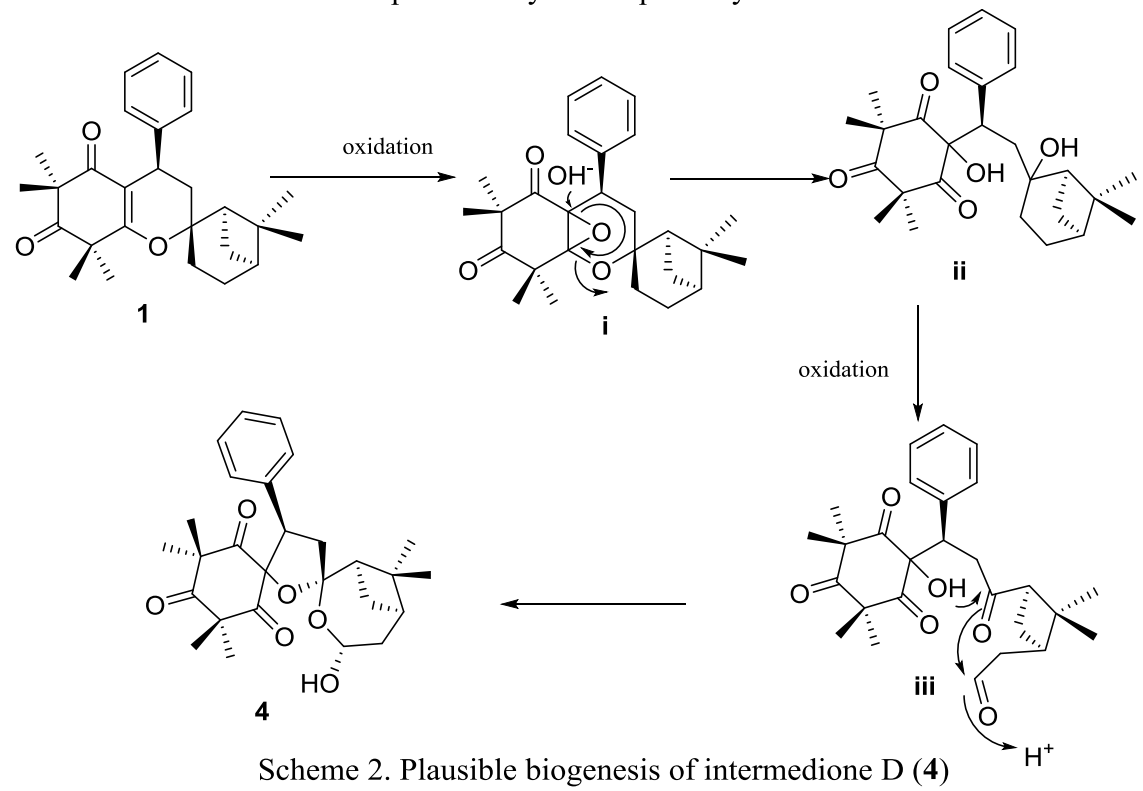
6.4 Experimental Section

6.4.1General Experimental Procedures

Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were obtained on Shimadzu UV-1800 UV spectrophotometer. IR data were obtained using Thermo Scientific Nicolet iS5 iD5 ATR spectrometer. NMR spectra were recorded on a Bruker BioSpin GmbH 800 MHz spectrometer at 25°C in $CDCl_3$ with solvent peaks referenced to δ_H 7.26 and δ_C 77.16. ESI-TOF data were recorded on an Agilent 6530-accurate mass Q-TOF LC/MS mass spectrometer. Altech Davisil 30-40 μm 60 Å C_{18} silica and diol-bonded silica 40-70 μm, 60 Å were used to adsorb the flower extract prior to HPLC separation. A Merck Hitachi L7100 pump equipped with a Merck Hitachi L7455 PDA detector and a Merck L7250 autosampler were used for HPLC. HPLC columns used were Betasil C_{18} 150 x 21.2 5μm 100 Å, Zorbax SB-Phenyl 21.2 mm i.d. x 25 cm and Diol YMC basic 150 x 20 mm i.d. 5 μm. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade and the H_2O was Millipore Milli-Q PF filtered.



Scheme 1. Proposed biosynthetic pathway for **1-3** and **5**



Scheme 2. Plausible biogenesis of intermedione D (**4**) from intermedione A (**1**)

6.4.2 Plant Material

Flowers of *C. intermedia* were collected from Clagiraba in south east Queensland. A voucher specimen, ACMYRT007 is deposited at the Griffith School of Environment, Griffith University, Gold Coast campus.

6.4.3 Extraction and Isolation

The air dried ground flowers of *C. intermedia* (87.8 g) were exhaustively extracted with MeOH to yield brown gum (5.5 g). The MeOH extract (5.5 g) was adsorbed onto the C_{18} silica gel (5.5 g) and a portion (1g) of it was filled into a refillable HPLC column (10 mm x 20 mm) and then connected in sequence to a C_{18} bonded silica HPLC column (21 mm x 150 mm) and eluted with a gradient from 100% H_2O to 100% MeOH over 60 minutes at a flow rate of 9 mL/min. The column was further eluted with MeOH for 10 minutes. Fractions were collected every minute. This procedure was repeated five times and every alternate fraction from the first HPLC run was analyzed by ^{1}H NMR. Based on NMR analysis, fractions 36 to 70 of each HPLC run were combined and evaporated to obtain a gum (1.7 g). An aliquot (1 g) of this sample was absorbed on to diol-bonded silica gel and filled into a refillable HPLC column (10 mm x 20 mm) then connected in sequence to a Diol bonded HPLC column (21 mm x 150 mm) and eluted with a gradient from hexane to dichloromethane over 60 minutes at a flow rate of 9 mL/min and then further eluted for 10 mins with 100% dichloromethane to yield 70 fractions. Based on the UV chromatogram data UV absorbing fractions were analyzed by NMR and fractions 12-14 (190 mg) and fractions 15-17 (54 mg) were recombined. Recombined fractions 12-14 (190 mg) were subjected to isocratic elution with 80% hexane/20% DCM on a Diol bonded HPLC column (21 mm x 150 mm) to yield intermedione A (**1**) (50 mg) and fractions 15-17 (54 mg) were further purified on a phenyl bonded silica gel HPLC column with a gradient from 40% H_2O/60% MeOH to 100% MeOH to obtain intermedione B (**2**) (1.8 mg), mixture of intermedione B (**2**) and C (**3**) (20 mg), intermedione D (**4**) (1.8 mg) and intermedione E (**5**) (1.9 mg). The mixture of **2** and **3** (20 mg) was further fractionated on a phenyl bonded silica gel HPLC column with a gradient from 40% H_2O/60% MeOH to 100% MeOH but this was ineffective at separating the mixture.

Intermedione A (1). Yellow gum (50 mg). $[\alpha]_D^{27}$ -28.8 (*c* 2.5, MeOH) UV λ_{max} (MeOH)/nm (log ε) 201 nm (4.02) FTIR ν_{max}: 3464, 2953, 2931, 2873, 1712, 1676, 1640, 1458, 1379, 1173, 1042 cm^{-1}. ^1H NMR (Table 1). ^{13}C NMR (Table 2). The HRESIMS (Positive mode) observed *m/z* 407.2608, $(M+H)^+$, (calculated for $C_{27}H_{35}O_3^+$ 407.2581).

Intermedione B (**2**). Colourless amorphous solid. (1.8 mg). $[\alpha]_D^{27}$ -3.6 (*c* 0.09, MeOH). UV (MeOH) λ_{max} (logε): 265 (3.7) nm .FTIR ν_{max}: 2976, 2922, 2870, 1712, 1654, 1612, 1463, 1381, 1352, 1168, 1055 cm^{-1}. ^1H NMR (Table 1). ^{13}C NMR (Table 2). The HRESIMS (Positive mode) observed *m/z* 407.2608, $(M+H)^+$, (calculated for $C_{27}H_{35}O_3^+$, 407.2581).

Intermedione C (**3**). Colourless amorphous solid. ^1H NMR (Table 1). ^{13}C NMR (Table 2). The HRESIMS (Positive mode) observed *m/z* 407.2605, $(M+H)^+$, (calculated for $C_{27}H_{35}O_3^+$, 407.2581).

Intermedione D (**4**). Colorless amorphous solid (1.9 mg). $[\alpha]_D^{27}$ +81 (*c* 0.095, MeOH). UV (MeOH) λ_{max} (logε): 265 (3.98) nm. FTIR ν_{max} 3738, 1707, 1514, 1462 cm^{-1}. ^1H NMR (Table 1). ^{13}C NMR (Table 2). The HRESIMS (Positive mode) observed *m/z* 437.2338, dehydrated protonated molecule, (calculated for $C_{27}H_{32}O_5^+$, 437.2323).

Intermedione E (**5**). Yellow color amorphous solid $[\alpha]_D^{27}$ -10 (*c* 0.095, MeOH). UV (MeOH) λ_{max} (logε): 203, 267 (6.30) nm. FTIR ν_{max} 3446 (br), 2926, 1682, 1445, 1394, 1209, 1139 cm^{-1}. ^1H NMR (Table 1). ^{13}C NMR (Table 2). The HRESIMS (positive mode) observed *m/z* 407.2584, $(M+H)^+$, (calculated for $C_{27}H_{35}O_3^+$, 407.2581).

6.4.4 Antimalarial Assay

Antimalarial activities of the compounds were evaluated according to the method described by Duffy and Avery.[29] Initially, the compounds (**1**-**5**) were incubated in the presence of 2 or 3% of

3D7 parasite and 0.3% hematocrit in a total assay volume of 50 µl, for 72 h at 37°C and 5% CO_2, in Poly-D-lysine coated Cell Carrier Imaging plates. Once the incubation was completed, plates were incubated with DAPI (4', 6-diamidino-2-phenylindole) in the presence of Saponin (from Quillaja Bark) and Triton X-100 and incubated for a further 5 h at room temperature in the dark before imaging on the OPERA^{TM} HTS confocal imaging system. The digital images gained were then investigated using the PerkinElmer Acapella spot detection software where spots which fulfill the criteria established for a stained parasite are counted. The % inhibition of parasite replication was calculated using DMSO and artemisinin control data.

6.4.5 Antibacterial Assay

The antibacterial activity of only the compounds **1**, **2** and the mixture of **2** and **3** were determined using a broth microdilution method against *S. aureus* ATCC 157293 bacterium. Compounds **4** and **5** were not tested for antibacterial activity due to insufficient quantity. The assay was conducted in 96 well-sterilized microtiter plate containing pure compounds at a concentration of 0.5 mM. Compounds were dissolved in 100% DMSO. To each well 25 µL of double strength broth was added and then 5 µL of compounds at 10mM of concentration was added to the well followed by 50 µL of inoculum and 20 µL of sterile water. Plates were incubated at 37°C while being shaken at 100 rpm, for 6 hours. After this period 10 µL of the resazurin stock solution (704 µmol) was added and the plate was incubated for a further hour. After the colour has been developed inhibition relative to resazurin was determined using a BMG Labtech FLUO star omega microplate fluorometer (excitation 570 nm; emission 620 nm). All the experiments were carried out in physical containment level 2 (PC2) laboratory facilities.

6.5 References and Notes

1. Beardsell, D.; Obrien, S.; Williams, E.; Knox, R.; Calder, D., *Aust. J. Bot.* **1993,** 41, 511-526.
2. Ghisalberti, E. L., *Phytochemistry* **1996,** 41, 7-22.
3. Pal Singh, I.; Bharate, S. B., *Nat. Prod. Rep.* **2006,** 23, 558-591.
4. Sidana, J.; Singh, S.; Arora, S. K.; Foley, W. J.; Singh, I. P., *Fitoterapia* **2011,** 82, 1118-1122.
5. Carroll, A. R.; Avery, V. M.; Duffy, S.; Forster, P. I.; Guymer, G. P., *Org. Biomol. Chem.* **2013,** 11, 453-458.
6. Carroll, A. R.; Urban, S.; Lamb, J.; Moni, R.; Guymer, G. P.; Forster, P. I.; Quinn, R. J., *J. Nat. Prod.* **2008,** 71, 881-883.
7. Hill, K. D.; Johnson, L. A., *Telopea* **1995,** 6, 185-504.
8. Carroll, A. R.; Lamb, J.; Moni, R.; Guymer, G. P.; Forster, P. I.; Quinn, R. J., *J. Nat. Prod.* **2008,** 71, 1564-1568.
9. Abraham, R. J.; Cooper, M. A.; Salmon, J. R.; Whittaker, D., *Org. Magn. Reson.* **1972,** 4, 489-507.
10. Siskos, M. G.; Tzakos, A. G.; Gerothanassis, I. P., *Org. Biomol. Chem.* **2015,** 13, 8852-8868.
11. Appendino, G.; Maxia, L.; Bettoni, P.; Locatelli, M.; Valdivia, C.; Ballero, M.; Stavri, M.; Gibbons, S.; Sterner, O., *J. Nat. Prod.* **2006,** 69, 251-254.
12. Tvaroska, I.; Taravel, F. R., *Adv. Carbohydr. Chem. Biochem.* **1995,** 51, 15-61.
13. Hwang, I. H.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T., *J. Nat. Prod.* **2016,** 79, 523-530.
14. Wu, A.; Cremer, D., *Int. J. Mol. Sci.* **2003,** 4, 158-192.
15. Chang, F.-R.; Yen, C.-T.; El-Shazly, M.; Yu, C.-Y.; Yen, M.-H.; Cheng, Y.-B.; Chen, S.-L.; Wu, Y.-C., *Bioorg. Med. Chem. Lett.* **2013,** 23, 2738-2742.
16. Wang, D.-Z., *Mar. Drugs* **2008,** 6, 349-371.
17. Holtzel, A.; Kempter, C.; Metzger, J. W.; Jung, G.; Groth, I.; Fritz, T.; Fiedler, H.-P., *J. Antibiot.* **1998,** 51, 699-707.

18. Blunt, J. W.; Copp, B. R.; Munro, M. H.; Northcote, P. T.; Prinsep, M. R., *Nat. Prod. Rep.* **2006**.
19. Haynes, R. K.; Chan, H.-W.; Lung, C.-M.; Ng, N.-C.; Wong, H.-N.; Shek, L. Y.; Williams, I. D.; Cartwright, A.; Gomes, M. F., *Chem. Med. Chem.* **2007,** 2, 1448-1463.
20. Proksch, P., *Toxicon* **1994,** 32, 639-655.
21. Karuso, P.; Bergquist, P.; Cambie, R.; Buckleton, J.; Clark, G.; Rickard, C., *Aust. J. Chem.* **1986,** 39, 1643-1653.
22. Suciati; Lambert, L. K.; Garson, M. J., *Aust. J. Chem.* **2011,** 64, 757-765.
23. Marchionni, C.; Vogel, P., *Helv. Chim. Acta.* **2001,** 84, 431-472.
24. Jiang, Z.; Chen, R.-Y.; Chen, Y.; Yu, D.-Q., *J. Nat. Prod.* **1998,** 61, 86-88.
25. Řezanka, T.; Hanuš, L.; Dembitsky, Valery M., *Eur. J. Org. Chem.* **2003,** 2003, 4073-4079.
26. Khambay, B. P. S.; Beddie, D. G.; Hooper, A. M.; Simmonds, M. S. J., *Tetrahedron* **2003,** 59, 7131-7133.
27. Makino, M.; Fujimoto, Y., *Phytochemistry* **1999,** 50, 273-277.
28. Wu, L.; Wang, X. B.; Li, R. J.; Zhang, Y. L.; Yang, M. H.; Luo, J.; Kong, L. Y., *Phytochemistry* **2016,** 131, 140-149.
29. Duffy, S.; Avery, V. M., *Am. J. Trop. Med. Hyg.* **2012,** 86, 84-92.

Five new antiplasmodial β-triketones from the flowers of Australian tree *Corymbia intermedia*

Sarath P. D. Senadeera,[a,b] Sandra Duffy,[b] Joshua Hayton,[a] Vicky M. Avery,[b] Anthony R. Carroll,*[,a,b]

[a] Environmental Future Research Institute, Griffith University, Gold Coast, QLD 4222, Australia.

[b] Eskitis Intitute, Griffith University, Brisbane, QLD 4111, Australia.

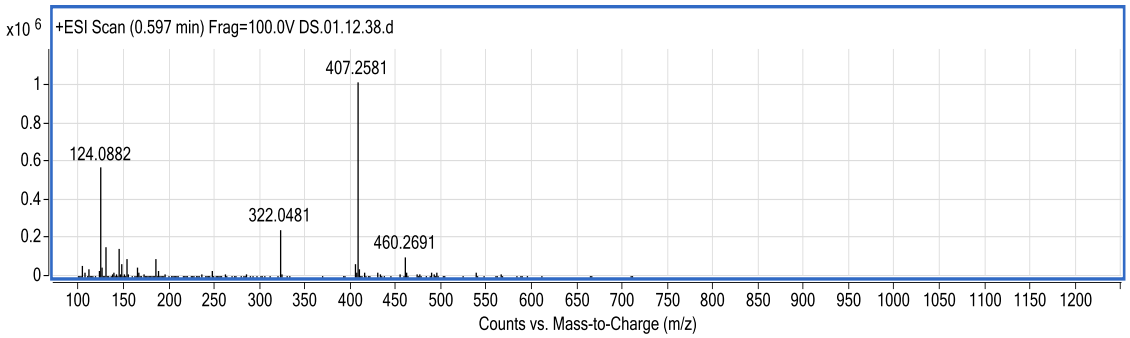


Figure S 1. Mass Spectrum of intermedione A

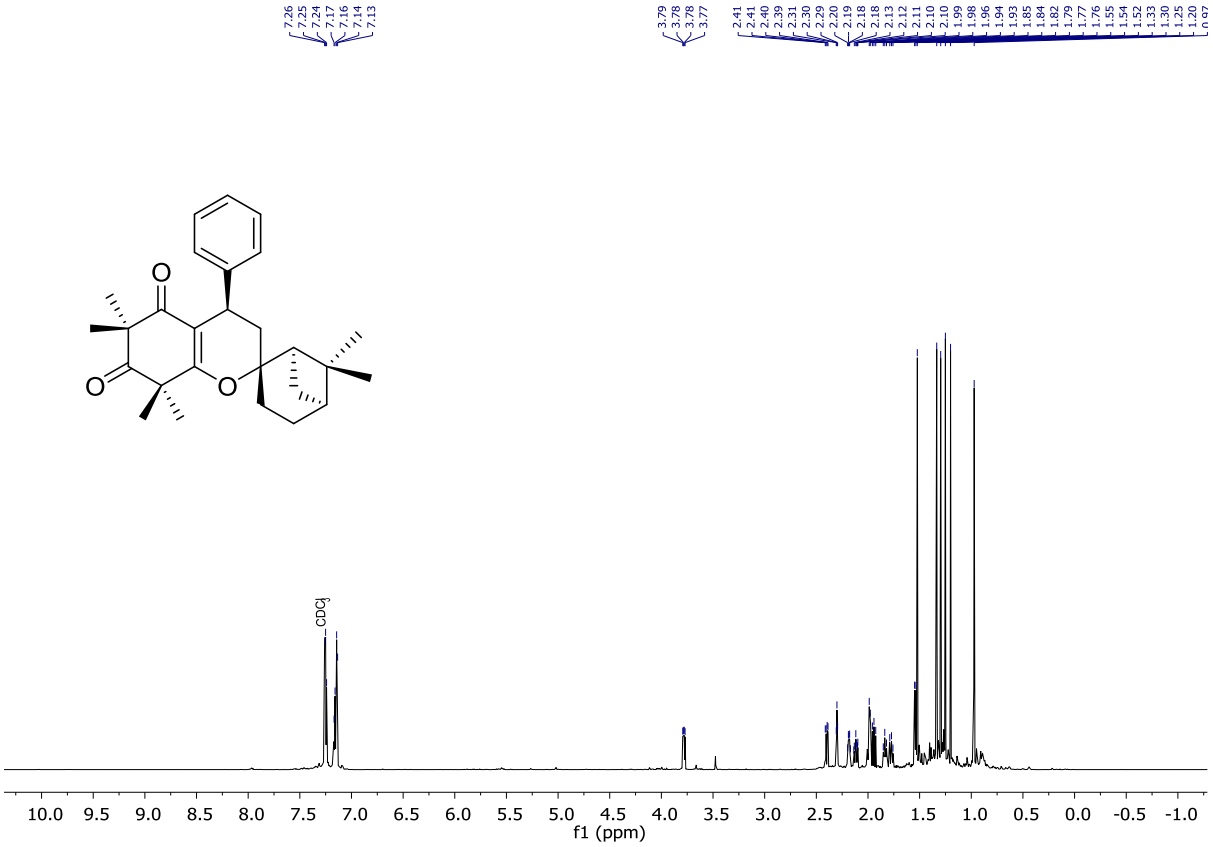


Figure S 2.^{1}H NMR spectrum of intermedione A in $CDCl_3$ (800 MHz)

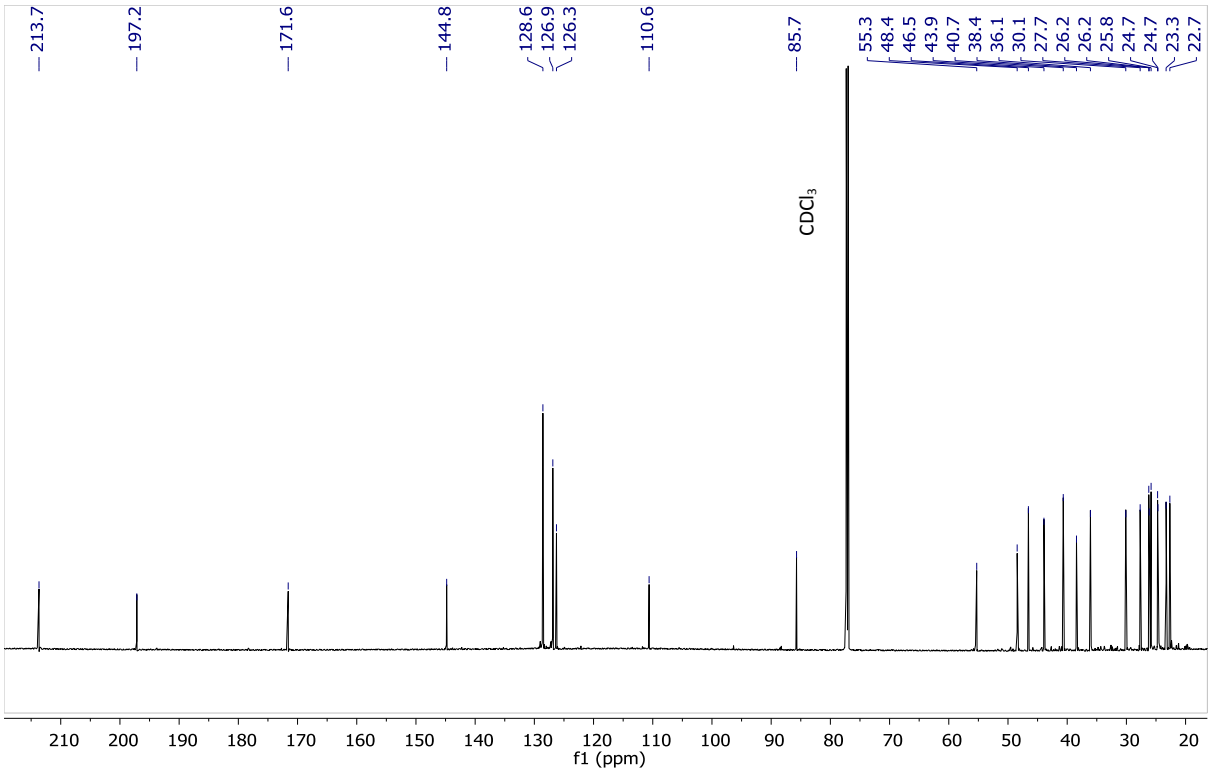


Figure S 3. ^{13}C NMR of intermedione A in $CDCl_3$ (800 MHz)

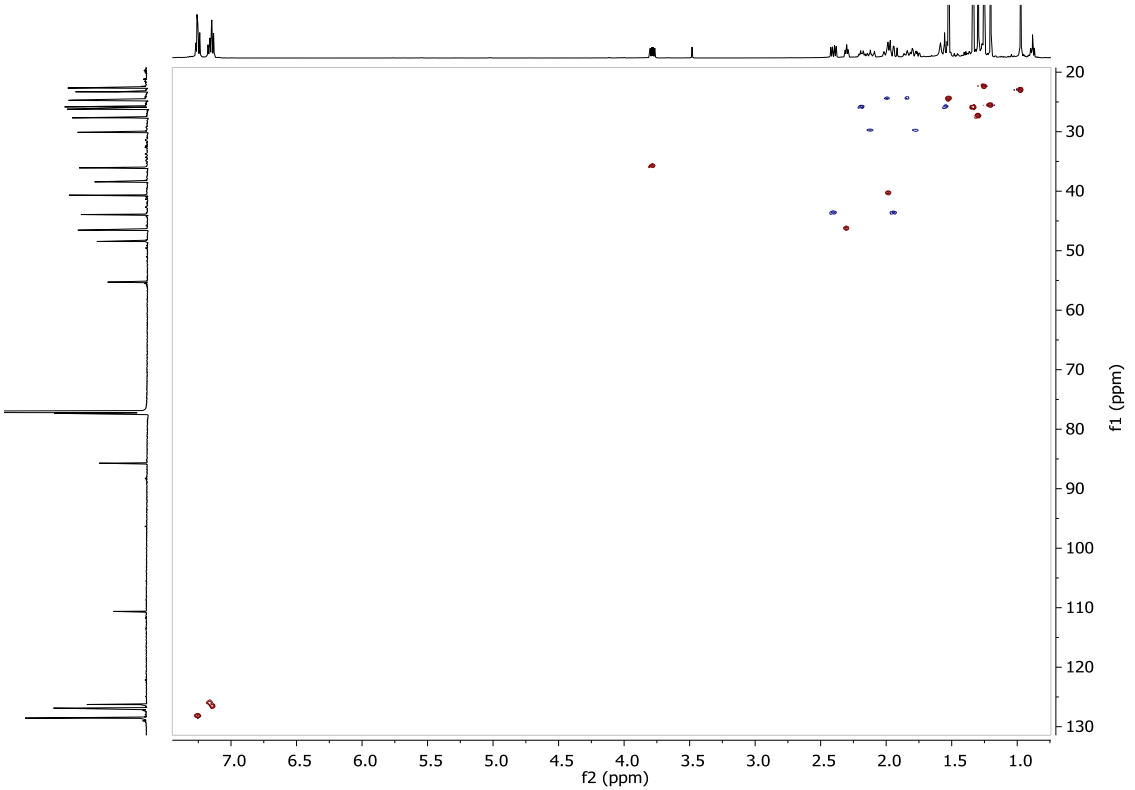


Figure S 4. HSQC spectrum of intermedione A in $CDCl_3$ (800 MHz)

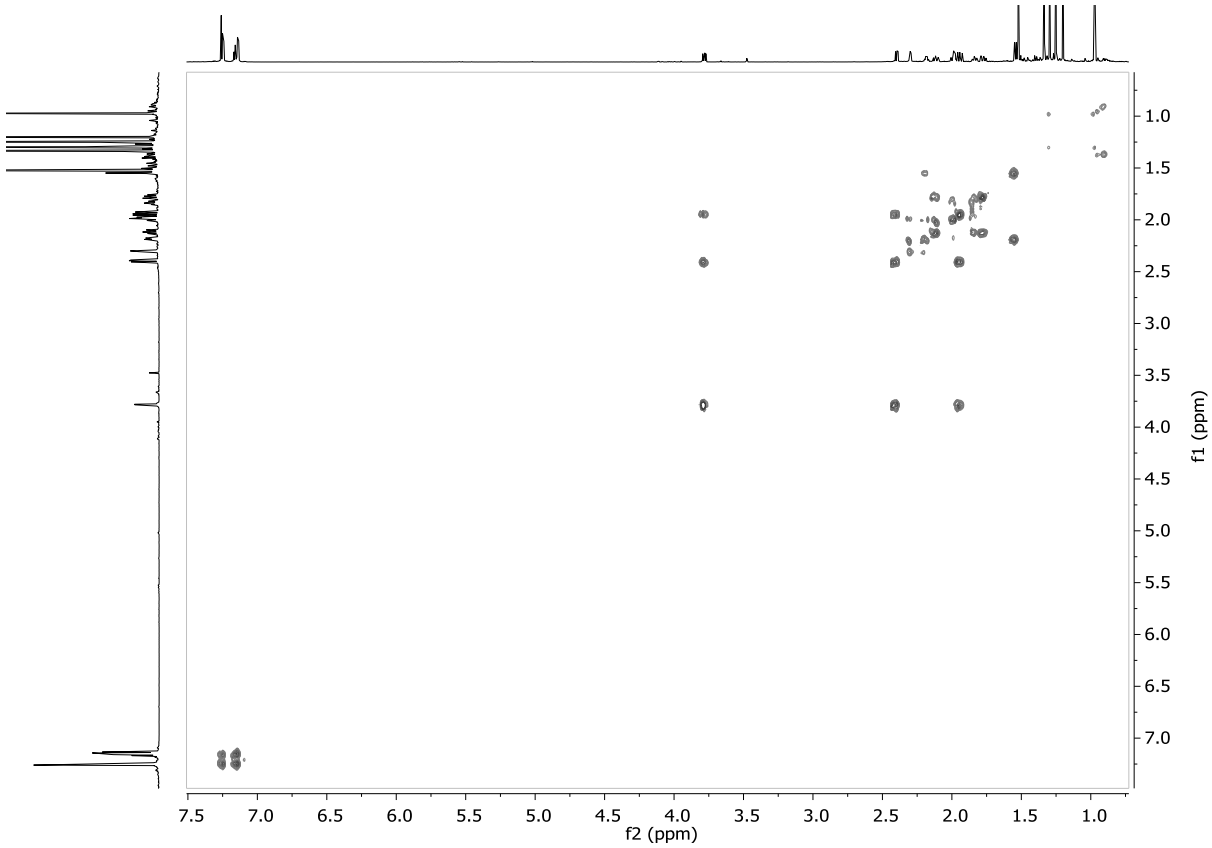


Figure S 5. COSY spectrum of intermedione A in $CDCl_3$ (800 MHz)

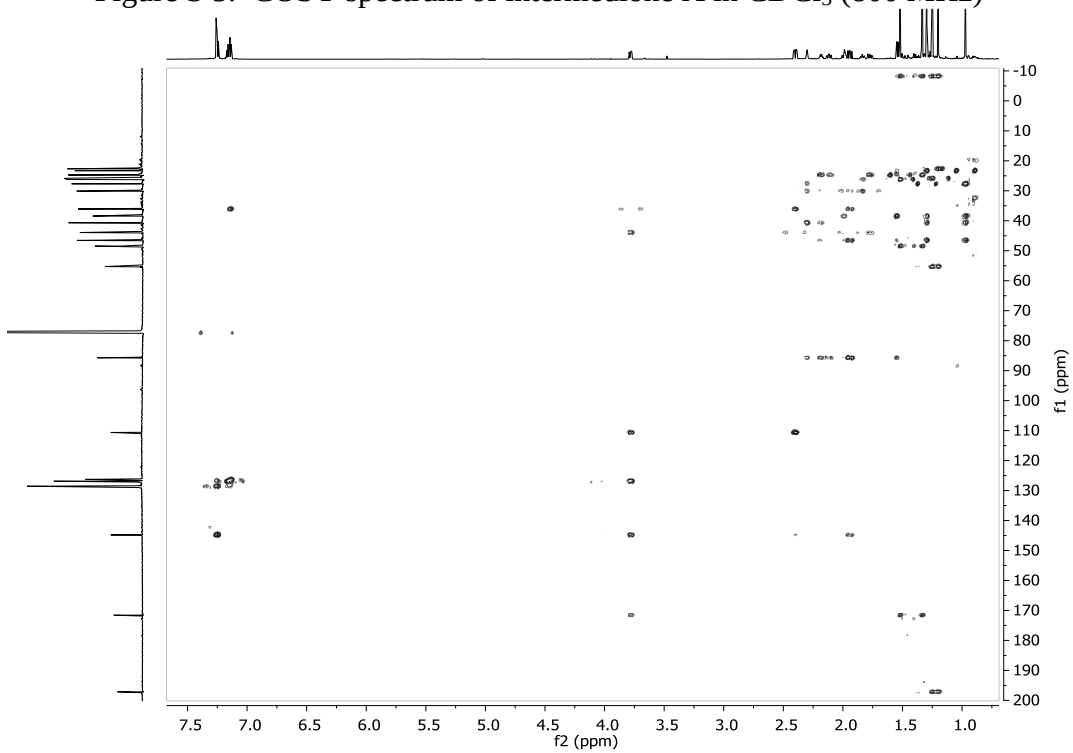


Figure S 6. HMBC spectrum of intermedione A in $CDCl_3$ (800 MHz)

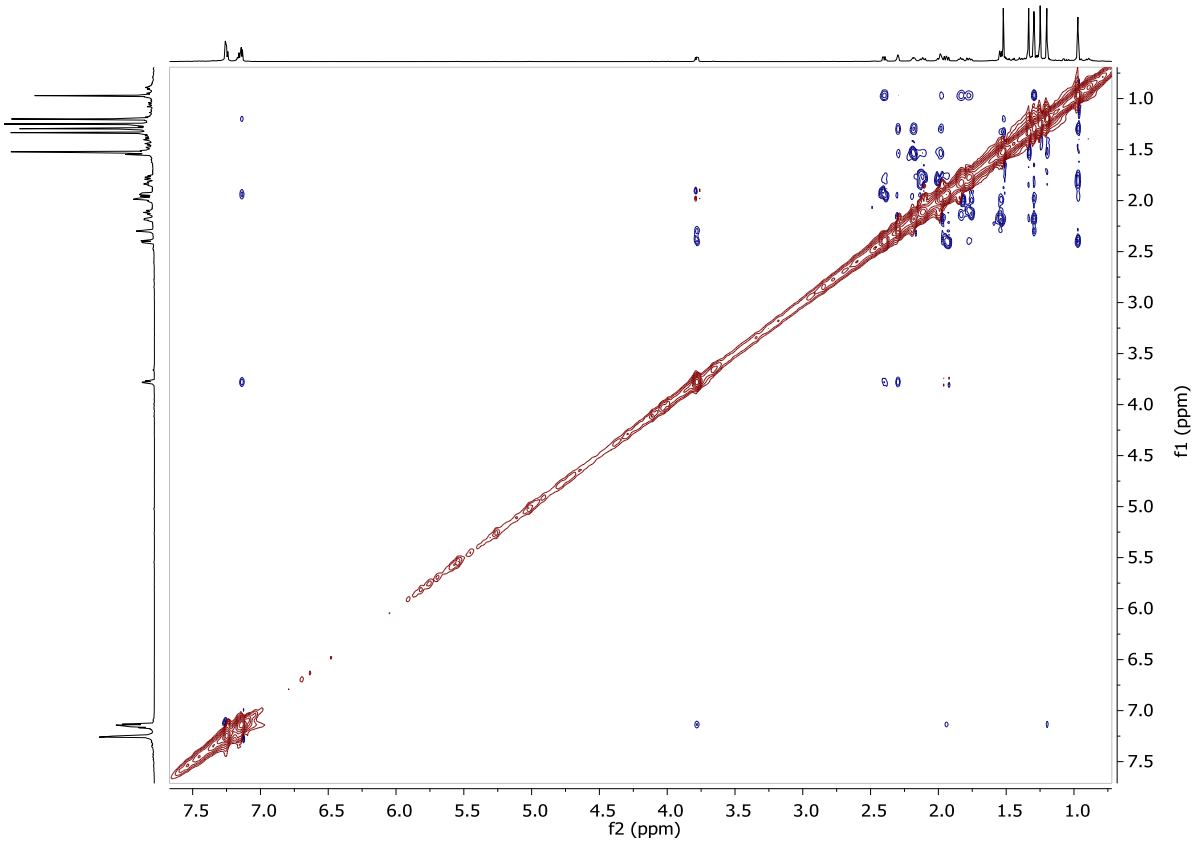


Figure S 7. ROESY spectrum of intermedione A in $CDCl_3$ (800 MHz)

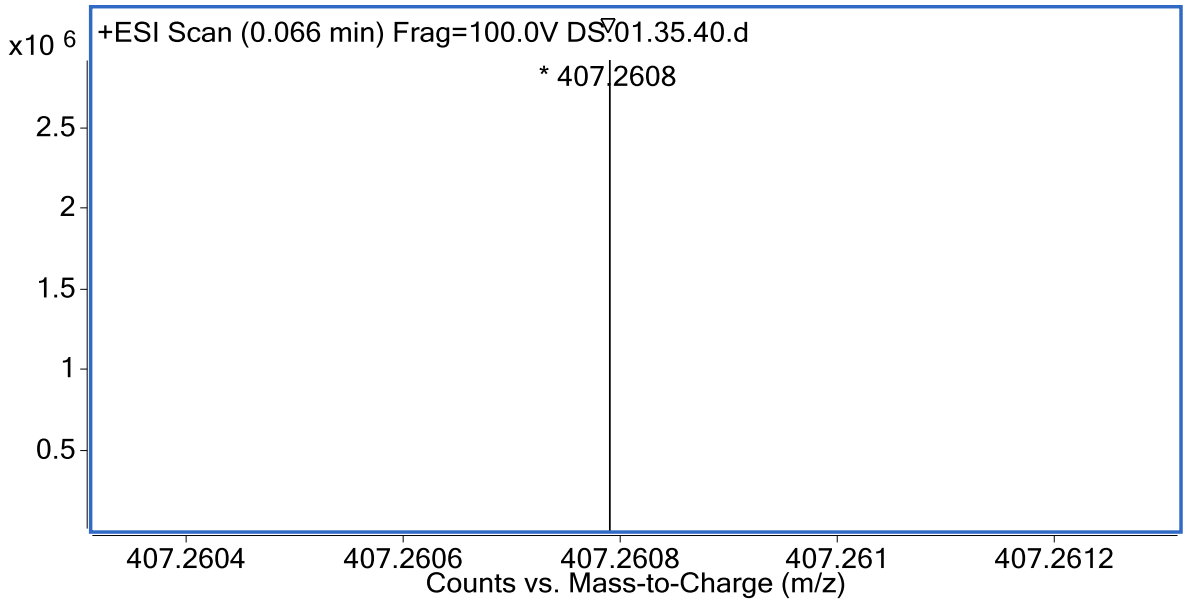


Figure S 8: Mass spectrum of intermedione B

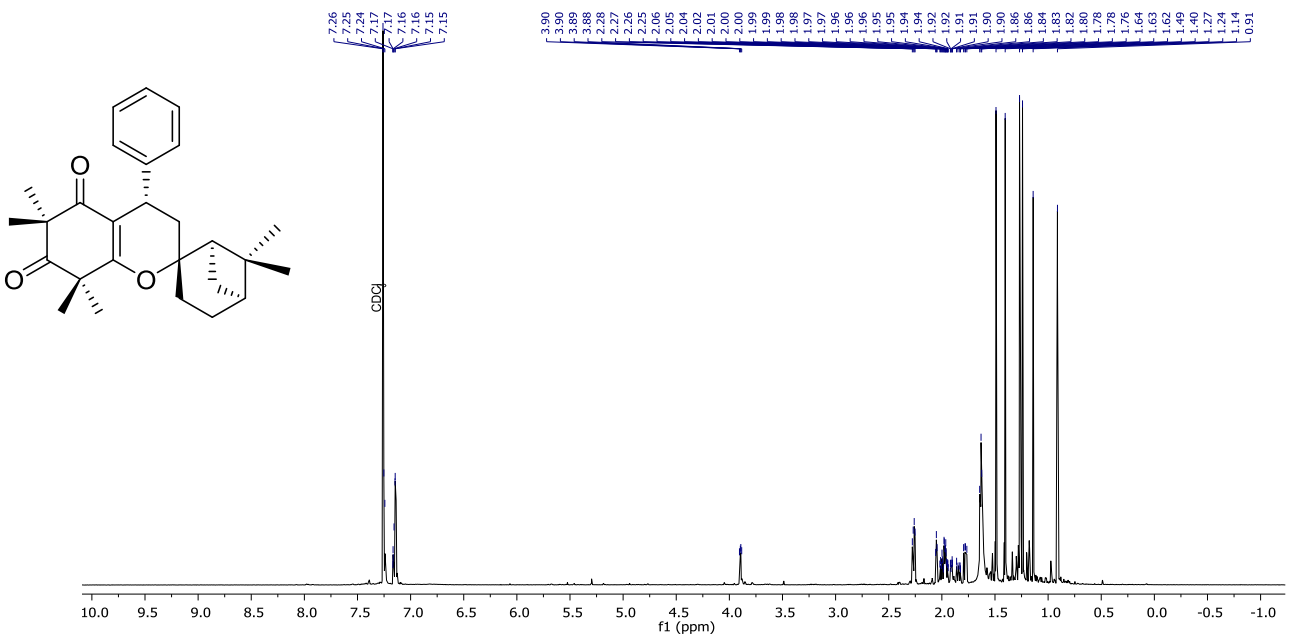


Figure S 9: ^{1}H NMR spectrum of intermedione B in $CDCl_3$ (800 MHz)

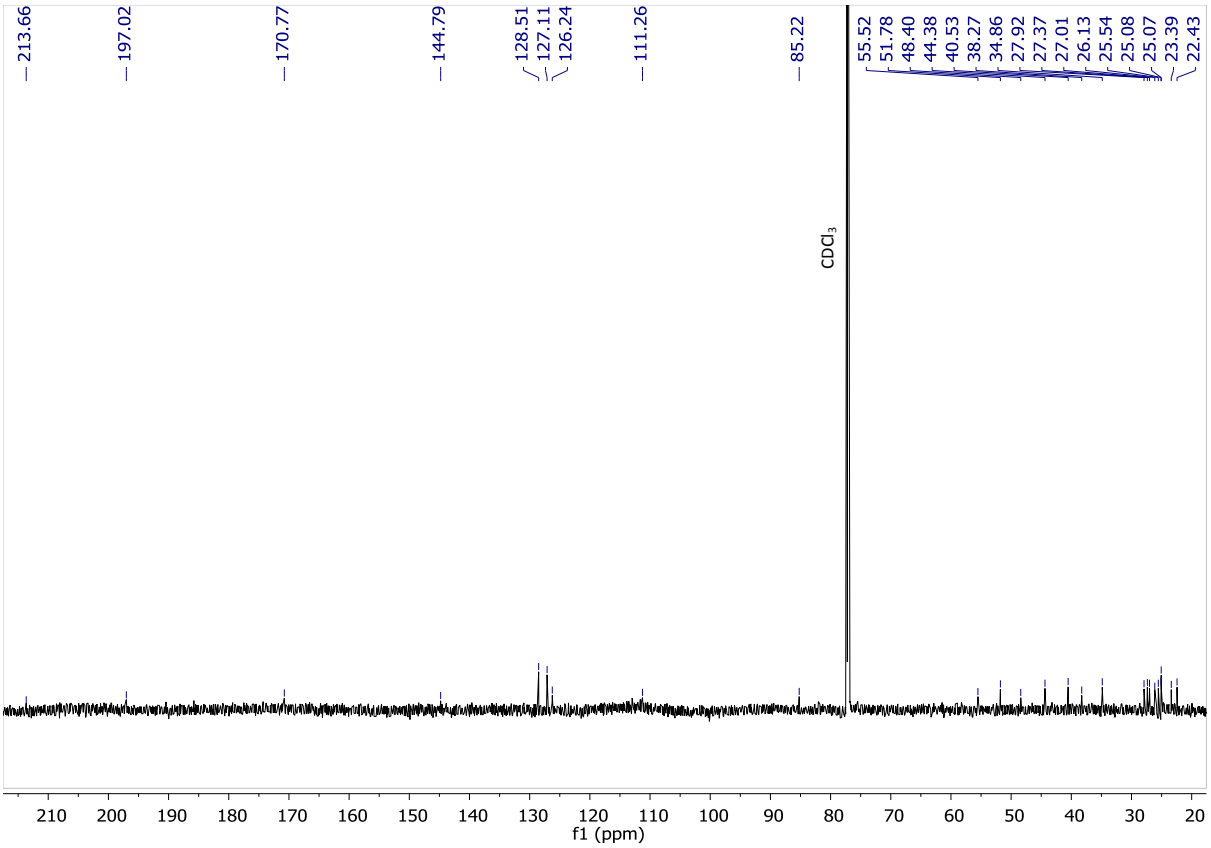


Figure S 10: ^{13}C NMR spectrum of intermedione B in $CDCl_3$ (800 MHz)

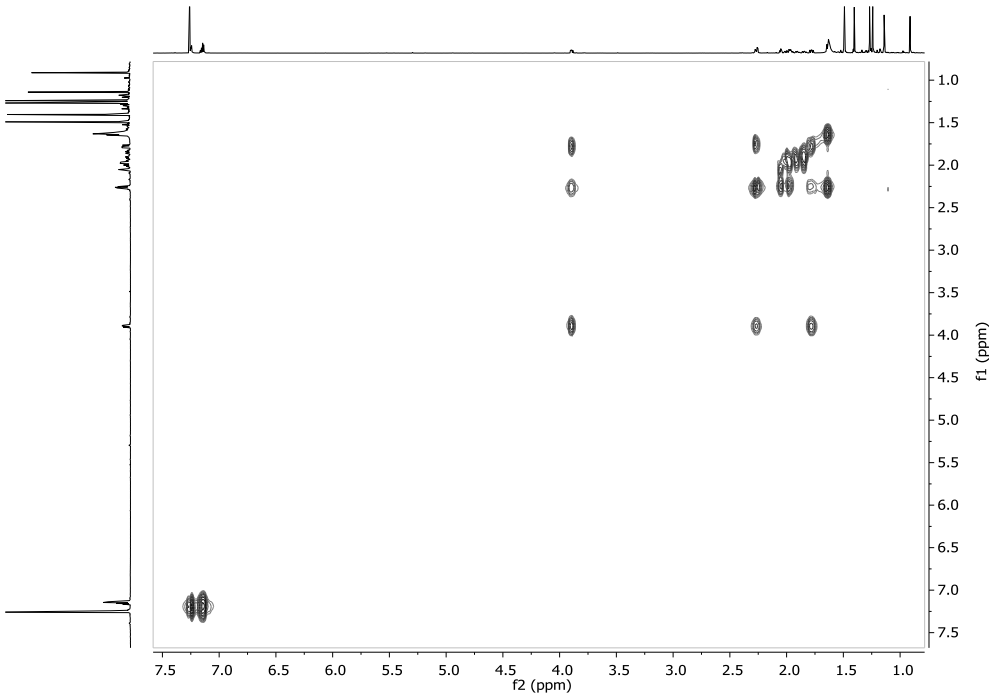


Figure S 11: COSY spectrum of intermedione B in $CDCl_3$ (800 MHz)

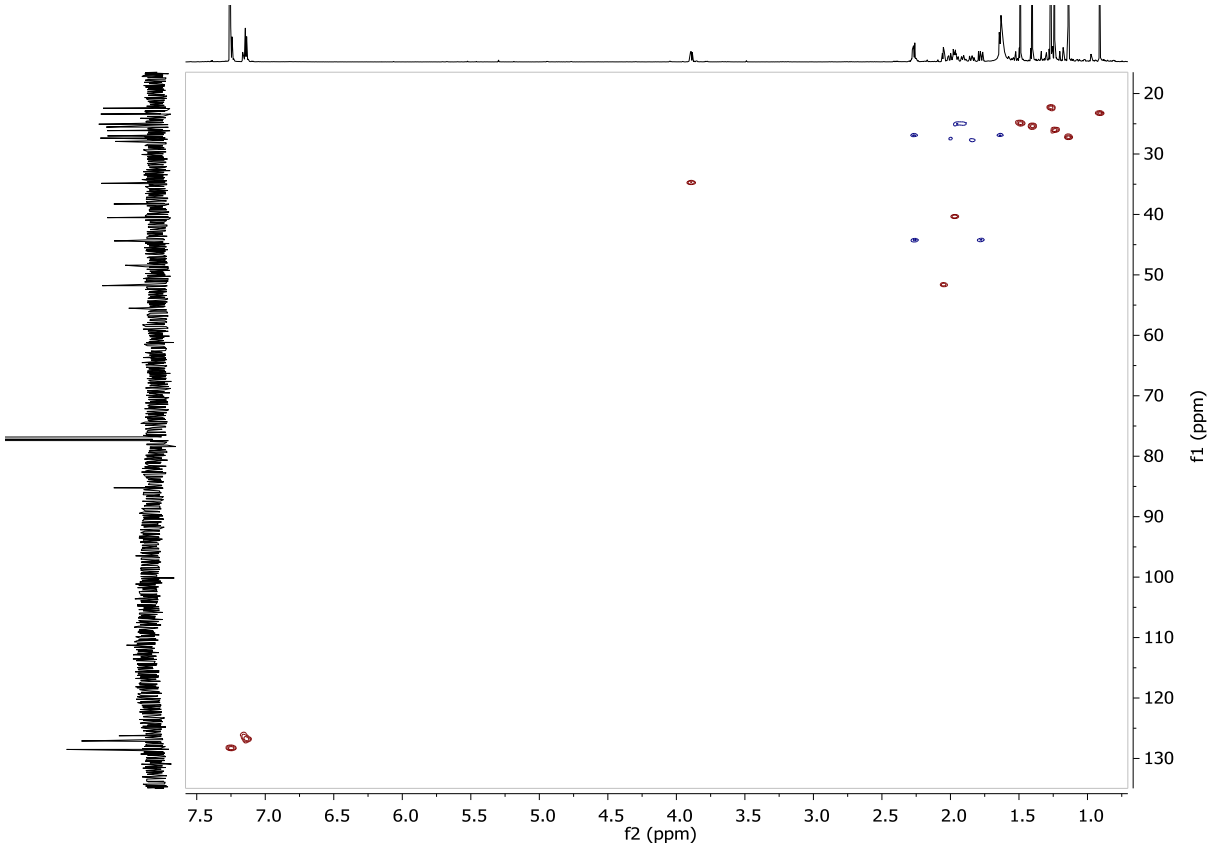


Figure S 12: HSQC spectrum of intermedione B in $CDCl_3$ (800 MHz)

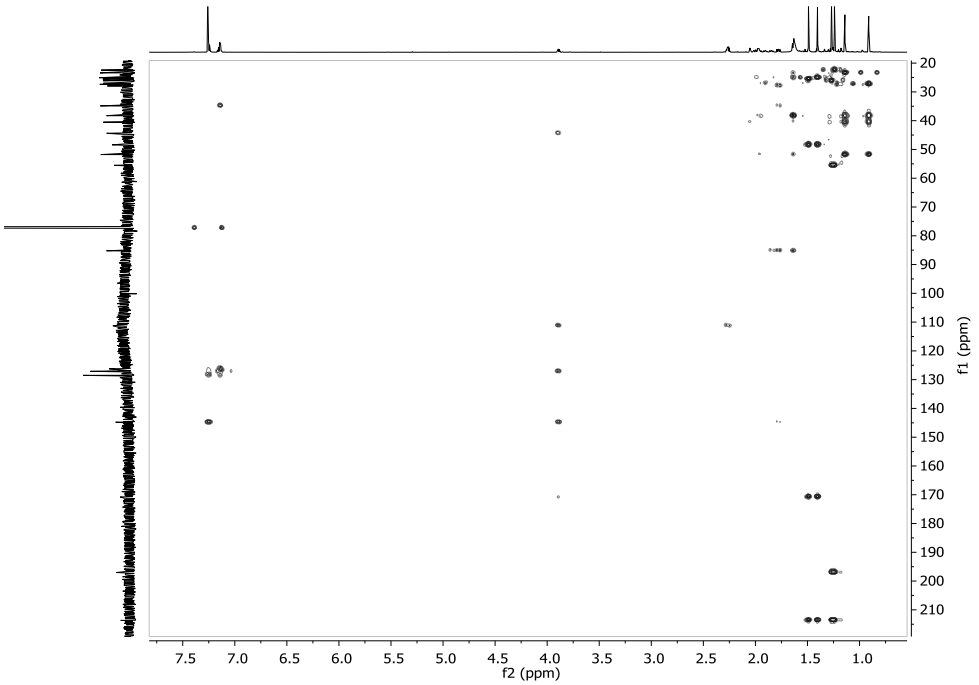


Figure S 13: HMBC spectrum of intermedione B in $CDCl_3$ (800 MHz)

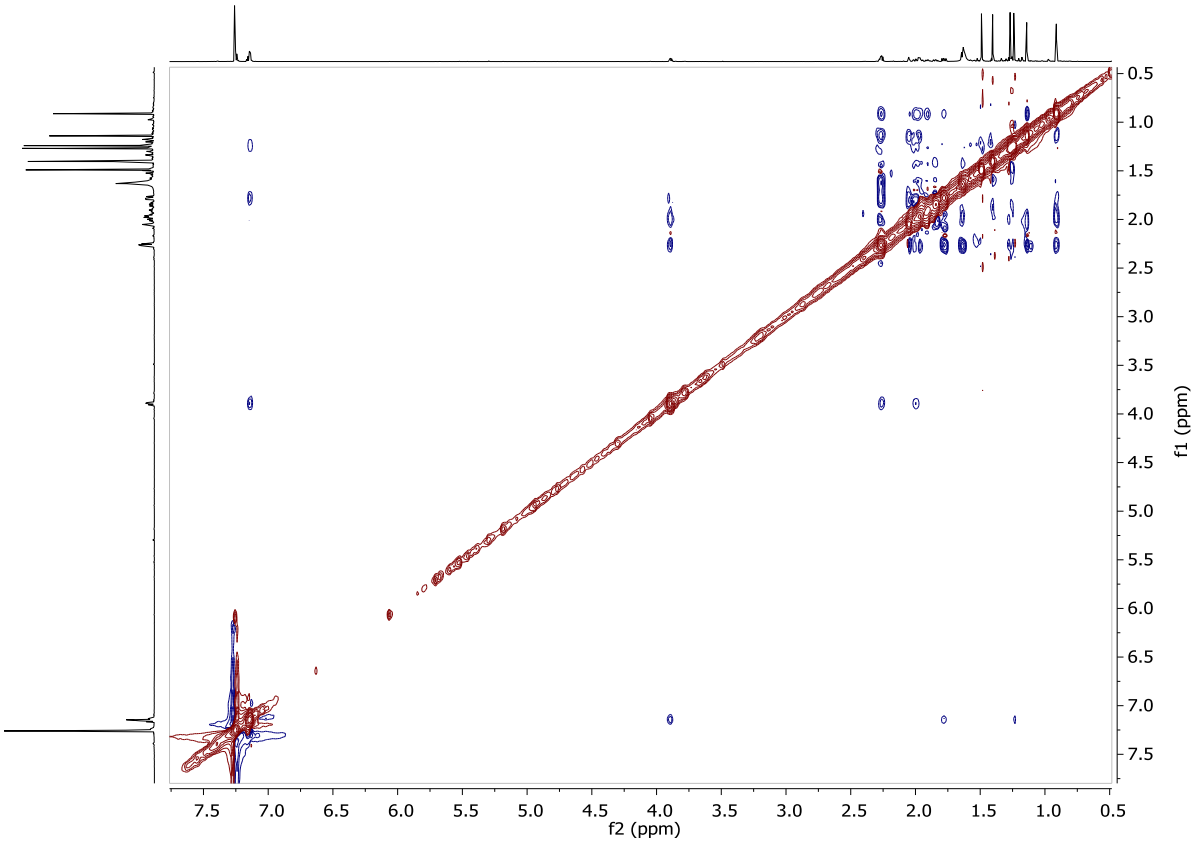


Figure S 14: ROESY spectrum of intermedione B in $CDCl_3$ (800 MHz)

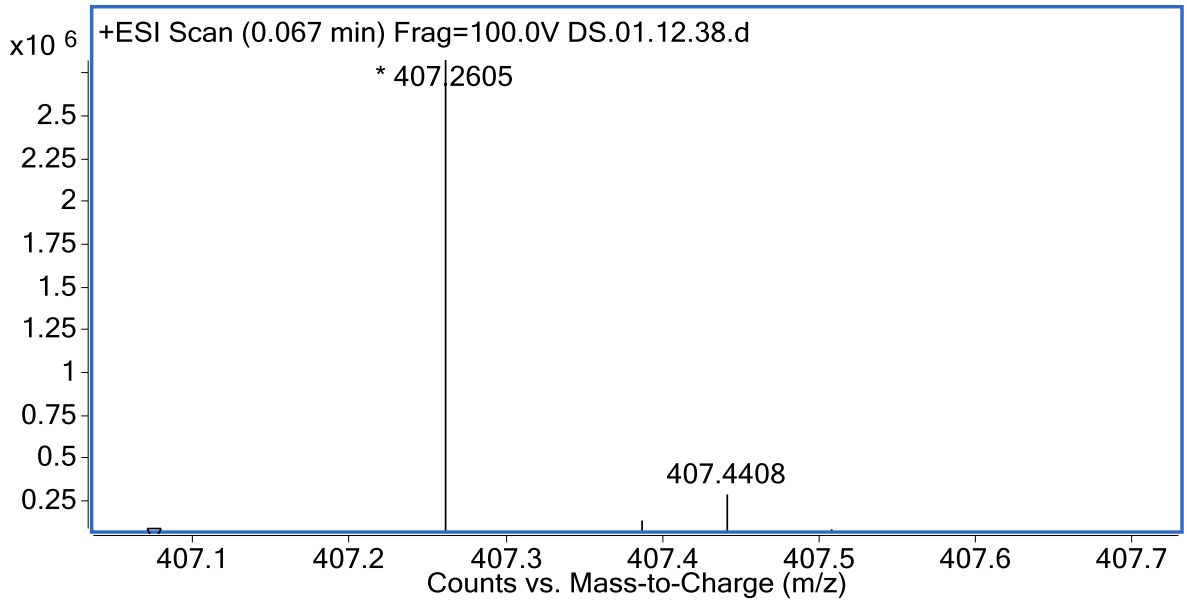


Figure S 15: Mass spectrum of compound C

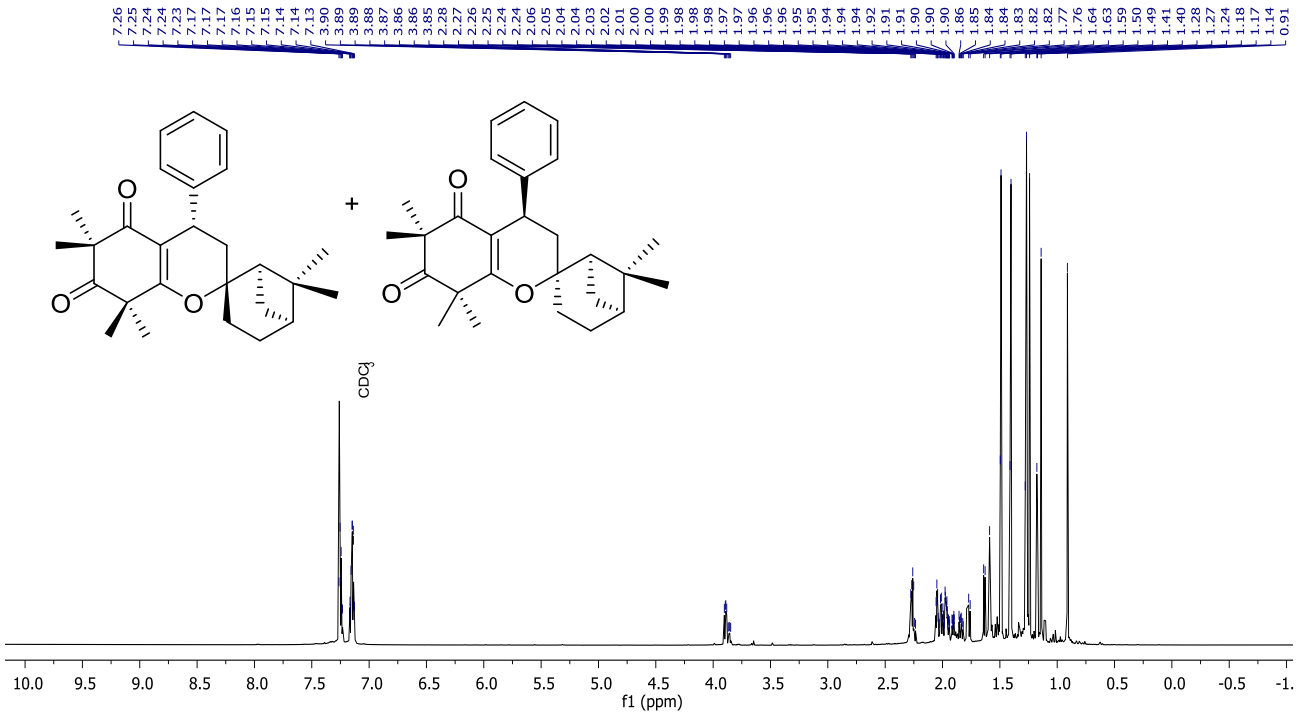


Figure S 16: ^{1}H NMR spectrum of intermedione B and C in $CDCl_3$ (800 MHz)

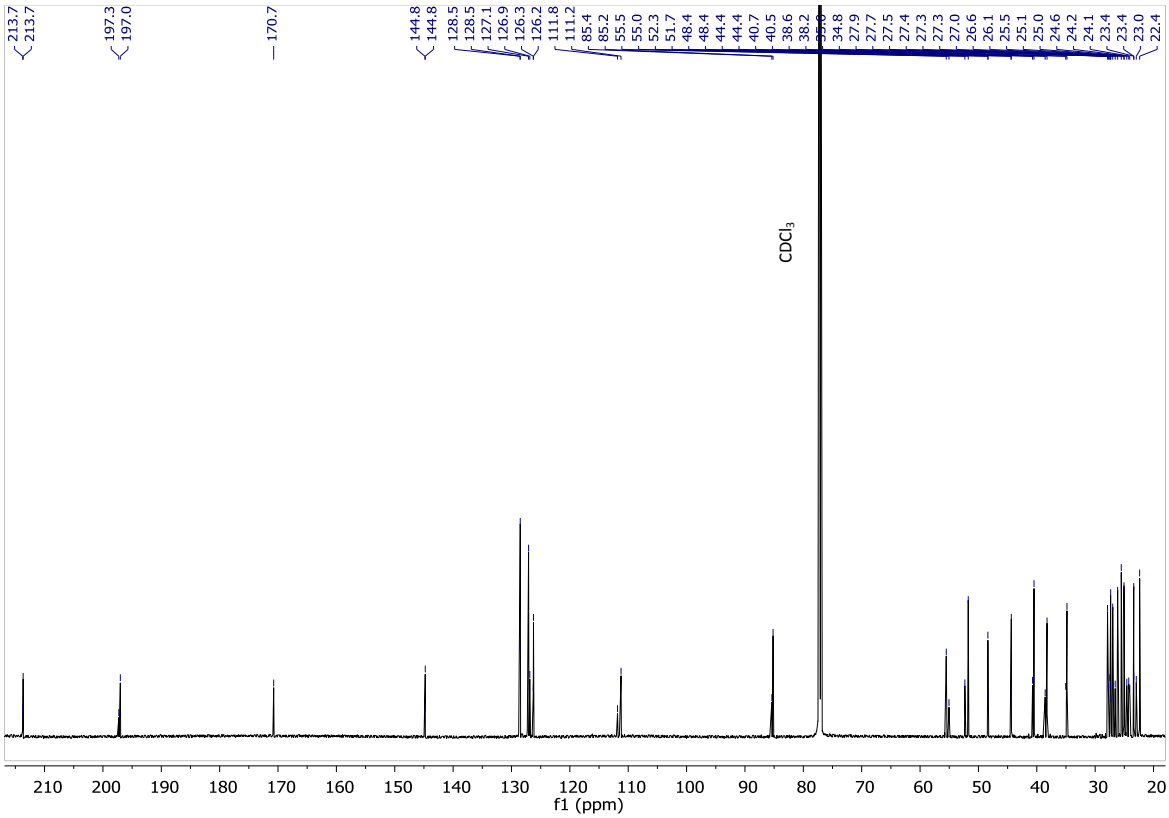


Figure S 17: ^{13}C NMR spectrum of intermedione B and C in $CDCl_3$ (800 MHz)

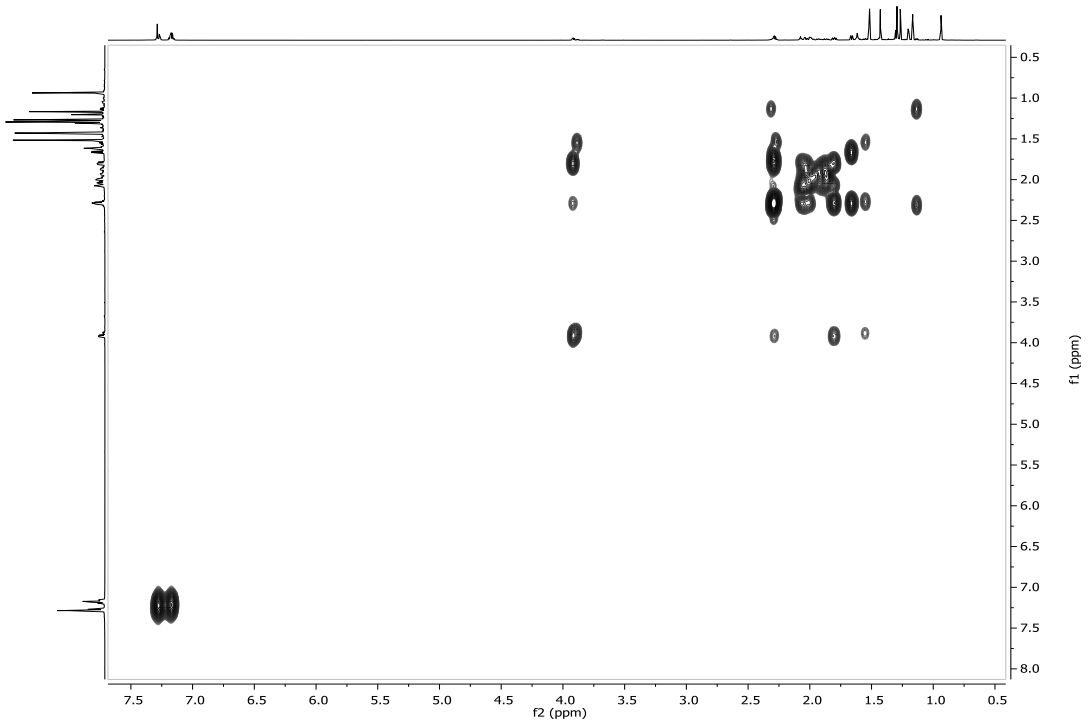


Figure S 18: COSY spectrum of intermedione B and C in $CDCl_3$ (800 MHz)

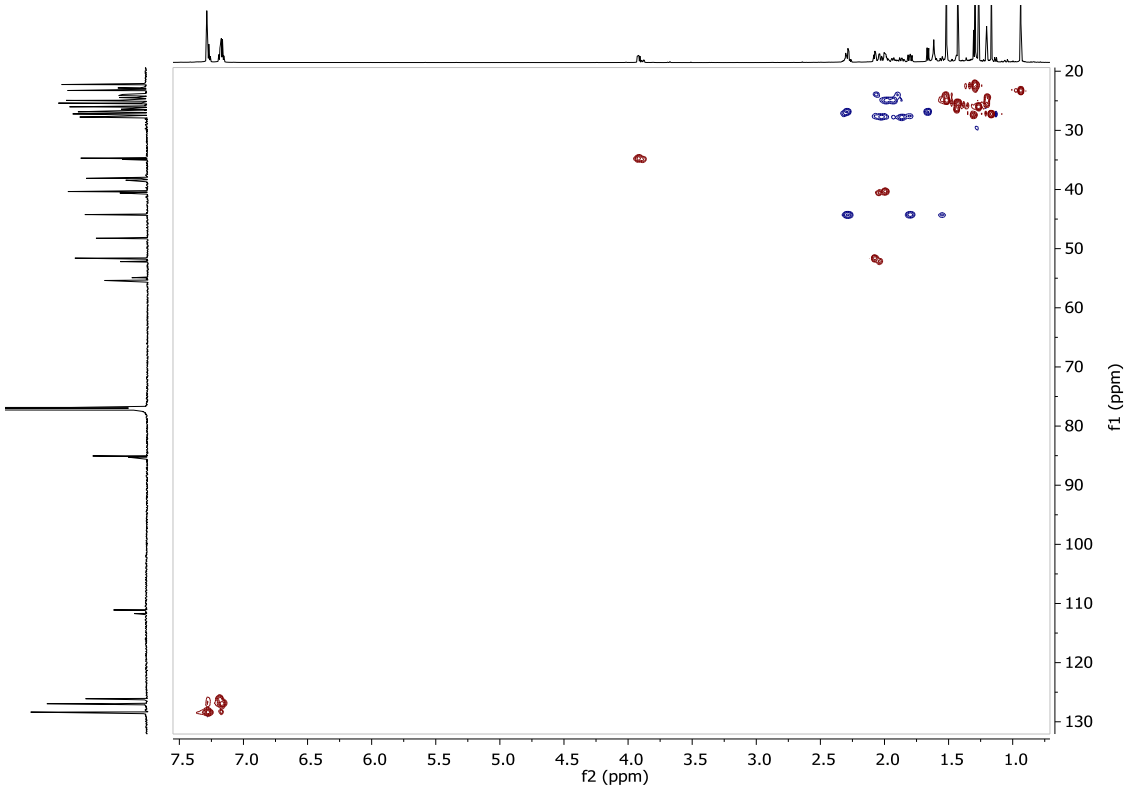


Figure S 19: HSQC spectrum of intermedione B and C in in $CDCl_3$ (800 MHz)

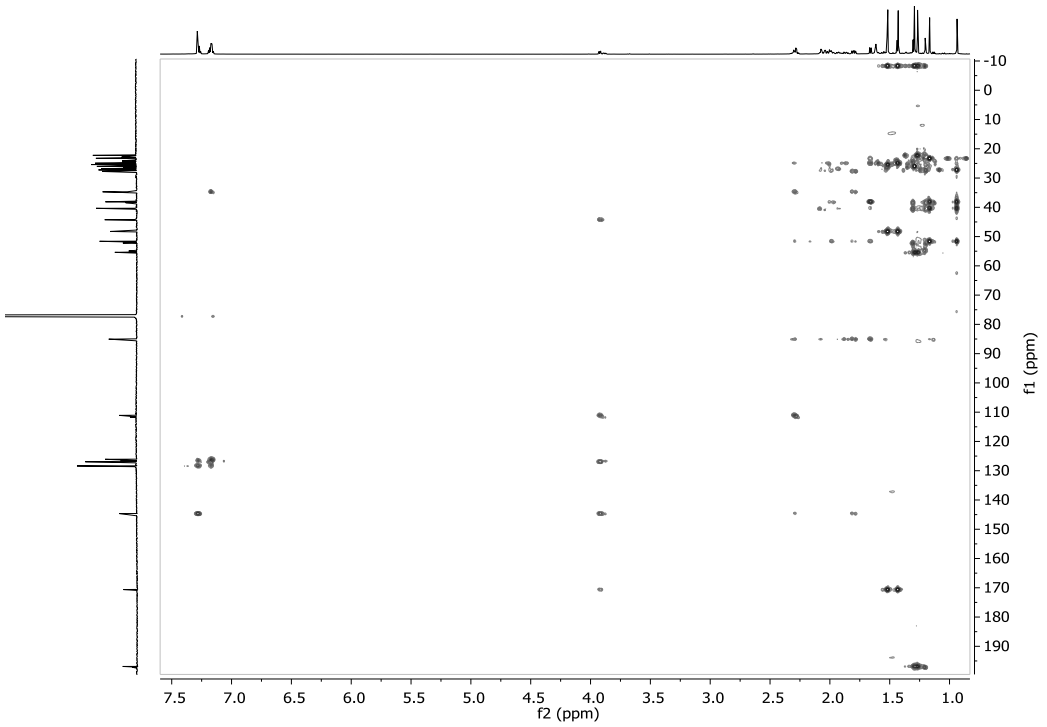


Figure S 20: HMBC spectrum of intermedione B and C in in $CDCl_3$ (800 MHz)

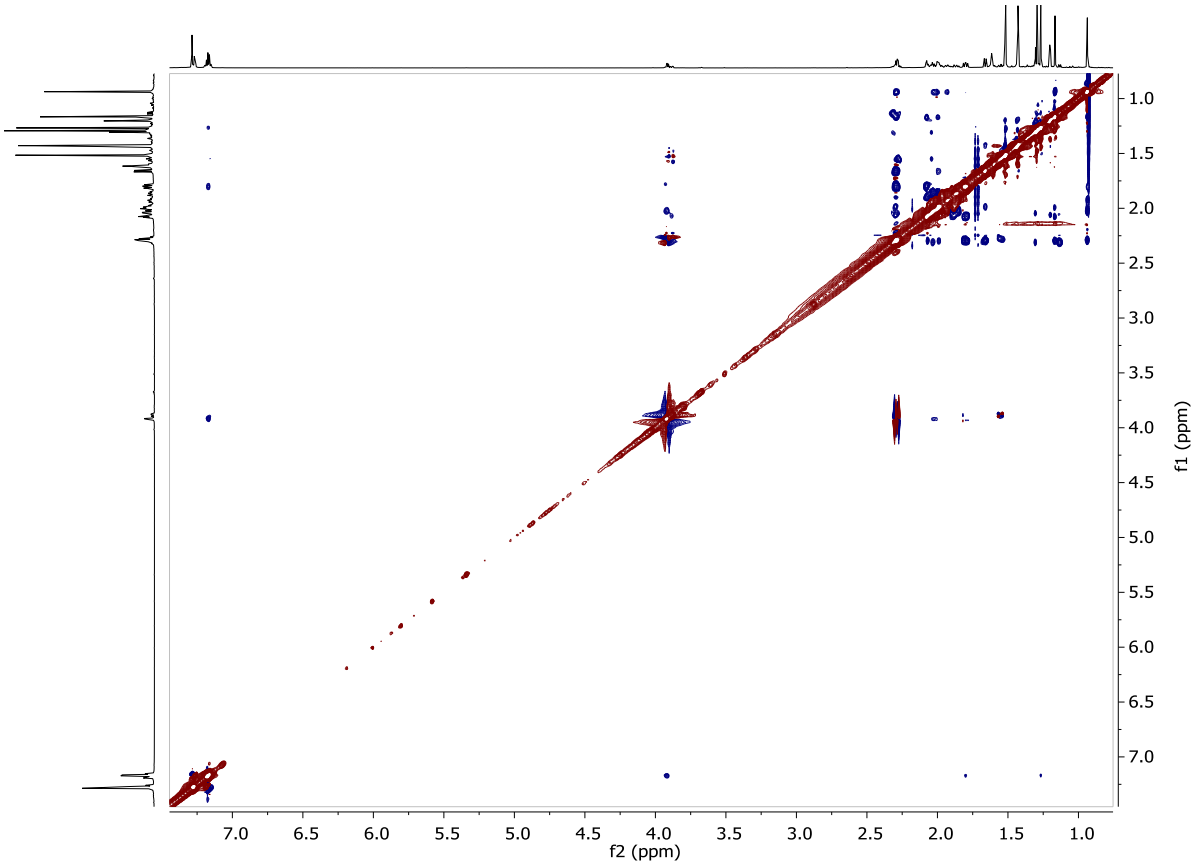


Figure S 21: ROESY spectrum of intermedione B and C in $CDCl_3$ (800 MHz)

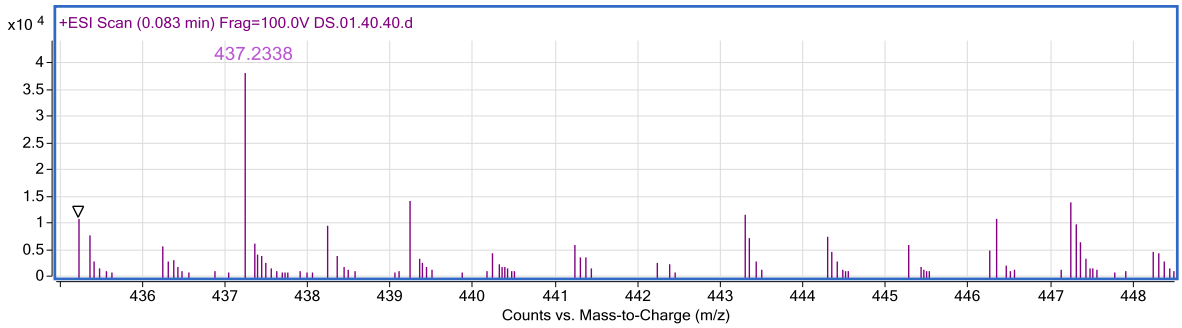


Figure S 22: Mass spectrum of intermedione D

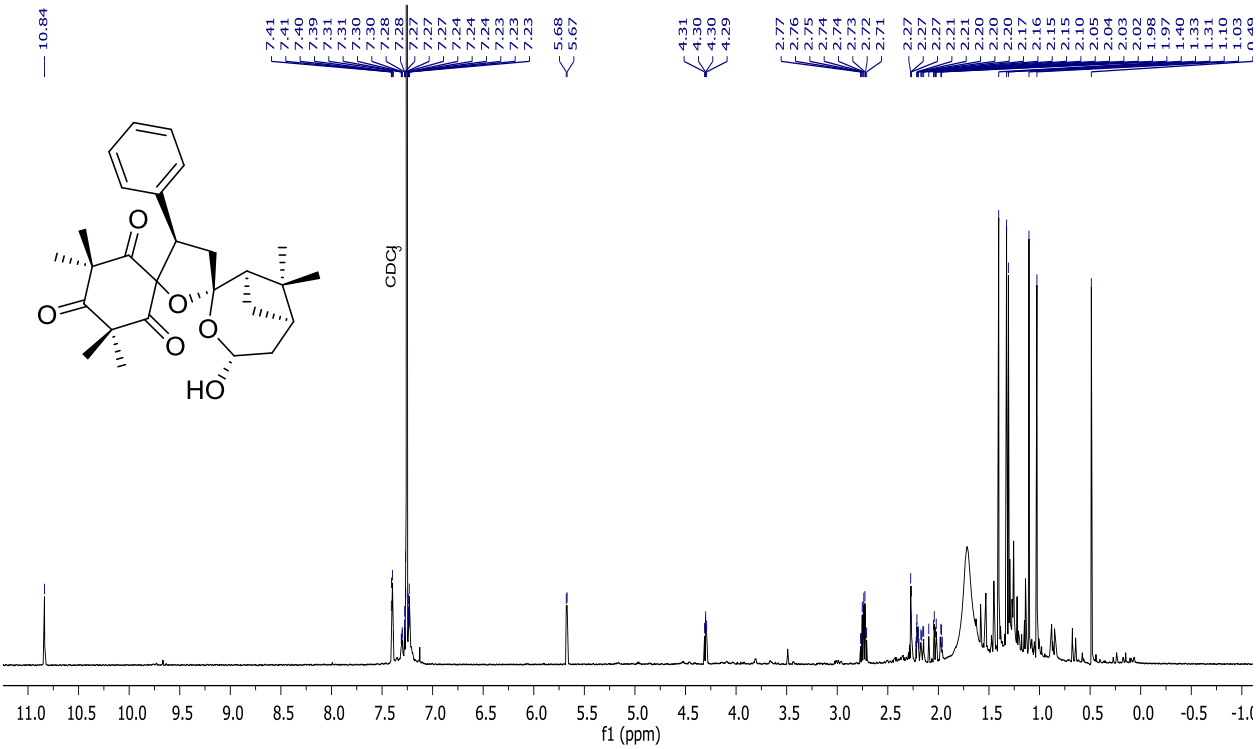


Figure S 23: ^{1}H NMR spectrum of intermedione D in $CDCl_3$ (800 MHz)

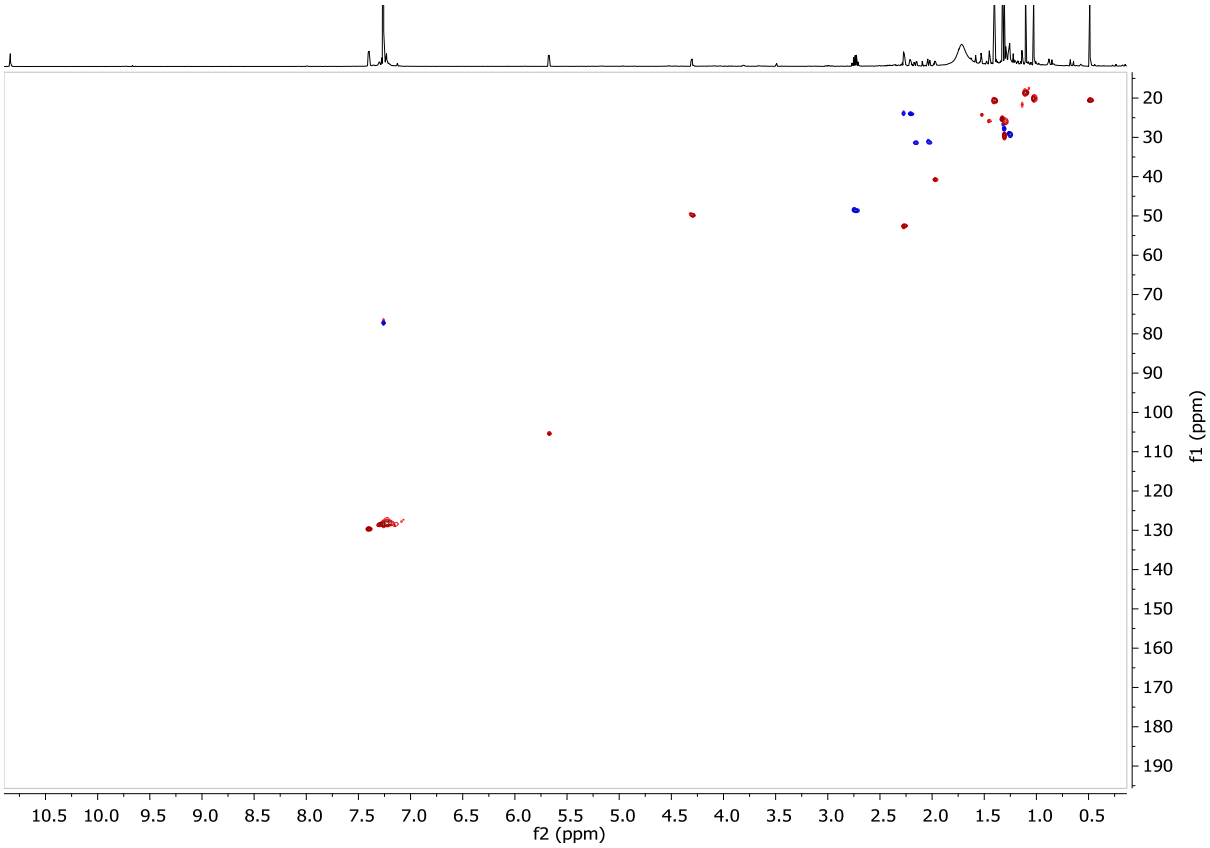


Figure S 24: HSQC spectrum of intermedione D in $CDCl_3$ (800 MHz)

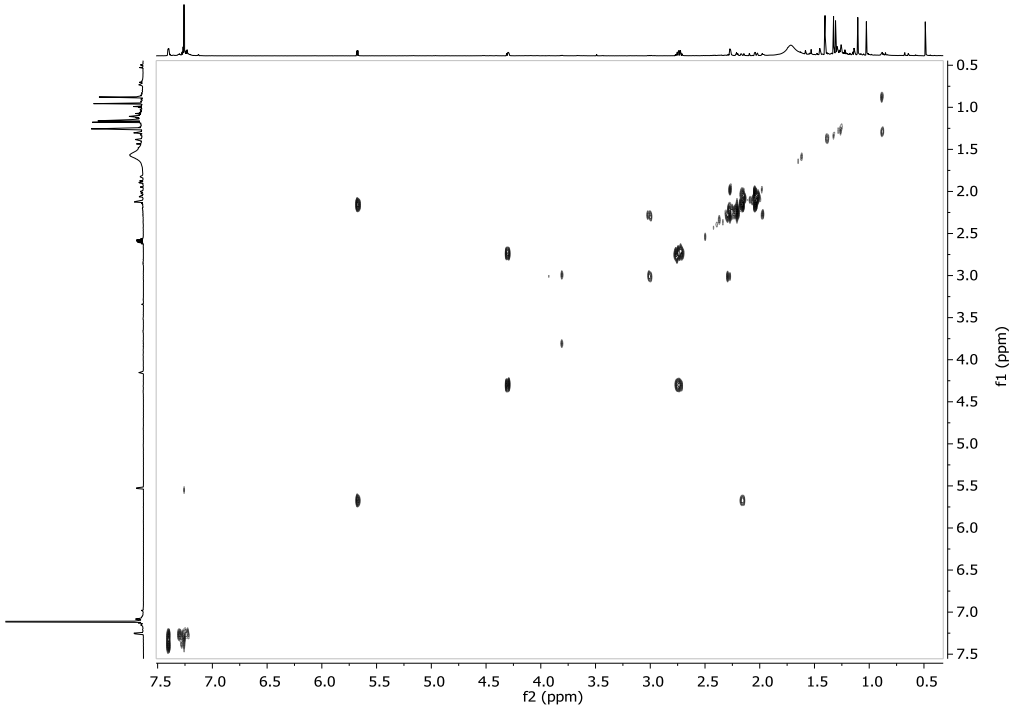


Figure S 25: COSY spectrum of intermedione D in $CDCl_3$ (800 MHz)

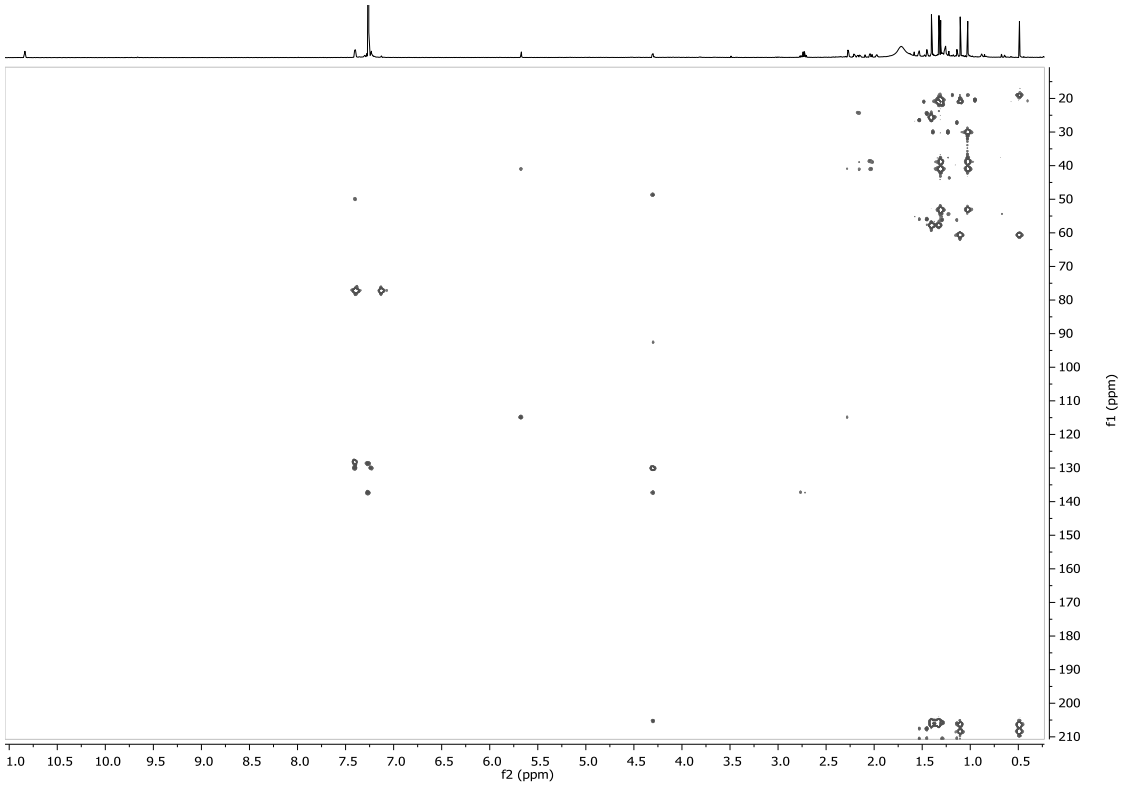


Figure S 26: HMBC spectrum of intermedione D in $CDCl_3$ (800 MHz)

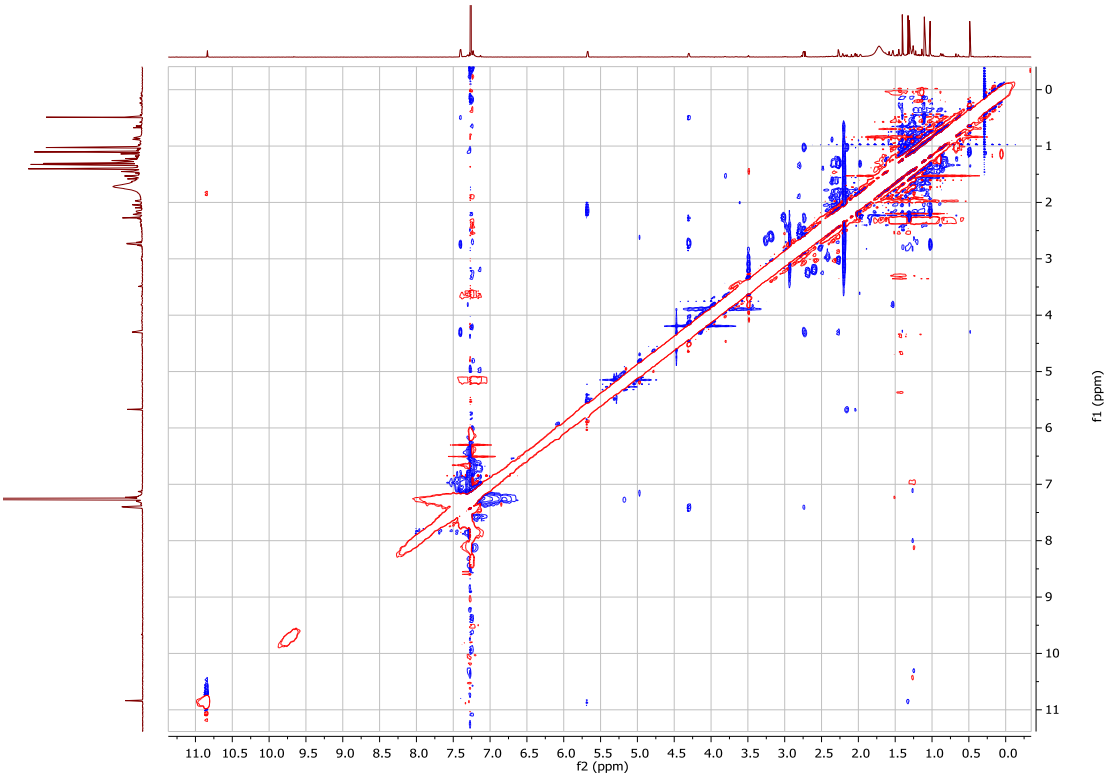


Figure S 27: ROESY spectrum of intermedione D in $CDCl_3$ (800 MHz)

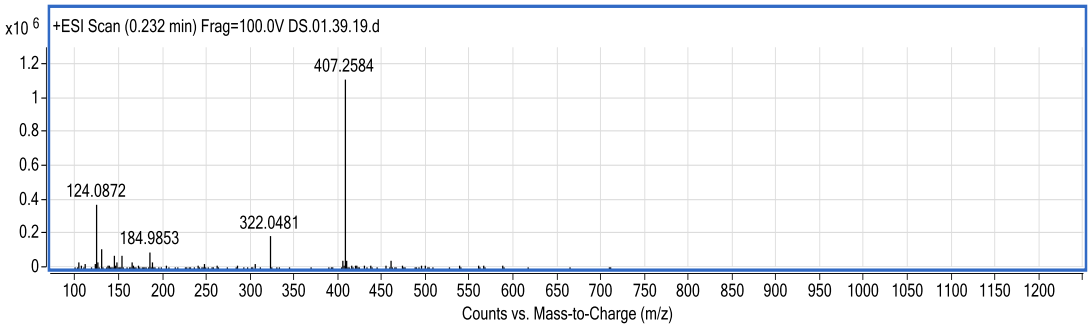


Figure S 28: Mass spectrum of intermedione E

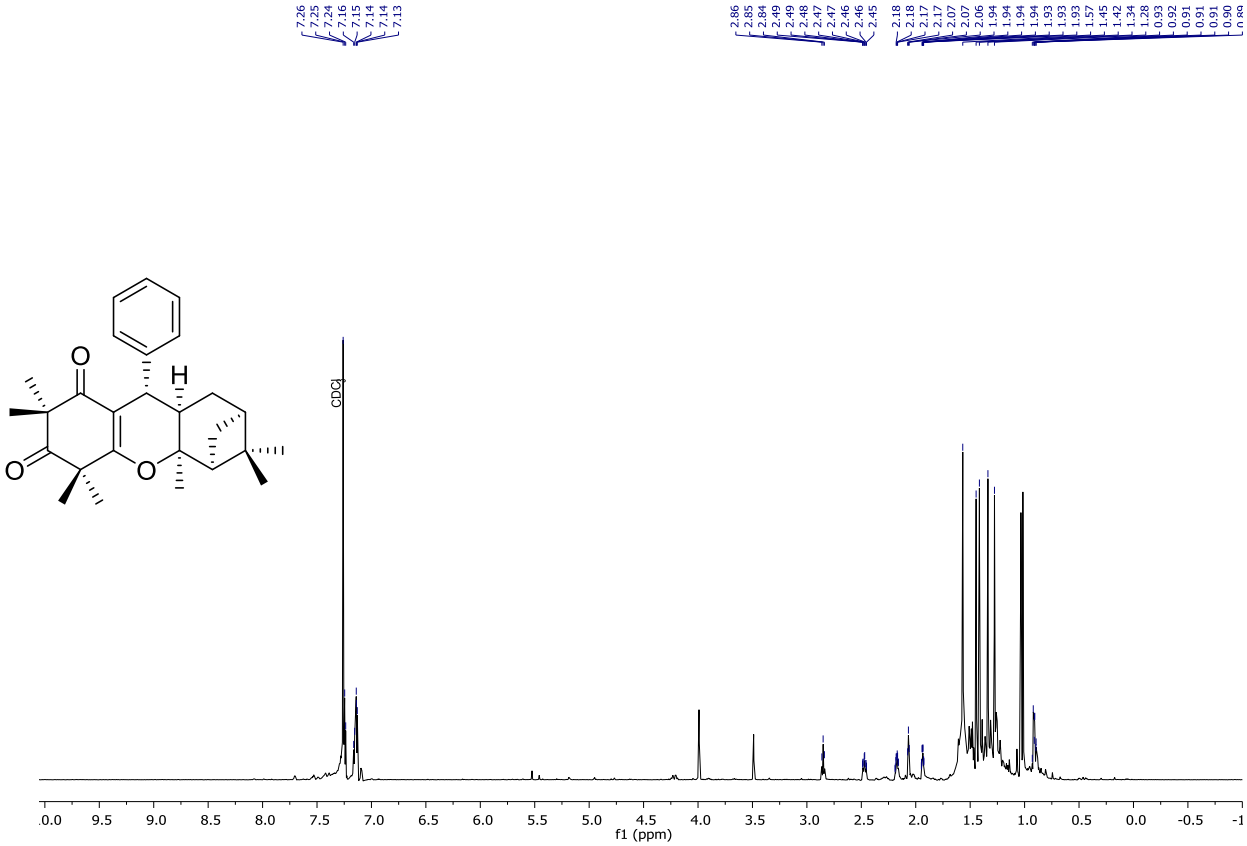


Figure S 29: ^{1}H NMR spectrum of intermedione E in $CDCl_3$ (800 MHz)

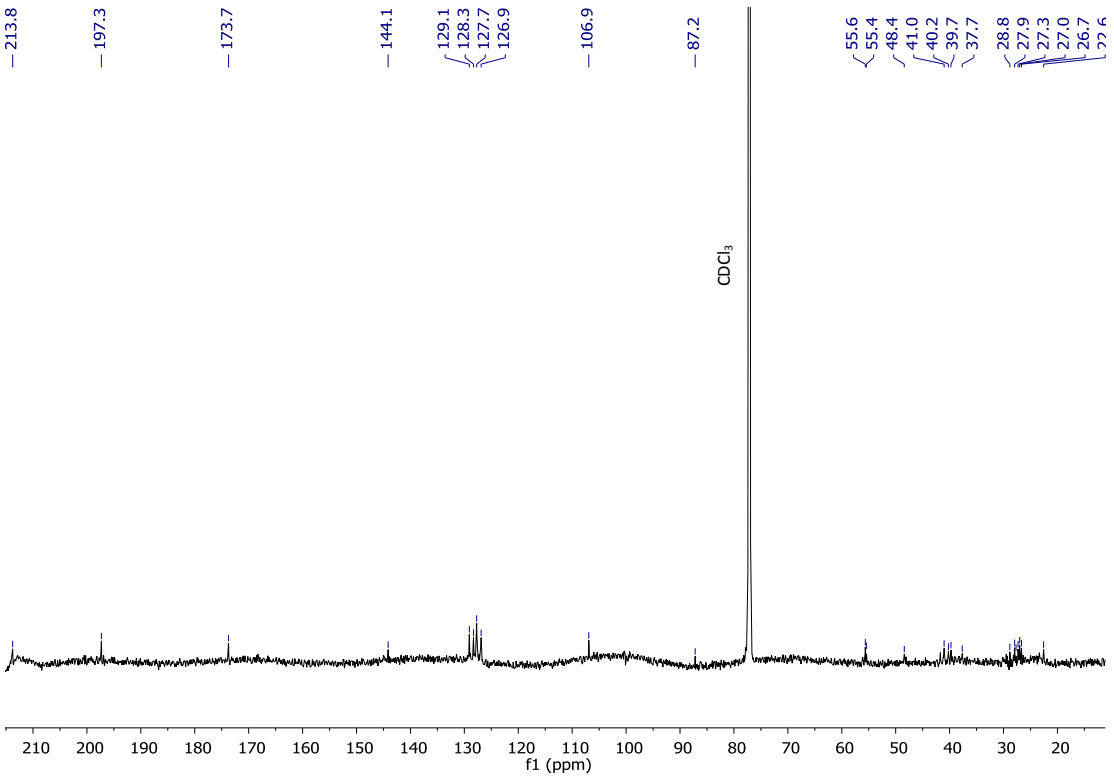


Figure S 30: ^{13}C NMR spectrum of intermedione E in $CDCl_3$ (800 MHz)

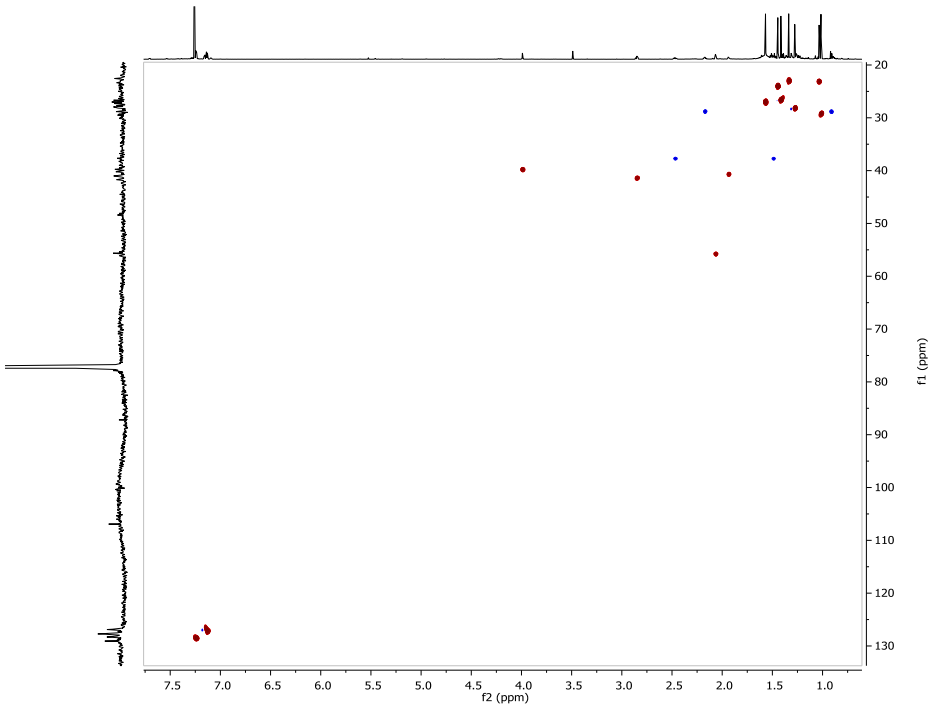


Figure S 31: HSQC spectrum of intermedione E in $CDCl_3$ (800 MHz)

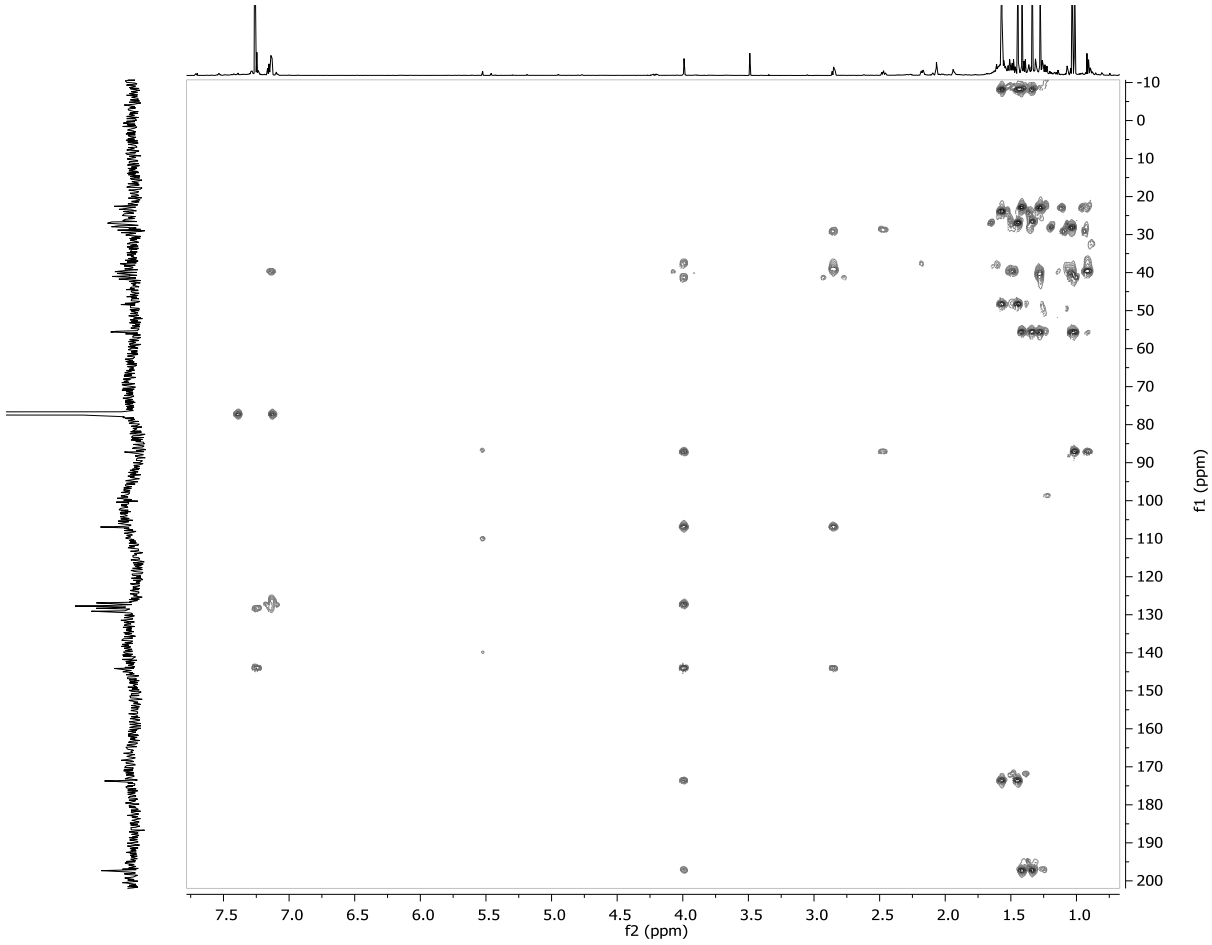


Figure S 32: HMBC spectrum of intermedione E in $CDCl_3$ (800 MHz)

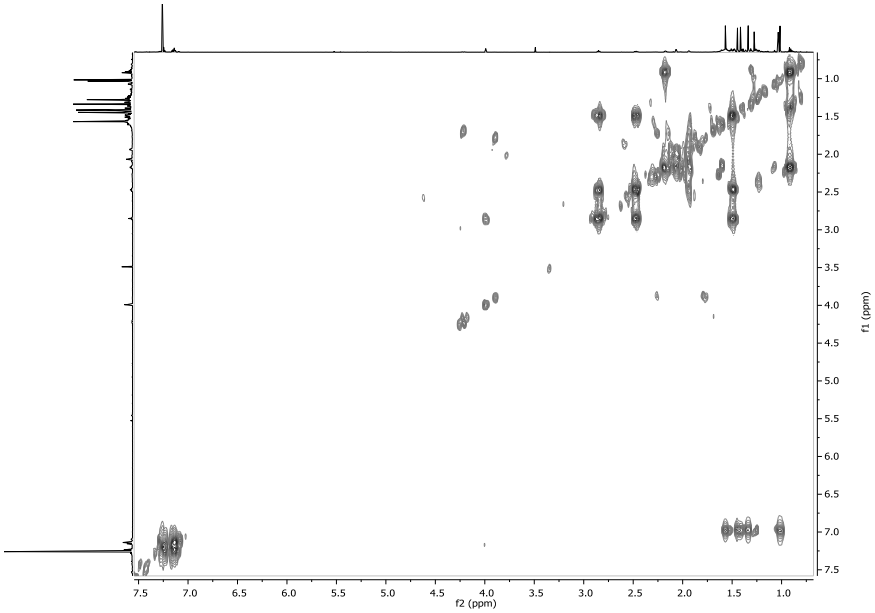


Figure S 33: COSY spectrum of intermedione E in $CDCl_3$ (800 MHz)

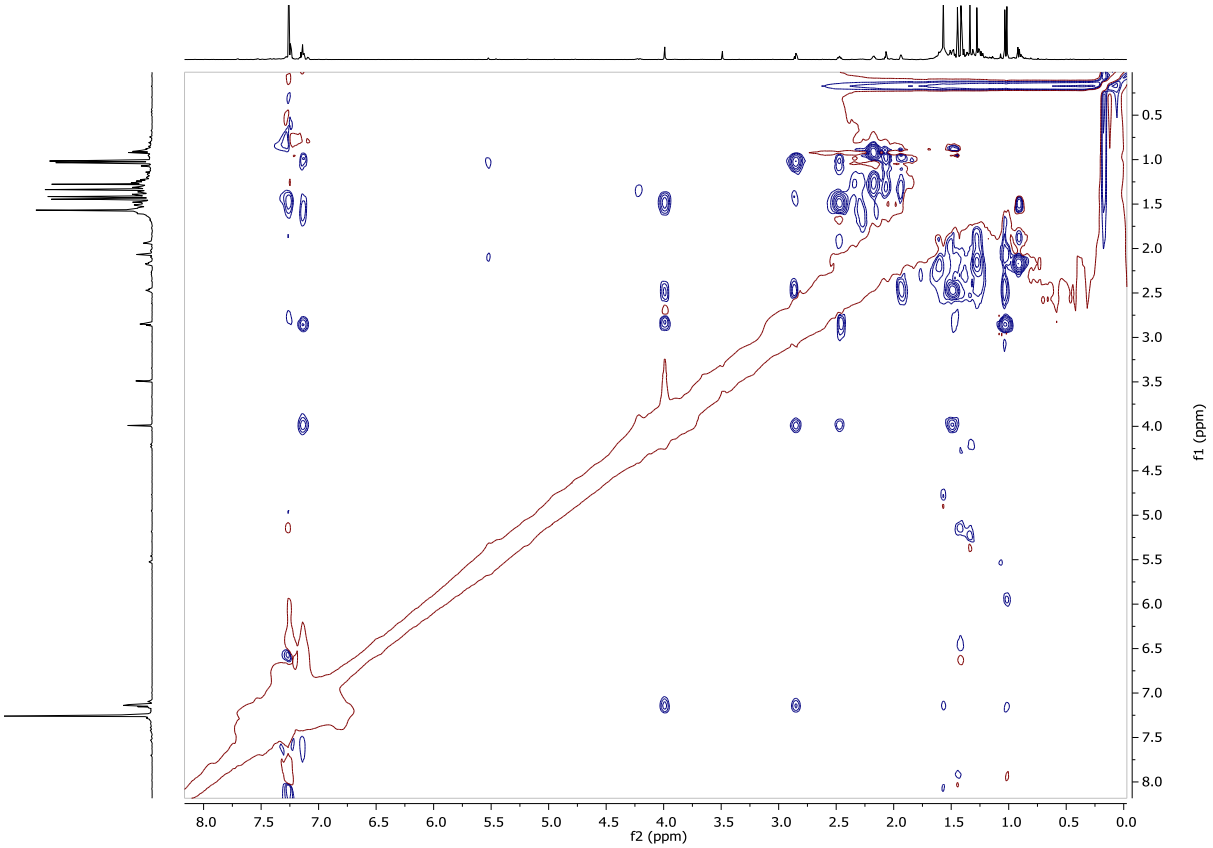


Figure S 34: ROESY spectrum of intermedione E in $CDCl_3$ (800 MHz)

CHAPTER 7

SEVEN NEW β-TRIKETONES FROM THE AUSTRALIAN PLANT *Corymbia torelliana*

SEVEN NEW β-TRIKETONES FROM THE AUSTRALIAN PLANT *Corymbia torelliana*

Flowers of *Corymbia torelliana* was investigated for β-trikeone chemistry. Extraction, isolation, and structure elucidation of seven new and four known β-triketones and their antiplasmodial and antibacterial activities are reported in this chapter.

STATEMENT OF CONTRIBUTION CO-AUTHORED UNPUBLISHED PAPER

This results chapter includes a co-authored prepared manuscript: 'Seven new β-triketones from the Australian plant *Corymbia torelliana*' which will be submitted to the Journal of Natural Products. It is formatted to meet the journal requirements.

The bibliographic details of the co-authored manuscript, including all authors are:

Sarath P. D. Senadeera, Leonardo Lucantino, Joshua Hayton, Vicky M. Avery, Anthony R. Carroll*

My contribution to the paper involved:

Collection and organization of information, data and references; Preparation of manuscript.

(Signed) [redacted] (Date) 14/03/2017

Name of student: Sarath Parakumge Dayani Senadeera

(Countersigned) [redacted] (Date) 14/03/2017

*Corresponding author: Anthony R. Carroll

(Countersigned) [redacted] (Date) 14/03/2017

Principle supervisor: Anthony R. Carroll

7.1 Abstract

The methanol extract of the flowers of *Corymbia torelliana* yielded six new β-triketones, torellianones A-F (**1**-**6**), the tetrahydroxycyclohexane, torellianol A (**7**) and four known β- triketones, 4*S*-ficifolidione (**8**), 4*R*-ficifolidione (**9**), kunzeanone A (**10**) and kunzeanone B (**11**). Torellianones A and B, C and D, and E and F were each isolated as inseparable diastereomeric mixtures. The chemical structures of **1**-**7** were elucidated from the analysis of 1D/2D NMR and MS data. Relative configurations of the compounds were determined using ROESY experimental data. All new compounds (**1**-**7**) and 4*S*-ficifolidione were tested for antiplasmodial and antibacterial activities. Diastereomeric mixtures of torellianones C, D and torellianones E, F and 4*S*-ficifolidione had antiplasmodial activity with IC_{50}'s of 7.2 ± 1.2 μM, 3.2 ± 0.3 μM, ~13.7 μM against the 3D7 chloroquine sensitive strain of *Plasmodium falciparum* respectively. Only the diastereomeric mixture of torellianones E and F inhibited the growth of *Staphylococcus aureus* ATCC 157293.

7.2 Introduction

The Myrtaceae is a flowering plant family of Gondwanan origin with 147 genera and more than 3000 species mainly occurring in the southern hemisphere. Australia is a center of diversity for the family, with more than 1500 species from 70 genera, mostly being endemic to the country.[1] Myrtaceae species are renowned for their abundance of terpene rich essential oils, but more recently non-volatile phloroglucinols and β-triketone constituents have received increasing attention in the literature.[2, 3] Both phloroglucinols and β-triketones exhibit biological activities including antimalarial, antibacterial, anti-fouling, antiprotozoal, Epstein Barr Virus inhibition, aldose reductase inhibition and HIV-RT inhibition.[3, 4] The Myrtaceae genus *Corymbia,* containing 113 species, are gum trees endemic to Australia. The genus was split from the larger genus *Eucalyptus* in 1995 based on molecular analysis of sRNA and morphological analysis of flowers.[5] Several species of *Corymbia* including *C. ficifolia*, *C. scabrida*, *C. peltata*, and *C. watsoniana* have

been reported to produce simple as well as complex β-triketone molecules and these compounds have been shown to possess interesting biological activities including insect repellent, antifeedant, thyrotropin releasing hormone receptor-2 binding affinity and antiplasmodial activities.[6-9] Many species of *Corymbia* produce very large flushes of flowers which attract bird, bat and insect pollinators. These flowers, high in pollen and nectar, are present for weeks at a time and do not appear to perish as a result of microbial infections. In light of these previous studies which have shown that the flowers are a rich source of β-triketones,[6-9] we anticipated that the flowers of *C. torelliana*, a large tree endemic to northern Australia would also be a rich source of bioactive β-triketones. This paper reports on the isolation, structure elucidation and biological activity of seven new β-triketone compounds, torellianones A-F (**1**-**6**) and a tetrhydroxycyclohexane, torellianol A (**7**) and the known β-triketones, 4*S*-ficifolidione (**8**), 4*R*-ficifolidione (**9**), kunzeanone A (**10**) and kunzeanone B (**11**) from the flowers of *Corymbia torelliana*.

7.3 Results and Discussion

The ground flowers of *C. torelliana* were extracted with MeOH and this extract was purified by HPLC on C_{18} silica gel eluting with a linear gradient from H_2O to MeOH over 60 minutes providing 60 fractions. Based on ^{1}H NMR analysis, fractions 48 to 70 were recombined and purified by HPLC on diol bonded silica gel using a gradient from 100% hexane to 100% dichloromethane (DCM). Three fractions appeared to contain single components from analysis of the HPLC chromatogram and the appearance of a single protonated molecule by electrospray MS, however, the ^{1}H NMR spectra for each of these fractions was complex and suggested that each appeared to contain mixtures of two very similar compounds because many regions of the spectrum has pairs of similar signals. This doubling of signals could either be associated with diastereomers or interconverting isomers. We have previously shown that the presence of exchange correlations observed in ROESY spectra (opposite phase correlations to ROESY correlations) can be a good diagnostic tool to confirm if doubling of signals in ^{1}H NMR spectra are associated with interconverting isomers.[10, 11] Applying this ROESY analysis tool to these three fractions indicated that each were diastereomeric mixtures of the new compounds, torellianones A and B (**1**, **2**) (0.33%), torellianones C and D (**3**, **4**) (0.3%), and torellianones E and F (**5**, **6**) (1.5%). Further

attempts to separate these mixtures using a number of reverse phase and normal phase HPLC packing and solvent conditions proved unsuccessful and the structure elucidation was undertaken

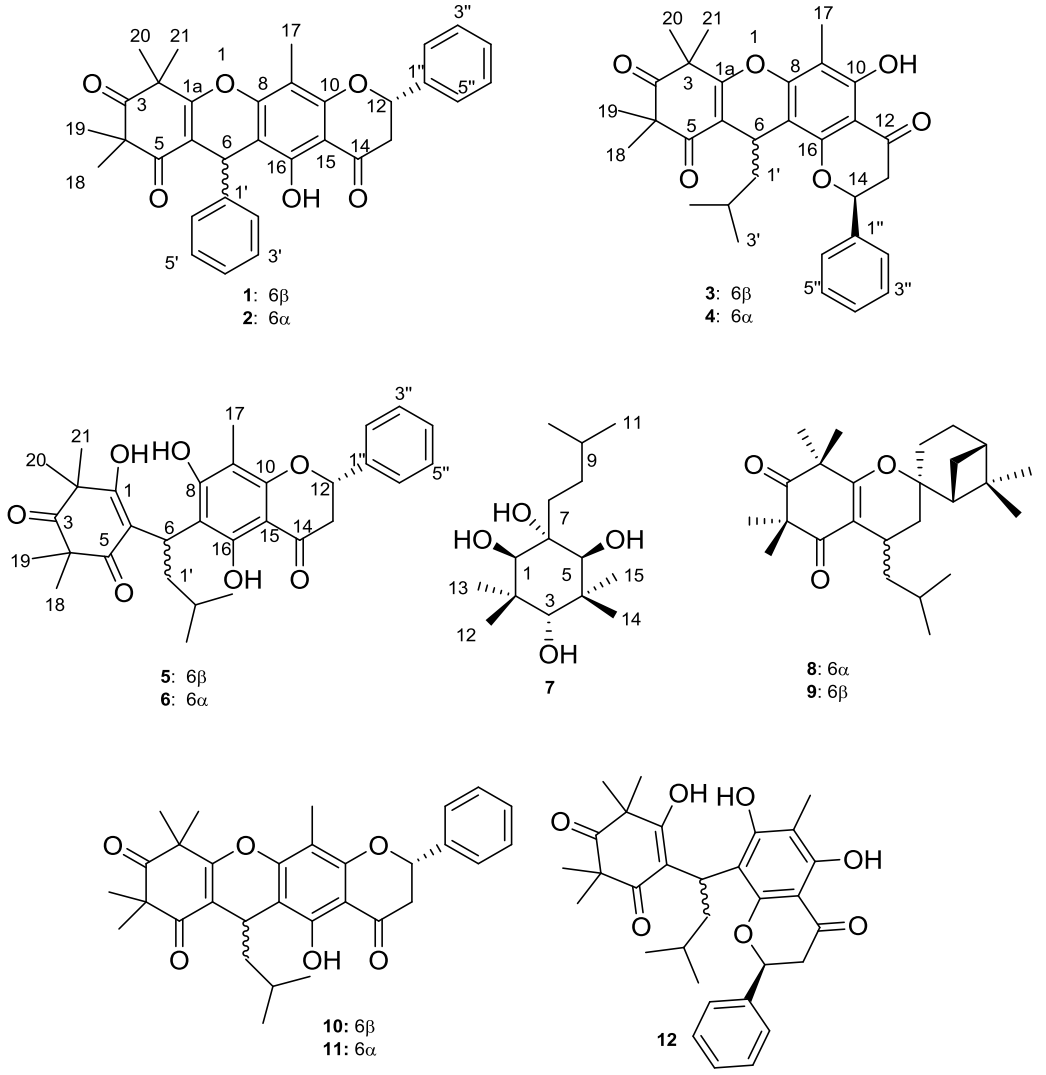


Figure 1. β-Triketone compounds isolated from *Corymbia torelliana*

on the diastereomeric mixtures. The known compounds 4*S*-ficifolidione (**8**) (1%), 4*R*-ficifolidione (**9**) (0.1%), kunzeanones A (**10**) (0.2%) and B (**11**) (0.2%) was also isolated and in the process of further purification of the above compounds, torellianol A (**7**) (0.6%) was obtained.

An equimolar mixture of torellianone A (**1**) and B (**2**) was obtained as a yellow amorphous solid. The (+) HRESIMS of **1** and **2** had a single ion at *m/z* 523.2131 assigned to the protonated mole-

cule indicating that both compounds had a molecular formula $C_{33}H_{30}O_6$. The IR spectrum of the mixture of **1** and **2** showed absorption bands at 1658 and 1714 cm^{-1} indicating carbonyls were present in the molecules. ^1H NMR spectrum contained signals for five quaternary methyl groups (all but one showing coincident chemical shifts between the two diastereomers), two monosubstituted phenyl groups, resonances for two mid field protons, one a singlet in each diastereomer (δ_H 5.283/5.275) and the other a doublet of doublets (δ_H 5.411/5.406), signals for two methylene protons (δ_H 3.05/3.02 and δ_H 2.857/2.847) and resonances for a downfield intramolecular hydrogen bonded phenolic proton in each diastereomer (δ_H 12.08/12.07). The doubling of signals was more evident in the ^{13}C NMR spectrum since 53 resonances were observed suggesting that all but three of the carbon signals (δ_C 211.73, 128.38 and 47.46) in each diastereomer were non-coincident. The difference in chemical shift however for the remaining 25 pairs of signals were between 0.01 and 0.33 ppm with only six pairs deviating from each other by 0.09 ppm or more. Analysis of edited HSQC and HMBC spectra indicated that **1** and **2** contained a syncarpic acid partial structure since HMBC correlations from two mutually $^3J_{CH}$ coupled geminal methyl singlets (1.65 and 1.546) to carbons at 211.7, 165.13, 47.46 and from another two mutually $^3J_{CH}$ coupled geminal methyl singlets (1.13 and 1.34) to carbons at 211.7, 56.51 and 197.0 are characteristic for this moiety.[6, 7, 9, 12] A phloroglucinol partial structure was suggested from HMBC correlations observed between the hydrogen bonded phenolic proton at δ_H 12.08/12.07 and two upfield aromatic quaternary carbons at δ_C 105.5/105.38 and 106.16/106.08 and an aromatic oxygenated quaternary carbon at δ_C 158.62/158.67, from the fifth methyl singlet at δ_H 2.22 to upfield aromatic quaternary carbons at δ_C 104.26/104.43 and two downfield oxygenated aromatic quaternary carbons at δ_C 154.84/154.89 and 158.62/158.67 and from the methine proton at δ_H 5.283/5.275 to δ_C 106.16/106.08, 154.84/154.89 and 158.62/158.67. The phloroglucinol was assigned to be part of a flavanone partial structure since HMBC correlations were observed from the mutually coupled methylene and oxygenated methine protons (determined from analysis of COSY correlations) to a ketone carbon at δ_C 197.0 and from the aromatic protons at δ_H 7.45 to the oxygenated methine carbon at δ_C 79.0. The methine protons at δ_H 5.283/5.275 also had HMBC correlations to the syncarpic acid carbons at δ_C 113.04/113.24, 165.13/165.15 and 197.00/197.02 and the phenyl carbons at 144.10/144.06 and 128.23/128.18 and this indicated that a benzyl group linked the syncarpic acid with the 8-methyl-flavanone. The molecular formu-

la assigned from MS analysis finally required an ether linkage between C-1a and C-8 to be present in the molecules. Torellianone A and B are therefore diastereomers, either epimeric at C-6 or C-12. Unfortunately since the majority of the proton resonances were coincident between the two diastereomers and even the most dissimilar (H-13β) being only 0.03 ppm different in chemical shift the definitive assignment of $^{1}H/^{13}C$ data to a specific diastereomer could not be made.

An inseparable mixture of torellianone C (**3**) and D (**4**) was obtained as a yellow amorphous solid (3.0 mg). The (+) HRESIMS for **3** and **4** contained a single ion at *m/z* 503.2452 assigned to a protonated molecule in both diastereomers indicating both compounds had a molecular formula $C_{31}H_{34}O_6$. The IR spectrum of the mixture of **3** and **4** showed absorption bands at 1654 and 1715 cm^{-1} indicating carbonyls were present in the molecules. Similar to **1** and **2**, analysis of the ^{1}H NMR spectrum for the mixture of **3** and **4** revealed doubling of the majority of signals in a 2:1 ratio suggesting that a mixture of two isomers were present. Since no exchange correlations were observed in a ROESY spectrum this suggested that the doubling of signals was associated with an inseparable mixture of diastereomers.[10] The ^{1}H NMR spectrum contained signals for a monosubstituted phenyl group, five quaternary methyl groups, two secondary methyl groups, two aliphatic methylenes, one aliphatic methine, one oxygenated methine and an intramolecular hydrogen bonded phenol. The ^{13}C NMR spectrum contained 55 resonances of which 52 were clearly pairs present in a 2:1 ratio while the remaining three were associated with resonances that were co-incident in the two diastereomers. Analysis of HSQC data demonstrated that both isomers **3** and **4** had 17 carbon bound protons (seven methyl, two methylene, three methine and five aromatic protons). There were a lot of similarities between the ^{1}H and ^{13}C NMR spectra of **1** and **2** with **3** and **4** indicating that the diastereomers **3** and **4** also contained syncarpic acid and C-methylated flavanone moieties and this conclusion was supported by HMBC correlations from the ^{1}H and ^{13}C resonances associated with these partial structures. COSY correlations indicated that **3** and **4** contained a $CHCH_2CH(CH_3)_2$ spin system and HMBC correlations from one of the methine protons of this spin system at δ_H 4.24/4.21 (H-6) to the syncarpic acid carbons at δ_C 197.58/197.65 (C-5), 167.33/167.27 (C-1a) and 114.48/114.42 (C-5a) indicated that an isobutyl group was directly attached to the syncarpic acid moiety at C-6 in **3** and **4** instead of the phenyl group found in **1** and **2**. This methine proton (H-6) also showed HMBC correlations to two oxygenated quaternary aromatic carbons at δ_C 156.54/156.26 (C-8) and 156.74/156.94 (C-16) and an

upfield aromatic quaternary carbon δ_C 105.86/106.10 (C-7) suggesting that the isobutyl group was also directly attached to a phloroglucinol. Mutual HMBC correlations from the intramolecular hydrogen bonded phenolic protons and the aromatic methyl resonances to upfield aromatic quaternary carbon resonance at δ_C 106.26/106.23 (C-9) and oxygenated aromatic quaternary carbon resonance at 159.58/159.4830 (C-10) indicated that the flavanone was methylated at C-6 in **3** and **4** compared to **1** and **2** each of which contained a C-8 methylated flavanone. The aromatic methyl resonances also showed HMBC correlations to carbons at δ_C 156.54/156.26 (C-8) which further substantiated the conclusion that **3** and **4** contained a 6-methylflavanone substituted at C-8 by the isobutyl group. An ether linkage between C-1a and C-8 fulfilled the final degree of unsaturation required from MS analysis. Torellianone C and D were therefore diasteromers epimeric at either C-6 or C-14. In torellianone C (**3**) the resonance associated with the isobutyl group (H-1′, H_2-2′, H_3-3′ and H_3-4′) were shifted between 0.09 and 0.14 ppm upfield relative to those in the minor diastereomer torellianone D (**4**) and this suggested that, in torellianone C, the phenyl group attached at C-14 was on the same face as the isobutyl group and thus shields the isobutyl protons. A ROESY correlation was observed between H-1′ and H-2″/H-6″ in torellianone C, but not in the torellianone D and this further supported this relative configurational assignment. Torellianone C (**3**) was therefore assigned a 6*R**, 14*S** relative configuration while torellianone D (**4**) possessed a 6*S**, 14*S** relative configuration.

A mixture of torellianone E (**5**) and F (**6**) was obtained as a yellow amorphous solid. The (+) HRESIMS of the mixture of **5** and **6** had a single ion at *m/z* 521.2537, assigned to a protonated molecule indicating a molecular formula $C_{31}H_{36}O_7$ in both diastereomers. The IR spectrum of the mixture of **5** and **6** showed absorption bands at 1654 and 1715 cm^{-1} indicating carbonyl groups were present in the molecules. The ^1H NMR spectrum (Table 1) for **5** and **6** had remarkable similarities to that of **3** and **4** including signals that could be assigned to five quaternary methyl groups, two secondary methyls, a monosubstituted phenyl, two methylenes and three methines, but most of the signals were broad. Twelve sharp singlets of varying intensity were also observed between δ_H 9.60 and 14.20 in the spectrum and this again suggested that the spectrum represented a mixture of several compounds. A ROESY spectrum displayed exchange correlations between protons at δ_H 4.34 and 4.41, 1.80 and 1.99 and six pairs of signals in the downfield region and this clearly demonstrated that part of the complexity in the ^1H NMR spectrum could

be explained by the presence of two isomers for each diastereomer interconverting on the NMR time scale.[10, 11] The ratio of these interconverting isomers was ~ 4:1. The ^{13}C NMR spectrum was

Table 1. ^{1}H and ^{13} C NMR data for **1**, **2** (600 MHz), **3** and **4** (800 MHz) in $CDCl_3$

	1		**2**		**3**		**4**	
Position	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)
1	-	-	-	-	-	-	-	-
1a	165.13	-	165.15	-	167.33	-	167.27	-
2	47.46	-	47.46	-	47.52	-	47.54	-
3	211.73	-	211.72	-	212.09	-	212.14	-
4	56.51	-	56.53	-	56.27	-	56.22	-
5	197.00	-	197.02	-	197.58	-	197.65	-
5a	113.04	-	113.24	-	114.48		114.42	
6	33.14	5.283 (s)	33.16	5.275 (s)	25.21	4.24 (dd, *J*=7.2, 5.4 Hz)	25.47	4.21 (dd, *J*=6.3, 6.3 Hz)
7	106.16	-	106.08	-	105.86	-	106.10	-
8	154.84	-	154.89	-	156.54	-	156.26	-
9	104.26	-	104.43	-	106.3	-	106.21	-
10	158.26	-	158.33	-	159.58	-	159.48	-
11	-	-	-	-	105.29	-	105.27	-
12	79.02	5.411 (dd, *J*=3.1, 12.7 Hz)	79.11	5.406 (dd, *J*=13.2, 2.9 Hz)	197.07	-	197.28	-
13α	43.50	2.857 (dd, *J*=3.1, 17.2 Hz)	43.83	2.847 (dd, *J*=17.2, 2.9 Hz)	44.10	3.09 (dd, *J*=17.2, 13.2 Hz)	43.97	3.13 (dd, *J*=17.2, 13.7 Hz)
13β		3.05 (dd, *J*=12.7, 17.2 Hz)	43.2	3.02 (dd, *J*=13.2, 17.2 Hz)		2.89 (dd, *J*= 17.2, 3.2 Hz)		2.88 (dd, *J*= 17.2, 2.9 Hz)
14	196.90	-	196.96	-	79.7	5.42 (dd, *J*=13.2, 3.2 Hz)	79.7	5.39 (dd, *J*=13.7, 2.9 Hz)
15	105.50	-	105.38	-	-	-	-	-
16	158.62	-	158.67	-	156.74	-	156.96	-
17-Me	7.92	2.22 (s)	8.01	2.22 (s)	7.30	2.18 (s)	7.30	2.19 (s)
18-Me	24.51	1.13 (s)	24.48	1.13 (s)	24.35	1.39 (s)	24.48	1.38 (s)
19-Me	23.44	1.34 (s)	23.40	1.34 (s)	24.63	1.37 (s)	24.54	1.35 (s)
20-Me	25.09	1.546 (s)	25.12	1.55 (s)	25.11	1.59 (s)	25.08	1.60 (s)
21-Me	25.19	1.65 (s)	25.17	1.65 (s)	25.22	1.481 (s)	25.24	1.477 (s)
1'	144.10	-	144.06	-	47.13	1.28 (m)	47.31	1.37 (m)
2'/6'	128.23	7.31 (dd, *J*=1.6, 7.3 Hz)	128.18	7.30 (dd, *J*=1.6, 7.3 Hz)	25.11	1.35 (m)	25.06	1.49 (m)
3'	128.38	7.24 (t, *J*=7.3 Hz)	128.38	7.22 (t, *J*=7.3 Hz)	22.92	0.67 (d, *J*=6.4 Hz)	23.44	0.79 (d, *J*=6.4 Hz)
4'	128.95	7.15 (tt *J*=1.2, 7.3 Hz)	128.97	7.14 (tt *J*=1.2, 7.3 Hz)	23.69	0.76 (d, *J*=6.5 Hz)	23.52	0.85 (d, *J*=6.5 Hz)
1"	138.55	-	138.58	-	138.31	-	138.2	-
*2"/6"	126.06	7.45 (m)	126.03	7.45 (m)	126.08	7.45 (d, *J*=7.6 Hz)	126.4	7.45 (d, *J*=7.6 Hz)
*3"/5"	129.03	7.45 (m)	129.04	7.45 (m)	128.99	7.47 (t, *J*=7.6 Hz)	128.93	7.47 (t, *J*=7.6 Hz)
*4"	126.79	7.41 (m)	126.76	7.41 (m)	128.99	7.41 (m)	128.93	7.41 (m)
16-OH/10-OH	-	12.08 (s)	-	12.07 (s)	-	12.14 (s)	-	12.17 (s)

* These signals showed second order couplings. The reported multiplicities and couplings are approximations.

even more complex with over 80 signals being observed between δ_C 7 and 214. The majority of these signals appeared as ~ 28 bands of four signals clustered together (less than 0.5 ppm apart) each in an approximate ratio of 10:5:3:2. This further suggested that the spectrum represent-edtwo diastereomers present in 2:1 ratio with each rotating between two conformations thus leading to signals for four isomers. Given that the molecular weight of **5** and **6** was 18 amu larger than torellianone C and D and contained all of the same features present in **1** and **2**, this suggest-ed that one less ether linkage was present in **5** and **6** compared to **1** and **2**. This would result in three downfield exchangeable protons in each isomer and 12 all up for the four isomers. Analysis of HSQC, COSY and HMBC correlations supported this hypothesis. A syncarpic acid moiety was assigned from the characteristic HMBC correlations from two geminal methyl resonances at δ_H 1.50/1.34 to carbons at ~ δ_C 212/176/49 and another two geminal methyl resonances at δ_H

1.40/1.34 to carbons at ~ δ_C 212/203/55. A 8-methylflavanone was clearly identified from HMBC correlations between the aromatic protons H-2'/H-6' and the oxygenated methine carbon, C-12, from the methylene protons (H_2-13) vicinal to H-12 to a ketone carbon (C-14), from the aromatic methyl resonance (17-CH_3) to two oxygenated (C-8, C-10) and one upfield (C-9) aromatic quaternary carbons, from the downfield hydrogen bonded phenolic resonances at δ_H 13.97/13.95 (16-OH) to an additional oxygenated (C-16) and two upfield (C-7, C-15) aromatic quaternary carbons and from the phenolic protons at δ_H 11.75/11.71 to two of the same aromatic carbons correlated to from 17-CH_3 (C-8, C-9). Analysis of COSY correlations established the partial structure –$CHCH_2CH(CH_3)_2$. HMBC correlations from the methine resonances at δ_H 4.33/4.36 to C-1, C-5 and C-5a of the syncarpic acid moiety and C-7, C-8 and C-16 of the phloroglucinol moiety established that C-6 linked the syncarpic acid with the 8-methyl flavanone. The third set of downfield exchangeable resonances at δ_H 9.91/9.84 showed HMBC correlations to C-1 and C-2 indicating that a hydroxyl group was attached to C-1. On the basis of this analysis, the 2D structure of the four isomers was established. ROESY analysis was undertaken to assign the sets of NMR data to each of the conformational and configurational isomers. ROESY correlations between 1-OH and 16-OH in all four isomers indicated that there was a hydrogen bond between 1-OH and 16-OH while the chemical shift of 8-OH in all isomers suggested that it was hydrogen bonded to the ketone oxygen, O-2.[7] Intense ROESY correlations from 8-OH to the methylene protons H-1′ and from 1-OH to H-6 in both major conformers of **5** and **6** suggested that the isobutyl side chain was on the same side as 8-OH. In the minor conformers of **5** and **6**, ROESY correlations were observed between 8-OH and H-6 and between 1-OH and H-1′, indicating that the isobutyl group was on the side closest to 1-OH in these conformers. A ~ 1ppm downfield shift of 8-OH and a ~ 0.7 ppm upfield shift of 1-OH in the major conformers relative to the minor conformers in both configurational isomers was also in keeping with the deshielding effect of the isobutyl group on the protons in close proximity to it. Unfortunately the protons associated with the flavanone stereogenic center were too far away from the isobutyl side chain to show ROESY correlations. However, a clear trend to chemical shift differences for the exchangeable protons provided evidence to establish the configuration at C-12 relative to C-6 in all isomers. If the four isomers are drawn with the same configuration at C-12, (with C-12 situated below the plane of the phloroglucinol ring, Figures 2 and 3), two isomers (**5a** and **6**) have the

β-triketone moiety situated above the plane of the phloroglucinol ring and in the other two isomers (**5** and **6a**) it is situated below the plane of the phloroglucinol ring. The chemical shift of 1-OH, 8-OH and 16-OH are downfield shifted (between 0.02 and 0.08 ppm) in the isomers with the β-triketone below the phloroglucinol ring compared to those above the plane. This can be explained by the slight deshielding effect of the O-11, C-12-C-13 bond (which also lies below the plane of the phloroglucinol ring) on resonances that are below the plane of the phloroglucinol. Likewise H-12 is downfield shifted in **5** compared to **6** because the β-triketone is on the same side of the phloroglucinol ring plane in **5** and thus deshields H-12. This evidence allowed **5** and **6** to be assigned 6*R**, 12*R** and 6*S**, 12*R** relative configurations respectively. The intractable separation of diastereomeric mixtures of β-triktone/flavanone adducts has been consistently reported in the literature.[13, 14] Likewise interconverting conformers of phloroglucinol/β-triketone adducts are not without precedent and in fact all ten compounds in this class that are not constrained by a pyran ring between the β-triketone and the phloroglucinol have been reported as interconverting conformers.[15] There have been three examples of β-triketone/flavanone adducts reported in the literature and all were isolated as mixtures represented by four sets of NMR signals.[12, 13, 15] The ^{13}C NMR data for the four isomers of one of these compounds (**12**) reported from *Kunzea ambigua* and *K. baxterii* was identical with that which we obtained.[13] Even the ratio of the isomers reported matched closely with our findings. No 2D NMR analysis was originally reported for **12** and since we observed no mutual HBMC correlations from the hydrogen bonded phenol (16-OH) and the aromatic methyl (17-CH_3) and since H-6 showed HMBC correlations to all three carbons substituted by hydroxyl groups we believe that the structure **12** was incorrectly assigned and should be revised to **5** and **6**.

To understand the unusual downfield chemical shift and sharpness of the hydroxyl protons of **5** and **6** molecular modelling was performed using Macromodel with conformational searching using a mixed MCMM/low-mode search. This modelling created the lowest energy conformations. In these conformers hydroxyl protons participate in hydrogen bonding with ketone oxygen as well as neighboring hydroxyls. (Figures 2 and 3). In the literature this type of hydrogen bonding has been reported for molecules such as corymbones and myrtucommulones.[9, 16]

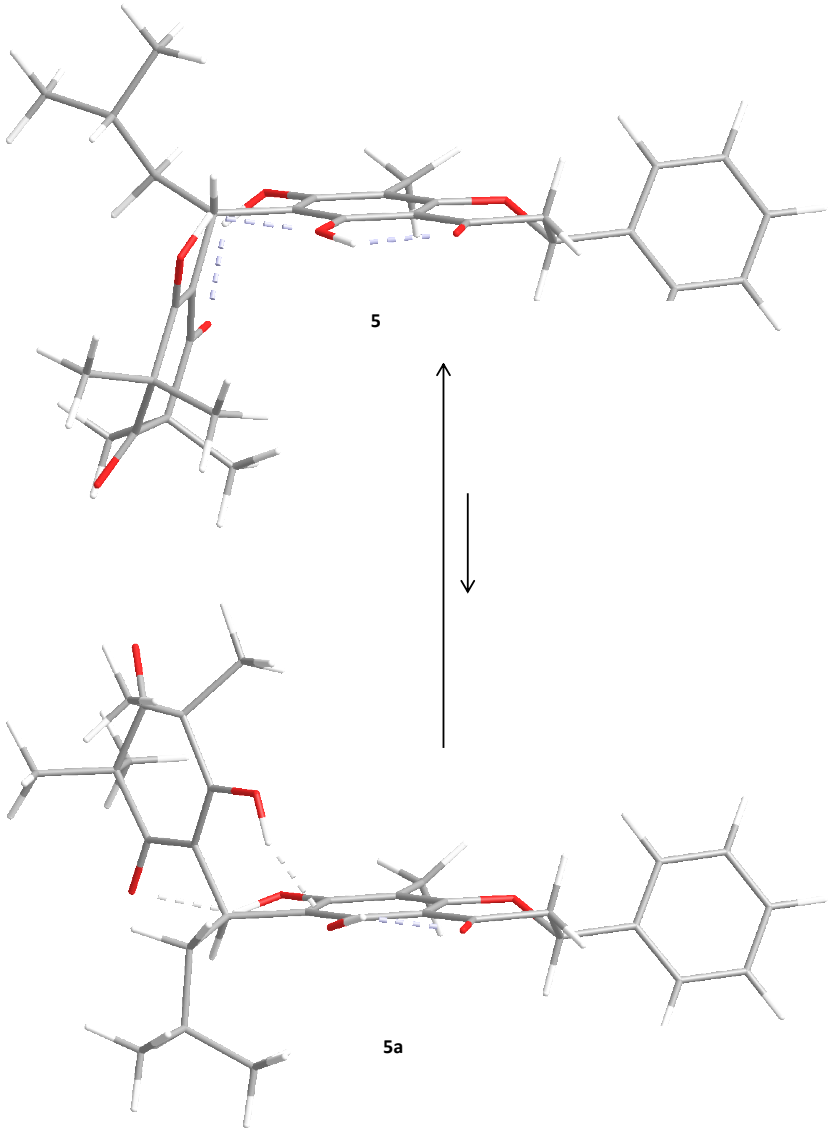


Figure 2. Three dimensional interconverting conformers of **5** and **5a**

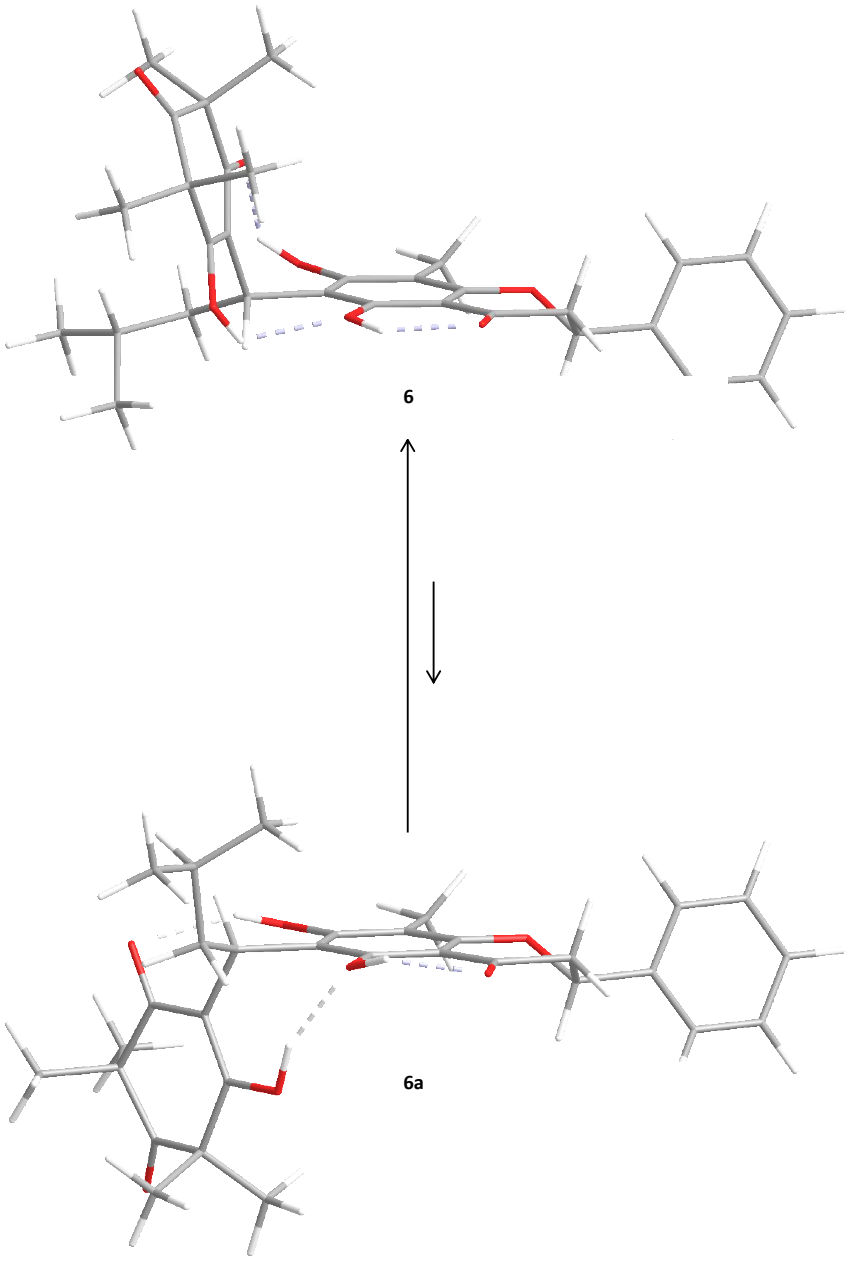


Figure 3. Three dimensional interconverting conformers of **6** and **6a**

Table 2. ^{1}H NMR and ^{13}C NMR data for **5** and **6** in $CDCl_3$ (800MHz)

Position	**5**		**5a**		**6**		**6a**	
	δ_C	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C	δ_H	δ_C	δ_H
1	176.3	-	176.8	-	176.1	-	176.9	-
1a	-	-	-	-	-	-	-	-
2	48.74	-	49.1	-	48.74	-	49.1	-
3	212.1	-	212.6		212.1	-	212.6	
4	55.37	-	54.42	-	55.37	-	54.52	-
5	203.4	-	203.6	-	203.5	-	203.4	-
5a	114.63	-	114.84		114.72	-	114.88	
6	27.8	4.33 (dd, *J*=7.8 Hz, 7.8 Hz)	29.48	4.42 (m)	27.7	4.36 (dd, 7.5 Hz, 7.5 Hz)	29.45	4.42 (m)
7	108.8	-	108.8	-	108.87	-	108.87	-
8	165.0	-	164.7	-	164.8	-	164.4	-
9	106.92	-	106.74	-	107.25	-	107.11	-
10	158.8	-	158.9	-	158.76	-	158.9	-
11	-	-	-	-	-	-	-	-
12	78.6	5.44 (dd, *J*=13.0, 3.2 Hz)	78.6	5.44 (dd, *J*=13.0, 3.2 Hz)	78.74	5.42 (dd, *J*=13.0, 3.6 Hz)	78.6	5.42 (dd, *J*=13.0, 3.6 Hz)
13α	43.02	3.04, (dd, *J*=17.2, 13.0 Hz)	43.02	3.04 (dd, *J*=17.2, 13.0 Hz)	43.09	3.04, (dd, *J*=17.5, 13.0 Hz)	43.09	3.04 (dd, *J*=17.5, 13.0 Hz)
13β	43.0	2.89 (dd, *J*=17.2, 3.2 Hz)	42.3	2.89 (dd, *J*=17.2, 3.2 Hz)	43.1	2.87 (dd, *J*=17.5, 3.6 Hz)	42.3	2.87 (dd, *J*=17.5, 3.6 Hz)
14	196.65	-	197.1	-	196.74	-	197.2	-
15	101.68	-	102.09	-	101.8	-	102.25	-
16	156.9	-	157.6	-	156.8	-	157.6	-
17-Me	8.04	2.07 (s)	8.32	2.08 (s)	8.12	2.08 (s)	8.42	2.08 (s)
18-Me	24.3	1.50 (s)	24.0	1.50 (s)	24.4	1.50 (s)	24.1	1.50 (s)
19-Me	22.24	1.34 (s)	22.24	1.34 (s)	22.15	1.34 (s)	22.15	1.34 (s)
20-Me	26.3	1.40 (s)	26.05	1.40 (s)	26.26	1.40 (s)	26.16	1.40 (s)
21-Me	27.27	1.34 (s)	27.12	1.34 (s)	27.34	1.34 (s)	27.17	1.34 (s)
1'	38.24	1.79 (ddd, *J*=14.3, 7.8 Hz, 6.9 Hz), 2.12 (ddd, *J*=14.3, 7.8 Hz, 6.9 Hz)	39.13	1.99 (m) 2.12 (m)	38.12	1.79 (tt, *J*=14.3, 7.8 Hz, 6.9 Hz), 2.12 (dt, *J*=14.3, 7.8 Hz, 6.9 Hz)	39.01	1.99 (m), 2.12 (m)
2'	26.95	1.40 (m)	27.01	1.40 (m)	26.98	1.39 (m)	27.01	1.40 (m)
3'	22.39	0.86 (d, *J*=6.7 Hz)	22.39	0.86 overlaps with **5**	22.44	0.88 overlaps with **5**	22.44	0.86 overlaps with **5**
4'	22.67	0.88 (d, *J*=6.7 Hz)	22.67	0.88 overlaps with **5**	22.70	0.88 overlaps with **5**	22.70	0.88 overlaps with **5**
1"	138.88	-	138.8	-	138.85	-	138.76	-
2"/6"	126.05	7.39 - 7.49 (m)	126.05	7.39 - 7.49 (m)	126.05	7.39 - 7.49 (m)	126.05	7.39 - 7.49 (m)
3"/5"	128.95	7.39 - 7.49 (m)	128.95	7.39 - 7.49 (m)	128.95	7.39 - 7.49 (m)	128.95	7.39 - 7.49 (m)
4"	128.77	7.39 - 7.49 (m)	128.77	7.39 - 7.49 (m)	128.77	7.39 - 7.49 (m)	128.77	7.39 - 7.49 (m)
1-OH	-	9.91	-	10.63	-	9.84	-	10.70
8-OH	-	11.75	-	10.72	-	11.71	-	10.78
16-OH	-	13.97	-	14.13	-	13.95	-	14.18

Torellianol A (**7**) was obtained as a yellow amorphous solid (3 mg). The (+) HRESIMS of **7** contained a sodiated molecule at *m/z* 297.2040, indicating a molecular formula $C_{15}H_{30}O_4$. Analysis of ^{1}H and ^{13}C NMR data for **7** in $CDCl_3$ (Table 3) revealed that the molecule had an element of symmetry since four ^{1}H resonances (δ_H 0.93, 1.16, 1.20 and 3.39) and six ^{13}C resonances (δ_C 20.9, 22.9, 28.5, 28.7, 40.9, 82.7) were double the intensity of the remaining signals. Correlations observed in the edited HSQC spectrum indicated that molecule has four methyls (δ_C 20.9, 22.9, 28.7), three methylenes (δ_C 28.5, 31.0, 36.6) and two methines (δ_C 76.8, 82.7). COSY correlations from δ_H 1.87-1.89 (H-7) to δ_H 1.30-1.33 (H-8) from (H_2-8) to δ_H 1.55 (H-9) and from H-9 to δ_H 0.93 (H-10/11) established that the molecule contained an isopentyl group. HMBC correlations from the methyl singlet at δ_H 1.16 (H-12/14) to δ_C 20.9, 40.9, 76.8, 82.7 (C-13/15, C-

2/4, C-3, C-1/5), the methyl singlet at δ_H 1.20 (H-13/15) to δ_C 28.7, 40.9, 77.8, 82.7 (C-12/14, C-2/4, C-3, C-1/C-5), the low field methine at δ_H 3.39 (H-1/H-5) to δ_C 20.9, 28.5, 36.6, 40.9, 75.9, 76.8, 82.7 (C-13/15, C-2/C-4, C-3, C-6, C-5/C-1) established that **7** contained a 4,4,6,6-tetramethylcyclohexane partial structure. The isopentyl side chain was attached to the cyclohexane moiety based on the HMBC correlation from δ_H 1.87-1.89 (H-7) to δ_C 75.9 (C-6). The NMR data acquired in $CDCl_3$ was inadequate to determine the functional groups attached to the three carbons (δ_C 75.9, 76.8, 82.7) that resonate at low field. Therefore NMR data for **7** was acquired in *d_6*-DMSO using a separate partially pure fraction that contained impurity signals at δ_H 2.3, 1.5 and 1.2 in the ^{1}H NMR spectrum (The more pure fraction which was used for NMR analysis in $CDCl_3$ has been used for biological activity testing). Inspection of the ^{1}H NMR data in *d_6*-DMSO revealed doublet couplings between methine protons at δ_H 3.41 and 3.12 (corresponding to δ_H 3.69 and 3.39 in $CDCl_3$) to the protons that resonate at δ_H 4.06 and 4.64 respectively. In the edited HSQC spectrum the protons at δ_H 4.06 and 4.64 did not show correlations to carbons and this indicated that **7** contained three hydroxymethines that were flanked by quaternary carbons. All of this information was used to establish gross chemical structure for **7**. The relative configuration of the four stereogenic centers in the molecule was assigned from analysis of ROESY data. In the $CDCl_3$ spectrum, correlations were observed from δ_H 3.69 to δ_H 1.16; δ_H 3.39 to δ_H 1.20, (1.30-1.33), (1.87-1.89); and δ_H 0.93 to δ_H (1.30-1.33). This suggested that the cyclohexyl group was in a chair conformation with H-3 in an axial position, while H_3-12/14, H-1/5, and the isopentyl side chain are in equatorial positions. Further to the ROESY correlations observed in the $CDCl_3$ spectrum ROESY correlations in *d_6*-DMSO (Figure 4) were also observed from δ_H 4.65 (1/5-OH) to δ_H 3.12, 3.41, 1.65-1.72, 0.95 (H-1/H-5, H-3, H-7, H_3-12/14). This indicated that 1/5-OH and H-3 were in axial positions on the β face of the molecule, while the isopentyl side chain and the methyls H_3-12/14 are also β but in equatorial positions. In addition, δ_H 0.95 (H_3-12/14) ROESY correlated to δ_H 4.06 (3-OH) and 3.41 (H-3) indicating that these methyl groups were equatorial and this was further supported by their downfield carbon chemical shift.[17] Torellianol A is therefore a tetrahydroxycyclohexane and is likely to be a reduced derivative of a β-triketone. Torellianol A possesses a plane of symmetry and is therefore achiral and thus had an optical rotation of zero. The closest related compounds to torellianol A reported in the literature are citriodorin isolated from *Corymbia citriodora*,[18] gallomyrtucommulones A - D isolated from *Myrtus com-*

munis,[19] gallomyrtucommulone E and F isolated from *Callistemon citrinus*,[20] and baeckenone F from *B. frutescens*[21] but in most of these compounds two of the syncarpic acid ketones are still intact. NMR data we reported for torellianol A was in close agreement to the NMR data reported for these known compounds. In particular the carbon resonances at δ_C 82.7 in $CDCl_3$ (C-1/5), δ_C 76.8 in $CDCl_3$ (C-3) and proton resonance at δ_H 3.69 in $CDCl_3$ (H-3) of torellianol A were also in the same range of δ_C 79-85 ppm, and δ_H 3.6-3.8 ppm of these known compounds.

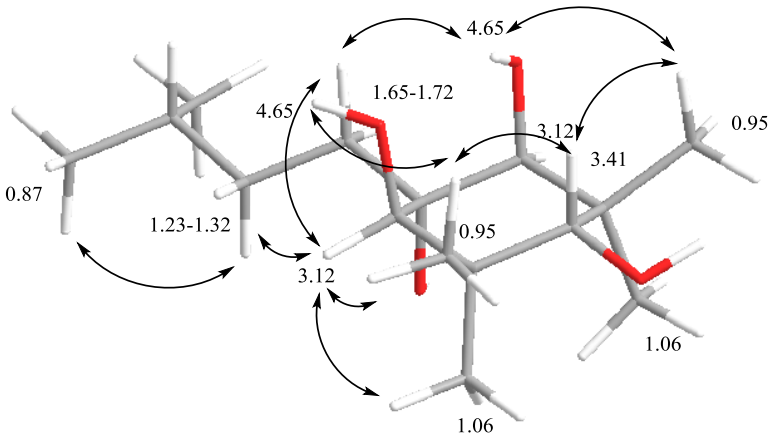


Figure 4. ROESY correlations observed for torellianol A in d_6-DMSO

Table 3.[1]H and [13] C NMR data for torellianol A (**7**) in $CDCl_3$ (800 MHz) and d_6-DMSO (800 MHz)

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$)	δ_C (d_6-DMSO)	δ_H (d_6-DMSO)
1/5	82.7	3.39 (s)	81.1	3.12 (d, *J*=7.7 Hz),
2/4	40.9	-	40.7	-
3	76.8	3.69 (s)	74.7	3.41 (d, *J*=6.2 Hz)
6	75.9	-	74.2	-
7	36.6	1.87-1.89 (m)	36.6	1.65-1.72 (m)
8	31.0	1.30-1.33 (m)	30.1	1.23-1.32 (m)
9	28.5	1.55 (n, *J*=6.8 Hz)	28.5	1.44 (n, *J*=6.7 Hz)
10/11	22.9	0.93 (d, *J*=6.7 Hz)	22.9	0.87 (d, *J*=6.7 Hz)
12/14	28.7	1.16 (s)	29.3	0.95 (s)
13/15	20.9	1.20 (s)	21.3	1.06 (s)
1/5-OH	-	-	-	4.65 (d, *J*=7.7 Hz)
3-OH	-	-	-	4.06 (d, *J*=6.2 Hz)
6-OH	-	-	-	4.00 (s)

Table 4. ^{1}H and ^{13}C NMR data for kunzeanone B (**8**) in $CDCl_3$ (800 MHz)

Position	δ_C	δ_H
1	166.9	-
2	47.7	-
3	212.2	-
4	56.3	-
5	197.5	-
6	114.6	-
7	25.0	4.30 (1H, dd, 6.3, 6.3 Hz)
8	46.7	1.38–1.46
9	25.3	1.38–1.50
10	23.2	0.879 (3H, d, 6.5 Hz)
11	23.5	0.88 (3H, d, 6.5 Hz)
12	24.4	1.37 (3H, s)
13	24.6	1.41 (3H, s)
14	25.2	1.47 (3H, s)
15	25.1	1.60 (3H, s)
2′	79.1	5.41 (1H, dd, 2.9, 13.2 Hz)
3′	44.0	3.11 (1H, dd,13.2, 17.0 Hz)
		2.90 (1H, dd, 2.9, 17.0 Hz)
4′	197.2	-
4′a	105.2	-
5′	158.4	-
6′	107.5	-
7′	156.5	-
8′	104.6	-
8′a	158.0	-
9′	7.9	2.16 (3H, s)
1″	138.7	-
2″, 6″	126.0	7.40–7.49 (m)
3″, 5″	129.0	7.40–7.49 (m)
4″	128.9	7.40–7.49 (m)
5′-OH	-	12.16 (1H, s)

In the original paper that reported the structure of kunzeanone B[13] the ^{1}H and ^{13}C NMR data presented in the experimental section was in fact identical to that reported for kunzeanone A. To correct this error, we have also provided the full ^{1}H and ^{13}C NMR data (Table 4) for kunzeanone B that we have assigned through detailed analysis of 2D NMR data.

Torellianones A-F are all examples of β-triketone/flavonone adducts. Related compounds in this class kunzeanone A, kunzeanone B, BF-4, BF-5, BF-6, leucadenones A-D, lumiaflavones A-C and Callistrilones A and B have been isolated from *Kunzea ambigua*, *Baeckea frutescens*, *Melaleuca leucadendron*, *Luma chequen* and *Callistemon rigidus* respectively, all plants from the Myrtaceae.[13-15, 22-24] Torellianones A and B are most closely related to kunzeanone A (**10**) and B (**11**) since they differ only by the replacement of the C-6 isobutyl group in **10** and **11** with a phenyl group in **1** and **2**. It is interesting that **10** and **11** are sufficiently structurally dissimilar that we and Ito *et al* could successfully separate the diastereomers, however, replacing the isobutyl group with a phenyl group makes separating the diastereomers intractable. Torellianones C and D (**3**, **4**) are the C-6 isobutyl analogs of BF-6. Torellianones E and F (**5**, **6**) are the pyran ring opened form

of kunzeanone A and B (**10**, **11**). Torellianol A is a tetrahydroxycyclohexane in which the three ketone groups of the syncarpic acid moiety have been reduced to hydroxyl groups.

Torellianones A-F (**1**-**6**) and torellianol A (**7**) were tested for antiplasmodial activity against the chloroquine sensitive 3D7 strain of *Plasmodium falciparum* and antibacterial activity against *Staphylococcus aureus* ATCC 157293. These compounds were also evaluated for cell cytotoxicity using the mammalian cell line HEK-293. A mixture of torellianone A and B and torellianol A were inactive towards 3D7 *Plasmodium falciparum* at a dose of 40 μM. Diastereomeric mixtures of torellianones C and D and torellianones E and F had antiplasmodial IC_{50} values of 7.2 ± 1.2 μM and 3.2 ± 0.3 μM respectively. In cell cytotoxicity testing, torellianones A and B and torellianol A were inactive towards the HEK-293 mammalian cell line at a dose of 100 μM. A mixture of torellianones C and D and a mixture of torellianones E and F inhibited HEK-293 cells by 32% and 75% at 100 μM respectively. Torellianones E and F, therefore had ~ 9-fold selectivity towards the malaria parasite. Reference compounds artesunate, chloroquine, dihydroartemisinin (DHA), puromycin, pyrimethamine and pyronaridine had IC_{50} values of ~4.2×10^{-4} μM, $4.4 \pm 0.15 \times 10^{-2}$ μM, $2.6 \pm 0.40 \times 10^{-4}$ μM, $9.4 \pm 2.6 \times 10^{-2}$ μM, $9.87 \pm 1.5 \times 10^{-4}$ μM, $7.33 \pm 3 \times 10^{-3}$ μM respectively against the 3D7 strain. Inhibition of HEK-293 cell growth by artesunate, chloroquine, dihydroartemisinin (DHA), puromycin, pyrimethamine and pyronaridine was 91%, 30%, 92%, 98%, 62% and 93% at 100 μM respectively. The lack of inhibition of *Plasmodium* growth by torellianol A indicates that the syncarpic acid moiety is likely to be essential for antiplasmodial activity. This was clearly supported by the IC_{50} of 3.37 μM activity of the simple β-triketone, woodsianone B isolated from *Angophora woodsiana.*[16] Among the active torellianone compounds; the mixture of torellianones E and F had the highest antiplasmodial activity of 3.2 ± 0.3 μM indicating that the uncyclized phloroglucinol contributes to higher antiplasmodial activity. The activity of torellianones E and F is similar to that of watsonianone A (5.3 μM) which also contains an uncyclized β-triketone.[6] In torellianones A and B, a phenyl ring is present at C-6 instead of the isobutyl group in torellianones E and F. The inactivity of the mixture of torellianone A and B could be due to the reduced solubility of these diastereomer as a result of increased lipophilicity. From the previous reports and from our recent study moderately potent antiplasmodial IC_{50} values of 0.29 μM, 1.0 μM and 1.8 μM against 3D7 *Plasmodium falciparum* have been reported from β-triketone compounds, watsonianone B, tomentosone A and rhodomyrtone re-

spectively. These results indicated that molecules containing a β-triketone moiety and a phloroglucinol group have higher antiplasmodial activity. In the torellianones, the presence of the flavonone part of the molecule, in addition to the β-triketone and phloroglucinol moieties might have reduced the polarity of the compounds. This non-polarity must have affected the solubility of the compounds, which in turn might have affected the antiplasmodial activity.

All the new and known compounds isolated from *Corymbia torelliana* were screened for antibacterial activity and only the mixture of torellianones E and F very weakly inhibited the growth of *Staphylococcus aureus* ATCC 157293 at a MIC of 630 μM. β-Triketone compounds, tomentosenol A, callistenone C, bullataketals A and B and semimyrtucommulone previously isolated from the species of Myrtaceae, *Rhodomyrtus tomentosa,*[25] *Callistemon lanceolatus,*[26] *Lophomyrtus bullata,*[27] *Myrtus communis*[28, 29] respectively had more potent antibacterial activities. All these compounds including torellianones C and D lacked pyran rings. These data provided further evidence that β-triketones containing a phloroglucinol but lacking a pyran ring show antibacterial activity.

7.4 Experimental Section

7.4.1 General Experimental Procedures

Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were obtained on a Shimadzu UV-1800 UV spectrophotometer. IR data were obtained using a Thermo Scientific Nicolet iS5 iD5 ATR spectrometer. NMR spectra were recorded on a Bruker BioSpin GmbH 800 MHz spectrometer and Varian Inova 600 MHz NMR spectrometers at 25°C with solvent peaks referenced to δ_H 7.26 and δ_C 77.16 in $CDCl_3$ and δ_H 2.49 and δ_C 39.5 in DMSO. ESI-TOF data were recorded on an Agilent 6530-accurate mass Q-TOF LC/MS mass spectrometer. Altech Davisil 30-40 μm 60 Å C_{18} silica and diol bonded silica 40-70 μm, 60 Å were used to adsorb the flower extract prior to HPLC separation. A Merck Hitachi L7100 pump equipped with a Merck Hitachi L7455 PDA detector and a Merck L7250 autosampler were used for HPLC separations. HPLC columns used were Betasil C_{18} 150 x 21.2 5 cm 100 Å Zorbax SB-Phenyl 21.2 mm i.d. x 25 cm and Diol YMC 150 x 20 mm, i.d. 5 mm. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade and the H_2O was Millipore Milli-Q PF filtered.

7.4.2 Plant Material

Flowers of *C. torelliana* were collected from Nerang in Southeast Queensland. A voucher specimen, ACMYRT001 is deposited at the Griffith School of Environment.

7.4.3 Extraction and Isolation

The air dried ground flowers of *C. torelliana* (100 g) were extracted with MeOH to yield a crude extract (15 g). An aliquot of the MeOH extract (4 g) was adsorbed on to the C_{18} silica gel (4 g) and a portion (1 g) of it was filled into the HPLC pre column cartridge (10 mm x 20 mm) then connected in sequence to a C_{18} bonded silica HPLC column (21 mm x 150 mm) (preconditioned in H_2O) and eluted with a gradient from 100% H_2O to 100% MeOH over 60 min. at a flow rate of 9 mL/min. After that the column was further eluted with MeOH for 10 min. Fractions were collected every min. This was repeated four times and every alternate fraction from the first HPLC separation was analyzed by ^{1}H NMR. Based on NMR analysis, fractions 48 to 70 from each HPLC separation were combined and evaporated to obtain a gum (0.95 g). This gum was absorbed on to diol bonded silica gel (1 g) and loaded into a refillable HPLC column (10 mm x 20 mm) then connected in sequence to the Diol bonded silica gel HPLC column (21 mm x 150 mm) and eluted with a gradient from hexane to DCM over 60 min at a flow rate of 9 mL/min and then further eluted for 10 minutes with 100% DCM yielding a 1:1 mixture of torellianones A and B (3.3 mg), a 2:1 mixture of torellianone C and D (3 mg), a mixture of torellianones E and F (23 mg), and the known compounds 4*R*-ficifolidione (10 mg), 4*S*-ficifolidione (1 mg), kunzeanone A (1.8 mg), kunzeanone B (2 mg). In the process of further isolation of torellianones A-F, another crude extract (10 g) was absorbed onto diol bonded silica gel (10 g) and loaded into a refillable HPLC column (25 mm x 75 mm), connected in sequence to the diol bonded silica gel MPLC column (150 mm x 50 mm) and eluted with gradient from 100% hexane to 100% DCM over 150 mins to give 150 fractions. Every other fraction was analyzed by ^{1}H NMR and similar fractions 98 -150 (284 mg) combined and adsorbed onto C_{18} (284 mg). This C_{18} adsorbed fraction was loaded into a refillable HPLC column (10 mm x 20 mm), connected in sequence to a phenyl bonded silica gel HPLC column and eluted with gradient from 100% H_2O to 100% MeOH to obtain torellianol A (3 mg).

7.4.4 Antiplasmodial Assay

Antiplasmodial activities of the new compounds (**1**-**7**) were evaluated according to the method described by Duffy and Avery, 2012.[30] Initially, the compounds (**1**-**7**) were incubated in the presence of 2 or 3% of 3D7 parasite and 0.3% hematocrit in a total assay volume of 50 µl, for 72 h at 37°C and 5% CO2, in Poly-D-lysine coated Cell Carrier Imaging plates. Once the incubation was completed, plates were incubated with DAPI (4', 6-diamidino-2-phenylindole) in the presence of saponin (from Quillaja Bark) and Triton X-100 and incubated for a further 5 h at room temperature in the dark before imaging on the OPERATM HTS confocal imaging system. The digital images gained were then investigated using the PerkinElmer Acapella spot detection software where spots which fulfill the criteria established for a stained parasite are counted. The % inhibition of parasite replication was calculated using DMSO and Artemisinin control data.

7.4.5 Antibacterial Assay

The antibacterial activities of the compounds **1**-**7** were determined using a broth micro dilution method against *S. aureus* ATCC 157293 bacterium. Assay was conducted in 96 well sterilized microtiter plate containing pure compounds at a concentration of 0.5 mM to 0.002 mM. Compounds were dissolved in 100% DMSO. To each well 25 µL of double strength broth was added and then 5 µL of compounds at 10 mM of concentration was added to the well followed by 50 µL of inoculum and 20 µL of sterile water. Plates were incubated at 37°C while being shaken at 100 rpm, for 6 hours. After this period 10 µL of the resazurin stock solution (704 µmol) was added and the plate was incubated for a further hour. After colour has been developed inhibition relative to resazurin was determined using a BMG Labtech FLUO star omega microplate fluorometer (excitation 570 nm; emission 620 nm). All the experiments were carried out in physical containment level 2 (PC2) laboratory facilities.

Torellianones A and **B** (**1**, **2**). Yellow amorphous solid (3.3 mg). FTIR ν_{max}: 3730, 2929, 2870, 1714, 1658, 1635, 1454, 1379, 1172, 1042 cm^{-1}. ^{1}H NMR (Table 1). ^{13}C NMR (Table 1). HRESIMS (Positive mode) observed *m/z* 523.2131 $(M+H)^{+}$ indicating a molecular formula $C_{33}H_{30}O_6$ (calcd for $C_{33}H_{31}O_6^{+}$ 523.2121).

Torellianones C and **D** (**3**, **4**). Yellow amorphous solid (3.0 mg). FTIR ν_{max}: 2950, 1650, 1635, 1450, 1370, 1160 cm^{-1}. ^{1}H NMR (Table 1). ^{13}C NMR (Table 1). HRESIMS (Positive mode) observed *m/z* 503.2452 $(M+H)^+$ indicating a molecular formula $C_{31}H_{34}O_6$ (calcd $C_{31}H_{35}O_6^+$ 503.2434).

Torellianones E and **F** (**5**, **6**). Yellow amorphous solid (23.0 mg). FTIR ν_{max}: 2948, 1635, 1467, 1350, 1155 cm^{-1}. ^{1}H NMR (Table 2). ^{13}C NMR (Table 2). HRESIMS (Positive mode) observed *m/z* 521.2534 $(M+H)^+$ indicating a molecular formula $C_{31}H_{34}O_6$ (calcd $C_{31}H_{35}O_6^+$ 521.2539).

Torellianol A (**7**). Yellow amorphous solid (3.0 mg). FTIR ν_{max}: 3730, 2958, 2926, 2872, 1712, 1514, 1454, 1105 cm^{-1}. ^{1}H NMR (Table 3). ^{13}C NMR (Table 3). HRESIMS (Positive mode) observed *m/z* 297.2040 $(M+Na)^+$ indicating a molecular formula $C_{15}H_{30}O_4$ (calcd $C_{15}H_{30}O_4Na^+$ 297.2042).

7.5 References and Notes

1. Johnson, L. A. S.; Briggs, B. G., Three old southern families - Myrtaceae, Proteaceae and Restionaceae. In *Ecological Biogeography of Australia*, Keast, A., Ed. The Hague : W. Junk: 1981; Vol. 1, pp 427-469.
2. Stefanello, M. É. A.; Pascoal, A. C. R. F.; Salvador, M. J., *Chem. Biodivers.* **2011,** 8, 73-94.
3. Pal Singh, I.; Bharate, S. B., *Nat. Prod. Rep.* **2006,** 23, 558-591.
4. Ghisalberti, E. L., *Phytochemistry* **1996,** 41, 7-22.
5. Hill, K. D.; Johnson, L. A., *Telopea* **1995,** 6, 185-504.
6. Carroll, A. R.; Avery, V. M.; Duffy, S.; Forster, P. I.; Guymer, G. P., *Org. Biomol. Chem.* **2013,** 11, 453-458.
7. Carroll, A. R.; Urban, S.; Lamb, J.; Moni, R.; Guymer, G. P.; Forster, P. I.; Quinn, R. J., *J. Nat. Prod.* **2008,** 71, 881-883.
8. Khambay, B. P. S.; Beddie, D. G.; Hooper, A. M.; Simmonds, M. S. J., *Tetrahedron* **2003,** 59, 7131-7133.

9. Carroll, A. R.; Lamb, J.; Moni, R.; Guymer, G. P.; Forster, P. I.; Quinn, R. J., *J. Nat. Prod.* **2008,** 71, 1564-1568.
10. Carroll, A. R., *Tetrahedron Lett.* **2016,** 57, 281-284.
11. Donald G. Davis, A. B., *J. Magn. Reson.* **1985,** 64, 533-535.
12. Hiranrat, A.; Mahabusarakam, W., **2008,** 64, 11193-11197.
13. Khambay, B. P. S.; Beddie, D. G.; Simmonds, M. S. J., *Phytochemistry* **2002,** 59, 69-71.
14. Ito, H.; Iwamori, H.; Kasajima, N.; Kaneda, M.; Yoshida, T., *Tetrahedron* **2004,** 60, 9971-9976.
15. Lee, C.-K., *Tetrahedron Lett.* **1999,** 40, 7255-7259.
16. Senadeera, S. P. D.; Duffy, S.; Avery, V. M.; Carroll, A. R., **2017,** 27, 2602-2607.
17. Nasipuri, D., *Stereochemistry of organic compounds: principles and applications*. New Age International: 1994.
18. Shen, Z.; Yu, Q.; Wang, Y.; Lin, G., *Linchan Huaxue Yu Gongye* **1987**, 35-43.
19. Appendino, G.; Maxia, L.; Bettoni, P.; Locatelli, M.; Valdivia, C.; Ballero, M.; Stavri, M.; Gibbons, S.; Sterner, O., *J. Nat. Prod.* **2006,** 69, 251-254.
20. Khanh, P. N.; Duc, H. V.; Huong, T. T.; Son, N. T.; Ha, V. T.; Van, D. T.; Tai, B. H.; Kim, J. E.; Jo, A. R.; Kim, Y. H.; Cuong, N. M., *Fitoterapia* **2016,** 109, 39-44.
21. Nisa, K.; Ito, T.; Kodama, T.; Tanaka, M.; Okamoto, Y.; Asakawa, Y.; Imagawa, H.; Morita, H., *Fitoterapia* **2016,** 109, 236-240.
22. Labbé, C.; Coll, J.; Connolly, J. D.; Faini, F.; Farrugia, L. J.; Rycroft, D. S., *Collect. Czech. Chem. Commun.* **2002,** 67, 115-123.
23. Makino, M.; Fujimoto, Y., *Phytochemistry* **1999,** 50, 273-277.
24. Cao, J.-Q.; Huang, X.-J.; Li, Y.-T.; Wang, Y.; Wang, L.; Jiang, R.-W.; Ye, W.-C., *Org. Lett.* **2016,** 18, 120-123.
25. Liu, H.-X.; Zhang, W.-M.; Xu, Z.-F.; Chen, Y.-C.; Tan, H.-B.; Qiu, S.-X., *RSC Adv.* **2016,** 6, 25882-25886.
26. Rattanaburi, S.; Mahabusarakam, W.; Phongpaichit, S.; Carroll, A. R., *Tetrahedron* **2013,** 69, 6070-6075.
27. Larsen, L.; Benn, M. H.; Parvez, M.; Perry, N. B., *Org. Biomol. Chem.* **2005,** 3, 3236-3241.

28. Appendino, G.; Bianchi, F.; Minassi, A.; Sterner, O.; Ballero, M.; Gibbons, S., *J. Nat. Prod.* **2002,** 65, 334-338.
29. Kashman, Y.; Rotstein, A.; Lifshitz, A., *Tetrahedron* **1974,** 30, 991-997.
30. Duffy, S.; Avery, V. M., *Am. J. Trop. Med. Hyg.* **2012,** 86, 84-92.

Four new β-triketones from Australian plant *Corymbia torelliana*

Sarath P. D. Senadeera,[a,b] *Leonardo Lucantino,*[b] *Joshua Hayton,*[a] *Vicky M. Avery* [b] *Anthony R. Carroll,**[,a,b]

[a] Environmental Future Research Institute, Griffith University, Gold Coast, QLD 4222, Australia.
[b] Eskitis Intitute, Griffith University, Brisbane, QLD 4111, Australia.

KEYWORDS β-triketones, *Corymbia torreliana, Myrtaceae,* antiplasmodial activity, antibacterial activity

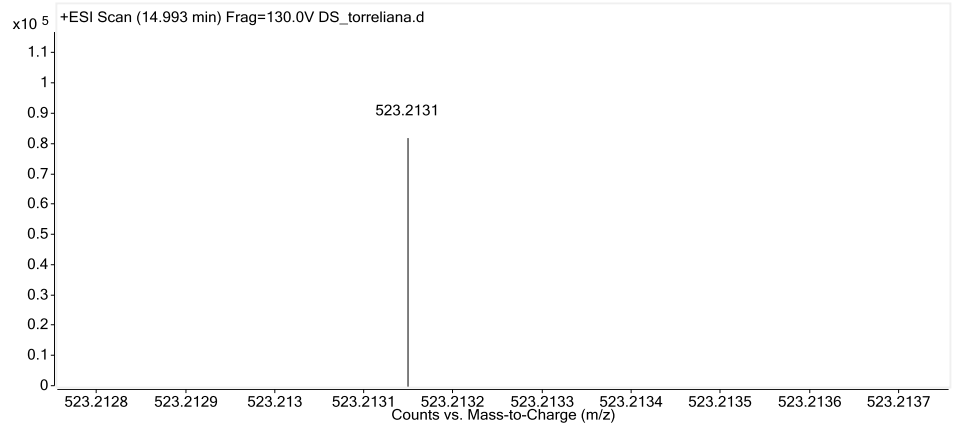


Figure S 1: Mass spectrum of torellianone A

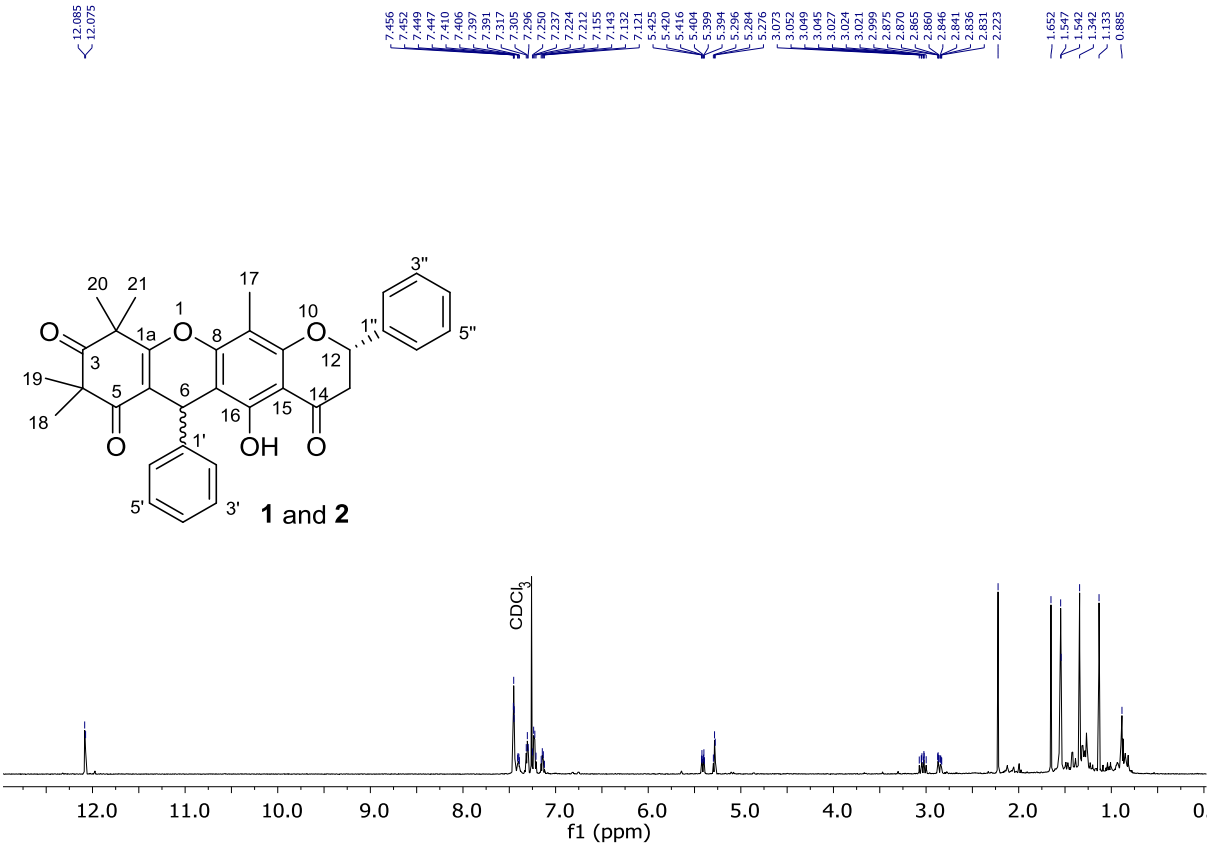


Figure S 2:[1]H NMR spectrum of torellianones A and B in $CDCl_3$ (600 MHz)

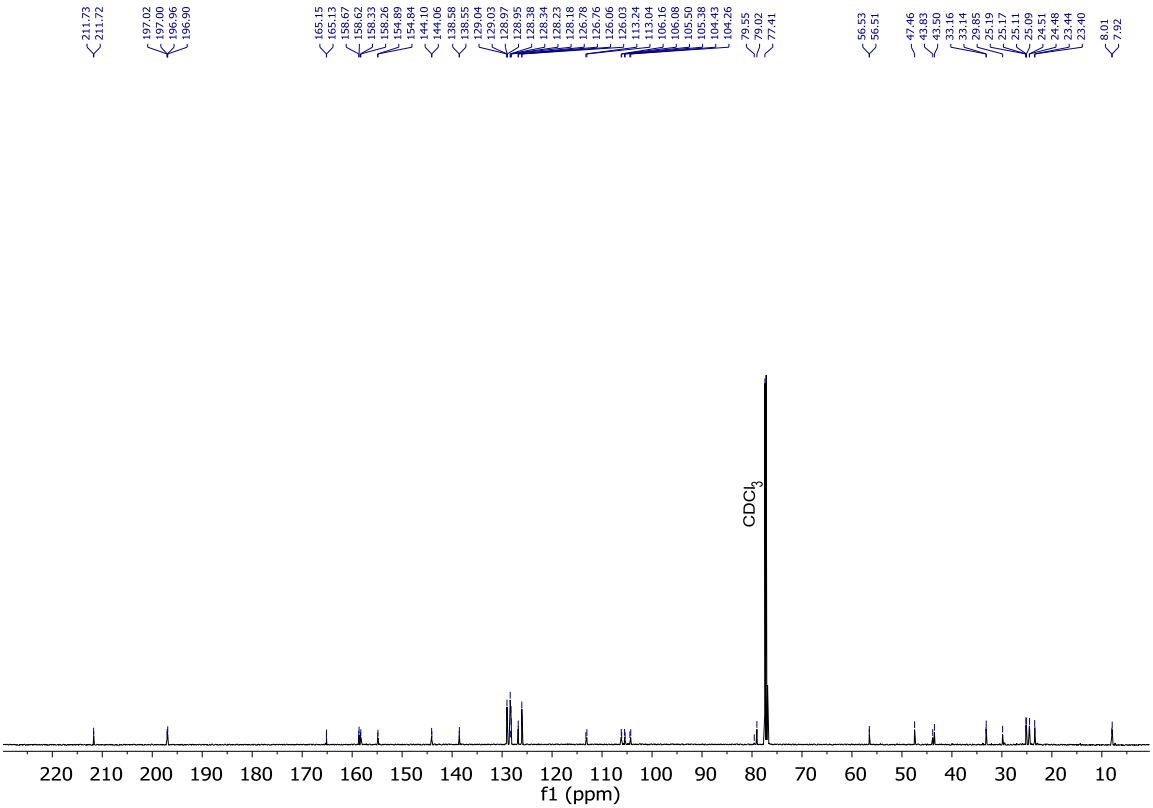


Figure S 3: ^{13}C NMR spectrum of torellianones A and B in $CDCl_3$ (600 MHz)

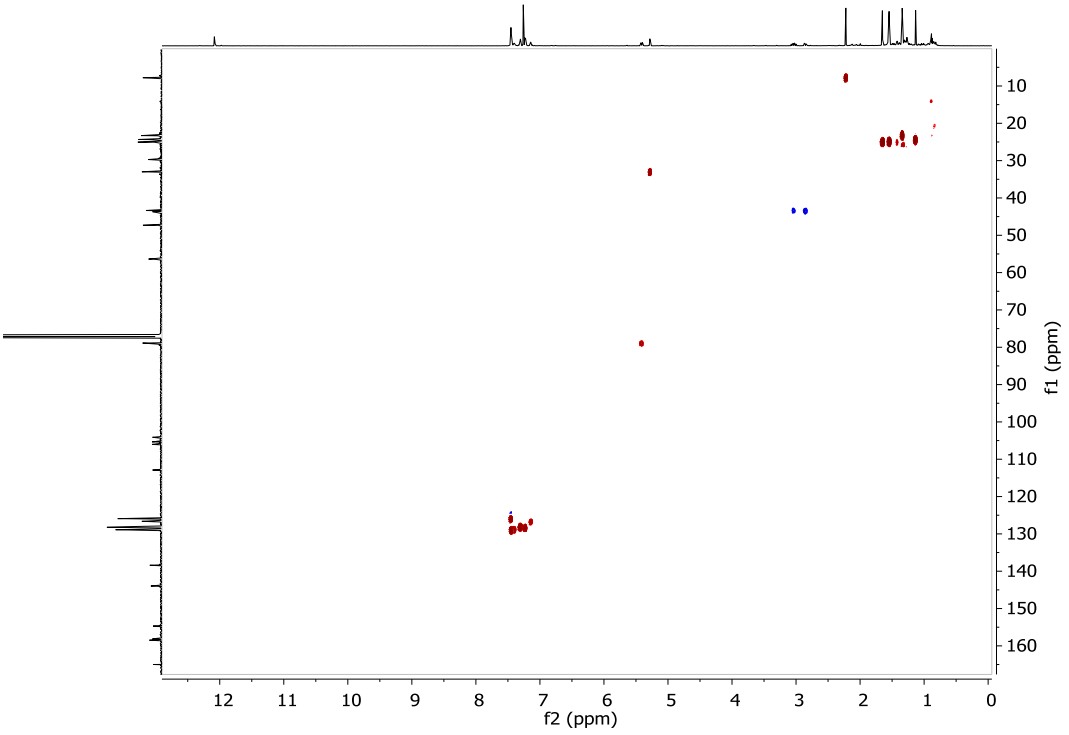


Figure S 4: HSQC spectrum of torellianone A and B in $CDCl_3$ (600 MHz)

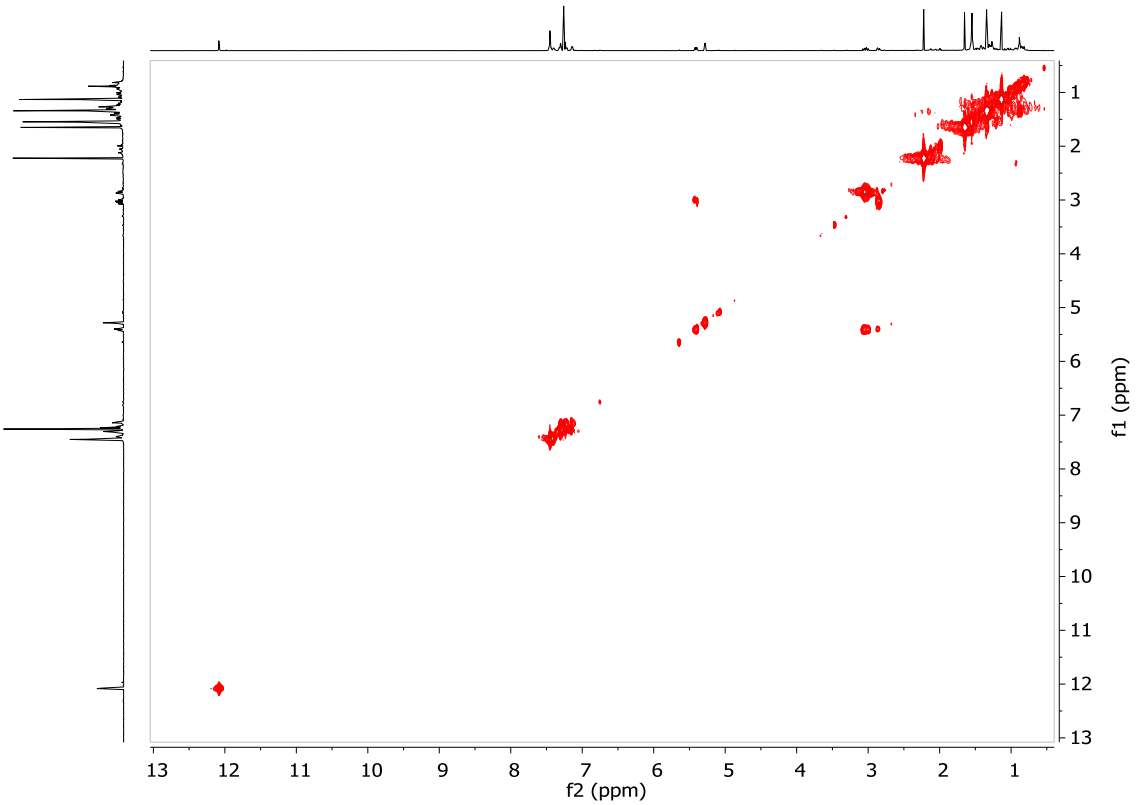


Figure S 5: COSY spectrum of torellianone A and B in $CDCl_3$ (600 MHz)

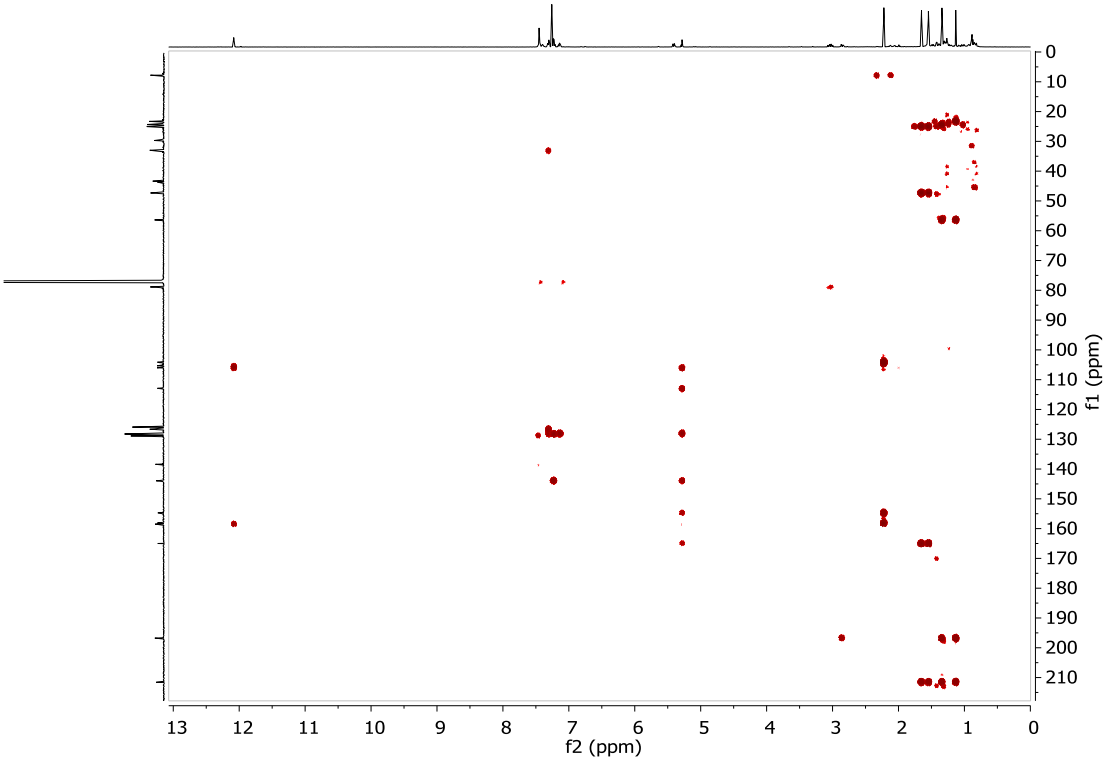


Figure S 6: HMBC spectrum of torellianone A and B in $CDCl_3$ (600 MHz)

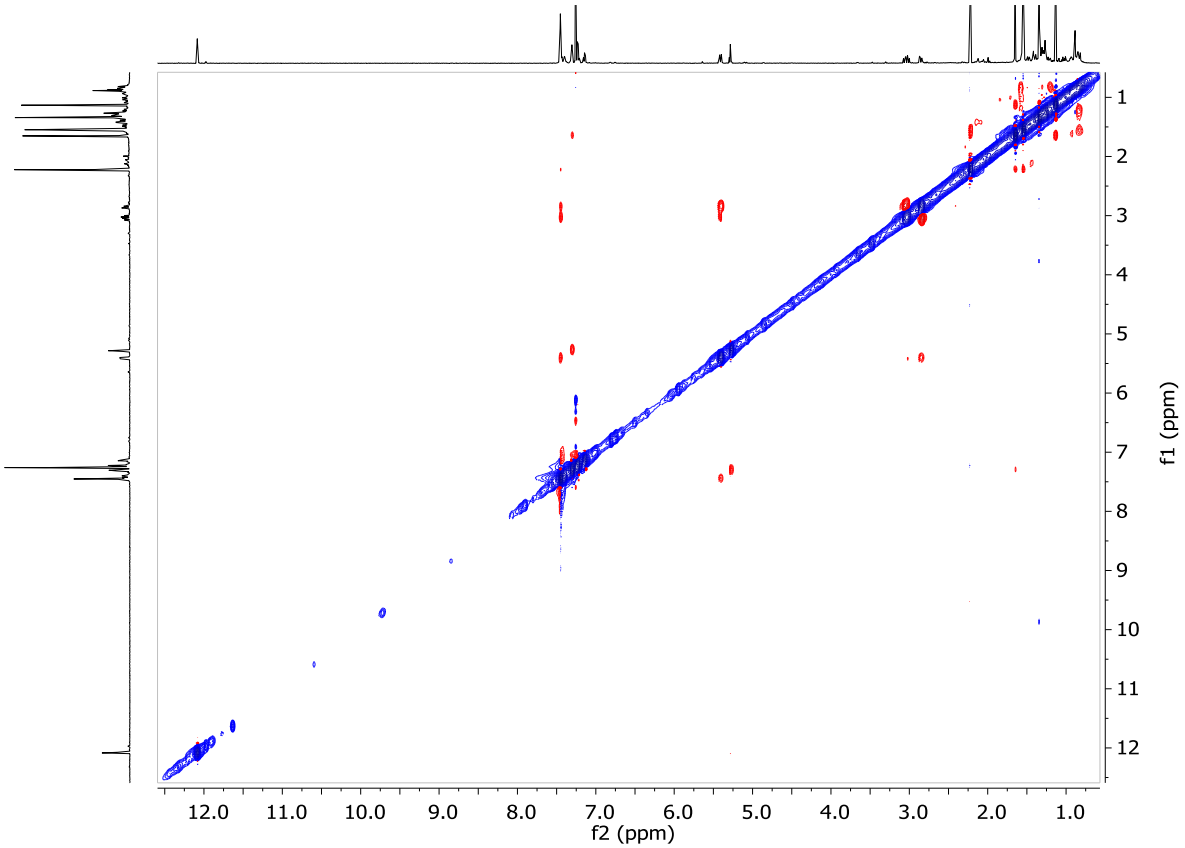


Figure S 7: ROESY spectrum of torellianone A and B in $CDCl_3$ (600 MHz)

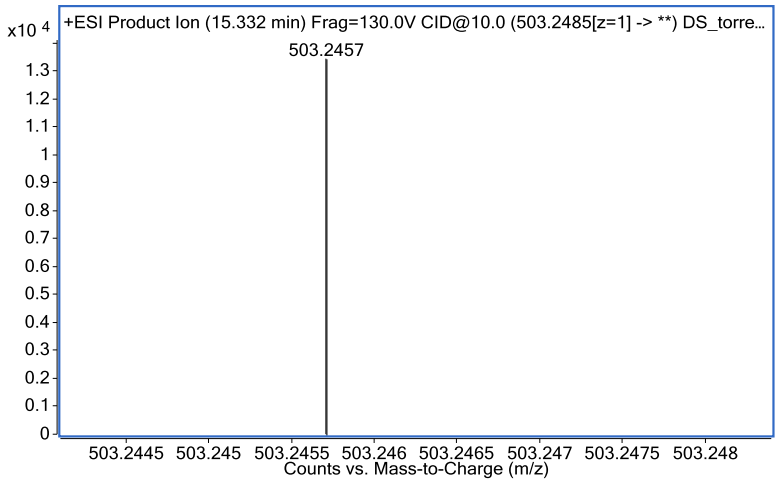


Figure S 8: Mass spectrum of torellianone C

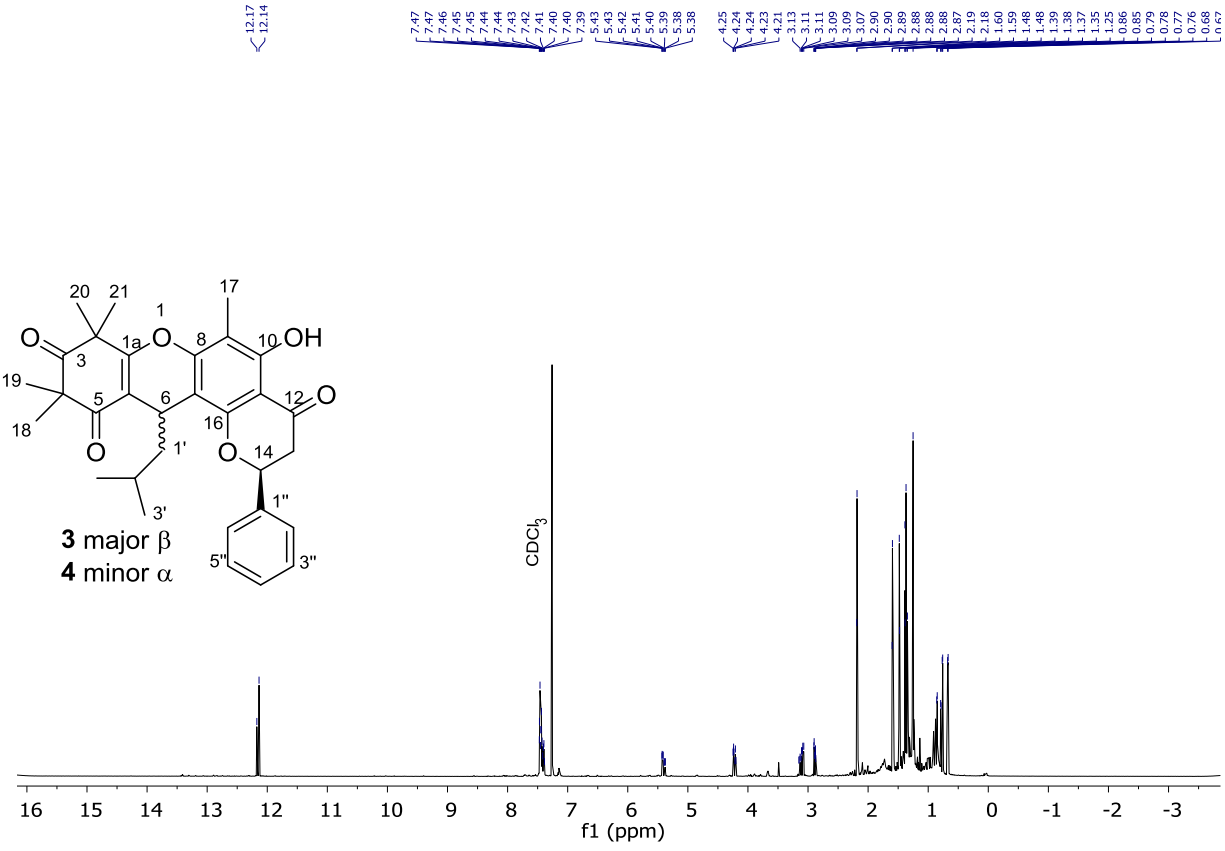


Figure S 9: ^{1}H NMR spectrum of torellianone C and D in $CDCl_3$ (800 MHz)

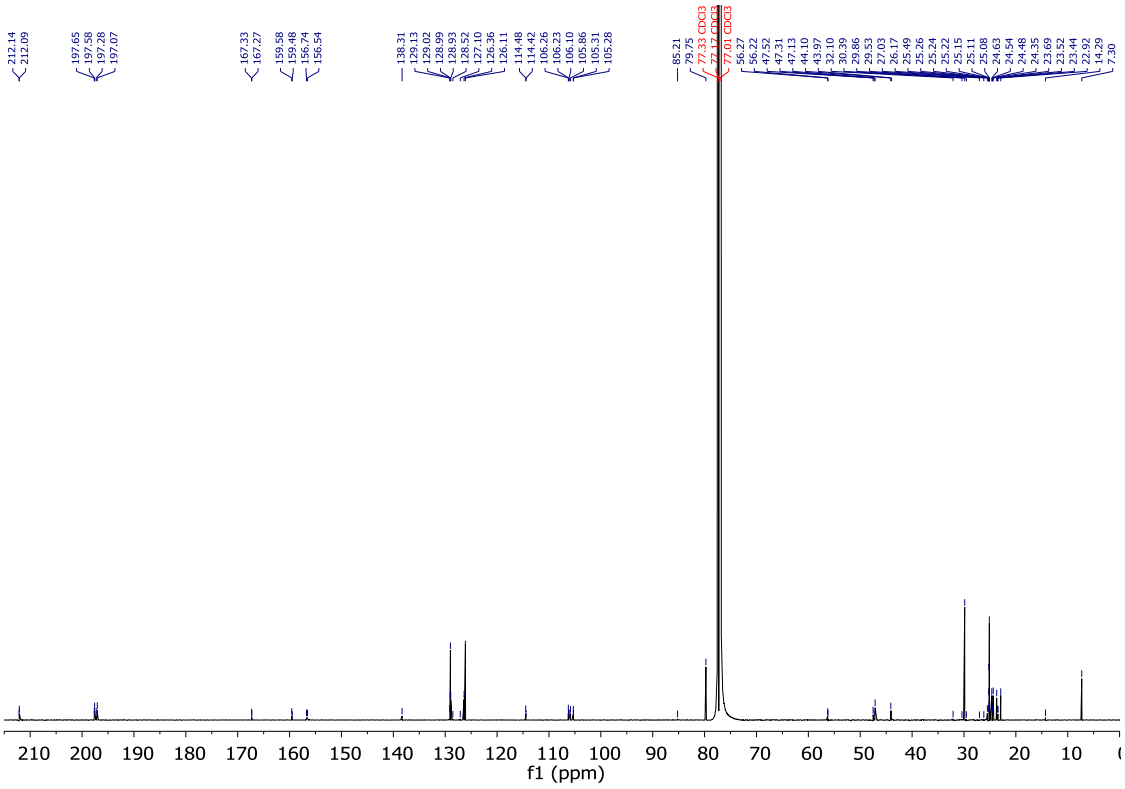


Figure S 10: ^{13}C NMR spectrum of torellianone C and D in $CDCl_3$ (800 MHz)

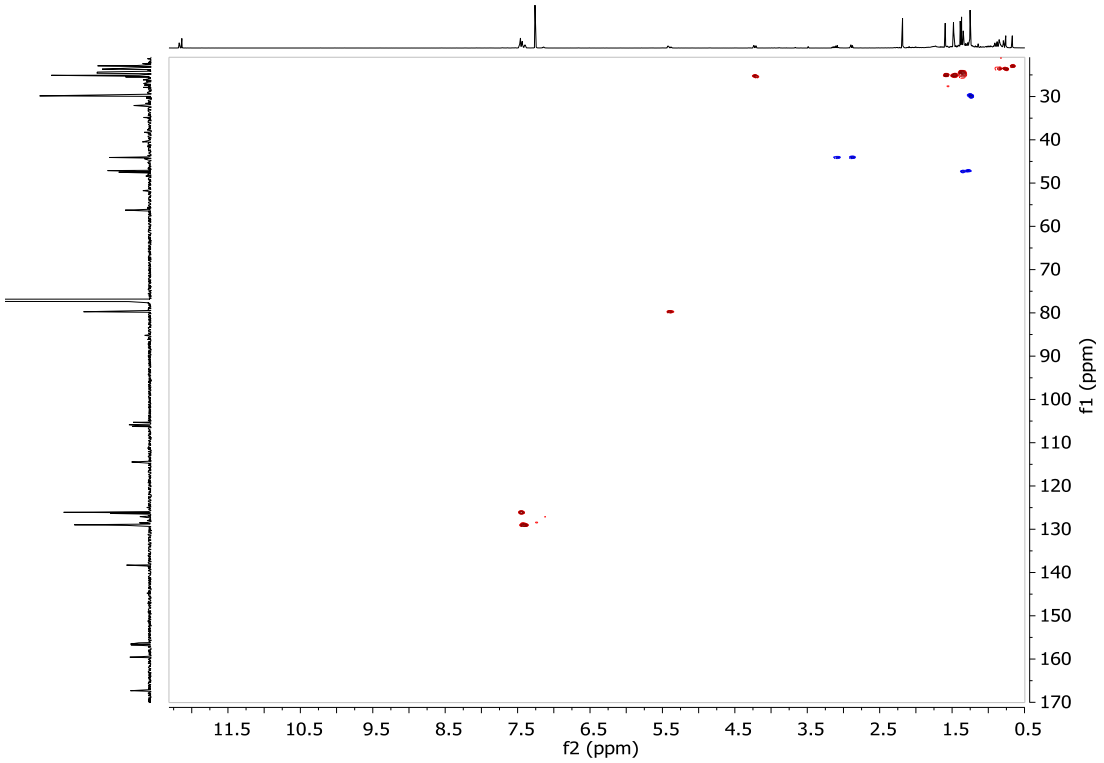


Figure S 11: HSQC spectrum of torellianone C and D in $CDCl_3$ (800 MHz)

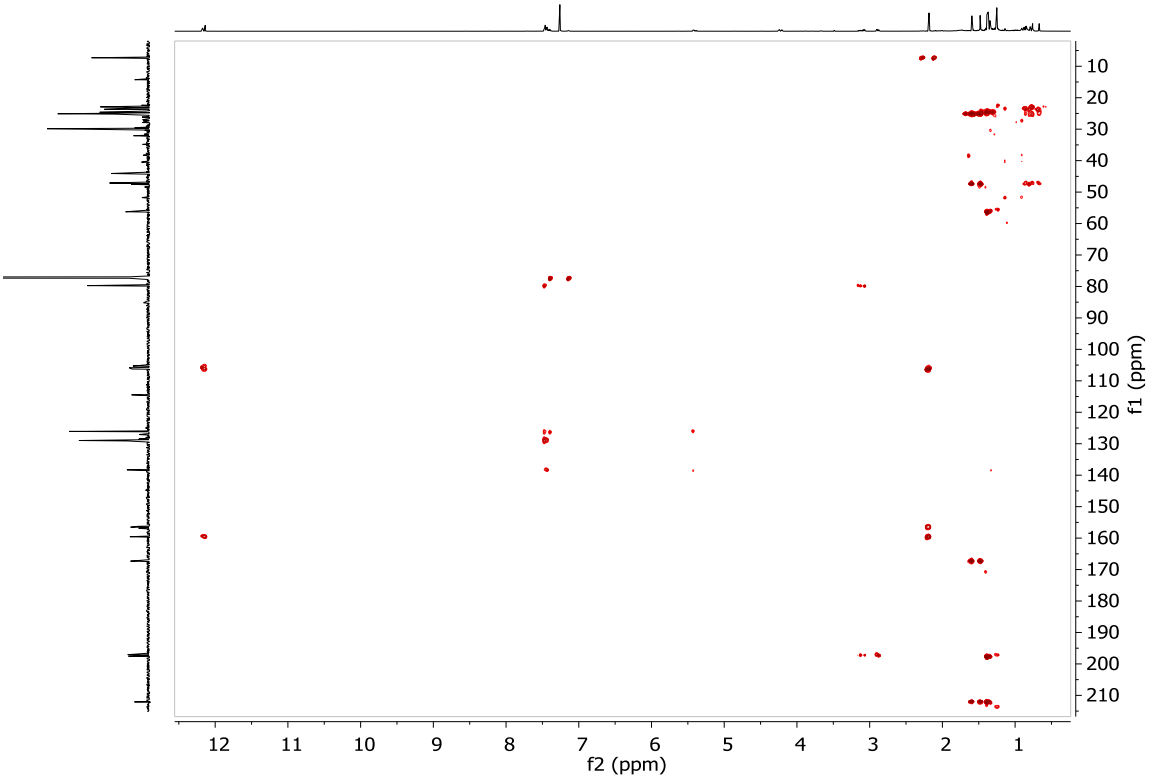


Figure S 12: HMBC spectrum of torellianone C and D in $CDCl_3$ (800 MHz**)**

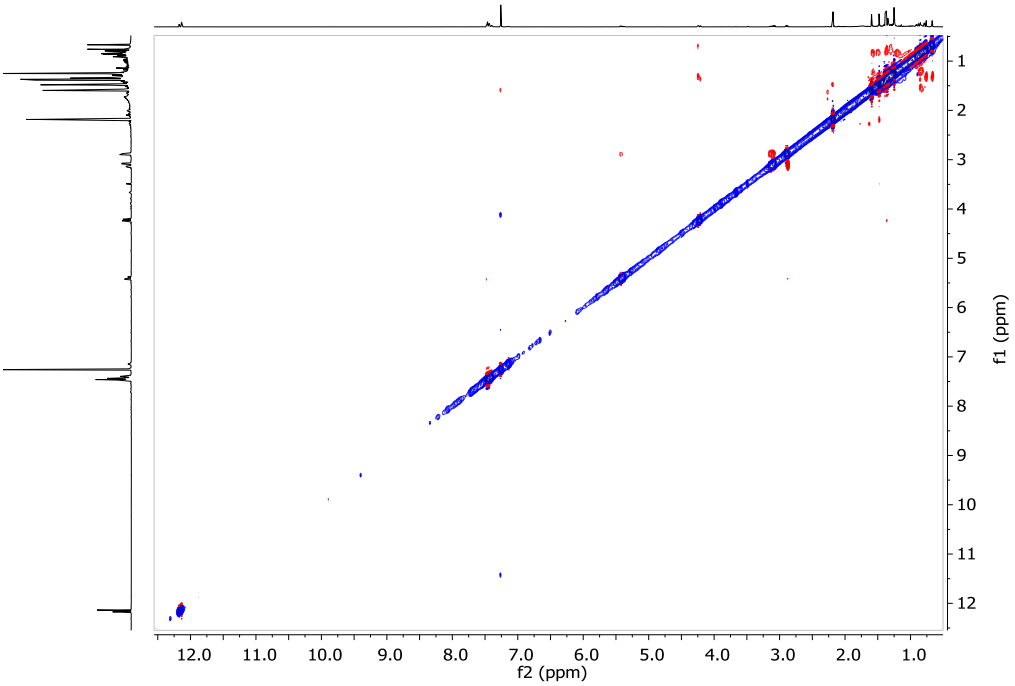


Figure S 13: ROESY spectrum of torellianone C and D in $CDCl_3$ (800 MHz)

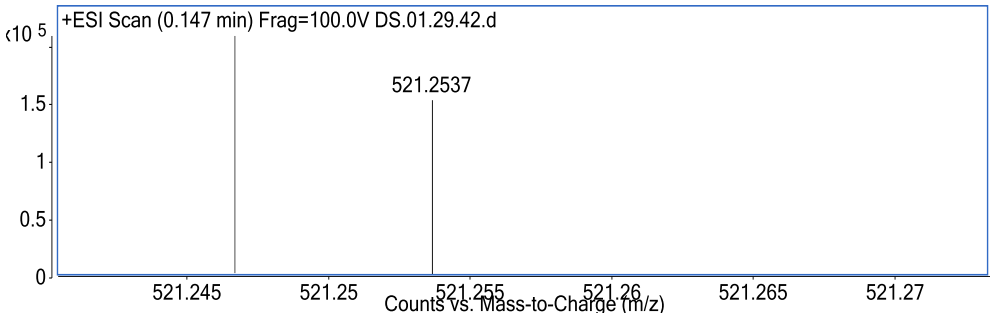


Figure S 14: Mass spectrum of torellianone E

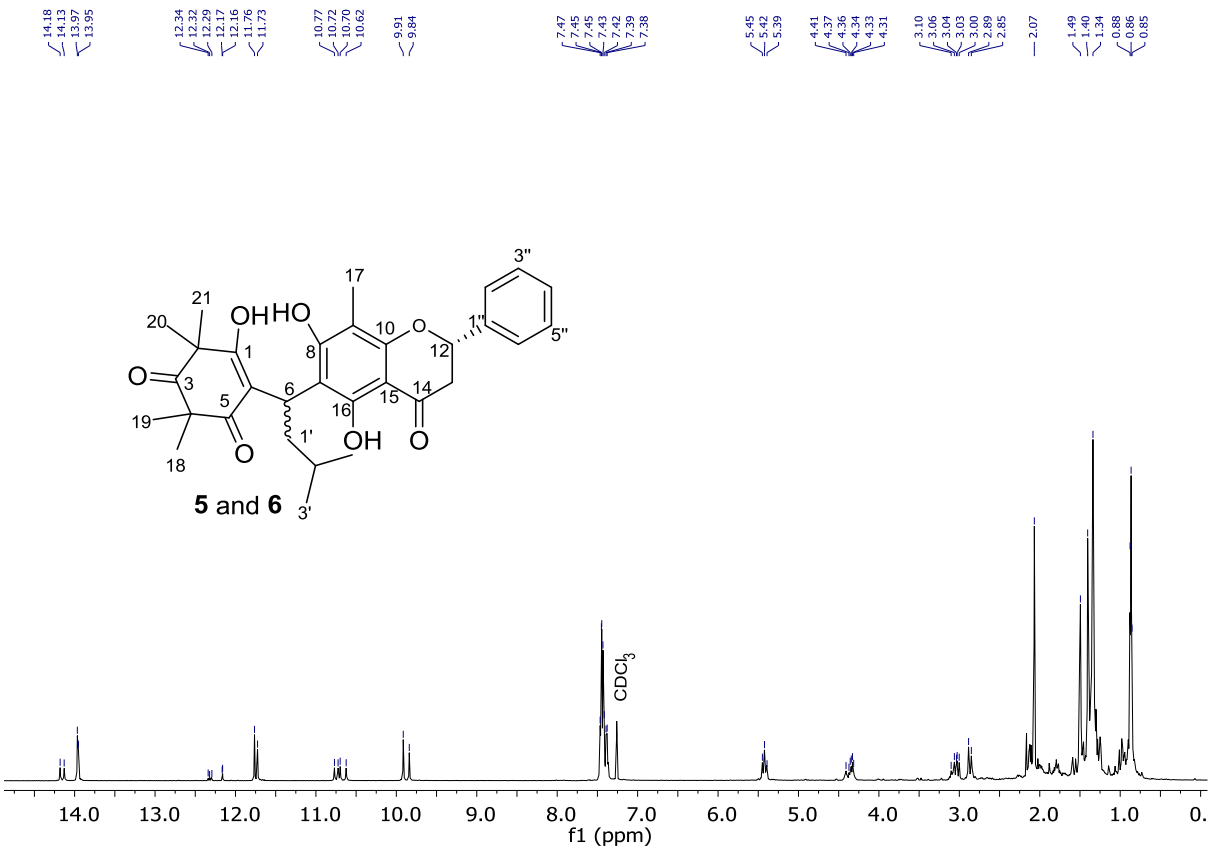


Figure S 15: ^{1}H NMR spectrum of torellianone E and F in $CDCl_3$ (500 MHz)

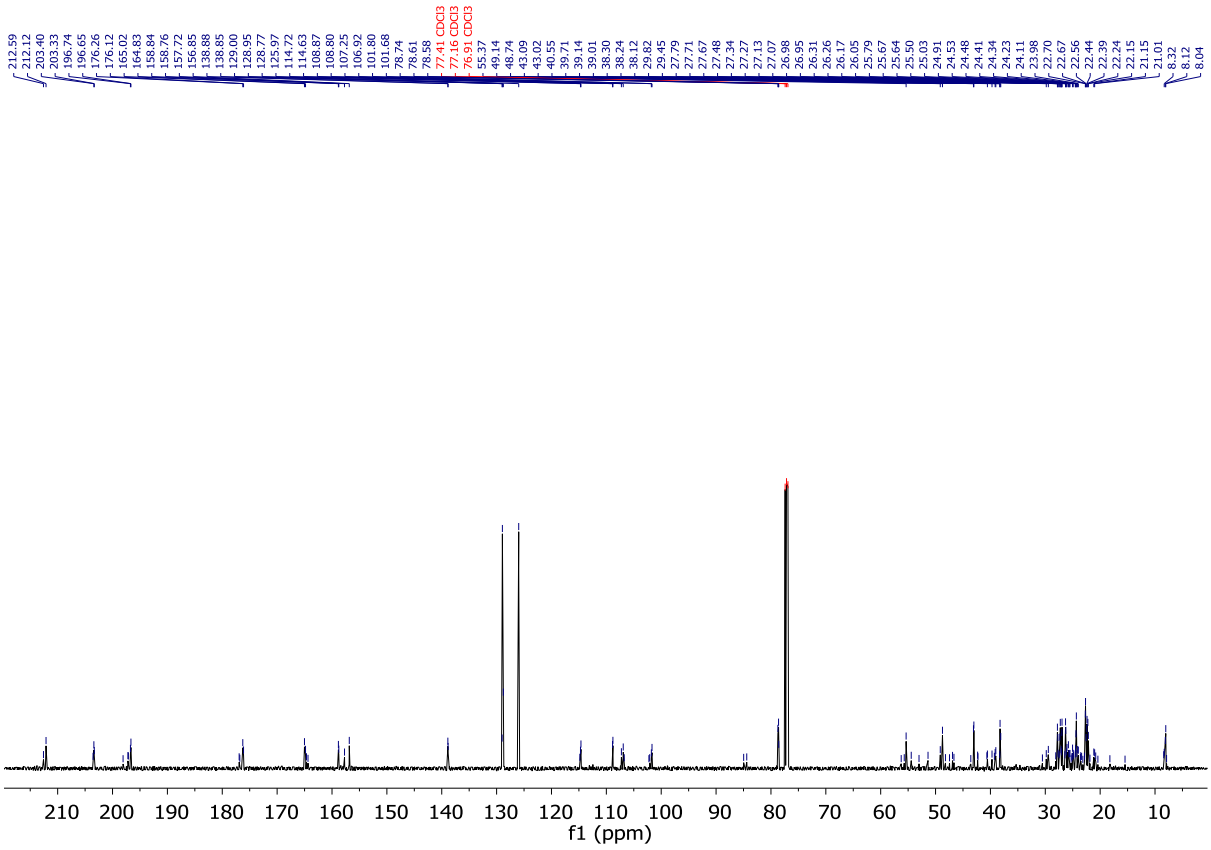


Figure S 16: ^{13}C spectrum of torellianone E and F in $CDCl_3$ (500 MHz)

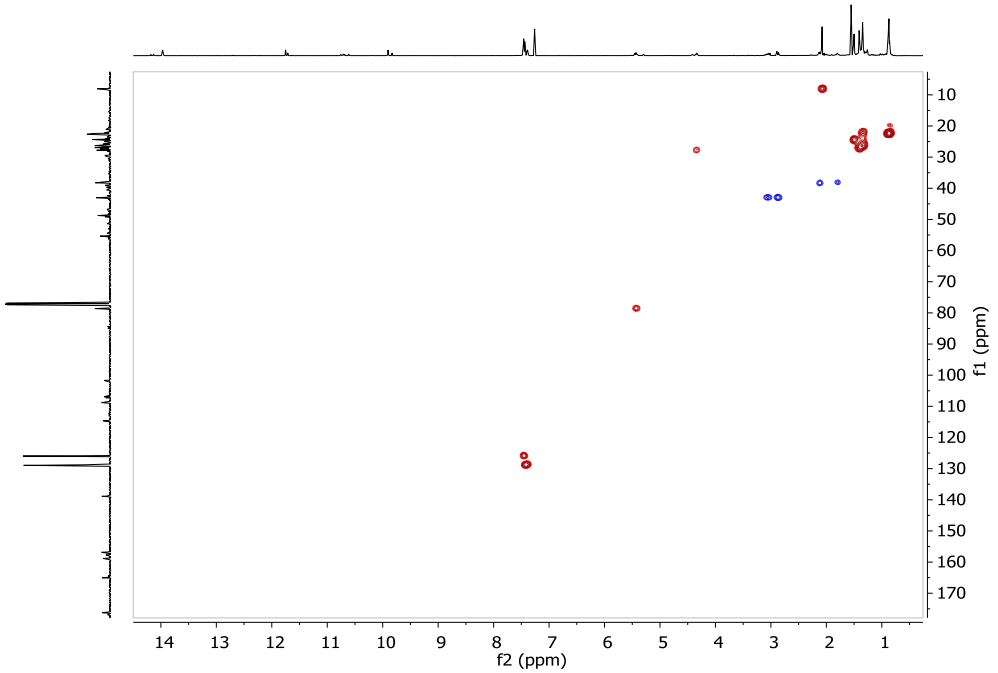


Figure S 17: HSQC spectrum of torellianone E and F in $CDCl_3$ (500 MHz)

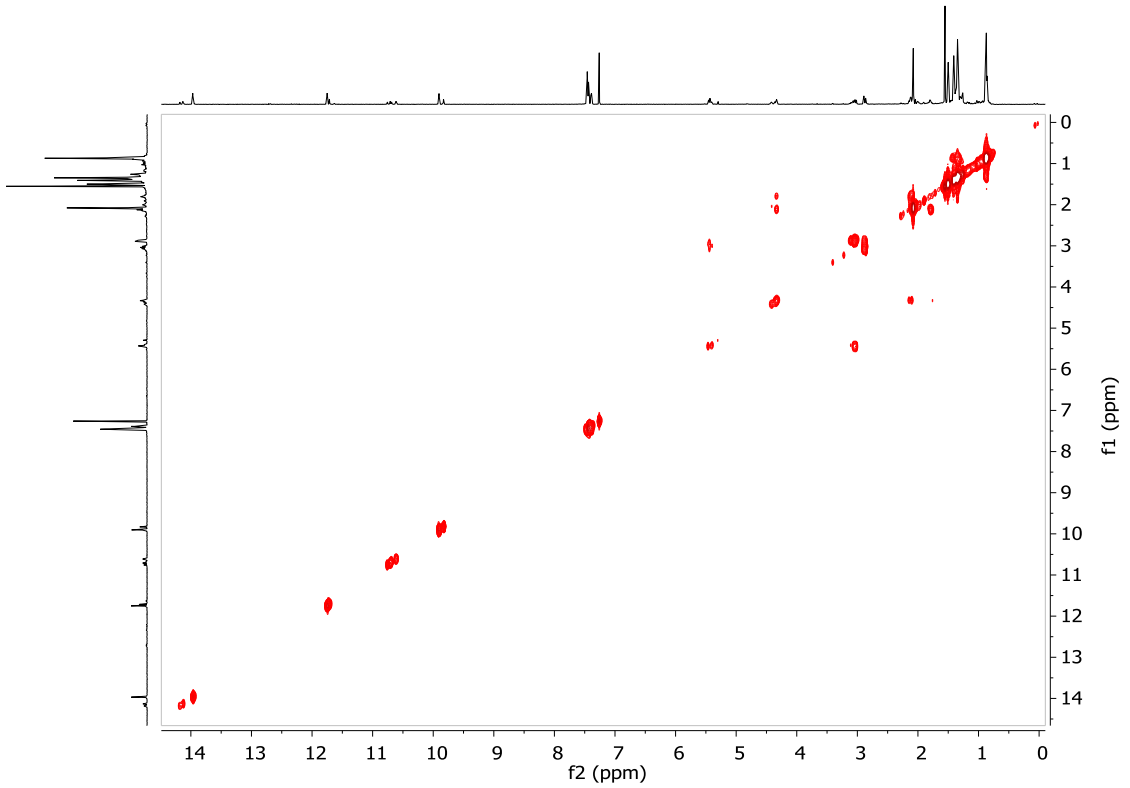


Figure S 18: HSQC spectrum of torellianone E and F in $CDCl_3$ (500 MHz)

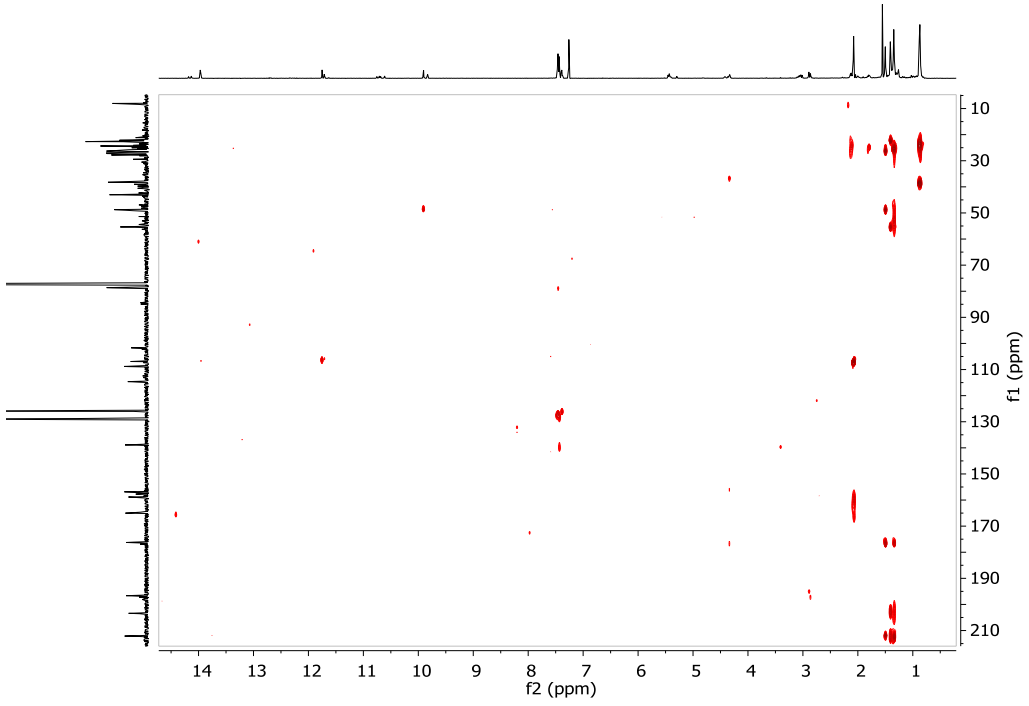


Figure S 19: HMBC spectrum of torellianone E and F in $CDCl_3$ (500 MHz)

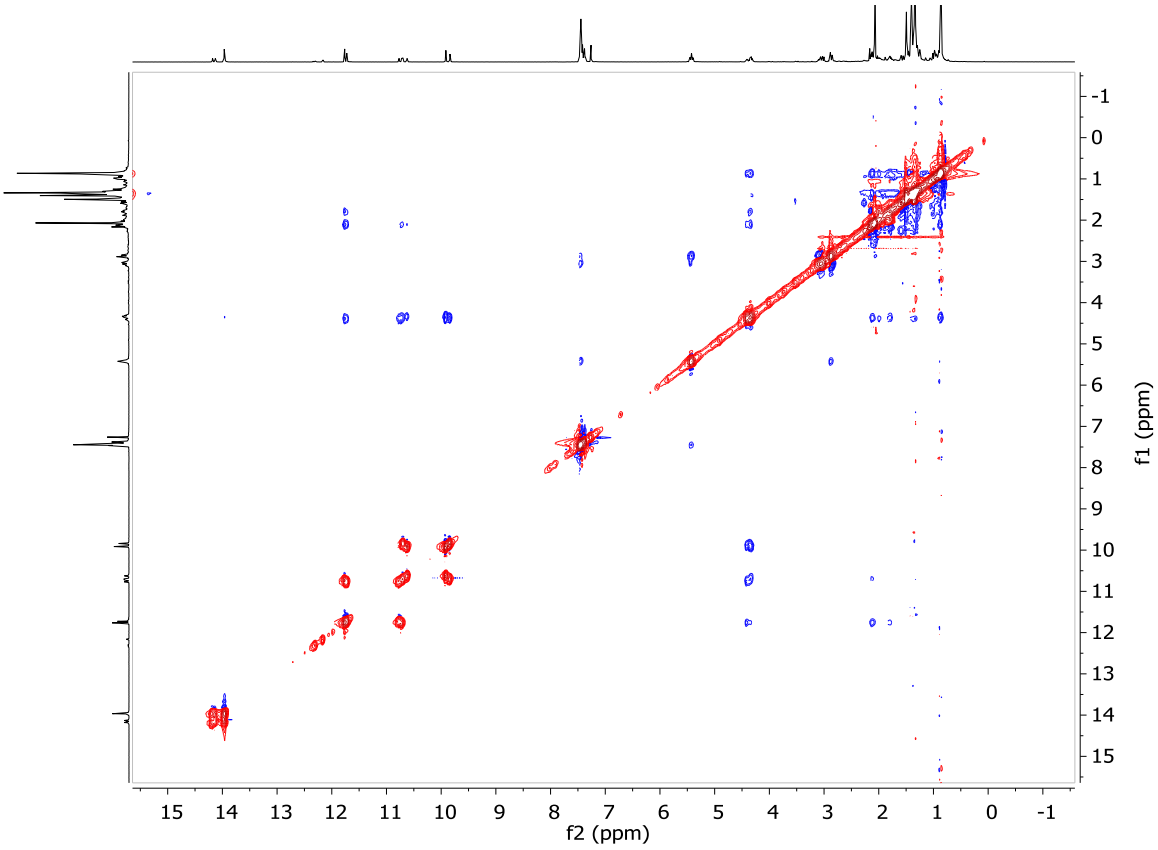


Figure S 20: ROESY spectrum of torellianone E and F in $CDCl_3$ (500 MHz)

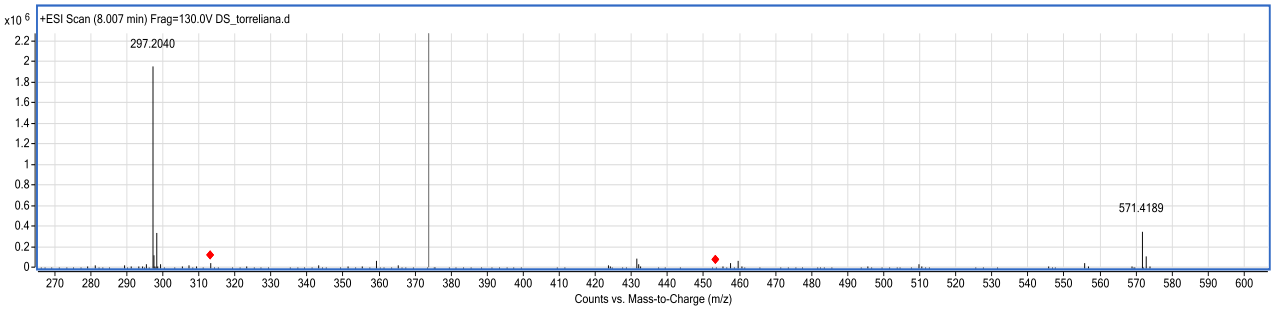


Figure S 21: Mass spectrum of torellianol A

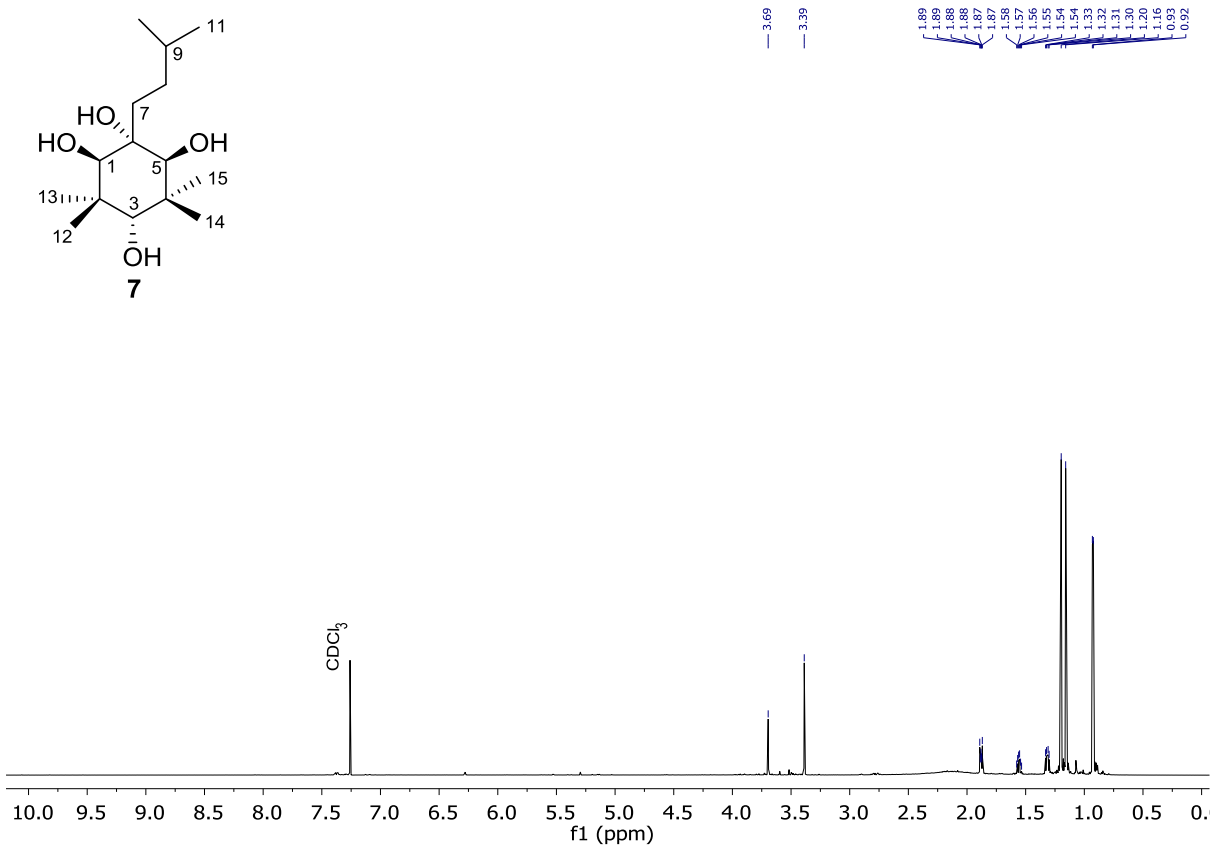


Figure S 22: ^{1}H NMR spectrum of torellianol A in $CDCl_3$ (800 MHz)

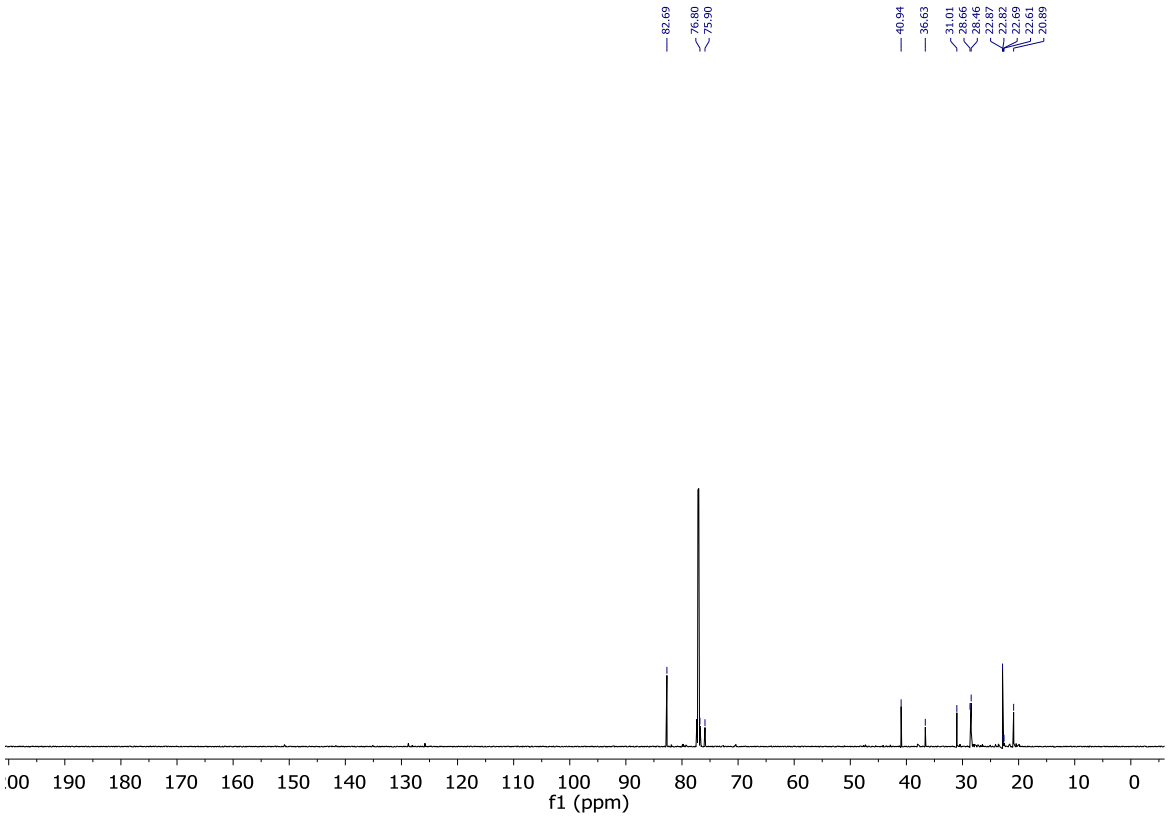


Figure S 23: ^{13}C NMR spectrum of torellianol A in $CDCl_3$ (800 MHz)

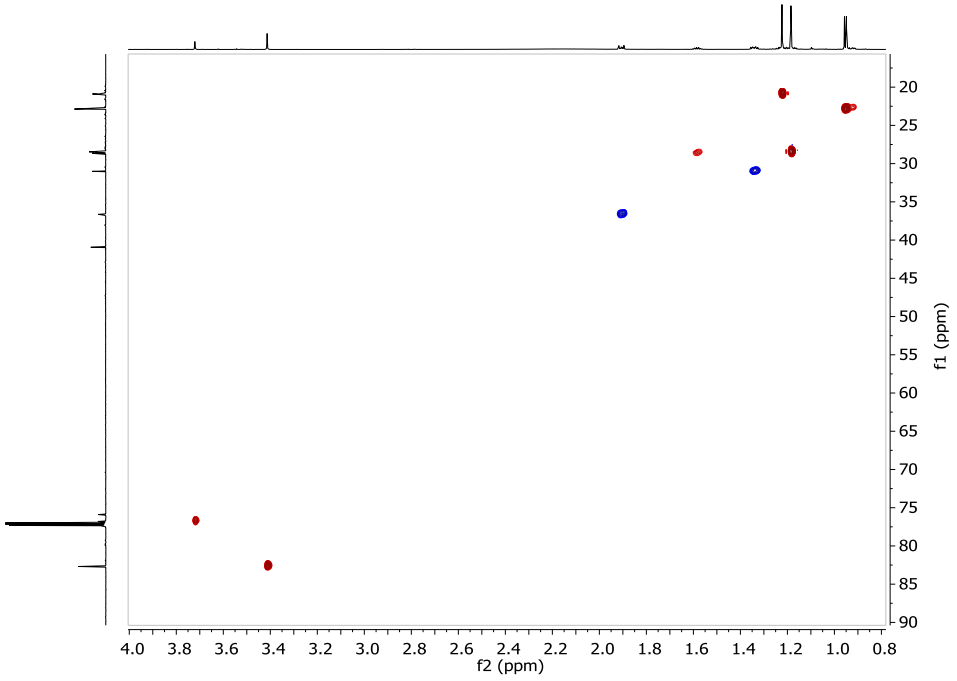


Figure S 24: HSQC spectrum of torellianol A in $CDCl_3$ (800 MHz)

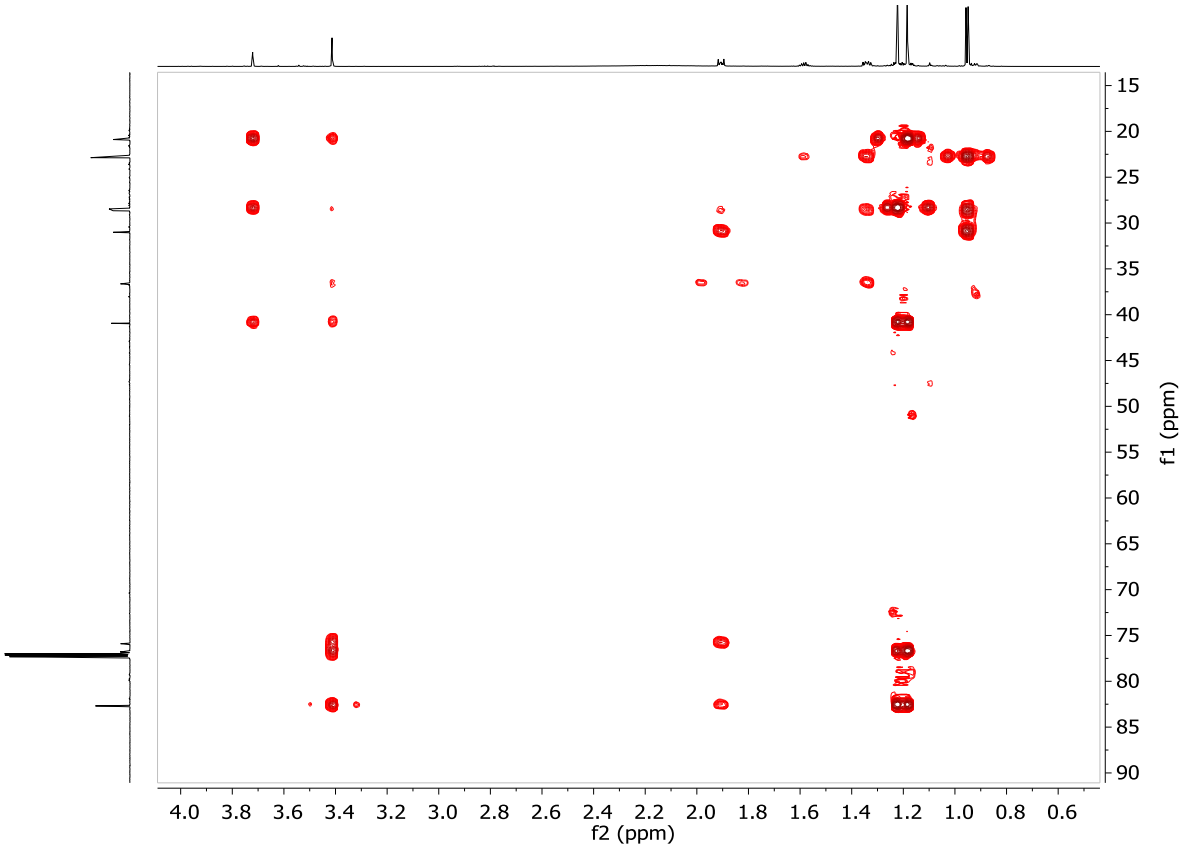


Figure S 25: HMBC spectrum of torellianol A in $CDCl_3$ (800 MHz)

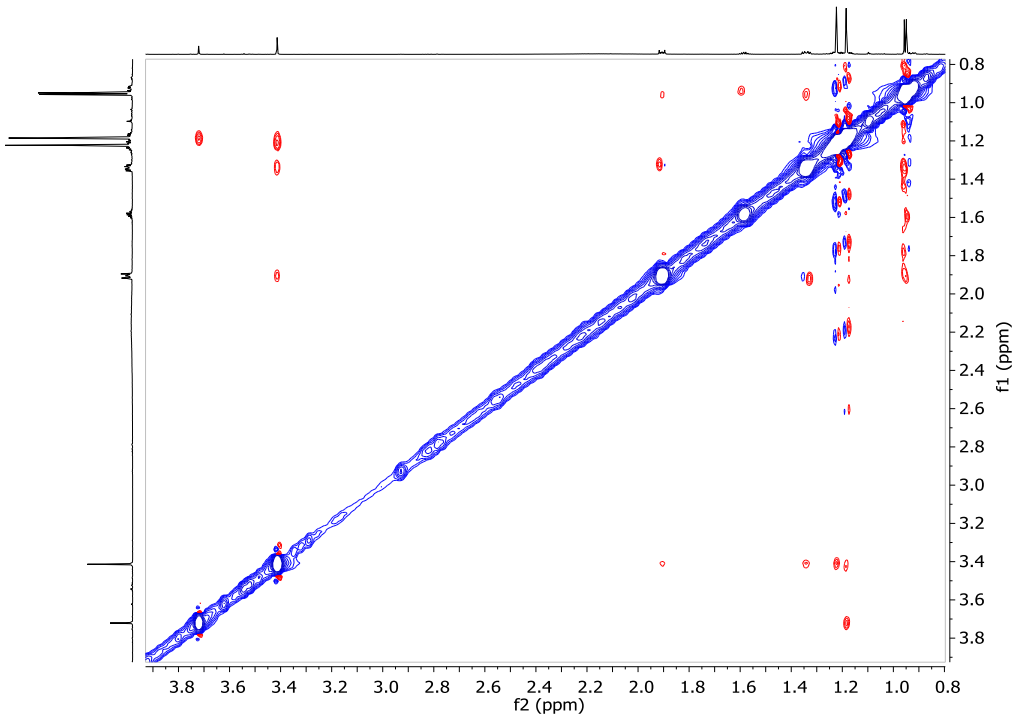


Figure S 26: ROESY spectrum of torellianol A in $CDCl_3$ (800 MHz)

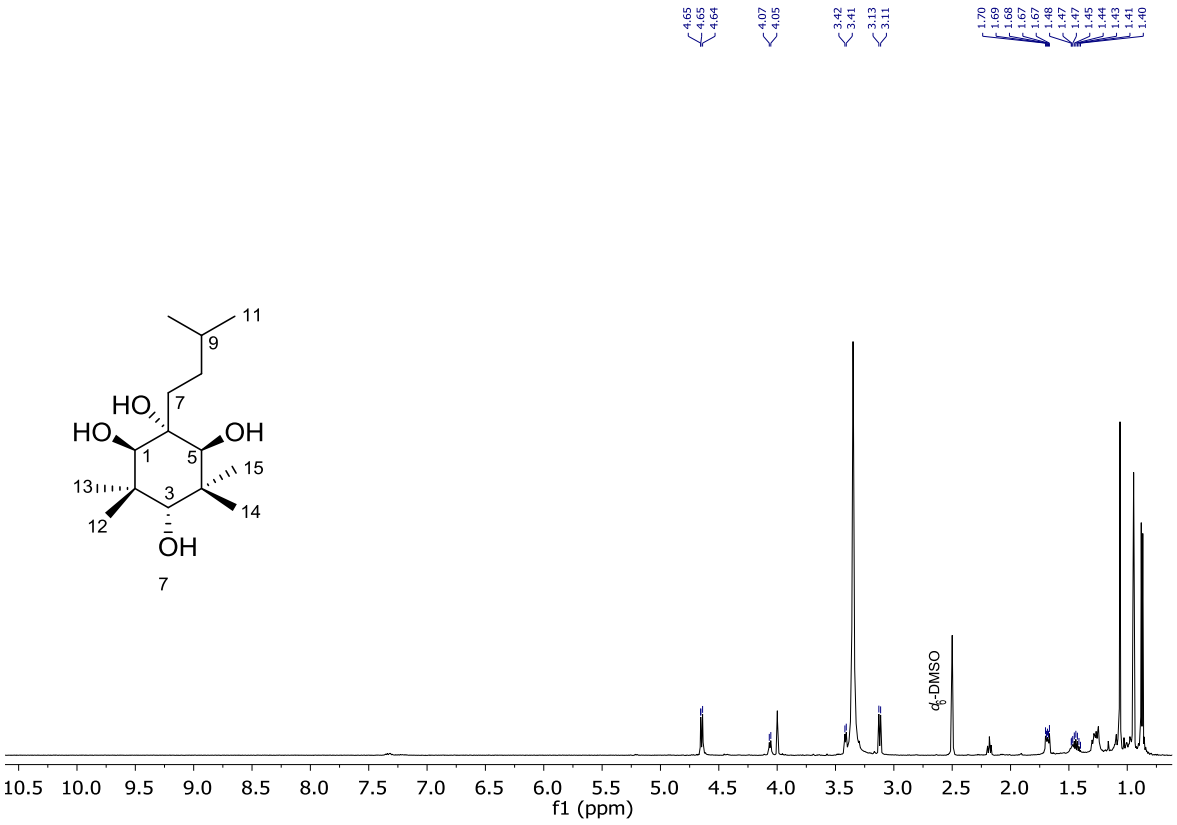


Figure S 27: ^{1}H NMR spectrum of torellianol A in d_6-DMSO (500 MHz)

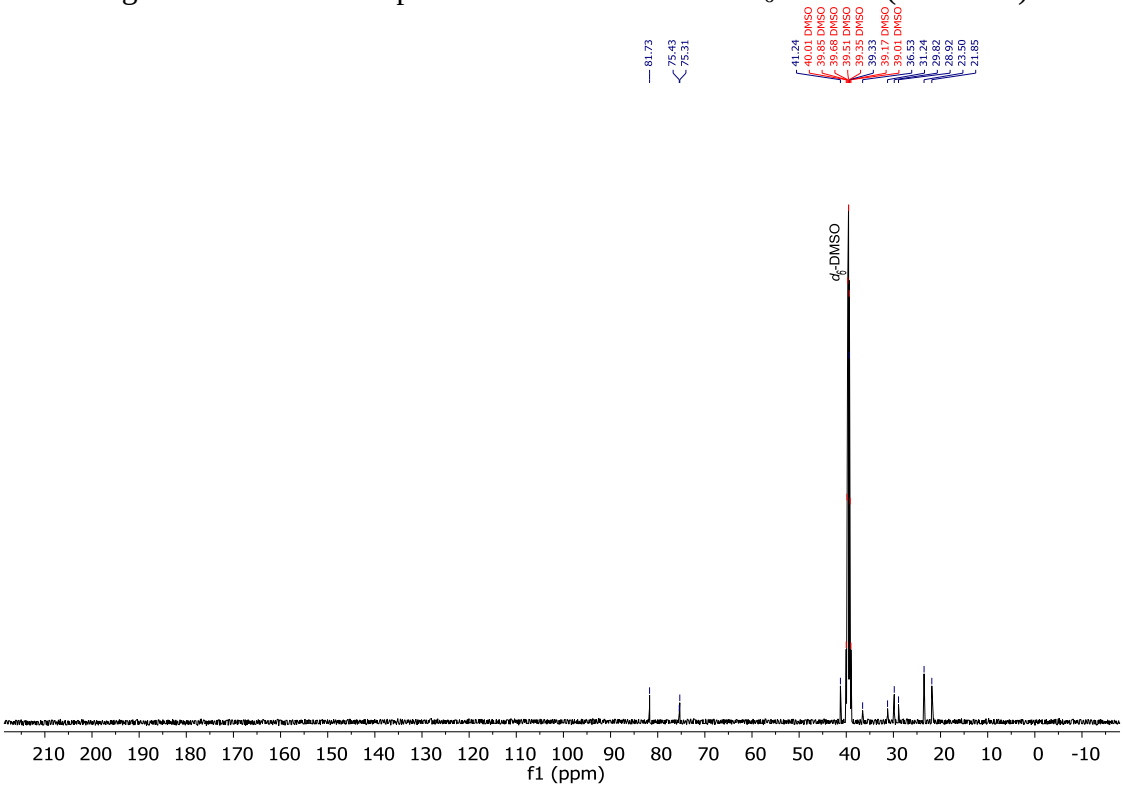


Figure S 28: ^{13}C NMR spectrum of torellianol A in d_6-DMSO (500 MHz)

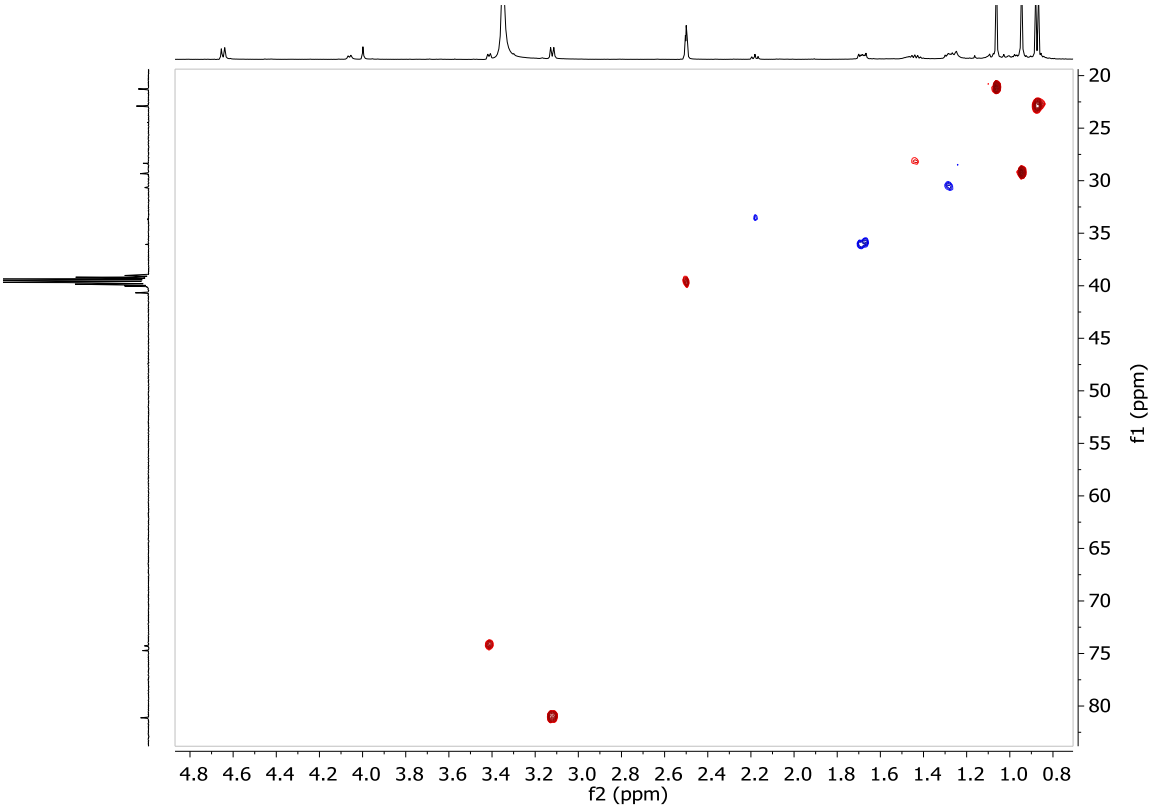


Figure S 29: HSQC spectrum of torellianol A in d_6-DMSO (500 MHz)

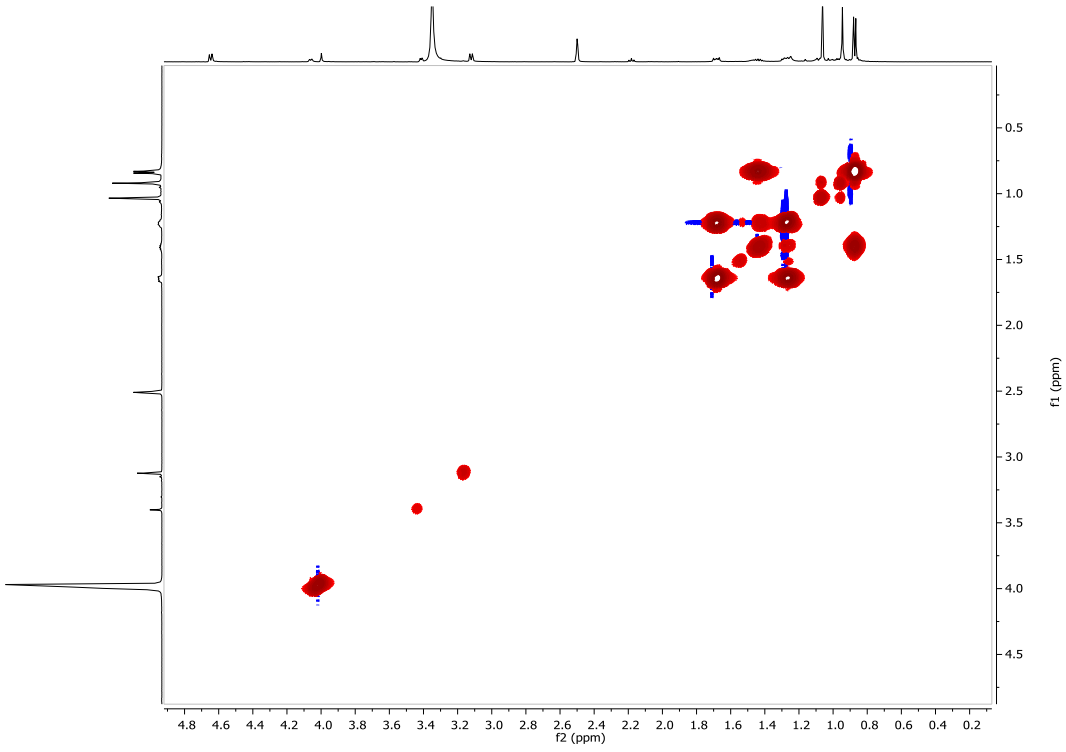


Figure S 30: COSY spectrum of torellianol A in d_6-DMSO (500 MHz)

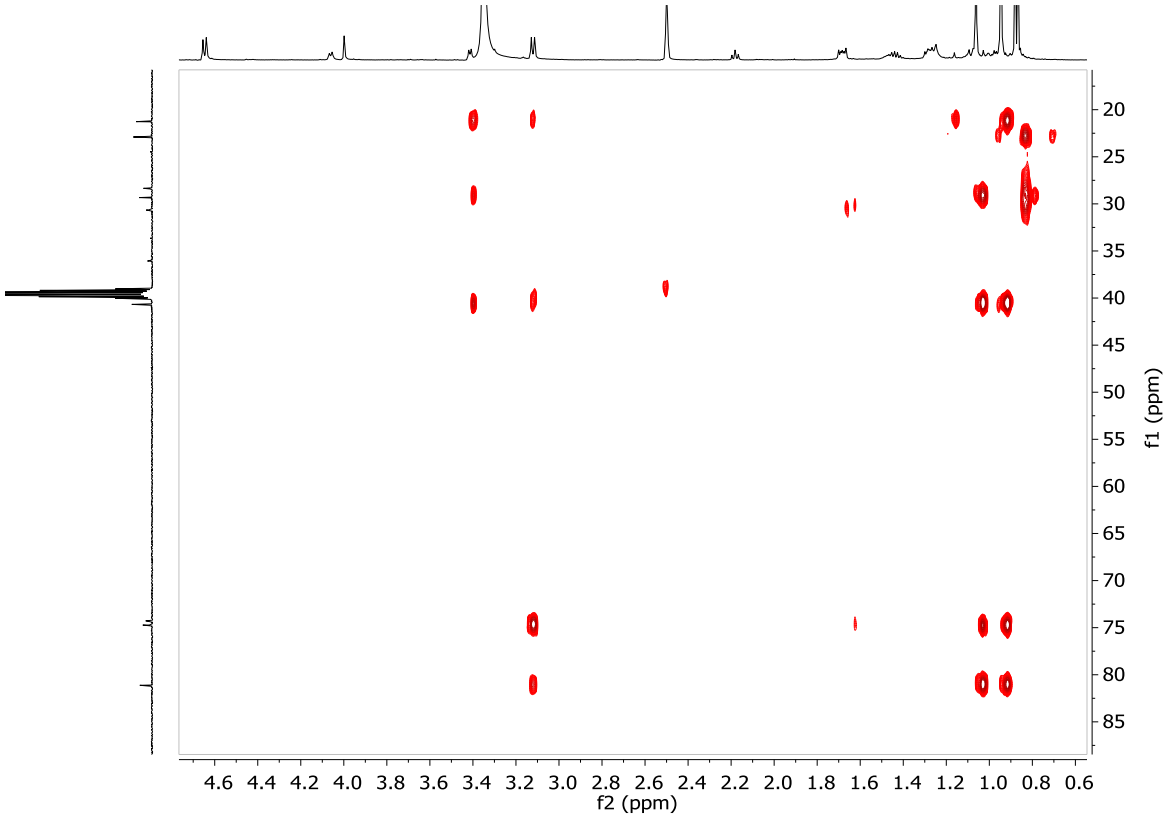


Figure S 31: HMBC spectrum of torellianol A in d_6-DMSO (500 MHz)

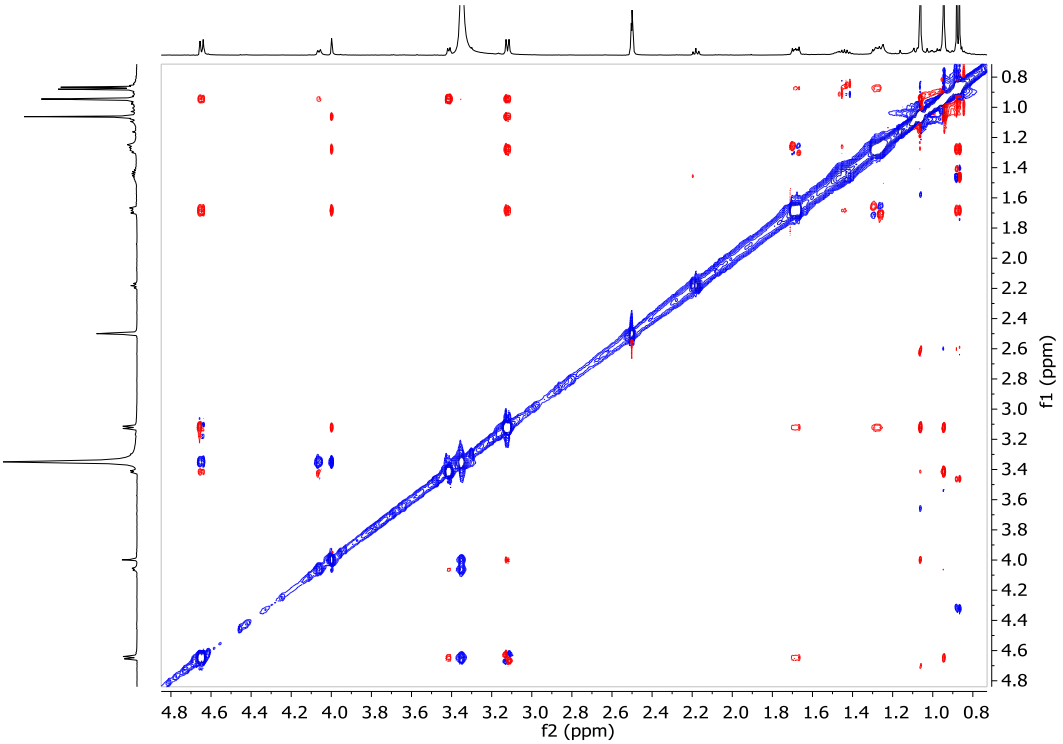


Figure S 32: ROESY spectrum of torellianol A in d_6-DMSO (500 MHz)

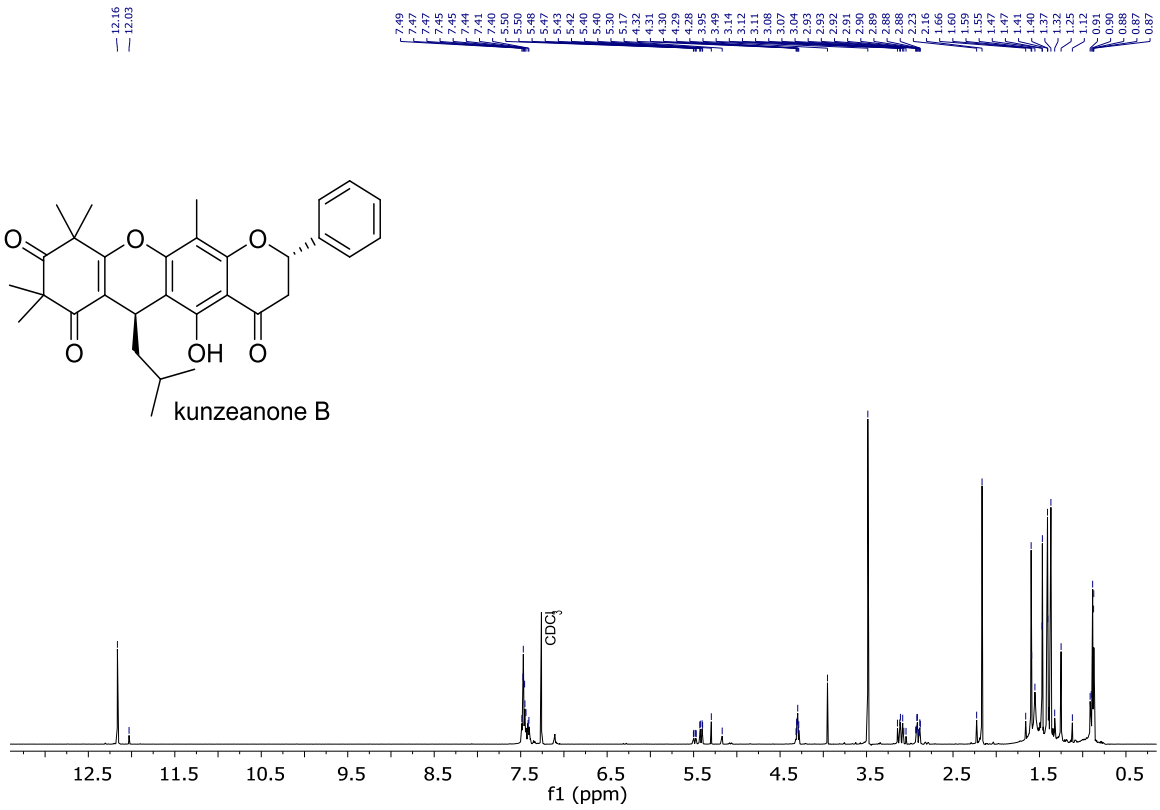


Figure S 33: ^{1}H NMR spectrum of kunzeanone B in $CDCl_3$ (500 MHz)

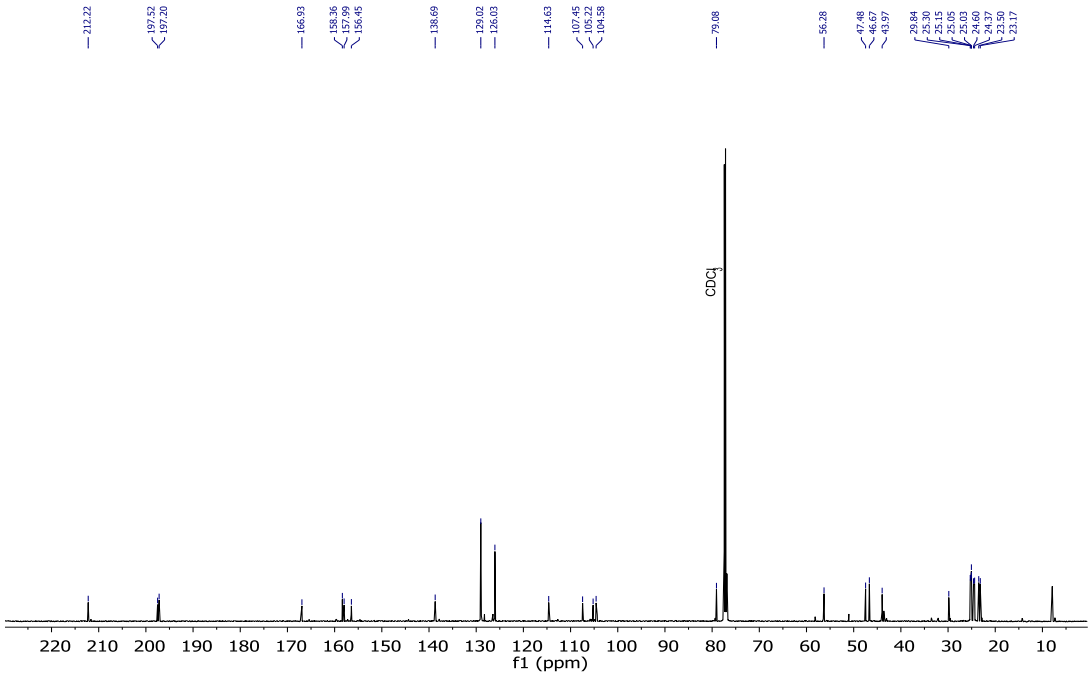


Figure S 34: ^{13}C NMR spectrum of kunzeanone B in $CDCl_3$ (500 MHz)

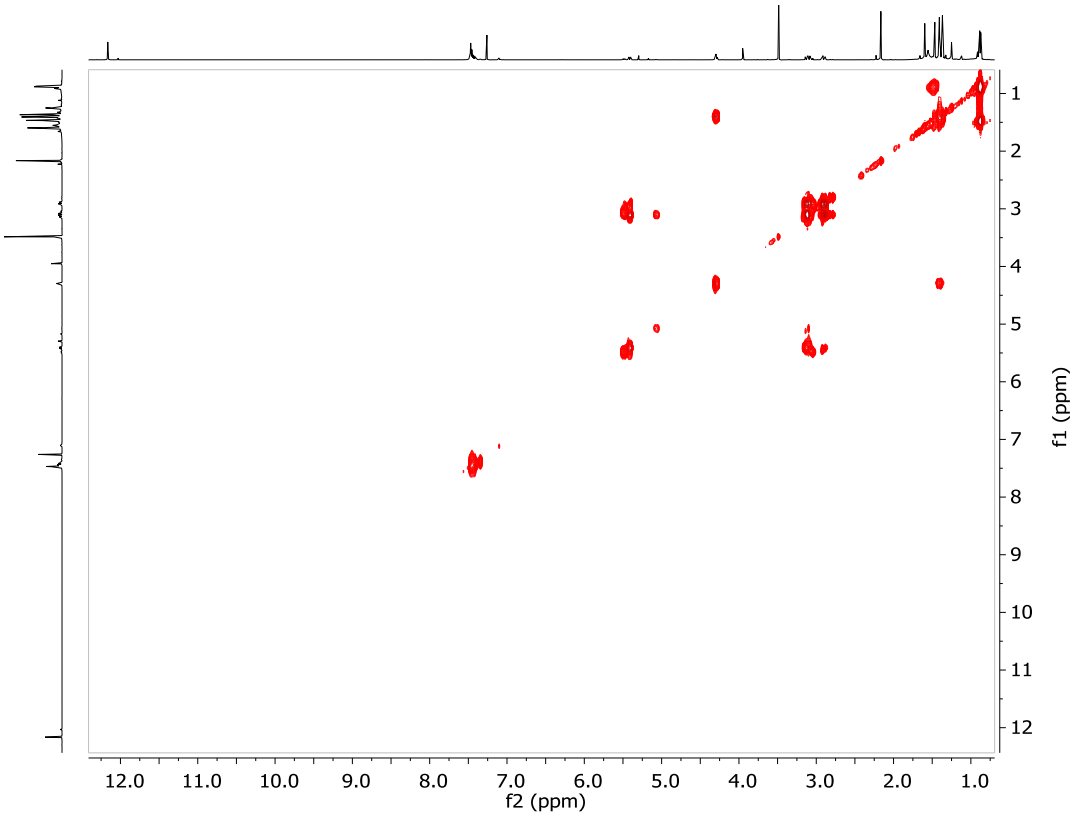


Figure S 35: COSY spectrum of kunzeanone B in $CDCl_3$ (500 MHz)

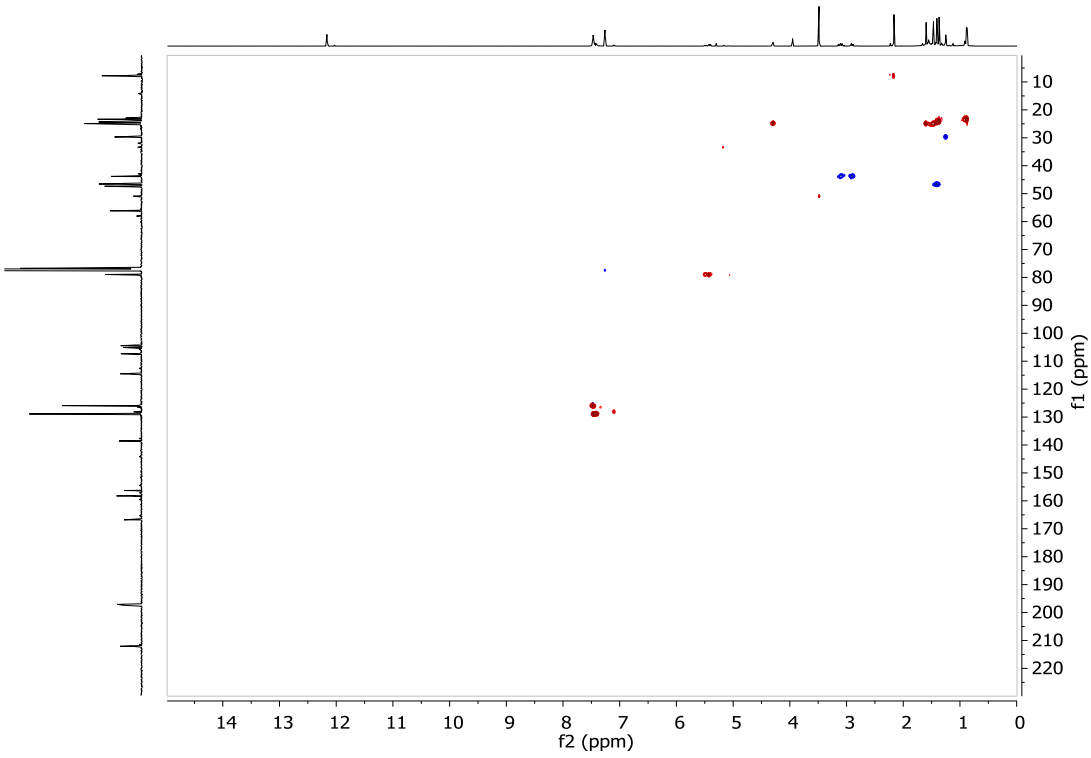


Figure S 36: HSQC spectrum of kunzeanone B in $CDCl_3$ (500 MHz)

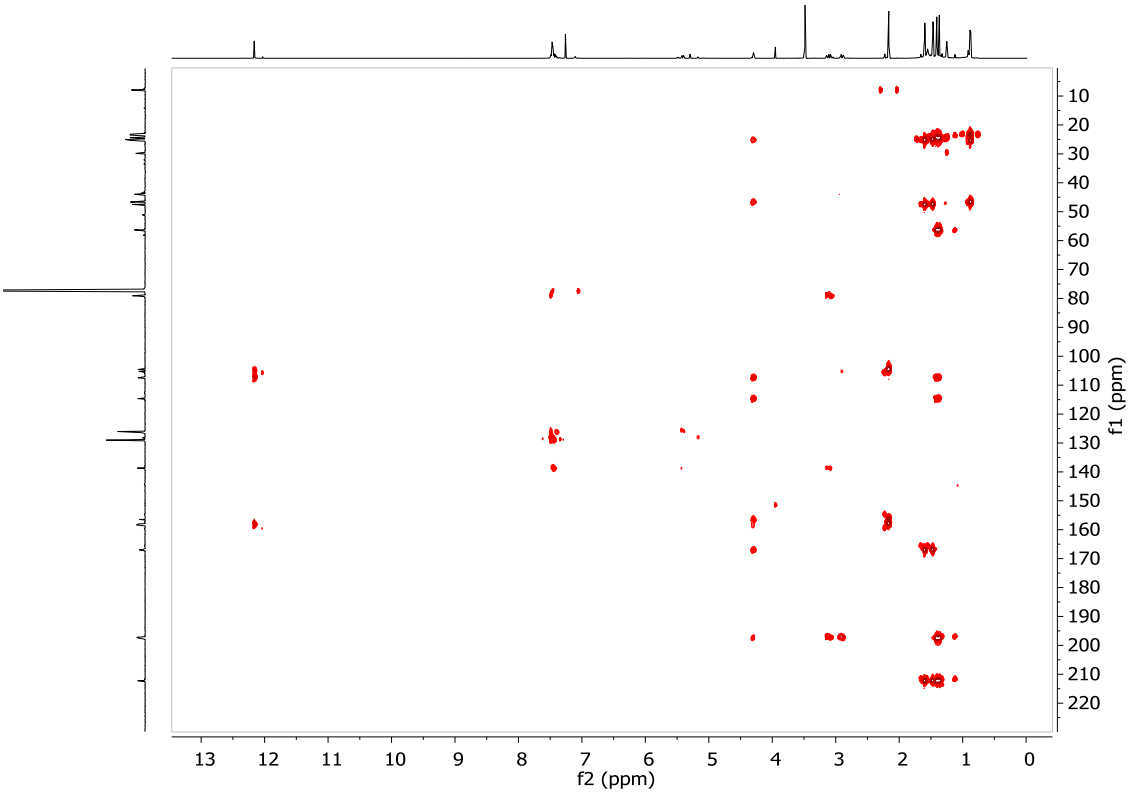


Figure S 37: HMBC spectrum of kunzeanone B in $CDCl_3$ (500 MHz)

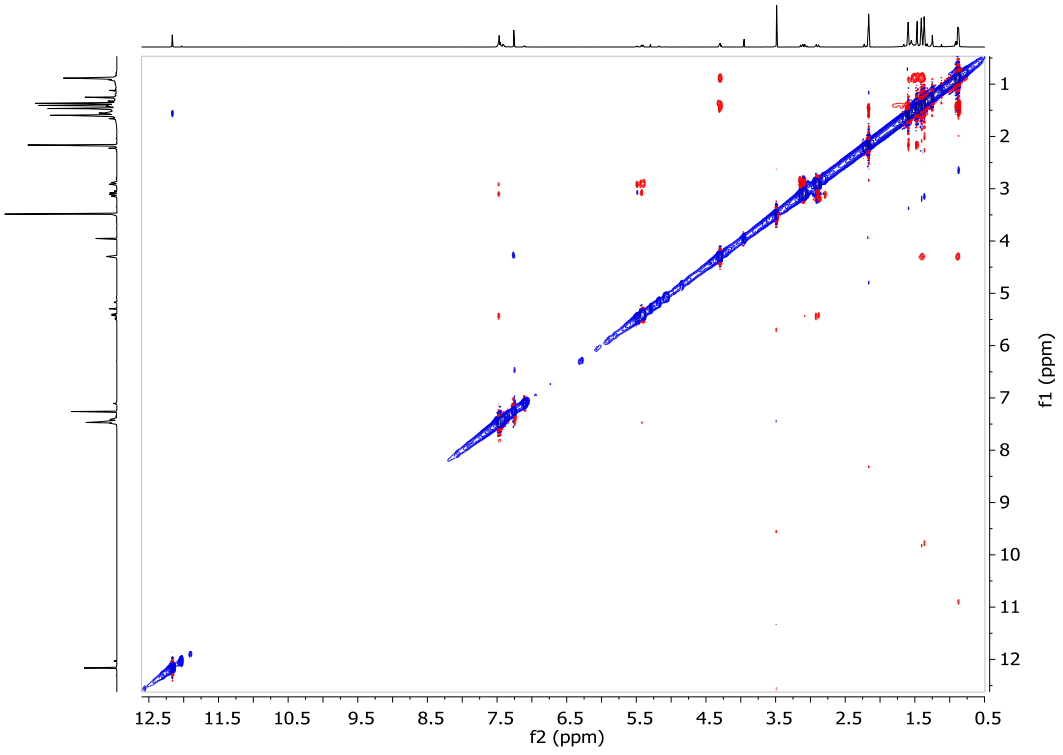


Figure S 38: ROESY spectrum of kunzeanone B in $CDCl_3$ (500 MHz)

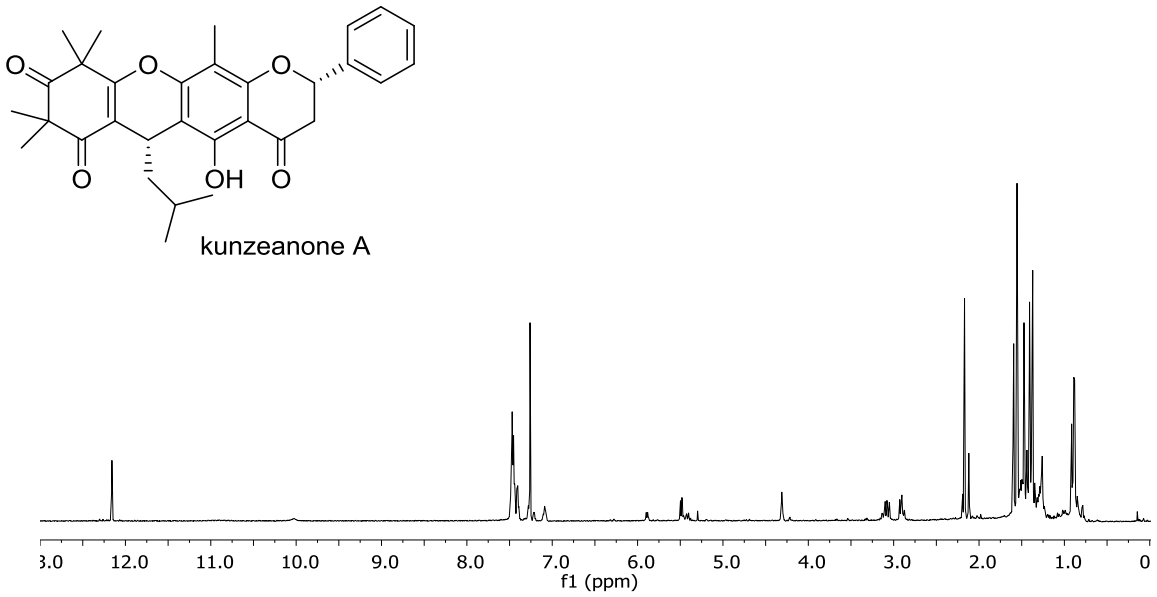


Figure S 39: ^{1}H NMR spectrum of kunzeanone A in $CDCl_3$ (500 MHz)

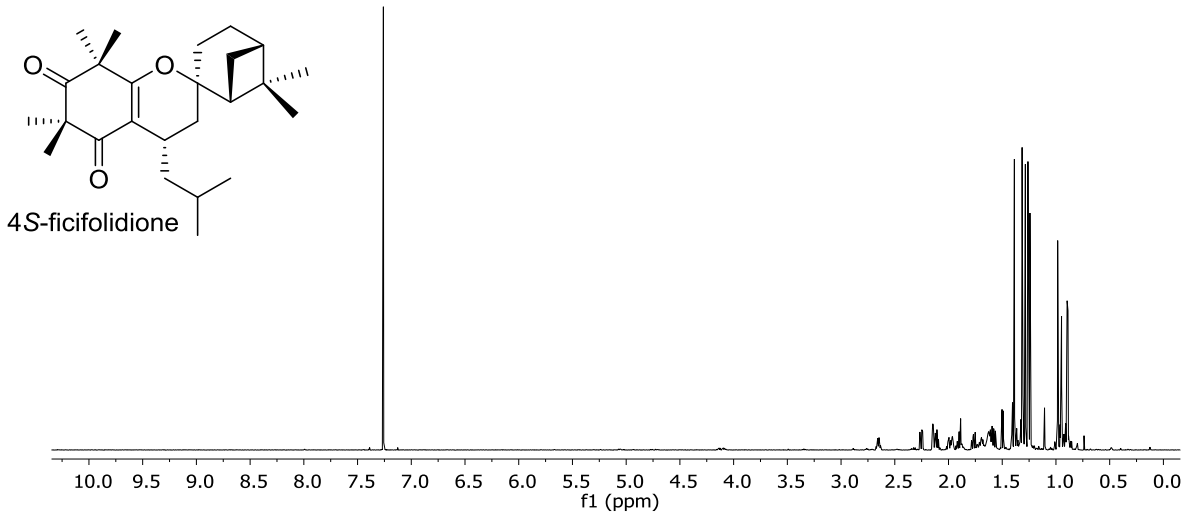


Figure S 40: ^{1}H NMR spectrum of 4*S*-ficifolidione in $CDCl_3$ (500 MHz)

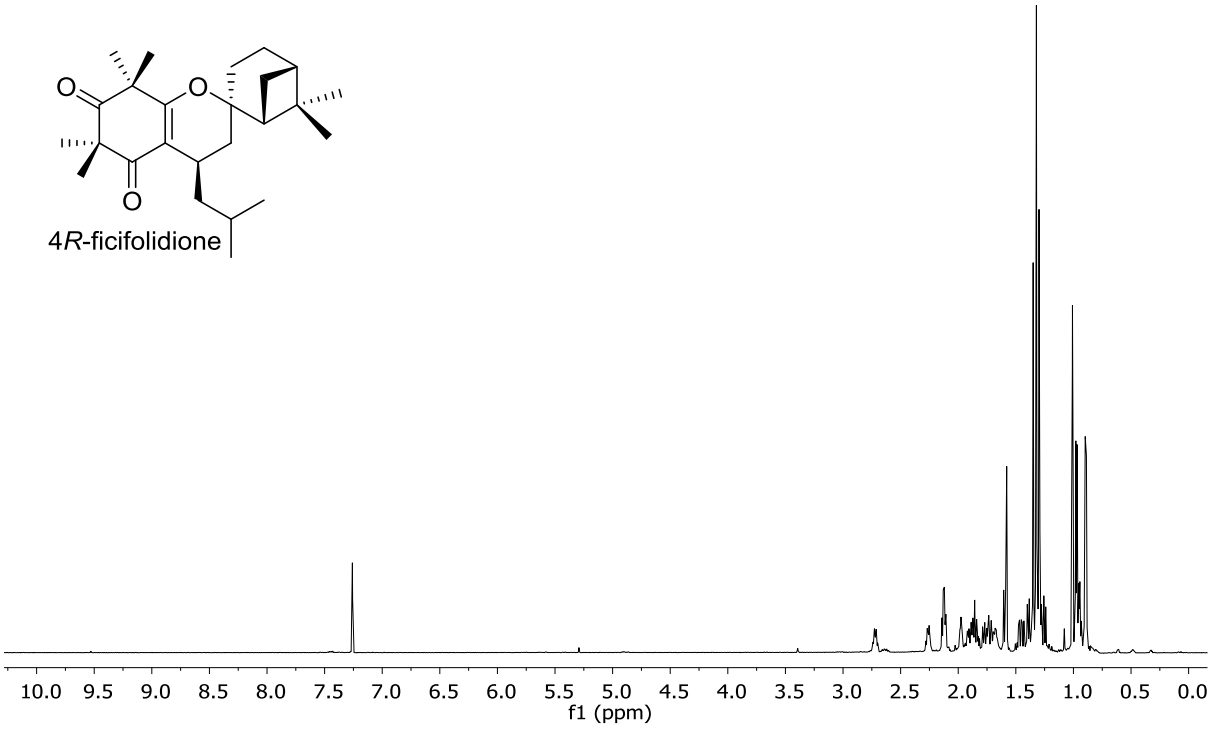


Figure S 41: ^{1}H NMR spectrum of 4*R*-ficifolidione in $CDCl_3$ (500 MHz)

CHAPTER 8

CONCLUSION

8.0 Conclusion

A comprehensive literature review on the chemistry and biological activities of compounds isolated from species of the Myrtaceae family, isolation, structure elucidation and antiplasmodial and antibacterial activity evaluation of twenty-four β-triketones, fourteen of which are new, from the flowers of two species of *Corymbia* and one species of *Angophora,* and investigation of chemotaxonomic relationships within eucalypts of Myrtaceae family were carried out successfully. The outcomes of the research fulfilled the primary objectives of the project and major findings are summarized here.

The literature review demonstrated that the Myrtaceae has two main groups of chemicals that can be divided based upon their physical properties; volatile components and non-volatile components. The volatile compounds are characterised predominantly as terpenoid constituents, mainly, mono and sesquiterpenes. The major non-volatile chemicals present in Myrtaceae species are β-triketones and phloroglucinols with research into β-triketones being an emerging area within recent years. Structural diversity of the β-triketones was significant and varied from simple molecules containing a syncarpic acid moiety linked to a ketone side chain to β-triketones conjugated to phloroglucinols, phloroglucinol/flavonone adducts, terpenes, and other β-triketones. During the last ten years, more than 100 new β-triketones have been isolated from Myrtaceae genera such as *Corymbia*, *Rhodomyrtus, Baeckea*, *Callistemon, Myrtus* and *Kunzea* and this literature review demonstrated that the Myrtaceae is a major natural source of β-triketones. Phloroglucinol compounds also found within Myrtaceae include acyl phloroglucinols, meroterpenes (euglobals and macrocarpals), sideroxylonals and dibenzofurans. All the compound classes, terpenes, β-triketones and phloroglucinols exhibited a wide spectrum of biological activities including antimicrobial, antioxidant, anti-inflammatory, antifeedant, and anti-fouling. In particular, β-triketones had potent biological activities towards drug-resistant pathogens such as *Plasmodium falciparum* and *Staphylococcus aureus* while also displaying low cytotoxicity. (Examples watsonianone B, rhodomyrtone and myrtucommulone A). The reviewed chemistry of the family established clear taxonomic relationships among genera and provides significant understandings for phylogenetic studies. For example, the existing debate about the taxonomic relationship among Eucalypts genera (*Corymbia, Angophora,* and *Eucalyptus*) can be addressed based on the chemotaxonomy of the family.

Angophora woodsiana, endemic to Australia was chemically investigated and this is the first time any plant from this genus has been studied. Two new β-triketones, woodsianone A (**8.1**) and woodsianone B (**8.2**), and nine known compounds rhodomyrtosone A (**8.3**), rhodomyrtosone D (**8.4**), rhodomyrtone (**8.5**), tomentodione A (**8.6**), tomentodione B (**8.7**) 4*S*-ficifolidione (**8.8**), kunzeanone A (**8.9**), watsonianone A (**8.10**) and watsonianone B (**8.11**) were isolated. (Figure 8.1). The new compound, woodsianone A, is only the third example of a β-triketone adducts containing a cadinene sesquiterpene. Woodsianone B is the first β-triketone epoxide derivative to be isolated from a natural source. The known compounds rhodomyrtosone A (**8.3**), rhodomyrtosone D (**8.4**), rhodomyrtone (**8.5**), tomentodione A (**8.6**) and tomentodione B (**8.7**) from *Rhodomyrtus tomentosa;* 4*S*-ficifolidione (**8.8**)**,** from *Corymbia ficifolia, Kunzea ericoides* and *R. tomentosa*, Kunzeanone A (**8.11**), from *Kunzea ambigua*, watsonianones A and B from *Corymbia watsoniana* have been reported previously. *Angophora* is another source of β-triketone molecules and the re-isolation of compounds previously reported from *Corymbia* species provides evidence for its close chemotaxonomic relationship with the genus *Corymbia.* Antiplasmodial activities of all the compounds were evaluated. The most potent were rhodomyrtone (IC_{50}1.8 ± 1.0 µM against3D7 strain) and woodsianone B (2.53 ± 0.11 µM against Dd2 strain). All the compounds were tested against *Staphylococcus aureus* ATCC 157293 for their antibacterial activity and woodsianone B and rhodomyrtone had antibacterial activity at MIC values of 0.02 mM and 0.63 mM respectively.

Chemical investigation of *Corymbia intermedia* yielded five new β-triketones, intermediones A-E (**8.15-8.19**)**.** (Figure 8.1 and Figure 8.2). Intermediones A-C are phenyl analogs of 4*S*/4*R*-ficifolidiones (**8.8**, **8.9**) isolated from *Corymbia ficifolia* and *Rhodomyrtus tomentosa*. Intermedione C was inseparable from intermedione B and its structure was determined for analysis of spectra acquired on the mixture. Intermediones A-C were diastereoisomers while intermedione E is a structural isomer of intermedione A. Intermedione D had a novel oxadispiro[bicyclo[4.1.1]octane-2,2'-furan-5',1''-cyclohexane tetracyclic ring system. To define the relative configuration of the compounds ^{1}H-^{1}H coupling constants and ROESY correlations were used. All the compounds showed moderate antiplasmodial activity against chloroquine sensitive (3D7) and resistant (Dd2) strains of *Plasmodium falciparum* with IC_{50} values ranging from 13-21 µM (3D7) and 15-26 µM (Dd2) respectively.

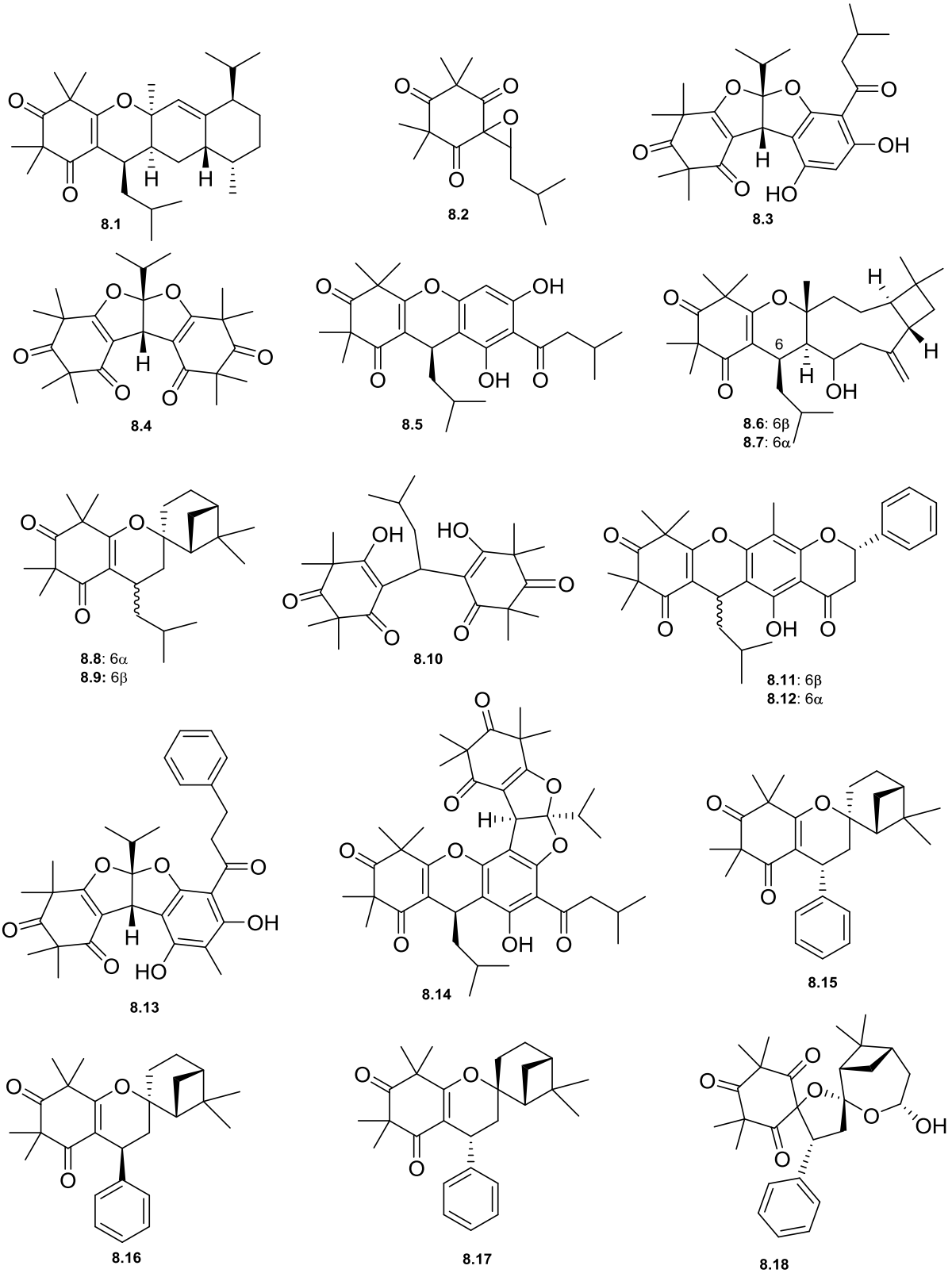


Figure 8.1. Compounds isolated from *Angophora woodsiana*, *Corymbia intermedia* and *Corymbia torelliana*

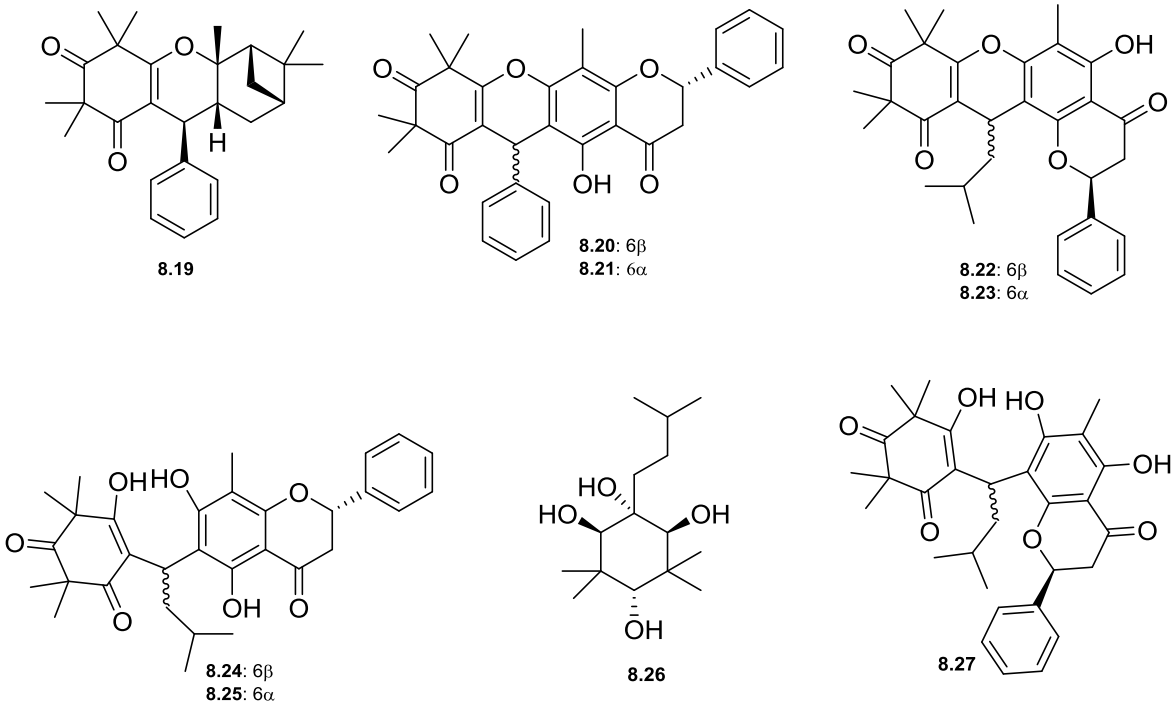


Figure 8.2. Compounds isolated from *Angophora woodsiana*, *Corymbia intermedia* and *Corymbia torelliana* (continued)

Only intermediones A, B and the mixture of intermediones B and C were evaluated for antibacterial activity against the bacteria *Staphylococcus aureus* ATCC 157293. Intermediones A, B and mixture of intermediones B and C were inactive at 0.5 mM against the bacteria *Staphylococcus aureus* ATCC 157293.

Another species from *Corymbia*, *C. torelliana* was also investigated for its chemical constituents and seven new β-triketones, torellianones A-F (**8.20**-**8.25**) and torellianol A (**8.26**) as well as four known compounds, 4*S*-ficifolidione (**8.8**), 4*R*-ficifolidione (**8.9**), kunzeanone A (**8.11**), and kunzeanone B (**8.12**) were isolated. (Figure 8.2). Torellianone A and B (1:1 mixture), torellianone C and D (2:1 mixture) and torrelianone E and F (13:7 mixture) were isolated as inseparable diastereomeric mixtures. The analysis of torellianone E and F was made even more complicated since each diastereomer was present as interconverting rotamers. This type of diastereomeric mixtures have been reported previously from *Kunzea ambigua*, *Baeckea*

frutescens, *Melaleuca leucadendron*, *Luma chequen* and *Callistemon rigidus*. In the literature, a regioisomer (**8.27**) of torellianone E and F had been reported, however, ^{1}H NMR data reported was identical with that of the torellianone E and F and it was therefore concluded that the structure previously reported was incorrect and it was corrected in this thesis. For the known compound kunzeanone B, ^{1}H and ^{13}C NMR data reported previously in the literature were same as that of kunnzeanone A and this was clearly an error in the original paper. Since we isolated both kunzeanone A and B, and acquired full 2D data sets on both compounds the ^{1}H and ^{13}C NMR data for kunzeanone B has now been corrected. Torellianol A (**8.26**), is the first completely reduced β-triketone analog to be reported. Antiplasmodial activity of new compounds torellianones A-F, torellianol A, and known compounds 4*S*/4*R*-ficifolidione, kunzeanone A were tested against chloroquine sensitive 3D7 strains of *Plasmodium falciparum* and only torrelianones C and D, E and F, 4*S*/4*R*-ficifolidiones and kunzeanone A had moderate antiplasmodial activity. Among the compounds tested for antibacterial activity, only mixture of torellianone E and F, lacking a pyran ring possessed activity (MIC 0.63 mM) against *Staphylococcus aureus* ATCC 157293.

8.1 β-Triketone Antiplasmodial Structure Activtiy Relationship

The isolated compounds were tested for their antiplasmodial activity against the chloroquine-sensitive 3D7 strain of *Plasmodium falciparum* and active compounds which exhibited potency less than 5 μM were further tested against the chloroquine resistant Dd2 strain of *P. falciparum*. As the β-triketones isolated were consisted of diverse structural features such as simple β-triketones, β-triketone/terpene adducts, β-triketone/flavonone adducts and β-triketone/phloroglucinol adducts, structure activity relationship of these compounds was studied. Interestingly, the IC_{50}s of woodsianone B (**8.2**) (3.0 ± 0.6 μM against 3D7 and 2.53 ± 0.11 against Dd2) and the lack of activity of torellianol A (**8.26**) at IC_{50} of 40 μM against chloroquine sensitive *P. falciparum* proved that the presence of β-triketone moiety as an essential part for

Table 8.1. Antiplasmodial and Antibacterial activities of β-triketones

Compound Class and Name	3D7 IC_{50} µM (n=3)	Dd2 IC_{50} µM (n=2)	HEK cytotoxicity IC_{50} µM (n=2)	Antibacterial Activity MIC mM
Simple β-Triketones				
Woodsianone B (**8.2**)	3.0 ± 0.6	2.53 ± 0.11	15.9 ± 5.7	0.02
Torellianol A (**8.26)**	IA	IA	IA	IA
β-Triketone terpene adducts				
Woodsianone A (**8.1**)	26.9 ± 2.0	IA	68%[a]	IA
Tomentodione A (**8.6**)	8.9[e]	IA	-	IA
Tomentodione B (**8.7**)	11.3[e]	IA	-	IA
4*S*-Ficifolidione (**8.8**)	16.7 ± 1.4	IA	IA	IA
4*R*-Fisifolidione (**8.9**)	~13.65	IA	4.89 ± 7.82	IA
Intermedione A (**8.15**)	12.45	18.5	64%	IA
Intermedione B (**8.16**)	9.93	-	-	IA
Intermedione D (**8.18**)	13.0	26.0	IA	IA
Intermedione E (**8.19)**	20.8	-	IA	IA
Intermediones B and C (**8.15** and **8.16)**	12.6	IA	85%	IA
β-Triketone phloroglucinol adducts				
Rhodomyrtosone A (**8.3**)	10.5 ± 0.9	-	59% [a]	IA
Rhodomyrtone (**8.5**)	1.8 ± 1.0	4.00 ± 0.30	11.4 ± 2.1	0.63
Watsonianone B (**8.13**)	0.29	0.44	85%	IA
Tomentosone A (**8.14**)	1.0	1.49	IA	IA
β-Triketone dimers				
Rhodomyrtosone D (**8.4**)	14.0 ± 6.5	-	IA[a]	IA
Watsonianone A (**8.10**)	5.3	8.8	42%	10
β-Triketone flavonone adducts				
Torellianone A and B (**8.20**, **8.21**)	IA	IA	IA	IA
Torellianone C and D (**8.22**, **8.23**)	7.2 ± 1.2		31.77 ± 6.84	IA
Torellianone E and F (**8.24**, **8.25**)	3.2 ± 0.332		75%	0.63
Kunzeanone A (**8.11**)	10.7 ± 1.6	-	IA[a]	IA
Kunzeanone B (**8.12**)	-	-	-	-
Pyrimethamine[f]	0.008 ± 0.009	62% at 40 mM	62% at 40 mM	-
Chloroquine[f]	0.0093 ± 0.0022	0.194 ± 0.038	IA	-
Pyronaridine[f]	0.0119 ± 0.0025	0.018 ± 0.003	5.22 ± 1.42	-
Puromycin[f]	0.062 ± 0.032	0.061 ± 0.0025	0.399 ± 0.001	-
Artesunate[f]	0.001 ± 0.0001	0.00067 ± 0.00018	94% at 20 mM	-
DHA[f]	0.0004 ± 0.0001	0.00043 ± 0.00007	83% at 20 mM	-

[a]% inhibition at the highest concentration tested (100 µM)
[b]activity reported from reference 12, HEK-293 inhibition at 120 mM
[c]activity reported from reference 26 HEK-293 inhibition at 40 mM
[d]Ligand efficiency LE = -1.36*p IC_{50}/(number of heavy atoms)
[e]n=1
[f]reference compounds

antiplasmodial activity. The strong IC_{50} of watsonianone B (**8.13**) (0.29 µM) against 3D7 and 0.44 µM against Dd2), rhodomyrtone (**8.5**) (1.8 ± 1.0 µM against 3D7, 4.00 ± 0.30 µM against Dd2), tomentosone A (**8.14**) (1.0 µM against 3D7, 1.49 µM against Dd2) indicated that the presence of β-triketone moiety conjugated to a phloroglucinol with an acyl side chain is more potent towards the parasite. In β-triketone/flavonone adducts torrelianone E and F (**8.22**) lacking pyran ring reported the potent IC_{50} (3.2 ± 0.332 µM). Kunzeanone A (**8.11**) and torellianone B (**8.12**) with isopropyl functionality at C-6 position had moderate IC_{50s} (10.7 ± 1.0 µM) while the torellianone A **(8.20**) with phenyl functionality at C-6 position was inactive. These results indicated that C-6 side chain too has effect on antiplasmodial activity.

The β-triketone/terpene adducts had moderate antiplasmodial activity ranging from 8.0 µM - 27.0 µM. The major issue with these molecules as well as with others was their low solubility in water. More potent activity could have been achieved from all the molecules if they had higher solubility in the testing mixture.

All the isolated β-triketones were screened for antibacterial activity against *Staphylococcus aureus* ATCC 157293. Among them, woodsianone B (**8.2**), rhodomyrtone (**8.5**) watsonianone A (**8.10**), and torellianone E and F (**8.24**, **8.25**) inhibited the growth of bacteria. The MIC values of them were 0.02 mM, 0.63 mM, 10 mM and 0.63 mM. The strong antibacterial activity (0.02 mM) of simple β-triketone, woodsianone B is interesting and it is required to further test against drug resistant bacterial strains. The special structural characteristic of watsonianone A and torellianone C was that they lacked pyran ring. Demonstration of strong antibacterial activity by woodsianone B (**8.2**) and the lack of activity found for torellianol A (**8.26**) proved that the β-triketone moiety is as an essential group for antibacterial activity. In the literature antibacterial activity of rhodomyrtone has been reported against drug sensitive as well as drug resistant strains.

8.2 Future work

1.0 In this thesis only three species of Myrtaceae were chemically investigated and it was proved that Myrtaceae species are a remarkable source of β-triketones with interesting anti-infective

properties. As only a tiny fraction of the Myrtaceae has been studied so far more research into the family will be carried out in the future to search for more β-triketones with interesting structural features and biomedical applications.

2.0 The literature review and current studies have shown that the chemistry present with specific. Myrtaceae genera have important taxonomic implications. However, since no comprehensive and systematic study has looked at the β-triketone/phloroglucinol chemistry within the family there is the potential through further future studies to complement our chemical knowledge of the family and this will be expected to fill some of these knowledge gaps.

3.0 During the antiplasmodial activity testing it was shown that solubility of β-triketones in water as a significant issue. It is anticipated that modification of these compounds to introduce more hydrophilic groups is likely to improve their water solubility and this could lead to more potently active structures. These studies would aid in expanding structure-activity relationships within this family of compounds.

4.0 Only antiplasmodial and antibacterial anti-infective properties have been investigated here. In the future these compounds should be tested against other drug targets such as prions, viruses and drug-resistant bacterial strains.