

Discovery of cytotoxic natural products from Red Sea sponges: Structure and synthesis

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Abstract:

Marine ecosystem continues to produce a great wealth of molecules endowed with cytotoxic, activity towards a large panel of tumor cells. Marine sponges, apparently defenseless organisms are endowed through evolution with a range of cytotoxic metabolites for self protection against predators and space competition. Interestingly, high biodiversity of sponges with demospongiae and *Calcarea* species that have yielded numerous bioactive compounds have been accorded in different regions of the Red Sea. This review for the first time provides a comprehensive overview of 123 cytotoxic agents derived from Red Sea sponges with diverse chemical structures covered till mid 2020 showing activities ranging from mildly active to very active against different panels of cancer cell lines. It has been divided according to the different classes of compounds including alkaloids, terpenoids (sesquiterpenes, diterpenes, triterpenes, sesterterpenes, norsesterterpenes), peptides and macrolides, lipids (steroids, fatty acids/amides and glycerides) etc. The enhancement in the cytotoxicity with respect to the molecular structure changes have been described in detail. We have also accounted total synthesis of cytotoxic molecules, subreamolline A, aerothionin, asmarine B, norrsolide and latrunculin B showing interesting activity against different cancer cell lines.

Key words: Cancer cell lines, Cytotoxicity, Marine Natural Products, Red Sea, Sponges

1. Introduction

Globally, cancer is the second deadliest disease that has gained huge attention by researchers who are trying to uncode its hidden aspects and to find different molecules for its effective treatment. Many such drug discovery programs are focusing on the exploration of natural products having rich source of molecular scaffolds with strong track record in the development of anti-cancer agents [1]. Marine sponges that belong to Phylum Porifera are known as the oldest multicellular animals being the richest source of pharmaceutical potential [2]. Marine sponges being sessile organisms, commonly soft bodied are master producers of cytotoxic secondary metabolites. Thereby, they are capable of inhibiting the growth of the surrounding competing organisms by diminishing their cell division, thus, taking advantage during space competition on a crowded reef substratum [3]. Hence, in sponges cytotoxicity of the compounds can be considered as a good proxy for their ability to overgrow in the coral fields and thus having potency to prevent or inhibit the growth of cancer cells which can be used for new drug development [4]. Anticancer drug development begins with the cytotoxicity assays of screening compounds against different cancer cell lines, as the ideal way to cure cancer is to kill all tumor cells and leave normal cells alive. Cytotoxicity assays are indeed quick methods to ascertain whether the molecule is effective against given human cancer cell lines. There are various assays described like dye exclusion, colorimetric, fluorometric and luminometric assays [5]. The most widely used is MTT colorimetric assay to assess the efficacy and interactions of anticancer agents. It is an indirect method that determines cell viability by effectively using the ability of living cells to catalyse reactions, affording measurable product where number of living cells is proportional to the quantity of the product [6]. The direct CVS method measures the DNA mass of living cells [7]. There are several papers that describe the differences between cytotoxicity and cell viability/proliferation test methods for e.g., Alamar blue, XTT, MTT, CVS, Trypan blue, CellTiter-Glo etc [8,9].

A synthetic analog, cytosine arabinoside (Ara C) of spongothymidine and spongouridine which was the first example of isolation from the Caribbean sponge *Tethya crypta* has led to the clinical anticancer agent [10]. Another FDA approved drug HalavenTM (*Eribulin mesylate*), a synthetic analogue of halichondrin B was isolated from the sponge *Halichondria okadai* [11]. Clinically, it shown potential activity against pre-treated metastatic breast cancer cells [12]. Till date among marine community, predominantly, marine sponges have been known to contribute around 30% of total natural products discovered [13]. Numerous reports have been found covering various biological activities of marine natural products from sponges found in different environmental and geographic conditions around the globe [14,15,16]. The Red Sea is the world's northern most tropical sea which acts as an inlet of the Indian Ocean, lying between Africa and Asia. It has a surface area of roughly 450,000 km² and is about

2250 km long, with a maximum depth of around 3000 m. The Red Sea is a unique body of water with extremely high temperatures (20-32°C) and salinity (37-42‰) [17]. Salinity-wise, the Red Sea is approximately 5% greater than the world's average salinity due to high rate of evaporation and lack of significant rivers or streams draining into it. It is inhabited by over 1000 invertebrate species, and 200 soft and hard corals. Thus, it is likely that Red Sea sponges produce different natural products as compared to the rest of the seas around the world. It constitutes one of the most promising area, being an extraordinary resource of pharmaceutical natural products as it harbours a diverse community of sponge species [18]. About 240 species of sponges have been recorded from the Red Sea by Michailan, 2004 [19]. More updates on the biodiversity of Red Sea sponges comprising of Demospongiae (225 species), Calcarea (32 species) and Homoscleromorpha (2 species) have been listed along with their distribution by Wooster et al. in 2019. Review papers describing novel anticancer drugs from marine sources [20] and cytotoxic compounds from sponge derived from micro-organisms have been previously reported in the literature [21]. Review article by El-Ezz et al (2017) covered natural products together with their biological activities isolated from Red Sea until year 2014 and described according to their chemical classes, including terpenes, alkaloids, sterols, steroidal glycosides and other metabolites [22]. Recently, Ebaa et al. (2020) presented comprehensive literature search until December 2019, with the inclusion of extra five years (from 2015 to 2019). They have described a total of 111 marine natural products from Red Sea marine organisms until the end of 2019 with potential biological activities [23]. However, there is no dedicated literature review reporting cytotoxic compounds isolated from Red Sea-sponges. In this review, we have presented cytotoxic compounds isolated from Red Sea sponges until mid of 2020 and described them according to the different classes of compounds they belong to like alkaloids, terpenoids, peptides and macrolides, lipids etc. and accounted for the total synthesis of some active molecules.

2. Cytotoxic natural products from Red Sea sponges

2.1 Alkaloids

Alkaloids are cyclic organic compounds containing nitrogen in a negative oxidation state and having limited distribution among living organisms [24]. Bromotyrosine derived alkaloids, subereamolline A (**1**) and aerotionin (**2**) were isolated from the sponge *Suberea mollis* (Hurghada, Egyptian Red Sea coast) [25]. Subereamolline A (**1**) exhibited strong cytotoxicity against migration and invasion of the breast adenocarcinoma cells (MDA-MB-231) (**Figure 1, Table 1**) [26]. The anti-migratory and anti-invasive activities were assessed using wound healing and cultures® BME cell invasion assay,

respectively. The activity was compared to a 30 μM dose of the antimetastatic lead 4-*S*-ethylphenylmethylene hydantoin (*S*-ethyl). Subereamolline A (**1**) showed significant anti-migratory activity at 10 μM . The percent migration of the MDA-MB-231 was about 52% with the expected IC_{50} value being around 0.4 μM . It displayed higher anti-migratory activity with the lower number of the cells migrated into the wound. The anti-invasive activity showed that subereamolline A (**1**) was cytotoxic at 2 μM dose showing 38% invasion of BME cells with IC_{50} value 1.7 μM , while, aerothionin (**2**) with terminal 1-oxa-2-azaspiro[4.5]deca-2,6,8-trienecarboxamide group at each side showed no activity. This clearly indicated the importance of the terminal ethyl carbamate moiety. It may also be that bulky 1-oxa-2-azaspiro[4.5]deca-2,6,8-trienecarboxamide group in aerothionin (**2**) may be hindering the binding at the target receptor. This displays the possibility of this potential compound as an excellent scaffold for the future design of breast cancer migration and invasion inhibitors. Another *Suberea* species collected off Yanbu yielded alkaloid aerothionin (**2**) along with other compounds aeroplysinin-2 (**3**) and subereaphenol C (**4**) [27]. Aeroplysinin-2 (**3**) was evaluated against MDA-MB-231 by wound healing assay which showed significant anti-migratory activity with IC_{50} 18 μM , as compared to 43.4 μM showed by the positive control Z-4-ethylthio-phenylmethylene hydantoin (*S*-Ethyl). Cervical carcinoma HeLa cell proliferation and cytotoxicity of compound (**3**) and (**4**) were evaluated using sulforhodamine B (SRB) assay. Aerothionin (**2**) and subereaphenol C (**4**) showed strong anti-proliferation activity against HeLa cell line with IC_{50} values of 29 and 13.3 μM , respectively. The IC_{50} value indicated that the isooxazoline ring system decreases anti-proliferative activity in aerothionin (**2**).

In the recent investigation by Drechsel, 2020 [28] anti-tumor activity of bromotysine alkaloids, aerothionin (**2**) and homoaerothionin on two mouse pheochromocytoma cells, MPC and MTT showed their promising application as anti-tumor agents. Aerothionin reduced the relative viability (RV) of MPC mouse pheochromocytoma cells after 24 h treatment, significantly starting with a concentration of 25 μM ($\text{RV}_{25\mu\text{M}} = 83.7 \pm 1.7\%$; $\text{RV}_{50\mu\text{M}} = 55.1 \pm 2.2\%$). Treatment with 10 μM , aerothionin diminished the number of proliferating cells under normoxic conditions significantly, while MPC cells under hypoxic conditions were not affected. In the MTT pheochromocytoma cell line, aerothionin showed a more aggressive cell behavior. Treatment with at least 25 μM ($\text{RV}_{25\mu\text{M}} = 82.8 \pm 3.9\%$; $\text{RV}_{50\mu\text{M}} = 48.3 \pm 4.0\%$) diminished RV under normoxic conditions significantly resulting in a half-maximal effective concentration (EC_{50}) of 48.1 μM in these cells. The study on 3D-pheochromocytoma cell spheroids, showed significant reduction or even complete inhibition of the spheroid growth when treated with 10 μM of aerothionin. The study also revealed acceptable toxicity

against normal tissue cells. The researchers suggest having continued investigation of this bromotyrosine to focus on the identification of the precise mechanism-of-action leading to the anti-tumor activity.

Shaala and Youssef (2019) reported brominated alkaloids, psammaplysin A (**5**), E (**6**), Z (**7**) and 19-hydroxypsammaplysin Z (**8**) from Red Sea sponge *Aplysinella* Species displaying potent cytotoxic activity [29]. The cytotoxic activities of compounds (**5–8**), were screened in an MTT assay against three human cancer cell lines, including MDA-MB-231, HeLa and colorectal carcinoma (HCT 116). Psammaplysin E (**6**) displayed the strongest activity against MDA-MB-231, HeLa and HCT-116 cell lines with IC₅₀ values of 0.29, 2.1 and 5.1 μ M, respectively, followed by psammaplysin A (**5**) with IC₅₀ values of 3.9, 8.5 and 5.1 μ M, respectively. 5-fluorouracil (5-FU) was used as the positive control which showed IC₅₀ values of 13.0 μ M (MDA-MB-231), 12.3 μ M (HeLa) and 4.6 μ M (HCT-116). In addition, psammaplysin Z (**7**) and 19-hydroxypsammaplysin Z (**8**) were moderately cytotoxic against MDA-MB-231, HeLa and HCT-116 cell lines with IC₅₀ values 19.4, 22.2 and 8.2 μ M and 13.2, 17.6 and 7.0 μ M, respectively. It was seen that the presence of spirooxepinisoxazoline moiety was important for the cytotoxicity in compounds (**5, 6, 7** and **8**). The best activity was observed, when the terminal amino group was attached to 2-(methylene)cyclopent-4-ene-1,3-dione moiety followed by when it was free amine. It was observed that the activity decreased when the terminal amine is attached to the urea moiety as seen in compounds (**7**) and (**8**). Brominated alkaloids, ceratinine H (**9**) and psammaplysin E (**6**) from Red Sea sponge *Pseudoceratina arabica* showed potent antiproliferative activity against the HeLa cells with IC₅₀ values of 2.56 and 2.19 μ M, respectively. In addition, psammaplysin E (**6**) had shown potent antimigratory activity against MDA-MB-231 with an IC₅₀ value of 0.31 μ M [30] comparable with recently reported IC₅₀ 0.29 μ M by Shaala and Youssef (2019). Psammaplysin E (**6**) and ceratinine H (**9**) contain 2-(methylene)cyclopent-4-ene-1,3-dione moiety attached to 1-o-propylamine dibromophenyl-4-ethylenamine group suggesting the importance of these moieties in the structure.

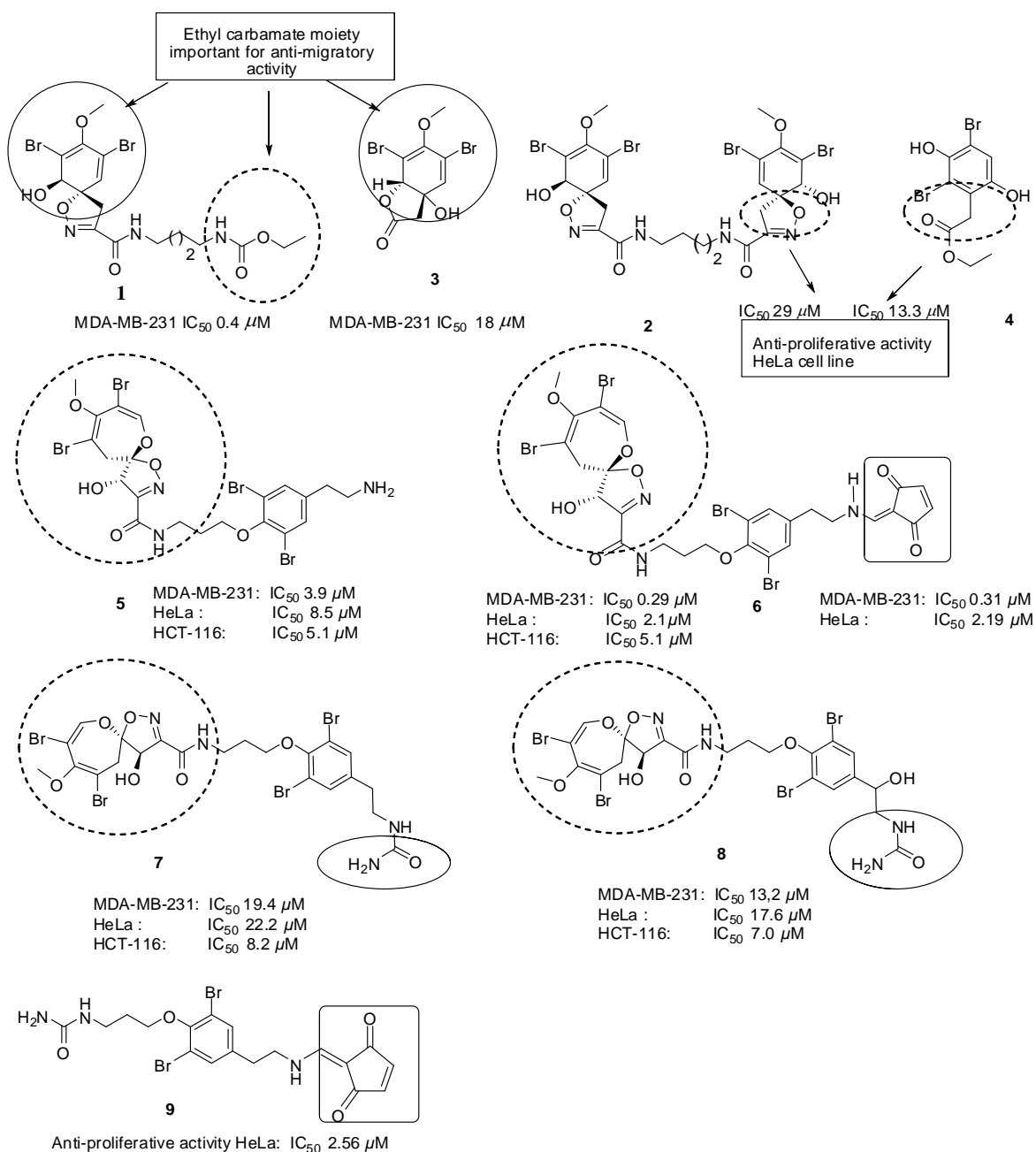


Fig 1: Chemical Structures of cytotoxic marine alkaloids (1-9).

A series of macrocyclic oxaquinolizidine alkaloids have been reported from the marine sponge *Xestospongia* species. Alkaloids, araguspongines A, C, K, L (**10-13**) and xestospongins B (**14**) (**Fig. 2**) were purified from the sponge *X. exigua* (Jeddah, Saudi Arabian Red Sea coast) [31]. These five oxaquinolizidine alkaloids were screened for their anticancer activity using the human epidermal growth factor receptor 2 (HER2)-over expressing breast cancer cell line BT-474 cells [32]. Suppression of BT-474 cell viability was most striking with araguspongine A (**10**) and C (**11**) treatments, with IC_{50} 9.3 and 15.2 μ M, respectively, while compounds (**12**), (**13**) and (**14**) showed IC_{50} values 29.5, 35.6 and 52.5 μ M, respectively. The study suggested that suppression of BT-474 cell was preferred when there

was a presence of mono-hydroxy and dihydroxy substitutions and decreased with the presence of N-oxides in the molecule. The molecular docking study of araguspongine C (**11**) on HER2 crystal structure suggested hydrogen bonding interaction between C-9'-tertiary hydroxyl group of the quinazolidine scaffold with the carboxylate side chain of Asp 863 in the DFG (Asp863-Phe864-Gly365) motif. The DFG motif of HER2 is located at the regulatory activation loop of the ATP binding pocket and is critical for HER2 protein kinase activity. The importance of hydrogen bonding interaction of araguspongine C with Asp 863 at the DFG motif was obvious when the C-9'-hydroxyl group was replaced by hydrogen as in araguspongine A. Therefore, C-9'-hydroxyl of araguspongine C is an important pharmacophoric group to retain HER2 inhibitory and anticancer activities. Araguspongine C (**11**) also exerted anti-proliferative effects when evaluated *in vitro* against a series of MDA-MB-231, MCF-7, BT-474, SKBR3 and T-47D breast cancer cell lines with different phenotypes and molecular characteristics in a dose-dependent manner. MDA-MB-231 and MCF-7 cells were the most sensitive with IC₅₀ 10.1 and 8.5 μ M, respectively, (**Table 1**) while, BT-474, SKBR3 and T47D were found to be less sensitive with IC₅₀ 15.2, 18.3, and 46.1 μ M, respectively. Determination of araguspongine C (**11**) effects on BT-474 cells clearly showed that at 10 μ M concentration, it causes accumulation of vacuoles in BT-474 cells and increases apoptotic cells. It was also capable of inhibiting c-Met phosphorylation with an IC₅₀ value of 19.9 μ M in a dose-dependent manner. It was found that araguspongine C induced suppression of BT-474 cancer cell growth mediated by induction of autophagic cell death. It was associated with down regulation of c-Met and HER2 receptor tyrosine kinases (RTKs) and suppression of receptor activation. Inhibition of these receptors was also confirmed by cell-free kinase assays and *in-silico* docking analysis. Suppression of RTK activity was also linked to PI3K/Akt/mTOR pathway inhibition. In addition, compound (**11**) caused a reduction in the expression levels of IP3 receptor.

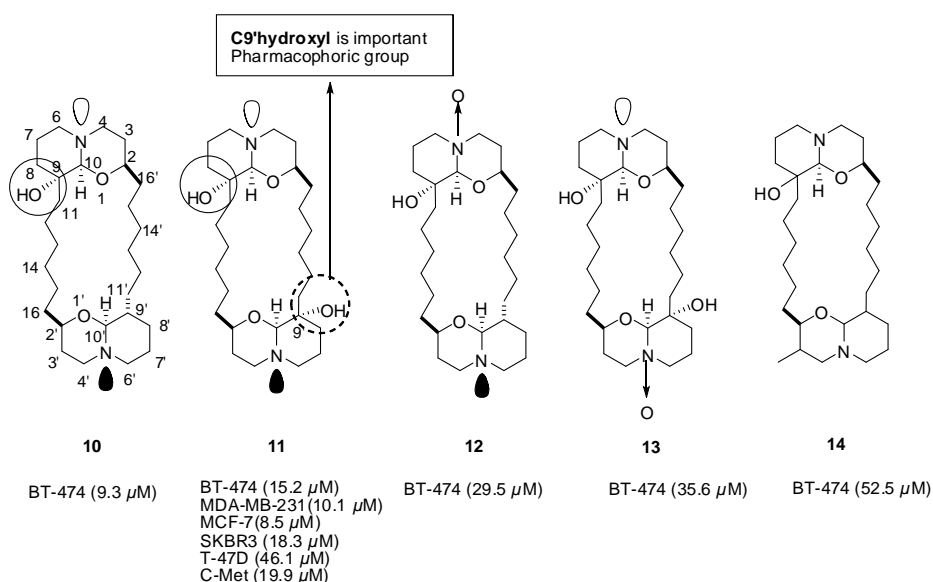


Fig. 2. Chemical Structures of cytotoxic marine alkaloids (10-14)

Hyrtioerectines A-C (**15-17**), belonging to tryptamine-derived alkaloids were isolated from *Hyrtios erectus* (Hurghada) [33] (**Fig. 3**). Hyrtioerectine A (**15**) possesses the carbon bond-linked moieties 6-hydroxy- β -carboline and 6-hydroxyindole. Compounds (**15-17**) exhibited moderate cytotoxicity against HeLa cell line with IC_{50} 0.026, 0.02, 0.02 μ M, respectively. The activity may be due to the presence of indole alkaloid group with 2-hydroxypropanoic acid side chain at 3-position. Better activity was observed in compound (**17**) where the indole alkaloid was attached to 2-hydroxypropionic acid group at 3-position. It can be also seen that 5-hydroxyindole was preferred over 6-hydroxyindole for cytotoxicity. Ashour et al. (2007) reported a series of indole alkaloids from sponge *H. erectus* collected from Egypt coast [34]. Of these alkaloids, indole-3-carbaldehyde (**18**) and 5-hydroxy-1H-indole-3-carboxylic acid methyl ester (**19**) showed 53% and 36% cytotoxic activity against L5178Y cell line at concentration of 0.069 and 0.052 μ M, respectively. In the reported study, it was found that the aldehydic group at 3-position of indole showed better activity. From the same source *H. erectus* (Safaga, Egyptian Red Sea coast) Sauleau et al. 2006 [35] have isolated hyrtiosulawesine (**20**) as a significant phospholipase A2 (PLA₂) inhibitor (IC_{50} 14 μ M) in a fluorometric assay. It belongs to the rare marine alkaloids in which the indole and β -carboline fragments of the molecule are linked through C-3/C-3 of both moieties. Youssef group have reported three similar alkaloids, hyrtioerectines D-F (**22-24**) (**Fig. 3**) from Red Sea sponge *Hyrtios* species [36]. All compounds (**21-23**) were evaluated for *in vitro* growth inhibition of A549, HT29 and MDA-MB-231 cells. Compounds (**21-23**) exhibited variable activity with GI_{50} values 25, 90 & 42 μ M; 30, 100 & 35 μ M and 28, 85 & 45 μ M for MDA-MB-231, A549 and HT29 cells, respectively (**Table 1**). The results indicated that compounds (**21**) and (**23**) were slightly more cytotoxic than compound (**22**). The sponge *Callyspongia siphonella* collected

from Hurghada along the Red Sea coast yielded two brominated oxindole alkaloids, 5-bromotrisindoline (**24**) and 6-bromotrisindoline (**25**) [37]. Compounds (**24**) and (**25**) revealed significant cytotoxicity against human colon cancer cell line (HT-29), human ovarian cancer cell line (OVCAR-3), and multiple myeloma cell line (MM.1S) with IC₅₀ values of 8, 7 and 9 μM, and 12.5, 9 and 11 μM, respectively. Better activity was observed when the bromine was located at 5-position.

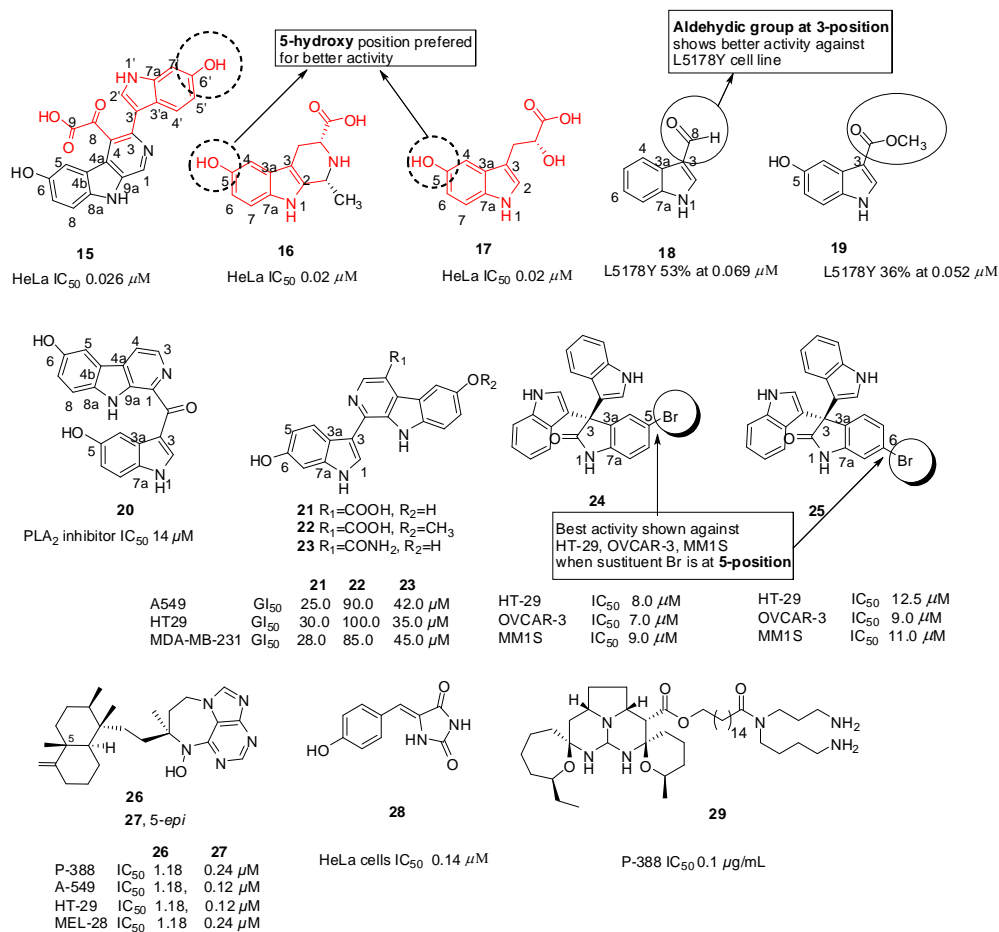


Fig. 3. Chemical Structures of cytotoxic marine alkaloids (15-29)

Sponge *Raspailia* sp, collected off Dahlak Archipelago, Eritrea [38] yielded, three alkaloids, asmarines A-C possessing a unique tetrahydro[1,4]diazepino[1,2,3-*g,h*] purine (THDAP) structure. Asmarine A (**26**) and B (**27**) (**Fig. 3**) showed cytotoxic activity against murine leukemia (P-388), human lung adenocarcinoma (A-549), human colon carcinoma (HT-29), and human melanoma cells (MEL-28). Asmarine B (**27**) was found to be more active with IC₅₀ 0.12–0.24 μM than asmarine A (**26**) with IC₅₀ 1.18 μM. All cell lines tested clearly indicated that a *cis*-ring junction of the decalin portion in (**27**) was important for better activity rather than *trans* in (**26**).

Lambrecht and co-worker (2018) [39] studied mechanism of action of the asmarine metabolite family by dissecting individual structural motifs to show why they are necessary and how they can be altered

(Fig 4). Considering the information like N-OH functionality and diazepine as the cyclic variants were critical for cytotoxic activity, they designed a structurally simplified compound termed “delmarine” (**26-2**) and showed that it is a similarly potent toxin as asmarines A and B. Delmarine (**26-2**) was found to arrest cells primarily in G1 phase in both the HeLa (cervical cancer) and HT1080 (fibrosarcoma). Examination of the influence of iron on delmarine-induced cell cycle arrest suggested that iron binding may be involved in the mechanism of delmarine. Further investigation, revealed that other than ferric iron including ferrous iron (Fe^{2+}), copper (Cu^{2+}), and zinc (Zn^{2+}), delmarine has ability to bind magnesium, manganese or calcium. However, except for iron, the addition of metals involved in cellular processes had little effect on cytotoxicity induced by delmarine. Treatment of HT1080 cells with delmarine showed a dose-dependent reduction of protein levels of iron storage protein ferritin (FTH1), whereas levels of the transferrin receptor (CD71) and the iron responsive element binding protein (IRP2) were found to be increased. Similar trends were also observed in HeLa cells. It was also found that delmarine-induced cytotoxicity was not driven by radical oxygen species (ROS). A series of analogs (**26a-h**) substituted at the N-6, N-7 N-9 positions were prepared having varying values of logP. N-7 substituted analogs (**26a-d**) were found to have little activity in HeLa or HT1080 cells even when their logP matched or exceeded that of delmarine. It was found that these compounds were unable to bind iron in aqueous buffer with anywhere near the affinity of delmarine. Due to the steric interactions between substituents at the N-6 and N-7 positions that do not exist in the diazepine structure force the NO bond out of plane in the simplified analogs. That is the reason that analogs substituted at N-7 lost the ability to bind iron efficiently as a result of this loss of planarity. Analogues with N-9 substituents (**26e-h**) showed iron binding ability and the potency of these compounds in HeLa and HT1080 cells was proportional to their logP. Compounds with higher logP values such as (**26g**) and (**26h**) were found to have cytotoxic activity on par with or exceeding that of delmarine.

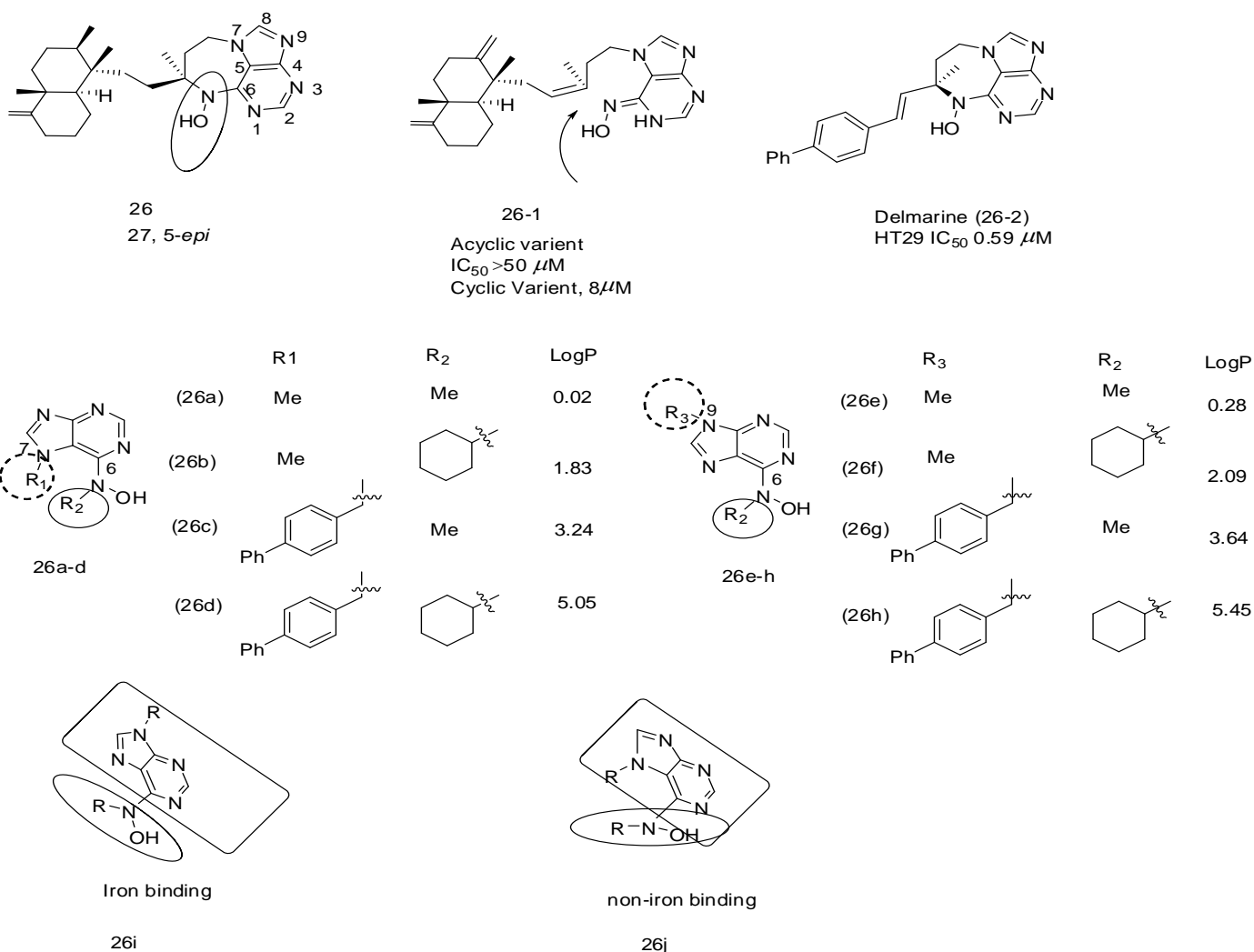


Fig. 4. Delmarine analogs

The differences in iron binding and cytotoxic activity between N-7 versus N-9 analogs (**26i-j**) was assigned to the asmarine diazepine i.e. the ring allows N6 and N7 alkyl groups to occupy the same plane by alleviating steric repulsion through covalent bond formation. The same effects can also be achieved by appendage of lipophilic groups to N9, but no similar purine metabolites have been isolated that contain an N9-terpenyl linkage, whereas N7 connections are common. Thus, through this study, it was concluded asmarine alkaloids are optimized for cytotoxic iron binding through three main structural features; 1) appendage of a terpenyl substituent for increasing the hydrophobicity, 2) N6 alkylation for an iron-binding and 3) the diazepine ring of the asmarines which allows the hydroxyl-aniline group to rotate into the plane of the purine without steric repulsion by the N7 substituent.

Investigation of *Hemimycale arabica* collected from Desha, south of Hurgada at the Egyptian coast afforded hydantoin alkaloid, (Z)-5-(4-hydroxybenzylidene)imidazolidine-2,4-dione (**28**) showing moderate activity against HeLa cells with IC₅₀ value of 0.14 μM [40]. It also showed potent *in vitro*

anti-growth and anti-invasive properties against PC-3M prostate cancer cells and significant anti-invasive activities in orthotopic xenograft and transgenic mice models [41]. A guanidine alkaloid, ptilomycalin A (**29**) was reported from Red Sea sponge *Hemimycale* sp. exhibiting significant cytotoxic activity against P-388 with IC₅₀ 0.1 µg/mL [42].

Table 1: Cytotoxic Alkaloids from Red Sea sponges

Alkaloids	Sponge	Cell line, IC ₅₀	Ref.
Subereamolline A (1) Aerothionin (2) Aerophysinin 2 (3) Subereaphenol C (4)	<i>Subereamol lis & Suberea sp</i>	(1): MDA-MB-231 (1.7 µM -anti- invasive activity; 0.4 µM - antimigratory activity) (2): HeLa (29 µM) (3): MDA-MB-231 (18 µM) (4): HeLa (13.3 µM)	Shaala et al. 2012; Shaala et al. 2015
Psammaplysin A (6) Ceratinine H (7) Psammaplysin E (8)	<i>Pseudocer- atina arabica</i>	(6): HeLa (8.50 µM); MDA-MB- 231 (3.90 µM); HCT-116 (5.1 µM); (7): HeLa (2.19 µM); MDA-MB- 231(0.31 µM); (8): HeLa (2.10 µM); MDA-MB-231 (0.29 µM); HCT-116 (3.7 µM)	Badr at al. 2008; Shaala & Youssef 2019
Psammaplysin Z (8) 19- hydroxypsammaplysi n Z (9)	<i>Aplysinella sp</i>	(8): HeLa (22.2 µM); MDA-MB- 231(19.4 µM); HCT-116 (8.2 µM) (9): HeLa (17.6 µM); MDA-MB- 231(13.2 µM); HCT-116 (17.0 µM)	Shaala & Youssef 2019
Araguspongines A,C,K,L (10,11, 12, 13) Xestospongine B (14)	<i>Xestospon- gia exigua</i>	(10): BT-474 (9.3 µM); (11): BT- 474 (15.2 µM); MDA-MB-231(10.1 µM); MCF-7(8.5 µM); SKBR3 (18.3 µM), T-47D (46.1 µM); C-Met (19.9 µM); (12): BT-474 (29.5 µM); (13): BT- 474 (35.6 µM); (14): BT-474 (52.5 µM)	Akl et al. 2015
Hyrzioerectines A–C (15-17)	<i>Hyrzios erectus</i>	(15, 16, 17): HeLa (0.026, 0.02, 0.02 µM)	Youssef, 2005;
Indole-3- carbaldehyde (18) 5-hydroxy-1 <i>H</i> -indole- 3-carboxylic acid methyl ester (19) Hyrziosulawesine (20)	<i>Hyrzios erectus</i>	(18): L5178Y 53% inhibition at 0.069 µM; (19): L5178Y 36% inhibition at 0.052 µM; (20): Anti- phospholipase A ₂ activity (14 µM)	Ashour et al. 2007; Sauleau et al. 2006
Hyrzioerectines D–F (21-23)	<i>Hyrzios sp</i>	(21, 22, 23): MDA-MB- 231(25,90,42 µM); A-549 (30,100, 35 µM); HT-29 (28,85,45 µM)	Youssef et al. 2013
5-bromotrisindoline (24) 6-bromotrisindoline (25)	<i>Callyspon- gia siphonella</i>	(24): HT-29 (8 µM);OVCAR-3 (7 µM); MM.1S (9 µM); (25): HT-29 (12.5 µM);OVCAR-3 (9 µM); MM.1S (11 µM)	El-Hawary et al. 2019

Asmarines A-B (26-27)	<i>Raspailia</i> sp	(26, 27): P-388 (1.18, 0.24 μ M); A-549 (1.18, 0.12 μ M); HT-29 (1.18, 0.12 μ M); MEL-28 (1.18, 0.24 μ M)	Yosief et al. 1998
(Z)-5-(4-hydroxybenzylidene)imidazolidine-2,4-dione (28)	<i>Hemimycalca arabica</i>	(28): HeLa (0.14 μ M)	Youseff et al. 2005; Mudit et al. 2009
Ptilomycalin A (29)	<i>Hemimycalca</i> sp.	(29) : P-388 (0.1 μ g/mL)	Kashman et al. 1989

2.1 Terpenoids

2.2.1 Sesquiterpenes: Systematic fractionation of an extract of *Diacarnus erythraenus* (Hurghada, Egypt) resulted in the isolation of a sesquiterpene, *O*-methyl guaianediol (30) (Fig. 5) [43], which exhibited a promising activity against P-388, A-549 and HT-29 cell lines with $IC_{50} > 0.004$ (Table 2). Derivatives of avarol, a sesquiterpenoid hydroquinone were purified from the sponge *Dysidea cinerea* collected near Ras Zaatir, in the Gulf of Eilat [44]. These compounds were identified as, 3'-hydroxyavarone (31), 3',6'-dihydroxyavarone (32), 6'-acetoxyavarol (33), 6'-acetoxyavarone (34) and 6'-hydroxy-4'-methoxyavarone (35) (Fig. 5) showing cytotoxic activity against P388 cells with IC_{50} values 0.0018, 0.0036, < 0.0016 , 0.03, > 0.054 , respectively. It was observed that the activity decreased when the benzoquinone group in these molecules was converted into enolic form *i.e* hydroquinone.

2.2.2 Diterpenes: Spongian-type diterpenes, norrsolide (36) and norrlandin (37) (Fig. 5) were isolated from the *Dysidea* species (Southern part of the Gulf of Suez) [45]. Norrisolide (36) and norrlandin (37) showed cytotoxic activity with IC_{50} 0.004 and 0.0027 μ M, respectively.

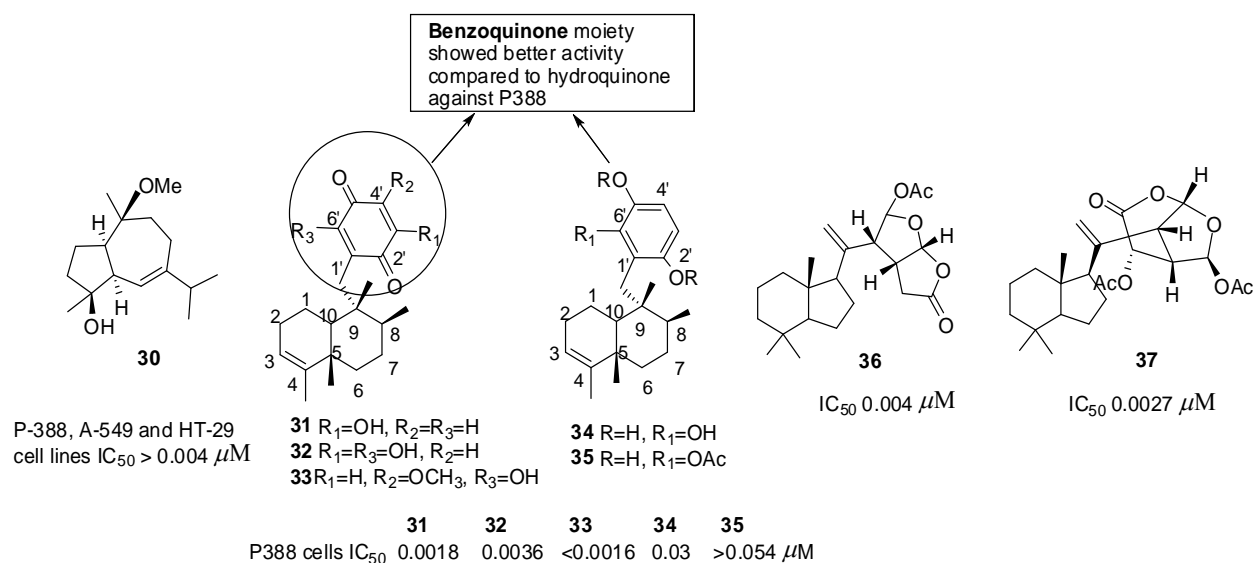


Fig. 5. Chemical Structures of sesquiterpenes & diterpenes (30-37).

2.2.3 Sesterterpenes

Several cytotoxic scalarane-type sesterterpenes were isolated from different collections of the Red Sea sponge *H. erecta* [46] (**Table 2**). Cytotoxic activity was shown by salmahyrtisol A (**38**), 3-acetylsesterstatin 1 (**39**), 19-acetylsesterstatin 3 (**40**) and salmahyrtisol B (**41**) (**Fig. 6**) towards P-388, A-549, and HT-29 cell lines with IC₅₀ value $\geq 0.0022 \mu\text{M}$. 16-hydroxyscalarolide (**42**) which was obtained from the same source collected off Hurghada, Egypt, exhibited growth inhibition activity against L5178Y cell line with 40.5% and 55.3% at concentrations of 0.0075 and 0.0249 μM , respectively [47]. Yet another collection same source from Jeddah, led to isolation of sesterterpene scalarinol, heteronemin (**43**) [48]. Heteronim (**43**) was cytotoxic with IC₅₀ values of 0.041, 0.027, 0.077 μM against HepG2, A549 and PC-3 cell lines, respectively.

They are many more reports on the isolation of cytotoxic compounds from Red Sea sponge *H. erectus*. Pentacyclic nitrogen-containing scalarane; 24-methoxypetrosaspongia C (**44**), scalaranes, sesterstatin 3 (**45**), 12-deacetyl-12-*epi*-scalaradial (**46**) and 12-deacetyl-12,18-di-*epi*-scalaradial (**47**) (**Fig. 6**) were characterized from *H. erectus* (Sharm el-Sheikh, Egypt) [49]. The compounds exhibited growth inhibitory effects against MCF-7, HepG2 and HCT-116. Significant activity was shown by 12-deacetyl-12,18-di-*epi*-scalaradial (**47**) with IC₅₀ 3.3, 1.7 and 3.4 μM against MCF-7, HepG2, and HCT-116 cell lines, respectively. Moderate activity was shown by compounds (**44**), (**45**) and (**46**) with IC₅₀ 55.4, 25.4, 26.5 μM , 24.0, 19.2, 26.1 μM , and 36.0, 23.4, 27.1 μM against MCF-7, HepG2, and HCT-116 cell lines, respectively. The activity improved when α,β -unsaturated butyrolactone/butyrolactam was opened to form -CHO groups at C-17 and C-18. For best activity, it is important to have α -CHO at C-18 and β -OH at C-17.

Re-investigation of *H. erectus* from different collection by the same research group gave two sesterterpenes analogs, namely 12-acetoxy,16-*epi*-hyrtiolide (**48**) and 12 β -acetoxy,16 β -methoxy-20 α -hydroxy-17-scalarin-19,20-olide (**49**) (**Fig. 5**), along with seven previously reported scalarane-type sesterterpenes, (**43**), (**45**), (**47**), 12 β ,20-dihydroxy-16 β -acetoxy-17-scalarin-19,20-olide (**50**), scalarolide 12-*epi*-24-deoxyscalarin (**51**) and scalarolide acetate (**52**) [50]. All compounds displayed anti-proliferative activity against tumor cell lines HepG2, HCT-116 and MCF-7. Compounds (**43**), (**47**) and (**50**) showed significant activity with IC₅₀ 1.1, 3.3 and 12.7 (MCF-7); 0.7, 3.4 and 3.5 μM (HCT-116) and 1.1, 1.7 and 9.6 μM (HepG2), respectively. In the study of programmed cell death against HCT-116 cells, compound (**43**) against HCT-116 cells showed significant increase in total cell death by 20 fold compared to control (doxorubicin). Cell death induced by compound (**43**) was attributed mainly to apoptosis induction; and to a much lesser extent to necrosis. Compound (**47**) significantly induced total cell death by 1.2 fold compared to control cells. Compound (**47**) also

induced cell killing effect *via* the activation of programmed cell death rather than non-specific necrosis cell death. Compounds **(48)**, **(49)**, **(51)** and **(52)** were cytotoxic against MCF-7 cell line with IC₅₀ of 32.6, 40.3, 30.7 and 20.9 μ M, respectively. Cytotoxicities against HCT-116 and HepG2 exhibited by compounds **(48)**, **(49)**, **(51)** and **(52)** were 57.5, 22.5, 14.4 and 15.4 μ M, and 21.8, 42.5, 17.3 and 15.5 μ M, respectively. Compound **(43)** was found to be significantly active with the presence of C16 (β -OAc), C19 (α -OAc) and C12 (-OH) groups. The next active compound was **(50)** which had C16 (β -OAc), C12 (-OH) groups and α,β -unsaturated γ -hydroxy butyrolactone. Similar compound **(45)** with absence of γ -hydroxy in the lactone ring did not show any activity indicating its importance for cytotoxicity.

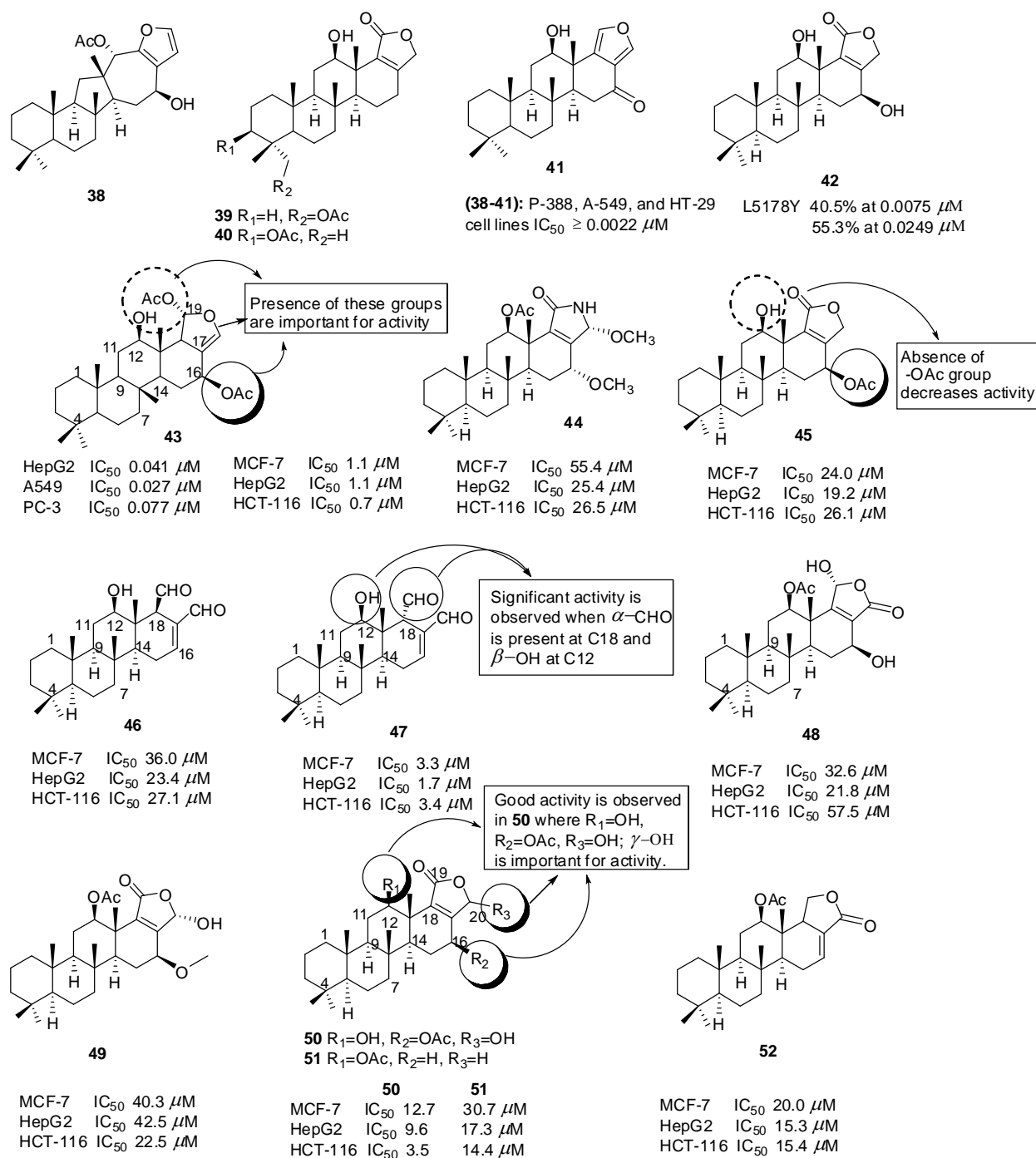


Fig. 6. Chemical Structures of sesterterpenes (38-52).

Two compounds bilospens A and B (**53-54**) (**Fig. 7**) with an unprecedented group of sesterterpenes, were isolated from sponge *Dysidea cinerea* collected in the Dahlak archipelago, Eritrea [51]. A mixture of (**53**) and (**54**) exhibited cytotoxic activity against P-388, A-549, HT-29 and MEL-28 cell lines with IC_{50} 0.0067 μM (**Table 2**). The marine sponge *Phyllospongia lamellosa* collected from Shaab Saad area, north of Hurghada Egypt resulted in the isolation and characterization of five scalarane sesterterpenes; phyllospongins A-E (**55-59**), together with other derivatives, 12 α -acetoxy-20,24-

dimethyl-25-norscalar-16-en-24-one (**60**), 12 α -acetoxy-13 β ,18 β -cyclobutan-20,24-dimethyl-24-oxoscalar-16-en-25 β -ol (**61**) and 12 α -acetoxy-24,25-epoxy-24-hydroxy-20,24-dimethylscalarane (**62**) [52]. Cytotoxic screening indicated that compounds (**55-59**) and (**62**) showed potent cytotoxic activity against HCT-116 with IC₅₀ ranging from 0.0006 to 0.0027 μ M, while (**59**) demonstrated cytotoxic activity against MCF-7 with IC₅₀ 0.0013 μ M. Compounds (**56**), (**58**) and (**59**) displayed cytotoxicity better than control with IC₅₀ 0.0013 μ M (HCT116), 0.0006 μ M (HCT116) and 0.0013 μ M (MCF-7), respectively. All the isolated compounds were less active against the HePG-2 cell line when compared to adriamycin (positive control).

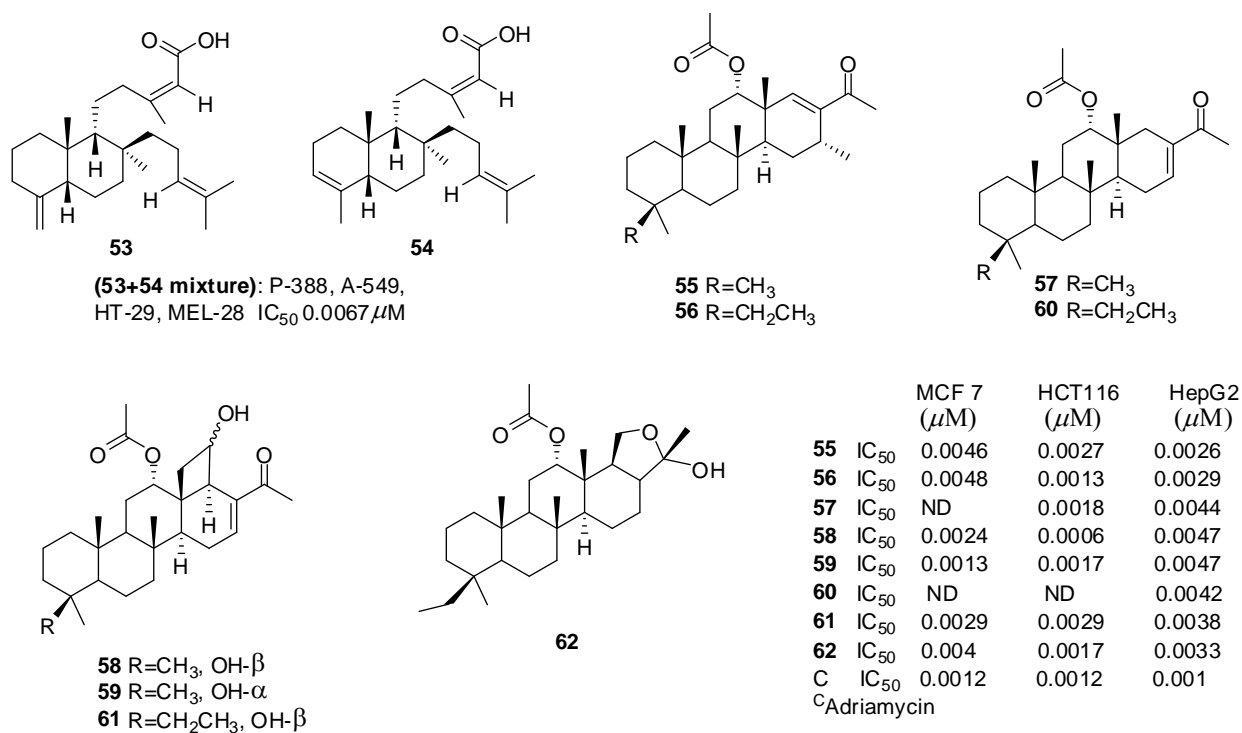


Fig. 7. Chemical Structures of sesterterpenes (53-62).

Table 2. Cytotoxic sesquiterpenes, diterpenes and sesterterpenes from Red Sea sponges

Terpenes	Sponge	Cell line, IC ₅₀	Ref.
<i>O</i> -Methyl guaianediol (30)	<i>Diacarnus erythraenus</i>	P-388, A-549, HT-29 (> 0.004 μM)	Youssef et al. 2001
3'-Hydroxyavarone (31) 3',6'-dihydroxyavarone (32) 6'-Acetoxyavarol (33) 6'-Acetoxyavarone (34) 6'-Hydroxy-4'-methoxyavarone (35)	<i>Dysidea cinerea</i>	(31, 32, 33, 34, 35): P-388 (0.0018, 0.0036, <0.0016, 0.03, >0.054 μM)	Minale et al. 1974; Hirsch et al. 1991
Norrsolide (36) Norrlandin (37)	<i>Dysidea sp</i>	(36, 37): cytotoxic (0.004, 0.0027 μM)	Rudi & Kashman 1990
Salmahyrtilol A (38) 3-acetylsesterstatin 1 (39) 19-acetylsesterstatin 3 (40) Salmahyrtilol B (41) 16- hydroxyscalarolide (42) Heteronemin (43)	<i>Hyrtios erecta</i>	(38-41): P-388, A-549, HT-29 (≥ 0.0022 μM) (42): L5178Y, 40.5% inhibition at 0.0075 μM and 55.3% inhibition at 0.0249 μM (43): HepG2 (0.041 μM); A549 (0.027 μM); PC-3 (0.077 μM)	Youssef et al. 2002; Ashour et al. 2007 Alarif et al. 2015
24-methoxypetrosaspongia C (44) Sesterstatin 7 (45) 12-deacetyl-12- <i>epi</i> -scalaradial (46) 12-deacetyl-12,18-di- <i>epi</i> -scalaradial (47) 12-acetoxy,16- <i>epi</i> -hyrtiolide (48) 12β -acetoxy,16β-methoxy,20 α -hydroxy-17-scalaren-19,20-olide (49) 12β,20-dihydroxy-16β-acetoxy-17-Scalaren-19,20-olide (50) 12- <i>epi</i> -24-deoxyscalarin (51) Scalarolide acetate (52)	<i>Hyrtios erectus</i>	(44, 45, 46, 47): MCF-7 (55.4, 24.0, 36.0, 3.3 μM); HepG2 (25.4, 19.2, 23.4, 1.7 μM); HCT-116 (26.5, 26.1, 27.1, 3.4 μM) (48, 49, 50, 51, 52): MCF-7 (32.6, 40.3, 12.7, 30.7, 20.9 μM); HCT-116 (57.5, 22.5, 3.5, 14.4, 15.4 μM); HepG2 (21.8, 42.5, 9.6, 17.3, 15.5 μM)	Elhady et al. 2016
Bilosespens A- B (53-54)	<i>Dysidea cinerea</i>	(53 & 54 Mixture): P-388; A-549, HT-29 & MEL-28 (0.0067 μM)	Rudi et al. 1999
Phyllospongins A-E (55-59) 12α-Acetoxy- 20,24-dimethyl-25 norscalar-16-en-24-one (60) 12α-Acetoxy-13β,18β-cyclobutan-20,24-dimethyl-24-oxoscalar-16-en-25β-ol (61) 12α-Acetoxy- 24,25-epoxy-24-hydroxy-20,24-dimethylscalarane (62)	<i>Phyllospongia lamellosa</i>	(55, 56, 57, 58, 59): MCF-7 (0.0046, 0.0048, ND*, 0.0024, 0.0013 μM); HepG2 (0.0026, 0.0029, 0.0044, 0.0047, 0.0047 μM); HCT-116 (0.0027, 0.0013, 0.0018, 0.0006, 0.0017 μM) (60, 61, 62): MCF-7 (ND, 0.0029, 0.004 μM); HepG2 (0.0042, 0.0038, 0.0033 μM); HCT-116 (ND, 0.0029, 0.0017 μM)	Hassan et al. 2015

ND-Not detected

2.2.4 Norsesterterpenes

Phytochemical constituents of the Red Sea sponge *Diacarnus erythraenus* collected from two different sites, Hurghada and El Qusier, Egypt reported several norsesterterpenes molecules. Of these, norsesterterpene cyclic peroxide, aikupikoxide A (**63**), three norditerpene cyclic peroxides, aikupikoxide B-D (**64-66**), muqubilin (**67**), nuapapuin A methyl ester (**68**) and sigmosceptrellin-B were later found to exhibit cytotoxicity (**Fig. 8, Table 3**) [53]. Compounds (**63-66**) showed cytotoxicity against P-388, A-549 and HT-29 with IC_{50} 0.024 μ M. Two norsesterterpene derivatives (-)-13,14-epoxymuqubilin A (**69**), (-)-9,10-epoxymuqubilin A (**70**) were isolated from another collection of the same sponge (Elfanadir, Hurghada Coast, Egypt), along with nuapapuin A methyl ester (**68**) [54], (-)-muqubilin (**67**), hurghaperoxide (**71**) [55], sigmosceptrellin-B and sigmosceptrellin B methyl ester (**72**) [56]. Compounds (**67-72**) were tested *in vitro* for growth inhibitory activity against seven human cancer cell lines, glioma (Hs683, U373, U251), melanoma (SKMEL28), carcinoma (A549, MCF-7, PC-3) using the MTT colorimetric assay. [57] Of these compounds, (-)-muqubilin (**67**), (-)-13,14-epoxymuqubilin A (**69**), (-)-9,10-epoxymuqubilin A (**70**) displayed IC_{50} values of <10 μ M. Compounds (**68, 71, 72**) were inactive IC_{50} values of >10 μ M. Compounds (**67-71**) displayed cytotoxic activities against MCF-7, A549 and PC3 cell lines. Compound (**67**) exhibited cytotoxicities with IC_{50} values 4.0 μ M (Hs683), 7.0 μ M (U373), 8.0 μ M (U251), 8.0 μ M (SKMEL28), 3.0 μ M (A549), 7.0 μ M (MCF-7) and 8.0 μ M (PC-3). Other potent compounds (**69, 70**) displayed cytotoxicities with IC_{50} values 3.0, 3.0 μ M (Hs683), 7.0, 4.0 μ M (U373), 22.0, 15.0 μ M (SKMEL28), 3.0, 3.0 μ M (A549), 6.0, 4.0 μ M (MCF-7) and 2.0, 1.0 μ M (PC-3), respectively. The observation suggested that the presence of free carboxylic acid function, epoxide and double bond at 9,10 position were critical for norterpenes to display cytotoxicity. In contrast, other metabolites with an esterified carboxylic group showed IC_{50} values of >40 μ M. Compounds (**68, 71**) displayed 38.0, 37.0 μ M (Hs683), 99.0, 83.0 (U373), 91.0, 87.0 μ M (U251), 80, 73.0 μ M (SKMEL28), 25.0, 31.0 μ M (A549), 51.0, 45.0 μ M (MCF-7) and 80.0, 73.0 μ M (PC-3), respectively. Further studies on (-)-muqubilin (**67**) indicated that it is a cytotoxic compound without selectivity between normal and cancer cells. It induces ROS production in cancer cells, while no apoptosis was observed in the same cancer cells treated by this compound. Re-investigation on the same sponge collected from Hurghada Egypt by Youssef and co-workers resulted in the isolation of three cytotoxic cyclic norsestertepene peroxides named tasnemoxides A-C (**73-75**) [58] (**Fig. 8**). The three compounds exhibited moderate cytotoxicity against three types of cancer cells including P-388, A-549, and HT-29.

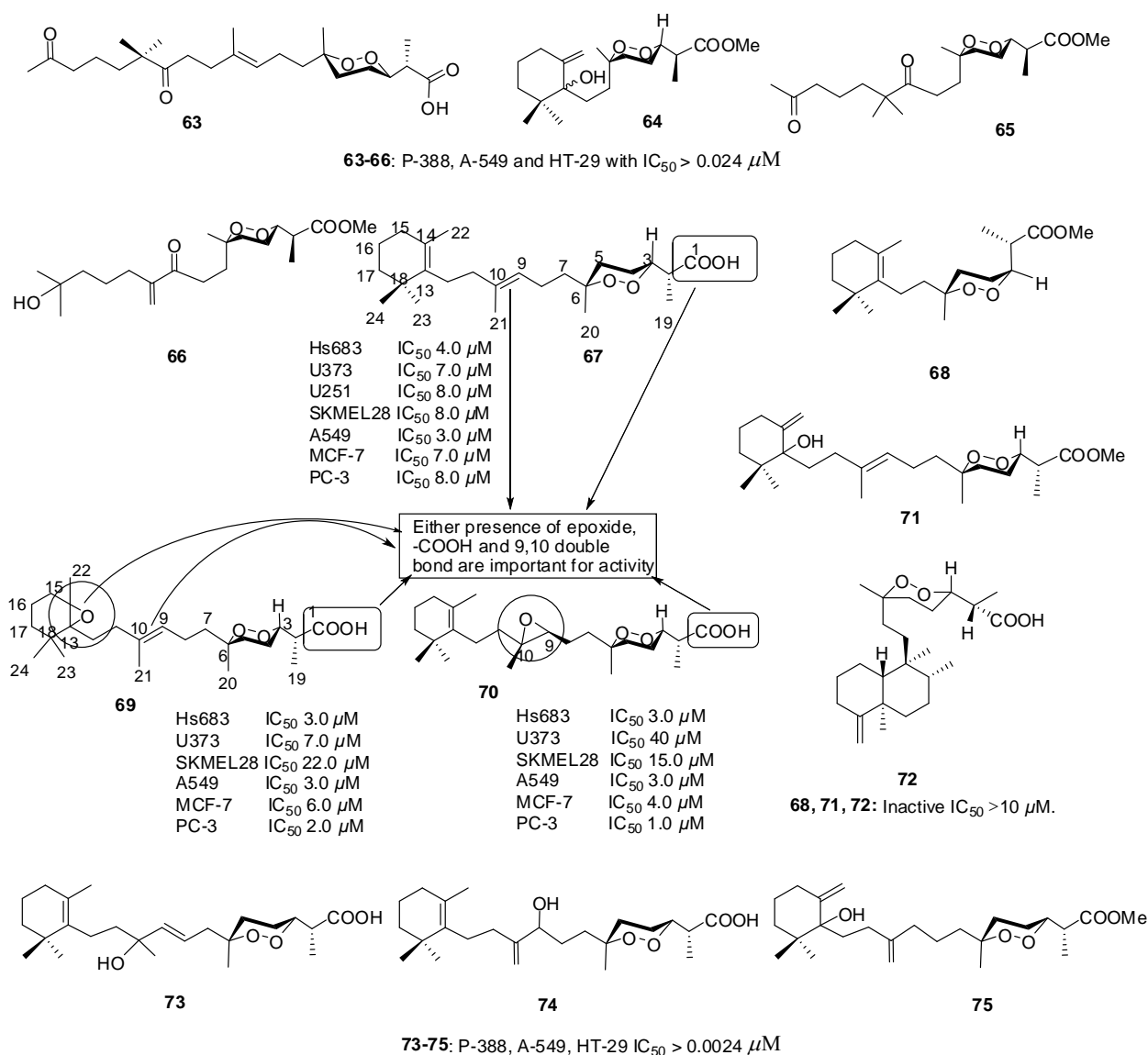


Fig. 8. Chemical Structures of norsesterterpenes (63-75).

2.2.5 Triterpenes: The Red Sea sponge *Siphonochalina siphonella* is one of few sponges known to produce squalene-derived cyclic ether triterpenes. Neviotanes, sipholenols and sipholenones were isolated from Red Sea sponge *S. Siphonella* collected from different locations [59,60,61,62]. Among these, sipholenol A (**76**), sipholenone A (**77**), neviotine A (**78**), siphonellinol C (**79**) and sipholenol I (**80**) (**Fig. 9**) were reported to exhibit cytotoxicity.

Sipholenol A (**76**) was found to potently reverse P-gp-mediated MDR to colchicine, paclitaxel, and vinblastine in the resistant KB-C2 and KB-V1 cells overexpressing P-gp (**Table 3**). A remarkable activity was shown by sipholenone A (**77**) against both MCF-7 and HepG2 cell lines with IC_{50} 0.0063 and 0.0069 μM , respectively. Sipholenol A (**76**) showed very good activity against HepG2 with IC_{50} 0.02 μM , followed by MCF-7 with IC_{50} 0.04 μM . Neviotine A (**78**) showed moderate cytotoxicity with

IC₅₀ 0.024 and 0.023 μ M against MCF-7 and Hep-G2, respectively. Ayyad et al (2014) also reported cytotoxicity for sipholenol A (**76**) towards human mammary carcinoma cell line MCF-7 cell line with IC₅₀ value of 8.8 μ M [63]. From the same geographical location Hurghada Egypt, Jain et al studied sponge *C. siphonella* which furnished terpenoids, (2*S*,4*aS*,5*S*,6*R*,8*aS*)-5-(2-((1*S*,3*aS*,5*R*,8*aS*,*Z*)-1-hydroxy-1,4,4,6-tetramethyl-1,2,3,3*a*,4,5,8,8*a*-octahydroazulen-5-yl)-ethyl)-4*a*,6-dimethyl-octahydro-2*H*-chromene-2,6-diol (**81**), sipholenone E (**82**), sipholenol J-M (**83-86**), siphonellinol D-E (**87-88**), siphonellinol C-23-hydroperoxide (**89**) [64] (**Fig. 9**). These compounds were evaluated for their ability to reverse P-glycoprotein (P-gp)-mediated multidrug resistance in human epidermoid cancer cells. The IC₅₀ values shown by compounds (**76**, **81-89**) on reversing P-gp-mediated MDR to colchicines for KB-3-1 cells were IC₅₀ 0.0048, 0.0056, 0.0047, 0.0047, 0.0071, 0.0051, 0.0047, 0.0042, 0.0046 and 0.0039 μ M, respectively.

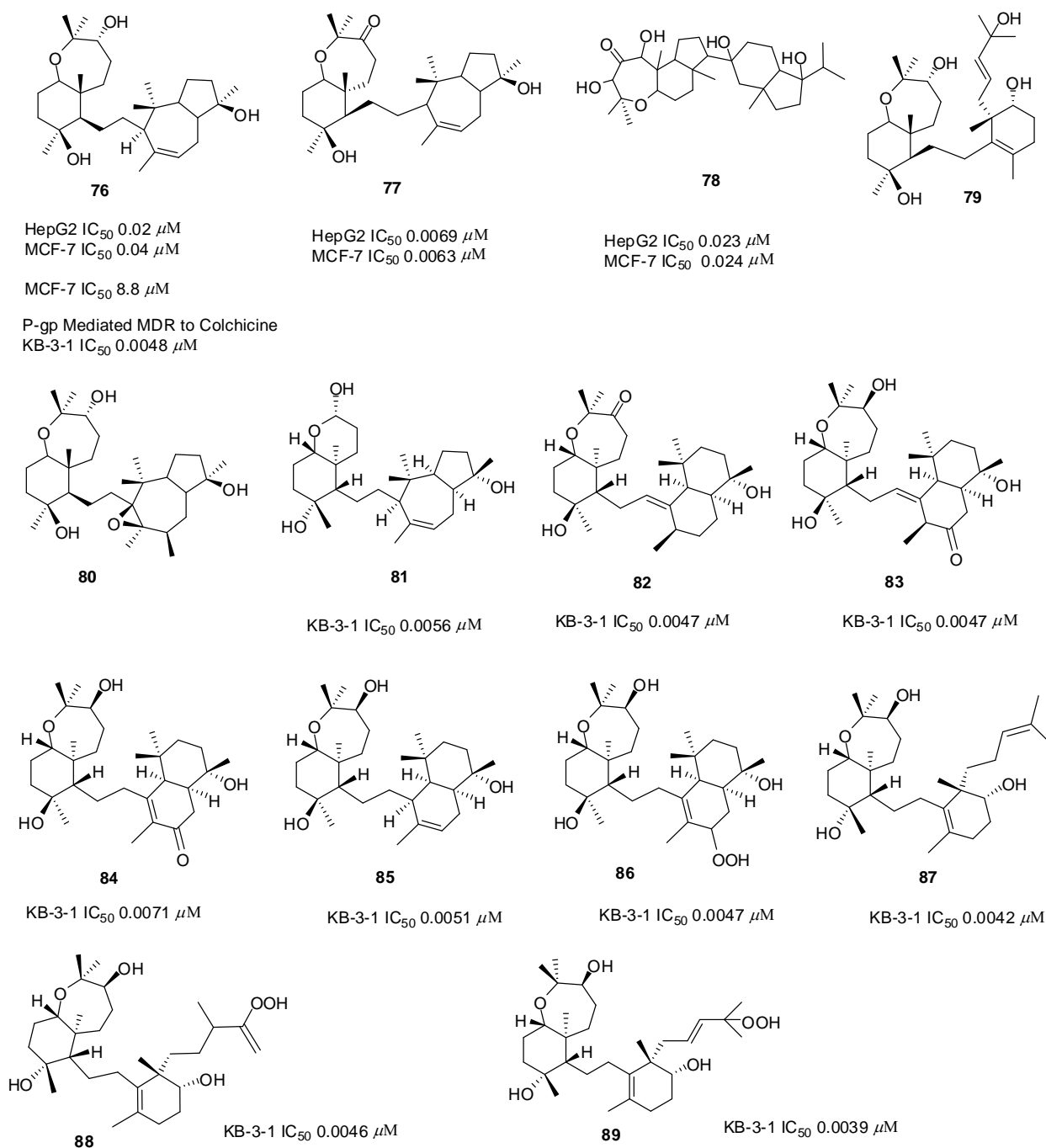


Fig. 9. Chemical Structures of norsesterterpenes (76-89).

Polyacetylene, callyspongenol-D (**90**) obtained from the sponge *S. siphonella* (Sharm Obhur, Jeddah, Saudi Arabia) (Ayyad et al 2014) was cytotoxic towards MCF-7 with IC₅₀ value of 11.7 μM (**Fig. 10**). Investigation of the organic extract of sponge, *Callyspongia* species (Hurghada, Egypt) yielded C₂₂-polyacetylenic alcohols, callyspongenols A-C (**91-93**) and dehydroisophonochalynol (**94**) [65]. Dehydroisophonochalynol (**94**) has also been reported from a Red Sea sponge *Siphonochalina* species

[66]. Compounds (**91-94**) showed moderate cytotoxicity with IC_{50} values of 0.0071, 0.0326, 0.0072, 0.0073 μM and 0.0146, 0.0326, 0.0072, 0.0169 μM against P388 and HeLa cell lines, respectively. Adriamycin was used as a positive control which showed IC_{50} values of $6.85e^{-5}$ and $1.14e^{-5}$ μM against P388 and HeLa cell lines.

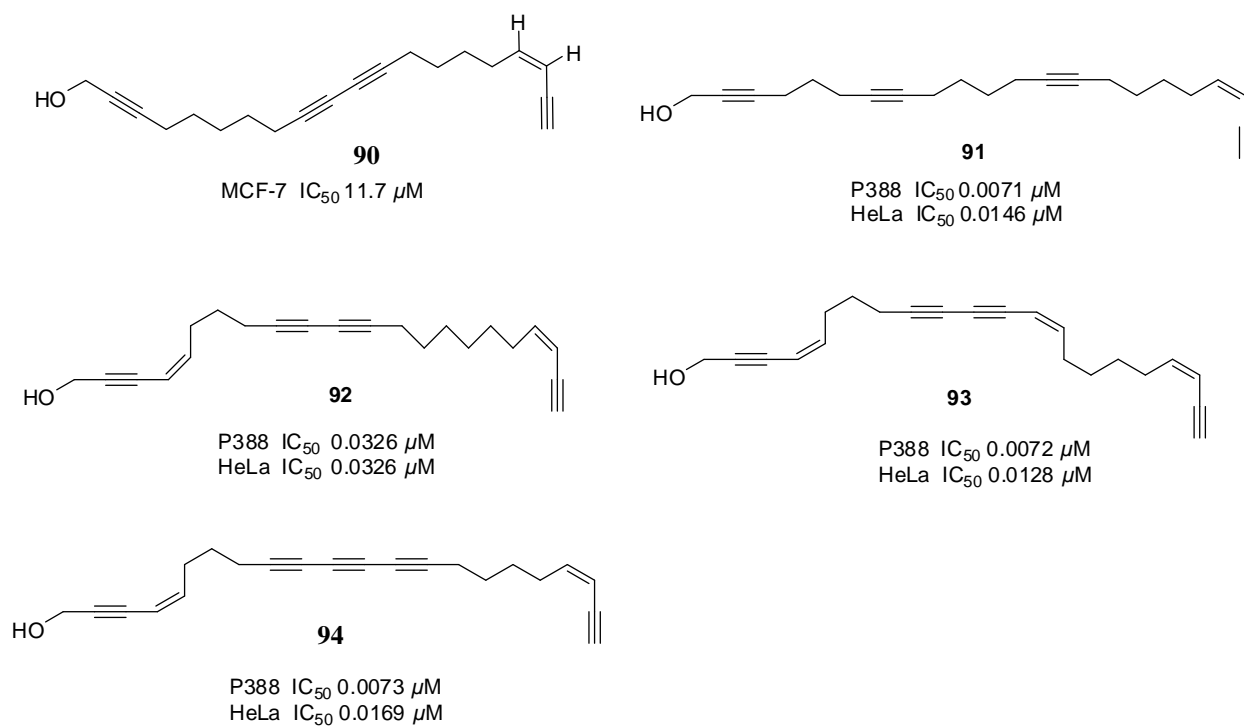


Fig. 10. Chemical Structures of norsesterterpenes (76-89).

Table 3. Cytotoxic norsesiterpenes and triterpenes from Red Sea sponges.

Terpenes	Sponge	Cell line, IC ₅₀	Ref.
Aikupikoxide A-D (63-66) Muquubilin (67) Nuapapuain A methyl ester (68) (-)-13,14-Epoxymuquubilin A (69) (-)-9,10 Epoxymuquubilin A (70) Hurghaperoxide (71) Sigmosceptrellin B methyl ester (72) Tasnemoxides A-C (73-75)	<i>Diacarnus erythraenus</i>	(63-66): P-388 , A-549, HT-29 ($\geq 1 \mu\text{g/mL}$) (67, 68, 69, 70, 71, 72): MCF-7 (7, 51, 6, 4, 45, 36 μM); A549 (3, 25, 3, 3, 31, 24 μM); PC3 (8, 80, 2, 1, 73, 44 μM) (73-75): P-388 , A-549, HT-29 ($>0.0024 \mu\text{M}$)	Kashman & Rotem 1979; Lefranc et al. 2013; Youssef 2004
Sipholenol A (76) Sipholenone A (77) Neviotine A (78) siphonellinol C (79) sipholenol I (80) (2 <i>S</i> ,4 <i>aS</i> ,5 <i>S</i> ,6 <i>R</i> ,8 <i>aS</i>)-5-(2-((1 <i>S</i> ,3 <i>aS</i> ,5 <i>R</i> ,8 <i>aS</i> , <i>Z</i>)-1-Hydroxy-1,4,4,6-tetramethyl-1,2,3,3 <i>a</i> ,4,5,8,8 <i>a</i> -octahydroazulen-5-yl)-ethyl)-4 <i>a</i> ,6-dimethyl-octahydro-2 <i>H</i> -chromene-2,6-diol (81) sipholenone E (82), sipholenols J-M (83-86), siphonellinols D-E (87-88), siphonellinol C-23-hydroperoxide (89)	<i>Siphonochalina siphonella</i>	(76): MCF-7 (0.04 μM); HepG2 (0.02 μM); A549 (8.9 μM); PC-3 (7.9 μM) (77, 78): MCF-7 (0.0063, 0.024 μM); HepG2 (0.0069, 0.023 μM) (76, 81, 82, 83, 84, 85, 86, 87, 88, 89): P-gp-mediated MDR to colchicines for KB-3-1 (0.0048, 0.0056, 0.0047, 0.0047, 0.0071, 0.0051, 0.0047, 0.0042, 0.0046 and 0.0039 μM) (85): HepG2 (24.01 μM); Caco-2 (26.64 μM); HT-29 (4.48 μM)	Jain et al. 2009; Al-Massarani et al. 2015; Ayyad et al. 2014; Youssef et al. 2003; Rotem & Kashman 1979
Callyspongenol D (90) Callyspongenols A-C (91-93) Dehydroisophonochalynol (94)	<i>Siphonochalina siphonella</i>	(90): MCF-7 (11.7 μM) (91-94): HeLa (0.0146, 0.0326, 0.0128, 0.0169 μM); P388 (0.0071, 0.0326, 0.0072, 0.0073 μM)	Rotem et al. 1979; Youssef et al. 2003

2.3 Peptides and macrolides

Sponges of the genus *Callyspongia* are prolific producer of structurally unique cyclic peptides [67]. The cytotoxic dichloromethane fraction of the sponge *Callyspongia* species collected off Obhur, Saudi Arabia, led to the isolation of a cytotoxic peptide, callyptide A (**95**) (Fig. 11). Callyptide A (**95**) was active with GI₅₀ values of 29, 18.5 and 30 μM against three cancer cell lines MDA-MB-231, A549 and HT-29, respectively (Table 4). The positive control doxorubicin showed IC₅₀ values of 0.30, 0.35 and 0.40 μM , respectively. A bicyclic glycopeptide, theonellamide G (**96**) was isolated from the sponge

Theonella swinhoei (Hurghada, Egypt) [68]. Theonellamide G (**96**) showed cytotoxic activity against HCT-16 cell line with IC₅₀ of 6.0 μM. Sponge *T. swinhoei* collected off Hurghada, Egypt yielded two cytotoxic macrolides, swinholide I (**97**) and hurghadolide A (**98**) from MeOH/CH₂Cl₂ extract [69] (**Fig. 11**). Compound (**97**) showed remarkable activity against HCT-116 cell line with an IC₅₀ of 0.0056 μM. While, compound (**98**) exhibited cytotoxicity with IC₅₀ 0.365 μM (**Table 5**). The presence of additional double bond at C2'-C3' and -OH at C26' in compound (**98**) might act as an important pharmacophore for showing remarkable cytotoxicity against HCT-116. On the contrary, in the actin-microfilament-disrupting assay, compound (**98**) was found to be 10 times more effective than compound (**97**) at disrupting microfilaments. The study revealed that, compounds (**97**) and (**98**) caused disruption of the actin cytoskeleton at concentrations of 0.07 and 0.0073 μM, respectively. Furthermore, the analysis suggested that (**97**) is more selective for cancer cells. It can also have additional mechanisms of initiating cytotoxicity in cancer cells as compared to the embryonic smooth muscle cells that are used in the microfilament-disrupting assay. Latrunculin A-B (**99A-B**) and oxalatrunculin B (**100**) (**Fig. 11**) were discovered from the Red Sea sponge *Negombata corticata* [70]. Latrunculins A and B displayed potent antimigratory activity against highly metastatic human prostate cancer PC-3M-CT⁺ cells and murine brain-metastatic melanoma B16B15b cells, respectively [71]. Similarly, latrunculin A showed potent anti-invasive activities (Cultrex1BME cell invasion assay) in the TNBC MDA-MB-231 cell line in a dose-dependent manner, with concentrations ranging from 0.1 μM to 1 μM. Cytotoxicity of oxalatrunculin B (**100**) was evaluated against several cell lines including HepG2 and HCT-116. It showed nonspecific cytotoxicity against solid tumor cells and hematopoietic cancerous cells. In addition, compound (**100**) was more potent against hepatocellular carcinoma than latrunculin B (**99**), with MIC of 16.34 μM and 19.27 μM, respectively. Compared to (**99**), compound (**100**) had presence of -SO₂ and -NOH groups in the molecule that may be responsible for its potent activity. Latrunculin B (**99**) was also isolated from the sponge *Negombata magnifica* collected off Hurghada, Egypt [72]. It displayed potent antimigratory activity in a wound healing assay and inhibited B16B15b tumor cell migration in a wound-healing assay at 1 μM.

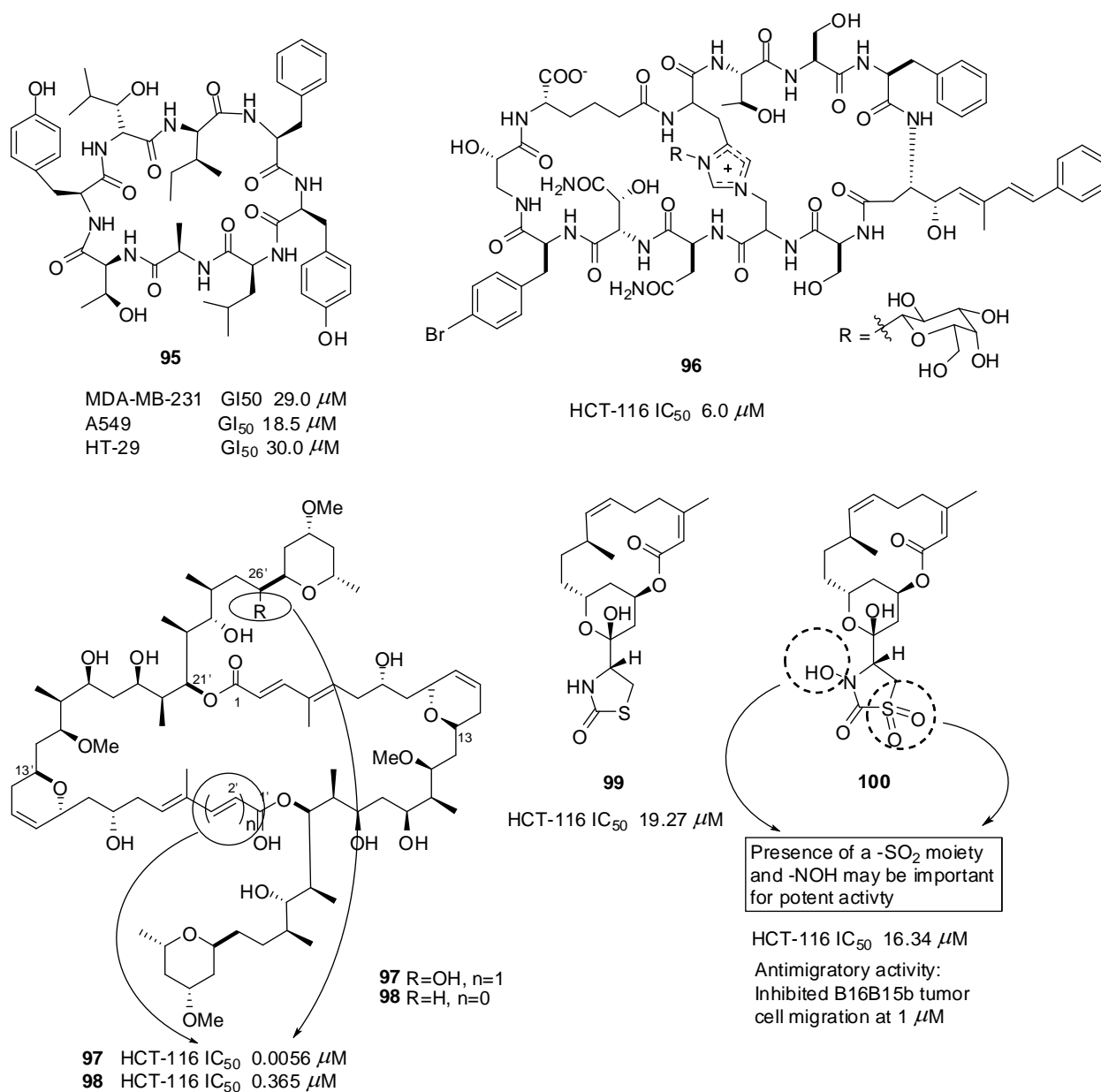
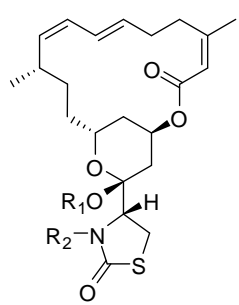

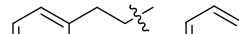





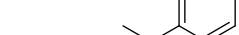







Fig. 11. Chemical Structures of peptides (95-96) and macrolides (97-100).

Khanfar et al [73] attempted SAR studies by optimization of the lactol hydroxy group (C17) and thiazolidinone NH of latrunculins and semi-synthesized 13 derivatives (**99A-2 to 99A-14**) (**Fig 12**). They were substituted by various aromatic and aliphatic groups. An actin polymerization kit was used to assess the direct actin binding activity of each analogue at two concentrations: 100 nm and 1 μm . Compounds (**99A-3 and 99A-14**) were profoundly more active than the parent latrunculin A in the inhibition of actin polymerization at both doses, whereas compounds (**99A-4, 99A-9, 99A-12, and 99A-13**) were nearly equipotent to latrunculin A. This specific binding assay proves that the antiproliferative and anti-invasive activities of latrunculin A derivatives are due to the disruption of actin polymerization, except in the case of compound **99A-11**. The anti-proliferative and anti-invasive

activities of (**99A**, **99A-2 to 99A-14**) were quantified using MTT and cell invasion assays, respectively. The anti-proliferative activities were measured against two cell lines, MCF7 and MDAMB- 231, at five different concentrations: 0.1, 0.5, 1, 10, and 50 μm . Compounds (**99A-3**) to (**99A-14**) were found to be more potent than parent latrunculin A (**99A**) against both cell lines, with 4.8- and 4.0-fold increases in activity against MCF7, and cytotoxic effects at doses greater than 1 μm . Although MDA-MB-231 cells were more resistant than MCF7 against most derivatives, compound (**99A-14**) retained potent activity against both cell lines. Although derivative (**99A-10**) was less active than (**99A**) in the invasion assay, this compound, along with (**99A-4**) and (**99A-9**), was nearly equipotent to the natural product (**99A**) in the proliferation assay.

Structure	R ₁	R ₂	Actin polymerization inhibition [%]	
			100 nM	1 μM
	H	H	30	70
	CH ₃	H	6	21
	a	H	31	94
	b	H	23	67
	CH ₃	CH ₃	11	19
	CH ₃	CH ₂ CH ₃	0	18
	CH ₃	c	20	49
	CH ₃	CH ₂ CH ₂ CH ₂ OH	0	21
	CH ₃	d	33	67
	CH ₃	b	17	53
	CH ₃	e	0	13
	H	CH ₂ CH ₃	31	70
	H	d	24	64
	H	CH ₂ OH	35	90

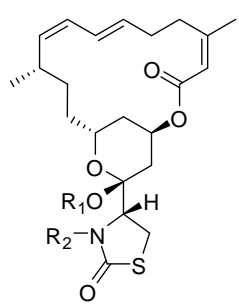

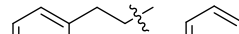





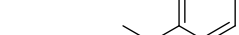





Structure	R ₁	R ₂	MCF (IC ₅₀ , μM)		MDA-MB-231 (IC ₅₀ , μM)	
			IC ₅₀ (nM)	IC ₅₀ (nM)	IC ₅₀ (nM)	IC ₅₀ (nM)
	H	H	284±72	9233± 825	0.48± 0.04	4.19± 1.49
	CH ₃	H	184±48	466±51	10.19± 1.72	23.42± 0.85
	a	H	>10000	>10000	0.10± 0.07	2.71± 0.52
	b	H	1049± 271	8548± 574	0.52± 0.14	5.05± 0.36
	CH ₃	CH ₃	333± 76	333± 76	13.07± 3.69	19.93± 3.27
	CH ₃	CH ₂ CH ₃	>10000	>10000	20.64± 2.77	>50.0
	CH ₃	c	1049± 271	8548± 574	5.30± 1.32	5.07± 0.72
	CH ₃	CH ₂ CH ₂ CH ₂ OH	8548± 574	333± 76	19.20± 3.91	>50.0
	CH ₃	d	333± 76	333± 76	0.78± 0.24	6.82± 1.41
	CH ₃	b	834± 109	834± 109	0.55± 0.091	4.48± 1.79
	CH ₃	e	>10000	>10000	15.4± 1.21	26.17± 3.91
	H	CH ₂ CH ₃	283± 51	283± 51	0.95± 0.28	7.19± 1.01
	H	d	445± 79	445± 79	0.63± 0.02 1	2.72± 0.39
	H	CH ₂ OH	210± 54	210± 54	0.12± 0.01	2.99± 0.15

Fig 12: Latrunculin analogues and their activity

Table 4: Cytotoxic peptides and macrolides from Red Sea sponges.

Peptides and macrolides	Sponge	Cell line, IC ₅₀	Ref.
Callyptide A (95)	<i>Callyspongia sp</i>	(95): MDA-MB-231 (29 μ M); A549 (18.5 μ M); HT-29 (30 μ M)	Shaala et al. 2016
Theonellamide G (96) Swinholide I (97) Hurghadolide A (98)	<i>Theonella swinhoei</i>	(96): HCT-16 (6.0 μ M) (97, 98): HCT-116 (0.0056, 0.365 μ M); actin-microfilament-disruption (0.07, 0.0073 μ M)	Youssef et al. 2014; Youssef & Mooberry, 2006
Latrunculin B (99) Oxalatrunculin B (100)	<i>Negombata corticata</i> <i>Negombata magnifica</i>	(99, 100): HepG2 (19.27, 16.34 μ M) (99): inhibited B16B15b tumor cell at 1 μ M.	Kashman et al. 1980; Ahmed et al. 2007; Elsayed et al. 2006

2.4 Lipids: steroids, fatty acids/amides and glycerides

2.4.1 Sterols: Crameli et al investigated the sponge *Erylus lendenfeldi* (Gulf of Eilat), which led to the isolation of oligoglycoside, eryloside A (**101**) [74]. The compound displayed activity against P388 with IC₅₀ 0.0059 μ M (**Table 5**). Sterols, ehrenasterol (**102**) and (22E)-ergosta-5,8,22-trien-7-one-3 β -ol (**103**) were obtained from the sponge *Biemna ehrenbergi* collected from Jeddah, Saudi Arabia (**Fig. 13**) [75]. Both sterols (**102, 103**) showed weak cytotoxicity against HCT-116 with IC₅₀ of 45 and 40 μ M, respectively. 4-methylene sterols, conicasterol (**104**) and swinhosterol B (**105**) were isolated from the sponge *Theonella mirabilis* collected off Sharm El-Sheikh, Egypt [76]. Both compounds were investigated for cytotoxicity against human breast adenocarcinoma cell line (MCF-7). The results showed that compound (**105**) was the most active compound with IC₅₀ value of 0.0067 μ M compared to doxorubicin (IC₅₀ 0.0093 μ M), while compound (**104**) was less active with IC₅₀ value of 0.015 μ M. The reduction of double bond at C8-14 to form carbonyl groups increases the activity in compound (**105**).

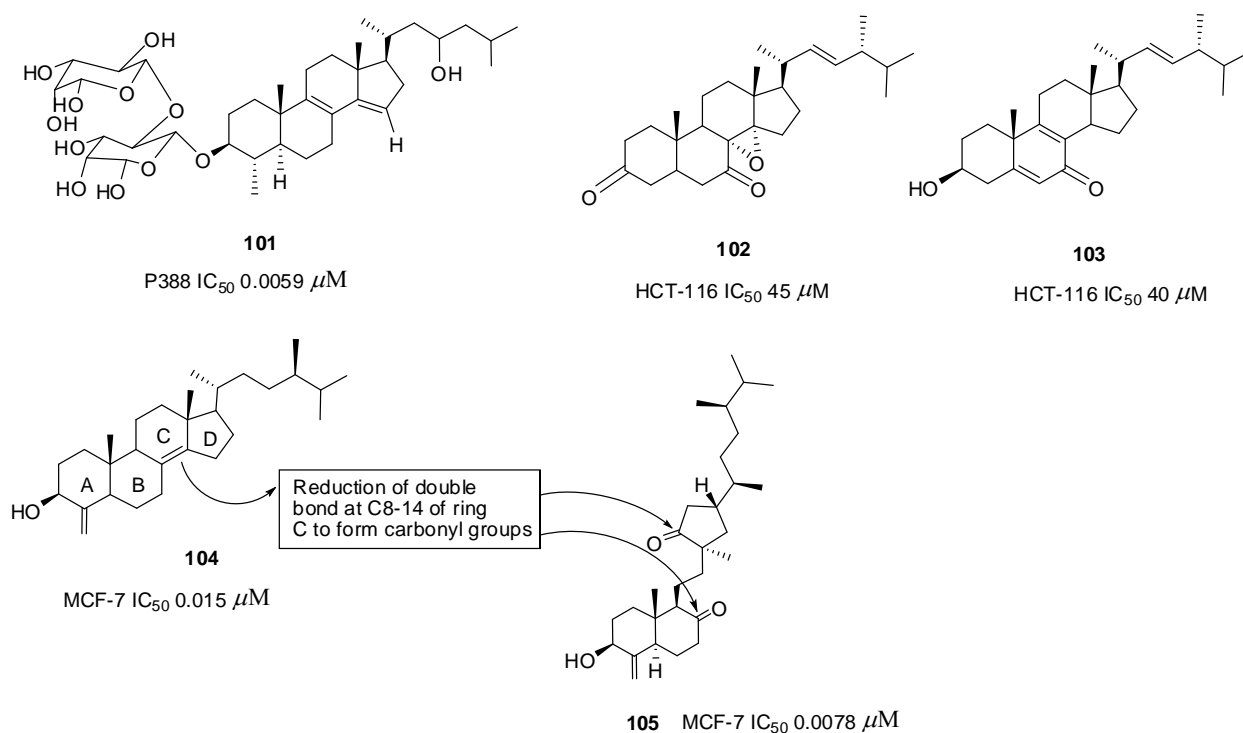


Fig. 13. Chemical Structures of steroids (101-105)

2.4.2 Fatty acid amides: The sponge *Callyspongia fistularis* (Hurghada, Egypt), led to the isolation of a cytotoxic polyacetylenic amide named as callyspongamide A (**106**) (**Fig. 14**), which showed a moderate cytotoxicity against HeLa cells with an IC₅₀ value of 0.0113 μM [77] (**Table 5**). Polyacetylene amides, siphonellamides A-B (**107-108**) and callyspongamide A (**106**), were characterized from sponge *Siphonochalina siphonella* (Southwest of Magawish Island, Hurghada, Egypt) [78]. All the compounds showed moderate cytotoxicity against HeLa, MCF-7, and A549 cancer cell lines. Compounds (**107**) exhibited cytotoxicity with IC₅₀ values of 18.0 μM and 24.2 μM against MCF-7 and A549 cell lines, respectively. Compound (**108**) displayed IC₅₀ values of 17.4 μM, 34.1 μM, and 25.9 μM against the HeLa, MCF-7, and A549 cancer cell lines, respectively. Callyspongamide A (**106**) possessed activities against the MCF-7 and A549 cell lines, with IC₅₀ values of 19.4 μM and 30.0 μM, respectively. The sponge *Spheciospongia vagabunda* collected from Ras Mohamed, Egypt, showed promising anticancer activity against HepG2 and MCF-7 cell lines [79]. Bioassay-guided fractionations yielded ceramides: *N* [(2*S*,3*S*,4*R*)-1,3,4-trihydroxytetradecan-2-yl]tridecanamide (**109**), (*R*)-2'-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxypentacosan-2-yl] octadecanamide (**110**) and (*R,Z*)-2'-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxytricosan-2-yl] nonadec-10-enamide (**111**). Compounds (**110**) and (**111**) displayed high potential cytotoxicity against HepG2 (IC₅₀ 24.7 and 21.3 μM) and MCF-7 (IC₅₀ 26.8 and 29.8 μM), respectively, while compound (**109**) showed lowest activity against HepG2 (IC₅₀ value 107 μM) and was inactive against MCF-7, IC₅₀ value >200 μM. Doxorubicin was used as

the positive control. Chemical investigation of the sponge *Echinoclathria* species (Safaga, Egypt) resulted in a ceramide, namely ((R)-2'-hydroxy-N-((2S,3S,4R)-1,3,4-trihydroxy-icosan-2-yl)cosanamide) (**112**) which displayed high potential cytotoxicity against HepG2 and MCF-7 cell lines with IC₅₀ values of 25.4 μM and 28.3 μM, respectively [80].

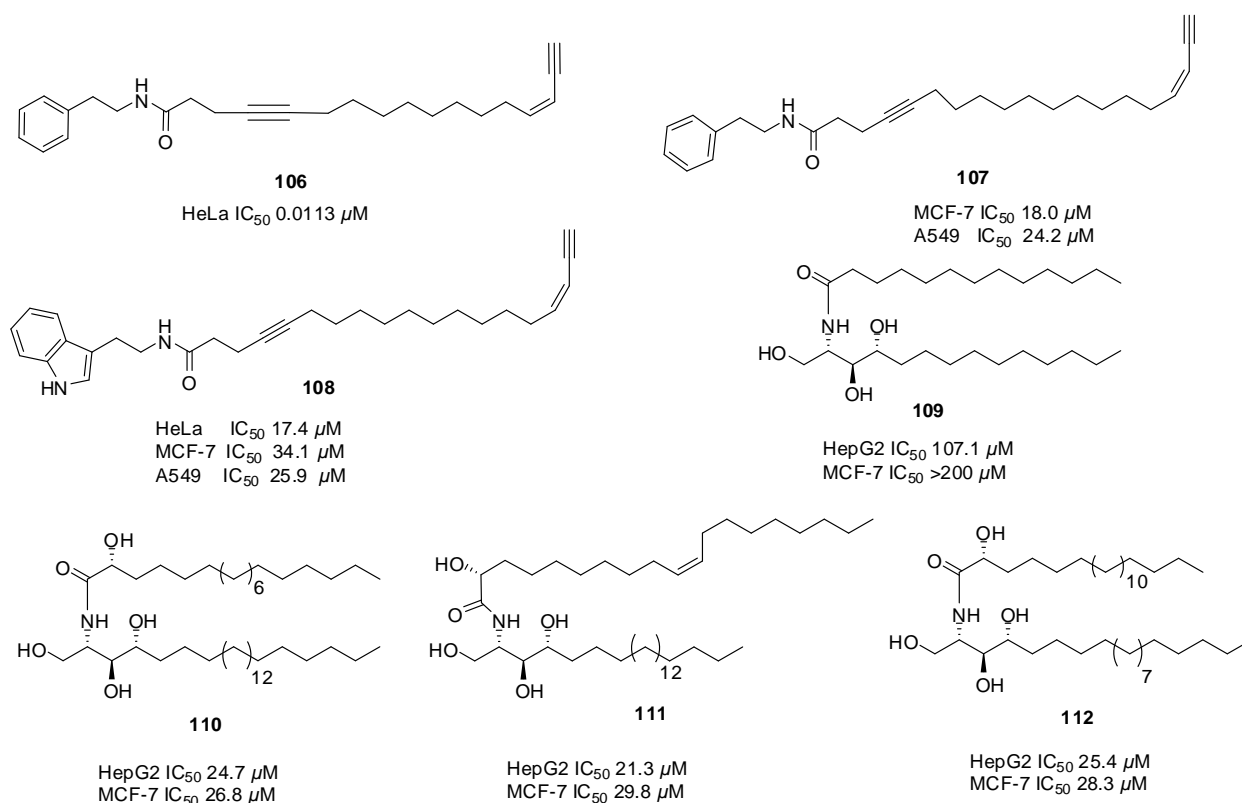


Fig. 14. Chemical Structures of fatty acid amides (106-112)

2.4.3 Fatty acids: Bioassay-guided fractionation of the methanolic extract of the sponge *Xestospongia testudinaria* (Ghurab reef, Jazan) yielded brominated acetylenic fatty acid derivatives, namely, 18,18-dibromo-(9E)-octadeca-9,17-diene-5,7-diyonic acid (**113**), 18-bromooctadeca-(9E,17E)-diene-7,15-diyonic acid (**114**), and 16-bromo (7E,11E,15E)hexadeca-7,11,15-triene-5,13-diyonic acid (**115**) (Fig. 15) [81]. Compound (**113**) exhibited broad spectrum inhibition of HeLa, HepG2 and Daoy cell lines with IC₅₀ values of 0.0535, 0.0412 and 0.0365 μM (Table 5), respectively. Compounds (**114**) and (**115**) showed moderate selectivity against HeLa and Daoy cells. Compound (**114**) was more potent than (**114**), since the IC₅₀ of (**114**) was 0.0865 and 0.0658 μM against HeLa and Daoy cells, respectively, compared to (**115**), which exhibited IC₅₀ values of 0.138 and 0.0765 μM against HeLa and Daoy cells, respectively.

2.4.4 Glycerides: Two new glycerides, mirabolides A and B (**116** and **117**) (**Fig. 15**) were isolated from the sponge *Theonella mirabilis* collected off Sharm El-Sheikh, Egypt [82]. Both compounds displayed cytotoxic activity against MCF-7 with IC₅₀ values of 0.0239 and 0.0072 μM, respectively.

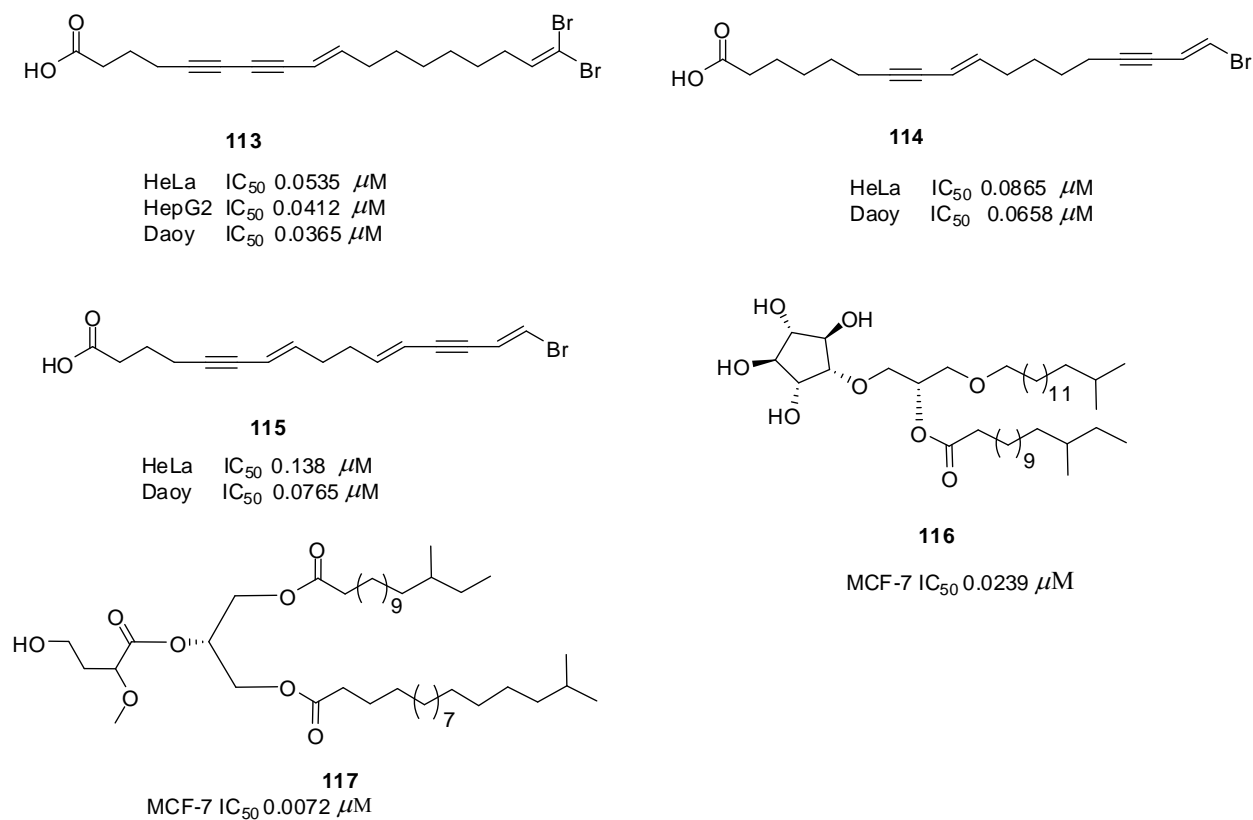


Fig. 15. Chemical Structures of fatty acids and glycerides (113-117)

2.5 Other compounds

Dibrominated phenols, subereaphenol A (**118**) and bromochloroverongiaquinol (**119**) were produced by sponge *Suberea mollis* (Egypt) [83] (**Fig. 16**). Subereaphenol A (**118**) showed moderate cytotoxicity against P388 leukemia cells at IC_{50} 0.0067 μ M (**Table 5**). While, bromochloroverongiaquinol (**119**) was cytotoxic to human cervical carcinoma HeLa cells with IC_{50} value of 0.0178 μ M. Similar compounds, aeroplysinin-2 (**120**) and a revised subereaphenol C (**121**) as ethyl 2-(2,4-dibromo-3,6-dihydroxyphenyl)acetate were isolated from *Suberea* species collected off Yanbu. Subereaphenol C (**121**) showed anti-proliferation activity against HeLa cell line with IC_{50} value 13.3 μ M [84]. In addition, aeroplysinin-2 (**120**) displayed potent antimigratory activity against MDA-MB-231 with IC_{50} of 18 μ M compared to 43.4 μ M showed by the positive control Z-4-ethylthio-phenylmethylenhydantoin (*S*-Ethyl). Shaala et al (2011) reported a brominated phenolic compound, subereaphenol D (**122**), and the known compound dichloroverongiaquinol (**123**) from the Red Sea sponge *S. mollis* (Hurghada) [85]. Compounds (**122**) and (**123**) were cytotoxic to HeLa cells, with IC_{50} values of 19 μ M and 13 μ M, respectively.

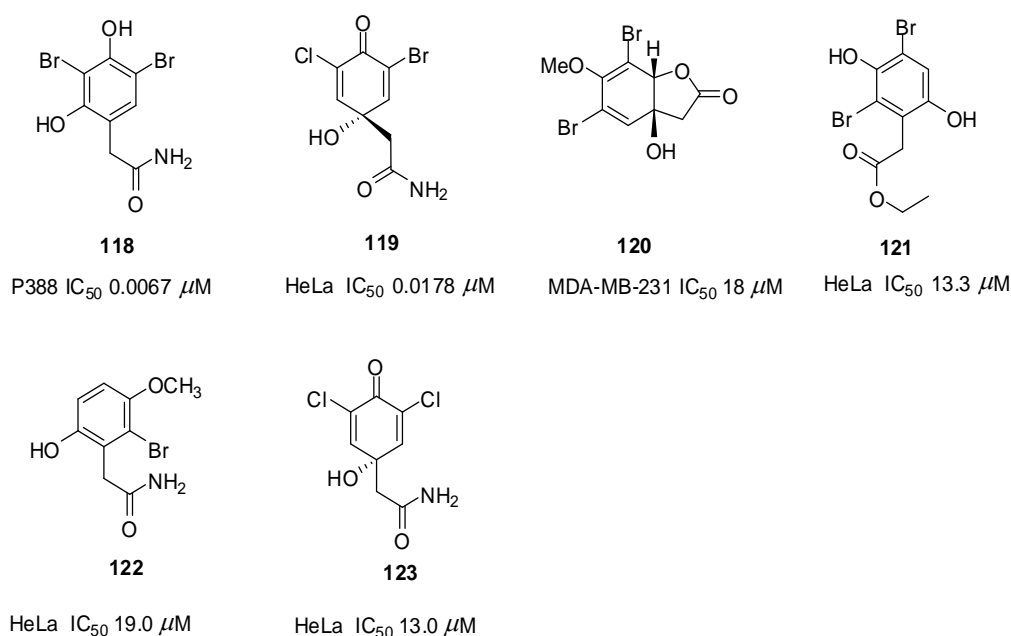


Fig. 16. Chemical Structures of other compounds (118-123)

Table 5: Cytotoxic lipids (steroids, fatty acids/amides, glycerides and other compounds)

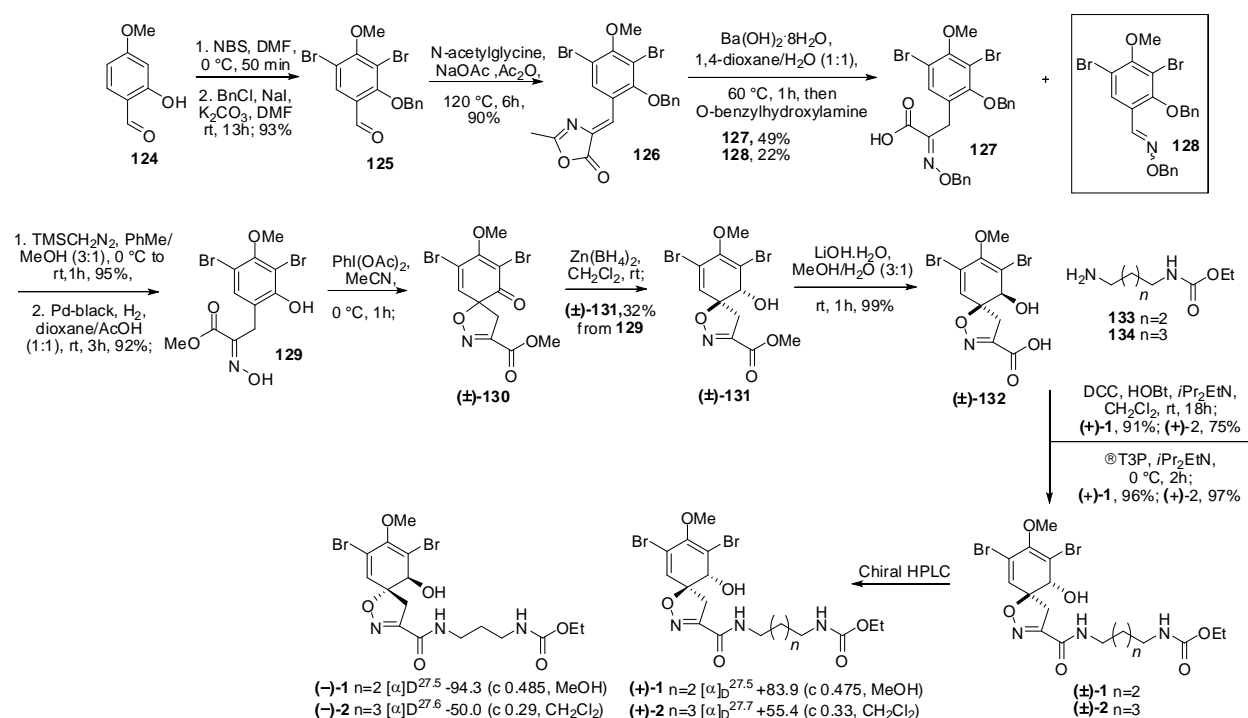
Natural Product	Sponge	Cell line, IC ₅₀	Ref.
Eryloside A (101)	<i>Erylus lendenfeldi</i>	(101): P388 (0.0059 μ M)	Carmely et al. 1989
Ehrenasterol (102) (22E)-ergosta-5,8,22-trien-7-one-3 β -ol (103)	<i>Biemna ehrenbergi</i>	(102, 103): HCT-116 (45, 40 μ M)	Youseff et al. 2015
Conicasterol (104) Swinhosterol B (105)	<i>Theonella mirabilis</i>	(104, 105): MCF-7 (0.015, 0.0067 μ M)	Abou-Hussein & Youseff 2016
Callyspongamide A (106)	<i>Callyspongia fistularis</i>	(106): HeLa (0.0113 μ M)	Youssef et al. 2003
Callyspongamide A (106) Siphonellamides A-B (107-108)	<i>Siphonochalin a siphonella</i>	(106): MCF-7 (19.4 μ M); A549 (30.0 μ M); HeLa (4.1 μ g/mL) (107): MCF-7 (18.0 μ M); A549 (24.2 μ M) (108): HeLa (17.4 μ M); MCF-7 (34.1 μ M); A549 (25.9 μ M)	Ki et al. 2020; Youssef et al. 2003
<i>N</i> [(2S,3S,4R)-1,3,4-trihydroxytetradecan-2-yl]tridecanamide (109) (<i>R,Z</i>)-20- hydroxy- <i>N</i> -[(2S,3S,4R)-1,3,4-trihydroxytricosan-2-yl) nonadec-10-enamide (110)	<i>Spheciospongia vagabunda</i>	(109, 110): HepG2 (24.7, 21.3 μ M); MCF-7 (26.8, 29.8 μ M)	Eltamany et al. 2015
((<i>R</i>)-2'- hydroxy- <i>N</i> -((2S,3S,4R)-1,3,4-trihydroxyicosan-2-yl) cosanamide) (112)	<i>Echinoclathria sp</i>	(112): HepG2(25.4 μ M); MCF-7 (28.3 μ M)	Abdelhameed et al. 2017
18,18-dibromo- (9E)-octadeca-9,17-diene-5,7-diyonic acid (113) 18-bromooctadeca-(9E,17E)-diene-7,15-diyonic acid (114) 16-bromo -7E,11E,15E) hexadeca- 7,11,15-triene-5,13-diyonic acid (115)	<i>Xestospongia testudinaria</i>	(113): HeLa (0.0535 μ M); HepG2 (0.0412 μ M); Daoy (0.0356 μ M); (114): HeLa (0.0865 μ M); Daoy (0.0658 μ M) (115): HeLa (0.138 μ M); Daoy (0.0765 μ M)	El-Gamal et al. 2016; Quinn & Tucker 1985; Hirsh et al. 1987
Mirabolides A-B (116, 117)	<i>Theonella mirabilis</i>	(116-117): MCF-7 (0.0239, 0.0072 μ M)	Abou-Hussein & Youssef, 2016
Subereaphenol A (118) Bromo-chloroverongiaquinol (119) Aeroplysinin-2 (120) Subereaphenol C (121) Subereaphenol D (122) Dichloroverongiaquinol (123)	<i>Suberea mollis</i> & <i>Suberea sp</i>	(118): P388 (0.0067 μ M) (119): HeLa (0.0178 μ M) (120): MDA-MB-231 (18 μ M) (121): HeLa (13.3 μ M) (122, 123): HeLa (19 μ M, 13 μ M)	Shaala et al. 2008; Shaala et al. 2011; D'Ambrosio et al. 1983

3. Synthesis of the potent cytotoxic molecules:

The total synthesis subereamolline A, aereothionin, asmarine B, norrsolide and latrunculin B has been presented here.

3.1 Subereamolline A (1)

Shearman et al in 2011 [86] reported the total synthesis of the potent cytotoxic molecule (+)-subereamolline A (**1**) and (-)-subereamolline A. The synthesis was initiated by treating 2-hydroxy-4-methoxybenzaldehyde (**124**) with N-bromosuccinimide (NBS) to obtain brominated aldehyde (**125**) (93% yield). The resulted aldehyde was further treated with N-acetyl glycine, sodium acetate and acetic anhydride to yield azlactone (**126**, 90%). Saponification and condensation using Ba(OH)₂ and O-benzylhydroxylamine yielded two products, carboxylic acid (**127**) in 49% and side product oxime (**128**) in low yield (22%). The corresponding methyl ester was synthesized on treating (**127**) with trimethylsilyldiazomethane. Oxime methyl ester (**129**) was generated on hydrogenolysis over Pd-black followed by oxidative cyclization with iodobenzene diacetate in acetonitrile solvent gave (±)-(**130**) and reduction yielded trans isomer (±)-(**131**) as a major product. Reaction of methyl ester (±)-(**131**) with LAH gave spiro acid (±)-(**132**). The reaction of spiro acid (±)-(**132**) with amine (**133/134**) in the presence of coupling reagent DCC/HOBt resulted (±)-**1** (91%). On subjecting it to chiral HPLC gave (+)-**1** with absolute configurations 'R' at C-1 and 'S' at C-6 chiral centers, respectively. While (-)-**1** is with 'S' and 'R' configurations at C-1 and C-6 chiral center, respectively (**Scheme 1**).

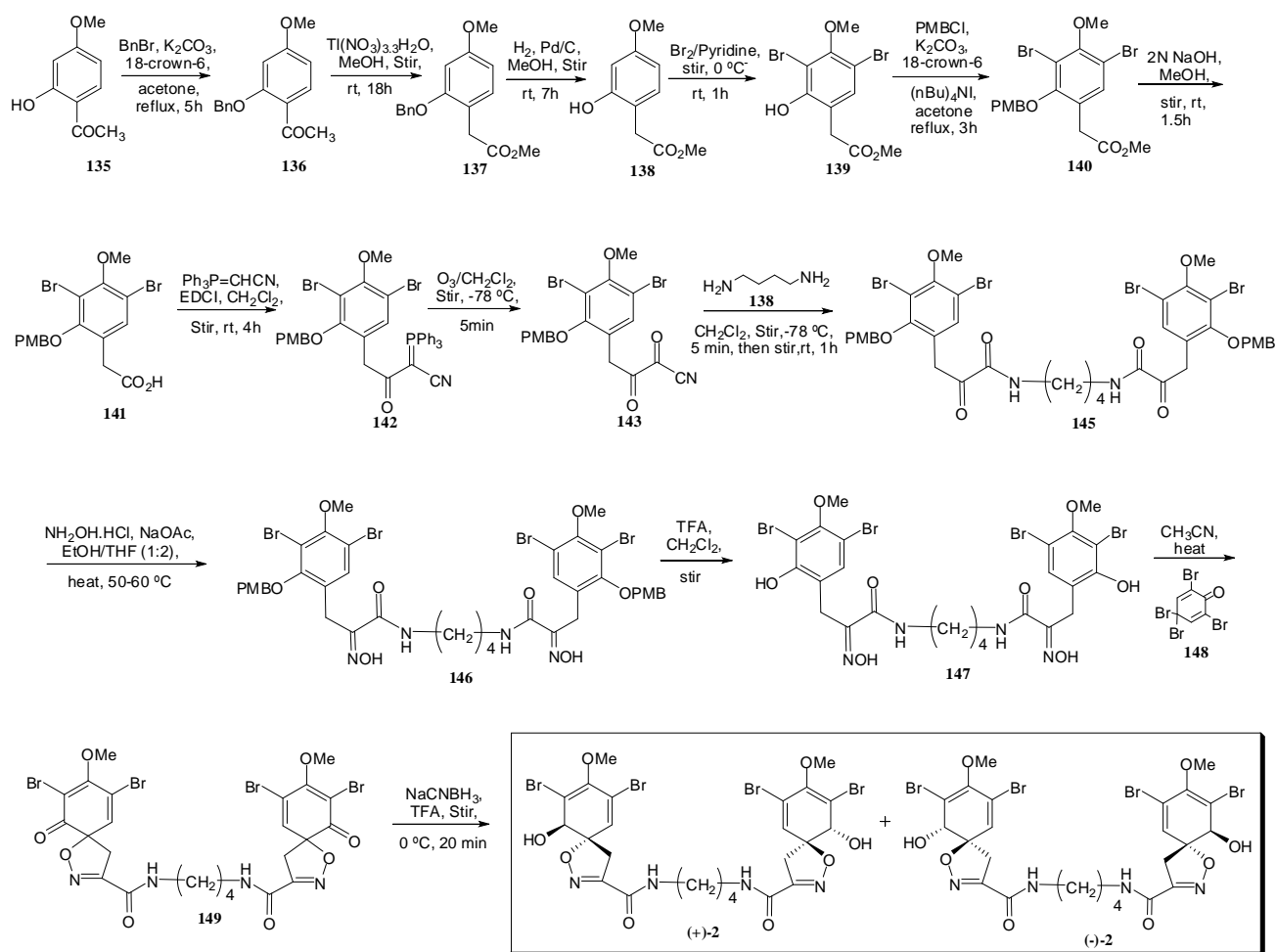


Scheme 1: Total synthesis of Subereamolline A (1)

3.2 Aerothionin (2)

Many research groups have reported total synthesis aerothionin (**2**) which are represented here.

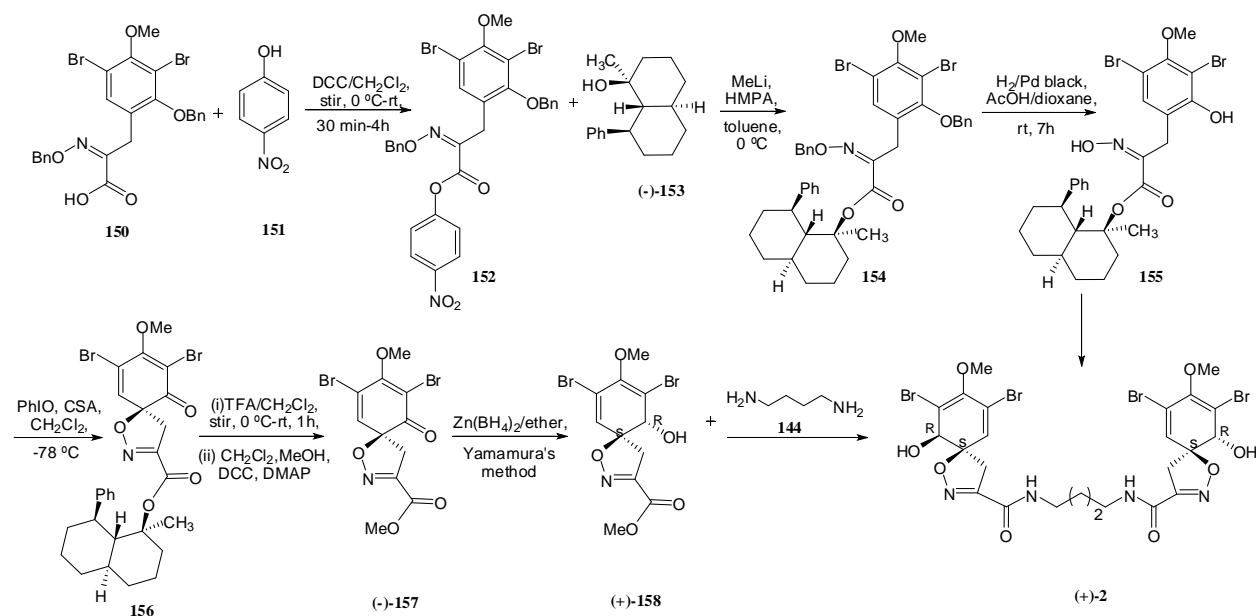
3.2.1 Synthesis by Wasserman et al. [87]: The synthesis of **2** was carried out using cyano ylide coupling methodology to yield (\pm)-*trans-trans*-aerothionins (**Scheme 2**). The synthesis was initiated by treating 2-hydroxy-4-methoxyacetophenone (**135**) with a mixture of benzyl bromide (BnBr), 18-crown-6 and K₂CO₃ using acetone to get benzyl ether (**136**) (97% yield) thereby protecting phenolic hydroxyl. Further, treatment with thallium nitrate trihydrate afforded methyl[2-(benzyloxy)-4-methoxyphenyl]acetate (**137**) in 76% yield. The deprotection using Pd/C catalyst under hydrogen atmosphere produced methyl-(2-hydroxy-4-methoxyphenyl)acetate (**138**). On reaction with bromine using pyridine yielded dibromo derivative (**139**) in 90% yield. Reaction of dibromo derivative (**139**) with *p*-methoxybenzyl chloride (PMBCl) in K₂CO₃, 18-crown-6 and tetra butyl ammonium iodide (TBAI) in acetone produced methyl-[3,5-dibromo-2-(*p*-methoxybenzyloxy)-4-methoxyphenyl]acetate (**140**). Upon hydrolysis using sodium hydroxide in methanol resulted in the formation of (**141**). Further coupling of (**141**) with (triphenylphosphoranylidene)acetonitrile using 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDCI) gave ylide (**142**). Further reaction with ozone in dichloromethane at a very low temperature produced (**143**). Treatment of (**143**) with 1,4-diaminobutane (**144**) afforded bis- α -ketoamide (**145**) which on reaction with hydroxylamine hydrochloride and sodium acetate gave bis-*E*-oxime (**146**) in 95% yield. Deprotection of (**146**) with TFA yielded (**147**) which on reaction with 2,4,4,6-tetrabromo-2,5-cyclohexadienone (**148**) in acetonitrile formed bis-spiro isoxazolidine (**149**) by intramolecular ring closure. Finally, the reduction of cyclohexadienone ketonic centers of (**149**) in a stereospecific manner generated both the hydroxyl groups in *trans* relationship with respect to the corresponding spiroisoxazoline rings to form racemic mixture of (\pm)-*trans-trans* aerothionins in 25% yield by using sodium cyanoborohydride in trifluoroacetic acid.



Scheme 2. Total synthesis of (±)-trans-trans aerothionins by Wasserman et al.

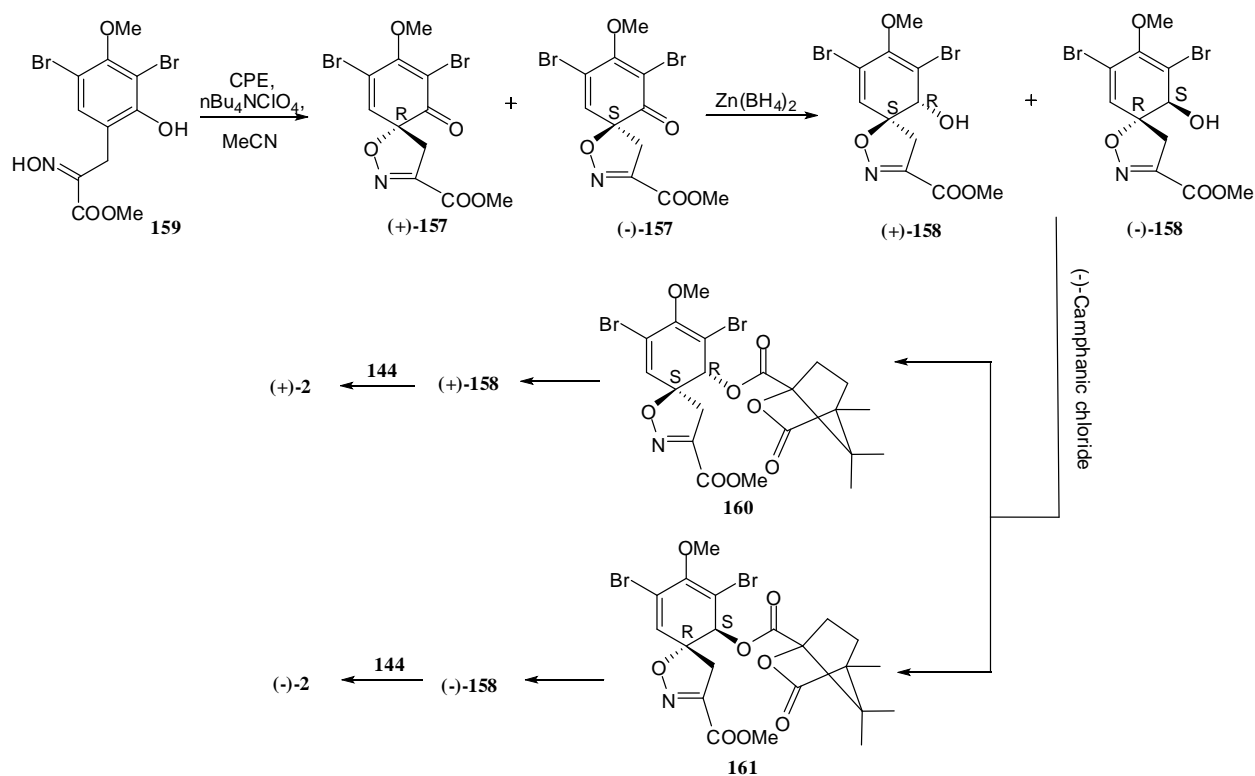
3.2.2 *Asymmetric synthesis by Murakata et al.* [88]: The synthesis began with the asymmetric preparation of enantiomerically enriched cyclohexadienone spiroisoxazoline (-)-(157). The reaction of 2-[(benzyloxy)imino]-3-[2-(benzyloxy)-3,5-dibromo-4-methoxyphenyl]-propanoic acid (150) with *p*-nitrophenol (151) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) using a mixture solvents dichloromethane/methanol afforded *p*-nitrophenyl ester (152) in 91% yield (Scheme 3). Further, trans-esterification with lithiated (-)-(153) as a chiral auxiliary gave 1-methyl-1-decalyl ester (154) in 85% yield. Hydrogenolysis using palladium catalyst in a mixture of acetic acid-dioxane (1:1) solvent system produced *o*-phenolic oxime ester (155) in 91% yield. Treating compound (155) with iodosylbenzene (PhIO) and camphor-sulfonic acid (CSA) using dichloromethane yielded spiroisoxazoline (156) in 83% yield. Further treatment with TFA at room temperature for 1 h and reaction with *N,N'*-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in the mixture of methanol and dichloromethane formed methyl ester (-)-(157) in 71% yield with 74% enantiomeric excess. Finally, reduction of (-)-(157) with $\text{Zn(BH}_4)_2$ in ether furnished

cyclohexadienylisoxazoline (+)-**(158)**, which on amidation with butanediamine **(144)** afforded (+)-**(2)** with *S*-configuration at both the spirocenters [89].



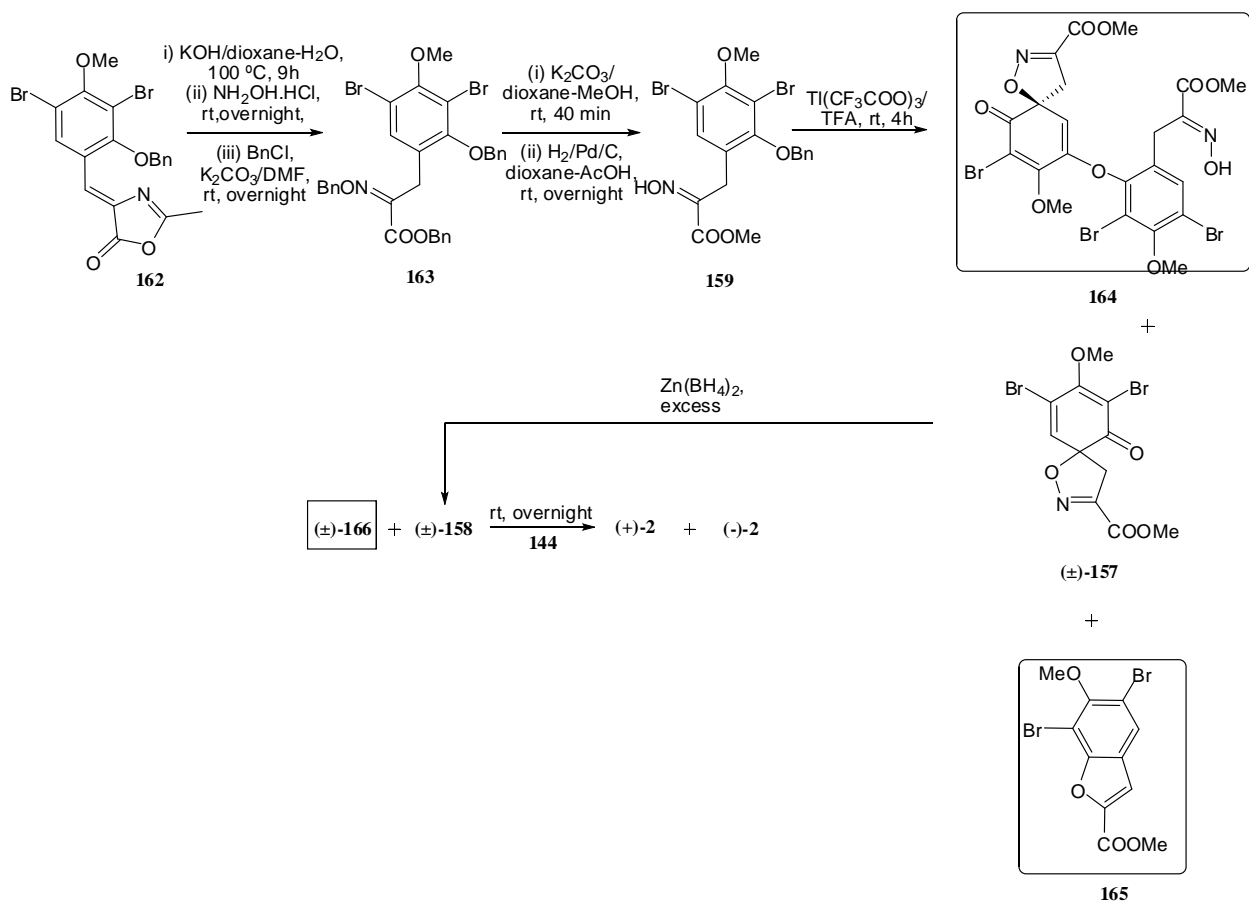
Scheme 3. Asymmetric synthesis of (+)-aerothionin.

3.2.3 Synthesis by Ogami *et al.* [90]: A highly efficient synthesis of optically pure (+)-**2** and (-)-**2** was reported from the racemic spiroisoxazoline derivative (\pm)-**(158)**. In this method, synthesis of compound (\pm)-**(158)** was efficiently achieved by electrochemical oxidation of **(153)** using constant potential electrolysis (CPE) to give (\pm)-**(157)** followed by its reduction with $\text{Zn}(\text{BH}_4)_2$ [91]. The reaction of (\pm)-**(158)** with (-)-camphanic chloride resulted in the production of camphanic acid esters **(160)** and **(161)** as two diastereomers in 46% and 47% yield, respectively which were easily separated by silica gel chromatography. The solvolysis of **(160)** and **(161)** using K_2CO_3 as a base furnished optically active spiroisoxazoline (+)-**(158)** and (-)-**(158)**, respectively. Finally, condensation of (+)-**(158)** and (-)-**(158)** with 1,4-diaminobutane produced optically pure (+)-**2** and (-)-**2** in 28% and 33% yield, respectively (**Scheme 4**).



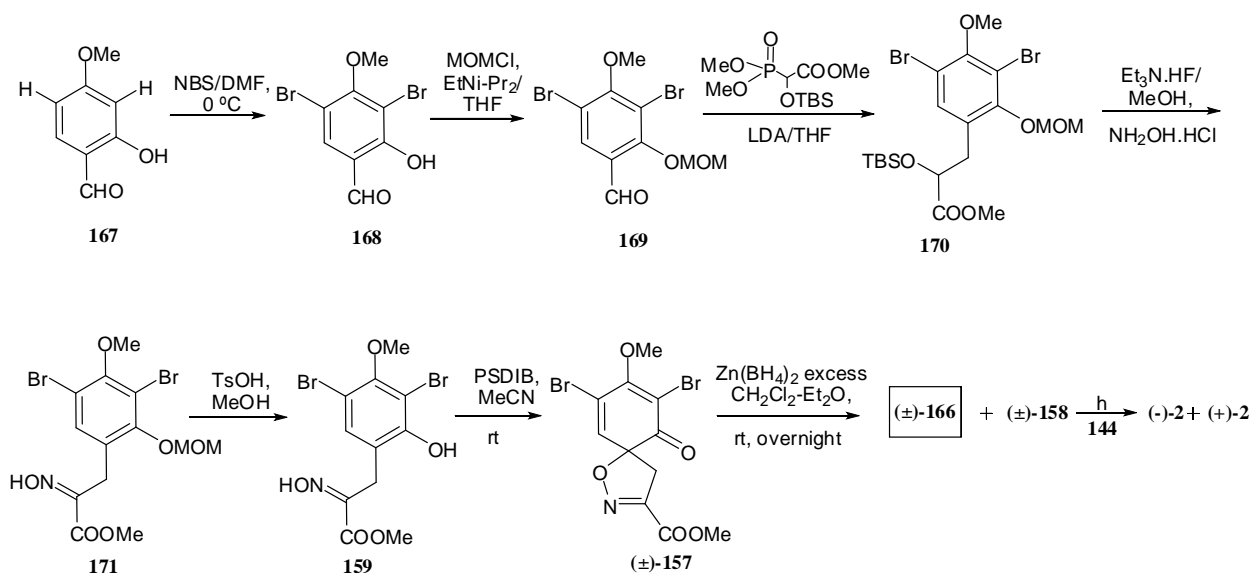
Scheme 4. Total synthesis of optically pure aerothionins.

3.2.4 *Synthesis by Yamamura et al.* [92]: In this synthesis of (\pm)-*trans-trans* aerothionins (**2**), phenolic oxidation of methyl pyruvate (**159**) with thallium (III) trifluoroacetate was used as a key step. Azalactone (**162**) was hydrolysed with KOH in a mixture of dioxane-water followed by the oximation with hydroxylamine hydrochloride, and benzylation using potassium carbonate (K_2CO_3) in *N,N*-dimethylformamide (DMF) yielded tribenzyl derivative (**163**) in 31% yield. Further *trans*-esterification in methanol and K_2CO_3 base gave corresponding methyl ester which after hydrogenolysis in the presence of catalytic palladium-carbon (10%) using a mixture of dioxane-acetic acid yielded the desired dihydroxy derivative (**159**) in moderate yield. The derivative (**159**) was oxidized by thallium (III) trifluoroacetate in TFA to afford compounds (**164**), (\pm)-(**157**) and (**165**) in 21, 27 and 3% yield, respectively. Excess of $\text{Zn}(\text{BH}_4)_2$ in a mixture of chloroform and ether was used for the reduction of racemate (\pm)-(**157**) which yielded a mixture of (\pm)-(**158**) *trans-trans* and (\pm)-(**166**) *cis-cis* products (1.3:1) with 70% yield. Finally, amidation of (\pm)-(**158**) with 1,4-butanediamine (**144**) furnished (\pm)-*trans-trans* aerothionins (**2**) in 18% yield (**Scheme 5**).



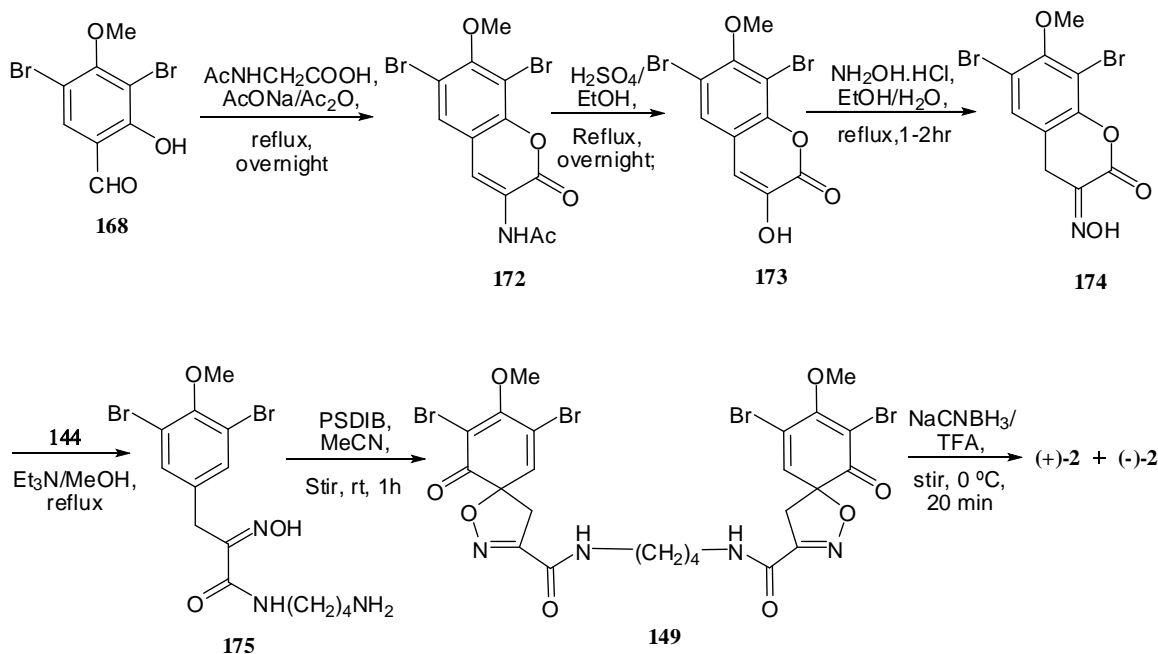
Scheme 5. Total synthesis of (±)-*trans-trans* aerothionins.

3.2.5 *Synthesis by Boehlow et al.* [93]: A new strategy for oxidative spirocyclization of phenolic oxime ester has been developed by Boehlow et al for the efficient synthesis of (±)-*trans-trans* aerothionins (**2**). The reaction of 2-hydroxy-4-methoxybenzaldehyde (**167**) with NBS in DMF yielded dibromoaldehyde (**168**) in 97% yield. Further treatment with methyl chloromethyl ether (MOMCl) in the presence of EtNi-Pr₂ using THF gave the protected aldehyde (**169**) in 99% yield. The reaction (**169**) with 2-(*tert*-butyldimethylsilyloxy)-2-(dimethylphosphono)acetate using LDA and THF afforded silyl enol ether (**170**) in 92% yield. Deprotection of (**170**) by Et₃N.HF in methanol, followed by immediate addition of hydroxylamine hydrochloride furnished oxime (**171**) in 90% yield. The treatment of (**171**) with catalytic amount of TsOH in methanol resulted in removal of MOM group to produce phenolic oxime (**159**) in 100% yield. The oxidative cyclization of oxime ether (**159**) using polymer-supported (diacetoxyiodo)benzene (PSDIB) reagent in acetonitrile afforded spiroisoxazoline (±)-(**157**) with high yield and purity. Spiroisoxazoline (±)-(**157**) so obtained was easily transformed into (±)-**2** by procedure as adopted by Yamamura *et al.* (**Scheme 6**).



Scheme 6. Total synthesis of (±)-trans-trans aerothionins.

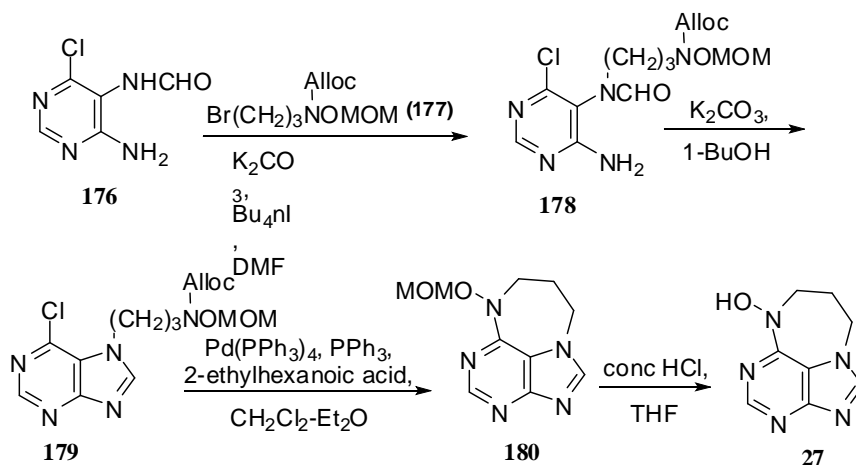
3.2.6 *Synthesis by Harburn et al.* [94]: A novel synthetic approach to (±)-(2) through acylation of amine with coumarin was adopted as a key step. The synthesis began with the treatment of dibromosalicylaldehyde (**168**) with *N*-acetylglycine in the presence of sodium acetate using acetic anhydride to yield acetamido coumarin (**172**) in 53-90% yield. Further hydrolysis using ethanolic solution of sulfuric acid gave enol (**173**) in 90% yield which on reaction with 8.5 equivalents of hydroxylamine hydrochloride in 80% ethanol under reflux furnished oximino coumarin (**174**) in 69% yield. The reaction of (**174**) with 1,4-butanediamine (**144**) using triethylamine as a base in methanol at 60 °C under reflux gave amide derivative (**175**) in 68% yield. The treatment of (**175**) with PSDIB using acetonitrile resulted in the oxidative spirocyclization which led to the formation of aerothionin precursor (**149**) and was converted into (±)-(2) by the same procedure as adopted by Wasserman *et al.* (**Scheme 7**).



Scheme 7. Total synthesis of (±)-aerotionins

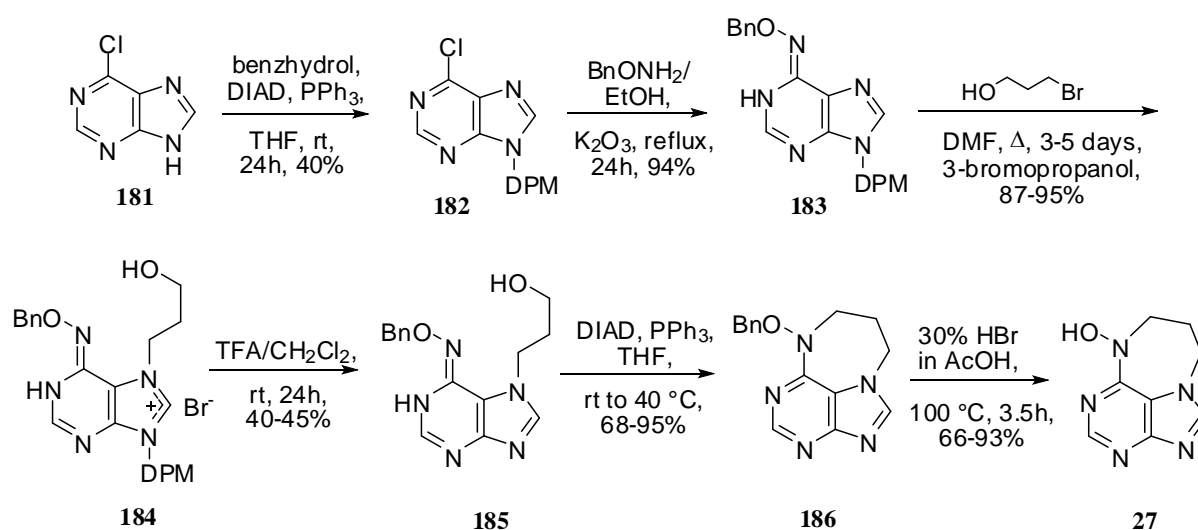
3.3 Asmarine B (27)

3.3.1 *Synthesis by Ohba and Tashiro (2002) [95]*: Ohba and Tashiro initiated total synthesis of asmarine B by using formamide (**176**) which on treatment with bromide (**177**), K_2CO_3 , Bu_4NI in DMF afforded N-alkylated formamide (**178**) in 74% yield. Further, base-promoted cyclization of (**178**) in 1-BuOH gave desired 7-alkylated 6-chloropurine (**179**) in 87% yield. The removal of Alloc group was carried out by reaction of (**179**) with Pd catalyst in the presence of 2-ethylhexanoic acid and intramolecular amination at the 6-position of the resulting amine using Et_3N (1-BuOH, reflux, 2h) gave tricyclic compound (**180**). In the final step, the deprotection of (**180**) using concentrated HCl in THF provided asmarine B (**27**) in 86% (**Scheme 8**).



Scheme 8: Total synthesis of asmarine B (27)

3.3.2 *Synthesis by Pappo and Kashman (2003)* [96]: Another, asmarine B synthesis started with reaction of 6-chloropurine (**181**) with triphenylphosphine and benzhydrol in dry THF and addition of diisopropyl azodicarboxylate (DIAD) at 0°C under argon atmosphere to obtain 9-diphenylmethyl-6-chloropurine (**182**) in 40% yield. Further, mixture of (**182**), O-benzyl hydroxylamine and K₂CO₃ were heated under reflux to produce N⁶-benzyloxy-9-diphenylmethyladenine (**183**). On treating (**183**) with 3-bromopropanol afforded compound (**184**) in 95% and diphenylmethyl (DPM) group was selectively removed under strong acidic condition, TFA in CH₂Cl₂ to get (**185**) in 45% yield. Further, using Mitsunobu reaction (DIAD, Ph₃P) [97] product (**186**) was achieved, which on warming in 30% HBr in AcOH solution at 100 °C for 3.5hr yielded final product asmarine B (93%) (**Scheme 9**). Subsequently, same research group [98] synthesized several asmarine B analogues by substituting different groups at 9-position.

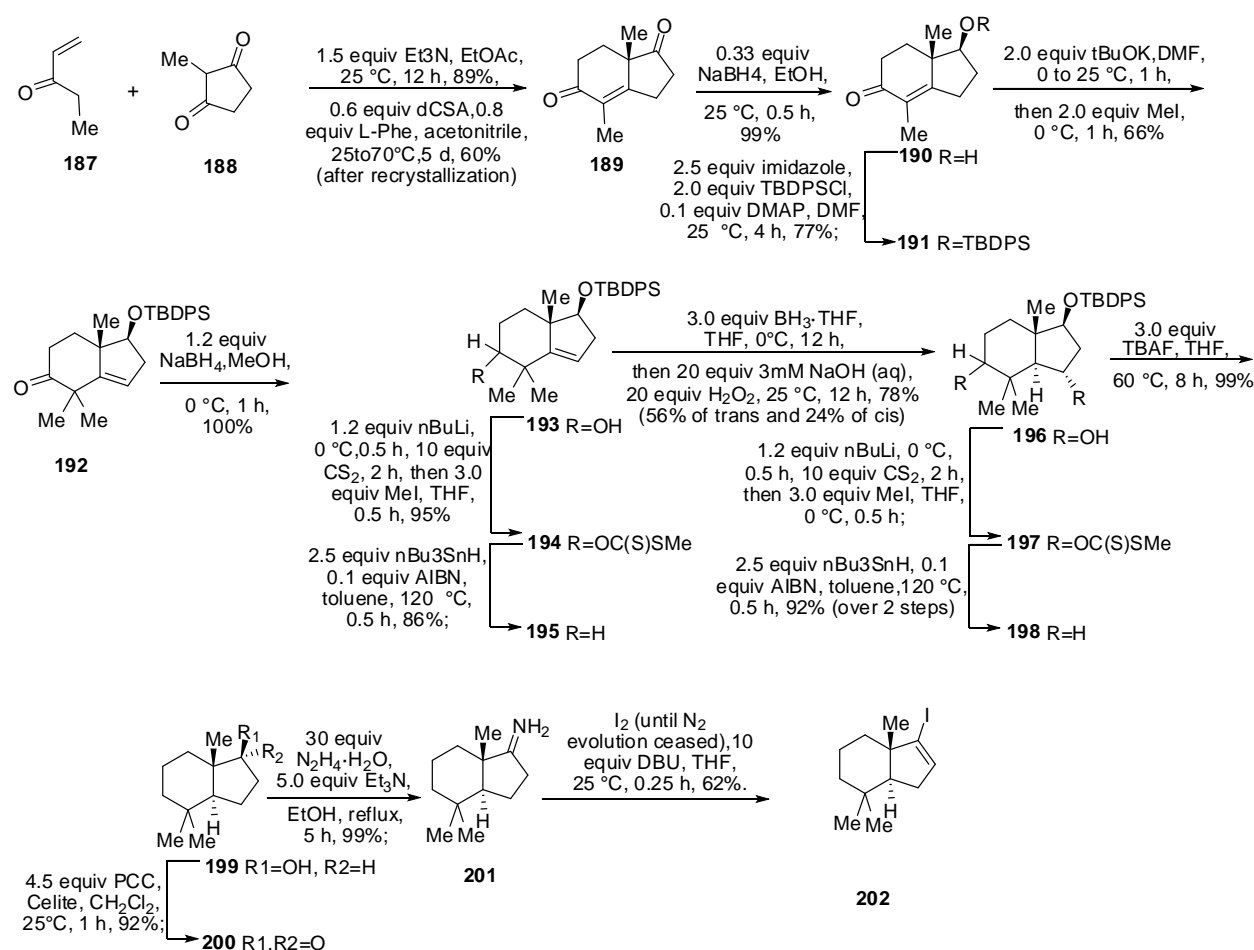


Scheme 9: Total synthesis of asmarine B (27)

3.3 Norrsolide (36)

3.4.1 *Synthesis by Brady et al. (2005)* [99]: The synthesis began with preparation of the hydrindane core fragment. Asymmetric Robinson annulations of enone (**187**) with diketone (**188**) [100] plus using D-CSA and L-phenylalanine in the reactions led to an enantioselective synthesis of ketone (+)-(**189**). Selective reduction of C9 carbonyl group followed by silylation afforded compound (**191**) in 76% yield. Treatment of (**191**) with *t*BuOK produced the extended enolate which upon methylation gave rise to ketone (**192**) in 66% yield. Reduction of the C4 carbonyl group of 16, followed by a Barton-McCombie radical deoxygenation of the resulting alcohol, produced alkene (**195**) in 82% yield. The hydroxylation of (**195**) using BH₃.THF/H₂O resulted in *trans*-bicycle (**196**) as the major product in 56% yield. (*trans/cis* 2.3:1). Ketone (**200**) was produced in 84 % yield by radical deoxygenation of

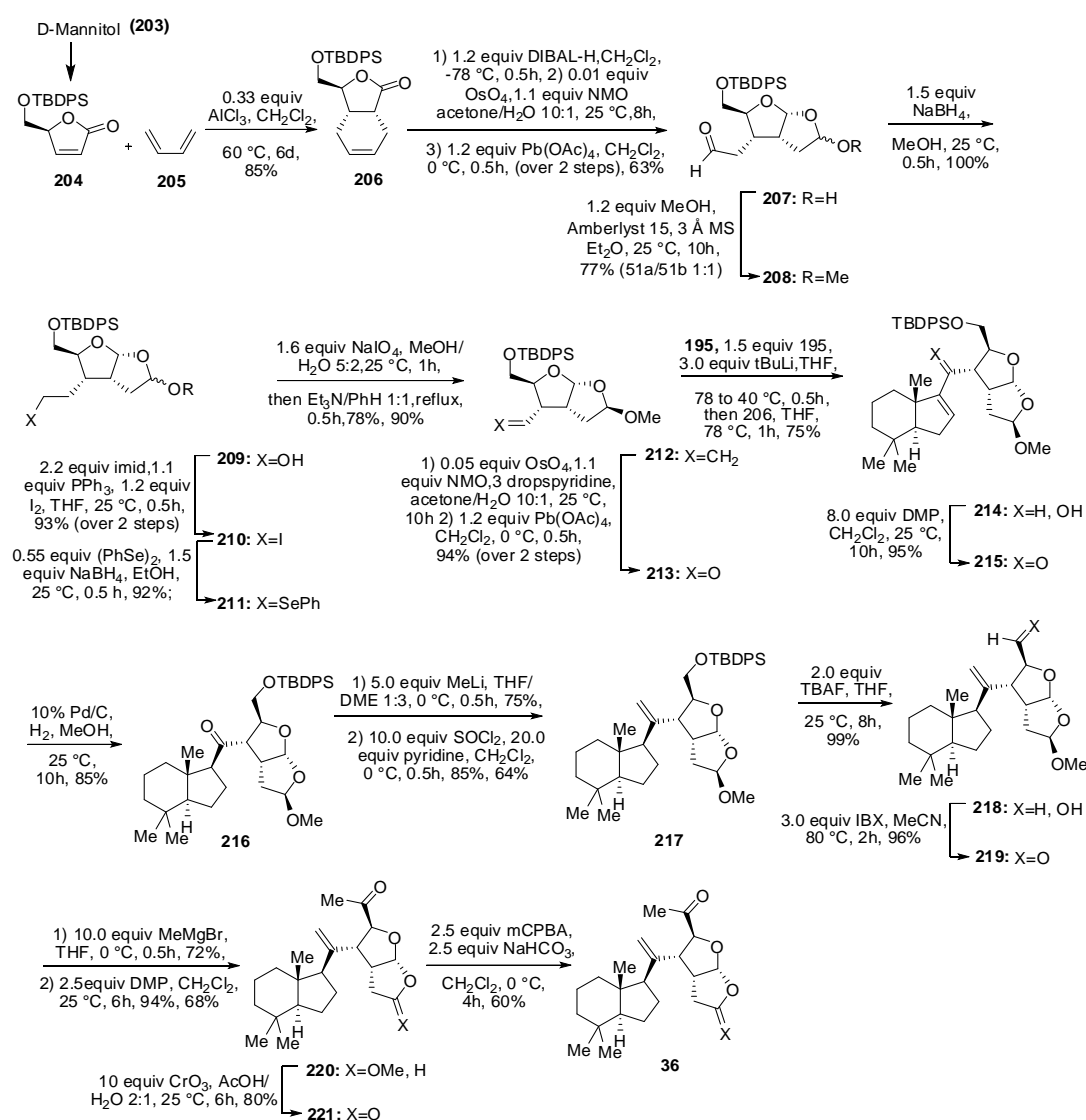
(**196**) and deprotection of the C9 silyl ether followed by oxidation. Reaction of (**200**) with hydrazine afforded hydrazone (**201**) and further treatment with I₂/Et₃N/DBU gave vinyl iodide (**195**) in 61% yield. The *trans* junction of the bicyclic scaffold of (**202**) was studied by a single-crystal X-ray diffraction analysis of hydrazone (**201**) (scheme 10).



Scheme 10. Preparation of bicyclic scaffold (202**) for synthesis of Norrsolide (**36**)**

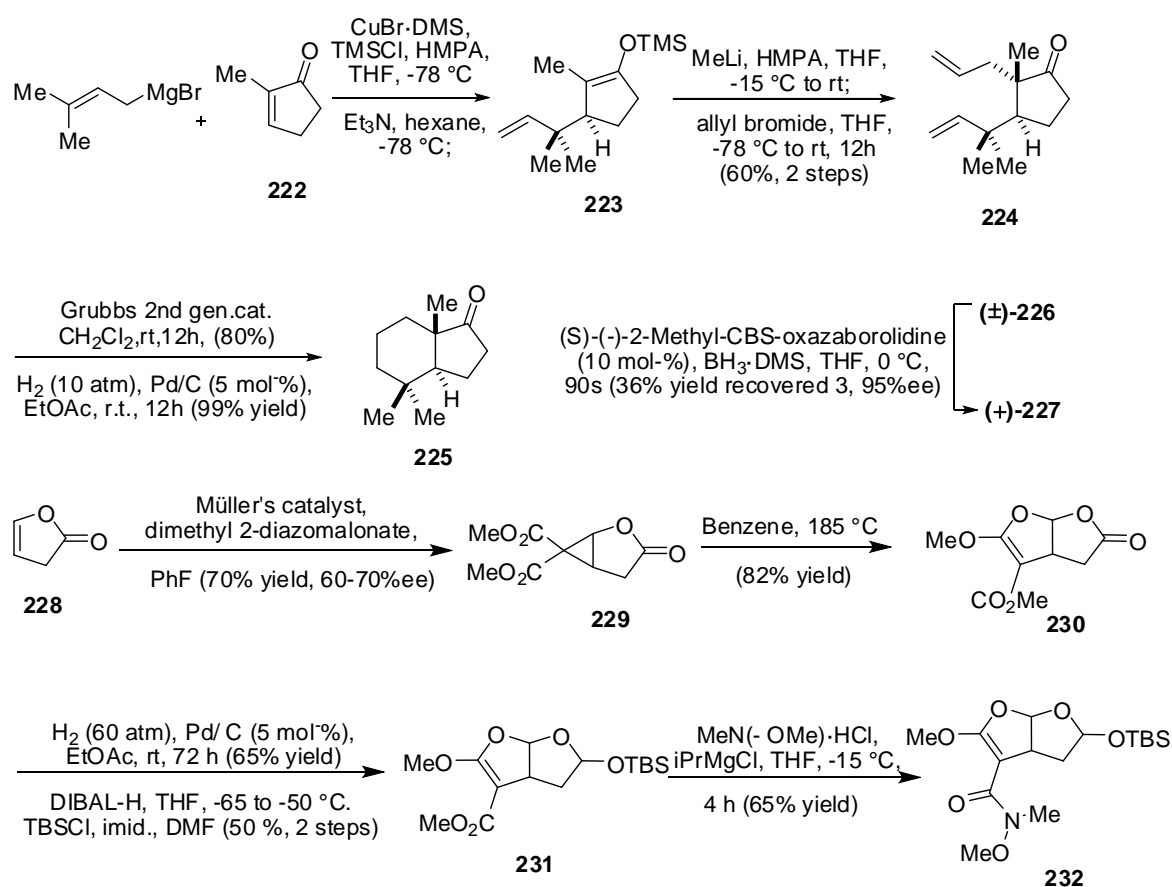
Enantiomerically pure butenolide (**204**) was prepared from D-mannitol (**203**) as described in the literature [101]. The Diels-Alder reaction of (**204**) and butadiene (**205**) under AlCl₃ catalysis afforded (**206**) in 85% yield. The reaction had proceeded exclusively from the opposite face of the bulky TBDPS group and formed single enantiomer. DIBAL-H reduction of (**206**) at C20 lactone followed by oxidative cleavage of double bond using OsO₄, NMO, Pb(OAc)₄ gave lactol (**207**, 63%) as 1:1 mixture of isomers at C-14 center. Further, lactol (**207**) on treatment with MeOH in the presence acid catalysis resulted in the formation of isomeric methyl ethers (**201**). Aldehyde (**208**), via intermediate (**209** and **210**) was converted to selenide (**211**) [102]. This was followed by oxidation (NaIO₄) and elimination to get alkene (**212**) in 61% yield. On dihydroxylation using OsO₄, NMO and oxidative cleavage resulted in aldehyde (**213**) in 94% yield. Further, treating vinyl iodide (**202**) with *t*-BuLi and quenching of the

anion with aldehyde **56** yielded desired allylic alcohol (**214**). This was followed by oxidation to form enone (**215**) using Dess-Martin periodinane. Enone (**215**) was hydrogenated across C8-C9 bond, exclusively from the α -face of the hydrindane core to produce ketone (**216**) in 85% yield. Reaction of (**216**) with excess MeLi and dimethoxyethane resulted in formation of corresponding tertiary alcohol which on treatment with thionyl chloride in pyridine gave terminal alkene (**217**) (85%). Fluoride-induced deprotection of the silyl ether and oxidation of the resulting alcohol (**218**) furnished aldehyde (**219**) (93%). Immediate reaction with excess of methyl Grignard produced (**220**) which on treatment with CrO₃ in aqueous acetic acid produced lactone (**221**) in 80% yield. In the final step, Baeyer-Villiger oxidation of (**221**) with *m*-CPBA in the presence of NaHCO₃ led to insertion of the oxygen between the C19 and C21 centers with complete retention of configuration produced norrisolide (**36**) in 60% yield (**Scheme 11**).



Scheme 11: Total synthesis of Norrisolide (36)

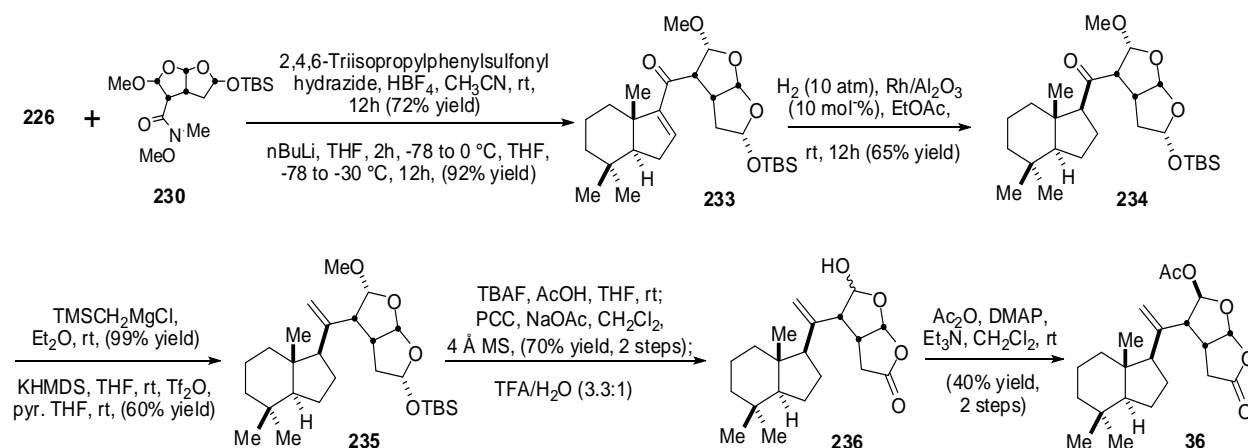
3.4.2 *Synthesis by Granger et al.* [103]: The synthesis initiates with the preparation of the hydrindane portion of norrsolide. Enone (**222**) was alkylated with the cuprate of isoprenyl Grignard in the presence of HMPA to get corresponding silyl enol ether (**223**) (**Scheme 12**). On treatment of (**223**) with MeLi and allyl bromide provided diene (**224**) in 60% yield. Further, ring closing metathesis with Grubbs second generation catalyst (0.5 mol %) gave the ring-closed product (**225**) in 80% yield. Hydrogenation of the olefin with Pd/C gave (\pm)-218-1. Specifically, treatment of (\pm)-(**226**) with (S)-(-)-2-methyl-DBS-oxazaborolidine (10 mol%) and BF₃.DMS yielded a 36% recovery of (+)-(**226**). The side chain furanyl derivative was prepared using furan-2(3H)-one (**228**), which was cyclopropanated with dimethyl 2-diazomalonate to give cycloadduct (**229**). Thermolysis of (**229**) in benzene at 185 °C resulted in the formation of adduct (**230**). Continuation of diastereoselective hydrogenation of intermediate (**230**) by using Pd/C under 60 atm H₂ with triethylamine and further reduction with DIBAL-H followed by protection as the TBS lactol provided compound (**231**). Finally, the methyl ester of intermediate (**231**) was transformed into Weinreb amide (**232**).



Scheme 12: Preparation of the norrisane side chain.

Condensation of ketone (**226**) with 2,4,6-triisopropylphenylsulfonyl hydrazide followed by treatment of resultant hydrazone with *n*-BuLi gave a vinyl anion which was treated with Weinreb amide (**230**). This provided enone (**233**) as a mixture of diastereomers. Hydrogenation of (**233**) by using rhodium on alumina in ethyl acetate provided ketone (**234**). Further addition of $\text{TMSCH}_2\text{MgCl}$ into ketone (**234**)

and activation of tertiary hydroxyl group with triflic anhydride and use of excess amount of pyridine cleanly generated desired 1,1-disubstituted olefin (**235**). The TBS acetal of (**235**) was deprotected with acetic acid buffered TBAF and oxidized with PCC. After TFA hydrolysis, intermediate (**236**) was obtained as a 1:1 mixture of lactol epimer. Finally upon acetylation, only the desired diastereomer matching natural norrisolide (**36**) was isolated in 40% yield (**Scheme 13**).

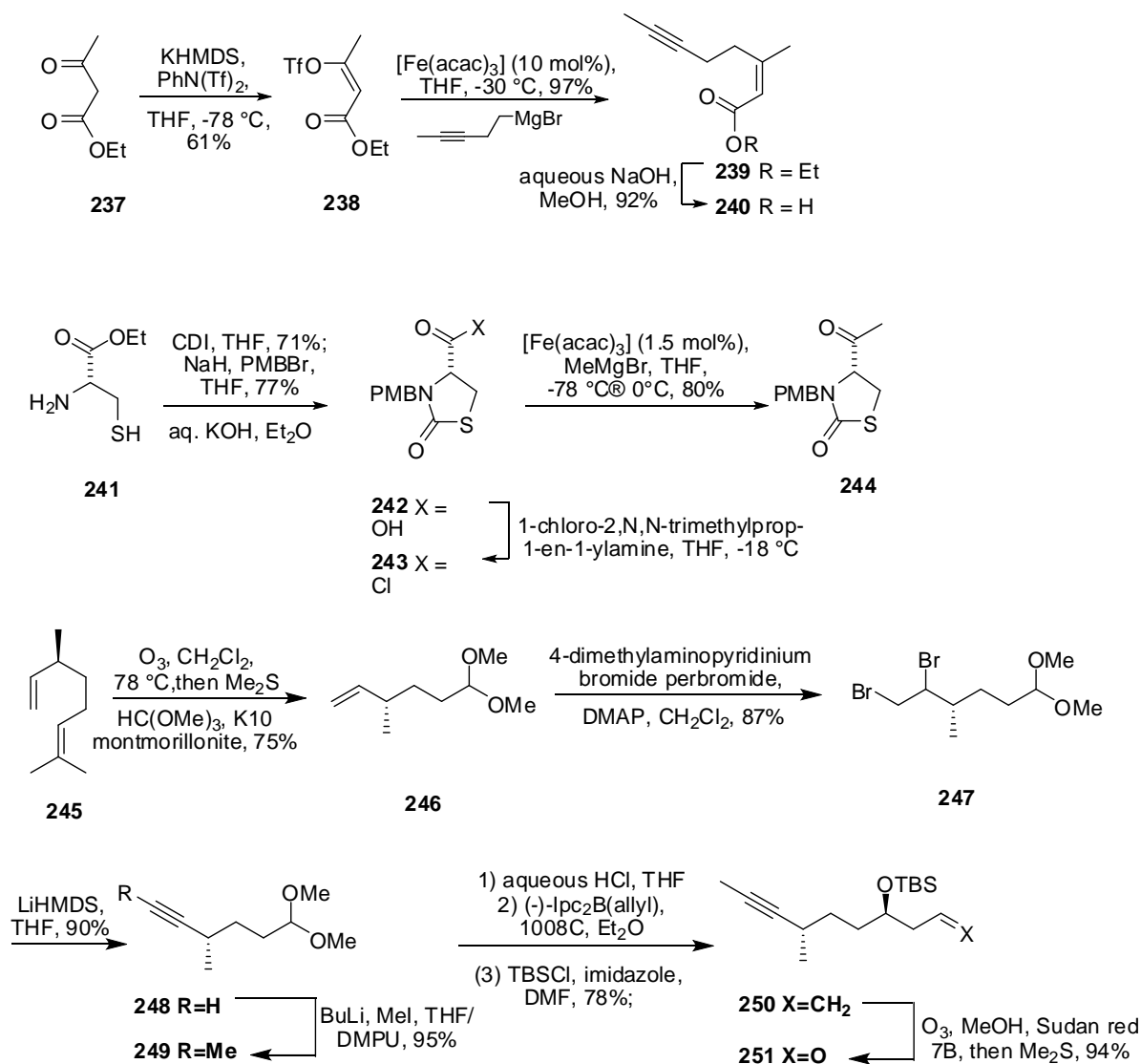


Scheme 13: Total synthesis of Norrisolide (36)

3.4 Latrunculin B (99B)

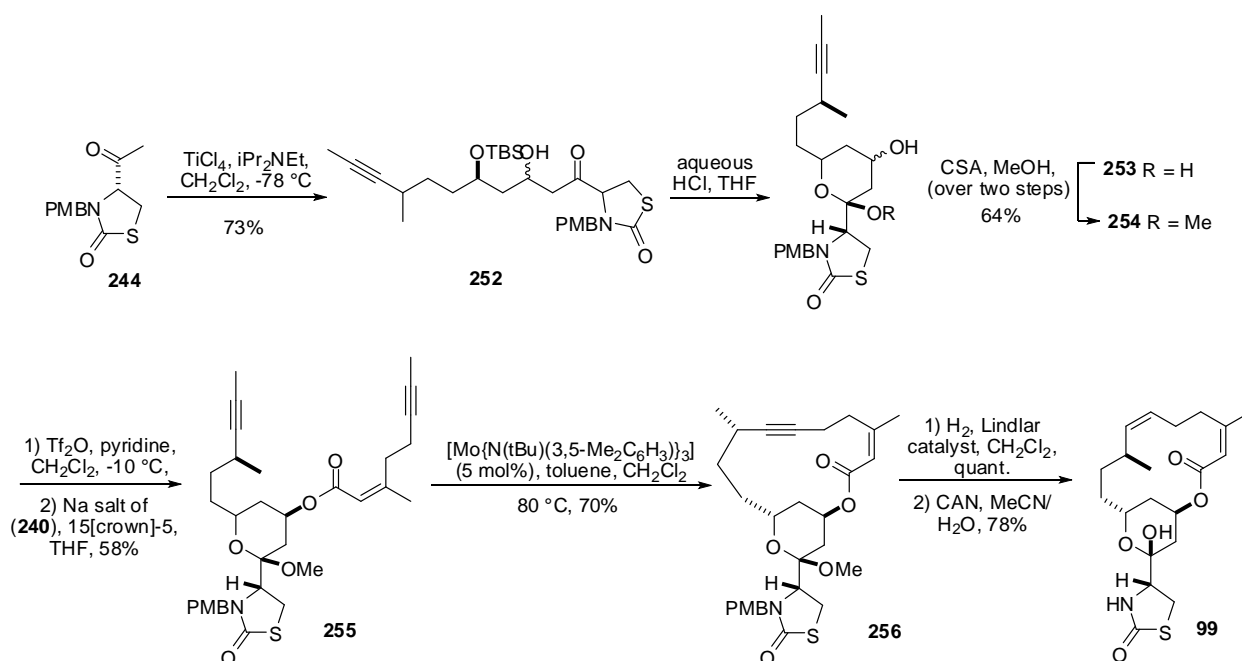
3.5.1 Synthesis by Fürstner, et al (2003) [104]: They used catalysis based synthesis which began with transformation of ethyl acetoacetate (**237**) to its corresponding triflate (**238**). Further, treatment using Grignard reagent derived from 1-bromo-3-pentyne in the presence of [Fe(acac)₃] as a precatalyst afforded the desired product (**239**) and under mild conditions yielded (**240**). This was the first case of using iron catalysed cross coupling reaction of an enol triflate. Other fragment cysteine derived ketone (**244**) was prepared from (R)-ethyl 2-amino-3-mercaptopropanoate (**241**) which was first converted to acid chloride (**243**) using standard method and using iron catalysis yielded (**244**) (**Scheme 14**).

The third building block was synthesized from (+)-citronelene (**245**, 91% ee) by selective ozonolysis and acetalization to get (**246**). Further, bromination of (**246**) provided (**247**) which on treatment with LiHMDS gave (**248**). Alkyne (**248**) was methylated with BuLi and MeI to yield (**249**). Deprotection of acetal group in (**249**) and allylation of the resulting aldehyde with (-)-Ipc₂B(allyl). Further, protection with a TBS group furnished (**250**) in diastereomerically pure form (d.R. > 99.5:0.5). Selective ozonolysis of its alkene entity afforded the required product (**251**) (**Scheme 14**).



Scheme 14: Preparation of desired products cysteine derived ketone (244) and aldehyde (251) for the synthesis of Latrunculin B (99B)

Reaction of aldehyde (**251**) with the titanium enolate derived from ketone (**244**) gave aldol (**252**) as a mixture (2:1) of diastereomers. Thus, acid catalysed cleavage of the TBS group in (**243**) delivered hemiacetal (**252**) (d.r. up to 7:1). Glycosylation with MeOH, conversion of product (**254**) into the corresponding triflate, and reaction with the sodium salt of acid (**240**) furnished diyne (**255**), the substrate for the envisaged macrocyclization by RCAM. The reaction proceeded well in the presence of precatalyst $[\text{Mo}\{\text{N}(\text{tBu})(3,5\text{-Me}_2\text{C}_6\text{H}_3)\}_3]$ and activated in CH_2Cl_2 to get cycloalkyne (**256**). This was further subjected to Lindlar reduction to ensure the stereoselective formation of Z-alkene entity, followed by cleavage of the N-PMB group and the methyl glycoside with CAN (ceric ammonium nitrate) to provide latrunculin B (**99B**) (Scheme 15).



Scheme 15: Total synthesis of Latrunculin B (99B)

Conclusion: Marine sponges being sessile are overgrown with different organisms/invaders and thus have evolved with a number of chemical defences. They produce a broad spectrum of cytotoxic compounds to cope successfully with this diverse variety of aggressors/invaders by killing or inhibiting the growth of their cells. Thus, tapping such marine natural products by understanding their structural chemistry will inspire chemists to design compounds with enhanced potency and selectivity to innovate novel anti-cancer agents. We have selected Red Sea as the geographical location which is a hotspot with huge sponge biodiversity and accounted cytotoxic compounds, representing different class of compounds, with maximum being terpenoids (65 compounds) followed by alkaloids (29 compounds) with the total reaching upto 124 including peptides, macrolides, fatty esters, amides etc. The most commonly used anticancer drug screen panels consisted of cultured cell lines like A-598 (renal), A-549 (lung), Daoy (medullocarcinoma), HER-2 (epidermal), MDA-MB-231, MCF-7, BT-474, SKBR3, T47D (Breast), HCT116, Caco-2 (colorectal), HT-29 (colon), HeLa (cervical), HepG2 (hepatomacellular), KB-16 (nasopharyngeal), P-388 (leukemia), L5187Y (lymphoma), MEL-28 (melanoma) etc. The biological activity of each compound against different cancer cell lines with IC_{50} values has been described. Surprisingly, several compounds isolated from Red Sea sponges have shown $IC_{50} < 10 \mu M$ which could be considered as possible candidates for additional mechanism-of-action studies. There are limited understanding of the structure-activity relationship (SAR) and mechanism of action studies of these molecules. In this review, we have discussed changes in the

activity profile with respect to the changes in the molecular structures which might give future directions for studying their mechanism-of-action taking into account different structural motifs. The progress in the SAR studies of aerothionin, asmarines, and latrunculin molecules in addition to the different strategies of total synthesis of subreamolline A, aerothionin, asmarine B, norrsolide and latrunculin B have been presented here. We believe that, detailed explanation on the cytotoxic activity and synthesis will definitely help the medicinal and synthetic chemists to build structure-activity relationship strategies in regard to further designing potential anticancer agents for the treatment of different cancers.

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List of abbreviations

Ac ₂ O	Acetic anhydride	Tl(CF ₃ COO) ₃	Thallium(III) trifluoroacetate
AlCl ₃	Aluminium chloride	SOCl ₂	Thionyl chloride
AIBN	Azobisisobutyronitrile	TiCl ₄	Titanium tetrachloride
Ba(OH) ₂	Barium hydroxide	TsOH	<i>p</i> -Toluenesulfonic acid
BnBr	Benzyl bromide	<i>n</i> Bu ₃ SnH	Tributyltin hydride
BnCl	Benzyl chloride	Et ₃ N	Triethylamine
BnONH ₂	O-Benzylhydroxylamine	TFA	Trifluoroacetic acid
PhN(Tf) ₂	<i>N,N</i> -Bis(trifluoromethyl-sulfonyl)aniline	HC(OMe) ₃	Trimethyl orthoformate
BH ₃ .THF	Borane-tetrahydrofuran	TMSCH ₂ MgCl	(Trimethylsilyl)methylmagnesium chloride
BH ₃ .DMS	Borane dimethyl sulfide complex	Zn(BH ₄) ₂	Zinc borohydride
BF ₃ .DMS	Boron fluoride-dimethyl sulfide	A-498	Renal cancer
NBS	<i>N</i> -bromosuccinimide	A-549	Human lung adenocarcinoma
TBS/TBSCl	<i>tert</i> -Butyldimethylsilyl chloride	BT-474	Breast cancer cells
TBDPS	<i>tert</i> -Butyldiphenylsilyl	Caco-2	human colorectal cancer cell lines
TBDPSCl	<i>tert</i> -Butyl(chloro)-diphenylsilane	CVS	Crystal violet staining
BuLi	Butyllithium	Daoy	Human medullocarcinoma cell
CSA	Camphorsulfonic acid	HCT 116	Human colorectal carcinoma
CS ₂	Carbon disulfide	Hela	Human cervical carcinoma
CDI	Carbonyldiimidazole	Hep G2	Human hepatomacellular carcinoma
CAN	Ceric ammonium nitrate	HER-2	Human epidermal growth factor receptor 2
CPE	Chlorinated polyethylene	HT-29	Human colon carcinoma
MOMCl	Chloromethyl methyl ether	KB-16	Human nasopharyngeal carcinoma

<i>m</i> -CPBA	<i>m</i> -Chloroperoxybenzoic acid	L5178Y	Mouse lymphoma
CrO ₃	Chromium trioxide	MCF-7	Breast adenocarcinoma cells
CuBr·DMS	Copper(I) bromide dimethyl sulfide complex	MDA-MB-231	Breast adenocarcinoma cells
PSIDB	(diacetoxyiodo)benzene	KHMDS	Potassium bis(trimethylsilyl)amide
DBU	1,8-Diazabicyclo(5.4.0)undec-7-ene	<i>t</i> BuOK	Potassium tert-butoxide
DCC	<i>N,N'</i> -Dicyclohexylcarbodiimide	®T3P	Propylphosphonic anhydride
DIBAL-H	Diisobutylaluminium hydride	PCC	Pyridinium chlorochromate
DIAD	Diisopropyl azodicarboxylate	Rh/Al ₂ O ₃	Rhodium on alumina
DIPEA	<i>N,N</i> -Diisopropylethylamine	NaHCO ₃	Sodium bicarbonate
DMAP	4-dimethylaminopyridine	NaH	Sodium hydride
DMF	<i>N,N</i> -Dimethylformamide	NaI	Sodium iodide
DMP	Dimethyl phthalate	NaIO ₄	Sodium periodate
DMPU	<i>N,N'</i> -Dimethylpropyleneurea	TBAF	Tetrabutylammonium fluoride
(PhSe) ₂	Diphenyl diselenide	TBAI/Bu ₄ N I	Tetrabutylammonium iodide
DPM	Diphenylmethyl	<i>n</i> Bu ₄ NClO ₄	Tetrabutylammonium perchlorate
EDCI/EDC/EDAC	1-ethyl-3-(3-dimethylaminopropyl) carbodimide	HBF ₄	Tetrafluoroboric acid
HMPA	Hexamethylphosphoramide	MEL-28	human melanoma cells
N ₂ H ₄ ·H ₂ O	Hydrazine hydrate	MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
HOBt	Hydroxybenzotriazole	NSCLC	Non-small cell lung cancer
IBX	2-Iodoxybenzoic acid	P-388	Murine leukemia cells
MeMgBr	Methylmagnesium bromide	PC12	Rat brain cancer cells
OsO ₄	Osmium tetroxide	PC-3M	Prostate cancer cell
Pd	Palladium	PI3K	Phosphatidylinositol-3-kinase
L-Phe	Phenylalanine	RTKs	Receptor tyrosine kinases
PSIDB	Polymer-supported (diacetoxyiodo)benzene	SKBR3	Human breast cancer cell
Bu ₄ NI	Tetra- <i>n</i> -butylammonium iodide	T47D	Human breast cancer cell
Tl(NO ₃) ₃	Thallium (III) nitrate	U373	human cancer cell line

References:

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- [1] A.L. Harvey, R.E. Ebel, R.J. Quinn, The re-emergence of natural products for drug discovery in the genomics era, *Nat. Rev. Drug. Discov.* 14 (2015) 111–129.
- [2] U. Hentschel, J. Piel, S.M. Degnan, M.W. Taylor, Genomic insights into the marine sponge microbiome, *Nature* 10 (2012) 641–654.
- [3] a) L.A.M. Aerts, Sponge-coral interactions on Caribbean reefs, *Mar. Ecol. Prog. Ser.* 175 (1998) 241–249. b) F. Folmer, M. Jaspar, M. Dicato, M. Diederich, Marine cytotoxins: callers for the various dances of death, *Gastroenterol. Hepatol.* 2 (2009) 34–50.
- [4] M.K. Wooster, O. Voigt, D. Erpenbeck, G. Wörheide, M.L. Berumen, *Sponges of the Red Sea. Coral Reefs of the World*, Springer Nature Switzerland AG (2019) 91–122.
- [5] Ö.S. Aslantürk, Book Chapter: In vitro cytotoxicity and cell viability assays: principles, advantages, and disadvantages, In book: *Genotoxicity-a predictable risk to our actual World.* (2016) 1–18.
- [6] B.E. Loveland, T.G. Johns, I.R. Mackay, F. Vaillant, Z.X. Wang, P.J. Hertzog, Validation of the MTT dye assay for enumeration of cells in proliferative and antiproliferative assays, *Biochem. Int.* 27 (1992) 501–510.
- [7] M.J.B. Fernandes, C. Limas, M.H. Rossi, E. Gonçalves, I.C. Simoni, Cytotoxicity of subfractions and compounds from *Polymnia sonchifolia*, *Braz. J. Microbiol.* 36 (2005) 338–341.
- [8] S. Uzunoglu, B. Karaca, H. Atmaca, A. Kisim, C. Sezgin, B. Karabulut, R. Uslu, Comparison of XTT and Alamar blue assays in the assessment of the viability of various human cancer cell lines by AT-101 (-/- gossypol), *Toxicol. Mech. Methods* 20 (2010) 482–486.
- [9] S. Lidia, W. Katarzyna, S. Piotr, M. Małgorzata, M. Szymon, L. Katarzyna, C. Tomasz, L. Piotr, K. Piotr, J. Anna, F. Anna, C. Zdzisław, The comparison of MTT and CVS assays for the assessment of anticancer agent interactions, *PLOS ONE.* 11 (2016) 0155772.
- [10] D.J. Newman, G.M. Cragg, K.M. Snader, The influence of natural products upon drug discovery, *Nat. Prod. Rep.* 17 (2000) 215–234.
- [11] Y. Hirata, D. Uemura, Halichondrins-antitumor polyether macrolides from a marine sponge, *Pure Appl.Chem.* 58 (1986) 701–710.
- [12] a) A. Zovko, M. Novak, P. Hååg, D. Kovalerchick, T. Holmlund, K. Färnegårdh, M. Ilan, S. Carmeli, R. Lewensohn, K. Viktorsson, Compounds from the marine sponge *Cribrochalina vasculum* offer a way totarget IGF-1R mediated signaling in tumor cells, *Oncotarget* 7 (2016) 50258–50276. b) J. Cortes, J. O’Shaughnessy, D. Loesch, J.L. Blum, L.T. Vahdat, K. Petrakova, P. Chollet, A. Manikas, V. Diéras, T. Delozier, Eribulin monotherapy versus treatment of physician’s choice in patients with metastaticbreast cancer (EMBRACE): A phase 3 open-label randomised study, *Lancet* 377 (2011) 914–923.
- [13] B.N. Han, L.L. Hong, B.B. Gu, Y.T. Sun, J. Wang, J.T. Liu, H.W. Lin, Natural products from sponges. In *symbiotic microbiomes of coral reefs sponges and corals*, Springer: Dordrecht, The Netherlands (2019) 329–463.

-
- [14] E.R. Abdelaleem, M.N. Samy, S.Y. Desoukey, M. Liu, R.J. Quinn, U.R. Abdelmohsen, Marine natural products from sponges (Porifera) of the order Dictyoceratida (2013 to 2019); a promising source for drug discovery, *RSC Adv.* 10 (2020) 34959–34976.
- [15] N.H. Shady, E.M. El-Hossary, M.A. Fouad, T.A.M. Gulder, M.S. Kamel, U.R. Abdelmohsen, Bioactive natural products of marine sponges from the genus *Hyrtios*, *Molecules* 22 (2017) 1–21.
- [16] M.F. Mehbub, J. Lei, C. Franco, W. Zhang, Marine sponge derived natural products between 2001 and 2010: trends and opportunities for discovery of bioactives, *Mar. Drugs* 12 (2014) 4539–4577.
- [17] D. Raitsos, I. Hoteit, P. Prihartato, T. Chronis, G. Triantafyllou, Y. Abualnaja, Abrupt warming of the Red Sea. *Geophys. Res. Lett.* (2011) 38.
- [18] M.K. Wooster, O. Voigt, D. Erpenbeck, G. Wörheide, M.L. Berumen, Sponges of the Red Sea. *Coral Reefs of the World* (2019) 91–122.
- [19] M. Ilan, J. Gugel, R.V. Soest, Taxonomy, reproduction and ecology of new and known Red Sea sponges, *Sarsia*, *North Atlantic Marine Science* 89 (2004), 388–410.
- [20] S.A.M. Khalifa, N. Elias, M.A. Farag, L. Chen, A. Saeed, M.-E.F. Hegazy, M.S. Moustafa, A.A. El-Wahed, S.M. Al-Mousawi, S.G. Musharraf, F.-R. Chang, A. Iwasaki, K. Suenaga, M. Alajlani, U. Göransson, H.R. El-Seedi, Marine natural products: A source of novel anticancer drugs, *Mar. Drugs* 17 (2019) 491.
- [21] H. Zhang, Z. Zhao, H. Wang, Cytotoxic Natural Products from marine sponge-derived microorganisms, *Mar. Drugs* 15 (2017) 68.
- [22] R.A. El-Ezz, A. Ibrahim, E. Habib, A. Wahba, H. Kamel, M. Afifi, H. Hassanean, S. Ahmed, Review of natural products from marine organisms in the Red sea. *Int. J. Pharm. Sci. Res.* 8 (2017) 940–974.
- [23] E.M. El-Hossary, M. Abdel-Halim, E.S. Ibrahim, S.M. Pimentel-Elardo, J.R. Nodwell, H. Handoussa, M.F. Abdelwahab, U. Holzgrabe, U.R. Abdelmohse, *Mar. Drugs* 18 (2020) 457.
- [24] S.W. Pelletier, In alkaloids: chemical and biological perspectives, Wiley: New York 1 (1983) 1–31.
- [25] M.I.A. Shoer, L.A. Shaala, D.T.A. Youssef, J.M. Badr, A.Z.M. Habib, Bioactive brominated metabolites from the Red Sea sponge *Suberea mollis*. *J. Nat. Prod.* 71 (2008), 1464–1467.
- [26] L.A. Shaala, D.T.A. Youssef, M. Sulaiman, F.A. Behery, A.I. Foudah, K.A. El Sayed, Subereamolline A as a potent breast cancer migration, invasion and proliferation inhibitor and bioactive dibrominated alkaloids from the Red Sea sponge *Pseudoceratina Arabica*, *Mar. Drugs* 10 (2012) 2492–2508.
- [27] L.A. Shaala, D.T.A. Youssef, J.M. Badr, M. Sulaiman, A. Khedr, Bioactive secondary metabolites from the Red Sea marine verongid sponge *Suberea* species, *Mar. Drugs* 13 (2015) 1621–1631.
- [28] A. Drechsel, J. Helm, H. Ehrlich, S. Pantovic, S.R. Bornstein, N. Bechmann, Anti-tumor activity vs. normal cell toxicity: Therapeutic potential of the bromotyrosines, aerothionin and homoaerothionin *In Vitro*, *Mar. Drugs* (2020), 18, 236.

-
- [29] L.A. Shaala, D.T.A. Youssef, Cytotoxic psammaphysin analogues from the Verongid Red Sea sponge *Aplysinella* species, *Biomolecules* 9 (2019) 1–10.
- [30] L.A. Shaala, D.T.A. Youssef, J.M. Badr, M. Sulaiman, A. Khedr, K.A. El Sayed, Bioactive alkaloids from the Red Sea marine verongid sponge *Pseudoceratina arabica*, *Tetrahedron* 71 (2015) 7837–7841.
- [31] K.Y. Orabi, K.A. El Sayed, M.T. Hamann, D.C. Dunbar, M.S. Al-Said, T. Higa, M. Kelly, Araguspongines k and l, new bioactive bis-1 oxaquinolizidine N-oxide alkaloids from Red Sea specimens of *Xestospongia exigua*, *J. Nat. Prod.* 65 (2002) 1782–1785.
- [32] M.R. Akl, N.M. Ayoub, H.Y. Ebrahim, M.M. Mohyeldin, K.Y. Orabi, A.I. Foudah, K.A. El Sayed, Araguspongine c induces autophagic death in breast cancer cells through suppression of c-Met and HER2 receptor tyrosine kinase signaling, *Mar. Drugs* 13 (2015) 288–311.
- [33] D.T.A. Youssef, Hyrtioerectines a-c, cytotoxic alkaloids from the Red Sea sponge *Hyrtios erectus*, *J. Nat. Prod.* 68 (2005) 1416–1419.
- [34] M.A. Ashour, E.S. Elkhayat, R. Ebel, R. Edrada, P. Proksch, Indole alkaloid from the Red Sea sponge *Hyrtios erectus*, *ARKIVOC* 15 (2007) 225–231.
- [35] P. Sauleau, M.T. Martin, M.E.T.H. Dau, D.T.A. Youssef, M.L.B. Kondracki, Hyrtiazepine, an azepino-indole-type alkaloid from the Red Sea marine sponge *Hyrtios erectus*, *J. Nat. Prod.* 69 (2006) 1676–1679.
- [36] D.T.A. Youssef, L.A. Shaala, H.Z. Asfour, Bioactive compounds from the Red Sea marine sponge *Hyrtios* species, *Mar. Drugs* 11 (2013) 1061–1070.
- [37] S.S. El-Hawary, A.M. Sayed, R. Mohammed, H.M. Hassan, M.E. Rateb, E. Amin, T.A. Mohammed, M. El-Mesery, A.B. Muhsinah, A. Alsayari, H. Wajant, M.A. Anany, U.R. Abdelmohsen, Bioactive brominated oxindole alkaloids from the Red Sea sponge *Callyspongia siphonella*, *Mar. Drugs* 17 (2019) 1–13.
- [38] T. Yosief, A. Rudi, Z. Stein, I. Goldberg, G.M.D. Gravalos, M. Schleyer, Y. Kashman, Asmarines a-c; three novel cytotoxic metabolites from the marine sponge *Raspailia* sp., *Tetrahedron Lett.* 39 (1998) 3323–3326.
- [39] M.J. Lambrecht, J.W. Kelly, R.A. Shenvi, Mechanism of action of the cytotoxic asmarine alkaloids, *ACS Chemical Biology* (2018), 13, 1299–1306.
- [40] D.T.A. Youssef, L.A. Shaala, K.Z. Alshali, Bioactive hydantoin alkaloids from the Red Sea marine sponge *Hemimycale Arabica*, *Mar. Drugs* 13 (2015) 6609–6619.
- [41] M. Mudit, M. Khanfar, A. Muralidharan, S. Thomas, G.V. Shah, R.W.M. van Soest, K.A. El Sayed, Discovery, design, and synthesis of anti-metastatic lead phenylmethylene hydantoins inspired by marine natural products, *Bioorg. Med. Chem.* 17 (2009) 1731–1738.
- [42] Y. Kashman, S. Hirsh, O.J. McConnell, I. Ohtani, I. Kusumi, H. Kakisawa, Ptilomycalin a: a novel polycyclic guanidine alkaloid of marine origin, *J. Am. Chem. Soc.* 111 (1989) 8925–8926.
- [43] D.T.A. Youssef, W.Y. Yoshida, M. Kelly, P.J. Scheuer, Cytotoxic cyclic norterpene peroxides from a Red Sea sponge *Diacarnus erythraenus*, *J. Nat. Prod.* 64 (2001) 1332–1335.

-
- [44] S. Hirsch, R.Y. Kashman, New avarone and avarol derivatives from the marine sponge *Dysidea cinerea*, *J. Nat. Prod.* 54 (1991) 92–97.
- [45] A. Rudi, Y. Kashman, Three new norrisolide related rearranged spongians, *Tetrahedron* 46 (1990) 4019–4022.
- [46] D.T.A. Youssef, R.K. Yamaki, M. Kelly, P.J. Scheuer, Salmahyrtisol a, a novel cytotoxic sesterterpene from the Red Sea sponge *Hyrtios erecta*, *J. Nat. Prod.* 65 (2002) 2–6.
- [47] M.A. Ashour, E.S. Elkhayat, R. Ebel, R. Edrada, P. Proksch, Indole alkaloid from the Red Sea sponge *Hyrtios erectus*, *ARKIVOC* 15 (2007) 225–231.
- [48] W.M. Alarif, S.S. Al-Lihaibi, M.A. Ghandourah, M.I. Orif, S.A. Basaif, S.E.N. Ayyad, Cytotoxic scalarane-type sesterterpenes from the Saudi Red Sea sponge *Hyrtios erectus*, *J. Asian Nat. Prod. Res.* 18 (2015) 1–7.
- [49] S.S. Elhady, A.M. El-Halawany, A.M. Alahdal, H.A. Hassanean, S.A. Ahmed, A new bioactive metabolite isolated from the Red sea marine sponge *Hyrtios erectus*, *Molecules* 21 (2016) 82.
- 50 S.S. Elhady, A.M. Al-Abd, A.M. El-Halawany, A.M. Alahdal, H.A. Hassanean, S.A. Ahmed, Antiproliferative scalarane-based metabolites from the Red Sea sponge *Hyrtios erectus*, *Mar. Drugs* 14 (2016) 1–14.
- [51] A. Rudi, T. Yosief, M. Schleyer, Y. Kashman, Bilosespens a and b: two novel cytotoxic sesterterpenes from the marine sponge *Dysidea cinerea*, *Org. Lett.* 1 (1999) 471–472.
- [52] M.H.A. Hassan, M.E. Rateb, M. Hetta, T.A. Abdelaziz, M.A. Sleim, M. Jaspars, R. Mohammed, Scalarane sesterterpenes from the Egyptian Red Sea sponge *Phyllospongia lamellose*, *Tetrahedron* 71 (2015) 577–583.
- [53] a) Y. Kashman, M. Rotem, Muqubilin, a new c₂₄-isoprenoid from a marine sponge, *Tetrahedron Lett.* 19 (1979) 1707–1708. b) K.A. El Sayed, M.T. Hamann, N.E. Hashish, W.T. Shier, M. Kelly, A.A. Khan, Antimalarial, antiviral, and antitoxoplasmosis norsesesterterpene peroxide acids from the Red Sea sponge *Diacarnus erythraeanus*, *J. Nat. Prod.* 64 (2001) 522–524. 3) D.T.A. Youssef, W.Y. Yoshida, M. Kelly, P.J. Scheuer, Cytotoxic cyclic norterperene peroxides from a Red Sea sponge *Diacarnus erythraenus*, *J. Nat. Prod.* 64 (2001) 1332–1335.
- [54] M. D'Ambrosio, A. Guerriero, C. Debitus, J. Waikedre, F. Pietra, Relative contributions to antitumoral activity of lipophilic vs. polar reactive moieties in marine terpenoids, *Tetrahedron Lett.* 38 (1997) 6285–6288.
- [55] Y. Guo, M. Gavagnin, E. Mollo, E. Trivellone, G. Cimino, N.A. Hamdy, I. Fakhr, M. Pansini, A new norsesesterterpene peroxide from a Red Sea sponge, *Nat. Prod. Lett.* 9 (1996) 105–112.
- [56] M. Albericci, J.C. Braekmand, D. Daloze, B. Tursch, The chemistry of three norsesesterterpene peroxides from the sponge *Sigmosceptrella laevis*, *Tetrahedron* 38 (1982) 1881–1890.
- [57] F. Lefranc, G. Nuzzo, N.A. Hamdy, I. Fakhr, L.M.Y. Banuls, G.V. Goietsenoven, G. Villani, V. Mathieu, R. van Soest, R. Kiss, M.L. Ciavatta, *In vitro* pharmacological and toxicological effects of norterperene peroxides isolated from the Red Sea sponge *Diacarnus erythraeanus* on normal and cancer cells, *J. Nat. Prod.* 76 (2013) 1541–1547.

-
- [58] D.T.A. Youssef, Tasnemoxides A-C, new cytotoxic cyclic norsesterterpene peroxides from the Red Sea sponge *Diacarnus erythraenu*, *J. Nat. Prod.* 67 (2004) 112–114.
- [59] S. Carmely, Y. Kashman, Neviotine-a, a new triterpene from the Red Sea sponge *Siphonochalina siphonella*, *J. Org. Chem.* 51 (1986) 784–788.
- [60] Y. Kashman, T. Yosief, S. Carmeli, New triterpenoids from the Red Sea sponge *Siphonochalina siphonella*, *J. Nat. Prod.* 64 (2001) 175–180.
- [61] S. Jain, S. Laphookhieo, Z. Shi, L.W Fu, S.I. Akiyama, Z.S. Chen, D.T.A. Youssef, R.W.M. van Soest, K.A. El Sayed, Reversal of p-glycoprotein-mediated multidrug resistance by sipholane triterpenoids, *J. Nat. Prod.* 70 (2007) 928–931.
- [62] a) S. Jain, I. Abraham, P. Carvalho, Y.H. Kuang, H.A. Shaala, D.T.A. Youssef, M.A. Avery, Z.S. Chen, K.A. El Sayed, Sipholane triterpenoids: chemistry, reversal of ABCB1/P-glycoprotein-mediated multidrug resistance, and pharmacophore modelling, *J. Nat. Prod.* 72 (2009) 1291–1298. b) S.M. Al-Massarani, A.A. El-Gamal, M.S. Al-Said, S.S. Al-Lihaibi, O.A. Basoudan, In vitro cytotoxic, antibacterial and antiviral activities of triterpenes from the Red Sea sponge, *Siphonochalina siphonella*, *Trop. J. Pharm. Res.* 14 (2015) 33–40.
- [63] S.E.N. Ayyad, R. Angawy, W.M. Alarif, E.A. Saqer, F.A. Badria, Cytotoxic polyacetylenes from the Red Sea sponge *Siphonochalina siphonella*, *Zeitschrift für Naturforschung C* 69 (2014) 117–123.
- [64] S. Jain, I. Abraham, P. Carvalho, H.E. Kuang, L.A. Shaala, D.T.A. Youssef, M.A. Avery, Z.S. Chen, K.A. El Sayed, Sipholane triterpenoids: chemistry, reversal of ABCB1/P-glycoprotein-mediated multidrug resistance, and pharmacophore modelling, *J. Nat. Prod.* 72 (2009) 1291–1298.
- [65] D.T.A. Youssef, R.W.M. van Soest, N. Fusetani, Callyspongenols a-c, new cytotoxic c22-polyacetylenic alcohols from a Red Sea sponge, *Callyspongia* species, *J. Nat. Prod.* 66 (2003) 679–681.
- [66] M. Rotem, Y. Kashman, New polyacetylenes from the sponge *Siphonochalina* sp, *Tetrahedron Lett.* 34 (1979) 3193–3196.
- [67] L.A. Shaala, D.T.A. Youssef, S.R.M. Ibrahim, G.A. Mohamed, Callyptide a, a new cytotoxic peptide from the Red Sea marine sponge *Callyspongia* species, *Nat. Prod. Res.* 30 (2016) 2783–2790.
- [68] D.T.A. Youssef, L.A. Shaala, G.A. Mohamed, J.M. Badr, F.H. Bamanie, S.R.M. Ibrahim, Theonellamide g, a potent antifungal and cytotoxic bicyclic glycopeptide from the Red Sea marine sponge *Theonella swinhoei*, *Mar. Drugs* 12 (2014) 1911–1923.
- [69] D.T.A. Youssef, S.L. Mooberry, Hurghadolide A and swinholide I, potent actin-microfilament disrupters from the Red Sea sponge *Theonella swinhoei*, *J. Nat. Prod.* 69 (2006) 154–157
- [70] a) Y. Kashman, A. Groweiss, U. Shmueli, Latrunculin, a new 2-thiazolidinone macrolide from the marine sponge *Latrunculia magnifica*, *Tetrahedron Lett.* 21 (1980) 3629–3632.; b) S.A. Ahmed, S. Odde, P.R. Daga, J.J. Bowling, M.K. Mesbah, D.T.A. Youssef, S.I. Khalifa, R.J. Doerksen, M.T. Hamann, Latrunculin with a highly oxidized thiazolidinone ring: structure assignment and actin docking, *Org. Lett.* 9 (2007) 4773–4776.

-
- [71] K.A. El Sayed, H.M. Shallal, M.A. Khanfar, A. Muralidharan, Bhushan Awate, D.T.A. Youssef, Y. Liu, Y-D. Zhou, D.G. Nagle, G. Shah, Latrunculin A and its C-17-O-carbamates inhibit prostate tumor cell invasion and HIF-1 activation in breast tumor cells. (2008) *J. Nat. Prod.* 71, 396-402.
- [72] K.A. El Sayed, D.T.A. Youssef, D. Marchetti, Bioactive natural and semisynthetic latrunculins, *J. Nat. Prod.* 69 (2006) 219–223.
- [73] M.A. Khanfar, D.T.A. Youssef, K.A. El-Sayed, Semisynthetic latrunculin derivatives as inhibitors of metastatic breast cancer: Biological evaluations, preliminary structure–activity relationship and molecular modeling studies, *Chem. Med. Chem.* (2010) 5, 274- 285.
- [74] S. Carmely, M. Roll, Y. Loya, Y. Kashman, The structure of eryloside a, a new antitumor and antifungal 4-methylated steroidal glycoside from the sponge *Erylus lendenfeldi*, *J. Nat. Prod.* 52 (1989) 167–170.
- [75] D.T.A. Youssef, J.M. Badr, L.A. Shaala, G.A. Mohameda, F.H. Bamanie, Ehrenasterol and biemnic acid; new bioactive compounds from the Red Sea sponge *Biemna ehrenbergi*, *Phytochem. Lett.* 12 (2015) 296–301.
- [76] D.R. Abou-Hussein, D.T.A. Youssef, Mirabolides a and b; new cytotoxic glycerides from the Red Sea sponge *Theonella mirabilis*, *Mar. Drugs* 14 (2016) 1–11.
- [77] D.T.A. Youssef, R.W.M. van Soest, N. Fusetani, Callyspongamide a, a new cytotoxic polyacetylenic amide from the Red Sea sponge *Callyspongia fistularis*, *J. Nat. Prod.* 66 (2003) 861–862.
- [78] a) D.W. Ki, A.H. El-Desoky, T. Kodama, C.P. Wong, M.A. Ghani, A.A. El-Beih, M. Mizuguchi, H. Morita, New cytotoxic polyacetylene amides from the Egyptian marine sponge *Siphonochalina siphonella*, *Fitoterapia* 142 (2020) 1–7. b) D.T.A. Youssef, R.W.M. van Soest, N. Fusetani, Callyspongamide a, a new cytotoxic polyacetylenic amide from the Red Sea sponge *Callyspongia fistularis*, *J. Nat. Prod.* 66 (2003) 861–862.
- [79] E.E. Eltamany, A.K. Ibrahim, M.M. Radwan, M.A. ElSohly, H.A. Hassanean, S.A. Ahmed, Cytotoxic ceramides from the Red Sea sponge *Sphaciospongia vagabunda*, *Med. Chem. Res.* 24 (2015) 3467–3473.
- [80] R.F. Abdelhameed, A.K. Ibrahim, T.A. Temraz, K. Yamada, S.A. Ahmed, Chemical and biological investigation of the Red Sea sponge *Echinoclathria* species, *J. Pharm. Sci. & Res.* 9 (2017) 1324–1328.
- [81] a) A.A. El-Gamal, S.M. Al-Massarani, L.A. Shaala, A.M. Alahdald, M.S. Al-Said, A.E. Ashour, A. Kumar, M.S. Abdel-Kader, W.M. Abdel-Mageed, D.T.A. Youssef, Cytotoxic compounds from the Saudi Red Sea sponge *Xestospongia testudinaria*, *Mar. Drugs* 14 (2016) 1–9. b) R.J. Quinn, D.J. Tucker, A brominated bisacetylenic acid from the marine sponge *Xestospongia testudinaria*, *Tetrahedron Lett.* 26 (1985) 1671–1672. c) S. Hirsh, S. Carmely, Y. Kashman, Brominated unsaturated acids from the marine sponge *Xestospongia* sp, *Tetrahedron* 43 (1987) 3527–3261.
- [82] D.R. Abou-Hussein, D.T.A. Youssef, Mirabolides a and b; new cytotoxic glycerides from the Red Sea sponge *Theonella mirabilis*, *Mar. Drugs* 14 (2016) 1–11.

-
- [83] L.A. Shaala, S.I. Khalifa, M.K. Mesbah, R.W.M. van Soest, D.T.A. Youssef, Subereaphenol a, a new cytotoxic and antimicrobial dibrominated phenol from the Red Sea sponge *Suberea mollis*, Nat. Prod. Commun. 3 (2008) 219–222.
- [84] L.A. Shaala, D.T.A. Youssef, J.M. Badr, M. Sulaiman, A. Khedr, Bioactive secondary metabolites from the Red Sea marine Verongid sponge *Suberea* species, Mar. Drugs 13 (2015) 1621–1631.
- [85] a) L.A. Shaala, F.H. Bamane, J.M. Badr, D.T.A. Youssef, Brominated arginine-derived alkaloids from the Red Sea sponge *Suberea mollis*, J. Nat. Prod. 74 (2011) 1517–1520. b) M. D'Ambrosio, A. Guerriero, R. de Clauser, G. de Stanchina, F. Pietra, Dichloroverongiaquinol, a new marine antibacterial compound from *Aplysina cavernicola*. Isolation and synthesis, Cell. Mol. Life Sci, 39 (1983) 1091–1092.
- [86] J.W. Shearman, R.M. Myers, J.D. Brenton, S.V. Ley, Total syntheses of subereamollines a and b, Org. Biomol. Chem. 9 (2011) 62–65.
- [87] H.H. Wasserman, J. Wang, Synthesis of the marine metabolite verongamine, hemibastadin-2 and aerothionin using the cyano ylide coupling methodology, J. Org. Chem. 63 (1998) 5581–5586.
- [88] M. Murakata, M. Tamura, O. Hoshino, Asymmetric oxidative cyclization of *O*-phenolic oxime esters: first synthesis of enantiomerically enriched spiroisoxazoline methyl esters, J. Org. Chem. 62 (1997) 4428–4433.
- [89] S. Nishiyama, S. Yamamura, Total syntheses of (±)-aerothionin and (±)-homoaerothionin, Tetrahedron Lett. 24 (1983) 3351–3352.
- [90] T. Ogamino, R. Obata, S. Nishiyama, Asymmetric synthesis of aerothionin, a marine dimeric spiroisoxazoline natural product, employing optically active spiroisoxazoline derivative, asymmetric synthesis of aerothionin, a marine dimeric spiroisoxazoline natural product, employing optically active spiroisoxazoline derivative, Tetrahedron Lett. 47 (2006) 727–731.
- [91] a) T. Ogamino, S. Nishiyama, A new ring opening access to aeroplysinin-1 a secondary metabolite of *Verongia aerophoba*, Tetrahedron 59 (2003) 9419–9423; b) T. Ogamino, Y. Ishikawa, S. Nishiyama, Electrochemical synthesis of spiroisoxazole derivatives and its application to natural products, Heterocycles 61 (2003) 73–78.
- [92] S. Nishiyama, S. Yamamura, Total syntheses of (±)-aerothionin, (±)-homoaerothionin and (±)-aerophobin-1, Bull. Chem. Soc. Jpn. 58 (1985) 3453–3456.
- [93] T.R. Boehlow, J. Harburn, C.D. Spilling, Approaches to the synthesis of some tyrosine-derived marine sponge metabolites: synthesis of verongamine and purealdin N, J. Org. Chem. 66 (2001) 3111–3118.
- [94] J.J. Harburn, N.P. Rath, C.D. Spilling, Efficient synthesis of tyrosine-derived marine sponge metabolites via acylation of amines with a coumarin, J. Org. Chem. 70 (2005) 6398–6403.
- [95] O. Masashi, T. Takahiro, Preparatory study for the synthesis of the marine sponge alkaloids asmarines a-f: synthesis of their heterocyclic portions, Heterocycles. 57 (2002) 1235–1238.
- [96] D. Pappo, Y. Kashman, Synthesis of 9-substituted tetrahydrodiazepinopurines-asmarine a analogues, Tetrahedron. 59 (2003) 6493–6501.

-
- [97] D.L. Hughes, *Organic Reactions*; Wiley: New York, 42 (1992)
- [⁹⁸] D. Pappo, S. Shimony, Y. Kashman, Synthesis of 9-Substituted tetrahydrodiazepinopurines: studies toward the total synthesis of asmarines, *J. Org. Chem.* 70 (2005) 199–206.
- [99] T.P. Brady, S.H. Kim, K. Wen, C. Kim, E.A. Theodorakis, Norrisolide: total synthesis and related studies, *Chem. Eur. J.* 11 (2005) 7175–7190.
- [100] B.H. Lipshutz, C. Hackmann, Conjugate Addition Reactions of Allylic Copper Species Derived from Grignard Reagents: Synthetic and Spectroscopic Aspects, *J. Org. Chem.* 59 (1994) 7437–7444.
- [101] a) C.R. Schmid, J.D. Bryant, M. Dowlatzedah, J.L. Phillips, D.E. Prather, R.D. Shantz, N.L. Sear, C.S. Vianco, Synthesis of 2,3-O-isopropylidene-D-glyceraldehyde in high chemical and optical purity: observations on the development of a practical bulk process *J. Org. Chem.* 56 (1991) 4056–4058; b) J. Mann, A. Weymouth-Wilson, A new approach to *cis*-chrysanthemic acid, *Carbohydr. Res.* 216 (1992) 511–515; c) F. Fazio, M.P. Scheider, A novel synthesis of 2-deoxy-L-ribose, *Tetrahedron: Asymmetry* 11 (2000) 1869–1876.
- [102] P.J. Kocienski, P. Raubo, C. Smith, F.T. Boyle, A formal synthesis of 18-O-methyl mycalamide b, *Synthesis* 1999 (1999) 2087–2095.
- [103] K. Granger, M.L. Snapper, Concise synthesis of norrisolide, *Eur. J. Org. Chem.* 2012 (2012), 2308–2311.
- [104] A. Fürstner, D. De Souza, L. Parra-Rapado, J.T. Jensen, Catalysis-based total synthesis of latrunculin b, *Angew. Chem. Int. Ed.* 42 (2003) 5358–5360.