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Review

Exploring chemical diversity of *Phorbas* sponges as a source of novel lead compounds in drug discovery

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Abstract: Porifera, commonly referred to as marine sponges, have stood out as major producers of marine natural products (MNPs). Sponges of the genus *Phorbas* have attracted much attention along years. They are widespread in all continents, and several structurally unique compounds have been identified from species of this genus. Terpenes, mainly sesterterpenoids, represent the great majority of secondary metabolites isolated from *Phorbas* species, even though several alkaloids and steroids have also been reported. Many of these compounds have shown a variety of biological activities. Particularly, *Phorbas* sponges have been demonstrated to be a source of cytotoxic metabolites. In addition, MNPs exhibiting cytostatic, antimicrobial and anti-inflammatory activities, have been isolated and structurally characterized. This work brings an overview of *Phorbas* secondary metabolites reported since the first study published in 1993 until 2020, and their biological activities.

Keywords: *Phorbas*; marine sponges; marine natural products (MNPs); bioactivity, cytotoxic metabolites, antimicrobial and anti-inflammatory activities, sesterterpene.

1. Introduction

Biodiversity of marine organisms that reflects on their rich chemical diversity is an important source of novel drug-lead skeletons. Sponges, among others, are one of the main sources of novel skeletons as well as of lead compounds [1,2], promising remedies in drug discovery [3,4], and biotechnological applications. It has been largely recognized that many of these marine natural products (MNPs) are produced by microorganisms associated with the sponge [5,6]. In fact, multicellular organisms, such as sponges, are now defined as "holobionts", i.e. the host and its associated microorganism community. Therefore, studies performed on sponge organic extracts are actually studies on holobiont organic extracts [7]. One of the most common classes of sponge-symbionts are cyanobacteria, an ancient group of microorganisms well known to produce a large array of secondary metabolites, including cyanotoxins [8–10].

Chemical diversity coming from marine sponges may drive drug discovery *research* [11–13]. The genus Phorbas is a suitable example to illustrate this point. Phorbas-derived natural products discovered until now include compounds belonging to four main classes: alkaloids, macrolides, steroids, and terpenoids. Many of them possess innovative structures that could play an important role in biotechnological and pharmaceutical applications.

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The genus *Phorbas* belongs to the class Demospongiae, order Poecilosclerida, family Hymedesmiidae [14], being the biggest representative of the family that contains 10 accepted genera, including beyond *Phorbas*, *Hamigera*, *Acanthancora*, *Hemimycale* [15–18]. The genus *Phorbas* stands out in the number of isolated MNPs, but also in relation to a large number of bioactive compounds, mainly displaying cytotoxic activity [19]. Sponges of the genus *Phorbas* are widespread, being present on all continents, including Antarctica (Figure 1) [20].

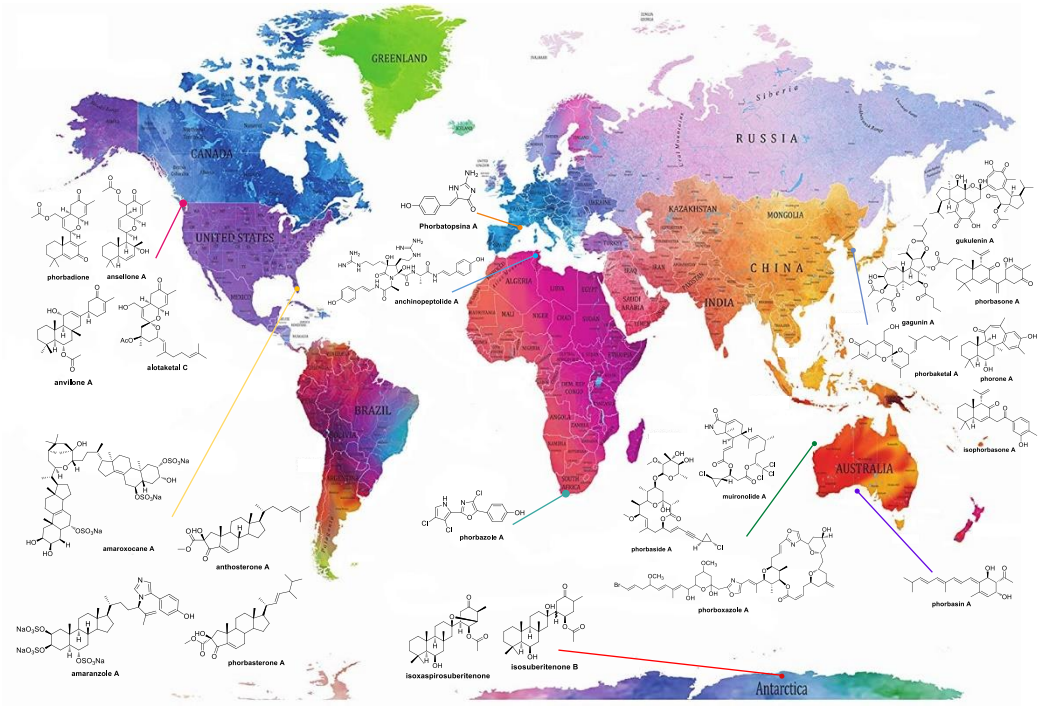

Figure 1. Global distribution of sponges of the genus *Phorbas* and their bioactive metabolites.

The first study has been carried out by Casapullo et al. (1993), reporting on the structural elucidation of four dimeric peptide alkaloids, anchinopeptolides [21,22] from a sample collected along the Tunisian coast. The following year, Rudi et al. (1994) analyzed a sample from South Africa highlighting for the first time the oxazole moiety as an uncommon feature of secondary metabolites from marine sponges [23]. This chemical feature is a distinctive marker of this species, being part of phorboxazoles, macrolides isolated by Searle and Molinsky (1995), from a sample collected in Western Australia [24], and of their minor analogue, hemi-phorboxazole A [25]. Secondary metabolites belonging to the alkaloid class have been also isolated from a sample collected along the coast of Marseille, France, and analyzed by Bouguet's group. With this regard, phorbatopsins are alkaloids with a benzylidene 2-aminoimidazolones structure [26]. Finally, the alkaloid-moiety was also present in the structure of a series of imidazolyl steroids, i.e. amaranzoles, isolated from a sample collected in shallow coral reef water of Key Largo, Florida [27,28].

Nitrogen incorporation seems to be a feature of *Phorbas* sponge's holobiome. Nitrogen is found also in MNPs belonging to the class of tetraterpenes, such as in gukulenin skeletons, isolated from Korean specimens [29,30], and possessing a tropolone-moiety. Eventually, the taurine unit, not very common in MNPs from sponges, has been found as a part of the terpenes gukulenins and phorbasins [31].

This review considers the potential of sponges of the genus *Phorbas* as sources of natural products and reviews what is known about their bioactivities. Secondary metabolites belonging to the above-mentioned four classes have been evaluated mainly for cytotoxic activity. However, sponges of the genus *Phorbas* have also been reported as a source a of compounds with antimicrobial, antioxidant, isocitrate lyase (ICL) inhibition activities [14].

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This work brings an overview of Phorbas secondary metabolites reported since the first study published in 1993 until 2020, and their biological activities.

2. Alkaloids

2.1 Anchinopeptolide e cicloanchinopeptolide

Anchinopeptolide A (**1**) is a dimeric peptide alkaloid isolated from *Phorbas tenacior* (previously *Anchinoe tenacior*) collected in Tunisia. This is the first study related for *Phorbas*, in 1993 [21]. To continue this work, Casapullo et al. (1994) described further four closely related compounds from the same sponge, the anchinopeptolides B-D (**2-4**) and cycloanchinopeptolide C (**6**), formed by an intramolecular cycloaddition of anchinopeptolide C (**3**) [22]. Compound **6** was indeed not detected in the sponge extract but could be synthesized by a photoinduced intramolecular [2+2] cycloaddition. This evidence strongly suggest that it has artefactual origin [32]. Silva et al. (2019) isolated anchinopeptolide E (**5**), the epimer at C-5′ of compound **3** (Figure 2), from a specimen of *P. tenacior* collected in France.

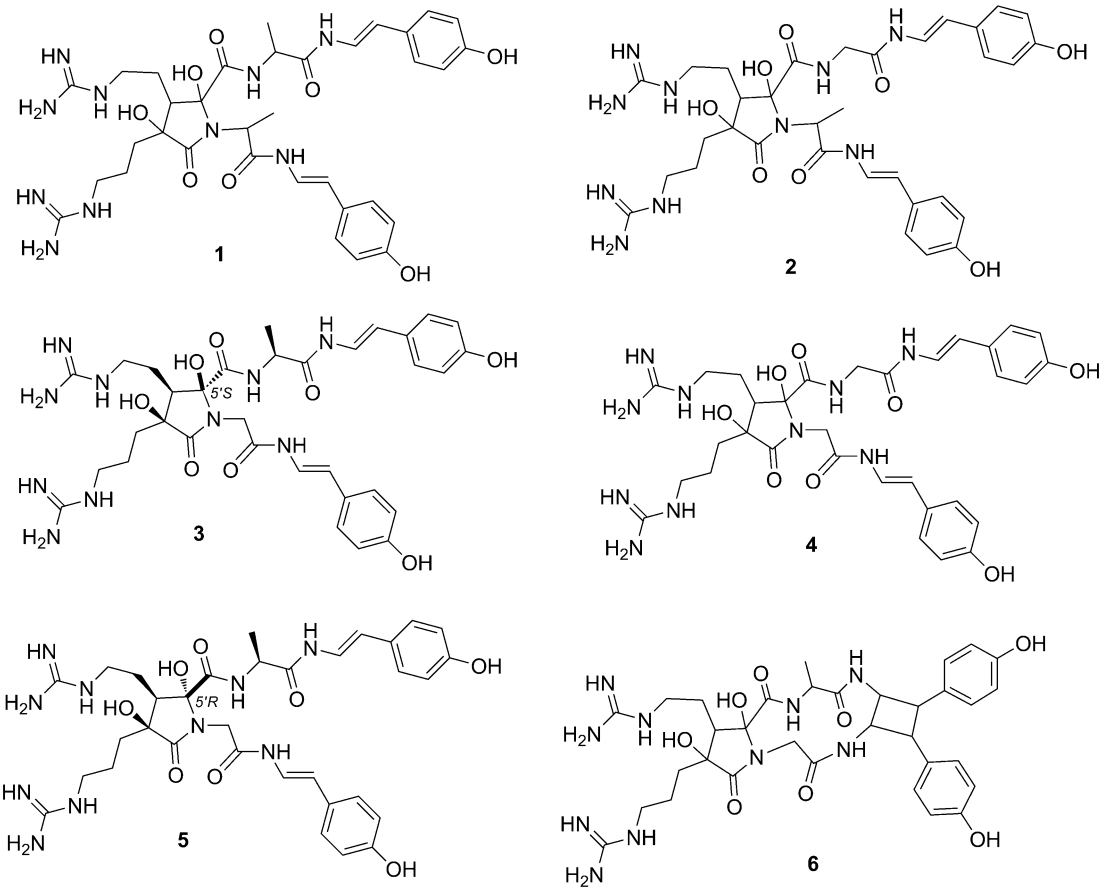

Figure 2. Chemical structures of anchinopeptolides A-E (**1-5**) and cicloanchinopeptolide C (**6**).

Further studies on the specimen *P. tenacior* allowed the identification of two more compounds, i.e. 6-(*p*-Hydroxyphenyl)-2H-3,4-dihydro-1,1-dioxo-1,4-thiazine (**7**) and l-glu-gly-4-hydroxystirylamine (**8**) (Figure 3) [33].

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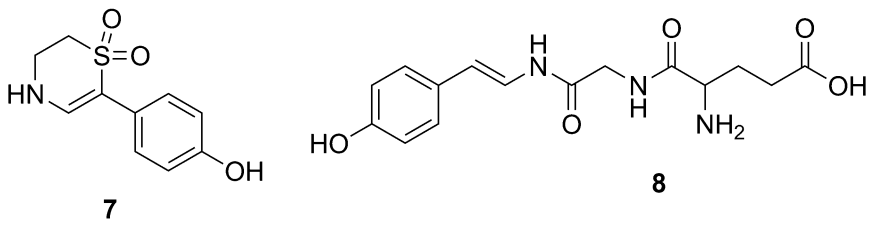

Figure 3. Chemical structures of compounds **7** and **8**.

2.2 Phorbazoles

The pioneering work of Rudi et al. (1994) allowed the first isolation of phorbazoles A-D (**9-12**) (Figure 4). They represent a new class of marine alkaloids embodying the chlorinated pyrrole moiety. Phorbazoles, isolated in small amounts, from a sample collected in South Africa, differ only by the presence and/or position of the chlorine atoms and their stereostructures were elucidated using mono- and bidimensional NMR data coupled with X-ray diffraction studies [23,34].

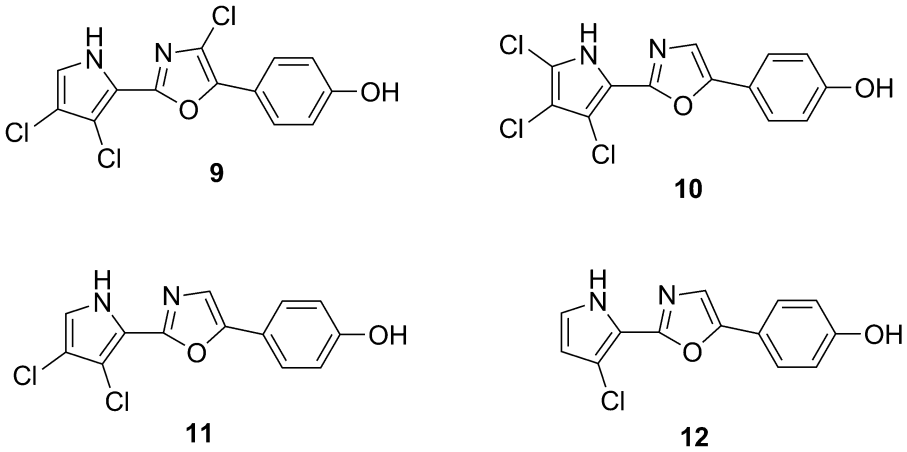

Figure 4. Chemical structures of phorbazoles A-D (**9-12**).

2.3 Zarzissine

Among the pioneering works, the study of Bouaicha et al. allowed the isolation of zarzissine (**13**), a pyridazine derivative (Figure 5). This small, highly nitrogenated alkaloid was isolated from *P. paupertas* (previously *Anchinooe paupertas*) collected in the Mediterranean Sea, Tunisia, by using Sephadex LH-20 column chromatography and HPLC, and identified by GC-MS and NMR [35].

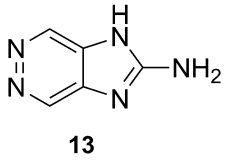

Figure 5. Chemical structure of zarzissine (**13**).

2.4 Phorbatopsins

Phorbatopsins A-C (**14-16**) were isolated from *P. topsenti* collected off Marseille at a depth of 5m, displaying an unprecedented 2-aminoimidazole core (Figure 6). The structure of phorbatopsins was easily elucidated through MS and mono- and bidimensional NMR analysis. The absolute configuration at C-6 of phorbatopsin B (**15**) was determined by using a modified procedure of Mosher's method [26].

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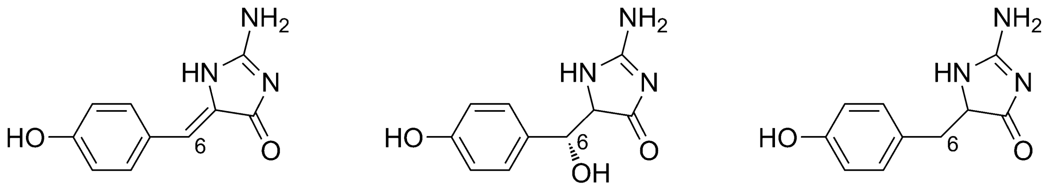


14 **15** **16**

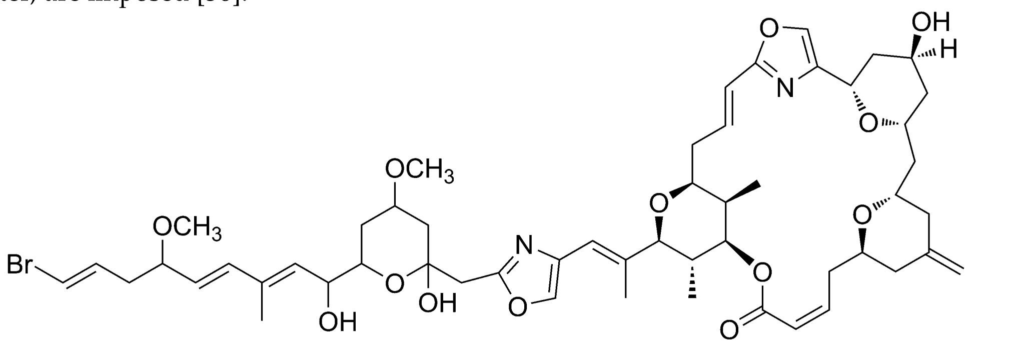
Figure 6. Chemical structures of Phorbatopsins A-C (**14-16**).

Phorbatopsins only differ in the degree of oxidation at C-6. Carbonyl or hydroxyl groups are known for their affinity for free radical, as well as a double bond seems to be essential for a good antioxidant activity. Biological data perfectly reflects this observation, since compound **14** showed the best activity as radical scavenger in the ORAC assays, followed by compound **15** and, finally, compound **16** that lacks both the double bond and the hydroxyl group at position 6 [26].

3. Macrolides

3.1 Phorboxazoles

In 1995, from a sample collected in western Australia, Searle and Molinsky [24] reported for the first time the isolation of two phenyl-pyrrolyloxazoles, phorboxazoles A-B (**17**-**18**) (figure 7). They belong to the macrolide class, containing seven rings including two oxazoles and four oxane rings, and represent the first members of a novel class of macrolides. Due to the presence of 15 stereogenic centers and 7 rings in their structure, the determination of conformation and relative configurations was not an easy task. This is a didactic example that shows as the assignment of both conformation and relative configuration in large macrolide-rings is possible, providing that some constraints, due to the presence of oxane rings and one oxazole ring, as in this case, along the macrolide's perimeter, are imposed [36].



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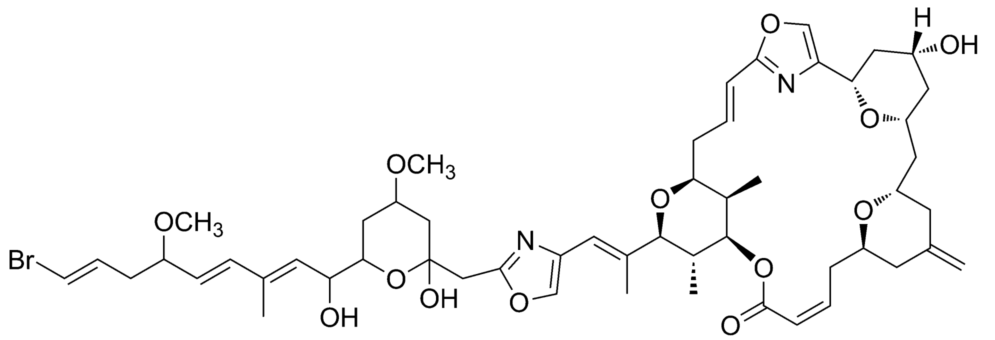

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Figure 7. Chemical structures of phorboxazoles A and B **(17-18).**

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3.2 Muironolides

One more example of the incredible *Phorbas*' chemodiversity is the isolation of muironolide A (**19**), a novel macrolide, bearing two unprecedented structural features: an hexahydro-1*H*-isoindolone-triketide ring and a trichlorocarbinol ester (Figure 8) [37].

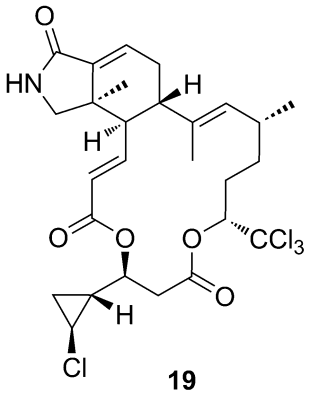
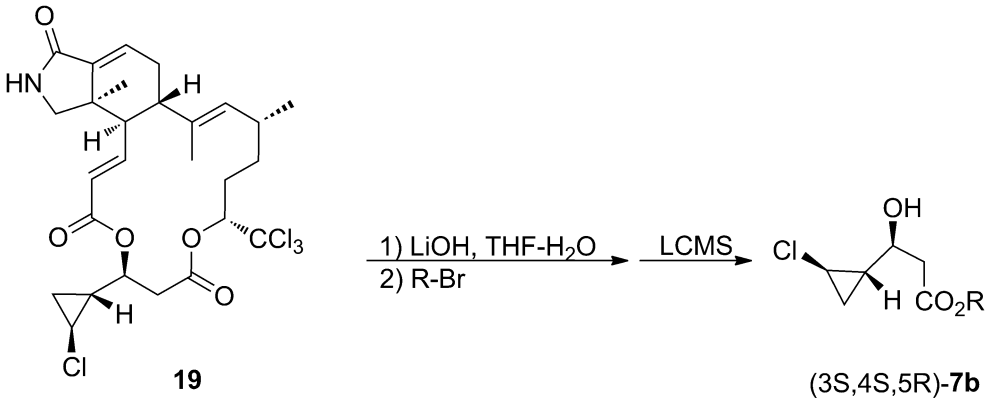


Figure 8. Chemical structure of muironolide A (**19**).

Muironolide A (**19**) also contains a cyclopropane ring, a structural motif already found in phorbasides. Interestingly, the configuration of cyclopropane ring is opposite compared to phorbaside A (**20**), but the same as that of callipeltin A, a depsipeptide isolated by Zampella et al. (1996) from the new Caledonian sponge *Callipelta* sp. [38].

The absolute configuration of the macrolide ring has been determined using CD spectroscopy, while the assignment of the absolute stereochemistry of the cyclopropane ring is a nice example of microscale degradation as a useful tool for natural product elucidation, when other powerful methods, such as NMR NOE data and *J*-based assignments, fail [39,40]. Molinsky's group succeeded in the microscale degradation of muironolide A (**19**) with basic hydrolysis to get the cyclopropane-unit derivative (scheme 1).



Scheme 1. Microscale degradation of muironolide A (**19**).

The four possible stereoisomers were synthesized starting from (-)-*O*-menthyl acrylate (Scheme 2) and used as standards to assign the stereochemistry of the cyclopropane unit of muironolide A (**19**), by comparison of their retention times using chiral LC-MS.

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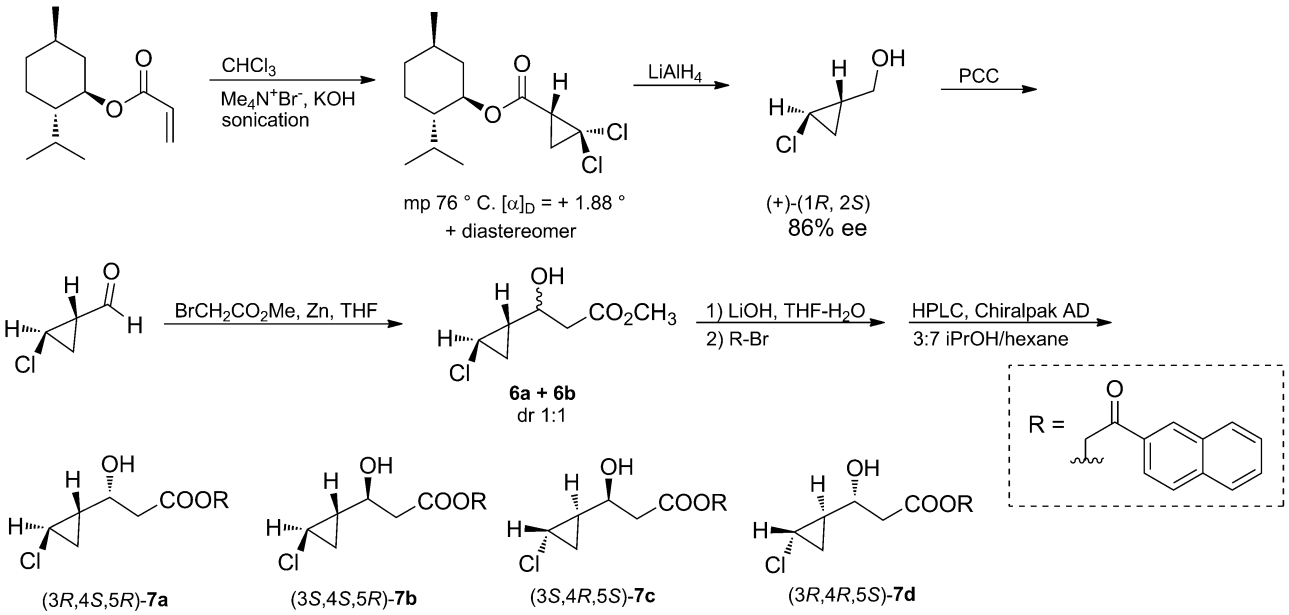

Scheme 2. Degradation of (-)-*O*-menthyl acrylate afforded compounds **7a-d**, used as standards for the establishement of the absolute stereochemistry of muironolide A (**19**).

3.3 Phorbasides

Phorbasides (**20-28**) are glycosylated macrolides possessing a unique structural motif: an ene-yne-trans-2-chlorocyclopropane moiety. They contain the same macrolide ring but different sugar units (Figure 9). Particularly, in phorbaside D (**23**) the sugar unit is fused with a oxazolone unit, while phorbaside B (**21**) and C (**22**) have a 1,4-linked disaccharide made of L-evalose [41,42] .

Phorbasides A-E (**20**-**24**), isolated from a *Phorbas* specimen collected off the Western Australian coastline which also provided phorboxazoles A-B (**17-18**), showed different cytotoxicity, which seems to be dependent upon the presence of a free hydroxyl group at position 2′-or 2′′ [41]. The repurification of minor fractions from the same sponge extract yielded phorbaside F (**25**) [43], only differing from phorbaside A (**20**) by the absence of a methyl group on the eastern side of the macrolide ring. Phorbaside G (**26**) is the only member of phorbasides family containing a C-4 oxidized pyranoside, while phorbaside I (**28**) is characterized by the presence of a formamide group instead of a hydroxyl group bearing by the pyranoside sugar unit (Figure 9) [44].

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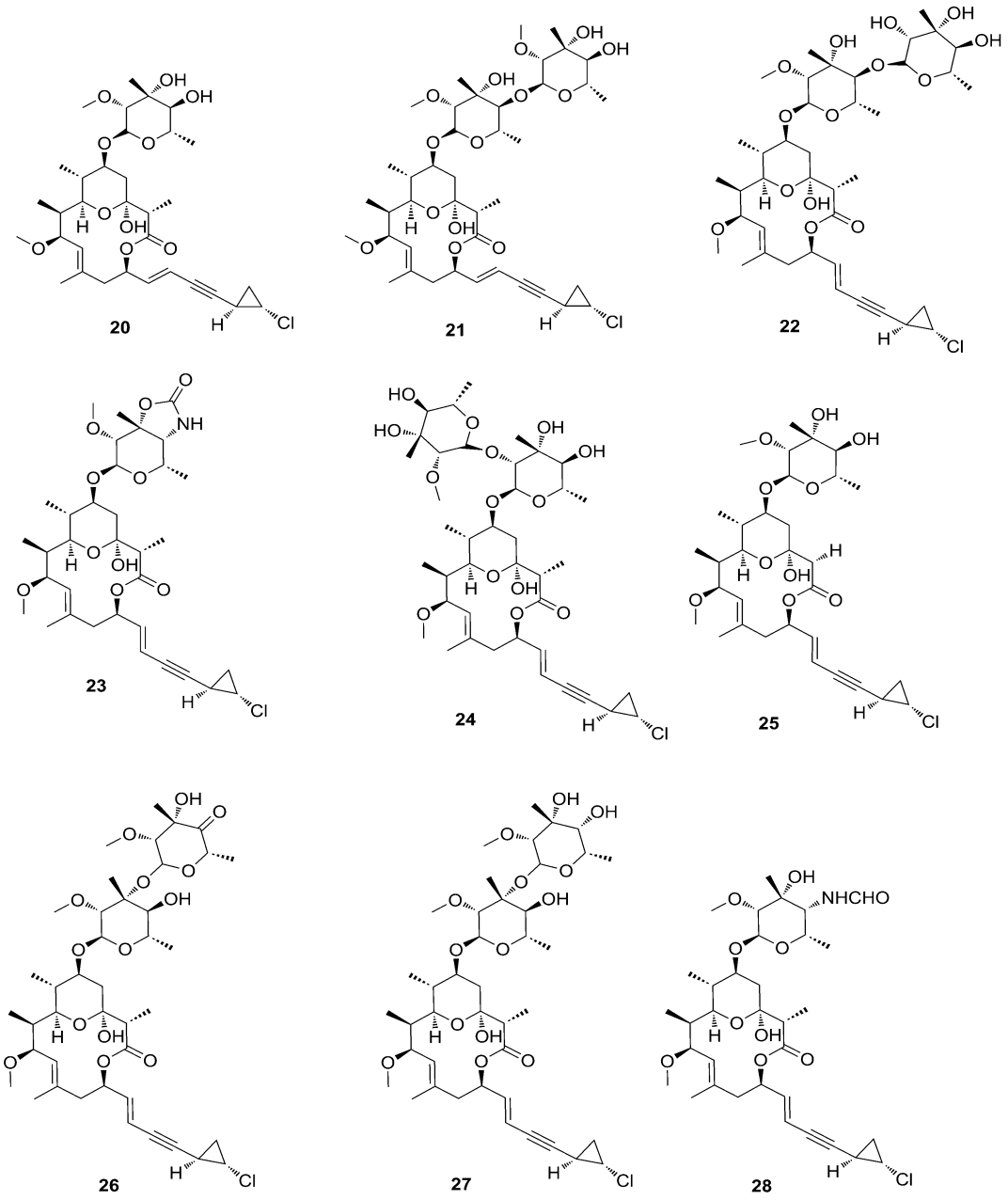

Figure 9. Chemical structure of phorbasides A-I **(20-28)**.

4. Steroids

4.1 Phorbasterones

Mazuno et al. reported for the first time the steroids phorbasterones A-D (**29-34**) from *Phorbas amaranthus*, collected in Florida [45]. These structures share a characteristic ring A-contracted steroid nucleus and differ from each other by the side chain: phorbasterone A (**29**) possesses a double bond at C-22 and a methyl group at C-24, phorbasterone B (**30**) has a fully saturated isoprenyl side chain, phorbasterone Ca and Cb (**31-32**) keep the double bond at C-22 and bear an ethyl group with opposite configuration at C-24; finally, phorbasterone Da and Db (**33-34**) lack the double bond at C-22 but keep the ethyl group at C-24 (Figure 10).

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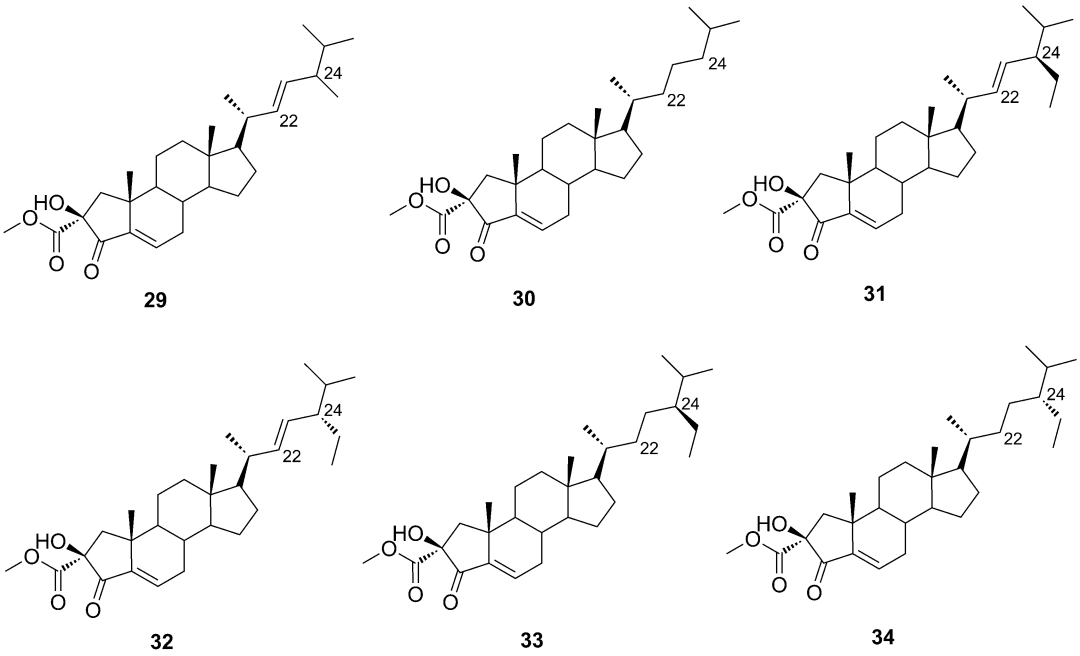

Figure 10. Chemical structures of phorbasterones A (**29**), B (**30**), Ca (**31**), Cb (**32**), Da (**33**) and Db (**34**).

4.2 Anthosterones

Althought anthosterones A-B (**35 -36**) have been isolated from *Anthoarcuata graceae* by Tishler et al. (1988) for the first time [46], they were isolated later also from the organic extract of *Phorbas amaranthus* by Mazuno et al. (2004), along with their congeners phorbasterones A-D (**29**-**34**) [45]. Anthosterones A-B (**35-36)** (Figure 11) represented the first examples of ring A-contracted steroids in which there is a one-carbon appendage at C-2 of the cyclopentane ring. The difference between the compounds resides in the side chain: anthosterone A (**35**) possess a double bond at C-24 as part of the side chain while anthosterone B (**36**) possess an additional olefinic methylene at C-24.

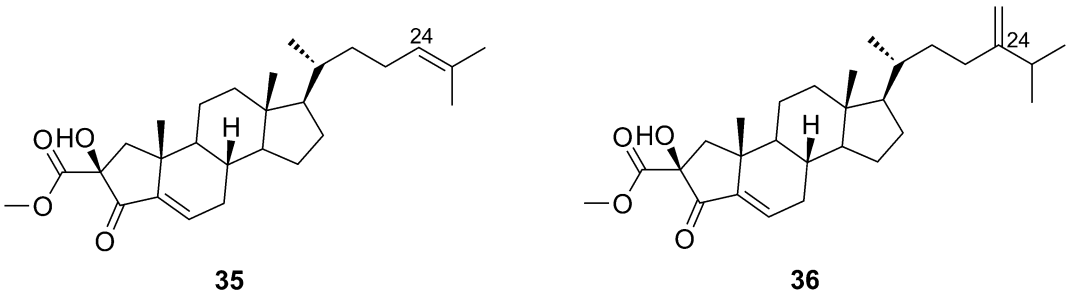

Figure 11. Chemical structures of anthosterones A-B (**35- 36**).

4.3 Amaroxocanes

Morinaka et al. (2009) reported amaroxocanes A and B (**37**-**38**), two dimeric sulfated steroids isolated from *Phorbas amaranthus*, collected on shallow coral reefs off Key Largo, Florida [47]. According to Nahar and Sarker (2012), these compounds are characterized by the two steroid moieties connected through an oxocane bridge (Figure 12), whose origin comes from different oxidative fusions of the side chains [48].

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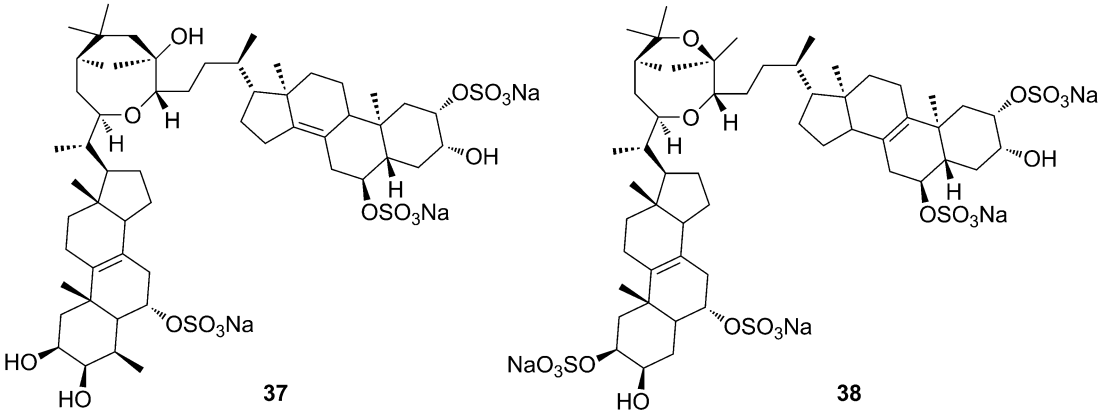

Figure 12. Chemical structures of amaroxocanes A- B (**37- 38**).

4.4 Amaranzoles

Amaranzole A (**39**) is the first imidazolyl steroidal alkaloid isolated from the tropical sponge *Phorbas amaranthus*, collected in Florida [27]. Extensive studies on the polar extracts of *P. amaranthus* led to the isolation, in 2010, of five additional steroidal alkaloids, amaranzoles B-F (**40**-**44**) [28]. Spectroscopic analysis allowed the structural elucidation of this family of compounds, highlighting the main structural difference consisting in the replacement of the C24-*N*-(4-p-hydroxyphenyl)imidazol-5-yl function with a C24-*O*-(4-p-hydroxyphenyl)imidazole-2- carboxylate motif (Figure 13).

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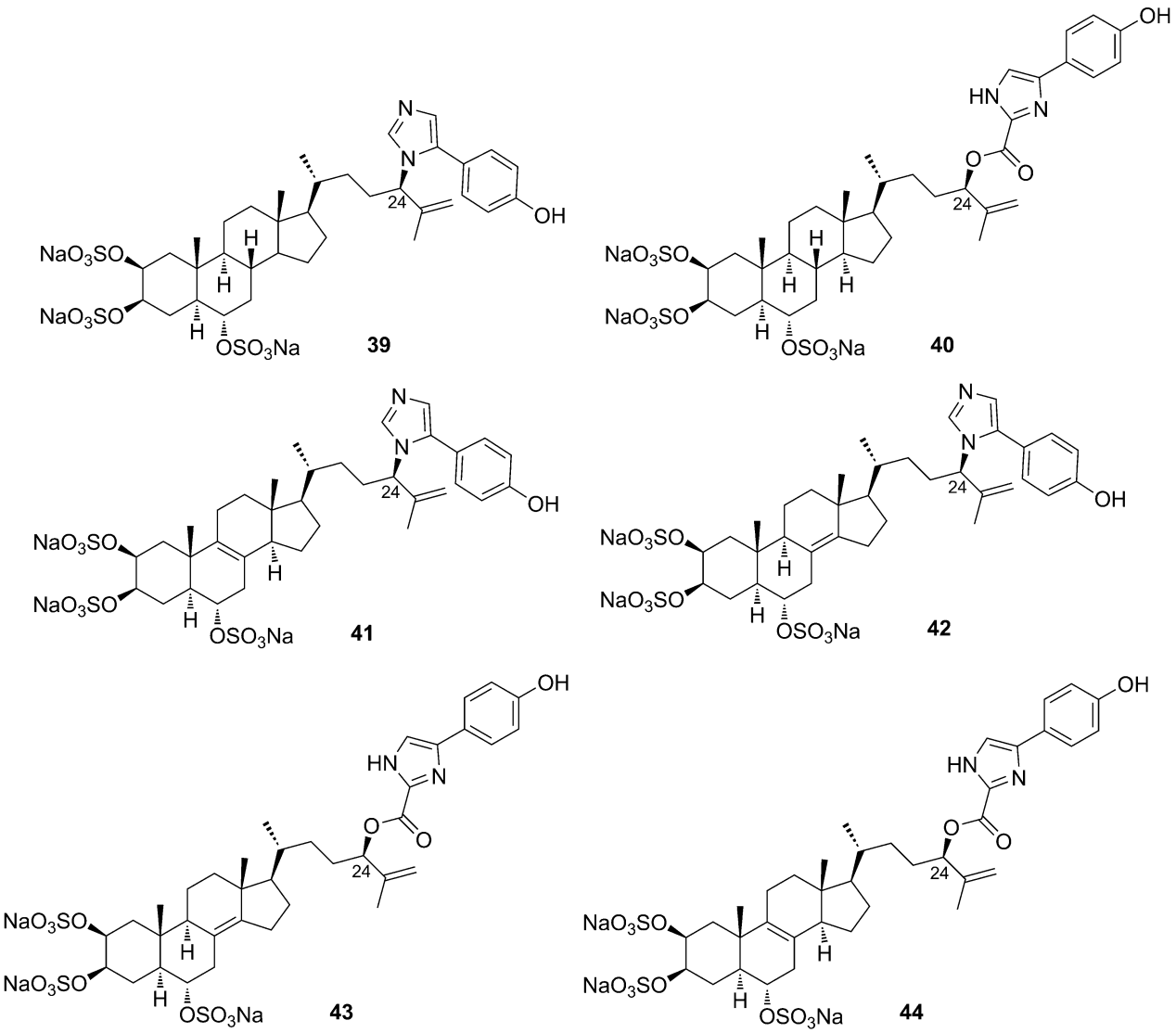

Figure 13. Chemical structures of amaranzoles A-F (**39**-**44**).

The authors suggest that the main structural difference in the amaranzole family may be due to an allylic rearrangement that interchange C-24-*N* and C-24-*O* bonds with concomitant loss of CO_2. The absolute configuration of the side chain at C-24 was assigned through the synthesis of a model ester and the chiroptical comparisons of its CD spectrum with that of an amaranzole B (**40**) derivative [28].

5. Terpenoids

5.1 Sesterterpenoids

5.1.1 Phorbaketals

In search of bioactive compounds from sponges of the genus *Phorbas* collected in South Korea, Rho et al. (2009), identified a new type of tricyclic sesterterpenoids, phorbaketals A-C (**45**-**47**) (figure 14), unprecedented in natural products [49]. They have a unique structure, with a spiroketal ring-containing hydrobenzopyran moiety. Phorbaketal A (**45**) has a carbonyl function at C-5, in ring A, while phorbaketals B (**46**) and C (**47**) both possess a hydroxyl group at C-5, but with opposite configuration. The absolute configuration of

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45 was determined by chemical conversion. Phorbaketal B (**46**) and C (**47**) are diastereomers and probably originate from a reductive process of phorbaketal A (**45**). Compound **45** has been isolated in large quantities; this observation suggests that its real producer may be a symbiont organism of the sponge. To confirm this hypothesis, the mixed microbial cultures obtained from the homogenized sponge were further cultivated and then re-extracted. As expected, phorbaketal A (**45**) was identified and isolated from the extract of the mixed bacteria culture. This allowed Rho et al. to suppose that compound **45** is produced by an endosymbiotic microorganism in the sponge [49].

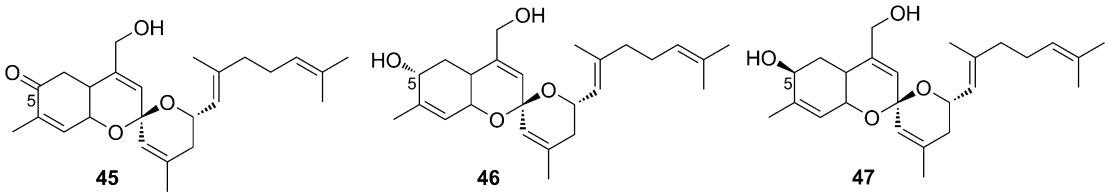

Figure 14. Chemical structures of phorbaketals A-C (**45**-**47**).

Some years later, three additional members of this family, phorbaketals L-N (**48**-**50**), have been elucidated by HRFABMS and NMR analysis from a specimen of *Phorbas* sp. collected in South Korea [50]. Phorbaketals L-N (**48**-**50**) share the tricyclic moiety with phorbaketal A (**45**) with the carbonyl group at C-5 but differ in the side chain at C-16 (Figure 15). While phorbaketal A (**45**) has a double bond at C-17 (Figure 14), phorbaketal L (**48**) differs for the position of this double bond (which is present between C-18/C-19) and for the presence of a hydroxyl group at C-17. Contrariwise, phorbaketal M (**54**) keeps the double bond at C-17 but shows the presence of a hydroperoxide group at C-22 and a double bond at C-23/C-25. Finally, phorbaketal N (**50**) shares the double bond at C-18/C-19 with compound **48** but possesses a bromine instead of a hydroxyl group at C-17; moreover, it possesses an epoxide ring at C-22/C-23.

Circular dichroism (CD) analysis allowed defining the absolute stereochemistry, suggesting phorbaketals L-N (**48**-**50**) to possess the same absolute configurations as phorbaketal A (**45**).

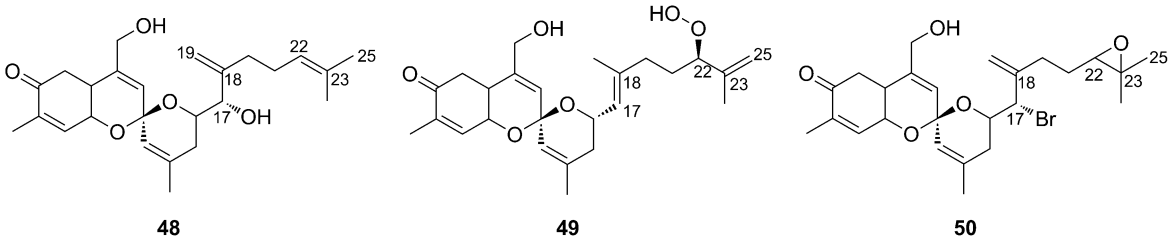

Figure 15. Chemical structure of phorbaketals L-N (**48**-**50**).

Wang et al. (2013) isolated from the organic extract of the Korean marine sponge *Monanchora sp*. eight new sesterterpenoids, namely phorbaketals D-K (**51**-**58**), along with their progenitors phorbaketals A-C (**45**-**47**), and phorbin A (**59**), a possible precursor of these sesterterpenoids (Figure 16) [51]. These compounds have not yet been identified in sponges of the genus *Phorbas*.

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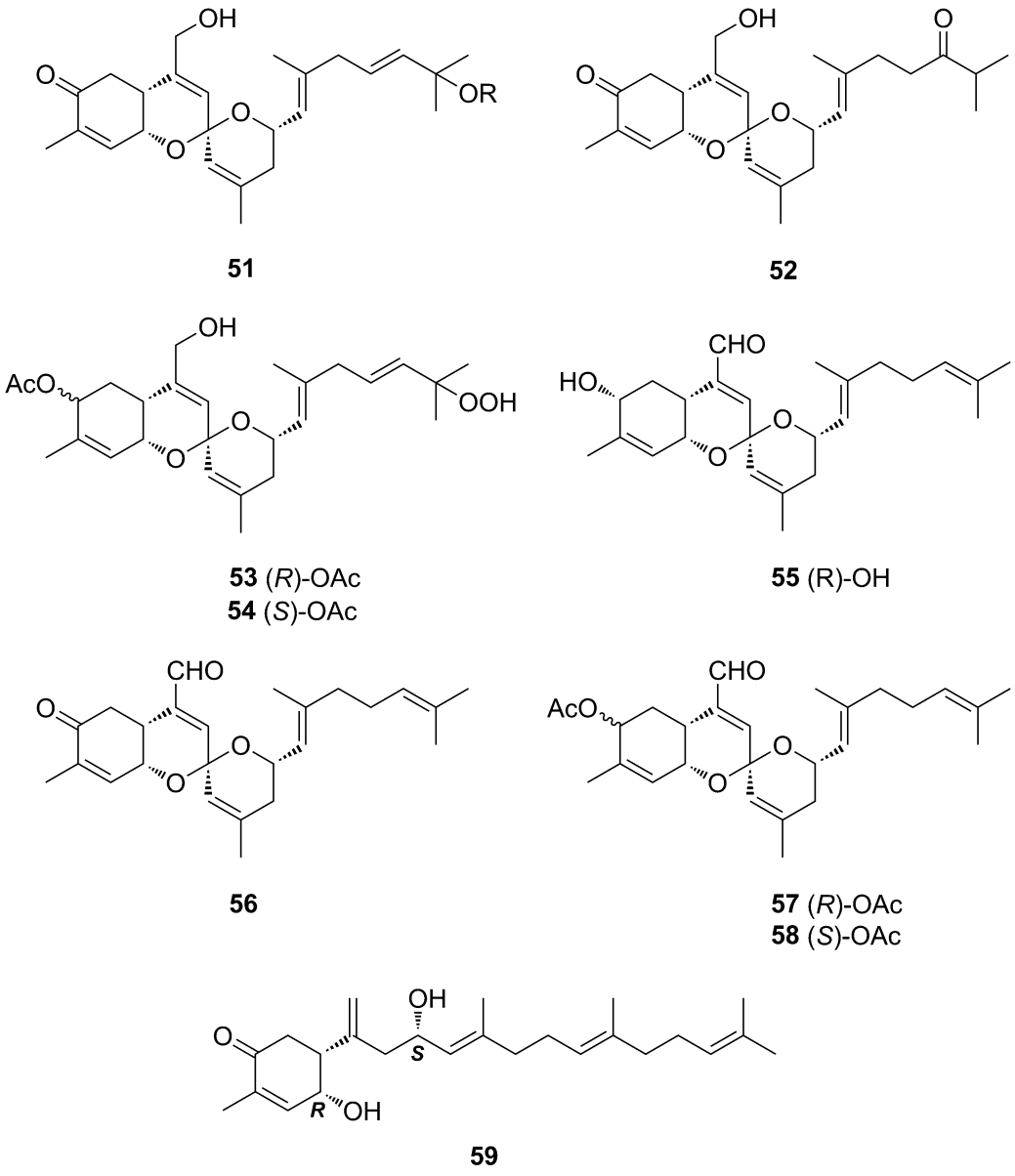

Figure 16. Chemical structures of phorbaketals D-K (**51**-**58**) and phorbin A (**59**) isolated from sponge *Monanchora* sp.

5.1.2 Alotaketals

Aloketals are sesterterpenoids with an unprecedented alotane carbon skeleton. Aloketal A (**60**) and B (**61**) (Figure 17) were isolated from the extracts of the sponge *Hamigera sp*., collected in Papua New Guinea [52]. They are the first members of a family of sesterterpenoids that may have the same "alotane" precursor, as other compounds identified in *Phorbas* such as phorbaketals (**45**-**47**) and suberitonones (**80**-**85**). Alotane sesterterpenoids have been isolated from sponges collected at three widely separated locations around the Pacific Rim (Papua New Guinea, Korea, and British Columbia). Alotaketal C (**62**) was isolated by Daoust et al. (2013) from a specimen of *Phorbas* collected in British Columbia (Figure 17) [53].

Further studies, aimed at an exhaustive search for minor sesterterpenoid compounds in the same *Phorbas* extract which already yielded alotaketal C (**62**), allowed to identify alotaketals D (**63**) and E (**64**) (Figure 17) [54].

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The basic skeleton of alotaketals is a tricyclic structure, very similar to phorbaketals (**45**-**58**). Alotaketals possess a carbonyl group at C-4 in ring A, except for alotaketal D (**63**), in which the carbonyl is substituted by an acetoxy function [54].

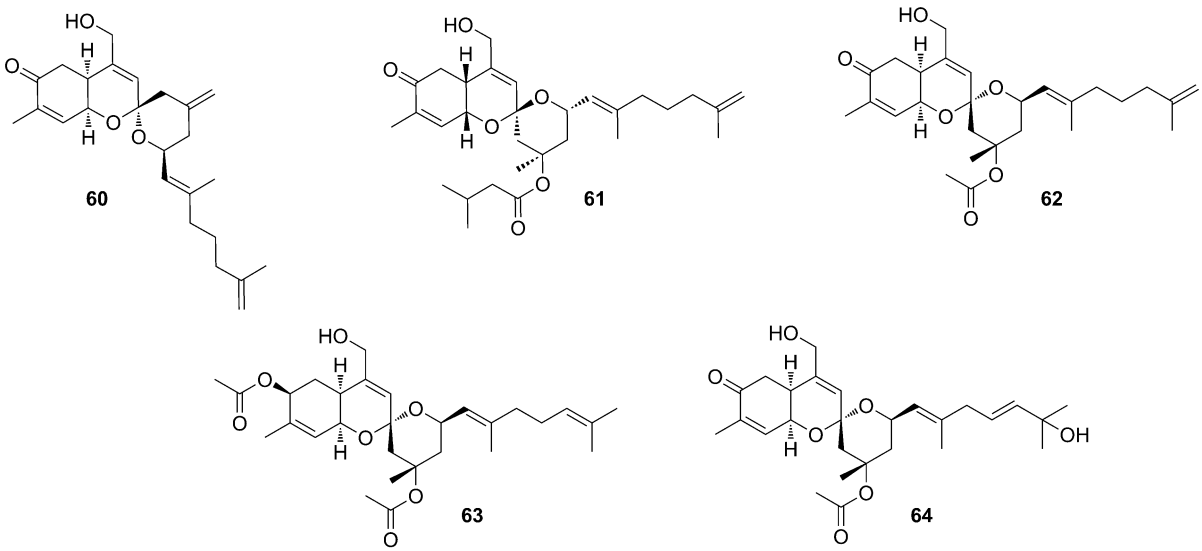

Figure 17. Chemical structures of alotaketals A-B (**60**-**61**) isolated from *Hamigera* sp., and alotaketals C-E (**62**-**64**) isolated from *Phorbas* sp.

The unique characteristics of the structures of phorbaketals (**45**-**58**) and alotaketals (**60**-**64**) have inspired many synthetic studies. Some authors also propose a possible biosynthetic route for the formation of these natural products (Scheme 3, paragraph 5.1.8) [55].

5.1.3 Ansellones

The first member of ansellone sesterterpenoid family is ansellone A (**65**) (Figure 18), identified in a study with a nudibranch *Cadlina luteomarginata* and the sponge *Phorbas sp.*, from which it fed, collected in British Columbia, Canada [56]. Compound **65** was identified as the main component in both organisms. It has an unprecedented ansellane tricycle skeleton. This new group of sesterterpenoids is biogenetically related to alotaketals and phorbaketals [56,57]. Through HRESIMS and NMR analyses, it was possible to identify the tetracyclic structure of ansellone A (**65**) and the positions of its double bonds and the positions of substitution of the rings. Ansellone A (**65**) is sequestered by *C. luteomarginata* from *Phorbas* sp. diet [56].

In addition to this, the same sponge provided phorone A (**79**) and isophorbasone A (**77**) (Figure 22). As previously mentioned, in the 2013 study by Daoust et al., where alotaketals were identified, ansellone B (**66**) and secaepoxyansellane A (**71**) were also identified [53]. Continuing this work, Wang et al. (2016), identified ansellones D-G (**67-70)** (Figure 18) [54].

The ansellones (**65-70**) are tetracyclic sesterterpenoids. The secoepoxyansellone A (**72**) has three rings of six members, one of them linked to an epoxide. Ansellones B (**66**) and C (**67**), epimers at C-13, feature an unprecedented heterocyclic skeleton that contains an oxocane ring [53]. Ansellone A (**65**) and E (**69**) are epimers whose difference lies in the configuration at C-11; ansellone D (**68**) has an *O*-methyl instead of hydroxyl in the same orientation as ansellone A (**65**). Ansellone F (**70**) displays two epoxide functions and ansellone G (**71**) has an α,β-unsaturated carbonyl function [54].

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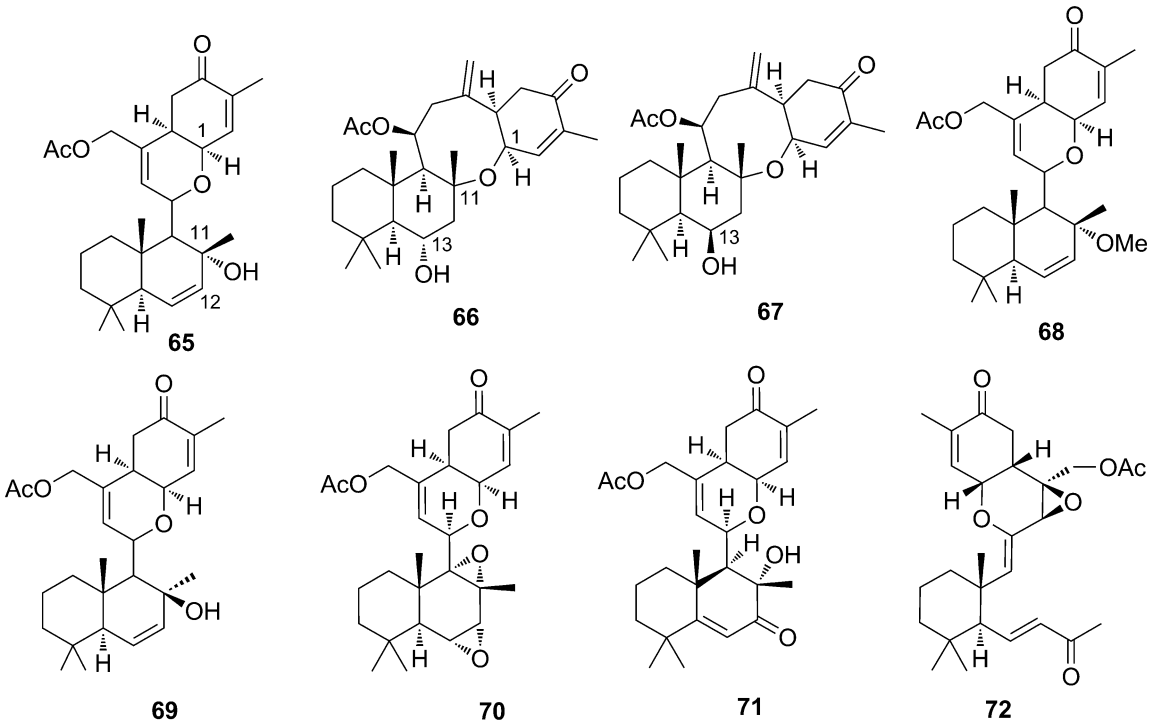

Figure 18. Chemical structures of ansellones A-G (**65-71**) and secoepoxyansellone A (**72**).

5.1.4 Anvilones

Anvilone is another group of sesterterpenoids identified by Wang et al. (2016), in their studies with *Phorbas* sp. collected in B.C., Canada. Anvilones A-B (**73-74**), have the unprecedented "anvilane" sesterterpenoide carbon skeleton [54]. They were identified by ESI-MS and NMR. They are tetracyclic sesterterpenoids. Anvilone B (**74**) differs from A (**73**) for the addition of an acetoxy functionality. Anvilones may originate from the same biosynthetic pathway as ansellones and alotaketals (Figure 19).

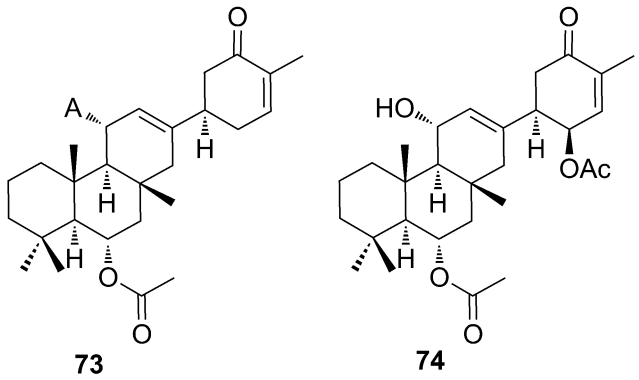

Figure 19. Chemical structures of anvilones A-B (**73-74**).

4.1.5 Phorbadione

The phorbadione (**75**) is a new compound identified by Daoust et al. (2013), together with ansellone B (**66**), secoepoxyansellone A (**72**) and alotaketal C (**62**). ESIMS and NMR data showed great similarity with ansellone A (**65**), with the presence of cyclohexenone and dihydropyran rings being observed. The difference between ansellone A (**65**) is in ring B with an extra unsaturation and an α,β-unsaturated carbonyl function in **75** [53] (Figure 20).

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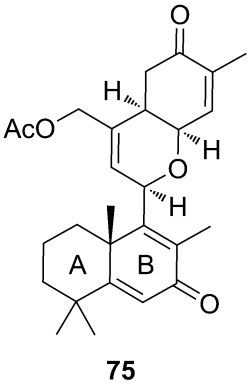
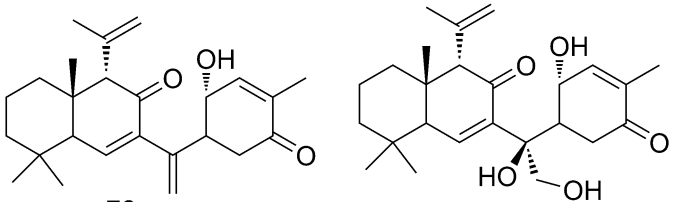


75

Figure 20. Chemical structure of phorbadione (**75**).

5.1.6 Phorbasones

Phorbasones A and B (**76-77**) (Figure 21) are sesterterpenoids identified from a specimen of *Phorbas* sp. collected in Korea, together with phorbaketals [58]. Probably ansellones (**65-71**), phorbaketals (**45-58**) and phorbasones (**76-77**) are biogenetically linked (Scheme 3, paragraph 5.1.8). Complete structures of phorbasones A-B (**76-77**) were elucidated by spectroscopic techniques and chemical reactions. They have a bicyclic structure connected by a small side chain to a cyclohexenone. The difference between them resides in the bridge-chain, with phorbasone B (**77)** having a hydroxymethylene and an additional hydroxyl group, and phorbasone A (**76)** possessing a methylene function at the same position. The absolute stereochemistry of compounds was determined by the Mosher's ester method and NMR.



76 77

Figure 21. Chemical structure of phorbasones A and B (**76-77**).

One year later, a further study on a *Phorbas* specimen collected in Korea, and from which ansellone B (**66**) was identified, allowed the identification of a new structure called isophorbasone A (**78**) (Figure 22) [59]. In compound **78** the bridge-chain contains one more carbon unit and the hexenone ring is replaced by phenol as compared to phorbasone A (**76**). Moreover, phorbasone A acetate (**79**), which is closely related to phorbasone A (**76**), was also structurally elucidated, showing an acetate function instead of the hydroxyl group on the hexenone ring [59]. This work also led to identification of phorone A (**80**) (Figure 22), in which FAB-MS and NMR data have shown a tetracyclic skeleton, consisting of a bicyclic system (i.e., decalin, similar to those of phorbasones) fused to a heptanone ring fused in turn to a fourth phenyl ring [59].

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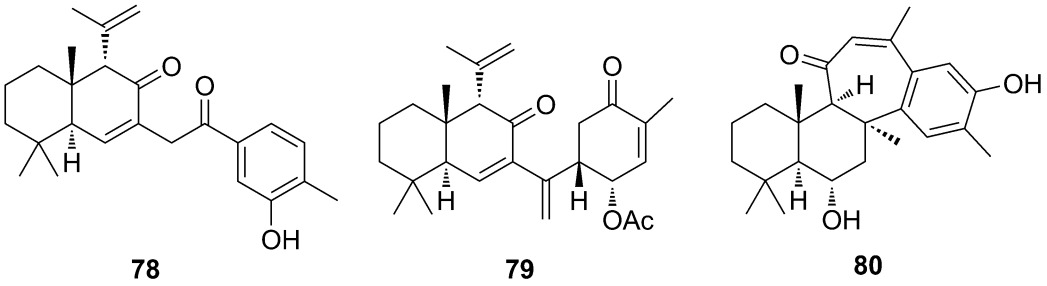

Figure 22. Chemical structures of isophorbasone A (**78**), phorbasone A acetate (**79**) and phorone A (**80**).

5.1.7 Suberitenones

Sesterterpenoids of the suberitone type were isolated and identified from the sponge *Phorbas areolatus*, collected in Antarctica. The main feature of these substances is the rare suberitane skeleton. In addition to the already known suberitenone A and B (**81-82**), and oxaspirosuberitenone (**83**), the new isosuberitenone B (**84**), 19-episuberitenone B (**85**), and isooxaspirosuberitenone (**86**) were isolated (Figure 23) [60]. Until then, these substances were considered markers of the *Suberites* genus, the only one in which they had been identified. The discovery from *Phorbas* excludes suberitanes as chemotaxonomic markers.

This sesterterpenoids were identified based on HRMS and NMR data analysis, by comparisons with those of the same group already reported. Suberitenone A (**81**) has a double bond at C-13 absent in B (**82**), which has a hydroxyl at this position. The HRMS data analysis showed that isosuberitenone B (**84**), and suberitenone B (**82**) are isomers. Analysis of ^{1}H-NMR and HMBC spectra, showed that the difference between the two compounds resides in the position of the acetoxy substituent, which in isosuberitenone B (**84**) is on the hexenone ring while in suberitenone B (**82**) is located on ring B. 19-episuberitenone B (**85**) is also an isomer of suberitenone B (**82**). 19-episuberitenone B (**85**) is indeed the epimer at C-19 of **82**. As observed for suberitenone B (**82**) and isosuberitenone B (**84**), isooxaspirosuberitenone (**86**) has NMR signals very similar to oxaspirosuberitenone (**83**), with the difference being the position of the acetoxy substituent group (Figure 23). Interestingly, these structures come from a 1,4 nucleophylic addition and cyclization process which involves the alcohol group at C-13 and the enone of the D ring, thus resulting into a rare structure in natural products [60].

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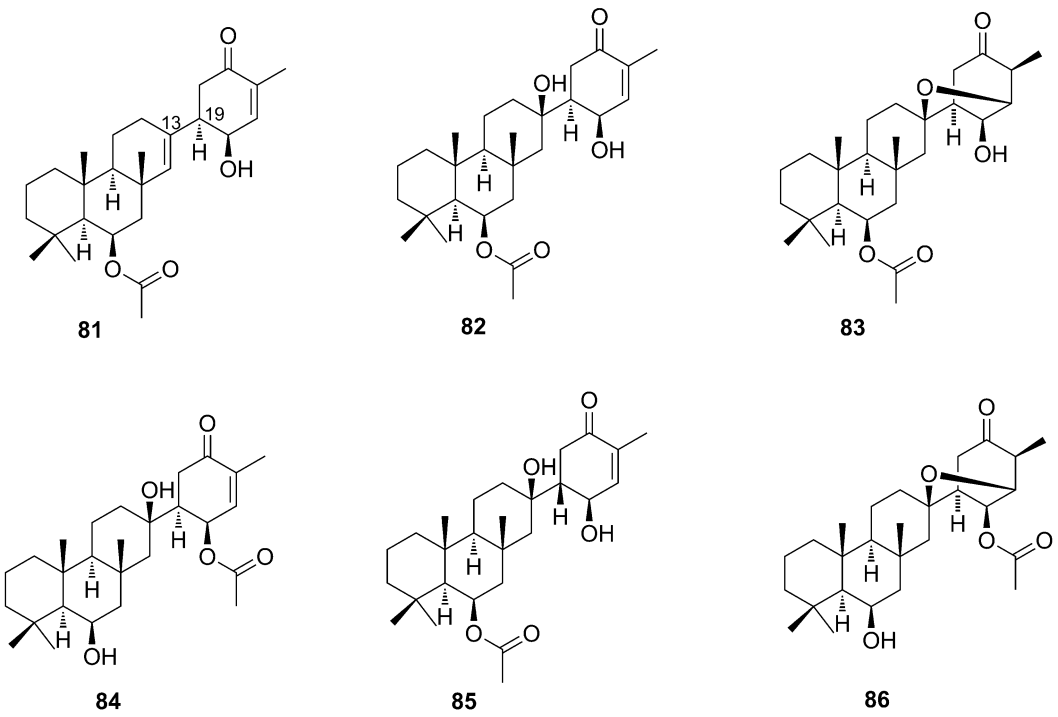

Figure 23. Chemical structures of suberitones and natural products derivatives (**81-86**).

5.1.8 Putative biosynthetic pathway of sesterterpenoids from *Phorbas*

Some authors have already proposed biosynthetic routes for the formation of sesterterpenoids found in *Phorbas*. Several of these natural products can share stages on the same route, the fact that several of them are found in the same species corroborates this idea. The main hypothesis is that they all start from the geranyl-farnesyl pyrophosphate, which through cationic cyclizations, oxidation, and rearrangements, would lead to different skeletons [59].

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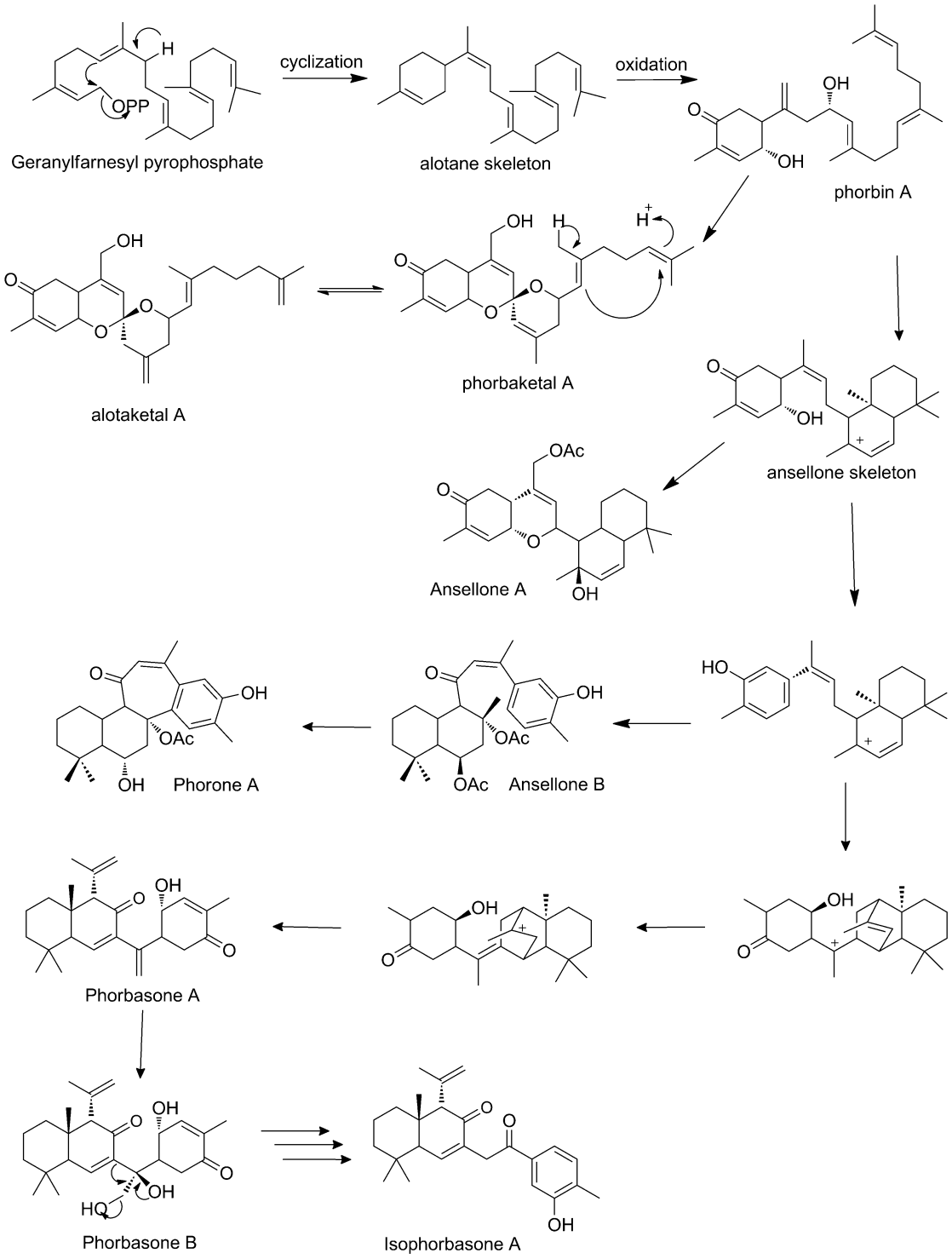

Scheme 3. Proposed biosynthesis of sesterterpenoids of *Phorbas* (adapted from Yao et al., 2020).

Phorbaketals (**45-58**) and alotaketals (**60-64**) are compounds with a spiral ring originated from the geranyl-farnesyl pyrophosphate unit. A pyrophosphate-generated allylic cation was attacked by proximal olefin to form the alotane skeleton and after successive oxidations produces phorbin A (**59**), phorbaketal (**45-58**) and alotaketal (**60-64**) [55].

Phorbin A (**59**) is considered a precursor of a series of *Phorbas* steroids, such as phorbasones (**76-77**). The farnesyl part of phorbin A (**59**) undergoes cationic cyclization, forming the skeleton of ansellone, a shared intermediate between phorbasones (**76-77**), ansellone (**65-71**) and phorone A (**80**), which has a common fused bicyclic ring. The ansellone skeleton reorganizes itself through cationic cyclization forming a bicyclic structure, which

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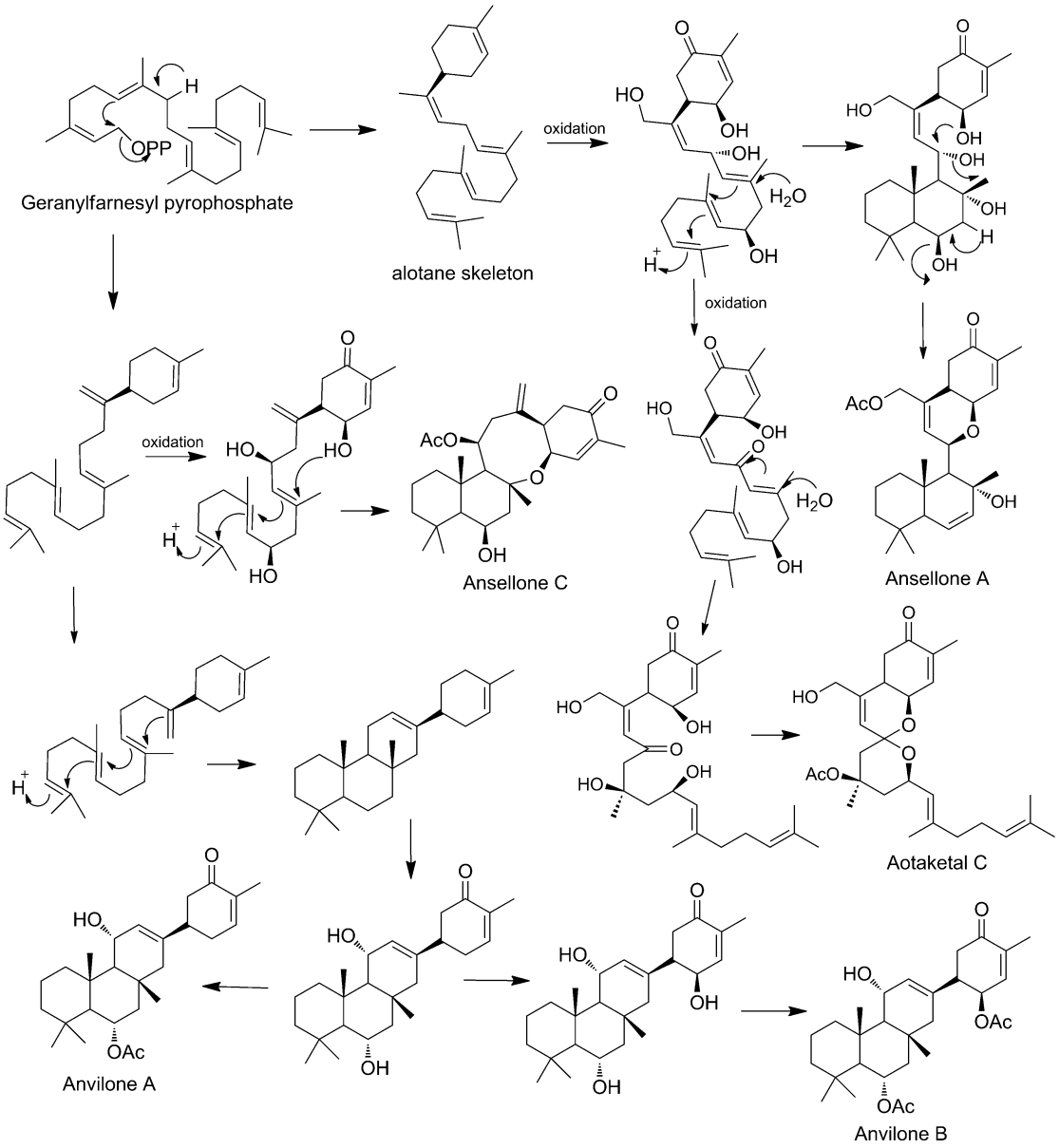
undergoes successive proton migrations and ring cleavage. This succession of reactions lead to the tricyclic structure of phorbasone A (**76**), which is hydroxylated to phorbasone B (**77**). For the formation of isophorbasone A (**78**), phorbasone B (**77**) pass through several stages, such as oxidation, dehydration, rearrangement and tautomerization of the hex-enone keto-enol (Scheme 3) [58,59].

The ansellone skeleton is the precursor of the ansellone (**65-71**) class and of the phorone A (**80**). Wang et al. relate the route of formation of the ansellones with the route of the anvillones (**73-74**) and alotaketals (**60-64**), found in *Phorbas* sp. collected in British Columbia (Scheme 4) [54] .



Scheme 4. Proposed biosynthesis of sesterterpenoids identified in *Phorbas* (adapted from Wang et al., 2016).

5.2 Diterpenoids

5.2.1 Phorbasins

Vuong and Capon (2000), identified a diterpene with a novel skeleton, namely phorbasin A (**87**) from a sponge collected in Australia [61]. Through NMR and ESI-MS analyses, it was possible to identify its structure (Figure 24) as a conjugated polyene attached

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to a monocyclic unit and classify it as a rearranged diterpene. The molecule was unstable and underwent decomposition during the analysis. It was therefore impossible to establish the absolute stereochemistry of the three chiral centers-of phorbasin A (**87**). The enantiomer portrayed in figure 24, displays the arbitrary absolute stereochemistry suggested by Vuong and Capon.

One year later, two additional phorbasins were identified, namely B and C (**88-89**) [62]. Phorbasin B (**88**) had its relative stereochemistry attributed based on coupling constant values and molecular modeling that provided theoretical measures of the dihedral angles. Furthermore, comparison with others natural products with the same ciclohexenone substructure contributed to the attribution. Phorbasin C (**89**) was spectroscopically very similar to phorbasin B (**88**). The molecular formula for phorbasin C (**89**) differed from phorbasin B (**88**) by 42 mass units (acetyl unit), and the ^{1}H NMR spectrum of phorbasin C (**89**) revealed characteristic acetate methyl resonance. Due to the lack of material and the instability of phorbasin A (**87**) and C (**89**), their absolute stereochemistry could not be determined in this study. One year later, the total synthesis of phorbasin C (**89**) was carried by Macklin et al. allowing the elucidation of the relative and the absolute configuration of compound **89** [63].The skeleton of phorbasins B-C (**88-89**) are very similar to phorbasin A (**87**) (Figure 24). They differ by the side chain and by some substituents on the cyclohexenone ring. They are likely to share a common biosynthetic pathway, even if not occurring as co-metabolites [62].

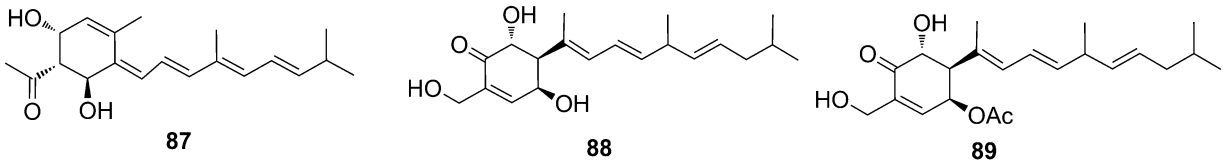

Figure 24. Chemical structures of phorbasins A-C (**87-89**).

Continuing these two studies, Zhang and Capon (2008), reisolated the phorbasins B-C (**88-89**) and confirmed their stereochemistry, and identified new analogs named phorbasins D-F (**90-92**) (Figure 25), which incorporate a somewhat unprecedented terpenyltaurine residue (2-aminoethanesulfonic acid) [64]. ESI-MS and NMR analysis allowed the identification of phorbasin D (**90**). The comparison of NMR data of phorbasins B-C (**88-89**) and D (**90**) showed the presence of different functional groups on the cyclohexenone ring. As in phorbasins B-C (**88-89**) the substituent is a hydroxymethylene while in phorbasin D (**90**) is a taurinyl group.

Phorbasins E-F (**91-92**) are actually dimers, probably derived from phorbasin B or C (**88-89**), fused by a seven-membered heterocyclic ring, which incorporates a taurinyl residue, an unprecedented feature in natural product skeletons [64]. Analysis of the structures that form the dimers, suggested that they may have the same biosynthetic origin and, therefore, a common absolute stereochemistry. The difference between these dimers is the presence of an acetate substituent linked to the phorbasin F (**92**), which is absent in E (**91**) [61,62,64].

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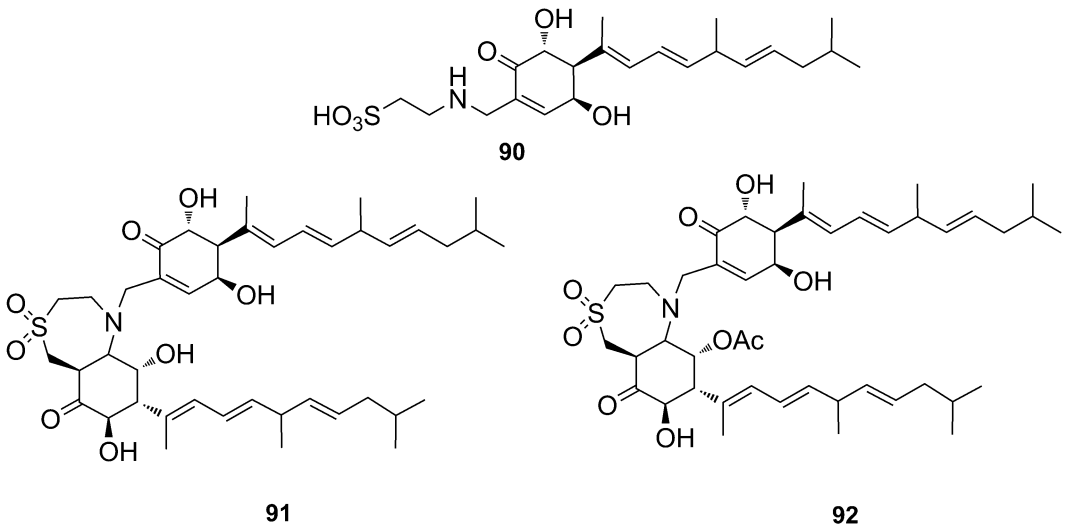

Figure 25. Chemical structures of phorbasins D-F (**90-92**).

Lee et al. (2008), isolated and identified three new diterpenes that were also named phorbasins G-I (**93-95**) [65] from *Phorbas gukhulensis*, collected in South Korea (Figure 26). These molecules, identified by FAB-MS and NMR experiments, feature a cyclohexane ring differently from their congeners previously discussed, which exhibit a cyclohexene moiety. Phorbasin G (**93**) has a taurine residue as a substituent on the ring, very similar to that found in phorbasin D (**90**). Phorbasin H (**94**) displays a carboxylic function, as well as phorbasin I (**95**) which differs from the former by the position of a double bond in the side chain [53]. Recently the synthesis of phorbasin H (**94**) was carried out and the absolute configuration of the natural product was determined as *S* [66] A dereplication work with LCMS, showed the presence of these phorbasins G-I in the extract of *P. amaranthus* [67].

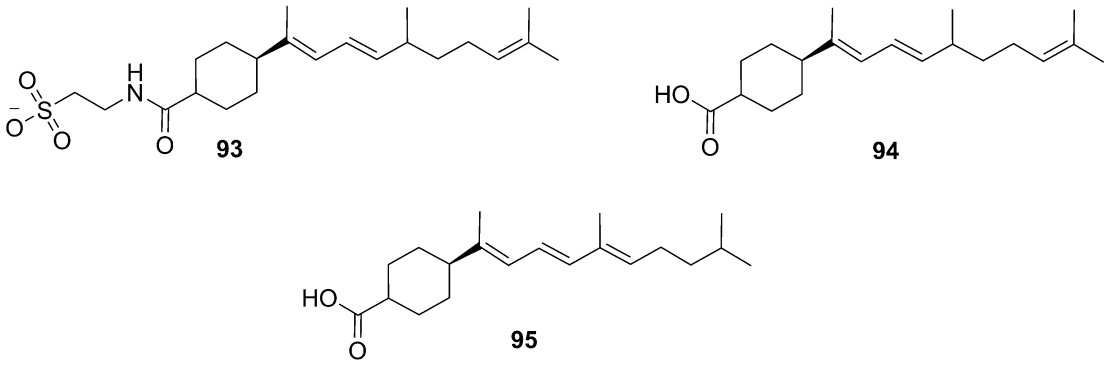

Figure 26. Chemical structures of phorbasins G-I (**93-95**).

Zhang et al. (2008), a few months later, identified five more phorbasins, i.e. phorbasins G-K (**96-100**) (Figure 27) [68]. Analysis of ESI-MS and NMR spectra revealed that phorbasin G (**96**) is a desoxy analogue of phorbasin B (**88**), while phorbasin H (**97**) is an acetate derivative of phorbasin G (**96**), with the same absolute stereochemistry. The ethoxy derivatives of phorbasin H (**97**) are a) phorbasin I (**98**) with an EtO group on C3 and b) phorbasin J (**99**) with EtO groups on C2 and C3. Phorbasin K (**100**) was reported as the dihydro analogue of phorbasin B (**88**). Phorbasins I and J (**98–99**) are likely solvolysis artifacts generated by storage in ethanol.

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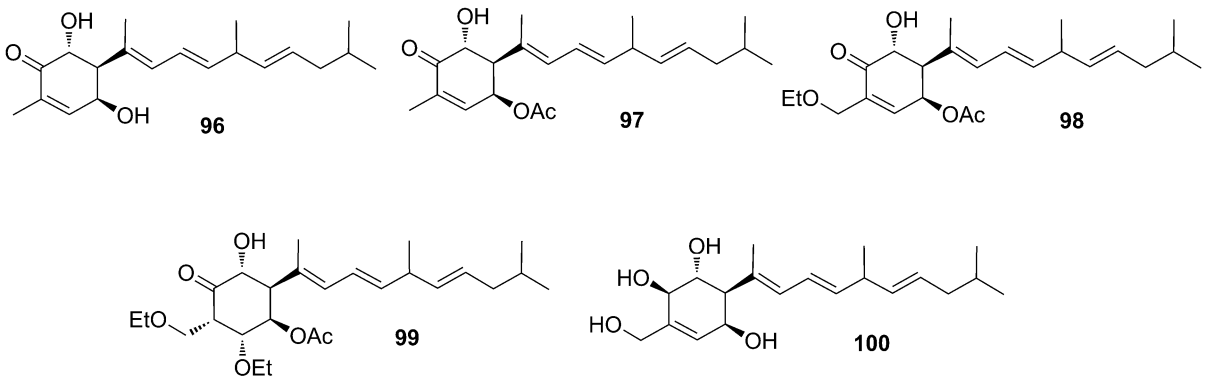

Figure 27. Chemical structures of phorbasins G-K (**96-100**) identified by Zhang and Capon, 2008.

5.2.2 Gagunins

Another diterpenoid group that occurs in the genus *Phorbas*, includes the gagunins. They were first identified in a study about the species collected in South Korea. Seven diterpenoids named gagunins A-G (**101-107**) (Figure 28) have been identified [69]. They have the core structure of homoverrucosane, with three fused carbon rings, i.e., cyclopentane, cyclohexane and cycloheptene. The main difference with the core structure of homoverrucosane is the stereochemistry of the junction between the five and six carbon rings. Another feature is that they are highly oxygenated structures with a variety of functions.

The ganunins structures was defined by FAB-MS, IR and NMR. Gagunin A (**101**) also underwent chemical modification, replacing all of its hydroxyl-substituted oxygen groups, which facilitated correct identification, as ester chains complicated the analysis by NMR. Gagunins A-C (**101-103**) differ from each other by the two substituents on the cyclopentane ring. Gagunins D-F (**104-106**) differ by the three substituents on the cycloheptene ring. Gagunin G (**107**) shares the substitution pattern of the cycloheptene ring with gagunin F (**106**) and differs from the latter by a substituent on the cyclopentane ring [69].

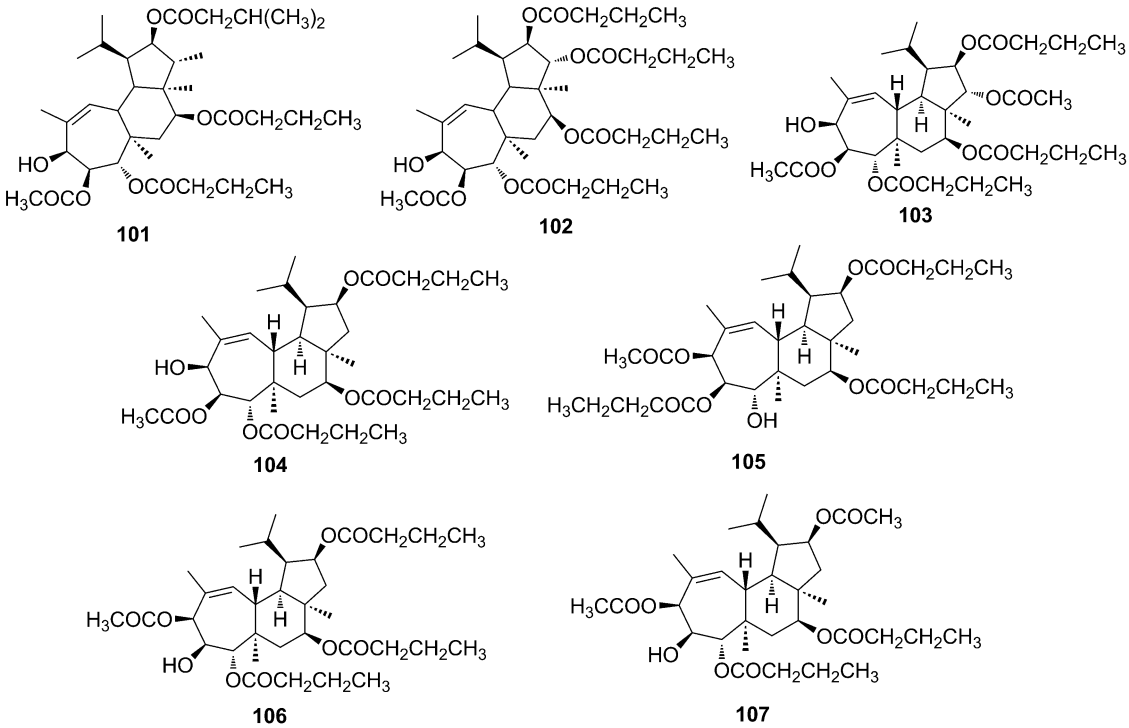

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Figure 28. Chemical structures of gagunins A-G (**101-107**).

Jang et al. (2008), when studying *Phorbas* sp. collected in Korea, identified phorbasin H (**94**), as previously mentioned, and also six known gagunins A-D (**101-104**) and F-G (**106-107**), and ten new gagunins H-Q (**108-117**) (Figure 29) [70]. The spectroscopic data of these substances were similar to that previously found for gagunins. In addition, IR absorption bands showed signals equivalent to ester and oxymethine groups found into gagunin structure. The combination of this information with NMR data allowed to identify the ten new members of this group.

Gagunins H-M (**108-113**) are very similar to gagunins A-D (**101-104**), showing changes in the substitution pattern of the cyclohexane ring differently from compounds **101-104**. Gagunins N-O (**114-115**) are similar to the F-G (**106-107**), but with a different substitution pattern on the cyclohexane ring. Gagunins P-Q (**116-117**) have identical substitution pattern in the cyclopentane and cyclohexane rings, and changes are in the substituent groups on the cycloheptene ring (Figure 29) [70].

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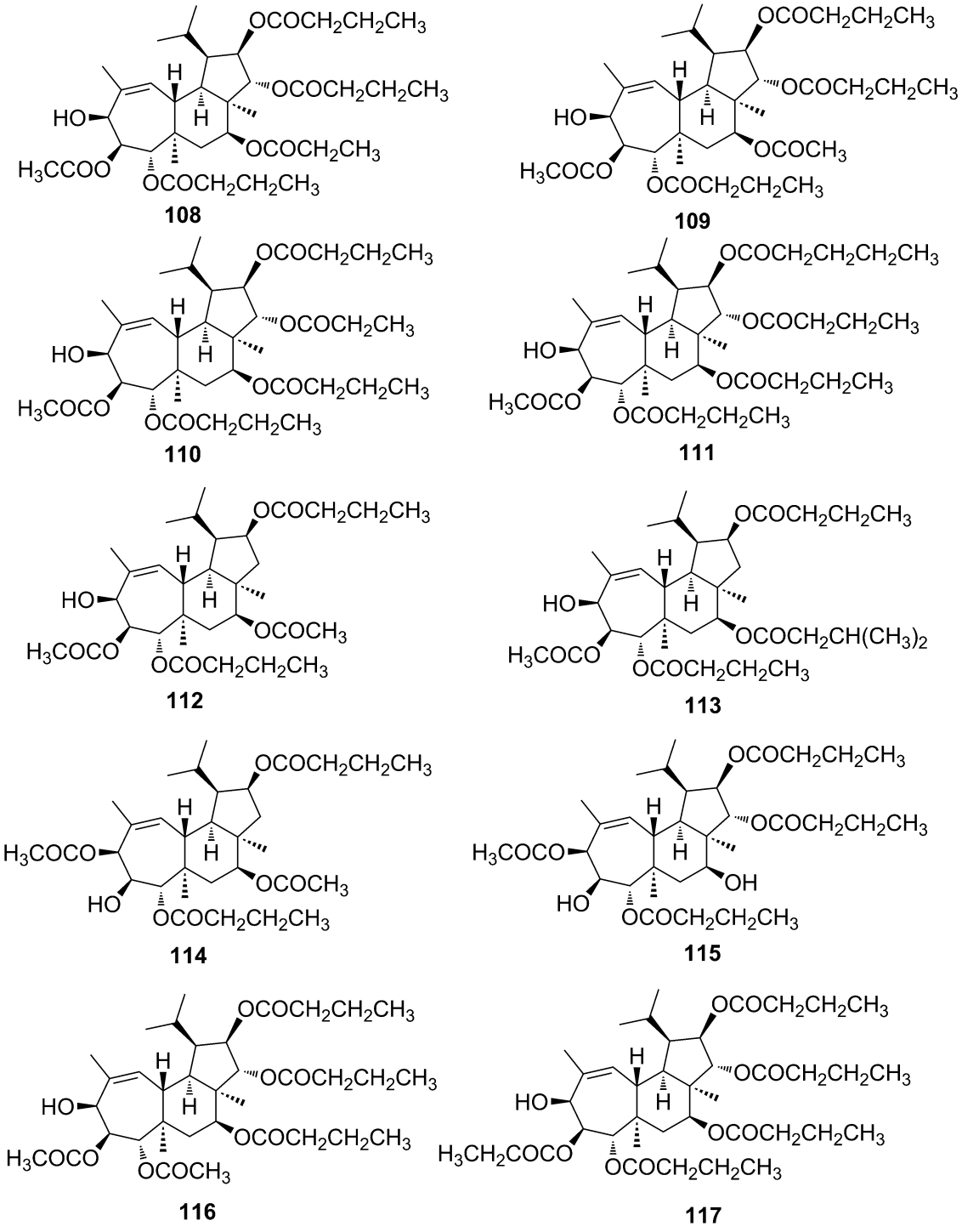

Figure 29. Chemical structures of gagunins H-Q (**108-117**).

5.3 Tetraterpenoids

5.3.1 Gukulenins

The only tetraterpenoids identified in the genus *Phorbas* to date have been the gukulenins, which have an unprecedented bis-tropolone skeleton. They were identified in the species *P. gukhulensis*, collected in South Korea. Gukulenins A-F (**118-123**) look like pseudodimers of the gagunines, also found in this species, but the different positions of substituting groups may indicate that they have another precursor. Hukulenins are linked by

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the substituents of the seven-membered carbon ring (tropolone), forming a new six-membered heterocyclic ring, except in gukulenin F (**123**) in which that ring does not form. They have very diverse oxygenated side chains, but smaller than the oxygenated groups found in gagunins, they also have nitrogen groups as substitutes [29,30] (Figure 30).

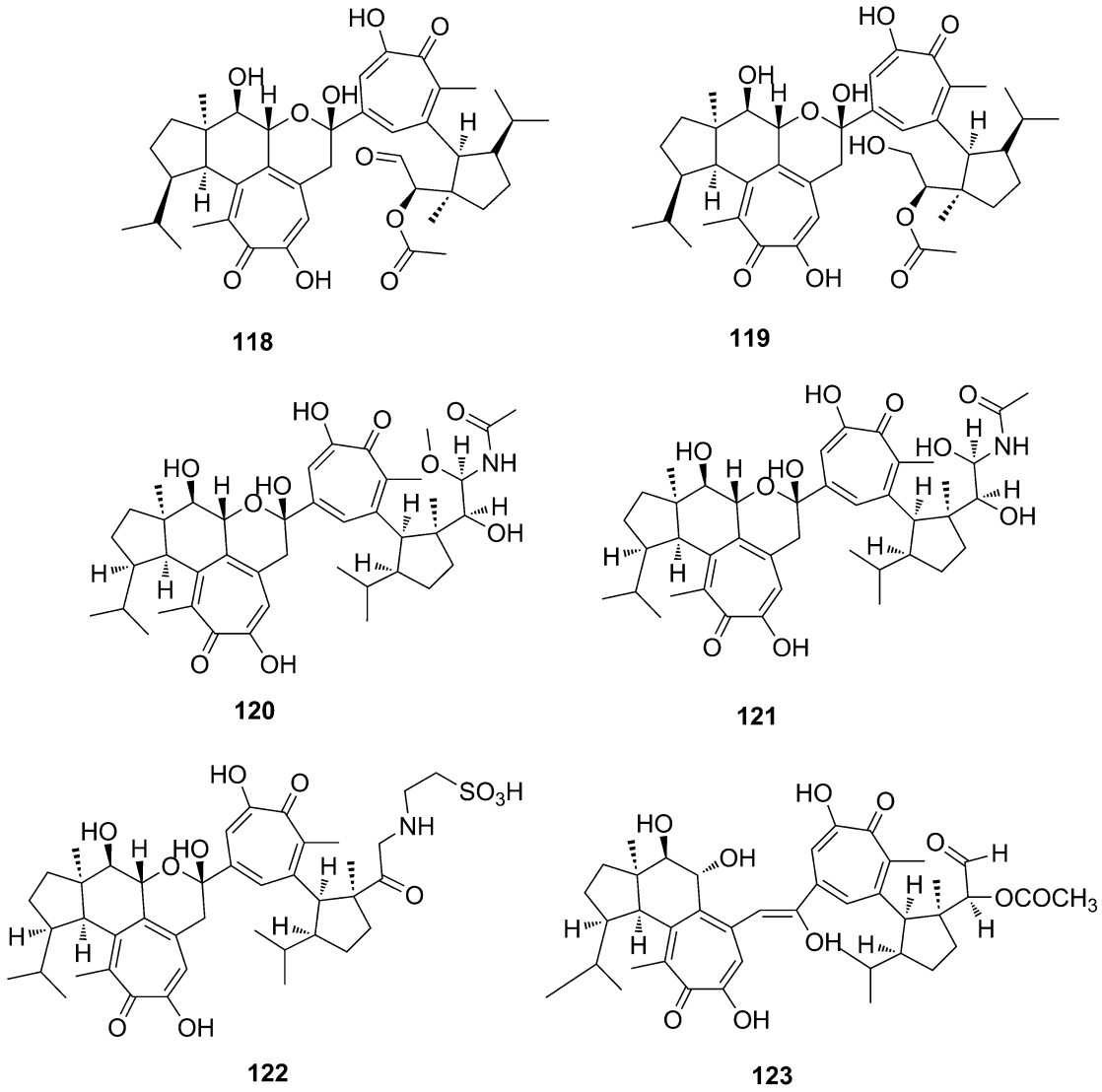

Figure 30. Chemical structures of gukulenins A-F (**118-123**).

6. Miscellaneous

6.1 Other compounds identified in P. paupertas

In the same study where the alkaloid zarzissine (**13**) was isolated, also the *p*-hydroxybenzaldehyde (**124**) was identified (Figure 31). This compound was identified in the sponge *P. paupertas* (previously *Anchinooe paupertas*) collected in Mediterranean, Tunisia [35].

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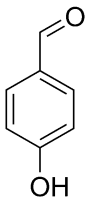
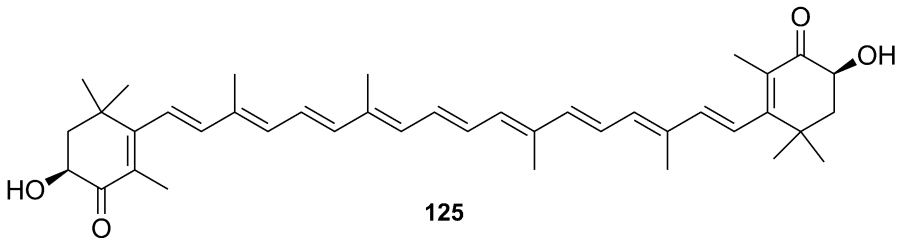


124

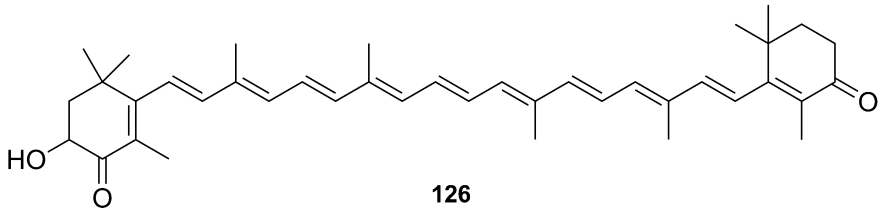
Figure 31. Chemical structure of the *p*-hydroxybenzaldehyde (**124**).

6.2 Other compounds identified in P. topsenti

The carotenoids astaxanthin (**125**) and adonirubin (**126**) were isolated from specie of *Phorbas topsenti* collected off Marseille (France), in addition to the alkaloids phorbatopsins (**14-16**) [26].



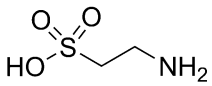
125



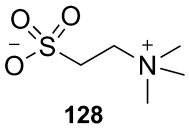
126

Figure 32. Chemical structures of the astaxanthin (**125**) and adonirubin (**126**).

In the framework of the study mentioned above, two sulfonic acids, taurine (**127**) and taurobetain (**128**), were isolated [26].



127



128

Figure 33. Chemical structures of taurine (**127**) and taurobetain (**128**).

6.3 Other compounds identified in P. amaranthus

Recently a dereplication study, based upon comparison of HR-MS fragmentation pathways, allowed the annotation of 18 known *Phorbas* metabolites, for the extract of *P. amaranthus*, collected in Brazil. Among the known compounds are: phorbasins (**87-89, 90-92, 93-95, 96-100**), phorbaketals (**45-47,49**), isophorbasone (**78**), ansellones (**72**), anvillones (**80**) and phorbasterones (**34-39**) [67].

Through Global Natural Product Social Molecular Networking (GNPS) were inferred 17 metabolites, among them most being lysophospholipids (LPL) (**129-130**), carotenoids (**131**) and sterols (**132-133**) (Figure 34). The GNPS also suggested other 29 metabolites annotated through a molecular subnetwork. Some examples of these metabolites are shown in Figure 34 [67].

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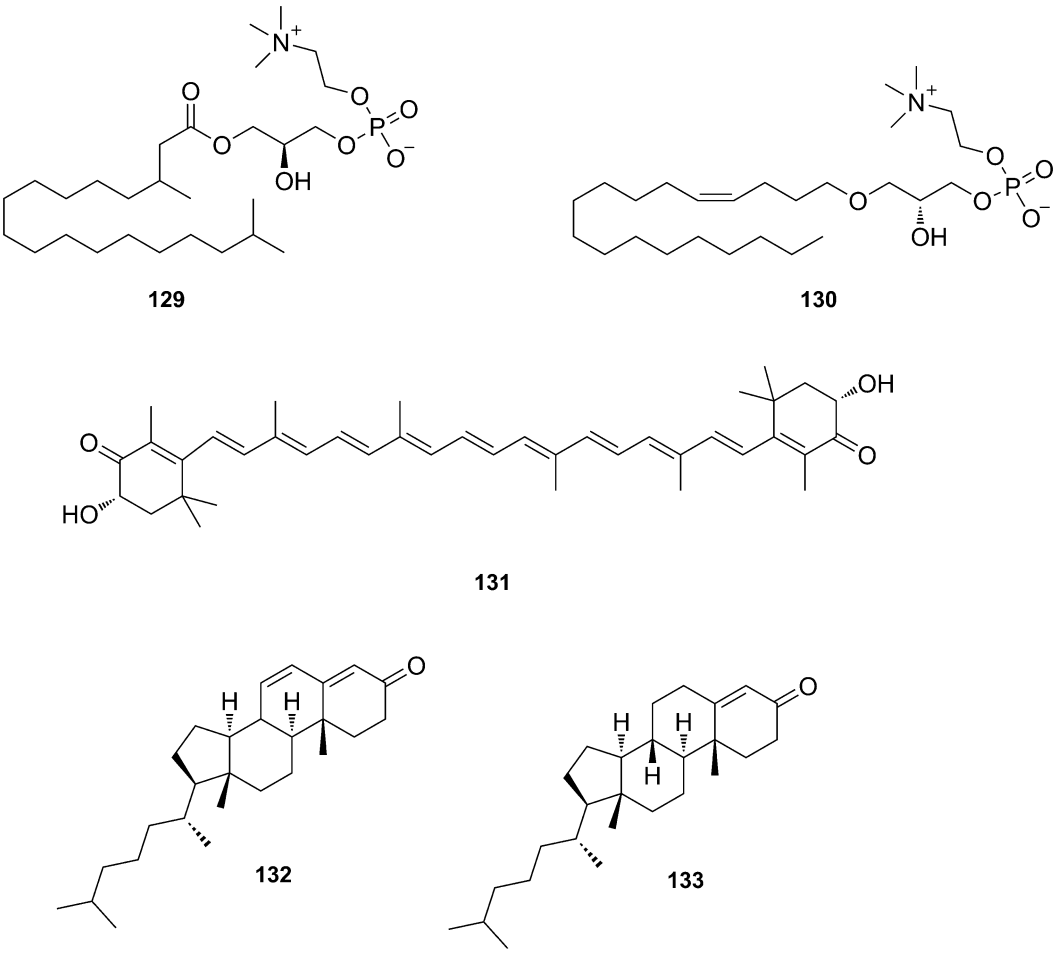

Figure 34. Chemical structures of compounds detected by GNPS in the organic extract *P. amaranthus* (**129-133**).

7. Bioactivity of compounds isolated from sponges of the genus *Phorbas*

The ocean is one of the places on the Earth that has a wide spectrum of natural resources. The advent of new technologies allowed the in deep study of marine biochemical diversity and the discovery of new bioactive marine natural products (MNPs). The complex habitats and exposure to extreme conditions of light, temperature, pH, salinity and others external factors induce marine organisms to produce a wide variety of specific and potent active substances that cannot be found elsewhere [71]. The genus *Phorbas*, as well as several other sponges found in the aquatic environment, are a rich source of bioactive natural products such as alkaloids, terpenes, macrolides, steroids and peptides.

Among the activities described for compounds identified from the genus *Phorbas*, cytotoxic activity (Table 1) stands out, some other activities (Table 2) besides this have also been analyzed. Bioactivity evaluation of pure compounds is often hampered by the low amount obtained from the natural source. Indeed, some compounds have been evaluated for their pharmacological properties only after being obtained on a larger scale by chemical synthesis.

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7.1 Cytotoxic and cytostatic activity

Cytotoxic properties assessed mainly by *in vitro* assays for compounds from *Phorbas* sponges were summarized in Table 1.

Table 1. List of MNPs originated from *Phorbas* with cytotoxic activity.

	Name	Class	Species	Cell Lines	Dose/ Concentration	Reference
13	Zarzissine	Alkaloid	*Phorbas tenacior*	P-388	IC_{50} 12 μg/mL	[35]
				KB	IC_{50} 5 μg/mL	
				NSCLC-N6	IC_{50} 10 μg/mL	
17	Phorboxazole A	Macrolide	*Phorbas sp.*	HCT-116	GI_{50} 4.36 × 10^{-10} M	[24]
				HT29	GI_{50} 3.31 × 10^{-10} M	
19	Muironolide A	Macrolide	*Phorbas sp.*	HCT-116	IC_{50} 96,5 μg/mL	[37]
20	Phorbaside A	Macrolide	*Phorbas sp.*	HCT-116	IC_{50} 30.0 μM	[41]
22	Phorbaside C	Macrolide	*Phorbas sp.*	HCT-116	IC_{50} 2 μM	[41]
23	Phorbaside D	Macrolide	*Phorbas sp.*	HCT-116	IC_{50} 61.9 μM	[41]
24	Phorbaside E	Macrolide	*Phorbas sp.*	HCT-116	IC_{50} 10.2 μM	[41]
29	Phorbasterone A	Steroid	*Phorbas amaranthus*	HCT-116	IC_{50} 1-3 μg/mL	[45,67]
30	Phorbasterone B	Steroid	*Phorbas amaranthus*	HCT-117	IC_{50} 1-3 μg/mL	[45,67]
31-32	Phorbasterone C	Steroid	*Phorbas amaranthus*	HCT-118	IC_{50} 1-3 μg/mL	[45,67]
33-34	Phorbasterone D	Steroid	*Phorbas amaranthus*	HCT-119	IC_{50} 1-3 μg/mL	[45,67]
45	Phorbaketal A	Sesterterpenoid	*Phorbas sp.*	A549	IC_{50} 11–12 μg mL^{-1}	[49,51]
				HT-29	IC_{50} 11–12 μg mL^{-1}	
				HepG2	IC_{50} 11–12 μg mL^{-1}	
46	Phorbaketal B	Sesterterpenoid	*Phorbas sp.*	A549	IC_{50} 12–460 μg/mL	[49,51]
				HT-29	IC_{50} 12–460 μg/mL	
				HepG2	IC_{50} 12–460 μg/mL	
47	Phorbaketal C	Sesterterpenoid	*Phorbas sp.*	A549	IC_{50} 12–460 μg/mL	[49,51]
				HT-29	IC_{50} 12–460 μg/mL	
				HepG2	IC_{50} 12–460 μg/mL	
50	Phorbaketal N	Sesterterpenoid	*Phorbas sp.*	HT-29	LG_{50} 5-15 μM	[50]
				PANC-1	IC_{50} 11.4 μM	
				A498	IC_{50} 18.7 μM	

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				ACHN	LC_{50} 24.4 μM	
84	Isosuberitenone B	Sesterterpenoid	*Phorbas areolatus*	A549	IC_{50} 8,8 μM	[60]
				HT-29	IC_{50} 9,0 μM	
				HepG2	IC_{50} 7,4 μM	
				MCF-7	IC_{50} 8,8 μM	
85	19-episuberitenone B	Sesterterpenoid	*Phorbas areolatus*	A549	IC_{50} 5,1 μM	[60]
				HT-29	IC_{50} 6,4 μM	
				HepG2	IC_{50} 5,0 μM	
				MCF-7	IC_{50} 5,1 μM	
88	Phorbasin B	Diterpene	*Phorbas sp.*	A549	LG_{50} 5-15 Mm	[64]
				HT-29	LG_{50} 5-15 μM	
89	Phorbasin C	Diterpene	*Phorbas sp.*	A549	LG_{50} 5-15 μM	[64]
				HT-29	LG_{50} 5-15 μM	
91	Phorbasin E	Terpenyl-tau-rine	*Phorbas sp.*	A549	LG_{50} 5-15 μM	[64]
				HT-29	LG_{50} 5-15 μM	
101	Gagunin A	Diterpenoid	*Phorbas sp.*	K-562	LC_{50} 50.1 μg/mL	[69]
102	Gagunin B	Diterpenoid	*Phorbas sp.*	K-563	LC_{50} 10.4 μg/mL	[69]
103	Gagunin C	Diterpenoid	*Phorbas sp.*	K-564	LC_{50} 0.71 μg/mL	[69]
104	Gagunin D	Diterpenoid	*Phorbas sp.*	K-565	LC_{50} 0.13 μg/mL	[69]
105	Gagunin E	Diterpenoid	*Phorbas sp.*	K-566	LC_{50} 0.03 μg/mL	[69]
106	Gagunin F	Diterpenoid	*Phorbas sp.*	K-567	LC_{50} 0.11 μg/mL	[69]
107	Gagunin G	Diterpenoid	*Phorbas sp.*	K-568	LC_{50} 2.0 μg/mL	[69]
108	Gagunin H	Diterpenoid	*Phorbas sp.*	K-569	LC_{50} 10.0 μg/mL	[70]
109	Gagunin I	Diterpenoid	*Phorbas sp.*	K-570	LC_{50}11.5 μg/mL	[70]
110	Gagunin J	Diterpenoid	*Phorbas sp.*	K-571	LC_{50}9.1 μg/mL	[70]
111	Gagunin K	Diterpenoid	*Phorbas sp.*	K-572	LC_{50} 17.5 μg/mL	[70]
112	Gagunin L	Diterpenoid	*Phorbas sp.*	K-573	LC_{50} 12.5 μg/mL	[70]
113	Gagunin M	Diterpenoid	*Phorbas sp.*	K-574	LC_{50} 0.71 μg/mL	[70]
114	Gagunin N	Diterpenoid	*Phorbas sp.*	K-575	LC_{50} > 50 μg/mL	[70]
115	Gagunin O	Diterpenoid	*Phorbas sp.*	K-576	LC_{50} 11.1 μg/mL	[70]
116	Gagunin P	Diterpenoid	*Phorbas sp.*	K-577	LC_{50} 8.5 μg/mL	[70]
117	Gagunin Q	Diterpenoid	*Phorbas sp.*	K-578	LC_{50} > 50 μg/mL	[70]
118	Gukulenin A	tetraterpenoid	*Phorbas gukulensis*	HCT-116	IC_{50} 62 nM	[30]
				FaDu	IC_{50} 57 nM	
				SN12C	IC_{50} 92 nM	
				MKN45	IC_{50} 0,13 nM	
				TOVG-21G	IC_{50} 0,04 μM	
				OVCAR-3	IC_{50} 0,13 μM	[72]
				A2780	IC_{50} 0,03 μM	
				SKOV3	IC_{50} 0,36 μM	
119	Gukulenin B	tetraterpenoid	*Phorbas gukulensis*	HCT-116	IC_{50} 0,55 μM	[30]

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				A2780		
				FaDu	IC_{50} 0,63 μM	
				SN12C	IC_{50} 0,61 μM	
				MKN45	IC_{50} 0,72 μM	
123	Gukulenin F	Tetraterpenoid	*Phorbas gukulensis*	K-562	LC_{50} 0,4 μM	[30]
				FaDu	IC_{50} 0,63 μM	
				SN12C	IC_{50} 0,61 μM	
				MKN45	IC_{50} 0,72 μM	

The alkaloid zarzissine (**13**) showed a potent cytotoxic activity against three cell lines, murine leukemia P-388, human nasopharyngeal carcinoma KB and human lung carcinoma NSCLC-N6 [35].

Macrolides exerting great cytotoxic effects against HCT-116 (human colon cancer cell line) in *in vitro* assays, include phorbasides A (**20**), C (**22**), D (**23),** and E (**24**). Phorbaside B (**21**) showed no activity. These results suggest that the presence of the free hydroxyl group at C-2 of the sugar moiety may play a key role to keep bioactivity [41]. Muironolide A (**19**) and phorboxazole A (**17**) were two other representatives of cytotoxic macrolides against colon tumor cells [37].

As regards steroids, phorbasterones A-D (**29**-**32**), displayed moderate cytotoxicity toward HCT-116 cells [45]. In support of these observations, more recently, the lipid fraction from *P. amaranthus*, likely enriched of sterols, was found to possess antiproliferative properties in HCT-116 cells [67].

The sesterterpenoids phorbaketals A–C (**45**-**47**) exhibited cytotoxic activity against human colorectal cancer HT-29, hepatoma cancer HepG2, and adenocarcinoma human alveolar basal epithelial cells lines A549 while phorbaketal N (**50**) were cytotoxic against human pancreatic cancer cell line PANC-1 and renal cancer cell lines A498 and ACHN [49,50]. In addition, the sesterterpenoids isosuberitenone B (**84**) and 19-suberitenone B (**85**) unveiled significant grow-inhibitory effects against A549, HepG2, HT-29 and MCF-7 tumor cell lines [60].

Putative anticancer lead compounds with a diterpenoid backbone were a) phorbasin B-C (**88-89**) and the terpenyl-taurine phorbasin E (**91**) tested in a colon cancer model (HCT-116 cell line) [64] and b) gagunins A-Q (**101-117**) in K-562 cells (leukemia cell line) [69]. Among the latter, gagunins A and B (**101-102**) turned out to be the less active compounds. The authors suggest that the presence of a bulky group at C-11 of the 5-membered ring negatively affects bioactivity, as compounds **107** and **108** are far less active than their congeners featuring either an acetoxyl group or hydrogen at the same position [69].

The tetraterpenoid gukulenin B (**119**) exhibited significant cytotoxicity against human pharynx cell carcinoma line FaDu , gastric carcinoma cell MKN45, colon carcinoma cell line HCT-116, and renal carcinoma cell SN12C and gukulenins C−F (**120-123**) showed potent cytotoxicity against K-562 and A549 [30]. Besides being active against all previously tested cancer cells, in a recent study gukulenin A(**118**) was shown to be a promising antitumor agent as a) inhibited tumor growth in an ovarian cancer xenograft mouse model and b) markedly reduced cell viability through apoptosis induction in four ovarian cancer cell lines [72].

7.2 Other biological activities

Secondary metabolites isolated from sponges of the genus *Phorbas* showed to possess a large array of biological activities other than cytotoxicity (Table 2). Anchinopeptolides B-D (**2-4**), peptide alkaloids from *P. tenacior*, exhibited high efficacy in displacing specific ligands from their relevant receptors, such as human B2 bradykinin, neuropeptide Y, and somatostatin receptors. On the other hand, anchinopeptolide A (**1)** was found to have

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weaker bioactivity in these binding assays [21]. The alkaloids zarzissine (**13**) and *p*-hydroxybenzaldehyde (**14**) showed slight antimicrobial activity against *Staphylococcus aureus* (Gram-positive bacterium) and *C. albicans* and *C. tropicalis* (yeasts) [35].

The crude extract of *Phorbas topsenti* was reported to have high antioxidant activity in Oxygen Radical Absorbance Capacity (ORAC) assay, thereby leading to the isolation of phorbatopsins A-C (**14-16**), i.e. the compounds responsible for the observed radical scavenging activity. Antioxidant capacity of the isolated compounds was also evaluated with ORAC assay measuring the loss of fluorescence of fluorescein in presence of the oxidative species AAPH [2,2′-azobis(2-amidino-propane) dihydrochloride] and compared with Trolox® used as the positive control. Phorbatopsin A (**14**) was the most active substance with an ORAC value comparable to Trolox®. These data clearly indicate the importance of the C5-C6 double bond in compound **14** to improve antioxidant properties of the phorbatopsin scaffold [26].

Macrolides phorboxazoles A-B (**17-18**) exhibited antifungal activity in the agar disc diffusion inhibition assay against *Candida albicans* and *Saccharomyces carlsbergensis* [24]. Another example is the macrolide muironolide A (**19**), which was reported to have antifungal activity against strains of *Cryptococcus neoformans* [73].

The genus *Phorbas* is also a source of other bioactive compounds, such the steroids amaroxocanes A-B (**37-38**), which were isolated and tested for chemical defense of the Caribbean Coral Reef Sponge *Phorbas amaranthus* from fish predators. Amaroxocane B (**38**) showed significant deterrent activity (3/10 pellets eaten), while amaroxocane A (**37**) elicited little feeding deterrence (8/10 pellets eaten) against a common reef predator, namely the bluehead wrasse. This study suggests that structural differences in the heterocycle moiety or the degree of sulfation may be responsible for differential anti-predatory activity [47].

Phorbaketal A (**45**), which also has cytotoxic activity, can promote osteogenic differentiation of human mesenchymal stem cells, which exhibited increased levels of differentiation markers such as osteocalcin, Dlx5, ALP, Runx2, and TAZ after drug exposure. This compound showed potential for bone reformation processes and new anabolic therapeutics in bone diseases. Moreover, as inhibiting mesenchymal stem cells differentiation into adipocytes, compound **45** may be a promising lead in designing novel drugs to treat obesity [74–76].

Sesterterpenoids phorbasones A-B (**76-77**) promote calcium deposition in mensenchymal C3H10T1/2 cells, thus inducing osteoblast differentiation. Authors concluded that phorbasone A (**76**) showed a distinct calcium deposition effect as compared to phorbasone B (**77**). Particularly, gene expression analysis of osteoblast differentiation markers unveiled compound **76** to increase Runx2 (a Runt protein), ALP (alkaline phosphatase), OSX (osterix), PTH (parathyroid hormone), and PTHrP (PTHrelated peptide) mRNA [58]. Another study reported on the potent inhibitory activity on nitric oxide (NO) production in RAW 264.7 LPS-activated mouse macrophage cells by phorbasone A acetate (**79**). This result indicated that effective suppression of NO production is a valuable strategy for the discovery of anti-inflammatory compounds [58].

Among sesterterpenoids, suberitenones A and B, oxaspirosuberitenone, isosuberitenone B, 19- episuberitenone B and isooxaspirosuberitenone (**81-86**) isolated from *Phorbas areolatus* (non-polar fraction) were tested against Gram positive (methicillin resistant and methicillin sensitive *Staphylococcus aureus*, MRSA and MSSA) and Gram negative (*Escherichia coli*, and *Klebsiella pneumoniae*) bacteria. This study reported oxaspirosuberitenone (**83**) as a significant antimicrobial compound against MRSA at the highest concentration tested [60,77].

Ansellone A (**65**) is able to activate cAMP signaling in HEK293 cells, derived from human embryonic kidney cells grown in tissue culture, which is a very important technique for the development of treatments for several diseases, such as heart failure, cancer, and neurodegenerative diseases. cAMP signaling activation by ansellone A (**65**) was comparable to that of forskolin, a natural product used for the treatment of cancer, obesity and allergies [53]. The latency reversal activity (LRA) of **65**, which has the function of reactivate

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the virus production in infected cells and produce an immune response or cell death, was also reported and determined by quantification of the changes in intracellular GFP expression in microplate [78]. The sesterterpenoid ansellone B (**66**) was reported as a potent inhibitor on nitric oxide production in RAW 264.7 LPS-activated mouse macrophage cells [79].

The compounds alotaketal C (**62**) and D (**63**) and anvilone A were reported to activate the latent proviral HIV-1 gene expression. Notably, alotaketal C (**62**) was more potent and gave a stronger effect than the control compound prostratin at the same concentration, while alotaketal D (**63**) and anvilone A elicited similar responses as prostratin [52, 77, 78].

The diterpen phorbasin H (**94**) was reported as an inhibitor of the yeast-to-hypha transition in *Candida albicans.* Growth experiments suggested that this compound does not inhibit yeast cell growth but inhibits filamentous growth in *C. albicans,* which means that the phorbasin H (**94**) induces a change in *C. albicans* morphology [81]. Another study reported the ethanolic extract rich in phorbasins (**87-89**) from the *Phorbas sp.* to exert growth inhibitory activity against Gram-positive bacteria *Staphylococcus aureus* and *Micrococcus luteus*. It was not possible to test pure compounds due to the low amount available and their instability [61,62].

The diterpenoids gagunins (**101-117**) were tested against Gram-positive and Gram-negative bacteria and pathogenic fungi [69]. The study about the cosmetic use of gagunin D (**104**) identified this compound as an anti-melanogenic agent. Gagunin D (**104**) inhibited the synthesis of melanin in mouse melan-a cells and a reconstructed human skin model. Suppression of tyrosinase expression, increased rate of tyrosinase degradation as well as inhibition of its enzymatic activity are putative mechanisms underlying the anti-melanogenic activity exhibited by gagunin D (**104**). These studies highlight the potential use of gagunin D (**104**) for skin lightening cosmetic formulations [82].

Table 2. List of MNPs originated from *Phorbas* with biological activities.

	name	class	species	biological activity	Reference
1	Anchinopeptolide A	alkaloids	*P. tenacior*	displacement of specific ligands from their receptors	[21]
2	Anchinopeptolide B	alkaloids	*P. tenacior*	displacement of specific ligands from their receptors	[19]
3	Anchinopeptolide C	alkaloids	*P. tenacior*	displacement of specific ligands from their receptors	[21]
4	Anchinopeptolide D	alkaloids	*P. tenacior*	displacement of specific ligands from their receptors	[21]
13	Zarzissine	alkaloids	*P. paupertas*	antimicrobial	[32]
14	*p*-hydroxybenzaldehyde	alkaloids	*P. paupertas*	antimicrobial	[32]
14	Phorbatopsin A	alkaloid	*P. topsenti*	antioxidant	[26]
15	Phorbatopsin B	Alkaloid	*P. topsenti*	Antioxidant	[26]
16	Phorbatopsin C	alkaloid	*P. topsenti*	antioxidant	[26]
17	Phorboxazole A	macrolide	*Phorbas sp.*	antifungal	[24]
18	Phorboxazole B	macrolide	*Phorbas sp.*	antifungal	[24]
19	Muironolide A	macrolide	*Phorbas sp.*	antifungal	[37]
22	Phorbaside C	Macrolide	*Phorbas sp.*	macrophage infectivity potentiator (Mip)	[83]
45	Phorbaketal A	sesterterpenoids	*Phorbas sp.*	anti-inflammatory	[74]
62	Alotaketal C	sesterterpenoid	*Phorbas sp.*	latency-reversing agents (LRAs)	[53,80]
63	Alotaketal D	Sesterterpenoid	*Phorbas sp.*	latency-reversing agents (LRAs)	[53,80]
65	Ansellone A	sesterterpenoid	*Phorbas sp.*	latency-reversing agents (LRAs) cAMP activator	[53] [78]
66	Ansellone B	sesterterpenoid	*Phorbas sp.*	inhibition of inducible NOS (iNOS)	[79]
73	Anvilone A	Sesterterpenoid	*Phorbas sp.*	latency-reversing agents (LRAs)	[54]
76	Phorbasone A	sesterterpenoid	*Phorbas sp.*	osteogenic properties	[58]
79	Phorbasone A acetate	sesterterpenoid	*Phorbas sp.*	inhibition of inducible NOS (iNOS)	[58]
83	oxaspirosuberitenone	sesterterpenoids	*P. areolatus*	antimicrobial	[60]
94	Phorbasin H	diterpenoid	*Phorbas sp.*	antifungal	[66,70]
104	Gagunin D	diterpenoid	*Phorbas sp.*	Anti-melanogenic	[82]
101-117	Gagunins	diterpenoid	*Phorbas sp.*	isocitrate lyase (ICL) inhibition	[69]
125	Astaxanthin	carotenoid	*P. topsenti*	antioxidant	[26]
126	Adonirubin	carotenoid	*P. topsenti*	antioxidant	[26]
127	Taurine	Sulfonic acid	*P. topsenti*	antioxidant	[26]
128	Taurobetain	Sulfonic acid	*P. topsenti*	antioxidant	[26]

Conclusions

Marine sponges, including *Phorbas*, still represent a prolific source of new molecules yet to be discovered. Novel and more powerful tools should be developed to a) ameliorate and accelerate the discovery process and b) reduce the risk of re-discovery of MNPs. *Dereplication* based upon liquid chromatography coupled with high-resolution tandem mass spectrometry, is a well-suited approach to solve these issues and allow to detect new metabolites even from well-known sponges such as *Phorbas*. With this regard, molecular networking [84,85] could represent a suitable means for a) fast detection, annotation and visualization of known compounds and their novel analogues and b) an in-depth re-examination of *Phorbas* species to unlock overlooked chemical entities. A fundamental aspect in bioactive natural products research is certainly the assignment of stereochemistry in order to identify the pharmacophore of a molecule and investigate drug-target interaction. Due to limited amounts of available compound, elucidation of the stereochemistry of natural products is very challenging. Therefore, as shown for many metabolites from natural sources including Phorbas sponges, chemical degradation of a molecule into simpler compounds can be exploited as a valuable approach to assist spectroscopic analysis in the assignment of relative and absolute configuration of natural products.

This article provides a comprehensive review of the literature on sponges of the genus *Phorbas,* throughout 1993–2020 and summarizes the discovery of one hundred and thirty-two compounds, including alkaloids, macrolides, terpenoids and steroids, and a brief insight into the putative biogenetic pathway and biosynthetic origin of sesterterpenoids from *Phorbas* sponges. Moreover, this review includes a survey on pharmacological activities shown by the reported metabolites.

Cytotoxic activity displayed by secondary metabolites from this genus, make them interesting MNPs for development of new drugs with antineoplastic activity. Thus, this article aims to be useful for the bioprospecting process of marine sponges of the genus *Phorbas* and bring attention to its biochemical diversity.

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