

A review of bioactive compounds from marine organisms with special mention on the potential of marine sponges in pharmacological applications

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Original Article

Abstract

A variety of biologically active compounds with unique structures and pharmacological properties have been reported to occur in marine organisms. Demospongiae, an important class of marine sponge is known for producing the largest number and diversity of secondary metabolites isolated till recent times. The review covers the diverse class of bioactive compounds isolated for therapeutic drug applications from different marine organisms. It is an attempt to update the marine research community with results of our ongoing search for bioactive secondary metabolites from marine sponge *Spongosorites halichondriodes* which has exhibited antibacterial, antifungal, cytotoxic, anti-inflammatory and immunomodulatory activity in our studies. *Spongosorites halichondriodes* (order Halichondrida, family Halichondriidae) is a predominant marine sponge collected from west coast of Mumbai, India. The sponge *S. halichondriodes* has shown presence of Octadecadienoic acid (Linoleic acid), ergostan tetraen-ol, dihydroxy cholanoic- methyl esters, C₂₈ saturated and unsaturated esters like 3 β , 4 β , 7 α , 12 α -tetrahydroxy-5 β -cholan-24-oic acid methyl ester, 7 α , 12 β - dihydroxy-5 β - cholan-24-oic acid methyl ester, novel isocoumarin citrinolactone A, a triterpenoid glycyrrhetic acid as well as other unknown compounds such as nucleoside inosine.

Keywords: Marine sponges, *spongosorites*, bioactive compounds, glycosylated sterol derivatives.

Introduction

Chemical substances derived from animals, plants and microorganisms have been used to treat human diseases since the dawn of medicine. The investigation of these chemical substances from natural products as source of human therapeutics reached its peak in the period 1970–1980, which resulted in strong influence of non-synthetic molecules to pharmaceutical industries (Koehn and Carter, 2005). Later, between 1981 and 2002, natural products or natural product-derived drugs comprised 28% of all new chemical entities (NCEs) (Newman *et al.*, 2003). In addition, 24% of these NCEs were synthetic or natural mimic compounds, based on the study of pharmacophores related to natural products (Newman *et al.*, 2000). This combined percentage (52% of all NCEs) proved that natural products are important sources for new drugs and are also good leads for further modification during drug development.

Over 20 new drugs were launched in the market between 2000 and 2005, originating from microorganisms, marine organisms, terrestrial plants, vertebrates and invertebrates. These drugs, together with several other natural products or their analogs undergoing clinical trials, continue to demonstrate the importance of compounds from natural

sources in modern drug discovery efforts (Chin *et al.*, 2006). Currently, over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infective. More interestingly, application of molecular biology techniques is increasing the availability of novel compounds that can be conveniently produced in bacteria or yeasts. Combinatorial chemistry approaches based on natural product like scaffolds to create are also helpful in screening libraries that closely resemble drug-like compounds. Various screening approaches were being developed to improve the ease with which natural products can be used in drug discovery campaigns and data mining. Virtual screening techniques were also applied to databases of natural products (Harvey, 2008).

Marine organisms have a shorter history of utilization in the treatment and prevention of human disease. Among the first bioactive compounds from marine sources, an anticancer drug (cytosine arabinoside, Ara-C) and an antiviral drug (adenine arabinoside, Ara-A) were approved in 1965. Their discovery led scientists to study marine organisms extensively over the past 30 years. Then, drug discovery research from marine organisms is accelerating and now involves interdisciplinary research including biochemistry, biology, ecology, organic chemistry and pharmacology (Capon, 2001; Haefner, 2003). Thousands of new marine natural products have been reported, belonging to chemical classes of steroids, terpenoids, isoprenoids, nonisoprenoids, quinones, brominated compounds, nitrogen heterocyclics, and nitrogen sulphur heterocyclics. Many new developments of the last five years in this field of research and important findings for bioactive compounds from *in vitro*, *in vivo* and clinical studies for therapeutic drug applications have been covered by Perdicaris *et al.*, 2013.

In the last five years, we have found huge potential in marine sponges of Mumbai coastal region. Herein, we are elaborating on the results of their compounds and pharmacological activity. Along with it, we have classified different pharmacological classes of compounds reported from marine organisms, and have tried to contribute in terms of the research findings from the marine sponge, *Spongosorites halichondriodes*. There are few reports about this species having cytotoxic property due to the presence of mono and bis indole alkaloids (Bao *et al.*, 2007a, b), but we contribute adding more detailed biological activity of *S. halichondriodes*.

Bioactive compounds from marine organisms

Antibacterial compounds from marine organisms

The antimicrobials derived from the sea organism belong to the class of phenol, lactone, sterol, terpenoid, phthalate, fatty acid, cyclic polysulphide, steroidal glycoside, polysaccharide,

sulphated glycoside, protein, acetylene, terpene, indole derivative, glycerol derivative, glycoproteins etc. Anti-microbial peptides are a "new" class of antimicrobial compounds with pharmaceutical potential, because of their broad spectrum activity for bacteria, fungi and virus. Their molecular targets are bacterial membranes and intracellular molecules like DNA, RNA and proteins (Sperstad *et al.*, 2011). Donia and Hamann (2003) have extensively reviewed potential anti-infective agents from marine flora. Squalamine, the first aminosterol isolated from the dogfish shark *Squalus acanthias* (Squalidae) showed potent antimicrobial activity, with an MIC of 1.0 µg/mL. It inhibited *Staphylococcus aureus*, and also exhibited antiangiogenic and antitumour properties. It is currently in clinical trials for the treatment of advanced non-small-cell lung cancer. Cribrostatins, another compound from a blue sponge *Cribrorhiza* sp. showed potent antineoplastic and antimicrobial activities. Cribrostatin 3 showed potent inhibitory activity against *Neisseria gonorrhoeae*, with an MIC of 0.09 µg/mL (Pettit *et al.*, 2000). A potent antibacterial diterpene, bromosphaerone was isolated from *Sphaerococcus coronopifolius*, which showed antibacterial activity against *S. aureus*, with an MIC of 0.047 µg/mL (Etahiri *et al.*, 2001) (Fig. 1). Pestalone, product of marine fungus was isolated from the surface of the brown alga *Rosenvingea* sp. of the genus *Pestalotia* had potent antibiotic activity against methicillin-resistant *S. aureus*, with an MIC of 0.037 µg/mL, and Vancomycin-resistant *Enterococcus faecium*, with an MIC of 0.078 µg/mL (Cueto *et al.*, 2001). Jorumycin, a dimeric isoquinoline alkaloid was isolated from the mantle and mucus of the Pacific nudibranch *Jorunna funebris*. It inhibited the growth of various Gram positive bacteria eg. *Bacillus subtilis*, *S. aureus* at a concentration of 0.050 µg/mL, with an inhibition zone of 16 mm. Despite its high potency, its cytotoxic effects at IC₅₀ of 0.012 µg/mL and the difficulty in obtaining a pure stable preparation hindered its development as a drug (Fontana *et al.*, 2000). Seven monoindole derivatives

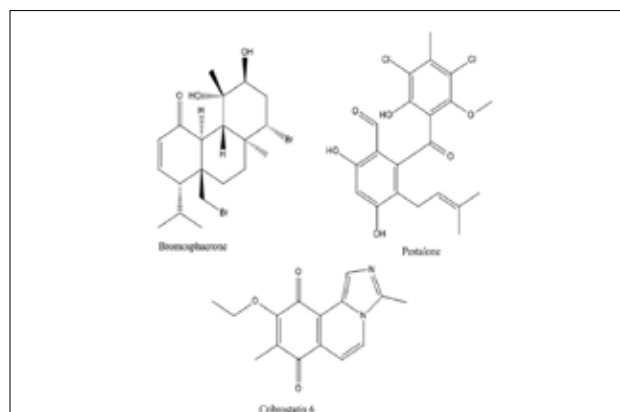


Fig. 1. Chemical structures of antibacterial compounds from marine organisms

have been isolated from *Spongosorites* sp. among which, five compounds were unique indole pyruvic acid derivatives (Bao *et al.*, 2007a). Bis (indole) alkaloids (topsentin and hamacanthin class) were also isolated from this species. Hamacanthin class exhibited more potent antibacterial activity than those of the topsentin class (Oh *et al.*, 2005) (Fig. 2). Symbiont microorganisms are also being explored in recent past as an alternative source for production of host bioactive metabolites. In one of the findings, a marine Gram-positive *Bacillus licheniformis* SAB1 bacterium identified by 16S rDNA, was isolated from sponge *Halichondria* sp. and showed significant antimicrobial activity (Devi *et al.*, 2010). In our own work, we have isolated a symbiont gram negative bacterium from marine sponge *Halichondria glabrata* which has shown promising antimicrobial activity with GenBank submission no. KF488586 (Jogani and Kumar, 2014).

Anti-fungal compounds from marine organisms

Antifungal compounds from marine organism cover marine macrolides, aminoalcohols and related heterocyclic structures,

including oxazoles and other alkaloids, peptides as well as a few representative terpenoids and polyketides. Jasplakinolide is the first example of a cyclodepsipeptide which was isolated from a sponge (Fig. 3) and was identified from a *Jaspis* sp. Also named jaspamide, is a 19-membered macrocyclic depsipeptide with selective in-vitro antimicrobial activity and a MIC of 25 µg/mL against *Candida albicans*. The in-vivo topical activity of a 2% solution of Jasplakinolide against a *Candida* vaginal infection in mice was similar to that of Miconazole nitrate (Crews *et al.*, 1986). Most antifungal compounds from marine origin are found to be cytotoxic. Consequently, they have not been considered promising for clinical applications. Table 1 enlists marine natural products that show potent antifungal activity, but the evidence of their cytotoxicity is not available (Donia and Hamam, 2003). Structures for these compounds are shown in Fig. 3. D-Phytosphingosine is the corresponding 4-hydroxy analog of D-sphinganine and the major sphingoid base found in the sphingolipids of higher plants and many invertebrates. The chain length of sphingoid bases is important for antifungal activity. In a comparative study, the antifungal activity of sphingolipids

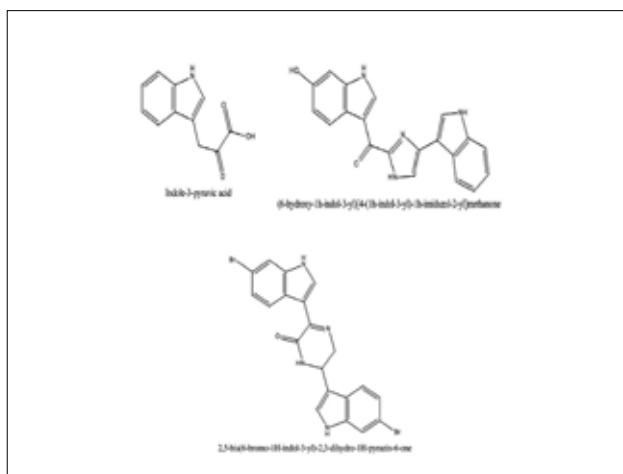


Fig 2. Chemical structures of alkaloids from *Spongosorites* sp.

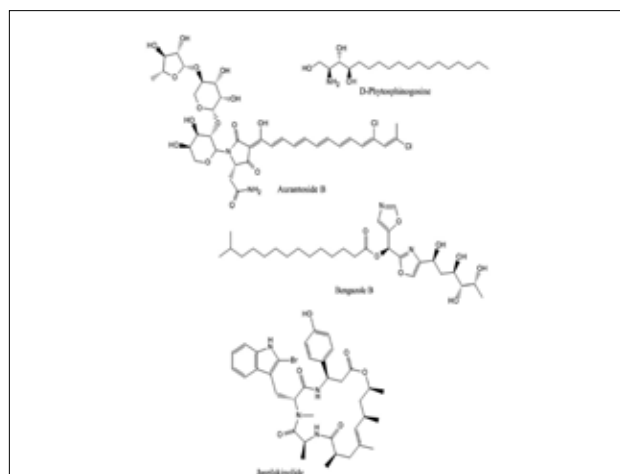


Fig 3. Chemical structures of antifungal compounds from marine organisms

Table 1: Antifungal compounds from marine organisms

Compound	Structure type	Source	Activity MIC	Cytotoxicity	Reference
Aurantioside	Polyketide	<i>Silquariaspongia japonica</i> , sponge	0.16 µg/ml 0.63 µg/ml (<i>A. fumigates</i>)	> 5 µg/ml (murine leukaemia cells)	Donia and Hamam, 2003
Halichon dramide	Macrolide	<i>Halichondria</i> sp. sponge	<i>C. albicans</i> 0.2 µg/mL	Highly toxic to mice at 1.4 mg/kg	
Fascaplysin	Bis (indole) alkaloid	<i>Fascaplysinopsis</i> sp. sponge	<i>S. cerevisiae</i> 0.1 µg/disk (20 mm zone) <i>C. albicans</i> at 1 µg/disk (11 mm zone)	L1210 IC ₅₀ 0.2 µg/mL	Mc Innes, 2008
Ptilomycalin A	Polycyclic guanidine alkaloid	<i>Ptilocaulis spiculifer</i> sponge	<i>C. albicans</i> 0.8 µg/mL HSV 0.2 µg/mL	P388 IC ₅₀ 0.1 µg/mL	Dalisay <i>et al.</i> 2011
Haliclona diamine	Alkaloid	<i>Haliclona</i> sp.	<i>C. albicans</i> 1 µg/disk	Not measured	Wattanadilok <i>et al.</i> 2007
Halishigamide A	Macrolide	<i>Halichondria</i> sp. sponge	<i>Trichophyton mentagrophytes</i> 0.1 µg/mL	L1210 IC ₅₀ 0.0036 µg/mL KB IC ₅₀ 0.012 µg/mL	Kobayashi <i>et al.</i> 1997

against *Candida glabrata* was found to be negligible for short-chain 2-amino-3-alkanol homologs, but increased with chain-length (C18) (Nicholas *et al.*, 2002). Kabiramide C and ulapualides A and B were the first described members of a unique family of trisoxazole macrolides in antifungal class of compounds. Two very potent antifungal macrolides, phorboxazoles A and B with novel parent ring structures were isolated in a sponge of the genus *Phorbas* from Western Australia (Molinski, 2004). Three new antifungal peroxides were isolated from two sponges, *Plakortis halichondriodes* from Jamaica and *Plakinastrella onkodes* collected in Florida, in addition to known plakortides E, F and H (Pomponi and Wright, 2002). The compounds like cyclic peptide Aciculitin B from *Aciculites ciliate*, tetramic acid glycoside aurantoside E from *Plakinolopha mirabilis*, 4 α -isocyanogorgon-11-ene from *Axinyssa terpinis*, and the biphenyl ether 3, 5-dibromo-2-(3, 5-dibromo-2-methoxyphenoxy) phenol from an unidentified sponge might be used as molecular probe to uncover novel antifungal drug targets or as lead for new compound with improved antifungal properties (Li *et al.*, 2012). Bioassay guided isolation and identification of marine red-sea sponge *Negombata magnifica* resulted in latrunculin B, a lead molecule for designing new antifungal for managing common fungal diseases in aquaculture (Devi *et al.*, 2013).

Anti-tumor compounds from marine organisms

Sponges have been a rich source of cytotoxic compounds like bryostatins. Other such compounds reported from sponges are the antineoplastic alkaloid Niphatesine D, which was extracted from a *Niphates* species (Kitagawa and Kobayashi, 1990); the Epinardins, isolated from an unidentified species (Ambrosion *et al.*, 1996); and the globostellatic acids A to D, which are isomalabaracane triterpene constituents of *Stelletta globostellata* (Ryu *et al.*, 1996). Probably the best known of the compounds with potential as anticancer drugs are the macrolides known as bryostatins, isolated primarily from the bryozoan, *Clavelina picta*, although some have been extracted from sponges and tunicates. Examples of active compounds from tunicates are the cytotoxic alkaloids clavelictine-A and -B, reported for *Clavelina picta* (Raub *et al.*, 1991)

and the cytotoxic dimeric disulphide alkaloid polycarpine (Fig. 4), isolated from *Polycarpa clavata* (Kang and Fenical, 1996). Anticancer agents (e.g., bryostatins, discodermolide, eleutherobin and sarcodictyin) have been isolated from bacteria of marine flora (Blunden, 2001). Arenicolides is a 26-membered polyunsaturated macrolactones, produced by the obligate marine actinomycete *Salinispora arenicola* strain CNR-005 isolated from marine sediment (Carlos *et al.*, 2009). Sponge derived compounds for treating cancer and inflammation in preclinical/ clinical trials are listed in Table 2. There is a large number of triterpenoid those exhibits cytotoxicity against a variety of tumor cells, as well as anticancer efficacy in preclinical animal models and has been reviewed (Li *et al.*, 2013).

Immunosuppressive compounds from marine organisms

There are very few marine natural products which have been described having immunosuppressive activity. The first time two immunosuppressive compounds, 4 alpha-methyl-5 alpha-cholest-8-en-3 beta-ol and 4, 5-dibromo-2-pyrrolic acid were isolated from a deep water marine sponge, *Agelas flabelliformis*. Both compounds were highly active in suppression of the response of murine splenocytes in the two-way mixed lymphocyte reaction (MLR) with little to no demonstrable cytotoxicity at lower doses (Gunasekera

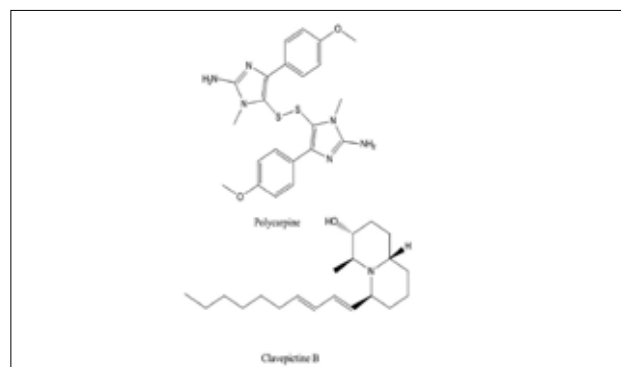


Fig 4. Antitumor compounds from marine organisms

Table 2: Sponge derived compounds for treating cancer and inflammation in preclinical/ clinical trials

Metabolite	Disease	Marine sponge source	Reference
Halichondrin B	Cancer	<i>Halichondria okadae</i>	Hirata and Uemura 1986
KRN 7000	Cancer	<i>Agelas mauritanus</i>	Nakagawa <i>et al.</i> 2000
LAF 389	Cancer	<i>Jaspis digonoxea</i>	Salomon <i>et al.</i> 2004
Laulimalide	Cancer	<i>Cacospongia mycofijiensis</i>	Mooberry <i>et al.</i> , 2004
IPL 512.602	Inflammation	<i>Petrosia contignata</i>	Hedner, 2007
Manoalide	Inflammation	<i>Luffariella valiallis</i>	Newman and Cragg, 2004
Salicylhalimide A and B	Cancer	<i>Haliclona</i> sp.	

et al., 1989). Till date, the most promising candidates for immunosuppressive therapy among marine natural products are discodermolide isolated from *Discodermia* species and microcolin A (Fig. 5). The isolated pure natural product discodermolide exhibited potent suppressive activity in the murine two-way MLR and concanavalin A stimulation of splenocyte cultures. In another study, FW63511 (Daidzein) and FW63512 (Genistein) were found to possess immunosuppressive and antitumor activities without any antimicrobial activities produced from *Micromonospora carbonacea* FIM 02-635 (Hong and Rong, 2006). In another attempt, components extracted from marine sponge *Aurora globostellata* and a total of 10 marine bacterial strains from *Callyspongia difusa* were evaluated. The bacterial strains showed potential to fight infections as well as production of

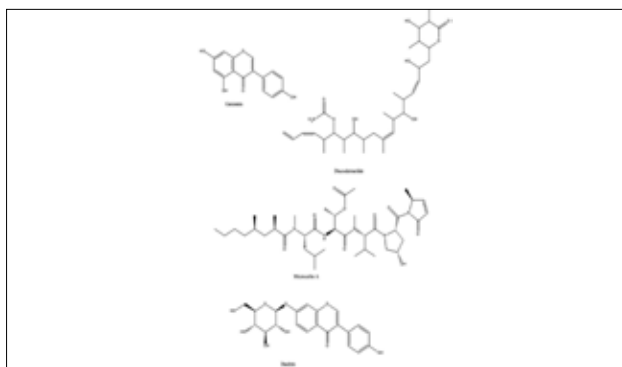


Fig 5. Immunosuppressive compounds from marine organisms

immunomodulators (Chairman *et al.*, 2013; Kalirajan *et al.*, 2013).

Anti-inflammatory compounds from marine organisms

A number of potential anti-inflammatory compounds have been isolated from marine invertebrates that exhibit phospholipase A2 (PLA2) inhibitory activity. A wide range of marine compounds have been investigated for their anti-inflammatory properties. Cacospongionolide B and petrosaspongiolide M are representative examples of anti-inflammatory compounds in experimental models of acute or chronic inflammation. The mechanisms of action of these compounds include phospholipase A, inhibition as well as the control of nuclear factor-B activation and inflammatory gene expression (Alcaraz and Paya, 2006). Other PLA2 inhibitors are the sesterterpenes variabilin from *Ircinia variabilis*, cacospongionolide B from *Fasciospongiaca vernosa* and petrosaspongiolide M from *Petrospongia nigra*, as well as the sesquiterpene bolinaquinone from *Dysidea* sp. Variabilin inhibits human synovial PLA2 (IC₅₀ 6.9 μ M) but not

5-lipoxygenase or cyclooxygenases 1 and 2 *in vitro* (Escrig *et al.*, 1997). These sesquiterpenes were found to inhibit human neutrophil degranulation, superoxide generation, leukotriene B4 (LTB4) production (variabilin), TPA-induced ear oedema (variabilin and bolinaquinone topically) as well as carrageenan induced paw oedema (variabilin, petrosaspongiolide M and bolinaquinone, p.o.) in mice (Giannini *et al.*, 2001; Haefner, 2003). By far, Manoalide, a non-steroidal sesterterpenoid isolated from a marine sponge, has proven to be a potent analgesic and anti-inflammatory compound. It inhibited phospholipase A2 from extracellular sources (snake venoms, bee, etc.), the release of arachidonic acid from rabbit polymorphonuclear leukocytes as well as calcium mobilization (Mayer 1989). The findings on IPL-576092, inhibiting the release of histamine from rat mast cells and also from the lung tissue of guinea pigs led its introduction into clinical trials (Fig. 6). Pseudopterisins are a constituent of the cosmetic "anti-wrinkle cream" sold by Estee Lauder under the brand name "Resilience" from the Caribbean gorgonian *Pseudopterogorgia elisabethae* (Newman and Cragg, 2004). In one of the recent studies, screening of marine sponge extracts led to anti-inflammatory

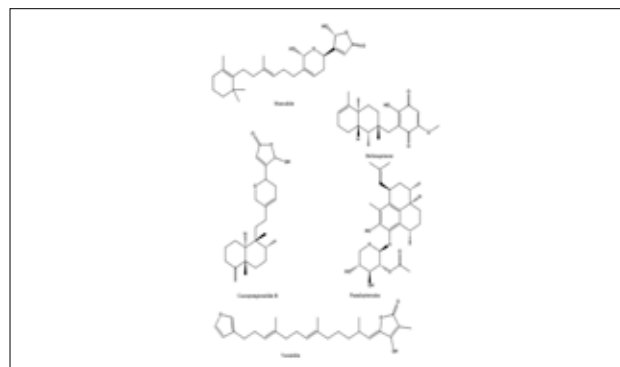


Fig 6. Anti-inflammatory compounds from marine organisms

agents Girolline as an inhibitor of protein synthesis targeting TLR signaling pathways (Fung *et al.*, 2014).

Taxonomystatus of *Spongosorites halichondriodes* (Dendy, 1905)

Kingdom: Animalia
Phylum: Porifera
Class: Demospongiae
Order: Halichondrida
Family: Halichondriidae
Genus: *Spongosorites*

Metabolites from the sponge, *Spongosorites* sp. from earlier work

Marine sponges are the most primitive multicellular animals and contain many metabolites, including lipids, in particular glycolipids especially unusual long-chain Δ^5 , 9 FA with no counterpart in the terrestrial world. They sometimes contain a third double bond or a bromine atom in the long chain structure (Litchfield and Morales, 1976). Sponges also contain special structural features in their cell membranes, in particular phospholipid FA and sterols, since sterol– phospholipid interactions are assumed to play a major role in cell membranes (Barnathan and Kornprobst, 1998). Phospholipids like methylene-interrupted PUFA, very long chain acids known as demospongic acids. Marine invertebrates, e.g. sponges, are filter feeders and consequently they can be associated with microorganisms. Thus, particular fatty acid appears as biomarkers for such organisms. Seven monoindole derivatives were isolated from the methanol extract of a marine sponge *Spongosorites* sp. by bioactivity-guided fractionation. Five compounds were unique indole pyruvic acid derivatives (Bao *et al.*, 2007a). Bis (indole) alkaloids, of the topsentin class and hamacanthin class, from the same sponge were investigated using several biological assays. In the evaluation of antimicrobial activity against various strains of bacteria and fungi, compounds of the hamacanthin class exhibited more potent antibacterial activity than those of the topsentin class (Oh *et al.*, 2006; Bao *et al.*, 2007b). These bis indole alkaloids showed the fibronectin- binding activity which highlighted the potential of these compounds for the treatment of *S. aureus* infections via inhibition of sortase activity (Oh *et al.*, 2005).

Bioactivities of metabolites from *Spongosorites halichondriodes*

We review here the various biological activities studied in different marine sponges worldwide. Along with we update with the recent research findings with all the studies done on the extracts of *S. halichondriodes*.

Antimicrobial and antifungal activity

Various metabolites from marine sponges and tunicates have been shown to possess antimicrobial, cytotoxic or antiparasitic properties, in agreement with the need for soft-bodied organisms to develop the art of chemicals against their predators. The interesting species of marine sponges reported in terms of different activities are *Ircinia felix*, *Topsentia ophiraphidites* and *Pandaros acanthifolium*. The active components from these species may yield useful candidates in the search for new pharmaceutical leads (Lutta *et al.*, 2008). A new bis (indole) alkaloid of the hamacanthin class along with topsentin class, from *Spongosorites* sp. has been studied for Sortase A inhibiting activity. The fibronectin- binding activity data highlighted the potential of these compounds

for the treatment of *S. aureus* infections via inhibition of sortase activity (Nondo *et al.*, 2011). In the evaluation of antimicrobial activity against various strains of bacteria and fungi, compounds of the hamacanthin class exhibited more potent antibacterial activity than those of the topsentin class. Deoxytopsentin and hamacanthin A also exhibited significant antibacterial activity against methicillin resistant *Staphylococcus aureus*, with MIC values of less than 12.5 $\mu\text{g}/\text{mL}$. In the antifungal activity test, hamacanthins, especially hamacanthin A, showed potent inhibitory activity against important pathogenic fungi. Bis (indole) alkaloid compounds also exhibited moderate cytotoxicity against cancer cell lines (Oh *et al.*, 2005). Compounds from a marine epiphytic bacterium *Pseudovibrio* sp. D323 produced tropodithietic acid (TDA) which was identified to be responsible for the antibacterial activity of the marine epiphytic bacterium *Pseudovibrio* sp. D323 and related strains. Interestingly, phenol was also produced by these bacteria but was not directly related to the antibacterial activity (Penesyan *et al.*, 2011). In another study by Ines *et al.*, 2007, extracts of the sponges *Agelas oroides* and *Axinella damicornis* were quite promising in inhibiting the growth of *Pseudomonas aeruginosa* and gentamycin resistant strains of *Listeria monocytogenes* and *Enterococcus faecalis* as well as broad spectrum activity against all the other bacteria. In a report by Ines *et al.*, 2007; comparatively only three among 9 sponge extracts show moderate capacity of growth inhibition against fungi strains. However, the antifungal activity of most of the sponge extracts was not so promising, which is not in agreement with our results from *S. halichondriodes* extracts (Kumar and Pal, 2012a). It showed moderate activity against both bacteria and fungi strains. The non-polar and polar extracts from *S. halichondriodes* have shown promising antimicrobial activity against both Gram negative and Gram positive bacteria. It also showed brine shrimp toxicity. The methanol and ethyl acetate extract showed presence of compounds like 5 α -Cholestane- 3 β - ol, Dihydrocholesterol, cholesterol, 3- β , 6- α Dihydroxy -5 α - cholan- 24- oic acid methyl ester. The hexane extract indicated presence of terpenoids like citronellyl acetate and butyrate along with pregnanediol. All these compounds have sufficient literature support of possessing antibacterial and anti-inflammatory activity (Kumar *et al.*, 2012c; Kumar and Pal, 2013).

Cytotoxic activity

In 1995 Murray *et al.*, reported the isolation of dragmacidin D from a deep water marine sponge *Spongosorites* sp. collected from the southern Australian coast. Dragmacidin D was found to be active against human lung tumor cell lines and inhibited *in vitro* growth of the P-388 murine and A-549 with IC₅₀ values of 1.4 and 4.5 $\mu\text{g}/\text{mL}$, respectively.

Four new bisindole alkaloids, nortopsentins A-D were isolated from the Caribbean deep sea sponge *Spongosorites ruetzleri* belonging to the same genus (Sakemi and Sun, 1991). The structures of nortopsentins A-D were established mainly on the basis of NMR spectroscopic data and were found to contain an imidazole ring between two indole units. Compounds exhibited cytotoxic activity against P-388 cells with IC₅₀ values of 7.6, 7.8, 1.7 and 0.9 µg/mL, respectively. There are reports on subclasses of pyrrole alkaloids and their anticancer nature from marine sponges. A dipyrroloquinone, *Zyzzyanone* A was isolated from the Australian marine sponge *Zyzzya fuliginosa* which showed mild cytotoxic activity against mouse Ehrlich carcinoma cells with IC₅₀ value of 25 µg/mL (Utkina *et al.*, 2004). Seven other novel pyrroloiminoquinones, the makaluvamines A-F were isolated from the Fijian sponge *Zyzzya cf. marsailis*. They exhibited potent *in vitro* cytotoxicity against the human colon tumor cell line HCT-116, topoisomerase II sensitive CHO cell line XRS-6, and also inhibited the catalytic activity of topoisomerase II (Radisky *et al.*, 1993). Pyrindemin A, a pyridine alkaloid from Okinawan marine sponge *Amphimedon* sp. demonstrated potent cytotoxicity *in vitro* against murine leukemia L-1210 and KB epidermoid carcinoma cells with IC₅₀ values of 0.058 and 0.5 µg/mL, respectively (Tsuda and Kobayashi, 1997). *Spongosorites halichondriodes* sponge extracts have also showed strong toxicity with LC₅₀ dose ranging from 5.24-1000 µg/ml speculating the toxic characteristic of the sponge. Guided by the brine shrimp test considered to be a preliminary cytotoxicity assay the extracts were tested in human cell lines. The ethyl acetate extract showed inhibition of % control growth at GI₅₀ of 19.7 µg/ml where for extracts, GI₅₀ value ≤ 20 µg/ml is considered to be active (Kumar and Pal, 2012a). The active extract has shown presence of inosine and 2, 2, 6, 6-Tetramethyl-4-piperidone in its GC-MS results. The earlier results of similar types of alkaloids have shown positive cytotoxicity activity in various cell lines. These alkaloids might be responsible for the positive cytotoxic activity in *S. halichondriodes* extracts which can be confirmed by further studies. In a recent review by Senthilkumar *et al.* (2013), antiangiogenic compounds and angiogenic regulators from marine sponge derived for cancer were explained for their role in inhibiting cancer cell proliferation and tumor angiogenesis.

Anti-inflammatory activity

In one of the studies, investigation of the in-vitro metabolism of glycyrrhetic acid by liver microsomes and to examine possible metabolic interactions that glycyrrhetic acid may have with other cytochrome P450 (CYP) substrates, it was incubated with rat and human liver microsomes. The results

suggested that glycyrrhetic acid has the potential to interact with a wide range of xenobiotics or endogenous chemicals that are CYP2C9, CYP2C19 and CYP3A4 substrates (Zhao *et al.*, 2012). It potentially inhibits the activity of mammalian p-450 including p-450 and also reduces TNF-α production and NF-κB activation and suppresses mouse ear inflammation stimulated by TPA. Thus, glycyrrhetic acid could be an anti-inflammatory agent based on p-450 inhibition (Ishida *et al.*, 2012).

The GC-MS has shown presence of moderate amounts of glycyrrhetic acid in marine sponge *S. halichondriodes*, which is a pentacyclic triterpenoid derivative of the beta-amyrin type obtained from the hydrolysis of glycyrrhizic acid, obtained from herb liquorice. This is the first report showing presence of glycyrrhetic acid in any marine sponge. In the study of anti-inflammatory activity in rats, the ethyl acetate extract of sponge has shown strong anti-inflammatory activity. The glycyrrhetic acid along with presence of compounds like 5 α-Cholestan-3 β-ol, Dihydrocholesterol, cholesterol, 3- β, 6-α Dihydroxy-5 α-cholan-24-oic acid methyl ester might be responsible for the activity which can be further confirmed by bioassay guided fractionation *in vitro* assay (Kumar *et al.*, 2014).

Immunosuppressive activity

In one of the studies by Purushottama *et al.* (2009) three crude extracts of *Halichondria panicea* had shown immunostimulative effects at lower concentrations. However, at higher concentrations, they exhibited immunosuppressive effects. The investigation of *S. halichondriodes* suggested that methanolic extract derived from the sponge may suppress both humoral and cellular immune responses. The extract not only suppressed nonspecific immune response, but also decreased humoral as well as cell-mediated immunity effectively. In the study, the nature of the compounds causing immunosuppressive activity was not known. However the presences of alkaloids, steroids, terpenoids and flavonoids were detected by preliminary pharmacognosy chemical tests. Later confirming presence of sterol compounds in the extract by LC-MS-MS they were understood to be responsible for the bioactivity (Kumar *et al.*, 2012c; Kumar *et al.*, 2012d).

Spongosorites halichondriodes

A variety of biologically active compounds with unique structures and pharmacological properties have been reported to occur in marine sponges. This review is an attempt to update the marine sponge researchers about bioactive compounds of *Spongosorites halichondriodes* a predominant marine sponge collected from western coast in Mumbai. This work investigated the antibacterial, antifungal,

cytotoxicity anti-inflammatory and immunological properties of *S. halichondriodes*. Antimicrobial studies used *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* and three pathogenic fungi, *Aspergillus flavus*, *Aspergillus niger* and *Metarhizium anisopliae* for the test. *Artemia salina* Brine shrimp assay was used for checking cytotoxic properties of the extracts before testing it on cell lines. All the extracts showed positive activity in all the three assays. Ethyl acetate extracts demonstrated activity on MDA-MB-435 human breast cell line (Kumar *et al.*, 2012a). The antioxidant and anti-inflammatory effects were also investigated. The anti-inflammatory effect was evaluated by carrageenan induced hind paw edema in rats. Hexane, ethyl acetate and butanol extract from *S. halichondriodes* (100, 200 and 500 mg/kg) and Diclofenac as positive control were studied and ethyl acetate extract reduced hind paw swelling significantly with increasing dose at 200 and 500 mg/kg (Kumar *et al.*, 2014). The present study also mentions immunomodulatory activity of methanolic extract of marine sponge *S. halichondriodes*. The extract was studied for haemagglutinating antibody titre, delayed-type hypersensitivity response and cyclophosphamide-induced myelosuppression for their immunomodulatory potential. The evaluation of immunomodulatory potential by oral administration of methanolic extract of marine sponge (200 mg/kg) evoked a significant decrease in total WBC count as compared to control, in antibody titre values, and also in delayed type hypersensitivity reaction induced by sheep red blood cells. Also it prevented myelosuppression in cyclophosphamide drug treated rats. The extract possessed immunosuppressant activity (Kumar *et al.*, 2012d). Further GC-MS and the fragmentation pattern of active extracts, showed presence of cholesterol and stigma- diene- ol which were the most abundant of 5 compounds identified in the butanol fraction. Octadecadienoic acid (Linoleic acid) and ergostan tetraen-ol and Dihydroxy cholanoic- methyl esters prevailed in the same fraction. C29 unsaturated stanols containing a 5 α -cholesterol nucleus were found to be present. Identification was based on NIST library search and mass bank, for matching the fragmentation pattern of the unknown compounds (Kumar *et al.*, 2012c). The composition of ethyl acetate and butanol extracts were also subjected to LC- MS/ MS identifying C₂₅-C₂₈ saturated and unsaturated esters like 3 β , 4 β , 7 α , 12 α -tetrahydroxy-5 β -cholan-24-oic acid methyl ester, 7 α , 12 β - dihydroxy-5 β - cholan-24-oic acid methyl ester, novel Isocoumarin citrinolactone A, a triterpenoid glycyrrhetic acid as well as other unknown compounds in this species such as nucleoside inosine was identified. Other compound identified was 3 β , 6 β , 7 α -trihydroxy-5 β -cholan-24-oic acid methyl ester (Kumar *et al.*, 2013). All the sterol ester derivatives are reported here for the first time in *S. halichondriodes*, family Halichondriidae supported by

literature reports for the occurrence of 3 β - hydroxy sterols considered as a biomarker for this family.

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