Novel Antitumor Agents: Marine Sponge Alkaloids, their Synthetic Analogs and Derivatives

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Abstract: Present review describes research on novel natural antitumor agents isolated from marine sponges. More than 90 novel cytotoxic antitumor compounds and their synthetic analogs have shown confirmed activity *in vitro* tumor cell lines bioassay and are of current interest to NCI for further *in vivo* evaluation. A great problem, to use directly the reservoir of marine organisms for therapy is the very low availability and the isolation of only very small amounts of the biologically active substances from the natural materials. Thus, the synthetic chemistry is required to develop high yield synthetic methods, which are able to produce sufficient marine alkaloids for a broad biological screening. This review will present some of the aspects of the medicinal chemistry developed recently to introduce such modifications. The structures, origins, synthesis and biological activity of a selection of N-heterocyclic marine sponge alkaloids are reviewed. The emphasis is on compounds poised as potential anticancer drugs: pyrroles, pyrazines, imidazole, and other structural families. With computer program PASS some additional biological activities are also predicted, which point toward new possible applications of these compounds. This review emphasizes the role of marine sponge alkaloids as an important source of leads for drug discovery.

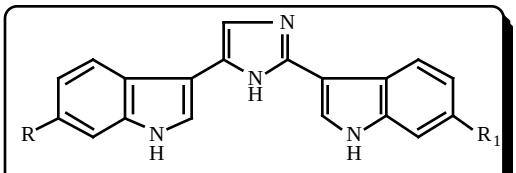
Keywords: Marine, sponge, alkaloids, antitumor, novel, prediction, activity.

1. STRUCTURE, BIOLOGICAL ACTIVITY AND SYNTHESIS

Sponges, belonging to type Porifera are an early branching event in the history of animals and separated the sponges from other metazoans [1]. As one would expect based on their phylogenetic position, fossil sponges are among the oldest known animal fossils, dating from the Late Precambrian. The approximately 5000 living sponge species are classified in the phylum Porifera, which is composed of three distinct groups: the Hexactinellida (glass sponges), the Demospongiae, and the Calcarea (calcareous sponges) [1-3]. Since 1950, over 3000 papers have been published reporting that the structures of more than 5500 metabolites, including more than 2000 contains nitrogen, unique to marine sponge species only few of which show high biological activity (see recent reviews, [1-9] and herein references cited).

Nortopsentins A **1**, B **2**, and C **3**, having a characteristic 2, 4-bis(3-indolyl)imidazole skeleton, were isolated from the deep water marine sponge *Spongsorites ruetzleri*. Nortopsentins A-C exhibited *in vitro* cytotoxicity against P388 cell: IC_{50} (μg/ml), 7.6, 7.8 and 1.7 respectively. [10-13].

Novel indolylthiazole compounds **4** – **13** analogs (Scheme **1**) of Nortopsentins were synthesized and evaluated for cytotoxicity in the NCI's *in vitro* disease-oriented antitumor screen against approximately a panel of 60 human tumor cell lines derived from leukemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. The results are shown in Table **1** as GI_{50} values. All compounds **4** – **13** exhibited cytotoxic activities against a variety of human cancer cell lines. The compound **4** selectively exhibited *in vitro* cytotoxicities against leukemia and ovarian cancer cell lines, affording GI_{50} of 3.27 μM in K562, 5.31 μM in Molt-4 and 8.14 μM in IGROV1 assay. In the other human tumor cell line assay, the GI_{50} of compound **4** exceeded 100 μM. To test the possibility that substitutes in the indole ring might result in a potency increase, most of the 2, 4-bis(3-substituted-indolyl)thiazoles showed broad effects on tumor cell lines from the leukemia, colon cancer, CNS cancer and breast cancer panels while unsubstituted counterpart **4** brought out highly selective activity against leukemia cell lines and IGROV1 ovarian cancer cell lines. The position of bromine in the indole ring plays an important role in cytotoxicity. Dibrominated compound **13** effectively exhibited MCF **4** of breast cancer, affording the GI_{50} of 0.888 μM. [14].



1 Nortopsentin A R = R_1 = Br
2 Nortopsentin B R = Br, R_1 = H
3 Nortopsentin C R = H, R_1 = Br

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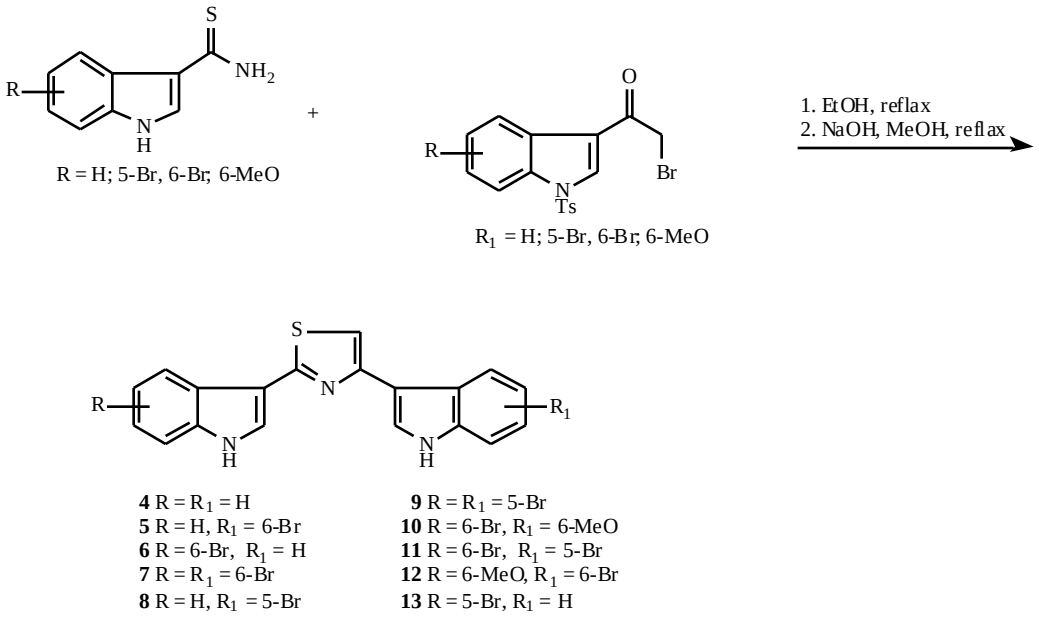
Table 1. Inhibition of *in Vitro* Tumor Cell Growth by Synthetic Analogs of Nortopsentins

Cell line	Cytotoxicity (*GI_{50} in μM)									
	4	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Leukemia										
CCRF-CEM	ND	14.6	10.9	10.1	2.11	2.40	27.7	2.58	2.66	2.99
HL-60(TB)	ND	ND	ND	0.95	2.43	2.76	ND	3.76	4.13	3.86
K-562	3.27	18.8	5.61	4.69	1.96	1.94	15.2	2.13	1.74	2.15
MOLT-4	5.31	19.9	31.2	5.80	1.41	1.75	23.0	1.55	2.95	2.26
RPMI8226	ND	19.4	12.2	11.4	1.97	1.95	27.1	2.24	2.03	1.84
Non-small cell lung cancer										
NCI-226	>100	14.4	24.4	3.3	2.10	3.14	45.4	2.48	2.24	7.23
NCIH322M	>100	16.7	18.9	18.4	2.01	1.99	70.8	2.51	3.09	2.99
NCI-H460	>100	16.1	7.31	16.4	2.55	1.93	23.2	2.31	1.58	2.00
EKVX	>100	15.6	29.5	28.6	ND	ND	32.1	0.48	0.29	0.55
Colon cancer										
HCT-15	>100	15.2	17.8	8.5	1.81	2.51	7.54	2.70	0.81	2.84
SW-620	>100	16.5	25.6	12.5	1.52	2.14	7.00	3.57	5.50	2.89
CNS cancer										
SF-295	33.6	14.6	9.23	4.81	1.98	2.71	73.5	4.56	ND	ND
SF-268	ND	18.3	32.1	ND	1.52	2.44	26.0	2.69	13.8	1.80
SNB-19	>100	17.8	41.9	17.2	2.11	3.75	>100	2.60	10.5	3.34
U21	>100	17.9	28.1	15.3	2.10	2.30	25.0	3.34	5.07	3.00
Ovarian cancer										
IGROV1	8.14	13.0	30.5	14.4	1.85	1.70	81.5	2.96	4.61	2.43
OVCAR-5	>100	16.1	37.1	23.4	1.96	2.14	42.7	2.16	3.44	2.35
Renal cancer										
786-0	>100	18.0	19.9	15.9	2.28	1.95	27.4	1.50	3.89	2.21
A498	>100	17.0	25.7	23.2	1.92	2.48	89.4	2.19	7.43	2.40
RXF 393	ND	22.4	7.6	18.4	1.81	1.69	11.9	2.42	2.03	1.66
Prostate cancer										
PC-3	>100	15.6	16.9	15.3	2.02	2.57	10.4	4.16	3.49	2.81
DU-145	>100	18.8	14.9	18.4	2.29	2.61	>100	3.74	5.46	2.03
Breast cancer										
MCF7	>100	16.7	27.2	6.5	2.13	2.70	54.1	0.88	4.36	3.82
MDAMB435	33.1	14.9	25.6	4.3	2.53	2.27	7.70	4.54	14.6	3.97
MDA-N	83.0	19.2	31.6	2.9	1.88	2.09	8.27	2.86	6.84	3.77
T-47D	>100	24.8	23.9	16.2	3.27	2.90	59.3	3.33	4.12	1.76
BT-549	>100	18.3	73.8	41.1	2.71	10.6	67.3	1.24	1.46	1.41
Melanoma										
LOX IWVI	21.8	15.5	6.5	11.3	1.97	1.69	64.1	2.32	2.41	1.87
MALME-3M	>100	16.9	>100	ND	1.28	1.72	17.5	1.45	1.50	2.83
M14	>100	14.2	32.4	10.6	1.61	1.63	>100	2.86	5.49	2.77
SK-MEL-2	>100	ND	37.5	68.8	1.81	1.98	19.1	3.25	7.30	2.91
SK-MEL-28	>100	14.2	7.7	32.0	3.02	3.05	>100	9.84	1.36	4.96
SK-MEL-5	ND	14.9	23.7	28.5	1.63	1.63	>100	2.82	5.13	3.36

*__Explanation for units activity__. The confidence intervals of best-fit values provided by nonlinear regression will be wide if you have not collected data over a wide enough range of X values to fully define the curve. One example is a sigmoid dose-response curve with no data defining the top and bottom plateau. When these data were fit to a sigmoid dose-response curve, the 95% confidence interval for the EC50 extended over fifteen orders of magnitude. The explanation is simple. The data were fit to a sigmoid equation with four variables: the top plateau, the bottom plateau, the slope, and the EC50 (the log (Dose) when response=50%). But the data do not form plateaus at either the top or the bottom, so the program is unable to fit unique values for the plateaus. The information is simply not in the data. Since the data do not define zero and one hundred, many curves (defined by different sets of parameters) would fit these data with similar sum of squares values. The 50 percent of: growth-inhibiting (GI_{50}); cytostatic (TGI_{50}); cytotoxic activity (LC_{50}), and inhibitory activity (IC50 or ED_{50}).



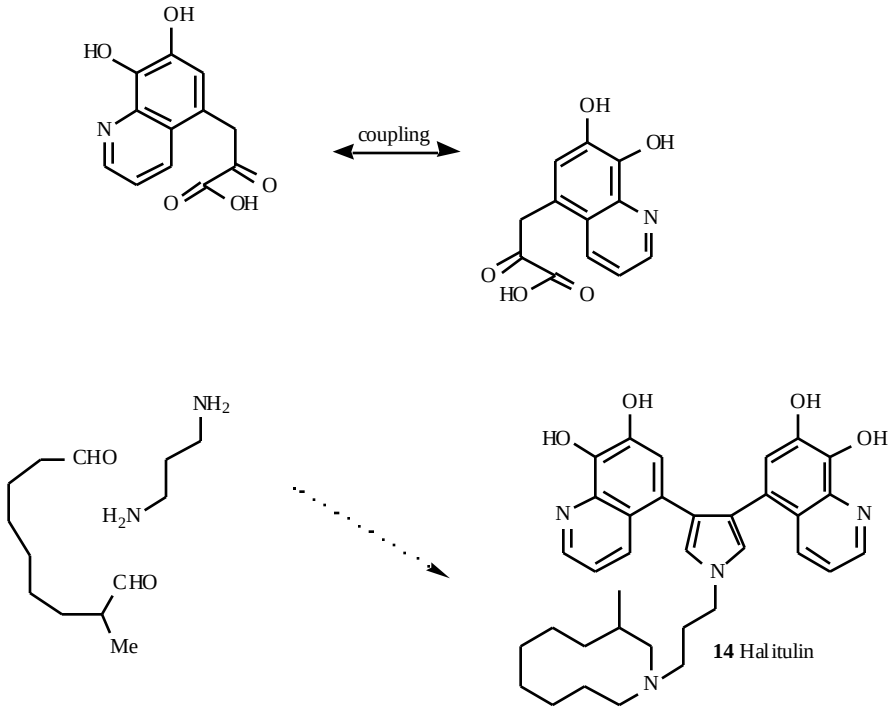
4 R = R_1 = H
5 R = H, R_1 = 6-Br
6 R = 6-Br, R_1 = H
7 R = R_1 = 6-Br
8 R = H, R_1 = 5-Br
9 R = R_1 = 5-Br
10 R = 6-Br, R_1 = 6-MeO
11 R = 6-Br, R_1 = 5-Br
12 R = 6-MeO, R_1 = 6-Br
13 R = 5-Br, R_1 = H

Scheme 1.

Halitulin **14**, a novel bisquinolinylpyrrole has been isolated from the sponge *Haliclona tulearensis*, and it found to have cytotoxic activity. The activity, IC_{50} values, against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma is 0.025, 0.012, 0.012 and 0.025 μg/ml respectively [15]. The two phenyl-C3 units of the latter's biogenic precursors, are suggested in this case, to be replaced by two 5-substituted-quinoline-C3 compounds, which subsequently will undergo decarboxylation. There does not seem to be a simple way to suggest the biogenesis of the latter C9N-C3 unit (Scheme **2**).

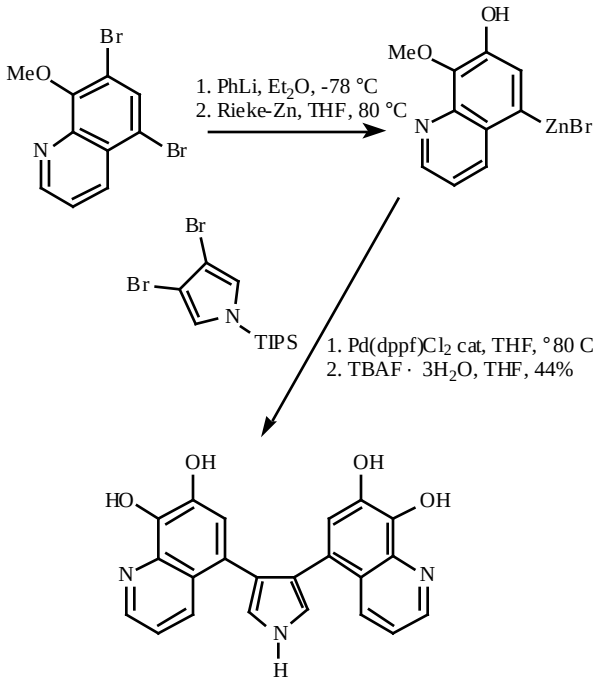
Palladium catalyzed Suzuki- and Negishi cross coupling reactions were used for synthesis of Halitulin core **15** (Scheme **3**) [16].

Motuporamines A-C **16**-**18**, cytotoxic alkaloids isolated from the marine sponge *Xestospongia exigua* (Kirkpatrick) [17]. These cytotoxic compounds **16**-**18** are the first example of a new family of macrocyclic alkaloids. Bioassay-guided fractionation of the *X. exigua* extracts led to the isolation of a mixture of motuporamines A **16**, B **17**, and C **18** along with known members of the petrosin and xestospongin/araguspongine class of 3-alkylpiperidine alkaloids. The motuporamines, which contain a spermidine-



Scheme 2.

like substructure, represent a new family of cytotoxic sponge alkaloids that appear to be biogenetically derived from the same basic building blocks, ammonia, acrolein, and a long-chain dialdehyde, involved in the Baldwin/Whitehead pathway to the 3-alkylpiperidine alkaloids isolated from marine sponges in the order Haplosclerida. The mixture of underivatized motuporamines showed modest *in vitro* cytotoxicity with a mean IC_{50} of 0.6 μg/ml against a panel of human solid tumor cancer cell lines. [18, 19].



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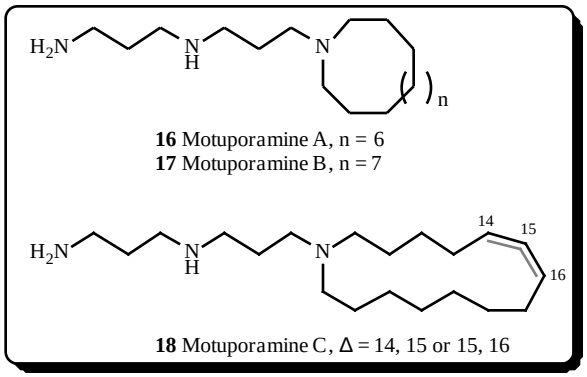
Scheme 3.

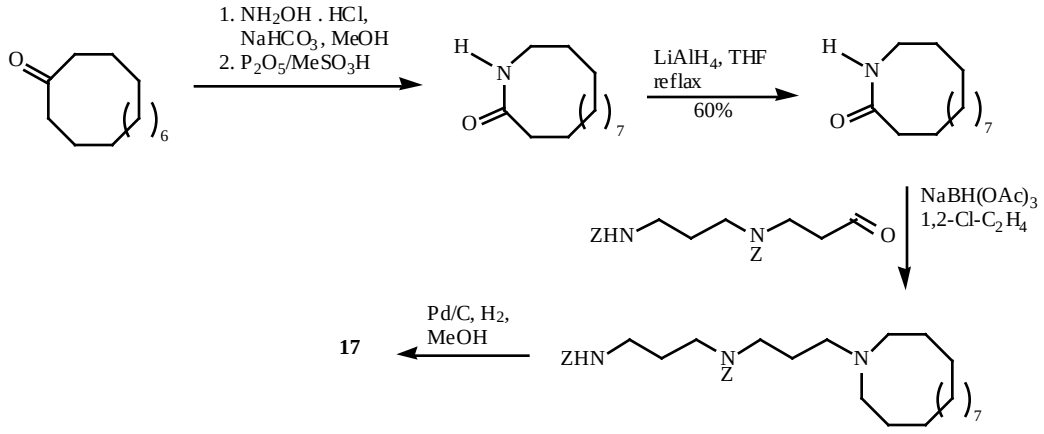
Two new cytotoxic bromotyrosine alkaloids, ma'edamines A **19** and B **20**, with unique 2(1H)pyrazinone ring have been isolated from the Okinawan marine sponge *Suberea* sp. [20]. Ma'edamines A **19** and B **20** are unique bromotyrosine alkaloids with a 2(1H)pyrazinone moiety between two bromotyrosine units. Biogenetically, ma'edamines A and B may be generated from 11, 12-dehydro form of aplysamine-2 or purpuramine H through formation of a 6-membered ring and dehydroxylation. Ma'edamines A **19** and B **20** exhibited cytotoxicity against murine leukemia L1210 cells (IC_{50}, 4.3 and 3.9 μg/ml, respectively) and epidermoid carcinoma KB cells (IC_{50}, 5.2 and 4.5 μg/ml, respectively) *in vitro*. Ma'edamine A **19** showed inhibitory activity against c-erbB-2 kinase *in vitro* with IC_{50} value of 6.7 μg/ml, while compound **20** did not show such activity (IC_{50} >10 μg/ml).



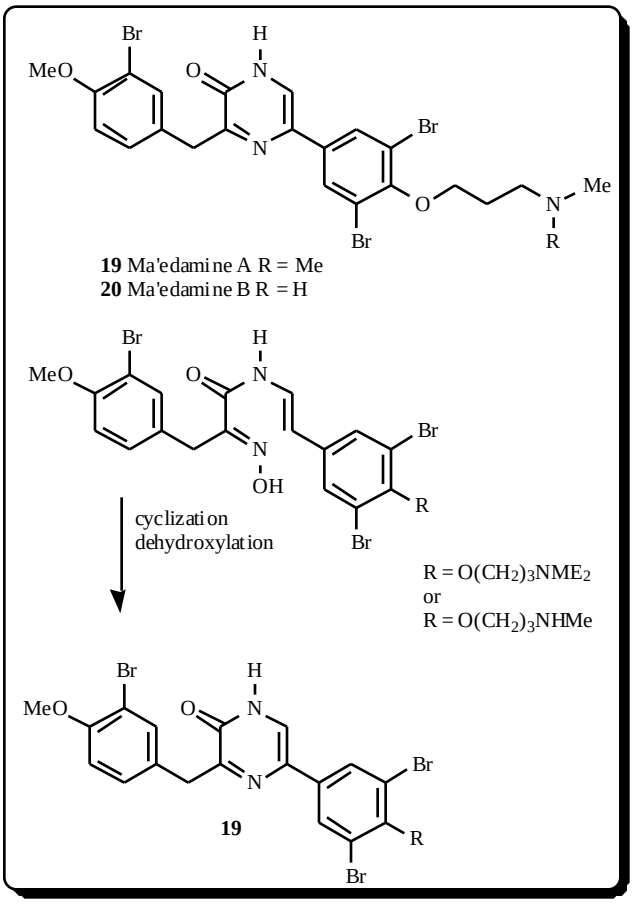
Total synthesis of motuporamines A and B **16** and **17** (Scheme **4**) have been reported recently [18].

A crude extract of a marine sponge designated PAL93-055 collected in Palau showed initial inhibitory bioactivities in a yeast assay for inhibitors of methionine aminopeptidase-2 (Met AP-2). Two isolated compounds **21** and **22** show cytotoxic activity against HeLa and B16F10 cell lines, consistent with previous reports for other alkaloids of the manzamine class. The greatest potency (IC_{50} 0.1 μg/ml) was observed for N-methyl-*epi*-manzamine D **21** against the B16F10 cell line [21].

Novel hexacyclic quaternary alkaloid, thorectandramine **23**, as well as fascaplysin **24** have been isolated from a Palauan sponge of the genus *Thorectandra* [22]. Both compounds **23** and **24** were tested for *in vitro* cytotoxicity against four human tumor cell lines, MALME-3M (melanoma), MCF-7 (breast), OVCAR-3 (ovarian) and A549 (non-small lung cell cancer) [23]. Thorectandramine **13** was only weakly active in MCF-7, OVCAR-3 and A549 cell lines (EC_{50} 27.0–55.0 μg/ml), but fascaplysin **24** was potently cytotoxic to all the cell lines tested: MALME-3M (EC_{50} 0.03 μg/ml), MCF-7 (EC_{50} 0.14 μg/ml), OVCAR-3 (EC_{50} 0.16 μg/ml and A549 (EC_{50} 0.38 μg/ml).

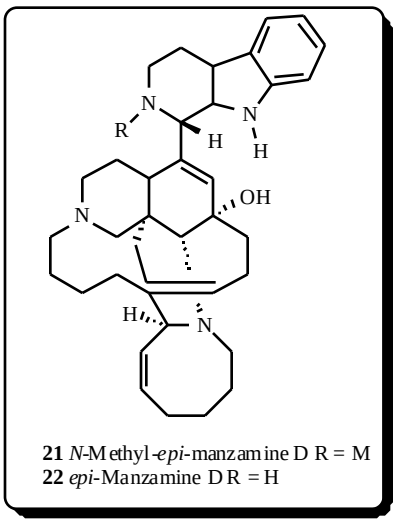


Scheme 4.

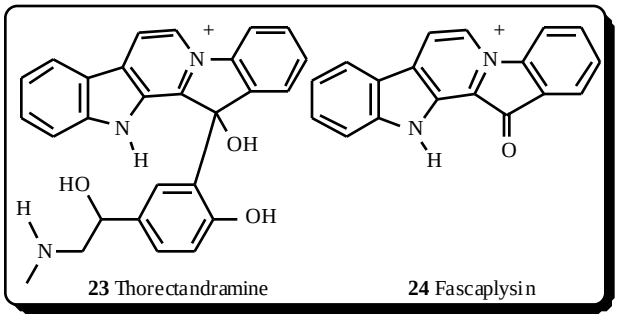


19 Ma'edamine A R = Me
20 Ma'edamine B R = H

Fascaplysin **24** is a planar fused-pentacyclic alkaloid was first isolated from a sponge, *Fascaplysinopsis* sp. [24]. Recently, other members of fascaplysin and the reticulatine **25** families were isolated from the sponge *Fascaplysinopsis reticulata* and two collections of the tunicate *Didemnum* sp. [25]: three new compounds: 3-bromofascaplysin **25**, 14-bromoreticulatine **27**, and 14-bromoreticulatate **29** along with reticulatate **28** previously known as a semi-synthetic product of **24**. Compounds **24** and **28** showed selectivity in a cell based cytotoxicity assay. Fascaplysin **24** exhibits a broad range of bioactivities including antibacterial, antifungal, antiviral, HIV-1-RT, p56 tyrosine kinase, antimalarial, potency to numerous cancer cell lines [26-31]. Compounds **24**, **25**, **26**-**29** were screened *in vitro* for solid tumor selectivity against a panel of human and murine tumor cells [32, 33]. Solid tumor selectivity is defined as a differential in kill zone units equal to or greater than 250 between any solid tumor cell line and either a normal or leukemia cell. Fascaplysin **24** showed murine solid tumor selectivity and it was the most cytotoxic of the compounds tested. 3-Bromofascapysin **25** was less cytotoxic and did not retain the selectivity observed for **24**. Other significant result was demonstrated that reticulatate **28** is excellent specificity against both murine and human cell lines as seen in the selectivity differences for the murine C38 versus the CFU cell lines and for the human H116 versus the CEM cell lines.

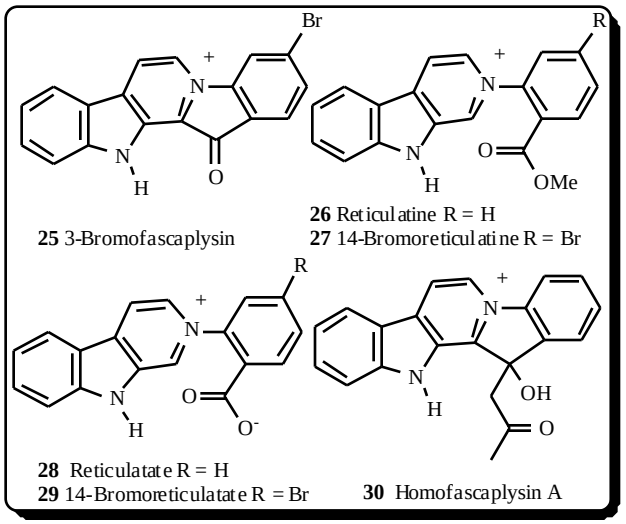


21 *N*-Methyl-*epi*-manzamine D R = M
22 *epi*-Manzamine D R = H



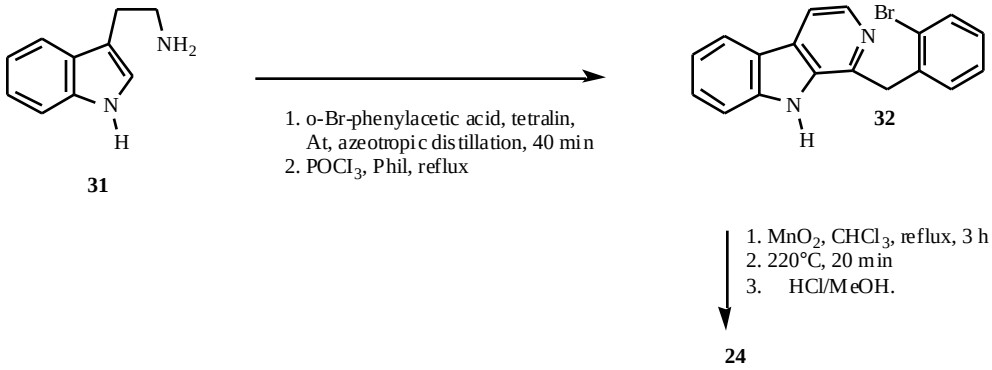
23 Thorectandramine **24** Fascaplysin

Fascaplysin **24** has been synthesized in five steps from tryptamine **31** in 44% overall yield. The key steps in the synthesis are (Scheme **5**): 1) dehydrogenation of the dihydro-β-carboline intermediate **32** simultaneously with its benzylic oxidation on treatment with MnO_2 and 2) the thermal cyclization of the resulting β-carboline. Similarly, indoloisoquinolines analogs of **24**, were prepared in six steps from α-amino ketone in 59 and 55% overall yields, respectively [34].



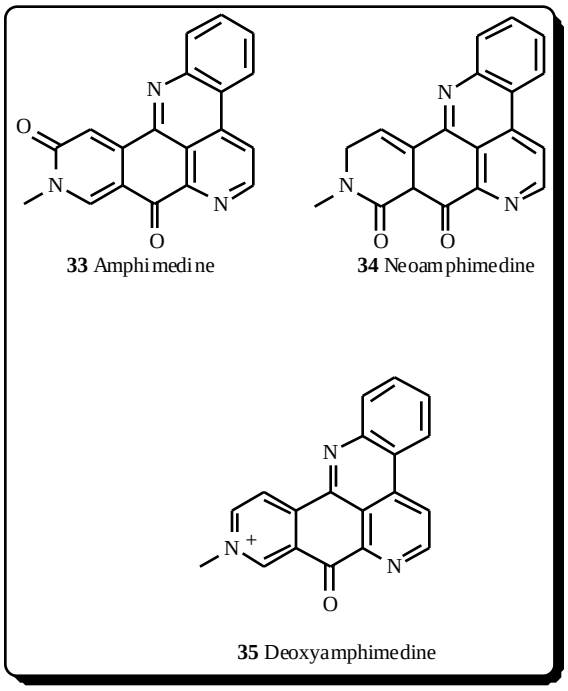
25 3-Bromofascaplysin
26 Reticulatine R = H
27 14-Bromoreticulatine R = Br
28 Reticulatate R = H
29 14-Bromoreticulatate R = Br
30 Homofascaplysin A

The pyridoacridines form an important class of marine metabolites, many of which exhibit significant cytotoxic activity [35, 36]. Amphimedine **33**, isolated in 1983 from an *Amphimedon* sp. sponge, was the first example of this type of alkaloid from a marine organism [37]. Recently, Ireland's group published the isolation of two structurally related compounds, neoamphimedine **34** [38] and deoxyamphimedine **35** [39]. Neoamphimedine **34** showed



Scheme 5.

potent anti-neoplastic activity in human xenograft tumors in athymic mice. Neoamphimedine was as effective as etoposide in mice bearing KB tumors and as effective as 9-aminocamptothecin in mice bearing HCT-116 tumors. Amphimedine **33** did not induce DNA aggregation or catenation *in vitro*, nor did it display any significant anti-neoplastic activity. These results suggest that neoamphimedine has a novel top2-mediated mechanism of cytotoxicity and anticancer potential [40].

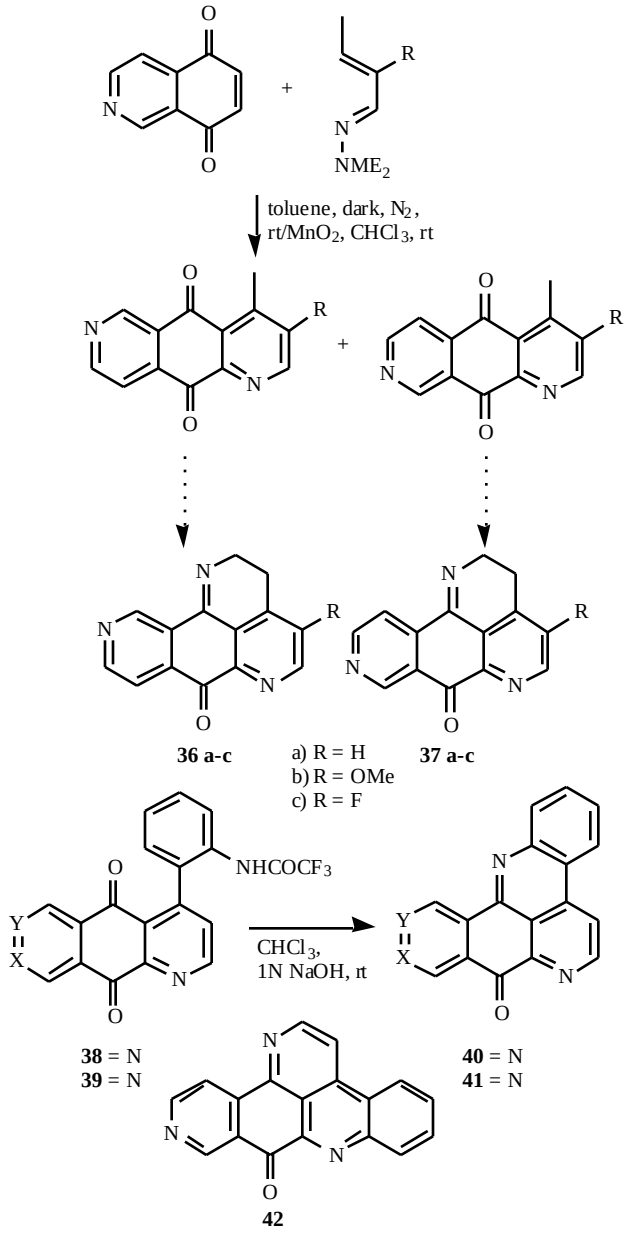


Synthetic analogs **33** and **34**, namely the tetra- and pentacyclic compounds **36(a-c)**, **37(a–c)**, **38, 39**, **40**, **41** and **42** (Scheme **6**) was tested on six distinct human cancer cell lines including various histopathological types (glioblastomas, colon, lung, and bladder cancers). The colorimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on overall growth of adherent cell lines was used. The IC_{50} values that is the concentration which reduced the mean growth value of the six cell lines by 50%, was determined for each drug, as compared to the mean control growth value. Table **2** illustrates the individual IC_{50} values of the different compounds obtained for each of the six cell lines under study. All those compounds present cytotoxic activity. The derivatives with the most close structure to the natural product amphimedine **33** exhibited IC_{50} values < 10^{-7}M and are the more active compounds of this series with the intermediates **38** and **39**. The substituted tetracycles **36b**, **36c** and **37b**, **37c** were sensibly less cytotoxic than the unsubstituted ones **36a** and **37a**. The difference in activity between the two isomers was relatively weak. More generally, the same cytotoxicity was observed from a cell line to another, marking the poor selectivity of these compounds, see Table **2** [41].

Table 2. Characterization of the *in Vitro* Cytotoxic-Related Antitumor Effects IC_{50} (μM)

Compound	Cell lines					
	U-87MG	U-373MG	J82	HCT-15	LoVo	A549
36a	2.2	0.8	2.4	0.7	2.5	0.9
37a	0.5	0.3	0.8	0.3	0.8	3.1
36b	0.5	0.7	3.1	0.5	2.9	0.8
37b	0.8	0.8	> 10	1.3	4.2	1.8
36c	3.2	3.0	3.0	2.8	2.5	> 10
37c	6.9	4.4	6.9	3.6	5.1	> 10
38	0.06	0.03	0.7	0.05	0.08	0.15
39	0.02	0.1	0.2	0.04	0.04	0.06
40	0.1	0.2	2.9	0.2	0.4	0.2
41	0.1	0.05	1.4	0.3	0.05	1.8
42	1.0	2.5	3.0	0.9	2.8	0.7

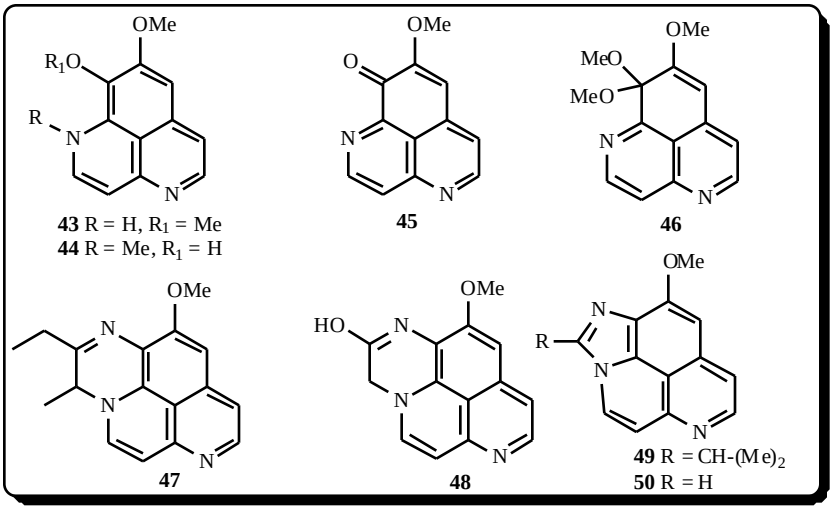
Four novel alkaloids of the aaptamine class have been isolated in addition to the known aaptamine **43**, isoaaptamine **44**, demethyl(oxy)aaptamine **45** and its dimethylketal from an unidentified species of Indonesian marine sponge of the genus *Xestospongia* sp.. Their antitumor activity against KB cells (ID_{50} μg/ml, **43** = 3.7; **44** = 0.5; **45** = 1.8, **46** = 3.5, **47-50** = > 10), and also antimicrobial activity was evaluated towards Gram (+) (*S. aureus*), Gram (-) (*E. coli*, *V. anguillarum*) bacterial strains, a fungus (*C. tropicalis*) [42]. A significant cytotoxic activity on KB cells was confirmed for aaptamine **43**, isoaaptamine **44**, demethyl(oxy)aaptamine **45**, according to the previous studies [43]; the new dimethylketal **46** also displayed interesting cytotoxic activity.

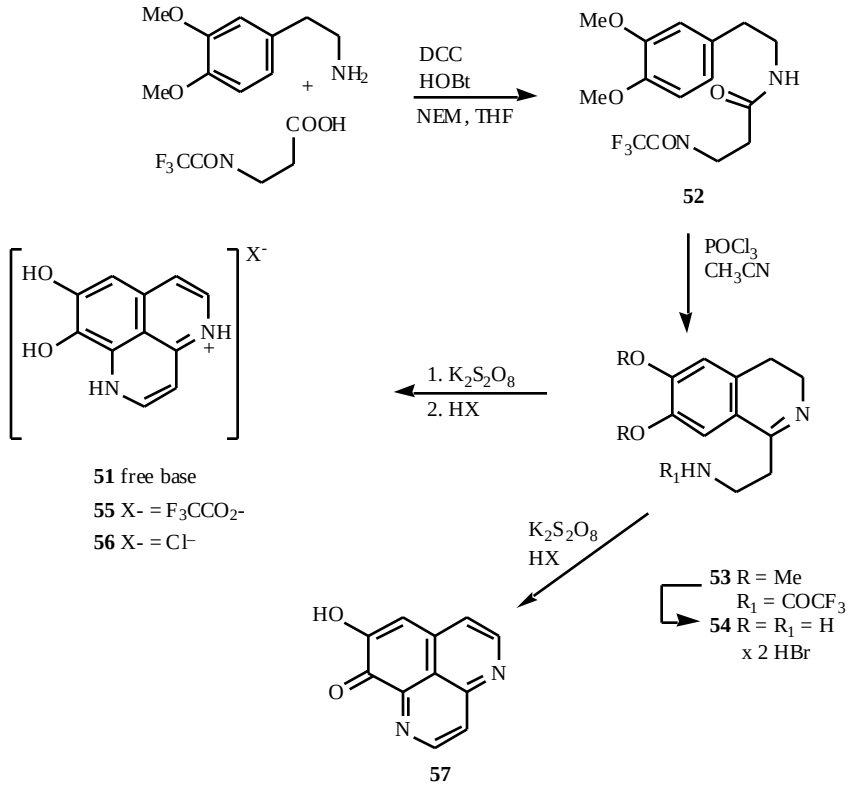


Scheme 6.

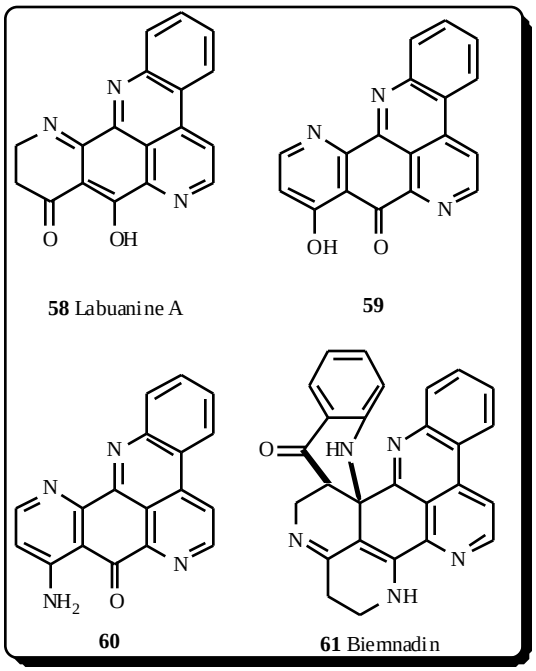
The bisdemethylaaptamine **51** was synthesized from homoveratrylamine and *N*-trifluoroacetyl-β-alanine (Scheme **7**) [44]. Coupling of the two components followed by Bischler–Napieralski cyclization of the resulting amide **52** afforded the dihydroisoquinoline **53** in high yield. On treatment with aqueous hydrobromic acid, **53** underwent cleavage of the methyl ether and trifluoroacetamido groups and afforded the catecholamine **54** in form of its stable and analytically pure bishydrobromide. As **54** precipitated from the reaction mixture, no cumbersome purification had to be carried out with this air-sensitive, amphoteric compound. Oxidative cyclization of **54** in 1% aqueous KOH with potassium peroxodisulfate or air yielded bisdemethylaaptamine besides minor amounts of its oxidation product bisdemethyloxyaaptamine **57** (analog of **45**). Due to the instability of the free base, **51** was purified as trifluoroacetate **55** or hydrochloride **56** by gel chromatography (20% overall yield).

Pyridoacridine alkaloids **58**–**61** were isolated from the Indonesian marine sponge *Biemna fortis* as neuronal differentiation inducers against a murine neuroblastoma cell line, Neuro 2A [45]. The chemical structure of the new compound, labuanine A **58**, was determined by spectroscopic study and chemical conversion. These pyridoacridine alkaloids induced multipolar neuritogenesis in more than 50% of cells at 0.03–3 μM concentration. Compound **60**, which showed the strongest neuritogenic activity among them, also induced increase of acetylcholinesterase, a neuronal marker in Neuro 2A and arrested cell cycle at the G2/M phase. Compounds **58**, **59** and **61** induced neurite outgrowth in more than 50% of cells at 1–3 μM concentration. The wide difference in neuritogenic activity between **59** and **60** implies the importance of the amino group at C-9 in 3 for neuritogenic activity. Compound **59** was a synthetic regio-isomer [46] of meridine [47], which was isolated from the ascidian *Amphicarpa meridiana*. Compound **60** was also a synthetic region-isomer [48] of cystodamine [49], which was isolated from the Mediterranean ascidian *Cystodytes delle chiajei*. It is noteworthy that compounds **59** and **60** were the first case of isolation from a natural source and compound **59** might be an artefact metabolite produced from labuanine A **58** by air oxidation.





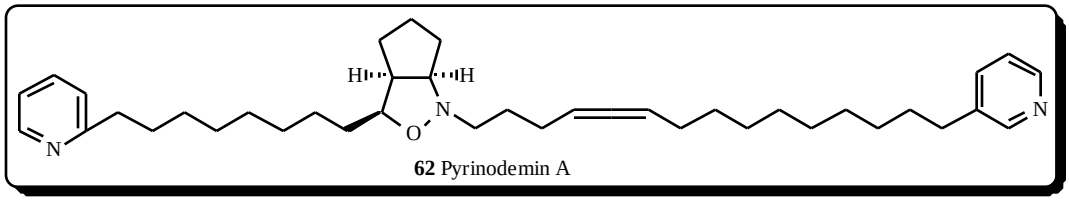
Scheme 7.

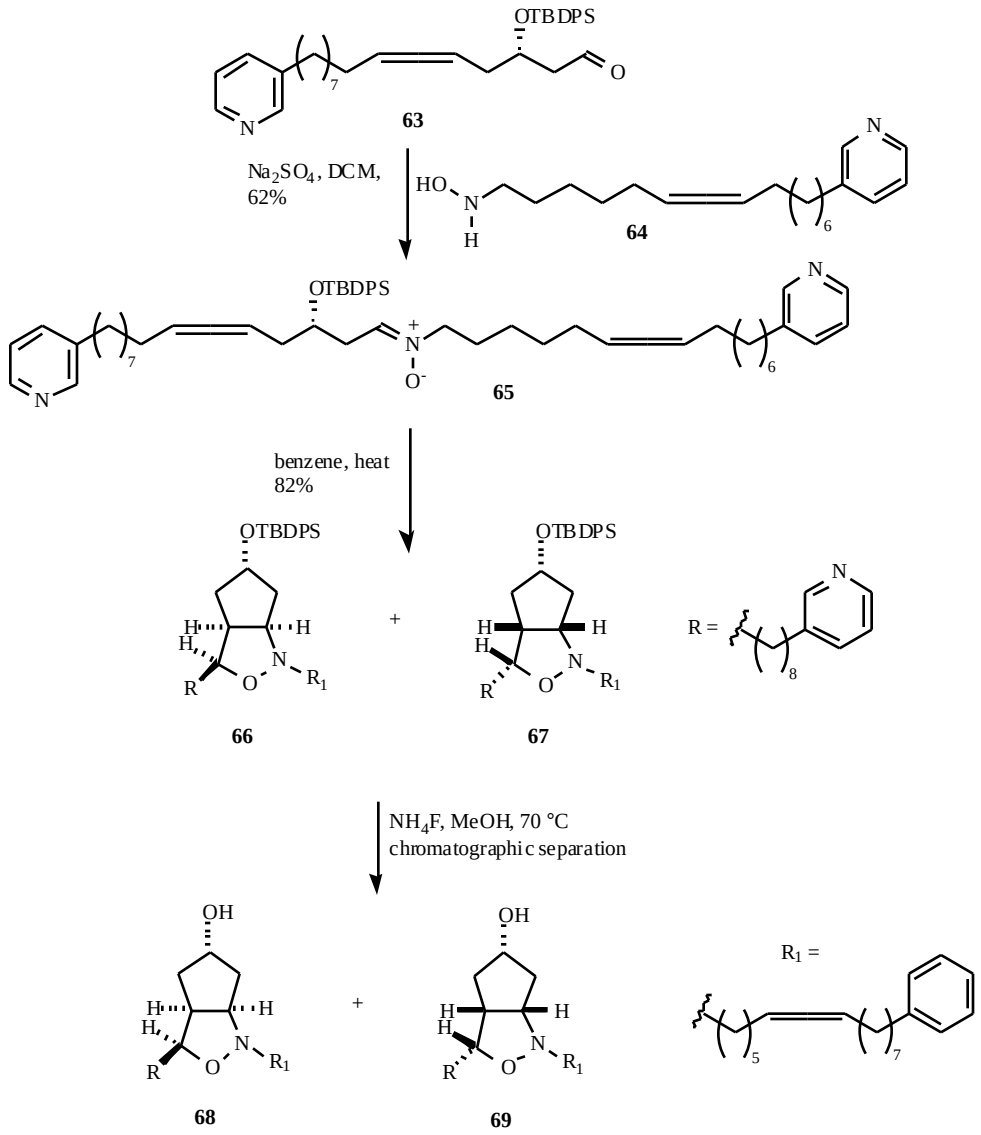


A novel cytotoxic pyridine alkaloid, pyrinodemin A **62**, with a unique *cis*-cyclopent[*c*]isoxazolidine moiety has been isolated from the Okinawan marine sponge *Amphimedon* sp. [50]. The compound is chiral and cytotoxic towards murine leukaemia L1210 and KB epidermoid carcinoma cells, and the absolute configuration of the marine sponge alkaloid pyrinodemin A **62** is established by organic synthesis [51].

Aldehyde **63** was condensed with known hydroxylamine **64** [52] to afford nitrone **65** in 62% yield. Thermal cyclization of nitrone **65** in benzene under reflux delivered an inseparable mixture of **66** and **67** in a combined yield of 82%. The mixture of **66** and **67** was deprotected with ammonium fluoride in methanol to give alcohols **68** (59% yield) and **69** (30% yield), which could be separated by flash chromatography (Scheme **8**).

New *bis*-pyridine alkaloids, pyrinodemins B-D **70**-**72**, have been isolated together with pyrinodemin A **62** and related 3-alkyl pyridine alkaloids from the Okinawan marine sponge *Amphimedon* sp. [53]. Pyrinodemins B-D **70**-**72** are unique *bis*-3-alkylpyridine alkaloids with a *cis*-cyclopent[*c*]isoxazolidine moiety like pyrinodemin A **62**. Pyrinodemins B-D **70**-**72** exhibited potent cytotoxicity against murine leukemia L1210 (IC_{50}, 0.07, 0.06, and 0.08 μg/ml, respectively) and KB epidermoid carcinoma cells (IC_{50}, 0.5 μg/ml, each) *in vitro*, comparable to pyrinodemin A **62**) [53].




OTBDPS
63
Na_2SO_4, DCM,
62%
64
65
benzene, heat
82%
66
67
NH_4F, MeOH, 70 °C
chromatographic separation
68
69
R =
R_1 =

Scheme 8.

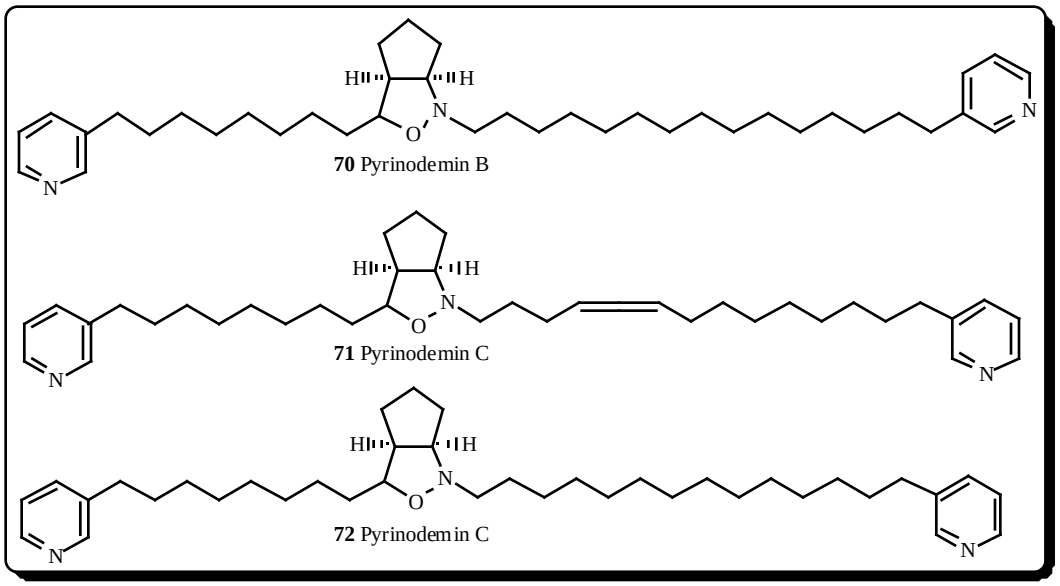
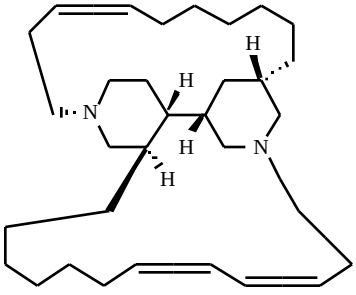
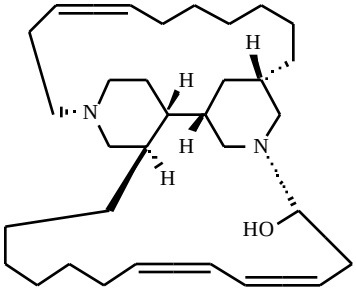

70 Pyrinodemin B
71 Pyrinodemin C
72 Pyrinodemin C

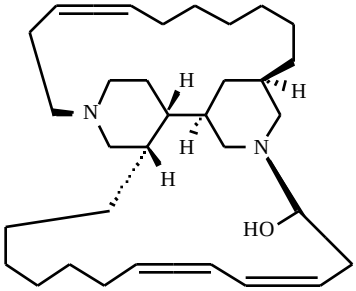
Table 3. Growth-Inhibitory Effects IC_{50} (μM) on Leukemia, Fibrosarcoma, Melanoma and Colon Cancer Cells



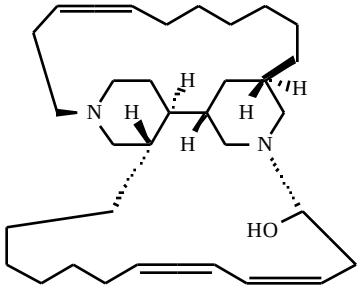
73 Haliclonacyclamine E



74 Arenosclerin A



75 Arenosclerin B



76 Arenosclerin C

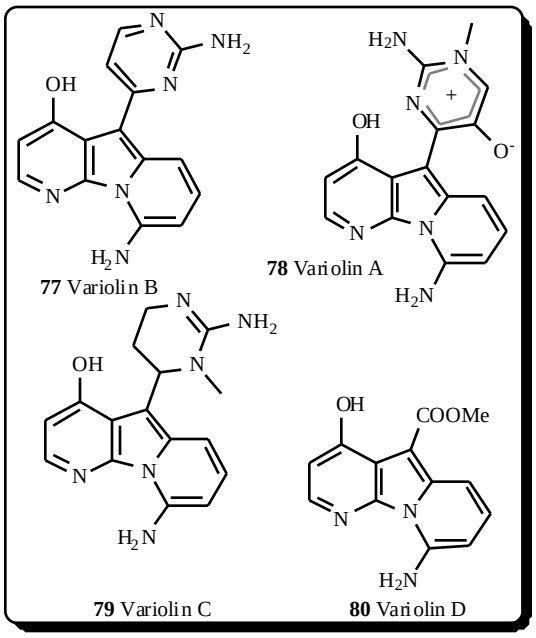
Compound	Cancer cell lines			
	HL60	B16	U138	L929
73	4.23	1.82	6.06	3.89
74	4.31	1.77	3.83	2.34
75	4.07	1.76	3.62	2.24
76	3.65	1.71	3.60	2.17

Haliclonacyclamie E **73**, and arenosclerins A **72**, B **73** and C **74**, novel tetracyclic alkylpiperidine alkaloids have been isolated from the marine sponge *Arenosclera brasiliensis* [54]. All isolated compounds displayed a similar range of cytotoxicity, irrespective of the cancer cell line tested (see Table **3**).

Variolins **77**-**80** comprise a group of marine heterocyclic substances isolated from the Antarctic sponge *Kirkpatrickia variolosa* [55, 56]. They have a common tricyclic skeleton, which has no precedents in either terrestrial or marine natural products, a pyrido[3', 2':4, 5]pyrrolo[1, 2-*c*]pyrimidine, substituted at position 5. Pharmacological evaluation of these compounds showed important antiviral and antitumor activity against P388 murine leukemia cells [55, 56]. Variolin B **77** compound has shown potent cytostatic and cytotoxic effects against different human leukemia cell lines, K-562, U-937, MOLT-4 and Jurkatt, human ovarian carcinoma cell lines, OVCAR-3, SKOV-3 and Igrov-1 and human intestinal carcinoma LoVo cell line. Variolin B **77** was found to be equally effective against LoVo carcinoma cell line and its multidrug resistant variant LoVo/Dx over expressing Pgp. By using biparametric BrdU/DNA flow cytometric analysis it was found that in LoVo cells, Variolin B concentrations in the nM range for 1 h cause an arrest of cells in G1 and a decrease of the rate of progression of Sphase cells to G2, whereas at concentrations in the mM range, even for a short time, the compound induced a blockade in G2 phase. In both leukemic and epithelial cancer cells Variolin B was found to be a strong activator of apoptosis, assessed by morphological and biochemical methods. Apoptosis occurred very rapidly, within 6-8 h following a short drug exposure. Variolin B induced apoptosis also in K562 erythroleukemia and epithelial ovarian cancer cell lines, which do not activate apoptosis after treatment with conventional anticancer drugs. While the *in vivo* antitumor activity of Variolin B is under investigation, studies have been initiated to elucidate the molecular mechanisms underlying the interesting biological activities of this new compound [57].

Variolin B **77** is the most active of the family, oxidation or reduction of the isolated D ring as in variolin A **78** or *N*-3'-methyl-3', 4', 5', 6'-tetrahydrovariolin B **79** reduces the activity. The importance of the aminopyrimidine ring at C5 of the above mentioned tricyclic system is corroborated by the lack of activity of variolin D **80**. The total synthesis of variolin B **77** from 4-methoxy-7-azaindole was described

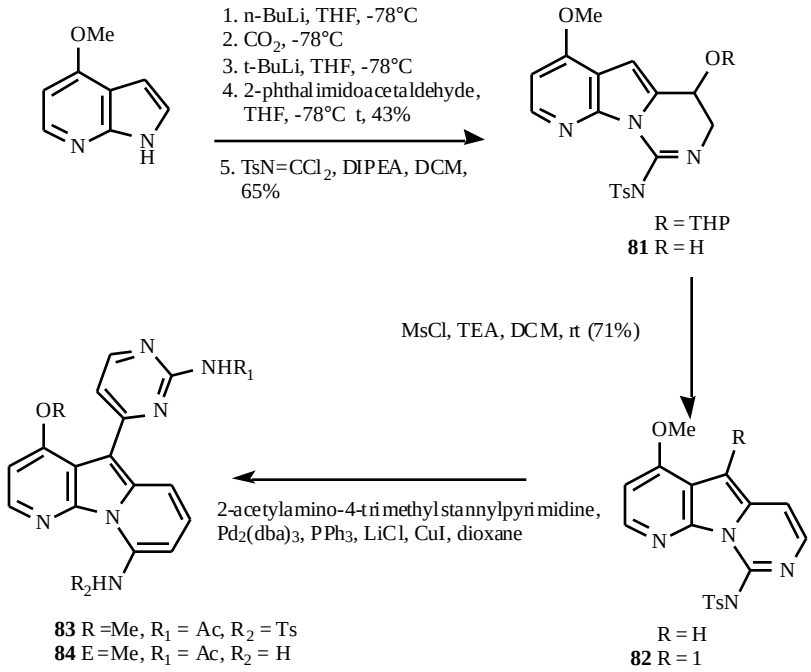
(Scheme **9**) [58]. The preparation of the protected amino derivative **81** and a coupling reaction of the iodo derivative **82** with 2-acetylamino-4-trimethylstannylpyrimidine are the key steps of the sequence. The use of *N*-tosyldichloro-methanimine for the cyclization step afforded a good entry to the 9-aminopyrido-[3', 2':4, 5]pyrrolo[1, 2-*c*]-pyrimidine system. Variolin B was obtained from the triply protected tetracyclic compounds **83** and **84** in two steps. Two total syntheses of variolin B **77**, and two syntheses of deoxyvariolin B, as well as several methods for the construction of the common tricyclic skeleton of these compounds have also been reported [59-65].



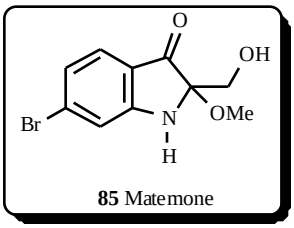
A new bioactive bromine-containing oxindole alkaloid, matemone **85**, was isolated from the Indian Ocean sponge *Iotrochota purpurea*, together with the known 6-bromoindole-3-carbaldehyde. Compound **85**, as 6-bromo-2-methoxy-2-hydroxymethane-3-indolinone and named matemone, shows mild cytotoxicity against three cancer cell lines and marginal antibacterial activity against *Staphyloccocus aureus*. Compound **85** also displayed mild cytotoxicity against the growth of the NSCLC-N6 L16 strain18 (lung cancer), Mia PaCa-2 cell line (pancreas cancer), and DU145 cell line (prostate cancer), with IC_{50} values of 30, 24, and 27 μg/ml, respectively [66]. Synthesis of **85** has not been reported.

The bromopyrrole longamide **86** [67, 68] was originally obtained from the Caribbean sponge *Agelas longissima* and more recently from a Homaxinella species of Japanese marine sponge [69]. Some antibacterial activity (MIC 60 μg ml^{-1}) has been ascribed to the optically active material obtained from the Caribbean sponge, while the racemic modification isolated from the Japanese source is inactive against P388 lymphocytic leukemia cells. Longamide B methyl ester **87** [69], which has been isolated, for the first time and in racemic form, from the Japanese sponge mentioned above, exhibits weak 30 μg ml^{-1}) cytotoxic activity, in ED_{50} vitro, against the same leukemia cell line. The corresponding acid (longamide B, **89**) [70] has been isolated, again in racemic form, from the Caribbean marine sponge *Agelas dispar* and shows modest (MIC 50 μg ml^{-1}) antibiotic activity against several strains of Gram-positive bacteria. "Semi-racemic" hanishin **89**, the ethyl ester of longamide B, was obtained from extracts of the highly polymorphic sponge *Acanthella carteri* and is cytotoxic towards NSCLC-N6 human non-small cell lung carcinoma of 9.7 (μg ml^{-1}) [71].

The first total syntheses (Scheme **10**) of the title compounds by routes capable of providing useful quantities of these materials as well as many derivatives have been reported [72].



Scheme 9.

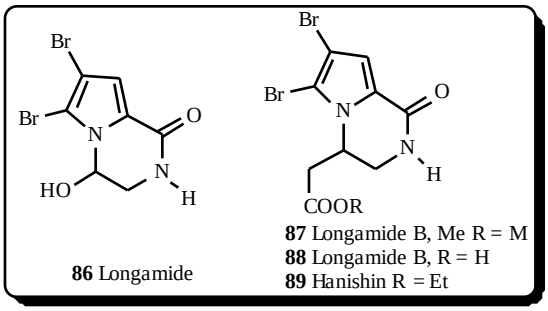


85 Matemone

Echinoclathrines A-C **90-92** were isolated from an Okinawan sponge, *Echinoclathria* sp. [73]. These compounds are a new class of pyridine alkaloids possessing a 4-aryl-2-methylpyfidine moiety as a common structural element. Echinoclathrine A **90** showed weak cytotoxicity against P388, A-549, and HT-29 cells at a level of IC_{50} 10 μg/ml. Echinodathfines A **90** and B **91** also exhibited weak immunosuppressive activity in a mixed lymphocyte reaction assay. Both **90** and **91** exhibited weak immunosuppressive activity in a mixed lymphocyte reaction assay with IC_{50} 7.9 and 9.7 μg/ml, respectively. Syntheses of these compounds have not been reported.

2. PREDICTION OF BIOLOGICAL ACTIVITY FOR MARINE SPONGE ALKALOIDS AND THEIR SYNTHETIC ANALOGS

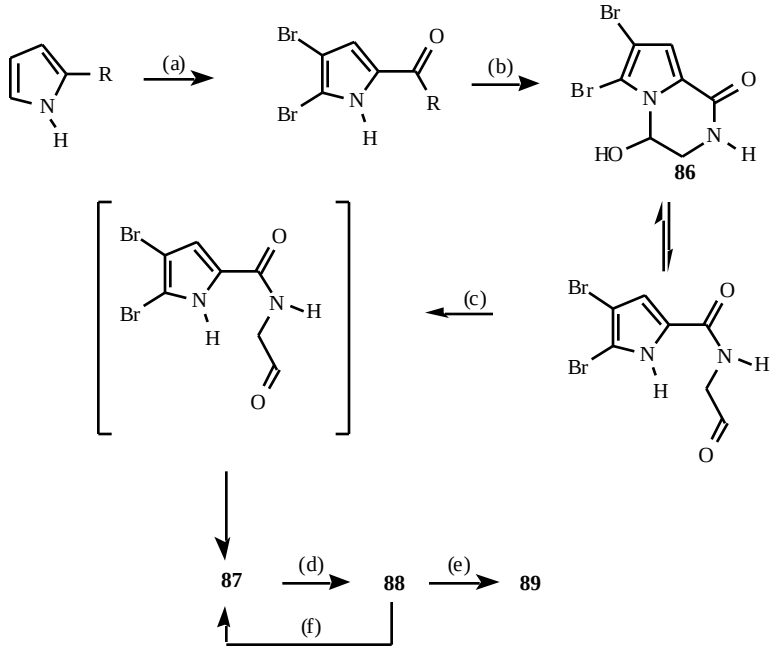
Keeping in mind that presented above data on biological activity of marine sponge alkaloids and their analogs characterize only a small part of possible biological potential in these molecules, we tried to estimate their biological activity spectra by computer prediction. For this purpose we used computer program PASS [74-77], which predicts more than 900 pharmacological effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity and embryotoxicity on the basis of structural formulae of compounds. PASS predictions are based on structure-activity relationships (SAR) analysis of the training set consisting of about 52, 000 of drugs, drug-candidates and lead compounds. Algorithm of PASS predictions is described in detail in several publications [74-78]. Using MOL or SD files as an input for PASS program, user may get a list of probable biological activities for any drug-like molecule as an output. For each activity Pa and Pi values are calculated, which can be interpreted either as the probabilities of a molecule belonging to the classes of active and inactive compounds respectively, or as the probabilities of the first and second kind of errors in prediction.



86 Longamide

87 Longamide B, Me R = M
88 Longamide B, R = H
89 Hanishin R = Et

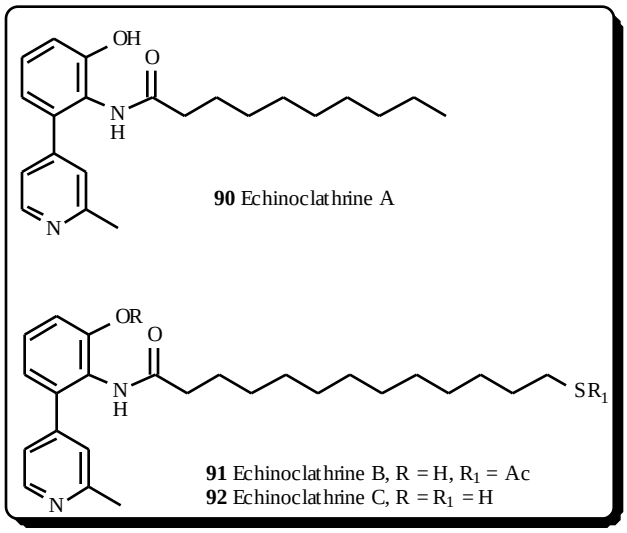
Interpretation of the prediction results and selection of the most prospective compounds are based on flexible criteria, which depend on the purpose of particular



(a) 1. $ClCOCl_3$, $(CH_3CH_3)_2O$, 18°C, 4h
2. Br_2, CH_3COOH, 18-60°C, 2h
(b) 1. $H_2NCH_2CH(OMe)_2CO$, CH_2Cl, 18°C, 20 h
2. 3 M aq HCl, $(CH_3)_2CO$, 18°C, 16 h
(c) $H_3CO_2CCH_2PO(OCH_2CH_3)_2$, $NaOCH_3$, CH_3OH, 18°C, 18h
(d) 10% aq NaOH, CH_3OH, reflux, 0.5 h
(e) CH_2N_2 (excess), $(CH_3CH_2)O$, 0-18 °C, 20 h
(f) CH_3CH_2OH, conc. HCl, reflux, 3h

Scheme 10.

investigation. If the user chooses rather higher value of Pa as a threshold for selection of probable activities, the chance to confirm the predicted activities by the experiment is high too, but many existing activities will be lost. For instance, if Pa>80% is used as a threshold, about 80% of real activities will be lost; for Pa>70%, the portion of lost activities is 70%, etc. By default, Pa=Pi value determined at the training is used as a threshold that provides the mean accuracy of prediction about 85% in leave one out cross-validation for all ~52000 compounds and 921 activities from the PASS training set. Average accuracy of PASS predictions obtained for heterogeneous evaluation set is almost 90% [79]. PASS also calculates so-called drug-likeness according to the method published in [80].



90 Echinoclathrine A

91 Echinoclathrine B, R = H, R_1 = Ac
92 Echinoclathrine C, R = R_1 = H

Example of biological activity spectrum predicted for Halitulin (compound **14**) is presented below. From 921, 23 activities taken into accoount by PASS 1.901 version, are predicted as probable with Pa>40% (Table **4**). Halitulin molecule is included into the PASS training set, however during the prediction it is removed from the PASS training set with both known activities "Antineoplastic" and "Antineoplastic alkaloid", to provide more objective results of prediction. Similar procedure is applied by default for any molecule that is included into the PASS training set.

As one may see from the Halitulin's are predicted activity spectrum, Antineoplastic activity is predicted with probability 42% (marked in bold). Also, several possible molecular mechanisms of antineoplastic action are predicted including Topoisomerase II inhibitor, Protein kinase inhibitor, Adenylate cyclase inhibitor, and Nucleotide metabolism regulator are predicted. However, the most probable activity for Halitulin, so-called *focal activity*, is Psychosexual dysfunction treatment that is predicted with Pa=81.4%. Several other activities are predicted with high probability including Calmodulin antagonist, Antiseborrheic, Antineurogenic pain, Cardiovascular analeptic, etc. These predictions specify the directions of further biological testing of this compound and, if they will be confirmed by the experiment, there were new possibilities for its pharmacotherapeutic applications.

In Table **5** part of PASS predictions for all 96 marine sponge alkaloids and their analogs is presented. Given results includes: Drug-likeness; Antineoplastic and Angiogenesis inhibitor actions for Pa>Pi threshold; mechanism of Antineoplastic action predicted with the highest probability; focal activities predicted for each compound. About 80% of compounds under consideration have drug-likeness values more than 0.5. Unfortunately, due to the limited size of the publication, we cannot present here the whole biological activities spectra for all 96 compounds however they can be easily obtained via Internet with PASS Inet version [77, 81].

Table 4. Prediction Activities Found for Alkaloid Halitulin 14

No.	Pa	Pi	for Activity
1.	0.814	0.014	Psychosexual dysfunction treatment
2.	0.637	0.022	Calmodulin antagonist
3.	0.636	0.034	Antiseborrheic
4.	0.607	0.015	Antineurogenic pain
5.	0.619	0.037	Cardiovascular analeptic
6.	0.524	0.027	Sigma receptor agonist
7.	0.575	0.142	Vascular (periferal) disease treatment
8.	0.502	0.070	Topoisomerase II inhibitor
9.	0.477	0.080	Antipruritic
10.	0.396	0.020	Protein kinase inhibitor
11.	0.461	0.099	Antidyskinetic
12.	0.471	0.110	Acetylcholine release stimulant
13.	0.470	0.119	Spermicide
14.	0.418	0.070	Rhinitis treatment
15.	0.430	0.087	Vasodilator, cerebral
16.	0.455	0.116	Acetylcholine M1 receptor antagonist
17.	0.451	0.122	Ovulation inhibitor
18.	0.405	0.079	Adenylate cyclase inhibitor
19.	0.420	0.097	Antineoplastic
20.	0.487	0.174	Arrhythmogenic
21.	0.406	0.124	Nucleotide metabolism regulator
22.	0.469	0.197	Hematotoxic
23.	0.401	0.140	Neurotrophic factor

By comparison of the predicted Antineoplastic activity with known experimental data we found that they coincided in almost 80% of cases (51/64). Known Antineoplastic activity was not predicted for 13 compounds including those with numbers **16**, **17**, **19**, **20**, **23**, **26**, **29**, **33**, **62**, **70**, **71**, **72**, **89**. From the compounds **62**, **70**-**72**, **89** have more than two new descriptors that is the reason for suggestion that the prediction results are not very reliable.

Despite the majority of known biological activities for respective marine sponge alkaloids is associated with Antineoplastic action, their number is less than 50% (42/96) among the predicted focal activities. Analysis of the predicted biological activity spectra with computer program PharmaExpert [81] shows that 55 different kinds of

biological activity are predicted with Pa>70%, 199 – with Pa>50%, 463 – with Pa>30%, and 810 – with Pa>Pi. Top twenty activities (Fig. **1**) predicted with Pa>Pi include: Interleukin antagonist (73 compounds); Vasodilator, cerebral (72 compounds); MAP kinase inhibitor (66 compounds); Liver fibrosis treatment, 5 HT release stimulant, Protein kinase inhibitor (65 compounds), Antineoplastic (63 compounds); Telomerase inhibitor, Antineoplastic alkaloid, Antiamyloidogenic, Benzodiazepine 1 receptor agonist, Interleukin 1 antagonist (62 compounds); etc.

Table 5. Prediction of Biological Activities for Marine Sponge Alkaloids and their Derivatives

Compound No	Drug-Likeness	Antineoplastic and Focal Activity Prediction
1	0.762	**0.783 0.007 Anxiolytic** 0.396 0.100 Angiogenesis inhibitor 0.382 0.112 Antineoplastic[3] 0.528 0.025 Cyclin-dependent kinase 2 inhibitor
2	0.724	**0.781 0.007 Anxiolytic** 0.390 0.104 Angiogenesis inhibitor 0.353 0.126 Antineoplastic[3] 0.526 0.026 Cyclin-dependent kinase 2 inhibitor
3	0.724	**0.781 0.007 Anxiolytic** 0.390 0.104 Angiogenesis inhibitor 0.353 0.126 Antineoplastic[3] 0.526 0.026 Cyclin-dependent kinase 2 inhibitor
4	0.606	**0.873 0.002 Telomerase inhibitor** 0.543 0.055 Antineoplastic 0.446 0.053 Cyclic AMP antagonist
5	0.339	**0.795 0.002 Telomerase inhibitor** 0.515 0.063 Antineoplastic[3] 0.425 0.068 Cyclin-dependent kinase 2 inhibitor
6	0.339	**0.795 0.002 Telomerase inhibitor** 0.515 0.063 Antineoplastic[3] 0.425 0.068 Cyclin-dependent kinase 2 inhibitor
7	0.361	**0.814 0.002 Telomerase inhibitor** 0.544 0.054 Antineoplastic[3] 0.420 0.071 Cyclin-dependent kinase 2 inhibitor
8	0.276	**0.795 0.002 Telomerase inhibitor** 0.515 0.063 Antineoplastic[3] 0.425 0.068 Cyclin-dependent kinase 2 inhibitor
9	0.287	**0.814 0.002 Telomerase inhibitor** 0.543 0.055 Antineoplastic[3] 0.432 0.064 Cyclin-dependent kinase 2 inhibitor
10	0.426	**0.644 0.003 Telomerase inhibitor** 0.531 0.058 Antineoplastic[3] 0.527 0.025 Cyclin-dependent kinase 2 inhibitor
11	0.314	**0.790 0.002 Telomerase inhibitor** 0.541 0.055 Antineoplastic[3] 0.409 0.079 Cyclin-dependent kinase 2 inhibitor

(Table 5) contd...

Compound No	Drug-Likeness	Antineoplastic and Focal Activity Prediction
12	0.426	**0.644 0.003 Telomerase inhibitor** 0.531 0.058 Antineoplastic 0.527 0.025 Cyclin-dependent kinase 2 inhibitor
13	0.276	**0.795 0.002 Telomerase inhibitor** 0.515 0.063 Antineoplastic[3] 0.425 0.068 Cyclin-dependent kinase 2 inhibitor
14[1]	0.975	**0.814 0.014 Psychosexual dysfunction treatment** 0.420 0.097 Antineoplastic[3] 0.502 0.070 Topoisomerase II inhibitor
15	0.933	**0.860 0.007 Antiseborrheic** 0.680 0.023 Antineoplastic 0.633 0.024 Topoisomerase II inhibitor
16[4]	0.963	**0.813 0.015 Systemic lupus erythematosus treatment** 0.615 0.011 Interferon agonist 0.293 0.199 Angiogenesis inhibitor
17[4]	0.963	**0.813 0.015 Systemic lupus erythematosus treatment** 0.615 0.011 Interferon agonist 0.293 0.199 Angiogenesis inhibitor
18	0.958	**0.683 0.027 Systemic lupus erythematosus treatment** 0.593 0.038 Phospholipase C inhibitor 0.247 0.197 Antineoplastic[3]
19[4]	0.599	**0.788 0.046 Arrhythmogenic** 0.449 0.078 Interferon agonist
20[4]	0.601	**0.620 0.008 Hallucinogen** 0.292 0.063 Dihydropteroate synthase inhibitor 0.266 0.231 Angiogenesis inhibitor
21	0.995	**0.971 0.000 Antineoplastic alkaloid** 0.796 0.008 Antineoplastic[3] 0.290 0.037 Protein kinase C stimulant
22	0.995	**0.974 0.000 Antineoplastic alkaloid** 0.779 0.010 Antineoplastic[3] 0.249 0.159 Tubulin antagonist
23[4]	0.984	**0.706 0.008 Antiobesity** 0.548 0.038 Adenylate cyclase inhibitor
24[1]	0.857	**0.701 0.012 Topoisomerase II inhibitor** 0.554 0.052 Antineoplastic[3]
25	0.571	**0.736 0.003 Cyclin-dependent kinase inhibitor** 0.543 0.055 Antineoplastic[3]
26[4]	0.610	**0.616 0.023 Acute neurologic disorders treatment** 0.559 0.039 Growth factor antagonist 0.296 0.195 Angiogenesis inhibitor
27	0.366	**0.543 0.010 Benzodiazepine 1 receptor agonist** 0.516 0.089 Serine protease unspecified inhibitor 0.177 0.065 Antineoplastic alkaloid[3]

(Table 5) contd.....

Compound No	Drug-Likeness	Antineoplastic and Focal Activity Prediction
28	0.670	**0.710 0.046 Mucomembranous protector** 0.565 0.037 Growth factor antagonist 0.211 0.043 Antineoplastic alkaloid[3]
29[4]	0.375	**0.701 0.048 Mucomembranous protector** 0.591 0.029 Growth factor antagonist 0.317 0.173 Angiogenesis inhibitor
30	0.881	**0.582 0.022 Interleukin 1 antagonist** 0.426 0.014 Protein kinase inhibitor 0.373 0.116 Antineoplastic
31[1]	0.732	**0.920 0.005 5 Hydroxytryptamine agonist** 0.689 0.106 Apoptosis agonist 0.375 0.115 Angiogenesis inhibitor 0.205 0.046 Antineoplastic alkaloid
32	0.510	**0.824 0.007 Spasmolytic** 0.510 0.057 Growth factor antagonist 0.434 0.079 Angiogenesis inhibitor 0.183 0.060 Antineoplastic alkaloid
33[4]	0.817	**0.683 0.015 Topoisomerase II inhibitor** 0.648 0.030 Antineoplastic
34	0.847	**0.712 0.030 Nootropic** 0.585 0.037 Topoisomerase II inhibitor 0.396 0.006 Antineoplastic alkaloid[3]
35	0.864	**0.718 0.010 Topoisomerase II inhibitor** 0.620 0.037 Antineoplastic
36a	0.966	**0.727 0.009 Topoisomerase II inhibitor** 0.469 0.078 Antineoplastic[3]
36b	0.952	**0.692 0.013 Topoisomerase II inhibitor** 0.443 0.088 Antineoplastic[3]
36c	0.877	**0.697 0.013 Topoisomerase II inhibitor** 0.432 0.092 Antineoplastic[3]
37a	0.966	**0.695 0.013 Topoisomerase II inhibitor** 0.489 0.072 Antineoplastic[3]
37b	0.951	**0.702 0.031 Nootropic** 0.662 0.018 Topoisomerase II inhibitor 0.459 0.082 Antineoplastic[3]
37c	0.875	**0.667 0.017 Topoisomerase II inhibitor** 0.449 0.085 Antineoplastic[3]
38	0.338	**0.768 0.007 Topoisomerase II inhibitor** 0.695 0.021 Antineoplastic[3]
39	0.338	**0.768 0.007 Topoisomerase II inhibitor** 0.695 0.021 Antineoplastic[3]
40	0.820	**0.749 0.007 Topoisomerase II inhibitor** 0.646 0.030 Antineoplastic[3]
41	0.824	**0.778 0.007 Topoisomerase II inhibitor** 0.641 0.031 Antineoplastic[3]
42	0.820	**0.749 0.007 Topoisomerase II inhibitor** 0.646 0.030 Antineoplastic[3]
43[1]	0.760	**0.847 0.029 Colony stimulating factor agonist** 0.585 0.031 Growth factor antagonist 0.654 0.029 Antineoplastic[3] 0.441 0.076 Angiogenesis inhibitor
44	0.896	**0.706 0.019 Antineoplastic[3]** 0.577 0.033 Growth factor antagonist 0.402 0.097 Angiogenesis inhibitor

(Table 5) contd.....

Compound No	Drug-Likeness	Antineoplastic and Focal Activity Prediction
45	0.695	**0.738 0.019 Antiseborrheic** 0.714 0.011 Topoisomerase II inhibitor 0.531 0.058 Antineoplastic[3] 0.402 0.097 Angiogenesis inhibitor
46[2]	0.437	**0.675 0.008 Interleukin 1 antagonist** 0.655 0.015 Growth factor antagonist 0.569 0.048 Antineoplastic[3] 0.455 0.070 Angiogenesis inhibitor
47	0.910	**0.676 0.075 Arrhythmogenic** 0.434 0.115 Topoisomerase II inhibitor 0.338 0.134 Antineoplastic[3]
48	0.885	**0.591 0.019 Interleukin 1 antagonist** 0.503 0.061 Growth factor antagonist 0.466 0.079 Antineoplastic[3]
49	0.752	**0.853 0.005 Antineoplastic enhancer** 0.538 0.010 Cyclic AMP antagonist 0.539 0.056 Antineoplastic[3]
50	0.896	**0.775 0.012 Leukotriene C antagonist** 0.516 0.063 Antineoplastic[3] 0.606 0.039 Nucleotide metabolism regulator 0.337 0.152 Angiogenesis inhibitor
51	0.928	**0.791 0.008 Antineoplastic** 0.702 0.012 Topoisomerase II inhibitor 0.362 0.126 Angiogenesis inhibitor
52	0.394	**0.893 0.002 Melatonin agonist** 0.643 0.134 Apoptosis agonist 0.442 0.076 Angiogenesis inhibitor
53	0.747	**0.872 0.022 Arrhythmogenic** 0.446 0.074 Angiogenesis inhibitor 0.452 0.087 Growth factor antagonist 0.227 0.215 Antineoplastic
54	0.975	**0.902 0.014 Arrhythmogenic** 0.590 0.041 Phospholipase C inhibitor 0.155 0.091 Antineoplastic alkaloid
55	0.748	**0.638 0.032 Antineoplastic** 0.608 0.030 Topoisomerase II inhibitor 0.375 0.116 Angiogenesis inhibitor
56	0.928	**0.791 0.008 Antineoplastic** 0.702 0.012 Topoisomerase II inhibitor 0.362 0.126 Angiogenesis inhibitor
57	0.827	**0.862 0.007 Antiseborrheic** 0.759 0.007 Topoisomerase II inhibitor 0.549 0.053 Antineoplastic 0.321 0.169 Angiogenesis inhibitor
58	0.929	**0.714 0.011 Topoisomerase II inhibitor** 0.531 0.058 Antineoplastic
59	0.894	**0.809 0.006 Topoisomerase II inhibitor** 0.691 0.022 Antineoplastic 0.367 0.122 Angiogenesis inhibitor
60	0.749	**0.741 0.008 Topoisomerase II inhibitor** 0.609 0.039 Antineoplastic

Table 5) contd.....

Compound No	Drug-Likeness	Antineoplastic and Focal Activity Prediction
61[2]	0.948	**0.627 0.013 Interleukin 1 antagonist** 0.614 0.029 Topoisomerase II inhibitor 0.369 0.007 Antineoplastic alkaloid
62[2, 4]	0.922	**0.618 0.050 Lipid metabolism regulator** 0.493 0.055 Cyclic AMP agonist 0.311 0.062 Antineoplastic enhancer
63[2]	0.620	**0.753 0.013 Antineoplastic** 0.318 0.006 Farnesyltransferase inhibitor
64	0.856	**0.772 0.014 Sickle-cell anemia treatment** 0.573 0.010 Gelatinase inhibitor 0.554 0.036 Angiogenesis inhibitor 0.212 0.148 Antineoplastic enhancer
65[2]	0.682	**0.708 0.006 Acetylcholine M1 receptor antagonist** 0.581 0.045 Antineoplastic 0.253 0.006 Farnesyltransferase inhibitor
66[2]	0.743	**0.712 0.005 Acetylcholine M1 receptor antagonist** 0.452 0.084 Antineoplastic 0.394 0.269 Integrin antagonist
67[2]	0.743	**0.712 0.005 Acetylcholine M1 receptor antagonist** 0.452 0.084 Antineoplastic 0.394 0.269 Integrin antagonist
68[2]	0.943	**0.616 0.054 Cholesterol synthesis inhibitor** 0.496 0.052 Cyclic AMP agonist 0.251 0.103 Antineoplastic enhancer
69[2]	0.943	**0.616 0.054 Cholesterol synthesis inhibitor** 0.496 0.052 Cyclic AMP agonist 0.251 0.103 Antineoplastic enhancer
70[2, 4]	0.892	**0.596 0.030 Cytokine modulator** 0.528 0.033 Cyclic AMP agonist 0.302 0.067 Antineoplastic enhancer
71[2, 4]	0.922	**0.618 0.050 Lipid metabolism regulator** 0.493 0.055 Cyclic AMP agonist 0.311 0.062 Antineoplastic enhancer
72[2, 4]	0.892	**0.596 0.030 Cytokine modulator** 0.528 0.033 Cyclic AMP agonist 0.302 0.067 Antineoplastic enhancer
73	0.992	**0.885 0.008 Nootropic** 0.480 0.009 Tubulin antagonist 0.444 0.005 Antineoplastic alkaloid[3]
74	0.992	**0.789 0.007 Cognition disorders treatment** 0.417 0.017 Tubulin antagonist 0.379 0.007 Antineoplastic alkaloid[3]
75	0.992	**0.789 0.007 Cognition disorders treatment** 0.417 0.017 Tubulin antagonist 0.379 0.007 Antineoplastic alkaloid[3]
76	0.992	**0.789 0.007 Cognition disorders treatment** 0.417 0.017 Tubulin antagonist 0.379 0.007 Antineoplastic alkaloid[3]
77	0.786	**0.848 0.002 P38 kinase inhibitor** 0.381 0.110 Angiogenesis inhibitor 0.358 0.124 Antineoplastic[3]

(Table 5) contd.....

Compound No	Drug-Likeness	Antineoplastic and Focal Activity Prediction
78	0.834	**0.675 0.004 P38 kinase inhibitor** 0.512 0.064 Antineoplastic[3]
79	0.921	**0.672 0.004 P38 kinase inhibitor** 0.213 0.146 Antineoplastic enhancer[3] 0.278 0.216 Angiogenesis inhibitor
80	0.590	**0.784 0.017 Myocardial ischemia treatment** 0.523 0.003 Adenosine A3 receptor antagonist 0.221 0.136 Antineoplastic enhancer[3]
81	0.704	**0.664 0.012 Antiprotozoal (Toxoplasma)** 0.486 0.058 Angiogenesis inhibitor 0.443 0.015 Dihydropteroate synthase inhibitor 0.398 0.105 Antineoplastic
82[2]	0.165	**0.803 0.006 Antiprotozoal (Toxoplasma)** 0.524 0.009 Dihydropteroate synthase inhibitor 0.447 0.074 Angiogenesis inhibitor 0.240 0.203 Antineoplastic
83	0.265	**0.754 0.011 Antiarthritic** 0.548 0.043 Growth factor antagonist 0.494 0.054 Angiogenesis inhibitor 0.224 0.132 Antineoplastic enhancer
84	0.475	**0.763 0.003 P38 kinase inhibitor** 0.422 0.086 Angiogenesis inhibitor 0.225 0.216 Antineoplastic
85[2]	0.551	**0.607 0.007 Vasodilator, cerebral** 0.535 0.031 Interferon agonist 0.469 0.078 Antineoplastic[3] 0.397 0.100 Angiogenesis inhibitor
86[2]	0.965	**0.862 0.025 Arrhythmogenic** 0.473 0.062 Interferon agonist
87[2]	0.862	**0.758 0.008 Antiamyloidogenic** 0.565 0.095 Integrin antagonist 0.205 0.159 Antineoplastic enhancer
88[2]	0.927	**0.733 0.009 Antiamyloidogenic** 0.736 0.024 Integrin antagonist
89[2, 4]	0.835	**0.788 0.063 Fibrinogen receptor antagonist** 0.552 0.104 Integrin antagonist
90	0.473	**0.621 0.054 Lysase inhibitor** 0.519 0.038 Cyclic AMP agonist 0.259 0.187 Antineoplastic[3]
91	0.500	**0.677 0.023 Chemoprotective** 0.437 0.008 Matrix metalloproteinase 1 inhibitor 0.258 0.188 Antineoplastic[3]
92	0.476	**0.717 0.019 Lysase inhibitor** 0.387 0.028 Gelatinase inhibitor 0.319 0.145 Antineoplastic[3] 0.284 0.210 Angiogenesis inhibitor

[1]Compound is included into the PASS training set, but is excluded from it with all associated information during the prediction.

[2]Number of new descriptors comparing to the compounds from the PASS training set is three or more.

[3]Predicted Antineoplastic activity coincided with the experimental data.

[4]Known Antineoplastic activity was not predicted by PASS.

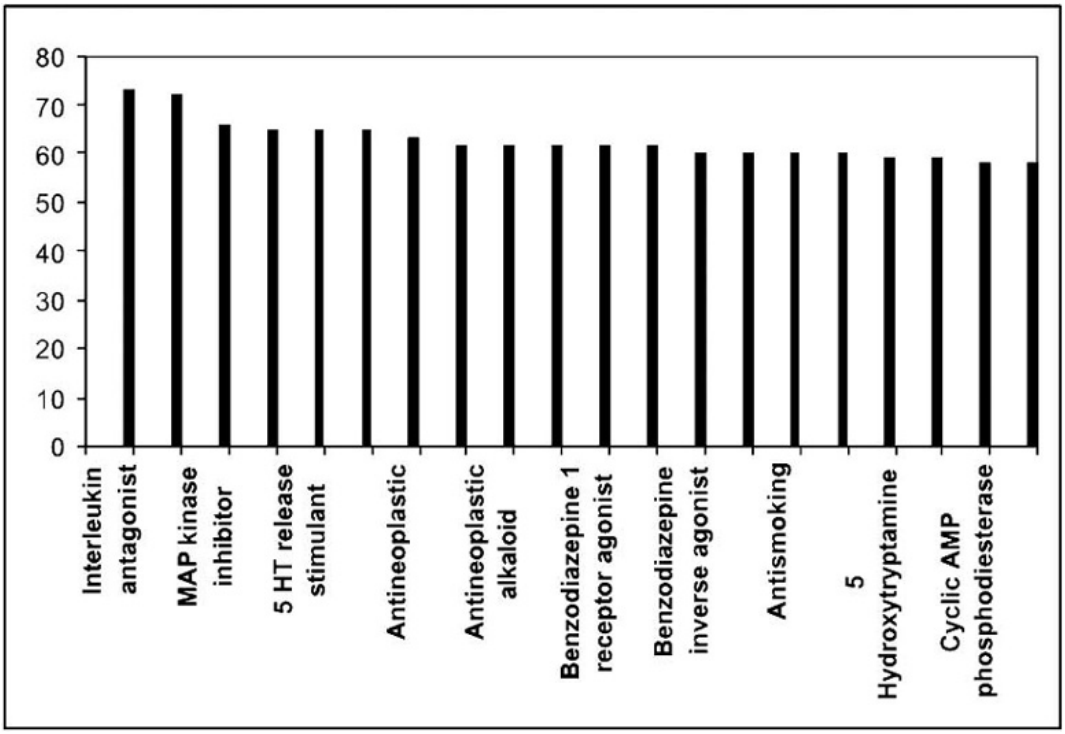


Fig. (1). Top 20 activities predicted with Pa > Pi.

Therefore, marine sponge alkaloids can be used as a source for selecting hits in many different pharmaco-therapeutic areas. By further application of SOSA (Selective Optimization of Side Activities) approach [82], it is possible to increase the desirable activities and to reduce the unwanted ones.

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