HETEROCYCLES, Vol. 74, 2007, pp. 53 - 88. © The Japan Institute of Heterocyclic Chemistry
Received, 30th August, 2007, Accepted, 23rd October, 2007, Published online, 26th October, 2007. REV-07-SR(W)2

BIOACTIVE NITROGENOUS METABOLITES FROM ASCIDIANS

Weifang Wang and Michio Namikoshi*

Department of Natural Product Chemistry, Tohoku Pharmaceutical University, Komatsushima, Aoba-ku, Sendai 981-8558, Japan

Abstract – Ascidians (class Ascidiacea) belong to the subphylum Urochordata (Tunicata) and are generally called as tunicates because the body is covered by tunic, a sack-like case, or sea squirts since they spurt water when disturbed. Nitrogenous secondary metabolites form more than 80% of new natural products obtained from ascidians and showed a wide variety of bioactivities. This review describes the structures and bioactivities of major classes of unique nitrogenous ascidian metabolites: cyclic peptides, in which aplidine is in the Phase II clinical trials, pyridoacridine alkaloids, β-carboline alkaloids, lamellarins and related pyrrole alkaloids, ecteinascidins (ETs, ET 743 completed the Phase III clinical trials), diterpene and steroidal alkaloids, and polysulfur alkaloids.

INTRODUCTION

Ascidians (Ascidiacea) belong to the subphylum Urochordata (Tunicata), which includes three classes, Ascidiacea, Thaliacea, and Appendiculata. The common feature of the animals in Urochordata is a tunic, a sack-like case covering their soft bodies. Therefore, these animals are called tunicates. Members in Ascidiacea are sessile filter feeders and those in the latter two classes are pelagic. These animals possess pharyngeal slits, dorsal hollow nerve cords, and notochords at their larval stages, but the latter two tissues are lost during development in Ascidiacea and Thaliacea. Ascidiacea is the largest and most diverse class in Urochordata, and ascidians take many different forms, which are conveniently divided into two types, solitary and colonial species. The solitary species generally live as isolated individuals, and the colonial species are characterized by many small individuals, called zooids, living together in a common tunic.

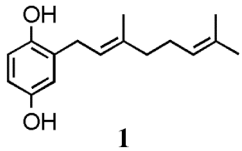
Unique color changes in ascidian blood, from yellow-green to deep blue by exposure to air, have been reported as early as 1847,[1,2] which prompted organic chemists to elucidate the molecular mechanism. Relatively large amounts of vanadium, sulfuric acid, and an uncharacterized nitrogenous compound were

---

This paper is dedicated to Professor Dr. Ekkehard Winterfeldt on the occasion of his 75th birthday.

deduced to cause the color changes.[3-5] Hemovanadins, oxygen-carrying proteins, were first supposed to function in the pigmentation, which was found to be wrong. However, the efforts have provided much knowledge on ascidian chemistry and biology,[6-8] and unstable hydroquinones, tunichromes, were obtained from the blood of several ascidian species.[9,10]

The first ascidian secondary metabolite, geranyl hydroquinone (**1**), was isolated from *Aplidium* sp. and reported in 1974.[11] It was about a quarter century later than the isolation of arabino nucleosides from a marine sponge *Tedania* sp.,[12] that is generally thought to be the beginning of marine natural product chemistry. Compound **1** exhibited chemo-preventive activity against some forms of leukemia, Rous sarcoma, and mammary carcinoma in test animals. Since malignant neoplasms have been the important targets for natural product chemists to search the medicines and their lead compounds, these findings encouraged the researchers for further studies on these organisms.[13]



1

Chemical studies on ascidians have grown considerably over the past several decades, and 761 new secondary metabolites have been isolated up to 2006 (Fig. 1). Ascidians remain unique among marine invertebrates in that they overwhelmingly produced nitrogen-containing metabolites (Fig. 1); most of these compounds were biosynthesized from amino acids. Of the 761 new compounds from ascidians, a vast majority, 621 (81.6%), contained nitrogen. A number of these nitrogenous compounds exhibited remarkable bioactivities, such as cytotoxic, antimicrobial, antiviral, and anti-inflammatory activities, and novel chemical structures originated in the various mechanisms of pharmacological activities. Six ascidian-derived drug candidates, didemnin B, ecteinascidin-743, aplidine, vitilevuamide, diazonamide, and asciddemin, entered or currently undergoing clinical or preclinical trails, are all nitrogenous compounds.[14] This review covers several major classes of unique nitrogenous ascidian-derived metabolites with pronounced biological activities.

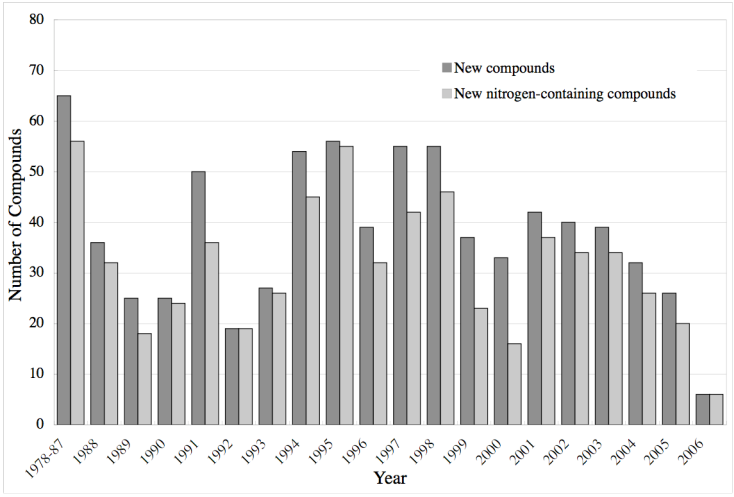
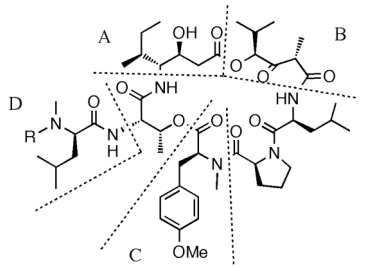
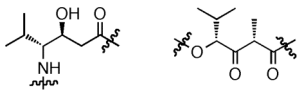


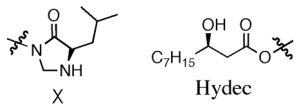
Fig. 1 Number of new compounds and new nitrogenous compounds from ascidians reported annually

CYCLIC PEPTIDES

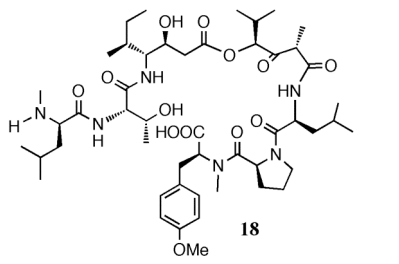
Cyclic peptides with novel structures and biological functions are one of the major structural classes isolated from ascidians. The discovery of didemnins, a well-known family of closely related highly bioactive cyclic depsipeptides, launched an exciting and intriguing chapter in marine natural product chemistry. The initial isolation of didemnins A (**2**), B (**3**), and C (**4**) from a Caribbean *Tridemnum solidum* was reported in 1981 as antiviral and antitumor agents.[15,16] Continuous studies on the large amounts of extracts of this ascidian provided 14 new congeners, didemnins D (**5**), E (**6**),[17,18] G (**7**),[19] M (**8**), N (**9**),[20] X (**10**), and Y (**11**),[20,21] nordidemnins A (**12**), B (**13**), C (**14**),[17,22] and N (**15**),[20] as well as methylenedidemnin A (**16**),[17] epididemnin A_1 (**17**), and a ring-opened form of didemnin A, acyclodidemnin A (**18**).[20] Didemnin M was previously described as didemnin H from *Tridemnum cyanophorum*.[23] A patent disclosed the structure and cytotoxic activity of dehydrodidemnin B (**19**), or aplidine, from the Mediterranean *Aplidium albicans*.[24] Two new didemnins with modified macrocyclic rings, [Try[5]]didemnin B (**20**) and [D-Pro[4]]didemnin B (**21**), were purified from *T. cyanophorum*,[25] which also afforded two new [Hip[2]]-modified didemnins, [Hysp[2]]didemnin B (**22**) and [Hap[2]]didemnin B (**23**).[26]

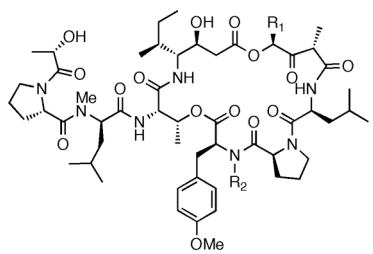




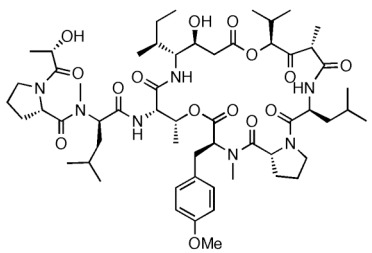


	A	B	C	D	R
2	Ist	Hip	Me_2Tyr	MeLeu	H
3	Ist	Hip	Me_2Tyr	MeLeu	Pro-Lac
4	Ist	Hip	Me_2Tyr	MeLeu	Lac
5	Ist	Hip	Me_2Tyr	MeLeu	Pro-Lac-$(Gln)_3$-pGlu
6	Ist	Hip	Me_2Tyr	MeLeu	Pro-Lac-$(Gln)_2$-pGlu
7	Ist	Hip	Me_2Tyr	MeLeu	CHO
8	Ist	Hip	Me_2Tyr	MeLeu	Pro-Lac-Gln-pGlu
9	Ist	Hip	Tyr	MeLeu	Pro-Lac
10	Ist	Hip	Me_2Tyr	MeLeu	Pro-Lac-$(Gln)_3$-Hydec
11	Ist	Hip	Me_2Tyr	MeLeu	Pro-Lac-$(Gln)_4$-Hydec
12	Norsta	Hip	Me_2Tyr	MeLeu	H
13	Norsta	Hip	Me_2Tyr	MeLeu	Pro-Lac
14	Norsta	Hip	Me_2Tyr	MeLeu	Lac
15	Norsta	Hip	Tyr	MeLeu	Pro-Lac
16	Ist	Hip	Me_2Tyr	X	—
17	Ist	epiHip	Me_2Tyr	MeLeu	H
19	Ist	Hip	Me_2Tyr	MeLeu	Pro-Pyruvate

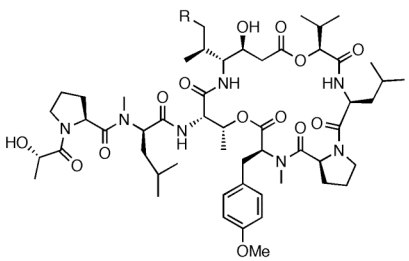




20: R_1 = $CHMe_2$, R_2 = H
22: R_1 = CH(Me)Et, R_2 = Me
23: R_1 = H, R_2 = Me



21

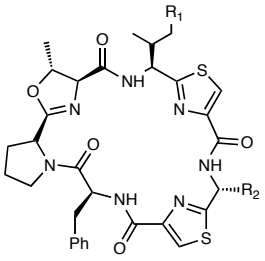


24: R = Me
25: R = H

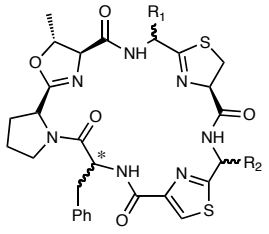
Two cytotoxic depsipeptides related to the didemnins, tamandarins A (**24**) and B (**25**), were discovered from a Brazilian ascidian of the family Didemnidae.[27] The cytotoxicity of tamandarin A (**24**) was evaluated against various human cancer cell lines and shown to be slightly more potent than didemnin B. Most of the didemnins exhibited remarkable antitumor, antiviral, and immunosuppressive activities.[28] Several structure-activity relationship studies on the didemnins have been reported.[29-32] More recently, the Illinois group reported systematic studies on the bioactivities of 42 natural and synthetic didemnin congeners and revealed that the native cyclic depsipeptide core was an essential structural requirement for most of the bioactivities, especially for cytotoxic and antiviral activities.[31] The linear side chain portion of the peptide can be altered with a gain of bioactivities in some cases. Didemnin B (**3**) was one of the most prominent members in this family, and its antitumor properties were reviewed in 1986.[33] Didemnin B (**3**) was advanced to the Phase II human clinical trials as the first marine natural product because of the high levels of in vivo antitumor activity but dropped from further studies due to the cardiotoxicity. Dehydrodidemnin B (aplidine) (**19**) showed no apparent cardiotoxicity and stronger antitumor activity than didemnin B and is now in Phase II clinical trials as a second-generation clinical candidate of the didemnin series.[14]

The genus *Lissoclinum* is an extraordinary source of cyclic peptides. Peptides from *Lissoclinum patella* were characterized by the presence of thiazole and oxazoline amino acids and divided into two general groups, heptapeptides and octapeptides. Within the past decade, collections of *L. patella* from a variety of locations have afforded heptapeptides, lissoclinamides 1-10 (**26**-**35**)[34-38] and ulicyclamide (**36**),[39] as well as octapeptides, patellamides A-G (**37**-**43**),[35,40-43] ulithiacyclamide (**44**),[39] and ulithiacyclamides B (**45**)[44] and E-G (**46**-**48**).[43] Most of the lissoclinamides exhibited mild cytotoxicity except for lissoclinamide 7 (**32**), which was highly cytotoxic against the human bladder carcinoma T24 cells, human fibroblasts MRC5CV1 cells, and peripheral blood lymphocytes with the IC_{50} values of 0.06, 0.04, and 0.08 μg/mL, respectively,[37] and lissoclinamide 4 (**29**), which was reported to reduce survival in both T24 and MRC5CV1 cells to approximately 5% of the untreated values at 5 μg/mL (IC_{50} = 0.8 μg/mL).[35] Ulithiacyclamide (**44**) showed the most potent cytotoxicity against the murine leukemia L1210 and human CEM acute lymphoblastic leukemia cell lines with the IC_{50} values of 0.35 and 0.01 μg/mL, respectively.[35] Patellamides A (**37**), B (**38**), and C (**39**) were about 10 times less cytotoxic having IC_{50} values of 2-4 μg/mL against L1210 cells, and the IC_{50} value of ulicyclamide was 7.2 μg/mL.[40] The structure-activity relationship (SAR) study revealed that the oxazoline group and/or disulfide bridge may be necessary to improve their cytotoxic activity.[45] While, the substitution of thiazolines with oxazolines led to the general decrease in cellular toxicity, and changes in the stereochemistry of amino acids in the macrocyclic ring influenced the cytotoxicity.[46] Patellamides B (**38**) and C (**39**) also reduced in vitro multi-drug resistance (MDR) of drug resistant lymphoblasts.[43] A metagenomic analysis revealed the

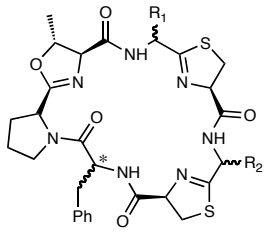
symbiotic cyanobacterium in *L. patella* synthesized patellamides by a ribosomal pathway.[47]



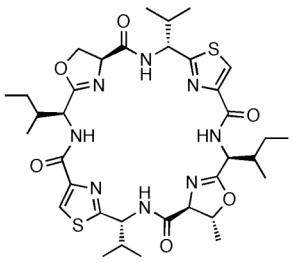
	R_1	R_2
26:	H	CH(Me)Et
30:	H	CH_2-Phe
36:	Me	Me



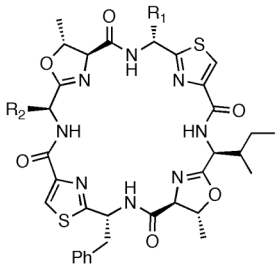
	R_1	R_2	*
27:	(*R*)-CH(Me)Et	(*R*)-Me	*S*
28:	(*R*)-CH(Me)Et	(*S*)-Me	*S*
29:	(*S*)-$CHMe_2$	(*R*)-CH_2-Phe	*S*
31:	(*R*)-$CHMe_2$	(*R*)-CH_2-Phe	*S*
33:	(*R**)-$CHMe_2$	(*R**)-CH_2-Phe	*S*
34:	(*S*)-CH(Me)Et	(*R*)-$CHMe_2$	*R*



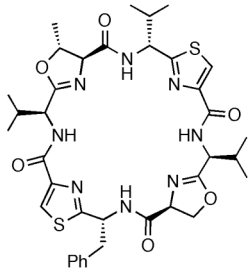
	R_1	R_2	*
32:	(*R*)-$CHMe_2$	(*R*)-CH_2-Phe	*S*
35:	(*S*)-CH(Me)Et	(*S*)-CH(Me)Et	*R*



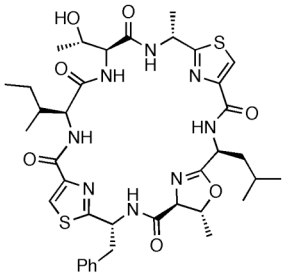
37



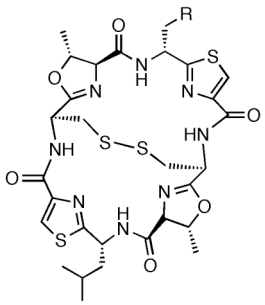
38: R_1 = Me, R_2 = CH_2CHMe_2
39: R_1 = Me, R_2 = $CHMe_2$
40: R_1 = Me, R_2 = CH(Me)Et
41: R_1 = $CHMe_2$, R_2 = $CHMe_2$



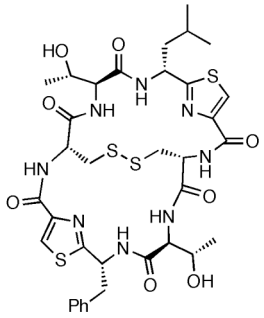
42



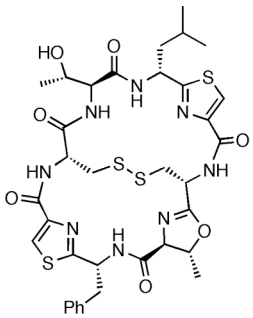
43



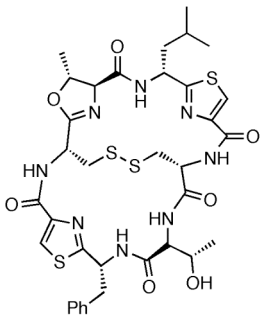
44: R = $CHMe_2$
45: R = Phe



46



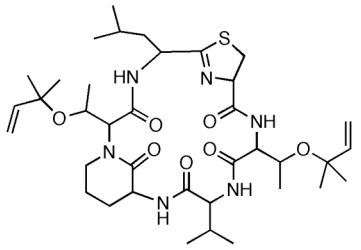
47



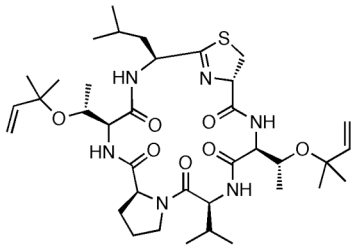
48

A new family of cyclic peptides, called patellins, were isolated from *L. patella* collected in Fiji and a *Lissoclinum* sp. from the Great Barrier Reef, Australia.[48,49] Patellins 1 (**49**) and 2 (**50**), cyclic hexapeptides, and patellins 3-6 (**51**-**54**), octapeptides, lacked thiazole residues normally associated with the peptides from *L. patella* and contained two serine or threonine residues, which were modified as dimethylallyl ethers. Several patellins have been shown to exist as mixtures of multiple conformers in their solutions.[49] Patellin 6 (**54**) showed modest cytotoxicity (IC_{50} = 2 μg/mL) against murine lymphocytic leukemia P388, human lung cancer A-549, human colon cancer HT-29, and monkey kidney CV-1 cell lines and inhibited the activity of topoisomerase II (IC_{50} = 2.5 μg/mL), while patellins 1-5 (**49**-**53**) were inactive in a series of in vitro cytotoxicity assays. Tawicyclamides A (**55**) and B (**56**) were isolated from the Philippine *L. patella* and represented a new family of cyclic octapeptides possessing thiazole and thiazoline residues.

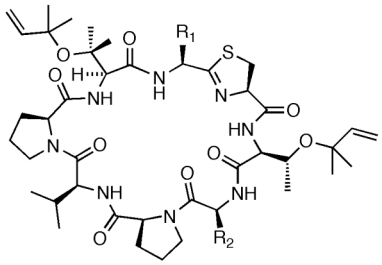
But these compounds lacked an oxazoline ring, which was also the characteristic moiety of cyclic peptides from *L. patella*.[50] Compounds **55** and **56** and their dehydro-analogs exhibited the weak cytotoxicity against human colon cancer cell lines at 31 μg/mL (IC_{50}'s).



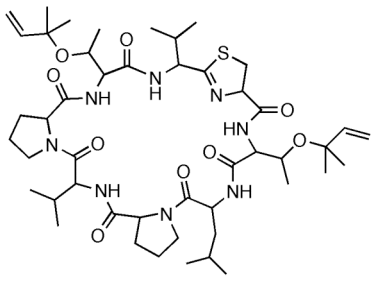
49



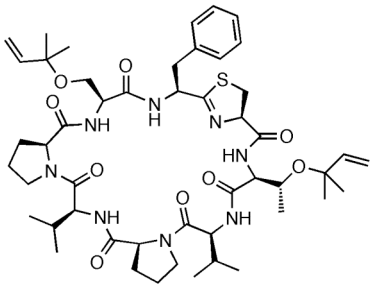
50



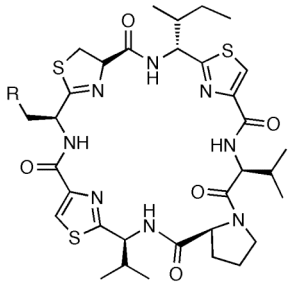
51: $R_1 = R_2 = CH_2CHMe_2$
54: $R_1 = CHMe_2$, R_2 = Phe



52

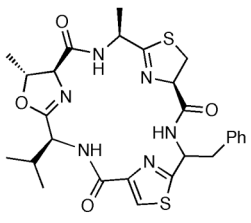


53

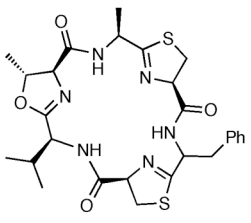


55: R = Phe
56: R = $CHMe_2$

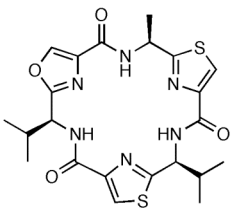
Bistratamides A-J (**57**-**66**), new cyclic hexapeptides, have been reported from *Lissoclinum bistratum*,[51-53] together with cycloxazoline (**67**),[54] a symmetric trimer containing three oxazoline rings formed by the condensation of valines and threonines. Cycloxazoline (**67**) showed cytotoxicity against MRC5CV1 and T24 cells (IC_{50} = 0.5 μg/mL), and the IC_{50} values of bistratamides A (**57**) and B (**58**) were 50 and >100 μg/mL, respectively. Bistratamides E-J (**61**-**66**) exhibited the cytotoxicity against human colon cancer HCT-116 cells (IC_{50}'s: 7.9, 28, 5, 1.7, 9, and 1 μg/mL, respectively).[53] Bistratamide D (**60**) was suggested to be a central nervous system depressant in a mouse bioassay.[52]



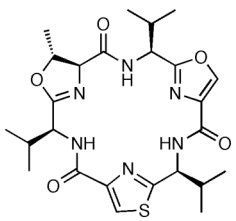
57



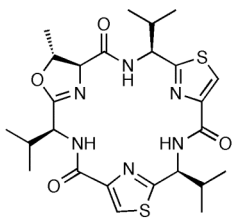
58



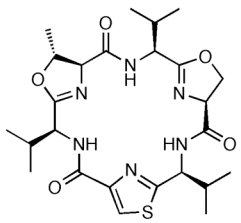
59



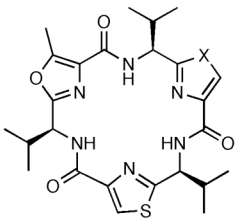
60



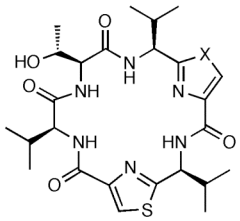
61



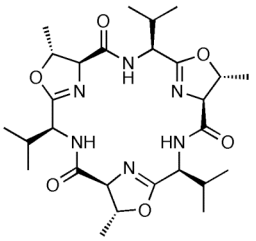
62



63: X = O
64: X = S

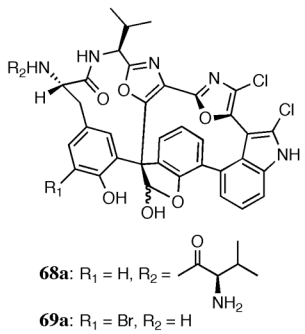


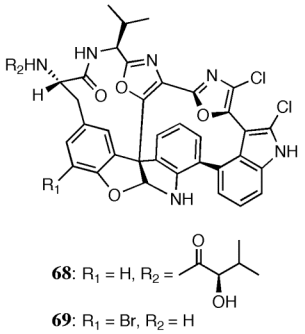
65: X = O
66: X = S

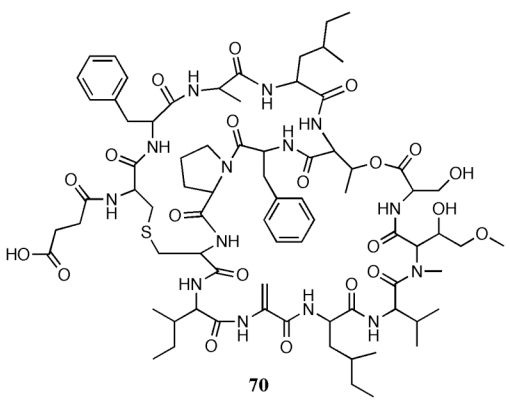


67

Two unique toxins, diazonamides A (**68**) and B (**69**), were isolated from *Diazona angulata*,[55] which was originally misidentified as *Diazona chinensis*.[56] Diazonamide A (**68**) had the cytotoxicity against HCT-116 and a murine melanoma cell line B-16 (IC_{50}'s < 15 ng/mL). Diazonamides A and B were first assigned as unusual halogenated highly unsaturated cyclic peptide structures **68a** and **69a**, respectively.[55] These unusual structures proposed for diazonamides, coupled with their impressive cytotoxic properties, immediately aroused intense interests in organic synthesis of these compounds. Although the synthesis of the proposed structure of diazonamide A (**68a**) was succeeded, the synthetic compound had little cytotoxic activity and was highly unstable.[57] Spectral data for diazonamide A was reevaluated and the structure of **68** was suggested.[57, 58] The polyheterocyclic core of the correct structure **68** was speculated to biosynthetically construct from an oxidized ditryptophan unit that was oxidatively coupled to a tyrosine residue. Diazonamide A inhibited the MAP-induced (IC_{50}: 0.30 and 0.47 μM with and without 0.5 mM $MgCl_2$, respectively) and glutamate-induced microtubule assembly (IC_{50}: 0.87 and 0.75 μM with and without preincubation, respectively), which were as potent as dolastatin 10 (IC_{50}: 0.49, 0.68, 1.2, and 1.3 μM, respectively) and stronger than dolastatin 15 (IC_{50}: 11, 17, 6.6, and 6.2 μM, respectively).[59] The inhibitory activity on microtubule assembly by diazonamide A was ascribed to the potent inhibition of tubulin-dependent GTP hydrolysis (IC_{50}: 2.4 ± 0.7 μM), which was also comparable with the effect observed by dolastatin 10 (IC_{50}: 3.4 ± 0.9 μM). Vitilevuamide (**70**), a bicyclic 13 amino acid peptide, was a tubulin polymerization inhibitor isolated from two ascidians *Didemnum cuculiferum* and *Polysyncranton lithostrotum*.[60,61] Compound **70** showed the cytotoxicity against several human tumor cell lines (LC_{50} = 6-311 nM), and the analysis in the 25-cell line panel revealed a weak correlation with several taxol analogs. At 9 μg/mL (5.6 μM), **70** showed the effect equivalent to that of colchicine at 25 μg/mL (62.5 μM) in a cell-based screen for tubulin assembly inhibitors and inhibited the polymerization of purified tubulin (IC_{50} = ca. 2 μM). A cell cycle analysis and competitive binding assays showed that **70** arrested cells in the G2/M phase and may interact with tubulin at a unique site distinct from vinblastine, colchicine, dolastatin 10, or GTP.[62] Diazonamide A (**68**) and vitilevuamide (**70**) are now under preclinical evaluations as potential antitumor agents.[14]



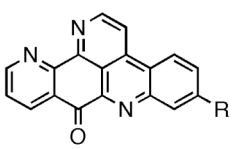




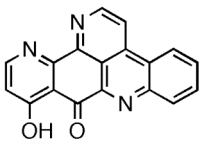
PYRIDOACRIDINE ALKALOIDS

The pyridoacridines are a class of colored polycyclic aromatic alkaloids possessing the pyrido[4,3,2-*mn*]acridine or acridone skeleton and probably the largest group in ascidian alkaloids. These compounds provide challenges in the structure assignment to natural product chemists but exhibit an array of interesting biological activities, especially the significant cytotoxicity against cultured cancer cells. The activity has often been attributed to the ability to intercalate into DNA, because their general planar structures are able to interpose between stacked base pairs. Interaction with DNA, inhibition of DNA metabolizing enzymes, and production of reactive oxygen species would play important roles in the cytotoxicity of the pyridoacridines.[63]

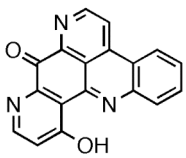
The first pyridoacridine alkaloid was isolated and identified as 2-bromoleptoclinidinone (**71**) from *Leptoclinides* sp. in 1987.[64] However, its structure was revised in 1989 on the basis of selective long-rang $^{1}H/^{13}C$ polarization transfer experiments and chemical correlation with ascididemin (**72**),[65] a congener isolated from the Okinawan *Didemnum* sp.[66] Compound **71** exhibited excellent cytotoxicity against 60 tumor cell lines in the NCI standard in vitro assay but was too toxic to yield significant antitumor responses in the xenograft models.[67] Ascididemin (**72**) was cytotoxic against L1210 cells (IC_{50} = 0.39 μg/mL),[66] inhibited the activity of topoisomerase II,[68-70] and caused the release of calcium in the sarcoplasmic reticulum with the potency of seven times greater than caffeine.[66] Compound **72** was one of the most potent anti-parasite agent in the 18 analogues tested against malaria and leishmania.[63] Studies performed at the NCI revealed the significant activity of **72** (T/C < 50%) against six of twelve human tumor cell lines in the preliminary in vivo hollow fiber assay, but a weak activity was observed in the xenograft models with the best response in the HCT-116 human colon tumor xenograft at a dose (i.p.) of 8 mg/kg on the fifth and ninth days (T/C = 58%). Although this level of response was not considered significant, **72** is now in the preclinical trials because HCT-116 is a relatively refractory tumor and a new understanding on SAR of **72** may improve the spectrum of in vivo antitumor activity.[63] Two related compounds, 11-hydroxyascididemin (**73**) and meridine (**74**), have been reported from *Leptoclinides* sp. and *Amphicarpa meridiana*, respectively.[68] Meridine (**74**) exhibited the cytotoxicity against P388 murine leukemia cells at 0.3-0.4 μg/mL and was highly active against the pathogenic yeasts *Candida albicans* (MIC and minimum fungicidal concentration (MFC) in the Sabouraud dextrose broth, 3.1 μg/mL; MIC and MFC in RPMI-1640, 0.2 μg/mL) and *Cryptococcus neoformans* (MIC = 0.8 and MFC = 6.2 μg/mL). Compound **74** also inhibited the growth of *Bacillus subtilis* (MIC = 3.1 μg/mL).[71]



71: R = Br
72: R = H

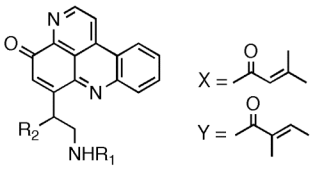


73

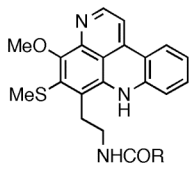


74

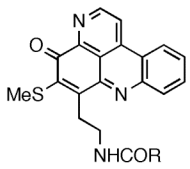
Ascidians of the genera *Cystodytes* and *Lissoclinum* afforded eleven cytotoxic tetracyclic pyridoacridines, cystodytins A-K (**75**-**85**), derivatives of 8*H*-pyrido[4,3,2-*mn*]acridone.[72-75] Cystodytins A (**75**) and B (**76**) showed cytotoxicity against L1210 cells (IC_{50}'s = 0.22 and 0.24 μg/mL, respectively) and powerful calcium-releasing activity in sarcoplasmic reticulum, which were respectively 36 and 13 times more potent than caffeine.[72] Cystodytins D-I (**78**-**83**) were active against L1210 and human epidermoid carcinoma KB cells (IC_{50} = 0.068-1.4 μg/mL). Cystodytin J (**84**) was cytotoxic against HCT cells (IC_{50} = 1.6 μM) and inhibited the topoisomerase II-mediated decatenation of kinetoplast DNA in a dose-dependent manner.[74] Varamines A (**86**) and B (**87**),[76] lissoclins A (**88**) and B (**89**),[77] and isodiplamine (**91**)[75] from *Lissoclinum* spp. and diplamine (**90**)[78] from *Diplosoma* sp. possessed the same carbon skeleton as that of cystodytins. Compounds **86**, **87**, and **90** showed cytotoxicity against L1210 cells (IC_{50}'s = 0.03, 0.05, and 0.02 μg/mL, respectively). Compounds **84**, **85**, **90**, and **91** exhibited the moderate to potent antimicrobial activity against a variety of microorganisms and cytotoxicity against P388 (IC_{50}'s = 3.5, 1.3, 1.9, and 2.1 μM, respectively).[75] Diplamine (**90**) showed cytotoxicity against HCT cells (IC_{50} < 1.4 μM) and inhibited the function of topoisomerase II and DNA intercalation.[74] The Indonesian *Eusynstyela latericius* gave styelsamines A-D (**92**-**95**).[79] Styelsamine D (**95**) was most potent in these alkaloids against HCT-116 (IC_{50} = 1.6 μM).



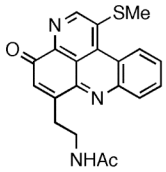
75: R_1 = X, R_2 = H
76: R_1 = Y, R_2 = H
77: R_1 = $COCH_2CMe_2OH$, R_2 = H
78: R_1 = X, R_2 = OH
79: R_1 = Y, R_2 = OH
80: R_1 = X, R_2 = OMe
81: R_1 = Y, R_2 = OMe
82: R_1 = X, R_2 = $OCO(CH_2)_7CH{=}CH(CH_2)_7Me$
83: R_1 = Y, R_2 = $OCO(CH_2)_7CH{=}CH(CH_2)_7Me$
84: R_1 = COMe, R_2 = H
85: R_1 = COMe, R_2 = OMe



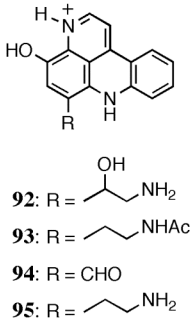
86: R = Et
87: R = Me



88: R = CH_2CHMe_2
89: R = (Me)C=CHMe(*E*)
90: R = Me



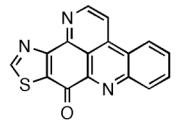
91



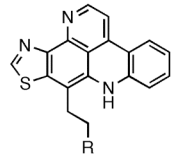
92: R =
93: R =
94: R = CHO
95: R =

Kuanoniamines A-F (**96**-**101**)[80,81] and dehydrokuanoniamine B (**102**)[74] are a series of pentacyclic aromatic alkaloids having a pyridoacridine-thiazole system. Compounds **96**-**99** weakly inhibited the proliferation of human epidermoid carcinoma (KB) cells in vitro, and the IC_{50} value of kuanoniamine A (**96**), the most active one, was 1 μg/mL.[80] Compounds **99** and **102** showed cytotoxicity against HCT cells (IC_{50} = ~8 μM).[74] The most recent study on the anticancer activity of **96** and **98** revealed that **96** was a potent growth inhibitor against all test tumor and non-tumor cell lines and that **98**, which was less potent than **96**, showed a high selectivity to the estrogen-dependent (ER+) breast cancer cell line.[82] Compound **96** also inhibited the DNA synthesis and caused an extensive reduction of the human breast cancer MCF-7 cells in G2/M phase as well as an increase in the apoptotic cells. Shermilamines A-E (**103**-**107**)[74,83-85] and

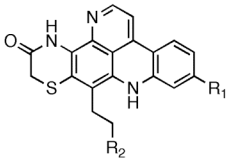
cycloshermilamine D (**108**),[86] thiazinone-fused pentacyclic pyridoacridines, have been obtained from *Trididemnum* sp. and *Cystodytes* spp. Shermilamine D (**100**) was patented as exhibiting cytotoxicity against P388 (IC_{50}, 0.53 μM), A-549 (0.27 μM), HT-29 (2.66 μM), and human melanoma SK-MEL-28 (0.53 μM) cell lines.[87] Arnoamines A (**109**) and B (**110**) were isolated from *Cystodytes* sp. and proposed to be the first members of a new family of pentacyclic aromatic alkaloids with a pyrrole ring fused to the pyridoacridine skeleton.[88] Compounds **109** and **110** showed the selective cytotoxicity against MCF-7 (GI_{50} = 0.3 and 5.0 μg/mL, respectively), A-549 (2.0 and 2.0 μg/mL), and HT-29 (4.0 and 3.0 μg/mL) and inhibited the activity of topoisomerase II through the intercalation into DNA.



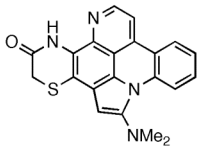
96



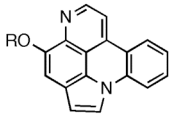
97: R = $NHCOCH_2CHMe_2$
98: R = NHCOEt
99: R = NHCOMe
100: R = $NHCOCHMe_2$
101: R = NHCOCH(Me)Et
102: R = $NHCOCH=CMe_2$



103: R_1 = Br, R_2 = NHCOMe
104: R_1 = H, R_2 = NHCOMe
105: R_1 = H, R_2 = $NHCOCH=CMe_2$
106: R_1 = H, R_2 = NMe_2
107: R_1 = H, R_2 = $N(O)Me_2$

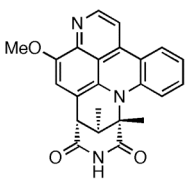


108

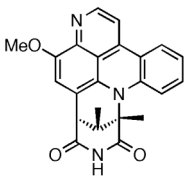


109: R = H
110: R = Me

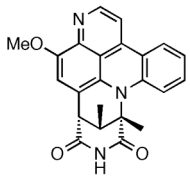
An interesting class of hexacyclic pyridoacridines, segolines A-C (**111**-**113**), possessed a unique bicyclic imide ring system.[89-91] A common characteristic trait of the segolines was the dramatic color changes, from orange to deep purple, associated with a pH shift from neutral to acidic. Isosegoline (**114**), a more polar isomer of segolines, had a succinimide ring instead of a glutarimide ring.[89] A Brazilian *Cystodytes dellechiajei* afforded two novel pyridoacridines, sebastianines A (**115**) and B (**116**).[92] These compounds had unprecedented ring systems: pyridoacridine-pyrrole in **115** and pyridoacridine-pyrrolidine condensed with α-hydroxyisovaleric acid in **116**. The profile of cytotoxicity against a panel of HCT-116 colon carcinoma cells indicated the p53-dependent mechanism for compounds **115** and **116**. Eilatin (**117**), the first heptacyclic fused-aromatic alkaloid with a unique symmetrical structure, was isolated from *Eudistoma* sp. and showed cytotoxic and antiproliferative activities in a broad range of tissue cultures.[93] The strong anti-HIV activity (IC_{50} = ca. 1 μM) in CD4+ HeLa cells and human peripheral blood monocytes was observed with **117**-Ru (II) complexes.[94] The plane structure of **117** was important for the activity.



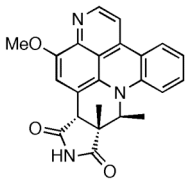
111



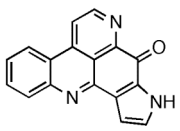
112



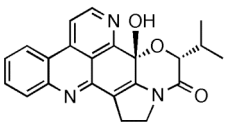
113



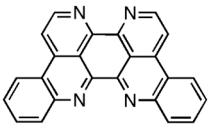
114



115



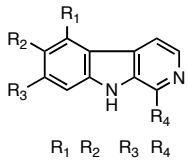
116



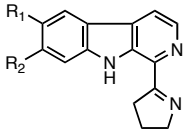
117

β-CARBOLINE ALKALOIDS AND RELATED COMPOUNDS

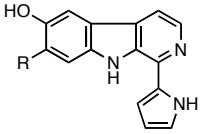
The β-carbolines form a large group of tryptophan-derived alkaloids and have been reported from several ascidian genera, such as *Eudistoma, Ritterella, Pseudodistoma*, and *Lissoclinum*. The colonial ascidian *Eudistoma olivaceum* has been the extraordinarily rich source of β-carbolines and yielded eudistomins A (**118**) and C-T (**119**-**136**).[95-98] Eudistomins were the first series of ascidian β-carbolines, and some of them exhibited the significant antiviral activity. A Caribbean *Lissoclinum fragile* gave eudistomin U (**137**) and its pseudo-isomer, isoeudistomin U (**138**).[99] Eudistomin V (**139**) was isolated from *Pseudodistoma aureum*,[100] and different collections of *Eudistoma* spp. afforded eudistomins W (**140**) and X (**141**)[101] and 19-bromoisoeudistomin U (**142**).[102] Eudistomin X (**141**) exhibited antimicrobial activities at 5 and 10 μg/disk against *B. subtilis* (17 and 18 mm), *Staphylococcus aureus* (11 and 12 mm), *Escherichia coli* (15 and 20 mm), and *C. albicans* (17 and 18 mm), respectively. Eudistomin W (**140**) was selectively active against *C. albicans* (13 mm at 10 μg/disk). *Eudistoma gilboverde* provided three *N*-methylated β-carboline alkaloids, 2-methyleudistomin D (**143**), 2-methyleudistomin J (**144**), and 14-methyleudistomin C (**145**).[103] Compound **145** showed the most potent cytotoxicity in the three compounds against four different human tumor cell lines (IC_{50}'s < 1.0 μg/mL).



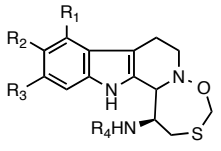
	R_1	R_2	R_3	R_4
120:	Br	OH	H	H
126:	H	OH	Br	H
130:	H	Br	H	H
131:	H	H	Br	H
134:	H	H	Br	$COCH_2Ph$
135:	H	Br	H	$COCH_2Ph$
136:	H	H	H	$COCH_2Ph$



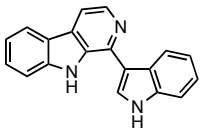
	R_1	R_2
123:	H	Br
124:	Br	H
125:	H	H
132:	OH	Br
133:	OH	H
139:	Br	Br



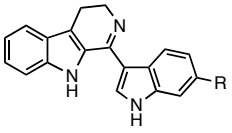
118: R = Br
129: R = H



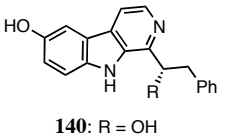
	R_1	R_2	R_3	R_4
119:	H	OH	Br	H
121:	Br	OH	H	H
122:	H	OH	Br	$-CO_2Me$
127:	H	H	Br	H
128:	H	Br	H	H



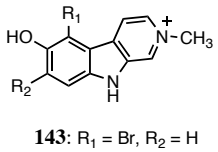
137



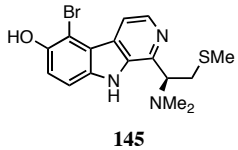
138: R = H
142: R = Br



140: R = OH
141: R = NMe_2 (*R*,*S*)

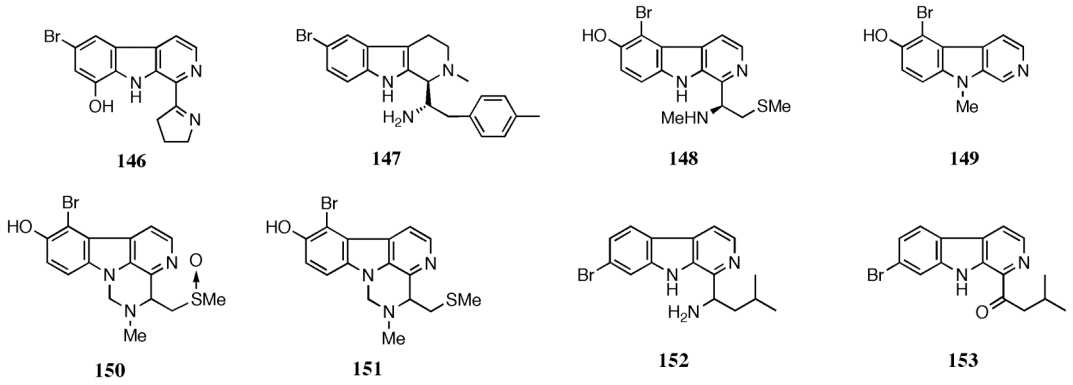


143: R_1 = Br, R_2 = H
144: R_1 = H, R_2 = Br

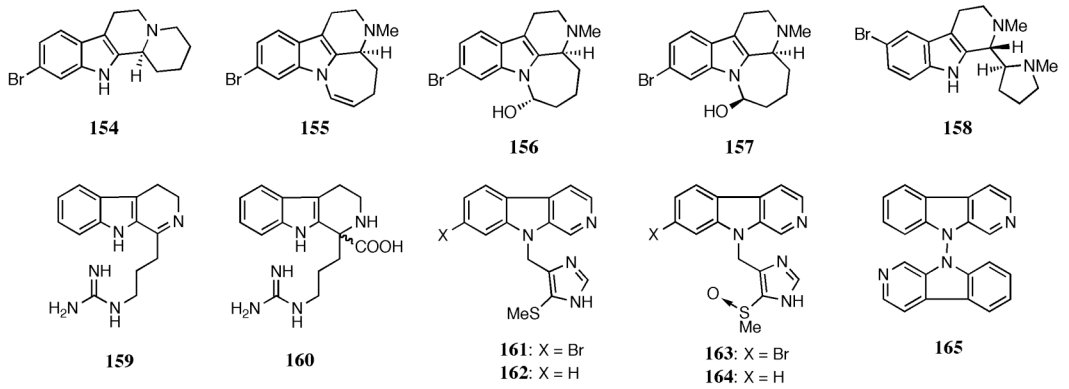


145

Related β-carbolines, eudistomidins A-F (**146**-**151**)[104-106] and eudistalbins A (**152**) and B (**153**),[107] were obtained from *Eudistoma glaucus* and *Eudistoma album*, respectively. Compounds **147**-**149** showed cytotoxicity against L1210 (IC_{50}'s = 3.4, 0.36, and 2.4 μg/mL, respectively) and mouse lymphoma L5178Y (3.1, 0.42, and 1.8 μg/mL) cells.[105] Compound **147** activated the rabbit heart muscle actomyosin ATPase by 93% at 30 μM, and **146** and **148** inhibited calmodulin in vitro (IC_{50} = 20 and 30 μM, respectively). Compound **149** induced the Ca^{2+} release from the sarcoplasmic reticulum, and this activity was about 10 times more potent than that of caffeine. Eudistomin E (**121**) and eudistalbin A (**152**) inhibited the growth of KB cells with the ED_{50} values of < 5.0 ng/mL and 3.2 μg/mL, respectively, whereas eudistalbin B (**153**) was inactive.[107]



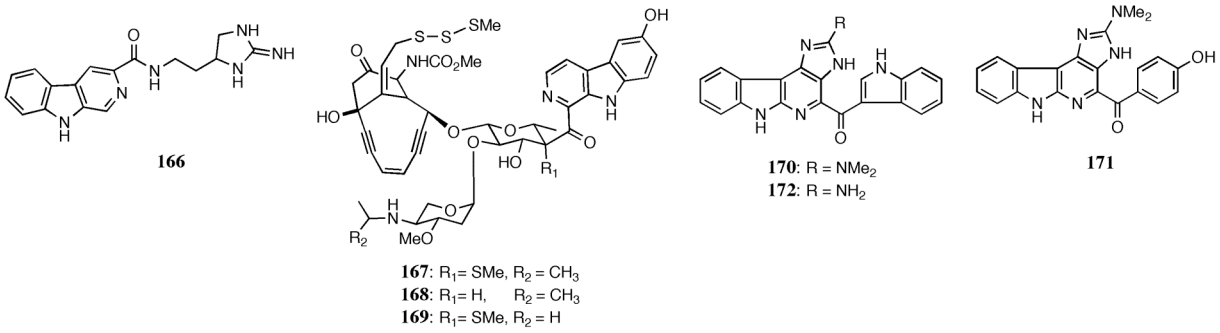
Arborescidines A-D (**154-157**) from *Pseudodistoma arborescens*[108] and woodinine (**158**) from *Eudistoma fragum*[109,110] were brominated tetrahydro-β-carbolines. Compound **158** exhibited antibacterial and antimycobacterial activities, and **157** showed weak cytotoxicity (IC_{50} = 3 μg/mL) against KB cells. An undescribed *Eudistoma* sp. afforded two trypargine derivatives, trypargimine (**159**) and 1-carboxytrypargine (**160**).[111] Compound **160** was proposed to be generated by enantioselective condensation of tryptamine with an α-keto acid derivative of arginine, which will be oxidatively decarboxylated to give **159**.



Unique N9-substituted β-carbolines, didemnolines A-D (**161**-**164**),[112] and a N9-linked β-carboline dimmer (**165**)[113] have been reported from different collections of *Didemnum* spp. Compounds **161**-**163** showed antimicrobial activity and moderate cytotoxicity against KB cells, and **163** was the most potent in three compounds (IC_{50} = 0.28 μg/mL) probably because of the sulfoxide moiety.

Tiruchanduramine (**166**) was the first ascidian-derived β-carboline-3-carboxylate and also the first natural product possessing the enduracididinamine moiety isolated from *Synoicum macroglossum* and inhibited the α-glucosidase activity.[114] Shishijimicins A-C (**167**-**169**) were novel enediyne antitumor antibiotics obtained from *Didemnum proliferum* possessing a novel sugar unit, which is a conjugated product of a hexose and a β-carboline moiety, attached to the calicheamicinone aglycone.[115] These compounds were highly cytotoxic against rat 3Y1 (IC_{50}'s = 2.0-4.8 pg/mL), human cervical carcinoma HeLa (1.8-6.3 pg/mL), and P388 (0.47-2.0 pg/mL) cell lines. Since **167**-**169** have the β-carboline moiety, recognition of DNA sequence by these compounds may be different from those of the other enediyne compounds.[115]

The first examples of α-carbolines were grossularines-1 (**170**) and -2 (**171**) isolated from a solitary ascidian *Dendrodoa grossularia*.[116,117] Compounds **170** and **171** showed potent cytotoxicity against human solid tumor WiDr (colon) and MCF-7 (breast) cell lines (active up to 10 ng/mL) but were less active against L1210 cells (ID_{50}'s = 6 and 4 μg/mL, respectively).[117] These compounds accumulated the cells in the G1 phase at the concentrations of 10 (**170**) and 1.5 μg/mL (**171**). Grossularine-2 (**171**) was suggested to act on DNA as a mono-intercalating agent. The Indonesian solitary *Polycarpa aurata* gave *N,N*-didesmethylgrossularine-1 (**172**), which inhibited the colony formation of Chinese hamster V79 cells.[118,119] Interestingly, **172** reduced the production of inflammatory cytokines IL-8 and TNF-α by inhibiting the activity of transcription factors AP-1 and NF-κB (unpublished data).

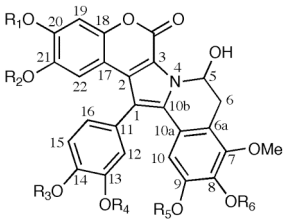


LAMELLARINS AND RELATED PYRROLE ALKALOIDS

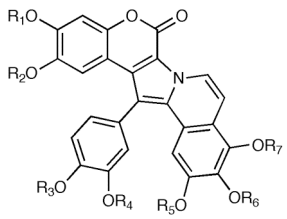
Lamellarins are a group of naturally occurring polyaromatic pyrrole alkaloids derived from L-tyrosine and L-DOPA. These compounds were named after a prosobranch mollusk *Lamellaria* sp., from which lamellarins A-D (**173**-**176**) were first isolated in 1985.[120] In 1988, lamellarins E-H (**177**-**180**) were reported from an ascidian *Didemnum chartaceum*.[121] Over forty lamellarins have been isolated from ascidians. The lamellarins contain a pentacyclic chromophore (6*H*-[1]benzopyrano[4',3':4,5]pyrrolo-[2,1-*a*]isoquinolinone) and are generally divided into two structural groups according to the presence or absence of Δ^5 olefin. These two groups can readily be distinguished by their ^{1}H NMR spectra.

Lamellarins I-M (**181**-**185**) and lamellarin N-triacetate (**186**-triacetate) were isolated from *Didemnum* sp., together with lamellarins A-C (**173**-**175**) and lamellarin D-triacetate (**176**-triacetate).[122] The isolation of lamellarins A-D from this ascidian will give further support on the idea that mollusks accumulated these compounds from dietary ascidian-derived metabolites. This *Didemnum* sp. also gave lamellarin S (**187**).[123] Sulfated lamellarins, 20-sulfate derivatives of lamellarins T-V and Y (**193**), and five new lamellarins T-X (**188**-**192**) were isolated from an unidentified Arabian ascidian, along with lamellarin N (**186**).[124] The first dimethoxy lamellarin, lamellarin Z (**194**), an 8-sulfate derivative of lamellarin G (**179**) and 20-sulfate derivatives of B (**174**), C (**175**), and L (**184**) were isolated from the Australian *D. chartaceum*.[125] Lamellarin β (**196**) was purified from *Didemnum* sp., together with lamellarins G (**179**) and L (**184**).[126] Lamellarins α (**195**), γ (**197**), and ε (**198**)[127] and lamellarins ζ (**199**), η (**200**), ϕ (**201**), and χ(**202**)[128] were

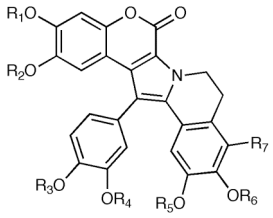
obtained from different collections of *Didemnum obscurum*.



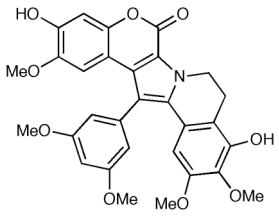
	R_1	R_2	R_3	R_4	R_5	R_6
173	H	Me	H	Me	Me	Me
190	H	Me	Me	H	Me	Me
190 20-sulfate	SO_3^-	Me	Me	H	Me	Me



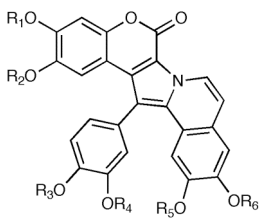
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
174	H	Me	H	Me	Me	Me	Me
174 20-sulfate	SO_3^-	Me	H	Me	Me	Me	Me
185	H	Me	H	Me	Me	Me	H
191	H	Me	Me	H	Me	Me	Me
192	H	Me	Me	H	Me	Me	H
198	H	Me	Me	Me	Me	Me	H
199	H	Me	Me	Me	Me	Me	Me
201	Ac	Me	Ac	Me	Ac	Me	Me



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
175	H	Me	H	Me	Me	Me	OMe
175 20-sulfate	SO_3^-	Me	H	Me	Me	Me	OMe
177	H	Me	Me	H	Me	Me	OH
178	H	Me	Me	Me	Me	Me	OH
179	Me	H	Me	H	Me	H	H
179 8-sulfate	Me	H	Me	H	Me	SO_3^-	H
181	H	Me	Me	Me	Me	Me	OMe
182	H	Me	Me	Me	Me	H	H
183	H	Me	H	Me	Me	Me	OH
184	H	Me	Me	H	Me	H	H
184 20-sulfate	SO_3^-	Me	Me	H	Me	H	H
187	H	H	H	H	Me	H	H
188	H	Me	Me	H	Me	Me	OMe
188 20-sulfate	SO_3^-	Me	Me	H	Me	Me	OMe
189	H	Me	Me	H	Me	Me	H
189 20-sulfate	SO_3^-	Me	Me	H	Me	Me	H
193	SO_3^-	Me	Me	H	H	Me	H
194	Me	H	H	H	Me	H	H
196	H	H	Me	H	H	H	H
202	Ac	Me	Ac	Me	Me	Ac	H



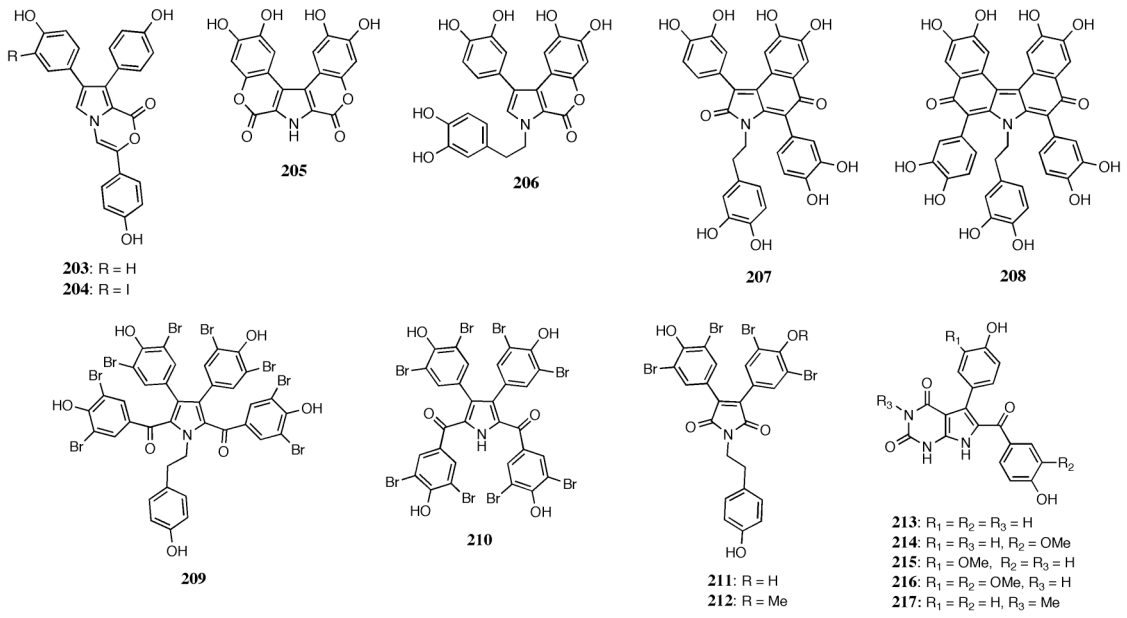
197



	R_1	R_2	R_3	R_4	R_5	R_6
176	H	Me	H	Me	Me	H
176-triacetate	Ac	Me	Ac	Me	Me	Ac
180	H	H	H	H	H	H
186	H	Me	Me	H	Me	H
186-triacetate	Ac	Me	Me	Ac	Me	Ac
195	H	Me	Me	H	Me	Me
200	H	Me	Me	Me	Me	Me

Lamellarins had the interesting structural features and exhibited a wide variety of remarkable bioactivities, such as inhibition of cell division, cytotoxicity, inhibition of HIV-I integrase, and immunomodulatory

activity. Lamellarins I (**181**), K (**183**), and L (**184**) were comparably cytotoxic against P388 and A-549 cells (IC_{50}'s = ~0.25 μg/mL), and **183** and **184** exhibited moderate immunomodulatory activity.[122] Lamellarin I (**181**) increased the cytotoxicity of doxorubicin, vinblastine, and daunorubicin in a concentration-dependent manner in multidrug-resistant (MDR) cells at non-toxic doses, and the potency of **181** as a MDR modulator was 9 to 16-fold higher than that of verapamil. It is suggested that **181** reversed MDR by directly inhibiting the P-glycoprotein-mediated drug efflux.[129] Lamellarin N (**186**) showed cytotoxicity against melanoma cell lines SK-MEL-5 (LC_{50} = 0.19 μM) and UACC-62 (9.88 μM).[124] Lamellarins F (**178**), ζ (**199**), χ (**202**), and L-triacetate (**184**-triacetate) strongly inhibited the proliferation of colorectal cancer COLO-205 cells (IC_{50} = 9.0, 5.6, 0.2, and 0.25 nM, respectively).[128] Sulfate derivatives of lamellarins had little or no cytotoxicity compared with the corresponding hydroxyl compounds but showed antiviral activity. For example, the IC_{50} values of lamellarin α (**195**) and its 20-sulfate against HeLa cells were 5.1 and 274 μM, respectively,[130] however, lamellarin α 20-sulfate strongly inhibited the HIV-1 integrase terminal cleavage and strand transfer activities in vitro, HIV-1 replication in cultured dental pulp p4-2 cells (IC_{50} = 88 μM), and the growth of HIV-1 in cells (IC_{50} = 8 μM).[131] Removal of the 20-sulfate group reinforced the cytotoxicity but completely abolished the activity against HIV-1 integrase. The sulfate group appeared to be a key structural element for the inhibition of HIV-1 integrase by lamellarins, which was also observed for the other sulfate-containing natural products, such as cyclodidemniserinol trisulfate from the Palauan *Didemnum guttatum*.[132]

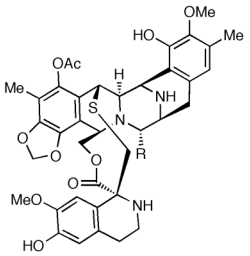


Lukianols, ningalins, polycitones/polycitrins, and rigidins are the other polyaromatic pyrrole alkaloids derived from dopamine. Two triphenylpyrrolo-oxazinones, lukianols A (**203**) and B (**204**), were isolated from an unidentified ascidian from Palmyra atoll, which resembled lamellarins O-R.[133] Lukianol A (**203**) exhibited moderate cytotoxicity against KB cells (IC_{50} = 1 μg/mL), and B (**204**) inhibited the activity of

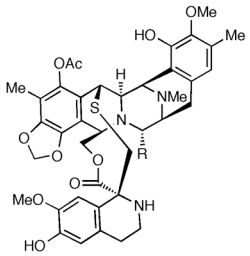
human aldose reductase (h-ALR2) at 0.6 μM, which was about 6-fold more potent than sobinil (IC_{50} = 3.6 μM), the known AR inhibitor.[134] Ningalins A-D (**205**-**208**), obtained from *Didemnum* sp., were believed to participate in the metal chelation phenomena due to the structural similarity to other metal binding *O*-catechols.[135] Polycitones A (**209**) and B (**210**) and polycitrins A (**211**) and B (**212**) were polybrominated pyrrole alkaloids produced by *Polycitor* sp., and **209** was a potent inhibitor of RNA and DNA-directed DNA polymerases.[136,137] Polycitone A (**209**) also inhibited three different retroviral reverse transcriptases as efficiently as cellular DNA polymerases.[138] Pyrrolopyrimidine alkaloids, rigidin (**213**)[139] and rigidins B-E (**214**-**217**),[140,141] were obtained from *Eudistoma* spp. and *Cystodytes* sp., respectively. Rigidin (**213**) was identified as a calmodulin antagonist, and **214**-**216** showed moderate cytotoxicity against L1210 cells.

ECTEINASCIDINS

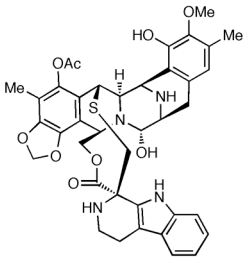
Ecteinascidia turbinata is the exceptional producer of exceedingly potent antitumor agents ecteinascidins (ETs). Powerful antitumor and immunomodulating activities of the extracts were reported as early as 1969,[142] but it was not until 1990 that six active alkaloids, ETs 729 (**218**), 743 (**219**), 745 (**220**), 759A (**221**), 759B (**222**), and 770 (**223**), were reported independently by two research groups.[143,144] Two additional analogues, ETs 722 (**224**) and 736 (**225**), were isolated from the Caribbean *E. turbinata.*[145] This species also provided ETs 597 (**226**), 583 (**227**), 594 (**228**), and 596 (**229**), putative biosynthetic precursors of previously described ETs,[146] and ETs 731 (**230**), 745B (**231**), 808 (**232**), and 815 (**233**).[147] The Thai *Ecteinascidia thurstoni,* the first example of the Asian ascidian contained the ETs, gave a new ET 786 (**234**) together with ET 770 (**223**).[148]



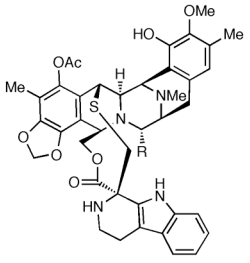
218: R = OH
230: R = H
231: R = OH, S-oxide



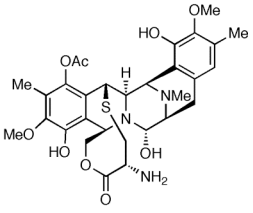
219: R = OH
220: R = H
221: R = =O (lactam)
222: R = OH, S-oxide
223: R = CN
233: R = C(CHO)=CHOH
234: R = CN, S-oxide



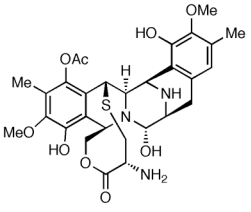
224



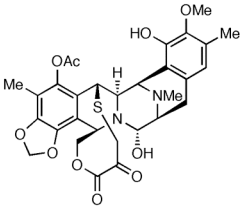
225: R = OH
232: R = $CH_2(CHO)_2$



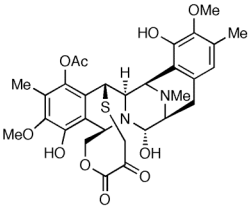
226



227



228



229

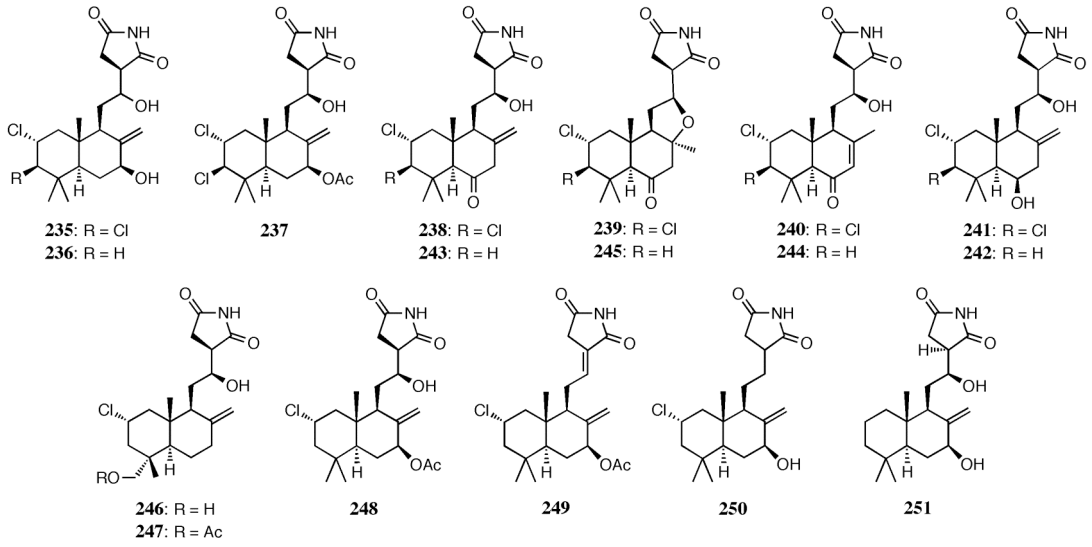
The common structural feature of the ETs is the fused pentacyclic moiety, composed of two tetrahydroisoquinoline units A and B. Compounds **218**-**223**, **230**, **231**, **233**, and **234** have one more tetrahydroisoquinoline moiety as unit C, and **224**, **225**, and **232** possessed a tetrahydro-β-carboline moiety at unit C. Compounds **226**-**229** lacked unit C. The unit C attached to the unit B by a 10-membered sulfide lactone ring, which is a quite distinctive structural feature of the ETs and different from the saframycin-class compounds, although the saframycins have very similar structures to the units A and B in ETs.[146] Two types of aromatic moieties at the unit C would be biosynthesized by the condensation of carbonyl groups with either dopamine or tryptamine equivalent.[149]

ETs possessed the strikingly potent cytotoxicity. ETs 722 (**224**) and 736 (**225**) inhibited the growth of L1210 cells (IC_{90}'s = 2.5 and 5.0 ng/mL, respectively), and the IC_{50} value of ET 743 (**219**) was 0.5 ng/mL. ETs 729 (**218**) and 743 (**219**) showed potent inhibitory activity against P388 cells (IC_{50}'s = 0.93 and 1.3 ng/mL, respectively).[143,144] ETs 770 (**223**) and 786 (**234**) exhibited cytotoxicity against breast cancer (IC_{50} = 2.5 and 7.6 nM, respectively) and nasopharynx carcinoma (0.034 and 0.15 μM) cells.[148] In vivo activities of ETs were evaluated by mouse tumor models and a variety of human tumor xenografts. ETs 729 (**218**), 743 (**219**), 722 (**224**), and 736 (**225**) demonstrated a remarkable in vivo efficacy in P388, B-16, M5076 (ovarian sarcoma), and Lewis lung carcinoma mouse models and several tumor xenograft models.[146] ET 729 (**218**) gave 8 survivors out of 10 test mice after 60 days following the inoculation of B-16.[147] These in vivo experiments indicated that the unit C in their structures affected the antitumor activity. ET 743 (**219**) is currently under registration for the treatment of soft tissue sarcomas and in Phase III trials for the ovarian cancer.[14] Besides the cytotoxicity, the other bioactivities, such as antimetabolic activity, enzyme inhibition, and antimicrobial activity, have been reported for some ETs.[146] ETs 770 (**223**) and 786 (**234**) inhibited the growth of *Mycobacterium tuberculosis* (MIC = 0.13 and 2.0 μM, respectively).[148] ET 743 (**219**) was revealed to have the inhibitory effects on macrophage viability, differentiation, and cytokine production.[150]

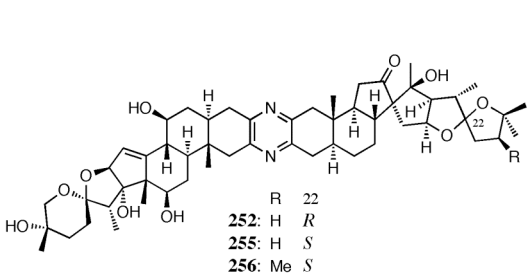
DITERPENE AND STEROIDAL ALKALOIDS

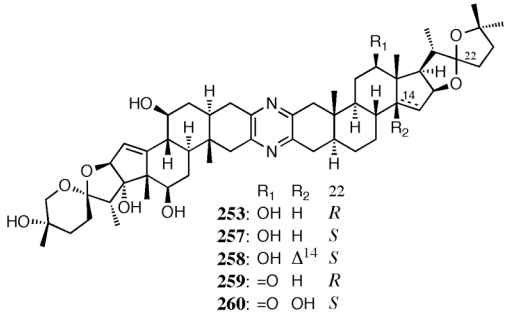
Terpenes are rarely encountered as the secondary metabolites from ascidians. Dichlorolissoclimide (**235**)[151,153] and chlorolissoclimide (**236**),[152] two labdane diterpenes with a unique succinimide moiety, were isolated from the New Caledonia *Lissoclinum voeltzkowi* and exhibited the remarkable cytotoxicity against non-small cell lung cancer NSCLC (IC_{50}'s = 9 and 10 ng/mL, respectively), KB (14 and 52 ng/mL), P388 (1 and 1.7 ng/mL), and doxorubicin-resistant P388 (300 and 200 ng/mL) cell lines.[152] Compounds **235** and **236** also inhibited the protein synthesis in the mammalian cells and the extracts of mammalian and plant cells.[154] An Okinawan *Lissoclinum* sp. afforded 15 labdane alkaloids, haterumaimides A-K (**237**-**247**)[155-157] and N-Q (**248**-**251**).[158] These compounds inhibited the first cleavage

of fertilized sea urchin eggs and exhibited moderate to potent cytotoxicity against P388 cells. Haterumaimides J (**246**) and K (**247**) were the most and second active compounds against P388 cells (IC_{50}'s = 0.23 and 0.45 ng/mL, respectively). The presence of the OH group at C-6, 7, 12, and/or 18, a chlorine atom at C-2 and an imido NH should be essential for the cytotoxicity against P388 cells.[158]



Ritterazines A-Z (**252**-**277**), isolated from *Ritterella tokioka*, were highly cytotoxic dimeric steroidal alkaloids structurally related to the cephalostatins.[159-162] Their structures consisted of two polyoxygenated steroidal skeletons linked by a pyrazine ring. Ritterazines exhibited modest to potent cytotoxicity against P388 cells (Table 1) according to the structural variations. Compounds **252**, **253**, **255**-**264**, and **276** showed the significant cytotoxicity, while **265**-**270**, **274**, **275**, and **277**, no 5/6 spiroketal, had marginal activity.[163] The contribution of the terminal 5/6 spiroketal to their cytotoxicity was also observed between **253** and **254**.[162] Ritterazine B (**253**) showed the most potent cytotoxicity to non-small cell lung cancer PC14 cells with a mean GI_{50} of 75.1 nM and arrested PC14 and the human promyelocytic leukemia HL-60 cells at the G2/M checkpoint. HL-60 cells became multinucleated, and, at 20 nM, this resulted in the onset of apoptosis. These results indicated that **253** would be a potent inducer of apoptosis, acting via a novel antimitotic mechanism.[164]





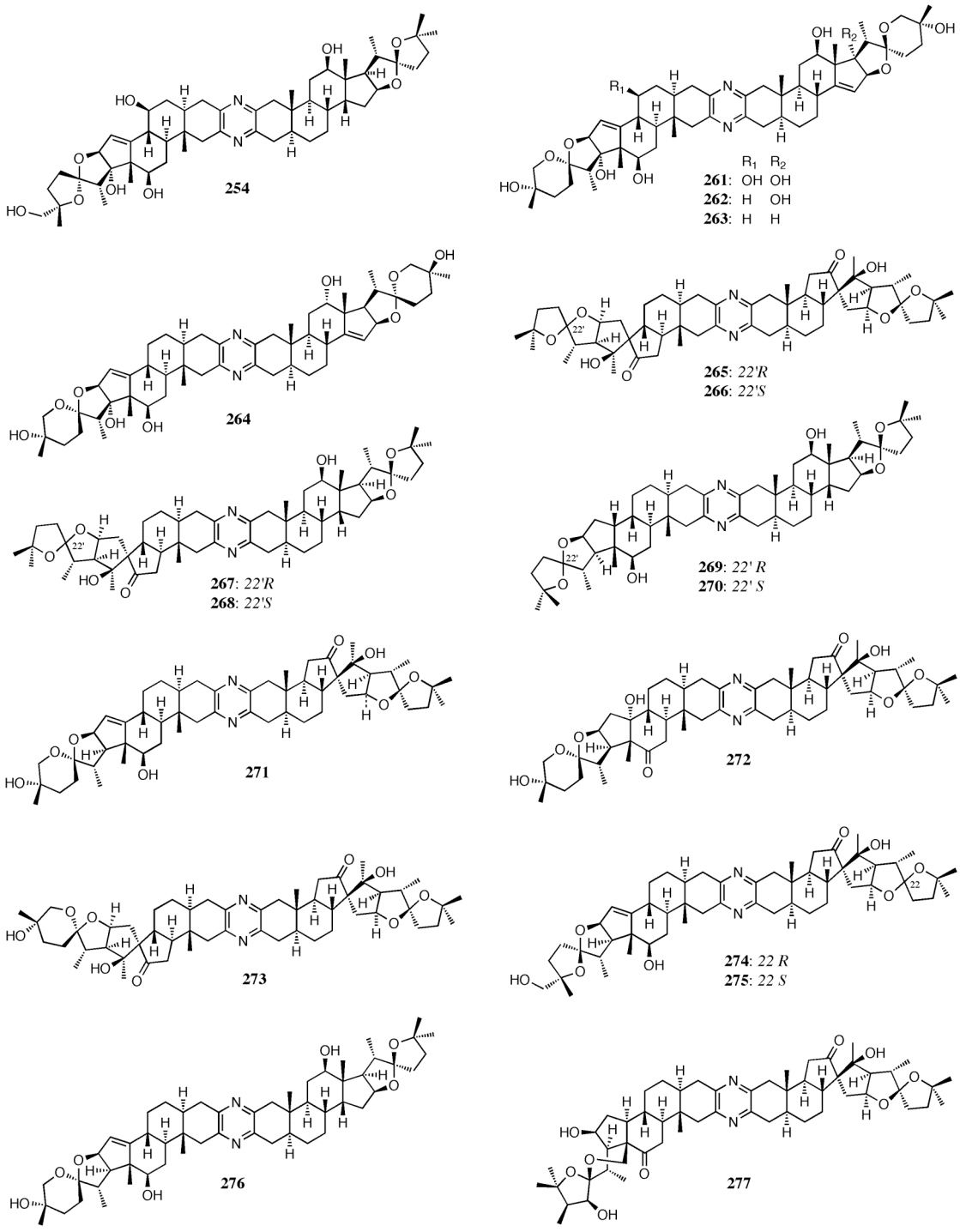
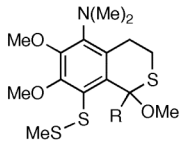


Table 1. Cytotoxicity (IC_{50}, ng/mL) of ritterazines against P388 cells[162]

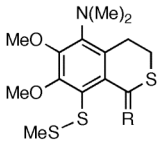
252	**253**	**254**	**255**	**256**	**257**	**258**	**259**	**260**	**261**	**262**	**263**	**264**
3.5	0.15	92	16	3.5	0.73	0.73	16	14	13	9.5	10	15
265	**266**	**267**	**268**	**269**	**270**	**271**	**272**	**273**	**274**	**275**	**276**	**277**
460	2100	710	570	2100	460	460	2100	2100	3200	3000	3.5	2000

POLYSULFUR ALKALOIDS

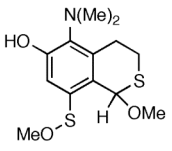
Ascidians have been proved to be rich sources of sulfur-containing natural products. The solitary *Polycarpa* spp. are rich producers of them. The Philippine *P. aurata* afforded polycarpamines A-E (**278**-**282**), which possessed rare sulfur-containing functional groups.[165] Compound **279** showed significant antifungal activity against *Saccharomyces cerevisiae* and *C. albicans*. A unique dimeric disulfide, polycarpine (**283**), was first isolated from *P. aurata* collected at Flinder Reef (Coral Sear) as a cytotoxic compound.[166] Subsequently, **283** and its dihydrochloride were discovered from a Micronesian *P. aurata*[118] and an Australian *Polycarpa clavata*,[167] respectively, together with some degradation products. Polycarpine (**283**) showed cytotoxicity against an array of human cancer cell lines and significant antitumor activity in mice against P388, L1210, and Ehrlich carcinoma cells.[168] High inhibitory activity against reverse transcriptases from Raus sarcoma and avian myeloblastosis viruses in vitro (IC_{50} = 3.5 μM) and Na^+/K^+-ATPase isolated from rat brain (IC_{50} = 0.5 μM) were also observed for **283**.[168] The inhibitory activity of **283** on the enzyme inosine monophosphate dehydrogenase could be reversed by the addition of excess dithiothreitol, which suggested that **283** reacted with thiol groups in the enzyme.[118] In addition, **283** activated p38 kinase, JNKs, and ERKs in mouse epidermal JB6 C1 41 cells and stimulated the p53-dependent transcriptional activity and phosporylation, and the dihydrochloride of **283** was cytotoxic against HCT-116 cells.[169] Recently, we have reported three new sulfur-containing alkaloids, polycarpaurines A-C (**284**-**286**), from the Indonesian *P. aurata*.[170] Polycarpaurine C (**286**) was the first example possessing the thiosulfate moiety obtained from marine organisms.[170] Polycarpaurines A (**284**) and C (**286**) inhibited the colony formation of V79 cells (EC_{50} = 6.8 and 8.6 μM, respectively), but B (**285**) showed modest inhibitory activity at 10 μM.



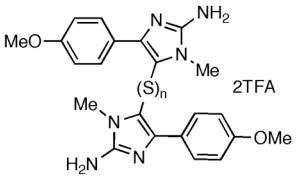
278: R = H
281: R = COMe



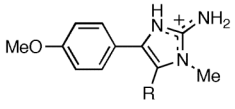
279: R = O
280: R = S



282



283: n = 2
284: n = 1



285: R = SO_3^-
286: R = S-SO_3^-

The first natural pentathiepin, varacin (**287**), was isolated from *Lissoclinum vareau* in 1991.[171] Since then, unusual dopamine-derived cyclic polysulfides have been emerged as the major class of sulfur-containing alkaloids from ascidians, and 30 homologues have been obtained from the genera *Lissoclinum, Eudistoma,* and *Polycitor*. The structures and bioactivities are listed in Tables 2 and 3.

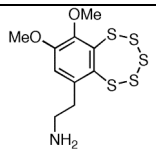
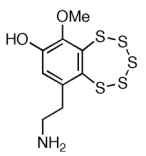
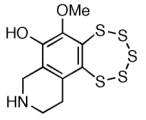
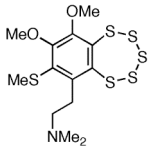
Lissoclinotoxins A (**288**) and B (**289**),[172,173] from *Lissoclinum perforatum*, *N,N*-dimethyl-5-(methylthio)-varacin (**290**) and 5-(methylthio)varacin (**291**) and their corresponding trithianes (**292** and **293**) from *Lissoclinum japonicum* and *Lissoclinum* sp., and 3,4-desmethylvaracin (**294**) from *Eudistoma* sp.[174] were reported. Varacin (**287**) exhibited the potent antifungal activity against *C. albicans* (14 mm at 2 μg/disk)

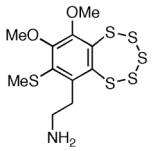
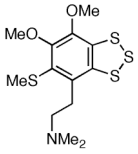
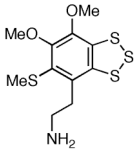
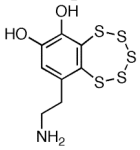
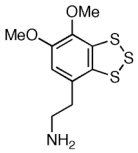
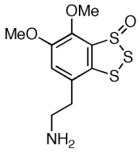
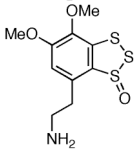
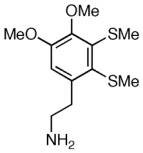
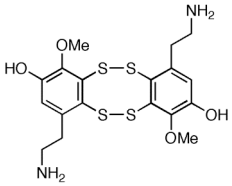
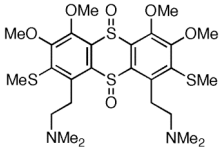
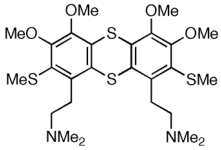
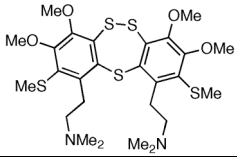
and cytotoxicity against HCT-116 cells (IC_{90} = 0.05 μg/mL), which was 100 times more active than 5-fluorouracil (5-FU). A profile of cytotoxicity provided preliminary evidence that **287** damages DNA.[171] Lissoclinotoxin B (**289**) was more potent than lissoclinotoxin A (**288**) against various Gram positive and negative bacteria. Compound **288** showed the cytotoxicity against L1210 (IC_{50} = 1 μg/mL) and toxicity against resistant strains of *Plasmodium falciparum*, which was more potent than the activity of quinine and chloroquine.[173] Lissoclinotoxin A (**288**) also inhibited the cell division of sea-urchin eggs at 16 μg/mL.[173] This antimitotic activity was not correlated with the polymerization or depolymerization processes.[173] Compounds **291** and **293** inhibited the protein kinase C activity (IC_{50}'s = 0.3 μg/mL).[174]

The far-eastern ascidian *Polycitor* sp. gave three potent antifungal and antibacterial polysulfides, varacins A-C (**295**-**297**).[175] Varacin C (**297**) showed more potent cytotoxicity than the antitumor agent, doxorubicin, against colon, prostate, breast, and lung cancer cells (IC_{50} = 2.4-48.2 nM).[177] Compound **297** was a thiol-dependent DNA-cleaving agent and suggested to cause the conversion of molecular oxygen to hydroxyl radical (•OH), which cleaves DNA under a physiological condition.[177] This mechanism may explain the potent cytotoxicity and antimicrobial activity of varacin C.

Lissoclinotoxin C (**298**) and the first dimer, lissoclinotoxin D (**299**), were isolated from an Australian *Lissoclinum* sp.[77] Lissoclin disulfoxide (**300**), a dimeric sulfoxide, was obtained from the South African *Lissoclinum* sp. as an inhibitor of IL-8 α and β receptors (IC_{50} = 0.6 and 0.82 μM, respectively) and also had inhibitory activity against PKC (IC_{50} = 1.54 μM).[176] A Philippine didemnid ascidian afforded two dimeric alkaloids, lissoclinotoxins E (**301**) and F (**302**).[178] These compounds displayed the cytotoxicity against the phosphatase and tensin homolog-deficient MDA-MB-468 cells with the IC_{50} values of 2.3 and 1.5 μg/mL, respectively.[178]

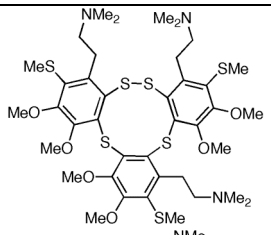
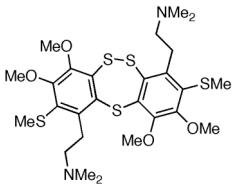
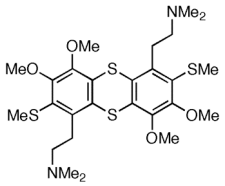
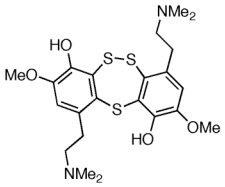
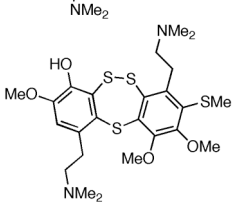
Table 2. Dopamine-derived polysulfur alkaloids from ascidians

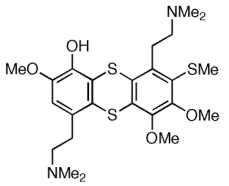
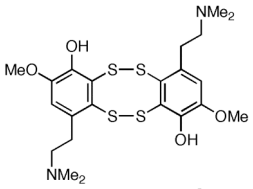
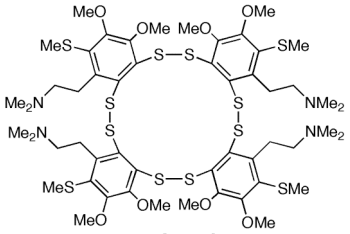
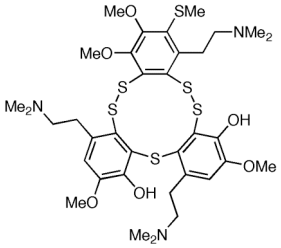
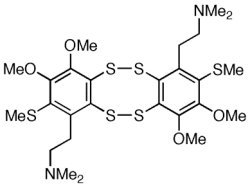
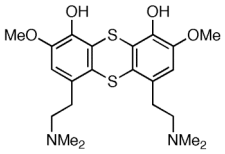
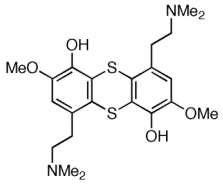
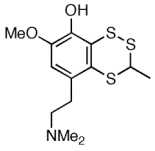
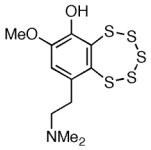
compound	structure	source	bioactivity	ref.
varacin **287**		*Lissoclinum vareau*	antifungal, cytotoxic	171
lissoclinotoxin A **288**		*Lissoclinum perforatum*	antifungal, antibacterial, antimalarial, cytotoxic	172 173
lissoclinotoxin B **289**		*Lissoclinum perforatum*	antimicrobial	173
290		*Lissoclinum japonicum*	antimicrobial, protein kinase C inhibitor	174 180 184

291		*Lissoclinum* sp.	protein kinase C inhibitor	174
292		*Lissoclinum japonicum*	antimicrobial, protein kinase C inhibitor	174, 180, 184
293		*Lissoclinum* sp.	protein kinase C inhibitor	174
294		*Eudistoma* sp.	protein kinase C inhibitor	174
varacin A **295**		*Polycitor* sp.	antimicrobial	175
varacin B **296**		*Polycitor* sp.	antimicrobial	175
varacin C **297**		*Polycitor* sp.	antimicrobial, acid-promoted DNA-cleavage	175, 177
lissoclinotoxin C **298**		*Lissoclinum* sp.	—	77
lissoclinotoxin D **299**		*Lissoclinum* sp.	antimicrobial	77
lissoclin disulfoxide **300**		*Lissoclinum* sp.	IL-8 Rα/Rβ inhibitor	176
lissoclinotoxin E **301**		Didemnid ascidian	cytotoxic	178
lissoclinotoxin F **302**		Didemnid ascidian	cytotoxic antimicrobial	178, 180, 184

We have reported a unique trimeric polysulfur alkaloid, lissoclibadin 1 (**303**), as a cytotoxic component from an Indonesian *Lissoclinum* cf. *badium*.[179,180] Further studies yielded the other 13 congeners, lissoclibadins 2-14 (**304**-**316**) (Table 3), including a tetramer, lissoclibadin 8 (**310**), a trimer, lissoclibadin 9 (**311**), and nine dimers, lissoclibadins 2-7 (**304**-**309**) and 10-12 (**312**-**314**).[119,180-182] The structures of lissoclibadins were assigned on the basis of their spectroscopic data, and the relative orientations of aromatic rings were defined by NOESY experiments and computational molecular modeling studies. Lissoclibadin 8 (**310**) was the first tetrameric dopamine-derived cyclic polysulfide alkaloid with four identical fully substituted dopamine derivatives connected through four disulfide bonds.[182] Although the structure of lissoclibadin 8 was first assigned as shown in Ref. 182, the exhaustive computational conformation analysis suggested that **310** is the only structure, which explains the every spectroscopic data, and that the other three possible structures, including the one shown in Ref. 182, showed inconsistencies with the data (manuscript in preparation). Lissoclibadin 9 (**311**), the second example of trimer, differed from lissoclibadin 1 (**303**) in both aromatic amine moiety and sulfur bridge.[182]

Table 3. Lissoclibadins isolated from *Lissoclinum* cf. *badium*

compound	structure	bioactivity	ref.
lissoclibadin 1 **303**		antibacterial, cytotoxic	179 180 183 184
lissoclibadin 2 **304**		antifungal, antibacterial, cytotoxic, induce IL-8 production	180 183 184
lissoclibadin 3 **305**		cytotoxic, induce IL-8 production	180 183 184
lissoclibadin 4 **306**		antibacterial, cytotoxic	181
lissoclibadin 5 **307**		antiyeast, antibacterial, cytotoxic	181

lissoclibadin 6 **308**		antiyeast, antibacterial, cytotoxic	181
lissoclibadin 7 **309**		antiyeast, antibacterial, cytotoxic	181
lissoclibadin 8 **310**		cytotoxic	119 182
lissoclibadin 9 **311**		cytotoxic	119 182
lissoclibadin 10 **312**		cytotoxic	119 182
lissoclibadin 11 **313**		cytotoxic	119 182
lissoclibadin 12 **314**		cytotoxic	119 182
lissoclibadin 13 **315**		cytotoxic	119 182
lissoclibadin 14 **316**		cytotoxic	119 182

Lissoclibadins 1-9 (**303**-**311**), 13 (**315**), and 14 (**316**) showed weak cytotoxicity against L1210 cells and remarkable inhibitory activity against the colony formation of V79 cells (Table 4). Lissoclibadins 11 (**313**) and 12 (**314**) were not active against L1210 at 20 μM, but **314** had a modest activity against V79. The V79 bioassay reflects the direct action of compounds on the cells.[184] The cytotoxicity of

lissoclibadins 1-3 (**303**-**305**) and 10 (**312**), **302**, **292**, and **290**, isolated from *L.* cf. *badium*, were examined against cultured human solid tumor cell lines to evaluate their potential anticancer efficacy (Table 5).[184] Lissoclibadins 1 (**303**) and 2 (**304**) showed similar activity against 9 human solid tumor cell lines and were more potent than cisplatin. Lissoclibadin 2 (**304**) was the most interesting compound because of the potent inhibitory activity against colon (DLD-1 and HCT 116), breast (MDA-MB-231), renal (ACHN), and large-cell lung (NCI-H460) cancer cell lines. This compound showed no apparent toxicity following the 50 mg/kg single i.v. treatment to mice and preferable stability in rat plasma.[184] Antitumor experiments using nude mice baring the human colon cancer (HCT-116) showed the maximum result on the sixth day after 5-day treatment with 25 and 50 mg/kg (i.v., once a day) (36 and 37% reduction, respectively), but a dose dependence was not observed (unpublished data).

Table 4. Cytotoxicity (IC_{50}, μM) against the murine leukemia cell L1210 and Chinese hamster V79 cell

	303	**304**	**305**	**306**	**307**	**308**	**309**	**310**	**311**	**313**	**314**	**315**	**316**
L1210	1.50	2.04	2.79	1.94	0.97	0.63	2.17	2.00	0.38	>20	>20	2.20	1.80
V79[a]	0.40	0.08	0.34										
V79[b]	0.20			0.71	0.06	0.06	0.17						
V79[c]	0.21							0.14	0.63	>20	7.90	0.44	0.70

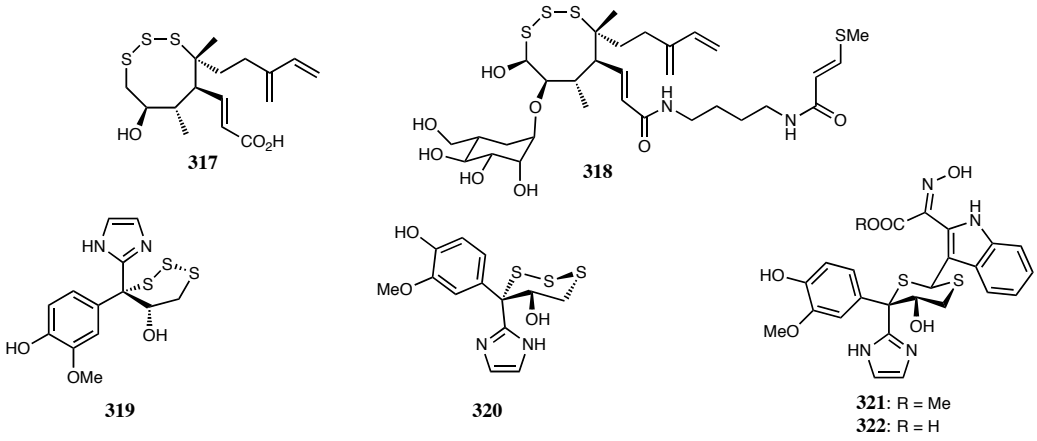
a,b,c: Three different experiments.

Lissoclibadins 2 (**304**) and 3 (**305**) and lissoclinotoxin F (**302**) induced the production of IL-8 in PMA-stimulated HL-60 cells, but lissoclinotoxin E (**301**), **292**, and **290** did not showed such activity.[183] Lissoclibadin 2 (**304**) inhibited the growth of the marine bacterium *Ruegeria atlantica* (12.2 mm at 5 μg/disk) and the fungus *Mucor hiemalis* (13.8 mm at 50 μg/disk),[180] and lissoclibadins 4-7 (**306**-**309**) showed weak antimicrobial activity against *S. aureus*, *E. coli*, and *S. cerevisiae*.[181]

Table 5. Cytotoxicity (IC_{50}, μM) of lissoclibadins 1-3 (**303**-**305**) and 10 (**312**) and compounds **302**, **292**, **290** isolated from *Lissoclinum* cf. *badium* against cultured human solid tumor cell lines[184]

Compd	Breast		Large-cell lung	Prostate	Renal		Colon		Melanoma
	T-47D	MDA-MB-231	NCI-H460	PC-3	ACHN	UO31	DLD-1	HCT-116	MALME-3M
Cisplatin	8.75	6.89	0.72	4.65	1.71	2.97	1.76	1.99	1.81
303	0.39	0.30	0.28	0.24	0.25	0.20	0.13	0.22	0.82
Cisplatin	6.12	5.71	0.67	3.36	1.57	2.65	2.88	2.46	3.27
304	0.57	0.20	0.27	0.64	0.24	0.58	0.10	0.14	0.77
305	3.18	3.59	2.32	7.02	3.26	2.22	1.12	1.49	2.96
312		0.54	0.53						
302	1.04	0.69	0.48	1.06	0.43	0.68	0.24	0.48	1.42
292	3.39	5.34	10.2	10.0	4.62	3.22	1.00	3.04	6.99
290	2.30	3.65	3.50	5.31	3.78	2.33	0.82	2.86	5.83

Trisulfur cyclic compounds **317** and **318** have been isolated from *Perophora viridis* collected off the Atlantic coast of North Carolina.[185] These compounds showed the toxicity to brine shrimp and inhibited the cell division of sea urchin eggs and the growth of *S. aureus* and *B. subtilis*.[185] *Cis*-1,2,3-trithiane derivative **319** was obtained from a New Zealand *Aplidium* sp.[186] Compound **319** was observed to interconvert to *trans*-isomer **320** in neutral or slightly basic solutions. *Hypsistozoa fasmeriana* collected at Tukukata and Leigh Harbor in New Zealand afforded **320**.[187] Two dithiane alkaloids **321** and **322** were also isolated from the Leigh Harbor collection. Compounds **319** and **320** showed identical bioactivities, such as modest cytotoxicity against P388 cells (IC_{50} = 21.6 μM) and antimicrobial activity to *B. subtilis* and *C. albicans* (4 mm at 120 μg/disk).[187]



BIOSYNTHESIS OF MARINE NATURAL PRODUCTS FROM ASCIDIANS

Although the intensive research efforts have identified the impressive numbers of bioactive metabolites from ascidians, biosyntheses of them are still unclear. Ascidians, like the other marine invertebrates, are known to exist in obligate or non-obligate symbiosis with microorganisms, particularly with cyanobacteria *Prochloron* spp. The structural similarities between ascidian and microbial metabolites let us speculate that symbiotic microorganisms are in many cases (at least in part) produce the compounds previously obtained from ascidians. The distribution of a specific family of natural products in unrelated ascidians or their seasonal variations will support this growing conjecture.[188] However, the origin of ascidian-derived metabolites has not been well demonstrated because of the complicated nature of marine symbiotic associations and the difficulties in isolation and cultivation of symbiotic microorganisms. With the aid of experimental strategies especially metagenomic analyses, some progresses have been made. Biosythesis of patellamides A (**37**) and C (**39**) have been elucidated to be performed by a symbiotic *Prochloron* sp. in the ascidian *L. patella* through a ribosomal pathway.[47] The other possible origin of ascidian-derived compounds will be the dietary microalgae and zoo planktons, and ascidians accumulate these compounds most probably for the chemical defense.[188]

The similarity of structures between ascidian and microbial metabolites proved to be of practical

importance in the case of ET 743, which is evaluated clinically as an antitumor agent. The content of ET 743 in the producing ascidian *E. turbinata* is low, and, therefore, a practical method for providing enough amounts of compound for clinical evaluations was required. Since the structure of ET 743 was similar to the bacterial metabolite cyanosafracin B, ET 743 has been produced by the semisynthesis from this compound.[189] Cyanosafracin B is easily obtained by fermentation of the bacterium *Pseudomonas fluorescens*.

The presence of unique endosymbiotic bacteria in *E. turbinata* has been demonstrated by 16S rDNA and in situ hybridization analysis.[190] The origin of ET 743 may be defined in the near future. A biosynthetic pathway involving the formation of tyrosine diketopiperazine followed by oxidation to the corresponding DOPA derivative has been suggested by isotope labeling studies.[191] This proposed biosynthetic pathway was different from that of the saframycins, structurally related myxobacterial metabolites from the terrestrial *myxococcus xanthus*.[191] Culture experiments of *E. turbinata* revealed that the production of ETs was observed with the colonies fed on a mixed diet of microalgae *Chaetoceros gracilis* and *Isochrysis galban*.[188]

There are a number of interesting marine natural products as potential drugs and their leads. However, the supply problem impedes the pharmacological and clinical evaluations. Studies on the biosynthesis and primary producers of natural products obtained from marine invertebrates are very important not only for chemical and ecological research but also the production of these secondary metabolites for further evaluations. These studies will elucidate the biosynthetic genes, and, in the future, a biotechnological production of marine natural and unnatural-natural products will be achieved.

CONCLUSION

During the past decades, ascidians have been thrust into the limelight of marine natural product chemistry. The increased attention has resulted in the isolation of numerous new classes of secondary metabolites, exhibiting significant biological activities. Although research on ascidians was initiated more recently than on some other marine invertebrates, the first marine natural product to enter human clinical trials, didemnin B, was the ascidian metabolite. Secondary metabolites of ascidians are interesting targets for isolation and organic synthesis because of their diverse structural features and remarkable bioactivities. Considering the unique structures and pronounced pharmacological properties of metabolites, especially the promising future in the clinical trials of ET 743 and aplidine, ascidians will continue to attract the attention of both chemists and pharmacologists.

REFERENCES

1. Harless, *Mueller's Arch.*, 1847, 148.

2. R. C. Bruening, E. M. Oltz, J. Furukawa, K. Nakanishi, and K. Kustin, *J. Nat. Prod.*, 1986, **49**, 193.
3. G. Prota, 'Marine Natural Products: Chemistry, Biology and Perspectives,' Vol. 1, ed. by P. J. Scheuer, Academic Press, New York, 1980, pp. 172-174.
4. M. Henze, *Hoppe-Seyler's Z. Physiol. Chem.*, 1911, **72**, 494.
5. M. Henze, *Hoppe-Seyler's Z. Physiol. Chem.*, 1912, **79**, 215.
6. I. G. Macara, G. C. McLeod, and K. Kustin, *Biochem. J.*, 1979, **181**, 457.
7. M. J. Smith, *Experientia*, 1989, **45**, 452.
8. M. J. Smith, D. Kim, B. Hornstein, K. Nakanishi, and K. Kustin, *Acc. Chem. Res.*, 1991, **24**, 117.
9. R. C. Bruening, E. M. Oltz, J. Furukawa, K. Nakanishi, and K. Kustin, *J. Am. Chem. Soc.*, 1985, **107**, 5298.
10. E. M. Oltz, R. C. Bruening, M. J. Smith, K. Kustin, and K. Nakanishi, *J. Am. Chem. Soc.*, 1988, **110**, 6162.
11. W. Fenical, 'Food-Drugs From the Sea, Proceedings, 1974,' ed. by H. H. Webber and G. D. Ruggieri, Marine Technology Society, Washington DC, 1976, pp. 388-394.
12. W. Bergmann and R. J. Feeney, *J. Am. Chem. Soc.*, 1950, **72**, 2809.
13. B. S. Davidson, *Chem. Rev.*, 1993, **93**, 1771.
14. D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2004, **67**, 1216.
15. K. L. Rinehart, Jr., J. B. Gloer, R. G. Hughes, Jr., H. E. Renis, J. P. McGovren, E. B. Swynenberg, D. A. Stringfellow, S. L. Kuentzel, and L. H. Li, *Science*, 1981, **212**, 933.
16. K. L. Rinehart, Jr., J. B. Gloer, J. C. Cook, Jr., S. A. Mizsak, and T. A. Scahill, *J. Am. Chem. Soc.*, 1981, **103**, 1857.
17. J. B. Gloer, Ph. D. Thesis, University of Illinois, Urbana, IL, 1983; *Chem. Abstr.*, 1983, **103**, 122692b; *Diss. Abstr. Int., B*, 1983, **45**, 188.
18. K. L. Rinehart, Jr., *U. S. Patent* 4493796, 1985 (*Chem. Abstr.*, 1985, **103**, 76241v).
19. R. E. Gutowsky, M. S. Thesis, University of Illinois, Urbana, IL, 1984.
20. R. Sakai, J. G. Stroh, D. W. Sullins, and K. L. Rinehart, *J. Am. Chem. Soc.*, 1995, **117**, 3734.
21. K. L. Rinehart, Jr., R. Sakai, and J. G. Stroh, *US Patent* 4948791, 1990 (*Chem. Abstr.*, 1991, **114**, 214413h).
22. K. L. Rinehart, Jr., *Eur. Patent*, No. 48149, 1982 (*Chem. Abstr.*, 1982, **97**, 133565n).
23. A. Boulanger, E. Abou-Mansour, A. Badre, B. Banaigs, G. Combaut, and C. Francisco, *Tetrahedron Lett.*, 1994, **35**, 4345.
24. K. L. Rinehart, Jr. and A. M. Lithgow-Bertelloni, *PCT Int. Appl.*, WO 9104985, 1991 (*Chem. Abstr.*, 1991, **115**, 248086q).
25. E. Abou-Mansour, A. Boulanger, A. Badre, I. Bonnard, B. Banaigs, G. Combaut, and C. Francisco,

Tetrahedron, 1995, **51**, 12591.

26. B. Banaigs, E. Abou-Mansour, I. Bonnard, A. Boulanger, and C. Francisco, *Tetrahedron*, 1999, **55**, 9559.
27. H. G. Chun, B. Davies, D. Hoth, M. Suffness, J. Plowman, K. Flora, C. Grieshaber, and B. Leyland-Jones, *Invest. New Drugs*, 1986, **4**, 279.
28. M. D. Vera and M. M. Joullié, *Med. Res. Rev.*, 2002, **22**, 102.
29. P. Jouin, J. Poncet, M.-N. Dufour, A. Aumelas, and A. Pantaloni, *J. Med. Chem.*, 1991, **34**, 486.
30. H. Kessler, S. Mronga, M. Will, and U. Schmidt, *Helv. Chim. Acta*, 1990, **73**, 25.
31. S. C. Mayer, J. Ramanjulu, M. D. Vera, A. J. Pfizenmayer, and M. M. Joullié, *J. Org. Chem.*, 1994, **59**, 5192.
32. R. Sakai, K. L. Rinehart, V. Kishore, B. Kundu, G. Faircloth, J. B. Gloer, J. R. Carney, M. Namikoshi, F. Sun, R. G. Hughes, Jr., D. G. Grávalos, T. G. de Quesada, G. R. Wilson, and R. M. Heid, *J. Med. Chem.*, 1996, **39**, 2819.
33. H. Vervoort, W. Fenical, and R. de A. Epifanio, *J. Org. Chem.*, 2000, **65**, 782.
34. J. M. Wasylyk, J. E. Biskupiak, C. E. Costello, and C. M. Ireland, *J. Org. Chem.*, 1983, **48**, 4445.
35. B. M. Degnan, C. J. Hawkins, M. F. Lavin, E. J. McCaffrey, D. L. Parry, A. L. van den Brenk, and D. J. Watters, *J. Med. Chem.*, 1989, **32**, 1349.
36. F. J. Schmitz, M. B. Ksebati, J. S. Chang, J. L. Wang, M. B. Hossain, D. van der Helm, M. H. Engel, A. Serban, and J. A. Silfer, *J. Org. Chem.*, 1989, **54**, 3463.
37. C. J. Hawkins, M. F. Lavin, K. A. Marshall, A. L. van den Brenk, and D. J. Watters, *J. Med. Chem.*, 1990, **33**, 1634.
38. L. A. Morris, J. J. K. van den Bosch, K. Versluis, G. S. Thompson, and M. Jaspars, *Tetrahedron*, 2000, **56**, 8345.
39. C. Ireland and P. J. Scheuer, *J. Am. Chem. Soc.*, 1980, **102**, 5688.
40. C. M. Ireland, A. R. Durso, Jr., R. A. Newman, and M. P. Hacker, *J. Org. Chem.*, 1982, **47**, 1807.
41. L. A. McDonald and C. M. Ireland, *J. Nat. Prod.*, 1992, **55**, 376.
42. M. A. Rashid, K. R. Gustafson, J. H. Cardellina, II, and M. R. Boyd, *J. Nat. Prod.*, 1995, **58**, 594.
43. X. Fu, T. Do, F. J. Schmitz, V. Andrusevich, and M. H. Engel, *J. Nat. Prod.*, 1998, **61**, 1547.
44. D. E. Williams, R. E. Moore, and V. J. Paul, *J. Nat. Prod.*, 1989, **52**, 732.
45. T. Shioiri, Y. Hamada, S. Kato, M. Shibata, Y. Kondo, H. Nakagawa, and K. Kohda, *Biochem. Pharmacol.*, 1987, **36**, 4181.
46. P. Wipf, P. C. Fritch, S. J. Geib, and A. M. Sefler, *J. Am. Chem. Soc.*, 1998, **120**, 4105.
47. E. W. Schmidt, J. T. Nelson, D. A. Rasko, S. Sudek, J. A. Eisen, M. G. Haygood, and J. Ravel, *Proc. Natl. Acad. Sci., USA*, 2005, **102**, 7315.

48. T. M. Zabriskie, M. P. Foster, T. J. Stout, J. Clardy, and C. M. Ireland, *J. Am. Chem. Soc.*, 1990, **112**, 8080.
49. A. R. Carroll, J. C. Coll, D. J. Bourne, J. K. MacLeod, T. M. Zabriskie, C. M. Ireland, and B. F. Bowden, *Aust. J. Chem.*, 1996, **49**, 659.
50. L. A. McDonald, M. P. Foster, D. R. Phillips, C. M. Ireland, A. Y. Lee, and J. Clardy, *J. Org. Chem.*, 1992, **57**, 4616.
51. B. M. Degnan, C. J. Hawkins, M. F. Lavin, E. J. McCaffrey, D. L. Parry, and D. J. Watters, *J. Med. Chem.*, 1989, **32**, 1354.
52. M. P. Foster, G. P. Concepción, G. B. Caraan, and C. M. Ireland, *J. Org. Chem.*, 1992, **57**, 6671.
53. L. J. Perez and D. J. Faulkner, *J. Nat. Prod.*, 2003, **66**, 247.
54. T. W. Hambley, C. J. Hawkins, M. F. Lavin, A. van den Brenk, and D. J. Watters, *Tetrahedron*, 1992, **48**, 341.
55. N. Linquist, W. Fenical, G. D. van Duyne, and J. Clardy, *J. Am. Chem. Soc.*, 1991, **113**, 2303.
56. H. C. Vervoort, Ph. D. Thesis, University of California at San Diego, 1999.
57. J. Li, S. Jeong, L. Esser, and P. G. Harran, *Angew. Chem. Int. Ed.*, 2001, **40**, 4765.
58. J. Li, A. W. G. Burgett, L. Esser, C. Amezcua, and P. G. Harran, *Angew. Chem. Int. Ed.*, 2001, **40**, 4770.
59. Z. Cruz-Monserrate, H. C. Vervoort, R. Bai, D. J. Newman, S. B. Howell, G. Los, J. T. Mullaney, M. D. Williams, G. R. Pettit, W. Fenical, and E. Hamel, *Mol. Pharmacol.*, 2003, **63**, 1273.
60. A. M. Fernandez, Ph. D. Thesis, University of Utah, 1996; Available from *Univ. Microfilms Int.*, Order No. DA971662, 1996; *Diss. Abstr. Int., B*, 1997, **57**, 7541.
61. C. M. Ireland and A. Fernandez, *PCT Int. Appl.*, WO 9813063, 1998 (*Chem. Abstr.*, 1998, **128**, 275065g).
62. M. C. Edler, A. M. Fernandez, P. Lassota, C. M. Ireland, and L. R. Barrows, *Biochem. Pharmacol.*, 2002, **63**, 707.
63. K. M. Marshall and L. R. Barrows, *Nat. Prod. Rep.*, 2004, **21**, 731.
64. S. J. Bloor and F. J. Schmitz, *J. Am. Chem. Soc.*, 1987, **109**, 6134.
65. F. S. de Guzman and F. J. Schmitz, *Tetrahedron Lett.*, 1989, **30**, 1069.
66. J. Kobayashi, J.-F Cheng, H. Nakamura, Y. Ohizumi, Y. Hirata, T. Sasaki, T. Ohta, and S. Nozoe, *Tetrahedron Lett.*, 1988, **29**, 1177.
67. F. Bracher, *Pharmazie*, 1997, **52**, 57.
68. F. J. Schmitz, F. S. DeGuzman, M. B. Hossain, D. van der Helm, *J. Org. Chem.*, 1991, **56**, 804.
69. S. S. Matsumoto, M. H. Sidford, J. A. Holden, L. R. Barrows, and B. R. Copp, *Tetrahedron Lett.*, 2000, **41**, 1667.

70. B. S. Lindsay, H. C. Christiansen, and B. R. Copp, *Tetrahedron*, 2000, **56**, 497.
71. P. J. McCarthy, T. P. Pitts, G. P. Gunawardana, M. Kelly-Borges, and S. A. Pomponi, *J. Nat. Prod.*, 1992, **55**, 1664.
72. J. Kobayashi, J.-F. Cheng, M. R. Wälchli, H. Nakamura, Y. Hirata, T. Sasaki, and Y. Ohizumi, *J. Org, Chem.*, 1988, **53**, 1800.
73. J. Kobayashi, M. Tsuda, A. Tanabe, M. Ishibashi, J.-F. Cheng, S. Yamamura, and T. Sasaki, *J. Nat. Prod.*, 1991, **54**, 1634.
74. L. A. McDonald, G. S. Eldredge, L. R. Barrows, and C. M. Ireland, J. *Med. Chem.*, 1994, **37**, 3819.
75. D. R. Appleton, A. N. Pearce, G. Lambert, R. C. Babcock, and B. R. Copp, *Tetrahedron*, 2002, **58**, 9779.
76. T. F. Molinski and C. M. Ireland, *J. Org. Chem.*, 1989, **54**, 4256.
77. P. A. Searle and T. F. Molinski, *J. Org. Chem.*, 1994, **59**, 6600.
78. G. A. Charyulu, T. C. McKee, and C. M. Ireland, *Tetrahedron Lett.*, 1989, **30**, 4201.
79. B. R. Copp, J. Jompa, A. Tahir, and C.M. Ireland, *J. Org. Chem.*, 1998, **63**, 8024.
80. A. R. Carroll and P. J. Scheuer, *J. Org. Chem.*, 1990, **55**, 4426.
81. Nilar; P. J. Sidebottom, B. K. Carté, and M. S. Butler, *J. Nat. Prod.*, 2002, **65**, 1198.
82. A. Kijjoa, R. Wattanadilok, N. Campos, M. S. J. Nascimento, M. Pinto, and W. Herz, *Mar. Drugs*, 2007, **5**, 6.
83. N. M. Cooray, P. J. Scheuer, L. Parkanyi, and J. Clardy, *J. Org. Chem.*, 1988, **53**, 4619.
84. A. R. Carroll, N. M. Cooray, A. Poiner, and P. J. Scheuer, *J. Org. Chem.*, 1989, **54**, 4231.
85. G. Koren-Goldschlager, M. Aknin, E. M. Gaydou, and Y. Kashman, *J. Org. Chem.*, 1998, **63**, 4601.
86. G. Koren-Goldschlager, M. Aknin, and Y. Kashman, *J. Nat. Prod.*, 2000, **63**, 830.
87. Y. Kashman, G. Koren-Goldshlager, M. Aknin, and D. G. Gravalos, *PCT Int. Appl.*, WO 9923099, 1999 (*Chem. Abstr.*, 1999, **130**, 332875p).
88. A. Plubrukarn and B. S. Davidson, *J. Org. Chem.*, 1998, **63**, 1657.
89. A. Rudi, Y. Benayahu, I. Goldberg, and Y. Kashman, *Tetrahedron Lett.*, 1988, **29**, 3861.
90. A. Rudi and Y. Kashman, *J. Org. Chem.*, 1989, **54**, 5331.
91. I. Viracaoundin, R. Faure, E. M. Gaydou, and M. Aknin, *Tetrahedron Lett.*, 2001, **42**, 2669.
92. Y. R. Torres, T. S. Bugni, R. G. S. Berlinck, C. M. Ireland, A. Magalhães, A. G. Ferreira, and R. M. da Rocha, *J. Org. Chem.*, 2002, **67**, 5429.
93. A. Rudi, Y. Benayahu, I. Goldberg, and Y. Kashman, *Tetrahedron Lett.*, 1988, **29**, 6655.
94. N. W. Luedtke, J. S. Hwang, E. C. Glazer, D. Gut, M. Kol, and Y. Tor, *ChemBioChem*, 2002, **3**, 766.
95. K. L. Rinehart, Jr., J. Kobayashi, G. C. Harbour, R. G. Hughes, Jr., S. A. Mizsak, and T. A. Scahill, *J. Am Chem. Soc.*, 1984, **106**, 1524.

96. J. Kobayashi, G. C. Harbour, J. Gilmore, and K. L. Rinehart, Jr., *J. Am. Chem. Soc*., 1984, **106**, 1526.
97. K. L. Rinehart, Jr., J. Kobayashi, G. C. Harbour, J. Gilmore, M. Mascal, T. G. Holt, L. S. Shield, and F. Lafargue, *J. Am. Chem. Soc*., 1987, **109**, 3378.
98. K. F. Kinzer and J. H. Cardellina, II., *Tetrahedron Lett*., 1987, **28**, 925.
99. A. Badre, A. Boulanger, E. Abou-Mansour, B. Banaigs, G. Combaut, and C. Francisco, *J. Nat. Prod*., 1994, **57**, 528.
100. R. A. Davis, A. R. Carroll, and R. J. Quinn, *J. Nat. Prod*., 1998, **61**, 959.
101. P. Schupp, T. Poehner, R. Edrada, R. Ebel, A. Berg, V. Wray, and P. Proksch, *J. Nat. Prod*., 2003, **66**, 272.
102. H. Kang and W. Fenical, *Nat. Prod. Lett*., 1996, **9**, 7.
103. M. A. Rashid, K. R. Gustafson, and M. R. Boyd, *J. Nat. Prod*., 2001, **64**, 1454.
104. J. Kobayashi, H. Nakamura, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett*., 1986, **27**, 1191.
105. J. Kobayashi, J.-F. Cheng, T. Ohta, S. Nozoe, Y. Ohizumi, and T. Sasaki, *J. Org. Chem*., 1990, **55**, 3666.
106. O. Murata, H. Shigemori, M. Ishibashi, K. Sugama, K. Hayashi, and J. Kobayashi, *Tetrahedron Lett*., 1991, **32**, 3539.
107. S. A. Adesanya, M. Chbani, M. Païs, and C. Debitus, *J. Nat. Prod*., 1992, **55**, 525.
108. M. Chbani and M. Païs, *J. Nat. Prod*., 1993, **56**, 99.
109. C. Debitus, D. Laurent, and M. Païs, *J. Nat. Prod*., 1988, **51**, 799.
110. S. Mahboobi, S. Dove, P. J. Bednarski, and S. Kuhr, *J. Nat. Prod*., 1997, **60**, 587.
111. R. M. van Wagoner, J. Jompa, A. Tahir, and C. M. Ireland, *J. Nat. Prod*., 1999, **62**, 794.
112. R. W. Schumacher and B. S. Davidson, *Tetrahedron*, 1995, **51**, 10125.
113. P. S. Kearns, J. C. Coll, and J. A. Rideout, *J. Nat. Prod*., 1995, **58**, 1075.
114. K. Ravinder, A. V. Reddy, P. Krishnaiah, P. Ramesh, S. Ramakrishna, H. Laatsch, and Y. Venkateswarlu, *Tetrahedron Lett*., 2005, **46**, 5475.
115. N. Oku, S. Matsunaga, and N. Fusetani, *J. Am. Chem. Soc*., 2003, **125**, 2044.
116. C. Moquin and M. Guyot, *Tetrahedron Lett*., 1984, **25**, 5047.
117. C. Moquin-Pattey and M. Guyot, *Tetrahedron*, 1989, **45**, 3445.
118. S. A. Abas, M. B. Hossain, D. van der Helm, and F. J. Schmitz, *J. Org. Chem*., 1996, **61**, 2079.
119. W.-F. Wang, Ph. D Thesis, Tokyo University of Marine Science and Technology, Tokyo, Japan, Sept. 2007.
120. R. J. Andersen, D. J. Faulkner, C. H. He, G. D. van Duyne, and J. Clardy, *J. Am. Chem. Soc*., 1985, **107**, 5492.

121. N. Lindquist, W. Fenical, G. D. Van Duyne, and J. Clardy, *J. Org. Chem.*, 1988, **53**, 4570.
122. A. R. Carroll, B. F. Bowden, and J. C. Coll, *Aust. J. Chem.*, 1993, **46**, 489.
123. S. Urban and R. J. Capon, *Aust. J. Chem.*, 1996, **49**, 711.
124. M. V. R. Reddy, D. J. Faulkner, Y. Venkateswarlu, and M. R. Rao, *Tetrahedron*, 1997, **53**, 3457.
125. R. A. Davis, A. R. Carroll, G. K. Pierens, and R. J. Quinn, *J. Nat. Prod.*, 1999, **62**, 419.
126. J. Ham and H. Kang, *Bull. Korean Chem. Soc.*, 2002, **23**, 163.
127. P. Krishnaiah, V. L. N. Reddy, G. Venkataramana, K. Ravinder, M. Srinivasulu, T. V. Raju, K. Ravikumar, D. Chandrasekar, S. Ramakrishna, and Y. Venkateswarlu, *J. Nat. Prod.*, 2004, **67**, 1168.
128. S. M. Reddy, M. Srinivasulu, N. Satyanarayana, A. K. Kondapi, and Y. Venkateswarlu, *Tetrahedron*, 2005, **61**, 9242.
129. A. R. Quesada, M. D. G. Gravalos, and J. L. F. Puentes, *Br. J. Cancer*, 1996, **74**, 677.
130. C. P. Ridley, M. V. Reddy, G. Rocha, F. D. Bushman, and D. J. Faulkner, *Bioorg. Med. Chem.*, 2002, **10**, 3285.
131. M. V. R. Reddy, M. R. Rao, D. Rhodes, M. S. T. Hansen, K. Rubins, F. D. Bushman, Y. Venkateswarlu, and D. J. Faulkner, *J. Med. Chem.*, 1999, **42**, 1901.
132. S. S. Mitchell, D. Rhodes, F. D. Bushman, and D. J. Faulkner, *Org. Lett.*, 2000, **2**, 1605.
133. W. Y. Yoshida, K. K. Lee, A. R. Carroll, and P. J. Scheuer, *Helv. Chim. Acta*, 1992, **75**, 1721.
134. S. Manazanaro, J. Salvá, and J. Á. de la Fuente, *J. Nat. Prod.*, 2006, **69**, 1485.
135. H.; Kang and W. Fenical, *J. Org. Chem.*, 1997, **62**, 3254.
136. A. Rudi, I. Goldberg, Z. Stein, F. Frolow, Y. Benayahu, M. Schleyer, and Y. Kashman, *J. Org. Chem.*, 1994, **59**, 999.
137. A. Rudi, T. Evan, M. Aknin, and Y. Kashman, *J. Nat. Prod.*, 2000, **63**, 832.
138. S. Loya, A. Rudi, Y. Kashman, and A. Hizi, *Biochem. J.*, 1999, **344**, 85.
139. J. Kobayashi, J.-F. Cheng, Y. Kikuchi, M. Ishibashi, S. Yamamura, Y. Ohizumi, T. Ohta, and S. Nozoe, *Tetrahedron Lett.*, 1990, **31**, 4617.
140. M. Tsuda, K. Nozawa, K. Shimbo, and J. Kobayashi, *J. Nat. Prod.*, 2003, **66**, 292.
141. R. A. Davis, L. V. Christensen, A. D. Richardson, R. M. da Rocha, and C. M. Ireland, *Mar. Drugs*, 2003, **1**, 27.
142. M. M. Sigel, L. L. Wellham, W. Lichter, L. E. Dudeck, J. L. Gargus, and A. H. Lucas, 'Food-Drugs From the Sea, Proceedings, 1969,' ed. by H. W. Youngken, Marine Technology Society, Washington DC, 1970, pp. 281-295.
143. A. E. Wright, D. A. Forleo, G. P. Gunawardana, S. P. Gunasekera, F. E. Koehn, and O. J. McConnell, *J. Org. Chem.*, 1990, **55**, 4508.
144. K. L. Rinehart, T. G. Holt, N. L. Fregeau, J. G. Stroh, P. A. Keifer, F. Sun, L. H. Li, and D. G.

Martin, *J. Org. Chem.*, 1990, **55**, 4512.

145. R. Sakai, K. L. Rinehart, Y. Guan, and A. H.-J. Wang, *Proc. Natl. Acad. Sci., USA*, 1992, **89**, 11456.
146. R. Sakai, E. A. Jares-Erijman, I. Manzanares, M. V. S. Elipe, and K. L. Rinehart, *J. Am. Chem. Soc.*, 1996, **118**, 9017.
147. K. L. Rinehart and R. Sakai, *US Patent* 2004059112, 2004 (*Chem. Abstr.*, 2004, **140**, 284677h).
148. K. Suwanborirux, K. Charupant, S. Amnuoypol, S. Pummangura, A. Kubo, and N. Saito, *J. Nat. Prod.*, 2002, **65**, 935.
149. I. Manzanares, C. Cuevas, R. Carcía-Nieto, E. Marco, and F. Gago, *Curr. Med. Chem. Anti-cancer Agents*, 2001, **1**, 257.
150. P. Allavena, M. Signorelli, M. Chieppa, E. Erba, G. Bianchi, F. Marchesi, C. O. Olimpio, C. Bonardi, A. Garbi, A. Lissoni, F. de Braud, J. Jimeno, and M. D'Incalci, *Cancer Res.*, 2005, **65**, 2964.
151. C. Malochet-Grivois, P. Cotelle, J. F. Biard, J.-P. Hénichart, C. Debitus, C. Roussakis, and J. F. Verbist, *Tetrahedron Lett.*, 1991, **32**, 6701.
152. J.-F. Biard, C. Malochet-Grivois, C. Roussakis, P. Cotelle, J.-P. Hénichart, C. Débitus, and J.-F. Verbist, *Nat. Prod. Lett.*, 1994, **4**, 43.
153. L. Toupet, J.-F. Biard, and J.-F. Verbist, *J. Nat. Prod.*, 1996, **59**, 1203.
154. F. Robert, H.-Q. Gao, M. Dnia, W. C. Merrick, M. T. Hamann, and J. Pelletier, *RNA*, 2006, **12**, 717.
155. M. J. Uddin, S. Kokubo, K. Suenaga, K. Ueda, and D. Uemura, *Heterocycles*, 2001, **54**, 1039.
156. M. J. Uddin, S. Kokubo, K. Ueda, K. Suenaga, and D. Uemura, *J. Nat. Prod.*, 2001, **64**, 1169.
157. M. J. Uddin, S. Kokubo, K. Ueda, K. Suenaga, and D. Uemura, *Chem. Lett.*, 2002, **10**, 1028.
158. J. Uddin, K. Ueda, E. R. O. Siwu, M. Kita, and D. Uemura, *Bioorg. Med. Chem.*, 2006, **14**, 6954.
159. S. Fukuzawa, S. Matsunaga, and N. Fusetani, *J. Org. Chem.*, 1994, **59**, 6164.
160. S. Fukuzawa, S. Matsunaga, and N. Fusetani, *J. Org. Chem.*, 1995, **60**, 608.
161. S. Fukuzawa, S. Matsunaga, and N. Fusetani, *Tetrahedron*, 1995, **51**, 6707.
162. S. Fukuzawa, S. Matsunaga, and N. Fusetani, *J. Org. Chem.*, 1997, **62**, 4484.
163. S. Fukuzawa, S. Matsunaga, and N. Fusetani, *Symposium Papers of the 38th Symposium on the Chemistry of Natural Products*, Sendai, Japan, 1996, pp. 73-78.
164. T. Komiya, N. Fusetani, S. Matsunaga, A. Kubo, F. J. Kaye, M. J. Kelley, K. Tamura, M. Yoshida, M. Fukuoka, and K. Nakagawa, *Cancer Chemother. Pharmacol.*, 2003, **51**, 202.
165. N. Lindquist and W. Fenical, *Tetrahedron Lett.*, 1990, **31**, 2389.
166. S. A. Fedoreyev, O. S. Radchenko, V. L. Novikov, V. V. Isakov, S. G. Ilyin, A. M. Popov, G. B. Elyakov, P. T. Murphy, R. H. Willis, and J. T. Baker, *Proceedings of the 8th Interernational Symposium on Marine Natural Products*, Santa Cruz de Tenerife, Tenerife, Canary Islands, Spain, 1995, pp. 196-197.

167. H. Kang and W. Fenical, *Tetrahedron Lett*., 1996, **37**, 2369.
168. O. S. Radchenko, V. L. Novikov, R. H. Willis, P. T. Mruphy, and G. B. Elyakov, *Tetrahedron Lett.*, 1997, **38**, 3581.
169. S. N. Fedorov, A. M. Bode, V. A. Stonik, I. A. Gorshkova, P. C. Schmid, O. S. Radchenko, E. V. Berdyshev, and Z. Dong, *Pharm. Res*., 2004, **21**, 2307.
170. W.-F. Wang, T. Oda, A. Fujita, R. E. P. Mangindaan, T. Nakazawa, K. Ukai, H. Kobayashi, and M. Namikoshi, *Tetrahedron Lett*., 2007, **63**, 409.
171. B. S. Davidson, T. F. Molinski, L. R. Barrows, and C. M. Ireland, *J. Am. Chem. Soc*., 1991, **113**, 4709.
172. M. Litaudon and M. Guyot, *Tetrahedron Lett*., 1991, **32**, 911.
173. M. Litaudon, F. Trigalo, M.-T. Martin, F. Frappier, and M. Guyot, *Tetrahedron*, 1994, **50**, 5323.
174. R. S. Compagnone, D. J. Faulker, B. K. Carté, G. Chan, A. Freyer, M. E. Hemling, G. A. Hofmann, and M. R. Mattern, *Tetrahedron*, 1994, **50**, 12785.
175. T. N. Makarieva, V. A. Stonik, A. S. Dmitrenok, B. B. Grebnev, V. V. Isakov, and N. M. Rebachyk, *J. Nat. Prod*., 1995, **58**, 254.
176. A. D. Patil, A. J. Freyer, L. Killmer, G. Zuber, B. Carte, A. J. Jurewicz, and R. K. Johnson, *Nat. Prod. Lett*., 1997, **10**, 225.
177. A. H. F. Lee, J. Chen, D. Liu, T. Y. C. Leung, A. S. C. Chan, and T. Li, *J. Am. Chem. Soc*., 2002, **124**, 13972.
178. R. A. Davis, I. T. Sandoval, G. P. Concepcion, R. M. da Rocha, and C. M. Ireland, *Tetrahedron*, 2003, **59**, 2855.
179. H. Liu, S. B. Pratasik, T. Nishikawa, T. Shida, K. Tachibana, T. Fujiwara, H. Nagai, H. Kobayashi, and M. Namikoshi, *Tetrahedron Lett*., 2004, **45**, 7015.
180. H. Liu, T. Fujiwara, T. Nishikawa, Y. Mishima, H. Nagai, T. Shida, K. Tachibana, H. Kobayashi, R. E. P. Mangindaan, and M. Namikoshi, *Tetrahedron*, 2005, **61**, 8611.
181. T. Nakazawa, J. Xu, T. Nishikawa, T. Oda, A. Fujita, K. Ukai, R. E. P. Mangindaan, H. Rotinsulu, H. Kobayashi, and M. Namikoshi, *J. Nat. Prod.*, 2007, **70**, 439.
182. W.-F. Wang, T. Nakazawa, K. Ukai, R. E. P. Mangindaan, D. Wewengkang, H. Rotinsulu, H. Kobayashi, S. Tsukamoto, and M. Namikoshi, *Symposium Papers of the 49th Symposium on the Chemistry of Natural Products*, Sapporo, Japan, 2007, pp. 359-364. The structure assigned for lissoclibadin 8 in this paper should be corrected as described in the text.
183. T. Oda, T. Fujiwara, H. Liu, K. Ukai, R. E. P. Mangindaan, M. Mochizuki, and M. Namikoshi, *Mar. Drugs*, 2006, **4**, 15.
184. T. Oda, K. Kamoshita, S. Maruyama, K. Masuda, M. Nishimoto, J. Xu, K. Ukai, R. E. P.

Mangindaan, and M. Namikoshi, *Biol. Pharm. Bull.*, 2007, **30**, 385.

185. T. Řezanka and V. M. Dembitsky, *Eur. J. Org. Chem.*, 2002, 2400.
186. B. R. Copp, J. W. Blunt, M. H. G. Munro, and L. K. Pannell, *Tetrahedron Lett.*, 1989, **30**, 3703.
187. A. N. Pearce, R. C. Babcock, C. N. Battershill, G. Lambert, and B. R. Copp, *J. Org. Chem.*, 2001, **66**, 8257.
188. B. S. Moore, *Nat. Prod. Rep.*, 2006, **23**, 615.
189. C. Cuevas, M. Pérez, M. J. Martín, J. L. Chicharro, C. Fernández-Rivas, M. Flores, A. Francesch, P. Gallego, M. Zarzuelo, F. de la Calle, J. García, C. Polanco, I. Rodríguez, and I. Manzanares, *Org. Lett.*, 2000, **2**, 2545.
190. G. M. König, S. Kehraus, S. F. Seibert, A. Abdel-Lateff, and D. Müller, *ChemBioChem*, 2006, **7**, 229.
191. K. L. Rinehart, *Med. Res. Rev.*, 2000, **20**, 1.

Michio Namikoshi was born in 1952, graduated from Shizuoka College of Pharmacy, Japan, in 1975, and obtained his Ph.D. from The University of Tokyo (Institute of Applied Microbiology) in 1981. He joined Prof. Kenneth L. Rinehart group at the Department of Chemistry, University of Illinois, Urbana-Champaign, USA, from August 1987 to February 1994 for his post-doctoral study. From March 1994 to March 2006, he served as Associate Professor and Professor at Tokyo University of Fisheries (from October 2003, Tokyo University of Marine Science and Technology). He moved to the present position on April 2006 as Professor of Natural Product Chemistry. His research interests are mainly in chemical ecology of echinoderms and search for new pharmacologically active substances through ecochemical study.

Weifang Wang, born in 1974, received her B.E. degree in Pharmaceutical for Chinese Materia Medica in 1996 and M.S. degree in Medicinal Chemistry in 1999 from Shenyang Pharmaceutical University. After a 5-year term appointment as Assistant Professor (1999-2001) and Lecturer (2001-2004) in Natural Products Chemistry at School of Pharmacy, Fudan University, she undertook doctoral research on Marine Natural Products Chemistry under the supervision of Prof. Michio Namikoshi at Tokyo Univeristy of Marine Science and Technology and obtained her Ph.D. there in 2007. Her research interest lies in the search for new bioactive secondary metabolites as chemical entities of potential drug candidates and their leads from marine invertebrates by bioassay-guided fractionation.