ISOLATION AND STRUCTURAL ELUCIDATION

OF

CYTOTOXIC AGENTS FROM MARINE

INVERTEBRATES AND PLANTS SOURCED

FROM

THE GREAT BARRIER REEF, AUSTRALIA

Thesis submitted by

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for the degree of Doctor of Philosophy

in the Department of Chemistry

James Cook University of North Queensland

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STATEMENT OF THE CONTRIBUTION OF OTHERS

I, the undersigned author of this work, acknowledge the contribution of others to this work. Substantial supervision was provided by Assoc. Prof. Bruce Bowden (School of Pharmacy and Molecular Sciences, JCU; primary supervision) and Dr Anna-Marie Babey (School of Biomedical Sciences, JCU). Editorial assistance in the preparation of this thesis was provided by Assoc. Prof. Bruce Bowden and Dr Anna-Marie Babey. Mr Rick Willis at the Australian Institute of Marine Sciences, Townsville, performed mass spectrometetry (ESI-MS) on samples. Mr Paul Gugger from the Research School of Chemistry, ANU, Canberra, performed the optical rotation ($[\alpha]_D$) experiments.

Madhavi Agrawal October 2007

DECLARATION ON ETHICS

I, the undersigned and author of this work, declare that the research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *James Cook University Policy on Experimentation Ethics*, *Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). Research involving the use of animals followed the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the Queensland Animal Care and Protection Act 2001*. The proposed research methodology for animal use received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A618_00)

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I DEDICATE THIS WORK TO MY MOTHER,

DR. MADHU. S. AGRAWAL

ABSTRACT

The interest in the marine environment has been stimulated by the array of biological activities of marine natural products and hence their potential biomedical applications. The advent of high throughput screening has allowed a large number of compounds to be tested for a range of biological activities, in order to assess their potential as pharmaceuticals. This study aimed to discover drug leads from marine invertebrates collected from the Great Barrier Reef (GBR) by screening extracts for pharmacological activity.

The pharmacological target was aimed to find novel cytotoxic compounds with potential as anticancer agents. Cytotoxicity was assessed *in vitro* using the P388D1 mouse lymphoma cell line. Thus 308 samples of marine invertebrates and plants were collected from the central section of the Great Barrier Reef (GBR). Bioassay guided fractionation led to the isolation and structural elucidation of seven new and twenty known cytotoxic metabolites. Structural elucidation was done via 1D and 2D NMR spectroscopy.

Two new brominated polyether triterpenes, Armatols G (**193**) and H (**194**) were isolated from the red alga *Chondria armata*. Compounds (**193**) and (**194**) exhibited moderate cytotoxicity, with IC_{50} values of 5.80 and 6.30 μg/ml. The relative stereochemistry throughout each molecule was determined via NOe experiments and by using Chem 3D and its MM2 energy minimization program to predict the lowest energy conformation. It is proposed that hydrolysis of the

acetate functionality and application of the Mosher's method for the resultant secondary alcohol should afford the absolute stereochemistry for compound **194**.

A new isomalabaricane triterpene, stelliferin D riboside (**195**) was isolated from the sponge *Rhabdastrella globostellata* along with the known isomalabaricanes, stelliferin A (**43**) and compound **51**. Stelliferin A (**43**) and stelliferin D riboside (**195**) exhibited IC_{50} values of 0.16 and 1.10 μg/ml respectively.

Five furanoditerpenes were isolated from the sponge *Spongia sp*. Spongiadiol diacetate (**97**) was most cytotoxic with an IC_{50} value of 2.10 μg/ml. This was followed by the new compound isospongiatriol (**196**) and the known compound epispongiatriol (**96**) which exhibited IC_{50} values of 14.0 μg/ml and 16.30 μg/ml respectively. Spongiatriol triacetate (**99**) was inactive in the assay. Isospongiadiol (**109**) was not tested due to decomposition of the compound.

A new pentabrominated phenolic diphenyl ether (**197**) was isolated from the sponge *Dysidea herbacea*. Compound (**197**) exhibited a moderate IC_{50} value of 2.20 μg/ml. All 4 positional isomers of diphenyl ethers that contain a 2,4-dibrominated B-ring and a 1-hydroxytribrominated A-ring with the ether linkage at the 2-position have now been reported from marine sponges.

A deaminated analogue of the known pyridoacridine alkaloid stellettamine (**188**) was isolated from the ascidian *Aplidium* sp. (cf *Aplidium cratiferum*). It was named nordehydrocyclodercitin (**200**). Cytotoxicity assays could not be

performed due to decomposition of the compound, but the cytotoxic activity of many pyridoacridine alkaloids is well documented

Two imidazole alkaloids, isonaamidine E (**225**) and its zinc complex, bis(isonaamidinato E)zinc (**408**) were isolated from a *Leucetta* sponge. The compound was assigned via spectroscopic methods. The electrospray results obtained for (**408**) were not readily interpreted in our hands, although molecular clusters that contained zinc and chlorine (from isotope patterns) were observed. The structure of **408** was verified by the addition of half an equivalent of $ZnCl_2$ to an isonaamidine E sample, which afforded a ^1H NMR spectrum that was identical to that observed for **408**.

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LIST OF ABBREVIATIONS

1D	one dimensional
2D	two dimensional
Aq	aqueous
br d	broad doublet
br s	broad singlet
C_6D_6	deuterated benzene
$CDCl_3$	deuterated chloroform
CH_2Cl_2	dichloromethane
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	Distortionless Enhancement by Polarisation Transfer
ESI-MS	Electrospray Ionisation Mass Spectrometry
EtOAc	ethyl acetate
EtOH	ethanol
FCS	foetal calf serum
HMBC	Heteronuclear Multiple-Bond Coherence
HSQC	Heteronuclear Single-Quantum Coherence
HPLC	High-performance Liquid Chromatography
i.d.	internal diameter
IR	Infrared
m	multiplet
MeOH	methanol
MeOH-d_4	deuterated methanol

m.p. melting point

NCI National Cancer Institute, Washington DC

NMR Nuclear Magnetic Resonance

nOe Nuclear Overhauser Effect

NOESY Nuclear Overhauser Effect Spectroscopy

OD optical density

PDA photodiode-array

ROESY Rotational Overhauser Effect Spectroscopy

S singlet

sp. species (singular)

spp. species (plural)

SRB sulforhodamine B

TCA trichloroacetic acid

t triplet

Tris tris(hydroxymethyl)aminomethane

UV Ultraviolet

Vis Visible Light

CHAPTER 1

Introduction-

Literature review of genera from which new metabolites have been isolated

1.1 General Introduction

Cancer is the second most common cause of death in the developed world, resulting in one out of five deaths worldwide. Currently, the most effective anti cancer compounds – anthracyclines, vinca alkaloids, taxanes and campothecins – have been developed from natural products, mainly from plants and microorganisms. These drugs, although extremely valuable are not ideal. They have numerous toxicities, principally myelosupression (bone marrow toxicity) and neurotoxicity. More importantly, many cancers are inherently resistant to these drugs or become so during prolonged treatment[1]. This implies the need to develop novel therapeutic agents, with innovative mechanisms of action. Thus scientists have turned to the marine ecosystem, which has already shown potential in the area of anticancer drug discovery. Numerous marine-derived metabolites have showed promising cytostatic activity, but have not progressed beyond preclinical/clinical trials. These include the bryostatins, halichondrins, spongiostatins, discodermolide, hemiasterlin and salinosporamide A to name a few[2].

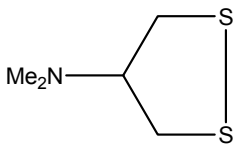
The most notable example is Yondelis™ or Et-743, isolated from a marine tunicate *Ecteinascidia turbinata*[3]. In 2005 The United States Food & Drug Administration (FDA) granted orphan drug designation to Yondelis™ for the treatment of soft tissue sarcoma (a cancer that arises in soft tissues such as fat, muscles, nerves etc.)[4]. Yondelis™ is an exciting new drug as it shows low potential for the cancer to become resistant to it, a condition that plagues the successful eradication of most cancers. In addition, it causes comparatively less damage to red blood cells than the current drugs in use for soft tissue sarcoma, implying that the drug may not cause

long term damage to the bone marrow (the site where blood cells are produced in the body)[5].

The revolutionary drug Aplidin™ derived from the sea hare *Aplidium albicans*[6] has also been granted orphan drug status by the FDA, for the treatment of multiple myeloma (MM)[4]. The survival rate for MM has not changed for the past three decades, with only about 28% of patients surviving for longer than five years. MM remains an incurable disease, with 10-year survival rates estimated to be less than 5%[7]. This is the second orphan drug designation granted to Aplidin™ by the FDA. The first was granted for the use of Aplidin™ in the treatment of acute lymphoblastic leukemia, a malignant disease of the bone marrow[7]. Other notable marine derived drugs in clinical and preclinical trials include Kahalalide F, isolated from the Hawaian nudibranch *E. rufescens* (Phase 2 for lung cancer)[8], ES-285, isolated from the mollusc *Spisula polynyma* (Phase 1 for advanced solid tumours)[9], Variolins, isolated from the Antarctic sponge *Kirckpatrickia varialosa* and Lamellarins, isolated from the ascidian *Didemnum chartaceum* (preclinical trials for ovarian, colon and other cancers)[10]. A rich pipeline of marine derived anicancer drugs have thus emerged in the recent past and these are opening potential new avenues for the treatment of cancer.

1.2 Literature review of metabolites reported from the red alga *Chondria armata*

Metabolites from the red algae of the genus *Chondria* are mainly known for their antimicrobial and antibiotic activity. They have been reported to produce cyclic polysulfides[11], terpenoids[12, 13], novel amino acids[14, 15] and amides[16]. Even though cyclic polysulfides are uncommon in nature they remain interesting because of the biological activity they exhibit. Amongst the genus *Chondria,* the only reported isolation of polysulfides (**1-7**) are from Mexican and Californian samples of *Chondria californica* which have demonstrated activity against the bacterium *Vibrio dignillarium*[11]. Of these cyclic polysulfides, 1,2,3,5,6-pentathiepane (**3**) and 1,2,4,6-tetrathiepane (**4**), which exhibit antibiotic properties, have also been isolated from the mushroom *Lentinus edodes*[17, 18]. Polysulfides in general are known to exhibit potent antimicrobial activity - for instance varacin (**8**)[19] isolated from a *Lissoclinum* sp., and varacins A-C (**9-11**) from an ascidian *Polycitor* sp.[20] all have demonstrated antibiotic activity in a number of antimicrobial assays. An arsenic compound (**12**) has been isolated from a sample of *Chondria crassicaulis*[21].



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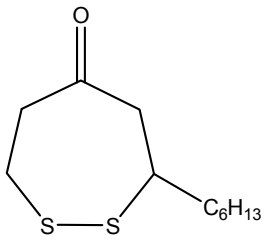

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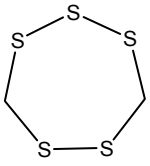

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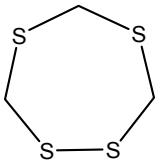

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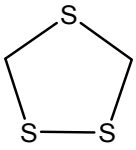

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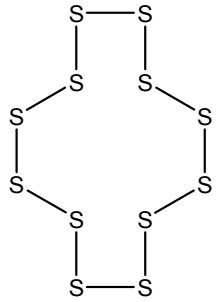

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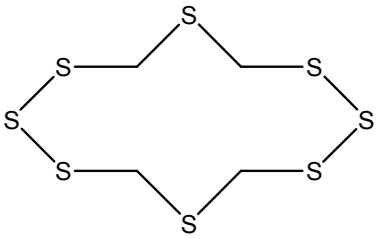

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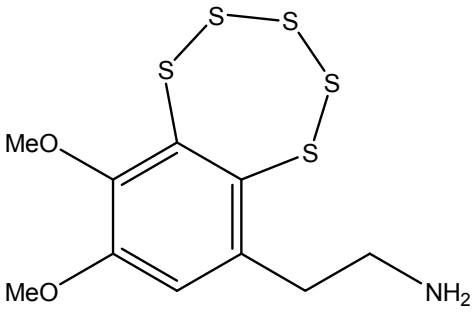

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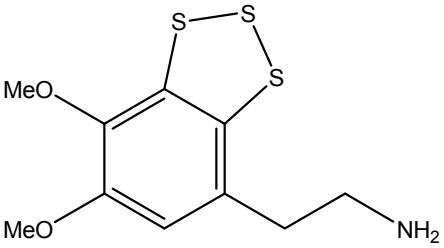

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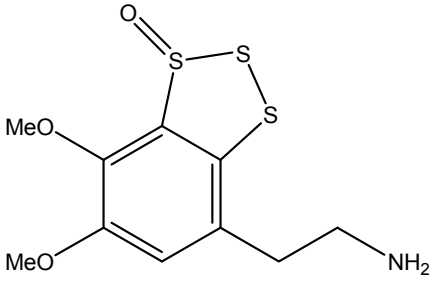

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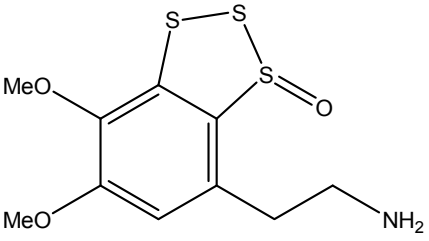

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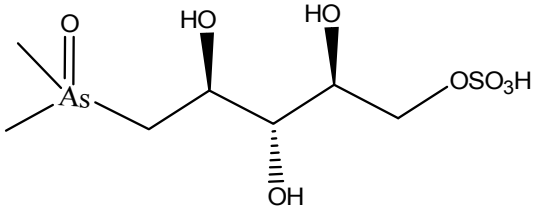

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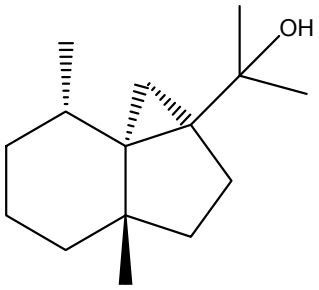

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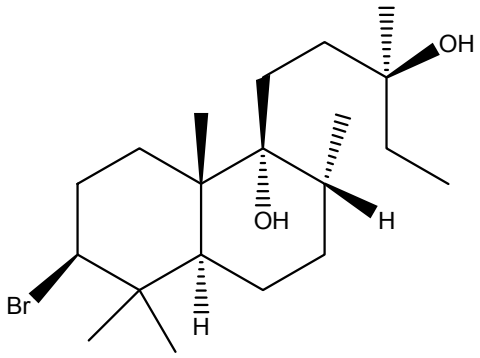
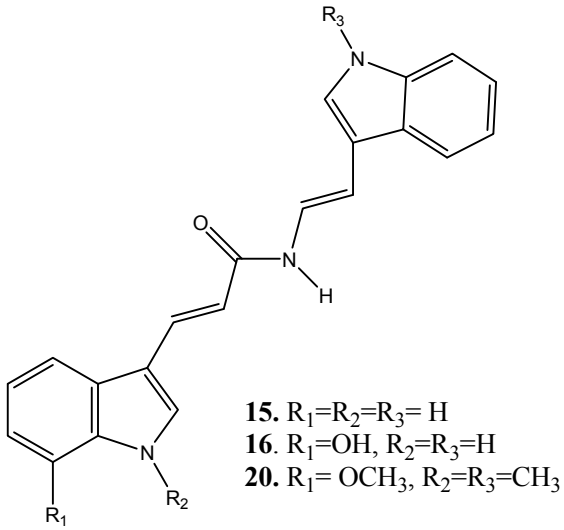


14.

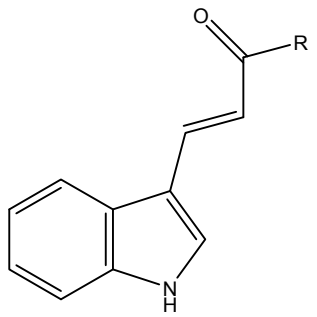
The occurrence of terpenes from the genus is limited to just two examples. These are the antibiotic sesquiterpenes- cycloeudesmol (**13**) from *Chondria oppossiticlada*[12] and ent-13-epiconcinndiol (**14**) from *Chondria tenuissima*[13]. Bisindole derivatives chondriamides A (**15**) and B (**16**) were isolated from an Argentinean *Chondria* sp[16]. Subsequently chondriamide C (**17**), the 10'-Z isomer of chondriamide A (**15**) was isolated from a Uruguayan sample of *Chondria atropurpurea*[22]. 3-Indoleacrylamide (**18**) and the known indoleacrylic acid (**19**) were also reported in the same paper. Compounds **15**, **17**, **18** and **20** (the O, N1, N1'-trimethyl derivative of compound **16**) showed *in vitro* anthelmintic activity against *Nippostrongylus brasiliensis*[22].

The amino acid derivatives from *Chondria* have received much attention due to their potent neurotoxic effects on humans and wildlife. Domoic acid (**21**), the first of these, a potent activator of the kainic receptors[16] in the central nervous system, was originally isolated from *Chondria armata* as an effective anthelmintic in the 1950's[23]. Years later, Maeda et al separated three isomers of this amino acid: isodomoic acids A (**22**), B (**23**) and C (**24**) from the same alga and investigated their

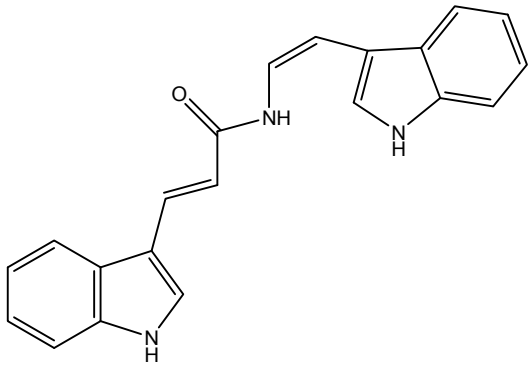
insecticidal activity[14, 23]. Domoic acid and its geometric isomers are now known to be the causative agent of amnesic shellfish poisoning (ASP), due to mussels that have ingested the toxin from their food source i.e. the diatom *Pseudonitzchia pungens forma multiseries*[24-27].



15. $R_1=R_2=R_3=H$
16. $R_1=OH$, $R_2=R_3=H$
20. $R_1=OCH_3$, $R_2=R_3=CH_3$



18. $R=NH_2$
19. R=OH



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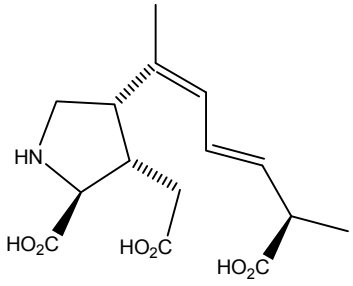
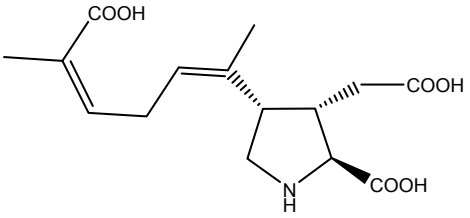


21.

In addition Wright et al have further separated geometrical isomers of domoic acid: isodomoic acids D (**25**), E (**26**), and F (**27**) as minor components from toxic

mussels[27], however the activity of some of them on kainate receptors was found to be weaker than that of domoic acid[28]. Isodomoic acids D, E and F have conjugated olefinic side chains, whereas isodomoic acids A, B, and C have non-conjugated olefinic bonds.



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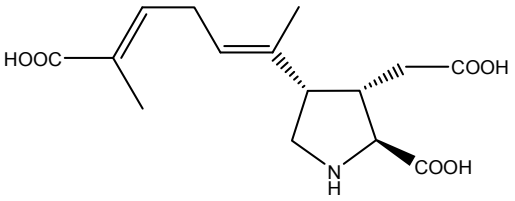

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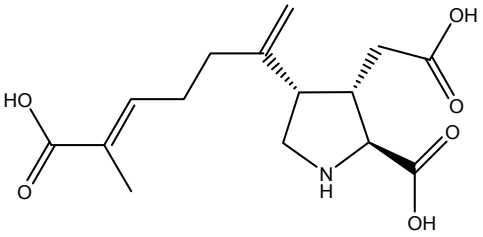

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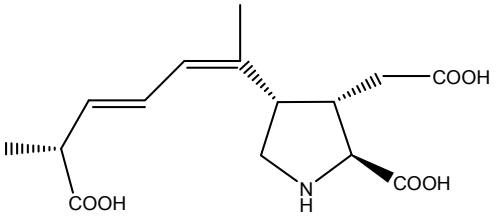

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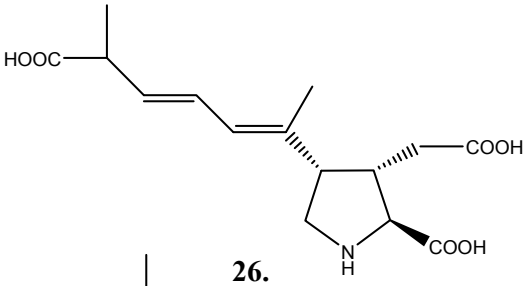

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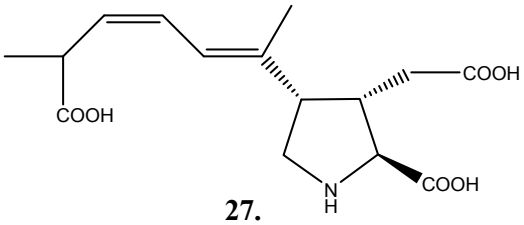

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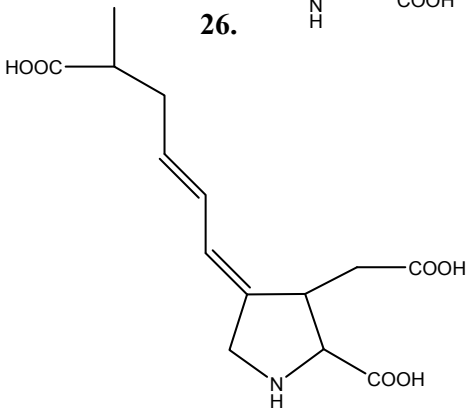

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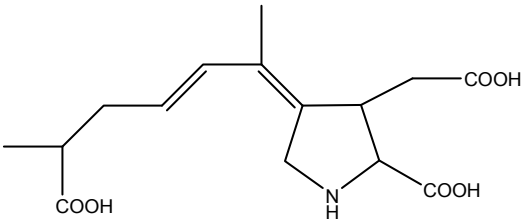
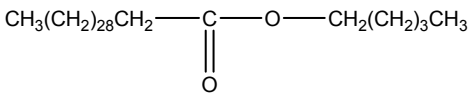


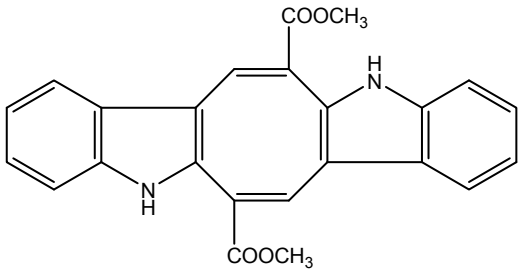
29.

Isodomoic acids A, B, E and F were re-isolated along with G (**28**) and H (**29**) from a *Chondria armata* sample collected from Kyushu Island, Japan. Isodomoic acids A, B, G and H seem to be specific to *C. armata* and have never been detected in the domoic acid producing diatoms[23]. An unusual metabolite, a long chain fatty ester, pentyl hentriacontanoate (**30**) along with an orange pigment, caulerpin (**31**) were isolated from a *Chondria armata* sample collected off the coast of Goa, India. Caulerpin is known to be a constituent of the green algae of genus *Caulerpa*[29].

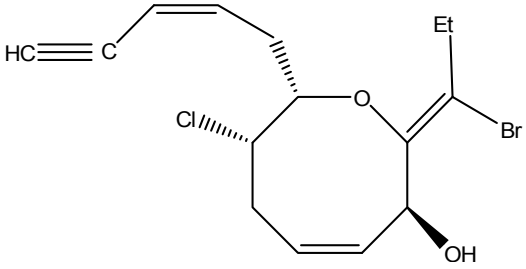
Chondriol (**32**) is an example of a halogenated acetylene and was isolated from a sample of *Chondria oppisiticlada*[30]. More recently, six new bromotriterpene polyethers, armatols A-F (**33-38**) were isolated from a sample of *Chondria armata* collected off the west coast of India[31]. Red algae are known to be a rich source of halogenated metabolites. Although the absolute stereochemistry for one centre was obtained using Mösher methodology, no evidence is presented in that report to correlate the relative stereochemistry for one ring system with the other[31]. Other examples of bromotriterpene polyethers are callicladol (**39**) isolated from a Vietnamese species of red algal genus *Laurencia*[32], enshuol (**40**) from a Japanese sample of *Laurencia*[33] and thyrsiferol (**41**) isolated from *Laurencia thyrsifera*[34].


CH3(CH2)28CH2
C
O
O
CH2(CH2)3CH3

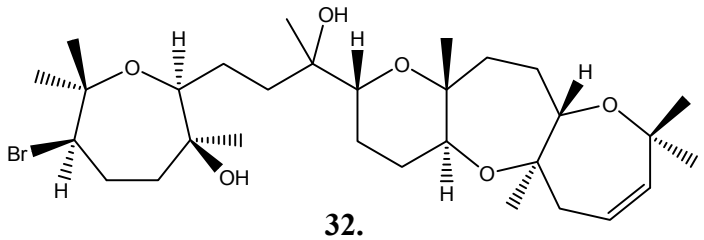
30.


COOCH3
H
N
N
H
COOCH3

31.


HC
C
Et
O
Cl
Br
OH

32.



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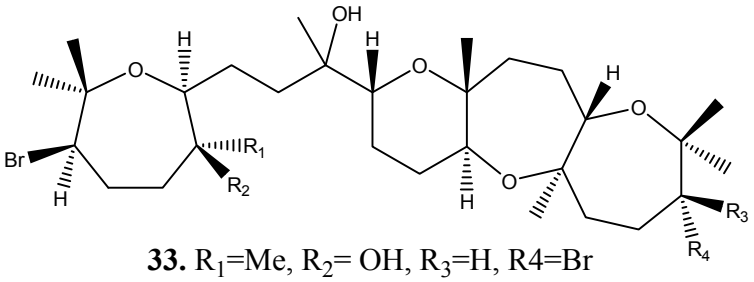
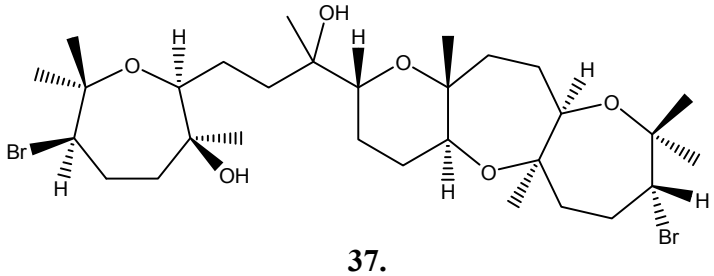
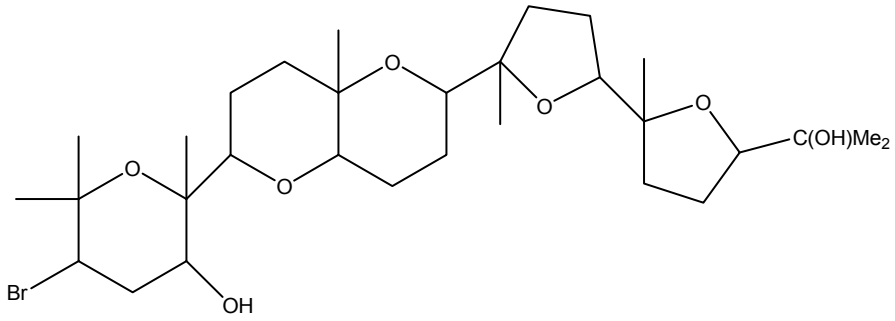


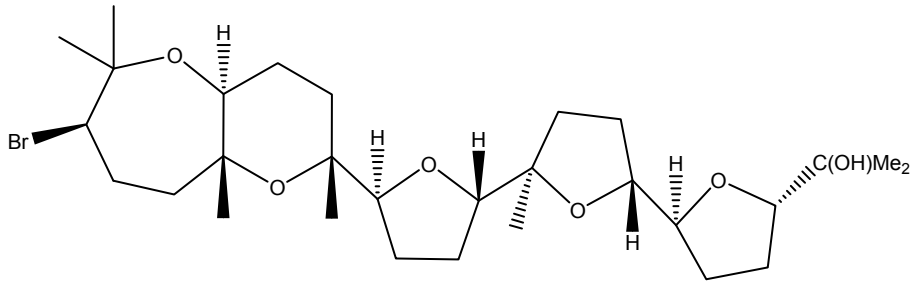
33. R_1=Me, R_2= OH, R_3=H, R4=Br
34. R_1=OH, R_2=Me, R_3=H, R_4=Br
35. R_1=Me, R_2=OH, R_3=Br, R_4=H
36. R_1=OH, R_2=Me, R_3=Br, R_4=H



37.



39.



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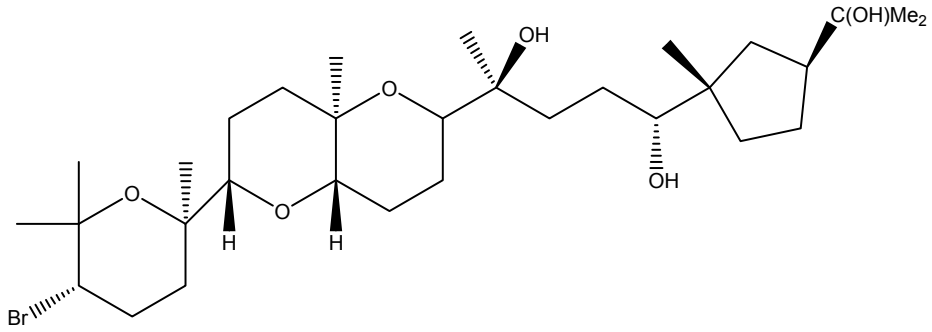
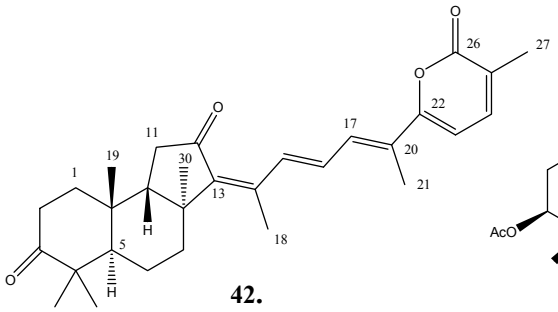


41.

1.3 Literature review of isomalabaricane triterpenes isolated from the sponge *Rhabdastrella globostellata*

A variety of highly cytotoxic isomalabaricane triterpenes have been characterised from *Rhabdastrella globostellata* and from sponges that have been reported as *Jaspis stellifera*, *Stelletta* sp., including *S. tenius* and *S. globostellata* and *Geodia globostellifera*. The nomenclature that has been used for isomalabaricane triterpenes

can be classified into three groups (A) the stelliferins, (B) the stelletins and (C) the globostellatic acids. Until the isolation of stelliferins the only triterpene that had been found in sponges was squalene[35, 36]. *Rhabdastrella globostellata* is a chocolate coloured sponge with a bright yellow interior that is commonly found in the following locations - on the reef flats of the Great Barrier Reef (GBR), New Caledonia and Fiji, off the coast of Somalia, Japan, and Indonesia. Its relatively soft tissue structure and lack of any calcareous or siliceous spicules for defence have led it to produce a rich array of cytotoxic triterpenes. Of these, structure (**42**) was first reported to possess the 6.6.5-*trans-anti-trans* ring system and erroneously assigned the rare malabaricane skeleton from spectral evidence[37]. Shortly after the first paper was published, an X-ray structure of compound (**42**) was reported which clearly showed that (**42**) had a 6.6.5-*trans-syn-trans* ring system. Since the ^{1}H and ^{13}C NMR spectral evidence was in close agreement with previously reported data, the authors proposed that the previous assignment be corrected and that structure (**42**) be classified as a member of the isomalabaricane triterpenes[38].



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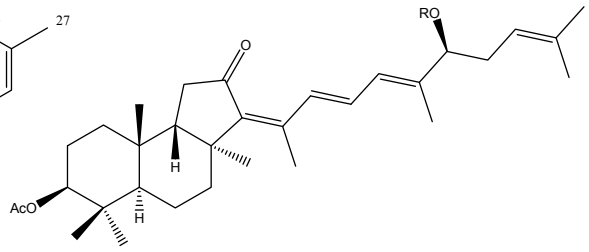
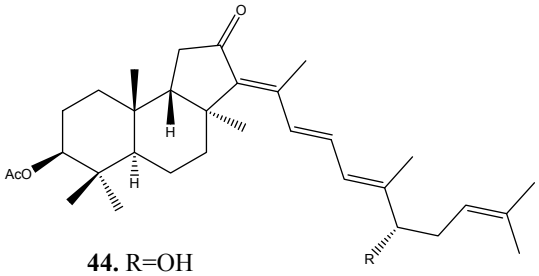
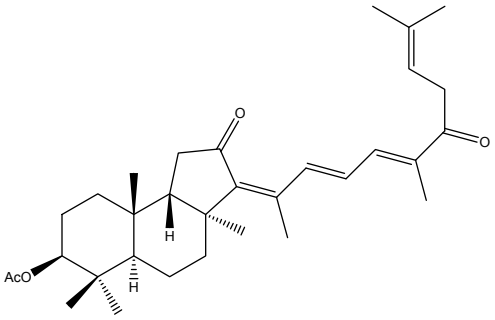


43. R=H
47. R=Ac

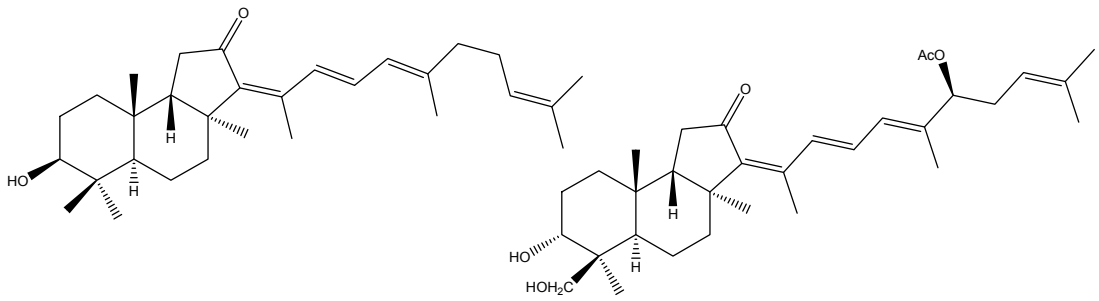


44. R=OH
48. R=OAc



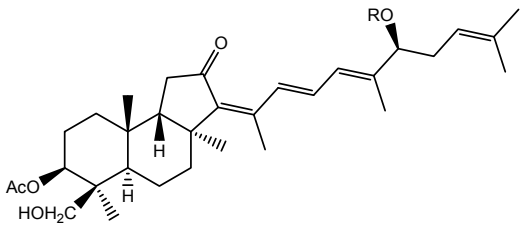
45.

The stelliferin derivatives A-F (**43-48**) were isolated in 1991 and their structures assigned by comparison of their spectral data with that of (**42**)[39]. It should be noted that isomerisation of the double bond adjacent to the ketone function in the presence of light has been observed and that the known isomalabaricane triterpenes can be divided into those with *E* and those with *Z* geometry. Other stelliferin derivatives include stelliferin G (**49**)[40], 29-hydroxystelliferin A (**50**)[40], compound (**51**)[41], 29-hydroxystelliferin D (**52**)[42], 29-hydroxystelliferin E (**53**)[40] and the epimers of **50** and **53**: 3-epi-29-hydroxystelliferin A (**54**) and 3-epi-29-hydroxystelliferin E (**55**)[42]. 3-Epi-29-hydroxystelliferin E (**55**) has shown moderate activity against A2780 ovarian cancer with an IC_{50} value of 27 μM[43] as well as antiproliferative activity against melanoma cells (MALME-3M) with an IC_{50} value of 1.20 μg/ml[40].

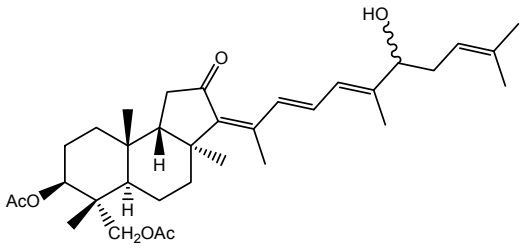


46. **49.**

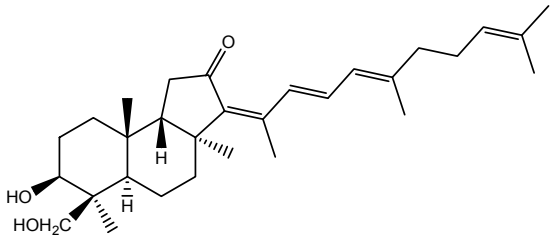
In the same assay 29-hydroxystelleferin E (**53**) showed similar activity at 2.27 μg/ml However the activity of mixtures of compound (**50**) and its 13*Z* isomer and compound **53** along with its 13*Z* isomer was found to be ten fold stronger with IC_{50} values of 0.11 μg/ml and 0.23 μg/ml respectively[40]. 3-Epi-29-hydroxystelliferin E (**55**) along with 29-hydroxystelliferin D (**52**) and 3-epi-29-hydroxystelliferin A (**54**) have also been tested on rat fibroblasts and at a concentration of 0.2 μM exhibited unusual morphological characteristics such as loss of pseudopodia, which affected their ability to attach to substrates followed by cell death in 5 days[42]. It must be noted that in the case of structure (**43**), the ketone at the C-3 position in the A-ring allows both of the rings to adopt the twist chair conformation in both 6-membered rings as evidenced in the reported X-ray structure, thus releasing the strain of the B-ring. The stelliferins with an alkoxy or hydroxy substituent at the C-3 position (e.g. stelliferins A-F), however, cannot adopt the twist-chair conformations so the B-ring must remain in the boat conformation[39].



50. R=H
53. R=Ac



51.



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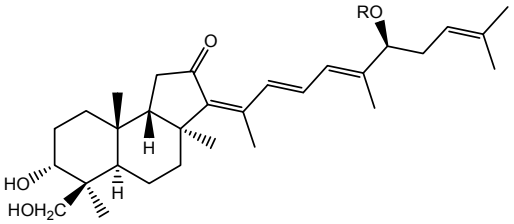
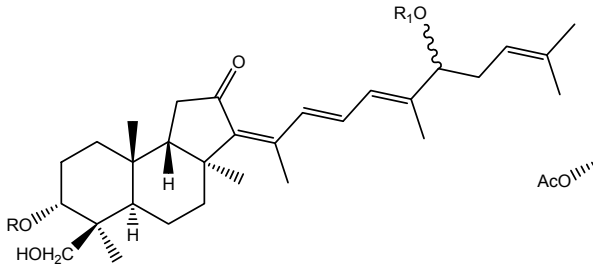
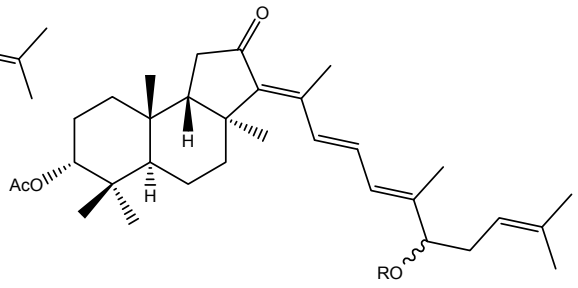


54. R=H
55. R=OAc



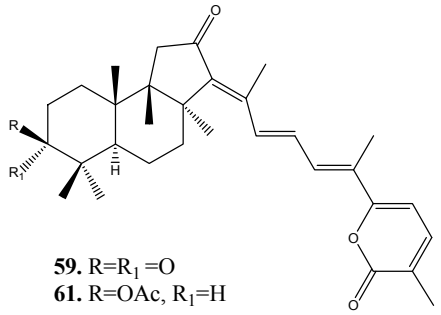
56. R=Ac, R_1=ribose
57. R=H, R_1=ribose



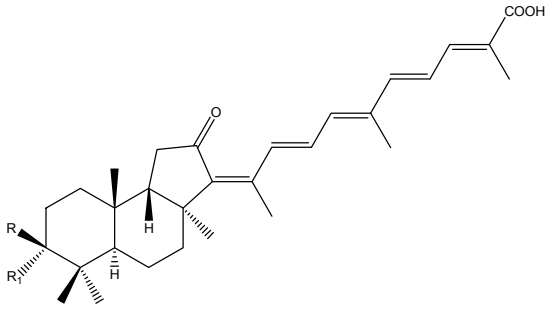
58. R=ribose

The first report of a saccharide derivative of stelliferin was from a Fijian sponge sample[44]. Stelliferin riboside (**56**) has shown moderate activity against ovarian cancer cells with an IC_{50} value of 38.1 μg/ml[44] and along with 3-epi-hydroxystelliferin E (**55**) induced 29% and 23% binding respectively in an assay measuring stabilization of binding of DNA with DNA polymerase β[43]. More recently

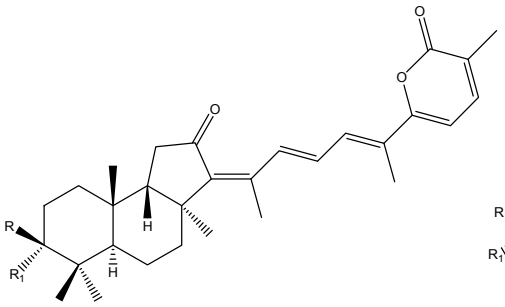
two more ribose congeners (**57-58**) have been reported[45]. Structures (**57**) and (**58**) have exhibited strong and specific activity against the mouse lymphoma L5178Y cell line with ED_{50} values of 0.22 nmol and 2.4 nmol respectively[45].



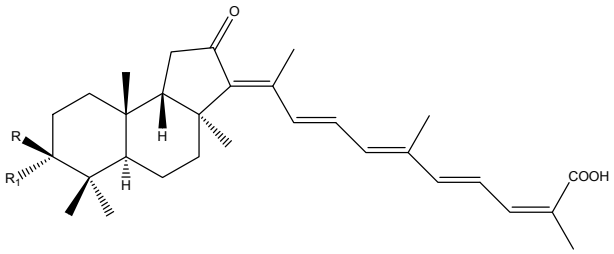
59. R=R_1 =O
61. R=OAc, R_1=H



63. R=R_1=O
66. R=OAc, R_1=H



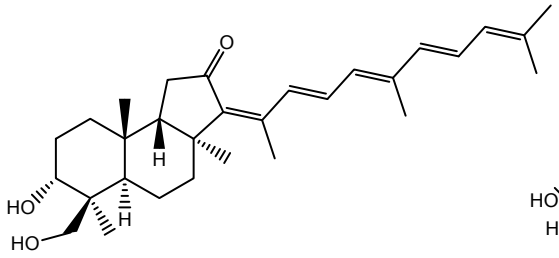
60. R=R_1=O
62. R=OAc, R_1=H



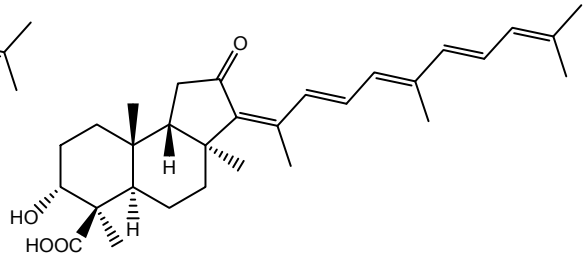
65 R=OAc, R_1=H
67. R=R_1=O

The stellettins usually consist of the γ-pyrone functionality which could be open in some of its congeners such that the olefinic chain terminates as a free carboxylic acid. The stellettins A (**59**)[46], B (**60**)[47], C (**61**) and D (**62**)[48] are examples of stellettins that have a lactone ring at the C-22 position (for numbering of the isomalabaricanes refer to results and discussion section). Stellettins E (**63**) and F (**64**) were isolated from the same sample that yielded stellettins B-D and are examples of stellettin congeners that have a free carboxylic acid at the C-27 position[48]. Other examples of stellettins that terminate in a free carboxylic group were reported in a subsequent

paper and named stellettins H (**65**), I (**66**) and rhabdastrellic acid-A (**67**) along with the known stellettins A-D (**61-64**)[49]. Rhabdastrellic acid-A (**67**) differs from structure (**65**) by the geometric position of the terminal carboxylic acid group. Stellettins A-D (**59-62**), H-I (**65-66**), stellettin E (**63**), and rhabdastrellic acid (**67**) have been tested against a set of isogenic colorectal cancer cells, wild type HCT-116 and the corresponding p21-deficient mutant cell line in which the p21 gene is disrupted through homologous recombination.

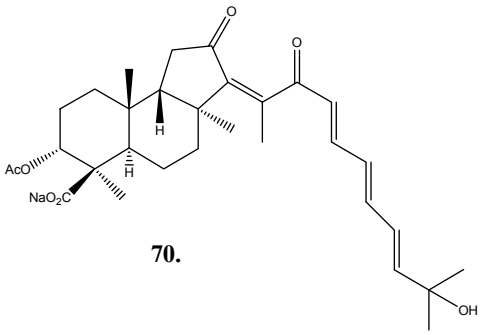


68.

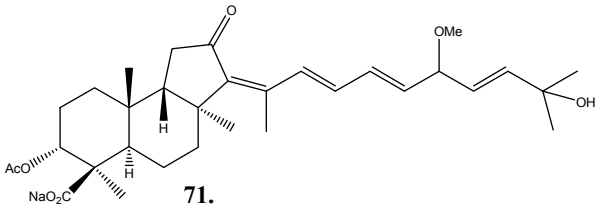


69.

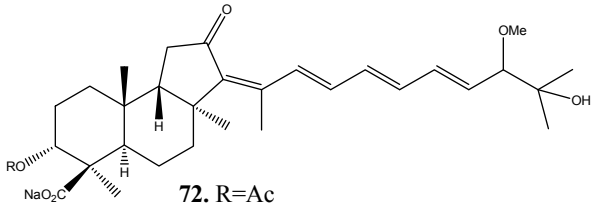
Stellettin B (**62**) and stellettin E (**63**) displayed selective toxicity toward the p21 deficient HCT cell line with IC_{50} values of 0.043 and 0.039 μM. The other isomalabaricanes were either very weakly active or inactive. It is interesting to note that both stellettin B (**60**) and stellettin E (**63**) contain a keto function at C-3 position and adopt 13Z geometry. It thus appears the terminus at the conjugated side chain does not play a major role for the activity[49]. Stellettin J (**68**) and K (**69**) have been isolated along with the known compound stelliferin riboside (**56**) from a Fijian sample. Stellettin J exhibited 5% DNA polymerase β binding at 28 μg/ml whereas stelettin K showed no activity in the same assay[43].



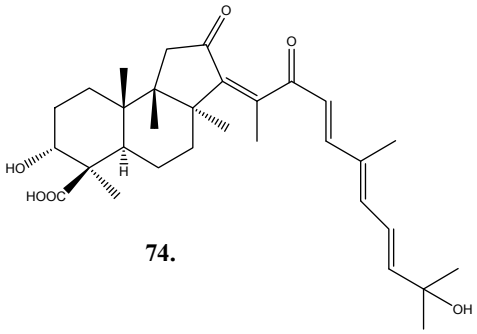
70.



71.

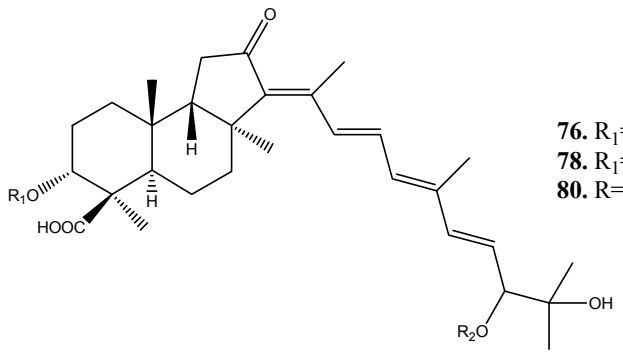


72. R=Ac
73. R= H

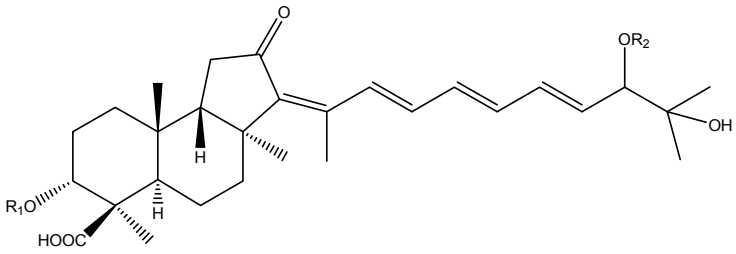


74.

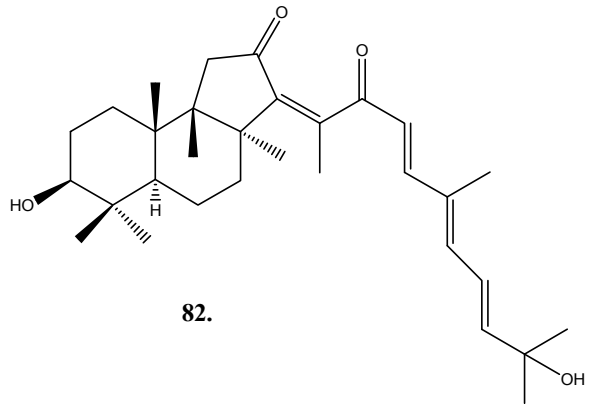
Unlike stelliferins which are oxygenated at position 19, globostellatic acids, like stellettin K, are carboxylated at the C-4 position. Globostellatic acids A-D (**70-73**) were isolated from a sponge sample from Japan[50]. Subsequently, globostellatic acids F-M (**74-81**) and globostelletin (**82**) were isolated along with known compounds globostellatic acid A (**70**) and D (**73**) from an Indonesian sample[45]. Globostellatic acids A-D (**70-73**) have exhibited cytotoxicity against P-388 murine leukemia cells with IC_{50} values of 0.1-0.46 μg/ml[50]. The globostellatic acids A-D (**70** and **73**), F-M (**74-81**) and globostelletin (**82**) have also been tested against different gram-positive and gram negative bacteria.



76. R_1=H, R_2=CH_2CH_3
78. R_1=$COCH_3$, R2=CH_2CH_3
80. R=H



75. R_1=H, R_2=CH_3
77. R_1=H, R2=CH_2CH_3
79. R_1=$COCH_3$, R_2=CH_2CH_3
81. R_1=H, R_2=H



82.

Globostelletin (**82**), globostellatic acids D (**73**) and G-I (**75-77**) showed moderate activity toward *Escherichia coli*, exhibiting inhibition zones of 10 mm at the loading concentration of 10 μg. At a similar concentration, the glycoside 3-deacetyl-stelleferin riboside (**57**) showed stronger antibacterial activity to *E. coli* (12 mm zone). For the gram-positive bacterium *Bacillus subtilis*, only globostelletin was found to be active, with inhibition zones of 12 and 13 mm at loading concentrations of 5 and 10 μg respectively. The globostellatic acids A-D (**70-73**), F-M (**74-81**)

globostelletin (**82**) and the ribosides (**57-58**) showed strong activity against the mouse lymphoma cell line L5178Y. In contrast globostellatic acids D/G (**73** and **75**), H-I (**76-77**), and L-M (**80-81**) and the riboside congeners (**57-58**) were weakly active against HeLa and PC-12 cells, while globostellatic acids A (**70**), F (**74**), J/K (**78-79**) and globostelletin (**82**) were inactive[45]. Much of the recent work on isomalabaricane triterpenes from *Rhabdastrella globostellata* has involved assays displaying their strong and often selective anti-tumour activity. However isomalabaricane terpenes have been shown to readily isomerize upon exposure to light during the isolation and characterization process and during storage they rapidly undergo equilibration to a 1:1 mixture of 13*E* and 13*Z* isomers, rendering their work up and characterisation difficult. Interest in these compounds remains however due to their significant cytotoxicity and biological activity.

1.4 Literature review of spongian diterpenes isolated from Dictyoceratid and Dendroceratid sponges

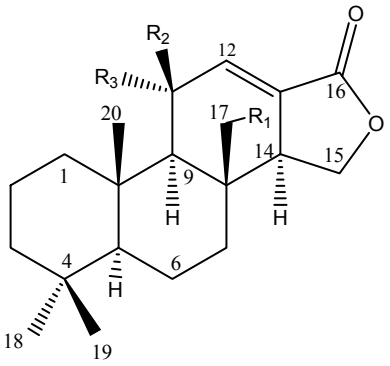
1.4.1 General introduction to furanoditerpenes

The terrestrial environment has yielded a number of furanoditerpenes and furanoditerpene lactones that exhibit biological activity such as antitumour activity[51], antiviral[52] and anti-inflammatory activities[53]. Crude extracts of plants that have been used in traditional and folklore medicine have afforded a number of these metabolites which in turn have been tested under modern laboratory environments for their potential in drug discovery research. The following paragraphs will focus on furanoditerpenoid metabolites from sponges of the orders Dictyoceratida and Dendroceratida that are based on the spongian diterpene framework. Even though a larger group of spongian diterpenes exists, some examples have undergone some form of skeletal rearrangement, and are not included in this review. Many examples of rearranged spongian diterpenes also come from nudibranchs, mainly belonging to the genus *Chromodoris*, and it is most likely that these metabolites are sequestered by the nudibranch from its sponge prey[54-56].

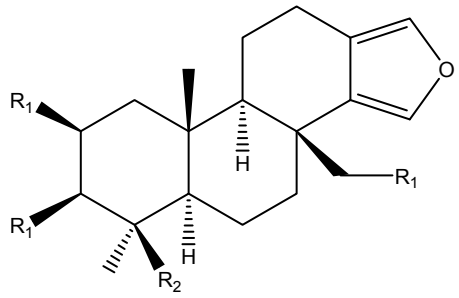
1.4.2 Spongian furanoditerpenes and derivatives

The first report of a spongian diterpene was the isolation of the lactone **(83)** named isoagatholactone from a collection of *Spongia officianalis*[57], the chemical structure and absolute stereochemistry of which were determined by the chemical correlation of the diterpene with grindelic acid[58]. Subsequently, a collection of *S. officianalis* from the Canary islands was found to produce the related spongian metabolites **(84-**

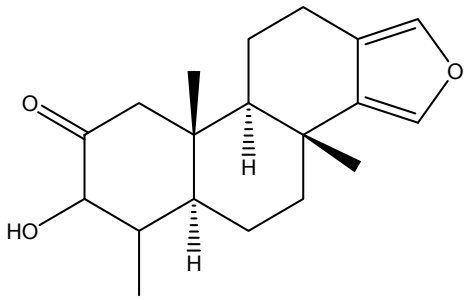
87)[59]. Compounds **(83)**, **(84)**, and **(85)** exhibited cytostatic activity at concentrations of 10, 1 and 5 μg/ml respectively against the HeLa cell line[60]. A specimen of *S. officinalis* collected in Papua New Guinea afforded three metabolites **(88-90)**[60]. The synthesis of **(88),** which afforded its absolute stereochemistry[61], has since been reported.



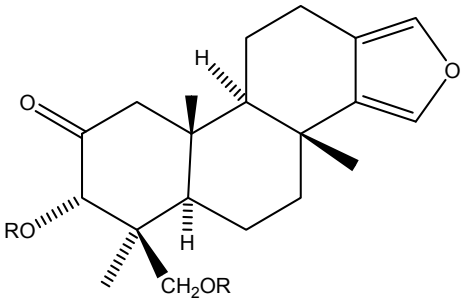
83. $R_1=R_2=R_3=H$
84. $R_1=R_3=H$, $R_2=OH$
85. $R_1=R_3=H$, $R_2=OAc$
86. $R_1=R_2=$ OH, $R_3=H$
87. $R_1=R_3=OH$, $R_2=H$



88. $R_1=H$, $R_2=COOH$
89. $R_1=H$, $R_2=CHO$
90. $R_1=H$, $R_2=CH_3$
91. $R_1=$ OH, $R_2=CH_2OH$



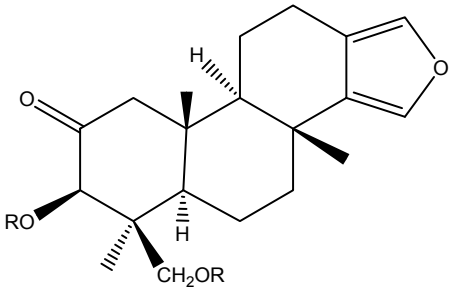
92.



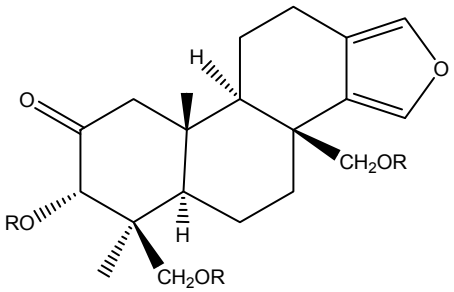
93. R=H
97. R=Ac

Structurally related compounds **(91)** and **(92)** were isolated from a collection of *Spongia* specimens from the Great Barrier Reef[62]. Structures **(88-91)** differ in the level of oxidation at the C-19 position. Other tetracyclic compounds that are

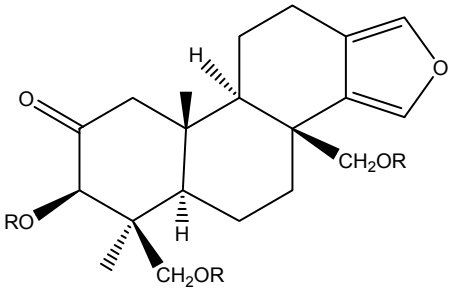
structurally characterized by a ring D furan and functionalised ring A include spongiadiol **(93)**, epispongiadiol **(94)**, spongiatriol **(95)**, epispongiatriol **(96)** and their corresponding diacetates **(97-98)** and triacetates **(99-100)**, all of which were isolated from a *Spongia sp.* collection from the GBR, Australia[63].



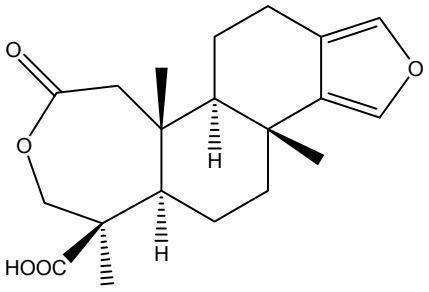
94. R=H
98. R=Ac



95. R=H
99. R=Ac



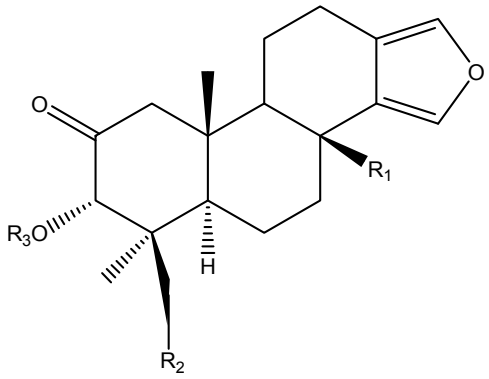
96. R=H
100. R=Ac



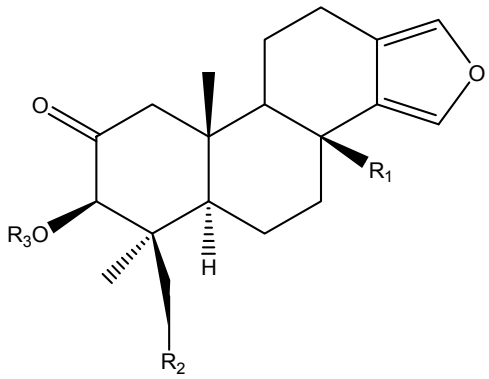
101.

Subsequently the mono-acetylated analogue of spongiadiol **(93)** was isolated from a collection of *Spongia arabica* from the Red Sea and was given the name spongialactone **(101)**[64]. Compounds **(93)** and **(94)** showed moderate cytotoxic activity against P388 cells at IC_{50} concentrations of 0.5 µg/ml and 8 µg/ml

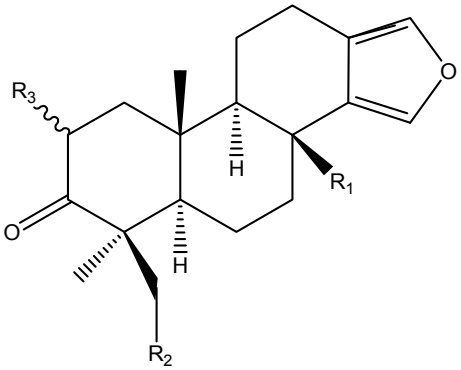
respectively. In addition antiviral activity against HSV-1 virus at IC_{50} concentrations of 0.25 μg/ml and 12.5 μg/ml was exhibited in the same study[65].



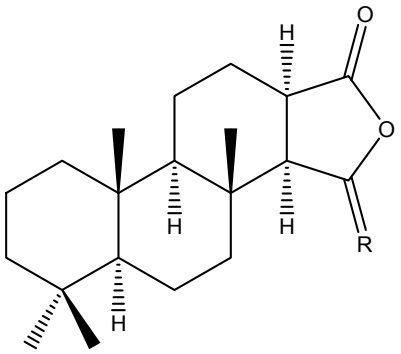
102. R_1=CH_2OH, R_2=R_3=H
103. R_1=CH_3, R_2=R_3=H



104. R_1=CH_2OH, R_2=R_3=H
107. R_1=CH_3, R_2=OH, R_2=Ac

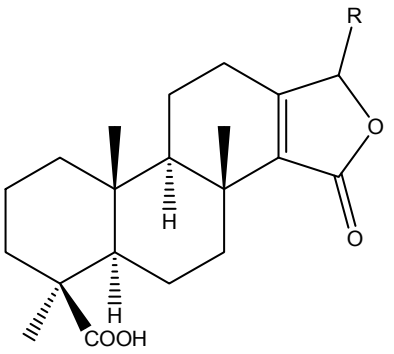


105. R_1= CH_2OH, R_2= H, R_3 =α-OH
106. R_1= CH_2OH, R_2= H, R_3= β-OH
108. R_1=CH_3, R_2=OH, R_3=H
109. R_1=CH_3, R2=OH, R_3=α-OH

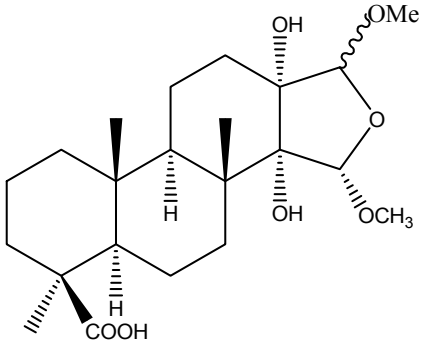


110. R= H, H
111. R= O

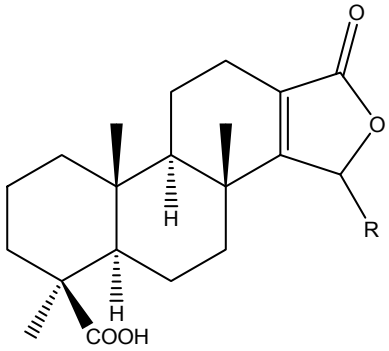
A collection of a *Spongia sp.* from Western Australia afforded five structures **(102-104), (105)** and its C-2 epimer **(106)**[66]. Related structures **(107-109)** have been isolated from *Hyatella intestinalis*, another member of the family Dictyoceratida[67]. Compound **(104)** has also been isolated from a deep water collection of *Spongia sp*. and exhibited IC_{50} concentrations of 5 μg/ml and 2 μg/ml against the P388 cell line and HSV-1 virus respectively [65].



112. R= β-OMe
113. R= α-OMe
114. R= H



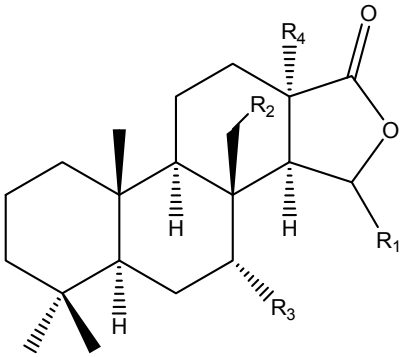
117.



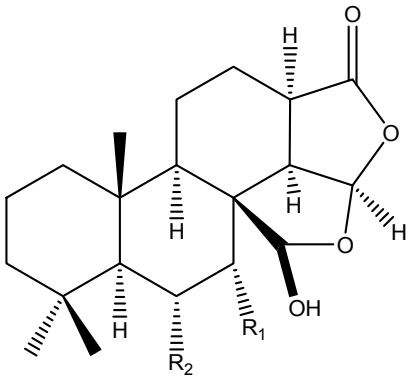
115. R=α-OMe
116. R=H
118. R=OH

A number of spongian diterpenes where the furan ring has been oxidised have been reported in the literature. The simplest examples are the lactone **(110)** and the anhydride **(111)**, isolated from the Dendroceratid sponge *Dictyodendrilla cavernosa*[68]. Moderate antineoplastic activities for compound **(110)** with IC_{50} values of 5.0 and 9.2 μg/ml with respect to L1210 and KB cell lines have been reported[69]. Other examples include structures **(112-117)** isolated from Micronesian specimens of *Spongia matamata*. These metabolites are examples wherein C-19 has been oxidised to a carboxylic acid functionality[70]. Subsequently a similar compound **(118)** was isolated from the Dictyoceratid sponge *Coscinoderma mathewsi*[71]. The Dendroceratid sponge *Aplysilla rosea* has yielded five acetylated lactones **(119-**

120)[72, 73] and **(121-123)**[74] named aplyroseols 7-16 and six hemiacetals **(124-130)** named aplyroseols 1-6[72].



119. R_1=R_4=H, R_2=R_3=OAc
120. R_1=R_2=R_4=H, R_3=OAc
121. R_1=R_3=R_4=H, R_2=OAc
122. R_1=OH, R_2=R_4=H, R_3=OAc
123. R_1=H, R_2=R_3=OAc, R_4=OH



124. R_1=OCOPr R_2=H
125. R_1=OAc, R_2=H
126. R_1 =OCOPr, R_2=OH
127. R_1=OCOPr, R_2=OAc
128. R_1= OH, R_2=OCOPr
129. R_1=OAc, R_2=OCOPr
130. R_1=OH, R_2= H
131. R_1=R_2=H
132. R_1=R_2=OAc

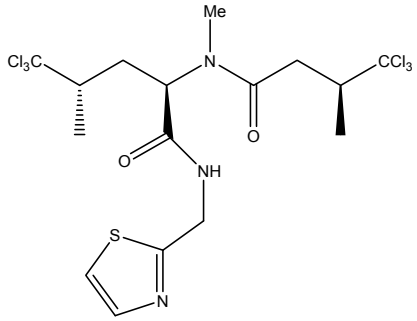
Compounds **124** and **125** were first isolated from the Caribbean Dendroceratid sponge *Igernella notabilis*[75]. The absolute stereochemistry of **124** was established from the X-ray crystal structure of its p-bromobenzoate derivative[76]. In addition **124** showed moderate cytotoxic activity against lymphocytic leukaemia cells (IC_{50} = 6.5 μg/ml)[75]. On the other hand compound **125**, isolated from the nudibranch spongivore *Chromodoris obsolete*, was cytotoxic to L1210 and KB cancer cell lines (IC_{50} = 1.9 and 2.5 μg/ml respectively)[69]. Two further compounds containing a hemiacetal bridge between C-15 and C-17 are compounds **131-132** (dendrillol-1 and 2), isolated from *Dendrilla rosea* collected off Sydney[73]. Compound **131** has been synthesized[77] and its structure was also confirmed from X-ray analysis[73].

1.5 Literature review of brominated diphenyl ethers isolated from the sponge *Dysidea herbacea*

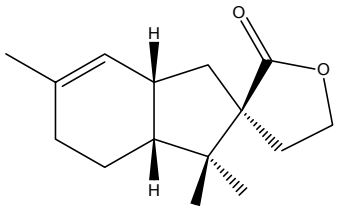
The recent reclassification of the family Dysideidae has led to the novel genus *Lamillodysidea*[78]. This newly proposed taxonomic nomenclature is synonymous with many sponges previously designated as *Dysidea herbacea*, and is not widely recognised in the literature. Thus, for the purpose of this review (and elsewhere in the thesis) the *Dysidea* nomenclature has been retained.

1.5.1 General introduction for *Dysidea herbacea* metabolites

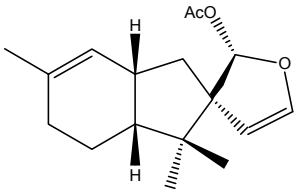
It is well documented that the marine sponge *Dysidea herbacea* occurs in two general chemotypes: one produces sesquiterpenes (usually furanosesquiterpenes) and polychlorinated amino acid derivatives, while the other produces only polybrominated diphenyl ethers[79]. The production of the chlorinated metabolites and the polybrominated diphenyl ethers has been reported to be due to the filamentous cyanobacterium *Oscillatoria spongeliae*[80-82]. For example a specimen of *Dysidea herbacea* from Heron Island, Australia contained 13-demethylisodysidenin (**133**) as the major chlorinated metabolite together with the sesquiterpenes herbadysidolide (**134**) and spirodysin (**135**)[80]. GC-MS analysis of the extracts revealed that the sesquiterpenes herbadysidolide (**134**) and spirodysin (**135**) were localised in the sponge cell fraction whereas 13-demethylisodysidenin (**133**) was localised in the cyanobacterial cells[80].



133.



134.

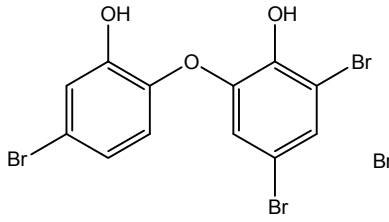


135.

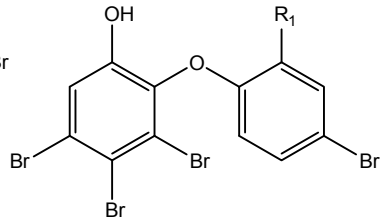
Recently, 16S-rDNA studies on *Oscillatoria* strains isolated from *Dysidea* samples that exhibited different colourmorph and growth characteristics indicated that each species of *Dysidea* hosted a distinct strain of *Oscillatoria*, which was interpreted to imply a high degree of host specificity and possible co-evolution between the symbiotic bacterium and its host sponge[83]. Therefore it is possible that the type of metabolites produced by the sponge may depend on the particular strain of cyanobacterium it harbours. For instance, a specimen of *Dysidea herbacea* from a shallow water lagoon from Palau contained 2-(2',4'-dibromophenyl)-4,6 dibromophenol (**136**) as the major metabolite. However, three other *Dysidea* "species" were found in the same location: two of these contained different polybrominated diphenyl ethers while the third contained only sesquiterpenes. The nudibranch *Chromodoris funerea* which is common in that location, contained a complex mixture of polybrominated diphenyl ethers and sesquiterpenes that are all obtained from the *Dysidea* spp. suggesting a dietary source of these metabolites[84].

1.5.2 Polybrominated diphenyl ethers

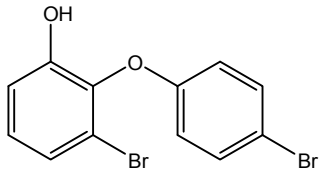
As a group, the polybrominated diphenyl ethers exhibit a wide range of activities in bioassays, ranging from antibacterial activity (against *S. aureus* and *T. mentagrophytes*), to cytotoxicity (Ehrlich ascite tumor cells)[85]. This cytotoxicity is exhibited by inhibition of a range of enzymes that are implicated in tumor development, such as inosine monophosphate dehydrogenase, guanosine monophosphate synthetase and 15-lipoxygenase[86].



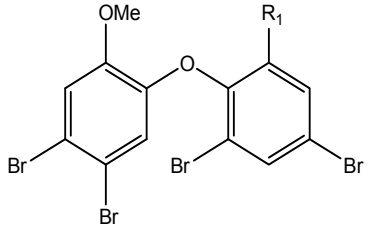
136.



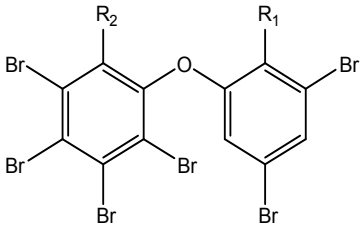
137. R_1=Br
150. R_1=OH



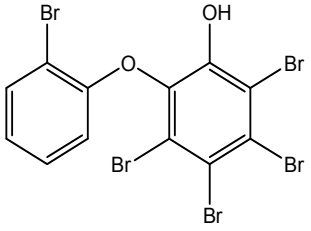
138.



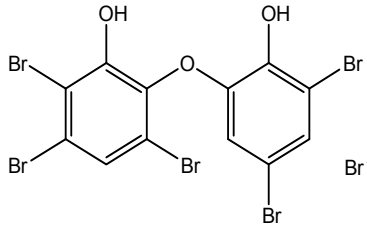
139. R_1= OH
140. R_1= OMe



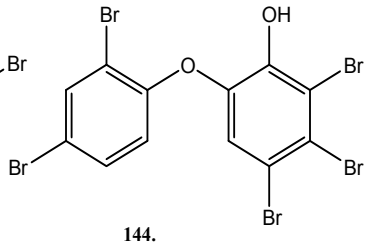
141. R_1=R_2=OH
142. R_1=R_2=OMe



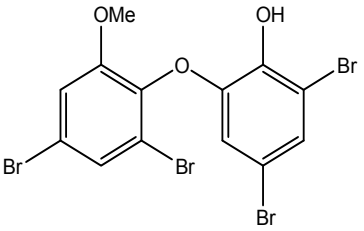
145.



143.

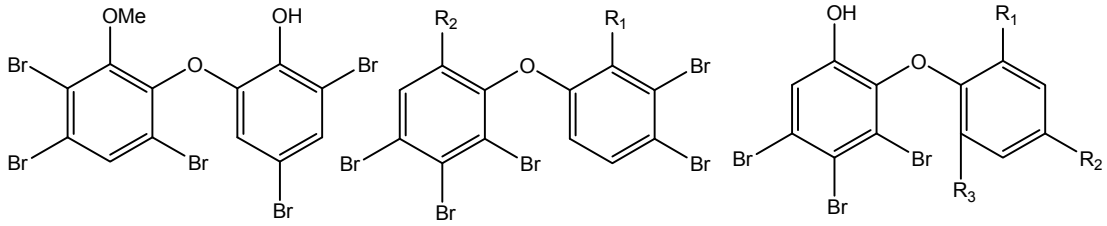


144.



146.

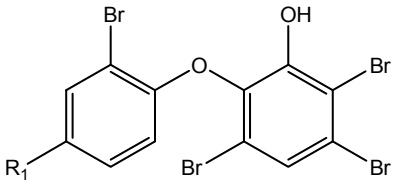
The earliest reports of brominated diphenyl ethers from *Dysidea herbacea* were in 1972 with the isolation of compounds (**137**) and (**138**)[87]. Subsequently structures **139-143**[88], **137-144**[89] and **145**[90] were reported. Compounds **146-150**[86] were isolated from a sample of *Dysidea herbacea* and shown to be active in a number of enzyme assays including inosine monophosphate dehydrogenase, guanosine monophosphate synthetase and 15-lipoxygenase. From the diphenyl ethers reported to date, structures **151-154**[91] are the only metabolites that have been reported to exhibit antifungal activity against the the phytopathogenic fungus *Cladosporium cucumerinum*. In addition, metabolites **151-154** also showed moderate activity against the gram positive bacteria *Bacillus subtilis*. More recent examples of diphenyl ethers from *Dysidea herbacea* are structures **155**[92] and **156**[93] isolated from samples collected from the GBR. Recent studies have also reported detection of polybrominated diphenyl ethers in higher trophic groups such as fish, turtles, birds and even marine mammals[94, 95], implying that these compounds are bioaccumulated in nature, and may persist in significant concentrations in such higher trophic organisms.



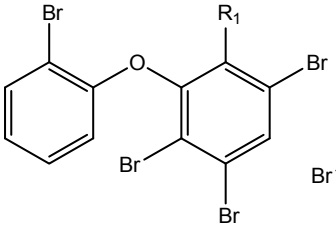
147.

148. R_1=R_2=OH
149. R_1=OH, R_2=OMe

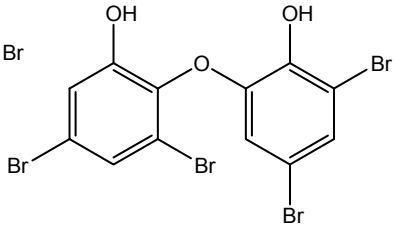
150. R_1=OH, R_2=R_3=Br
151. R_1=Br, R_2=R_3=H



152. R_1=H
155. R_1=Br

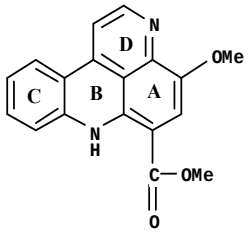


153. R_1=OH
154. R_1=OMe

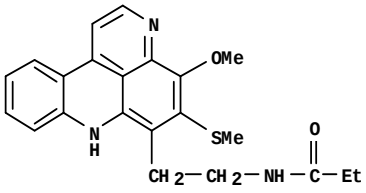


156.

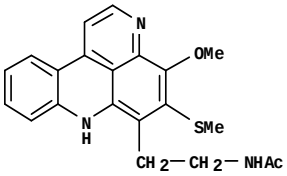
1.6 Literature review of pyridoacridine alkaloids isolated from marine invertebrates.



157.



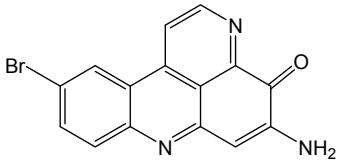
158.



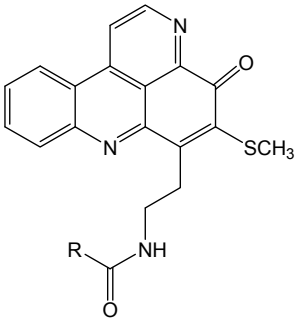
159.

The pyridoacridines form one of the largest groups of metabolites to be isolated from marine invertebrates. They are a family of heterocyclic alkaloids that have been reported from ascidians, sponges, anemones, and a prosobranch mollusc, although it is likely that the latter sample had accumulated the alkaloids from dietary intake. They are notable for their biological activity in a variety of biological assays (anti-neoplastic, anti-HIV, intercalation of DNA, insecticidal and antifouling)[96] and as a group have generated interest as a source of new lead structures for the development of therapeutic agents[97, 98]. However, biological evaluation has shown the superiority of analogues as therapeutic agents in the ascididemnin and meridine series when compared to the natural products[97].

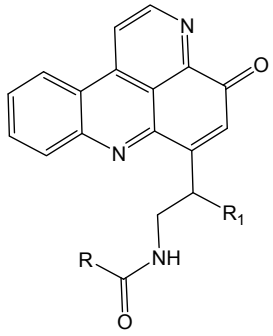
The pyridoacridines are highly coloured and their structures are based on 11-H pyrido [4, 3, 2-mn] acridine or 8H-pyrido [4, 3, 2-mn] acridone skeletons. Structurally they vary due to the different substituents located on ring A or due to the mode of ring fusion. Amongst the tetracyclic pyridoacridines the first group contains norsegoline (**157**)[99, 100] and varamines A (**158**) and B (**159**)[101], whereas the second group contains pantherinine (**160**)[101], cystodytins A-J (**161-170**)[102-104], diplamine (**171**)[105] and lissoclins A (**172**) and B (**173**)[106].

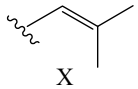


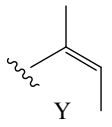
160.

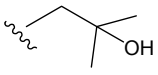


171. R = CH_3
172. R = i-Bu
173. R = Y







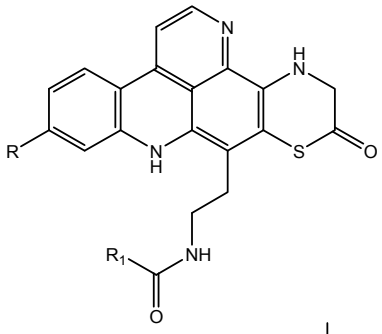


Z

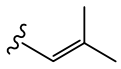
161. R = X, R_1 = H
162. R = Y, R_1 = H
163. R = Z, R_1 = H
164. R = X, R_1 = OH
165. R = Y, R_1 = OH
166. R= X, R_1 = OCH_3
167. R = Y, R_1 = OCH_3
168. R = X, R_1 = $OOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$
169. R = Y, R_1 = $OOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$
170. R = CH_3, R_1 = H

Norsegoline (**157**) was isolated from Red Sea sample of a *Eudistoma* sp.[99], whereas varamines A (**158**) and B (**159**) were isolated from a Fijian collection[101]. Norsegoline (**157**) has a methyl ester group at C-1 whereas the varamines have an amide-substituted side chain at C-1. Pantherinine (**160**) which was isolated from the south

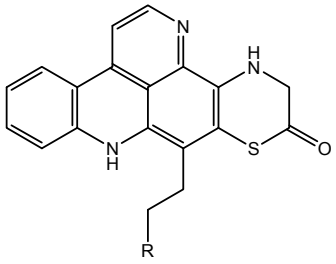
Australian ascidian *Aplidium pantherinum* is the only example in the tetracyclic group to contain a bromine atom[101]. Amongst the tunicates, *Cystodytes dellechiajei* has yielded a variety of tetracyclic pyridoacridine derivatives – the Cystodytins A-J (**161-170**), which again differ in the functionality at ring A. The most complex of these are cystodytins H (**168**) and I (**169**), which are lipid acylated. Diplamine (**171**) and lissoclins A (**172**) and B (**173**) contain a SCH_3 group at position C-2 and an amide group at C-1, however the three differ in the nature of the acyl group on the amide side chain.



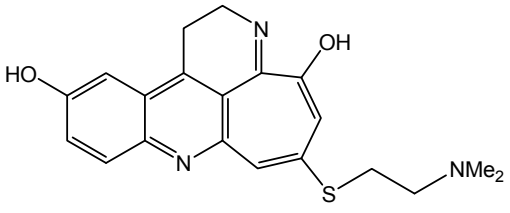
174. R = Br, R_1 = CH_3
175. R = H, R_1 = CH_3
176. R = H, R_1 = X



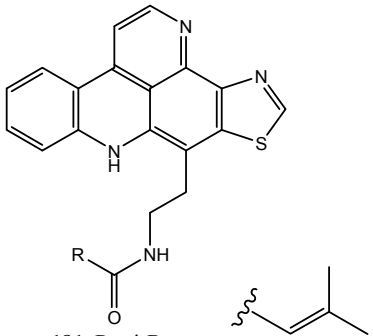
X



177. R=NMe_2
178. R=N(O)Me_2

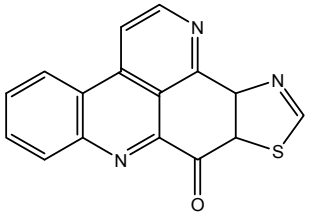


179.



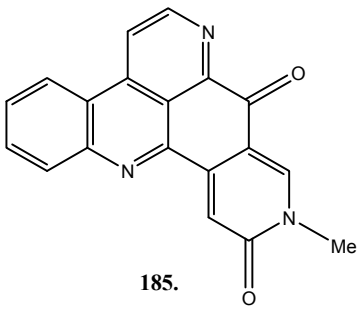
181. R = i-Bu
182. R = CH_2CH_3
183. R = CH_3
184. R = X

X



180.

Addition of a cysteine residue to cystodytins affords pentacyclic pyridoacridines, such as the shermilamines[104, 107-109] and thiazole-containing pyridoacridines, such as the kuanoniamines[110] (see Fig 1.). Shermilamines A (**174**) and B (**175**) were isolated from a purple *Tridemnum* sp. Subsequently shermilamine C (**176**) was reported from a Cystodytes sp[104]. Shermilamines D (**177**), E (**178**) and tintamine (**179**) were isolated from *Cystodytes violatinctus*[111]. Kuanoniamines A-D (**180-183**) have been isolated from the mollusc, *Chelynotus semperi* from Pohnpei[110]. Kuanoniamines B and C have also been isolated from the sponge *Oceanapia saggittaria*[112]. Subsequently, dehydrokuanoniamine B (**184**) was isolated from a Fijian specimen of *Cystodytes* sp.[104]. Amphimedine (**185**), isolated from the Pacific sponge *Amphimedon* sp.[113], was the first marine-derived pyridoacridine alkaloid reported and is also considered to arise from a cystodytin precursor. It was subsequently reported from a *Didemnum* collected in Okinawa[114] and from a Mediterranean ascidian *Cystodytes delle chiajei*[115]. Meridine (**186**) was isolated from a marine sponge *Corticium* sp. and reported to have antifungal properties[116]. Ascididemin (**187**) was initially isolated from an Okinawan *Didemnum* sp.[114].



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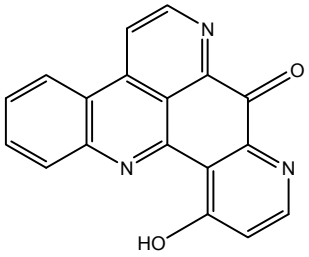
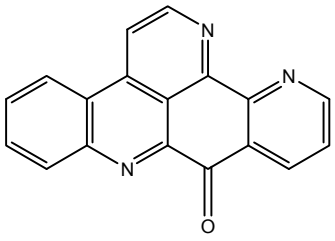
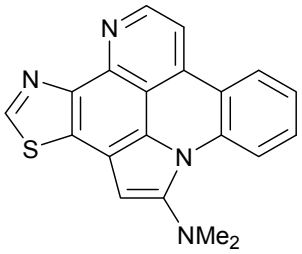


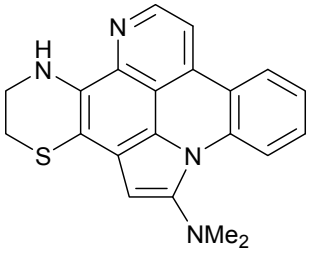
186.



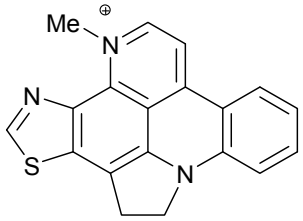
187.



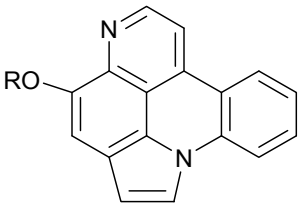
188.



189.



190.



191. R = H
192. R = OMe

Further cyclisation of the ethylamine side chain of shermilamine-like and kuanoniamine-like precursors, followed by aromatisation leads to hexacyclic pyridoacridines such as stellettamine (**188)**[117] and cycloshermilamine D (**189**)[111]. Loss of the dimethylamino functionality after cyclisation is observed in pentacyclic cyclodercitin (**190**)[118], and the arnoamines A and B (**191, 192**)[119] (see Fig 1.). Stellettamine (**188**) was isolated from a deep water sponge collection collected off the coast of California. Cycloshermilamine D (**189**) was isolated from the tunicate *Cystodytes violatinctus*[111]. Arnoamines A (**191**) and B (**192**) have a pyrrole ring fused to the pyridoacridine ring system and were isolated from an ascidian *Cystodytes sp.*[119]. Cyclodercitin (**190**) was isolated from a sponge *Stelletta sp*[118].

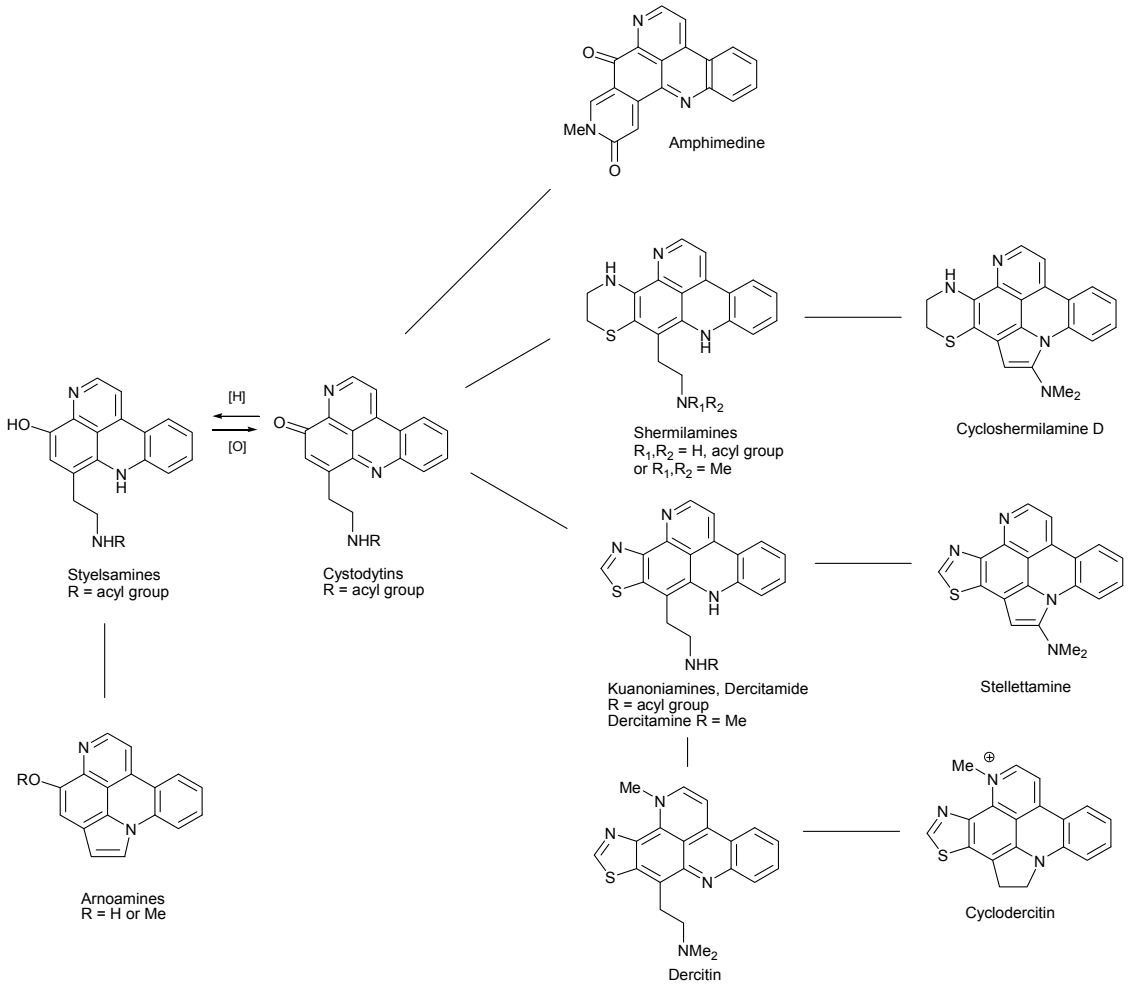


Fig 1. Proposed family tree for the pyridoacridine alkaloids (adapted from Skyler and Heathcock[120])

CHAPTER 2

Results, Discussion and Experimental-

New Metabolites

(and known compounds isolated from the same organisms)

2.1 Results and discussion of metabolites from the red alga *Chondria armata*

2.1.1 Armatol G (193)

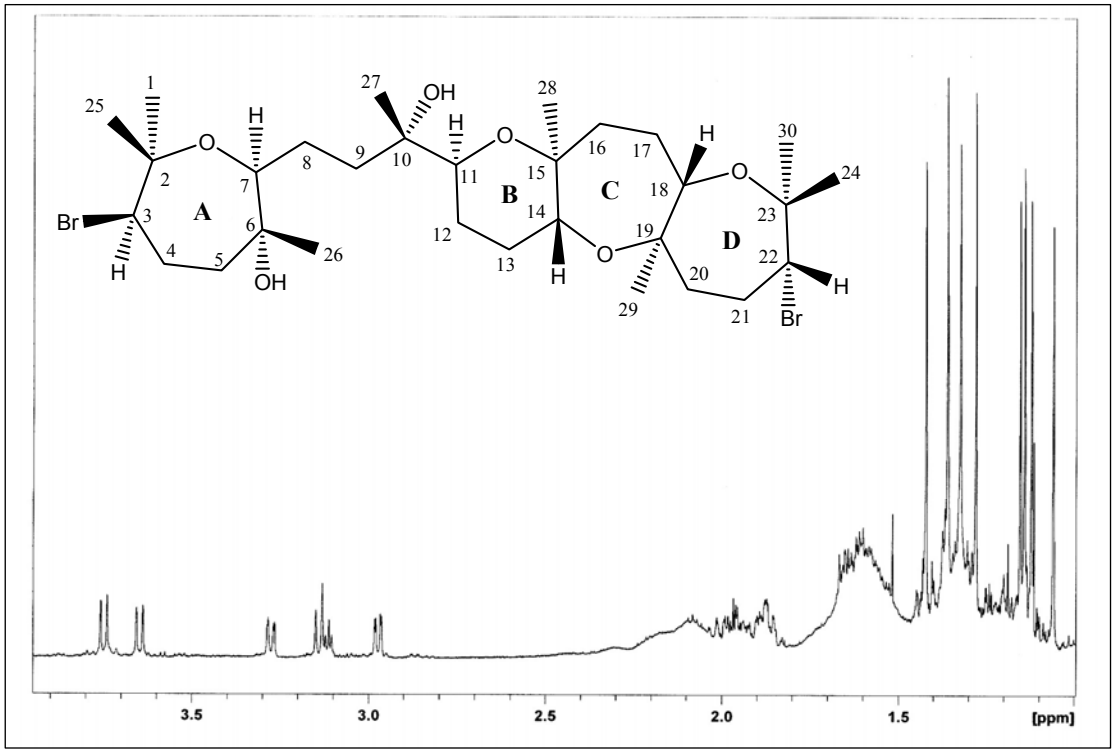


Fig 2. ^{1}H NMR spectrum of (193) in C_6D_6

A freeze dried sample of *Chondria armata* collected from Orpheus Island, GBR was extracted with dichloromethane (DCM) and chromatographed via silica gel vacuum chromatography to yield two brominated polyethers, armatols G (**193**) and H (**194**). Armatol G had a molecular formula $C_{30}H_{52}Br_2O_6$ which was deduced by high resolution ESI positive ion mass spectrometry and from ^{13}C NMR data. The ^{1}H NMR spectrum (see Fig 2) revealed the presence of eight methyl singlets, six downfield methines and two D_2O exchangeable resonances attributable to two OH groups. The presence of two bromine atoms was indicated by the characteristic 1:2:1 isotope

distribution for the molecular ion in the mass spectrum and by two resonances at δ 59.9 and 60.6 in the ^{13}C NMR spectrum (see Fig 3). The ^{13}C NMR spectrum accounted for thirty carbon atoms, the multiplicities of which were deduced via an HSQC experiment. Thus, the molecule contained eight methyl groups, six oxygenated tertiary carbon atoms, four oxygenated methine groups, two brominated methines and ten methylene groups.

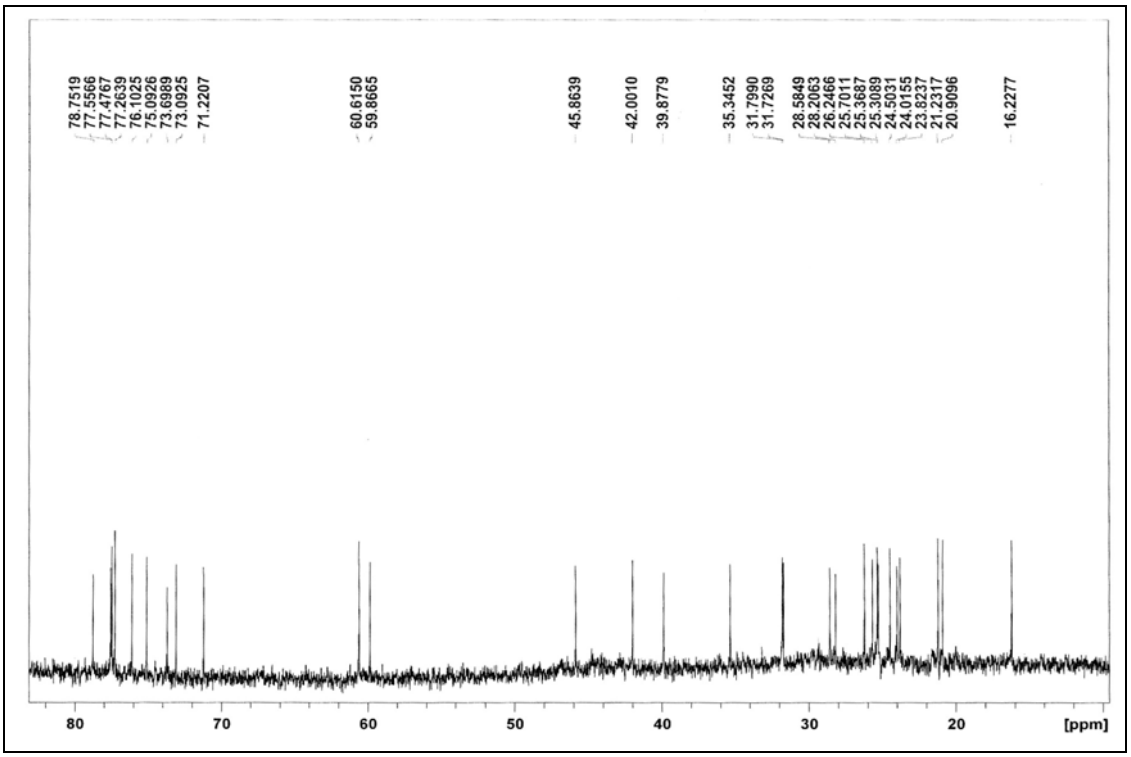


Fig 3. ^{13}C NMR spectrum of (193) in C_6D_6

In addition, the presence of ten oxygenated carbons which were at δ77.3 (C-2), 73.7 (C-6), 77.5 (C-7), 73.1 (C-10), 75.1 (C-11), 71.2 (C-14), 77.5 (C-15), 76.1 (C-18) 78.8 (C-19), and 77.6 (C-23) suggested a brominated triterpene polyether diol, and together with the number of double bond equivalents indicated by the molecular formula, implied the presence of four rings.

Gradient selective TOCSY and COSY data showed that the four methine signals at δ 2.97, 3.14, 3.65 and 3.75 were each linked to two different contiguous methylene groups while the methine signals at δ 3.12 and 3.28 were each coupled to another pair of adjacent methylene signals. Therefore in the selective gradient TOCSY spectrum the signal resonating at δ 2.97 (H-7) correlated with the methylene signals at δ 1.60, 1.93 (H_2-8) and δ 1.98, 1.32 (H_2-9); the signal at δ 3.12 (H-14) correlated with the methylene signals at δ 1.59, 1.67 (H_2-13), 1.55, 1.65 (H_2-12) and the methine at δ 3.28 (H-11); the signal at δ 3.14 (H-18) correlated with the methylene signals at δ 1.39, 1.87 (H_2-17) and δ 1.42, 1.64 (H_2-16); the methine at δ 3.65 (H-22) showed correlations with the methylene signals at δ 1.95, 1.86 (H_2-21), and δ 1.29, 1.21 (H_2-20) and finally the methine signal at δ 3.75 (H-3) showed correlations with the methylene signals at δ 1.89, 2.01 (H_2-4) and δ1.14, 1.43 (H_2-5).

Furthermore HSQC and HMBC data enabled the subunits to be linked together. A long range correlation between C-2 (δ 77.3) and H-7 (δ 2.97) confirmed the presence of the ether ring A and the presence of the ether linkages in rings C and D were similarly established by the long range correlations between C-19 (δ 78.8) and H-14 (δ 3.12) and between C-23 (δ 77.6) and H-18 (δ 3.14) respectively. On the other hand, selective gradient NOESY correlations between H-11 (δ 3.28) and H_3-28 (δ 1.12) indicated the location of the B ring despite the absence of an HMBC correlation between H-11 (δ 3.28) and C-15 (δ 77.6). Furthermore long range HMBC correlations between C-6 (δ 73.7) and H_3-26 (δ 1.06), H-5 (δ 1.43) and H-4 (δ 1.89) and correlations from C-10 (δ 73.1) to H_3-27 (δ1.28) and H-9 (δ 1.98) implied that C-6 and C-10 were oxygenated quaternary carbon atoms, and hence the location of the

two -OH groups were deduced. This was confirmed by the D_2O exchange experiment in $CDCl_3$ as replacement of a proton by deuterium affects the magnetic environment of nearby atoms, causing a small chemical shift change. The shifts observed for both the ^1H NMR methyl signals (H_3-26 and H_3-27), and for the ^{13}C signals in the vicinity of C-6 and C-10 confirmed the positions for the –OH groups. Thus, an upfield shift of 0.2 and 0.1 ppm at C-6 and C-10 respectively were observed in the ^{13}C NMR spectra after the D_2O exchange experiment was performed. Similarly upfield shifts of 0.03 ppm for both H_3-26 and H_3-27 were observed in the ^1H NMR spectra.

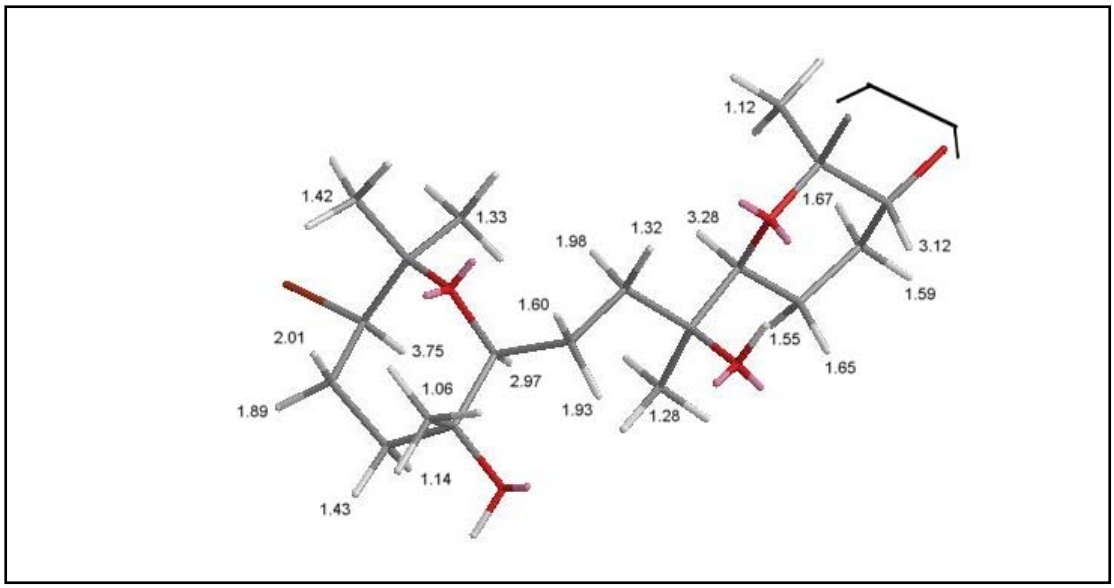
The relative stereochemistry was inferred by selective gradient NOESY experiment as follows: H-3 showed correlations with one H-4 proton (δ 1.89), one H-5 proton (δ 1.14), H_3-1 (δ 1.33), and H-7 (δ 2.97), while H-7 showed correlations with H-3, H_3-1, one of the H-5 protons (δ 1.14), one H-8 proton (δ 1.93) and one of the H-9 protons (δ 1.98). The stereochemistry of the exchangeable OH group at C-6 was deduced from NOESY correlations observed from H_3-26 (δ 1.06) to the other H-5 proton (δ 1.43), the other H-4 proton (δ 2.01) and the other H-8 proton (δ 1.60). Thus the relative stereochemistry of ring A was identical to that of armatols C and E. Similarly, correlations from H-22 (δ 3.65), to both H-21 protons (δ 1.86 and 1.95), H_3-24 (δ 1.16), H-18 (δ 3.14) and one of the H-20 protons (δ 1.21) showed that the relative stereochemistry at ring D was identical to armatols B and C.

The NOESY data confirmed that the stereochemistry at both ring junctions was *trans* in nature. Thus correlations from H_3-24 (δ 1.16) to H-18 (δ 3.14) and H-22 (δ 3.65) and from H_3-30 (δ 1.36) to H_3-29 (δ 1.14) were observed. In addition correlations from H-14 (δ 3.12) to H-16_a (δ 1.42) and H-20_a (δ 1.21), and from H-16_a (δ 1.42) to

H-18 (δ 3.14), and H-20_a (δ 1.21) to H-22 (δ 3.65) confirmed the *trans* nature of the C/D ring junction. Furthermore, correlations from H_3-28 (δ 1.12) to H-11 (δ 3.28) and H_3-29 (δ 1.14) indicated the *trans* nature of the B/C ring junction. More interestingly, as previously discussed, cross peaks between H-11 (δ 3.28) and H_3-28 (δ 1.12) indicated that the stereochemistry of the ring B ether link had H-11 and H_3-28 in a *cis* configuration.

The ^{1}H NMR signal observed for H-7 (dd, J = 9.6, 2.2 Hz) clearly indicated the lack of rotation around single bonds in the uncyclised portion of the structure. This potentially enables the relative stereochemistry of the two cyclised portions to be related to each other. Proton H-11 showed a gradient selective NOESY correlation with H_3-27 (δ 1.28) which in turn had correlations with H_b-9 (δ 1.98) and H_b-8 (δ 1.93). The latter two signals in turn had correlations with H-7 (δ 2.97) which enabled the relative stereochemistry throughout the molecule to be determined using Chem 3D and its MM2 energy minimization program to predict the lowest energy conformation (see Fig 4). The critical NOe's observed were from H-7 to H_b -8 and H_b -9, the same protons as have NOe's to H_3-27 (which related the stereochemistry at C-10 and C-7), and from H-11 to H_b-9 (which related the stereochemistry at C-11 to C-10 and C-7).

The stereochemistry at C-10 was not determined for the previously reported armitols A-F (**32-37**)[31]. The *syn-trans-anti-trans* geometry for ether linkages and ring junctions (rings B-D) contrasts with *syn-trans-syn-trans* geometry reported for ether linkages and ring junctions in armitols A-F (**32-37**).



Proton	^{1}H Shift (δ)	NOe	Distance (Å)	Proton	^{1}H Shift (δ)	NOe	Distance (Å)
H-3	3.75	H_a -4: δ1.89	2.7	H_3-1	1.33	H_3-25: 1.42	2.4
		H_a -5: δ1.14	2.3			H-7: δ2.97	2.3
		H-7: δ2.97	2.3			H-3: δ3.75	2.4
		H_3-1: δ1.33	2.4			H_a -16: δ1.42	2.4
H-7	2.97	H-3: δ3.75	2.3			H_b -16: δ1.64	2.6
		H_b -8: δ1.93	2.65	H_3-25	1.42	H_3-1: δ1.33	2.4
		H_b -9: δ1.98	2.5			H_b -4: δ2.01	2.3
H-11	3.28	H_3-27: δ1.28	2.4	H_3-26	1.06	H_b -4: δ2.01	2.3
		H_a -12: δ1.55	2.4			H_b -5: δ1.43	2.4
		H_3-28: δ1.12	2.2			H_a-8: δ1.60	2.4
		H_b -9: δ1.98	2.4	H_3-27	1.28	H_b -8: δ1.93	2.4
						H_b -9: δ1.98	2.6
						H-11: δ3.28	2.4
				H_3-28	1.12	H-11: δ3.28	2.2

Fig 4. Chem3D MM2 energy minimized structure of Armatol G (193) showing ^{1}H NMR chemical shifts for relevant signals and distances calculated for all observed NOESY correlations.

2.1.2 Armatol H (194)

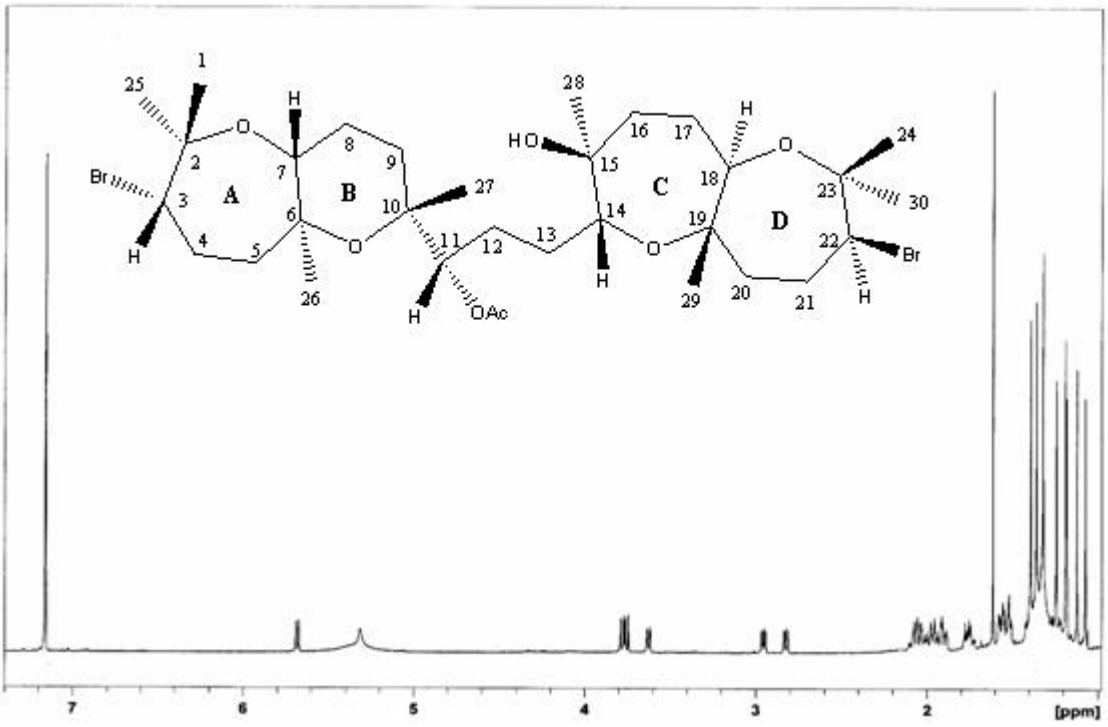


Fig 5. ^{1}H NMR spectrum of (194) in C_6D_6

Armatol H had the molecular formula $C_{32}H_{53}O_7Br_2$ deduced by ESI positive ion mass spectrometry and from ^{13}C NMR spectral data. The molecular formula thus suggested the presence of an acetylated triterpene polyether with four rings. The presence of two bromine atoms was suggested by the presence of the characteristic 1:2:1 isotope pattern of the molecular ion in the mass spectrum and by the resonances at δ 60.1 and 59.8 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum (see Fig 5) in the low field region exhibited six methine signals at δ 2.82, 2.95, 3.62, 3.75, 3.78, and 5.68. The two signals at δ 3.75 and 3.78 were from protons connected to brominated carbons as shown by the HMQC data which connected those protons

with the carbon signals at δ 60.1 and 59.8 respectively. In addition the presence of the downfield doublet at δ 5.68 and a three proton singlet at δ 1.61 (in C_6D_6) in the ^1H NMR spectrum together with a ^{13}C NMR signal at δ 170.4 indicated the presence of a secondary acetate group. The ^{13}C NMR (see Fig 6) and HSQC experiment accounted for 32 carbons: nine methyl groups, ten methylene groups, six methine groups, six quarternary carbons α to oxygen atoms, and the previously mentioned carbonyl group (not included in Fig 6).

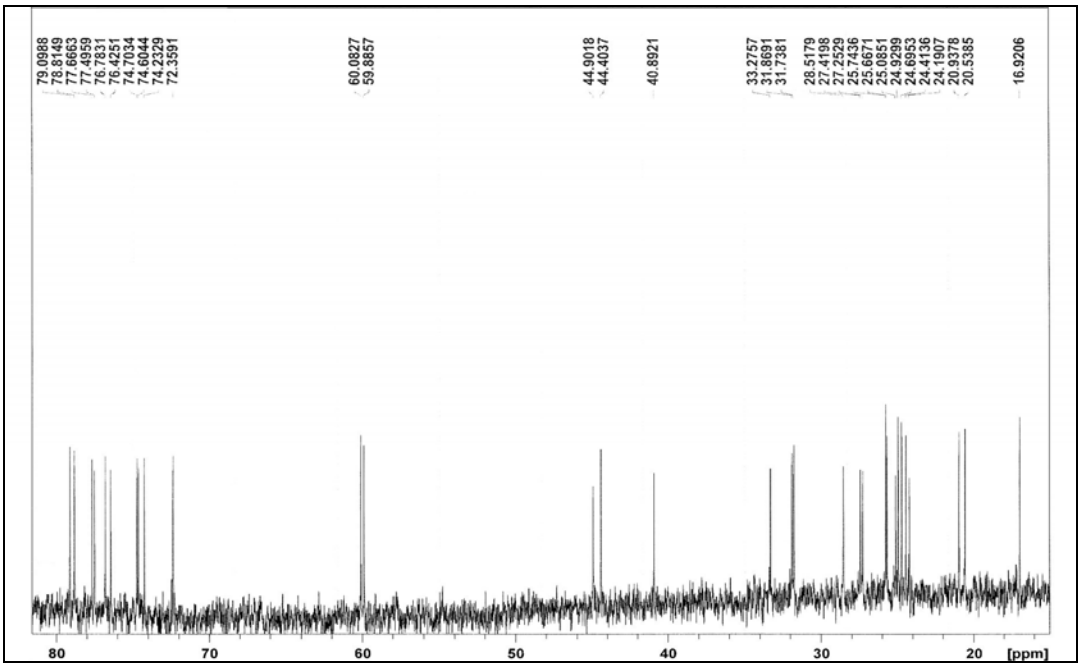


Fig 6. ^{13}C NMR spectrum of (194) in C_6D_6

Two selective gradient TOCSY experiments (which utilized the signals at δ 3.78 and 3.75), together with HSQC and COSY data enabled assignments of the protons on the two brominated rings A and D. Thus irradiation at the brominated methine signal at δ 3.78 (H-3) afforded correlations with signals at δ 1.98 (H_a-4), 1.90 (H_b-4), 1.32

(H_a-5), and 1.57 (H_b-5); while irradiation of the brominated methine signal at δ3.75 (H-22) showed correlations with signals at δ 1.90 (H_a-21), 2.03 (H_b-21), 1.56 (H_a-20) and 1.41 (H_b-20). Four additional selective gradient TOCSY experiments permitted assignments of the proton signal for the remainder of the molecule. Thus the methine signal at δ 2.95 (H-7) showed correlations with δ 1.39 (H_a-8), 2.04 (H_b-8), 1.25 (H_a-9) and 1.76 (H_b-9), while the methine signal at δ 2.82 (H-18) showed correlations with δ 1.48-1.55 (H_2-16), 1.32 (H_a-17) and 1.96 (H_b-17). Irradiation of the methine proton at δ 5.68 (H-11) revealed correlations with δ 1.95 (H_a-12), 2.08 (H_b-12), 1.54 (H_a-13), 1.74 (H_b-13), and δ 3.62 (H-14). The same correlations were observed by irradiation of the methine signal at δ 3.62 (H-14).

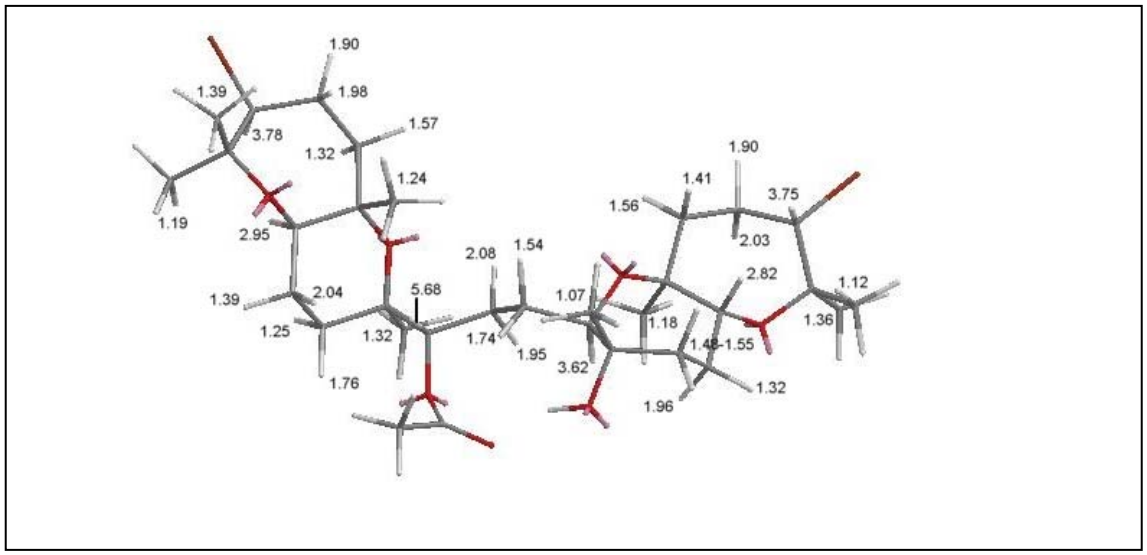
The HMBC data revealed correlations from δ 3.78 (H-3) to C-2, C-4, C-5 and C-25, whereas the methyl signal at δ 1.24 (H_3-26) showed correlations to C-5, C-6 and C-7. The remaining information provided by the TOCSY data could be linked through HSQC and HMBC correlations to derive all other carbon linkages in the triterpene structure. Thus the methyl-substituted oxygenated carbons C-10 and C-15 linked C-9 to C-11 and C-14 to C-16 respectively (evident from HMBC correlations between H_3-27 and C-9, C-10, C-11 and between H_3-28 and C-14, C-15 and C-16). Finally, the methyl-substituted oxygenated carbon C-19 linked C-18 and C-20 (evident from HMBC correlations between H_3-29 and C-18, C-19, C-20). HMBC and selective gradient NOESY experiments were used to obtain evidence in support of ether linkages. Thus, an HMBC correlation from H-7 to C-2 and a clear NOESY correlation between H-7 (δ 2.95) and H_3-1 (δ 1.19) confirmed the presence of ring A. On the other hand, an HMBC correlation from H-18 to C-23 together with NOESY correlations between H-18, H_3-30 and H-22 confirmed the presence of the 7-

membered brominated ring D. Also, NOESY correlations from H-14 (δ 3.62) to H_3-29 (δ 1.18) allowed the closure of ring C, and provided evidence for relative stereochemistry. An –OH group was determined to be at C-15 from deuterium induced ^{1}H NMR and ^{13}C NMR shifts after exchange of the –OH group with D_2O. Thus, an upfield shift of 0.1 ppm at C-15 and 0.002 ppm for H_3-28 were observed in the ^{13}C NMR and ^{1}H NMR spectra respectively, after the D_2O exchange experiment was performed. Note that although the ^{1}H NMR shift is very small, at 600 MHz this was 1.2 Hz, and no shift whatsoever was observed for any other methyl signal. Since the molecular formula indicated the presence of four rings, the oxygenated carbons at C-6 and C-10 must be linked by an ether bridge to complete ring B. This was supported by strong NOESY/ ROESY correlations between H_3-26 and H-11, which also defines the stereochemistry for the two substituents on C-10 in relation to the relative stereochemistry of ring A.

The relative stereochemistry for the ring systems were inferred from results of the selective gradient NOESY and ROESY experiments. H-3 had cross peaks with one H-5 proton, H-7, H_3-1 (δ 1.19) and one H-4 proton (δ 1.90) suggesting that ring A had assumed an ordinary crown conformation. H-7 had cross peaks with H_a-8 (δ 1.39) and H_a-9 (δ 1.25), indicative of a chair conformation. The acetate proton at δ 5.68 showed cross peaks with the methyl at δ 1.24 and H_b-12 at δ 2.08 and H_b-13 at δ 1.74. Since H_3-1 had cross peaks with H-7 and H_b-4 had cross peaks with H_3-26 a *trans* junction was established between rings A and B. NOESY cross peaks from H-18 (2.82) to H_3-28 (1.07) and H_3-30 (1.12) were observed. In addition ROESY cross peaks from H_3-30 (1.12) to H-22 (3.75) and from H_3-29 (1.18) to H-14 (3.62) established a *trans* junction between rings C and D.

The ^{1}H NMR signals observed for H-11 (dd, *J* =11.1, 1.2 Hz) and H-14 (dd, *J* =10.0, 1.5 Hz) clearly indicated the lack of rotation around single bonds in the uncyclised portion of the structure. This enabled the relative stereochemistry of the two cyclised portions to be related to each other. Proton H-14 had correlations with H_a-13 (δ 1.54) and H_a-12 (δ 1.95) and H-11 (δ 5.68); the latter signal in turn had correlations with H_3-26 (δ 1.24) which enabled the relative stereochemistry throughout the molecule to be determined using Chem 3D and its MM2 energy minimization program to predict the lowest energy conformation. The distances predicted for all observed NOe correlations from the energy minimized structure are presented in Fig 7. With one exception (H-14 to H_b-12) all predicted distances are equal to or less than 2.7 Angstroms. The H-14 to H_b-12 distance (3.3 Angstroms) can be significantly reduced by slight angular changes within the non-cyclised portion of the carbon chain without large increases in distances for other observed NOe's.

The diagnostic NOe correlations that relate the relative stereochemistries of rings A/B and rings C/D to the uncyclised portion of the carbon chain (C-11 to C-13) are from H_3-29 (δ 1.18) to both H-12 protons and to H-14, which also exhibits NOe correlations to both H-12 protons. The NOe from H-11 to H_3-26 defines its relative orientation in relation to the ring A/B junction, while its NOe to H_b-12 relates the ring C/D stereochemistry to that of C-11 and that of the A and B rings, defining the relative stereochemistry throughout the molecule. It is proposed that hydrolysis of the acetate functionality and application of the Mosher's method[121] for the resultant secondary alcohol should afford the absolute stereochemistry (note the previous comment regarding the absolute stereochemistries of armitols A-F on page 9).



Proton	^{1}H Shift (δ)	NOe	Distance (Å)	Proton	^{1}H Shift (δ)	NOe	Distance (Å)
H-3	3.78	H_3-1: δ1.19	2.4	H_3-1	1.19	H-3: δ3.78	2.5
		H-7: δ2.95	2.2			H-7: δ2.95	2.3
		H_a -4: δ1.90	2.7			H_3-25: δ1.39	2.1
		H_a -5: δ1.32	2.4	H_3-25	1.39	H_3-1: δ1.19	2.1
H-7	2.95	H-3: δ3.78	2.4			H_b -4: δ1.98	2.7
		H_a -8: δ1.39	2.3				
		H_b -9: δ1.25	2.4	H_3-26	1.24	H_b -8: δ2.04	2.5
		H_3-1: 1.19	2.4			H_b -4: δ1.98	2.1
		H_a -5: δ1.32	2.3			H-11: δ5.68	2.3
H-11	5.68	H_3-26: δ1.24	2.2			H_b -5: 1.57	2.7
		H_b -13 : δ1.74	2.5	H_3-27	1.32	H_a -12: δ1.95	2.3
		H_b -12 : δ2.08	2.5	H_3-28	1.07	H_b -13: δ1.74	2.3
		H_b -8: δ2.04	2.3			H_a -16: δ1.51	2.5
H-14	3.62	H_3-29: δ1.18	2.2	H_3-29	1.18	H-14: 3.62	2.2
		H_b -13: δ1.74	2.7			H_a -12: δ1.95	2.3
		H_a -12: δ1.95	2.2			H_b -12: δ2.08	2.7
		H_b -12 : δ2.08	3.3			H_b -20: δ1.56	2.6
H-18	2.82	H-22: δ3.75	2.2			H_b -17: δ1.96	2.3
		H_3-30: 1.12	2.3	H_3-30	1.12	H-22: δ3.75	2.4
		H_a -20: δ1.41	2.5			H-18: δ2.82	2.3
		H_a -16: δ1.51	2.2	H_3-24	1.36	H_b -21: δ2.03	2.3
		H_a -17: δ1.32	2.6			H_3-30: 1.12	2.4
H-22	3.75	H-18: δ2.82	2.2				
		H_a -21: δ1.90	2.7				
		H_3-30: 1.12	2.4				
		H_a -20: δ1.41	2.4				

Fig 7. Chem3D MM2 energy minimized structure of Armatol H (194) showing ^{1}H NMR chemical shifts for relevant signals and distances calculated for all observed NOESY correlations.

	Armatol G (193)			Armatol H (194)		
C #	δ^1H, mult., J (Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C	δ ^{1}H, mult., J (Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C
1	1.33, s	25.4	H_3-25	1.19, s	24.9	H_3-25
2		77.3	H_3-1, H_3-25		77.7	H-3
3	3.75, dd , 10.9, 1.1	60.6	H_3-1, H_2-5, H_3-25	3.78, d, 10.6	60.1	
4	1.89, m	31.7		1.90, m	31.7	H-3
	2.01, m			1.98, m		
5	1.14, m	45.9		1.32, m	44.9	H-3, H_3-26
	1.43, m			1.57, m		
6		73.7	H_3-26		76.8	H_3-26
7	2.97, dd, 9.6, 2.2	77.5	H_a-5, H_a-8	2.95, dd, 11.6, 4.7	72.4	H_b-9, H_3-26
8	1.60, m	24.0	H_b-9	1.39, m	24.2	H_3-26
	1.93, m			2.04, m		
9	1.32, m	35.3	H-11	1.25, m	33.3	H_3-27
	1.98, m			1.76, m		
10		73.1	H_b-9		74.6	H-11, H_3-27
11	3.28, dd, 10.9, 2.2	75.1		5.68, dd, 11.1, 1.2	74.2	H_3-27
12	1.55, m	28.2	H-11, H_b-13	1.95, m	27.4	
	1.65, m			2.08, m		
13	1.59, m	25.3	H_a-12	1.54, m	28.5	H-11
	1.67, m			1.74, m		
14	3.12, dd, 11.0, 4.5	71.2	H_3-28	3.62, dd, 10.0, 1.5	76.4	H_3-28
15		77.5	H-14, H_3-28		74.7	H_3-28
16	1.42, m	42.0	H-14, H-17, H_3-28	1.48-1.55	40.9	H_3-28
	1.64, m					
17	1.39, m	28.6		1.32, m	27.3	
	1.87, m			1.96, m		
18	3.14, dd, 11.5, 1.1	76.1	H_2-16	2.82, dd, 11.3, 2.9	78.8	H_3-29
19		78.8	H_3-29		79.1	H_3-29
20	1.21, m	40.0	H-22, H3-29	1.41, m	44.4	H-22, H_3-29
	1.29, m			1.56, m		
21	1.86, m	31.8	H_3-22	1.90, m	31.9	
	1.95, m			2.03, m		
22	3.65, dd, 11.2, 1.2	59.9	H_3-24, H_3-30	3.75, d, 11.2	59.8	
23		77.6	H-22, H_3-24, H_3-30		77.5	H-22
24	1.16, s	24.5	H_3-30	1.36, s	25.7	H-22
25	1.42, s	26.2	H_3-1	1.39, s	25.7	H_3-1
26	1.06, s	21.2		1.24, s	20.9	
27	1.28, s	23.8		1.32, s	24.9	
28	1.12, s	16.2	H_2-16, H_2-17	1.07, s	25.1	
29	1.14, s	20.9	H_2-20, H_2-21	1.18, s	16.9	
30	1.36, s	25.7	H_3-24, H_3-30	1.12, s	24.4	H-22
OAc				1.61, s	20.5	
C=O					170.4	H-11

Table 1. ^{1}H NMR and ^{13}C NMR data of (193) and (194) in C_6D_6

2.2 Results and discussion of metabolites isolated from the sponge *Rhabdastrella globostellata*

2.2.1 Stelleferin D riboside (195)

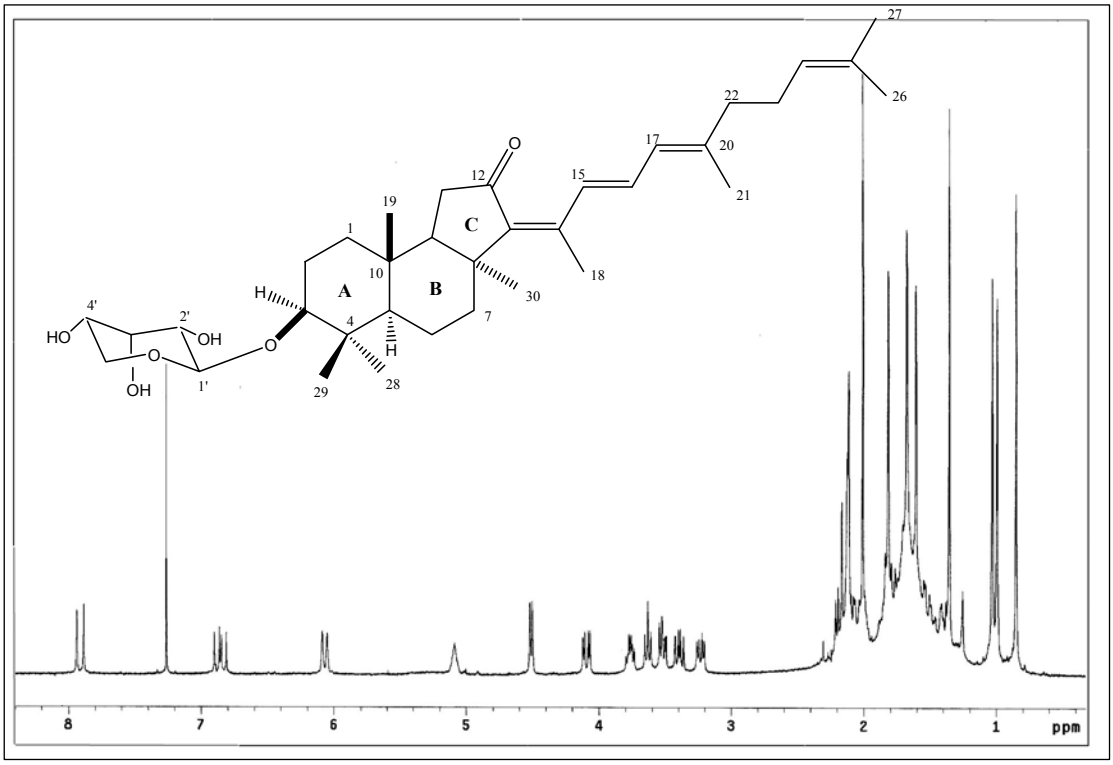


Fig 8. ^{1}H NMR spectrum of (195) in $CDCl_3$

The DCM extract of a freeze dried sample (10.89g) of *Rhabdastrella globostellata* was fractionated via vacuum chromatography on a silica gel column. The less polar fraction of 100% DCM yielded the known isomalabaricanes**,** stelliferin A (**43**, 4.4 mg) and **51** (3.4 mg). The 100% MeOH fraction afforded a bright yellow crystalline compound (2.9 mg) which was assigned structure **195**. The molecular formula of $C_{35}H_{54}O_6$ was suggested for compound **195** by negative ion high resolution electrospray MS measurement of the $(M-H)^-$ and $(M+Cl)^-$ ions (m/z 569.3847, 605.3614 respectively). The ^{1}H NMR spectrum (see Fig 8.) of compound **195**

indicated the presence of 8 methyl groups (δ 0.85, 0.99, 1.03, 1.35, 1.61, 1.67, 1.81, 2.02), four olefinic protons (δ 5.10, 6.07, 6.85, and 7.91) five oxygenated methine groups (δ 3.23, 3.53, 3.63, 3.75, and 4.50) and an oxygenated methylene group (δ 3.39, 4.10).

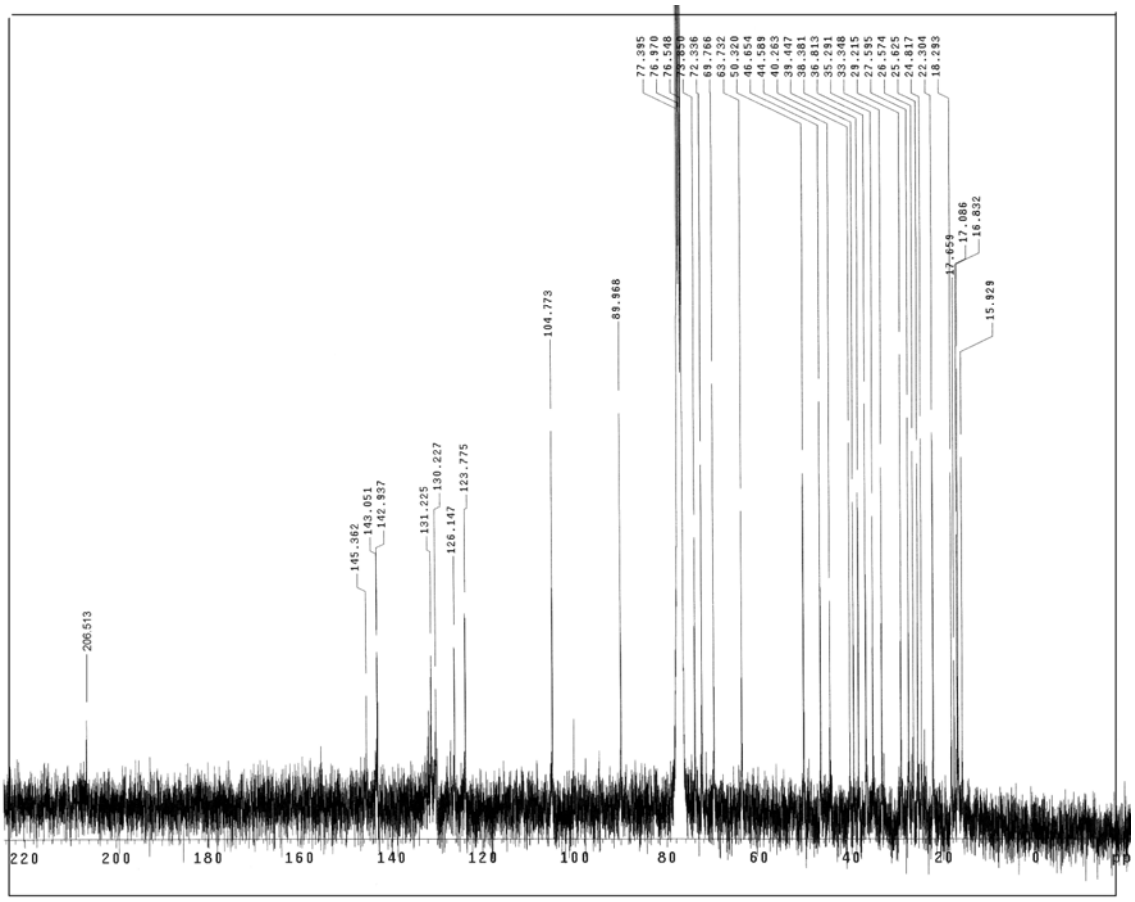


Fig 9. ^{13}C NMR spectrum of (195) in $CDCl_3$

The ^{13}C NMR (see Fig 9.) and DEPT experiment confirmed the presence of the eight methyl groups, eight methylene groups, eleven methine and eight quarternary carbons. In addition, HMQC correlations from δ 4.50 to a carbon at δ 104.7 was indicative of compound **195** containing a dioxygenated methine. Furthermore the

presence of four oxygenated methines and an oxygenated methylene suggested the molecule possessed a pentose sugar.

The protonated carbons were all assigned using HMQC in combination with the DEPT experiment. A signal at δ 206.4 in the ^{13}C NMR spectrum was indicative of a ketone functionality. This was supported by an IR absorption band at 1600 cm^{-1}. In addition to the ketone functionality a further eight carbons downfield of 120 ppm (in the ^{13}C NMR spectrum) suggested that the molecule contained four double bonds. Thus, the molecular formula indicated that the molecule possessed nine elements of unsaturation which in combination with the above data suggested that compound **195** had four rings.

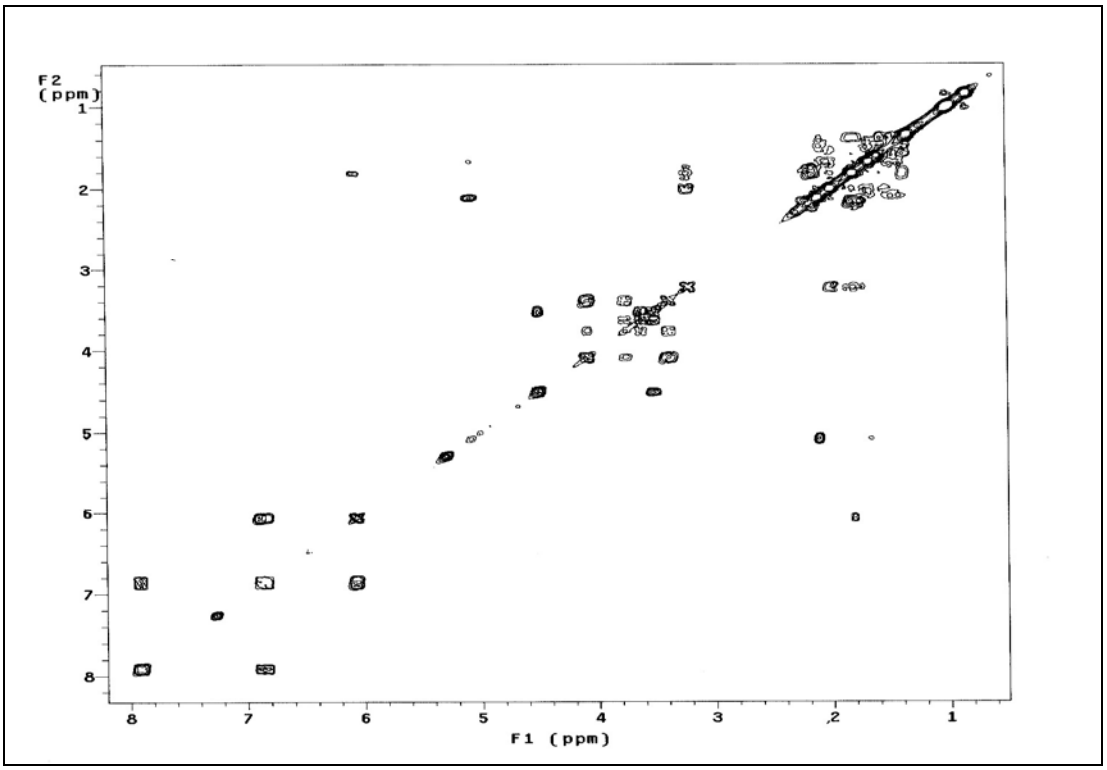


Fig 10. COSY spectrum of (195)

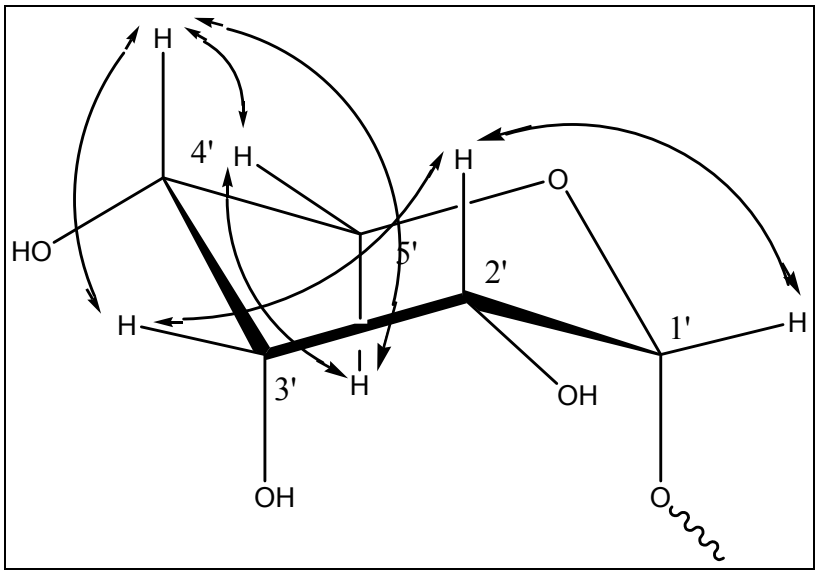


Fig 11. COSY correlations for the β-ribose moiety in compound (195)

Thus analysis of the COSY data revealed correlations from δ 3.75 (H-4') to 4.10 (H_a-5'), 3.39 (H_b-5') and δ 3.63 (H-3'). The latter signal in turn correlated to δ 3.53 (H-2') (see Fig 10 and 11) and δ 3.53 (H-2') also showed a correlation to δ 4.50 (H-1'). HMQC and HMBC correlations for the ribose confirmed the cyclic nature of the sugar. Thus, HMBC correlations were observed from δ 104.7 (C1', anomeric carbon) to δ 3.53 (H-2'), 3.23 (H-3), 3.63 (H-3'), 4.10 (H-5'a) and 3.39 (H-5'b). In addition, a correlation from δ 90.0 (C-3) to δ 4.50 confirmed the point of attachment of the sugar. Comparison of the *J* value of H-1' (δ 4.50, d, *J* 5.4 Hz) confirmed that the stereochemistry at the anomeric carbon was α, as the value for $J_{(H\text{-}1'\text{-}H\text{-}2')}$ for an α-riboside is observed (4.8 Hz suggested from published data)[44].

HMBC correlations mainly from four methyl groups at δ 1.03 (H_3-28), 0.85 (H_3-29), 1.35 (H_3-30) and 0.99 (H_3-19) enabled assignment of the remaining three rings (see Fig 12 and 13.). Thus correlations from δ 1.03 (H_3-28) and 0.85 (H_3-29) to C-3, C-4 and C-5; from δ 0.99 (H_3-19) to C-1, C-5, and C-10 and from δ1.30 and 2.20 (H_2-1) to C-2 allowed the assignment of ring A. A further correlation from δ 0.99 (H_3-19) to C-9, in addition to correlations from δ 1.35 (H_3-30) to C-7, C-8 and C-9 and C-13 were observed. In addition, COSY correlations between H-5 and H-6, and between H-9 and H_2-11 allowed the complete assignment of ring B. The chemical shift of H_2-11 (δ 2.11) suggested it was next to the ketone. The ketone carbonyl was placed between C-11 and C-13 to form ring C and this accounted for the remaining degree of unsaturation in the molecule.

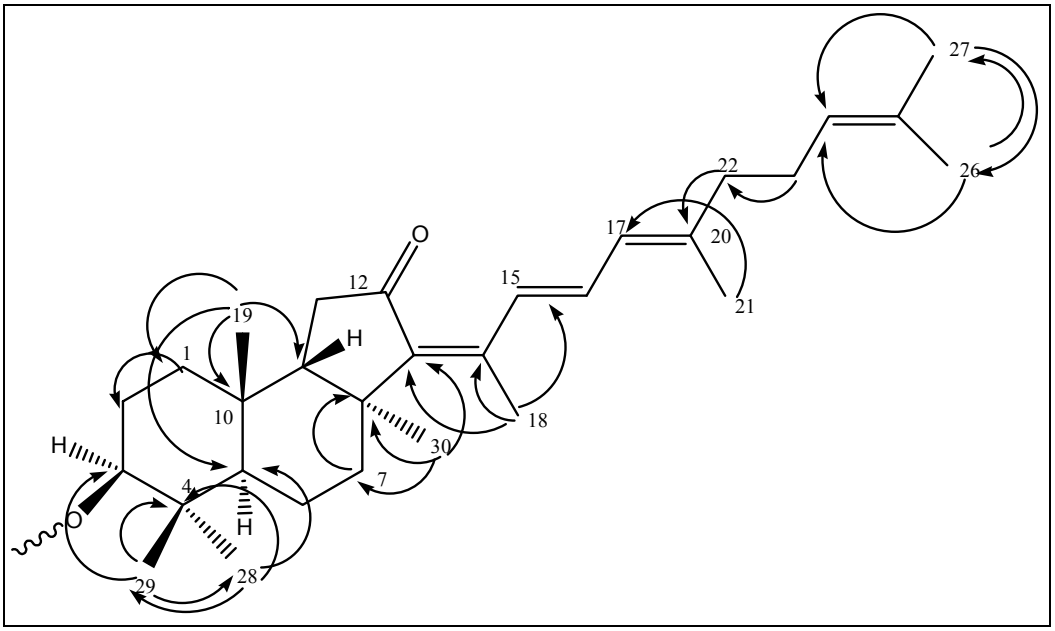


Fig 12. Selected HMBC correlations for Stelliferin D riboside (195)

The olefinic side chain was attached at the C-13 position since the methyl at δ 2.02 (H_3-18) correlated with C-13 in the HMBC spectrum. The signals for the side chain were assigned with the help of COSY and HMBC data (see Fig 10, 12 and 13). Thus additional HMBC correlations from H_3-18 (δ 2.02) to C-14 and C-15; from H_3-21 (δ 1.81) to C-17, C-20 and C-22; and from H_3-26 (δ1.61) and H_3-27 (δ 1.67) to C-24 and C-25 together with COSY correlations from H-16 (δ 6.85), to H-15 (δ 7.91) and H-17 (δ 6.07) enabled the assignment of the olefinic side chain. Comparison of *J* values and splitting patterns of protons along the side chain with published data suggested *Z*, *E, E* geometry at the C-13, C-15 and C-17 positions respectively. Thus a doublet at δ 7.91 (*J*=15.6 Hz), a doublet of doublets at δ 6.85 (*J*=15.6, 11.1) and a doublet at δ 6.07 (*J*=10.8 Hz) for protons on C-15, C-16 and C-17 respectively were in close agreement to published data for structure **56** (see table 2)[44].

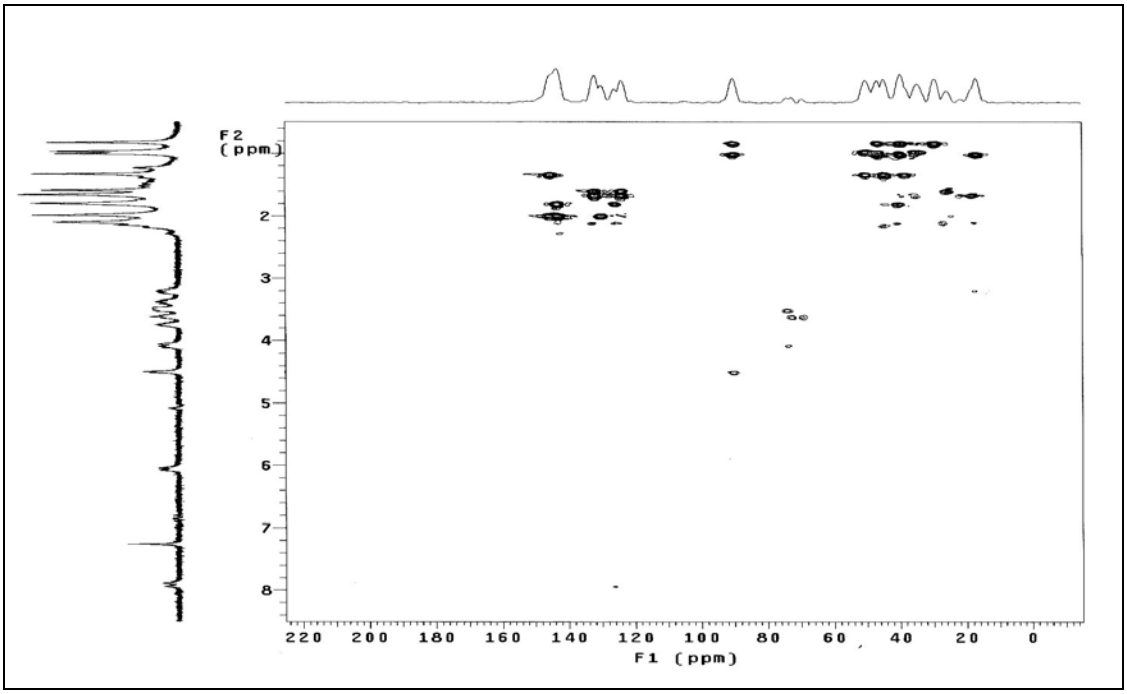


Fig 13. HMBC spectrum of (195)

In addition the low field resonances of H-15 (δ 7.91) and H-16 (δ 6.85) in the ^{1}H NMR spectrum suggested that they were in the deshielding region of the carbonyl group which suggested that the geometry of the C-13–C-14 double bond was *Z* [44]. The stereochemistries for the ring junctions were determined by comparison of ^{13}C NMR data with that of published data for known isomalabaricanes. Therefore, ^{13}C chemical shift values of δ 46.7, 22.3, 24.8, and 50.3 were indicative of C-5 (α), C-19 (β), C-30 (α) and C-9 (β) respectively (see table 2)[44]. Hence compound **195** was identified as the 3β(α-riboside) of stelliferin D

	Stelliferin D riboside (195)			Stelliferin riboside (56)[44]	
C #	δ ^{1}H, mult., *J* (Hz)	δ ^{13}C	HMBC ^{1}H to 13C	δ ^{1}H, mult., *J* (Hz)	δ ^{13}C
1	2.20, m	33.1	H_3-19	1.05, m	31.5
	1.30, m			0.90, m	
2	2.0, m	26.5	H_2-1	1.90, m	25.4
	1.80, m			1.68, m	
3	3.23, dd, 11.7, 5.4	90.0	H_3-28, H_3-29, H-1'	4.72. dd, 3.2	79.0
4		39.5	H_3-28, H_3-29		38.4
5	1.70, m	46.7	H_3-19, H_3-28, H_3-29	2.32, m	42.6
6	1.48, m	18.3		0.96, m	19.1
				1.4, m	
7	2.12, m	38.4	H_3-30	1.30, m	39.6
8		44.6	H_2-7, H_3-30		45.7
9	1.81, m	50.3	H_3-19, H_3-30	1.78, m	51.5
10		35.3	H_3-19		36.6
11	2.11, m	36.8		2.14, m	37.7
12		206.4	H-15		208.1
13		145.4	H_3-18, H_3-30		147.8
14		143.2	H_3-18		143.2
15	7.91, d, 15.6	130.1	H_3-18	7.96, d, 15.6	134.2
16	6.85, dd, 15.6, 11.1	131.3		6.76, dd, 15.3, 11.0	130.4
17	6.07, d, 10.8	126.2	H_3-21	6.20, d, 11.2	131.7
18	2.02, s	16.0		1.98, s	17.0
19	0.99, s	22.3		0.98, s	23.4
20		143.0	H_3-21, H_2-22		139.1
21	1.81, s	17.1		1.73, s	12.8
22	1.63, m	40.3	H_3-21	4.15, t, 7.2	83.0
23	2.28, m	34.5	H_2-22	2.36, m	33.5
				2.18, m	
24	5.10, bt	123.7	H_3-26, H_3-27	5.0, bm	120.5
25		131.9	H_3-26, H_3-27		135.4
26	1.61, s	17.7	H-24, H_3-27	1.64, s	26.8
27	1.67, s	25.6	H-24, H_3-26	1.57, s	19.0
28	1.03, s	29.2		0.88, s	28.8
29	0.85, s	16.9		0.84, s	22.5
30	1.35, s	24.8		1.36, s	25.2
OAc				2.02, s	22.3
C=O					171.5
1'	4.50, d, 5.4	104.7	H-2', H-3, H-3', H-5'a, H-5'b	4.37, d, 4.8	98.9
2'	3.53, dd, 5.7, 3.6	72.2	H-3'	3.57, dd, 6.4, 4.6	72.2
3'	3.63, t, 6.9	73.7	H-2', H-5'a	3.67, t, 6.0	73.7
4'	3.75, m	69.7	H-3', H-5'b	3.71, dm, 5.5	70.8
5'	4.10, dd, 11.7, 3.9	63.7		4.0, dd, 12.1, 3.4	64.0
	3.39, dd, 12.3, 7.0			3.34, dd, 12.1, 6.0	

Table 2. ^{1}H NMR and ^{13}C NMR data of (195) and (56) in $CDCl_3$

2.2.2 Known isomalabaricane triterpenes isolated from the same *Rhabdastrella globostellata* sample

2.2.2.1 Stelliferin A (43)

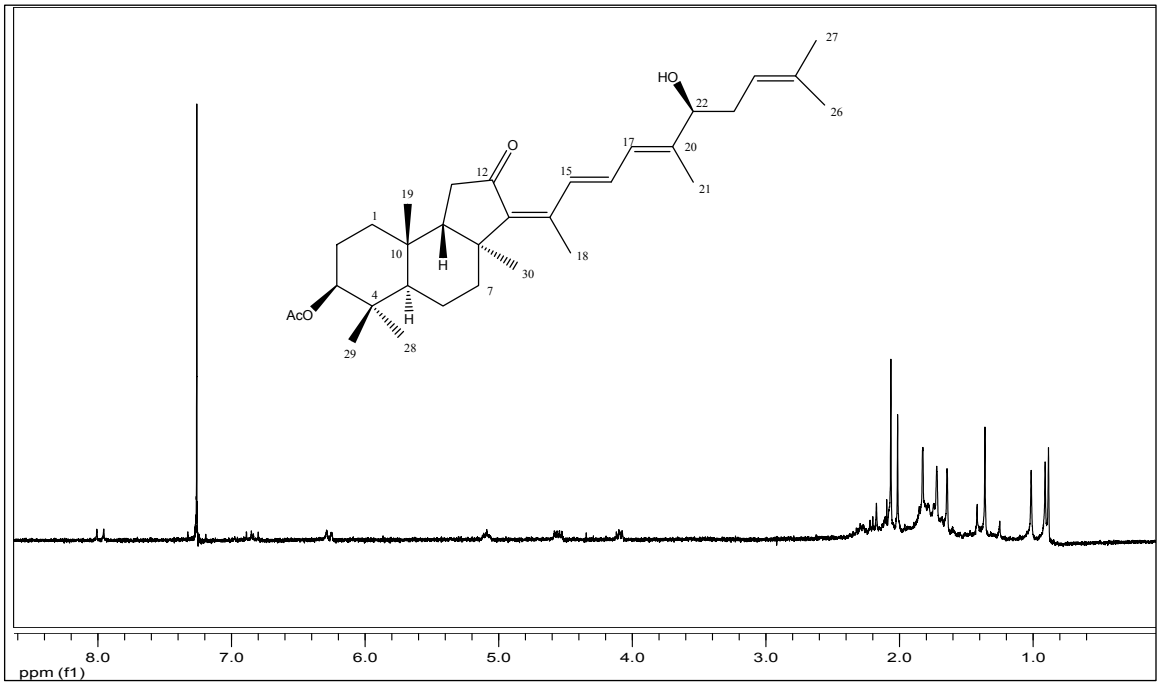


Fig 14. ^{1}H NMR spectrum of (43) in $CDCl_3$

The ^{1}H NMR spectrum of compound **43** (see Fig 14 and table 3) exhibited four olefinic protons. Three were conjugated, with signals at δ 7.99, 6.84, 6.28, and one was in an isopropylene group with a signal at δ 5.10. Further, signals for four olefinic methyl groups at δ 2.02, 1.83, 1.65, 1.72, oxygenated methine signals at δ 4.56 and δ 4.10, and four aliphatic methyl groups at δ 0.89, 0.92, 1.02 and 1.37 were observed. This data together with a ketone signal at δ 206.3 in the carbon spectrum and signals for an acetate substituent implied the isolated compound was a stelliferin type isomalabaricane triterpene. The ^{13}C NMR data disclosed the presence of nine

methyls, six methylene, four sp^3 methines including two carbinyl ones, four sp^2 methines, three sp^3 and four sp^2 quaternary carbons, an ester carbonyl and a conjugated ketone. The ^1H NMR and ^{13}C NMR spectra were identical with published data for stelliferin A (**43**)[39].

2.2.2.2 (13*Z*,15*E*,17*E*)-3β,28-diacetoxy-22-hydroxyisomalabarica-13,15,17,24-tetraen-12-one (51)

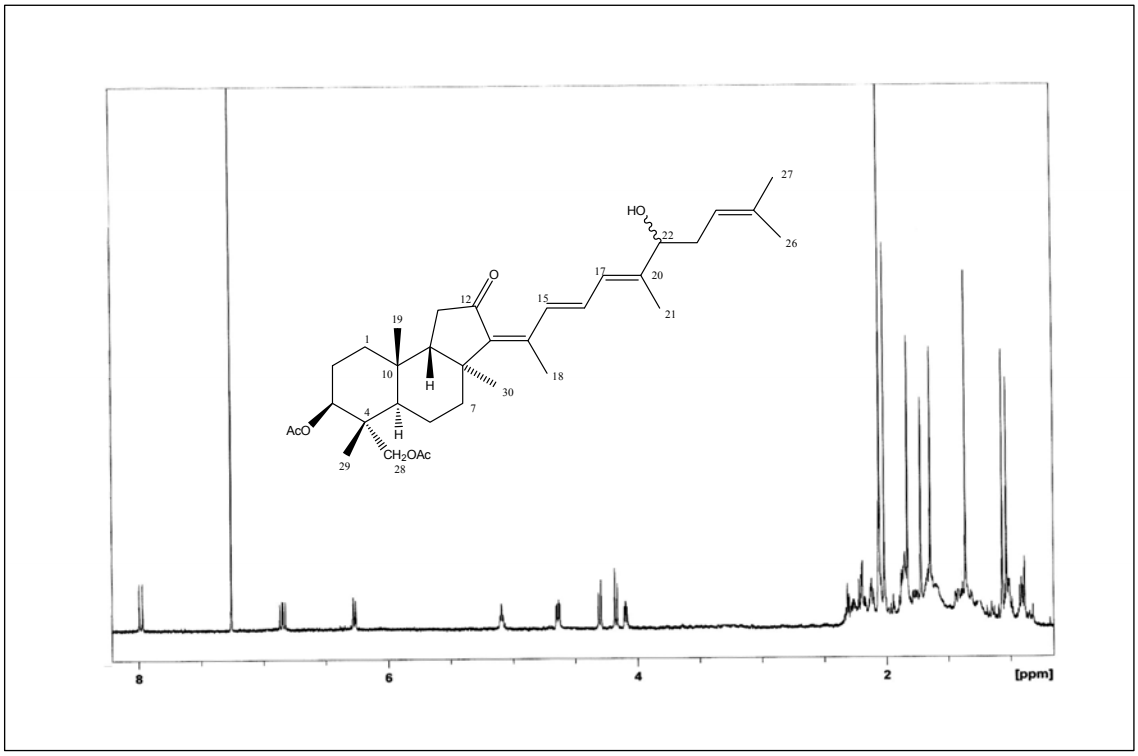


Fig 15. ^1H NMR spectrum of (51) in $CDCl_3$

The ^1H NMR spectrum of **51** (see Fig 15) was similar to that of stelliferin A (**43**) in the olefinic region. Thus three methine signals reminiscent of a conjugated trienone system as seen in reported isomalabaricanes were seen in the ^1H HMR spectrum. These were at δ 7.95, 6.26, and 6.85. In addition two triplets at δ 5.09 and 4.10 and a

doublet of doublets at δ 4.65 were assigned to the olefinic methine α to the isopropyl group, and the oxygenated methines at H-22 and H-3 respectively. There were two additional doublets in the downfield region at δ 4.32 and 4.17 and an additional signal at δ 2.01. Thus one methyl group in stelliferin A was replaced by a methylene group substituted by an acetate moiety. This was confirmed by the HMBC data and the structure was confirmed as (13*Z*,15*E*,17*E*)-3β,28-diacetoxy-22-hydroxyisomalabarica-13,15,17,24- tetraen-12-one (**51**). Since published data for the above compound is incomplete[41], assignment of chemical shifts were obtained from ^{13}C NMR and 2D NMR data (see table 3).

Thus, HMBC correlations mainly from four methyl groups at δ 4.17 (H_2-28), 1.06 (H_3-29), 1.36 (H_3-30) and 1.03 (H_3-19) enabled assignment of the remaining three rings (see Table 3). Correlations from δ 4.17 (H_2-28) and 1.06 (H_3-29) to C-3, C-4 and C-5; from δ 1.03 (H_3-19) to C-5, and C-10 and from δ 0.90 and 1.03 (H_2-1) to C-2 allowed the assignment of ring A. A further correlation from δ 1.03 (H_3-19) to C-9, in addition to correlations from δ 1.36 (H_3-30) to C-7, C-8 and C-9 and C-13 were observed. In addition, COSY correlations between H-5 and H-6, and between H-9 and H_2-11 allowed the complete assignment of ring B. The chemical shift of H_2-11 (δ 2.20) suggested it was next to the ketone. The ketone carbonyl was placed between C-11 and C-13 to form ring C.

The olefinic side chain was attached at the C-13 position since the methyl at δ 2.01 (H_3-18) correlated with C-13 in the HMBC spectrum. The signals for the side chain were assigned with the help of COSY and HMBC data (see Table 3). Thus additional HMBC correlations from H_3-18 (δ 2.01) to C-14 and C-15; from H_3-21 (δ 1.83) to C-

17, C-20 and C-22; and from H_3-26 (δ1.65) and H_3-27 (δ 1.73) to C-24 and C-25 together with COSY correlations from H-16 (δ 6.85), to H-15 (δ 7.95) and H-17 (δ 6.26) enabled the assignment of the olefinic side chain.

(13*Z*,15*E*,17*E*)-3β,28-diacetoxy-22-hydroxyisomalabarica-13,15,17,24-tetraen-12-one (51)				**Stelliferin A (43)**	
C #	^{1}H, mult., *J* (Hz)	^{13}C	HMBC ^{1}H to ^{13}C	^{1}H, mult., *J* (Hz)	^{13}C
1	0.90, m 1.03, m	32.7		1.59, m 1.40, m	32.9
2	1.68, m 1.90, m	25.4	H_2-1	1.80, m 1.56, m	25.0
3	4.65, dd, 11.1, 5.4	80.2	H-1, H-5, H_2-28, H_3-29	4.56. dd, 11.0, 5.4	80.7
4		41.4	H-2b, H_2-28, H_3-29		38.1
5	1.84, m	47.2	H_2-6, H_3-19, H_2-28	1.73, m	46.5
6	2.04, m	21.0	H-5, H-7, H_3-19	1.48, m	18.2
7	2.12, m	38.7	H_3-30	2.1, m	38.1
8		44.4	H_2-6, H_3-30		44.5
9	1.86, m	50.0	H_2-11, H_3-19, H_3-30	1.85, m	50.2
10		35.4	H_3-19		35.4
11	2.20, m	36.7		2.11, m	36.8
12		208.2	H_2-11		206.3
13		145.7	H_2-7, H_3-18, H_3-30		145.8
14		142.7	H-15, H_3-18		142.4
15	7.95, d, 15.3	131.8	H-17, H_3-18	7.99, d, 15.3	132.1
16	6.85, dd, 15.3, 10.8	130.3	H-17	6.84, dd, 15.6,11.1	130.2
17	6.26, 10.8	126.3	H-16, H_3-21	6.28, d, 11.1	126.2
18	2.01, s	16.0	H-15	2.02, s	15.9
19	1.03, s	21.4		1.02, s	22.3
20		143	H-16, H_3-21		143.2
21	1.83, s	12.8	H-17	1.83, s	12.6
22	4.10, t, 6.6	76.8	H-17, H_3-21, H_2-23	4.10, t, 6.0	76.8
23	2.28, m	34.5	H-24	2.30, m	34.2
24	5.09, t, 6.9	119.5	H_3-26, H_3-27	5.10, t, 6.9	119.7
25		135.4	H_2-23, H_3-26, H_3-27		134.6
26	1.65, s	18.01	H-24, H_3-27	1.65, s	17.9
27	1.73, s	25.9	H-24, H_3-26	1.72, s	25.8
28	4.17, d, 11.4 4.32, d, 11.7	66.9	H-3, H-5, H_3-29	0.92, s	28.9
29	1.06, s	24.2	H-5, H_2-28	0.89, s	16.9
30	1.36, s	24.9		1.37, s	24.6
OAc	2.05, s	21.1	H_2-28		21.1
OAc	2.01, s	21.3	H-3	2.06, s	
C=O		170.4	H-3		170.8
C=O		171	H_2-28		

Table 3. ^{1}H NMR and ^{13}C NMR data of (43) and (51) in $CDCl_3$

2.3 Results and discussion of metabolites isolated from the sponge *Spongia* sp.

2.3.1 Isospongiatriol (196)

The DCM soluble portion of extracts from a specimen of *Spongia sp*. was found to be cytotoxic towards P388 cells at concentrations of 20 μg/ml. Flash chromatography of this extract afforded five components which were further purified via reversed-phase HPLC. The more polar fractions from flash chromatography (100% MeOH) afforded the new compound, isospongiatriol (**196**), together with known metabolites isospongiadiol (**109**) and epispongiatriol (**96**). The 25% EtOAc fractions yielded spongiadiol diacetate (**97**) and spongiatriol triacetate (**99**).

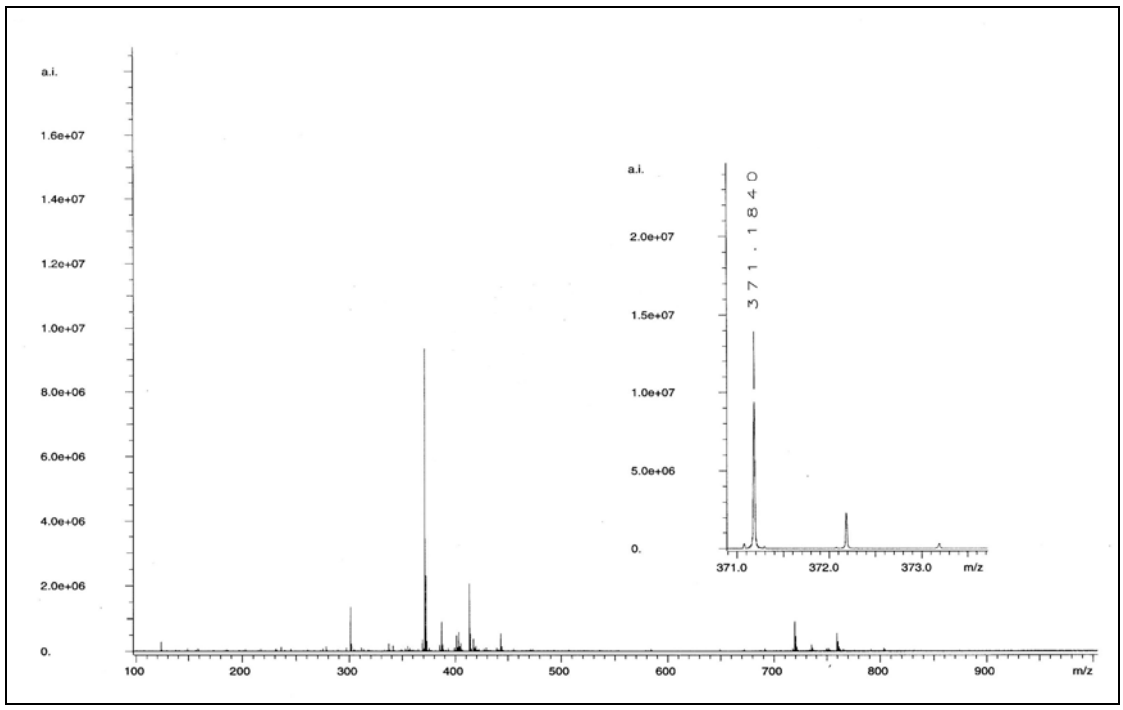


Fig 16. HRESIMS of (196) showing the MNa^+ ion at 371.1830

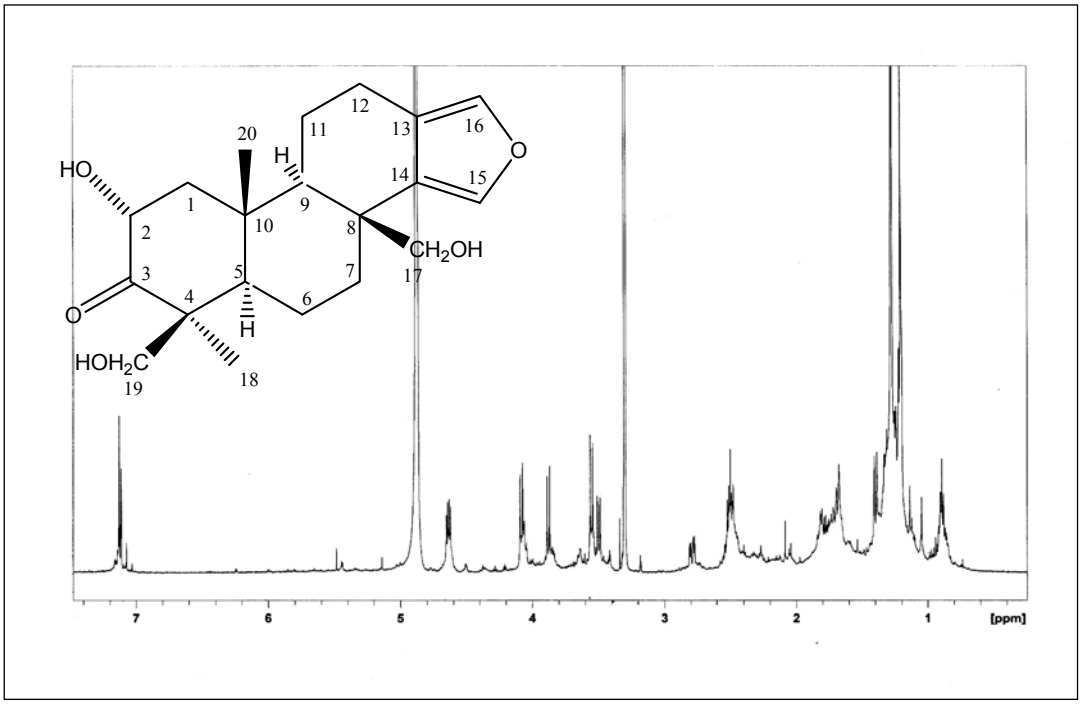


Fig 17. ^{1}H NMR spectrum of (196) in MeOH-d_4

A MNa^{+} ion at 371.1830 in the (+) HRESIMS allowed a molecular formula of $C_{20}H_{28}O_5$ to be assigned to isospongiatriol (**196,** see Fig 16). The ^{1}H NMR spectrum (see Fig 17) showed two methyl signals at δ 1.22 and 1.28 and two fine doublets in the aromatic region at δ 7.13 and 7.12. Four one proton doublets at δ 4.09, 3.87, 3.56 and 3.49 in the ^{1}H NMR spectrum were assigned to two oxygenated methylene groups. Selective irradiation at each of these signals showed that protons at δ 3.49 and 3.87 and protons at δ 3.56 and 4.09 were coupled to each other respectively. In addition HSQC data revealed that the methylene groups were linked to oxygen atoms (see fig 17 and table 4). Thus analysis of the HSQC data indicated that these signals belonged to two sets of un-equivalent protons on two oxygenated carbons (^{1}J correlations from δ 4.09 and 3.56 to δ 65.4; δ 3.87 and 3.49 to δ 62.8). An oxygenated methine proton signal at δ 4.65, which correlated with δ 70.9 in the HSQC spectrum was also present. The fact that two fine doublets at δ 7.13 and 7.12

were observed in the ^{1}H NMR spectrum in addition to four sp^2 carbon signals, two of which at δ 138.8 and 138.2 belonged to the α-carbons and the other two at δ 120.2 and δ 130.5 to the β-carbons, indicated the presence of a furan moiety. A carbon signal at δ 214.1 indicated the presence of a ketone group.

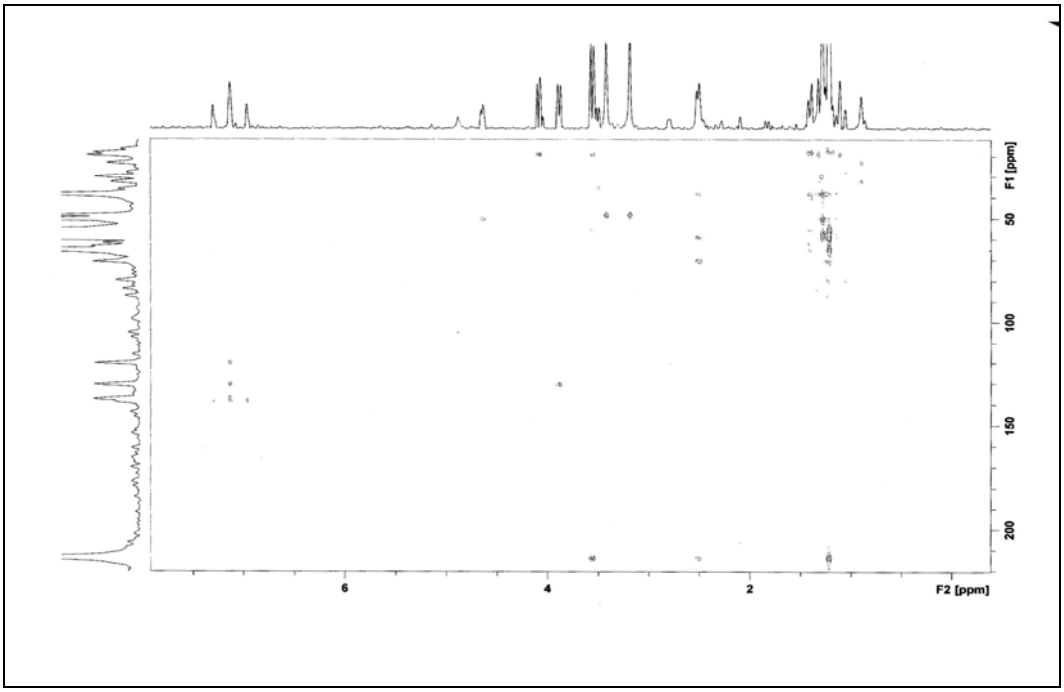


Fig 18. HMBC spectrum of (196)

A literature search for furanoditerpene triols led to two structures i.e. spongiatriol (**95**) and its C-3 epimer epispongiatriol (**96**). However, the doublet of doublets at δ 4.65 (in $CDCl_3$) was replaced by a singlet at δ 4.77 (H-3) in spongiatriol (**95**) and at δ 4.03 (H-3) in epispongiatriol (**96**)[63]. This suggested the compound was a positional isomer of spongiatriol (**95**), wherein the -OH group was at the C-2 position and the carbonyl group was at the C-3 position. This was confirmed by the HMBC experiment (see Fig 18.) where correlations from δ 4.65 (H-2) to δ 50.5 (C-1), and more importantly ^{3}J couplings from δ 4.09, 3.56 (H_2-19) and δ 1.23 (H_3-18) to δ

214.1 (C-3) were observed. Other long range correlations which enabled the molecule to be linked together were from δ 1.27 (H_3-20) to δ 50.5 (C-1), δ 59.7 (C-5), δ 58.0 (C-9) and δ 38.9 (C-10); δ 1.39 (H-5) to δ 56.2 (C-4), δ 18.4 (C-6), δ 17.7 (C-18), and δ 65.4 (C-19); δ 1.40 (H-9) to δ 41.0 (C-8), 38.9 (C-10), δ 19.7 (C-11) and δ 62.8 (C-17) and from δ 3.87 and δ 3.49 (H_2-17) to δ 36.1 (C-7) and δ 130.5 (C-14) (see Fig 18.). The relative stereochemistry of the two methyl groups attached at C-4 and C-10 were confirmed by NOESY correlations. Thus cross peaks from δ 1.23 (H_3-18) to δ 1.39 (H-5) and from δ 1.27 (H_3-20) to δ 3.87 (H-17_b) and δ 3.56 (H-19_a) indicated that the relative stereochemistry of A-ring was similar to that of spongiatriol (**95**), isospongiadiol (**109**) and that all junctions were *trans* in nature. Hence, the new metabolite (**196**) is similar to spongiatriol but has the functional groups at C-2 and C-3 interchanged, so it has been named isospongiatriol.

Isospongiatriol (196)			
C #	δ ^{1}H, mult., *J* (Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C
1	1.23, m	50.5	H-2, H_3-20
	2.49, m		
2	4.65, dd, 12.5, 6.2	70.9	H_2-1,
3		214.1	H_2-1, H-2, H_3-18, H_2-19
4		56.2	H-5, H_3-18
5	1.39, m	59.7	H_b-1, H_3-18, H_3-20
6	1.28, m	18.4	H-5
	1.40, m		
7	1.24, m	36.1	H_2-17
	2.49, m		
8		41.0	H_a-6, H-9
9	1.40, m	58.0	H_2-11, H_3-20
10		38.9	H_2-1, H-9, H_3-20
11	1.67, m	19.7	H-9
	1.88, m		
12	2.51, m	21.4	
	2.82, m		
13		120.2	H-11, H-12, H-16
14		130.5	H-12, H-15, H_2-17
15	7.12, d, 1.38	138.2	H-16
16	7.13, d, 1.56	138.8	H-15
17	3.49, d, 11.0	62.8	H-9
	3.87, d, 11.3		
18	1.23, s	17.7	H_2-19
19	3.56, d, 11.3	65.4	H-5, H_3-18
	4.09, d, 11.5		
20	1.27, s	18.8	

Table 4. ^{1}H NMR and ^{13}C NMR data of (196) in MeOH-d_4

2.3.2 Known furanoditerpenes isolated from the same *Spongia* sp. sample

2.3.2.1 Epispongiatriol (96), spongiadiol diacetate (97) and spongiatriol triacetate (99) and isospongiadiol (109)

The structures of known compounds (**96**), (**97**), (**99**), and (**109**) were confirmed by comparison of their NMR data with published data[63, 67].

2.4 Results and discussion of metabolites isolated from the sponge *Dysidea herbacea*

2.4.1 1-Hydroxy-3,4,6,2',4'-pentabromodiphenyl ether (197)

The ^{1}H NMR spectrum of the crude dichloromethane extract from a *Dysidea herbacea* sample collected from -11m at Pelorus Island in December 2003 contained, in addition to the signals characteristic of the pentabrominated diphenyl ether (**155**)[92], a singlet at δ 7.74 indicative of the presence of a minor metabolite. The brominated diphenyl ether fraction was isolated by vacuum liquid chromatography, and the major and minor metabolites were separated by reverse-phase HPLC. The minor metabolite (**197**) was found by high-resolution electrospray mass spectrometry (negative ion mode) to have the same molecular formula as (**155**), $C_{12}H_5Br_5O_2$ (see Fig 19).

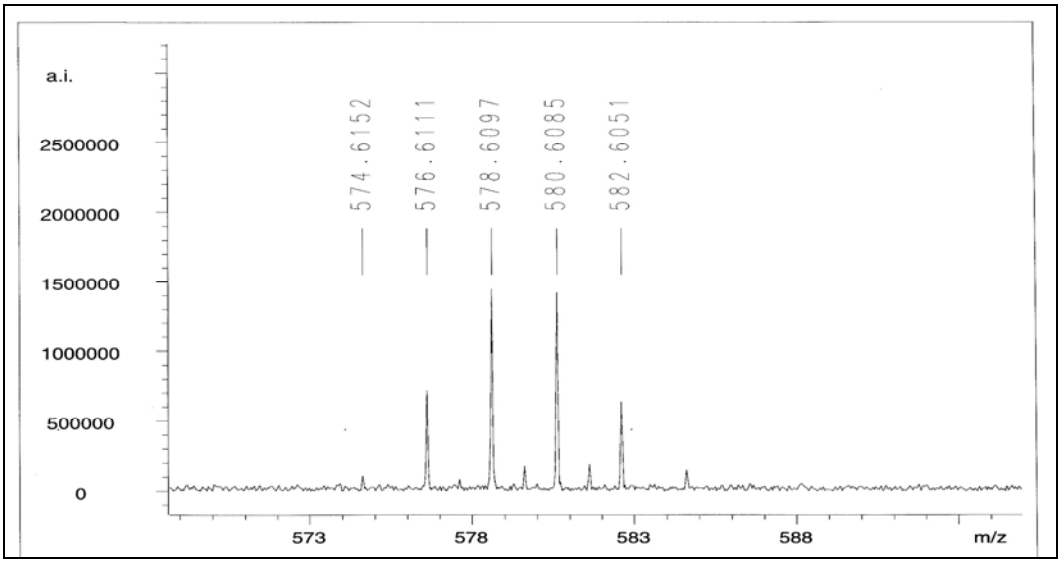


Fig 19. HRESIMS Mass spectrum of (197)

The ^{1}H and ^{13}C NMR spectra (see Fig 20 and 21) were consistent with a phenolic diphenyl ether that contained a tribrominated phenolic A-ring, and a dibrominated B-ring. This meant that the A ring substitution pattern was isomeric with that of (**155**), but two (**137** and **144**) of the other three positional isomers for the sole hydrogen atom on the A-ring had already been reported from *Dysidea* and *Phyllospongia* samples[86, 89].

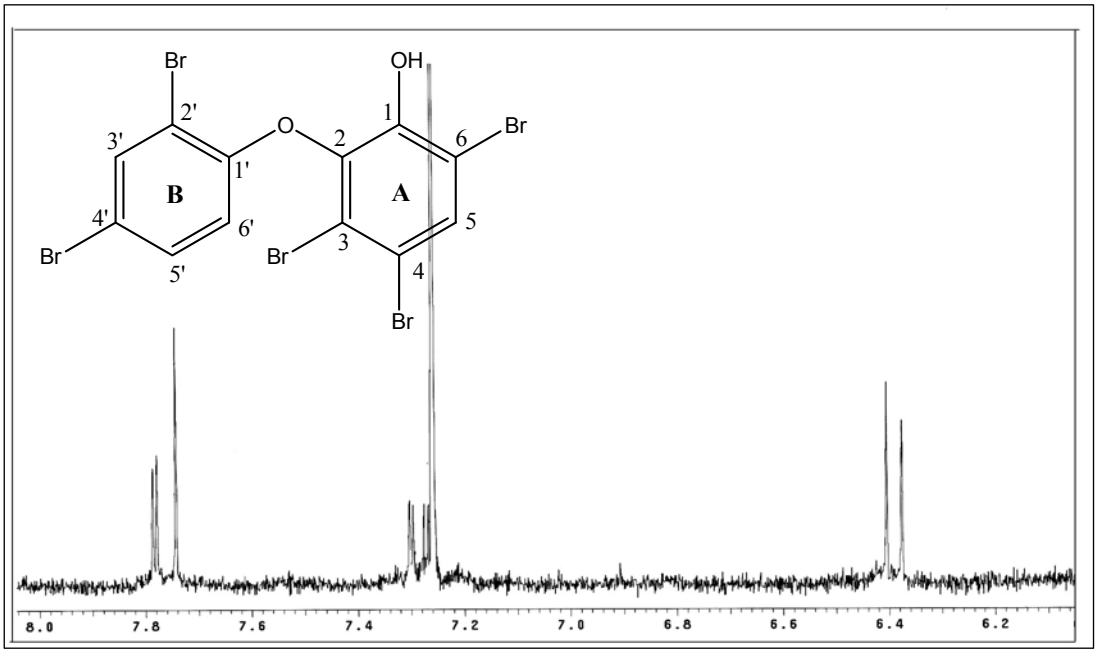


Fig 20. ^{1}H NMR spectrum of (197) in $CDCl_3$

The ^{1}H NMR signal for the sole hydrogen atom on ring A for (**197**) resonated at δ 7.74, but at δ 7.42 for (**137**)[89, 92], δ 7.01 for (**144**)[95]and δ 7.55 for (**155**)[92]. The ^{1}H NMR shifts for the protons on the B ring of (**197**) (δ 6.40, 7.29 and 7.78) were quite similar to those reported for (**155**) (δ 6.41, 7.28 and 7.78) and (**137**) (δ 6.41, 7.29 and 7.79), but the shifts reported for H5' and H6' for (**144**) (δ 7.45 and 6.89 respectively

in $CDCl_3$[95], 7.4 and 6.82 respectively in CCl_4[89]) were significantly different. The structure of (**197**) was clearly 1-hydroxy-3,4,6,2',4'-pentabromodiphenyl ether, and indeed the ^1H NMR and ^{13}C NMR shifts observed in the A ring were in good agreement with those reported for 1-hydroxy-3,4,6,2'-tetrabromodiphenyl ether (**153**)[91] (see table 5).

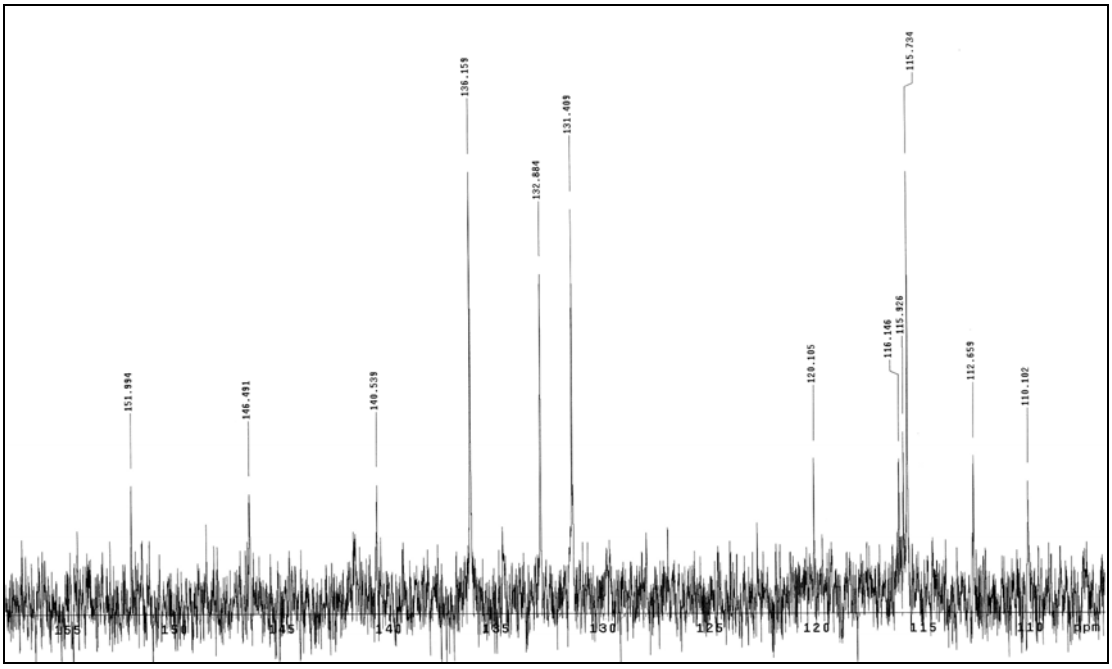
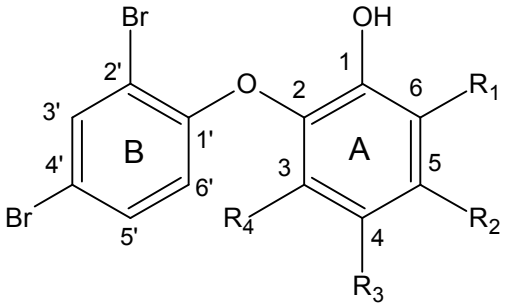


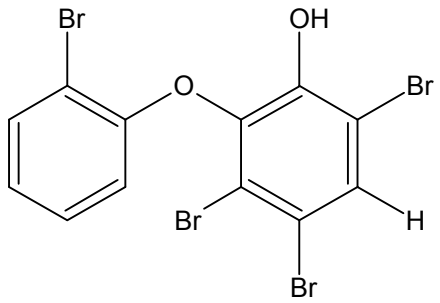
Fig 21. ^{13}C NMR spectrum of (197) in $CDCl_3$

	197		**153**[91]	**155**[92]		**137**[92]		**144**[95]
C #	δ^1H,mult.,*J*(Hz)	δ^{13}C	δ^{13}C	δ^1H,mult.,*J*(Hz)	δ^{13}C	δ^1H,mult.,*J*(Hz)	δ^{13}C	δ^1H,mult.,*J*(Hz)
1		146.4	146.7		148.1		148.9	
2		140.5	141.0		138.7		139.9	
3		120.1	120.2		116.7		113.6	7.01, s
4		116.1	116.1	7.55, s	128.0		119.3	
5	7.74, s	132.8	132.8		122.2		122.9	
6		110.1	109.8		113.4	7.42, s	120.8	
1’		151.9			152.1		151.8	
2’		112.6			112.6		112.8	
3’	7.78, d, 2.4	136.1		7.78, d, 2.2	136.1	7.79, d, 2.2	136.4	7.81, d, 2.4
4’		115.7			115.8		116.3	
5’	7.29, dd, 8.8, 2.4	131.4		7.28, dd, 8.8, 2.2	131.3	7.29, dd, 8.8, 2.2	131.6	7.45, dd, 8.6, 2.4
6’	6.40, d, 8.8	115.9		6.41, d, 8.8	115.8	6.41, d, 8.8	115.8	6.89, d, 8.6

Table 5. ^{1}H NMR and ^{13}C NMR data of (197, 153, 155, 137 and 144) in $CDCl_3$



	R_1	R_2	R_3	R_4
(**137**)	H	Br	Br	Br
(**144**)	Br	Br	Br	H
(**155**)	Br	Br	H	Br
(**197**)	Br	H	Br	Br



(**153**)

2.4.2 Known diphenyl ethers isolated from a second *Dysidea herbacea* sample

2.4.2.1 1,2'-Dimethoxy-3,4,5,3',5'-pentabromodiphenyl ether (198)

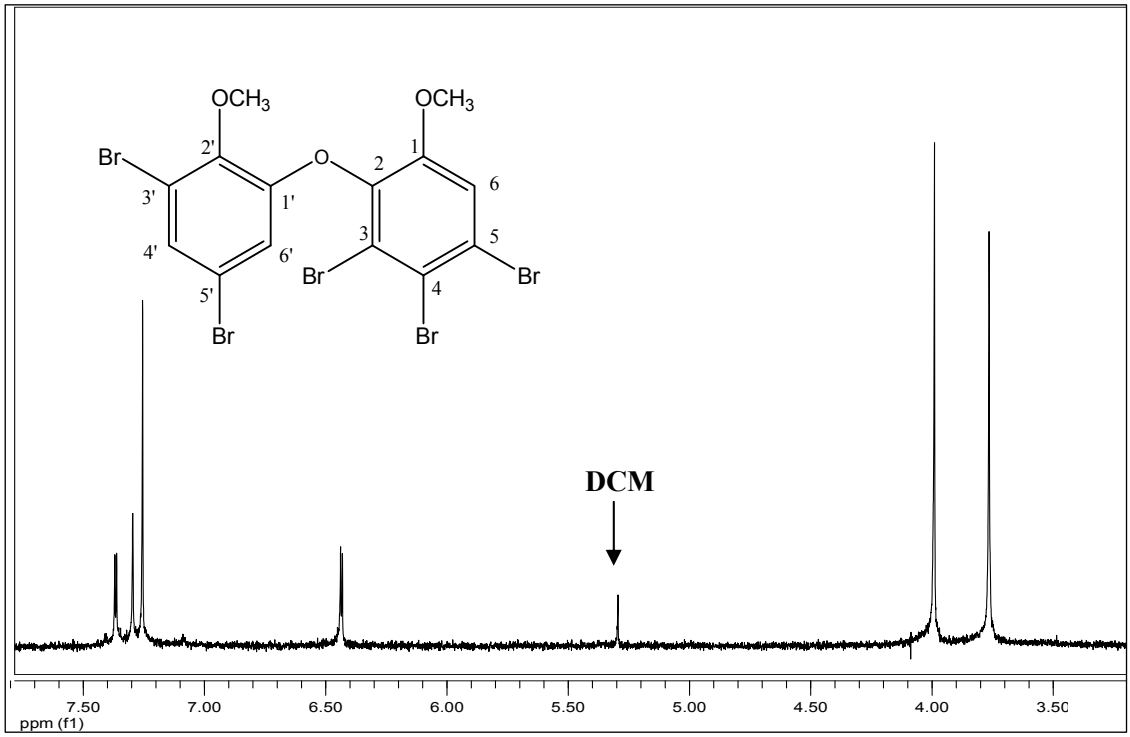


Fig 22. ^{1}H NMR spectrum of (198) in $CDCl_3$

The DCM extract of a sample of *Dysidea herbacea* (see comments on page 34 on revised taxonomy of *Dysidea herbacea*) collected at Arab Reef (12m) in the central section of the GBR contained a mixture of three brominated diphenyl ethers. Application of the crude extract on a silica gel column yielded compounds (**198**) and (**199**) as the less polar fractions and compound (**144**) as the more polar fraction. The ^{1}H NMR spectrum of compound **198** (see Fig 22) showed signals due to meta coupled aromatic protons at δ 6.44 and 7.37 and two methoxy singlets at δ 3.77 and 3.99. In addition a singlet at δ 7.30 was also present. The ^{13}C NMR spectrum showed

resonances for all fourteen carbons which included four brominated carbons, four oxygenated carbons and three aromatic carbons. Comparison to published data revealed that the compound was the known metabolite 1,2'-dimethoxy-3,4,5,3',5'-pentabromodiphenyl ether (**198**), isolated from the sponge *Phyllospongia dendyi*[122].

2.4.2.2 1,2'-Dihydroxy-3,4,5,6,3',5'-hexabromodiphenyl ether (141)

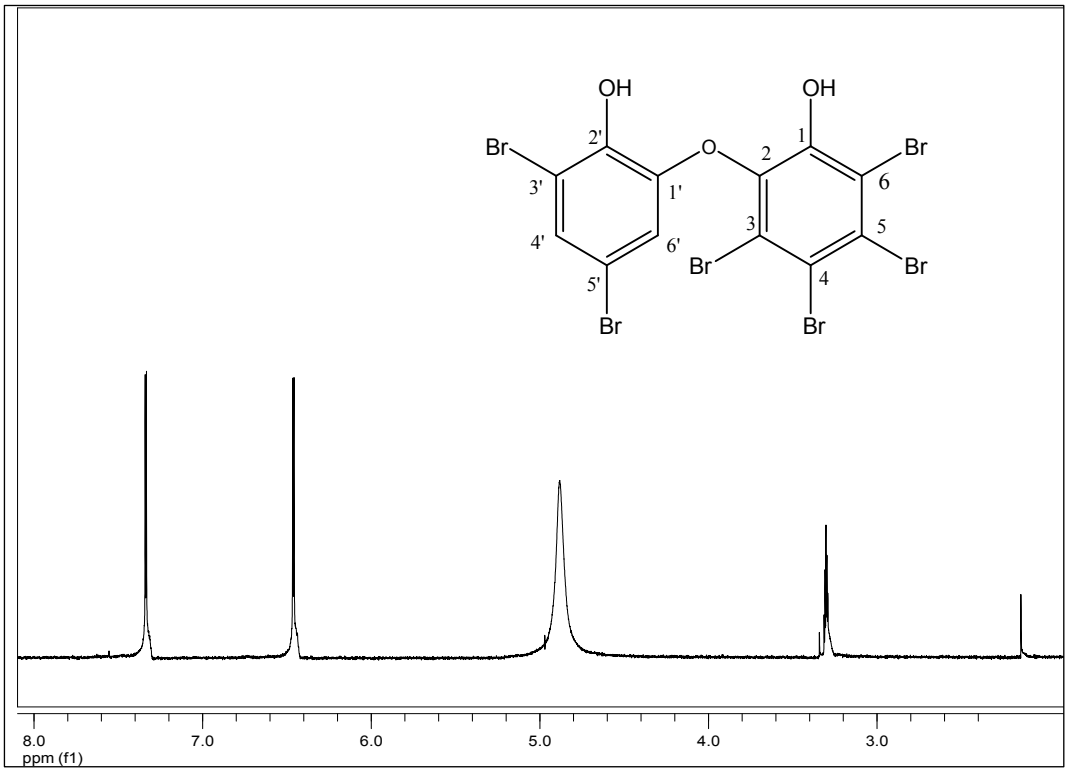


Fig 23. ^{1}H NMR spectrum of (141) in MeOH-d_4

The ^{1}H NMR spectrum (see Fig 23) contained only two fine doublets at δ 6.47 and 7.34 and a broad singlet at δ 4.89. The doublets at δ 6.47 and 7.34 were indicative of meta coupled aromatic protons and the broad singlet at δ 4.89 was indicative of presence of H_2O / exchanged OH protons in the MeOD-d_4 solvent. The ^{13}C NMR

spectrum revealed the presence of two aromatic protonated carbon atoms (δ 126.9 and 130.0), four oxygenated carbons (δ 141.4, 145.5, 146.9 and 150.6), and six brominated carbons (δ 111.3, 112.4, 116.5, 117.1, 119.1 and 121.5). Comparison to published data revealed that the metabolite was 1,2'-dihydroxy-3,4,5,6,3',5'-hexabromodiphenyl ether (**141**)[89].

2.4.2.3 1,2'-Dimethoxy-3,5,3',5'-tetrabromodiphenyl ether (199)

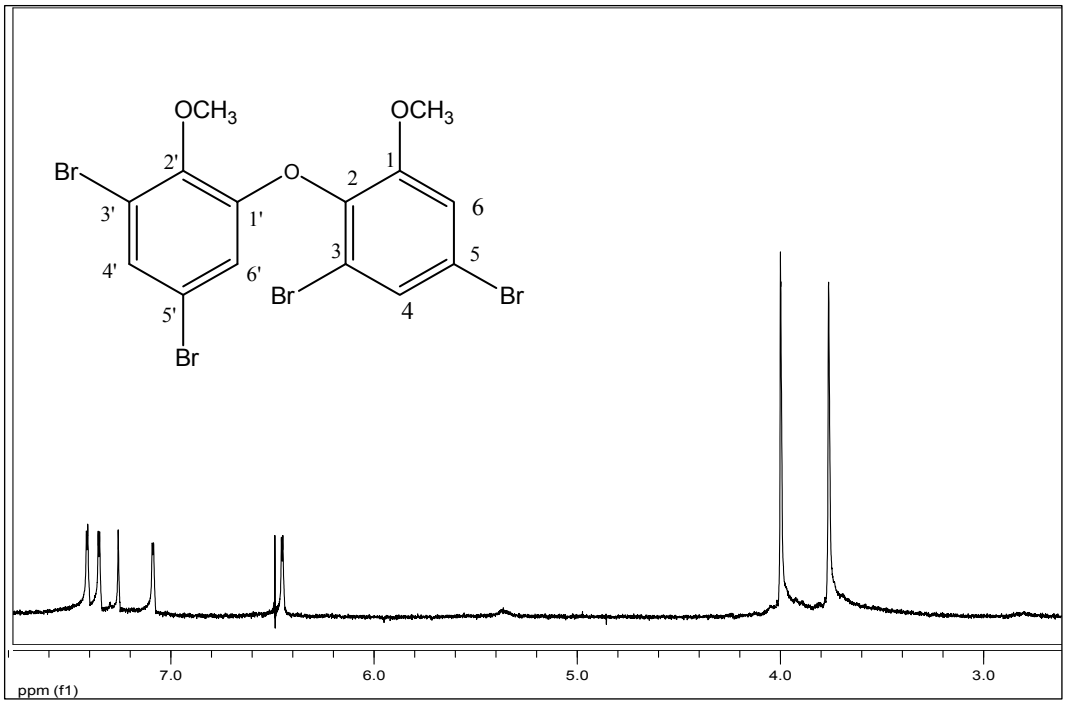


Fig 24. ^{1}H NMR spectrum of (199) in $CDCl_3$

The ^{1}H NMR spectrum (see Fig 24) afforded four fine doublets in the aromatic region at δ 6.45, 7.09, 7.35 and 7.41 and two singlets δ 3.74 and 4.0. Decoupling experiments showed that the protons at δ 6.45 and 7.35 belonged to two meta protons in ring B and δ 7.09 and 7.41 belonged to the meta protons in ring A respectively. The ^{13}C NMR spectrum revealed signals for fourteen carbons, four of which were

assigned to brominated carbons; four to oxygenated carbons and four to protonated aromatic carbons. The remaining two signals were assigned to OCH_3 groups. Comparison with literature NMR data indicated that the compound was the known metabolite 1,2'-dimethoxy-3,5,3',5'-tetrabromodiphenyl ether (**199**) isolated from the sponge *Phyllospongia dendyi*[122].

The group at James Cook University has noted previously that populations of *D. herbacea* found on the lee side of the Palm Island group contain a different range of brominated diphenyl ethers from those found in an open reef environment on the GBR.

2.5 Results and discussion of an alkaloid isolated from the ascidian *Aplidium* sp. (cf *Aplidium cratiferum*)

2.5.1 Nordehydrocyclodercitin (200)

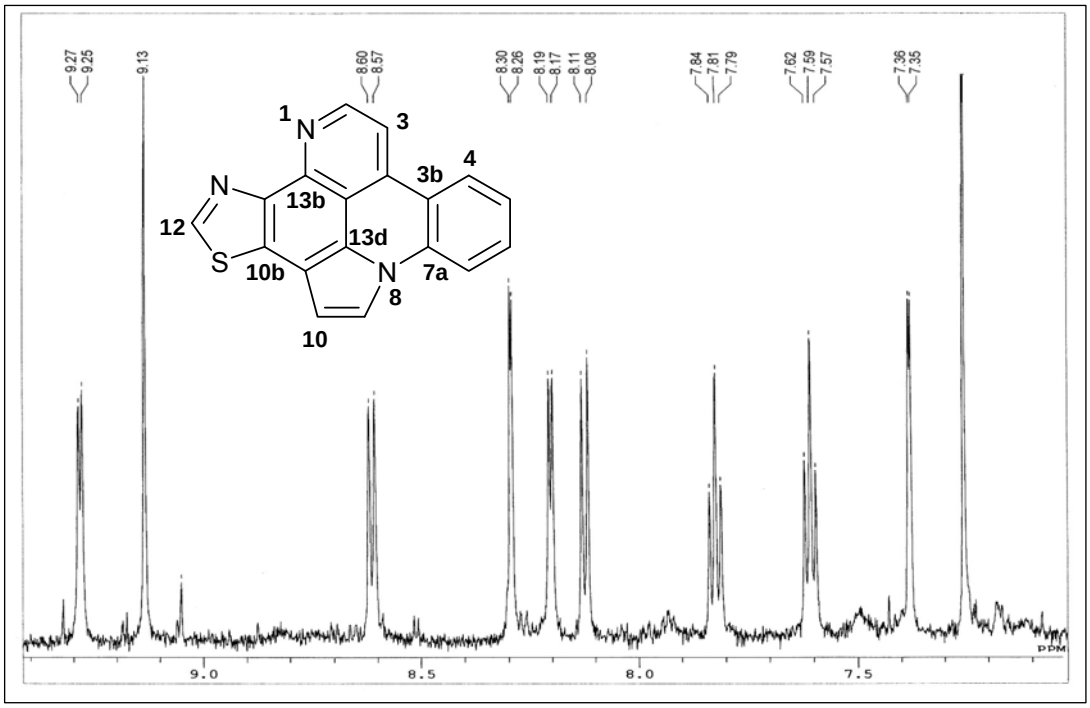


Fig 25. ^{1}H NMR spectrum of (200) in $CDCl_3$

The ascidian *Aplidium* sp. which was collected at Arab Reef, after methanol extraction and chromatography of the extract on alumina, afforded nordehydrocyclodercitin (**200**). The ^{1}H NMR spectrum showed nine signals, all resonating in the low-field region between δ 7.39 and 9.28 (see Fig 25). High resolution electrospray mass spectrometry (see Fig 26) and ^{13}C NMR data (see Fig 27) indicated the formula $C_{18}H_9N_3S$. The formula indicated the presence of sixteen

double bond equivalents, so the metabolite was clearly a polycyclic aromatic alkaloid. These signals represented four spin systems. Two of these spin systems were characteristic of a typical pyridoacridine alkaloid: the first was a pair of mutually coupled doublets at δ 9.28 and 8.20, J = 5.4 Hz, typical for the α and β protons of a pyridine system, while the second was diagnostic of an ortho-disubstituted aromatic ring (δ 8.61, brd, J = 8.25 Hz; 7.59, brt, J = 7.55 Hz; 7.81, brt, J = 7.55 Hz; 8.12, d, J = 8.25 Hz). The third spin system consisted of a pair of doublets at δ8.29 and 7.39, J = 2.8 Hz, and the small magnitude of the coupling suggested vicinal protons on a 2,3-substituted 5-membered heterocyclic ring (pyrrole, furan or thiophene). The fourth system consisted of a proton singlet at δ 9.13.

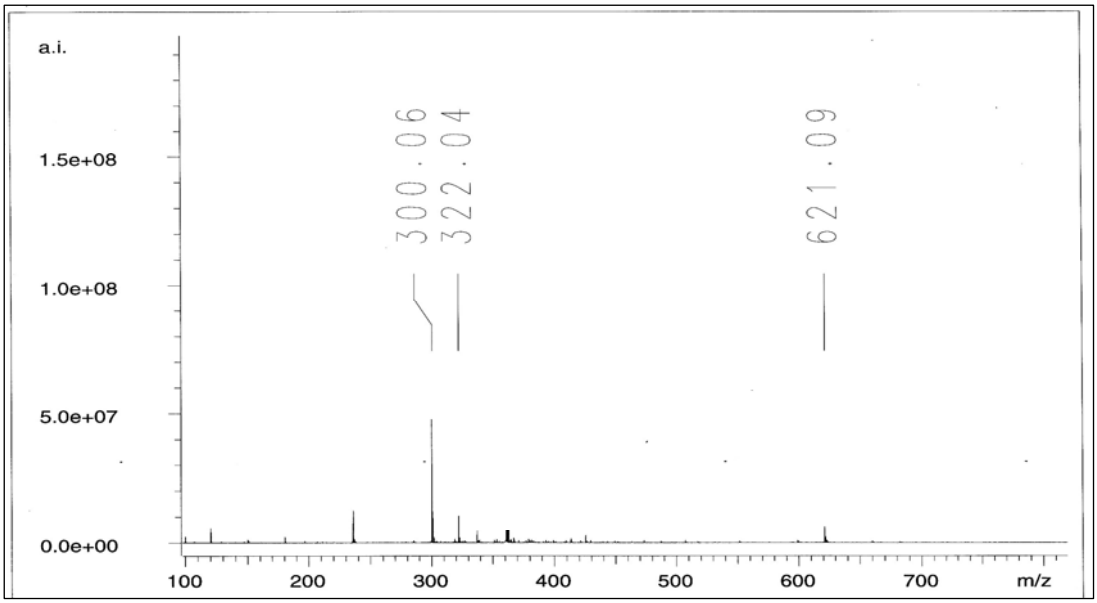


Fig 26. ESIMS of (200) showing the $[M+H]^+$, $[M+Na]^+$ and $[2M+Na]^+$ ions

The downfield chemical shift was suggestive of a thiazole proton, a common feature of tunicate alkaloids and peptides. As the heteroatom's observed in the formula (N_3S)

were accounted for by a pyridoacridine nucleus (N_2) plus a thiazole ring (NS), the 5-membered heterocyclic ring was deduced to be a fused pyrrole ring that incorporated one of the pyridoacridine nitrogen atoms.

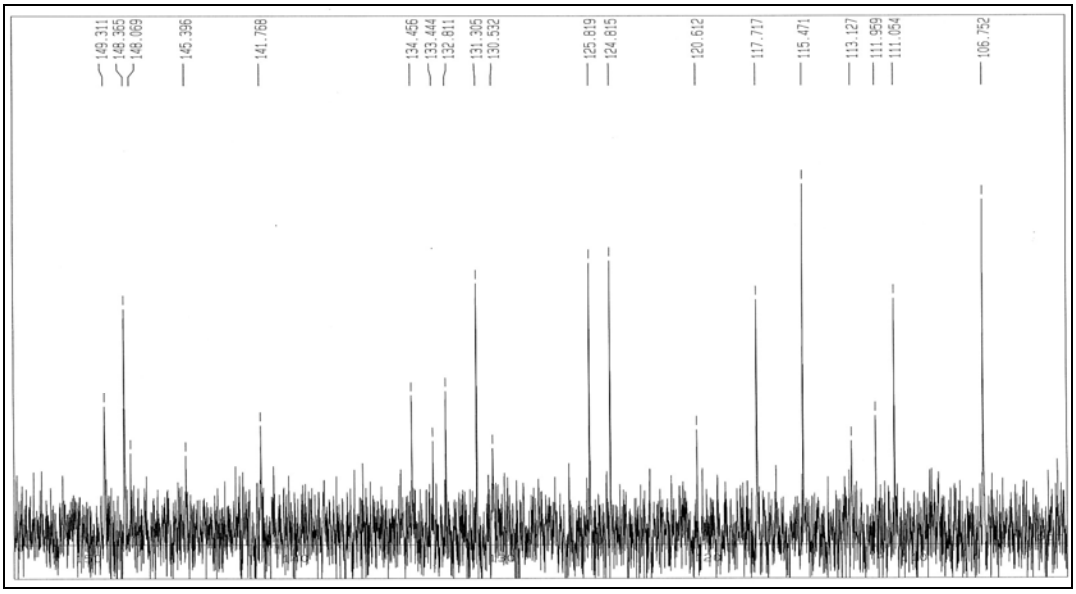


Fig 27. ^{13}C NMR spectrum of (200) in $CDCl_3$

A literature search for structures that contained these features returned stellettamine (**188**) and cyclodercitin (**190**)[117], both of which had been isolated from marine sponges. Cycloshermilamine D (**189**)[111], which was isolated from the marine ascidian *Cystodytes violatinctus*, is an analogue of stellettamine with a 6-membered tetrahydro-heterocycle in place of the thiazole ring.

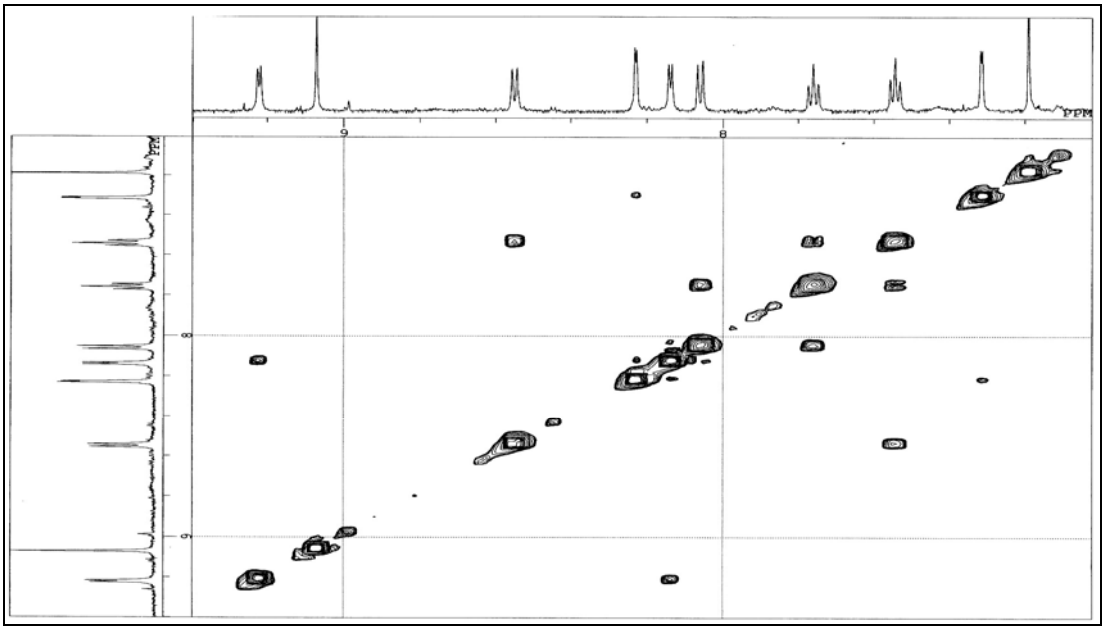


Fig 28. COSY spectrum of (200)

The COSY spectrum (see Fig 28) showed clear correlations between protons in each spin system, and this readily enabled proton assignments. Thus correlations between H-2 (δ 9.28d) and H-3 (δ 8.20d) confirmed the previously mentioned assignment to α and β protons of a pyridine nucleus. In addition correlations from protons H-4 to H-5, H-5 to H-6 and from H-6 to H- 7 confirmed the ortho di-substituted aromatic ring system. Correlations from H-9 to H-10 indictated coupling between the vicinal protons of a pyrrole ring.

The metabolite (**200**) was thus proposed to be the deaminated analogue of stellettamine, and indeed, the ^{1}H chemical shifts and coupling constants observed showed great similarities with those published for stellettamine, except for signals from the pyrrole protons and the aromatic proton H-7 that are significantly deshielded by the dimethylamino group in stellettamine (see Table 6). Because of the large number of quarternary carbon atoms, structural confirmation and full NMR

assignment relied heavily on the results from an HMBC experiment. The HMBC experiment showed proton correlations to all quarternary carbon signals except C-10b (δ 132.8). Observed HMBC correlations (see Fig 29) confirmed the hexacyclic structure (**200**) for nordehydrocyclodercitin.

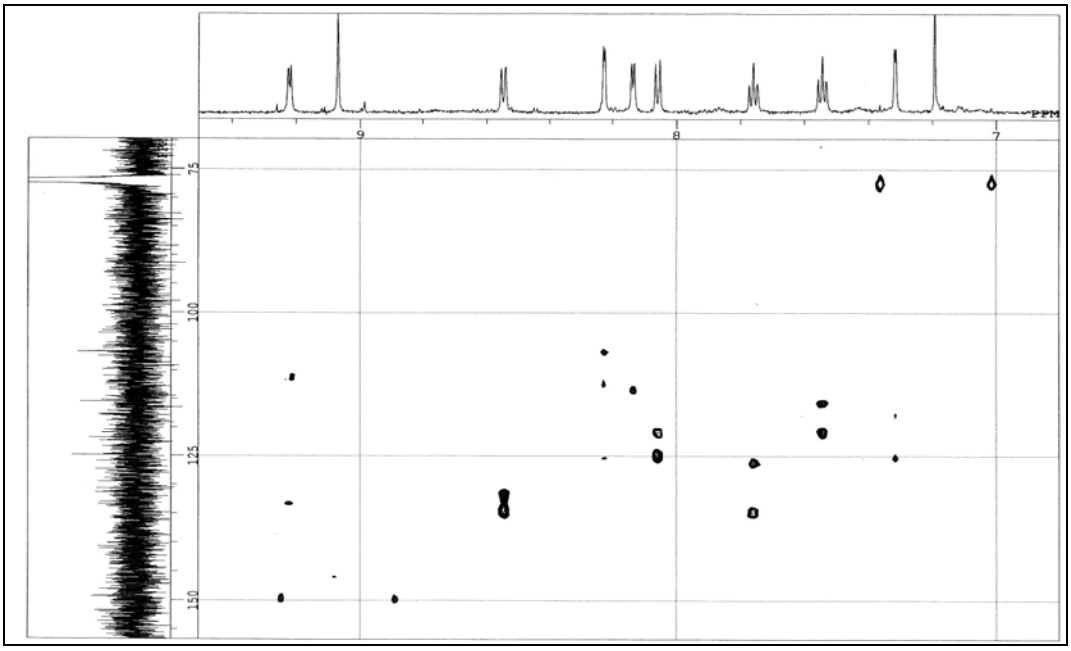


Fig 29. HMBC spectrum of (200)

	Nordehydrocyclodercitin (**200**)			Stellettamine (**188**)[117]	
C #	δ ^{1}H, mult., *J*(Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C	δ ^{1}H, mult., *J*(Hz)	δ^{13}C
2	9.28, d, 5.4	148.4	H-3	9.16, d, 5.3	147.5
3	8.20, d, 5.4	111.1	H-2	8.10, d, 5.3	110.5
3a	-	133.4	H-2, H-4	-	133.0
3b	-	120.6	H-3, H-5, H-7	-	121.4
4	8.61, brd, 8.25	125.8	H-6	8.55, dd, 8.0, 1.9	124.3
5	7.59, brt, 7.55	124.8	H-6, H-7	7.51, ddd, 8.0, 7.9, 1.4	124.8
6	7.81, brt, 7.55	131.3	H-4	7.67, ddd, 8.6, 7.9, 1.8	130.8
7	8.12, d, 8.25	115.5	H-5	9.18, dd, 8.6, 1.4	118.4
7a	-	134.5	H-4, H-6	-	136.4
9	8.29, d, 2.8	117.7	H-10		149.4
10	7.39, d, 2.8	106.8	H-9	6.86, s	94.2
10a	-	112.0	H-9	-	110.6
10b	-	132.8	-	-	129.7
12	9.13, s	149.3	-	9.06, s	149.1
13a	-	145.4	H-12	-	145.0
13b	-	141.8	H-2	-	140.8
13c	-	113.1	H-3	-	113.3
13d	-	124.8	H-9, H-10	-	122.0

Table 6. ^{1}H NMR and ^{13}C NMR data of (188, 200) in $CDCl_3$

2.6 Experimental

2.6.1 General

2.6.1.1 High-Performance Liquid Chromatography

Preparative high-performance liquid chromatography (HPLC) was performed using a GBC LC 1150 HPLC pump, a Rheodyne 7725i manual injector with a 2.5 ml sample loop, and various C-18 columns (described under individual separation procedures). Samples were observed at multiple UV/Vis wavelengths simultaneously with a GBC LC 5100 Photodiode Array Dector (PDA) and WinChrom Chromatography Data System (Version 1.3.1) software. Flow rates ranged from 1-3 ml/min. Distilled water and LR grade methanol (MeOH) were filtered through a Millipore 0.45 μm HA (water) or FH (methanol) membrane filter, and degassed by sparging with helium for 15 minutes.

2.6.1.2 Spectroscopy

Melting points (m.p.) were determined with a Stuart Scientific SMP1 melting point apparatus. Ultraviolet (UV) spectra were gathered in 100% methanol (MeOH) on a Varian Cary SE UV-Vis-NIR spectrophotometer. Infrared (IR) spectra were taken in AR grade $CHCl_3$ using a Nicolet Nexus Fourier Transform IR spectrometer. Nuclear Magnetic Resonance (NMR) spectra were obtained on a 300 MHz Varian Mercury spectrometer and 600 MHz Bruker spectrometer. Electrospray ionisation mass spectrometry (ESI-MS) was performed in HPLC grade MeOH with a Bruker BioApex 47e Fourier Transform Ion Cyclotron Resonance Mass Spectrometer by Mr. Rick Willis at the Australian Institute of Marine Science, Townsville.

2.6.2 Isolation and bioassay guided fractionation- *Chondria armata*.

2.6.2.1 Plant material and extraction

Plant material (40.5g, wet weight of *Chondria armata*) was collected from Pioneer Bay, Orpheus Island and frozen until extraction. The freeze-dried alga was extracted four times with 400 ml. of CH_2Cl_2 in a 500 ml. conical flask and the four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 0.45 g of crude extract.

2.6.2.2 Bioassay guided fractionation

The cytotoxic fraction (organic extract) was resuspended in a minimal amount of Hexane:CH_2Cl_2 (1:1) and applied to a glass column (i.d. 1.5 cm, length 12 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 10% methanol (MeOH) in EtOAc (fractions 1-10). The solvent was removed from each fraction at 30-40°C under vacuum on a rotary evaporator. Fractions 9 (100% EtOAc, 9.6 mg) and 10 (10% MeOH/CH_2Cl_2, 3.9mg) were cytotoxic and were further separated using column chromatography.

Fraction 9 (5.6 mg) was applied to a glass column (i.d. 1.0 cm, length 8 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated

with CH_2Cl_2. The column was eluted with Hexane: EtOAc: Acetone (2:1:0.5) which afforded fractions 9.1 - 9.12. The cytotoxic fractions 9.6 (3.1 mg) and 9.7 (4.1 mg) were combined and were once more put through the column and eluted with the same solvent mixture which afforded fractions 9.6.1 - 9.6.10. This procedure yielded the cytotoxic fraction 9.6.8 (5.4 mg) which was assigned structure **193** (armatol G). Fraction 10 (3.9 mg) was combined with fractions 9.8 - 9.11 (2.7 mg) and applied to a glass column (i.d. 1.0 cm, length 9 cm) packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with CH_2Cl_2. Stepwise gradient elution from 20%, 40% 60%, 80%, 90%, 95% CH_2Cl_2/EtOAc and 100% EtOAc afforded fractions 10.1 - 10.7. The major component from the cytotoxic fraction 10.5 (1.3 mg) was assigned structure **194** (armatol H)

Physical properties of pure metabolites isolated

Armatol G (193):

Clear oil; (Found: $[M+Na]^+$, 689.2024. $C_{30}H_{52}O_6{}^{79}Br_2Na$ requires $[M+Na]^+$, 689.2023); IR λ_{max} (Chloroform) cm^{-1}: 3477, 2988, 2880, 1135, 1073.

^{1}H NMR spectrum (C_6D_6): Refer to table 1

^{13}C NMR spectrum (C_6D_6): Refer to table 1

^{1}H NMR spectrum ($CDCl_3$): 4.17, d, *J* 10.2 Hz, H-22; 3.98, d, *J* 10.2 Hz, H-3; 3.51, d, *J* 10.2 Hz, H-18; 3.26-3.24, m, H-11 and H-7; 2.10, m, H_2-4; 2.1, m, H_2-21; 1.93, m, H-8a, 1.60, m, H-8b; 1.86, m, H-12a, 1.53, m, H-12b; 1.75, m, H-20a, 1.54, m, H-20b; 1.68, m, H-16a, 1.43, m, H-16b; 1.67, m, H-13a, 1.59, m, H-13b; 1.63, m, H-

17a, 1.36, m, H-17b; 1.19, m, H-9a, 1.18, m, H-9b; 1.35, s, H_3-1; 1.35, s, H_3-24; 1.34, s, H_3-25; 1.34, s, H_3-30; 1.19, s, H_3-26; 1.12 s, H_3-28; 1.11, s, H_3-27; 1.11, s, H_3-29.

^{13}C NMR spectrum ($CDCl_3$): 78.7, C-19; 77.7, C-23; 77.3, C-2; 77.0, C-7; 75.9, C-18; 74.8, C-11; 74.2, C-6; 73.1, C-10; 71.2, C-14; 60.3, C-3; 59.4, C-22; 45.6, C-5; 41.8, C-16; 39.9, C-20; 34.0, C-9; 31.6, C-21; 31.3, C-4; 28.1, C-17; 27.2, 26.0, C-1; C-12; 25.4, C-30; 25.3, C-25; 24.5, C-24; 23.6, C-8; 23.6, C-27; 21.3, C-26; 20.6, C-29; 16.1 , C-28.

Armatol H (194):

Clear oil; (Found: $[M+Na]^+$, 731.2128. $C_{32}H_{54}O_7{}^{79}Br_2Na$ requires $[M+Na]^+$, 731.2133); IR λ_{max} (Chloroform) cm^{-1}: 3489, 2880, 1135, 1073, 764.

^{1}H NMR spectrum (C_6D_6): Refer to table 1

^{13}C NMR spectrum (C_6D_6): Refer to table 1

^{1}H NMR spectrum ($CDCl_3$): 5.28, dd, *J* 10.4, 1.6 Hz, H-11; 4.14, dd, *J* 11.0, 1.1 Hz, H-22; 4.12, dd, *J* 10.9, 1.2 Hz, H-3; 3.51, dd, *J* 10.0, 2.5 Hz, H-14; 3.26, dd, *J* 11.2, 4.9 Hz, H-7; 3.13, dd, *J* 11.3, 3.0 Hz, H-18; 2.24, m, H_2-4; 2.08, m, H_2-21; 1.91, m, H-17a, 1.41, m, H-17b; 1.78, m, H-8a, 1.45, m, H-8b; 1.77, m, H-12a, 1.63, m, H-12b; 1.70, m, H-9a, 1.34, m, H-9b; 1.75, m, H-16a, 1.65, m, H-16b; 1.74, m, H-5a, 1.49, m, H-5b; 1.53, m, H_2-20; 1.44, m, H-13a, 1.20, m, H-13b; 1.34, s, H_3-25; 1.34, s, H_3-24; 1.33, s, H_3-1; 1.21, s, H_3-30; 1.20, s, H_3-26; 1.19, s, H_3-29; 1.09, s, H_3-27; 1.08, s, H_3-28.

^{13}C NMR spectrum ($CDCl_3$): 170.4, OAc; 20.5, OAc; 79.1, C-19; 78.9, C-6; 78.4, C-18; 77.6, C-2; 77.5, C-23; 75.7, C-14; 74.2, C-10; 74.0, C-11; 72.0, C-7; 60.0, C-4; 59.8, C-22; 44.7, C-5; 44.3, C-20; 40.6, C-16; 32.6, C-9; 31.7, C-4; 31.6, C-21; 27.6, C-13; 26.8, C-17; 26.4, C-12; 25.5, C-25; 25.5, C-24; 25.0, C-28; 24.7, C-27; 24.6, C-1; 24.4, C-30; 23.4, C-8; 20.8, C-20; 16.8, C-29.

2.6.3 Isolation and bioassay guided fractionation- *Rhabdastrella globostellata*.

2.6.3.1 Animal material and extraction

The sponge *Rhabdastrella globostellata* was collected by hand using scuba (from 5m) from Pith Reef in the central section of the Great Barrier Reef Marine Park, Australia. The sample was frozen immediately after collection and kept frozen until used. The animal material was freeze dried (14.63 g) and extracted four times with 400 ml of CH_2Cl_2 in a 500 mL conical flask. The four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 0.728g of crude extract.

2.6.3.2 Bioassay guided fractionation

The cytotoxic fraction (organic extract) was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 8 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2

in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 50% MeOH in EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator, and fractions throughout were protected from exposure to light.

Fractions 5 (12.2mg) and 10 were cytotoxic. Fraction 10 was pure and was identified as stelliferin D riboside, **195** (2.9mg). Fraction 5 was further purified by applying to a glass column packed with silica gel (60 H, thin layer chromatography; Merck). The column was eluted with 15 ml of 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% CH_2Cl_2 in hexane and 100% CH_2Cl_2 to collect fractions 5.1-5.10. Fractions 5.4 (3.4mg) and 5.8 (4.4mg) were cytotoxic and identified as (13*Z*,15*E*,17*E*)-3β,28-diacetoxy-22-hydroxyisomalabarica-13,15,17,24-tetraen-12-one (**51**) and stelliferin A (**43**) respectively.

2.6.3.3 Physical properties of pure metabolites isolated

Stelliferin D riboside (195):

Yellow amorphous powder, m.p. 232-240° C; $[\alpha]_{436}$ = -156° (*c* 0.23, MeOH); (Found: $[M-H]^-$, 569.3847 $C_{35}H_{53}O_6$ requires $[M-H]^-$, 569.3849); IR λ_{max} (Chloroform) cm^{-1}: 2920, 1660, 1580, 1562, 1449, 1033, 986; UV (EtOH) nm: 344 (ε 37563).

^{1}H NMR spectrum ($CDCl_3$): Refer to table 2.

^{13}C NMR spectrum ($CDCl_3$): Refer to table 2.

Stelliferin A (43):

^{1}H NMR spectrum ($CDCl_3$): Refer to table 3.

^{13}C NMR spectrum ($CDCl_3$): Refer to table 3.

(13*Z*,15*E*,17*E*)-3β,28-diacetoxy-22-hydroxyisomalabarica-13,15,17,24-tetraen-12-one (51)

^{1}H NMR spectrum ($CDCl_3$): Refer to table 3.

^{13}C NMR spectrum ($CDCl_3$): Refer to table 3.

2.6.4 Isolation and bioassay guided fractionation- *Spongia* sp.

2.6.4.1 Animal material and extraction

The sponge *Spongia* was collected by hand using scuba (7.6m) from Arab Reef in the central section of the Great Barrier Reef Marine Park, Australia. The sample was frozen immediately after collection and kept frozen until used. The freeze-dried animal material (17.64 g) was extracted four times with 400 mL of CH_2Cl_2 in a 500 mL conical flask. The four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 0.105g of crude extract.

2.6.4.2 Bioassay guided fractionation

The cytotoxic fraction (organic extract) was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.0 cm, length 11 cm) that had been packed with alumina gel and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 25% MeOH/ EtOAc, 100% MeOH (fractions 1-11). The solvent was removed from each fraction at 30-40°C under vacuum on a rotary evaporator.

Fractions 7 (6.1mg) and 11 (6.6mg) were cytotoxic and were further purified. Fraction 7 was purified via HPLC on a Hewlett-Packard RP-18 semi-preparative column (7µm, i.d 9.5mm, length 250mm) eluted with 90% $MeOH_{(aq)}$ at a flow rate of 1.5 ml/min. The MeOH was removed from fractions 7.1 (retention time 11.4 min) and 7.2 (retention time 14.1 min) and each was transferred to a separating funnel and extracted with dichloromethane. Removal of the dichloromethane solvent from fraction 7.1 afforded spongiatriol triacetate (**99**, 1.4 mg)**.** The major metabolite spongiadiol diacetate (**97**, 3.0 mg) was isolated from fraction 7.2.

Fraction 11 was injected on a Hewlett-Packard RP-18 semi-preparative column (7µm, i.d 9.5mm, length 250mm) eluted with 80% $MeOH_{(aq)}$ at a flow rate of 1.0 ml/min. Fractions 11.2 (retention time 6.42 min) and 11.4 (retention time 9.39 min) were cytotoxic and once again were purified via HPLC by injection on the above

mentioned column and elution with 65% MeOH. Rechromatography of fraction 11.2 afforded isospongiatriol (**196**, 1.1 mg) and epispongiatriol (**96**, 1.6mg). Cytotoxic fraction 11.4 yielded isospongiadiol (**109**, 4.1 mg).

2.6.4.3 Physical properties of pure metabolites isolated

Isospongiatriol (196)

Crystalline solid, m.p. 163-165° C; $[\alpha]_D$ = -76° (*c* 0.18, MeOH); (Found: $[M+Na]^+$, 371.1830. $C_{20}H_{28}O_5Na$ requires $[M+Na]^+$, 371.1829); IR λ_{max} (Chloroform) cm^{-1}: 3440, 1710, 1045, 895; UV (EtOH) nm: 220 (ε 4768).

^{1}H NMR spectrum (MeOH-d_4): See table 4.

^{13}C NMR spectrum (MeOH-d_4): See table 4.

Isospongiadiol (109)

^{1}H NMR spectrum ($CDCl_3$): 7.07, brs, H-15; 7.05, brs, H-16; 4.62 dd, *J* 12.0 6.3 Hz, H-2; 4.14, d, *J* 11 Hz, H-19a; 3.66, d, *J* 11 Hz, H-19b; 2.80, m, H-12a; 2.46, m, H-12b; 2.64, m, H-1a; 1.20, m, H-1b; 2.17, m, H-7a; 1.58, m, H-7b; 1.84, m, H-11a; 1.70, m, H-11b; 1.71, m , H_2-6; 1.40, m, H-5; 1.24, m, H-9; 1.30, s, H_3-18; 1.26, s, H_3-17; 1.25, s, H_3-20.

^{13}C NMR spectrum ($CDCl_3$): 214.0, C-4; 137.0, C-16; 136.7, C-14; 135.1, C-15; 119.4, C-13; 69.9, C-2; 65.7, C-19; 58.7, C-5; 55.9, C-9; 54.5, C-4; 49.5, C-1; 41.2, C-7; 38.0, C-10; 34.2, C-8; 26.4, C-17; 20.5, C-12; 20.0, C-6; 19.3, C-18; 18.8, C-11; 17.6, C-20.

Spongiadiol diacetate (97)

^{1}H NMR spectrum ($CDCl_3$): 7.11, brs, H-16; 7.05, brs, H-15; 5.46, s, H-3; 4.03, s, H_2-19; 2.62, d, *J* 17 Hz, H-1a; 2.20, *J* 17 Hz, H-1b; 2.16, s, CH_3CO; 2.08, s, CH_3CO; 1.26, s, CH_3; 1.24, s, CH_3; 1.04, s, H_3-20.

Spongiatriol triacetate (99)

^{1}H NMR spectrum ($CDCl_3$): 7.13, brs, H-16; 7.10, brs, H-15; 5.46, s, H-3; 4.36, d, *J* 11 Hz, H-17a; 4.09, d, *J* 11 Hz, H-17b; 4.00, s, H_2-19; 2.62, d, *J* 17 Hz, H-1a; 2.17, d, *J* 17 Hz, H-1b; 2.15, s, CH_3CO; 2.08, s, CH_3CO; 2.02, s, CH_3CO; 1.30, s, H3-18; 1.03, s, H3-20.

Epispongiatriol (96)

^{1}H NMR spectrum ($CDCl_3$): 7.23, brs, H-16; 7.23, brs, H-15; 4.03, s, H-3; 3.78, d, *J* 10 Hz, H-17a or H-19a; 3.70, d, *J* 11Hz, H-19a or H-17a; 3.48, d, *J* 10 Hz, H-17b or H-19b; 3.35, d, *J* 11 Hz, H-19b or H-17b; 2.75, d, *J* 12 Hz, H-1a; 2.10, d, *J* 12 Hz, H-1b; 1.40 H_3-18, 0.85, H_3-20.

2.6.5 Isolation and bioassay guided fractionation- *Dysidea herbacea*

2.6.5.1 Animal material and extraction

The sponge *Dysidea herbacea* was collected by hand using scuba (from11m) near Pelorus Island in the central section of the Great Barrier Reef Marine Park, Australia. The sample was frozen immediately after collection and kept frozen until used. A taxonomic sample (registered sample No.G25097) is lodged with the Museum of Tropical Queensland, Flinders Street, Townsville, Qld. 4810. The freeze-dried sponge (10.89 g) was successively extracted with dichloromethane (3 × 100 ml). The solvent was removed on a rotary evaporator to afford a crude extract (0.448g)

2.6.5.2 Bioassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 10 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 50% MeOH in EtOAc (fractions 1-11). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator, and fractions throughout were protected from exposure to light.

Fraction 3 was further purified by reverse phase HPLC on a Hewlett-Packard C18 column (10 x 250 mm) eluted with acetonitrile / 1% aqueous ammonium acetate (9:1) at a flow rate of 1.5 ml/min. The acetonitrile was removed from fractions that contained the metabolite 1-hydroxy-3,4,6,2',4'-pentabromodiphenyl ether**, 197** (retention time 3.23 min) and compound **155** (retention time 2.845 min) and each was transferred to a separating funnel and extracted with dichloromethane. Removal of the dichloromethane solvent afforded minor **197** (1.7 mg) and major metabolite **155** (6.2 mg) in a 1:10 ratio (w.r.t peak area) respectively.

2.6.5.3 Physical properties of pure metabolites isolated

1-Hydroxy-3,4,6,2',4'-pentabromodiphenyl ether (197)

Crystalline solid, m.p. 120-121° C; (Found: $[M-H]^-$, 578.6097. $C_{12}H_4{}^{79}Br_3{}^{81}Br_2O_2$ requires $[M-H]^-$, 578.6093); IR λ_{max} (Chloroform) cm^{-1}: 3515, 3019, 2927, 2854, 1727, 1466, 1392, 1299, 1257, 1091, 1043, 932, 871, 807; UV (EtOH) nm: 211 (ε 27193), 292 (ε 2379), sh 317 (ε 1022).

^{1}H NMR spectrum ($CDCl_3$): Refer to table 5.

^{13}C NMR spectrum ($CDCl_3$): Refer to table 5.

2.6.5.4 Animal material and extraction

Another sample of the sponge *Dysidea herbacea* was collected by hand using scuba (from 12m) from Arab reef in the central section of the Great Barrier Reef Marine

Park, Australia. The sample was frozen immediately after collection and kept frozen until used. The freeze-dried sponge (10.89 g) was successively extracted with dichloromethane (3 × 100 ml). The solvent was removed on a rotary evaporator to afford a crude extract (0.206g)

2.6.5.5 Bisoassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 10 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 50% MeOH in EtOAc (fractions 1-11). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator, and fractions throughout were protected from exposure to light.

Fractions 2-5 were cytotoxic. Fraction 2 and 3 were identified as 1,2'-dimethoxy-3,4,5,3',5'-pentabromodiphenyl ether, **198** (3.1 mg) and 1,2'-dimethoxy-3,5,3',5'-tetrabromodiphenyl ether, **199** (2.7 mg) respectively. Fraction 5 was identified as 1,2'-dihydroxy-3,4,5,6,3',5'-hexabromodiphenyl ether, **141** (3.9 mg).

2.6.5.6 Physical properties of pure metabolites isolated

1,2'-Dihydroxy-3,4,5,6,3',5'-hexabromodiphenyl ether (141)

^{1}H NMR spectrum (MeOH-d_4): 7.33, d, *J* 2.1 Hz, H-4'; 6.45, d, *J* 2.1 Hz, H-6'.

1,2'-Dimethoxy-3,4,5,3',5'-pentabromodiphenyl ether (198)

^{1}H NMR spectrum ($CDCl_3$): 7.36, d, *J* 2.1 Hz, H-4'; 7.29, s, H-6; 6.43, d, *J* 2.1Hz, H-6'.

1,2'-Dimethoxy-3,5,3',5'-tetrabromodiphenyl ether (199)

^{1}H NMR spectrum ($CDCl_3$): 7.41, d, *J* 2.5 Hz, H-4; 7.35, d, *J* 2.1 Hz, H-4'; 7.10, d, *J* 2.4 Hz, H-6; 6.45, d, *J* 2.1 Hz, H-6'.

2.6.6 Isolation and bioassay guided fractionation-*Aplidium* sp.

2.6.6.1 Animal material and extraction

The ascidian *Aplidium* sp.(cf *Aplidium cratiferum*) was collected by hand using scuba from Arab Reef (11 m) in the central section of the Great Barrier Reef Marine Park, Australia. The sample was frozen immediately after collection and kept frozen until used. A taxonomic sample (registered sample No. G21286) is lodged with the Museum of Tropical Queensland, Flinders Street, Townsville, Qld. 4810. The wet

ascidian (7.576g) was successively extracted with MeOH (3 ×100 ml). The solvent was removed on a rotary evaporator and further partitioned between water and DCM. The DCM was removed on a rotary evaporator to afford a crude extract (0.532g).

2.6.6.2 Bioassay guided fractionation

The cytotoxic fraction (organic extract) was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 11 cm) that had been packed with alumina (thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 25% MeOH/ EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator. Fraction 8, which was obtained as a yellow crystalline solid (1.9 mg; 0.025% of wet weight), and was named nordehydrocyclodercitin (**200**)

2.6.6.3 Physical properties of pure metabolites isolated

Nordehydrocyclodercitin (200)

Crystalline solid, m.p. 286-287° C; (Found: $[M+H]^{+\bullet}$, 300.0587. $C_{18}H_{10}N_3S$ requires $[M+H]^+$, 300.0589); IR λ_{max} ($CHCl_3$)/cm-^{1}: 1603, 1458; UV/Vis (MeOH) λ_{max}/nm (log ε) 256 (4.37), 293 (4.11), 338 (3.63), 360 (3.67).

^{1}H NMR spectrum ($CDCl_3$): Refer to table 6.

^{13}C NMR spectrum ($CDCl_3$): Refer to table 6.

CHAPTER 3

Introduction-

Literature review of genera from which known metabolites have been isolated

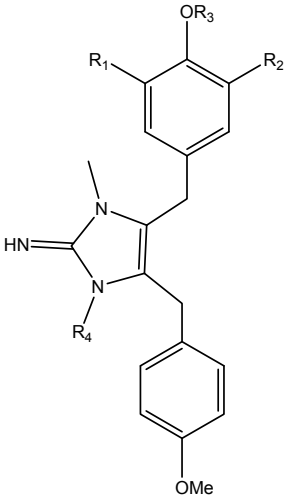
3.1 Literature review of metabolites reported from the sponge *Leucetta* sp.

3.1.1 General introduction

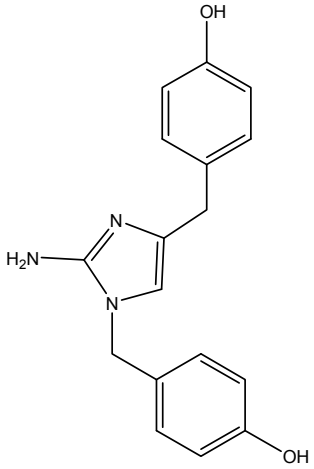
Chemical investigation of sponges belonging to the genus *Leucetta* has led to the isolation of a number of interesting imidazole alkaloids such as the naamines, isonaamines, naamidines, isonaamidines and their corresponding Zn-complexes. The alkaloids of these groups are similar in that naamines and isonaamines possess a central 2-aminoimidazole ring to which two substituted benzyl moieties are attached, while naamidines and isonaamidines are similarly substituted, with another oxidized imidazole ring attached to the 2-amino group. Biosynthetically related compounds have also been isolated from *Leucetta* sponges and these include dorimidazole[123], leucettamine[124], clathridines[125], preclathridines[125], and kealiiquinone[126]. In addition a Fijian collection of the sponges has yielded a unique chiral 2-amino-imidazole and spiro-cyclopentimidazolidines known as calcaridine and spirocalcaridine[127]. An interesting study conducted on nine different collections of calcareous *Leucetta* sp. for variation in their alkaloid constituents showed that the species either contained the polyunsaturated fatty amino alcohol-leucettamol A or imidazoles. Thus these two classes of alkaloids never occurred in the same sponge sample. In other words *Leucetta* sponges appear to contain either 2-amino imidazoles or the amino polyene leucettamol A but not both[128].

3.1.2 The naamines, isonaamines, naamidines and isonaamidines

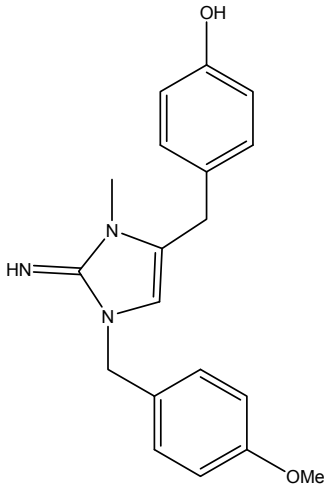
The naamines and their positional isomers, the isonaamines are the simplest of these imidazole alkaloids. Lead compounds are naamine A (**201**) and isonaamine A (**202**) which have para oxygenated benzyl substituents at the 4 and 5 positions, or the 3 and 5 positions respectively of the 2-aminoimidazole[127]. Naamine B (**203**), isolated from collections of the sponge from the Red Sea on the other hand has a 3-hydroxy-4-methoxybenzyl group[129]. The isomer (**204**) of naamine A (**201**) has also been reported[130]. Naamine C (**205**), isolated from a *Leucetta* sponge sample collected in Micronesia is an oxygenated analogue of naamine A (**201**) and has a tetra substituted ring A and a disubstituted ring B[131]. Naamine D (**206**) was isolated from an Egyptian sample as the antifungal and nitric oxide synthase inhibitor component of the sponge[132]. More recently the N,N dimethyl derivative (**207**) of naamine D has been reported[128]. Naamine E (**208**) was isolated from a sponge sample collected in the GBR[133]. In addition, Naamines F (**209**) and G (**210**) have been isolated from an Indonesian sample of the sponge[134]. Naamine G (**210**) exhibited strong antifungal activity against the phytopathogenic fungus *Cladosporium herbarum* and also showed mild cytotoxicity against mouse lymphoma (L5178Y) and human cervix carcinoma (HeLa) cell lines[134].



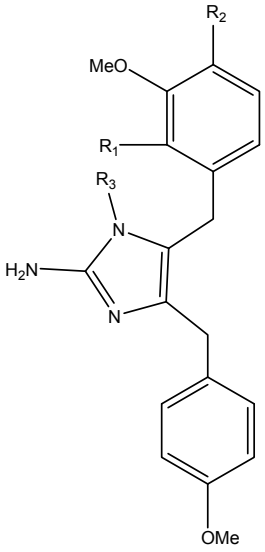
201. R_1=H, R_2=H, R_3=H, R_4=H
203. R_1=H, R_2=OMe, R_3=H, R_4=Me
207. R_1=H, R_2=H, R_3=Me, R_4=Me
209. R1=OMe, R_2=H, R_3=H, R_4=H
210. R_1=OMe, R_2=OMe, R_3=H, R_4=H



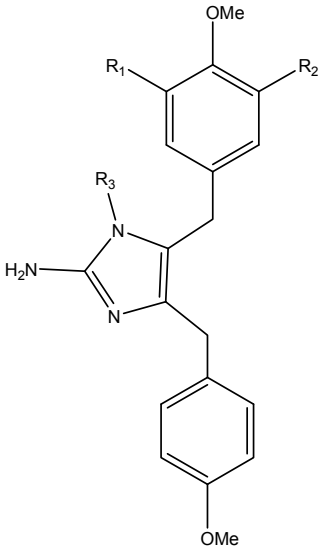
202.



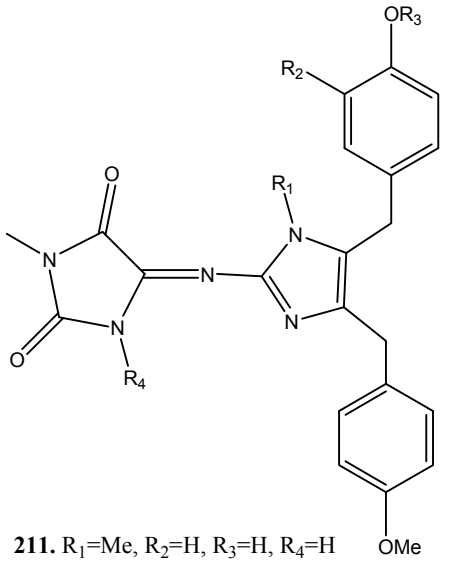
204.



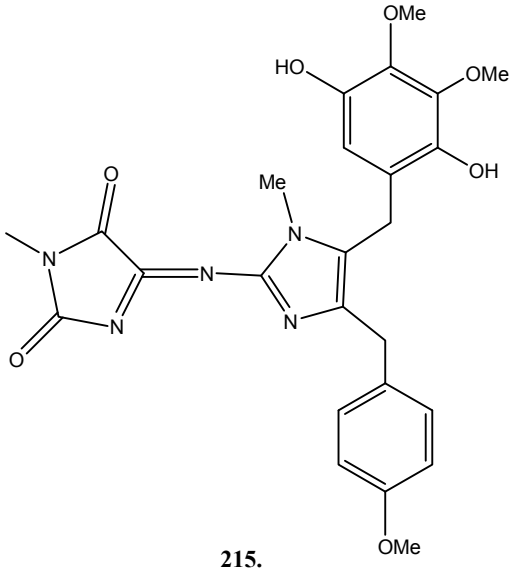
205. R_1=OH, R_2=OMe, R_3=Me



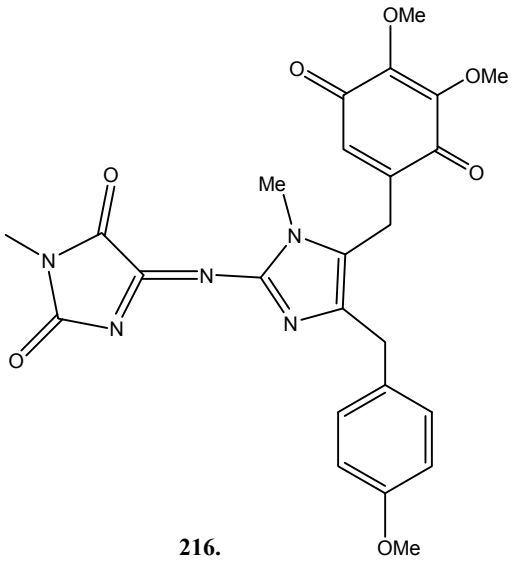
206. R_1=H, R_2=H, R_3=H
208. R_1=OH, R_2=OH, R_3=Me



211. R_1=Me, R_2=H, R_3=H, R_4=H
212. R_1=Me, R_2=OH, R_3=Me, R_4=H
213. R_1=Me, R_2=H, R_3=H, R_4=Me
214. R_1=H, R_2=H, R_3=Me, R_4=H
217. R_1=Me, R_2=H, R_3=Me, R_4=H



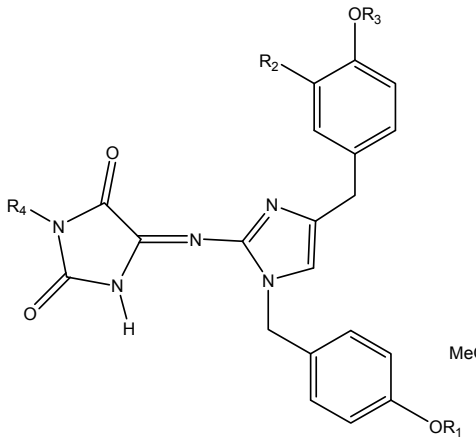
215.



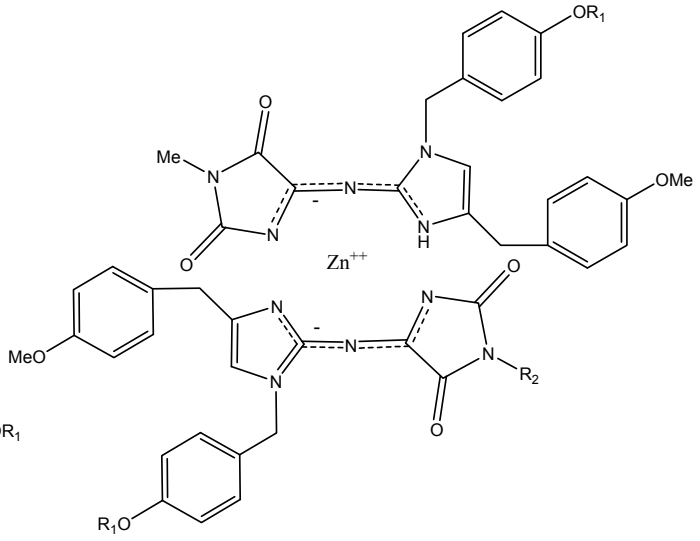
216.

The naamidines are 4,5 dibenzylated 2-aminoimidazole alkaloids where the amino group is substituted with another oxidised imidazole ring. Naamidines A-D (**211-214**) were isolated from a Red Sea sample of the sponge *Leucetta chagosensis*[129]. Naamidine A (**211**) was found to be selective in inhibiting the growth of tumor cells

which are dependent upon epidermal growth factor for their growth, while showing acceptably low general cytotoxicity. Such selectivity was established through *in vitro* and *in vivo* comparative testing using tumor cells which over express EGF receptor sites. The *in vivo* tests used nude athymic mice that were implanted with squamous cell carcinoma[135]. Subsequently, Naamidines E (**215**), F (**216**)[132], and G (**217**)[136] were isolated from a *Leucetta* sponge collected from GBR and Red Sea respectively.



218. R_1=H, R_2=H, R_3=H, R_4=Me
219. R_1=Me, R_2=H, R_3=Me, R_4=H
220. R_1=Me, R_2=H, R_3=Me, R_4=Me
222. R_1=H, R_2=H, R_3=Me, R_4=H
225. R_1=Me, R_2=OMe, R_3=Me, R_4=Me



221. R_1=R_2=Me
223. R1=H, R_2=Me
224. R_1=H, R_2=H

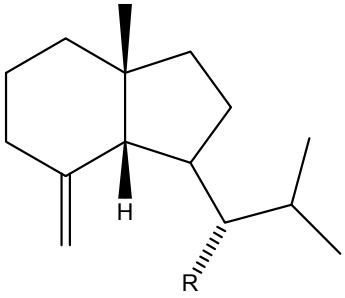
The isomers (**218, 219**) of naamidine A (**211**) and B (**212**) have been reported[129]. Isonaamidine C (**220**) was synthetically produced[137], however the Zn-complex (**221**) of isonaamidine C (**220**) has been isolated from a *Leucetta* sponge and its nudibrach prey *Notodoris gardineri*[138]. Three imidazole alkaloids along with known isonaamidine B (**219**) and isonaamine B (**204**) were isolated from a Pacific sample of *Leucetta*. These included isonaamidine D (**222**), and zinc complexes (**223, 224**) of

isonaamidine B and isonaamidine D respectively[130]. Isonaamidine D (**222**) showed weak antifungal activity against *Aspergillus niger* with MIC value of 100μg/ml[130]. Isonaamidine E (**225**), which was isolated from a *Leucetta* sponge collected from the GBR showed moderate cytostatic activity with GI_{50} values of 7.0 μg/ml and 1.3 μg/ml against HepG2 and Huh7 cell lines respectively[133].

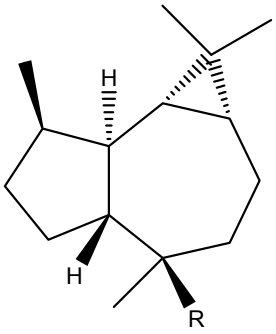
3.2 Literature review of terpenoid isocyanides, isothiocyanates, and formamides isolated from sponges *Axinella cannabina* and *Acanthella acuta*

Sesquiterpenoid and diterpenoid isocyanides, isothiocyanates, and formamides are of closely related groups of compounds that are produced by sponges[139, 140] and often found in nudibranchs, although it is most likely that the nudibranchs derive these metabolites from their sponge diet[141, 142]. The Mediterranean sponge *Axinella cannabina* is a prolific source of such metabolites with these rare functionalities. Further, the isolation of isocyanides are usually accompanied by their corresponding isothiocyanates and formamides, most likely biogenetically derived from them[143]. *Axinella cannabina* initially yielded two metabolites with an axane skeleton. The compounds included an isonitrile (axisonitrile-1, **226**), and isothiocyanate; (axisothiocyanate-1, **227**). Prior to this the only known naturally occuring isonitrile was the antibiotic xanthocillin[139]. This axane skeleton was also found to be present in oppositol (**228**), a brominated sesquiterpene alcohol from the marine red alga *Laurencia subopposita*[144]. In addition axisonitrile-2 (**229**) and axisothiocyanate–2 (**230**) along with axamide-1 (**231**) and axamide-2 (**232**) have been reported from

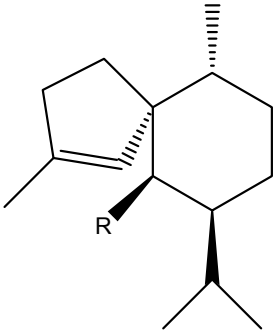
Axinella cannabina[140]. Axisonitrile-3 (**233**), axisothiocyanate-3 (**234**) and axamide-3 (**235**) were subsequently isolated and feature a novel spiroaxane carbon skeleton[140].



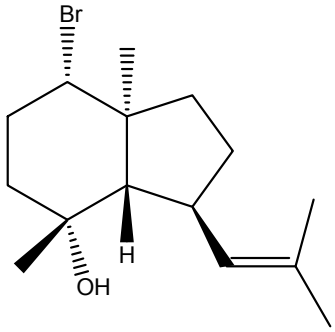
226. R=NC
227. R=NCS
231. R=NHCHO



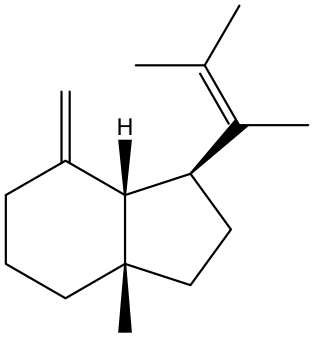
229. R=NC
230. R=NCS
232. R=NHCHO



233. R=NC
234. R=NCS
235. R=NHCHO



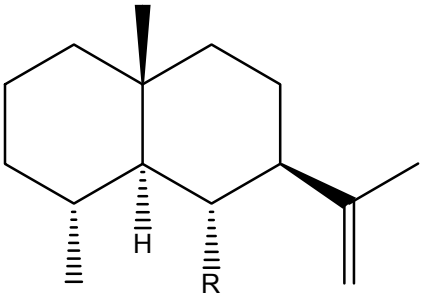
228.



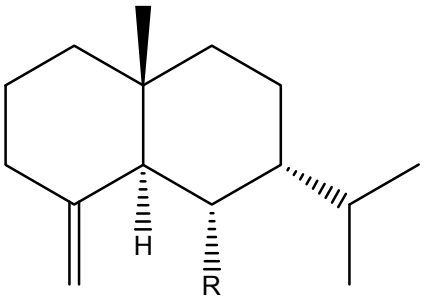
236. R=NC
237. R=NCS
238. R=NHCHO

Three more axanes (**236-238**) have been isolated from a Mediterranean *Axinella* sp.[145] and their structures have been confirmed by total synthesis[146]. Acanthellin-1 (**239**) another sesquiterpenoid isonitrile was isolated from *Acanthella acuta*, a sponge that belongs to the same family (Axinellidae)[147]. It features a 4-epi-eudesmane skeleton. Related structures (**240-244**) were isolated from a Mediterranean sample of *Axinella cannabina*[148]. Further study on the sponge *Axinella cannabina* afforded a

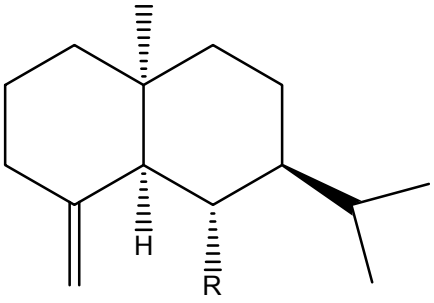
new isocyanide-isothiocyanate-formamide triad (**245-247**) based on the cis-eudesmane skeleton[149].



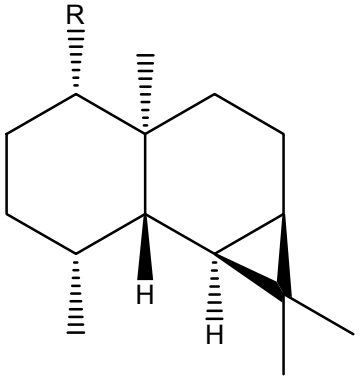
239. R=NC
240. R=NCS
241. R=NHCHO



242. R=NC
243. R=NCS
244. R=NHCHO



245. R=NC
246. R=NCS
247. R=NHCHO



248. R=NC
249. R=NCS
250. R=NHCHO

Two of these compounds (**245-246**) have also been isolated from *Acanthella acuta* [149]. The isolation of acanthellin-1(**239**) from both these sponges suggests that these nitrogenous sesquiterpenes could be of taxonomical interest[149]. Further study on this sponge led to the isolation of three novel sesquiterpenes (**248-250**) based on the epi-

maaliane skeleton that carried an isonitrile, isothiocyanate and formylamino functions respectively. Bicyclogermacrene was found to co-occur with these metabolites[150].

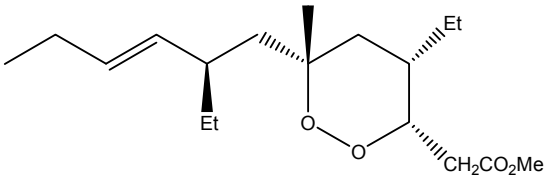
3.3 Literature review of metabolites reported from the sponge *Plakortis* sp.

3.3.1 General introduction

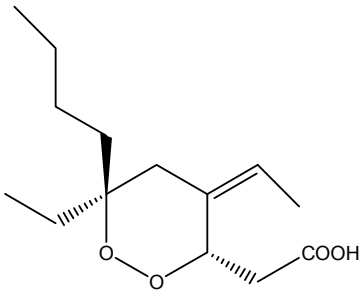
Sponges from the genus *Plakortis* have yielded a wide variety of metabolites that include plakortin, plakinic acids, plakorin, plakortolide, plakinidone, peroxyplakoric acids, manadic acids, plakortones and plakortides[151]. They are a prolific source of polyketide peroxides. Many *Plakortis*-derived peroxides have a 1,2-dioxan ring with such groups as an acetic acid moiety at C-3 and an aliphatic chain (sometimes terminating with a phenyl ring) at C-6[152].

3.3.2 Cyclic peroxides and related metabolites from *Plakortis* sp.

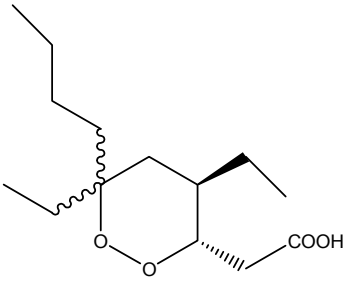
Plakortin (**251**), isolated from the Caribbean *Plakortis halichondriodes* was the first such compound to be described (in 1978)[153]. Subsequently plakortin derivatives (**252**) and (**253**) from sponge *Callyspongia sp.*[154] and (**254-256**) from *Plakortis lita* have been reported [155].



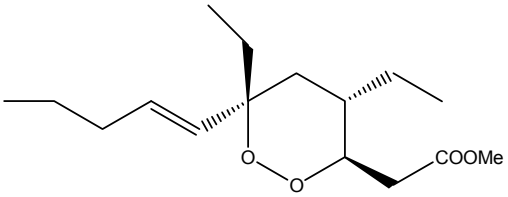
251.



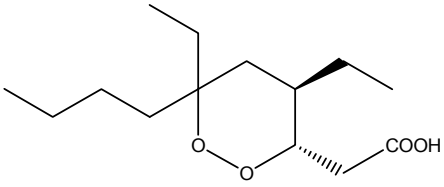
252.



253.



254.



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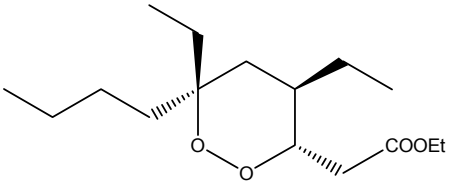
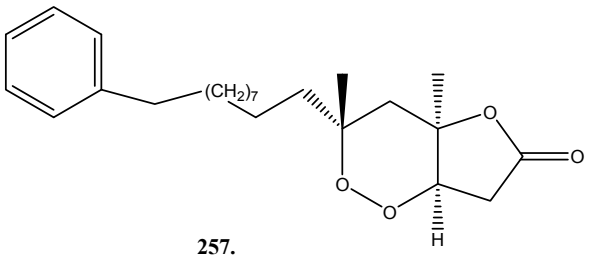


256.

The structural pattern was further extended by the isolation of the unstable metabolites, plakortolide E (**257**) and plakoric acid (**258**)[152]. Further, studies on this species from Truk and Okinawa afforded the polyketide butenolides (**259-261**), the chondrillins (**262-263**) which are cyclic peroxides related to plakortin, and a series of novel peroxy aliphatic esters (**264-267**) related to the chondrillins[156].



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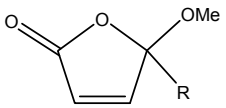
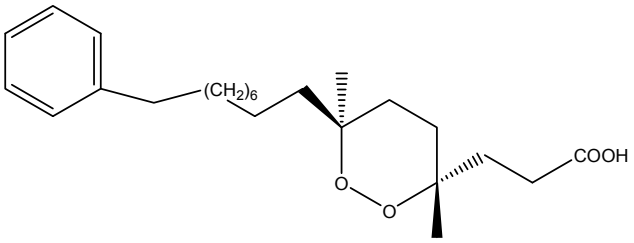


259. R=-$(CH_2)_{15}$-Me

260. R=-$(CH_2)_{13}$

261. R=-$(CH_2)_{11}$



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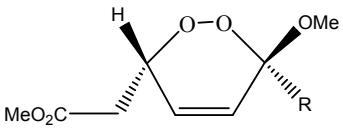
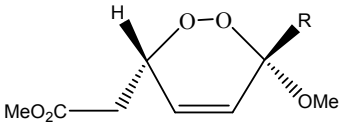


262. R= -$(CH2)_{15}$-Me

264. R= -$(CH_2)_{13}$

266. R=-$(CH_2)_{11}$

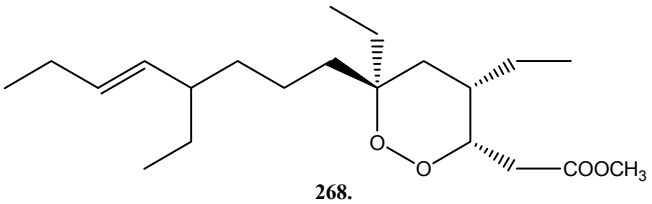


263. R= -$(CH_2)_{15}$-Me

265. R= -$(CH_2)_{13}$

267. R= -$(CH_2)_{11}$

A search for novel Ca^{2+} activators led to the isolation of another three novel metabolites, the plakortides F-H (**268-270**), the known metabolite 3-epiplakortin (**271**) and the α,β-unsaturated ester (**272**)[157]. Related compounds plakortides M (**273**) and N (**274**) have also been reported[158]. Further endoperoxides haterumadioxins A (**275**) and B (**276**) were isolated from the Okinawan sponge *Plakortis lita*[151].



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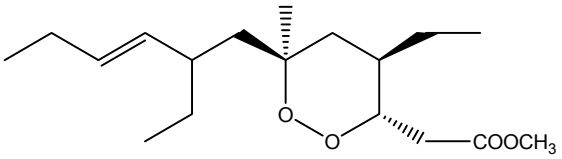

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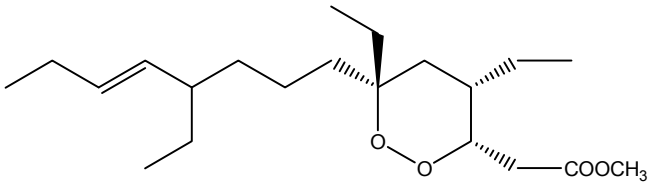

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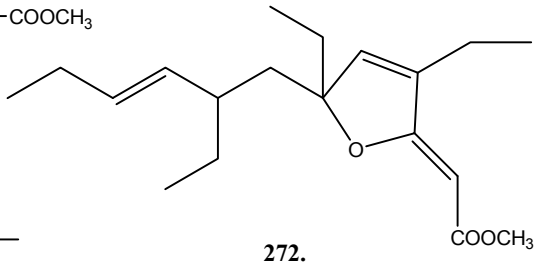

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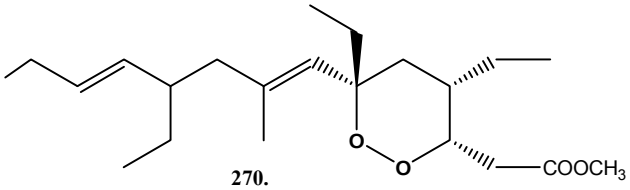

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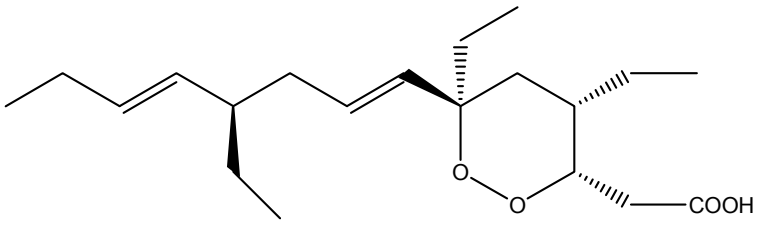

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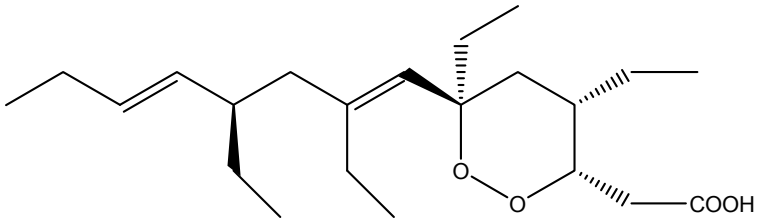

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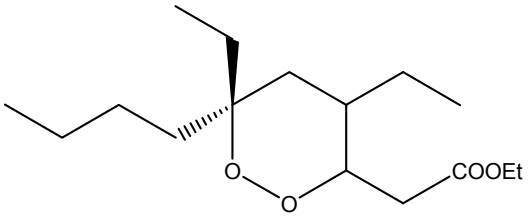

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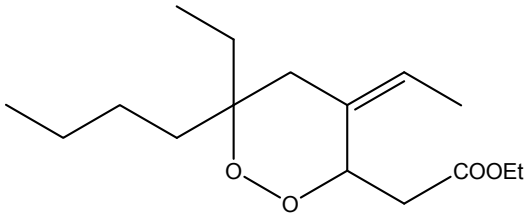


276.

3.4 Literature review of scalarane type metabolites isolated from Dictyoceratid sponges

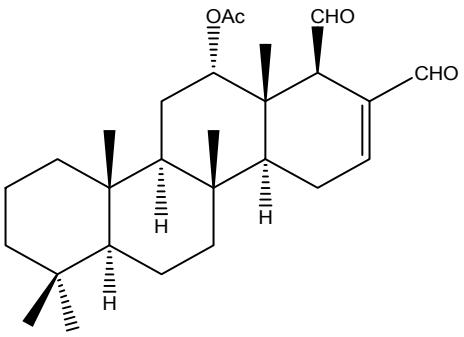
3.4.1 General introduction to Dictyoceratid sponges

Dictyoceratid sponges are soft sponges that do not contain any spicules for defense from predators. The two families *Thorectidae* and *Spongiidae* are a rich source of scalarane sesterterpenes and not surprisingly, scalaranes have been shown to exhibit ichthyotoxic and antifeedant properties[159]. Molluscs feeding on these sponges may sequester the scalarane metabolites for protection, or they may chemically transform them in order to store defense chemicals in a less toxic form.

3.4.2 Sesterterpenes from Dictyoceratid sponges

On Guam, some populations of *Hyrtios erecta* were found to produce scalaradial (**277**) while other populations were found to produce heteronemin (**278**)[160]. *Glossodoris* nudibranchs that predated on the former population contained scalaradial (**277**), deoxyscalarin (**279**) and scalarin (**280**) while the nudibranchs that fed on the latter population contained 12-episcalaradial (**281**) as well as heteronemin (**278**)[160]. It should be noted that rings C, D and E of the sesterterpene heteronemin (**278**) are very similar to the structure of the sesquiterpene olepupuane (**282**)[161], the precursor of polygodial (**283**) isolated from *Dendrodoris* nudibranchs[162]. Some Dendrodorid nudibranchs have been shown to be capable of *de novo* biosynthesis of sesquiterpenes which are protected analogues of polygodial[163].

The related structures 12-deacetoxy-12-oxo-deoxoscalarin (**284**) and 12-deacetyl-12-epi-deoxoscalarin (**285**) have been isolated along with heteronemin (**278**) from the dorid nudibranch *Glossodoris atromaginata* and its sponge prey[164]. The metabolites (**284**) and heteronemin (**278**) showed selective cytotoxicity against human thyroid carcinoma. The presence of the compound (**285**) along with heteronemin (**278**) supported the hypothesis that the mollusc had derived the compound from its prey.



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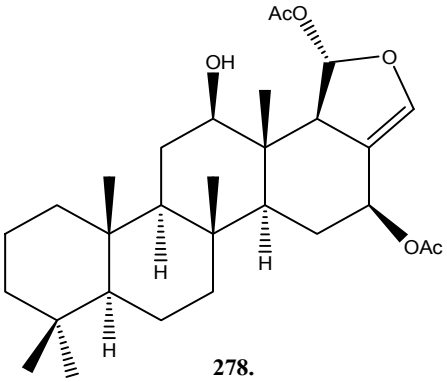

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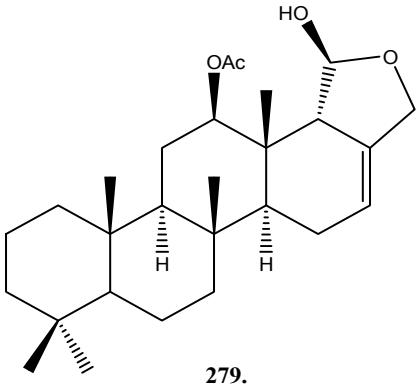

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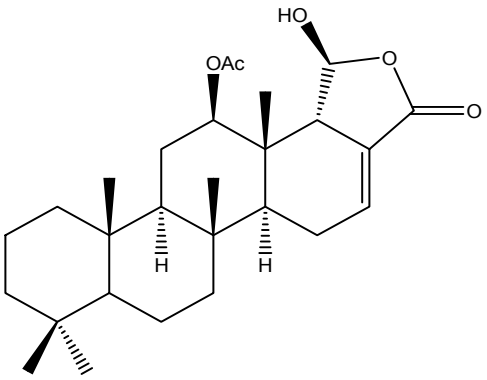

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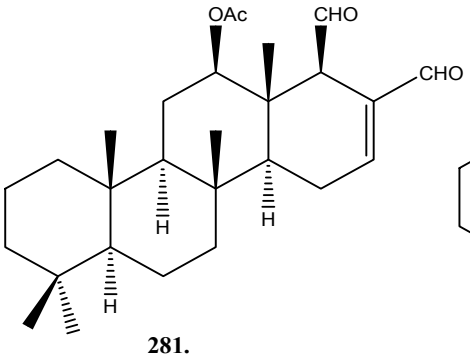

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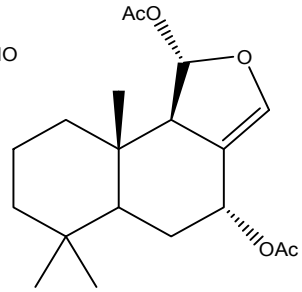

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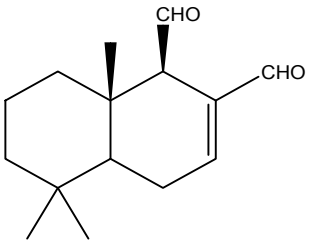

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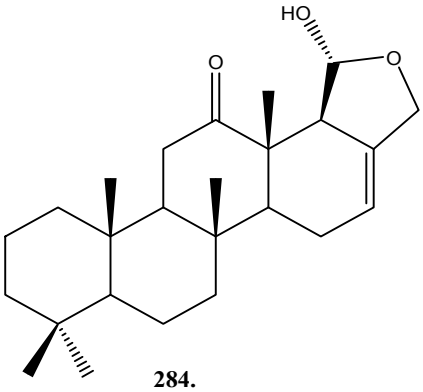

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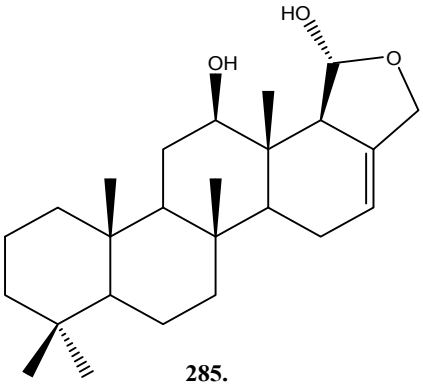

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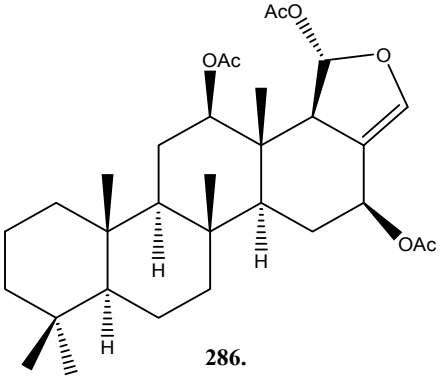
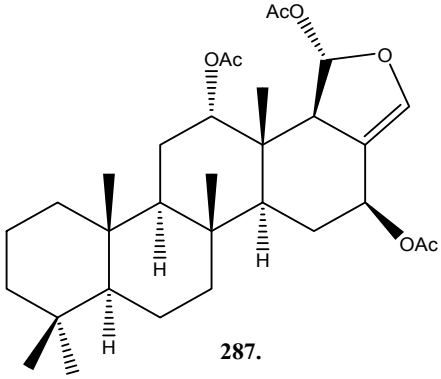


286.



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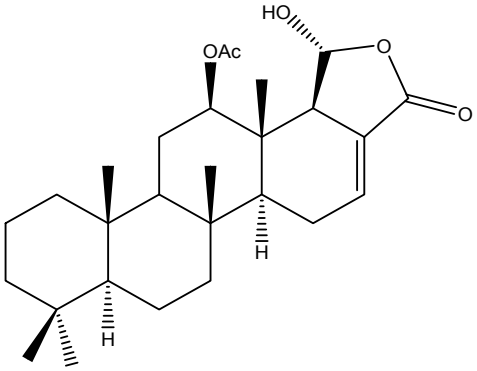

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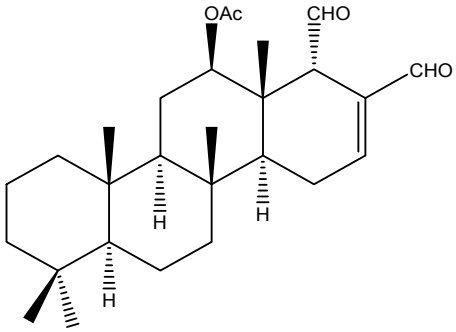
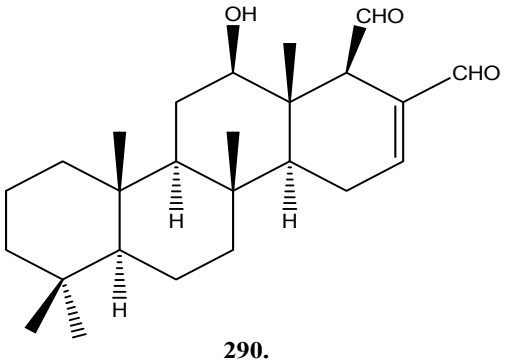


289.

Eleven sesterterpenes were isolated from a *Hyrtios erecta* specimen collected in Tonga: heteronemin acetate (**286**) and its epimer 12-epi heteronemin acetate (**287**), 12-episcalarin (**288**), heteronemin (**278**), 12-epi-scalaradial (**281**), scalaradial (**277**), 12,18-di-epi-scalaradial (**289**), 12-deacetyl-12-epi-scalaradial (**290**), scalarafuran (**291**) and hyrtial (**292**)[165].



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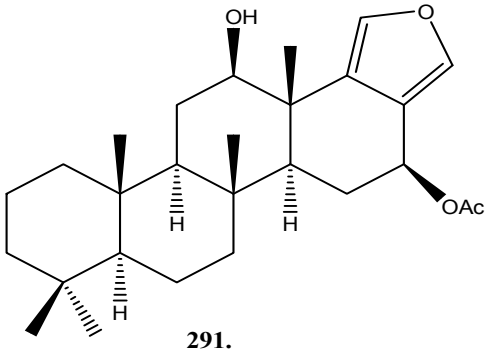

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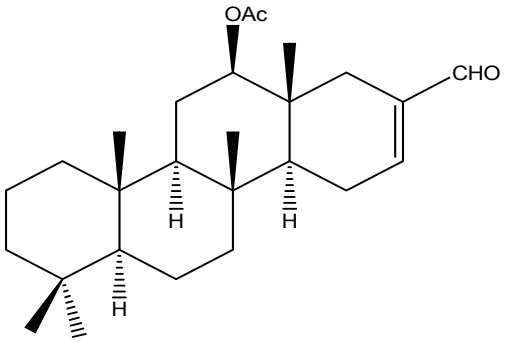

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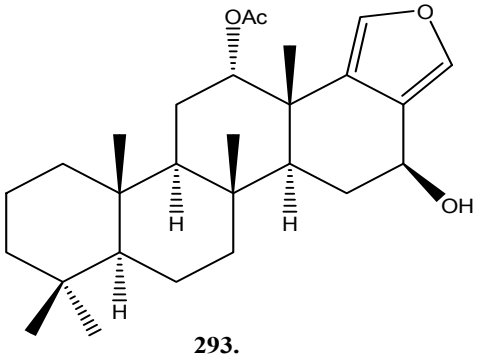

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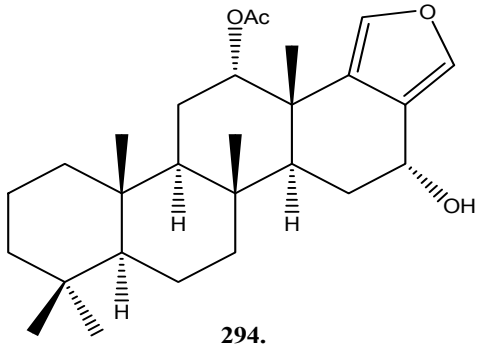
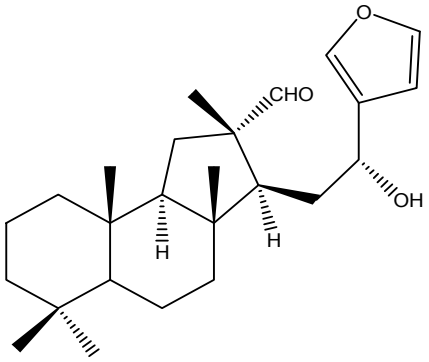


294.

Isoscalarafuran A (**293**) and B (**294**) were isolated from a sponge sample along with hyrtiosal (**295**) [166]. Hyrtiosal (**295**), a novel ring C contracted sesterterpene, was first described in early 1992 from an Okinawan specimen of *Hyrtios erecta* and was reported to inhibit the proliferation of KB cells *in vivo*[167].



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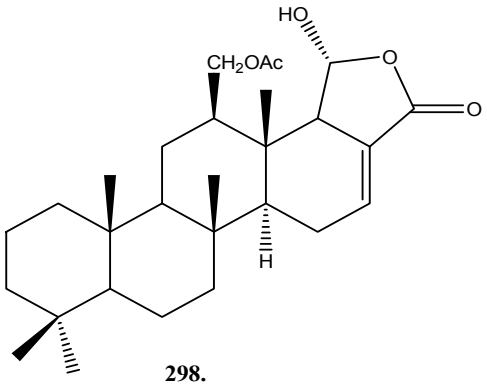

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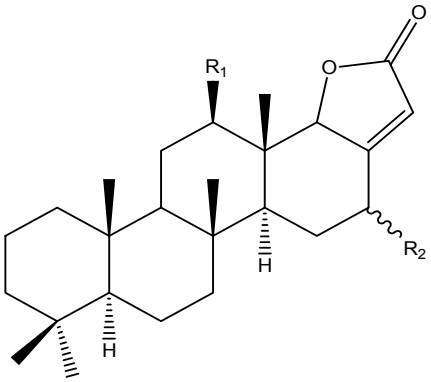
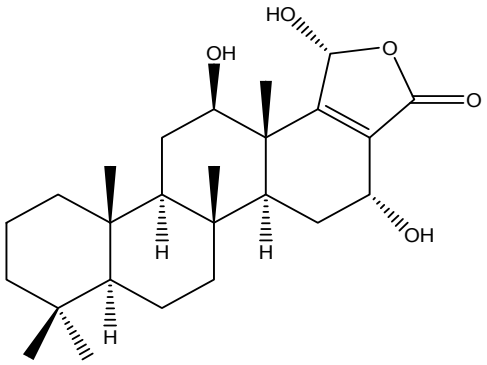


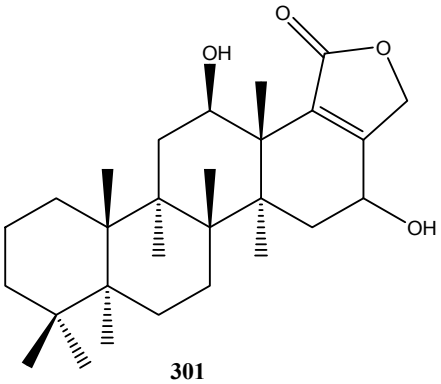
296. R_1=OH, R_2=β-OH
297. R_1=OAc, R_2=β-OH
299. R_1=OH, R_2=α-OH



300.

16-Episcalarolbutenolide (**296**) and its 12-acetate derivative (**297**) which are similar to scalarolbutenolide (**299**)[168] were reported along with compound **298**, which is an isomer of **297** with the same ring D and E geometry as scalarin (**279**). Compounds (**296-298**) were cytotoxic against P-388 leukemia cells with IC_{50} values of 0.4, 2.1, and 0.9 μg/ml respectively [169]. Hyrtiolide (**300**), 16-hydroxyscalarolide (**301**) and 12-

deacetyl-Δ^{17}-hyrtial (**302**) were isolated from an Okinawan sample along with scalarolide (**303**) and 12-deacetylhyrtial (**304**) [170].



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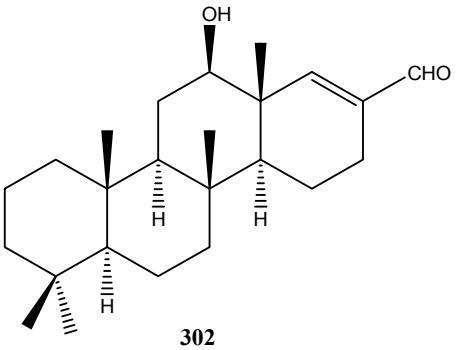

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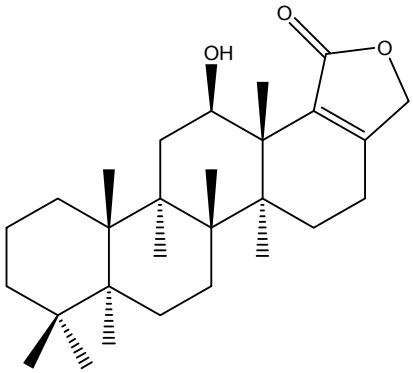

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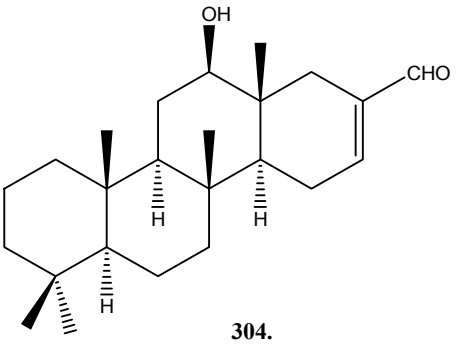
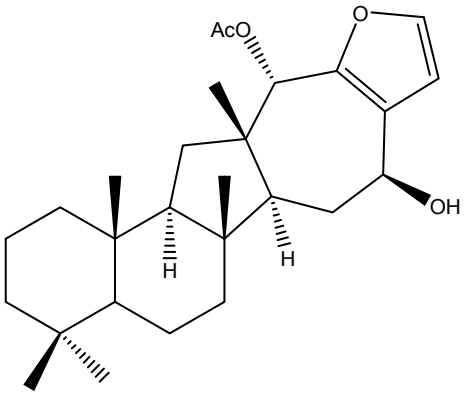


304.

Salmahyrtisol A (**305**), B (**306**) and C (**307**) together with 3-acetylsesterstatin-1 (**308**) and 19-acetylsesterstatin-3 (**309**) were reported from a Red Sea collection of *Hyrtios erecta*[171]. Since hyrtiosal and salmahyrtisol A, which both contain a ring-contracted 5-membered C ring, were isolated from the same sponge sample, it is likely that the former is the biosynthetic intermediate to the latter[171].



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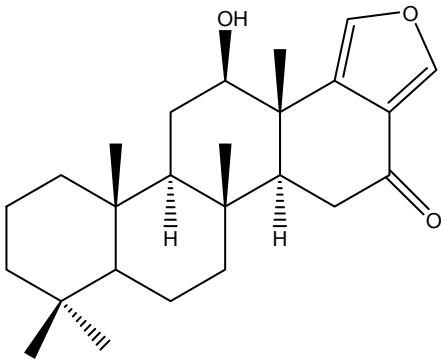
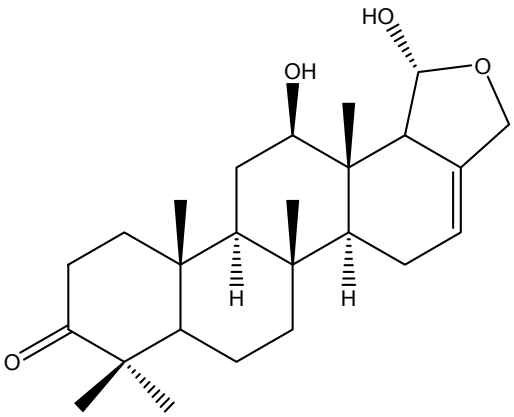


306.

Compounds **308** and **309** are derivatives of sesterstatin-1(**310**), -2 (**311**) and -3 (**312**) which were reported from *Hyrtios erecta* collected from the Republic of Maldives[172]. Sesterstatin–2 (**311**) was shown to specifically inhibit the gram-positive bacteria *Staphylococcus aureus*[172]. All three compounds showed significant cytotoxicity to P388, human lung carcinoma (A-549), and human colon carcinoma (HT-29)[171, 172]. Subsequently, sesterstatin-4 (**313**) and -5 (**314**) were isolated from a specimen of *Hyrtios erecta*. Sesterstatin-4 (**313**) exhibited an ED_{50} of 4.9µg/ml against P388 cells whereas sesterstatin-5 (**314**) exhibited a GI_{50} of 1.9 µg/ ml against the DU-145 prostrate cell line[173].



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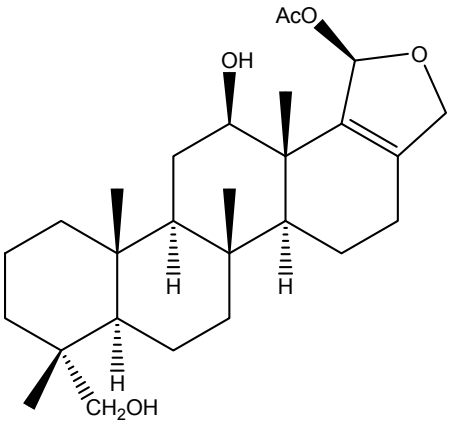
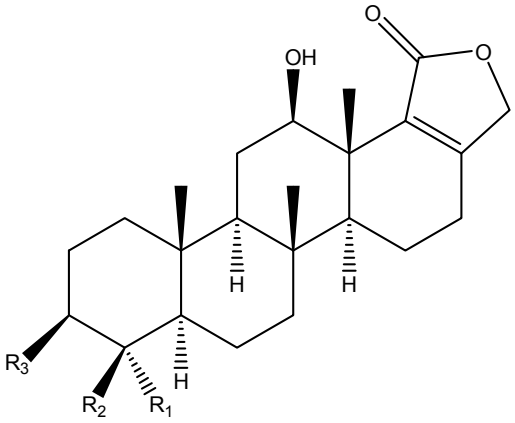
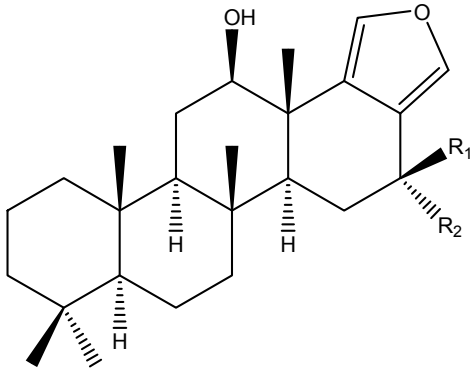


308.

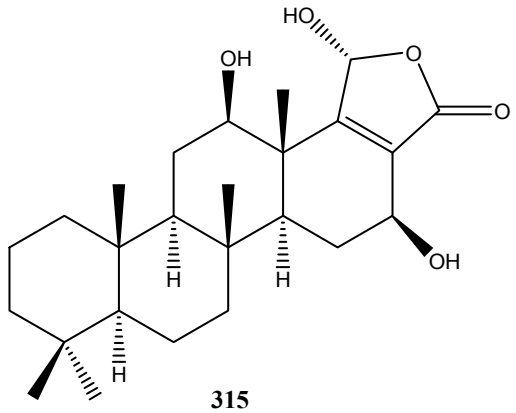


309. R_1=R_2=Me, R_3=OAc
310. R_1=R_2=Me, R_3=OH
311. R_1=CH_2OH, R_2=CH_3, R_3=H
312. R_1=Me, R_2=CH_2OH, R_3=H

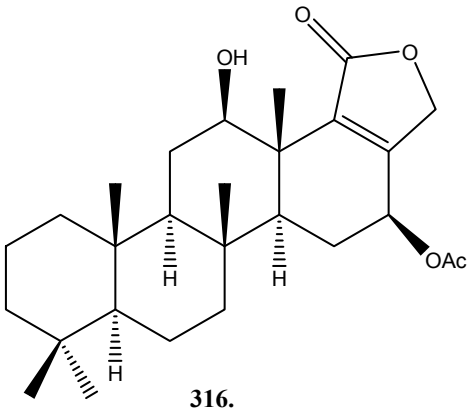


313. R_1=H, R_2=OH
314. R_1=OH, R_2= H

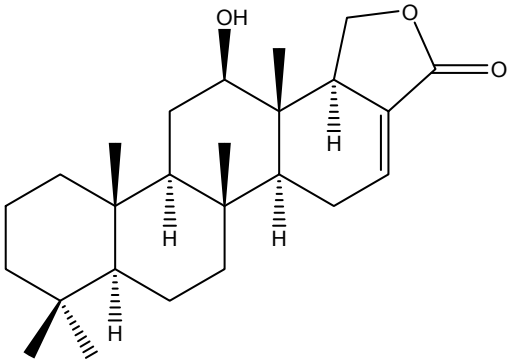
Sesterstatin-6 (**315**), which was isolated from a collection from the Maldives showed significant cancer cell growth inhibition against murine P388 lymphocytic leukemias and exhibited an ED_{50} value of 0.17 μg/ml which proved to be the most inhibitory of the series. Sesterstatins-1, -2, -3 and -6 have α,β unsaturated lactone rings whereas sesterstatins-4 and -5 have a furan moiety[174]. Sesterstatin-7 (**316**) together with 16-epi-scalarolbutenolide (**296**), 25-dehydroxy-12-*epi*-deacetylscalarin (**317**), 3-acetylsesterstatin-1 (**309**) and 21-acetoxydeoxyscalarin (**318**) were isolated from a Red Sea sample of *Hyrtios erecta*[175].



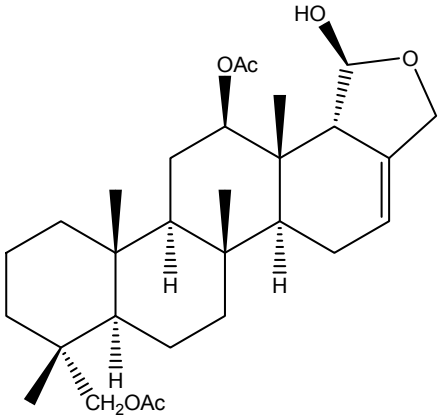
315



316.



317



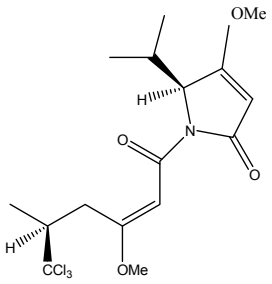
318.

Sesterstatin-7 (**316**) showed 63% inhibition of growth for *Mycobacterium tuberculosis* at a concentration of 6.25 μg/ml. Compound **296** displayed moderate activity whereas **317** and **309** were weakly active against the same biological target[175].

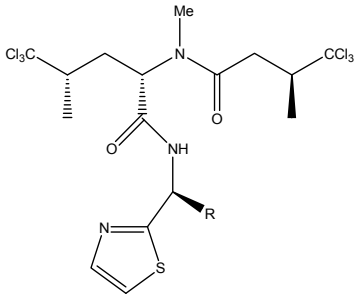
3.5 Literature review of polychlorinated amino acid derivatives isolated from the sponge *Dysidea herbacea*

3.5.1 Chlorinated peptide-derived metabolites

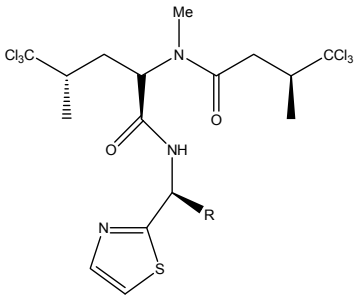
The range of metabolite classes found in *Dysidea herbacea* were discussed in Chapter one. The polychlorinated amino acid derivatives are exemplified by dysidin (**319**)[176], dysidenin (**320**)[177], and its 5-epimer, isodysidenin (**321**)[178]. Dysidenin is reported to be a potent inhibitor of the iodine-sodium co-transporter in bovine thyroid[179]. A specimen from the GBR yielded four additional polychlorinated compounds. These were demethyldysidenin (**322**) and demethylisodysidenin (**323**) which are demethylated homologues of dysidenin and isodysidenin respectively[180].



319.



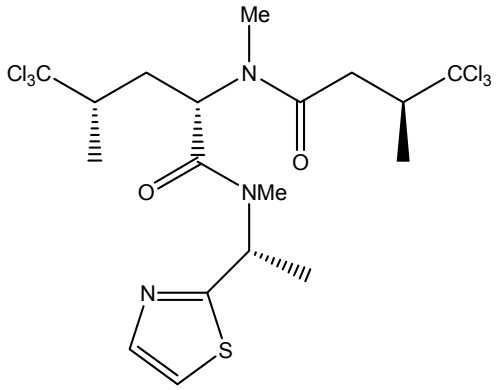
320. R=Me
322. R=H



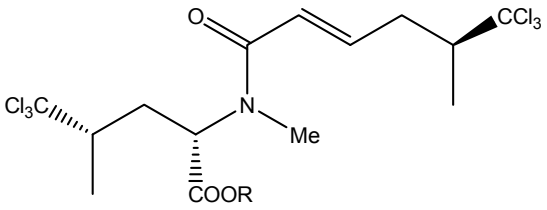
321. R=Me
323. R=H

Neodysidenin (**324**) was isolated from the marine sponge *Dysidea herbacea* collected on the Great Barrier Reef. Neodysidenin belongs to the L-series of trichloroleucine peptides, and the configuration of the N-methyl thiazolyl alanine residue (13R) was shown to be opposite to that of dysidenin[181]. Simple N-acyl tricholoroleucine derivatives herbaceamide (**325**)[182] and herbacic acid (**326**)[183] have

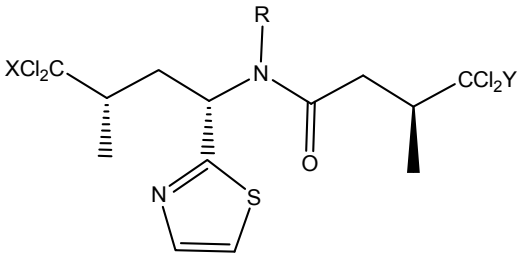
been isolated from a GBR collections. The absolute stereochemistry of all chiral centres was shown to be the S-configuration as seen in related chlorinated natural products having a tricholoroisopropyl group[183].



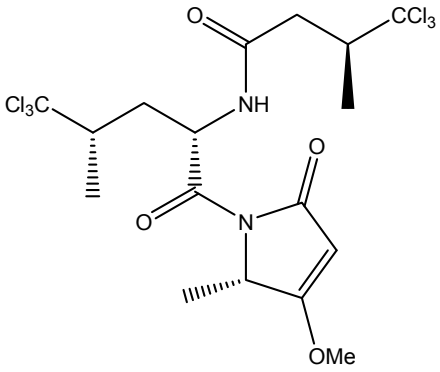
324.



325. R=Me
326. R= H



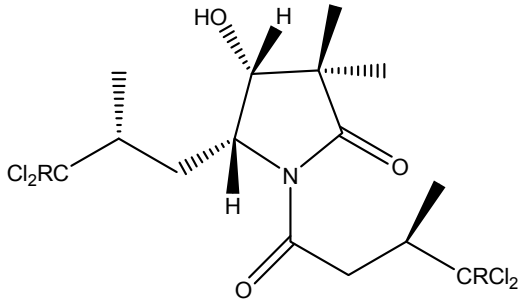
327. R=H, X=Cl, Y=Cl
328. R=Me, X=Cl, Y=Cl
329. R=Me, X=Cl, Y=H
330. R=H, X=Cl, Y=H
331. R=Me, X=H, Y=H



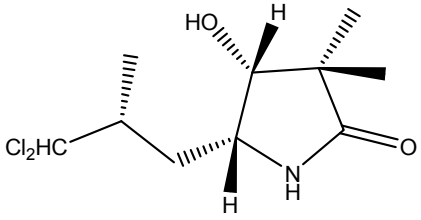
332.

Thus it is suggested that herbacic acid may be a prototype intermediate that arises shortly after biohalogenation of the pro-4S methyl group of L–leucine that leads to the precursor (2S,4S)-5,5,5-trichloroleucine before the steps leading to the more complex trichloromethyl natural products such as metabolites belonging to the

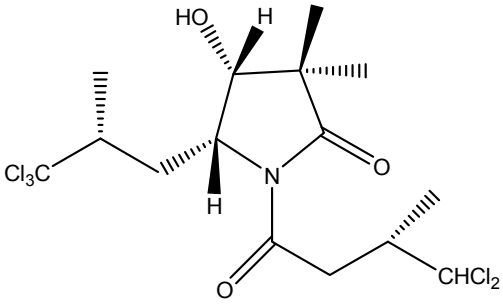
dysidenin family. Two other hexachlorinated metabolites, dysideathiazole (**327**) and N-methyldysideathiazole (**328**) and a minor compound 10-dechloro-N-methyldysideathiazole (**329**) were isolated from a specimen from Pohnpei[184]. 10-dechlorodysideathiazole (**330**) was isolated as a minor metabolite of a Palauan specimen, together with the major metabolites 9,10-didechloro-N-methyldysideathiazole (**331**) and 10-dechloro-N-methyldysideathiazole (**329**). A second specimen from Palau contained dysideapyrrolidone (**332**), the absolute stereochemistry of which was determined by X-ray crystallography to be S at all chiral centres[184].



333. R = Cl
334. R = H



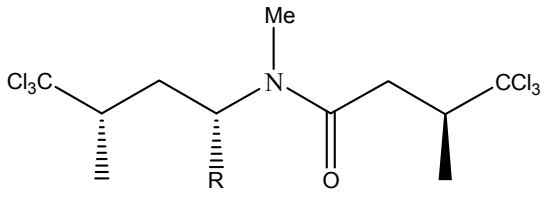
335.



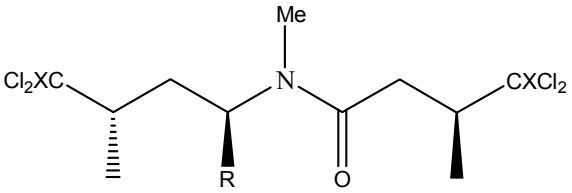
336.

A hexachloro metabolite, named dysidamide (**333**) was isolated from a Red Sea sponge specimen of *Dysidea herbacea* and its structure was confirmed by an X-ray analysis of the crystal structure[185]. Subsequently, specimens of *Dysidea herbacea*

collected off the coast of Ethiopia yielded an unusual and diverse array of metabolites which included two nitrogenous metabolites, dysidamides B (**334**) and C (**335**) and D (**336**)[186]. *Dysidea* sponge samples collected from the GBR have yeilded three new polychlorinated dipeptide methyl esters (**337-339**) and their related acids (**340-341**)[187].



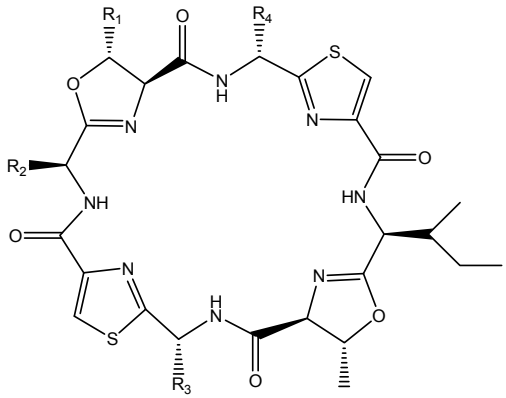
337. R=COOMe
340. R=COOH

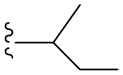


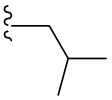
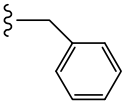
338. X=Cl, R=COOMe
339. X=H, R=COOMe
341. X=Cl, R=COOH

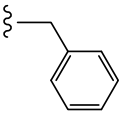
3.6 Literature review of cyclic peptides isolated from the ascidian *Lissoclinum patella*

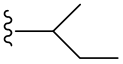
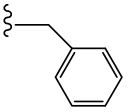
Colonial ascidians from the genus *Lissoclinum* are a prolific sources of biologically active metabolites which include cyclic peptides[188, 189], macrolides[190, 191], alkaloids[192, 193] and diterpenoids[194]. *Lissoclinum*-derived cyclic peptides characteristically contain thiazole and oxazoline moieties, and many of these peptides show cytotoxic activity. The cyclic peptides isolated from *Lissoclinum patella* are classified according to the following nomenclature – the patellamides, the patellins, the lissoclinamides , the ulithiacyclamides and the tawicyclamides.

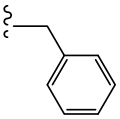


342. R_1=H, R_2=  , R_3=R_4= 

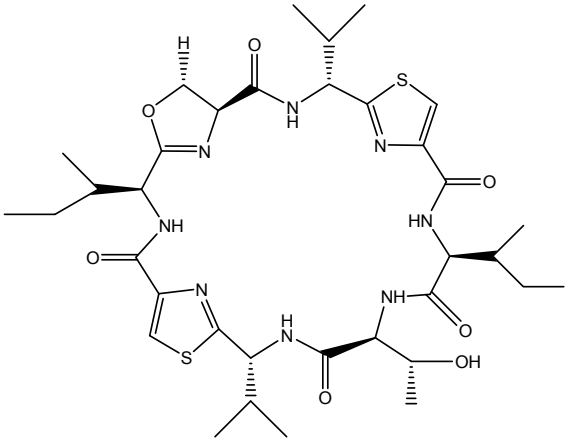
343. R_1=R_4=Me, R_2=  , R_3= 

344. R_1=R_4=Me, R_2=  , R_3= 

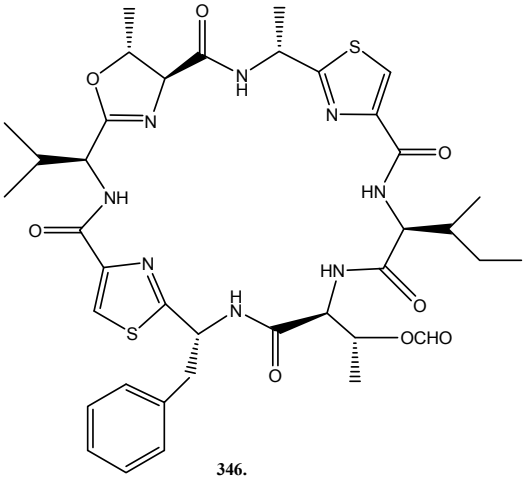
347. R_1=R_4=Me, R2=  , R_3= 

348. R_1=Me, R_2=  , R_3= 

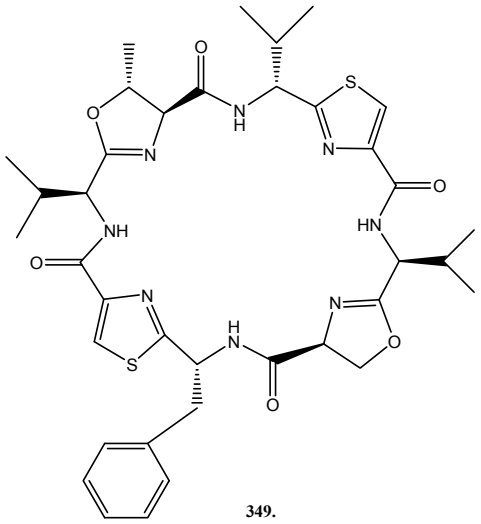
R_4= 



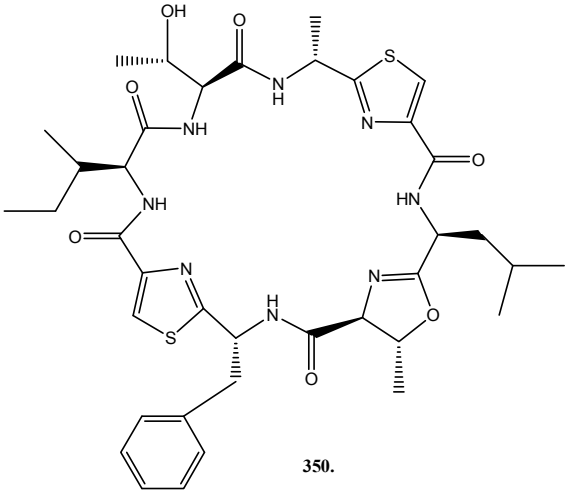
345.



346.

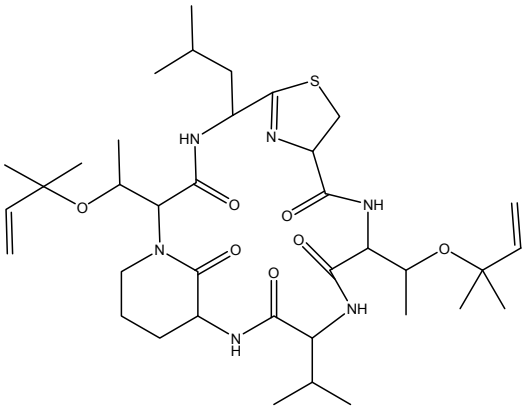


349.

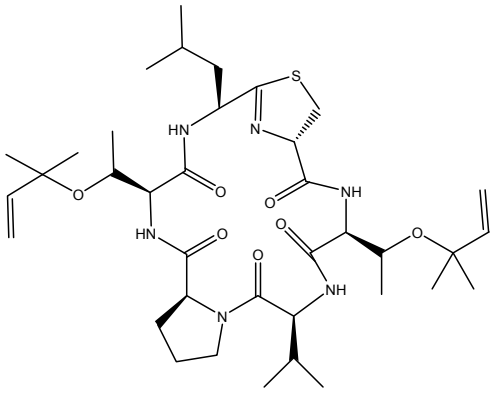


350.

Patellamides A (**342**), B (**343**) and C (**344**) were reported by Ireland et al in 1982 and they were described as cyclic octapeptides with thiazole and oxazoline rings representing linkage points for amino acids[195]. The structures **342-344** exhibited IC_{50} values of 2-4 μg/ml towards L1210 murine leukemia cells[195]. The original structures have subsequently been revised and it is known that the thiazole and oxazoline rings in all the patellamides are separated by a single amino acid residue[196]; diagrams presented here are the revised structures. Prepatellamide A (**345**)[197] and prepatellamide B formate (**346**)[198] have since been reported. Patellamide D (**347**), which was found to reverse multidrug resistance (MDR) of CEM/VLB100 human leukemic cell line was isolated from a GBR collection of the ascidian [199, 200]. Patellamide E (**348**) was isolated from a collection at Pulau Salu, Singapore along with known patellamides A and B. It was found to be weakly cytotoxic with an IC_{50} value of 125 μg/ml against human colon tumour cells *in vitro*[188]. A collection from Western Australia yielded a new patellamide, patellamide F (**349**) along with the known patellamide B (**343**)[189]. Patellamide G (**348**) was isolated from a Micronesian sample of the ascidian. Acid hydrolysis of the peptide yielded the same amino acid equivalents as Patellamide B (**343**) (L-threonine- two equivalents, L-leucine, L-Isoleucine, D-alanine, and D-phenylalanine), however, its molecular formula differed from that of patellamide B by eighteen mass units. This suggested that one of the threonine units in patellamide G (**350**) was not cyclised to an oxazoline ring and the structural features of a threonine, a substitued oxazoline, alanine, phenylalanine, and two thiazole rings were identified from spectroscopic data[201]. Patellins 1-6 (**351-356**) were isolated from a Fijian sample of *Lissoclinum patella*[202]. Patellins 1and 2 are cyclic hexapeptides, while patellins 3-6 are cyclic octapeptides.



351.



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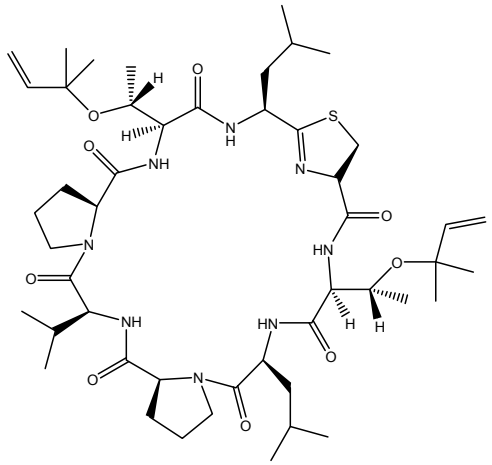

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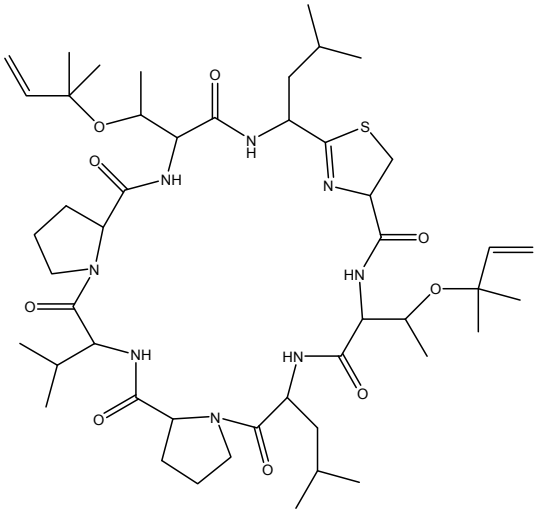

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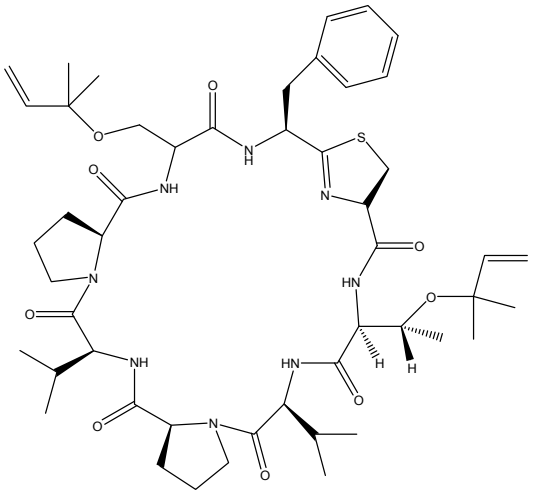

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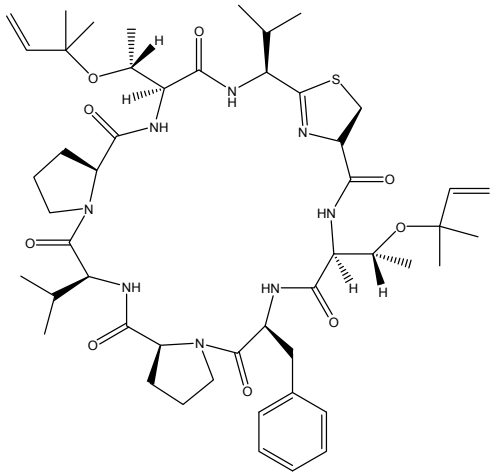
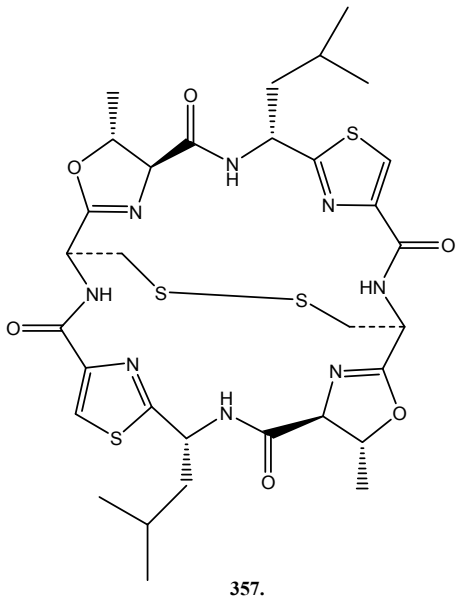
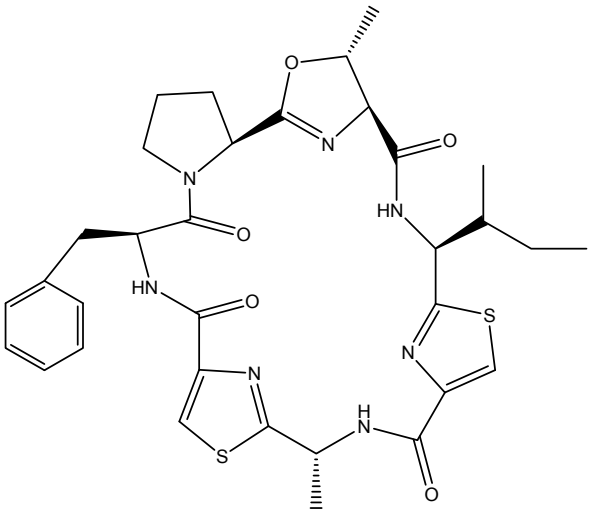


356.

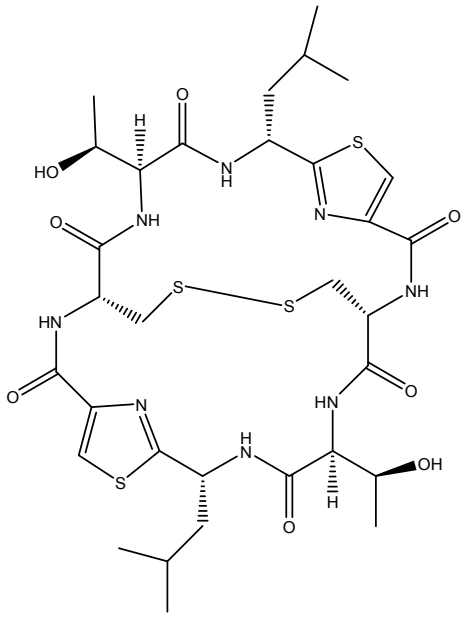
Ulithiacyclamide (**357**) and ulicyclamide (**358**) were the first cyclic peptides to be isolated from *Lissoclinum patella*[203]. Subsequently Preulitiacyclamide (**359**)[204] and preulicyclamide (**360**)[198] were isolated from *Lissoclinum patella* samples collected in the Pacific. Ulicyclamide (**358**) is an octapeptide which features nitrogen-containing heterocycles. Ulithiacyclamide (**357**) on the other hand is a symmetric octapeptide with a cysteine S-S linkage bridging the peptide ring. Ascidiacyclamide (**361**) is the only other known symmetric cyclic peptide to be isolated from *Lissoclinum patella*[205]. The synthesis was subsequently reported[206], and the absolute stereochemistry determined by X-ray crystallography[207]. Note that the stereochemistry of the leucine moieties is incorrectly depicted in the latter paper. Of the known *Lissoclinum* cyclic peptides, ulithiacylamide (**357**) is the most potent cytotoxic compound. It has IC_{50} values of 0.35 and .01μg/ml for murine leukemia L1210 and human CEM cell lines respectively.



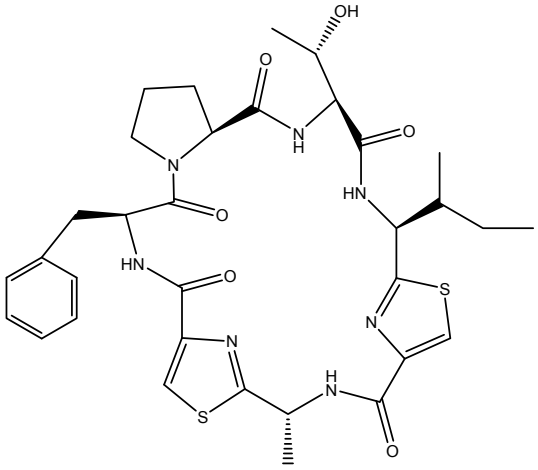
357.



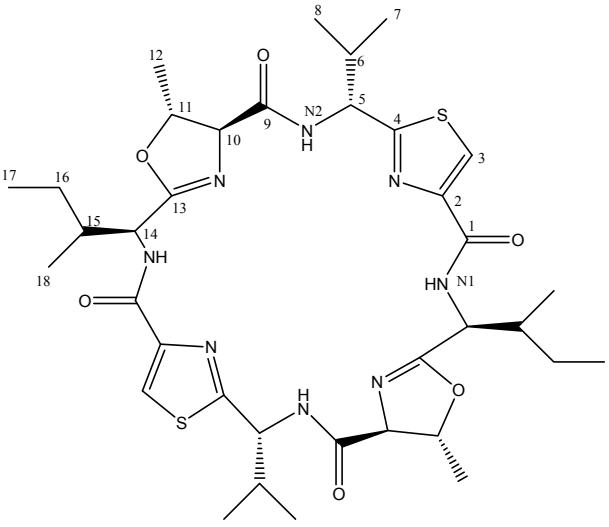
358.



359.

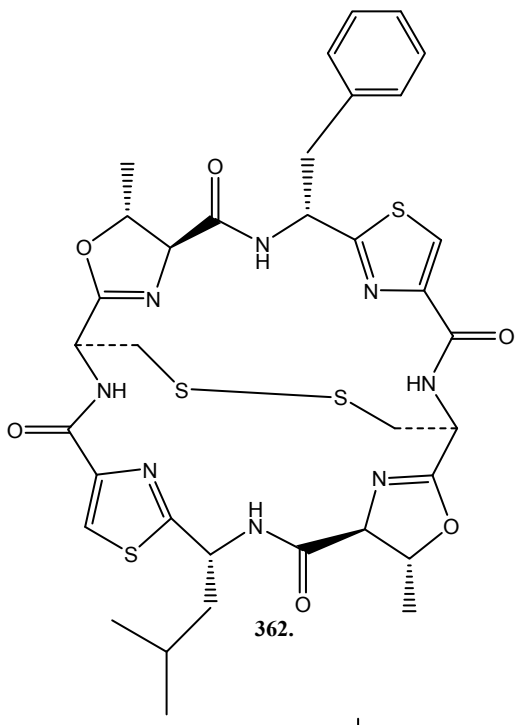


360.

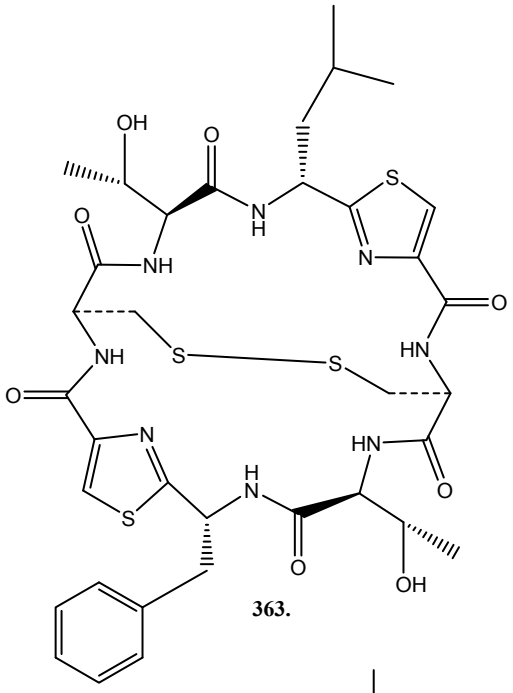


361.

Patellamides A (**342**), B (**343**) and C (**344**) are approximately 10 times less cytotoxic having IC_{50} values of 2-4 μg/ml for L1210 cells[199]. Ulithiacyclamide B (**362)** was isolated along with patellamides A (**342**) , B (**343**) and C (**344**)[208]. Ulithiacyclamide B (**362**) is closely related to ulithiacyclamide (**357**) except that the molecule lacks the symmetry associated with ulithiacyclamide as it contains a phenylalanine unit. Ulithiacylamide B (**362**) showed an IC_{50} value of 17 μg/ml against KB cells[208].



362.



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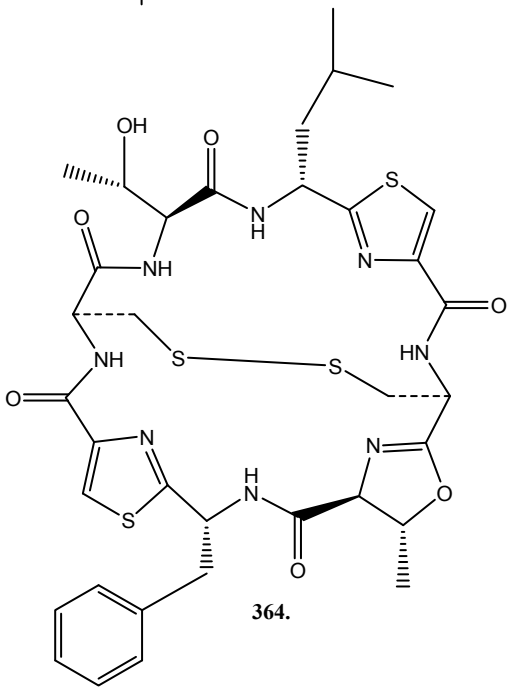

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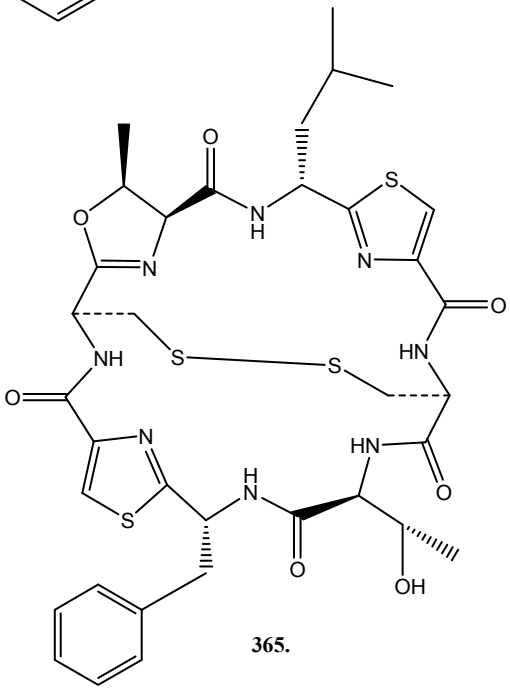
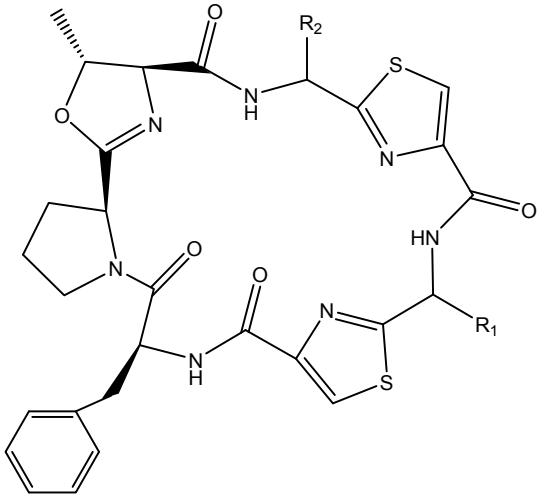
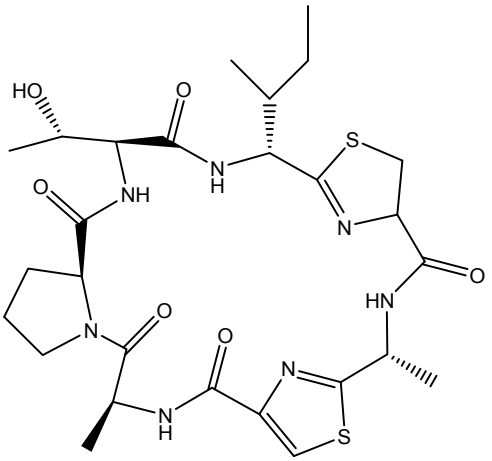


365.

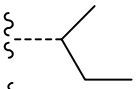
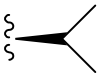
Related structures are ulithiacyclamides E-G (**363-365**); Ulithiacyclamide E (**363**) is very similar to ulithiacyclamide B (**362**) except for the fact that two oxazoline rings are replaced by free threonine residues[130]. Ulithiacyclamide F (**364**) and G (**365**) are isomers that contain one oxazoline and one free threonine residue, and are hydrated

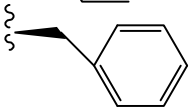
forms of ulithiacyclamide E (**363**). Thus the oxazoline ring in ulithiacyclamide F (**354**) is located between C-18 and C-23[130]. A total of 11 lissoclinamides which are cyclic heptapeptides have been reported to date. Extraction of *Lissoclinum patella* collected from the Caroline islands yielded the lissoclinamides 1-3 (**366-368**)[209]. Prelissoclinamide-2 (**369**) was subsequently isolated from a sample of the ascidian collected in the Pacific[198]. Lissoclinamides 4 (**370**), 5 (**371**) and 6 (**372**) were isolated along with patellamide D (**347**)[210]. Lissoclinamides 4 (**370**) and 5 (**371**) have similar structures except that lissoclinamide 4 (**370**) contains a thiazoline instead of one of the two thiazole rings found in lissoclinamide 5 (**371**). Lissoclinamides 7 (**373**) and 8 (**374**) have the same structural features as lissoclinamides 4 (**370**) and 6 (**372**) and thus contain one oxazoline ring, one proline, one valine, one phenylalanine and one alanine residue in addition to thiazole and/or thiazoline rings. All four peptides have the same sequence of amino acids around the ring and differ from one another only in their stereochemistry or the number of thiazole and thiazoline rings[211]. Lissoclinamides 9 (**375**) and 10 (**376**) were isolated from the Indo-Pacific samples of the ascidian. MS and CD competition studies show that lissoclinamide 10 (**376**) shows selectivity for Cu^{2+} in the presence of an excess of Zn^{2+} whereas lissoclinamide 9 (**375**) is less selective for copper ions[212]. Two new cyclic octapeptides tawicyclamides A (**377**) and B (**378**) have been isolated from *Lissoclinum patella* collected in the Philippines. Structures (**377**) and (**378**) possess a thiazoline ring instead of the characteristic oxazoline rings seen in the other cyclic peptides isolated from *Lissoclinum patella* and thus represent a new class of cyclic peptides from this ascidian[213].

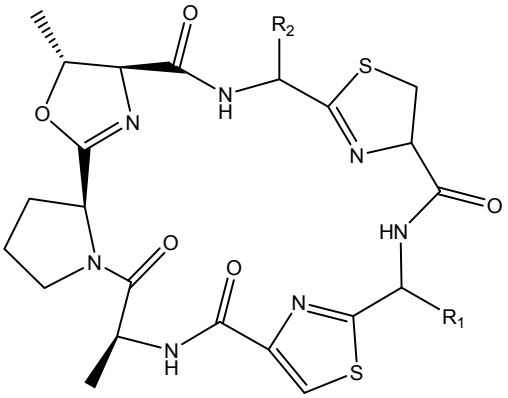


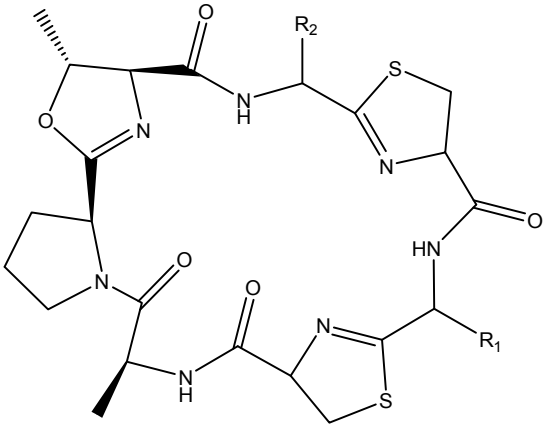


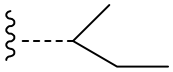
369.

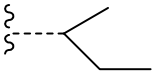
366. R_1=  , R_2= 

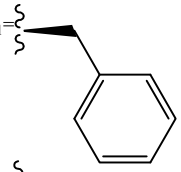
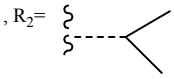
371. R_1=  , R_2= 

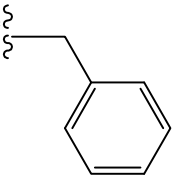
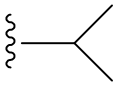


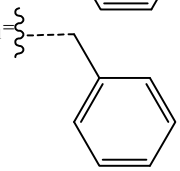
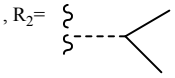


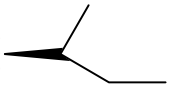
367. R_1=  , R_2= 

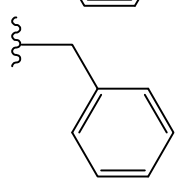
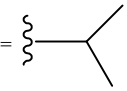
368. R_1=  , R_2= 

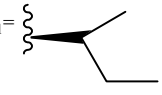
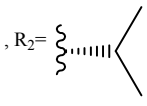
370. R_1=  , R_2= 

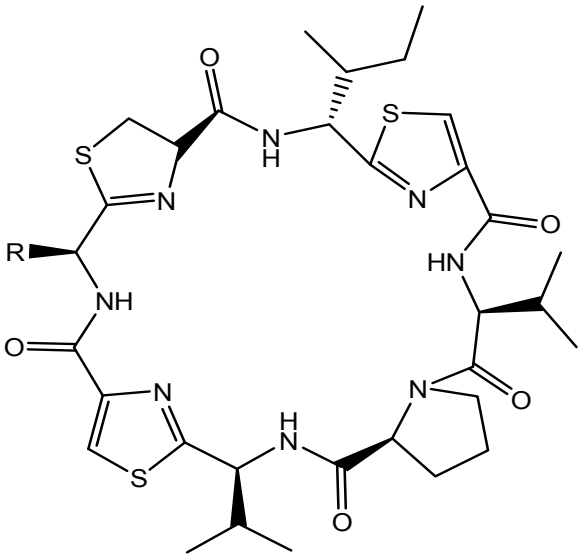
373. R_1=  , R_2= 

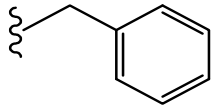
372. R_1=  , R_2= 

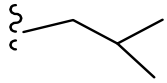
376. R_1=  , R_2= 

374 R_1=  , R_2= 

375. R_1=  , R_2= 



377. R= 

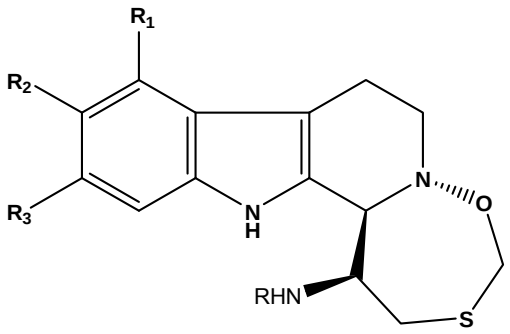
378. R= 

3.7 Literature review of β–carbolines, dihydro-β-carbolines and tetrahydro-β-carbolines reported from ascidians

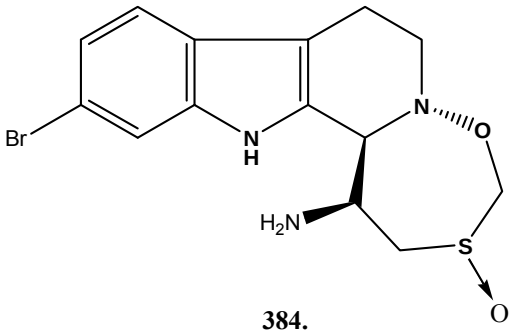
The eudistomin-eudistomidins are a group of ß-carbolines, dihydro-β-carbolines and tetrahydro-β-carbolines which often are phenolic, brominated or contain both functionalities on the aromatic ring. The simplest ones are C-1 unsubstituted but may also for example contain a pyrrolyl substituent at C-1, a C-1 pyrrolinyl group and a C-1 phenyl acetyl group. Other C-1 substituted β carbolines have also been recently isolated and contain other amino acid or carbohydrate derived units at the C-1 position. This general class of metabolites has been isolated from tunicate genera which include *Eudistoma*, *Ritterella*, *Pseudodistoma* and *Lissoclinum*.

Eudistomin alkaloids were first isolated from the Caribbean tunicate *Eudistoma olivaceum* and generated interest due to their antiviral activity[214, 215]. Subsequent biological evaluation revealed that they exhibit a range of biological activity including antibacterial[216], antifungal[216, 217] and antitumor activity[218, 219]. Hence eudistomins have been a focus of synthetic effort by a number of research groups. The highest levels of antiviral activity are expressed by those metabolites that have a 1,3,7-oxathiazepine ring. These include eudistomins C (**379**), E (**380**), F (**381**), K (**382**), L (**383**), K-sulfoxide (**384**) and debromoeudistomin K(**385**)[220]. Of these E (**380**) and K (**381**) show most promising biological activity. Eudistomin E (**380**) showed potent activity towards the human nasopharyngeal carcinoma KB cell line at an ED_{50} value of less that 5.0 ng/ml[221]. Eudistomin K (**382**) exhibited activity against

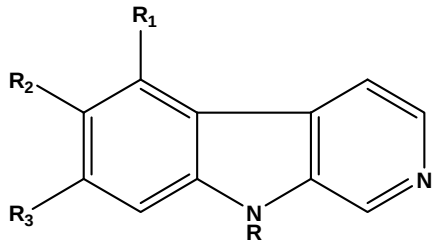
a range of human tumor cell lines and an IC_{50} value of .01μg/ml against P-388 cells[220]. In addition, eudistomin C (**379**) isolated from the Okinawan tunicate *Eudistoma glaucus* showed activity against murine leukemia L1210 at an IC_{50} value of 0.36μg/ml[218].



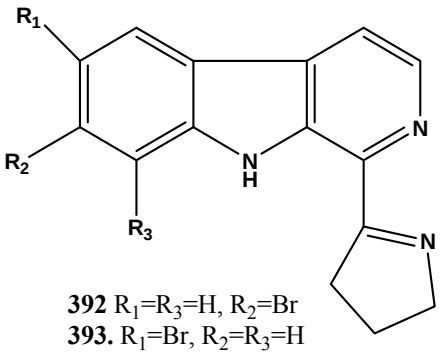
379. R=R_1=H, R_2=OH, R_3=Br
380. R=R_3=H, R_1=Br, R_2=OH
381. R=$COCH_3$, R_1=H, R_2=OH, R_3=Br
382. R=R_1=R_2=H, R_3=Br
383. R=R_1=R_3=H, R_2=Br
385. R=R_1=R_2=R_3=H



384.

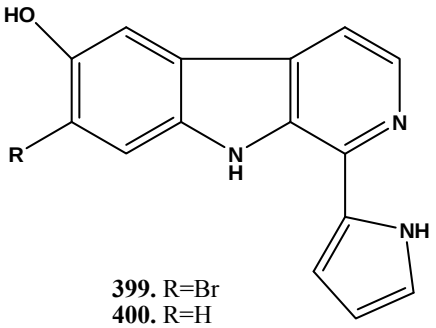


386. R= R_3= H, R_1= Br, R_2= OH
387. R= R_1= H, R_2 = OH, R_3= Br
388. R= R_1= R_3= H, R_2= Br
389. R= R_1= R_2= H, R_3= Br
390. R=CH_3, R_1=Br, R_2=OH, R_3=H
391. R=R_1=R_2=R_3=H

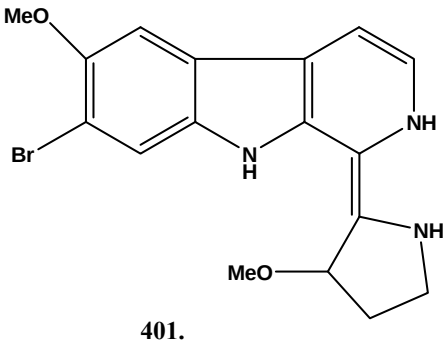


392 R_1=R_3=H, R_2=Br
393. R_1=Br, R_2=R_3=H
394. R_1=R_2=R_3=H
395. R_1=OH, R_2=Br, R_3=H
396 R_1=OH, R_2=R_3=H
397. R_1=R_2=Br, R_3=H
398. R_1=Br, R_2=H, R_3=OH

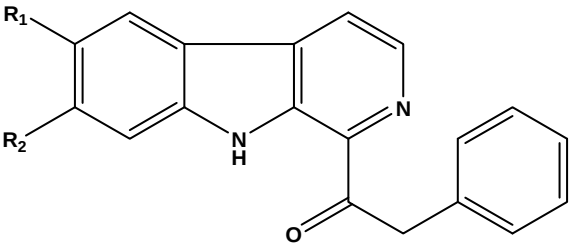
The simplest of the β-carbolines are those that are unsubstitued at the C-1 position. These include eudistomidin D (**386**) and eudistomins J (**387**), N (**388**), and O (**389**) which were isolated from a Caribbean sample of *E. olivaceu*[222]. Eudistomin O (**389**) was also isolated from the New Zealand ascidian *Riterella sigillinoides* along with β-carboline[220]. Subsequently, a specimen of *E. glaucus* from Okinawa afforded the N-methylated analogue of eudistomidin D (**390**)[218]. Eudistomin O (**389**) and β-carboline (**391**) showed moderate activity towards Herpes simplex Type 1 and polio vaccine Type 1 viruses. Eudistomidin D (**386**) is moderately active towards murine leukemia L1210 cells at an IC_{50} value of 2.4 μg/ml[223]. Eudistomins G (**392**), H (**393**), I (**394**), P (**395**) and Q (**396**) are examples of β-carbolines with a C1 pyrrolinyl group that were isolated from *E. olivaceum*[214]. Eudistomins H (**393**) and I (**394**) have subsequently been isolated from the Australian ascidian *Pseudodistoma aureum* along with Eudistomin V (**397**)[224]. The Okinawan tunicate *E. glaucus* afforded the metabolite eudistomidin A (**398**). Related structures eudistomins A (**399**) and M (**400**) which feature a pyrrole group at the C-1 position of the β-carboline skeleton have been reported[214, 222] along with eudistomin B (**401**), although the structure of **401** was not fully resolved[225]. Eudistomins R (**402**), S (**403**) and T (**404**) contain a 2-phenylacetyl residue attached to C-1 of the β-carboline[226, 227]. Structurally related eudistomidin B (**405**) was isolated from the Okinawan tunicate *E. glaucus*. It showed moderate activity towards the leukemia L1210 cell line at an IC_{50} value of 3.4 μg/ml[218].



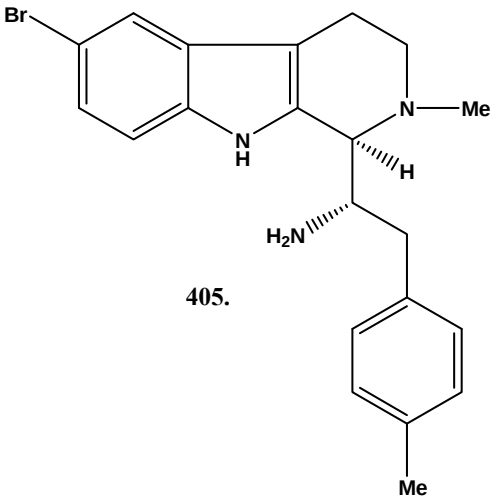
399. R=Br
400. R=H



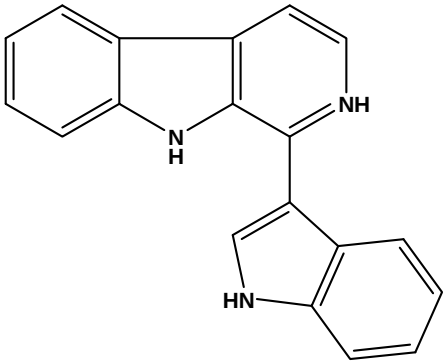
401.



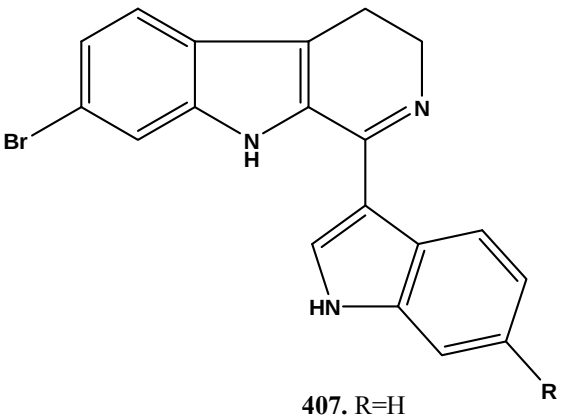
402. R_1=H, R_2=Br
403. R_1=Br, R_2=H
404. R_1=H, R_2=H



405.

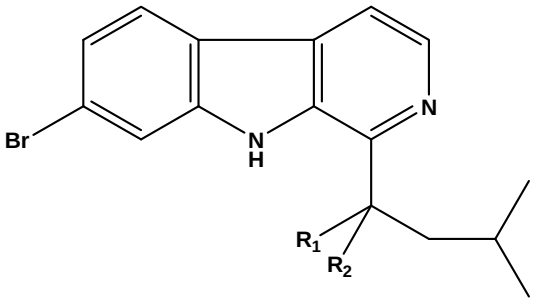


406.

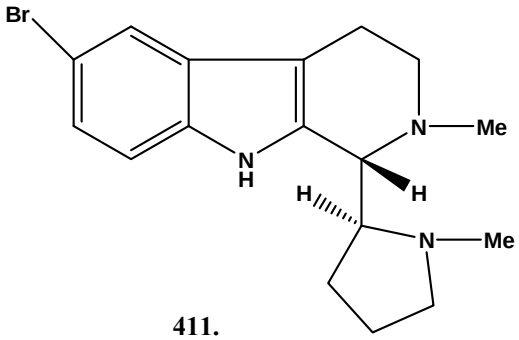


407. R=H
408. R=Br

Eudistomin U (**406**) is an example of a β-carboline which has other substituents at C-1. Two related compounds isoeudistomin U (**407**) and bromoisoeudistomin U (**408**) have been isolated from an Australian *Eudistoma sp*[228]. Isoeudistomin U (**407**) was initially reported as a 4-substituted dihydro-α-carboline[229] but its structure was revised after total synthesis to be 3,4-dihydroeudistomin U, and is the only example of a dihydro-β-carboline[230]. Other examples of β-carbolines which have other substituents at C-1 include eudistalbins A (**409**) and B(**410**) from the New Caledonian ascidian *Eudistoma album*[221].



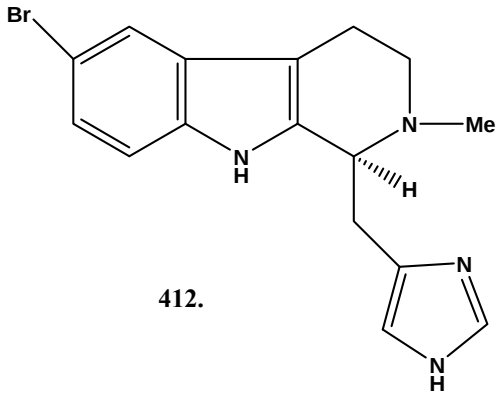
409. R_1=H, R_2= NH_2
410. R_1, R_2=O



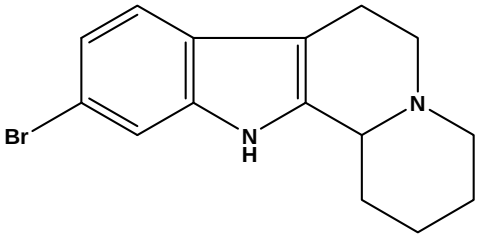
411.

Tetrahydro- β-carbolines with various substituents at C-1 have been reported. They include woodenine (**411**), from the New Caledonian ascidian *Eudistoma fragum*[231] and lissoclin C (**412**) from a *Lissoclinum sp.*[106]. Arborescidines A-D (**413-416**) are

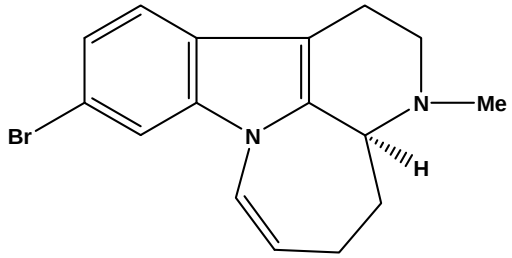
examples of tetrahydro-β-carbolines which are brominated at C-7 that have been reported from the tunicate *Pseudodistoma arborescens*[232].



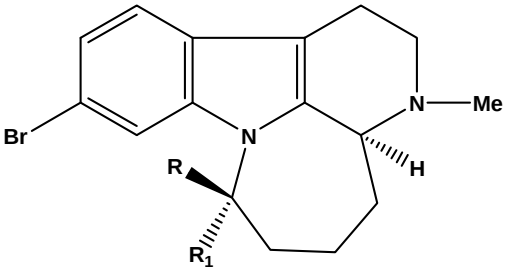
412.



413.



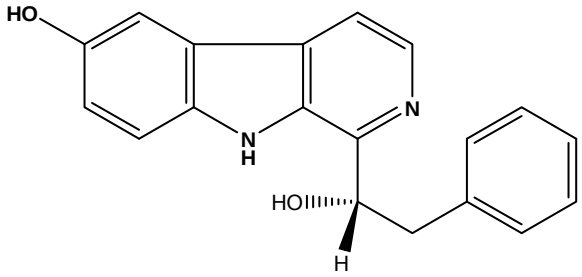
414.



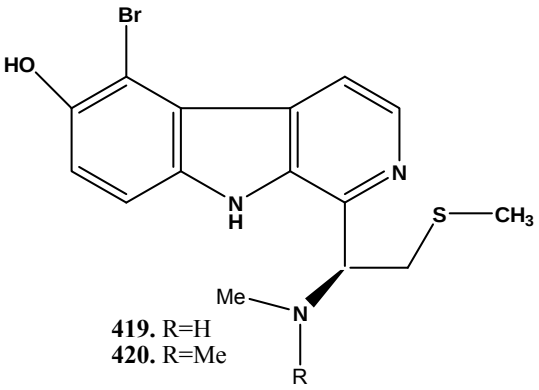
415. R=H, R_1=OH
416. R=OH, R_1=H

Eudistomins W (**417**) and X (**418**) were isolated from a Micronesian specimen of *Eudistoma sp.* Eudistomin W is a hydroxyl phenyl ethyl derivative and eudistomin X is an N, N-dimethylated phenyl ethyl derivative. Eudistomin X was found to have antibacterial and anti-fungicidal properties. It showed activity against *E.coli*, *B. subtilis, S. aureus* and *C. albicans* at loading doses of 5 and 10 μg per disk. On the other hand eudistomin W was selectively active towards *C. albicans* giving a zone of inhibition of 13 mm at 10 μg[216]. The N, N dimethyl function at C-10 in eudistomin X

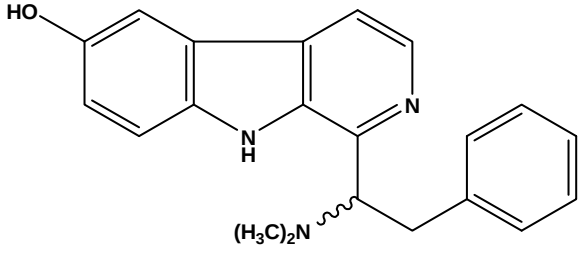
has already been observed in eudistomidin C (**419**)[218] and in 14-methyl eudistomin C (**420**), but the latter carries a methylthio substituent at C-11 instead of the phenyl group present in eudistomin X. 14-Methyl eudistomidin C (**420**) was isolated along with 2-methyleudistomin D (**421**) and 2-methyleudistomin J (**422**) and the known β-carbolines eudistomins C (**379**), E (**380**), J (**387**), K (**385**) and L (**383**) from *Eudistoma gilboverde*. 14-Methyl eudistomidin C (**420**) showed the most potent cytotoxic activity with an IC_{50} value of less than 1.0 μg/ml against four different human tumour cell lines[219].



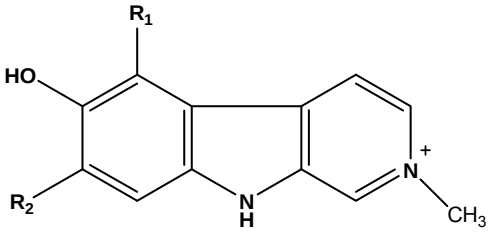
417.



419. R=H
420. R=Me



418.



421. R_1=Br, R_2=H
422. R_1=H, R_2=Br

CHAPTER 4.

Results, Discussion and Experimental –

(Known metabolites isolated from different genera)

4.1 Results and discussion of metabolites isolated from *Leucetta* sp.

4.2.1 Isonaamidine E (225)

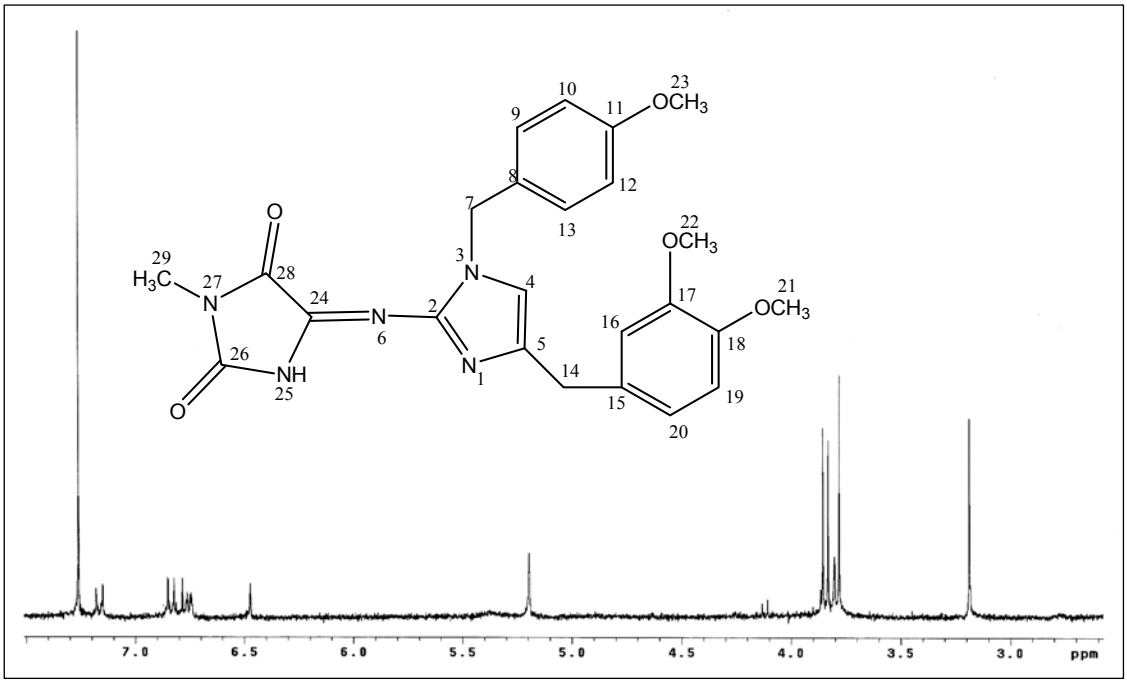


Fig 30. ^{1}H NMR spectrum of Isonaamidine E (225) in $CDCl_3$

The ^{1}H NMR spectrum (see Fig 30) of (**225**) contained three singlets at δ 3.78, 3.84 and 3.85 attributed to three methoxy groups. In addition a singlet resonating at δ 3.19 was indicative of an N-CH_3 group. The presence of two aromatic rings was also suggested in the ^{1}H NMR spectrum. The ^{1}H NMR shifts and *J* values were consistent with a 1,3,4-substituted and 1,4 substituted ring system (see table 7). An olefinic proton at δ 6.49 was also indicated in the ^{1}H NMR spectrum. Comparison with published spectral data revealed that compound (**225**) was isonaamidine E[133].

4.1.2 Bis(isonaamidinato E)zinc (423)

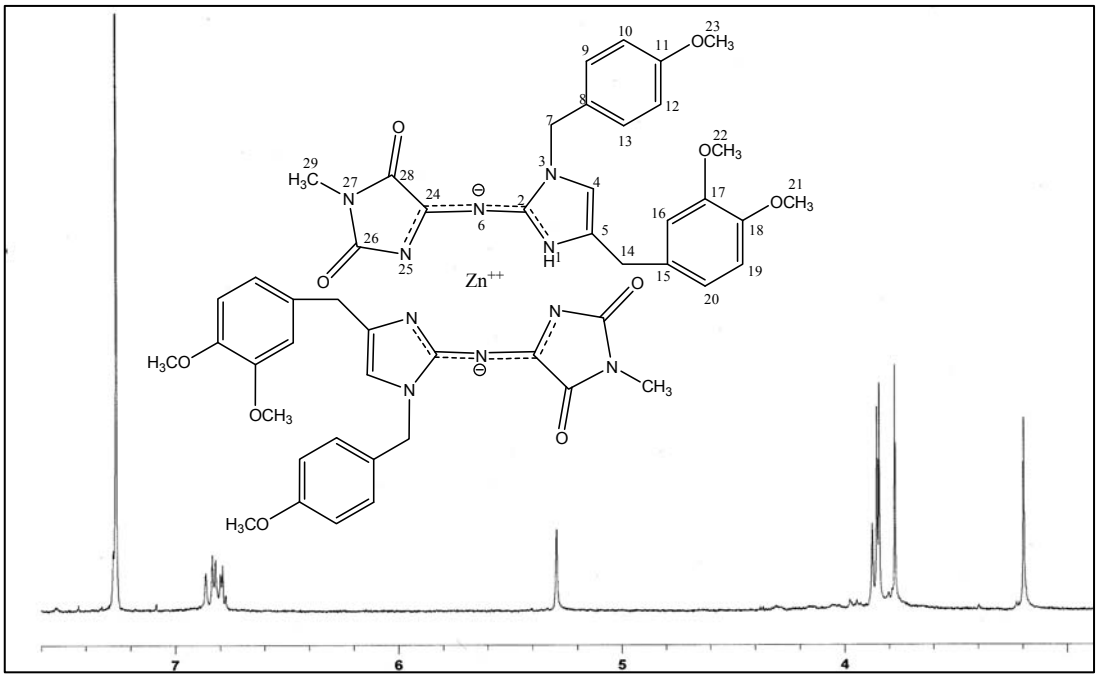


Fig 31. ^{1}H NMR spectrum of Bis(isonaamidinato E)zinc (423) in $CDCl_3$

The ^{1}H NMR spectrum of compound **423** (see Fig 31) was extremely similar to that of isonaamidine E (**225**) except that there was a downfield shift for the olefinic singlet at δ 6.49 (for **225**) to δ 6.77 for compound **423**. In addition there was a downfield shift for the aromatic signal at δ 7.17 (for **225**) to δ 7.27 (for **423**). Addition of $ZnCl_2$ (half equivalent) to compound **225** revealed a ^{1}H NMR spectrum identical to that of compound **423**. This indicated that compound **423** was a zinc complex of compound **225**. Zinc complexes have been reported from *Leucetta* sponges for a few isonaamidines[130, 138], however this is the first time the zinc complex of isonaamidine E has been isolated.

Isonaamidine E (255)[133]			Bis(isonaamidinato E)zinc (423)			
C #	δ^1H, mult., J (Hz)	δ^{13}C	C #	δ^1H, mult., J (Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C
2		144.0	2		143.7	
4		140.0	4		138.8	H-14
5	6.49, brs	116.1	5	6.77, brs	115.3	H-7
7	5.30, brs	48.6	7	5.31, brs	46.7	
8		128.4	8		127.3	H-7, H-12
9	6.84, d, 8.4	129.7	9	7.27, d , 8.3	129.5	
10		114.4	10		113.7	
11		159.7	11		159.0	H-9, H-10, H_3-21
12	6.84, d, 8.4	114.4	12	6.84. m	114.0	
13	7.17, d, 8.3	129.7	13	7.27, d, 8.3	129.5	H-7
14	3.81, brs	34.4	14	3.9, brs	32.0	
15		131.4	15		130.4	H-14
16	6.81, d, 2.0	112.2	16	6.86, m	111.7	H-14
17		147.9	17		148.1	H-16
18		149.1	18	6.8, m	149.0	H-19, H_3-22
19	6.79, d, 8.3	111.5	19	6.82, m	111.0	
20	6.79, dd, 8.3, 2.0	120.9	20	6.82, m	120.3	H-19
21	3.78, s	55.5	21	3.77, s	55.4	
22	3.85, s	56.1	22	3.87, s	56.2	
23	3.84, s	56.1	23	3.85, s	56.1	
24		148.8	24		148.8	
26		155.2	26		153.0	H_3-29
28		159.7	28		160.6	H_3-29
29	3.19, s	24.9	29	3.19, s	25.0	

Table 7. ^{1}H NMR and ^{13}C NMR data for Isonaamidine E (255) and Bis(isonaamidinato E)zinc (423) in $CDCl_3$

4.2 Results and discussion of metabolites isolated from *Axinella* sp.

4.2.1 Axisonitrile-3 (233)

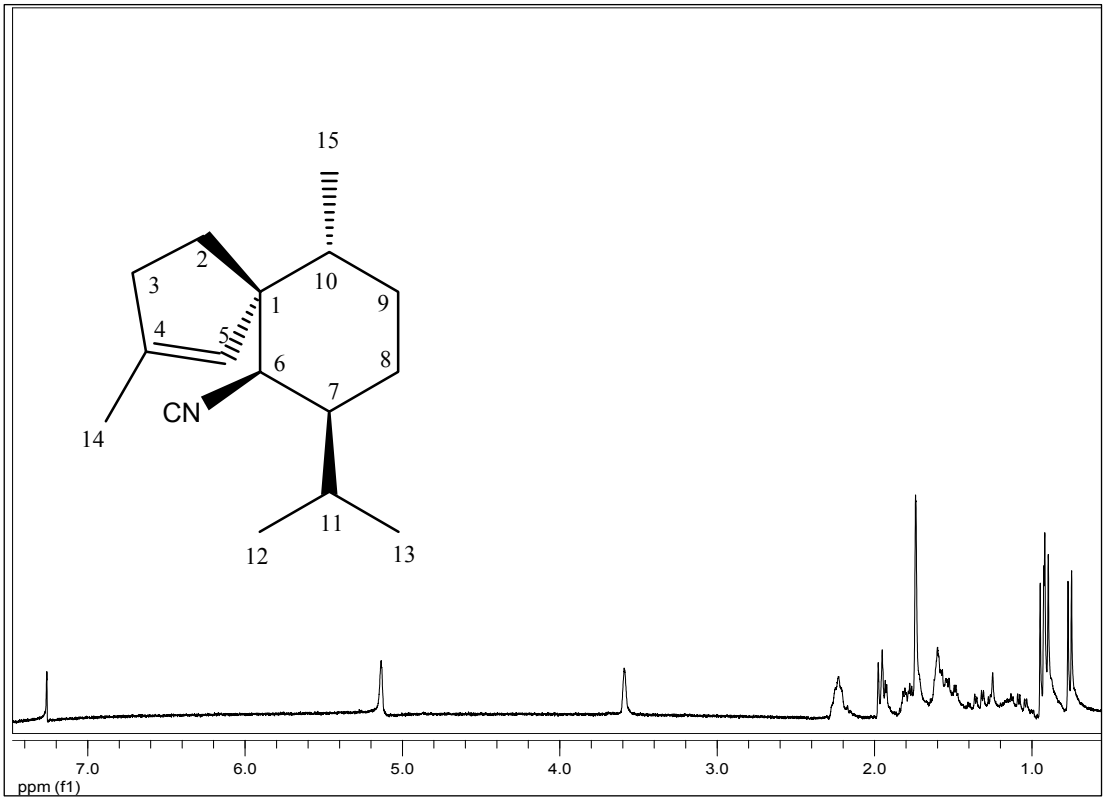


Fig 32. ^{1}H NMR spectrum of Axisonitrile-3 (233) in $CDCl_3$

A sample of the sponge *Axinella* sp. was extracted with DCM and further chromatographed on a silica gel column to yield two sesquiterpenes; Axisonitrile-3 (**233**) and Axisothiocyanate-3 (**234**). The ^{1}H NMR spectrum of compound **233** (see Fig 32) showed the presence of four methyl signals. Out of these, two at δ 0.92 and 0.95 belonged to an isopropyl group. Another methyl signal was also a doublet, at δ 0.76. The more downfield methyl signal was at δ 1.74 so was located on a double bond. In addition, a broad signal, at δ 3.59 indicated the presence of a methine

proton attached to an isonitrile group (which resonated at δ 155.7 in the ^{13}C NMR spectrum). Another broad signal at δ 5.13 indicated the presence of an unsaturation. This was reinforced by the ^{13}C NMR spectrum (see Fig 33) which indicated the presence of two olefinic carbons at δ 145.0 (C-4) and 123.7 (C-5). Other ^{13}C signals accounted for a further 13 carbons. The HMBC and HMQC data (see Table 8) enabled deduction of the structure. Particularly important HMBC correlations were from δ 1.74 (H_3-14) to δ 123.7 (C-5), δ 145.0 (C-4), δ 36.0 (C-3); δ 0.76 (H_3 -15) to δ 34.6 (C-10), δ 31.4 (C-9) and δ 57.2 (C-1), and from δ 0.94 (H_3-13) to δ 29.9 (C-11), and δ 43.9 (C-7) and δ 20.5 (C-12) which enabled assignment of the methyl groups and the skeleton. In addition COSY correlations from δ 1.91 (H-10) to δ 1.56 (H_2-9) and from δ 1.56 (H_2-9) to δ 1.81 (H_2-8) enabled assignment of the protons to C-10, C-9 and C-8 respectively. This was also confirmed by the HMQC experiment. For full assignments refer to table 8.

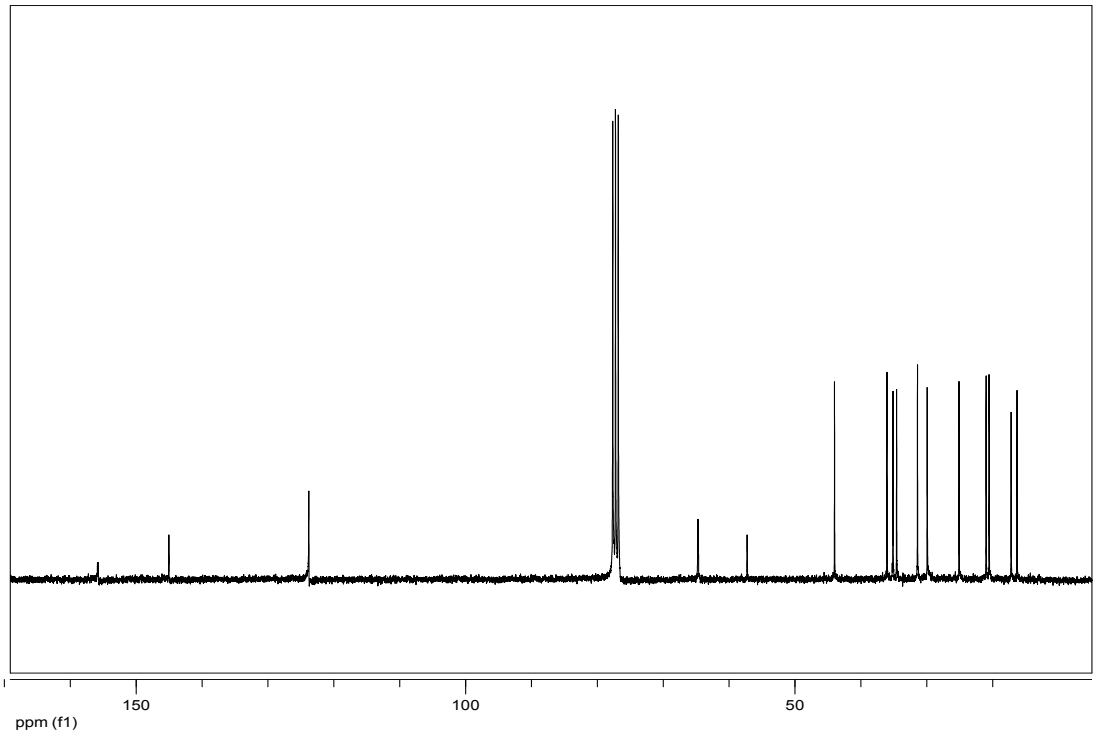


Fig 33. ^{13}C NMR spectrum of Axisonitrile-3 (233) in CDCl

4.2.2 Axisothiocyanate-3 (234)

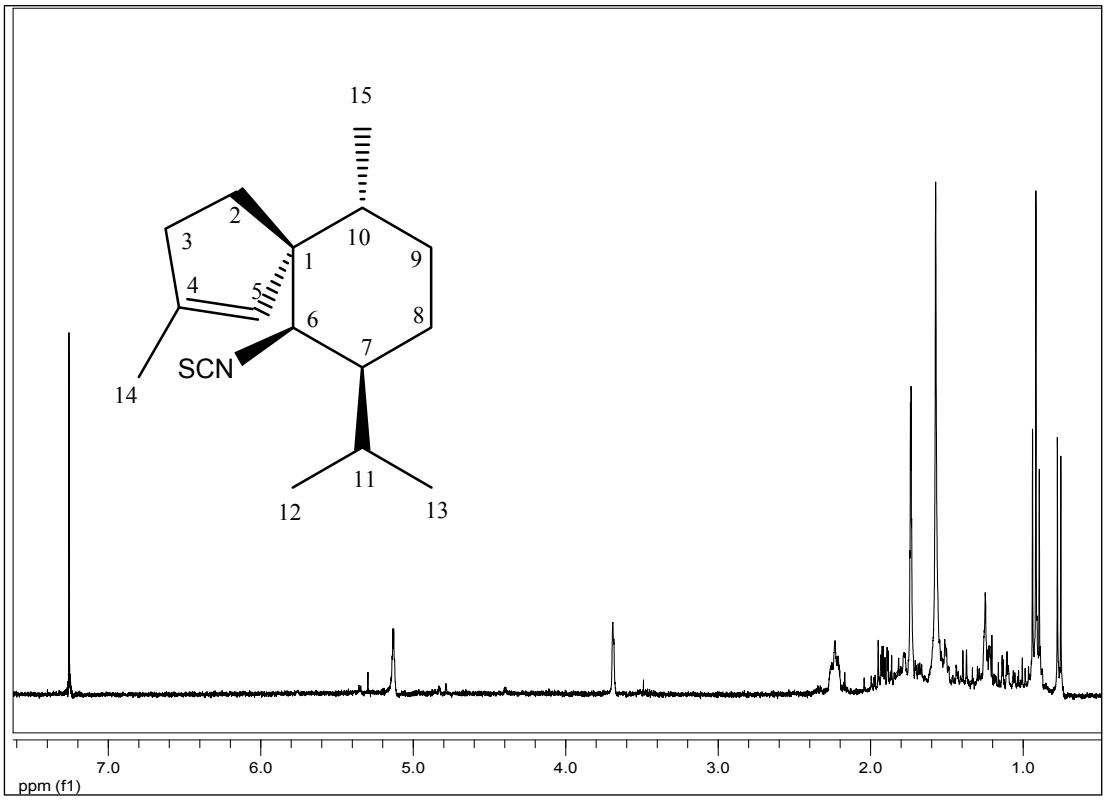


Fig 34. ^{1}H NMR spectrum of Axisothiocyanate-3 (234) in $CDCl_3$

The ^{1}H NMR spectrum of compound **234** (see Fig 34) was nearly identical to that of the isonitrile except there was a downfield shift of the methine singlet from δ 3.59 to δ 3.69. The ^{13}C NMR spectrum (see Fig 35) lacked the NC signal at δ 155.7. The ^{13}C NMR spectrum afforded signals for all the carbons except for the isothiocyanate carbon. These included four methyl signals at δ 20.6 (C-12), δ 21.0 (C-13), δ 17.2 (C-14) δ 16.3 (C-15). Signals for two olefinic carbons were seen at δ 145.1 (C-4) and δ 124.1 (C-5). In addition there were signals for four methylene groups at δ 35.2 (C-2), δ 36.1 (C-3), δ 25.6 (C-8) and δ 31.5 (C-9), four methines at δ 67.6 (C-6), δ 45.6 (C-7), δ 35.2 (C-10) and δ 30.3 (C-11) and a quaternary carbon at δ 59.1.

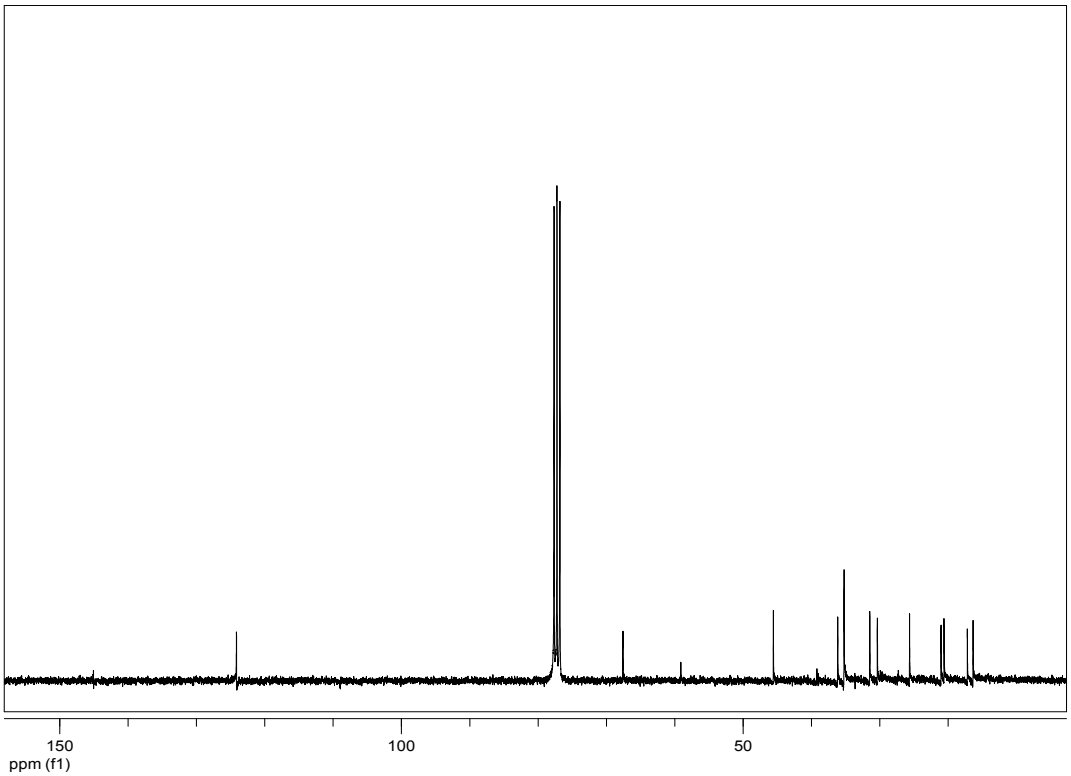


Fig 35. ^{13}C NMR spectrum of Axisothiocyanate-3 (234) in $CDCl_3$

The molecule was fully assigned with the help of HMQC, HMBC and COSY data (see Table 8). Comparison of the obtained data with published data for axisonitrile-3 and for axisothiocyanate-3[140, 233] confirmed the identification of the two metabolites.

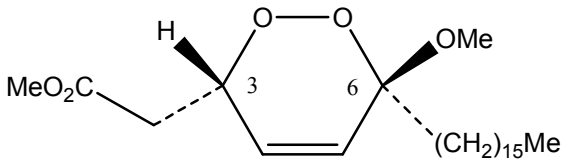
Axisonitrile-3 (233)				Axisothiocyanate-3 (234)			
C #	δ^{1}H, mult., J (Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C	C #	δ^{1}H, mult., J (Hz)	δ^{13}C	HMBC ^{1}H to ^{13}C
1		57.4	H-2, H_3-15	1		59.1	H_3-15
2	1.95, m	35.1		2	1.93, m	35.2	
3	2.25, m	36	H_3-14	3	2.26, m	36.1	H_3-14
4		145	H_3-14	4		145.1	H_3-14
5	5.13, brs	123.7	H_3-14	5	5.13, brs	124.1	H_3-14
6	3.59, brs	64.7	H-2	6	3.69. brs	67.6	
7	1.90, m	43.9	H_3-12, H_3-13	7	1.88, m	45.6	H_3-12, H_3-13
8	1.81, m	25.1		8	1.80, m	25.6	
9	1.56, m	31.4	H_3-15	9	2.14, m	31.5	H-10, H_3-15
10	1.91, m	34.5	H_3-15	10	1.95, m	35.2	H_3-15
11	2.23, m	29.9	H_3-12, H_3-13	11	2.21, m	30.3	H_3-12, H_3-13
12	0.91, d, 6.6	20.5	H_3-13	12	0.91, d, 6.3	20.6	H_3-13
13	0.94, d, 6.9	20.9	H_3-12	13	0.93, d, 6.6	21	H_3-12
14	1.74, s	17.2		14	1.74, s	17.2	
15	0.76, d, 6.9	16.3		15	0.76, d, 6.6	16.3	
NC		155.7		NCS		*	

* not observed

Table 8. ^{1}H NMR and ^{13}C NMR data of Axisonitrile-3 (233) and Axisothiocyanate-3 (234) in $CDCl_3$

4.3 Results and Discussion of metabolites isolated from *Plakortis lita*

4.3.1 Chondrillin (262)



The ^{1}H NMR data indicated the presence of two methoxy singlets at δ 3.35 and 3.68, a derivatised straight chain fatty acid moiety that was indicated by the presence of a broad singlet at δ1.23 and a three proton triplet at δ 0.84. In addition the signals at δ 6.17 and 5.86 were indicative of a double bond. The carbon spectrum revealed more information. Thus two methoxy carbons were assigned at δ 50.9 and 51.9. The ester carbonyl carbon resonated at δ 170.8 and the two other oxygenated carbons were at δ 73.7 and 78.8. Two sp^2 carbons were also indicated due to the presence of signals at 129.2 (C-4) and 126.4 (C-5). All these assignments were identical to published data for chondrillin (**262**)[156].

4.4 Results and discussion of metabolites isolated from *Hyrtios erecta*

4.4.1 Heteronemin (278)

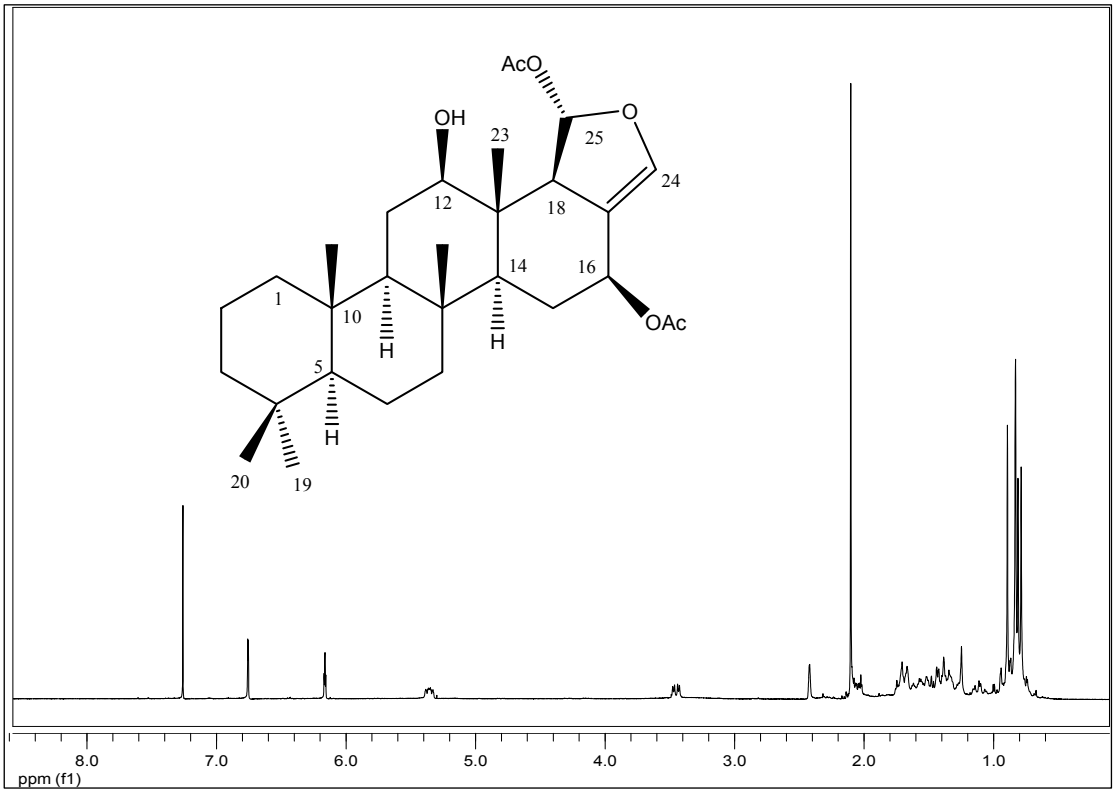


Fig 36. ^{1}H NMR spectrum of Heteronemin (278) in $CDCl_3$

The ^{1}H NMR spectrum (see Fig 36) indicated the presence of two acetate groups (δ 2.10, singlet, 6H), five tertiary methyl groups (δ 0.89, singlet, 3H; δ 0.83, singlet, 6H; δ 0.80 singlet, 6H), and a vinyl methine triplet at δ 6.17. The presence of one double bond was confirmed from the ^{13}C NMR spectrum (see Fig 37: signals at δ 135.3 (C-20) and 114.4 (C-17). The ^{13}C NMR indicated a scalarane framework due to the presence of signals at δ 56.5 (C-5), δ 58.8 (C-9), δ 54.7 (C-14), and δ 64.2 (C-18) as has been previously reported for other scalaranes[159]. In addition the presence of the two acetate groups was established by carbonyl signals at δ 171.3 and 170.0.

The ^{13}C NMR spectrum also indicted the presence of a doubly oxygenated carbon atom at δ 101.7 (C-19) and two oxygenated carbon atoms at δ 80.5 (C-12) and δ 69.3 (C-16). Comparison of the ^{1}H NMR and ^{13}C NMR data with published data enabled the compound to be identified as heteronemin[234].

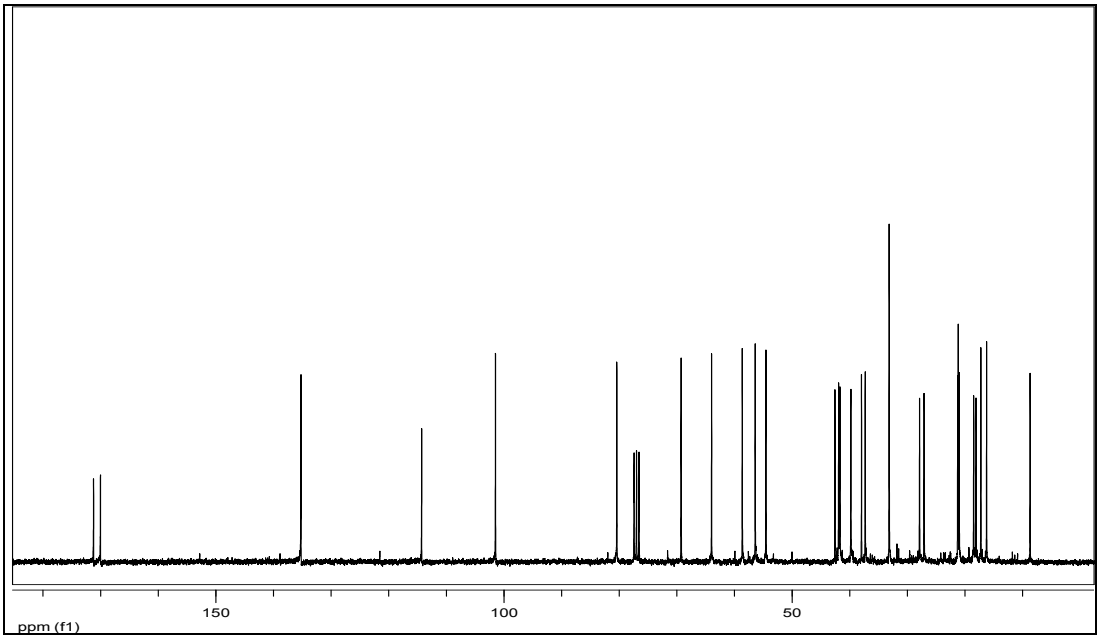
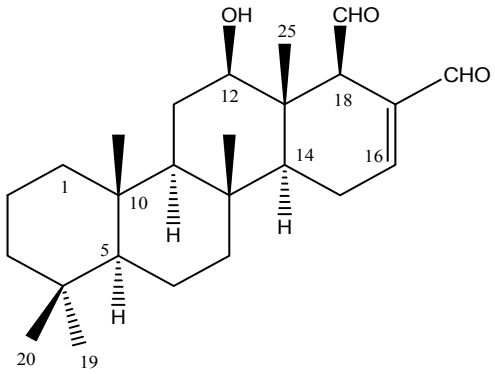


Fig 37. ^{13}C NMR spectrum of Heteronemin (278) in $CDCl_3$

4.4.2 Scalaradial (277)



The known compound scalaradial (**277**) was isolated from the same sponge sample and the confirmed by comparison of its NMR data with published spectral data[160].

4.5 Results and discussion of metabolites isolated from the sponge *Dysidea herbacea*

4.5.1 Dysideathiazole (327)

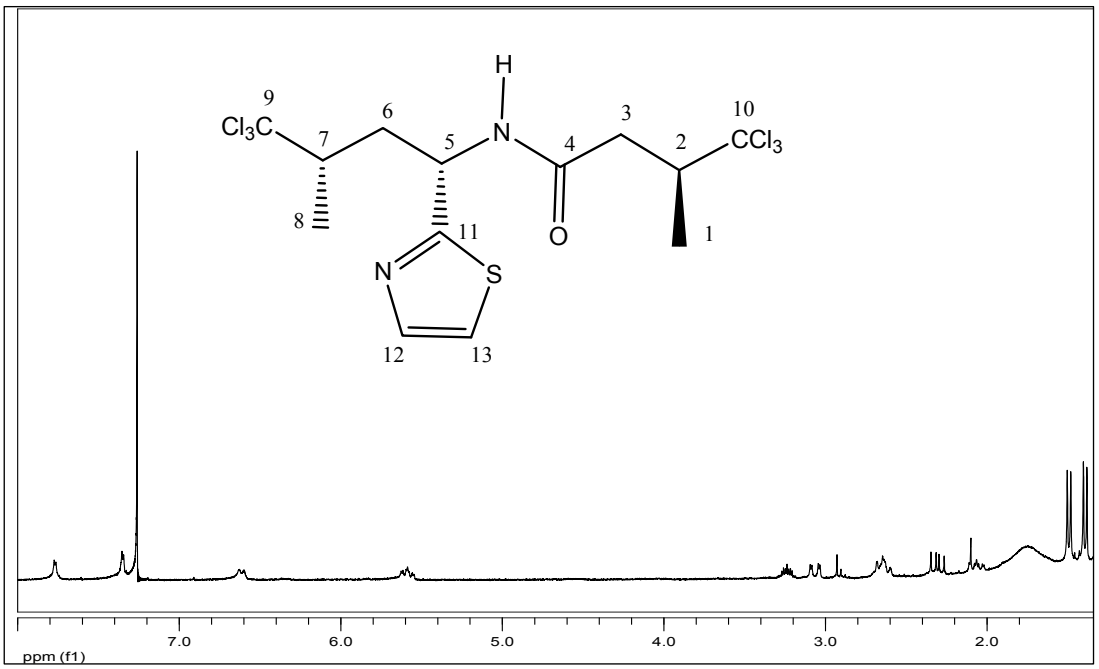


Fig 38. ^{1}H NMR spectrum of Dysideathiazole (327) in $CDCl_3$

The ^{1}H NMR spectrum of **327** (see Fig 38) showed two methyl doublets at δ 1.29 and 1.50. Two fine doublets at δ 7.74 and 7.34 suggested the presence of a thiazole ring. The ^{13}C NMR spectrum showed the amide carbonyl signal at δ 170.1, together with signals at δ 168.3, 142.3, and 119.9 which are characteristic of a 2-substituted thiazole ring. In addition signals at δ 104.8 and 105.5 were indicative of a

trichloromethyl group in an amino acid derivative. The metabolite was identified as dysideathiazole (**327**) by comparison of this data with published spectral values[184].

4.5.2 N-methyldysideathiazole (328)

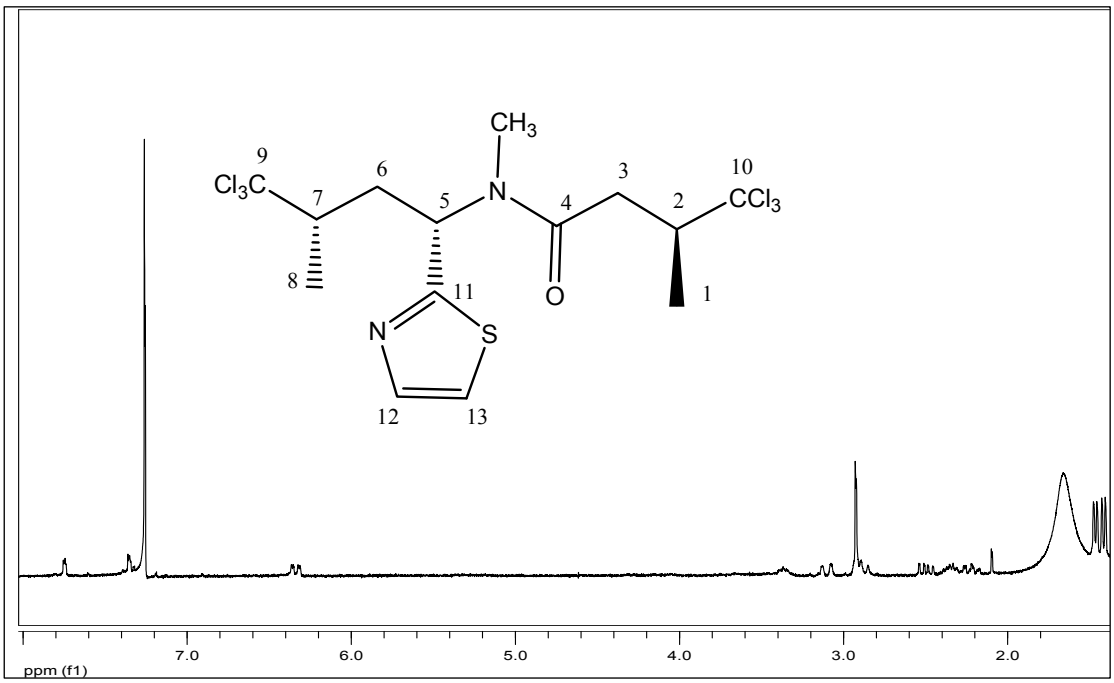


Fig 39. ^{1}H NMR spectrum of N-methyldysideathiazole (328) in $CDCl_3$

The ^{1}H NMR spectrum (see Fig 39) showed resonances that indicated the presence of three methyl groups. Two upfield doublets at δ 1.41 (H-1) and δ 1.44 (H-4) were assigned to two secondary methyl groups and a downfield signal at δ 2.93 was assigned to an N-methyl group. Two fine doublets at δ 7.74 (H-12, 3Hz) and δ 7.34 (H-13, 3Hz) suggested the presence of a thiazole ring. The ^{13}C NMR yielded signals for 14 carbons. These included three methyl signals at δ 17.2 (C-1), 16.3 (C-8) and 30.0 which was assigned to the N-Me, two methylene groups at δ 37.4 (C-3) and

33.6 (C-6), three methines at δ 51.4 (C-2), 52.0 (C-7) and 51.6 (C-5) and four quaternary carbons at δ 171.3 (C-4), 168.2 (C-11), 105.6 (C-9) and 105.1 (C-10). The signal at δ 168.2, together with signals at 142.3, and 119.9 were characteristic of a 2-substituted thiazole ring while the signals at δ 105.6 and 105.1 were characteristic of trichlorinated methyl groups. The signal at δ 171.3 was assigned to the amide carbon. The structure was confirmed as N-methyldysideathiazole (**328**) by comparison of this data with published spectral values[184].

4.6 Results and discussion of metabolites isolated from *Lissoclinum patella*

4.6.1 Ulithiacyclamide (357)

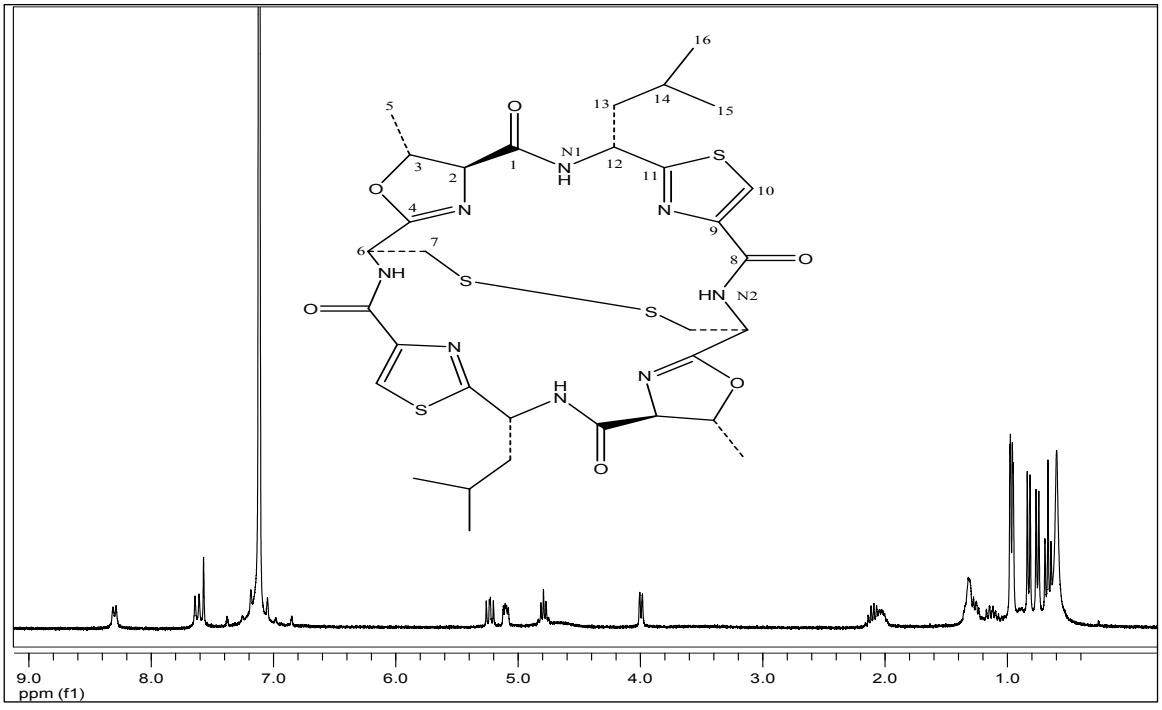


Fig 40. ^{1}H NMR spectrum of Ulithiacyclamide (357) in $CDCl_3$

The ^{1}H and ^{13}C NMR spectra exhibited signals for only four amino acid residues as it was symmetrical in nature. Two NH signals at δ 7.70 and 8.50 and two signals in the ^{13}C NMR spectrum at δ 170.5 (C-1) and δ 170.0 (C-10) indicated peptide linkages. The ^{1}H NMR spectrum (see Fig 40) indicated the presence of a methyl-substituted oxazoline ring due to signals at δ 4.05 and 4.71 and a large coupling constant of 8 Hz typical of a *trans* 1, 2 disubstituted oxazoline ring. In addition a thiazole proton singlet at δ 8.10 in the ^{1}H NMR spectrum and signals resonating at δ 170.5, 149.2 and 124.1 in the ^{13}C NMR spectrum were indicative of a thiazole ring. Signals for a leucine unit and a cystine unit were also observed. These features were consistent with those for ulithiacyclamide (**357**), and the data matched literature values reported[203].

4.6.2 Ascidiacyclamide (361)

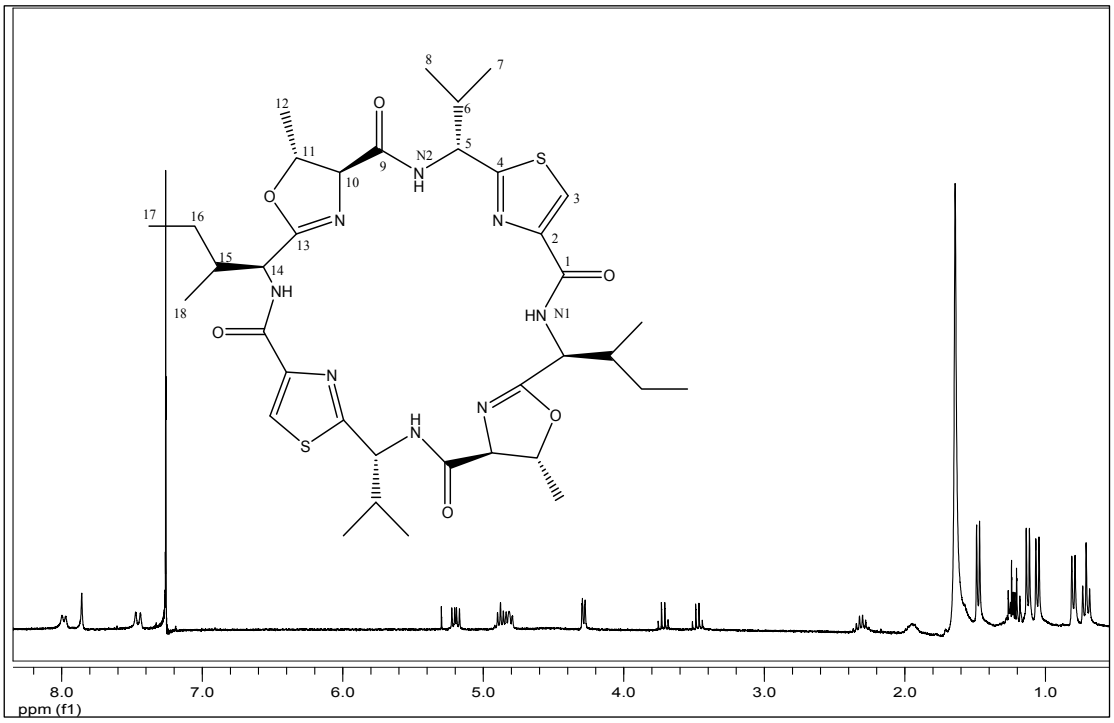


Fig 41. ^{1}H NMR spectrum of Ascidiacyclamide (361) in $CDCl_3$

A symmetrical cyclic peptide structure was indicated by the ^{1}H NMR (see Fig 41) and ^{13}C NMR spectral data for the isolated metabolite **361**. The ^{1}H NMR spectrum (see Fig 39) was similar to that of ulithiacyclamide (**357**), however no signals for a cysteine residue were observed. Thus the molecule had a methyl substituted oxazoline ring, an isoleucine unit (instead of a leucine), a thiazole ring and a valine unit (instead of a cysteine). ^{1}H NMR and ^{13}C NMR data were identical to literature values of ascidiacyclamide (**361**)[205].

4.7 Results and discussion of metabolites isolated from *Eudistoma gilboverde*

4.7.1 Eudistomin H (393)

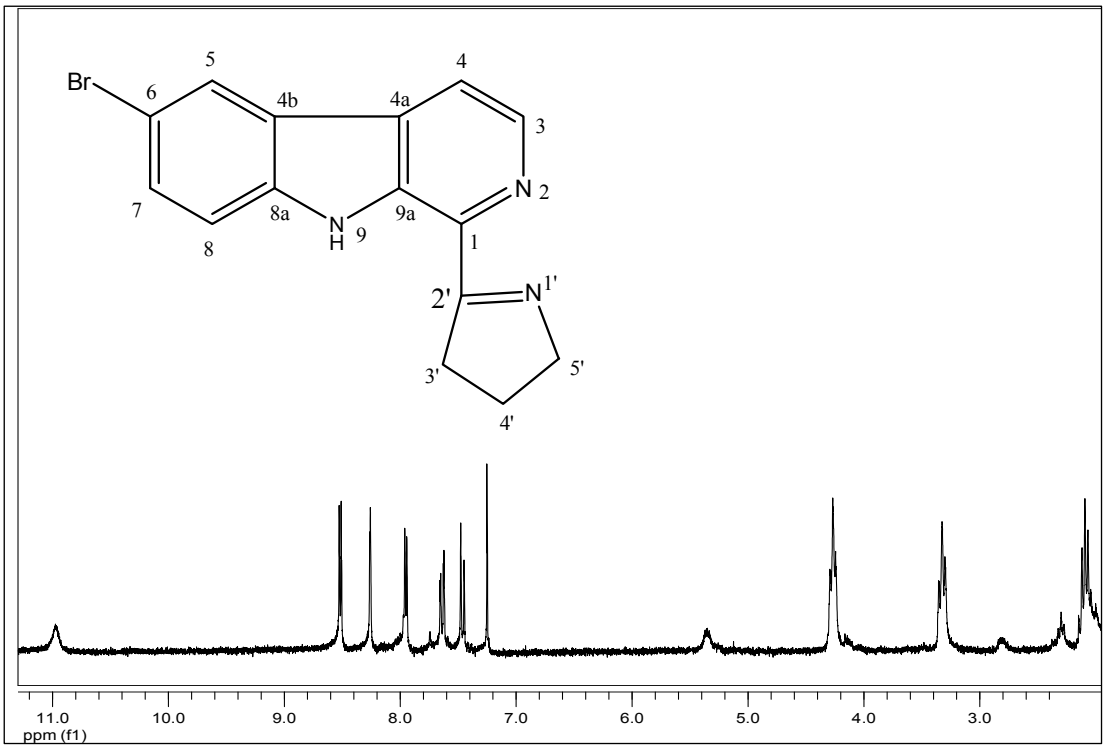


Fig 42. ^{1}H NMR spectrum of Eudistomin H (393) in $CDCl_3$

Eudistomin H (**393**) was isolated from the methanol extract of the tunicate *Eudistoma gilboverde*. The crude extract was subjected to chromatography on an alumina column to afford a yellow crystalline solid. Interpretation of the ^{1}H NMR data (see Fig 42) indicated the presence of a disubstituted β-carboline and a pyrroline group. The protons resonating at the aromatic region ie δ 7.48, 7.65, 7.96, 8.27 and 8.53 were indicative of a 1,6-disubstituted β-carboline moiety, whereas the three multiplets at δ 3.30, 2.09 and 4.23 were indicative of a 2'-substituted pyrroline moiety. The ^{13}C NMR spectrum (see Fig 43) of **393** displayed resonances for 15 carbons: 5 methines, 7 quarternary carbons and three methylene groups. Interpretation of the ^{1}H NMR, ^{13}C NMR, H-H COSY and HMQC spectra allowed the assignment of protonated methines. The quarternary carbons were unambiguously assigned by HMBC data (see Table 9). All three methylene proton resonances showed HMBC correlations to the imino carbon at δ 177.0 in the ^{13}C NMR spectrum.

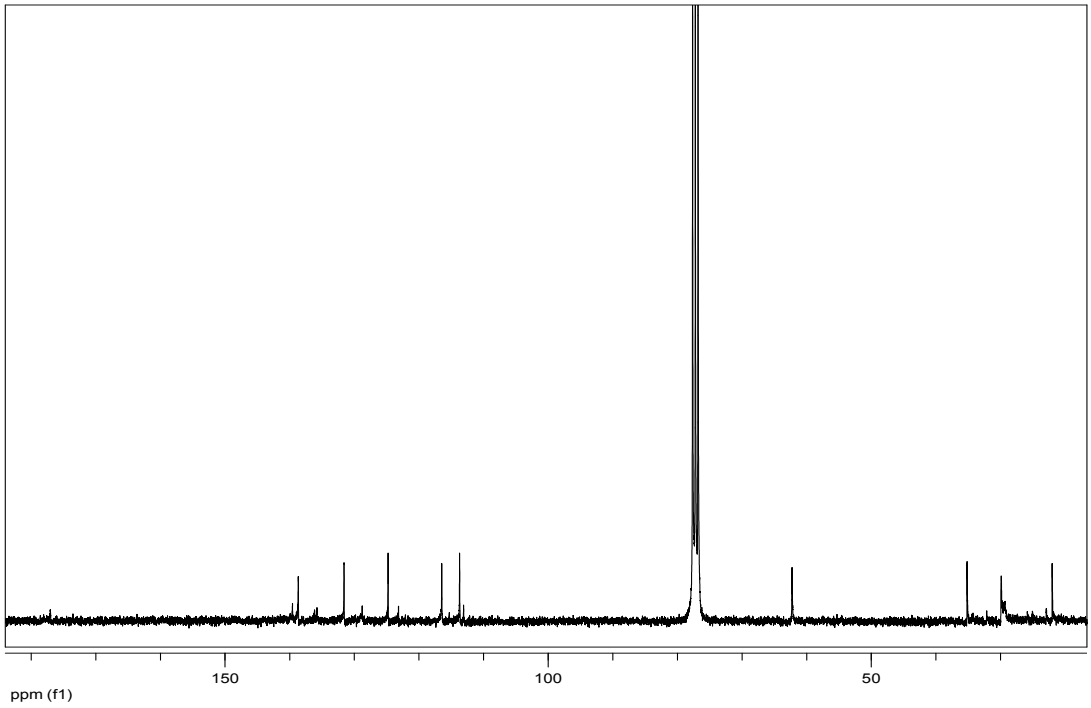


Fig 43. ^{13}C NMR spectrum of Eudistomin H (393) in $CDCl_3$

Hence the 2'substituted pyrroline ring was confirmed. The β-carboline moiety was assigned from HMBC correlations observed from five aromatic protons to the remaining 10 carbons. Thus, correlations from δ 7.99 to C-4_a and C-9_a; from δ 8.31 to C-8_a; from δ 7.52 to C-4_b and from δ 8.50 to C-1 enabled the assignment of all the quarternary carbons with the exception of the brominated carbon at C-6 (see Table 8), and the data was in agreement with published data for eudistomin H[214].

Eudistomin H (393)			
C #	δ^1H, mult., J (Hz)	δ^{13}C	HMBC 1H to ^{13}C
1		135.8	H-3
3	8.53, d, 5.1	138.7	
4	7.96, d, 5.1	116.5	H-3
4a		128.8	H-3, H-4
4b		123.2	H-8
5	8.27, d, 1.4	124.8	
6		113.0	
7	7.65, d, 8.7, 1.4	131.6	H-5, H-8
8	7.48, d, 8.7	113.7	
8a		139.6	H-5
9-NH	11.0, brs		
9a		136.1	H-4
2'		177.0	H-3', H-4', H-5'
3'	3.30, m	35.2	
4'	2.09, m	22.0	
5'	4.03, m	62.6	

Table 9. ^{1}H NMR and ^{13}C NMR data of Eudistomin H (393) in $CDCl_3$

4.8 Experimental

4.8.1 Isolation and bioassay guided fractionation- *Leucetta* sp.

4.8.1.1 Animal material and extraction

Animal material (12.3g, dry weight) of *Leucetta* sp. was collected from Shrimp reef (12 m) and stored at 4°C until extraction. The freeze-dried sponge was extracted four times with 400 mL of CH_2Cl_2 in a 500 mL conical flask and the four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 3.6 g of crude extract.

4.8.1.2 Bioassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane: CH_2Cl_2 (1:1) and applied to a glass column (i.d. 2.0 cm, length 9 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 10 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl

acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 10% methanol (MeOH) in EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator. Fractions 3 (1.7 mg) and 4 (2.3 mg) were identified as Isonaamine E (**225**) and Bis(isonaamidinato E)zinc (**423**) respectively.

4.8.1.3 Physical properties of purified metabolites isolated

^{1}H NMR spectrum ($CDCl_3$): Refer to Table 7.

^{13}C NMR spectrum ($CDCl_3$): Refer to Table 7.

4.8.2 Isolation and bioassay guided fractionation- *Axinella* sp.

4.8.2.1 Animal material and extraction

Animal material (13.8g, dry weight) of *Axinella* sp. was collected from Shrimp reef (5 m) and stored at 4°C until extraction. The freeze-dried sponge was extracted four times with 400 ml of CH_2Cl_2 in a 500 ml conical flask and the four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 4.4 g of crude extract.

4.8.2.2 Bioassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane: CH_2Cl_2 (1:1) and applied to a glass column (i.d. 2.0 cm, length 9 cm) that had been packed with

silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 10 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 10% methanol (MeOH) in EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator. Fractions 2 (4.7 mg) and 4 (5.3 mg) were purified and identified as axisonitrile-3 (**233**) and axisonthiocyanate-3 (**234**) respectively.

4.8.2.3 Physical properties of purified metabolites isolated

^{1}H NMR spectrum ($CDCl_3$): Refer to Table 8.

^{13}C NMR spectrum ($CDCl_3$): Refer to Table 8.

4.8.3 Isolation and bioassay guided fractionation- *Plakortis lita*

4.8.3.1 Animal material and extraction

The sponge *Plakortis lita* was collected by hand using scuba from Shrimp reef (5 m).The sample was frozen immediately after collection and kept frozen until used. The animal material (16.723 g, dry weight) was extracted four times with 400 mL of CH_2Cl_2 in a 500 ml conical flask. The four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 0.25 g of crude extract.

4.8.3.2 Bioassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 9 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 15 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 25% MeOH/ EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator. Fraction 4 (8.9mg) was purified and identified as chondrillin (**262**).

4.8.3.3 Physical properties of purified metabolites isolated

Chondrillin (262)

^{1}H NMR spectrum ($CDCl_3$): 6.17, dd, *J* 10.2, 4.2 Hz, H-4; 5.86, dd, *J* 10.2, 2.7 Hz, H-5; 4.78, m, H-3; 3.71, s, ester CH_3O; 3.38, s, ketal CH_3O; 2.91, dd, *J* 16.2, 8.1 Hz, H-2a; 2.60, dd, *J* 16.2, 5.4 Hz, H-2b; 1.62, m, H-7; 1.23, brs, H-8–21; 0.84, t, *J* 6.6 Hz, H_3-22.

^{13}C NMR spectrum ($CDCl_3$): 170.8, C-1; 129.2, C-4; 126.4, C-5; 78.8, C-6; 73.7, C-3; 51.9, ester CH_3O; 50.9, ketal CH_3O; 37.2, C-2; 34.2, C-7; 22.7-31.9, C-8–21; 14.1, C-22.

4.8.4 Isolation and bioassay guided fractionation- *Hyrtios erecta*

4.8.4.1 Animal material and extraction

The sponge *Hyrtios erecta* was collected by hand using scuba from Flinders reef (5 m). The sample was frozen immediately after collection and kept frozen until used. The animal material (21.4 g) was extracted four times with 400 mL of CH_2Cl_2 in a 500 mL conical flask. The four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 0.927g of crude extract**.**

4.8.4.2 Bioassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 12 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 25% MeOH/ EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator.

Fractions 6 (29.9 mg) and 7 (15.6 mg) were cytotoxic and further separated via chromatography. Fractions 6 and 7 were combined and applied to a glass column packed with alumina and eluted with 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 25% MeOH/ EtOAc (fractions 6.1-6.10). Fraction 6.3 (9.9 mg) was cytotoxic and identified as heteronemin (**278**). Fraction 6.7 (1.1 mg) was identified as scalaradial (**277**).

4.8.4.3 Physical properties of purified metabolites isolated

Scalaradial (277)

^{1}H NMR spectrum ($CDCl_3$): 9.53, d, *J* 3.5 Hz, H-19; 9.45, s, H-20; 7.10, m, H-16; 2.81, brs, H-18; 2.42, m, H-15; 1.20, m, H-12; 1.74, m, H-7; 1.69, m, H-1;; 1.56, m, H-2; 1.51, m, H-6; 1.34, m, H-11; 122, m, H-14; 0.80, m, H-9; 0.79, m H-5; 0.95, s, H_3-25; 0.91, s, H_3-24; 0.84, s, H_3-23; 0.84, s, H_3-21; 0.80, s, H-22; 2.1, s, CH_3CO.

Heteronemin (278)

^{1}H NMR spectrum ($CDCl_3$): 6.77, d, *J* 1.5 Hz, H-19; 6.17, t, *J* 3 Hz, H-20; 5.38, dd, *J* 10, 4 Hz, H-16; 3.46, dd, *J* 5.1, 4.6 Hz, H-12; 2.42, brs, H-18; 2.10, s, CH_3CO; 2.10, s, CH_3CO; 0.89, s, CH_3; 0.83, s, CH_3; 0.83, s CH_3; 0.80, s, CH_3; 0.80, s, CH_3.

^{13}C NMR spectrum ($CDCl_3$): 171.3, $COCH_3$; 170.0, $COCH_3$; 135.3, C-20; 114.4, C-17; 101.7, C-19; 80.5, C-12; 69.3, C-16; 64.2, C-18; 58.8, C-9; 56.5, C-5; 54.7, C-14; 42.7, C-13; 42.0, C-3; 41.8, C-7; 39.9, C-1; 38.1, C-10; 37.4, C-8; 33.3, C-21; 33.1, C-4; 28.0, C-15; 27.2, C-11; 21.4, CH_3CO; 21.3, CH_3CO; 21.1, C-22; 18.6, C-6; 18.2, C-2; 17.4, C-24; 16.4, C-23; 8.8, C-25.

4.8.5 Isolation and bioassay guided fractionation-*Dysidea herbacea*

4.8.5.1 Animal material and extraction.

The sponge *Dysidea herbacea* was collected by hand using scuba from Flinders reef (12.6 m). The sample was frozen immediately after collection and kept frozen until used. The freeze dried animal material (15.5 g) was extracted four times with 400 ml of CH_2Cl_2 in a 500 mL conical flask. The four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 0.691g of crude extract.

4.8.5.2 Bioassay guided fractionation

The cytotoxic fraction was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 12 cm) that had been packed with silica gel (60 H, thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, (fractions 1-9). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator.

Fraction 5 was cytotoxic and was further purified via HPLC on a Hewlett-Packard RP-18 column (7µm, i.d 9.5mm, length 250mm) eluted with 75% MeOH (aq) at a flow rate of 1.5 ml/min. The MeOH was removed from fractions that contained the metabolites dysideathiazole (retention time 4.97 min) and N-methyldysideathiazole (retention time 6.13 min) which were present in the ratio of 1:3 w.r.t. peak areas. Each fraction was transferred to a separating funnel and extracted with dichloromethane. Removal of the dichloromethane solvent afforded dysideathiazole (4.1 mg**, 327**) and N-methyldysideathiazole (3.0 mg, **328**).

4.8.5.3 Physical properties of purified metabolites isolated

Dysideathiazole (327):

^{1}H NMR spectrum ($CDCl_3$): 7.74, d, *J* 3 Hz, H-12; 7.34, d, *J* 3 Hz, H-13; 5.58, ddd, *J* 12, 9, 3 Hz, H-5; 3.25, dqd, *J* 9.5, 6.5, 3 Hz, H-2; 3.05, dd, *J* 15, 3 Hz, H-3a; 2.99, dd, *J* 15, 9.5 Hz, H-3b; 2.60, dd, *J* 14, 12 Hz, H-6a; 2.08, m, H-6b; 2.64, dq, *J* 10, 6.5 Hz, H-7; 1.49, d, *J* 6.5 Hz, H_3-8; 1.39, d, *J* 6.5 Hz, H_3-1; 6.39, brd, *J* 9 Hz, NH.

^{13}C NMR spectrum ($CDCl_3$): 170.1, C-4; 168.3, C-11; 142.3, C-12; 119.9, C-13; 105.5, C-9, 104.8, C-10; 52.1, C-7; 51.6, C-2; 48.9, C-5; 40.2, C-3; 39.9, C-6; 16.9, C-1; 16.6, C-8.

N-methyldysideathiazole (328):

^{1}H NMR spectrum ($CDCl_3$): 7.74, d, *J* 3.0 Hz, H-12; 7.34, d, *J* 3.0 Hz, H-13; 6.33, dd, *J* 12.0, 3.0 Hz, H-5; 3.35, dqd, *J* 9.0, 6.5, 3.0 Hz, H-2; 3.09, dd, *J* 16, 3 Hz, H-3a; 2.49, dd, *J* 16, 9 Hz, H-3b; 2.85, dd, *J* 13, 12 Hz, H-6a; 2.24, m, H-6b; 2.31, m, H-7; 1.44, d, *J* 6.5, H_3-8; 1.41, d, *J* 6.5, H_3-1; 2.93, s, N-CH_3.

^{13}C NMR spectrum ($CDCl_3$): 171.3, C-4; 168.2, C-11; 142.3, C-12; 119.9, C-13; 105.6, C-9; 105.1, C-10; 52.0, C-7; 51.6, C-5; 51.4, C-2; 37.4, C-3; 33.6, C-6; 17.2, C-1; 16.3, C-8; 30.0, N-CH_3.

4.8.6 Isolation and bioassay guided fractionation- *Lissoclinum patella*

4.8.6.1 Animal material and extraction

The sponge *Lissoclinum patella* was collected by hand using scuba from Arab reef (12 m). The sample was frozen immediately after collection and kept frozen until used. The frozen animal material (10.1 g) was extracted four times with 400 ml of MeOH in a 500 mL conical flask. The four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 2.7 g of crude extract.

4.8.6.2 Bioassay guided fractionation

The cytotoxic fraction (organic extract) was resuspended in a minimal amount of hexane and applied to a glass column (i.d. 1.5 cm, length 9 cm) that had been packed with alumina (thin layer chromatography; Merck) and solvated with hexane. The column was eluted under vacuum with 25 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2, 100% EtOAc, 25% MeOH/EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator.

Fraction 8 (6.9 mg), which was cytotoxic was further separated by chromatography through a glass column that had been packed with silica gel (60 H, thin layer chromatography; Merck). The column was eluted with 40 ml of diethyl ether and 10 fractions were collected i.e fractions 8.1-8.10. The column was finally washed with 10 ml of 100 % EtOAc to afford fraction 8.11. Fraction 8.6 (2.0 mg) and 8.11 (1.8 mg) were cytotoxic and identified as ascidiacyclamide (**351**) and ulithiacyclamide (**349**) respectively.

4.8.6.3 Physical properties of purified metabolites isolated

Ulithiacyclamide (357):

^{1}H NMR spectrum ($CDCl_3$): 7.72, s, H-10; 5.36, m, H-12; 5.24, m, H-6; 4.71, dq, *J* 8, 7 Hz, H-5; 4.05, dd, *J* 8, 2 Hz, H-2; 3.22, dd, *J* 14, 6 Hz, H-7a; 3.02, dd, *J* 14, 4 Hz, H-7b; 1.66, m, H-13; 1.35, m, H-14; 1.10, d, *J* 7 Hz, H_3-5; 0.90, d, *J* 7 Hz, H_3-15; 0.75, d, *J* 7 Hz, H_3-16; 8.50, d, *J* 9 Hz, NH-2; 7.70, d, *J* 9 Hz**,** NH-1**.**

^{13}C NMR spectrum ($CDCl_3$): 170.5, C-11; 170.0, C-1; 167.3, C-4; 160.1, C-8; 149.2, C-9; 124.1, C-10; 81.7, C-3; 74.1, C-2; 48.5, C-12; 48.4, C-6; 46.4, C-13; 46.5, C-8; 25.3, C-14; 22.8, C-16; 22.7, C-15; 22.1, C-5

Ascidiacyclamide (361):

^{1}H NMR spectrum ($CDCl_3$): 7.91, s, H-3; 5.22, dd, *J* 10.0, 6.3 Hz, H-5; 4.86, dq, *J* 6.3, 6.3 Hz, H-11; 4.83, ddd, *J* 8.1, 6.1, 1.2 Hz, H-14; 4.27, dd, *J* 6.3, 1.2 Hz, H-10;

2.31, m, H-6; 1.95, m, H-15; 1.27, m, H-16a; 1.17, m, H-16b; 1.49, d, *J* 6.3 Hz, H_3-13; 1.13, d, *J* 6.1 Hz, H_3-8; 1.07, d, *J* 6.1 Hz , H_3-7; 0.80, d, *J* 6.8 Hz, H_3-18; 0.72, dd, *J* 6.8, 6.8 Hz, H_3-17; 8.01, d, *J* 8.1 Hz, NH-1; 7.40, d, *J* 10.0 Hz, NH-2.

^{13}C NMR spectrum ($CDCl_3$): 171.2, C-1; 168.9, C-9; 168.4, C-13; 160.3, C-2; 149.5, C-4; 123.0, C-3; 81.5, C-11; 73.5, C-10; 54.7, C-5; 52.0, C-14; 37.0, C-15; 33.4, C-6; 24.6, C-16; 21.7, C-12; 19.1, C-7; 17.9, C-8; 14.9, C-18; 10.7, C-17.

4.8.7 Isolation and bioassay guided fractionation- *Eudistoma gilboverde*

4.8.7.1 Animal material and extraction.

Animal material (13.6 g) of *Eudistoma gilboverde* was collected from by hand using scuba from Flinders reef (12 m) and stored at 4°C until extraction. The frozen animal material was extracted four times with 400 ml of MeOH in a 500 ml conical flask and the four extractions were combined and filtered. The solvent was removed at 40°C under vacuum on a rotary evaporator (Büchi) to give 4.32 g of crude extract.

4.8.7.2 Bioassay guided fractionation:

The cytotoxic fraction (organic extract) was resuspended in a minimal amount of Hexane: CH_2Cl_2 (1:1) and applied to a glass column (i.d. 2.0 cm, length 9 cm) that had been packed with alumina gel (thin layer chromatography) and solvated with hexane. The column was eluted under vacuum with 10 ml each of 100% hexane, 10% CH_2Cl_2 in hexane, 25% CH_2Cl_2/Hexane, 50% CH_2Cl_2/Hexane, 100% CH_2Cl_2, 10% ethyl acetate (EtOAc) in CH_2Cl_2, 25% EtOAc/CH_2Cl_2, 50% EtOAc/CH_2Cl_2,

100% EtOAc, 10% methanol (MeOH) in EtOAc (fractions 1-10). The solvent was removed from each fractions at 30-40°C under vacuum on a rotary evaporator. Fractions 10 (5.1 mg) was identified as Eudistomin H (**393**)

4.8.7.3 Physical properties of purified metabolites isolated

Eudistomin H (393):

^{1}H NMR spectrum ($CDCl_3$): Refer to Table 9.

^{13}C NMR spectrum ($CDCl_3$): Refer to Table 9.

CHAPTER 5

Results and Discussion-

Cytotoxicity Assay for isolated metabolites

5.1 Introduction

The P388D1 mouse lymphoma cell line was the first screening tool to be utilised by the National Cancer Institute (NCI) in the United States[235], and has become a widely used preliminary drug screening tool to identify potential anticancer agents. Once potential cytotoxic agents are identified they are then tested in a secondary screen using other cancer cell lines. The P388D1 mouse lymphoma cell lines value as a probe in identifying new anticancer agents in preliminary studies is appreciated the world over, mainly due to its sheer sensitivity to cytotoxic molecules and the fact that the cytotoxic assay employing the P388D1 mouse lymphoma cell line is quick owing to the short doubling time for the cells (16 hours)[235].

5.2 Results and discussion

Cytotoxicity assays involving the P388D1 mouse lymphoma cell line revealed that ulithiacyclamide was the most cytotoxic of all the purified metabolites tested with an effective concentration that inhibited cell growth by half (IC_{50}) of 0.25 μg/ml (see Table 10, Appendix 1.1). By contrast, the related ascidiacyclamide (**361**) was 10-fold less potent, with an IC_{50} of 4.07 μg/ml (see Table 10, Appendix 1.1). Of all the *Lissoclinum* cyclic peptides, ulithiacyclamide (**357**) is known to be the most cytotoxic and it could be proposed that the S-S bridge in the molecule gives it this unique ability[199]. Published data does suggest this as ulithiacyclamide (**357**) has IC_{50} values of 0.35 and 0.01μg/ml for murine leukemia L1210 and human CEM cell lines

respectively[199]. On the other hand patellamides A, B and C (which are comparable to ascidiacyclamide (**361**) in the absence of the S-S bridge) are approximately 10 times less cytotoxic, having IC_{50} values of 2-4 μg/ml for L1210 cells[199]. It should be noted however that ulithiacyclamide B, which is closely related to ulithiacyclamide (**357**) except that the molecule lacks the symmetry associated with ulithiacyclamide (**357**) and possesses a phenyl group that forms a part of a phenylalanine unit, has an IC_{50} value of 17μg/ml against the human cervix carcinoma (KB) cell-line[208].

Compound	IC_{50} ± s.e.m. (μg/ml)
Ulithiacyclamide (**357**)	0.25 ± 0.01
Ascidiacyclamide (**361**)	4.07 ± 0.36
Scalaradial (**277**)	0.30 ± 0.02
Heteronemin (**278**)	0.32 ± 0.02
Chondrillin (**262**)	2.50 ± 0.99
Dysideathiazole (**326**)	18.20 ± 0.66
N-methyldysideathiazole (**327**)	19.0 ± 0.14
Eudistomin H (**393**)	21.10 ± 0.22

Table 10: Cytotoxic Potential of Known Compounds Isolated in this Study:

Compound structures were elucidated as described. Purified compounds were tested against the mouse lymphoma cell line, P-388D1, to determine the effective concentration that killed half of the cells (IC_{50}). Data is presented as the mean ± standard error of the mean (s.e.m.).

Compound	IC_{50} ± s.e.m. (µg/ml)
Armatol G (**193**)	5.80 ± 0.27
Armatol H (194)	6.30 ± 0.1
Stelliferin A **(43)**	0.16 ± 0.03
Stelliferin D riboside (195)	1.10 ± 0.09
Epispongiatriol **(96)**	16.30 ± 2.32
Spongiadiol diacetate (**97**)	2.10 ± 0.08
Isospongiatriol (196)	14.0 ± 0.99
1,2'-Dihydroxy-3,4,6,2',4'-hexabromodiphenyl ether (**141**)	4.10 ± 0.03
1-Hydroxy-3,4,6,2',4'-pentabromodiphenyl ether (197)	2.20 ± 0.04
1,2'-Dimethoxy-3,4,5,6,3',5'-pentabromodiphenyl ether (**198**)	3.80 ± 0.03

Table 11: Cytotoxic Potential of New Compounds and their Known Analogues: Compound structures were elucidated as described. Purified compounds were tested against the mouse lymphoma cell line, P-388D1, to determine the effective concentration that killed half of the cells (IC_{50}). Data is presented as the mean ± standard error of the mean (s.e.m.). The compounds listed in bold print represent those that are new compounds.

Another striking difference in biological activity was evidenced by the 10 fold difference in IC_{50} values for the isomalabaricane triterpenes- stelliferin A (**43**) and the new compound stelliferin D riboside (**195**) (see Table 11, Appendix 1.6). This is the first reported cytotoxicity for both the compounds, even though it is well established that the isomalabaricanes as a class are quite cytotoxic in nature[40, 45]. The activity profile of stelliferin A (**43**) against the P388D1 cell line resembles the

globostellatic acids A-D, which have shown IC_{50} values of 0.1-0.46 μg/ml against the same cell line[50].

Armatols G (**193**) and H (**194**), the new brominated polycyclic ethers isolated from the red alga *Chondria armata*, showed moderate activity in this assay exhibiting IC_{50} values of 5.8 and 6.3 μg/ml respectively (see Table 11, Appendix 1.5). Again, this is the first reported cytotoxicity for this group of polycyclic ethers. Prior to this the related armatols were only reported to have antimicrobial activity[31]. Two new metabolites isospongiatriol (**196**) and 1-hydroxy-3,4,6,2',4'-pentabromodiphenyl ether (**197**) showed moderate activity of 14 μg/ml and 2.2 μg/ml respectively (see Table 11, Appendices 1.7 and 1.9 respectively) .

Finally, the scalaranes- scalaradial (**277**) and heteronemin (**278**) exhibited strong activity against the P388D1 cell line with IC_{50} values of 0.30 and 0.32 μg/ml respectively (see Table 10, Appendix 1.2). Published data has shown heteronemin to exhibit strong activity toward human breast carcinoma (MCF-7), human colon carcinoma (HT-29), human cervix carcinomas (HeLa) and (KB) cell lines with IC_{50} values ranging between 0.29-0.45 μg/ml. Interestingly, the same study showed that heteronemin acetate had an IC_{50} value of 6.45 μg/ml against the MCF-7 cell line and was inactive towards the HT-29, HeLa and human cervix carcinoma KB cell lines, suggesting that the hydroxyl functionality at the C-12 position could be a defining factor in the antitumour activity of these molecules[236]. Compound **51**, isospongiadiol (**109**), nordehydrocyclodercitin (**200**), isonaamidine E (**225**) and bis(isonaamidinato E)zinc (**408**) were not tested due to decomposition of compounds.

5.2.1 Future scope

As mentioned before, the P388D1 mouse lymphoma cell-line is relatively sensitive to cytotoxic agents and thus a useful tool in discovering and tracking active compounds from the marine extracts tested. In the future it is worth investigating whether these compounds target specific types of cancer. In order to do so it is proposed that panels of at least 6 different cancer cell lines should be assessed for growth in the presence of each compound. These cell lines would ideally cover a range of cancer-types: breast cancer (MCF7), acute promyeolocytic leukemia (HL-60), glioma (SF-268), a large cell lung cancer (NCI-H460), mouse lymphoma (P388D1), and mouse neuroblastoma/glioma hybrid (NG-108) as these are a standard 6 cell line panel for preliminary screening and for tracking the active compounds during isolation and purification as indicated by the National Cancer Institute (NCI)[237].

5.3 Experimental

5.3.1 Cell culture and maintenance

The P388D1 mouse lymphoma cell-line was donated by the National Cancer Institute (NCI), Washington DC. Cells were maintained at approximately 10^5-10^6 cells/ml in antibiotic-free RPMI media containing 5% foetal calf serum (FCS) at 37°C in a humidified atmosphere containing 5% CO_2 in an incubator, and passaged every 2-3 days. Cells were frozen down for storage in 1 ml of a freezing media consisting of 10% dimethyl sulfoxide (DMSO) in RPMI-1640 with 5% FCS at 1×10^7 cells/ml.

5.3.2 Cytotoxicity Screening and Seeding Plates

The culture media used for seeding plates and for sample preparation was RPMI-1640, containing 5% FCS and 50 μg/ml of gentamicin to prevent bacterial contamination as extracts were not sterilized prior to use. Aliquots (100 μl) of a 5×10^4 cells/ml suspension in media or media alone (background controls) were pipetted into designated wells (Fig 1: unshaded and shaded, respectively) of a flat-bottomed 96-well plate. The 96-well plate was incubated (37°C, 5% CO_2) for 24 hours to stabilise the cells prior to addition of extract preparations.

5.3.3 Extract Preparation and Application

Each crude extract was resuspended in the drug-vehicle (20% aqueous methanol) at 2 mg/ml by first dissolving in methanol (MeOH) at 20 mg/ml and diluting to 2 mg/ml with water. A portion of the resuspended extract was removed and diluted to 100 µg/ml with media, then further diluted to 20 µg/ml with media.

Aliquots (100 µl) of the 100 µg/ml and 20 µg/ml of extract in media were added to the designated wells (see Fig 44: D1a, D1b) of a 96-well plate that had been seeded with cells and incubated for 24 hours, to give a final test concentration of 50 µg/ml and 10 µg/ml, respectively. A total of seven extract-preparations at the two concentrations were added to the designated wells (see Fig 44: D1a-D7a, D1b-D7b) of the 96-well plate. Aliquots (100 µl) of media containing 5% drug-vehicle, in the absence of extract, were added into the designated wells (see Fig 1: Veh) of the same 96-well plate to serve as a vehicle control. Aliquots (100 µl) of media, in the absence of extract and drug-vehicle, were added to the designated wells (see Fig 44: Med) of the same 96-well plate to serve as an additional negative control, and for comparison with media containing 5% drug-vehicle to establish that the drug-vehicle was not affecting the growth of cells at the concentration employed. The just-described extract preparations and controls were simultaneously aliquoted using an 8-channel multipipette, on a per-column basis. Variation between columns pipetted was allowed for by placing the treatments in rows (see Fig 44). The 96-well plate was incubated (37°C, 5% CO_2) for a further 48 hours (3 cell-doubling times) and then fixed.

5.3.4 Cell Fixation

Each 96-well plate was removed from the incubator and placed on a bench for 5 minutes to allow the cells to settle. In order to fix the cells to the bottom of the wells, 50 µl of cold (4°C) 50% aqueous trichloroacetic acid was gently layered onto the media in each well. The plates were kept at room temperature for a further 5 minutes, then gently transferred to a refrigerator and incubated at 4°C for 1 hour. Plates were then removed from the refrigerator and flicked over a sink to remove all liquid contents. The wells were rinsed five times with tap water, flicking to remove all liquid contents after each rinse, then allowed to dry overnight.

5.3.5 Cell Staining and Measurement

Aliquots (100 µl) of 0.4% (w/v) sulforhodamine B (SRB) in 1% aqueous acetic acid were pipetted into each well to stain the cells that were fixed to the plate. The plates were stained for at least 30 minutes, and then flicked over the sink to remove all excess stain. The wells were rinsed five times with 1% aqueous acetic acid, flicking to remove all liquid contents after each rinse, then allowed to dry overnight.

The stain was solubilised by adding 100 µl of 10 mM Tris buffer to each well and mixing on an orbital shaker (Edwards Bio-Line) for 10 min. The optical density (OD) of the solution in each well was measured at 492nm using a Labsystems Multiskan EX 96-well plate reader, with Labsystems Genesis Version 3.00 software. The OD data was imported into Microsoft Excel for manipulation.

5.3.6 Determination of Cytotoxicity

The mean background-corrected OD for each extract at each concentration was expressed as a percentage of the mean background-corrected OD for the drug-vehicle control. Since the OD is directly proportional to cell number, this percentage represents the mean number of cells present in each well for each extract at each concentration, relative to the control. All 308 organic extracts were tested for cytotoxicity and were retested in the P388D1 cytotoxicity assay at 25 μg/ml and 5 μg/ml to confirm activity and assist prioritisation.

5.3.7 IC_{50} Determination of Isolated Compounds

Selected isolated compounds were assessed for cytotoxicity against the P388D1 cell-line as per the previously described procedure and were tested at six concentrations from 100 μm to 1nm. Incubations were performed on three different passages of cells for replication purposes and to allow for variation in cell populations. The IC_{50} values were determined from the graphical examination of the data from each passage, and the mean IC_{50} and standard error of the mean (s.e.m.) calculated.

	1	2	3	4	5	6	7	8	9	10	11	12
A	D1a	D1a	D1a	D1a	D1a	D1b	D1b	D1b	D1b	D1b	D1b	D1b
B	D2a	D2a	D2a	D2a	D2a	D2b	D2b	D2b	D2b	D2b	D2b	D2b
C	D3a	D3a	D3a	D3a	D3a	D3b	D3b	D3b	D3b	D3b	D3b	D3b
D	Veh	Veh	Veh	Veh	Veh	Med	Med	Med	Med	Med	Med	Med
E	D4a	D4a	D4a	D4a	D4a	D4b	D4b	D4b	D4b	D4b	D4b	D4b
F	D5a	D5a	D5a	D5a	D5a	D5b	D5b	D5b	D5b	D5b	D5b	D5b
G	D6a	D6a	D6a	D6a	D6a	D6b	D6b	D6b	D6b	D6b	D6b	D6b
H	D7a	D7a	D7a	D7a	D7a	D7b	D7b	D7b	D7b	D7b	D7b	D7b

Fig 44. A sample of the 96-well plate design, showing position of wells A1–H12, for cytotoxicity testing of seven extracts at two concentrations (D1a-D7a and D1b-D7b). Negative controls, lacking extracts and drugs, contained media with 5% drug vehicle (Veh) and media alone (Med). Unshaded areas represent wells with 100 µl of 5×10^4 cells/ml, while shaded areas represent background controls that contained no cells.

REFERENCES

1. Rowinsky, E. K., *Annu. Rev. Med.* **1997,** 48, 353-374.
2. Sehgal, R.; Sharma, C.; Nandave, M.; Ojha, S. K., *Pharma Rev.* **2006,** 4, (143-147).
3. Pommier, Y.; Kohlhagen, G.; Bailly, C.; Waring, M.; Mazumder, A.; Kohn, K. W., *Biochemistry* **1996,** 35, 13303-13309.
4. Jimeno, J.; Aracil, M.; Tercero, J. C., *J. Trans. Med.* **2006,** 4, 1-9.
5. Henriques, J. A. P.; Soares, D. G.; Bonatto, D.; Salvador, M.; Schwartsmann, G.; Larsen, A. K., *Drug Design Reviews* **2005,** 2, 501-525.
6. Urdiales, J. L.; Morata, P.; Nunez De Castro, I.; Sanchez-Jimenez, F., *Cancer Lett.* **1996,** 102, 31-37.
7. Rawat, D. S.; Joshi, M. C.; Joshi, P.; Atheaya, H., *Anti-Cancer Agents in Med. Chem.* **2006,** 6, 33-40.
8. Henriquez, R.; Faircloth, G.; Cuevas, C., *Antican. Agents Nat. Prod.* **2005**, 215-240.
9. Den Brok, M. W. J.; Nuijen, B.; Meijer, D. M.; Millan, E.; Manada, C.; Beijnen, J. H., *J. Pharm. Sci. Tech.* **2005,** 59, 246-257.
10. Tardy, C.; Facompre, M.; Laine, W.; Baldeyrou, B.; Garcia-Gravalos, D.; Francesch, A.; Mateo, C.; Pastor, A.; Jimenez, J. A.; Manzanares, I.; Cuevas, C.; Bailly, C., *Bioorg. Med. Chem.* **2004,** 12, 1697-1712.
11. Wratten, S. J.; Faulkner, D. J., *J. Org. Chem.* **1976,** 41, 2465-2467.
12. Fenical, W.; Sims, J. J., *Tetrahedron Lett.* **1974,** 13, 1137-1140.
13. Oztunc, A.; Imre, S.; Lotter, H.; Wagner, H., *Phytochemistry* **1989,** 28, 3403-3404.
14. Maeda, M.; Kodama, T.; Tanaka, T.; Yoshizumi, H.; Takemoto, T.; Nomoto, K.; Fujita, T., *Chem. Pharm. Bull.* **1986,** 34, 4892-4895.
15. Maeda, M.; Kodama, T.; Tanaka, T.; Yoshizumi, H.; Takemoto, T.; Nomoto, K., *Tetrahedron Lett.* **1987,** 28, 633-636.
16. Palermo, J. A.; Flower, P. B.; Seldes, A. M., *Tetrahedron Lett.* **1992,** 33, 3097-3100.

17. Morita, K.; Kobayashi, H., *Tetrahedron Lett.* **1966**, 573-575.
18. Morita, K.; Kobayashi, S., *Chem. Pharm. Bull.* **1967,** 15, 988-990.
19. Davidson, B. S.; Molinski, T. F.; Barrows, L. R.; Ireland, C. M., *J. Amer. Chem. Soc.* **1991,** 113, 4709-4710.
20. Makarieva, T. N.; Stonik, V. A.; Dmitrenok, A. S.; Grebnev, B. B.; Isakov, V. V.; Rebachyk, N. M., *J. Nat. Prod.* **1995,** 58, 254-258.
21. Edmons, J.; Shibata, T.; Yang, F., *Tetrahedron Lett.* **1997,** 38, 5819-5820.
22. Davyt, D.; Entz, W.; Fernandez, R.; Mariezcurrena, R.; Mombru, A. W.; Saldana, J.; Dominguez, L.; Coll, J.; Manta, E., *J. Nat. Prod.* **1998,** 61, 15601563.
23. Zaman, L.; Arakawa, O.; Shimosu, A.; Onoue, Y.; Nishio, S.; Shida, Y.; Noguchi, T., *Toxicon* **1997,** 35, 205-212.
24. Holland, P. T.; Selwood, A. I.; Mountfort, D. O.; Wilkins, A. L.; McNabb, P.; Rhodes, L. L.; Doucette, G. J.; Mikulski, C. M.; King, K. L., *Chem. Res. Toxicol.* **2005,** 18, 814-816.
25. Wright, J. L., *Nat. Toxins* **1998,** 6, 91-92.
26. Erickson, D., *Sc. Am.* **1992,** 266, 129-130.
27. Wright, J. L. C.; Boyd, R. K.; de Freitas, A. S. W.; Falk, M.; Foxall, R. A.; Jamieson, W. D.; Laycock, M. V.; McCulloch, A. W.; McInness, A. G.; Odense, P.; Pathak, V. P.; Quilliam, M. A.; Ragan, M. A.; Sim, P. G.; Thipault, P.; Walter, J. A., *Can. J. Chem.* **1989,** 68, 22-25.
28. Hampson, D. R.; Huang, X. P.; Wells, J. W.; Walter, J. A.; Wright, J. L. C., *Eur. J. Pharmac.* **1992,** 218, 1-8.
29. Govenkar, M. B.; Wahidulla, S., *Phytochemistry* **2000,** 54, 979-981.
30. Fenical, W.; Sims, J. J., *Tetrahedron Lett.* **1973,** 14, 313-316.
31. Ciavatta, M. L.; Wahidulla, S.; D'Souza, L.; Scognamiglio, G.; Cimino, G., *Tetrahedron* **2001,** 57, 617-623.
32. Suzuki, M.; Matsuo, Y.; Takahashi, Y.; Masuda, M., *Chemistry lett.* **1995,** 11, 1045-1046.
33. Matsuo, Y.; Suzuki, M.; Masuda, M., *Chemistry lett.* **1995,** 11, 1043-1044.
34. Blunt, J. W.; Hartshorn, M. P.; McLennan, T. J.; Munro, M. H. G.; Robinson, W. T.; Yorke, S. C., *Tetrahedron Lett.* **1978**, 69-72.
35. Carmely, S.; Kashman, Y., *J. Org. Chem.* **1983,** 48, 3517-3525.

36. Fernández, J. J.; Souto, M. L.; Norte, M., *Nat. Prod. Rep.* **2000,** 17, 235-246.
37. Ravi, B. N.; Wells, R. J., *J. Org. Chem.* **1981,** 46, 1998-2001.
38. McCabe, T. C., J.; Minale, L.; Pizza, C.; Zollo, F.; Riccio, R., *Tetrahedron Lett.* **1982,** 23, 3307-3310.
39. Tsuda, M.; Ishibashi, M.; Agemi, K.; Sasaki, T.; Kobayashi, J., *Tetrahedron* **1991,** 47, 2181-2194.
40. Meragelman, K. M.; McKee, T. C.; Boyd, M. R., *J. Nat. Prod.* **2001,** 64, 389-392.
41. Ravi, B. N.; Wells, R. J., *Aust. J. Chem.* **1982,** 35, 39-50.
42. Oku, N.; Matsunaga, S.; Wada, S.; Watabe, S.; Fusetani, N., *J. Nat. Prod.* **2000,** 63, 205-209.
43. Clement, J. A.; Li, M.; Hecht, S. M.; I., K. D. G., *J. Nat. Prod.* **2006,** 69, 373-376.
44. Tabudravu, J. N.; Jaspars, M., *J. Nat. Prod.* **2001,** 64, 813-815.
45. Fouad, M.; Edrada, R. A.; Ebel, R.; Wray, V.; Muller, W. E. G.; Lin, W. H.; Proksch, P., *J. Nat. Prod.* **2006,** 69, 211-218.
46. Su, J. Y.; Meng, Y. H.; Zeng, L. M.; Fu, X.; Schmitz, F. J., *J. Nat. Prod.* **1994,** 57, 1450-1451.
47. Ravi, B. N.; Wells, R. J.; Croft, K. D., *J. Org. Chem.* **1981,** 46, 1998-2001.
48. McCormick, J. L.; McKee, T. C.; Cardellina, J. H.; Leid, M.; Boyd, M. R., *J. Nat. Prod.* **1996,** 59, 1047-1050.
49. Tasdemir, D.; Mangalindan, G. C.; Concepcion, G. P.; Verbitski, S. M.; Rabindran, S.; Miranda, M.; Greenstein, M.; Hooper, J. N. A.; Harper, M. K.; Ireland, C. M., *J. Nat. Prod.* **2002,** 2002, 210-214.
50. Ryu, G.; Matsunaga, S.; Fusetani, N., *J. Nat. Prod.* **1996,** 59, 512-514.
51. Satoh, M.; Satoh, Y.; Isobe, K.; Fujimoto, Y., *Chem. Pharm. Bull.* **2003,** 51, 341-342.
52. Jiang, R. W.; Ma, S. C.; But, P. P. H.; Mak, T. C. W., *J. Nat. Prod.* **2001,** 64, 1266-1272.
53. Moody, J. O.; Robert, V. A.; Connolly, J. D.; Houghton, P. J., *J. Ethnopharmac.* **2006,** 104, 87-91.

54. Molinski, T. F.; Faulkner, D. J.; He, C. H.; Van Duyne, G. D.; Clardy, J., *J. Org. Chem.* **1986,** 51, 4564-4567.
55. Bobzin, S. C.; Faulkner, D. J., *J. Org. Chem.* **1989,** 54, 3902-3907.
56. Gavagnin, M.; Vardaro, R. R.; Avila, C.; Cimino, G.; Ortea, J., *J. Nat. Prod.* **1992,** 55, 368-371.
57. Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J., *Tetrahedron Lett.* **1976,** 30, 1331-1333.
58. Cimino, G.; De Rosa, D.; De Stefanos, S.; Minale, S., *Tetrahedron.* **1974**, 645-649.
59. Gonzalez, A. G. E., D. M.; Martin, J. D.; Martin, V. S.; Perez, C.; Perez, R. , *Tetrahedron* **1984,** 40, 4109-4113.
60. Keyzers. R. A.; Northcote, P. T. D.-C., M. T., *Nat. Prod. Rep.* **2006,** 23, 321-334.
61. Arno, M.; Gonzalez, M. A.; Zaragoza, R. J., *Tetrahedron.* **1999,** 55, 12419-12428.
62. Gunasekera, S. P.; Schmitz, F. J., *J. Org. Chem.* **1991,** 56, 1250-1253.
63. Kazalauskas, R.; Murphy, P. T.; Wells, R. J.; Noack, K.; Oberhansli, W. E.; Schonholzer, P., *Aust. J. Chem.* **1979,** 32, 867-880.
64. Hirsch, S.; Kashman, Y., *J. Nat. Prod.* **1988,** 51, 1247-1249.
65. Kohmoto, S.; McConnell, O. J.; Wright, A.; Cross, S., *Chemistry lett.* **1987,** 9, 1687-1690.
66. Searle, P. A.; Molinski, T. F., *Tetrahedron* **1994,** 50, 9893-9908.
67. Cambie, R. C.; Craw, P. A.; Stone, M. J.; Bergquist, P. R., *J. Nat. Prod.* **1988,** 51, 293-297.
68. Cambie, R. C.; Craw, P. A.; Bergquist, P. R.; Karuso, P., *J. Nat. Prod.* **1987,** 50, 948-949.
69. Miyamoto, T. S., Kenichiro; Arao, Kazuyoshi; Komori, Tetsuya; Higuchi, Ryuichi; Sasaki, Takuma., *Tetrahedron* **1996,** 52, 8187-8198.
70. Li, C.; Schmitz, F. J.; Kelly-Borges, M., *J. Nat. Prod.* **1999,** 62, 287-290.
71. Hyosu, M.; Kimura, J., *J. Nat. Prod.* **2000,** 63, 422-423.
72. Karuso, P.; Taylor, W. C., *Aust. J. Chem.* **1986,** 39, 1629-1641.
73. Karuso, P.; Bergquist, P. R.; Cambie, R. C.; Buckleton, J. S.; Clark, G. R.; Rickard, C. E. F., *Aust. J. Chem.* **1986,** 39, 1643-1653.

74. Taylor, W. C.; Toth, S., *Aust. J. Chem.* **1997,** 50, 895-902.
75. Schmitz, F. J.; Chang, J. S.; Hossain, M. B.; Van der Helm, D., *J. Org. Chem.* **1985,** 50, 2862-2865.
76. Hambley, T. W.; Taylor, W. C.; Toth, S., *Aust. J. Chem.* **1997,** 50, 903-909.
77. Abad, A.; Arno, M.; Cunat, A. C.; Marin, M. L.; Zaragoza, R. J., *J. Org. Chem.* **1992,** 57, 6861-6869.
78. Flatt, P. M.; Gautschi, J. T.; Thacker, R. W.; Musafija-Girt, M.; Crews, P.; Gerwick, W. H., *Mar. Biol.* **2005,** 147, 761-774.
79. Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Princep, M. R., *Nat. Prod. Rep.* **2004,** 21, 1-49.
80. Faulkner, D. J.; Unson, M. D.; Bewley, C. A., *Pure. Appl. Chem* **1994,** 66, 1983-1990.
81. Unson, M. D.; Faulkner, D. J., *Experientia* **1993,** 49, 349-353.
82. Unson, M. D.; Holland, N. D.; Faulkner, D. J., *Mar. Biol.* **1994,** 119, 1-11.
83. Thacker, R. W.; Starnes, S., *Mar. Biol.* **2003,** 142, 643-648.
84. Carte, B.; Kernan, M. R.; Barrabee, E. B.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J., *J. Org. Chem.* **1986,** 51, 3528-3532.
85. Popov, A. M.; Stekhova, S. I.; Utkina, N. K.; Rebachuk, N. M., *Pharm. Chem.* **1999,** 33, 71-73.
86. Fu, X.; Schmitz, F. J.; Govindan, M.; Abbas, S. A.; Hanson, K. M.; Horton, P. A.; Crews, P.; Laney, M.; Schatzman, R. C., *J. Nat. Prod.* **1995,** 58, 1384-1391.
87. Sharma, G. M., Vig, B., *Tetrahedron Lett* **1972,** 17, 1715-1718.
88. Norton, R. S.; Croft, K. D.; Wells, R. J., *Tetrahedron.* **1981,** 37, 2341-2349.
89. Carté, B.; Faulkner, D. J., *Tetrahedron.* **1981,** 37, 2335-2339.
90. Salva, J. F., D. J., *J. Nat. Prod.* **1990,** 53, 757-760.
91. Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Van Soest, R. W.; Kunzmann, A.; , *J. Nat. Prod* **1997,** 60, 1313-1316.
92. Bowden, B. F.; Towerzey, L.; Junk, P. C., *Aust. J. Chem.* **2000,** 53, 299-301.
93. Utkina, N. K.; Denisenko, V. A., *Chem. Nat. Comp.* **2006,** 42, 606-607.
94. Vetter, W.; Stoll, E.; Garson, M. J.; Farhey, S. J.; Gaus, C.; Muller, J. F., *Environ. Toxic. Chem.* **2002,** 21, 2014-2019.

95. Marsh, G.; Stenutz, R.; Bergman, A., *Eur. J. Org. Chem.* **2003**, 2566-2576.
96. Nilar; Sidebottom, P. J.; Carte, B. K.; Butler, M. S., *J. Nat. Prod.* **2002,** 65, 1198-1200.
97. Delfourne, E.; Darro, F.; Bontemps-Subielos, N.; Decaestecker, C.; Bastide, J.; Frydman, A.; Kiss, R., *J. Med. Chem.* **2001,** 44, 3275-3282.
98. Gomez-Bengoa, E.; Echavarren, A. M., *J. Org. Chem.* **1991,** 56, 3497-3501.
99. Rudi, A.; Benayahu, Y.; Goldberg, I.; Kashman, Y., *Tetrahedron Lett.* **1988,** 29, 3861-3862.
100. Rudi, A.; Kashman, Y., *J. Org. Chem.* **1989,** 54, 5331-5337.
101. T. F. Molinski, C. M. I., *J. Org. Chem* **1989,** 54, 4256.
102. Kobayashi, J.; Cheng, J.; Walchi, M. R.; Nakamura, S.; Hirata, Y.; Sasaki, T.; Ohizumi, Y., *J. Org. Chem.* **1988,** 53, 1800-1804.
103. Kobayashi, J.; Tsuda, M.; Tanabe, A.; Ishibashi, M.; Cheng, J. F.; Yamamura, S.; Sasaki, T., *J. Nat. Prod.* **1991,** 54, 1634-1638.
104. McDonald, L. A.; Eldredge, G. S.; Barrows, L. R.; Ireland, C. M., *J. Med. Chem.* **1994,** 37, 3819-3827.
105. Charyulu, G. A.; McKee, T. C.; Ireland, C. M., *Tetrahedron Lett.* **1989,** 30, 4201-4202.
106. Searle, P. A.; Molinski, T. F., *J. Org. Chem.* **1994,** 59, 6600-6605.
107. Carroll, A. R.; Cooray, N. M.; Poiner, A.; Scheuer, P. J., *J. Org. Chem.* **1989,** 54, 4231-4232.
108. Cooray, N. M.; Scheuer, P. J.; Parkanyl, L.; Clardy, J., *J. Org. Chem.* **1988,** 53, 4619-4620.
109. Eder, C.; Schupp, P.; Proksch, P.; Wray, V.; Steube, K.; Mueller, C. E.; Frobenius, W.; Herderich, M.; Van Soest, R. W. M., *J. Nat. Prod.* **1998,** 61, 301-305.
110. Carroll, A. R.; Scheuer, P. J., *J. Org. Chem.* **1990,** 55, 4426-4431.
111. Koren-Goldshlager, G.; Aknin, M.; Kashman, Y., *J. Nat. Prod.* **2000,** 63, 830-831.
112. Salomon, C. E.; Faulkner, D. J., *Tetrahedron Lett.* **1996,** 37, 9147-9148.
113. Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P.; Schmidt, P. G.; Shoolery, J. N., *J. Amer. Chem. Soc.* **1983,** 105, 4835-4836.

114. Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S., *Tetrahedron lett.* **1988,** 29, 1177-1180.
115. Bontemps, N.; Bonnard, I.; Banaigs, B.; Combaut, G.; Francisco, C., *Tetrahedron Lett.* **1994,** 35, 7023-7026.
116. McCarthy, P. J.; P;, P. T.; Gunawardana, G. P.; Kelly-Borges, M.; Pomponi, S. A., *J. Nat. Prod.* **1992,** 55, 1664-1668.
117. Gunawardana, G. P.; Koehn, F. E.; Lee, A. Y.; Clardy, J.; He, J. H.; Faulkner, D. J., *J. Org. Chem.* **1992,** 57, 1523-1526.
118. Gunawardana, G. P.; Kohmoto, S.; Burres, N. S., *Tetrahedron lett.* **1989,** 30, 4359-4362.
119. Plubrukarn, A.; Davidson, B. S., *J. Org. Chem.* **1998,** 63, 1657-1659.
120. Skyler, D.; Heathcock, C. H., *J. Nat. Prod.* **2002,** 65, 1573-1581.
121. Dale, J. A.; Mosher, H. S., *J. Amer. Chem. Soc.* **1973,** 95, 512-519.
122. Liu, H.; Namikoshi, M.; Meguro, S.; Nagai, H.; Kobayashi, H.; Yao, X., *J. Nat. Prod.* **2004,** 67, 472-474.
123. Alvi, K. A.; Crews, P.; Loughhead, D. G., *J. Nat. Prod.* **1991,** 54, 1509-1515.
124. Kong, F.; Faulkner, D. J., *J. Org. Chem.* **1993,** 58, 970-971.
125. Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P., *Tetrahedron* **1992,** 49, 329-336.
126. Akee, R. K.; Carroll, T. R.; Yoshida, W. Y.; Scheuer, P. J.; Stout, T. J.; Clardy, J., *J. Org. Chem.* **1990,** 55, 1944-1946.
127. Edrada, R.; Stessman, C. C.; Crews, P., *J. Nat. Prod.* **2003,** 66, 939-942.
128. Crews, P.; Clark, D. P.; Tenney, K., *J. Nat. Prod.* **2003,** 66, 177-182.
129. Carmely, S.; Ilan, M.; Kashman, Y., *Tetrahedron.* **1989,** 45, 2193-2200.
130. Fu, X.; Schmitz, F. J.; Tanner, R. S.; Kelly-Borges, M., *J. Nat. Prod.* **1998,** 61, 384.
131. Fu, X.; Barnes, J. R.; Do, T.; Schmitz, F. J., *J. Nat. Prod.* **1997,** 60, 497-498.
132. Dunbar, D. C.; Rimoldi, J. M.; Clark, A. M.; Kelly, M.; Hamann, M. T., *Tetrahedron* **2000,** 56, 8795-8798.
133. Gross, H.; Kehraus, S.; Koenig, G. M.; Woerheide, G.; Wright, A. D., *J. Nat. Prod.* **2002,** 65, 1190-1193.

134. Hassan, W.; Edrada, R.; Ebel, R.; Wray, V.; Berg, A.; Van Soest, R.; Wiryowidagdo, S.; Proksch, P., *J. Nat. Prod.* **2004,** 67, 817-822.
135. Copp, B. R.; Fairchild, C. R.; Cornell, L.; Casazza, A. M.; Robinson, S.; Ireland, C. M., *J. Med. Chem.* **1998,** 41, 3909-3911.
136. Carroll, A. R.; Bowden, B. F.; Coll, J. C., *Aust. J. Chem.* **1993,** 46, 1229-1234.
137. Nakamura, S.; Kawasaki, I.; Yamashita, M.; Ohta, S., *Heterocycles* **2003,** 60, 583-598.
138. Alvi, K. A.; Peters, B. M.; Hunter, L. M.; Crews, P., *Tetrahedron* **1993,** 49, 329-336.
139. Cafieri, F.; Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D., *Tetrahedron.* **1973,** 29, 4259-4262.
140. Di Blasio, B.; Fattorusso, E.; Magno, S.; Mayol, L.; Pedone, C.; Santacroce, C.; Sica, D., *Tetrahedron.* **1976,** 32, 473-478.
141. Thompson, J. E.; Walker, R. P.; Wratten, S. J.; Faulkner, D. J., *Tetrahedron.* **1982,** 38, 1865-1873.
142. Burgoyne, D. L.; Dumdei, E. J.; Andersen, R. J., *Tetrahedron.* **1993,** 49, 4503-4510.
143. Ciminiello, P.; Fattorusso, E.; Magno, S.; Mayol, L., *Experientia* **1986,** 42, 625-627.
144. Wratten, S. J.; Faulkner, D. J., *J. Org. Chem.* **1977,** 42, 3343-3349.
145. Iengo, A.; Mayol, L.; Santacroce, C., *Experientia.* **1977,** 33, 11-12.
146. Chenera, B.; Chuang, C. P.; Hart, D. J.; Lai, C. S., *J. Org. Chem.* **1992,** 57, 2018-2029.
147. Minale, L.; Riccio, R.; Sodano, G., *Tetrahedron.* **1974,** 30, 1341-1343.
148. Ciminiello, P.; Fattorusso, E.; Magno, S.; Mayol, L., *J. Org. Chem.* **1984,** 49, 3949-3951.
149. Ciminiello, P.; Magno, S.; Mayol, L.; Piccialli, V., *J. Nat. Prod.* **1987,** 50, 217-220.
150. Ciminiello, P.; Fattorusso, E.; Magno, S.; Mayol, L., *J. Nat. Prod.* **1985,** 48, 64-68.
151. Takada, N.; Watanabe, M.; Yamada, A.; Suenaga, K.; Yamada, K.; Ueda, K.; Uemura, D., *J. Nat. Prod.* **2001,** 64, 356-359.

152. Varoglu, M.; Peters, B. M.; Crews, P., *J. Nat. Prod.* **1995,** 58, 27-36.
153. Higgs, M. D.; Faulkner, D. J., *J. Org. Chem.* **1978,** 43, 3454-3457.
154. Toth, S. I.; Schmitz, F. J., *J. Nat. Prod.* **1994,** 57, 123-127.
155. Harrison, B.; Crews, P., *J. Nat. Prod.* **1998,** 61, 1033-1037.
156. de Guzman, F. S.; Schmitz, F. J., *J. Nat. Prod.* **1990,** 53, 926-931.
157. Patil, A. D.; Freyer, A. J.; Carte, B.; Johnson, R. K.; Lahouratate, P., *J. Nat. Prod.* **1996,** 59, 219-223.
158. Jimenez, M.; Garzon, S. P.; Rodriguez, A. D., *J. Nat. Prod.* **2003,** 66, 655-661.
159. Bowden, B. F.; Coll, J. C.; Li, H., *J. Nat. Prod.* **1992,** 55, 1234-1240.
160. Rogers, S. D.; Paul, V. J., *Mar. Ecol. Prog. Ser.* **1991,** 77, 221-232.
161. Avila, C.; Cimino, G.; Crispino, A.; Spinella, A., *Experientia.* **1991,** 47, 306-310.
162. Cimino, G.; Sodano, G.; Spinella, A., *J. Nat. Prod.* **1988,** 51, 1010-1011.
163. Cimino, G.; De Rosa, S.; De Stefano, S.; Morrone, R.; Sodano, G., *Tetrahedron* **1985,** 41, 1093-1100.
164. Fontana, A.; Cavaliere, P.; Ungur, N.; D'Souza, L.; Parameswaram, P. S.; Cimino, G., *J. Nat. Prod.* **1999,** 62, 1367-1370.
165. Crews, P.; Bescansa, P., *J. Nat. Prod.* **1986,** 49, 1041-1052.
166. Davis, R.; Capon, R. J., *Aust. J. Chem.* **1993,** 46, 1295-1299.
167. Iguchi, K.; Shimada, Y.; Yamada, Y., *J. Org. Chem.* **1992,** 57, 522-524.
168. Cimino, G.; De Rosa, S.; De Stefano, S., *Experientia* **1981,** 37, 214-216.
169. Ryu, G.; Matsunaga, S.; Fusetani, N., *J. Nat. Prod.* **1996,** 59, 515-517.
170. Miyaoka, H.; Nishijima, S.; Mitome, H.; Yamada, Y., *J. Nat. Prod.* **2000,** 63, 1369-1372.
171. Youssef, D. T. A.; Yamaki, R. K.; Kelly, M.; Scheuer, P. J., *J. Nat. Prod.* **2002,** 65, 2-6.
172. Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Hoard, M. S.; Melody, N.; Pettit, R. K., *J. Nat. Prod.* **1998,** 61, 13-16.
173. Pettit, G. R.; Tan, R.; Melody, N.; Cichacz, Z. A.; Herald, D. L.; Hoard, M. S.; Pettit, R. K.; Chapuis, J. C., *Bioorg. & Med. Chem.* **1998,** 8, 2093-2098.
174. Pettit, G. R.; Tan, R.; Cichacz, Z. A., *J. Nat. Prod* . **2005,** 68, 1253-1255.

175. Youssef, D. T. A.; Shaala, L. A.; Emara, S., *J. Nat. Prod.* **2005,** 68, 1782-1784.

176. Hofheinz, W.; Oberhansli, W. E., *Helv. Chim. Acta.* **1977,** 60, 660-669.

177. Kazlauskas, R.; Lidgard, R. O.; Wells, R. J.; Vetter, W., *Tetrahedron lett.* **1977,** 18, 3183-3186.

178. Charles, C.; Braekman, J. C.; Daloze, D.; Tursch, B.; Karlsson, R., *Tetrahedron Lett.*

1978, 19, 1519-1520.

179. Van Sande, J., Denenbourg, F., Beauwens, R., Braekman, J.C., Daloze, D., Dumont, J.E., *Molecular Pharmacology* **1990,** 37, 583-589.

180. Erickson, K. L.; Wells, R. J., *Aust. J. Chem.* **1982,** 35, 31-38.

181. MacMillan, J. B., Trousdale, E.K., Molinski, T.F., *Organic Lett.* **2000,** 2, 2721-2723.

182. Lee, G. M., Molinski, T.F., *Tetrahedron Lett.* **1992,** 33, 7671-7674.

183. MacMillan, J. B., Molinski, T.F., *J. Nat. Prod* **2000,** 63, 155-157.

184. Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J., *J. Org. Chem.* **1993,** 58, 6336-6343.

185. Carmely, S. l.; Gebreyesus, T.; Kashman, Y.; Skelton, B. W.; White, A. H.; Yosief, T., *Aust. J. Chem.* **1990,** 43, 1881-1888.

186. Sauleau, P.; Retailleau, P. l.; Vacelet, J.; Bourguet-Kondracki, M., *Tetrahedron* **2005,** 61, 955-963.

187. Stapleton, B. L.; Cameron, G. M.; Garson, M. J., *Tetrahedron* **2001,** 57, 4603-4607.

188. McDonald, L. A.; Ireland, C. M., *J. Nat. Prod.* **1992,** 55, 376-379.

189. Rashid, M. A.; Gustafson, K. R.; Cardellina, J. H.; Boyd, M. R., *J. Nat. Prod.* **1995,** 58, 594-597.

190. Abriskie, T. M.; Mayne, C. L.; Ireland, C. M., *J. Amer. Chem. Soc.* **1988,** 110, 7919-7920.

191. Ueda, K.; Hu, Y., *Tetrahedron Lett.* **1999,** 40, 6305-6308.

192. Liu, H.; Fujiwara, T.; Nishikawa, T.; Mishima, Y.; Nagai, H.; Shida, T.; Tachibana, K.; Kobayashi, H.; Mangindaan, R. E. P.; Namikoshi, M., *Tetrahedron.* **2005,** 61, 8611-8615.

193. Uddin, J.; Ueda, K.; Siwu, E. R. O.; Kita, M.; Uemura, D., *Bioorg. & Med. Chem.* **2006,** 14, 6954-6961.

194. Uddin, M. J.; Kokubo, S.; Ueda, K.; Suenaga, K.; Uemura, D., *Chem. Lett.* **2002,** 10, 1028-1029.

195. Ireland, C. M.; Durso, A. R. J.; Newman, R. A.; Hacker, M. P., *J. Org. Chem.* **1982,** 47, 1807-1811.

196. Sesin, D. F.; Gaskell, S. J.; Ireland, C. M., *Bulletin des Societes Chimiques Belges.* **1986,** 95, 853-867.

197. Faulkner, D. J., *Nat. Prod. Rep.* **2002,** 19, 1-48.

198. Sesin, D. F.; Gaskell, S. J.; Ireland, C. M., *Bulletin des Societes Chimiques Belges* **1986,** 95, 853-867.

199. Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J., *J. Med. Chem.* **1989,** 32, 1349-1354.

200. Williams, A. B.; Jacobs, R. S., *Cancer Lett.* **1993,** 71, 97-102.

201. Fu, X.; Do, T.; Schmitz, F. J.; Andrusevich, V.; Engel, M. H., *J. Nat. Prod.* **1998,** 61, 1547-1551.

202. Carroll, A. R.; Coll, J. C.; Bourne, D. J.; MacLeod, J. K.; Zabriskie, T. M.; Ireland, C. M.; Bowden, B. F., *Aust. J. Chem.* **1996,** 49, 659-667.

203. Ireland, C.; Scheuer, P. J., *J. Amer. Chem. Soc.* **1980,** 102, 5688-5691.

204. Patil, A. D.; Freyer, A. J.; Killmer, L.; Chambers-Myers, C.; Johnson, R. K., *Nat. Prod. Lett.* **1997,** 9, 181-187.

205. Hamamoto, Y.; Endo, M.; Nakagawa, M.; Nakanishi, T.; Mizukawa, K., *Journal of the Chemical Society, Chemical Communications.* **1983,** 6, 323-324.

206. Hamada, Y.; Kato, S.; Shioiri, T., *Tetrahedron Lett.* **1985,** 26, 3223-3226.

207. Ishida, T.; Inoue, M.; Hamada, Y.; Kato, S.; Shioiri, T., *J. Chem. Soc., Chem. Comm.* **1987,** 5, 370-371.

208. Williams, D. E.; Moore, R. E.; Paul, V. J., *J. Nat. Prod.* **1989,** 52, 732-739.

209. Wasylyk, J. M.; Biskupiak, J. E.; Costello, C. E.; Ireland, C. M., *J. Org. Chem.* **1983,** 48, 4445-4449.

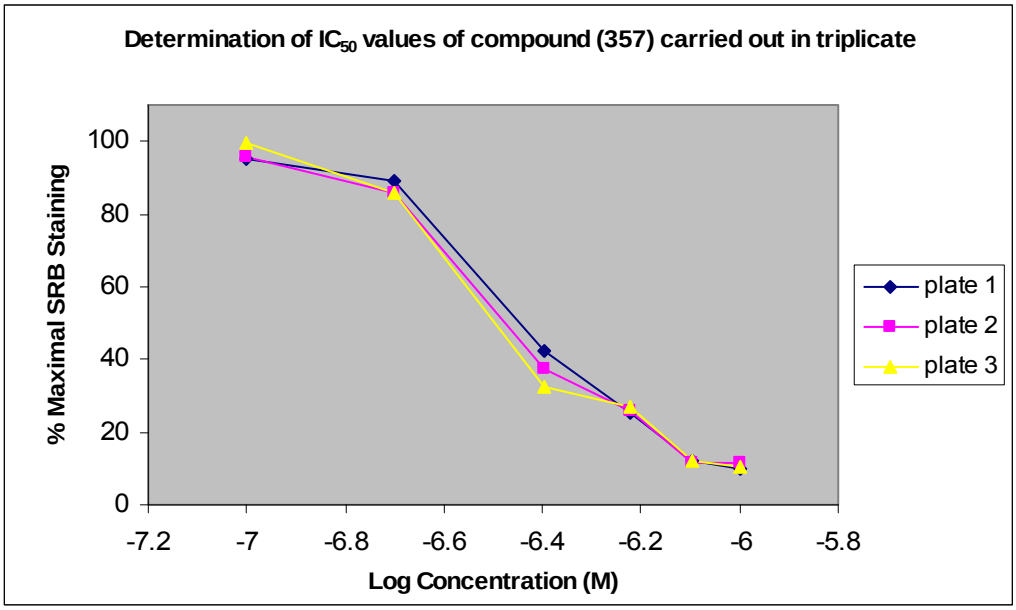
210. Schmitz, F. J.; Ksebati, M. B.; Chang, J. S.; Wang, J. L.; Hossain, M. B.; D., V. d. H.; Engel, M. H.; Serban, A.; Silfer, J. A., *J. Org. Chem.* **1989,** 54, 3463-3472.

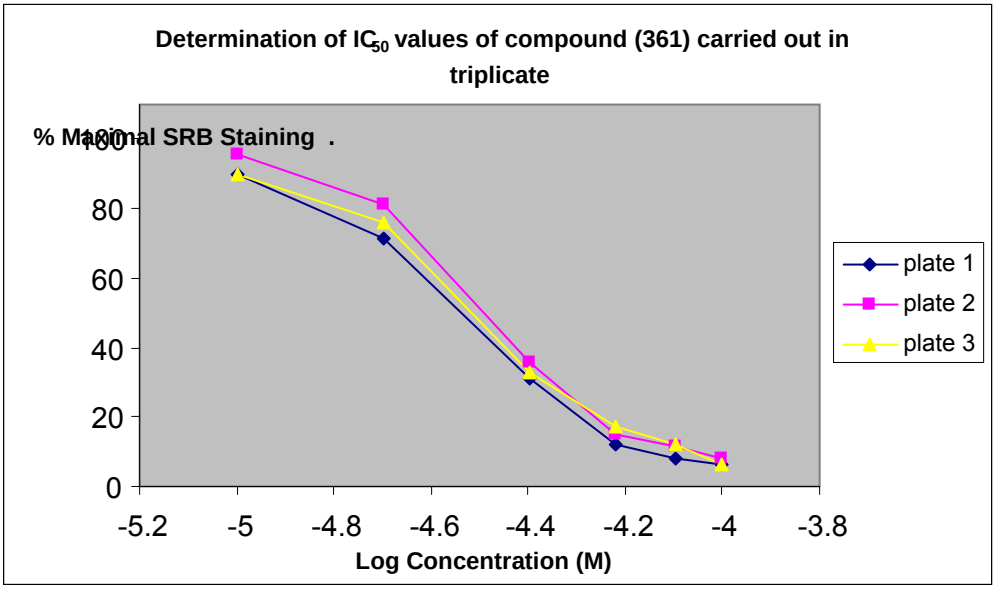
211. Hawkins, C. J.; Lavin, M. F.; Marshall, K. A.; L., V. d. B. A.; Watters, D. J., *J. Med. Chem.* **1990,** 33, 1634-1638.
212. Morris, L. A.; Jaspars, M. l.; Kettenes-van den Bosch, J. J.; Versluis, K.; Heck, A. J. R.; Kelly, S. M.; Price, N. C., *Tetrahedron* **2001,** 57, 3185-3197.
213. McDonald, L. A.; Foster, M. P.; Phillips, D. R.; Ireland, C. M.; Lee, A. Y.; Clardy, J., *J. Org. Chem.* **1992,** 57, 4616-4624.
214. Rinehart, K. L.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G.; Mizsak, S. A.; Scahill, T. A., *J. Amer. Chem. Soc.* **1984,** 106, 1524-1526.
215. Lake, R. J.; Brennan, M. M.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K., *Tetrahedron lett.* **1988,** 29, 2255-2256.
216. Schupp, P.; Poehner, T.; Edrada, R.; Ebel, R.; Berg, A.; Wray, V.; Proksch, P., *J. Nat. Prod.* **2003,** 66, 272-275.
217. Agarwal, A.; Agarwal, S. K.; Shukla, P. K.; Khan, Z. K., *Med. Chem. Res.* **1994,** 4, 396-405.
218. Kobayashi, J.; Cheng, J.; Ohta, T.; Nozoe, S.; Ohizumi, Y.; Sasaki, T., *J. Org. Chem.* **1990,** 55, 3666-3670.
219. Rashid, M. A.; Gustafson, K. R.; Boyd, M. R., *J. Nat. Prod.* **2001,** 64, 1454-1456.
220. Lake, R. J.; Blunt, J. W.; Munro, M. H. G., *Aust. J. Chem.* **1989,** 42, 1201-1206.
221. Adesanaya, S. A.; Chibani, M.; Pais, M.; Debitus, C., *J. Nat. Prod.* **1992,** 55, 525-527.
222. Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L., *J. Amer. Chem. Soc.* **1984,** 106, 1526-1528.
223. Kobayashi, J.; Taniguchi, M.; Hino, T.; Ohizumi, Y., *Journal of Pharm. and Pharmac.* **1988,** 40, 62-63.
224. Davis, R. A.; Carroll, A. R.; Quinn, R., *J. Nat. Prod.* **1998,** 61, 959-960.
225. Rinehart, K. L.; Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F., *J. Amer. Chem. Soc.* **1987,** 109, 3378-3387.
226. Kinzer, K. F.; Cardellina, J. H., *Tetrahedron lett.* **1987,** 28, 925-926.
227. VanWagenen, B. C.; Cardellina, J. H., *Tetrahedron lett.* **1989,** 30, 3605-3608.

228. Badre, A.; Boulanger, A.; Abou-Mansour, E.; Banaigs, B.; Combaut, G.; Franscisco, C., *J. Nat. Prod.* **1994,** 57, 528-533.
229. Massiot, G.; Nazabadioko, S.; Bliard, C., *J. Nat. Prod.* **1995,** 58, 1636-1639.
230. Kang, H.; Fenical, W., *Nat. Prod. lett.* **1996,** 9, 7-12.
231. Debitus, C.; Laurent, D.; Pais, M., *J. Nat. Prod.* **1988,** 51, 799-801.
232. Chbani, M.; Pais, M.; Delaunaux, J.-M.; Debitus, C., *J. Nat. Prod.* **1992,** 55, 525-527.
233. Hirota, H.; Tomono, Y.; Fusetani, N., *Tetrahedron* **1996,** 52, 2359-2368.
234. Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J., *Tetrahedron Lett.* **1976,** 30, 2631-2634.
235. Dawe, C. J.; Potter, M., *Amer. J. Path.* **1957,** 33, 603-610.
236. Wonganuchitmeta, S.; Yuenyongsawad, S.; Keawpradub, N.; Plubrukarn, A., *J. Nat. Prod.* **2004,** 67, 1767-1770.
237. Boyd, M. R., *PPO updates.* **1989,** 3, 1-12.

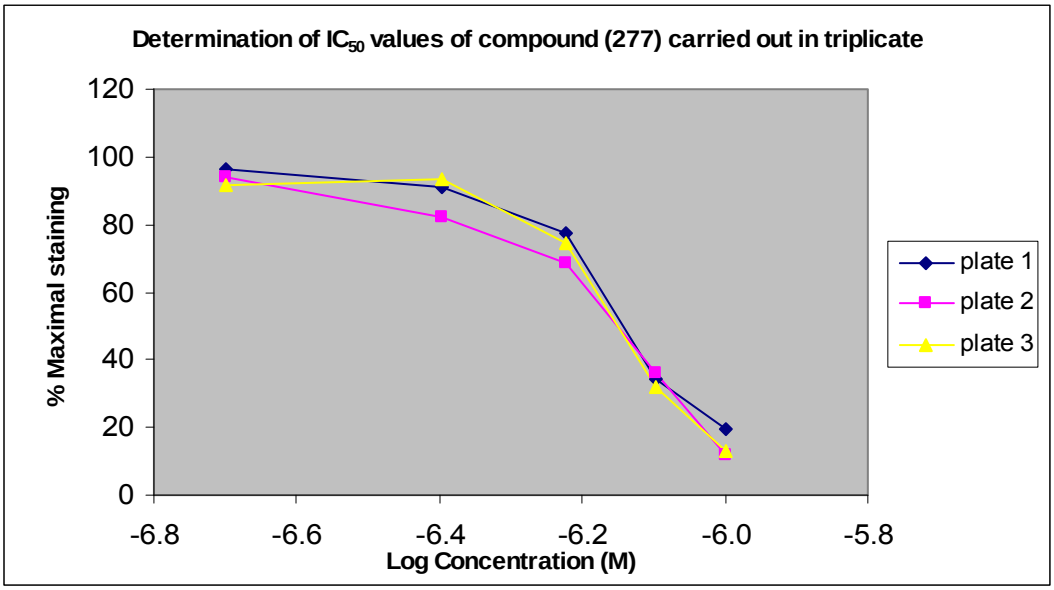
APPENDICES

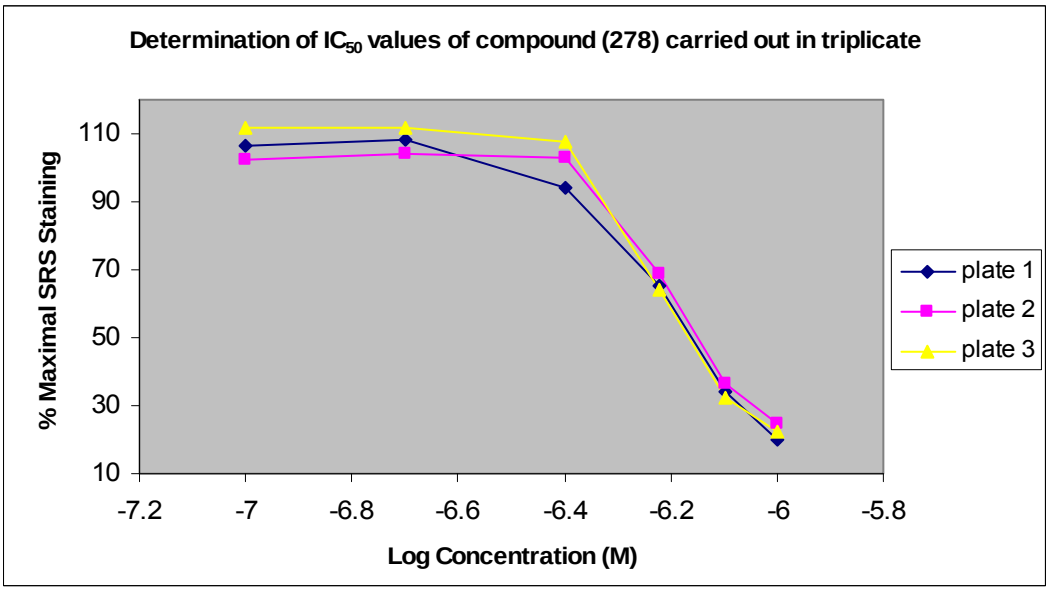
APPENDIX 1.1


Determination of IC50 values of compound (357) carried out in triplicate
% Maximal SRB Staining
100
80
60
40
20
0
-7.2
-7
-6.8
-6.6
-6.4
-6.2
-6
-5.8
Log Concentration (M)
plate 1
plate 2
plate 3

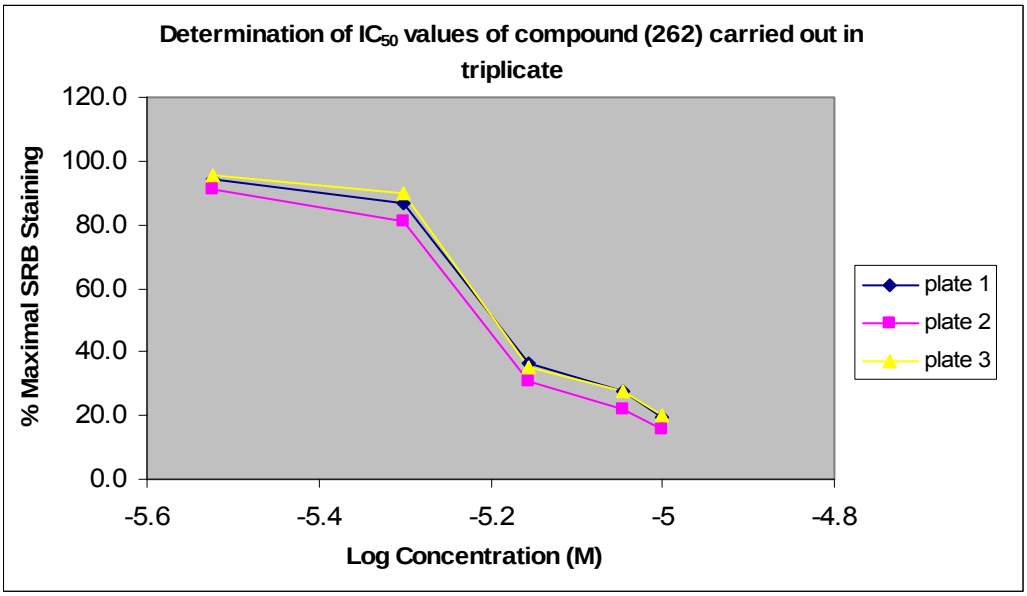

Determination of IC50 values of compound (361) carried out in triplicate
% Maximal SRB Staining .
100
80
60
40
20
0
-5.2
-5
-4.8
-4.6
-4.4
-4.2
-4
-3.8
Log Concentration (M)
plate 1
plate 2
plate 3

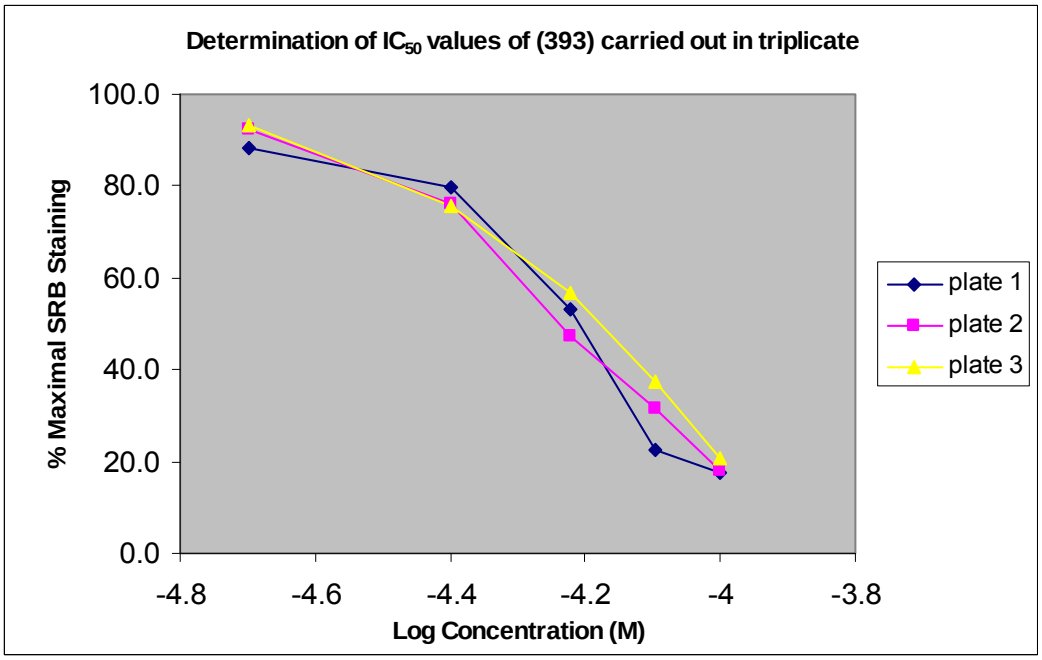
APPENDIX 1.2


Determination of IC50 values of compound (277) carried out in triplicate
% Maximal staining
120
100
80
60
40
20
0
-6.8
-6.6
-6.4
-6.2
-6.0
-5.8
Log Concentration (M)
plate 1
plate 2
plate 3

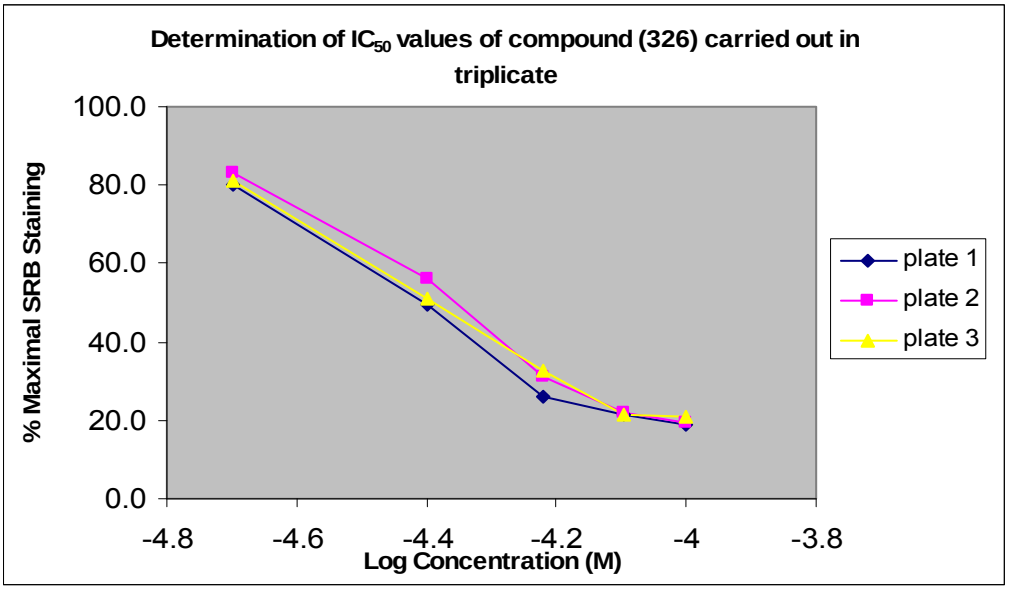

Determination of IC50 values of compound (278) carried out in triplicate
% Maximal SRS Staining
110
90
70
50
30
10
-7.2
-7
-6.8
-6.6
-6.4
-6.2
-6
-5.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 1.3


Determination of IC50 values of compound (262) carried out in triplicate
% Maximal SRB Staining
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-5.6
-5.4
-5.2
-5
-4.8
Log Concentration (M)
plate 1
plate 2
plate 3


Determination of IC50 values of (393) carried out in triplicate
% Maximal SRB Staining
100.0
80.0
60.0
40.0
20.0
0.0
-4.8
-4.6
-4.4
-4.2
-4
-3.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 1.4


Determination of IC50 values of compound (326) carried out in triplicate
% Maximal SRB Staining
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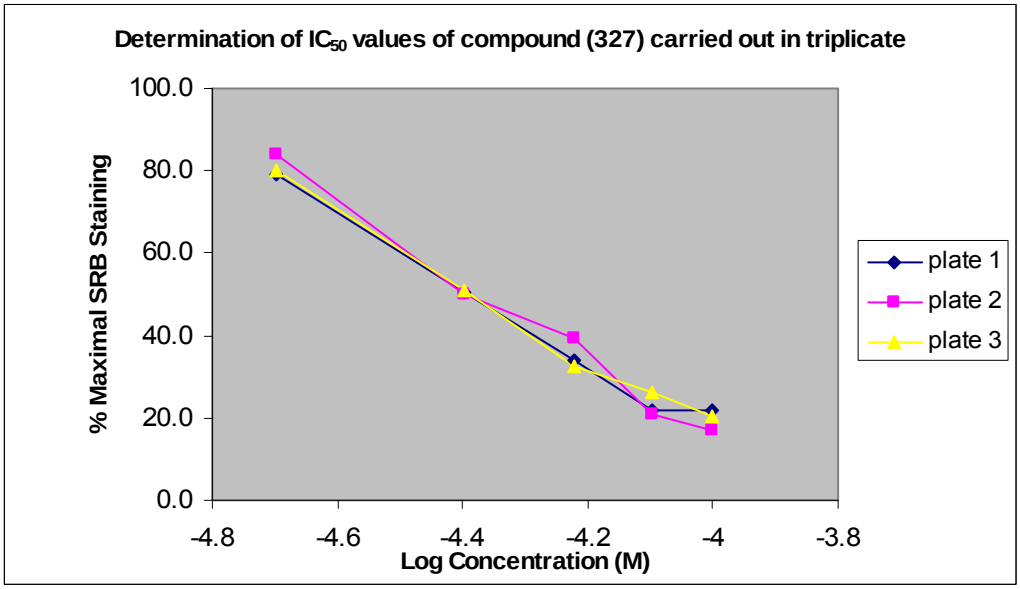
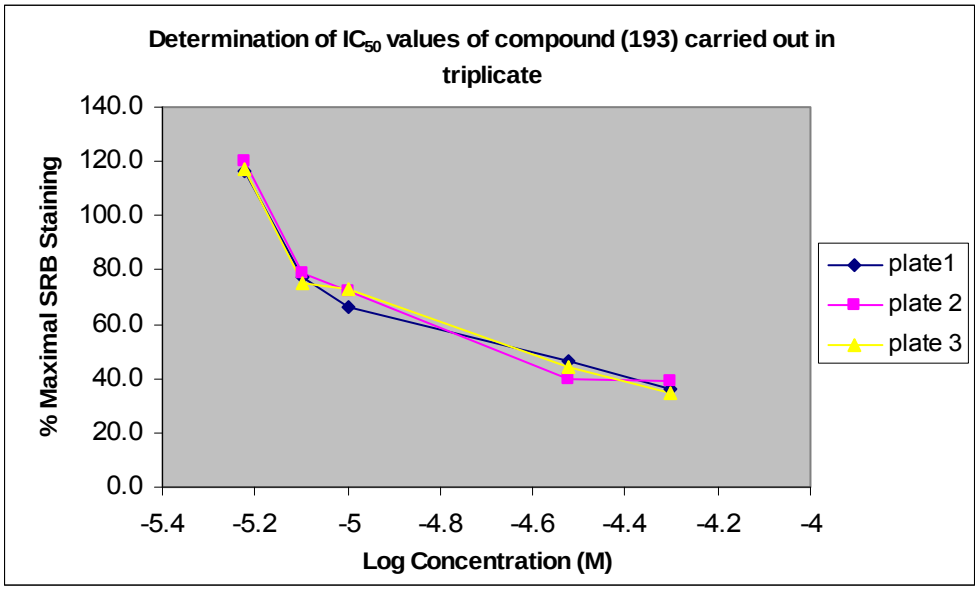
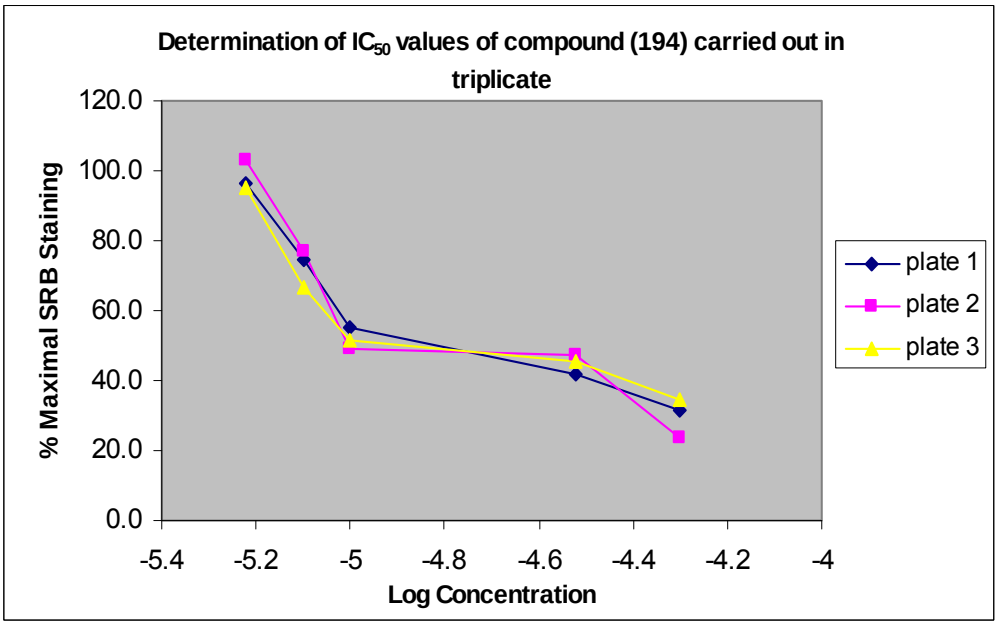
80.0
60.0
40.0
20.0
0.0
-4.8
-4.6
-4.4
-4.2
-4
-3.8
Log Concentration (M)
plate 1
plate 2
plate 3

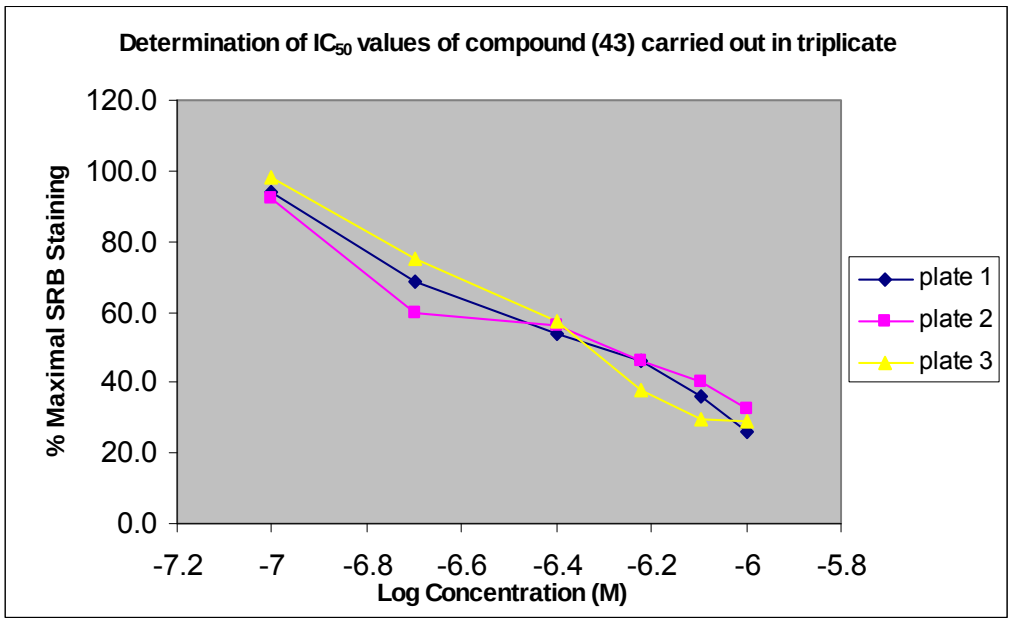

Determination of IC50 values of compound (327) carried out in triplicate
% Maximal SRB Staining
100.0
80.0
60.0
40.0
20.0
0.0
-4.8
-4.6
-4.4
-4.2
-4
-3.8
Log Concentration (M)
plate 1
plate 2
plate 3

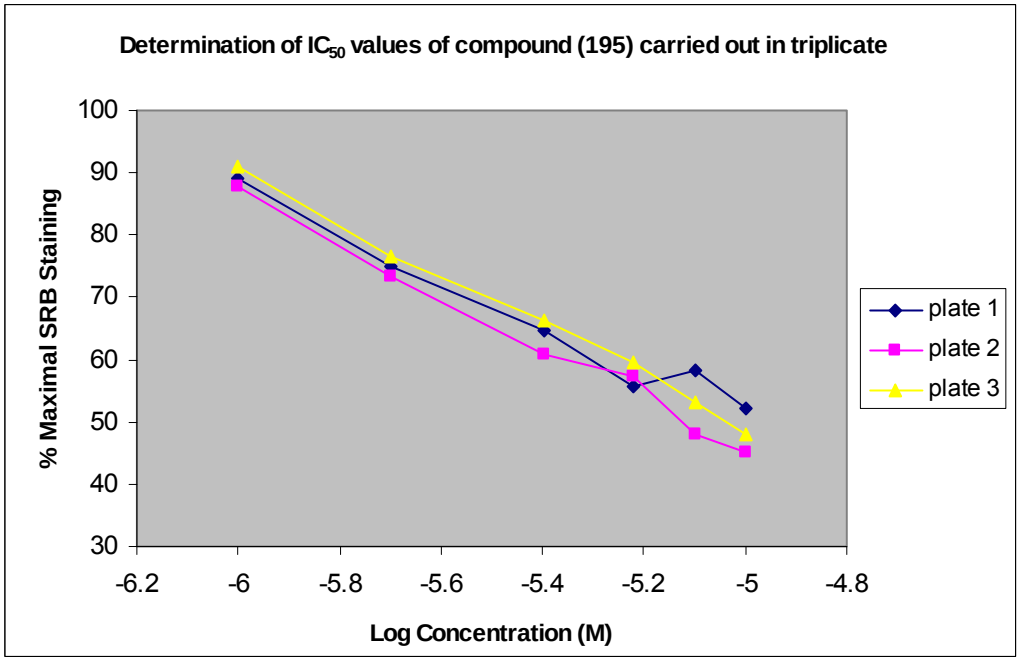
APPENDIX 1.5


Determination of IC50 values of compound (193) carried out in triplicate
% Maximal SRB Staining
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-5.4
-5.2
-5
-4.8
-4.6
-4.4
-4.2
-4
Log Concentration (M)
plate1
plate 2
plate 3

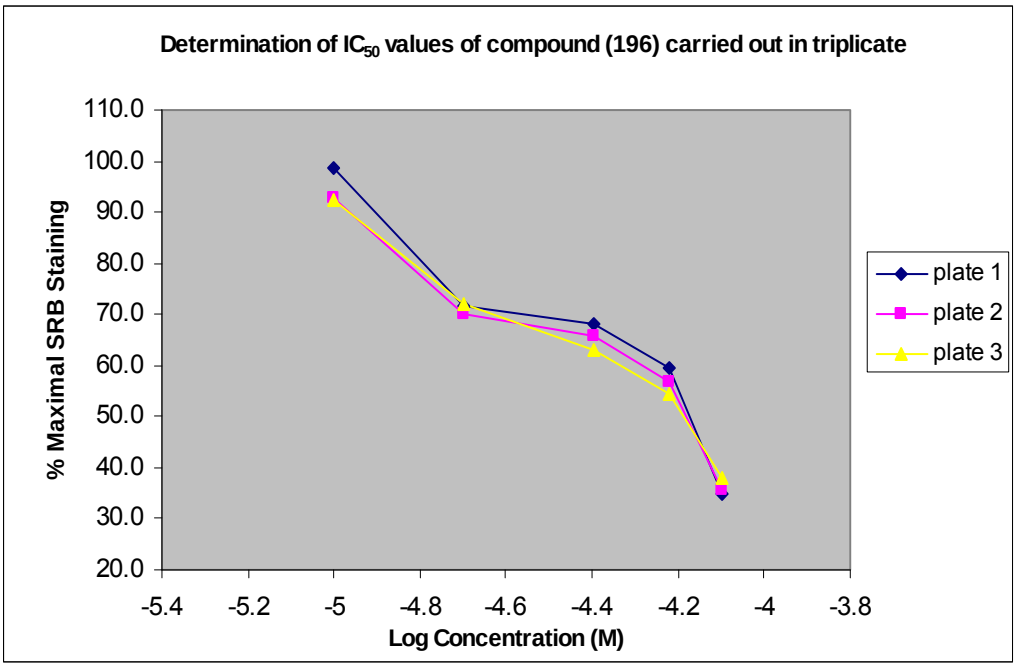

Determination of IC50 values of compound (194) carried out in triplicate
% Maximal SRB Staining
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-5.4
-5.2
-5
-4.8
-4.6
-4.4
-4.2
-4
Log Concentration
plate 1
plate 2
plate 3

APPENDIX 1.6

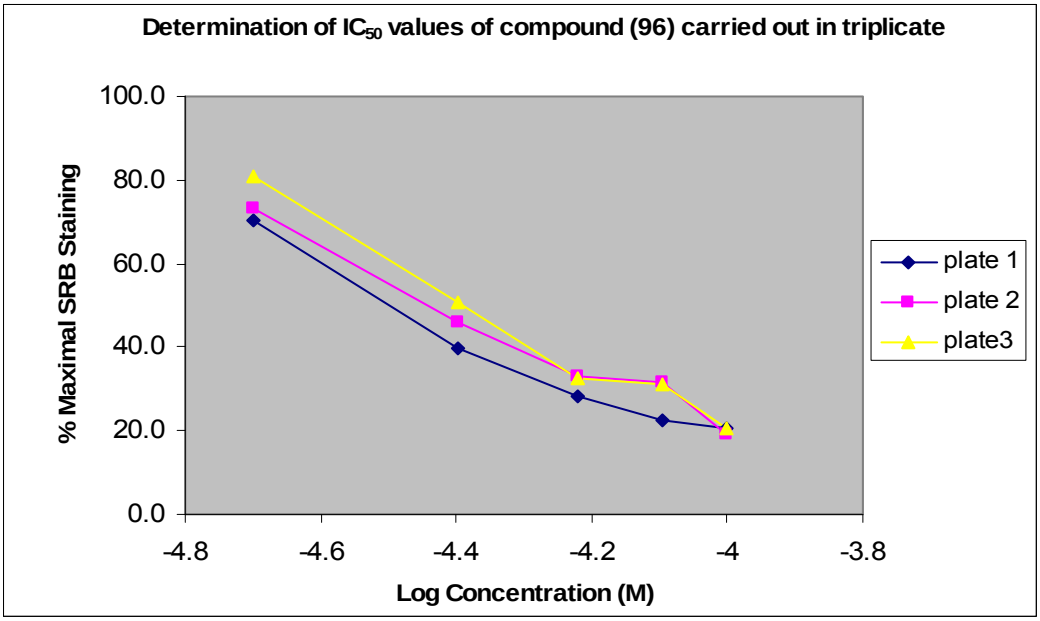

Determination of IC50 values of compound (43) carried out in triplicate
% Maximal SRB Staining
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-7.2
-7
-6.8
-6.6
-6.4
-6.2
-6
-5.8
Log Concentration (M)
plate 1
plate 2
plate 3

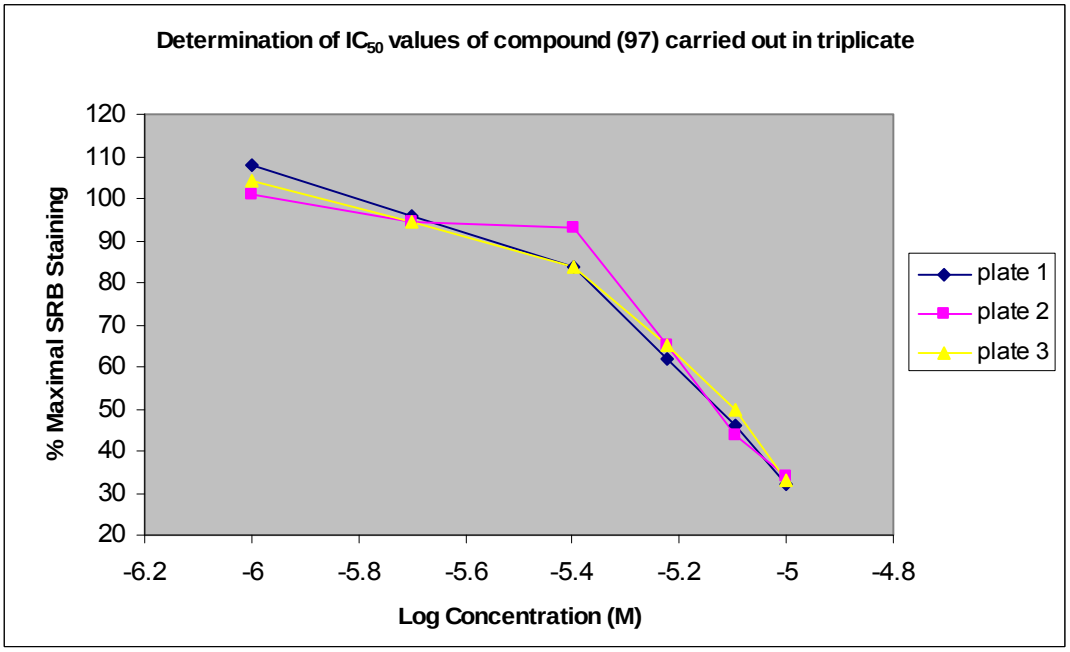

Determination of IC50 values of compound (195) carried out in triplicate
% Maximal SRB Staining
100
90
80
70
60
50
40
30
-6.2
-6
-5.8
-5.6
-5.4
-5.2
-5
-4.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 1.7

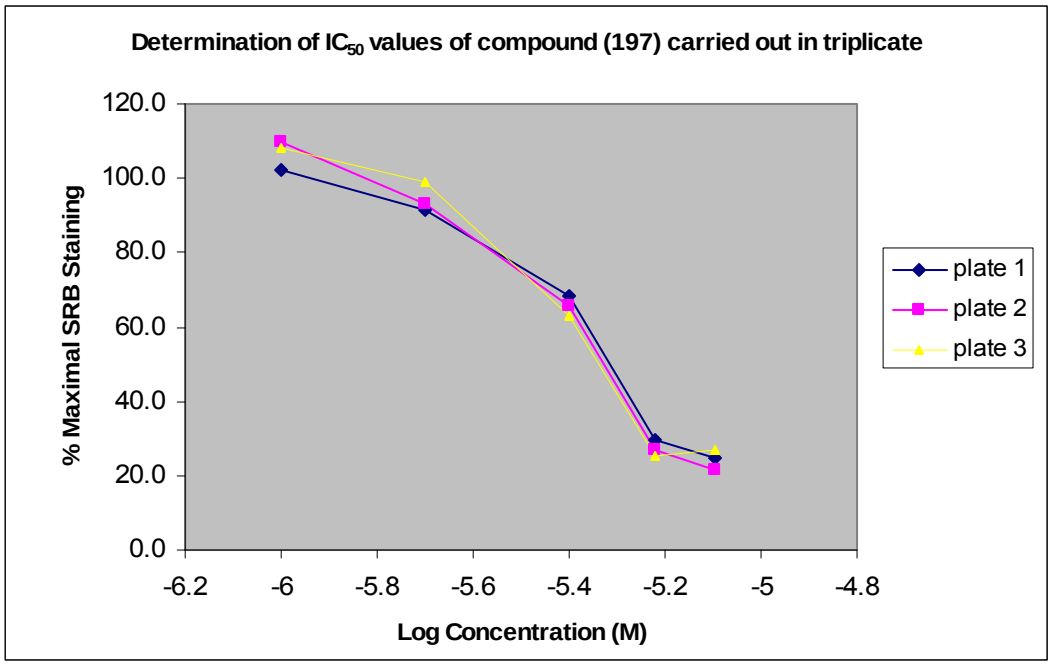

Determination of IC50 values of compound (196) carried out in triplicate
% Maximal SRB Staining
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
-5.4
-5.2
-5
-4.8
-4.6
-4.4
-4.2
-4
-3.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 1.8

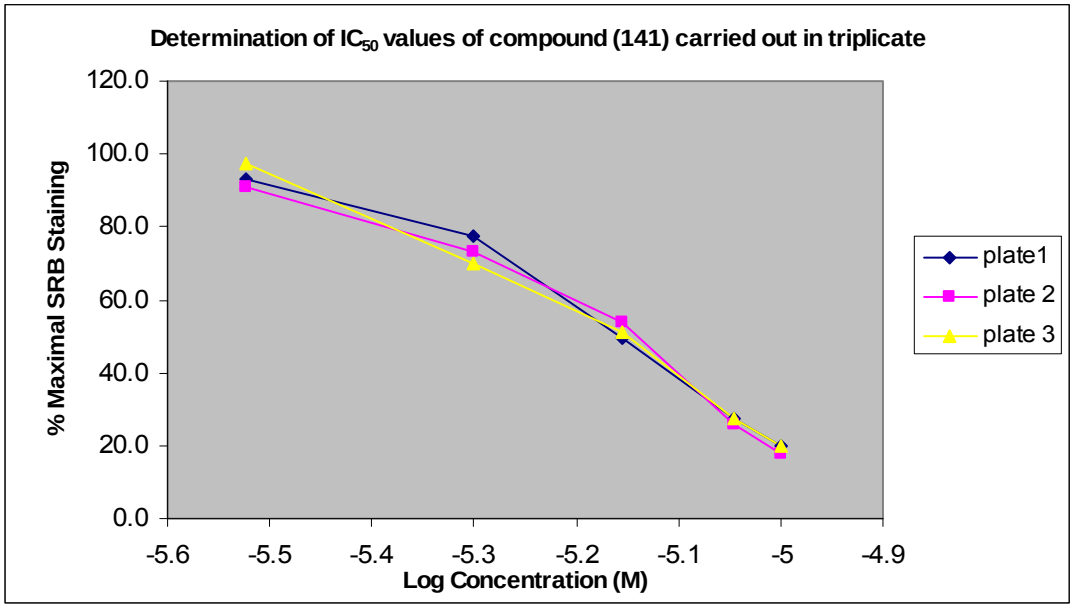

Determination of IC50 values of compound (96) carried out in triplicate
% Maximal SRB Staining
100.0
80.0
60.0
40.0
20.0
0.0
-4.8
-4.6
-4.4
-4.2
-4
-3.8
Log Concentration (M)
plate 1
plate 2
plate3

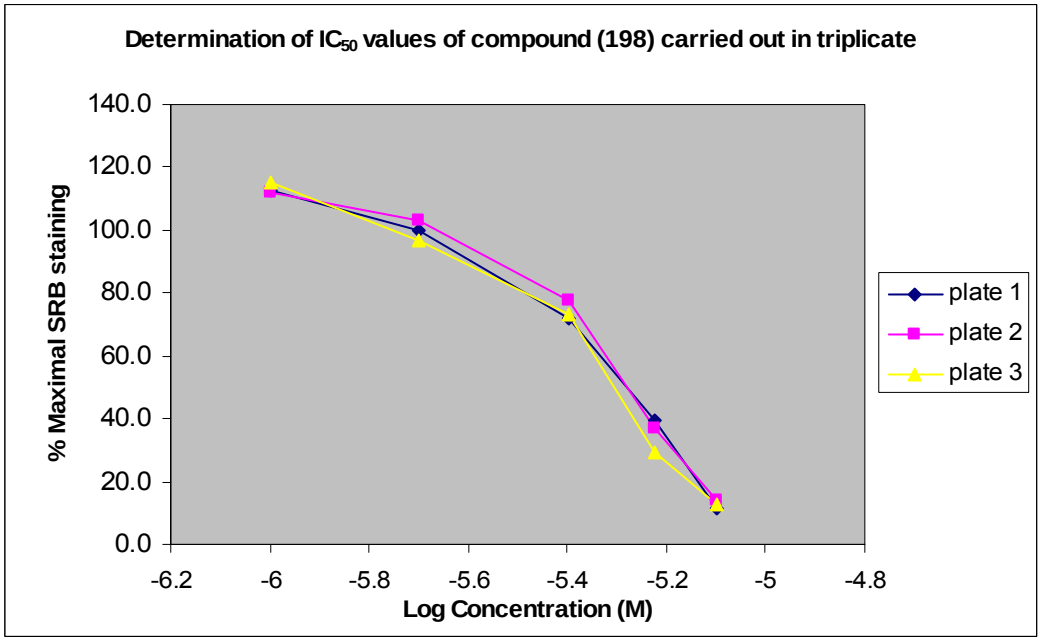

Determination of IC50 values of compound (97) carried out in triplicate
% Maximal SRB Staining
120
110
100
90
80
70
60
50
40
30
20
-6.2
-6
-5.8
-5.6
-5.4
-5.2
-5
-4.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 1.9


Determination of IC50 values of compound (197) carried out in triplicate
% Maximal SRB Staining
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-6.2
-6
-5.8
-5.6
-5.4
-5.2
-5
-4.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 2.0


Determination of IC50 values of compound (141) carried out in triplicate
% Maximal SRB Staining
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-5.6
-5.5
-5.4
-5.3
-5.2
-5.1
-5
-4.9
Log Concentration (M)
plate1
plate 2
plate 3


Determination of IC50 values of compound (198) carried out in triplicate
% Maximal SRB staining
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-6.2
-6
-5.8
-5.6
-5.4
-5.2
-5
-4.8
Log Concentration (M)
plate 1
plate 2
plate 3

APPENDIX 2.1

Catalogue no	Taxonomy	Organic extract		Aqueous extract	
		25µg/ml	5µg/ml	25µg/ml	5µg/ml
2001	sponge	active	inactive		
2008	ascidian	active	inactive		
2009	ascidian	inactive	inactive		
2011	green alga	active	inactive	inactive	inactive
2012	green alga	active	inactive	inactive	inactive
2013	green alga	active	inactive	inactive	inactive
2014	sponge	inactive	inactive	inactive	inactive
2015	green alga	active	inactive	inactive	inactive
2023	soft coral	inactive	inactive		
2024	sponge	inactive	inactive		
2025	ascidian	inactive	inactive	inactive	inactive
2026	sponge	inactive	inactive		
2031	soft coral	inactive	inactive		
2032	sponge	active	inactive		
2033	sponge	active	inactive		
2034	bryozoan	active	inactive		
2035	sponge	active	inactive		
2036	soft coral	active	inactive		
2038	sponge	active	inactive	inactive	inactive
2039	alga	active	inactive	inactive	inactive
2040	ascidian	active	inactive		
2041	ascidian	active	inactive		
2042	sponge	active	inactive		
2043	sponge	active	inactive		
2044	green alga	active	inactive		
2045	soft coral	active	inactive		
2046	cyanobacteria	active	inactive		
2047	green alga	active	inactive		
2049	sponge	active	inactive	inactive	inactive
2050	soft coral	active	inactive		
2051	sponge	active	inactive		
2052	ascidian	active	inactive		
2053	sponge	active	inactive		
2054	alga	active	active		
2055	sponge	active	active		
2056	ascidian	active	active		
2057	ascidian	active	inactive		
2058	soft coral	active	inactive		
2059	ascidian	inactive	inactive		
2060	soft coral	active	inactive		
2061	sponge	inactive	inactive		
2062	sponge	active	inactive		
2063	ascidian	active	inactive	inactive	inactive
2064	sponge	active	active	inactive	inactive

2065	sponge	active	inactive	inactive	inactive
2069	ascidian	active	inactive	inactive	inactive
2070	soft coral	active	inactive	inactive	inactive
2071	soft coral	active	inactive		
2072	ascidian	inactive	inactive		
2073	green alga	inactive	inactive		
2074	sponge	inactive	inactive		
2077	sponge	inactive	inactive		
2079	ascidian	active	inactive		
2080	sponge	active	inactive		
2084	sponge	active	active	inactive	inactive
2085	bryozoan	active	inactive	inactive	inactive
2088	sponge	inactive	inactive		
2089	soft coral	inactive	inactive		
2097	soft coral	inactive	inactive		
2098	sponge	inactive	inactive		
2099	sponge	inactive	inactive		
2101	sponge	inactive	inactive		
2106	ascidian	inactive	inactive		
2107	soft coral	inactive	inactive		
2108	sponge	inactive	inactive	inactive	inactive
2112	green alga	inactive	inactive		
2114	sponge	inactive	inactive		
2115	sponge	inactive	inactive		
2117	ascidian	inactive	inactive		
2120	ascidian	active	inactive		
2121	ascidian	inactive	inactive		
2122	sponge	active	inactive		
2124	ascidian	inactive	inactive		
2125	ascidian	active	inactive		
2126	ascidian	active	inactive		
3003	gorgonian	active	inactive		
3004	gorgonian	active	inactive		
3006	hard coral	active	inactive		
3007	green alga	active	active		
3008	cyanobacteria	active	inactive	inactive	inactive
3009	sponge	active	active		
3011	soft coral	active	inactive		
3015	sponge	inactive	inactive		
3017	ascidian	active	inactive		
3018	ascidian	inactive	inactive		
3021	soft coral	active	inactive		
3022	sponge	active	inactive	inactive	inactive
3023	sponge	active	inactive	inactive	inactive
3024	sponge	active	inactive		
3026	sponge	inactive	inactive		
3027	sponge	active	inactive		
3028	sponge	active	inactive	inactive	inactive
3033	ascidian	active	inactive		
3034	sponge	active	inactive		
3035	sponge	active	inactive		

3036	sponge	active	active		
3037	sponge	active	inactive	inactive	inactive
3040	sponge	inactive	inactive	inactive	inactive
3041	sponge	inactive	inactive	inactive	inactive
3042	ascidian	inactive	inactive		
3046	gorgonian	inactive	inactive		
3049	green alga	active	active		
3050	soft coral	inactive	inactive		
3051	sponge	inactive	inactive		
3052	sponge	active	inactive		
3053	soft coral	inactive	inactive		
3054	soft coral	active	inactive		
3056	soft coral	inactive	inactive		
3057	sponge	active	inactive		
3058	sponge	inactive	inactive		
3059	sponge	active	inactive		
3060	sponge	inactive	inactive		
3061	sponge	active	active		
3062	sponge	inactive	inactive		
3063	sponge	inactive	inactive		
3064	sponge	active	inactive		
3065	ascidian	active	inactive		
3066	sponge	active	inactive		
3068	sponge	active	inactive		
3069	soft coral	inactive	inactive		
3070	gorgonian	inactive	inactive		
3071	sponge	inactive	inactive		
3073	sponge	active	inactive		
3074	ascidian	active	inactive		
3077	gorgonian	active	active		
3078	ascidian	inactive	inactive		
3079	sponge	inactive	inactive		
3080	sponge	inactive	inactive		
3082	soft coral	inactive	inactive		
3088	sponge	active	inactive		
3089	sponge	inactive	inactive		
3090	gorgonian	inactive	inactive		
3091	sponge	inactive	inactive	inactive	inactive
3092	sponge	inactive	inactive	inactive	inactive
3095	sponge	inactive	inactive	inactive	inactive
3096	sponge	inactive	inactive	inactive	inactive
3099	sponge	inactive	inactive		
3100	sponge	inactive	inactive		
3109	sponge	inactive	inactive		
3110	sponge	inactive	inactive		
3111	ascidian	inactive	inactive		
3124	sponge	inactive	inactive		
3130	sponge	inactive	inactive		
3133	soft coral	active	inactive		
3142	gorgonian	inactive	inactive		
3142	ascidian	inactive	inactive		

3152	sponge	active	inactive		
3164	sponge	active	inactive		
3165	sponge	active	inactive		
3175	sponge	inactive	inactive		
3182	sponge	inactive	inactive		
3188	sponge	inactive	inactive		
3195	green alga	inactive	inactive		
3204	gorgonian	inactive	inactive		
3205	sponge	active	inactive		
3206	sponge	active	inactive		
3210	alga	active	inactive		
3214	ascidian	active	inactive	inactive	inactive
3216	sponge	inactive	inactive		
3219	sponge	active	inactive		
3221	sponge	inactive	inactive		
3222	sponge	inactive	inactive		
3226	sponge	inactive	inactive		
3230	sponge	inactive	inactive		
3231	sponge	active	inactive		
3232	soft coral	active	inactive		
3233	sponge	active	inactive		
3233	sponge	active	inactive		
3235	sponge	active	inactive		
3236	sponge	active	inactive		
3237	soft coral	active	inactive		
3238	sponge	active	inactive		
3239	sponge	active	inactive		
3240	sponge	active	inactive		
3242	sponge	active	inactive		
3243	sponge	inactive	inactive	inactive	inactive
3243	sponge	inactive	inactive	inactive	inactive
3244	sponge	inactive	inactive		
3247	sponge	active	inactive		
3248	soft coral	active	inactive		
3249	soft coral	active	inactive		
3250	sponge	active	inactive		
3251	heliopora	active	inactive		
3256	sponge	active	inactive		
3257	sponge	inactive	inactive		
3258	sponge	inactive	inactive		
3262	sponge	active	inactive		
3263	ascidian	active	inactive		
3265	sponge	active	inactive		
3266	soft coral	active	inactive		
3267	sponge	active	inactive		
3268	sponge	inactive	inactive		
3269	sponge	inactive	inactive	inactive	inactive
3271	sponge	inactive	inactive		
4001	ascidian	inactive	inactive		
4002	sponge	active	inactive		
4003	ascidian	active	inactive		

4005	ascidian	active	inactive		
4006	ascidian	active	inactive		
4007	sponge	inactive	inactive		
4008	sponge	inactive	inactive		
4009	sponge	inactive	inactive		
4010	soft coral	inactive	inactive		
4011	sponge	inactive	inactive		
4012	sponge	active	inactive		
4017	sponge	inactive	inactive		
4018	ascidian	inactive	inactive		
4019	gorgonian	active	inactive		
4020	ascidian	inactive	inactive	inactive	inactive
4021	sponge	inactive	inactive	inactive	inactive
4022	sponge	inactive	inactive		
4023	sponge	inactive	inactive		
4024	sponge	active	inactive		
4025	sponge	inactive	inactive		
4026	sponge	inactive	inactive		
4027	sponge	inactive	inactive		
4028	sponge	active	inactive		
4029	sponge	inactive	inactive		
4030	sponge	active	inactive		
4031	sponge	active	inactive		
4032	sponge	active	inactive		
4033	sponge	active	inactive		
4034	ascidian	inactive	inactive		
4035	sponge	inactive	inactive		
4036	sponge	inactive	inactive		
4037	sponge	inactive	inactive		
4038	sponge	inactive	inactive		
4039	sponge	active	inactive		
3039	sponge	active	inactive		
4040	sponge	active	inactive		
2067	sponge	active	inactive		
4041	sponge	active	inactive		
4042	alga	active	inactive		
4043	sponge	active	inactive		
4044	ascidian	inactive	inactive		
4045	soft coral	active	inactive		
4046	gorgonian	inactive	inactive		
4047	sponge	inactive	inactive		
4048	soft coral	inactive	inactive		
4049	soft coral	active	inactive		
4050	sponge	inactive	inactive		
4051	sponge	inactive	inactive		
4053	sponge	inactive	inactive		
4054	sponge	inactive	inactive		
4058	sponge	inactive	inactive		
4060	sponge	inactive	inactive		
4061	sponge	inactive	inactive		
4062	sponge	active	inactive		

4063	sponge	inactive	inactive		
4065	sponge	inactive	inactive		
4066	red alga	active	active		
4068	sponge	inactive	inactive		
4069	sponge	active	inactive		
4070	sponge	inactive	inactive		
4071	ascidian	active	inactive		
4072	soft coral	active	inactive		
4073	gorgonian	inactive	inactive		
4074	sponge	active	inactive		
4075	sponge	inactive	inactive		
4076	sponge	active	inactive	inactive	inactive
4077	gorgonian	active	inactive		
4078	gorgonian	active	inactive		
4079	cyanobacteria	active	inactive		
4080	soft coral	active	inactive		
4081	sponge	active	inactive		
4082	sponge	inactive	inactive		
4083	sponge	active	inactive		
4084	sponge	active	inactive		
4086	soft coral	active	inactive		
4090	sponge	active	inactive		
4091	soft coral	inactive	inactive		

Table 12. Crude extracts of all the specimen extracts tested in this study at the two concentrations (25µg/ml and 5µg/ml) in the P388D1 assay. Blank rows indicate that the extracts were not tested at the respective concentrations.