REVIEW

Marine Pyridoacridine Alkaloids: Biosynthesis and Biological Activities

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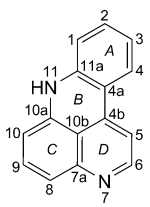
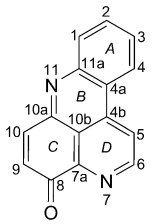
Pyridoacridines are a class of strictly marine-derived alkaloids that constitute one of the largest chemical families of marine alkaloids. During the last few years, both natural pyridoacridines and their analogues have constituted excellent targets for synthetic works. They have been the subject of intense study due to their significant biological activities; cytotoxic, antibacterial, antifungal, antiviral, insecticidal, anti-HIV, and anti-parasitic activities. In the present review, 95 pyridoacridine alkaloids isolated from marine organisms are discussed in term of their occurrence, biosynthesis, biological activities, and structural assignment.

Contents

1. Introduction. – Marine natural products are molecules rich in diverse biological activities. Pyridoacridine alkaloids are a class of strictly marine-derived alkaloids that constitute one of the largest chemical families of marine alkaloids. They have been isolated from marine sponges, tunicates, anemone, and molluscs which are often ornately decorated with bright colors and patterns [1 – 3]. Tropical tunicates (ascidians) in particular are generally richly pigmented in colors which vary from yellow to deep red, orange, blue, and purple. It is often found that pyridoacridines isolated from such tunicates are the pigments responsible for their coloration. They are generally obtained as crystalline solids with melting points above 300°. They have also been isolated as hydrochloride salts. Few pyridoacridines are found to be optically active. The optical activity of these compounds is due to the presence of an additional asymmetric side chain. The majority of pyridoacridine alkaloids have a planar heterocyclic system. Structurally, pyridoacridines have a common tetracyclic heteroaromatic parent-11*H*-pyrido[4,3,2*nm*]acridine (**1a**) or 4*H*-pyrido[2,3,4-*kl*]acridone skeleton (**1b**), referred to as pyridoacridines. They vary in structure by appendage 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 C(2) in ring *A*.



1a



1b

Pyridoacridines can be divided into tetracyclic, pentacyclic, hexacyclic, heptacyclic, and octacyclic [4] (*Tables 1 – 4*). Amphimedine, the first pyridoacridine analogue from a marine organism, was isolated from an *Amphimedon* sp. sponge [5]. Since then, many pyridoacridines have been identified or synthesized. There has been considerable interest on the biological activity of these compounds, mainly on their potential as antitumor agents [1][6]. Almost all natural pyridoacridines have been reported to possess significant cytotoxic activity against tumor cells, in part due to their capacity to intercalate into DNA [1]. However, the most intriguing property of the pyridoacridine compounds is their capacity to interfere with the catalytic functions of topoisomerase (TOPO) II; the great chemical diversity in this family of alkaloids provided a

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large set of tools to manipulate the activity of this enzyme, either stimulating cleavage of DNA through stabilization of covalent TOPO II-DNA complexes or promoting the catenation of DNA [7].

During the past years, both natural pyridoacridines and their analogues have constituted excellent targets for synthetic works [6], confirming that this family of alkaloids as a whole is of interest as a source of new lead structures for drug development. This work presents a review of the literature describing 95 marine pyridoacridine alkaloids. This alkaloid class was chosen for their structural diversity. Here, we have listed the pyridoacridines that have appeared in the literature over the past few decades with their biological activities, structural assignment, and references (*Tables 1 – 4*). The main aim of this review is to provide knowledge to researchers for rapid identification of the isolated marine pyridoacridines.

2. Biosynthesis of Pyridoacridine Alkaloids. – It has been proposed that the pyridoacridine alkaloids are biosynthesized using a pathway that involves the utilization of tryptophan and DOPA as precursors [3]. As shown in *Scheme 1*, the condensation of *O*-quinone derived from DOPA, and kynuramine, a catabolic product from tryptophan could lead to the pyridoacridine skeleton. The condensation was supported by the biomimetic synthesis of the pyridoacridine skeleton by the *Kashman* group using kynuramine and *O*-quinone [53] (*Scheme 1*).

Scheme 1. *Biosynthetic Formation of the Pyridoacridine Skeleton* [53]

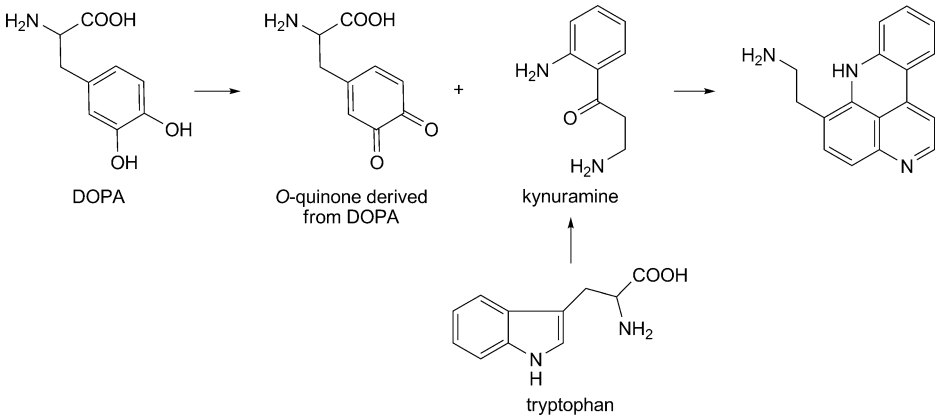

Table 1. *Tetracyclic Pyridoacridine Alkaloids* **1 – 26**

No.	Alkaloid name	Source	Ref.
1	Pantherinine	South Australia ascidian *Aplidium pantherinum*	[8]
2	Pantherinine acetate	South Australia ascidian *Aplidium pantherinum*	[8]
3	Cystodytin A	Okinawan tunicate *Cystodytes dellechiajei*	[9]
4	Cystodytin B	Okinawan tunicate *Cystodytes dellechiajei*	[9]
5	Cystodytin C	Okinawan tunicate *Cystodytes dellechiajei*	[9]
6	Cystodytin D	Okinawan tunicate *Cystodytes dellecbiajei*	[10]
7	Cystodytin E	Okinawan tunicate *Cystodytes dellecbiajei*	[10]
8	Cystodytin F	Okinawan tunicate *Cystodytes dellecbiajei*	[10]
9	Cystodytin G	Okinawan tunicate *Cystodytes dellecbiajei*	[10]
10	Cystodytin H	Okinawan tunicate *Cystodytes dellecbiajei*	[10]
11	Cystodytin I	Okinawan tunicate *Cystodytes dellecbiajei*	[10]
12	Cystodytin J	Fijian ascidian *Cystodytes* sp.	[11]
13	Cystodytin K	Zealand ascidian *Lissoclinum notti*	[12]
14	Iminoquinone	Indonesian ascidian *Eusynstyela latericius*	[13]
15	Styelsamine A	Indonesian ascidian *Eusynstyela latericius*	[13]
16	Styelsamine B	Indonesian ascidian *Eusynstyela latericius*	[13]
17	Styelsamine C	Indonesian ascidian *Eusynstyela latericius*	[13]
18	Styelsamine D	Indonesian ascidian *Eusynstyela latericius*	[13]
19	Diplamine	Fiji Islands tunicate *Diplosomra* sp.	[14]
20	Isodiplamine	New Zealand ascidian *Lissoclinum notti*	[12]
21	Diplamine B	Papua New Guinea ascidian *Lissoclinum* cf. *badium*	[15]
22	Norsegoline	Red Sea tunicate *Eudistom*a sp.	[16][17]
23	Tintamine	Indian Ocean tunicate *Cystodytes violatinctus*	[18]
24	Tintamine diacetate	Indian Ocean tunicate *Cystodytes violatinctus*	[18]
25	Varamine A	Fiji Island tunicate *Lissoclinum vareau*	[19]
26	Varamine B	Fiji Island tunicate *Lissoclinum vareau*	[19]

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Table 2. *Pentacyclic Pyridoacridine Alkaloids* **27** – **81**

No.	Alkaloid name	Source	Ref.
27	Kuanoniamine A	Micronesian tunicate and its predator, a prosobranch mollusk *Chelynotus semperi*	[20]
28	Kuanoniamine B	Micronesian tunicate and its predator, a prosobranch mollusk *Chelynotus semperi*	[20]
29	Dehydrokuanoniamine B	Fijian ascidian *Cystodytes* sp.	[11]
30	Kuanoniamine C	Micronesian tunicate and its predator, a prosobranch mollusk *Chelynotus semperi*	[20]
		Indonesian sponge *Oceanapia* sp.	[21]
31	Kuanoniamine D	Micronesian tunicate and its predator, a prosobranch mollusk *Chelynotus semperi*	[20]
32	*N*-Deacetylkuanoniamine C	Micronesian sponge *Oceanapia* sp.	[22]
33	Kuanoniamine F	Singaporean ascidian	[23]
34	Dehydrokuanoniamine F	South-Pacific Ocean ascidian *Cystodytes violatinctus*	[24]
35	Sagitol	Indonesian sponge *Oceanapia* sp.	[21]
36	Sagitol C	Indonesian sponge *Oceanapia* sp.	[21]
37	Petrosamine	Thai sponge *Petrosia* n. sp.	[25]
38	Debromopetrosamine trifluoroacetate salt	Sponge *Xestospongia* cf. *carbonaria*	[26]
39	Ecionine A	Australian sponge *Ecionemia geodides*	[27]
40	Ecionine B	Australian sponge *Ecionemia geodides*	[27]
41	Cystodimine A	Western Mediterranean ascidian *Cystodytes dellechiajei*	[28]
42	Cystodimine B	Western Mediterranean ascidian *Cystodytes dellechiajei*	[28]
43	Shermilamine A	Tunicate *Tridemnum* sp.	[29]
44	Debromoshermilamine A =	Tunicate *Eudistoma* sp.	[17]
	Shermilamine B	Tunicate *Tridemnum* sp.	[29]
45	*N*-Deacetylshermilamine B	Western Mediterranean ascidian *Cystodytes dellechiajei*	[28]
46	Shermilamine C	Fijian ascidian *Cystodytes* sp.	[11]
47	Shermilamine D	Tunicate *Cystodytes violatinctus*	[30]
48	Shermilamine E	Indian Ocean tunicate *Cystodytes violatinctus*	[18]
49	Shermilamine F	South-Pacific Ocean ascidian *Cystodytes violatinctus*	[24]
50	Sebastianine A	Brazilian ascidian *Cystodytes dellechiajei*	[31]
51	Lissoclinidine trifluoroacetate salt	New Zealand ascidian *Lissoclinum notti*	[12]
52	Lissoclinidine B	Papua New Guinea ascidian *Lissoclinum* cf. *badium*	[15]
53	Labuanine A	Indonesian sponge *Biemna fortis*	[32]
54	Ascididemin	Okinawan tunicate *Didemnum* sp.	[33]
		Western Mediterranean ascidian *Cystodytes delle chiajei*	[34]
55	3-Hydroxyascididemin	Anemone *Calliactis parasitica* (Actiniaria)	[35]
56	8,9-Dihydro-11-hydroxyascididemin	Okinawan sponge *Biemna* sp.	[36]
57	11-Hydroxyascididemin	Okinawan sponge *Biemna* sp.	[36]
		Ascidian *Amphicarpa meridiana* and *Leptoclinides* sp.	[37]
58	12-Deoxyascididemin	Australian ascidian *Polysyncraton echinatum*	[38]
59	9-Hydroxyisoascididemin	Indonesian sponge *Biemna fortis*	[32]
		Australian sponge *Ancorina geodides*	[39]
60	Meridine	South Australia ascidian *Amphicarpa meridiana* and *Leptoclinides* sp.	[37]
61	Meridin-12(13*H*)-one	South Australia ascidian *Amphicarpa meridiana* and *Leptoclinides* sp.	[37]
62	9-Aminobenzo[*b*]pyrido[4,3,2-*de*][1,10]-phenanthrolin-8(8*H*)-one	Indonesian sponge *Biemna fortis*	[32]
63	Ancorine A	Australian sponge *Ancorina geodides*	[39]
64	Cnemidine A	Australian tunicate *Cnemidocarpa stolonifera*	[39]
65	Arnoamine A	Ascidian *Cystodytes* sp.	[40]
66	Arnoamine B	Ascidian *Cystodytes* sp.	[40]
67	Arnoamine C	South-Pacific Ocean ascidian *Cystodytes violatinctus*	[24]
68	Arnoamine D	South-Pacific Ocean ascidian *Cystodytes violatinctus*	[24]
69	Dercitin	Sponge *Dercitus* sp.	[41]
70	Nordercitin	Sponges *Dercitus* sp. and *Stetleta* sp.	[42]
71	Dercitamine	Sponges *Dercitus* sp. and *Stetleta* sp.	[42]
72	Dercitamide	Sponges *Dercitus* sp. and *Stetleta* sp.	[42]
73	Amphimedine	Pacific Ocean sponge *Amphimedon* sp.	[5]
74	2-Bromoamphimedine	Thai sponge *Petrosia* n. sp.	[25]
75	Demetyldeoxyamphimedine	Western Mediterranean ascidian *Cystodytes dellechiajei*	[43]
76	1-Hydroxydeoxyamphimedine trifluoroacetate salt	Palau sponge *Xestospongia* cf. *carbonaria*	[26]
77	3-Hydroxydeoxyamphimedine trifluoroacetate salt	Sponge *Xestospongia* cf. *carbonaria*	[26]
78	Neoamphimedine	Philippine sponge *Xestospongia* sp. and Micronesian sponge *Xestospongia* cf. *carbonaria*	[44]
79	Deoxyamphimedine	Sponges *Xestospongia* sp. and *Xestospongia* cf. *carbonaria*	[45]
80	Cystodamine	Mediterranean ascidian *Cystodytes delle chiajei*	[34]
81	2-Bromoleptoclinidinone	Ascidian *Leptoclinides* sp.	[46]

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Table 3. *Hexacyclic Pyridoacridine Alkaloids* **82** – **91**

No.	Alkaloid name	Source	Ref.
82	Nordehydrocyclodercitin	Australian ascidian *Aplidium* sp., cf. *Aplidium cratiferum*	[47]
83	Stellettamine	Australian ascidian *Aplidium* sp., cf. *Aplidium cratiferum*	[47][48]
84	Cycloshermilamine D	Tunicate *Cystodytes violatinctus*	[49]
85	13-Didemethylaminocycloshermilamine D	Western Mediterranean ascidian *Cystodytes dellechiajei*	[43]
86	Sebastianine B	Brazilian ascidian *Cystodytes dellechiajei*	[31]
87	Segoline A	Tunicate *Eudistoma* sp.	[16]
		Indian Ocean tunicate *Eudistoma bituminis*	[50]
88	Isosegoline A	Tunicate *Eudistoma* sp.	[16][17]
89	Segoline B	Tunicate *Eudistoma* sp.	[17]
90	Segoline C	Tunicate *Eudistoma bituminis*	[50]
91	Cyclodercitin	Sponge *Dercitus* sp.	[42]

Table 4. *Heptacyclic* **92** *and Octacyclic Pyridoacridine Alkaloids* **93** – **95**

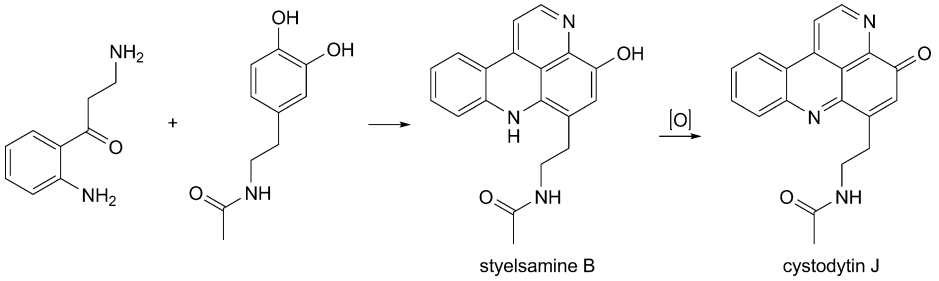
No.	Alkaloid Name	Source	Ref.
	Heptacyclic		
92	Eilatin	Tunicate *Eudistoma* sp.	[17][51]
	Octacyclic		
93	Biemnadin	Okinawan sponge *Biemna* sp.	[36]
		Indonesian sponge *Biemna fortis*	[32]
94	Eudistone A	Seychelles tunicate *Eudistoma* sp.	[52]
		Okinawan sponge *Biemna* sp.	[36]
95	Eudistone B	Seychelles tunicate *Eudistoma* sp.	[52]

The arnoamines were the first two pentacyclic pyridoacridine alkaloids isolated that have a pyrrole ring attached to the skeleton. Other related pyridoacridines that have a pyrrole ring attached at the same position include cyclodercitin (**91**) [42] and stelletamine [48]. The ethylene bridge of the pyrrole ring in the pyrrole-containing pyridoacridines suggests a biosynthetic origin similar to the pyridoacridines possessing an aminoethyl side chain as cystodytins [9] and varamines [19]. It can be proposed that the pyrrole ring could be formed by the cyclization of the aminoethyl side chain, presumably through its imine-enamine oxidation state. The biosynthetic similarity among the pyrrole- and aminoethyl-containing pyridoacridines also support the hypothesis that DOPA is the biosynthetic precursor of pyridoacridines.

Biosynthetically, it has been hypothesized that pyridoacridine alkaloids belonging to the sulfur-containing chemotype such as shermilamine B (**44**) were formed from tryptophan, dopamine, and cysteine. *Skyler* and *Heathcock* suggested that the free amine styelsamine D (**18**) can be the starting point to simple dopamine-derived pyridoacridines and to the more complex thiazole- or thiazinone-containing pyridoacridines [54] (*Scheme 2*). Accordingly, 13-didemethylaminocycloshermilamine D (**85**) could be obtained by oxidative coupling between the ethylamine side chain and the acridine NH group of *N*-deacetylshermilamine B (**45**) [43][49][54]. A biosynthetic link between demethyldeoxyamphimedine (**75**) and styelsamine D (**18**) by a formylation/cyclization/oxidation pathway was postulated. The cyclization of the ethylamine side chain occurred in this case at the unsubstituted C(9) of styelsamine D, leading to a new pyridine ring. Also, different biosynthetic pathways for pyridoacridines were discussed [24][43][47][54].

3. Pyridoacridines and Their Biological Activities. – 3.1. *Anticholinesterase Activity.* Petrosamine (**37**) displayed potent AChE inhibitory activity (IC_{50} 0.091 μM) about six times more potent than galanthamine (positive control, IC_{50} 0.590 μM), whereas 2-bromoamphimedine (**74**)

Scheme 2. *Biosynthetic Pathway of Cystodytin J* [54]



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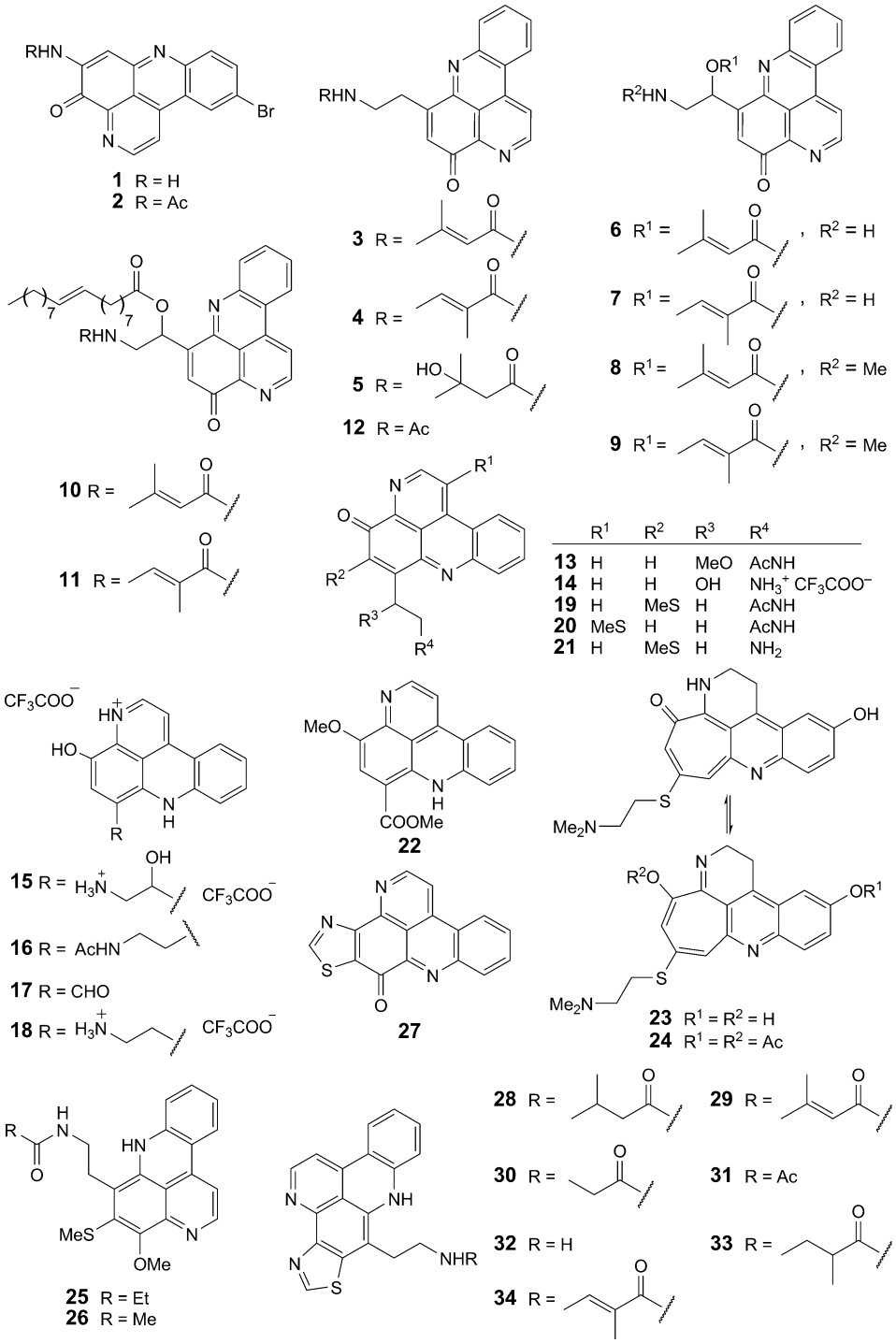

showed very weak potency with IC_{50} higher than 300 μM [25].

3.2. *Cytotoxic Activity.* Pantherinine (**1**) showed mild cytotoxic activity ED_{50} 4.5 μg/ml against P388 murine leukemia cells [8]. Cystodytins A (**3**) and B (**4**) showed potent cytotoxicity, exhibiting IC_{50} values of 0.22 and 0.24 μg/ml against mouse leukemia cell lines L1210, respectively [9]. Cystodytins D – I (**6** – **11**) were cytotoxic against murine lymphoma L1210 cells with IC_{50} values of 1.1 (**6** and **7**), 0.068 (**8** and **9**), and 0.080 (**10** and **11**) μg/ml, and 1.4 (**6** and **7**), 0.078 (**8** and **9**), and 0.092 (**10** and **11**) μg/ml against human epidermoid carcinoma KB cells *in vitro* [10]. The cytotoxic effect of kuanoniamines A (**27**) and C (**30**) had been evaluated against MCF-7 ER (+), MDAMB-231 ER (−), NCI-H460, SF-268, and UACC-62 and one non-tumour cell line MRC-5 by the SRB method. Compound **27** was a potent growth inhibitor of all the human tumor cell lines, as well as the non tumor cell line. Though **30** was found to be much less potent than **27**, it was found to possess a high selectivity towards the estrogen-dependent (ER +) breast cancer cell line [55]. Moreover, kuanoniamines A (**27**) and D (**31**) and shermilamine B (**44**)

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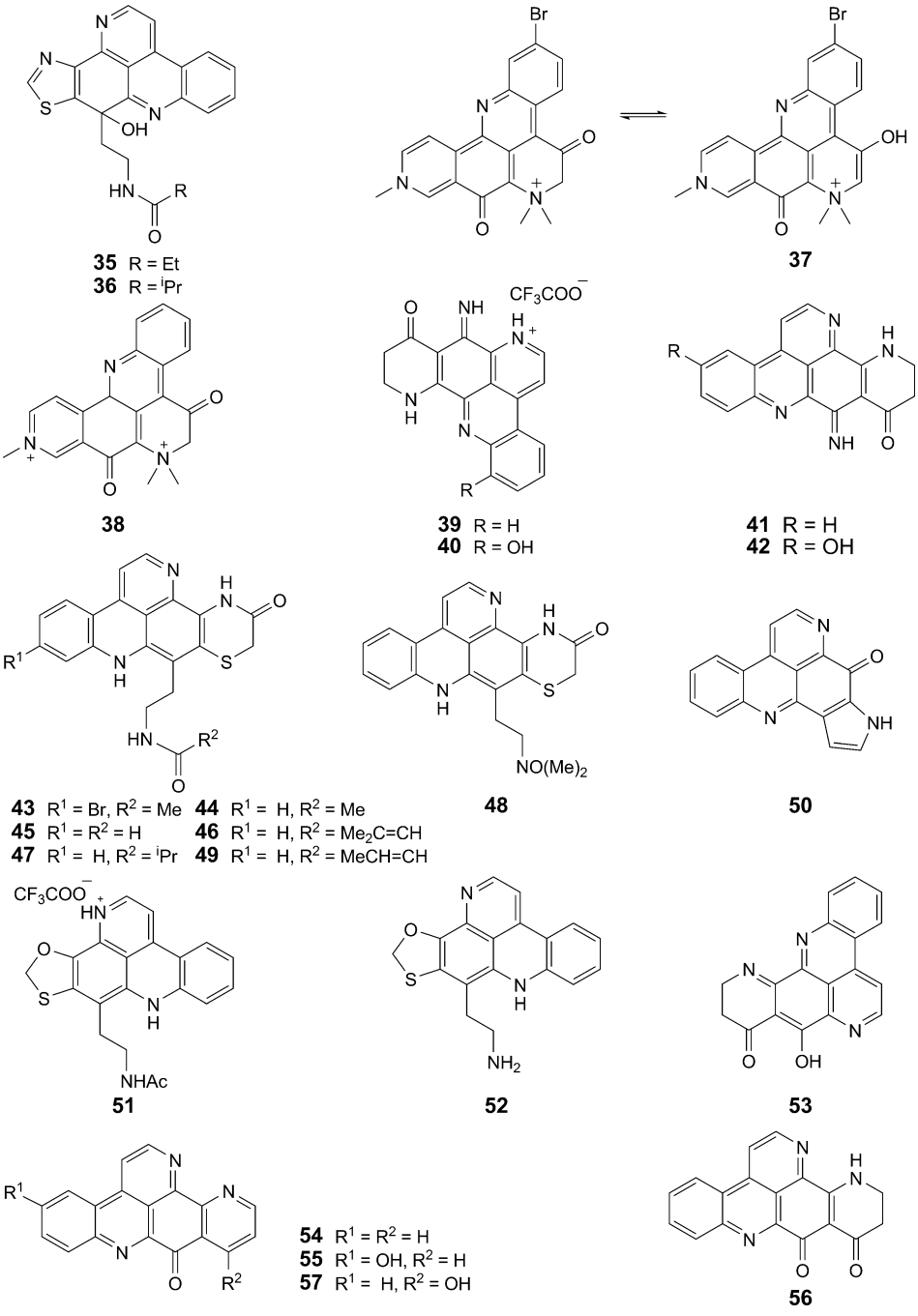

showed cytotoxic activity against KB cells (IC_{50} 5 μg/ml), while kuanoniamine B (**28**) showed weak activity (IC_{50} > 10 μg/ml) [20]. Cystodytins A (**3**) and J (**12**), diplamine (**19**), dehydrokuanoniamine B (**29**), kuanoniamine D (**31**), shermilamines B (**44**) and C (**46**), and eilatin (**92**) showed dose-dependent inhibition of proliferation in human colon tumor (HCT) cells *in vitro.* All these compounds inhibited the topoisomerase (TOPO) II-mediated decatenation of kinetoplast (*k*DNA) in a dose-dependent manner, which correlated with their cytotoxic potencies and their ability to intercalate into calf thymus DNA [11]. The cytotoxic effect of sagitol C (**36**) was tested against L5178Y, PC12, and Hela cell lines. At a concentration of 24.6l μM, it gave 93, 88, and 76% growth suppression against the tested cell lines and 81, 74, and 37% at a concentration of 12.3l μM with ED_{50} values of 0.7, 0.9, and 2.3 μM, respectively [21]. Ecionines A (**39**) and B (**40**), 11-hydroxyascididemin (**57**), 9-hydroxyisoascididemin (**59**), ancorine A (**63**), and cnemidine A (**64**) were tested against PC3 and NFF cell lines. The tested compounds (excluding **39**, IC_{50} 17 μM) exhibited potent activity towards prostate cancer PC3 cells with comparable IC_{50} values from 0.97 to 3.3 μM. Compound **63** had a weak cytotoxic effect on PC3 cells (IC_{50} of 17 μM). Compound **59** has been found to inhibit the growth of P-388 (IC_{50} 4.18 μM), A-549 (human lung cancer, IC_{50} 0.03 μM), HT-29 (human colon cancer, IC_{50} 0.40 μM), and

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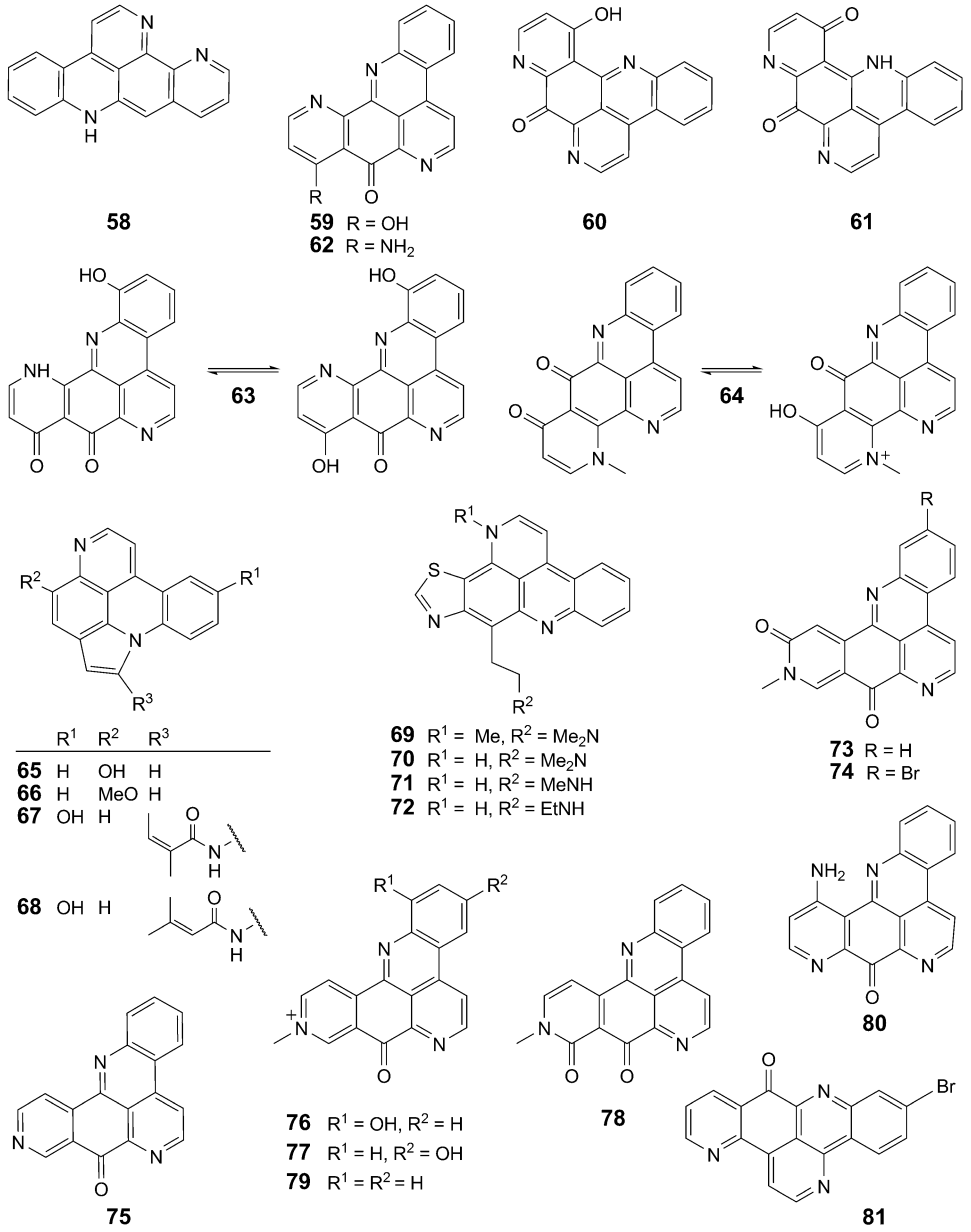

MEL-28 (IC_{50} 0.17 μM). Compound **39** showed moderate cytotoxicity against bladder cancer cell lines with IC_{50} values of 6.48 (TSU-Pr1), 6.49 (TSU-Pr1-B1), 3.55 (TSU-Pr1-B2), and 3.66 μM (5637), respectively [27]. Compound **40** had a modest cytotoxic effect on 5637 and TSU-Pr1-B2 cells at 10 μM with cell growth inhibitions of 54% and 51% cells, respectively, but did not have an effect on TSU-Pr1-B1 cells at 10 μM [27]. 11-Hydroxyascididemin (**57**) displayed *in vitro* cytotoxicity against a panel of cancer cell lines with similar IC_{50} values ranging from 1 to 6 μM [6]. A cytotoxic activity of 11-hydroxyascididemin (**57**) against PC3 cells (IC_{50} 1.9 μM) comparable to that reported by *Delfourne* and *Bastide* (IC_{50} 5 μM) [6]. Meridine (**60**) had comparable activity against the invasive bladder cancer TSU-Pr1 cell line with IC_{50} values of 3.77 (TSU-Pr1), 4.56 (TSU-Pr1-B1), and 3.76 μM (TSU-Pr1-B2) but had a smaller effect on the superficial bladder cancer cell line 5637. Biemnadin (**93**) displayed weak cytotoxic effects against the superficial bladder cancer cell line 5637 (10 μM induced cell death in 22% cells), but had no effect on TSU-Pr1 cells [27]. In toxicity studies using the human embryonic kidney cell line HEK293, ascididemin (**54**) and 12-deoxyascididemin (**58**) had cytotoxic activity with IC_{50} values of 1.48 and 7.63 μM, respectively. However, eilatin (**92**) exhibited a plateau of 62% inhibition against HEK293 [38]. The IC_{50} values of dehydrokuanoniamines B (**29**) and F (**34**), shermilamines C (**46**) and F (**49**), and arnoamines C (**67**) and D (**68**) were evaluated on A375 (melanoma), HCT116, and SW480 (colon) cancer cell lines using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Compound **67** was the most active, with IC_{50} values 4.32, 8.48, and 6.00 μM, respectively. Compound **34** showed selective activity to the SW480 cell line (IC_{50} 3.30 μM) [24]. Shermilamines A

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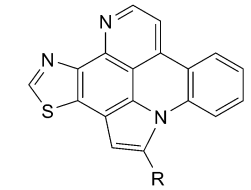
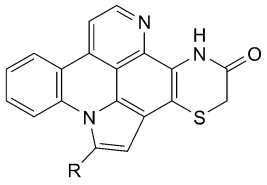
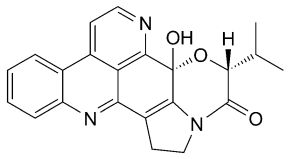


82 R = H
83 R = Me_2N



84 R = Me_2N
85 R = H



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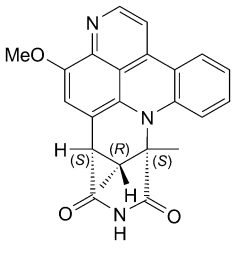

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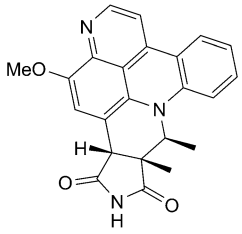

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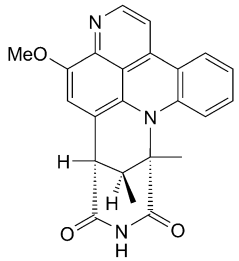

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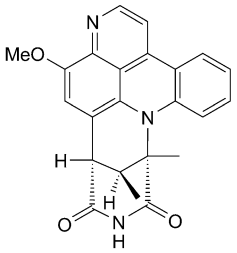

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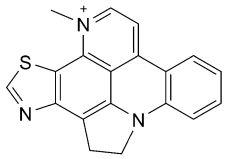

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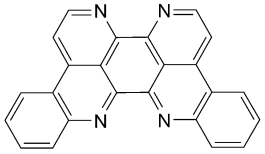

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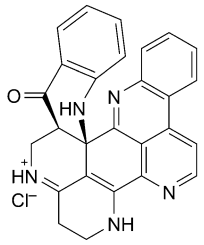

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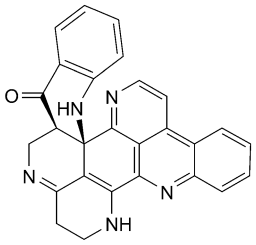

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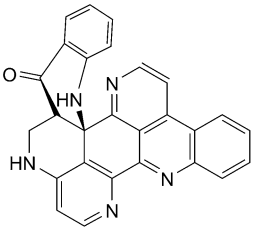

95

(**43**) and B (**44**), ascididemin (**54**), and amphimedine (**73**) showed cytotoxicity to cultures of murine leukemia cells (P388) at 0.344 μg/ml. Compounds **44** and **54** inhibited toposiomerase II at concentrations of 30 and 75 μM, respectively. However, compounds **43** and **73** were inactive [37].

Sebastianines A (**50**) and B (**86**) were screened against four human colon tumor (HCT) cell lines comprised of p53 and p21 knockouts, as well as the parental cell line of each. Both alkaloids displayed a cytotoxic profile against a panel of HCT-116 colon carcinoma cells. Both **50** and **86** showed a slightly lower IC_{50} in the $p21^{-/-}$ *versus* the $p21^{+/+}$ [31]. Diplamine B (**21**) and lissoclinidine B (**52**) were tested in the Hdm2 electrochemiluminescence assay. They had IC_{50} values of 101.3 and 98.1 μM, respectively. Diplamine B (**21**) and lissoclinidine B (**52**) are new inhibitors of Hdm2 auto-ubiquitylation and clearly stabilize both p53 and Hdm2 in cells at low micro-molar concentrations. On the other hand, **21** and **52** increased p53 and Hdm2 in a dose-dependent manner and exhibited the greatest increase in p53 and Hdm2 at 10 μM, similar to *N*-acetyl-leucyl-leucyl-norleucinal (ALLN) (50 μM, proteasome inhibitor) [15].

Arnoamine A (**65**) exhibited selective cytotoxicity against MCF-7 breast cancer cell lines with a GI_{50} value of 0.3 μg/ml, *versus* GI_{50} values 2.0 and 4.0 μg/ml against A-549 lung and HT-29 colon cancer cell lines, respectively. Arnoamine B (**66**) was less active against the MCF-7, A-549, and HT-29 cancer cell lines (GI_{50} values 5.0, 2.0, and 3.0 μg/ml, respectively) [40]. Diplamine (**19**), isodiplamine (**20**), and lissoclinidine (**51**) possessed moderate to high activity towards P388, HCT-116, and BSC-1. Compound **19** was the most cytotoxic compound towards BSC-1, although interestingly, this potency was not for P388 or HCT-116 tumour cells. Lissoclinidine (**51**) was evaluated against the NCI 60-cell line panel, but was found to exhibit only moderate activity and selectivity (panel average values: GI_{50} 1.0 μM, 6.9 μM, LC_{50} 29 μM) [12]. Diplamine (**19**) is cytotoxic towards L1210 murine leukemia cells with an IC_{50} of 2×10^{-2} μg/ml [14]. Ascididemin (**54**) was cytotoxic with an IC_{50} value of 0.39 μg/ml against L1210 murine leukemia cells [33]. Nordercitin (**70**), dercitamine (**71**), and dercitamide (**72**) inhibited *in vitro* proliferation of P388 murine leukemia cells [42]. Cystodamine (**80**) showed activity

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against CEM human leukemic lymphoblasts (IC_{50} 1.0 μg/ml) [34]. Neoamphimedine (**78**) was cytotoxic to normal CHOAA8 cells with an IC_{50} of 2 μg/ml. In quantitative DNA cleavage assays, it stimulated topoisomerase II-dependent cleavage 3% compared to etoposide, which stimulated 38% cleavage at the same concentration. No stimulation of DNA cleavage was seen with amphimedine (**73**) in the presence of topoisomerase II [44]. Deoxyamphimedine (**79**) showed cytotoxic activity against human colon tumor cells (HCT-116) with an IC_{50} of 335 nM. Also, it was tested in Chinese hamster ovary cells, AA8 (wild type), and EM9 (sensitive to single strand (ss) DNA break damage). EM9 cells were 4-fold more sensitive to damage from deoxyamphimedine (**79**) than the AA8 cells, with IC_{50} values of 6 and 25 μM, respectively [45]. 2-Bromoleptoclinidinone (**81**) shows mild cytotoxicity against lymphocytic leukemia cells (PS; ED_{50} 0.4 μg/ml) [46]. Varamine A (**25**) or varamine B (**26**) showed cytotoxic activity towards L1210 murine leukemia cells with IC_{50} of 0.03 and 0.05 μg/ml, respectively [19].

3.3. *Antimicrobial Activity.* Diplamine (**19**) showed antimicrobial against *E. coli* and *S. aureas* [14]. Compounds **19**, **20**, and **51** exhibited modest to potent antimicrobial activity towards a variety of microorganisms including the bacteria *B. subtilis* and *E. coli* and the fungi *C. albicans* and *T. mentagrophytes* [12]. Demetyldeoxyamphimedine (**75**) and 13-didemethylaminocycloshermilamine D (**85**) were tested against the marine bacterial strain *L. anguillarum* and the terrestrial bacterial strain *M. luteus* using a liquid growth inhibition assay based on a NCCLS method. They showed an activity against the two strains in the order of *micro*-molar with less activity on the marine strain. The *MIC* values ranged from 6.5 to 7 μM toward *M. luteus* and from 7.0 to 9.0 μM toward *L. anguillarum* [43]. Kuanoniamines C (**30**) and D (**31**) and *N*-deacetylkuanoniamine C (**32**) did not show any growth inhibiting activity against *B. subtilis* 168, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922, nor any antifungal activity against *C. cucumerinum* using the agar plate diffusion assay [22]. The antimicrobial activities of cystodytins (**3** – **11**), diplamine (**19**), isodiplamine (**20**), and lissoclinidine (**51**) were determined by a liquid growth inhibition assay based on an NCCLS method. They exhibited activity against a panel of bacteria and fungi strains (*MIC* values from 1 to 29 μM). Also, ascididemin (**54**) was found to be active against *E. coli*, *C. resinae*, and *B. subtilis* but inactive towards *P. aeruginosa* and *T. mentagrophytes* [28].

3.4. *Antiviral Activity.* Dercitin (**69**) exhibited strong inhibition of HSV-1 at 5 μg/ml with moderate cytotoxicity. It also completely inhibited murine A59 coronavirus at 1 μg/ml with no cytotoxicity [56]. On the other hand, kuanoniamine A (**27**) and ascididemin (**54**) appeared inactive [57]. Eilatin (**92**)-Ru(II) complexes displayed strong anti-HIV activity in CD4+ HeLa cells and human peripheral blood monocytes [58].

3.5. *Insecticidal Activity.* Kuanoniamines C (**30**) and D (**31**) exhibited insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis* (LC_{50} 156 and 59 ppm, resp.), when incorporated into artificial diet [22].

3.6. *Antitrypanosomal Activity.* The antitrypanosomal activity of 12-deoxyascididemin (**58**), ascididemin (**54**), and eilatin (**92**) was evaluated *in vitro* against *T. brucei brucei*. They exhibited potent activity against *T. brucei brucei* with IC_{50} values of 0.077, 0.032, and 1.33 μM, respectively [38]. Amphimedine (**73**) had no activity against *T. brucei brucei* while, neoamphimedine (**78**) was quite active with an IC_{50} of 0.065 μg/ml (*ca.* 0.21 μM) [4].

3.7. *Other Activities.* There are several miscellaneous activities reported for pyridoacridines in the literature. This section presents those disparate data.

Ascididemin (**54**) can facilitate calcium release from the sarcoplasmic reticulum. It is also seven times more potent than caffeine, a well-known Ca-releaser, in the Ca-releasing activity in sarcoplasmic reticulum [33]. Cystodytins A (**3**) and B (**4**) showed exceptional activity in this assay, while cystodytin C (**5**) and ascididemin (**54**) were not as effective [4]. The affinity of kuanoniamines for adenosine and GABA receptors compared to theophylline and caffeine was tested. Kuanoniamine D (**31**) showed affinity to A1- and A2A-adenosine receptors with K_i values of 2.94 and 13.7 μM, respectively. Kuanoniamine C (**30**) was less active than kuanoniamine D (**31**), whereas *N*-deacetylkuanoniamine C (**32**) showed no affinity toward adenosine receptors. In addition, compounds **30** – **32** exhibited moderate affinity to benzodiazepine binding sites of $GABA_A$ receptors [22]. The pyridoacridine alkaloids labuanine A (**53**), 9-hydroxyisoascididemin (**59**), 9-aminobenzo[*b*]pyrido[4,3,2-*de*][1,10]phenanthrolin-8(8*H*)-one (**62**), and biemnadin (**93**) induced multipolar neuritogenesis in more than 50% of cells at 0.03 – 3 μM concentration. Compound **62** 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 [32]. 1-Hydroxydeoxyamphimedine (**76**), 3-hydroxydeoxyamphimedine (**77**), debromopetrosamine (**38**), amphimedine (**73**), neoamphimedine (**78**), and deoxyamphimedine (**79**) were evaluated in a zebrafish phenotype-based assay. Amphimedine (**73**) was the only compound that caused a phenotype in zebrafish embryos at 30 μM. No phenotype other than death was observed for compounds **38** and **76** – **79** [26]. Nordercitin (**70**), dercitamine (**71**), and dercitamide (**72**) exhibited immunosuppressant activity [42].

4. Structural Assignment of Pyridoacridines. – Currently, standard spectroscopic methods for investigating the structure of natural products comprise nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and ultraviolet spectroscopy (UV), and these are often combined with mass spectrometry (MS). Single-crystal X-ray diffraction is a powerful technique used for determining the molecular topology. The assignment of structure in general by NMR in highly condensed heterocyclic aromatic compounds is complicated because of the difficulty in defining the correct regioisomer from many possibilities. However,

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these problems can be solved by employing new powerful multipulse NMR techniques like HMQC/HSQC, HMBC, INADEQUATE, INAPT. J_{CH} Coupling constant analysis has been helpful in the resolution of ambiguous structural assignments. When suitable crystals of the compound are available, single crystal X-ray diffraction analysis has given definitive structures. Because the ring system is highly conserved, some general features in the appearance of the ^{1}H-NMR spectra are common to most of these alkaloids and useful in identifying a member of this class of compounds. The di-substituted benzene ring *A* gives rise to a distinctive linear four H-atom coupled spin network (H–C(1 – 4), 7.0 – 9.0 ppm, $J = 8 – 9$ Hz) with H–C(1) resonating at lowest field due to the deshielding acridine N-atom. A second *AB* spin system (8.5, 9.0 ppm, $J = 5.6$ Hz) is assignable to H–C(5,6), the H-atoms of a tri-substituted pyridine ring. A strong NOE is seen between H–C(4) and H–C(5), thus, linking these two non-scalar-coupled substructures.

5. Conclusions. – Pyridoacridines, a class of marine-derived alkaloids fulfill all the requirements of being lead compounds in their respective therapeutic category. They possess varied chemical compositions and conformations. They present a wide array of biological activities. In this review, 95 naturally occurring marine pyridoacridines were listed, and their biosynthetic pathways and biological activities were discussed.

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