7. Marine natural alkaloids as anticancer agents

Deepak Kumar and Diwan S. Rawat
Department of Chemistry, University of Delhi, New Delhi-110007, India

Abstract. Alkaloids are naturally occurring nitrogen containing biologically active heterocyclic compounds. Over the last few years, a large number of biologically important alkaloids with antiviral, antibacterial, anti-inflammatory, antimalarial, antioxidant and anticancer activities have been isolated from marine source. Present article summarizes the isolation and anticancer activity evaluation of natural marine alkaloids and their synthetic analogues that includes pyridoacridine, indole, pyrrole, pyridine, Isoquinoline, guanidine and steroidal alkaloids.

Introduction

Since ancient times nature has been a source of medicines to cure many deadly diseases. Majority of drugs in use today are either natural products (NP), their derivatives (ND), natural products mimics (NPD) or semisynthetic derivatives (SSD) [1-4]. In natural sources, plants, animals and microorganisms have been the main source of biologically important molecules. Ocean has been considered as the main source of medicines and during the past two decades thousands of compounds and their metabolites with several different type of biological activity such as antimicrobial, anti-inflammatory, antimalarial, antioxidant, anti HIV and anticancer activity have been isolated from marine microorganisms [5-12]. But till date only few anticancer drugs such as citarabine,

'Dedicated to Dr. DS Bhakuni and Prof. Deepak Pental
Correspondence/Reprint request: Prof. Diwan S. Rawat, Department of Chemistry, University of Delhi New Delhi-110007, India. E-mail: dskumar@chemistry.du.ac.in
vidarabine etc have been commercially developed from marine compounds while several others are currently in different stages of clinical trials [13]. Over 18000 compounds have been isolated from marine source and approximately 150 compounds are cytotoxic against the different tumor cells [14,15]. Some of the prominent anticancer compounds which are in different stages of clinical trials include aplidine, ecteinascidin-734 (Yondelis), bryostatin-1, squalamine, dolastatin-10, ILX651, and KRN7000 (α-galactosylceramide) [16].

The present article summarises the recent development in the area of marine alkaloids that includes pyridoacridine, indole, pyrrole, pyridine, isoquinoline, guanidine and steroidal alkaloids.

1. Pyridoacridine alkaloids

Pyridoacridines are highly coloured marine natural products having polycyclic planar heteroaromatic 11\(H\)-pyrido[4,3,2,\(mn\)]acridine system (1) [17]. They are probably the largest class among marine alkaloids and are almost universally isolated from sponges, ascidians as well as from a mollusc and a coelenterate [18]. Pyridoacridine alkaloids show significant biological activity primarily cytotoxicity and certain specific biological properties viz. fungicidal and bactericidal properties, inhibition of topoisomerase II, anti HIV, intercalation of DNA property, \(Ca^{+2}\) releasing activity, production of reactive oxygen species [19-22]. These activities depends upon the substitution pattern of the basic structure of pyridoacridine, therefore many synthetic analogues have also been synthesized keeping the basic skeleton of pyridoacridine in mind. The synthesis of these analogues and their biological activity evaluation revealed that in most of the cases cytotoxicity of the analogues has improved compared to the parent molecule [23, 24]. During the last few years, numerous additional compounds of this family were isolated; most of them are polycyclic with different substituents such as shermilamine, kuanoniamine, neoamphimedine, arnoamines and styelsamines.

It has been observed that almost all the pyridoacridines shows promising cytotoxicity against different type of tumors. Therefore a great interest was developed to modify the pyridoacridine moiety for developing a new generation of therapeutic agents. The first review article on marine pyridoacridines alkaloids was published by Molinski in 1993 [25] followed by Ding et al. in 1999 [26]. The cytotoxicity of the compounds of this family is a manifestation of their DNA binding properties, topoisomerase II inhibition and the production of reactive oxygen species.

Pyridoacridines vary structurally by attachment of different side chains or fusion of different rings to ring C of the basic structure (1) and less often to the acridine nitrogen. Halogen substitution in pyridoacridines is quite rare; even if it is present, then it is always bromine at C2 in ring A. Oxidation states of the rings are variable and in some cases ring D is partially saturated. Additional rings are
often attached to ring C. Pyridoacridines can be divided into tetracyclic, pentacyclic, hexacyclic, heptacyclic and octacyclic alkaloids.

1.1. Tetracyclic alkaloids

In 1988, Kobayashi et al. reported the isolation of three tetracyclic alkaloids, cystodytins A-C (3-5) from tunicate Cystodytes dellechiajei collected from Okinawa [27]. Then in 1991, the same group reported six other novel tetracyclic alkaloids of cystodytin family, cystodytins D-I (6-11) along with cystodytins A (3) and B (4) [28]. Thus cystodytins A-C (3-5) are the first pyridoacridine alkaloids isolated from a marine tunicate and therefore the first tetracyclic member of this class. The common heterocyclic nucleus of cystodytins A-C (3-5) is an iminoquinone substituted at C10 with a 2-amidoethyl side chain. The N-acyl groups are derived from \( \beta,\beta' \)-dimethylacrylic, tiglic and 3-hydroxy-3-methylbutanoic acids, respectively. Cystodytins D-I (6-11) are chiral, levorotatory compounds. Cystodytins F-I (7-10) are substituted with an O-methyl ether or O-9-octadecenoate ester. The isomeric pairs of cystodytin \( \beta,\beta' \)-dimethylacrylate and tiglate amides could not be separated and were characterized as 7:2 mixtures. Hydration of cystodytin A (3) in presence of 6% aq. HC1 at 100 °C gives cystodytin C (5). When treated with diazomethane, it afforded monomethyl ether (12) with 23% yield. This transformation is unusual as it constitutes a formal reductive methylation. Cystodytin A (3) is readily reduced in the ionization stage of a mass spectrometer as observed for quinones. Cystodytin A (3), when hydrogenated over Adams catalyst in acetic acid yielded (13) by reduction of the side chain and dissubstituted benzene ring, but the iminoquinone part remains intact. Compounds 3, 4 and 5 showed potent cytotoxicity against L-1210 with IC\(_{50}\) values of 0.22, 0.22 and 0.24 \( \mu \)g/mL, respectively. Cystodytins D-I (6-11) were also found to be cytotoxic against murine lymphoma L-1210 cells with IC\(_{50}\) values of 1.1 (6 and 7), 0.068 (8 and 9) and 0.080 (10 and 11) \( \mu \)g/mL and values of 1.4 (6 and 7), 0.078 (8 and 9) and 0.092 (10 and 11) \( \mu \)g/mL against human epidermoid carcinoma KB cells in vitro.
Cystodytin J (14) was isolated from a ascidian Cystodytes sp. [29]. Cystodytin J (14) showed cytotoxic activity against HCT and xrs-6 with IC$_{50}$ values of 1.6 and 135.6 µM, respectively. It also inhibited the topoisomerase (TOPO) II-mediated decatenation with IC$_{90}$ value of 8.4 µM. Recently, Appleton et al. reported isolation of cystodytins K (15), a new member of cystodytins, from the extract of an ascidian Lissoclinum notti collected near Leigh Harbour, Northland, New Zealand [30]. Structure of compound was determined by spectroscopic techniques, including 2D $^1$H-$^{15}$N NMR experiments and was found to be 12-methoxy derivative of cystodytin J (14). Cystodytins K (15) exhibited cytotoxic activity against P-388 murine leukaemia cell line with IC$_{50}$ value of 1.3 µM.

Two bright crimson pigments, Varamine A (16) and B (17) were isolated from the Fijian ascidian Lissoclinum vareau [31]. Varamines A (16) and B (17) have parent tetracyclic aromatic ring system at the same oxidation level as the methylation product of cystodytin A (12). Varamines also contain a methyl thioether substituent at C9. Varamine A (16) was readily oxidised by aq. ceric ammonium nitrate to imonoquinone (18) with 90% yield. Varamine A (16) and B (17) exhibited cytotoxicity towards L-1210 murine leukemia cells with IC$_{50}$ values of 0.03 and 0.05 µg/mL, respectively.

In 1989, Ireland et al. isolated a new tetracyclic alkaloid, diplamine (19) from the tunicate Diplosoma sp. collected from the Fiji Island [32]. The structure was established by interpretation of spectral data and chemical analysis. Diplamine (19) was found to be cytotoxic towards L-1210 murine leukemia cells with IC$_{50}$ value of 0.02 µg/mL. Recently, two novel alkaloids, isodiplamine (20) and lissoclindine (21) along with known diplamine (19) were isolated from an ascidian Lissoclinum notti collected near Leigh Harbour, Northland, New Zealand.

All the compounds (19-21) were tested for their cytotoxicity against murine leukaemia (P-388), human colon tumour (HCT-116) and non-malignant African Green Monkey kidney (BSC-1) cell lines. Diplamine (19) was found to be the
most active compound among the three and it was observed that movement of the thiomethyl group from C-9 (diplamine) to C-5 (isodiplamine) decreases cytotoxicity against all the cell lines and the same pattern also observed, when the thiomethyl group is cyclised into a benzoxathiole ring (lissoclinidine). These results were also found to be consistent with the proposed mechanism of cytotoxicity of diplamine, which includes DNA intercalation, inhibition of topoisomerase II and other DNA processing enzymes and bioreductive activation. Lissoclinidine (21) was also evaluated against the NCI 60 cell line panel and demonstrated moderate activity and selectivity with panel average values of GI$_{50}$ = 1.0 mM, TGI = 6.9 mM and LC$_{50}$ = 29 mM.

In 1998, Copp et al. reported the isolation of four new tetracyclic pyridoacridine alkaloids, styelsamines A-D (22-25) from an extract of the ascidian *Eusynstyela latericius* [33]. The structures of all the compounds were determined on the basis of 1D and 2D NMR spectroscopy. Styelsamines A-D (22-25) exhibited mild cytotoxicity toward the human colon tumor cell line (HCT-116) with IC$_{50}$ values of 33, 89, 2.6 and 1.6 $\mu$M, respectively.
1.2. Pentacyclic alkaloids

Amphimedine (26) was the first example of pyridoacridine alkaloids to be fully characterized [34]. In 1983, Schmitz et al. isolated amphimedine as a sparingly soluble yellow pigment from *Amphimedon* sp. The structure of amphimedine was established on the basis of spectroscopic data analysis. High resolution mass spectral analysis established the molecular formula C$_{19}$H$_{11}$N$_3$O$_2$ ($m/e = 313.08547$, + 0.35 mass error) for amphimedine. In mass spectrum very few fragments were observed corresponding to loss of CH, CO, CHO and HCN. The UV spectra of compound (26) in absolute ethanol showed absorption at $\lambda_{\text{max}}$ 210 nm (19690), 233 nm (39393), 281 nm (9099), 341 nm (6060). Significant changes were observed upon addition of NaBH$_4$ [$\lambda_{\text{max}}$ 235 nm (12879), 280 nm (9090)], indicating the presence of $\alpha,\beta$-unsaturated ketone, which was further supported by the strong absorption at 1690 cm$^{-1}$. Further presence of amide functionality was confirmed by IR and $^{13}$C NMR. No OH or NH absorptions were observed in the IR and due to low solubility of compound (26) in common organic solvents, NMR spectral data were obtained in trifluoroacetic acid-d$_4$ and CDCl$_3$ (2:1). The 2D NMR techniques ($^1$H-$^1$H correlation and $^{13}$C-$^{13}$C INADEQUATE NMR) were also used to confirm the structure of amphimedine (26).

In 1999, Ireland et al. reported the isolation of a new pyridoacridine, neoamphimedine (27) along with amphimedine (26) from *Xestospongia* sp. from the Philippines and *Xestospongia cf. carbonaria* from Micronesia [35]. He deduced the molecular formula for neoamphimidine as C$_{19}$H$_{11}$N$_3$O$_2$ by high-resolution fast atom bombardment (FAB) mass spectral analysis. Both amphimedine and neoamphimedine have the same molecular formula hence they are isomers. Recently, deoxyamphimedine (28) along with two known compounds (26 and 27) was isolated from two tropical *Xestospongia* sponges [36]. Amphimedine, neoamphimedine and deoxyamphimedine have the same skeleton, but they differ in biological activities and this is probably due to the the differences in their structures. Literature survey revealed that amphimidine relatively inactive compared to neoamphimedine and deoxyamphimedine. Neoamphimedine inhibits topoisomerase II while amphimedine is relatively nontoxic at the same dose level [37] and deoxyamphimedine damages DNA independent of topoisomerase enzymes through the generation of reactive oxygen species [38].
Schmitz et al. reported the isolation of three new alkaloids 29-31 from two ascidians. The meridine (29) and a relatively stable tautomer of meridine i.e. 30 were isolated from Amphicarpa meridiana collected at Stenhouse bay, South Australia [39]. The structure of meridine (29) was determined by X-ray analysis while that of 31 was established by spectral analysis. The third alkaloid, 11-hydroxyascididemin (31) was isolated from a Leptoclinides sp. from Truk Lagoon. All three alkaloids (29-31) were found to be cytotoxic. Recently, Menendez et al. synthesized a regioisomer of meridine named as 9 Hydroxybenzo[b]pyrido[4,3,2-de](1,10)-phenantrolin-8-one (32) from 5,8-dimethoxy-6-nitro-4(1H)-quinolinone in eight steps with 23% overall yield [40]. Compound (32) was tested for cytotoxicity against different tumor cell lines and exhibited mild to strong cytotoxic activity against P-388, A-549, HT-29 and MEL-28 with IC$_{50}$ values of 4.18, 0.03, 0.40 and 0.17, whereas IC$_{50}$ values for meridine were 0.08, 0.08, 0.84 and 0.08, respectively. Compound 32 and the natural meridine (29) were also tested in vitro for Topoisomerase II inhibitory activity. Meridine showed mild activity (IC$_{50}$ = 3 mM), whereas compound 32 was found to be inactive even at the highest concentration (33 mM). In 1988, a novel pentacyclic alkaloid, ascididemin (33) was isolated from brown colored tunicate Didemnum sp. collected at Kerama Islands, Okinawa [41]. The structure of compound was elucidated on the basis of spectroscopic data. Aascididemin (33) was found to be cytotoxic against L-1210 murine leukemia cells in vitro with IC$_{50}$ value of 0.39 µg/mL. Delfourne et al. synthesized an isomer of ascididemin, named as 9H-quino[4,3,2-de][1,7]phenanthroline-9-one (34) starting from 1,4-dimethoxyacridine with an overall yield of 12% along with other derivatives (35-39) of compound 34 [42]. These compounds were tested in vitro at six different concentrations on 12 different human cancer cell lines such as glioblastomas, breast, colon, lung, prostate and bladder cancers. Almost all the compounds showed significant cytotoxic activity and compound 34 was found as much potent or slightly less potent as the natural ascididemin (33). Aascididemin (33) and the isomer (34) exhibited cytotoxicity against U-87MG (0.07, 0.8 µM), U-373MG (0.5, 0.8 µM), SW-1088 (0.6, 3 µM), T-47D (0.6, 0.7 µM), MCF-7 (0.07, 0.9 µM), Lovo (0.9, 0.7 µM), HCT-15 (0.06, 0.4 µM), A-549 (0.2, 7 µM), A-427 (0.06, 0.08 µM), PC-3 (0.008, 0.09 µM), T-24 (0.8, 0.1 µM) and J-82 (0.3, 1 µM), respectively.

A new pentacyclic alkaloid, cystodamine (40) was isolated from a mediterranean ascidian Cystodytes dellechiajei collected near the bay of Gabes, at Skhira, Tunisia [43]. The structure was determined by extensive 2D NMR data analysis and was found to contain a phenanthroline unit fused with 7 aminopyridine moiety. Cystodamine (40) showed cytotoxic activity against CEM human leukemic lymphoblasts with IC$_{50}$ value of 1.0 µg/mL. Later, Delfourne et al. revised the structure of cystodamine (40) to 11-hydroxyascididemin (31) by comparison of the spectroscopic data with those of synthetic cystodamine, meridine and 11-hydroxyascididemin [44]. 11-Hydroxyascididemin had been previously isolated by Schmitz et al. from the other marine source Amphicarpa meridiana.
In 1988, Scheuer et al. reported the isolation of a new pentacyclic alkaloid, shermilamine A (41) from purple colonial tunicate *Trididemnum* sp. [45]. After one year, shermilamine B (42) was reported by two groups simultaneously Scheuer [46] and Kashman [47]. In 1994, McDonald et al. isolated shermilamine C (43) from a Fijian ascidian *Cystodytes* sp. [48]. Shermilamine A (41) contains a pentacyclic pyridoacridine thiazinone system while shermilamine B (42) is a debromo analogue of shermilamine A (41).

Two novel shermilamine alkaloids, shermilamine D (44) and E (45) were isolated from the Indian Ocean tunicate *Cystodytes violatinctus* collected at the Mayotte Lagoon, Comoros Islands, northwest of Madagascar [49]. Shermilamine D (44) exhibited cytotoxicity against P-388, A-549, HT-29 and MEL-28 cancer cell lines with IC₅₀ values of 0.53, 0.27, 2.66 and 0.53 µM, respectively [50]. A new member of shermilamines, cycloshermilamine D (46) was isolated from the same marine tunicate *Cystodytes violatinctus* [51]. The structure of cycloshermilamine D (46) was established mainly on the basis of NMR spectroscopic data and was found to be closely related to shermilamine D (44) having hexacyclic structure.

Kuanoniamines A-D (47-50) were isolated along with the known shermilamine B (42) from a tunicate and its prosobranch mollusc predator *Chelynotus simperi* [52]. The structures were established by extensive NMR analysis and correlations spectroscopy. Kuanoniamines C (49) and D (50) were also isolated from another tunicate of the genus *cystodytes* collected in Pohnpei [53]. Kuanoniamines B (48) and D (50) are homologues of kuanoniamine C (49) having isovaleramide and acetamide side chains, respectively. Kuanoniamine A (47) is structurally different from the other three alkaloids and lacks the 2-amidoethyl side chain and contains an iminoquinone moiety. In 1994,
McDonald *et al.* isolated dehydrokuanoniamines B (51) from a Fijian ascidian *Cystodytes* sp. [54]. More recently, the N-deacyl derivative (52) was isolated from the sponge *Oceanapia* sp. collected at Truk Lagoon, Micronesia along with its two parent molecule (49) and (50) [55]. Kuanoniamines A-D (47-50) showed weak cytotoxicity. Kuanoniamine A (47) was found to be the most active compound of the group and inhibits the proliferation of KB (human pharyngeal cancer) cell lines *in vitro* with IC$_{50}$ value of 1 µg/mL. Dehydrokuanoniamine B (51) and kuanoniamines D (50) were found to have comparative potentials *in vitro* against HCT (IC$_{50}$ = 8.3 and 7.8 µM) and xrs-6 cells (IC$_{50}$ = 80 and 88.9 µM). The N-deacyl derivative (52), kuanoniamine C (49) and D (50) were tested *in vitro* against two human cancer cell lines, HeLa cells and MONO MAC-6 cells. Kuanoniamine C (49), D (50) and N-deacyl derivative (52) exhibited IC$_{50}$ values of 5.1, 1.4 and 1.2 µg/mL (HeLa) and values of 1.2, 0.8 and 2.0 µg/mL (MONO MAC-6).

In 1988, Gunawardanda *et al.* isolated dercitin (53) from the deep water marine sponge *Dercitus* sp. collected from Bahamas [56]. The structure of dercitin was assigned on the basis of spectroscopic data. This structure (53) was subsequently revised to structure (54) by the interpretation of the magnitude of long range proton-carbon coupling constants. Dercitin (54) exhibited *in vitro* antitumor activity against P-388 (IC$_{50}$ = 0.05 µg/mL) and human tumor cells (HCT 8, A-549, T47D) with IC$_{50}$ value of 1.0 µg/mL. Dercitin (54) also showed *in vivo* activity against P-388 (T/C 170%, 5 mg/kg). One year later, the same group isolated three new pentacyclic pyridoacridine alkaloids, nordercitin (55), dercitamine (56) and dercitamide (57) from the extract of a red coloured sponge *Stelletta* sp. collected in Bahamas [57]. Later dercitamide (57) was found to be identical to kuanoniamine C (49). Compounds (55-57) inhibited the proliferation of P-388 murine leukemia cells *in vitro* with IC$_{50}$ values of 4.79, 26.7 and 12.0 µM, respectively.

Two new pyridoacridine alkaloids, arnoamines A (58) and B (59) were isolated from the ascidian *Cystodytes* sp. collected in the vicinity of Arno Atoll, Republic of Marshall Islands [58]. They were supposed to be the first members of
pentacyclic pyridoacridine alkaloids having a pyrrole ring fused with the pyridoacridine ring system. The structures of 58 and 59 were established on the basis of spectroscopic data, particularly those obtained from HMBC and NOE NMR experiments. The arnoamines A (58) and B (59) displayed a much unexpected chemical reactivity. The pyrrole ring hydrogens labelled as Ha and Hb showed deuteration exchange, when NMR were recorded in CDCl₃/TFA-d4. Arnoamine A (58) exhibited cytotoxicity against the MCF-7, A-549 and HT-29 cell lines with GI₅₀ values of 0.3, 2.0 and 4.0 µg/mL, respectively, whereas Arnoamine B (59) showed GI₅₀ values of 5.0, 2.0 and 3.0 µg/mL against the MCF-7, A-549 and HT-29 cell lines, respectively.

The methanol extract of the ascidian Cystodytes dellechiaijei, collected in Brazil yielded two novel alkaloids, sebastianine A (60) and B (61) [59]. The structures of both the compounds were established by analysis of spectroscopic data. Sebastianine A (60) was found comprising of a pyridoacridine system fused with a pyrrole unit and sebastianine B (61) is having a pyridoacridine system fused with a pyrrolidine system condensed with R-hydroxyisovaleric acid. Sebastianine A (60) and B (61) showed cytotoxic activity against a panel of HCT-116 colon carcinoma cells.

Recently, Davis et al. isolated two new pyridoacridine alkaloids, ecionines A (62) and B (63) from Australian sponge Ecionemia geodides [60]. Both the compounds were found to contain an imine moiety, which is very rarely found in pyridoacridine class of compounds. Both the compounds were tested against a panel of human bladder cancer cell lines (TSU-Pr1, TSU-Pr1-B1 and TSU-Pr1-B2) and the superficial bladder cancer cell line 5637. Compound (63) showed moderate cytotoxicity against all the cell lines, with IC₅₀ values
of 6.48 mM (TSU-Pr1), 6.49 mM (TSU-Pr1-B1), 3.55 mM (TSU-Pr1-B2) and 3.66 mM (5637), whereas Compound (64) showed cytotoxic effect on 5637 and TSU-Pr1-B2 cells at 10 mM, with cell growth inhibitions of 54% and 51% cells, respectively, but did not have any effect on TSU-Pr1-B1 cells at 10 mM.

1.3. Hexacyclic alkaloids

The extracts of a deep violet sponge Dercitus sp. collected in the Bahamas yielded a hexacyclic alkaloid cyclodercitin (64). The sixth ring in cyclodercitin (64) is formally derived by cyclization of the 2-aminoethyl side chain to the acridine nitrogen, while the pyridine ring is substituted with an N-methyl group. Cyclodercitin (64) inhibited the proliferation of P-388 murine leukemia cells \textit{in vitro} with IC$_{50}$ value of 1.9 $\mu$M.

Recently, stellettamine (65) was isolated from a deep water marine sponge Stelleta sp. [61]. The molecular formula, C$_{20}$H$_{14}$N$_{4}$S was determined by high resolution FAB mass spectroscopy. The structure of the compound was established on the basis of $^1$H-$^1$C correlation spectroscopy except the orientation of thiazole ring. Therefore complete structure of stellettamine (65) was determined by a single-crystal X-ray diffraction experiment.

1.4. Heptacyclic alkaloids

Eilatin (66) is the only known heptacyclic pyridoacridine alkaloid of the marine origin [62]. Molecular formula of eilatin (66) was determined as C$_{24}$H$_{12}$N$_{4}$ by high-resolution EIMS. $^1$H NMR spectrum showed only six aromatic protons that could agree with the common six protons of the benzodiazaphenanthroline system. The $^{13}$C NMR spectrum exhibited only 12 carbon lines (6 for monoprotonated carbons and 6 nonprotonated carbons). This suggests a symmetrical dimeric structure for eilatin (66). Various 2D NMR experiments such as $^1$H-$^{13}$C correlations and a HETCOSY experiment were failed to deduce the structure and finally it was determined by a single-crystal X-ray analysis. Eilatin (66) was found to exhibit cytotoxic activity against HCT cell line with IC$_{50}$ value of 5.3 $\mu$M.
1.5. Octacyclic alkaloids

In 1991, Faulkner et al. isolated two novel optically active octacyclic alkaloids, eudistones A (67) and B (68) from the Seychelles tunicate Eudistoma sp. [63]. Eudistone A (67) was obtained as an amorphous yellow powder. The molecular formula C_{27}H_{19}N_{5}O for eudistone A (67) was determined by high resolution mass spectroscopy, which implies 21 degrees of unsaturation. The $^{13}$C NMR signal at 191.8 ppm and an IR band at 1660 cm$^{-1}$ indicated the presence of an unsaturated ketone and the broad bands at 3360 and 3220 cm$^{-1}$ attributed for primary or secondary amines. The complete structure of the compounds was determined on the basis of other correlations NMR techniques such as COSY, NOE, HMBC and HMQC. Eudistone B (68) was obtained as a white amorphous powder. The molecular formula C_{27}H_{17}N_{5}O for eudistone B (68) was determined and has one more degree of unsaturation than that present in eudistone A (67). Therefore eudistone B (68) is a dehydrogenation product which was also supported by air oxidation of eudistone A (67) to eudistone B (68). When air is bubbled through a solution of eudistone A (67) in DMSO at 60°C for 48 hrs, the dihydropyridine ring of eudistone A (67) is aromatized to yield eudistone B (68).

![Chemical structures of eudistones A, B, and octacyclic analogue](image)

Recently, Demeunynck et al. synthesized an octacyclic analogue (69) of eilatin [64]. The compound (69) was tested against two cancer cell lines, HT-29 (human colon adenocarcinoma) and A-431 (human epithelial carcinoma). Unfortunately due to its low solubility in water, the compound could only be tested at low concentration (5 µM) and did not show any activity against HT-29 and 85% survival on A-431 cell lines.

2. Indole alkaloids

Indole-containing alkaloids have frequently been isolated from diverse marine invertebrates including bryozoans, coelenterates, sponges, tunicates, algae, symbiotic bacteria and fungi [65-72]. Indole alkaloids show different type of biological activities such as cytotoxic, antitumor, antiviral, antimicrobial,
antiparasitics, antiserotonin and anti-inflammatory activities [73]. Due to the interesting biological activities and unique structural features, the indole series have become an attractive research field for the development of new pharmacological lead compounds. In the past few years, some of the isolated natural organic compounds and their derivatives have been synthesized by chemists and evaluated for their biological activity to find new lead compounds against different infectious diseases [74-79].

2.1. Bisindole alkaloids

In 1988, Kohmoto et al. isolated a bisindole alkaloid, dragmacidin (70) from a deep water marine sponge *Dragmacidin* sp. [80]. Dragmacidin was found to contain two indole groups joined by a piperazine ring system which had not been found before in marine natural products. The molecular formula of dragmacidin was deduced as C_{21}H_{19}Br_{3}N_{4}O from FAB HRMS data analysis. Several 2D NMR experiments such as COSY, HETCOR, COLOC and HETCOSY were performed in order to determine the structure of the compound. Dragmacidin (70), when treated with excess acetic anhydride and pyridine overnight at room temperature yielded the triacetate derivative (71). An ethanolic solution of dragmacidin (70) on treatment with 10% Pd/C at room temperature under 20 psi of hydrogen gives tridebromodragmacidin (72). Dragmacidin (70) exhibited *in vitro* cytotoxicity with IC_{50} values of 15 µg/mL against P-388 cell lines and 1-10 µg/mL against A-549 (human lung), HCT-8 (human colon) and MDAMB (human mammary) cancer cell lines.

The pacific sponge *Hexadella* sp. collected from the coast of British Columbia yielded two other members of dragmacidin family, dragmacidon A (73) and dragmacidon B (74) along with a new alkaloid, topsentin C (75) [81]. The structures of the compounds 73-75 were proposed on the basis of spectroscopic analysis. Dragmacidon A (73) showed *in vitro* cytotoxicity in the L-1210 assay with ED_{50} value of 10 mg/mL, whereas topsentin C (75) and dragmacidon B (74) were found to be inactive.

In 1995, Capon et al. reported the isolation of dragmacidin D (76) from a deep water marine sponge *Spongosorites* sp. collected from the southern Australian coast [82]. Dragmacidin D (76) 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 IC_{50} values of 1.4 and 4.5 µg/mL, respectively.

Four new bisindole alkaloids, nortopsentins A-D (77-80) were isolated from the Caribbean deep sea sponge *Spongosorites ruetzleri* [83]. The structures of nortopsentins A-D (77-80) were established mainly on the basis of NMR spectroscopic data and were found to contain an imidazole ring between two indole units. Compounds (77-80) exhibited cytotoxic activity against P-388 cells with IC_{50} values of 7.6, 7.8, 1.7 and 0.9 µg/mL, respectively.
The sponge *Topsentia genitrix*, collected from Banyuls (France) yielded two bisindole alkaloids, topsentin (81) and bromotopsentin (82). They were found to contain 2-acyl imidazole moiety inserted between two indole units with different substitution on benzene rings [84]. In 1995, Capon *et al.* reported the isolation of isobromotopsentin (83) from the deep water sponge *Spongosorites* sp. collected from the coast of southern Australia [85].

Topsentin (81) inhibited proliferation of cultured human and murine tumor cells. It exhibited *in vitro* activity against P-388 with IC50 value of 3 μg/mL, human tumor cell (HCT-8, A-549, T47D) with IC50 value of 20 μg/mL and *in vivo* activity against P-388 (T/C 137%, 150 mg/kg) and B16 melanoma (T/C 144%, 37.5mg/kg) [86]. Bromotopsentin (82) showed antiproliferative activity against human broncopulmonary cancer cells (NSCLC-N6) with an IC50 = 12 μg/mL [87]. Deoxytopsentin (84) was isolated from the sponge *Hexadella sp* collected in Jervis Inlet, British Columbia [88]. In 1999, bromodeoxytopsentin (85) and isobromodeoxytopsentin (86) were isolated from sponge *Spongosorites genitrix* collected from Jaeju Island Korea by Shin *et al.* [89]. Structurally topsentin (81) and deoxytopsentin (84) are the same except the indole ring which is unsubstituted in case of deoxytopsentin (84). Deoxytopsentin (84) showed the antiproliferative activity against human broncopulmonary cancer cells (NSCLC-N6) with an IC50 value of 6.3 μg/mL. It also displayed moderate activity against breast cancer and hepatoma (HepG2) with an IC50 of 10.7 and 3.3 μg/mL, respectively.
Recently, Kobayashi et al. isolated a new cytotoxic bis-indole alkaloid, hyrtinadine A (87) from Okinawan marine sponge *Hyrtios* sp. [90]. The structure elucidation was achieved on the basis of spectroscopic data. Hyrtinadine A (87) was supposed to be the first example of a bisindole alkaloid with a 2,5-disubstituted pyrimidine ring between two indole units. Hyrtinadine A (87) exhibited *in vitro* cytotoxicity against murine leukemia L-1210 and human epidermoid carcinoma KB cells with IC$_{50}$ values of 1.0 and 3 $\mu$g/mL, respectively.

Hyrtiosins A (88) and B (89) were also isolated together with known 5-hydroxyindole-3-aldehyde (90) from the Okinawan marine sponge *Hyrtios erecta* [91]. Compound (90) exhibited cytotoxic activity against human epidermoid carcinoma KB cells *in vitro* with IC$_{50}$ value of 4.3 $\mu$g/mL, while hyrtiosins A (88) and B (89) were less cytotoxic than 5-hydroxyindole-3-aldehyde (90) and showed 21% and 16% inhibition, respectively, at 10 $\mu$g/mL against KB cells.

### 2.2. Indolocarbazoles

Staurosporine (91) was first isolated from *Streptomyces staurosporeus* Awaya (AM-2282) [92,93] and subsequently from other actinomycetes e.g. *Streptomyces actuosus* [94] and *Streptomyces species* strain M-193 [95]. The structure and stereochemistry of the compound in its MeOH-H$_2$O solvate form was deduced by X-ray crystallography. Staurosporine (91) exhibited *in vitro* activity against several different type of tumors such as human neuroblastoma cell line (NB-1), HeLa S3 cells, B16 melanoma cells and P-388 leukemia cells [96,97]. Cordell et al. evaluated the cytotoxicity of staurosporine (91) towards the murine P-388 lymphocytic leukemia and human carcinoma KB cell lines. Staurosporine (91) showed potent cytotoxic activity with ED$_{50}$ value of 0.0024 $\mu$g/mL for the KB system and <0.08 $\mu$g/mL for the P-388 system.
Schupp et al. isolated two new indolocarbazole alkaloids, 3-hydroxy-3′-demethoxy-3′-hydroxystaurosporine (92) and 11-hydroxy-4′-N-demethylstaurosporine (93) from the marine ascidian Eudistoma toealensis and its predator, Pseudoceros sp. along with four known congeners (94-97) and staurosporine (91) in their protonated states [98]. Recently, a natural staurosporine analogue, ZHD-0501 (98) was isolated from the fermentation broth of a marine-derived Actinomadura sp. 007 through a bioassay-guided separation procedure [99]. ZHD-0501 (98) was supposed to be the first example of staurosporine analogue carrying a heterocycle fused to the pyran ring.

Schupp et al. evaluated the potential of these staurosporine derivatives as inhibitors of cell proliferation and macromolecule synthesis [100]. Compound (94) was found to be the most active staurosporine derivative both as MONO-MAC-6 cells inhibitor and inhibitor of RNA and DNA synthesis. The IC_{50} values of staurosporine (91) and the derivatives, 94, 95 and 96 for inhibiting MONO-MAC-6 cells were 24.4, 13.3, 33.3 and 29.7 ng/mL, respectively, while those of 92 and 93 was >100 ng/mL each. The percentage inhibition of RNA and DNA synthesis of compounds 91 and 94 were 93 and >98, 98 and >98, respectively. Compound (98) inhibited the proliferation of human cancer A-549, BEL-7402, HL-60 cells and mouse leukemia P-388 cells with the percentage inhibition of 82.6%, 57.3%, 76.1%, 62.2% in the SRB assay [101]. It also inhibited the proliferation of mouse cancer tsFT210 cells with the inhibition rates of 28.3% at 21 μM and 20.5% at 2.1 μM in the SRB assay. Analysis of structure activity relationship demonstrated that hydroxylation of staurosporine at position 3 of the indolocarbazole moiety causes an increase in antiproliferative activity, while hydroxylation at 11th position resulted in a decrease in activity. All these data suggested that not only the presence or absence of hydroxyl group, but also the position of OH group is crucial to determine the antiproliferative properties of the various staurosporine analogues.
A novel carbazole alkaloid, coproverdine (99) was isolated from an unidentified ascidian *Anchorina* sp. collected from the north Island of New Zealand [102]. The structure of 99 was established on the basis of extensive spectroscopic data analysis. Coproverdine (99) was evaluated against a variety of murine and human tumor cell lines such as P-388, A-549, HT-29, MEL-28 and DU-145 exhibiting IC$_{50}$ values of 1.6, 0.3, 0.3, 0.3 and 0.3 $\mu$M, respectively.

### 2.3. Ergoline alkaloids

Makarieva *et al.* isolated pibocin A (100) from the far-eastern ascidian *Eudistoma* sp. [103]. Its structure and absolute stereochemistry were established on the basis of spectroscopic and X-ray data analysis and was supposed to represent the first example of marine ergoline alkaloids. Pibocin A (100) exhibited moderate cytotoxicity against mouse Ehrlich carcinoma cells with ED$_{50}$ value of 12.5 $\mu$g/mL. Recently, pibocin B (101) was isolated from the colonial ascidian *Eudistoma* sp. [104]. Its structure was established as (8$\beta$)-2-bromo-N-O-methyl-6,8-dimethylergoline on the basis of NMR, FAB and MALDI TOF MS data and chemical means. Pibocin B (101) exhibited moderate cytotoxic activity against mouse Ehrlich carcinoma cell with an ED$_{50}$ value of 25 $\mu$g/mL.

### 2.4. Peptidoindoles

Styelin D, a 32-residue, C-terminally amidated peptide was isolated from the blood cells of the solitary ascidian *Styela clava* [105]. It was found to contain two novel amino acids, dihydroxyarginine and dihydroxylysine, and two distinctly unusual amino acids including, 6-bromotryptophan and 3,4-dihydroxyphenylalanine. Styelin D exhibited cytotoxicity against HCT-116 cells with IC$_{50}$ value of 10.1 $\mu$g/mL, and human ME-180 cervical epithelial cells with ED$_{50}$ value of 50 $\mu$g/mL.

Nakao *et al.* isolated kapakahine B (102) from the marine sponge *Cribrochalina olemda* collected at Pohnpei, Micronesia [106]. Kapakahine B (102) was found having a cyclic hexapeptide with an $\alpha$-carboline ring system and showed moderate cytotoxicity against P-388 murine leukemia cells with an IC$_{50}$ value of 5.0 $\mu$g/mL.
Two isomeric cycloheptapeptides, phakellistatin 3 (103) and isophakellistatin 3 (104), were isolated from the Western Indian marine sponge *Phakellia carteri* [107]. They were supposed to represent the first examples of photo-Trp serving as a natural peptide unit. A significant difference in the activity was also observed with the photo-Trp indole ring juncture. Phakellistatin (*trans*-ring juncture) exhibited inhibition of P-388 (ED$_{50}$ = 0.33 µg/mL) while isophakellistatin (*cis*-ring juncture) showed no significant effects.

### 2.5. β-Carbolines

Eudistomin K (105) was isolated from the Caribbean ascidian *Eudistoma olivaceum* and found to exhibit antitumor activity against L-1210, A-549, HCT-8 and P-388 cell lines with IC$_{50}$ of 0.01 µg/mL against P-388 cell line [108]. Recently Kobayashi *et al.* reported the isolation and structure elucidation of a new β-carboline alkaloid, eudistomidin G (106) from the Okinawan marine tunicate *Eudistoma glaucus* [109]. Eudistomidins G (106) exhibited significant cytotoxic activity against L-1210 murine leukemia cells with IC$_{50}$ value of 4.8 µg/mL *in vitro*.

Adesanya *et al.* reported the isolation of two novel brominated β-carbolines, eudistalbin A (107) and B (108) from the marine tunicate *Eudistoma album* along with the known compound eudistomin E (109) [110]. The cytotoxicity of these compounds was tested using the human nasopharyngeal carcinoma KB cell lines. Eudistomin E (109) exhibited 100% cytotoxicity at seven concentrations ranging from 10 to 0.005 µg/mL (ED$_{50}$ <5.0 ng/ml). Eudistalbin A (107) showed 100% cytotoxicity at 10, 92% at 5, and 0% at 1 µg/mL (ED$_{50}$ = 3.2 µg/mL), whereas eudistalbin B (108) exhibited 0% cytotoxic activity at 10 and 1 µg/mL.
Three new alkaloids, hyrtioerectines A-C (110-112) were isolated from a red coloured marine sponge *Hyrtios erectus* [111]. The structure of the compounds 110-112 were established on the basis of their spectral data including 1D (1H and 13C) and 2D (1H-1H COSY, NOESY, ROESY, HMQC and HMBC) NMR experiments and compound 110 was found to contain 6-hydroxy β-carboline and 6-hydroxyindole units linked through C3-C3’ carbon bond. Hyrtioerectines A-C (110-112) were evaluated for their cytotoxicity against HeLa cells and showed moderate cytotoxic activity with IC50 values of 10, 5.0 and 4.5 µg/mL, respectively.

Foderaro *et al.* reported the isolation of a new tetrahydro-β-carboline alkaloid, bengacarboline (113) from the Fijian ascidian *Didemnum* sp. [112]. The structure of the compound was determined by 1H, 13C NMR and HRMS-FAB data analysis and was found to contain one indole and one tryptamine units attached to C-1 of a tetrahydro-β-carboline system through C-3 and C-2 of the indole and tryptamine moieties. Bengacarboline (113) was found to be cytotoxic towards a 26 cell line human tumor panel *in vitro* with a mean IC50 value of 0.9 µg/mL and also inhibited the catalytic activity of topoisomerase II at 32 µM.
More recently, a new 1-imidazoyl-3-carboxy-6-hydroxy-β-carboline alkaloid, named as hyrtiocarboline (114) was isolated from a marine sponge *Hyrtios reticulates* [113]. The structure was elucidated on the basis of spectroscopic data such as $^1$H-$^1$C and $^1$H-$^1$N HMBC NMR experiments. Hyrtiocarboline (114) was tested for antiproliferative activity against 13 cancer cell lines and showed selective activity against three cancer cell lines, non-small cell lung (H522-T1), melanoma (MDA-MB-435) and lymphoma (U937) with IC$_{50}$ values of 1.2, 3.0 and 1.5 µg/mL, respectively. Hyrtiocarboline (114) also exhibited 57% inhibition of HeLa cells at 230 µM.

Two new β-carboline alkaloids, 6-hydroxymanzamine A (115) and 3,4-dihydromanzamine A (116) were isolated from the marine sponge *Amphimedon* sp collected from the Kerama Islands, Okinawa, Japan [114]. The structures of the compounds were elucidated on the basis of NMR spectral data. Compounds 115 and 116 were found to be cytotoxic *in vitro* against L-1210 with IC$_{50}$ values of 1.5 and 0.48 µg/mL, respectively and KJ3 cells  with IC$_{50}$ values of 2.5 and 0.61 µg/mL, respectively.

![Chemical structures](image)

Edrada *et al.* reported the isolation of four new manzamine congeners 117-120 and four known compounds 121-124 from the marine sponge *Xestospongia ashmorica* collected from the shores of Mindoro Island, Philippines [115].

The structures of the compounds were established on the basis of NMR spectroscopic and mass spectrometric data analysis. The $N$-oxide structures for compounds 118-120 were confirmed by conversion to the corresponding tertiary bases by reduction with Zn/HCl. All compounds (117-124) were tested for their *in vitro* cytotoxicity against L-5178 mouse lymphoma cells using the microculture tetrazolium (MTT) assay at different concentrations ranging from 0.3 to 20 µg/mL. All the compounds, except 121 were found to be active against L-5178 cell lines. From the activity profile, structure activity relationship between the different manzamine derivatives was also established. The $N$-oxide compounds 119 and 120 were the most active compounds with ED$_{50}$ value of 1.6 µg/mL followed by compounds 117 and 122.
(ED$_{50}$ = 1.8 µg/mL each). The other compounds 118, 123 and 124 also exhibited significant cytotoxic activity with ED$_{50}$ values of 3.2, 6.6 and 2.3 µg/mL, respectively.

Two years later, three new manzamine congeners, manzamine M (125), 3,4-dihydromanzamine J (126) and 3,4-dihydro-6-hydroxymanzamine A (127) were isolated from the Okinawan marine sponge *Amphimedon* sp. [116]. The structures and relative stereochemistry were determined on the basis of spectroscopic data. Manzamine M (125), 3,4-dihydromanzamine J (126) and 3,4-dihydro-6-hydroxymanzamine A (127) showed cytotoxicity against murine leukemia L-1210 cells with IC$_{50}$ values of 1.4, 0.5 and 0.3 µg/mL, respectively.
2.6. Trisindole alkaloids

In 1994, Bifulco et al. reported the isolation of two tris-indole alkaloids, Gelliusines A (128) and B (129) from a deep water new Caledonian sponge *Gellius* or *Orina* sp. [117]. Gelliusin A (128) and B (129) were found to be diastereomeric compounds made up by the coupling of three indole units. In compounds 128 and 129, two 6-bromo tryptamine units are linked through their aliphatic chains to the C-2 and C-6 position of a central serotonin moiety. The coupling of the indole unit appears to be non stereoselective giving two enantiomeric pairs, having different relative configuration at C-8 and C-8" named (±) Gelliusines A (128) and B (129). Gelliusines A (128) and B (129) showed cytotoxicity with an IC₅₀ value of between 10 and 20 μg/mL against KB, P-388, P-388/dox, HT-29 and NSCLCN-6 cell lines.

![Gelliusine A and B](image)

2.7. Miscellaneous indole alkaloids

Kondo et al. reported the isolation of two new indole alkaloids, isoplysin A (130) and D6-bromohypaphorine (131) from the Okinawan marine sponge *Aplysina* sp. [118]. The structures of both the compounds were established by spectral and chemical means. Isoplysin A (130) was found to be weakly cytotoxic against murine lymphoma L-1210 (IC₅₀ = 11.5 μg/mL) and human epidermoid carcinoma KB cells (31% inhibition at 20 μg/mL), while D-6-bromohypaphorine (131) showed no significant effects.

In 2007, four new prenylated indole alkaloids, notoamides A-D (132-135) were isolated from marine-derived fungus *Aspergillus* sp. which was separated from the mussel *Mytilus edulis* collected off Noto Peninsula in the Sea of Japan [119]. The structures and absolute stereochemistry of the compounds were determined mainly on the basis of spectroscopic data analysis and were found comprising of pyranoindole ring system. Compounds (132) and (133) contain the bicyclo[2.2.2]diazaoctane ring system also. Notoamides A-C (132-134) exhibited weak cytotoxicity against HeLa and L-1210 cells with IC₅₀ values in the range of 22–52 μg/mL but the IC₅₀ value of notoamide D (135) was greater than 100 μg/mL and it was believed that the dihydroxypyrano-2-oxindole ring system, that is common to compounds 132-134 is responsible for the remarkable differences in cytotoxic activity. Notoamide D (135) contains a pyrroloindole
instead of dihydroxypyrano-2-oxindole ring system. Recently, six new prenylated indole alkaloids, notoamides F-K (136-141) were isolated from a marine-derived Aspergillus sp [120]. Notoamide I (139) showed weak cytotoxicity against HeLa cells with an IC$_{50}$ value of 21 $\mu$g/mL, whereas for notoamides F (136), J (140) and K (141), the IC$_{50}$ values were more than 50 $\mu$g/mL.

Three new indole alkaloids, shearinines D-F (142-144) along with the known shearinine A (145) were isolated from marine-derived fungus Penicillium janthinellum [121]. The structures of all the compounds were established by 1D and 2D NMR such as HSQC, HMBC, COSY, NOESY and HREIMS data analysis. Shearinines A, D, E and F were tested for cytotoxicity against mouse epidermal JB6 P+ Cl 41 cells using the MTS method. The compounds displayed no cytotoxicity up to 200 $\mu$M, whereas some of the compounds showed cancer preventive and antileukemic properties. Shearinine E (143) inhibited EGF-induced malignant transformation of JB6 P$^+$ Cl 41 cells in a soft agar with INCC$_{50}$ (inhibition of number of the colonies) value of 13 $\mu$M. The Shearinines A (145), D (142) and E (143) induced apoptosis in human leukemia HL-60 cells at 100 $\mu$M concentration by 10%, 39% and 34% of the apoptotic cells when compared to control cells, respectively.
Reyes et al. reported the isolation and structure elucidation of six new bromoindole alkaloids, aplicyanins A-F (146–151) from CH$_2$Cl$_2$/MeOH extract of the tunicate *Aplidium cyaneum* collected in Antarctica [122]. Aplicyanins A-F (146-151) were tested for cytotoxicity against three human tumor cell lines, including colon (A-549), lung (HT-29) and breast (MDA-MB-231). Compounds 147, 149, 150 and 151 showed cytotoxicity against these cell lines, whereas compounds 146 and 148 were found to be inactive. Compounds 147, 149, 150 and 151 demonstrated IC$_{50}$ values of 0.66, 0.63, 8.70 and 1.31 (A-549), 0.39, 0.33, 7.96 and 0.47 (HT-29) and 0.42, 0.41, 7.96 and 0.81 (MDA-MB-231). From the activity profile it is clear that compound 150 shows the least activity and this was explained on the basis of presence of the acetyl group at N-16 in compounds 147, 149 and 151 which is crucial to exhibit the activity.

Amade et al. reported the isolation and structure elucidation of new bromine containing oxindole alkaloid, matemone (152) along with a known compound, 6-bromoindole-3-carbaldehyde from the Indian Ocean sponge *Iotrochota purpurea* [123]. Compound 152 showed weak cytotoxicity against NSCLC-N6 L16 strain18 (lung cancer), Mia PaCa-2 cell line (pancreas cancer) and DU145 cell line (prostatecancer) with IC$_{50}$ values of 30, 24 and 27 µg/mL, respectively.

Dendridine A (153), a unique C2-symmetrical 4,4'-bis(7-hydroxy)indole alkaloid was isolated from an Okinawan marine sponge *Dictyodendrilla* sp. [124]. The structure of compound was elucidated by spectroscopic data including 2D NMR data such as the $^1$H-$^1$H COSY, ROESY and HMBC spectra. Dendridine A (153) exhibited moderate cytotoxicity against murine leukemia L-1210 cells with IC$_{50}$ value of 32.5 µg/mL.

Four novel brominated indole alkaloids, arborexidines A-D (154–157) were isolated from the extract of a marine tunicate *Pseudodistoma arborescens* [125]. Out of four, only arborescidine D (157) showed *in vitro* cytotoxic activity against the growth of KB human buccal carcinoma cells with IC$_{50}$ value of 3 µg/mL.
3. Pyrrole alkaloids

3.1. Bromopyrrole alkaloids

Kuramoto et al. isolated two novel alkaloids, cylindradines A (158) and B (159) from the marine sponge *Axinella cylindratus* collected at the Seto inland sea near Sada Cape in Ehime prefecture [126]. The chemical structures and absolute stereochemistry of these compounds were assigned by spectroscopic and X-ray data analysis. Cylindradines A (158) and B (159) displayed moderate cytotoxicity against the murine leukemia cell line P-388 with IC₅₀ value of 7.9 and 33 μg/mL, respectively.

In 1993, a novel alkaloid, agelastatin A (160) was isolated from the deep water marine sponge *Agelas dendromorpha* collected in the Coral Sea near New Caledonia [127]. Agelastatin A (160) showed significant *in vitro* activity against L-1210 and KB tumor cells [128]. He also studied the structure activity relationship of agelastatins and found that the C-8a hydroxyl group and both NH
groups are necessary for optimal activity. Alkylation or acylation of these functional
groups, as well as removal of the C-1 pyrrole bromine, leads to a significant loss of
potency. Recently, Tilvi et al. isolated three related pyrrole-imidazole alkaloids,
named agelastatins E (161), F (162) and benzosceptrin C (163) along with agelastatin
A (160) from marine sponge *Agelas dendromorpha* [129]. The structures of the
compounds were established on the basis of spectroscopic data interpretation. The compounds 160-163 were evaluated for cytotoxic activity against the KB cell lines.
All the compounds lacked significant bioactivity at 30 μM except for agelastatin A
(160) which showed 100% activity at 30 and 3 μM.

A new pyrrole alkaloid, clathrodin (164) was isolated from the MeOH extract
of the Caribbean sea sponge *Agelas clathrodes* [130] and showed significant
cytotoxicity against CHO-K1 cells with ED$_{50}$ value of 1.33 μg/mL.

The tetracyclic pyrrole-imidazole alkaloid, dibromophakellstatin (165) was
isolated from the marine sponge *Phakellia mauritiana* [131]. The structure and
absolute stereochemistry of the compound was determined by interpretation of
NMR and X-ray crystal data analysis. Dibromophakellstatin (165) showed inhibitory
activity against a panel of human cancer cell lines, ovary (OVCAR-3), brain (SF-295),
kidney (A-498), lung (H-460), colon (KM20L2) and melanoma (SK-MEL-5) with
ED$_{50}$ values of 0.46, 1.5, 0.21, 0.62, 0.11 and 0.11 μg/mL, respectively.

Four new alkaloids 3-bromomaleimide (166), 3,4-dibromomaleimide (167),
12-chloro-11-hydroxydibromoisophakellin (168) and $N$-methylmanzacidin C
(169) were isolated from the marine sponge *Axinella brevistyla* collected in
western Japan [132]. Their structures were determined on the basis of spectroscopic data analysis. Compounds 166-168 exhibited cytotoxicity against
L-1210 cells with IC$_{50}$ values of 1.1, 0.66 and 2.5 μg/mL, respectively, whereas
$N$-methylmanzacidin C (169) was found to be inactive.

Umeyama et al. reported the isolation and structure elucidation of a novel
bromopyrrole alkaloid (170) along with (±)-171 and (±)-longamide (172) from
the marine sponge *Homaxinella* sp. collected in japan [133]. Compounds 170 and
(±)-171 showed mild cytotoxic activity in vitro against P-388 lymphocytic
leukemia cells with ED$_{50}$ values of 21.5 and 30 μg/mL, respectively, while
compound (172) was inactive (ED$_{50}$ = >100 μg/mL).
More recently, Hertiani et al. reported the isolation of 11 new brominated pyrrole alkaloids from the Indonesian marine sponge *Agelas linnaei* [134]. These alkaloids includes a new dibromophakellin derivative (173), 4-(4,5-dibromo-1-methylpyrrole-2-carboxamido)-butanoic acid (174), agelanin A and B (175 and 176), agelanesins A–D (177-180) and mauritamide B-D (181-183).

All the compounds (173-183) were tested for cytotoxicity against the murine L-1578Y mouse lymphoma cell line. Agelanesins A–D (177-180) showed prominent activity while others were found to be inactive. The IC50 values for agelanesins A–D (177-180) were 9.55, 9.25, 16.76 and 13.06 µM, respectively. Compounds 177 and 178 were the most potent concluding that cytotoxicity of the
agelanesins is related to the degree of bromination of the pyrrole ring. Increase in bromination decreases the activity as observed for 179 and 180 compared to 177 and 178. While the presence of an iodide substituent on the tyramine moiety causes a small differences in activity as 177 and 179 have similar activity compared to 178 and 180.

3.2. Pyrroloquinones

A new dipyrroloquinone, zyzzyanone A (184) was isolated from the Australian marine sponge Zyzya fuliginosa [135]. Zyzzyanone A (184) showed mild cytotoxic activity against mouse Ehrlich carcinoma cells with IC$_{50}$ value of 25 µg/mL. One year later Zyzzyanones B-D (185-187), three related dipyrroloquinones were isolated from the same sponge Zyzya fuliginosa along with the known zyzzyanone A (184) [136]. The structures of the compounds 185-187 were established by extensive NMR spectroscopic data. Zyzzyanones B-D (185-187) also pronounced weak cytotoxicity against mouse Ehrlich carcinoma cells with IC$_{50}$ value of 25 µg/mL.

![Chemical structures of zyzzyanones A (184), B (185), C (186), and D (187).](image)

3.3. Pyrroloquinoline alkaloids

In 1986, Landini et al. reported the isolation of discorhabdins C (190) from the extract of red-brown sponge Latrunculia du [137]. The structure of the compound was determined by a single crystal X-ray diffraction study and it was found to contain a new tetracyclic iminoquinone chromophore with a spiro 2,6-dibromocyclohexadienone. Two years later, discorhabdins A (188) and (189) B along with known discorhabdins C (190) were isolated from the three species of Latrunculia sponge collected in New Zealand [138]. A related compound, discorhabdins D (191) was isolated from Latrunculia brevis collected in New-Zeeland [139]. Discorhabdins A (188), B (189) and C (190) showed in vitro cytotoxicity against P-388 assays with ED$_{50}$ values of 0.05, 0.1 and 0.03 µg/mL. Discorhabdin A (188) and discorhabdin C (190) showed no cytotoxicity against P-388 system in vivo but were found to be toxic to mice at about 2 mg per kg of
body weight. Discorhabdin B (189) showed some antitumour effect with a T/C of 117% at a dose of 0.25 mg/kg, but this did not reach the significance level of 120%. Discorhabdins C (190) was also found to be active toward L-1210 tumor cells at very low levels (ED$_{50}$ < 100 ng/mL). Discorhabdin D (191) exhibited mild cytotoxicity against P-388 in vitro with IC$_{50}$ value of 6 µg/mL, however in vivo it showed significant activity against P-388 (T/C 132% at 20 mg/kg).

Two other members of discorhabdin family, discorhabdins L (192) and I (193) were isolated from *Latrunculia brevis* and their structures were assigned on the basis of spectroscopic data analysis and comparison with the known discorhabdins A (188) and B (189) [140]. Discorhabdins L (192) and I (193) were tested against a panel of 14 tumor cell lines including prostate (DU-145 and LN-caP), ovary (SK-OV-3, IGROV and IGROV-ET), breast (SK-BR3), melanoma (SK-MEL-28), endothelio (HMEC1), NSCL (A549), leukemia (K-562), pancreas (PANC1) and colon (HT-29, LOVO and LOVO-DOX). Both the compounds exhibited potent cytotoxic activity in most of the cases. The HT-29 colon cell line was found to be the most sensitive with GI$_{50}$ values of 0.12 and 0.35 µM for compounds 192 and 193, respectively.

Recently, Lang et al. isolated a novel alkaloid, discorhabdin W (194) from a marine sponge *Latrunculia sp* [141]. It is a symmetrical dimer of discorhabdin in which two discorhabdin units are linked by a disulfide linkage. The structure and stereochemistry were assigned by 1D and 2D NMR experiments and mass spectrometry. Discorhabdin W (194) exhibited potent cytotoxicity against P-388 cells with IC$_{50}$ value of 0.09 µg/mL.
Sun et al. reported the isolation of three highly functionalized pyrroloquinoline alkaloids, batzelline A-C (195-197) from the deep water sponge *Batzella sp* collected in Bahamas [142]. The structure of 195 was determined by X-ray and those of 196 and 197 by comparison of their spectral data with that of 195 and by chemical transformations. One year later same group isolated four related alkaloids, isobatzellines A-D (198-201) from the sponge *Batzella sp*. [143]. Isobatzellines A-D (198-201) were found to exhibit *in vitro* cytotoxicity against P-388 leukemia cell lines, whereas batzellines (195-197) were inactive and it was explained on the basis of difference in their structure. Both, isobatzellines and batzellines have the same pyrrolo[4,3,2-de]quinoline ring system but isobatzellines contain an aminoiminoquinone moiety that is different from the aminoquinone moiety in the batzellines which could be responsible for the activity of isobatzellines.

In 1999, two novel batzelline analogues, named as secobatzellines A (202) and B (203) were isolated from a deep water marine sponge *Batzella sp*. The structure of the compounds were determined by NMR, HR FABMS data analysis and chemical analysis and was found to contain pyrroloaminoiminoquinone moiety previously reported in isobatzellines. Secobatzellines A (202) and B (203) exhibited *in vitro* cytotoxicity against the cultured murine P-388 tumor cell line with IC$_{50}$ values of 0.06, 1.22 µg/mL and against human lung carcinoma A-549 cell line with IC$_{50}$ values of 0.04, 2.86 µg/mL, respectively.

Kobayashi et al. reported the isolation and structure elucidation of a sulphur containing alkaloid, prianosin A (204) from the Okinawan marine sponge *Prianos melanos* [144]. Prianosin A (204) was found to be cytotoxic against L-1210 and L-5178Y murine leukemia cells with IC$_{50}$ values of 37 and 14 ng/mL *in vitro*. In 1988, same group reported the isolation of three related alkaloids, prianosins B-D (205-207) from the same sponge *Prianos melanos* [145]. All the alkaloids were found having the same tetrahydrothiophene ring as prianosin A (204). Prianosins B-D (205-207) were evaluated for cytotoxic activity *in vitro* against murine lymphomas L-1210 and L-5178Y cells and human epidermoid carcinoma KB cells. The IC$_{50}$ values were found to be 2.0, 1.8 and >5.0 µg/mL (24% inhibition at 5.0 µg/mL) for prianosins B (205), 0.15, 0.024 and 0.57 µg/mL for prianosins C (206) and 0.18, 0.048 and 0.46 µg/mL for prianosins D (207).
Radisky et al. reported the isolation and structure elucidation of seven novel pyrroloiminoquinones, the makaluvamines A-F (208-213) from the Fijian sponge Zyzzya cf. marsailis [146]. The makaluvamines A-F (208-213) 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. Makaluvamine A (208) and C (210) also exhibited in vivo antitumor activity against the human ovarian carcinoma ovcar-3 implanted in athymic mice. Makaluvamine F (213) was found to be the most active compound followed by makaluvamine E and A. Makaluvamine D and C were less potent than A, E and F, whereas Makaluvamine B was found not active against HCT-116. The same activity pattern was also observed against xrs-6, a Chinese hamster ovary (CHO) cell line being makaluvamine F (213) the most potent compound, while makaluvamine B least active. However, the metabolite cytotoxicity trends are substantially different than the hypersensitivity factors (HF) obtained by comparison of the cytotoxicity against xrs-6 versus BR1 (a DNA-repair proficient CHO line). Makaluvamine A (208) exhibited the largest hypersensitivity factor of 9, followed by makaluvamines F, E, C, and D. These results give a clue about the mechanism of action of Makaluvamines that involves DNA double-stranded breakage, an activity characteristic of topoisomerase II inhibitors.

Makaluvamine G (214) was isolated from a sponge of the genus Histodennella collected in Indonesia [147]. The structure of the compound was determined on the basis of 1D and 2D NMR experiments. Makaluvamine G (214) pronounced significant cytotoxicity to several tumor cell lines exhibiting an IC_{50} value of 0.50 µg/mL against P-388 (murine leukemia), A-549 (human nonsmall cell lung cancer), HT-29 (human colon cancer) and MCF-7 (human breast cancer) and value of 0.35 µg/mL against KB (human oral epidermoid carcinoma). It was also found to be a moderate inhibitor of topoisomerase-I (IC_{50} = 3.0 µM) and did not significantly inhibit topoisomerase-II. It also inhibited RNA (IC_{50} = 15 µM), DNA (15 µM) and protein (21 µM) synthesis.

In 1997, a new related alkaloid, makaluvamine N (215) was isolated from the Philippine sponge Zyzzya fuliginosa [148]. Compound 215 showed in vitro cytotoxicity against the human colon tumor cell line HCT-116 with LC_{50} value of 0.6 µg/mL. Makaluvamine N (215) also demonstrated an ability to inhibit the
catalytic activity of topoisomerase II and exhibited 90% inhibition of topoisomerase II unwinding of pBR-322 at 5 µg/mL. Casapullo et al. reported the isolation of a new member of makaluvamine family, makulavamine P (216) from the sponge Zyzya cf. fuliginosa collected in the Vanuatu Islands [149]. The compound was characterized on the basis of its spectral data and comparison with the other related compounds. Makulavamine P (216) exhibited moderate cytotoxicity to KB tumor cells (64% inhibition of cell growth at 3.2 µg/mL).

Recently Shinkre et al. synthesized two series of makaluvamine analogs 217 (a-g) and 218 (c-g) by introducing different substituents at the 7-position of the pyrroloiminoquinone ring present in makaluvamines. These compounds were obtained in two steps by treatment of the methoxypyrroloiminoquinone with different primary amine derivatives and subsequent removal of tosyl protecting group [150]. Compounds 217 (a-g) and 218 (c-g) were evaluated for their cytotoxicity against human breast cancer cell lines MCF-7 and MDA-MB-468 and human colon cancer cell line HCT-116 using etoposide and m-AMSA as standard drugs. HCT-116 cells were shown to be the most sensitive to etoposide and m-AMSA with IC50 values of 1.7 and 0.7 µM, respectively and MDA-MB-468 cells showed IC50 values of 13.6 and 8.5 µM for etoposide and m-AMSA, respectively, whereas MCF-7 cells were found to be the least sensitive with IC50 values of 35.6 and 21.7 µM for etoposide and m-AMSA, respectively. Most of the makaluvamine analogs have shown significantly better inhibition than the control drugs in these assays. Compounds (217c, 218d, 218f, and 218g)
Marine natural alkaloids as anticancer agents

exhibited better activity (IC$_{50}$ = 1.3, 0.5, 1.0 and 0.8 μM, respectively) against HCT-116 as compared to control drug etoposide (IC$_{50}$ = 1.7 μM). Compound 218d exhibited better IC$_{50}$ value against HCT-116 as compared to m-AMSA (IC$_{50}$ = 0.7 μM). All the compounds exhibited better IC$_{50}$ values against MCF-7 and MDA-MB-468 as compared to etoposide as well as m-AMSA. Compounds 217 (a-g) and 218 (c-g) were also evaluated for their ability to inhibit topoisomerase II enzymatic activity and found that five makaluvamine analogs (217c, 217d, 217f, 218c and 218e) exhibited inhibition of topoisomerase II comparable to etoposide and m-AMSA. Three of these compounds (217f, 218c and 218e) showed the strongest inhibition of catalytic activity of topoisomerase II.

In 1997, the methanol extract of the Fijian sponge Zyzzya fuliginosa yielded a new pyrroloiminoquinone derivative, veiutamine (219) [151]. The structure of the compound was determined by 1D and 2D NMR experiments and was found bearing a p-oxy benzyl substituent at carbon 6 of the basic pyrroloiminoquinone system. Veiutamine (219) exhibited cytotoxicity against the human colon tumor cell line HCT-116 with IC$_{50}$ value of 0.3 μg/mL. Wakayin (220) was isolated from the ascidian Clauelinus sp [152]. It was supposed to represent the first example of pyrroloiminoquinone alkaloid to be isolated from an ascidian. Wakayin (220) exhibited in vitro cytotoxicity against the human colon tumor cell line (HCT-116) with IC$_{50}$ value of 0.5 μg/mL. Preliminary studies such as Inhibition of topoisomerase II enzyme (250 μM) and the observation of a 3-fold differential toxicity toward the CHO cell line EM9 (sensitive to DNA-damaging genotoxic agents) versus BR16 (resistant to BCNU) provided evidences that the activity of wakayin could be related to interfering with or damaging DNA.
Two new bispyrroloiminoquinone alkaloids, tsitsikammamme A (221) and tsitsikammamine B (222) were isolated from the South African Latrunculid sponge *Tsitsikamma favus* [153]. Reinvestigation of the extracts of the sponge *Tsitsikamma favus* yielded two N-18 oxime analogues of tsitsikammamine A and B, 223 and 224 [154]. Compounds 223 and 224 exhibited significant cytotoxic activity against human colon tumor (HCT-116) cell line with IC\textsubscript{50} values of 128.2 and 16.5 μM, respectively, when compared with their parent alkaloids 221 and 222 (IC\textsubscript{50} = 1.4 and 2.4 mM, respectively).

Recently, two aza-analogs, 225 and 226 of tsitsikammamine and wakayin were synthesized based on a 1,3-dipolar cycloaddition reaction between indole 4,7-dione and a diazo-aminopropane derivative in which the pyrrole ring of the pyrroloquinoline moiety was replaced by a pyrazole ring [155]. The ability of the compounds 225 and 226 to inhibit the DNA cleavage activities of human topoisomerases I and II was assayed in a cell-free assay. Both the compounds exhibited 0% inhibition of topoisomerase II. Compound 225 inhibited partially topoisomerase I at 100 μM, whereas no inhibitory activity was observed for compound 226.

![Chemical Structures](image_url)

### 3.4. Pyrroloacridine

Two novel alkaloids, plakinidine A (227) and B (228) were isolated from Vanuatuan red sponge *Plakortis* sp. [156]. Their structures were determined by 1D and 2D NMR experiments and were found to contain a pyrrolo (2,3,4-\textit{k}-\textit{l}) acridine system. In the same year, IreIend et al. reported the isolation and structure elucidation of a new compound plakinidine C (229) together with plakinidine A (227) and B (228) from the MeOH extract of *Plakortis* sp. collected
in Fiji [157]. Plakinidine A-C (227-229) exhibited cytotoxic activity towards L-1210 murine leukemia cell lines with IC\textsubscript{50} values of 0.1, 0.3 and 0.7 \(\mu\text{g/mL}\), respectively.

\[
\begin{align*}
227, & \quad R = H \\
228, & \quad R = \text{CH}_3 \\
229, & \quad R = H, 9,10\text{-didehydro}
\end{align*}
\]

### 3.5. Miscellaneous pyrrole alkaloids

Ircinamine B (230) was isolated from the marine sponge \textit{Dactylia} sp. collected at Cape Sada in Japan and showed moderate cytotoxic activity against the murine leukemia cell line P-388 with IC\textsubscript{50} value of 0.28 \(\mu\text{g/mL}\) [158].

A novel tetracyclic alkaloid, perinadine A (231) was isolated from the cultured broth of the fungus \textit{Penicillium citrinum} separated from the gastrointestinal tract of a parrot fish \textit{Scalus ovifrons} collected at Hedo Cape, Okinawa Island [159]. Perinadine A (231) exhibited mild cytotoxicity against murine leukemia L-1210 cell line with IC\textsubscript{50} value of 20 \(\mu\text{g/mL}\).

In 1994, Perry \textit{et al.} isolated a new alkaloid, Variolin B (233) from the Antarctic sponge \textit{Kirkpatrickia varialosa}. The structure was determined by X-ray crystallography and interpretation of spectral data [160]. Variolin B (233) was supposed to be the first examples of natural products with a pyridopyrrolopyrimidine moiety. In the same year two other pyridopyrrolopyrimidine alkaloids, variolin A (232) and \(N(3')\)-methyl tetrahydrovariolin B (234) were isolated from the same sponge \textit{Kirkpatrickia varialosa} [161]. Variolins (232-234) were tested \textit{in vitro} against P-388 cell lines. Variolin A (232) and variolin B (233) showed \textit{in vitro} activity against P-388 cell lines with IC\textsubscript{50} value of 3.8 \(\text{ng/mL}\) and 210 \(\mu\text{g/mL}\), respectively. Compound 234 was found to be inactive against P-388 but showed \textit{in vivo} activity against P-388 leukemia (T/C 125% at 10 mg/Kg). Compound 234 also showed significant \textit{in vitro} activity against the HCT-116 cell line with IC\textsubscript{50} value of 0.48 \(\mu\text{g/mL}\).

Kashman \textit{et al.} isolated a novel bisquinolinylpyrrole alkaloids, halitulin (235) from a marine sponge \textit{Haliclona tulearensis} collected in Sodwana Bay, Durban, South Africa [162]. Its structure was established mainly on the basis of spectroscopic data and chemical means. Halitulin (235) was considered as the first natural
compound to be discovered that has a 7,8- dihydroxyquinoline system and found to be cytotoxic against several tumor cell lines such as P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma with IC$_{50}$ value of 0.025, 0.012, 0.012 and 0.025 µg/mL, respectively.

### 4. Pyridine alkaloids

In 1999, Kobayashi et al. isolated a novel pyridine alkaloid, pyrinodemin A (236) from the Okinawan marine sponge *Amphimedon* sp. [163]. The structure of compound 236 was assigned from 2D NMR data and EIMS fragmentation and was found to contain two 3-alkyl-substituted pyridine rings with a cis-cyclopent[c]isoxazolidine moiety. Pyrinodemin A (236) demonstrated potent cytotoxicity *in vitro* against murine leukemia L-1210 and KB epidermoid carcinoma cells with IC$_{50}$ values of 0.058 and 0.5 µg/mL, respectively. One year later, three new bis-pyridine alkaloids, pyrinodemins B-D (237-239) were isolated together with pyrinodemin A (236) from the same sponge *Amphimedon* sp. [164]. Pyrinodemins B-D (237-239) exhibited potent cytotoxicity *in vitro* against murine leukemia L-1210 with IC$_{50}$ values of 0.07, 0.06 and 0.08 µg/mL, respectively and KB epidermoid carcinoma cells (IC$_{50}$ = 0.5 µg/mL each).
A novel pyridine alkaloid, pyrinadine A (240) was isolated from the marine sponge *Cribrochalina* sp. collected from the Unten Port, Okinawa [165]. The structure was established by spectroscopic data and chemical conversions. When treated with zinc/acetic acid, pyrinadine A yielded compound (241), generated by cleavage at the azoxy moiety of pyrinadine A. Pyrinadine A (240) exhibited *in vitro* cytotoxicity against L-1210 murine leukemia (IC$_{50}$ = 2 $\mu$g/mL) and KB human epidermoid carcinoma cells (IC$_{50}$ = 1 $\mu$g/mL).

In 2006, Takekawa *et al.* reported the isolation of amphimedosides A-E (242-246) from a marine sponge *Amphimedon* sp. [166]. The structures of compounds 242-246 were determined by NMR, FABMS data interpretation. The site of glycosylation in compound 242 was confirmed by the $^1$H-$^{15}$N HMBC experiment and the location of the double bond in 246 was assigned on the basis of tandem FABMS data. Amphimedosides (242-246) were the first examples of $\beta$-D-glucosylated 3-alkylpyridine alkaloids till the date and exhibited mild to strong cytotoxicity against P-388 murine leukemia cells with IC$_{50}$ values of 11, 11, 5.0, 0.45 and 2.2 $\mu$g/mL, respectively.
Echinoclathrines A-C (247-249), a new class of pyridine alkaloids having 4-aryl-2-methylpyridine unit, were isolated from an Okinawan sponge, *Echinoclathria* sp. [167]. The structures of compounds were established by interpretation of spectral data. Only echinoclathrine A (247) displayed weak cytotoxicity (IC\(_{50}\) = 10 µg/mL) against P-388, A-549 and HT-29 cell lines, while others were found to be inactive.

5. Isoquinoline alkaloids

Two new isoquinolinequinones alkaloids, cribrostatins 1 (250) and 2 (251) were isolated from a deep blue colored sponge *Cribrochalina* sp. [168]. The structures of the compounds were determined by extensive NMR data analysis and single-crystal X-ray diffraction experiment. Cribrostatins 1 and 2 were found to be active against lymphocytic leukemia cell line (P-388) with ED\(_{50}\) values of 1.58 and 2.73 µg/mL, respectively. Pettit *et al.* reported the isolation of cribrostatins 3 (252), 4 (253) and 5...
Marine natural alkaloids as anticancer agents

(254) from the same sponge Cribrochalina sp. [169]. Compounds 251-254 were evaluated for cytotoxicity against several cancer cell lines. Mouse leukemia P-388 cell line was found to be the most sensitive to Cribrostatins 3 (252), 4 (253) and 5 (254) exhibiting with ED50 values of 2.5, 2.2 and 0.045 µg/mL, respectively.

Cribrostatin 6 (255) was also isolated from the same marine sponge Cribrochalina sp. [170]. The structure of compound was assigned on the basis of 1H, 13C, 15N NMR and HRMS data interpretation and finally structure was confirmed by X-ray crystal data analysis. Cribrostatin 6 (255) was found to inhibit the growth of murine P-388 lymphocytic leukemia (GI50 = 0.29 µg/mL) and a panel of human cancer cell lines. Among human cancer cell lines, the best activity in terms of potency was obtained against MCF-7 (GI50 = 0.21) followed by SF-268 (GI50 = 0.24) and DU-145 (GI50 = 0.38), whereas GI50 value of >1µg/mL was observed against BXPC-3, NCI-H460 and KM20L2 cell lines.

A new isoquinoline alkaloid, jorumycin (256) was isolated from the mantle and the mucus of the pacific nudibranch Jorunna funebris [171]. The structure of compound was established on the basis of ESIMS data and of an extensive 2D NMR analysis. Jorumycin (256) showed very interesting activity against NIH 3T3 tumor cells (100% of inhibition at 50 ng/mL) and also exhibited promising cytotoxic activity against P-388, A-549, HT-29 and MEL-28 with IC50 value of 12.5 µg/mL each.

6. Guanidine alkaloids

In 1989, Kashman et al. reported the isolation of a novel guanidine alkaloid ptilomycalin A (257) from the Caribbean sponge Ptilocaulis spiculifer and the red sea sponge Hemimycale sp. [172]. Ptilomycalin A (257) consists of a pentacyclic guanidine unit and a spermidine unit linked by a linear long-chain fatty acid.
Ptilomycalin A (257) exhibited significant cytotoxic activity against P-388, L-1210 and KB cell lines with IC₅₀ values of 0.1, 0.4 and 1.3 mM, respectively.

Recently, Black et al. synthesized three novel analogues, 258, 259 and 260 of ptilomycalin A (257) [173]. Compounds 258-260 were tested against four cancer cell lines including human chronic myelogenous leukaemia (K-562), human ovarian carcinoma (A-2780), human large cell carcinoma (H-460) and mouse lymphoid neoplasm (P-388). Compound 258 showed the best activity against all the cell lines comparable to the parent compound (257). The IC₅₀ values of 0.52, 0.92, 0.52 and 0.69 µg/mL were obtained against K-562, A-2780, H-460 and P-388, respectively for compound 258, whereas compound 259 was found to be less potent than compound 258. Compound 260 was the least active compound of the three, which indicated that the presences of a spacer chain and spermidine residue are essential for the compounds to demonstrate the biological activity.
Seven new tricyclic guanidine alkaloids, netamines A-G (261-267) were isolated from the extract of the poeciloscleridae sponge *Biema laboutei* collected near the Sainte-Marie Island on the east coast of Madagascar [174]. The structures of compounds were determined on the basis of 1D, 2D NMR and HRFABMS data interpretation. All the compounds 261-267 were evaluated for cytotoxicity against three human tumor cell lines: NSCL (A-549), colon (HT-29) and breast (MDA-MB-231). Only netamines C (263) and D (264) showed promising activity against A549 (GI₅₀ = 4.3 and 6.6 µM) HT29 (GI₅₀ = 2.4 and 5.3 µM) and MDA-MB-231 (GI₅₀ = 2.6 and 6.3 µM), whereas other compounds were found to be inactive or very less toxic.

7. Aminoimidazole alkaloids

Ralifo *et al.* reported the isolation and structure elucidation of two novel alkaloids, leucosolenamines A (268) and B (269) from the marine sponge *Leucosolenia* sp. [175]. Compound 268 was found to contain a 2-aminoimidazole unit substituted at C-4 and C-5 by an N,N-dimethyl-5,6-diaminopyrimidine-2,4-dione and a benzyl group, respectively. Although, compound 269 has the same core structure but C-4 is substituted by a 5,6-diamino-1,3-dimethyl-4-(methylimino)-3,4-dihydropyrimidin-2(1H)-one moiety. This substitution pattern is unique and had never been observed in imidazole alkaloid chemistry. Leucosolenamine A (268) exhibited mild cytotoxicity against the murine colon adenocarcinoma C-38 cell line, whereas compound 269 was inactive. In the same year the other group isolated two new imidazole alkaloids, naamidines H (270) and I (271) from the marine sponge *Leucetta chagosensis* collected in North Sulawesi, Indonesia [176]. The compounds 270 and 271 demonstrated weak cytotoxicity against HeLa cells with IC₅₀ values of 5.6 and 15 µg/mL, respectively.
8. Steroidal alkaloids

Four novel steroidal alkaloids, plakinamine G (272), plakinamine H (273), 4R-hydroxydemethylplakinamine B (274) and tetrahydropakinamine A (275) were isolated from the marine sponge *Corticia* sp. [177]. The structures of these compounds were established spectroscopically mainly by 1D, 2D NMR and mass spectrometry (HR-EIMS). Compounds 272-275 were tested for cytotoxicity against rat glioma (C6) and murine macrophages (RAW-264) cell lines. Compounds 272 and 275 found to be the most active against C6 cells with IC$_{50}$ values of 6.8 and 1.4 µg/mL, respectively, whereas they showed no activity against RAW-264 cell line. Compounds 273 and 274 were cytotoxic against both the cell lines with compound 273 being more active against C6 cells (IC$_{50}$ = 9.0 µg/mL) than to RAW-264 (IC$_{50}$ = 61 µg/mL), while compound 274 showed greater value of IC$_{50}$ (16.2 µg/mL) against RAW-264 cell line than to C6 cells (IC$_{50}$ = 26.1 µg/mL). One year later four new related steroidal alkaloids, plakinamine I-K (276-278) and dihydroplakinamine K (279) were isolated from the same sponge *Corticia* niger [178]. Compounds (276-279) as their hydrochloride salts were evaluated for cytotoxicity against the human colon tumor cell line (HCT-116). Compounds 278 and 279 were found to be the most active in terms of potency with an IC$_{50}$ value of 1.4 µM each. Compounds 276 and 277 were moderately active with IC$_{50}$ values of 10.6 and 6.1 µM, respectively.

Ritterazines B (280) and C (281), two dimeric steroidal alkaloids were isolated from the tunicate *Ritterella tokioka* collected off the Izu Peninsula [179]. Their structures including absolute stereochemistry were assigned by spectral and chemical methods. Ritterazines B (280) and C (281) displayed potent cytotoxicity against the P-388 murine leukemia cells with IC$_{50}$ values of 0.018 and 9.4 ng/mL, respectively.

Three novel steroidal alkaloids, cortistatins J-L (282-284) were isolated from the Indonesian marine sponge *Corticia simplex* [180]. The structures of compounds 282-284 were established by 1D and 2D NMR (COSY, HMQC and HMBC) data analysis. Cortistatin J (282) demonstrated potent cytostatic anti-proliferative activity
against human umbilical vein endothelial cells (HUVEC) with IC$_{50}$ value of 8 nM and also inhibited migration and tubular formation of HUVEC induced by VEGF or bFGF, whereas cortistatins K (283) and L (284) were less potent than cortistatin J (282) with IC$_{50}$ values of 40 and 23 nM, respectively.
9. Miscellaneous alkaloids

Four novel alkaloids 285-288, related to aaptamines were isolated from the MeOH extract of the Indonesian marine sponge *Xestospongia* sp. collected from Jakarta along with the known aaptamine (289), iso-aaptamine (290), demethyl(oxy)aaptamine (291) and its dimethylketal (292) [181]. Their structures were determined on the basis of 1D and 2D NMR spectroscopic data. All the compounds 285-292 were evaluated for cytotoxic activity against KB cell lines. Compounds (289-292) exhibited moderate cytotoxicity against KB cells with ID$_{50}$ values of 3.7, 0.5, 1.8 and 3.5, respectively, while compounds (285-288) were less potent with ID$_{50}$ value of >10 µg/mL.

Four tetracyclic alkyl-piperidine alkaloids, Halichlonacyclamie E (293) arenosclerins A (294), B (295) and C (296) were isolated from the marine sponge *Arenosclera brasiliensis* [182]. All the compounds were tested for their cytotoxicity against HL-60, B-16, U-138 and L-929 cancer cell lines. Compound 293-296 exhibited almost the same range of cytotoxicity with IC$_{50}$ values of 4.23, 4.31, 4.07, 3.65 (HL-60), 1.82, 1.77, 1.76, 1.71 (B-16), 6.06, 3.83, 3.62, 3.60 (U-138) and values of 3.89, 2.34, 2.24, 2.17 (L-929), respectively.

Four bis-piperidine alkaloids, madangamine F (297), halichlonacyclamine F (298), arenosclerins D (299) and E (300) were isolated from the marine sponge *Pachychalina alcoidifera* [183]. Compounds 297-300 were evaluated for cytotoxicity against SF-295 (human CNS), MDA-MB-435 (human breast), HCT-8 (colon) and HL-60 (leukemia) cancer cell lines. Halichlonacyclamine F (298) and arenosclerin D (299) were found to be the most active compounds with IC$_{50}$ values of 4.5 and 5.9 µg/mL (SF-295), 1.0 and 1.2 µg/mL (MDA-MB-435), 8.6 and 6.2 µg/mL (HCT-8), 2.2 and 6.2 µg/mL (HL-60), whereas compounds 297
and 300 showed IC<sub>50</sub> values of 19.8 and 8.7 µg/mL (SF-295), 16.2 and 3.1 µg/mL (MDA-MB-435), 16.7 and 6.9 µg/mL (HL-60), >25 and >25 µg/mL (HCT-8) cell lines.

Matsunaga et al. reported the isolation and structure determination of two new 3-alkylpiperidine alkaloids, tetradehydrohalicyclamine A (302) and 22 hydroxyhalicyclamine A (303) along with a known halicyclamine A (301) from a marine sponge *Amphimedon* sp. collected in southern Japan [184]. Compounds 301, 302 and 303 were found to be cytotoxic against P-388 cells with IC<sub>50</sub> values of 0.45, 2.2 and 0.45 µg/mL, respectively.

Three new diketopiperazine alkaloids, 6-methoxyspirotryprostatin B (304), 18-oxotryprostatin A (305) and 14-hydroxyterezine D (306) along with other metabolites were isolated from the ethyl acetate extract of a marine-derived fungal strain *Aspergillus sydowi* [185]. All the compounds were evaluated for Cytotoxicity against A-549 and HL-60 cell lines. Compounds 304-306 exhibited weak cytotoxicity against A-549 cells with IC<sub>50</sub> values of 8.29, 1.28 and 7.31 µM, respectively. In addition, compound 304 also demonstrated significant cytotoxicity against HL-60 cells with an IC<sub>50</sub> value of 9.71 µM.

Two novel pyrazine alkaloids botryllazine A (307) and botryllazine B (308) along with the new imidazole alkaloid 2(p-hydroxybenzoyl)-4-(p-hydroxyphenyl)-imidazole (309) were isolated from the red ascidian *Botryllus leachi* [186]. The structures of compounds 307-309 were elucidated by interpretation of spectral data and botryllazine A (307) was supposed to represents the first example of a marine alkaloid containing a pyrazine nucleus derived from three tyrosine precursors. All the compounds (307-309) were tested in vitro for cytotoxicity against P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma. Botryllazine A (307) was inactive with ED<sub>50</sub> value of 10 µg/mL each.
Botryllazine B (308) exhibited weak cytotoxicity ($ED_{50} = 5 \mu g/mL$) against A-549 and MEL-28 cell lines, whereas compound 309 was mildly active against all the four tumor cell lines with $ED_{50}$ of $5 \mu g/mL$.

Kobayashi et al. isolated novel bromotyrosine alkaloids, maedamines A (310) and B (311) from Okinawan marine sponge Suberea sp. [187]. Structures were elucidated on the basis of spectroscopic data and these compounds were found containing a $2(1H)$-pyrazinone moiety between two bromotyrosine units. Maedamines A (310) and B (311) exhibited in vitro cytotoxicity against murine leukemia L-1210 cells with $IC_{50}$ values of 4.3 and 3.9 $\mu g/mL$, respectively and epidermoid carcinoma KB cells with $IC_{50}$ values of 5.2 and 4.5 $\mu g/mL$, respectively. Maedamine A (310) also demonstrated inhibitory activity against c-erbB-2 kinase with $IC_{50}$ value of 6.7 $\mu g/mL$, while compound 311 being inactive against c-erbB-2 kinase ($IC_{50} >10 \mu g/mL$).
Two new bromotyrosine alkaloids, purealidin S (312) and purpuramine J (313) were isolated from the Fijian marine sponge *Druinella* sp. [188]. Compound 313 contains a bromotyrosine N-oxide unit which is very rarerly found in marine natural products. Both the compounds were tested for cytotoxicity against A-2780 (Ovarian tumor) and K-562 (leukaemia) cell lines. Compounds 312 and 313 showed mild cytotoxicity against these two cell lines with IC$_{50}$ values of 7.44 and 6.77 µg/mL (A-2780) and values of 6.02 and 1.24 µg/mL (K-562), respectively.

Two new dimeric polysulfide alkaloids, lissoclinotoxins E (314) and F (315) were isolated from the MeOH extract of a Philippine didemnid ascidian [189]. The polysulfide structures for compounds 314 and 315 were determined by interpretation of spectroscopic data and chemical means. Computational chemistry studies suggested the trans- and cis- orientations of N-alkyl chains about the tricyclic systems of lissoclinotoxins E (314) and F (315), respectively. Compounds 314 and 315 exhibited significant cytotoxicity against PTEN-deficient human breast carcinoma, MDA-MB-468 cell lines with IC$_{50}$ values of 2.3 and 1.5 µg/mL, respectively.

Williams *et al.* isolated motuporamines A-C (316-318) from the marine sponge *Xestospongia exigua* [190]. The crude mixtures of motuporamines A-C could not readily be separated and they were obtained as a mixture of three (316-318). The mixture of motuporamines A-C (316-318) showed significant cytotoxicity against a panel of human solid tumor cancer cell lines with IC$_{50}$ value of 0.6 µg/mL.
Two novel alkaloids, pterocellins A (319) and B (320) were isolated from the New Zealand marine bryozoans *Pterocella vesiculosa* [191]. The structures were assigned by NMR and mass spectral data analysis and finally structure was confirmed by single-crystal X-ray diffraction experiments. Pterocellins A (319) and B (320) were evaluated for cytotoxicity against P-388 murine leukemia cell lines and exhibited relatively potent activity with IC$_{50}$ values of 477 and 323 ng/mL, respectively.

The cytotoxicity of pterocellins A (319) and B (320) was also evaluated by the NCI in their 60 cell line panel, which represents a variety of human tumor cell types such as leukemia, non-small cell lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate and breast cancers. Compounds 319
and 320 exhibited potent cytotoxicity with panel average values of GI₅₀ = 1.4 μM, TGI = 4.8 μM, LC₅₀ = 17.0 μM for pterocellin A (319) and GI₅₀ = 0.7 μM, TGI = 2.1 μM, LC₅₀ = 6.9 μM for pterocellin B (320). The leukemia cell line (CCRF-CEM) was found to be the most sensitive cell line to pterocellin A (319) with GI₅₀ value of 0.05 μM and TGI value of 0.8 μM, although the high LC₅₀ value of >100 μM implied that pterocellin A (319) is cytostatic rather than cytotoxic to this cell line. The most sensitive cell line to pterocellin B (320) was the melanoma cell line MALME-3M with GI₅₀ value of 0.03 μM and TGI value of 0.1 μM, whereas cell lines such as NCI-H23, melanoma MALME-3M, M14, SK-MEL-5, breast MDA-MB-435 and MDA-N were found to be sensitive to both the compounds.

References
Marine natural alkaloids as anticancer agents


