THEMATICAL REVIEW

SULFATION PATHWAYS

Sources and biological activities of marine sulfated steroids

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This paper is part of a thematic section on Sulfation Pathways. The guest editors for this section were Jonathan Wolf Mueller and Paul Foster.

Abstract

Marine environment is rich in structurally unique molecules and can be an inspiring source of novel drugs. Currently, six marine-derived drugs are in the market with FDA approval and several more are in the clinical pipeline. Structurally diverse and complex secondary metabolites have been isolated from the marine world and these include sulfated steroids. Biological activities of nearly 150 marine sulfated steroids reported from 1978 to 2017 are compiled and described, namely antimicrobial, antitumor, cardiovascular and antifouling activities. Structure–activity relationship for each activity is discussed.

Introduction

The marine biota represents one of the most unexplored extreme environments in the world. Oceans cover about 70% of the Earth’s surface (Giddings & Newman 2015) and approximately one half of the Earth’s species is present in the marine world (Glaser & Mayer 2009). Marine biota has a higher taxonomic diversity composition than its terrestrial counterpart (Appeltans et al. 2012, Pimm 2012), which combined with its unique environmental conditions and organisms, offers an enormous resource of novel compounds for industrial development such as pharmaceuticals, food ingredients, cosmeceuticals, drug delivery systems and industrial enzymes (Martins et al. 2014).

In the past 50 years, progressive improvements have been made in exploration of new marine habitats, leading to the isolation of thousands of unique marine natural products (Martins et al. 2014). Currently, there are six U.S. Food and Drug Administration (FDA)-approved marine-derived drugs in the market and several compounds in different phases of the clinical trials (Mayer et al. 2017). The first marine drug launched in the market was a synthetic analog of the natural spongthyminde nucleoside originally isolated from a Caribbean marine sponge (Cytosar-U, FDA approved for cancer in 1969). Following, in 2004, omega-3 fatty acids, isolated from fish oils (Lovaza), granted the FDA approval as an anti-hypertriglyceridemia as well as a synthetic derivative (Prialt) inspired by a naturally occurring peptide, isolated from the venom of the cone snail Conus magus, for the management of severe chronic pain associated with cancer, AIDS and neuropathies. More recently, FDA has approved a macrolide (Halaven, 2010), derived from...

Within the marine-derived compounds which are in clinical trials, a marine steroid (squalamine, Fig. 1) is in phase III of drug development. Squalamine is a sulfated aminosterol derived from the internal organs (primarily the liver) of the dogfish shark and although was first described as an antimicrobial agent against fungi, protozoa and gram-negative and gram-positive bacteria (Moore et al. 1993) is now recognized as an anti-angiogenic drug with a novel intracellular mechanism of action. The drug acts against the development of aberrant neovascularization by inhibiting multiple growth factors, including vascular endothelial growth factor (VEGF) (Connolly et al. 2006). Clinical evidence has shown that these growth factors play a role in angiogenesis and ocular neovascular disease. OHR Pharmaceuticals Inc. evaluated the safety and effectiveness of squalamine for the treatment of neovascular age-related macular degeneration (IMPACT phase II study), showing an improvement in visual function when used in combination with an anti-VEGF agent (https://www.ohrpharmaceutical.com/media-center/press-releases/detail/446/ohr-pharmaceutical-presents-data-from-ohr-102-phase-ii, October 28, 2017). Presently, a phase III trial of squalamine is underway (NCT02727881, https://clinicaltrials.gov/ct2/show/NCT02727881, October 28, 2017). Squalamine has been targeted of several reviews highlighting its potential as a drug candidate (Abd et al. 2017, Hussain & Ciulla 2017), which puts forward the potential of sterol sulfates derived from marine organisms as models for development of new drugs.

Sulfur, in the form of sulfur salt, is the third most abundant element in seawater (Pinet 2011). So, it is not a surprise that sulfated metabolites are extensively distributed in marine organisms (Kornprobst et al. 1998). Sulfated compounds are found in a wide variety of organisms, from prokaryotes to multicellular species, being flavonoids the phenolic small molecules with more sulfated derivatives described from plants and seagrass (Correia-da-Silva et al. 2014). In marine biota, several sulfated secondary metabolites from different chemical classes (e.g., terpenoids, carotenoids, steroids, alkaloids, etc.) have been identified (Kornprobst et al. 1998). Sulfated steroids constitute one of the most numerous classes of these secondary metabolites; however, their biological functions are still poorly understood (Stonik 2001). Some of them are known for their use in chemical protection against predators (Morinaka et al. 2009), pathogenic (Smirniotopoulos et al. 2015) or fouling organisms (Iorizzi et al. 1995). Other marine products, structurally close to bile acids and alcohols of higher animals, were described to facilitate food absorption and digestion in some invertebrates (Levina et al. 2004). Some sulfated steroids were also found to inhibit the digestive enzymes of marine molluscs, *endo*-1,3-β-D-glucanases, therefore functioning as a chemical defence mechanism (Zvyagintseva et al. 1986, Makarieva et al. 1995, Guzii et al. 2008). While regulation of sulfation and desulfation are vital processes for vertebrate endocrine system (Mueller et al. 2015), little is known concerning the influence of sulfated steroids on endocrine system of marine organisms. Considering that sulfated steroid derivatives can act by interaction with nuclear receptors (genomic pathways) or membrane surface (non-genomic) (Falkenstein et al. 2000, Fiorucci et al. 2012), it is possible to explain the interference with endocrine systems (Mueller et al. 2015). It is interesting to add that some sulfated steroids were found as pheromones in marine species (Fine & Sorensen 2005, Stacey 2015). The hypothesis of intervention at the endocrine level may also be corroborated if we take into account the intervention of some sulfated marine steroids in hormone-dependent human tumor cell lines (Malyarenko et al. 2015).

The investigation of biological/pharmacological activities of these marine-derived sulfated steroids can lead to novel structures for the treatment of important pathologies. This review covers the biological activities of 142 marine sulfated steroids reported from 1978 to 2017. The majority of reported marine sulfated steroids are secondary metabolites of sterols, a subgroup of steroid compounds, which present a C-3 hydroxyl group and an aliphatic side chain at C-17 position additionally to the cyclopentane perhydrophenanthrene core (Moss 1989). The most important biological activities found in sulfated steroids can be categorized as antimicrobial, antitumor...
and cardiovascular. However, other activities were also reported, namely antifouling, showing their potential for different applications other than therapeutic drugs. Some structure–activities relationships (SAR) were also tentatively established.

**Therapeutic applications**

**Antimicrobial activity**

**Antibacterial**

Several reasons contribute to the imperative need for new antibiotics. The fact that infectious diseases still represent one of the greatest threats to human health worldwide is due to the rapid development of resistance by bacteria to therapeutic agents available (Spellberg et al. 2011, Butler et al. 2017).

Thirteen marine sulfated sterols (1–13, Fig. 2), mostly isolated from sponges, were shown to have antibacterial activity. Sponges are sessile organisms that endure over the years, due to their capability to engaging in chemical battles for defence or food competition against other animals, making them a reservoir of diverse and unique compounds. The study of their metabolites is of great importance to the discovery of new solutions to combat the development resistance by bacteria.

Carboxylated steroid 3β-sulfates (compounds 1–3), isolated from the marine sponge *Toxadocia zumi*, showed antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* at 100 μg/disk and 50 μg/disk, respectively (Nakatsu et al. 1983), whereas the respective 3β-hydroxy sterols were not active, suggesting that 3β-sulfate group was necessary for the antibacterial activity (Nakatsu et al. 1983).

Compound 4, isolated from the starfish *Luidia clathrata*, also inhibited the growth of both bacterial species (*B. subtilis* and *S. aureus*) at 50 μg/disk (Iorizzi et al. 1995). The monosulfate polymastamide A (5), isolated from the marine sponge *Polymastia boletiformis*, was the first example of a marine natural product with an uncommon side chain containing an amide bond linking the steroid part to a non-proteinaceous amino acid. Compound 5 showed an *in vitro* activity against *S. aureus* at 100 μg/disk (Kong & Andersen 1993).

Two disulfated sterols (6 and 7), isolated from the brittle star *Ophioderma longicauda* (Riccio et al. 1985), were tested for their activity against *S. aureus*, and the results were compared with other sulfated and non-sulfated sterols. While non-sulfated sterols exhibited inhibitory activity at 200 μg/disk, sulfated sterols 6 and 7 were much more potent against *S. aureus* (20 μg/disk) (Andersson et al. 1989).

Fusetani and coworkers first described isolation of halistanol trisulfate (8) from the marine sponge *Halichondria cf. moorei* Bergquist and have found that this compound was able to inhibit the growth of gram-positive and gram-negative bacteria. However, the acid hydrolysis product of 8 exhibited no antimicrobial activity showing that the sulfate groups were necessary for this activity (Fusetani et al. 1981). More recently, 8 was also found to have a bactericidal activity against *S. aureus*, with a minimum inhibitory activity (MIC) value of 512 μg/mL (Marinho et al. 2012). Moreover, compound 8 not only exhibited antibacterial activity against *Streptococcus mutans* CI (clinical isolated from an active caries) and *S. mutans* UA159 strains at 3.0 μg/mL, but also inhibited the biofilm formation by these strains approximately 85 and 99%, respectively (Lima et al. 2014). From the sponge *Topsentia* sp., five trisulfated sterols, topsentiasterol sulfates A–E (9–13), were isolated and shown to be active against *Pseudomonas aeruginosa* and *Escherichia coli* at 10 μg/disk (Fusetani et al. 1994). Compounds 9–12 were
the first examples of marine sulfated steroids possessing a butenolide or a furan functionality at the end of the side chain (Fusetani et al. 1994).

From the aforementioned 13 antibacterial sulfated steroids, it is possible to observe that six were trisulfated steroids, five were monosulfated and only two were disulfated steroids. Some studies have highlighted the sulfate groups as crucial groups for the activity (Fusetani et al. 1981, Nakatsu et al. 1983, Andersson et al. 1989).

**Antifungal**

There are four major classes of antifungal agents available for clinicians today: the allylamines, the azoles, the echinocandins and the polyenes (Lockhart & Warnock 2015). Due to the host toxicity and interactions with other drugs of the currently used antifungal agents, and the significant morbidity and mortality associated with invasive fungal infections in immune-compromised patients, new agents with different mechanism of action and with extended activity against pathogens are needed (Perfect 2016).

Antifungal activity was described for 26 sulfated steroids (5, 8, 12–35, Fig. 3), namely against Candida albicans, Mortierella remannianus, Saccharomyces cerevisiae, Aspergillus flavus and marine/plant fungal pathogens (Pythium ultimum, Cladosporium cucumerinum, Pycnularia oryzae, Lindra thallasiae).

Several sulfated steroids such as 5, 8, 12–16 have the ability to inhibit Candida sp. Compound 5 and another monosulfated sterol conjugated with an amino acid, 14, exhibited inhibitory activity against C. albicans at 75 μg/disk (Kong & Andersen 1993) and at 100 μg/disk (Smyrniotopoulos et al. 2015), respectively. Topsentiasterol trisulfates D and E (12 and 13, Fig. 3) also showed activity against C. albicans at 10 μg/disk (Fusetani et al. 1994), while 8 exhibited activity against two strains of Candida sp., i.e. sensitive (C. parapsilosis) and azole resistant (C. krusei) to antibiotics belonging to the class of azoles. Halistanol trisulfate (8) was more active against C. krusei (IC<sub>50</sub> of 26 μg/mL) than C. parapsilosis (IC<sub>50</sub> of 60 μg/mL) (Kossuga et al. 2007). Compounds 12 and 13 also showed antifungal activity against M. remannianus (at 10 μg/disk) (Fusetani et al. 1994). Eurysterols A and B (15 and 16, Fig. 3), two monosulfated steroids isolated from a marine sponge of the genus Euryspongia, were tested for their inhibitory activity against amphotericin B-resistant strains of C. albicans. Despite their structural similarities, eurysterol A (15) was more active (MIC value of 16 μg/mL) than eurysterol B (16) (MIC value of 63 μg/mL), allowing to postulate that the presence of the double bond in 16 caused a decrease of the activity when compared to 15 (Boonlarpraprad & Faulkner 2007).

Some sulfated steroids, including the monosulfates 17–22 and the trisulfate 23, also showed growth inhibitory activity against the yeast Saccharomyces cerevisiae: antifungal tests were carried out for acantherol I (17) and acantherol J (18) and both compounds showed growth inhibitory activity against S. cerevisiae strain A364A and its mutants (Tsukamoto et al. 1998). Compound 17 had an inhibitory zone of 7 mm against the strain A364A while 18 showed 11 mm effect (paper disks impregnated with 0.1mg of each compound), and both of them showed higher inhibitory zone against the mutant than the wild-type strains (Tsukamoto et al. 1998). Four sulfated steroids were examined for their fluconazole reversal activity by a checkerboard assay that measures the combination treatment effect of two agents on a microbe, and the fractional inhibitory concentration (FIC) value was used to define the effect as synergistic (≤0.5), additive (0.51–1.0), indifferent (1.1–2.0) or antagonist (>2.0). Two S. cerevisiae strains that overexpress C. albicans CDR1 (DSY 415) and MDR1 (DSY 415) efflux pumps were used in tests with capisterones A (19) and B (20), that significantly enhanced fluconazole activity in the CDR1 and MDR1 efflux pump-overexpressing strains, with FIC values of 0.33 and 0.08 for 19 and 0.33 and 0.15 for 20 (Li et al. 2006). Geodisterol-3-O-sulfite (21) and 29-demethylgeodisterol-3-O-sulfite (22), the first described naturally occurring sulfated steroids with an aromatic ring system, were isolated from the marine sponge Topsentia sp. These compounds were also found to enhance the activity of fluconazole in a S. cerevisiae strain overexpressing the C. albicans efflux pump MDR1 (FIC values of 0.08 and 0.15, respectively), as well as in a fluconazole-resistant C. albicans clinical isolate known to overexpress MDR1 (FIC values of 0.2 for both compounds) (DiGirolamo et al. 2009). A trisulfated steroid having a rare side chain with a diethyl substitution at C-24, Sch 575867 (23), isolated from a marine sponge belonging to the family Astroscleridae, was found to exhibit the antifungal activity with MIC value of 15 μg/mL against a modified strain of the wild-type S. cerevisiae, PMS03 (Yang et al. 2003a).

Three glycosylated steroids, minutosides A (24) and B (25), and pycnopodioside (26), were active against A. flavus with inhibition zones of 5–10 mm at the highest concentration of 20–60 μg/disk. Compound 25, the first example of a natural steroidal xyloside containing an amide function in the aglycone, showed activity only at...
Figure 3
Antifungal sulfated steroids.
the highest concentration (20–60 μg/disk), while 24 and 26 exhibited activity at 20–60 μg/disk (inhibition zones of 5–7.5 mm) (Chludil & Maier 2005). The desulfated analogs of 24 and 26 were inactive against A. flavus at all tested concentrations, allowing to conclude that the presence of a sulfate group in the aglycone moiety could play an important role in the antifungal activity against A. flavus (Chludil & Maier 2005).

The growth of four plant pathogenic fungi, P. ultimum, C. cucumerinum, P. oryzae and the marine-derived L. thallasiae, was inhibited by a few sulfated sterols. Compound 5 exhibited in vitro activity against P. ultimum at 25 μg/disk (Kong & Andersen 1993). The two amino acid-conjugated sterols 14 and 27, isolated from the sponge Polymastia boletiformis, showed inhibition zones of 10 and 8 mm at 30 and 60 μg/disk, respectively, against C. cucumerinum (Smyrniotopoulos et al. 2015). Several glycosylated sterol sulfates were also reported with antifungal activity against C. cucumerinum. Anasteroside A (28, Fig. 3), an asterosaponin (a 3β,6α-dihydroxysteroid with a Δ9(11) unsaturation, a sulfate group at C-3, and a carbohydrate chain with four to six monosaccharide units attached to C-6) which was isolated from the starfish Anasterias minuta, showed an inhibition zone of approximately 12 mm (at 10 μg/disk) against C. cucumerinum, while anasteroside B, with a shorter side chain and lack of the hydroxyl group at C-20, was inactive at all tested concentrations (Chludil et al. 2002).

To evaluate the influence of the oligosaccharide moiety in the antifungal activity, versicoside A (29), which is structurally similar to anasterosides A and B, but with a different side chain, was enzymatically hydrolyzed to the triglycoside forbesoside H and the pentaglycoside thornasteroside A (30). While the hexaglycosylated sterol 29 and the pentaglycosylated sterol 30 were active (inhibition zone of approximately 8 mm and 5 mm at 10 μg/disk, respectively), the triglycosylated sterol forbesoside H showed no antifungal activity in all concentration ranges (Chludil et al. 2002). Desulfation of versicoside A (29) by solvolysis afforded an inactive saponin. The results obtained in this work suggested that the side chain in the steroidal aglycone moiety, the structure of the sugar portion and the presence of a sulfate group at C-3, might play an important role in the antifungal activity of these sulfated steroidal saponins (Chludil et al. 2002). Other three sulfated glycosylated compounds, isolated from the starfish A. minuta, were studied. Compounds 24 and 26 exhibited antifungal activity against C. cucumerinum (inhibition zones of 7–10 mm at 10–60 μg/disk) (Chludil & Maier 2005), and compound 25 showed activity only at the highest concentration (20–60 μg/disk) against C. cucumerinum (inhibition zones of 3–4 mm) (Chludil & Maier 2005). The desulfated analogs of 24 and 26 were inactive against C. cucumerinum at all tested concentrations, allowing to conclude that the presence of a sulfate group in the aglycone moiety could play an important role in the antifungal activity against C. cucumerinum (Chludil & Maier 2005).

The plant pathogenic fungus P. oryzae has been used as a test for the primary screening of antineoplastic and antifungal agents (Tang et al. 2005a), and five asterosaponins isolated from the starfish Cucilla novaeguineae exhibited antifungal activity in this screening. Asterosaponins 31 and 32 showed significant antifungal inhibitory activity with minimum morphological deformation concentration (MMDC) values of 8 and 4 μg/mL, respectively (Tang et al. 2005a). Thornasteroside A (30), asterosaponin 33, marthasteroside A1 (34) and regularoside A (35) exhibited moderate activity inducing morphological deformation of P. oryzae mycelia with MMDC values of 64, 16, 32 and 64 μg/mL, respectively (Tang et al. 2006).

Compounds 19 and 20, isolated from the tropical green alga Penicillus capitatus, showed potent inhibitory activity against the marine fungal pathogen L. thallasiae, with median lethal dose (LD50) concentrations of 0.03 and 0.94 μg/mL, respectively (Puglisi et al. 2004).

From the 26 antifungal sulfated steroids, 13 were isolated from sponges, 11 from starfishes and only two were obtained from a marine alga. It is possible to observe that almost all sulfated steroids with antifungal activity are monosulfated with a sulfate group in position C-3 (mainly with β-configuration) and almost half of them are glycosylated (eight asterosaponins and three monoglycosides). It is noteworthy to mention that none of the glycosylated steroids have the sulfate group on the glycosidic portion.

These antifungal studies were mostly in vitro preliminary tests and only few used resistance models. Overall, these studies put forward the potential of sulfated steroids as antifungal agents.

**Antiviral**

Several sulfated flavonoids and other polyphenols have been highlighted for their antiviral activity (Sousa et al. 2008, Correira-da-Silva et al. 2014). Twenty-five marine sulfated steroids (8, 36–59, Fig. 4) were studied against several viruses: human immunodeficiency virus (HIV-1 and HIV-2), feline leukemia virus (FeLV), polio virus...
(PV and PV-3), junin virus (JV), respiratory syncytial virus (RSV) and herpes simplex virus (HSV-1 and HSV-2), as discussed below.

Weinbersterol disulfate A (36), which was isolated from the marine sponge *Petrosia weinbergi*, exhibited HIV-inhibitory activity with half maximum effective concentration (EC50) value around 1.0μg/mL (Sun et al. 1991). Protection against the cytopathic effects of HIV-1 infection was evaluated in the National Cancer Institute (NCI) primary screen for several sulfated sterols, namely: sterols with sulfates on A and B rings; sterols with oxygen or sulfate substituent on the D ring and sterols with

Figure 4
Antiviral sulfated steroids.
sulfation at C-21 (McKee et al. 1994). Promising activity was obtained with four trisulfated sterols with a common 2α, 3β and 6α-trisulfate substitution pattern (McKee et al. 1994), i.e. compound 8 (EC_{50}=6μM), ibisterol trisulfate (37, EC_{50}=13μM) isolated from the Caribbean sponge Topsentia sp. (McKee et al. 1993), halistanol trisulfate F (38, EC_{50}=3μM), and halistanol trisulfate G (39, EC_{50}=6μM), isolated from the marine sponge Pseudoxinissa digitata (Bifulco et al. 1994). Compounds 8, 37, 38 and 39 were also tested against a strain of HIV-2, which generally showed up to 70–80% protection (McKee et al. 1994). Cytopathic effects of HIV-1 infection were also obtained for four disulfated sterols (40–43), which were isolated from Tremaster novaceaeldonaei, and have 3β and 6α substitution pattern and additional sites of oxygenation located on the sterol side chain. Compounds 40–43 exhibited antifungal activity with EC_{50} between 13 and 48μM (McKee et al. 1994). It is interesting to note that sterols with sulfate groups in the A and B rings were the most active, whereas sterols with oxygenation in the D ring did not showed antifungal activity (McKee et al. 1994).

Haplosamates A (44) and B (45), isolated from a Philippine sponge Xestospongia sp., represent the first examples of marine sterols with the sulfamate functionality and an unprecedented six-membered ether ring. Compounds 44 and 45 inhibited HIV type 1 integrase with IC_{50} values (Qureshi & Faulkner 1999) higher (50μg/mL and 15μg/mL, respectively) than those of ibisterol sulfates B (46) and C (47) (2.3μg/mL and 1.8μg/mL, respectively), also isolated from the same sponge (Lerch & Faulkner 2001).

Clathsterol (48), isolated from the Red Sea sponge Clathria sp., was found to inhibit an activity against HIV-1 reverse transcriptase at 10μg/mL (Rudi et al. 2001).

Two hydroxylated sterols disulfates, weinbersterol sulfates A (36) and B (49), which were isolated from the sponge Petroia weinbergi, showed an in vitro activity against FeLV (EC_{50} of 4.0 and 5.2μg/mL, respectively). The cyclopropane-containing side chain of these compounds shows the side chain diversity of the sterols from sponges (Sun et al. 1991).

Compounds 50–53, which only differ in the side chain, were isolated from brittle stars (D’Auria et al. 1987, Roccatagliata et al. 1996) and were tested for their inhibitory effects on the replication of RNA (PV, JV, RSV) and DNA (HSV-1) viruses (Roccatagliata et al. 1996). Compounds 50 and 52, with a Δ^{22} double bond, showed a marked RSV inhibitory activity (75% inhibition of RSV replication at 40μg/mL) and were also active against PV (65% reduction of the cytopathic effect at 68μg/mL); however, both exhibited weaker activity against the other viruses investigated (<50% reduction of virus plaque formation). Compound 53, with Δ^{24(28)} double bond, inhibited 54% of JV replication, but was less active against the other viruses (<50% reduction of virus plaque formation). On the contrary, 51 was weakly active to all the viruses tested (Roccatagliata et al. 1996). Even though HSV-1 was described as the less sensitive virus to these sulfated sterols (Roccatagliata et al. 1996), four sulfated sterols (8, 54, 55, 56) were recently tested against HSV-1. Monosulfated sterol 54, extracted from the China sea starfish Asterina pectinifera (Peng et al. 2010), and the polyhydroxylated sterol asterosaponin P2 (55), with the sulfate group only in the side chain, isolated from the Far-Eastern starfish Patiria (Asterina) pectinifera (Kicha et al. 2000), exhibited activity against HSV-1, with MIC values of 0.2 and 0.07μM, respectively (Peng et al. 2010). Similarly, compound 8 and halistanol trisulfate C (56) were tested for their activity against HSV-1 (KOS strain) and both showed activity with IC_{50} values of 5.63 and 6.09μg/mL, respectively (Guimaraes et al. 2013).

Three sulfated sterols (57–59) found in the Antarctic brittle star Astrotoma agassizii (Roccatagliata et al. 1998), were tested against three pathogenic viruses of humans, HSV-2, JV and PV-3. Compounds 58 and 59 exhibited marked HSV-2 inhibitory activity (IC_{50} values of 18.4 and 24.3μg/mL), while 57 was active at the highest tested concentration (80μg/mL) with an IC_{50} of 51.5μg/mL. However, only 59 presented inhibitory activity (IC_{50} of 67.4μg/mL) toward JV virus, while 58 was only active against PV-3 at 80μg/mL (IC_{50} of 58.5μg/mL). Therefore, these researchers concluded that sterols with sulfate groups at C-21 and C-2/C-3 were very effective against HSV-2, PV-3 and JV viruses (Comin et al. 1999).

From the 25 compounds with the antiviral activity, more than half of them were disulfated. It is noteworthy to observe that disulfated sterols with the sulfate groups in C-3 and C-21 positions (50–53, and 59) were active against PV viruses while almost all trisulfated sterols (8, 37–39) possessed anti-HIV-1 activity. Moreover, it is interesting to highlight that the majority of the marine antiviral sulfated sterols were isolated from sponges, and only a few are from brittle stars and starfishes.

**Anti-parasitic**

Halistanol trisulfate 8 (Fig. 2) previously described for its antibacterial activity, was shown to inhibit adenosyl phosphoribosyl transferase in a dose-dependent manner, with 92% inhibition at 25μg/mL (IC_{50} of 2.87μg/mL).
The inhibitory activity of the desulfated analog of 8 was also tested, but no activity was observed. To study the influence of sulfate counter-cations, different sulfate salts of Na\(^+\), K\(^+\) and Mg\(^{2+}\) were submitted to the assay, and no activity was exhibited. Therefore, the presence of sulfate groups and their spatial position were proved to be essential for the enzymatic inhibitory activity (Kossuga et al. 2007).

**Antitumor activity**

Nearly 80 marine sulfated steroids with potential antitumor activity have been reported and are compiled in Supplementary Table 1 (see section on supplementary data given at the end of this article) in chronological order (Supplementary data). Among these, 52 marine sulfated steroids were investigated as potential antitumor agents after 2000, whereas only 23 were described before the year 2000 (Supplementary Table 1). Most part of the studies focused on the growth inhibitory effects of the tumor cells, namely against BEL-7402, K-562, Hep-G2, SK-Mel2, KB, PC-3, SNU-C5, RPMI-795, HCT-116, HT-29, Hela, QGY-770 and JURCAT cancer cell lines. It is interesting to mention that molecular targets/mechanisms have been explored and disclosed mainly in the last decade and will be following presented.

**Apoptosis**

The monosulfate glycosylated sterol, leptaochotensoside A (60, Fig. 5A), isolated from the starfish *Leptasterias ochotensis* (Malyarenko et al. 2015), was found to significantly reduce the colony formation of breast cancer T-47D cells using a soft agar method with 48% inhibition of the colony formation at 50μM. This compound also reduced the epidermal growth factor (EGF)-induced colony formation of mouse epidermal JB6 Cl41 cells up to 44% relative to control (200μM) (Malyarenko et al. 2015). The cancer preventive action of 60 was investigated through a non-genomic mechanism of regulation of mitogen-activated protein kinase (MAPK) signaling pathway and was showed to inhibit effectively the EGF-induced phosphorylation of extracellular signal-regulated protein (ERK1/2) and mitogen- and stress-activated protein (MSK-1) kinases. MAPK pathways are evolutionarily conserved kinase modules that link extracellular signals to the machinery that controls fundamental cellular processes such as growth, proliferation, differentiation, migration and apoptosis (Malyarenko et al. 2015).

Astrosteroside D (61, Fig. 5A), an astersaponin with two ketones in the side chain, isolated from the edible Vietnamese starfish *Astrostephon monacanthus* (Thao et al. 2013), was shown to induce apoptosis of HL-60, PC-3 and SNU-C5 cells via the inactivation of phosphatidylinositol 3-kinase (PI3K)/AKT and ERK 1/2 MAPK pathways and the downregulation of C-myc (Thao et al. 2014).

Astrosterosinovaeguineoside II (62, Fig. 5A), which contains an epoxide at C-22 in the side chain, was isolated from the starfish *Culcita novaeguineae* (Tang et al. 2005b). This compound was shown to induce apoptosis of human glioblastoma U87MG cells by the mitochondrial apoptotic pathway (Zhou et al. 2011).

Antitumor effects of 31 (Fig. 5A) against U87MG cells may result from interfering with cell cycle progression and inducing apoptosis, possibly by decreasing Bcl-2 protein expression (Cheng et al. 2006). Moreover, it was also found that 31 inhibited the proliferation of A549 human lung cancer cells through induction of endoplasmic reticulum stress-associated apoptosis (Zhao et al. 2011).

Astrosterosin archasterosides B (63, Fig. 5A), isolated from the Vietnamese starfish *Archaster typicus*, exhibited cytotoxic activity against HeLa and JB6 P+ Cl41 cell lines. This compound was also shown to induce basal AP-1- and p53-transcriptional activations in JB6 Cl41 (Kicha et al. 2010).

Coscinasterioside B (64, Fig. 5A), a glycosylated sterol monosulfate isolated from the starfish *Coscinasterias tenuispina* (Riccio et al. 1986), was investigated for its p53 inhibitory activity in a yeast two-hybrid test system. This compound was found to inhibit p53 up to 59% relative to control (Levina et al. 2010). Gamma irradiation and anticancer therapy induce p53-dependent apoptosis in several normal tissues where the p53 gene is highly expressed such as lymphoid tissues, hematopoietic organs, intestinal epithelia and testis. Thus, temporary suppression of p53 might be a workable therapeutic strategy to prevent adverse effects of cancer therapy and other drugs (Nayak et al. 2009).

**Angiogenesis**

Sokotrasterol sulfate (65, Fig. 5B), a trisulfated steroid isolated from the sponge of the family Halichondriidae (Makarieva et al. 1983), was evaluated as an agent that promotes endothelial sprouting. The compound showed a dose-dependent endothelial sprouting activity, which peaked at 5μg/mL (approximately 0.9 sprouts per bead), and was also able to promote angiogenesis in vivo (vascular density of ±5 vessels per mm, at 60μg/mL). The action of
Figure 5
Antitumor sulfated steroids. (A) Apoptosis. (B) Angiogenesis. (C) Antiproliferative. (D) Tubulin polymerization. (E) PKCζ inhibition. (F) PTK inhibition. (G) GDPX inhibition. (H) MT1-MMP inhibition. (I) PXR agonism. (J) FXR antagonism.
65 is dependent on cyclooxygenase-2 (COX-2) activation, vascular endothelial growth factor (VEGF) induction and the α5β3 integrin. It was also found that the sulfate groups played a critical role in angiogenic activity of 65 (Murphy et al. 2006).

Trisulfated lembsterols A (66) and B (67) (Fig. 5B), isolated from the marine sponge Petrosiastrongylata, showed inhibitory activity against thymidine phosphorylase (at 41 and 45μM, respectively), an enzyme related to angiogenesis that has an important role in growth and metastasis of solid tumors (Aoki et al. 2002). Thymidine phosphorylase is expressed at higher levels in a wide variety of solid tumors than that in the adjacent non-neoplastic tissues. A derivative of 66 without the sulfate group was tested and did not show any inhibition even at 230μM, suggesting the importance of the sulfate group for the inhibitory activity against thymidine phosphorylase. Compounds 66 and 67 were the first thymidine phosphorylase inhibitors with a non-nucleoside skeleton (Aoki et al. 2002).

Antiproliferative

Two disulfated sterols 68 and 69 (Fig. 5C), first isolated from the Ophioplos aculeata (Fedorov et al. 1994) and Ophiura sarsi (Levina et al. 1990), respectively, were investigated for their cytotoxic activity against Ehrlich carcinoma cells and mouse spleen lymphocytes, inhibitory activity of 3H-thymidine incorporation into DNA, and the influence of both compounds on transmembrane transport of calcium ions in Ehrlich carcinoma (Aminin et al. 1995). Although they did not exhibit cytotoxic activity against the carcinoma cells, both compounds inhibited the incorporation of 3H-thymidine around 60 and 50%, at 100μg/mL. This inhibition may reflect several mechanisms by which steroids affect macromolecular synthesis (Aminin et al. 1995). Ehrlich carcinoma cells are capable of accumulating radioactive calcium from external medium. Addition of sterols 68 and 69 at 100μg/mL to the incubation medium led to an increase of Ca2+ uptake in tumor cells of about 260 and 150% above the control, respectively. Compound 69 showed almost half of the activity to stimulate calcium transport compared to 68, which can be related to the presence of an unsaturated side chain in compound 68 (Aminin et al. 1995).

Tubulin polymerization

Novaeguinosides A (70), B (71), C (72) and D (73) (Fig. 5D), isolated from the starfish Culcita novaeguineae, were tested for in vitro activity of tubulin polymerization promotion (Tang et al. 2009). Compounds 70 and 72 contain sulfated side chains not previously found in other asterosapinins. On the other hand, the 26-carboxylic acid function in the side chains of 71 and 73 is conjugated with an amide derivative of taurine. Compounds 70, 72 and 73 exhibited significant activity with P<sub>e</sub> (percent end-point promote coefficient) values of 28, 42 and 23% and P<sub>a</sub> (percent kinetic promote coefficient) values of 94, 149 and 85% at 12, 9 and 9μM, respectively. Compounds 70–73 also displayed in vitro cytotoxicity against leukemia K-562 and human hepatoma BEL-7402 cell lines with IC<sub>50</sub> ranging from 0.7 to 9.5μM, 72 being the most potent. The author attributed their cytotoxic activity to the presence of the Δ9(11)-3β,6α-dioxysteroidal aglycone with a sulfate group attached at C-3 and an oligosaccharide moiety at C-6 (Tang et al. 2009).

PKCζ inhibition

The isoform ζ (zeta) of protein kinase C (PKC) has been implicated as an integral factor in several types of cancer (Whitson et al. 2009). The trisulfated sterols spheciosterol sulfates A-C (74–76, Fig. 5E) were isolated from the sponge Spheciospongia sp., along with the known sterol trisulfate topsentiasterol sulfate E (13). Compounds 74, 75, 76 and 13 inhibited not only PKCζ with IC<sub>50</sub> values of 1.6, 0.5, 0.1 and 1.2μM, respectively, but also NF-κB activation, in a cell-based assay, with EC<sub>50</sub> values of 12–64μM (Whitson et al. 2008).

Fibrosterol sulfates A (77) and B (78) (Fig. 5E), two sterol dimmers isolated from the sponge Lissodendoryx (Acanthodoryx) fibrosa, were shown to exhibit PKCζ inhibition with IC<sub>50</sub> values of 16 and 6μM, respectively. Both 77 and 78 composed of two cholestene monomers, with different configuration at C-3 and oxygenation at C-22 in only one monomer. Additionally, the number of sulfate groups in both sterols is also different being four in 77 and five in 78 (Whitson et al. 2009).

PTK inhibition

The oncogenic protein tyrosine kinase (PTK) comprises a family of enzymes that regulate cell growth and intracellular pathways. Halistanol trisulfate (8, Fig. 5F) showed activity against PTK pp60c-src with an IC<sub>50</sub> value of 4μM. Acid hydrolysis of 8 to remove the sulfate gave desulfated halistanol, which exhibited no activity. Kinetic studies revealed that 8 is a competitive inhibitor for the peptide-binding site of the enzyme and both competitive and non-competitive for ATP (Slate et al. 1994).
The disulfated sterol 79, isolated from the green brittle star Ophiophracta incrassata, and compounds 7, 51, 80 and 81 (Fig. 5F) were found to inhibit the PTK pp60v-src with IC50 of 65, 31, 62, 11 and 12μM, respectively (Fu et al. 1994). These sterol sulfate PTK inhibitors represented a different chemotype from other known marine natural products PTK inhibitors (Fu et al. 1994).

GDPX inhibition
Ophirastanol trisulfate (82, Fig. 5G), isolated from a deep water marine sponge Topsentia ophiraphidites, exhibited significant inhibition in the guanosine diphosphate/G-protein RAS exchange assay (GDPX) (Gunasekera et al. 1994). This assay determined the ability of inhibitors to affect the exchange of guanine nucleotides bound to p21RAS (mutant form of RAS protein). RAS is able to transform cells when it is in the GTP-bound conformation, and mutant forms of RAS lead to unregulated cell proliferation which are found in human cancer (Gunasekera et al. 1994).

MT1-MMP inhibition
Membrane-type matrix metalloproteinases (MT-MMPs) are known as key enzymes in tumor metastasis and inhibitors of MT-MMPs are believed to be potential anticancer drugs (Fujita et al. 2001). Compound 83 (Fig. 5H), a steroid monosulfate with a methylphosphate functionality isolated from the marine sponge Cribrochalin sp., was found as a MT1-MMP inhibitor, showing an IC50 value of 160mg/mL (Fujita et al. 2001).

PXR agonism
Pregnane X Receptor (PXR) is a member of the nuclear receptors (NRs) superfamily involved in the expression of a wide family of genes (Imperatore et al. 2014). Solomonsterols A (84) and B (85) (Fig. 5I), two trisulfated sterols isolated from the marine sponge Theonella swinhoei, were the first examples of natural marine PXR agonists (Festa et al. 2011). Both sterols effectively increased the expression of two well characterized PXR target genes, CYP3A4 and MDRI, in a human hepatocyte cell line (Festa et al. 2011).

Phallusialaster A monosulfate (86, Fig. 5I), the first sulfated sterol isolated from a tunicate, Phallusia fumigata, was shown to induce PXR transactivation in liver carcinoma cells HepG2 cells and stimulated the expression of the PXR target genes CYP3A4 and MDRI in the same cell line (Imperatore et al. 2014). Recent studies suggested that the feature and/or the shape of A/B ring junction is critical for the structure-PXR regulating activity relationship in the case of sulfated steroids, since two other compounds, one differing in the configuration at C-6, and another featuring a double bond Δ5(6), lacked PXR modulatory activity (Imperatore et al. 2016).

FXR antagonism
The farnesoid-X-receptor (FXR) is a ligand-regulated transcription factor involved in the regulation of lipid and glucose homeostasis in mammals. A disulfated sterol 87 (Fig. 5J), isolated from the brittle star Ophiolepis superba (D’Auria et al. 1989), was tested as an antagonist of FXR and showed potent antagonist activity (80% of FXR transactivation inhibition at 10μM) in HEpG2 cells transfected with FXR (Sepe et al. 2011).

From the 75 steroids that exhibited antitumor activity (Supplementary data) 41 were glycosylated, from which 28 were asterosaponins (with a sulfate in C-3p, 6α-penta- or hexasaccharide, and a Δ9(11) unsaturation) and the remaining 13 were mono- (seven), di (four) or triglycosylated (two). In only two cases the sulfate group was in the sugar portion. Concerning the 34 non-glycosylated steroids, four were dimers with more than three sulfate groups, 12 were trisulfated, eight were disulfated and ten were monosulfated (including one that had phosphate a phosphate group simultaneously). From the trisulfated compounds that exhibited antitumor activity, most of them have the three sulfate groups in the ring system, with the substitution pattern 2β-3α-6α-trisulfate.

Cardiovascular activity
Antihypertensive activity
Hymenosulfate (88, Fig. 6A), the first sulfated sterol isolated from a marine microalgae (Haptophyte Hymenomonas sp.), showed a potent Ca2+-releasing activity in sarcoplasmic reticulum, i.e., ten times more potent than caffeine (Kobayashi et al. 1989).

Halistanol disulfate B (89, Fig. 6B), isolated from the sponge Pachastrella sp., was able to inhibit the endothelin converting enzyme (ECE) with an IC50 of 2.1μM, while the non-sulfated counterpart was totally inactive. ECE is associated with the conversion of an inactive intermediate residue into a potent vasoconstrictive peptide endothelin-1 (ET-1) and the overproduction of ET is associated with hypertension and renal failure (Patil et al. 1996).
**Antiplatelet activity**

Sch 572423 (90, Fig. 6C), a disulfated sterol with hydroxylated side chain, and 8 were identified as P2Y12 inhibitors with IC$_{50}$ of 2.2 and 0.48 μM, respectively (Yang et al. 2003b). P2Y$_{12}$ is an important protein receptor regulator of blood clotting evolved in aggregation (Yang et al. 2003b). Both compounds have in common the presence of sulfate groups at C-2β and C-3α and a side chain of ten carbons.

**Hemolytic activity**

The hemolytic activity of some sulfated steroids (30–32, 34, 91–100, Fig. 6D) was evaluated. From a series...
Bioactive marine sulfated steroids 30, 34, 91, 92, 93, and 94 showed activity with hemolytic index values of 22.000, 11.400, 31.400, 21.500, 17.700, and 10.700, respectively, allowing to suspect that a sulfate group at C-3 is important for the hemolytic activity (Fusetani et al. 1984). From the starfish Aphelasterias japonica, quinovoside aphelasteroside C (95), aphelaketotriol (96), cheliferoside L1 (97), forberoside E3 (98), and 3-O-sulfothornasterol A (99) were isolated and tested for hemolytic activity against mouse erythrocytes (Ivanchina et al. 2000). Steroids 95, 96, 97, 98, and 99 exhibited ED50 values of 1.9 × 10^{-4} M, 4.0 × 10^{-5} M, 1.8 × 10^{-4}, 3.3 × 10^{-4} M, and 1.1 × 10^{-4} M, respectively (Ivanchina et al. 2000).

Triseramide (100, Fig. 6D), a sulfonated sterol, caused 50% hemolysis of erythrocytes at a minimal concentration of 1.2 × 10^{-4} M/L (Levina et al. 2004). Eight asterosaponins having in common a 3β-SO3, 6α-penta- or hexasaccharide, Δ1(11) unsaturation, a hydroxyl at C-20, and a carbonyl at C-24 of the side chain (Hwang et al. 2011, Thao et al. 2013). Compounds 29 and 30 (Fig. 7) inhibited the production of nitric oxide (NO) in Raw 264.7 cells (Hwang et al. 2011). When compared to control cells, treatment with 30 in a range of 0.1–4 µM showed a reduction in NO production up to 19%, while 29, at 8 µM, presented an inhibition of 41% (Hwang et al. 2011). Four asterosaponins, astrosteroisides A (101), C (102) and D (61), which were isolated for the first time from the edible Vietnamese starfish Astropecten monacanthus (Thao et al. 2013), and marthasteroside B (103) (Fig. 7), were reported for their effects on the inflammatory response of bone marrow-derived dendritic cells, by measuring the production of three pro-inflammatory cytokines, tumor necrosis factor (TNF-α), interleukin 6 (IL-6) and interleukin 6 subunit p40 (IL-12 p40) (Thao et al. 2013). Compound 61 exhibited potent inhibitory effects on the production of TNF-α, IL-6 and IL-12 p40 with IC50 values of 1.2, 3.5 and 0.60 µM, respectively.

Anti-inflammatory

The anti-inflammatory activity was reported for six ‘classical’ asterosaponins, with a sulfate group at C-3, a carbohydrate portion, with five or six sugars, at C-6, a Δ9(11) unsaturation, and a carbonyl group at C-24 of the side chain (Hwang et al. 2011, Thao et al. 2013). Compounds 29 and 30 (Fig. 7) inhibited the production of nitric oxide (NO) in Raw 264.7 cells (Hwang et al. 2011). When compared to control cells, treatment with 30 in a range of 0.1–4 µM showed a reduction in NO production up to 19%, while 29, at 8 µM, presented an inhibition of 41% (Hwang et al. 2011). Four asterosaponins, astrosteroisides A (101), C (102) and D (61), which were isolated for the first time from the edible Vietnamese starfish Astropecten monacanthus (Thao et al. 2013), and marthasteroside B (103) (Fig. 7), were reported for their effects on the inflammatory response of bone marrow-derived dendritic cells, by measuring the production of three pro-inflammatory cytokines, tumor necrosis factor (TNF-α), interleukin 6 (IL-6) and interleukin 6 subunit p40 (IL-12 p40) (Thao et al. 2013). Compound 61 exhibited potent inhibitory effects on the production of TNF-α, IL-6 and IL-12 p40 with IC50 values of 1.2, 3.5 and 0.60 µM, respectively.

Figure 7
Anti-inflammatory sulfated steroids.
respectively. Compounds 101 and 103 presented significant inhibitory effects on IL-6 production with IC\textsubscript{50} values of 3.17 and 4.37 µM, respectively, while 102 showed moderate inhibitory effects with IC\textsubscript{50} of 30.5 µM. The author concluded that the presence of an α,β-unsaturated carbonyl in the side chains of 101 and 103 and the two ketone groups in 61 were important in the inhibitory effects of these compounds (Thao et al. 2013).

All sulfated steroids described with anti-inflammatory activity (Fig. 7) were asterosaponins that were isolated from starfishes. With exception of 103, all anti-inflammatory sulfated steroids also displayed antitumor activity, which could increase the potential of these compounds as models for new antitumor agents (Rayburn et al. 2009).

Others

Inhibition of BACE1

From an undescribed species of Topsentia, topsentinelin K trisulfated (104, Fig. 8A), a rare 24-isopropyl sulfated sterol was isolated. Compound 104 inhibited the aspartic protease BACE1-mediated cleavage of amyloid precursor protein in a dose-dependent manner (IC\textsubscript{50} value of 1.2 µM), although in a detergent-dependent manner suggestive of non-specific aggregation (Dai et al. 2010). The activity of 104 was attributed to the presence of the sulfate esters, since the desulfated compounds did not show activity (Dai et al. 2010).

Anti-osteoblast proliferation

The high prevalence of osteoporosis has been regarded as a major public health problem all over the world. The effects on osteoblastic proliferation of some sulfated sterols isolated from the starfish Asterias amurensis were evaluated in the in vitro model UMR-106 cell lines. Compound 105 (Fig. 8B) significantly promoted the osteoblastic proliferation, in a range of concentrations of 0.01–100 µM, while 106 (Fig. 8B) was found to promote osteoblastic proliferation at higher concentrations. In contrast, a non-glycosylated non-sulfated derivative of 105 and a sulfated and non-glycosylated compound, also isolated from the starfish, exhibited no activity, showing that the β-D-xylene residue of saponins is necessary for activity (Liu et al. 2008). Both sulfated saponins have in common the presence of the sulfate group at C-15α, which is rare among the sulfated sterols reported, two hydroxyl groups at C-3β and C-6α, and a β-D-xylene in the side chain.

Environmental applications

Antifouling activity

Fouling on ships represents serious economic and environmental problems. These problems are associated with frictional resistance resulting in generated roughness which leads to speed loss and accelerated fuel consumption, in addition to increasing costs and emission of harmful gases as well as money and time for cleaning and maintenance (Schultz 2007). Biofouling in ships may also lead to transport of non-native species, causing a major human health and environmental impact (Abbott et al. 2000). Thus, the search for antifouling products becomes a fast growing research field and some sulfated compounds have been reported as potential nontoxic antifouling agents (Almeida et al. 2017, Vilas-Boas et al. 2017).

Carboxylated sterol sulfates (1–3, Fig. 9), showed antifouling activity against Salmacinsia tribanchiata at 10 µg/mL (Nakatsu et al. 1983). Compounds 4, 107, and 108 (Fig. 9), isolated from the starfish Luidia clathrata, were able to inhibit the attachment of barnacle larvae at 0.2 mg/mL, while asterosaponins 30, 103, ophidiansomide F (109), and regularoside B (110) (Fig. 9) showed complete inhibition of larvae attachment at the same concentration (Iorizzi et al. 1995). This study was the first demonstration that bioactive compounds from echinoderms may play a role in the prevention of fouling. It is believed that echinoderms have evolved an effective mechanism of protection from fouling (Iorizzi et al. 1995). Compound 8 showed antifouling activity, and no toxicity, against cypris larvae of the barnacle Balanus amphitrite, with an IC\textsubscript{50} of 5.0 µg/mL (Tsukamoto et al. 1997, Fusetani 2004).

Goniopectenosides A-C (111–113, Fig. 9), three asterosaponins that possess the same pentasaccharide moiety linked to C-6 of the sterol nucleus and with a 22-oxo functionality in the side chain, were isolated from the starfish Gongipecten demonstrans, and were found to

![Figure 8](http://jme.endocrinology-journals.org) (A) Sulfated steroid with BACE1 inhibitory activity. (B) Sulfated steroids with anti-osteoblast activity.
inhibit significantly the settlement of the biofouling marine brown macroalga *Hincksia irregularis* at 1.0 mg/mL. Moreover, the compounds were also nontoxic to *Deleya marina*, a biofouling marine bacterium model specie, showing that these compounds may be useful in biofouling control applications with specificity to marine algae (Marino et al. 2000).

Antifouling activity (mainly against macrofouling organisms) was shown by 14 compounds (Fig. 9), 13 of which were monosulfated (only one trisulfated compound was described for this activity). Furthermore, from these 14 sulfated sterols, most part possesses a side chain of eight carbons and half of them were asterosaponins with a terminal fucose in the polysaccharide moiety and a carbonyl group in the side chain. Among the asterosaponins, a β-hydroxyl group at C-20 was a common feature.

Most part of the antifouling sulfated steroids was isolated from sponges with only a few isolated from starfish species.

**Figure 9**
Antifouling sulfated steroids.

**Conclusions and perspectives**

Nearly 150 sulfated steroids isolated from various marine sources were investigated for biological activity. From this compilation, it is possible to verify that marine sulfated steroids were mainly isolated from two large phyla of marine invertebrates, echinoderms (Echindermata) and sponges (Porifera). Moreover, monosulfated steroids were more abundant than disulfated, which, in turn, were more abundant than trisulfated. A few dimers with four or more sulfates were also reported (Whitson et al. 2009, Ushiyama et al. 2012). It is also possible to observe that most part of the isolated marine sulfated steroids belong to the sterols class and the sulfate group is prevalent at C-3 position.

The majority of monosulfated steroids possessed the sulfate group at C-3 and only a few in the side chain. Monosulfated steroids presented a large structural diversity, from the simplest sterol sulfate, with a sulfate
group at C-3, to polyhydroxysteroids monosulfates, with both sulfate and hydroxyl groups. Monosulfates of glycosylated steroids were also found with the carbohydrate moieties in the ring system or in the side chain and few with the carbohydrates in both. The most common of these steroidal glycosides sulfates were the classical astеразонин, with the sulfate group at C-3 and the carbohydrate chain of five or six sugars attached at C-6. An unusual compound, which is an inhibitor of MT1-MMP, having both sulfate and phosphate groups in the ring system, was also isolated (Fujita et al. 2001).

Disulfated steroids frequently have one sulfate group in the ring system (usually at C-3) and other in the side chain (usually at C-21). However, some sulfated steroids presented both sulfate groups in the ring system, with a large structural diversity, e.g. both sulfates in ring A, one sulfate in ring A and another in ring B, and one sulfate in ring A and a sulfonamide in ring D. Disulfated steroids were mainly simultaneously hydroxylated and sulfated and few were glycosylated.

In the case of trisulfated steroids, the three sulfate groups appear more frequently with the common 2β, 3α, 6α-trisulfate substitution pattern and only few presented sulfation at C-2, C-3 and C-21.

These secondary metabolites showed a broad spectrum of complementary biological activities, namely antimicrobial and/or anti fouling activities, and antitumor, cardiovascular and/or anti-inflammatory activities. Although more extensive assays were precluded by the limited availability of pure compounds, halistanol trisulfate (8) was the most well-studied sulfated steroid, followed by thornasteroside A (30). Both compounds are remarkable for their wide variety of bioactivities. New enzyme inhibitory activities are still being reported, for example, inhibitory activity against NAD+-dependent lysine deacetylase (KDAC) – sirtuins (SIRT) – of three halistanol sulfates was reported during the review process of this work (Nakamura et al. 2018).

It is also worth mentioning that due to their hydrophilic scaffold, sulfated steroids can reach intracellular targets and improve bioavailability, which represents a tremendous advantage in therapeutics. On the other hand, the nontoxicity associated to the sulfated molecules raises the probability of discovering novel and safer therapeutic agents (Correia-da-Silva et al. 2014). From the marine compounds currently in the market, only Prialt and Yondelis, became drugs without any modification of the original natural molecule, while all the others suffered lead optimization, in different steps of their development. Therefore, optimization of these marine sulfated steroids by structural modifications or synthetic supply of unmodified natural molecule could be worth to explore to develop new solutions for some diseases as also for environmental problems.

Supplementary data
This is linked to the online version of the paper via free access.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work was supported through national funds provided by FCT/MCTES – Foundation for Science and Technology from the Minister of Science, Technology and Higher Education (PIDDAC) and European Regional Development Fund (ERDF) through the COMPETE – Programa Operacional Factores de Competitividade (POFC) programme, under the projects PTDC/ MAR-BIO/4694/2014 (reference POCI-01-0145-FEDER-016790; Project 3599 PPCDT – Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas) and PTDC/AAG-TEC/0739/2014 (reference POCI-01-0145-FEDER-016793; Project 9471 RIDTI – Reforçar a Investigação, o Desenvolvimento Tecnológico e a Inovação) in the framework of the programme PT2020.

Acknowledgments
The author acknowledges the Strategic Funding UID/Multi/04423/2013 provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of the programme PT2020.

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Received in final form 21 December 2017
Accepted 3 January 2018