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Minireview

Indole alkaloid marine natural products: An established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases

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Abstract

The marine environment produces natural products from a variety of structural classes exhibiting activity against numerous disease targets. Historically marine natural products have largely been explored as anticancer agents. The indole alkaloids are a class of marine natural products that show unique promise in the development of new drug leads. This report reviews the literature on indole alkaloids of marine origin and also highlights our own research. Specific biological activities of indole alkaloids presented here include: cytotoxicity, antiviral, antiparasitic, anti-inflammatory, serotonin antagonism, Ca-releasing, calmodulin antagonism, and other pharmacological activities.

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Keywords: Indole alkaloids; Bioactive marine natural products; New drug leads

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Introduction

The marine environment, covering 70% of the earth’s surface and 95% of its tropical biosphere represents 34 of the 36 phyla of life and provides a fascinating variety of biodiversity exceeding
that of the terrestrial environment. Not surprising is that marine organisms produce an unprecedented molecular diversity by the incorporation of elements like bromine that are not readily available to terrestrial species. Partially responsible for the unique secondary metabolism of marine life are the ecological pressures in the marine ecosystem including significant competition for space, deterrence of predation and a high level of symbiosis between different species (Konig et al., 1994). Due to the biogenetic origin, marine organism secondary metabolites possess a number of structural differences as compared to terrestrial natural products. In addition, marine plants, mostly of the algal class (nonvascular), are unrelated to the majority of the terrestrial flora; similarly, the marine invertebrates such as sponges (Porifera), coelenterates (Cnidaria) and mollusks (Mollusca) are also not closely related to their terrestrial counterparts (Pelletier, 1986). Over 12,000 compounds from marine invertebrates (phyla: Annelida, Arthropoda, Brachiopoda, Bryozoa, Chordata, Cnidaria, Echinodermata, Hemichordata, Mollusca, Nemotoda, Platyhelminthes, Porifera), algae (phyla: Chlorophycota, Cryptophycota, Chromophyta, Cyanophyta, Euglenophyta, Rhodophyta), and microorganisms (bacteria, fungi and protozoa), have been discovered by a relative few marine research groups (Blunt and Munro, 2001). Natural products from the oceans have been reviewed by infectious disease such as for antiparasitic drugs (Kayser et al., 2002), antituberculosis agents (El Sayed et al., 2000), and anti-HIV agents (Gochfeld et al., 2003). Furthermore, there are some excellent general and extensive reviews focusing on marine natural products such as “Marine bioprospecting—trawling for treasure and pleasure” (Capon, 2001), “The influence of natural products upon drug discovery” (Newman et al., 2000), “Marine natural products” (Faulkner, 2002), and Marine Pharmacology 1999 and 2000 (Mayer and Hamann, 2002, 2004). The application of synthetic modifications and biocatalysis on the production of marine natural product libraries has been reviewed in: “Enhancing marine natural product structural diversity and bioactivity through semisynthesis and biocatalysis” in Current Pharmaceutical Design (Hamann, 2003).

In this paper we have focused on the marine indole alkaloids due to the significant activity that they have elicited in cancer or cytotoxicity assays and would also like to draw attention to the tremendous unexplored potential this class of secondary metabolites may have on neurological targets and behavioral diseases.

A variety of marine sources including sponges, tunicates, red algae, acorn worms, and symbiotic bacteria have been shown to generate indole alkaloids, which represent the largest number and most complicated of the marine alkaloids (1/4 of total alkaloids) (Kobayashi et al., 1990). The alkaloids obtained from marine organisms frequently possess novel frameworks while in other cases terrestrially related compounds clearly exist. Marine metabolites often possess complexities such as halogen substituents. Their structure elucidation, chemical modification, stereochemistry, synthesis, and pharmacology have received a great deal of interdisciplinary attention from areas of research other than chemistry and include pharmacology, physiology, and medicine.

In this report we have focused on the pharmacologically active indole alkaloid marine natural products that have been shown to have biological activity, including cytotoxic, antiviral, antimicrobial, antiparasitics, anti-inflammatory, antiserotonin, Ca\(^{2+}\), calmodulin-antagonistic activity and antitopoisoerase-I activity, along with in vivo activity when available.

**Cytotoxic indole alkaloids**

**Dragmacidin** [1], a bisindole alkaloid isolated from a deep water marine sponge *Dragmacidin* sp. and showed cytotoxicity with an IC\(_{50}\) of 15 \(\mu\)g/mL against P-388 cell lines and 1–10 \(\mu\)g/mL against A-549 (human lung), HCT-8 (human colon), and MDAMB (human mammary) cancer cell lines in vitro (Kohmoto et al., 1988). **Dragmacidin** [1] and a number of marine natural products including 2 and 3 contain two indole groups joined by a piperazine ring system.

**Staurosporine** [4], an indole [2,3-\(\alpha\)-carbazo] alkaloid, possesses cytotoxicity against a number of experimental tumor cell lines (Mekuriyen and Cordell, 1988). Staurosporine was first identified from *Streptomyces staurosporeus* Awaya (AM-2282) and other actinomycetes, including *Streptomyces actuosus* and *Streptomyces* strain M-193. The ED\(_{50}\) values of staurosporine were shown to be 0.0024 and <0.08 \(\mu\)g/mL against KB and P-388, respectively. This study also revealed that staurosporine did not inhibit tubulin polymerization or disrupt micro tubular function at concentrations of 6 and 32 \(\mu\)g/mL, whereas the other antitumor agents, vincristine, vinblastine, and colchicine, exhibit a characteristic inhibition of cell mitosis in metaphase and prevents assembly of microtubules. **Eudistomin K** [5] isolated from the Caribbean ascidian *Eudistoma olivaceum*, was described as an antitumor lead against L-1210, A-549, HCT-8 and P-388 cell lines, with an in vitro IC\(_{50}\) of P-388 reported as 0.01 \(\mu\)g/mL (Lake et al., 1989). *Grossularine* 1 and 2 [6, 7], isolated from the tunicate *Dendrodoa grossularia*, possess cytotoxic properties, against L-1210 (ID\(_{50}\) 6 and 4 \(\mu\)g/mL, respectively), WiDr (colon) and MCF7 (breast) (both are <0.01 \(\mu\)g/mL) (Moquin-Pathey and Guyot, 1989), and also appear to act as a monointercalating agent of DNA. **Halocamazine A** and **B** [8, 9] are tetrapeptide-like metabolites isolated from the solitary ascidian *Halocynthia roretzi* and exhibited cytotoxic activity against neuronal cells cultured from fetal rat brain, mouse neuroblastoma N-18 cells, and human hepatoma Hep-G2 cells. The distribution of halocamazine A and B was only in the “morula”-like cells, the most plentiful cell type (more than 50%) among the *H. roretzi* hemocytes (Azumi et al., 1990). From the Caribbean deep-sea sponge *Spongiosorites ruetzleri*, topsentin [10] was shown to possess a bis (indolyl) imidazole structure and exhibits in vitro activity against P-388 (IC\(_{50}\) 3.0 \(\mu\)g/mL) and human tumor cells (HCT-8, A-549, T47D, 20 \(\mu\)g/mL) and in vivo activity against P-388 (T/C137%, 150 mg/kg) and B16 melanoma (T/C 144%, 37.5 mg/kg) (Tsujii et al., 1988). The evaluation of topsentin analogues for biological activity showed that the introduction of a hydroxyl group enhances the cytotoxicity while bromination diminishes activity. **Hytriosins A** and **B** [11, 12], isolated from the Okinawan sponge
*Hyrtios erecta* exhibited cytotoxic activities greater than 5-hydroxyindole-3-aldehyde, which was reported to be active in vitro against human epidermoid carcinoma KB cells (IC\textsubscript{50} 4.3 μg/mL) (Kobayashi et al., 1990). Nortopsentins A, B, and C [13, 14, 15], isolated from the Caribbean deep-sea sponge *S. ruetzleri*, possess the unique imidazolediaryl[indole] skeleton and inhibits activity against P-388 cells with IC\textsubscript{50} values of 7.6, 7.8, and 1.7 μg/mL, respectively (Sakemi and Sun, 1991). Methylated derivatives of the nortopsentins had improved activity against P-388 cells when compared to that of the parent compound. Eudistomin E and Eudistalin B [16, 17] were shown to possess cytotoxic activities with ED\textsubscript{50} <0.005 and 3.2 μg/mL, respectively, in vitro against KB cells (Adesanya et al., 1992) and were extracted from the marine tunicate *Eudistoma album* with EtOH. Dragmacidin D [2], a new bis (indole)-derived sponge metabolite, was isolated from the sponge *Spongodoritis* sp. and exhibits anticancer activity in vitro against human epidermoid carcinoma KB cells (IC\textsubscript{50} 4.3 μg/mL), respectively, against L-1210, and 3.5 μg/mL (Makarieva et al., 2001). Convolvatamydine A [29], extracted from *Amathia convolute* collected in the Gulf of Mexico near Florida, was shown to have significant activity against HL-60 human plasmacytoid leukemia cells, including a change in culture plate adhesion, growth arrest, and phagocytosis of particles at 0.1–25 μg/mL (Kamano et al., 1995). Manzamine B, E, and F, [30, 31, 32] collected from various sponges including an *Amphimedon* sp. throughout the world, have been shown to have IC\textsubscript{50} values of 6.0, 5.0, and 5.0 μg/mL, respectively, against P-388 murine leukemia cells. These metabolites have recently been discussed in our detailed review included in the series the “Alkaloids” 2003 which discusses their biological activity against cancer, infectious diseases and in particular the highly promising activity against malaria and tuberculosis (Hu et al., 2003).

**Antiviral activity**

Eudistomin K and L [5, 33], contain a novel oxazahexazepine ring and were isolated from the Caribbean tunicate *E. olivaceum* (Rinehart et al., 1984). They inhibit HSV-1 growth at 0.25 and 0.10 μg/disk, respectively. In further investigations, seventeen *eudistomin* (A–Q) from *E. olivaceum* were divided into four groups (group 1: simple β-carbolines, including eudistomin D [34], J [35], N [36], and O [37]; group 2: pyrrolyl-β-carbolines, including *eudistomin* A [38], B [39] (Benson et al., 2000), and M [40]; group 3: pyrrolyl-β-carbolines, including *eudistomin* G [41], H [42], I [43], P [44], and Q [45]; and group 4: tetrahydro-β-carbolines with an oxazahexazepine ring, including *eudistomin* C [46], E [16], F [47], K [5], and L [33]) (Rinehart et al., 1987). The eudistomin containing the oxazahexazepine ring (C, E, F, K, and L) showing the most significant antiviral activity against HSV-1, of which C and E, with a phenolic group were active down at 0.005–0.01 μg/disk. The trend of antiviral potency of eudistomin was in the order of group 4 (C, E, F, and L) >> group 3 (H and P)= group 1 [D and N (O)] > group 2 (A and B). The substituent (Br and/or OH) on the β-carboline also affects the antiviral activity of eudistomin, which can be expressed as E (5-Br, 6-OH)>C (6-OH)>L (6-Br)=K (7-Br) and P (6-OH, 7-Br)=H (6-Br)>C (7-Br)>Q (6-OH)=0 (no substitution). *Eudistomin* C and E possess the activities against RNA viruses such as Coxsackie A-21 virus and equine rhinovirus and against DNA viruses such as HSV-1, HSV-2, and Vaccinia virus. The acetylation on the phenol and primary
amine functional groups of eudistomin C reduces its activity by 100-fold. Eudistomin A was also isolated from the New Zealand ascidian Ritterella sigillinaeoides (Lake et al., 1989) and showed antiviral activity in vitro against Herpes simplex Type 1 and Polio vaccine Type 1 viruses. The similar activity trend was also reported as eudistomin C and K (0.04–0.05 µg/disc) > debrromoeudistomin K and eudistomin K sulfoxide (0.4 µg/disc) > eudistomin O (0.5 µg/disc) > β-carboline (2 µg/disc). Topsentin and bromotopsentin [10, 48] were isolated from the Caribbean deep-sea sponge S. ruetzleri collected in the Bahamas and possess Bis (indolyl) imidazole structures (Tsujii et al., 1988). They exhibited antiviral activities in vitro against HSV-1, Vesivular stomatitis virus (VSV), and the corona virus A-59. Fascaplysin and homofascaplysin A cation [49, 50], isolated from the Benga Lagoon sponge Fascaplysinopsis reticulata, possess antiviral properties, with the inhibitions against reverse transcriptase (at 1 mg/mL): 81%, 58%, and 94%, respectively (Jimenez et al., 1991). Dragmacidin D (2), a bis (indole)-derived sponge metabolite, isolated from sponge Spongosorites sp. inhibited in vitro replication of feline leukemia virus (FeLV) with an IC50 of 6.25 µg/mL (Wright et al., 1992). Coscinamide A, B, and C [51, 52, 53], isolated from the organic extract of Coscinoderma sp. collected in Papua, New Guinea, showed activity in the NCI’s XTT-based assays (0.5 mg/disc). Extracts that were isolated from Flustra foliacea using crude petroleum ether were shown to strongly inhibit influenza virus plaque growth (Morales-Rios et al., 2001).

Antimicrobial/antiparasitic activity

Dihydroflustramine C and Flustramine D [54, 55], are interesting in regard to the marine origin and belonging to the phystostigmine structural class of alkaloids, reported from the marine bryozoan F. foliacea (Laycock et al., 1986). The alkaloid fraction mainly containing these two compounds exhibited a wide antibacterial spectrum including activity against Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus and Staphylococcus epidermidis in disc diffusion assays (0.5 µg/disc). Eudistomin A-Q, isolated from the Caribbean tunicate E. olivaceum, displayed a widely differing degree of antimicrobial activities (Rinehart et al., 1987). The eudistomin A-Qs contain the oxathiazepine rings are generally most active. Some eudistomins are active against Bacillus subtilis (D, I, and Q) or Saccharomyces cerevisiae (H or both (P)), while others are active against E. coli and Penicillium atrovenetum [C, K, L, and N(O)]. Fascaplysin [49], biogenetically derived from trypamine and structurally belonging to the pentacyclic quaternary salt, was isolated from the Fijian sponge Fascaplysinopsis sp. (Roll et al., 1988). Fascaplysin showed activities against the growth of several microbes, with a 15-mm zone at 0.1 µg/disc against S. aureus, 8-mm zone at 5 µg/disc against E. coli, 11-mm zone at 1 µg/disc against Candida albicans, and 20-mm zone at 0.1 µg/disc against S. cerevisiae. Halocyanine A and B [8–9], modified tetrapeptides, were isolated from the solitary ascidian H. roretzi from Japan (Azumi et al., 1990). Halocyanine A inhibited the growth of several microbes, including the Gram-positive bacteria B. subtilis (MIC 150 µg/mL), Bacillus megaterium (MIC 50 µg/mL), Bacillus cereus (MIC 100 µg/mL), and the yeast Candida neoformans (MIC 100 µg/mL). Halocyanine B displayed the comparative antimicrobial activities as A. Chelonin A, B and bromochelonin B [56, 57, 58], isolated from the marine sponge Chelonaplysilla sp., are another example of antimicrobial tryptophan derivatives (Bobzin and Faulkner, 1991). They exhibited activity against B. subtilis at a concentration of 100 µg/disk. Dragmacidin D [2], a new bis (indole)-derived sponge metabolite, isolated from the sponge Spongosorites sp. (Wright et al., 1992) inhibited the growth of several microbes, including E. coli (MIC 15.6 µg/mL), B. subtilis (MIC 3.1 µg/mL), Candida aeruginosa (MIC 62.5 µg/mL), C. albicans (MIC 15.6 µg/mL), and C. neoformans (3.9 µg/mL). Styelin D, a peptide isolated from the blood cells (hemocytes) of the solitary ascidian S. clava, displayed antimicrobial activity against methicillin-resistant and susceptible strain of S. aureus (Taylor et al., 2000). Styelin D has an effect on the outer and inner membranes on E. coli, making the outer membranes permeable to nitrocefin (a β-lactamase substrate) and the inner membranes permeable to o-nitrophenyl β-D-galactopyranoside (a β-galactosidase substrate). Notopsentins A, B, and C [13–15], isolated from the Caribbean deep-sea sponge S. ruetzleri, exhibited antifungal activity against C. albicans (Sakemi and Sun, 1991). From the Bahamian deep-water marine sponge Discodermia sp., antifungal alkaloids discobahamian A and B [59, 60] were isolated (Gunasekera et al., 1994). They showed weak activity against the yeast C. albicans. Pibocin B [28], isolated from the ascidian Eudistoma sp. obtained from the Sea of Japan, was shown to have antimicrobial activity against B. subtilis, C. albicans, and S. aureus (Makarieva et al., 2001). Hamacanthin B [61] was obtained from Hamacantha sp., a deep-water marine sponge, and was shown to inhibit activity of C. albicans and C. neoformans (Jiang et al., 2002). Dichloromethane extractions from the chelostome bryozoan F. foliacea exhibited antimicrobial activity against B. subtilis (Morales-Rios et al., 2001). Rhopaladin C [62], isolated from the Okinawan tunicate Rhopalaea sp., was shown to have antimicrobial activity against Corynebacterium xerosis and Sarcina lutea (MIC, 16 µg/mL each) (Sato et al., 1998). The manzamine alkaloids are now represented by approximately 40 different reported structures which can be divided into those which contain an indole moiety (as part of a beta-carboline system and represented by 30–32) and those without a beta-carboline system. The β-carboline system has been shown to play a prominent role in the very promising antimarial activity for this class of alkaloids (reviewed in Hu et al., 2003). Jasplakinolide [63], obtained from a sponge collected in Fiji, was shown to have an MIC of 25 µg/mL against C. albicans. For a review of marine natural products with significant activity against infectious diseases please see our review “Marine natural products and their potential applications as anti-infective agents” in Lancet Infectious Diseases 2003 by Donia and Hamann (2003).
Anti-inflammatory indole alkaloids

Cheloni A [56], isolated from the sponge Chelonaphysilla sp. found in a marine lake in Palau exhibited antiinflammatory activity in addition to the antimicrobial activity discussed earlier (Bobzin and Faulkner, 1991). Cheloni A showed a 60% inhibition against PMA-induced inflammation in a mouse ear model (50 µg/ear), but shows no in vitro inhibition against bee venom phospholipase A₂, Dragmacidin [1], isolated from the sponges Dragmacidin sp. and Hexadella sp. has been shown to have anti-inflammatory activity against bee venom PLA₂ and mouse ear edema in vitro and in vivo assays, respectively (Jiang et al., 1994).

Activity of indole alkaloids on serotonin receptors

Gelliusine A [22], is a brominated tris-indole alkaloid occurring as enantiomeric pairs, isolated from a deep water New Caledonian sponge Gelli or Orina sp. (Bifulco et al., 1994). At higher concentrations (5–70 µg/mL), gelliusine A causes a serotonin-like and methysergide-sensitive contraction; while at low concentration, it is able to antagonize serotonin-induced contraction, indicating the ability to block the serotoninergic receptor. In a later investigation, gelliusines A, B, E and F [22–23, 64–65] were submitted to a series of receptor binding assays at a concentration of 5 µg/mL (Bifulco et al., 1995), including the somatostatin receptor site (the inhibition of specific ligand binding were 100%, 100%, 87% and 91%, respectively), the neuropeptide Y receptor (90%, 62%, 63% and 67%, respectively), and the human B2 bradykinin receptor site (100%, 93%, 63% and 89%, respectively) (Bifulco et al., 1995). A number of halogenated indoles related to isoaphyllin A [21] have shown interesting selectivity for serotonin receptors when evaluated for affinity toward the 5-HT₂A and 5-HT₂C receptors. Clearly one of the more interesting and unexplored applications for marine natural products is their potential selective affinity for various neurological targets like the 5-HT subtypes and the resulting impact on behavior in whole animal studies (see our discussion in Hu et al., 2002).

Ca-releasing activity

9-Methyl-7-bromoeudistomin D [66], isolated from the Caribbean tunicate E. olivaceum, was found to induce Ca²⁺ release from the sarcoplasmic reticulum (Kobayashi et al., 1989). The comparison of several eudistomin analogues suggested that the 9-methyl group is quite important to the activity, and the bromine substituent in the β-carboline is better than chlorine or iodine. The SAR also indicated that the alkyl substitution to N-2 in the β-carboline lead to decrease the activity. Compared to caffeine, 9-methyl-7-bromoeudistomin was approximately 1000 time more potent in causing Ca²⁺ release from the sarcoplasmic reticulum of skeletal muscle. 7-Bromo-eudistomin D [67], obtained by demethylation of 7-bromo-O-methyleudistomin D, was found to be 400 times more potent than caffeine in Ca²⁺ releasing action (Rinehart et al., 1987). Isolated from F. foliacea, flustramines A and B [68–69], were found to cause relaxation of both skeletal and smooth muscle (Morales-Rios et al., 2001). Barretin [70], isolated from the Swedish cold-water sponge Geodia baretti, was shown to inhibit contractions of a Guinea-pig ileum (Lidgren et al., 1986).

Calmodulin-antagonistic activity

The Okinawan tunicate Eudistoma glaucus afforded eudistomidin A [71] and was shown to be the first calmodulin antagonist from marine origin (Kobayashi et al., 1986). IC₅₀ (2 × 10⁻⁵M) against calmodulin-activated brain phosphodiesterase was 15 times more potent than W-7 (3 × 10⁻⁴M), a well-known calmodulin antagonist.

Enzyme inhibitors

Isolated from the Okinawan tunicate Rhopalaea sp., Rhopaladin B [72], was the only compound from the series Rhopaladin A–D that was shown to inhibit cyclin dependent kinase 4 and c-erbB-2 kinase with an IC₅₀ of 12.5 and 7.4, respectively (Sato et al., 1998). Dragmacidin D and E [2, 3], isolated from Dragmacidin sp., were shown to inhibit serine-threonine protein phosphatases. Dragmacidin D (2) was also shown to inhibit neural nitric oxide synthase (ßNOS), which may prove to help in the treatment of Huntington’s, Parkinson’s, and Alzheimer’s diseases (Yang et al., 2002).

Anti-topoisomerase-I activity

The evaluation of an Indonesian sponge Histodermella sp., collected from Manado Bay, afforded makaluvamine G [18], a structural analog to the discorhabdin compounds, and exhibited moderate inhibition of topoisomerase-I (IC₅₀ 3.0 µM) (Carney et al., 1993). The activity of inhibition of RNA (IC₅₀ 15 µM), DNA (IC₅₀ 15 µM), and protein (IC₅₀ 21 µM) synthesis was also reported.

Discussion

A wide diversity of biologically active indole alkaloids have been reported from marine sources. Indole alkaloids derived from Dragmacidin sp. have been shown to exhibit cytotoxic activity, anti-inflammatory activity, and to inhibit enzyme activity. The Fijian sponge Fascaplysinopsis sp. produced cytotoxic and antiviral compounds. S. staurosporeus and D. grossularia, which yielded staurosporine and grossularine 1 and 2, respectively, both were shown to possess cytotoxic properties. Eudistomin A–Q were obtained from the ascidian E. olivaceum. Cytotoxic activity was characteristic of these alkaloids, as well as antiviral, Ca²⁺, and antimicrobial. Indole alkaloids isolated from H. roretzi were shown to exhibit cytotoxicity and antimicrobial activity. S. ruetzleri afforded compounds that possessed cytotoxic, antiviral, and antimicrobial activity. The Okinawan sponges H. erecta and Aplysina sp. gave compounds that exhibited cytotoxic activity.
nesian sponge *Histodermella* sp. yielded makaluvamine G, which was shown to have cytotoxic and anti-topoisomerase-1 activity. Several indole alkaloids were isolated from *Eudistoma* sp. and were shown to exhibit cytotoxic, antimicrobial, and calmodulin-antagonistic activities. *P. carteri*, *Ircinia* sp., and *Cribochalina olemda* all possessed indole alkaloids that had cytotoxic activity. Indole alkaloids isolated from the New Caledonian sponge *Gellius* or *Orina* sp. was shown to have cytotoxic activity, as well as activity against serotonin receptors.

**Conclusion**

The biological activity of marine indole alkaloids is clearly a product of the unique functionality and elements involved in the biosynthesis of marine natural products. For instance, the bromination of many of the mentioned natural products has the potential to increase the biological activity significantly. The cytotoxic activity of *grossularine 1* is increased as compared to *grossularine 2* due to the extra indole group in place of the benzene ring. As seen with the nortopsentin compounds, bromination does not always result in better activity. *Nortopsentin B* with only one R group brominated is cytotoxic at 0.2 μg/mL and more active than *nortopsentin A* with two brominated R groups and *nortopsentin C* which also has one bromine opposite that of *nortopsentin B* and this change decreases its activity to 1.7 μg/mL (against P-388 cells.) The decrease in cytotoxic activity can also be observed with *topsentin B1* and B2 where the addition of bromine reduces the activity by half. In antiviral activity, the specific placement of substituents is vital as can be seen with *eudistomin K* and *L*. In *eudistomin K*, with the bromine placed in the R3 position, as opposed to the R2 position as in *L*, the growth inhibition was shown to be 0.25 μg/mL, 0.15μg/mL more active than *L*. *Eudistomin K* was also shown to be active against Herpes simplex Type I and Polio vaccine Type I viruses, while *L* was not. Concerning the activity of indole alkaloids on serotonin receptors, the addition of an indole group, as seen in *gelliusine A* and *B*, to the main Gelliusine structure causes a significant increase in the inhibition of ligand binding, the neuropeptide Y receptor, and the human B2 bradykinin receptor site. *Gelliusine E* shows the poorest activity, which may be due to the lack of the extra indole group of *gelliusine A* and *B* and the extra bromine carried by *gelliusine F*. In the case of the *isoaplysin* related alkaloids the addition of bromine to the indole system elicited very significant improvements in binding selectivity to the various 5-HT subtypes. This clearly indicates that these halogenated marine indole alkaloids are certain to help define the function of the various 5-HT subtypes in addition to the identification of drug leads with potential clinical applications.

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[46] Eudistomin C: R₁ = H, R₂ = OH, R₃ = Br, R₄ = H
[16] Eudistomin E: R₁ = Br, R₂ = OH, R₃ = H, R₄ = H
[47] Eudistomin F: R₁ = H, R₂ = OH, R₃ = Br, R₄ = C₂H₃O₂
[5] Eudistomin K: R₁ = H, R₂ = H, R₃ = Br, R₄ = H
[33] Eudistomin L: R₁ = H, R₂ = Br, R₃ = H, R₄ = H

Grossularine 1

[6]

Grossularine 2

[7]

Halocyamine A

[8]

[10] Topsentin: R = H
[48] Bromotopsentin: R = Br

Hyrtiosin A

[11]

Hyrtiosin B

[12]

[13] Nortopsentin A: R₁ = R₂ = Br
[14] Nortopsentin B: R₁ = Br, R₂ = H
[15] Nortopsentin C: R₁ = H, R₂ = Br

Eudistalbin A

[17]
[29] Convolutamydine A

[30] Manzamine B

[31] Manzamine E: \( R = H \)

[32] Manzamine F: \( R = \text{OH} \)

[33] Eudistomin D: \( R_1 = \text{Br}, R_2 = \text{OH}, R_3 = \text{H} \)

[34] Eudistomin J: \( R_1 = \text{H}, R_2 = \text{OH}, R_3 = \text{Br} \)

[35] Eudistomin N: \( R_1 = \text{H}, R_2 = \text{Br}, R_3 = \text{H} \)

[36] Eudistomin O: \( R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{Br} \)

[37] Eudistomin A: \( R_1 = \text{OH}, R_2 = \text{Br} \)

[38] Eudistomin M: \( R_1 = \text{OH}, R_2 = \text{H} \)

[39] Eudistomin G: \( R_1 = \text{H}, R_2 = \text{Br} \)

[40] Eudistomin H: \( R_1 = \text{Br}, R_2 = \text{H} \)

[41] Eudistomin I: \( R_1 = \text{H}, R_2 = \text{H} \)

[42] Eudistomin P: \( R_1 = \text{OH}, R_2 = \text{Br} \)

[43] Eudistomin Q: \( R_1 = \text{OH}, R_2 = \text{H} \)

[44] Eudistomin B: \( R_1 = \text{OH}, R_2 = \text{H} \)

[45] Eudistomin C: \( R_1 = \text{Br}, R_2 = \text{H} \)

[46] Homofascaplysine A

[47] Coscinamide A: \( R_1 = \text{Br}, R_2 = \text{H} \)

[48] Coscinamide B: \( R_1 = R_2 = \text{H} \)

[49] Coscinamide C: \( R_1 = \text{Br}, R_2 = \text{OH} \)
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