Chapter 36
Pyridoacridine Alkaloids from Marine Origin: Sources and Anticancer Activity

Anake Kijjoa

Abstract The pyridoacridines are a group of highly colored, polycyclic aromatic natural products isolated from marine invertebrates, especially marine sponges and tunicates. As a class, the pyridoacridines are considered DNA binding molecules and have been characterized mainly on the basis of their cytotoxicity, even though they may possess other interesting biological activities such as antibacterial, antifungal, antiviral, antiparasitic and insecticidal activities. Recent reports have demonstrated that the individual pyridoacridines can vary dramatically in their molecular mechanism of cell killing. It was suggested that both core structure and substituents can have significant influences on the electronic distribution and topology of the molecule of these compounds, which ultimately affect the mechanism underlying their biological activities. This chapter covers structures and sources of the isolated marine pyridoacridine alkaloids, as well as the mechanisms underlying the cytotoxicity of certain naturally occurring marine pyridoacridines.

Keywords Anticancer activities · Apoptosis · Caspase-3 activation · Cytotoxicity · DNA intercalator · Iminoquinone · Pyridoacridine alkaloids · ROS generation · Topoisomerase II poisoning · Ubiquitylation inhibitor

36.1 Introduction

Cancer is a constant and major burden on the human population, and epidemiological evidence shows that current treatment of cancer with chemotherapy and surgery are still far from optimal. Therefore, further research is required to look for alternative drugs that are more effective and have less side-effect than the drugs currently used in chemotherapy. One of these avenues is to search for new chemical entities, *via* genetic mining from the marine environment, which can be potential for the development of arsenals in cancer chemotherapy [1].

A. Kijjoa (✉)
ICBAS-Instituto de Ciências Biomédicas Abel Salazar and Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
e-mail: ankijjoa@icbas.up.pt

S.-K. Kim (ed.), *Handbook of Anticancer Drugs from Marine Origin,*
DOI 10.1007/978-3-319-07145-9_36

Marine organisms constitute an important source of bioactive compounds with continuing interest in the pharmaceutical in biotechnological sectors. In recent years, the chemistry of natural products derived from marine organisms, along with the development of new *in vitro* bioassay tools, has become the focus of a much greater research effort. Currently there are around 15 marine natural products in various phases of clinical development, mainly in the oncology area, with more on the way and several products already on the market [2]. Among the myriad of the secondary metabolites from marine sources, marine alkaloids represent a major group of bioactive marine natural products. Specifically, the pyridoacridines, with most of the compounds being isolated from the marine sponges and tunicates, constitute the largest group of the marine alkaloids which exhibit relevant biological activities such as antibacterial, antifungal, antiviral, antiparasitic, insecticidal and anti-tumor activities [3]. Almost all natural marine pyridoacridines have been reported to possess significant cytotoxicity against cultured cells, and the family as a whole seems to be of great interest as a source of new lead structures for the development of future generation of therapeutic agents [4]. Due to the importance and the volume of work published for the pyridoacridine alkaloids, this chapter will focus only on the structures of the naturally occurring marine pyridoacridines and their sources, as well as the mechanisms underlying the cytotoxicity of some relevant compounds. Although excellent reviews on chemistry and biological activities of the pyridoacridine alkaloids have been previously published by Molinski [5], Ding et al. [6], Delfourne and Bastide [4] and Marshall and Barrows [3], this chapter covers the pyridoacridine alkaloids isolated from the marine sources and the mechanisms underlying the cytotoxicity of certain naturally occurring marine pyridoacridines, reported until October 2013.

36.2 Pyridoacridine Alkaloids

Pyridoacridines are planar polyheterocyclic compounds possessing a common 11H-pyrido[4, 3, 2-*mn*]acridine skeleton (**1**) (Fig. 36.1), even though the majority are more accurately described as “pyridoacridone”, derivatives of the hypothetical iminoquinone, 8H-pyrido[4,3,2-*mn*]acridone (**2**) (Fig. 36.1). Among marine alkaloids, the pyridoacridines seem to be the largest group, with most of the compounds being isolated from marine sponges and ascidians and, in lesser extent, from certain mollusks and one coelenterate [5]. While the pyridoacridine alkaloids found in the mollusk *Chelynotus semperi* were undoubtedly produced from an ascidian dietary source, their distribution across three other phyla led to the suggestion that the alkaloids might be produced by the same or similar organisms associated with the different hosts [7].

The structures of the pyridoacridine alkaloids vary depending on the nature and position of the side chains on ring C and on the acridine nitrogen, as well as on the presence of the rings fused with ring C. The oxidation states of the rings can also vary, with partial saturation frequently observed in the ring attached to ring

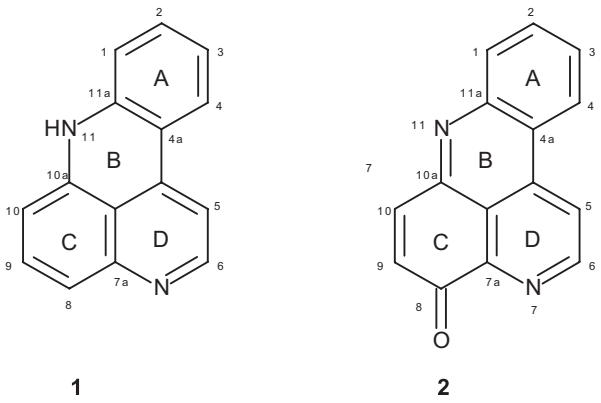


Fig. 36.1 Structures of 11H-pyrido[4, 3, 2-*mn*]acridine (**1**) and 8H-pyrido[4,3,2-*mn*] acridone (**2**) skeleton

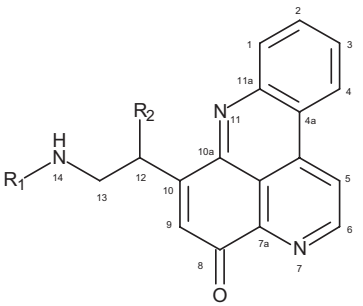
C and, occasionally in ring D [5]. Based on the number of rings attached to the 11H-pyrido[4, 3, 2-*mn*]acridine (**1**) or 8H-pyrido[4,3,2-*mn*]acridone (**2**) skeleton, pyridoacridines can be divided into tetracyclic, pentacyclic, hexacyclic, heptacyclic and octacyclic alkaloids.

36.2.1 Tetracyclic Alkaloids

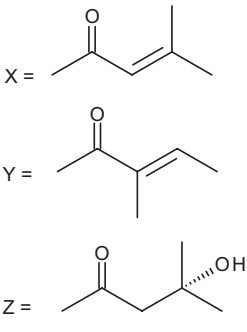
The first group of the pyridoacridines possesses the oxidized ring C, i.e., an iminoquinone subunit (**2**). Kobayashi et al. [8] reported isolation of cystodytin A–C (**3–5**) (Fig. 36.2), the first members of the tetracyclic pyridoacridine alkaloids, from the Okinawan marine tunicate *Cystodytes dellechiajei*. Later on, the same group isolated cystodytin D–I (**6–11**) (Fig. 36.2), also from the same tunicate [9]. McDonald et al. [10] reported isolation of another cytodytin analogue which they have named cytodytin J (**12**), in addition to cytodytin A (**3**), from the methanol extract of a Fijian *Cystodytes* ascidian (Fig. 36.2). Appleton et al. [11] reported isolation of cystodytins J (**12**) and cystodytin K (**13**), from the ascidian *Lissoclinum notti*, collected in New Zealand (Fig. 36.2).

Halogenated tetracyclic pyridoacridine alkaloids have been also reported from the marine sources. Kim et al. [12] reported isolation of pantherinine (**14**), a brominated pyridoacridine, from the ascidian *Aplidium pantherinum*, collected at Stenhouse Bay, South Australia. Pantherinine (**14**) has, in addition to a bromine substituent on C-3 of the aromatic ring A, the amino group on C-9 of the quinone moiety (Fig. 36.3).

The thiomethyl substituent is also common in the pyridoacridine alkaloids. Lissoclins A and B (**15**, **16**), iminoquinones with the thiomethyl substituent on C-9, were isolated from *Lissoclinum sp*., collected from the Great Barrier Reef [13]. Lissoclin A (**15**) differs from lissoclin B (**16**) only in the side chain at C-10, with isovaleryl in the former and tigloyl in the latter (Fig. 36.3).



3, R_1 = H, R_2 = X
4, R_1 = H, R_2 = Y
5, R_1 = H, R_2 = Z
6, R_1 = OH, R_2 = X
7, R_1 = OH, R_2 = Y
8, R_1 = OMe, R_2 = X
9, R_1 = OMe, R_2 = Y



10, R_1 = O-C-$(CH_2)_7CH$=$CH(CH_2)_7CH_3$, R_2 = X

11, R_1 = O-C-$(CH_2)_7CH$=$CH(CH_2)_7CH_3$, R_2 = Y

12, R_1 = CHO, R_2 = H

13, R_1 = CHO, R_2 = OMe

Fig. 36.2 Structures of cystodytins A–K (**3–13**)

Another group of structurally related thiomethyl substituted iminoquinones are diplamine (**17**), isodiplamine (**18**) and diplamine B (**19**) (Fig. 36.3). Diplamine (**17**) was first isolated as a cytotoxic alkaloid from the tunicate *Diplosoma* sp. [14], and later, together with isodiplamine (**18**), from the New Zealand ascidian *Lissoclinum notti* [11] while diplamine B (**19**) was reported from the ascidian *Lissoclinum* cf. *badium*, collected in the Coral Sea, Papua New Guinea [15].

The second group of the tetracyclic alkaloids possesses the 11H-pyrido[4, 3, 2-*mn*]acridine skeleton (**1**). The most simple tetracyclic pyridoacridines of this group are styelamines A–D (**20–23**) (Fig. 36.4), which were isolated from an extract of the ascidian *Eusynstyela latericius*, collected from Ujung Pandang, Indonesia [16]. The common feature of these compounds is the presence of the hydroxyl group at C-8 of the aromatic ring C. Styelamines C (**22**) and D (**23**) were later isolated from the purple morph of the ascidian *Cystodytes dellechiajei*, collected in Catalonia [17]. The structurally related varamines A (**24**) and B (**25**) (Fig. 36.4), with the methoxyl group at C-8 and the thiomethyl substituent at C-9, were reported as metabolites of the Fijian ascidian *Lissoclinum vareau* by Molinski and Ireland [18], and norsegoline (**26**) (Fig. 36.4) was reported as a metabolite of the marine tunicate *Eudistoma* sp. [19, 20].

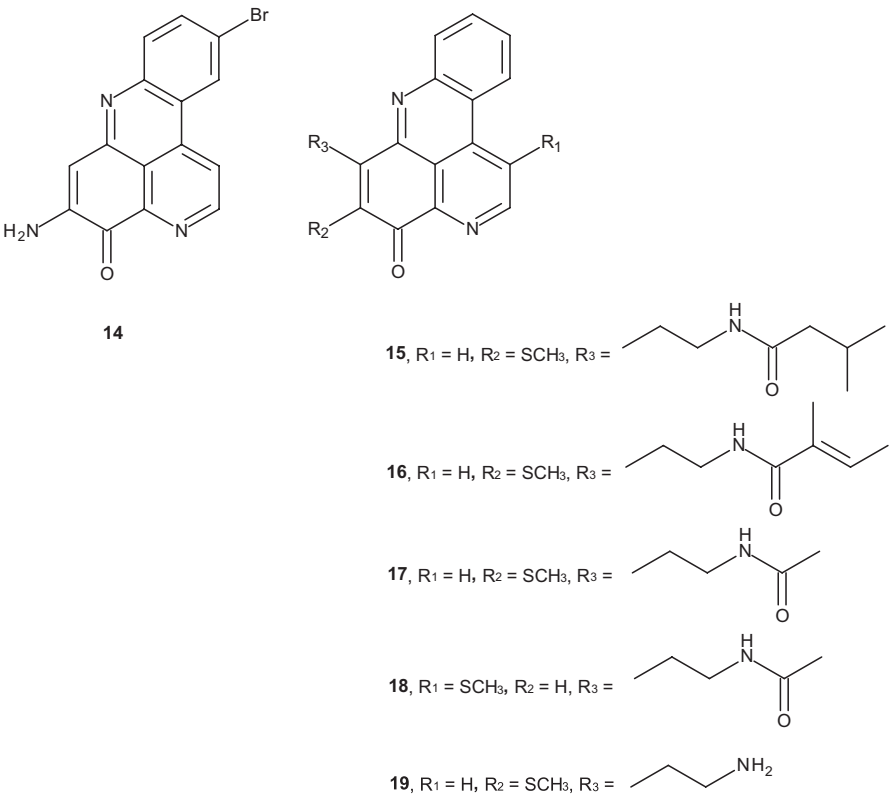


Fig. 36.3 Structures of pantherinine (**14**), lissoclin A (**15**) and B (**16**), diplamine (**17**), isodiplamine (**18**) and diplamine B (**19**)

36.2.2 Pentacyclic Alkaloids

Amphimedine (**27**) (Fig. 36.5), the first marine-derived pentacyclic pyridoacridine alkaloid, was isolated from a Pacific sponge *Amphimedon* sp. [21]. It was later isolated, together with neoamphimedine (**29**) (Fig. 36.5), from the marine sponge *Xestospongia* sp., collected from Surigao, Phillipines [22]. Tasdemir et al. [23] reported isolation of amphimedine (**27**), neoamphimedine (**29**), and deoxyamphimedine (**31**) (Fig. 36.5), from two specimens of the marine sponge *Xestospongia* sp., collected from the Philippines and Palau. Thale et al. [24] described isolation of neoamphimedine (**29**) and 5-methoxyneoamphimedine (**30**) (Fig. 36.5) from the marine sponge *X.* cf. *exigua*, collected from Indonesia, as well as of neoamphimedine (**29**), 5-methoxyneoamphimedine (**30**), neoamphimedine Y (**35**) and neoamphimedine Z (**36**) (Fig. 36.5), from *X.* cf. *carbonaria,* collected from Papua New Guinea. Wei et al. [25] isolated three new analogues of amphimedine, i.e., 1-hydroxy-deoxyamphimedine (**32**), 3-hydroxy-deoxyamphimedine (**33**), debromopetrosamine (**37**), along with amphimedine (**27**), neoamphimedine (**29**) and deoxyamphimedine (**31**)

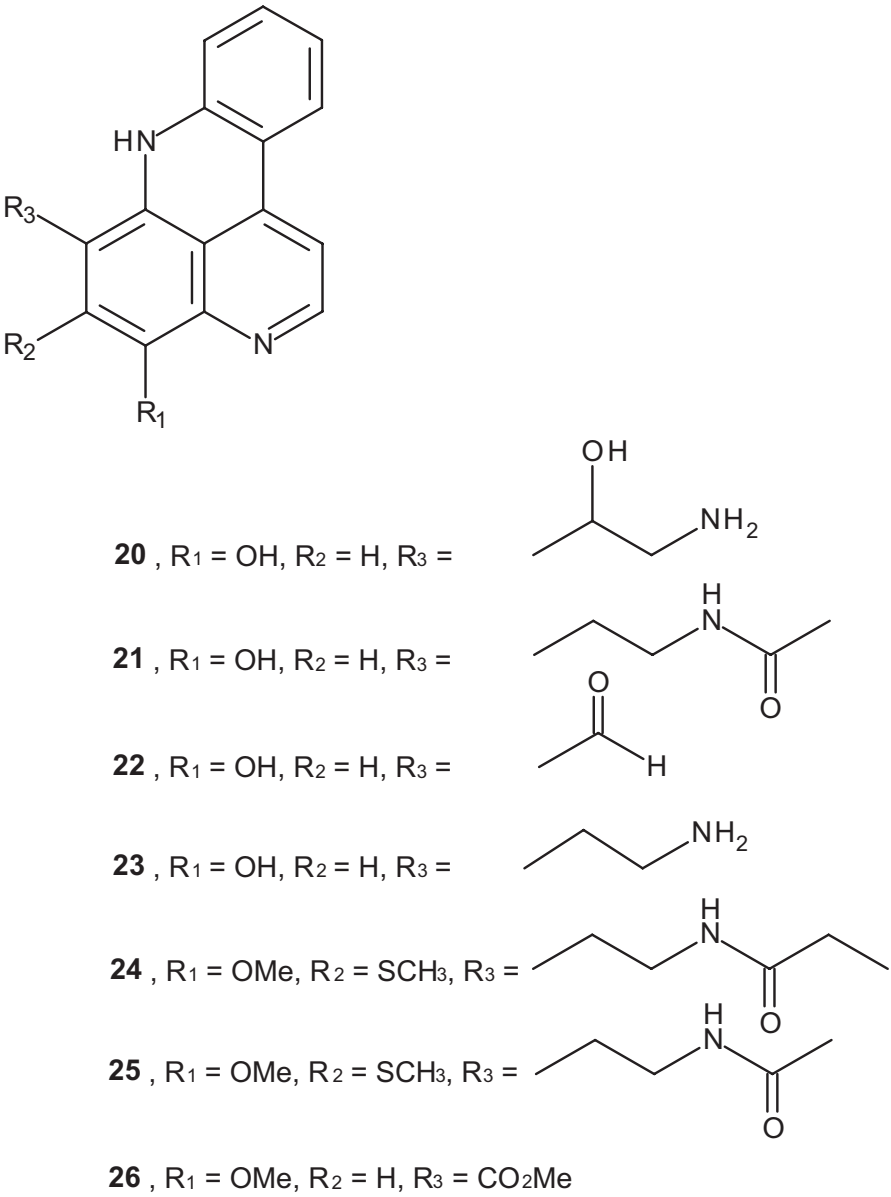
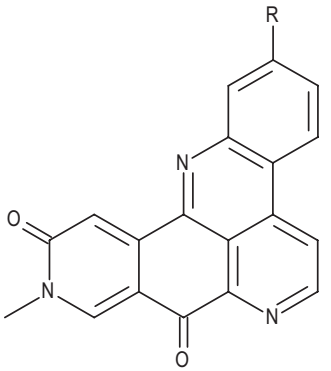
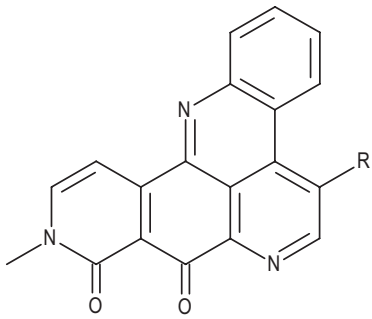


Fig. 36.4 Structures of styelamines A–D (**20**–**23**), varamines A (**24**) and B (**25**), and norsegoline (**26**)

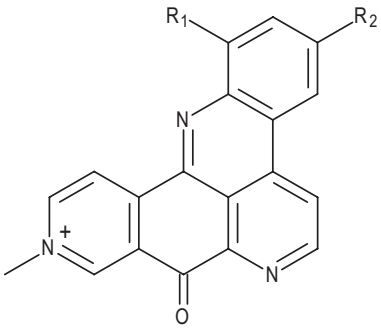
(Fig. 36.5), from the marine sponge *X.* cf. *carbonaria*. Recently, Bry et al. [17] reported isolation of, among other pyridoacridine alkaloids, demethyldeoxyamphimedine (**34**) (Fig. 36.5), from the purple morph of the Mediterranean ascidian *Cystodytes dellechiajei*.



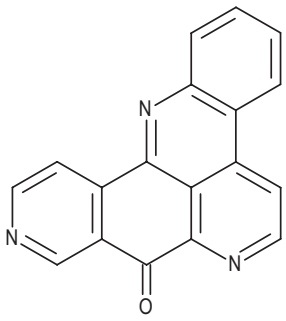
27, R = H
28, R = Br



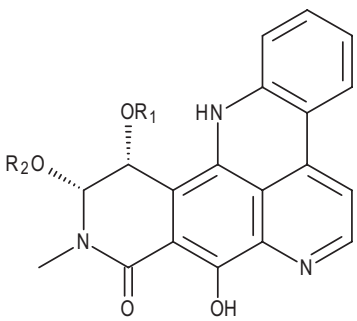
29, R = H
30, R = OMe



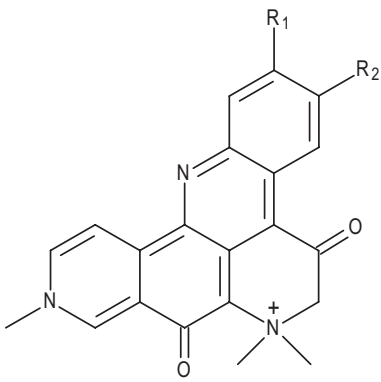
31, $R_1 = R_2 = H$
32, $R_1 = OH$, $R_2 = H$
33, $R_1 = H$, $R_2 = OH$



34



35, $R_1 = R_2 = H$
36, $R_1 = R_2 = Me$



37, $R_1 = R_2 = H$
38, $R_1 = Br$, $R_2 = H$
39, $R_1 = H$, $R_2 = Br$

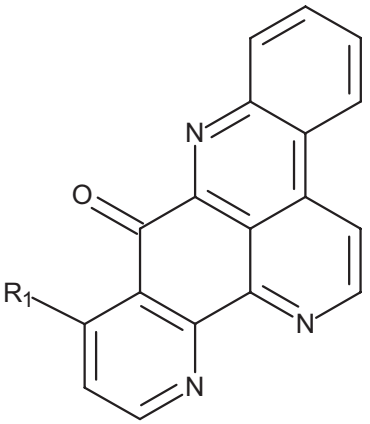
Fig. 36.5 Structures of amphimedine (**27**), 2-bromoamphimedine (**28**), neoamphimedine (**29**), 5-methoxyamphimedine (**30**), deoxyamphimedine (**31**), 1-hydroxyamphimedine (**32**), 3-hydroxy-deoxyamphimedine (**33**), demethyldeoxyamphimedine (**34**), neoamphimedine Y (**35**), neoamphi-medine Z (**36**), debromopetrosamine (**37**), petrosamine (**38**) and petrosamine B (**39**)

Another interesting pentacyclic pyridoacridine alkaloid is petrosamine (**38**) (Fig. 36.5). This compound was first isolated from the methanol extract of the marine sponge *Petrosia* sp., collected in Belize [26]. In a crystalline form, petrosamine (**38**) exists as a diketone with the carbonyl groups on C-5 and C-8. However, in solution, the C-5 carbonyl exists in an *enol* form.

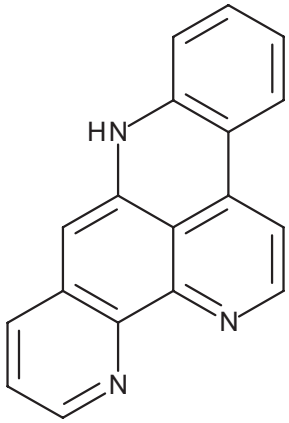
In a search for compounds with anticholinesterase activity, Nukoolkarn et al. [27] reported isolation of petrosamine (**38**) and 2-bromoamphimedine (**28**), from the marine sponge *Petrosia* n. sp., collected from the intertidal zone of Phuket Island, Thailand. They have found that only petrosamine (**38**) exhibited strong anti-acetylcholinesterase activity approximately six times higher than that of the reference galantamine A. Computational docking study of petrosamine (**38**) with the enzyme from the electric eel *Torpedo californica* (TcAChE) revealed that the major contribution to the petrosamine-TcAChE interaction was due to the quaternary ammonium group of petrosamine (**38**). Using the *Helicobacter pylori* enzyme aspartyl semialdehyde dehydrogenase (ASD) to detect antibacterial activity for bioassay-guided fractionation, Carroll et al. [28] reported isolation of petrosamine B (**39**) from the methanol extract of the marine sponge *Oceanapia* sp., collected from North Queensland, Australia. Petrosamine B (**39**) is isomeric with petrosamine (**38**); the only difference between the two compounds is the position of the bromine atom. Petrosamine B (**39**) was found to be weak inhibitor of ASD with an IC_{50} of 306 μM.

Ascididemin (**40**) (Fig. 36.6), a member of the pentacyclic pyridoacridines with a pyridine ring fused with C-8 and C-9 of ring C of the iminoquinone moiety, was reported as a potent antileukemic pentacyclic aromatic alkaloid from the Okinawan tunicate *Didemnum* sp. [29]. It was later isolated from various sources such as the Mediterranean ascidian *Cystodytes dellechiajei* [30], a previously undescribed red *Didemnum* sp. and *Didemnum rubeum*, collected from Indonesia [31], an unidentified Singaporean ascidian [32], and together with 12-deoxyascididemin (**42**) (Fig. 36.6) from the Australian ascidian *Polysyncraton echinatum* [33]. In contrast to ascididemin (**40**), meridine (**43**) (Fig. 36.6) possesses a pyridine ring fused with C-9 and C-10 of ring C of the iminoqunone moiety. Meridine (**43**) was isolated, together with its relatively stable tautomer (**44**) and 11-hydroxyascididemin (**41**) (Fig. 36.6), from the ascidian *Amphicarpa meridiana*, collected from South Australia [34], and later from the marine sponge *Ecionemia geodides*, also collected from Australia [35]. It is interesting to mention also that 11-hydroxyascididemin (**41)** was also isolated from the Mediterranean ascidian *Cystodytes dellechiajei*, however its structure was wrongly assigned as cystodamine [36, 37].

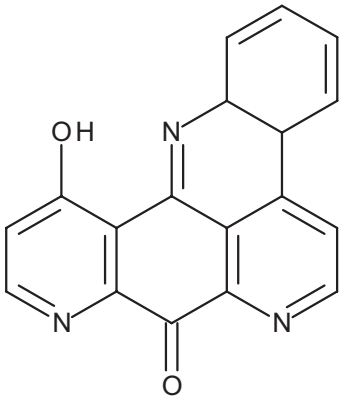
Shermilamines are pentacyclic pyridoacridines whose ring C of the 11H-pyrido[4, 3, 2*mn*]acridine skeleton is fused with the 3-thiomorpholinone ring through C-8 and C-9. While different analogues of aminoethyl substituents can be found at C-10, C-2 can have bromine substituent. The group of Sheuer has first reported isolation of shermilamines A (**45**) and B (**46**) (Fig. 36.7) from a purple colonial tunicate *Trididemnum* sp., collected in Guam [38, 39]. Shermilamine B (**46**) was later isolated from several sources, including an unidentified purple colonial tunicate and its prosobranch mollusk predator *Chelynotus semperi*, also by Sheuer's group [7], and together with *N*-deacetylshermilamine B (**50**) (Fig. 36.7), from the Mediterranean



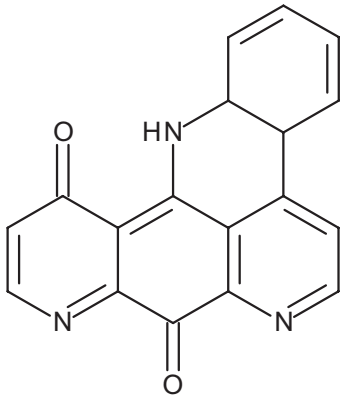
40, R = H
41, R = OH



42



43



44

Fig. 36.6 Structures of ascididemin (**40**), 11-hydroxyascididemin (**41**), 12-deoxyascididemin (**42**), meridine (**43**) and meridine tautomer (**44**)

purple morph of the ascidian *Cystodytes dellechiajei* [17, 40], as well as from the Fijian *Cystodytes* sp. ascidian, together with shermilamine C (**47**) (Fig. 36.7) [10]. Koren- Goldshlager et al. [41] reported isolation of shermilamines D (**48**) and E (**49**) (Fig. 36.7) from the Indian Ocean tunicate *Cystodytes violatinctus*, collected at the Mayotte Lagoon, Comoros Islands, northwest of Madagascar.

The kuanoniamines are members of the pentacyclic pyridoacridine alkaloids whose ring C is fused with a 1,3-thiazole ring through C-8 and C-9. The name kuanoniamine is derived from the Hawaiian word "*kuanoni*" meaning change when

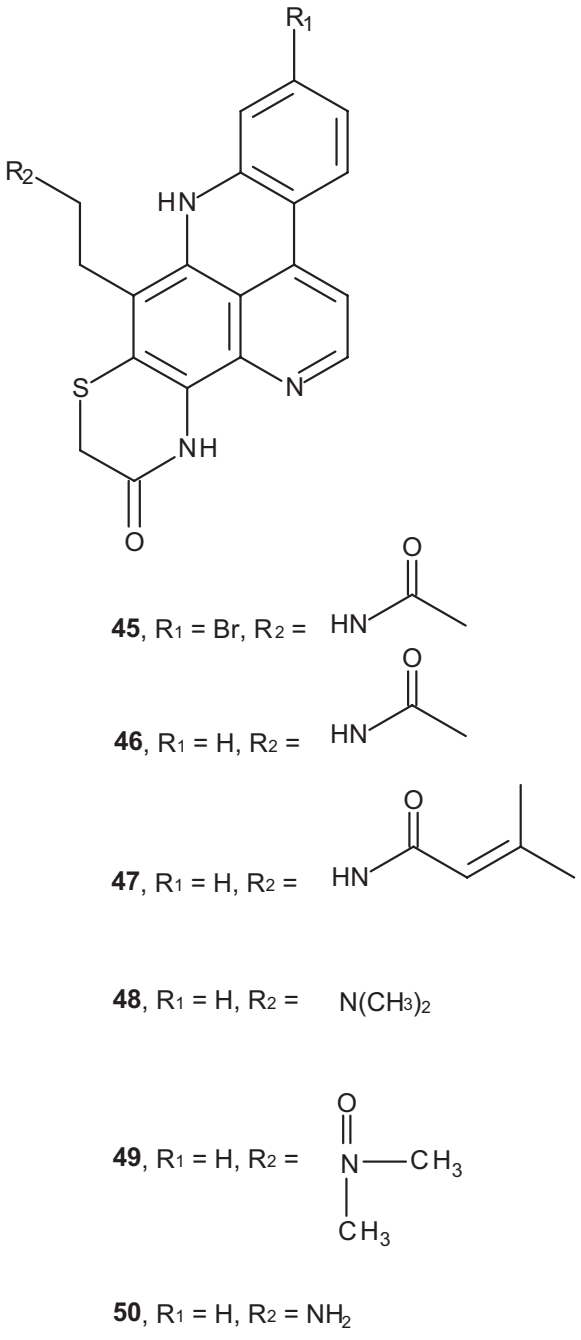


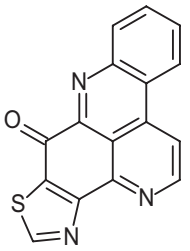
Fig. 36.7 Structures of shermilamines A–E (**45–49**) and *N*-deacetylshermilamine (**50**)

pertaining to color since the colors of these compounds are extremely sensitive to changes in pH. They are yellow in neutral and basic solution while in acidic solution they change to brilliant purple [7]. Kuanoniamines have been isolated from both marine sponges, mostly from the genus *Oceanapia*, and ascidians. Carrol and Sheuer [7] first described isolation of kuanoniamines A–D (**51–54**) (Fig. 36.8) from both the lamellariid mollusk *Chelynotus semperi* and an unidentified purple colonial tunicate, collected from Mante Channel, Pohnpei. Kuanoniamines C (**53**) and D (**54**) were later isolated from the purple tunicate, tentatively identified as *Cystodytes* from Pohnpei, by Gunawardana et al. [42]. McDonald et al. [10] isolated, among other pyridoacridine alkaloids, kuanonamine D (**54**) and dehydrokuanoniamine B (**55**) (Fig. 36.8) from a Fijian *Cystodytes* sp. ascidian. Eder et al. [43] described isolation of kuanoniamine C (**53**) and *N*-deacetylkuanoniamine D (**56**) (Fig. 36.8) from the marine sponge *Oceanapia* sp., collected from Truk Lagoon, Micronesia. The new kuanoniamines, kuanoniamines E (**57**) and F (**58**) (Fig. 36.8) were isolated, together with kuanoniamines A (**51**), C (**53**) and D (**54**), from an ascidian collected in Singapore [32]. During a search for bioactive compounds from marine sponges from the Gulf of Thailand, Kijjoa et al. [44] also isolated kuanoniamines A (**51**) and C (**53**), from the marine sponge *Oceanapia sagittaria*. Kuanoniamine D (**54**) and *N*-deacetylkuanoniamine D (**56**) were also isolated from the purple morph of Mediterranean ascidian *Cystodytes dellechiajei* [17, 40].

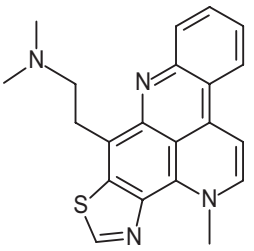
A structurally related pentacyclic pyridoacridine dercitin (**61**) (Fig. 36.8) was isolated from the deep-water marine sponge *Dercitus* sp., collected from Bahamas by Gunawardana et al. [45]. The same group also reported isolation of nordercitin (**59**), dercitamine (**60**) (Fig. 36.8) and dercitamide from the marine sponges *Dercitus* sp. and *Stelletta* sp. [46]. Later on, the same group has revised the structure of dercitamide to kunoniamine C (**53**) [42]. Lissoclinidine (**62**) (Fig. 36.8), a pentacyclic alkaloid containing a 1,3-oxathiolane ring fused with C-8 and C-9 of ring C, was first isolated from the ascidian *Lissoclinum notti*, collected in New Zealand [11] while its *N*14-deacetyl derivative, lissoclinidine B (**63**) (Fig. 36.8) was isolated together with diplamine B (**19**) and isolissoclinotoxin B, from *Lissoclinum* cf. *badium*, collected of the coast of Papua New Guinea [15]. Solomon and Faulkner [47] also reported isolation of dercitamide (kuanoniamine C), as a major metabolite, and another structurally related sagitol (**64**) (Fig. 36.8) as a minor metabolite, from the marine sponge *Oceanapia sagittaria* in Palau.

Sebastianine A (**65**) (Fig. 36.9), a pentacyclic alkaloid comprising of a pyrole ring fused with C-9 and C-10 of the ring C of a pyridoacridine system, was isolated from the methanol extract of the deep blue morphs of the ascidian *Cystodytes dellechiajei*, collected near São Sebastião Island, Brazil [48]. Another pyrole containing pentacyclic pyridoacridines are arnoamines A (**66**) and B (**67**) (Fig. 36.9), however in these compounds the pyrole ring is fused with C-10 of ring C and N-11 of ring B. Arnoamines A (**66**) and B (**67**) were also reported from the ascidian *Cystodytes* sp, collected in the vicinity of Arno Atoll, Republic of Marshall Islands [49].

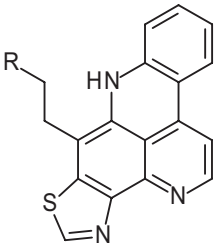
Besides the iminoquinone moiety, 1,4-diimine moiety can be found in the pyridoacridines, although they are rare for the pyridoacridine structure class. Examples are ecionines A (**68**) and B (**69**) (Fig. 36.9), which were isolated from the marine sponge *Ecionemia geodides*, collected from Tasmania, Australia [35].

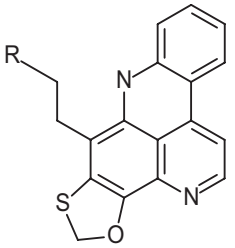


51

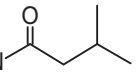


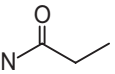
61



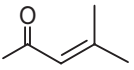


62, R = NHCOMe
63, R = NH_2

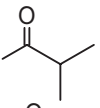
52, R = 

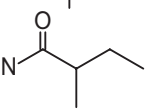
53, R = 

54, R = 

55, R = 

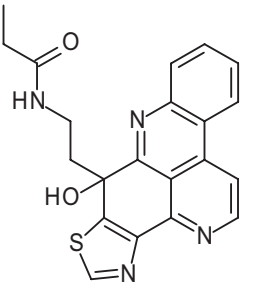
56, R = NH_3

57, R = 

58, R = 

59, R = $N(CH_3)_2$

60, R = $NHCH_3$



64

Fig. 36.8 Structures of kuanoniamines A–D (**51–54**), dehydrokuanoniamine B (**55**), *N*-deacetylkuanoniamine D (**56**), kuanoniamines E and F (**57**, **58**), nordercitin (**59**), dercitamine (**60**), dercitin (**61**), lissoclinidine (**62**), lissoclinidine B (**63**) and sagitol (**64**)

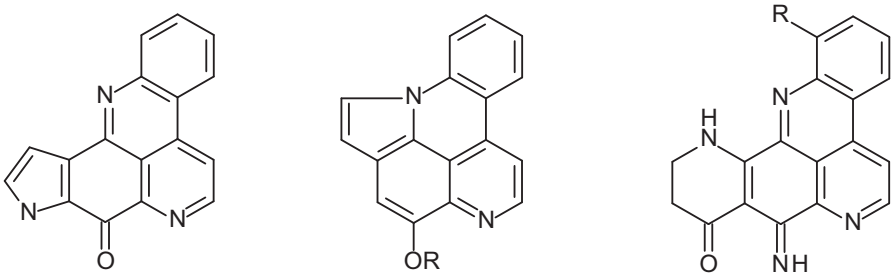


Fig. 36.9 Structures of sebastianine A (**65**), arnoamines A (**66**) and B (**67**), ecionines A (**68**) and B (**69**)

36.2.3 Hexacyclic Alkaloids

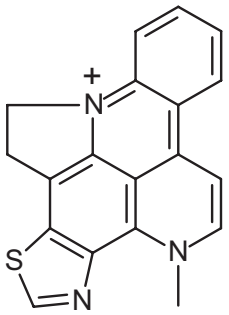
So far, only few hexacyclic pyridoacridine alkaloids have been isolated from marine sources. These alkaloids can be considered as derived from cyclization of the side chain on C-8 or C-10 of the pentacyclic pyridoacridine derivatives. Thus, while cyclodercitin (**70**) (Fig. 36.10) was isolated, together with nordercitin (**59**), dercitamine (**60**) and kuanoniamine C (**53**), from the marine sponges *Dercitus* sp. and *Stelletta* sp. [46], a structurally related stellettamine (**71**) (Fig. 36.10) was isolated from a *Stelletta* sp. [42].

Similarly, cycloshermilamine D (**72**) (Fig. 36.10) and shermilamine D (**48**) were isolated from the same tunicate (*Cystodytes violatinctus*) [50]. On the other hand,13-didemethylaminocycloshermilamine D (**73**) (Fig. 36.10) was isolated, together with shermilamine B (**46**) and *N*-deacetylshermilamine B (**50**), from the purple morph of the Mediterranean ascidian *Cystodytes dellechiajei* [17]. In the same way, sebastiamine B (**74**) (Fig. 36.10) was isolated together with sebastiamine A (**65**) from the deep blue morphs of the ascidian *Cystodytes dellechiajei*, collected near São Sebastião Island, Brazil [48].

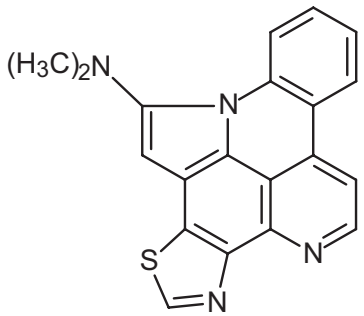
A group of hexacyclic pyridoacridine alkaloids possessing the benzo 1,6-phenanthroline ring system are the segolines. Segoline A (**75**), isosegoline A (**76**) and segoline B (**77**) (Fig. 36.11) were isolated, together with norsegoline (**26**), from the marine tunicate *Eudistoma* sp. [19, 20]. Later on, Viracaoundin et al. [51] reported isolation of segoline A (**75**) and segoline C (**78**) (Fig. 36.11) from the purple tunicate *Eudistoma bituminis*, collected from the Mayotte lagoon, in the Comoros Islands, northwest of Madagascar.

36.2.4 Heptacyclic Alkaloids

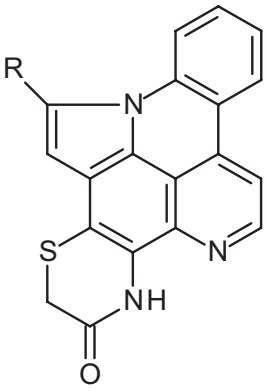
Eilatin (**79**) (Fig. 36.12) is the only known marine heptacyclic pyridoacridine alkaloid. It was first isolated from the tunicate *Eudestoma* sp. by Rudi et al. [20]. Later



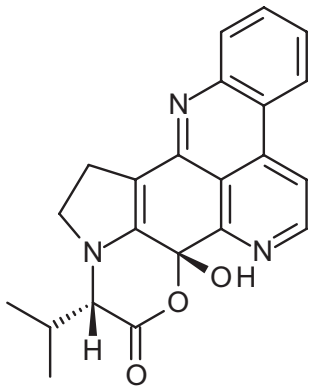
70



71



72, R = N(CH$_3$)$_2$
73, R = H



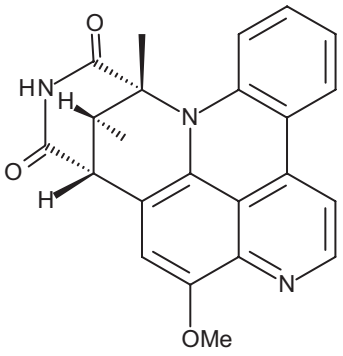
74

Fig. 36.10 Structures of cyclodercitin (**70**), stellettamine (**71**), cycloshermilamine D (**72**), 13-dedimethylaminocycloshermilamine D (**73**) and sebastianine B (**74**)

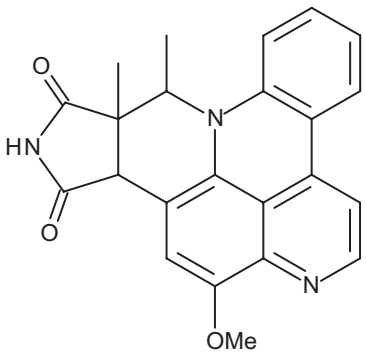
on, the same group described isolation of eilatin (**79**), together with norsegoline (**28**), segoline A (**75**), isosegoline A (**76**), segoline B (**77**), and debromoshermilamine A, from the same organism [19]. Eilatin (**79**) was also isolated from a Fijian *Cystodytes* sp. ascidian [10] and the Australian ascidian *Polysyncraton echinatum* [33].

36.2.5 Octacyclic Alkaloids

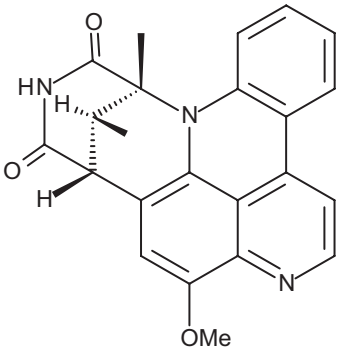
Up to date, eudistones A (**80**) and B (**81**) (Fig. 36.12) are the only described marine-derived octacyclic pyridoacridine alkaloids. They were both isolated, together with ascididemin (**40**), from the Seychelles tunicate *Eudistoma* sp. [52].



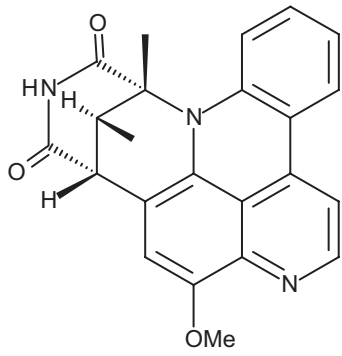
75



76



77

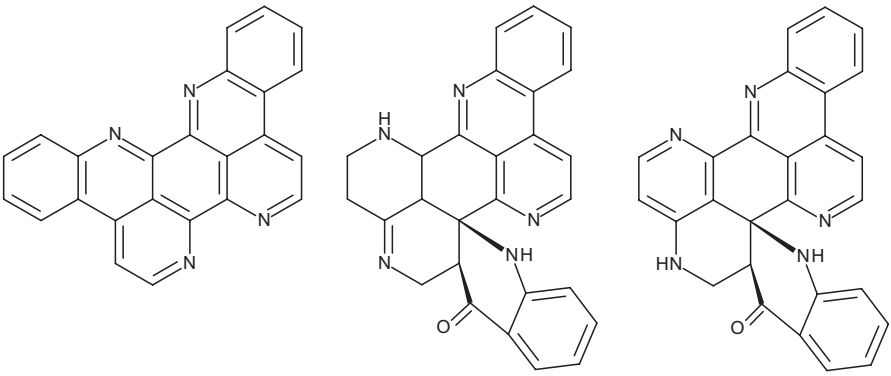


78

Fig. 36.11 Structures of segoline A (**75**), isosegoline A (**76**), segolines B (**77**) and C (**78**)

36.3 Anticancer Activities of Pyridoacridine Alkaloids

As a class, the pyridoacridines are considered DNA binding molecules and have been characterized mainly on the basis of their cytotoxicity. Consequently, their cytotoxicity is often attributed to their ability to intercalate DNA and thereby interact with, or inhibit, DNA metabolizing enzymes. Some pyridoacridines which are cytotoxic to cultured mammalian tumor cell lines have demonstrated excellent antitumor activity in various models, while others have proven too toxic to be useful. Recent work has demonstrated that the individual pyridoacridines can vary dramatically in their molecular in cell killing [3]. Although some excellent reviews on antitumor activity of naturally occurring pyridoacridine alkaloids from marine sources and their synthetic analogues have been previously published [3, 4, 6], there is still



79 80 81

Fig. 36.12 Structures of eilatin (**79**), eudistones A (**80**) and B (**81**)

no review that covers recent developments of the studies of mechanisms of the antitumor activity of particular groups of these marine-derived alkaloids. The fact that almost all of the pyridoacridine alkaloids, isolated from the marine sources, have been tested for their *in vitro* cytotoxicity on human tumor cell lines but only a few of them have been further investigated for their mechanism of action, has prompted us to focus on relevant groups of these alkaloids whose mechanisms of cytotoxicity have been thoroughly investigated.

36.3.1 Mechanism for Apparent Intercalator-Induced Inhibition of Topoisomerase II by Pyridoacridine Alkaloids from a Cystodytes sp. Ascidian

One of the early works on the mechanisms of cytotoxicity of the marine pyridoacridine alkaloids was carried out by the group of Chris Ireland. McDonald et al. [10] have evaluated the *in vitro* cytotoxic activity of cystodytin A (**3**), cystodytin J (**12**), diplamine (**17**), shermilamine B (**46**), shermilamine C (**47**), kuanoniamine D (**54**), dehydrokuanoniamine D (**55**) and eilatin (**79**), against the human colon tumor cell line HCT-116. The result showed that all compounds were active to varying degree. Diplamine (**17**, $IC_{50} < 1.4$ μM) and cystodytin J (**12**, $IC_{50} = 1.6$ μM) were the most potent; both inhibited HCT-116 replication with IC_{50} values less than 2 μM, while shermilamine B (**46**, $IC_{50} = 13.8$ μM) and shermilamine C (**47**, $IC_{50} = 16.3$ μM) were the least active. DNA intercalation studies using an ethidium bromide displacement assay showed that diplamine (**17**, $K_{disp} = 21$ μM) and cystodytin J (**12**, $K_{disp} = 51$ μM) were the most efficient intercalators. Interestingly, shermilamine C (**47**), the least cytotoxic pyridoacridine, was the poorest intercalator ($K_{disp} > 100$ μM) followed shermilamine B (**46**), which was the second to last in term of cytotoxicity and intercalative ability ($K_{disp} > 100$ μM). These pyridoacridines were also evaluated for

their capacity to inhibit topoisomerase II catalytic activity. All compounds were found to be capable of inhibiting decatenation. Like the classic cleavable complex-stabilizing etoposide, diplamine (**17**) was able to effect dose-dependent inhibition of topoisomerase II-catalyzed kinetoplast DNA (kDNA) decatenation *in vitro*. Interestingly, diplamine (**17**, IC_{90} = 9.2 μM) was found to be more potent than etoposide (IC_{90} = 68 μM). Cystodytin J (**12**) and diplamine (**17**) were found to be the most potent (IC_{90} = 8.4 and 9.2 μM, respectively) inhibitors while shermilamine C (**47**) and shermilamine B (**46**) were among the least potent (IC_{90} = 138 μM and 118 μM, respectively). Furthermore, these compounds were also tested for their ability to exert enhanced toxicity toward the double strand break repair-deficient xrs-6 Chinese hamster ovary (CHO) mutant cell line relative to the repair-proficient BR1 cell line, and none of them showed significant BR1/xrs-6 differential toxicity. Since enhanced toxicity toward the DNA double strand break repair-deficient CHO xrs-6 cell line *versus* the repair-competent BR1 line indicates "cleavable complex" mediated toxicity, they suggested that no cleavable complex formation had occurred and that these compounds did not cause double strand breaks in DNA. On the other hand, cystodytin J (**12**) and diplamine (**17**) were found to cause dramatic effects on RNA and DNA synthesis. However, no effect was observed on protein synthesis during the 9 h exposure period. On the basis of the observed correlations between HCT-116 cytotoxicity, topoisomerase II inhibition, and DNA intercalation, they hypothesized that these pyridoacridines brought about cell death by inhibiting DNA interactive protein (e.g., topoisomerase II) following intercalation and as a result of intercalation, the pyridoacridines inhibited proliferation of HCT-116 cells by interfering with nucleic acid structure and function. This interference was brought about partly by disruption of the function of topoisomerase II. They also suggested that by intercalating into DNA, the pyridoacridine interrupted the interaction between topoisomerase II and DNA, and consequently, the enzyme could not carry out its normal function during replication. The fact that these compounds showed neither enhanced toxicity toward DNA double strand break repair-deficient CHO cell lines nor produced cleavable complexes led the authors to suggest that these alkaloids inhibit topoisomerase II catalytic activity not by producing a cleavable complex but by interacting with DNA itself. They argued that since the pyridoacridines intercalated into DNA with high affinity and may change the topology of the molecule, it is likely that they inhibit other DNA binding enzymes necessary for replication. Therefore, other enzymes such as polymerases or topoisomerase I may be unable to bind or function properly due to the presence of intercalator molecules in the DNA. Based on the relative cytotoxic, intercalative, and topoisomerase II inhibitory activities of the pyridoacridines tested, the authors suggested the structural characteristics for these activities. The tetracyclic alkaloids cystodytin J (**12**) and diplamine (**17**), which possessed the iminoquinone portion, were found to be the best intercalators and topoisomerase II inhibitors and also the most potent growth inhibitors of the series. They suggested also that the diminished potencies of the other pyridoacridines may result, in part, from steric effects of the additional ring (s), although electronic effect of the iminoquinone may also be important since the pentacyclic alkaloids shermilamine B (**46**) and shermilamine C (**47**) which lack the

iminoquinone moiety, were among the least active in all the assays. The authors also proposed that the heptacyclic alkaloid eilatin (**79**) may cleave DNA by producing hydroxyl radical, which can damage DNA, following intercalative binding with DNA. Structurally, eilatin (**79**), a phenantroline possessing a double nitrogen bay region to chelate metal, is able to facilitate redox cycling to generate ROS. Other pyridoacridines may have less hydroxyl radical-generating ability due in part to a diminished ability to chelate metal ions and are less able to nick DNA in a manner similar to eilatin (**79**).

36.3.2 Influence of N-14 Substitution on the Observed Antitumor Activity of Cystodytins and Styelsamines

Although most of naturally occurring cystodytins and styelsamines have been evaluated for their cytotoxicity, exhibiting a range of potency (IC_{50} = 0.12–2.9 μM), only the DNA binding ability of cytodytin J (**12**) has been reported [10]. The fact that the antitumor activity of the pyridoacridine alkaloids is often attributed to DNA binding, Marshall and others have noted that such correlation is compound specific [3]. Consequently, Fong and Copp [53] have explored the influence of N-14 substitution on the antitumor activity of the cystodytin and styelsamine alkaloids by preparing library of natural and non-natural analogues to evaluate their DNA affinity, by ethidium bromide displacement assay, and cytotoxicity towards a panel of human tumor cell lines. The ethidium bromide displacement assay revealed that styelsamines B (**21**) and D (**23**) exhibited the highest affinity for calf thymus (CT) DNA within the styelsamine compound library (Fig. 36.13), with the apparent binding constant (K_{app}) 5.33×10^6 and 3.64×10^6 M^{-1}, respectively, while other styelsamine analogues showed mild to low affinity for CT-DNA. These results prompted them to suggest that various sidechains could hinder DNA binding. For the cystodytin library (Fig. 36.13), they have found that cystodytins A (**3**) and J (**12**) exhibited slightly higher apparent binding constants than their non-natural analogues.

Using the preliminary one dose (10 μM) testing against 57 human tumor cell lines, they have found that, of the styelsamine analogues, N^{14}-3-phenylpropanamide (**84**) (Fig. 36.13) was the most active, having the 10 μM dose resulting in mean cell killing (negative growth), and the remaining analogues were considered either mildly active, or inactive in the case of palmitamide (**85**) (Fig. 36.13). Of the four cystodytin analogues tested (**3**, **12**, **86**, **87**), cystodytin J (**12**) was considered to be inactive, while the 3-phenylpropanamide (**87**) (Fig. 36.13) was observed to be the most active. Interestingly, sub-panel selectivity was observed for both natural and some synthetic analogues of styelsamine. Thus, styelsamine B (**21**) was found to be more selective towards melanoma, non-small cell lung cancer and ovarian panels; styelsamine D (**23**) towards non-small cell lung cancer, CNS and renal; styelsamine-N^{14}-3-methylbut-2-enamide (**82**) towards leukemia and renal; styelsamine-N^{14}-benzamide (**83**) towards melanoma and renal, and styelsamine-N^{14}-3-palmitamide (**85**) towards colon and renal cancer cell lines. In contrast, cytodytins

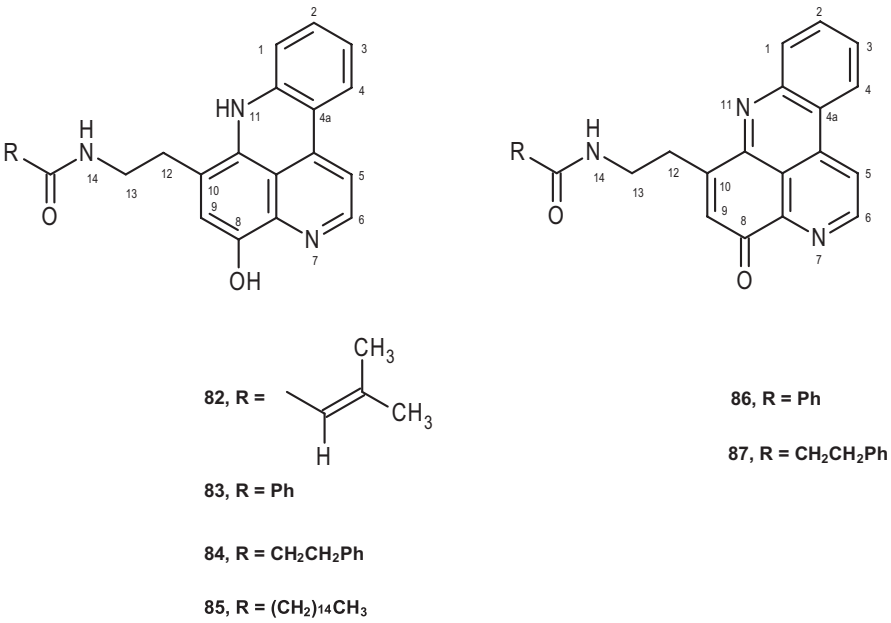


Fig. 36.13 Structures of styelsamine-N^{14}-3-methylbut-2-enamide (**82**), styelsamine-N^{14}-benzamide (**83**), styelsamine N^{14}-3-phenylpropanamide (**84**), styelsamine-N^{14}-3-palmitamide (**85**), cystodytin-N^{14}-benzamide (**86**), cystodytin-N^{14}-phenylpropanamide (**87**)

A (**3**), J (**12**) and cystodytin-N^{14}-benzamide (**86**) were essentially non-selective, while cystodytin-N^{14}-phenylpropanamide (**87**) exhibited selectivity towards colon, melanoma and renal cancer cell line sub-panels. They have found also that, in general, most compounds exhibited poor cytotoxicity, unable to reach LC_{50} (50% lethality) or TGI (total growth inhibition, cytostatic) levels of activity. Interestingly, they also found that there was no correlation between DNA affinity (K_{pp} values) and cytotoxicity (GI_{50} values) for these compounds since styelsamines B (**21**), D (**23**) and styelsamine-N^{14}-3-methylbut-2-enamide (**82**), all exhibited almost the same level of growth inhibition (GI_{50} = 3.2–4.0 μM), whereas styelsamines B (**21**) and D (**23**) bound approximately ten times more strongly to DNA than styelsamine-N^{14}-3-methylbut-2-enamide (**82**). Similarly, styelsamine-N^{14}-3-palmitamide (**85**) and cystodytin-N^{14}-phenylpropanamide (**87**), which exhibited approximately the same level of DNA affinity, showed different levels of antiproliferative activity (**85** was inactive; GI_{50} of **87** was 0.32 μM). More importantly, they have suggested that lipophilicity was an important determinant of cell based antiproliferative activity since cell penetration is clearly a requisite condition for molecules that exert biological activity by targeting DNA. By plotting one dose mean cell growth inhibition activities against clog P, they have verified that, for styelsamine and cytodytin series, the optimal activity occurred with alkaloid having clog P around 4.0 to 4.5. Consequently, they concluded that sidechain modified analogues of styelsamines and/or cystodytins may have potential as new classes of antitumor agents.

36.3.3 Mechanism of Antitumor Activity of Ascididemin

Among the pyridoacridine alkaloids isolated from marine sources, ascididemin (**40**) was the most extensively investigated for the mechanism of its antitumor activity. It was found to be highly toxic to human colon (HCT-116) and breast (MCF-7) cancer cell lines [54] as well as to different leukemic cell lines [30]. Most importantly, ascididemin (**40**) was equally toxic to drug-sensitive and multidrug-resistant cell lines. Ascididemin (**40**) was also found to be able to intercalate DNA, preferentially at GC-rich sequences [30]. Dassonneville et al. [55] have investigated the effect of ascididemin (**40**) on the activity of topoisomerases and have found that, in the presence of ascididemin (**40**), a band corresponding to linear DNA could be clearly seen proving that ascididemin (**40**), like etoposide, inhibited the relegation of DNA once the double helix had been cleaved by the enzyme; however, the intensity was much weaker than that caused by etoposide. These data suggested that ascididemin (**40**) is a weak inhibitor of topoisomerase enzymes. By using the radiolabelled DNA substrate, they have found that ascididemin (**40**) effectively functioned as a topoisomerase II poisoning, stabilizing DNA-topoisomerase II covalent complex. Furthermore, ascididemin (**40**) was proved to be less toxic to P338 (sensitive to camptothecin) cells than to P388CPT5 (resistant to camptothecin) cells, suggesting that topoisomerase I was not a cellular target for ascididemin (**40**). Moreover, ascididemin (**40**) was found to be equally toxic to both human leukemia sensitive (HL-60) and resistant (HL-60/MX2) to mitoxantrone cell lines, and since HL-60/MX2 cells display atypical multidrug resistance with the absence of P-glycoprotein overexpression and altered topoisomerase II catalytic activity and reduced levels of topoisomerase IIα and IIβ, they suggested that topoisomerase II did not contribute to the cytotoxic action of ascididemin (**40**). This finding was corroborated by the immunoblot assay which showed the absence of DNA-topoisomerase II covalent complexes in HL-60 leukemia cells, in the presence of ascididemin (**40**). They have also investigated the effects of ascididemin (**40**) on the cell cycle of HL-60 leukemia cells and have found that the increase of the sub-G1 population (apoptotic cells) was up to 70% in the ascididemin-treated HL-60 leukemia cells whereas it represented only 3% in the cell in the control. Interestingly, the increase in the sub-G1 population was concomitant with the decrease of the G1 population (from 45 to 10%). Additionally, they have shown also that ascididemin (**40**) was able to cleave PARP (poly(ADP-ribose) polymerase), an enzyme involved in DNA repair by caspase-3. Thus, they concluded that the induction of apoptosis is associated with an activation of caspase-3 by ascididemin (**40**).

In order to clarify the conflicting data about the mechanism of antitumor activity of ascididemin (**40**), Matsumoto et al. [56] attempted to investigate the mechanism responsible for DNA damage of ascididemin (**40**), together with two structurally related synthetic alkaloids **88** and **89** (Fig. 36.14). It is interesting to note that these compounds share two common structural motifs, i.e., a double nitrogen bay region and a reducible, iminoquinone heterocyclic ring.

The results showed that ascididemin (**40**), **88** and **89** significantly inhibited topoisomerase II catalytic activity and generated some minor topoisomerase II dependent

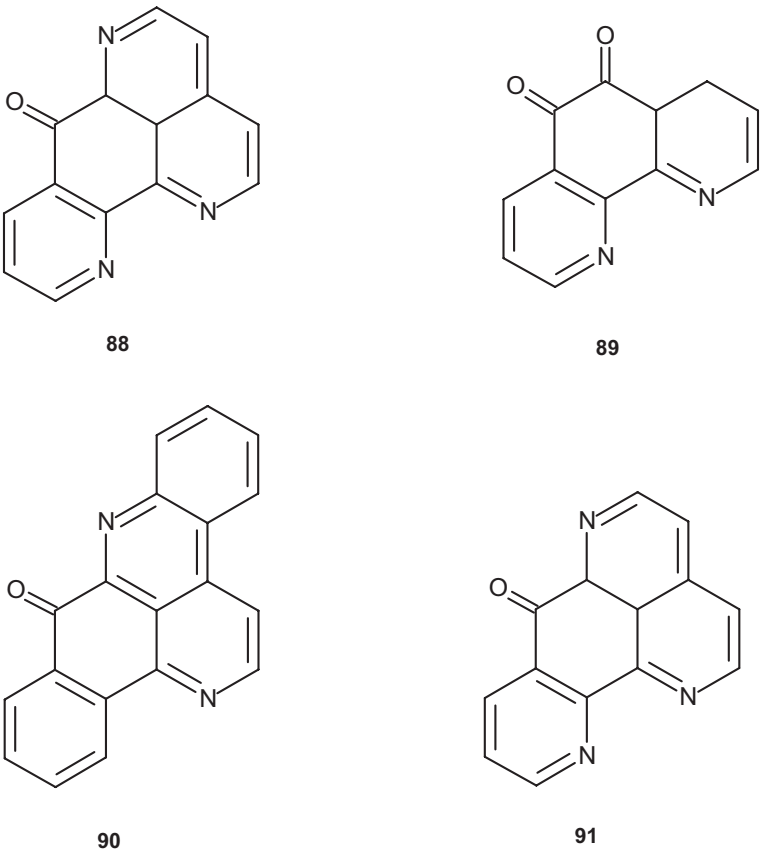


Fig. 36.14 Structures of DNA cleaving (**88**, **89**) and DNA non-cleaving (**90**, **91**) synthetic alkaloids

DNA cleavage, while no cleavage was found in the presence of **90** and **91**. However, it was shown that only 5 mM dithiothreitol (DDT) and 91 μM of ascididemin (**40**), **88** or **89** in H_2O was required to stimulate maximal DNA cleavage, independent of topoisomerases. Moreover, they have observed that DNA cleavage by these compounds was both concentration and time dependent. Assessment of DNA cleavage under anoxic conditions revealed that oxygen was a necessary component for *in vitro* DNA cleavage stimulation by these compounds. Consequently, they hypothesized that the DNA cleavage was caused by reactive oxygen species (ROS), which was substantiated by the electron paramagnetic resonance (EPR) study. Additionally, they have found also that addition of metal ions ($FeSO_4$ or NiCl) did not facilitate DNA cleavage by these compounds, and addition of chelators did not protect against the cleavage, thus excluding Fenton-type reaction as the primary ROS generation. The fact that the anti-oxidative enzyme catalase was able to extensively protect against DNA cleavage by ascididemin (**40**), **88** or **89**, it was suggested that H_2O_2 could be the possible reactive species initially generated. Thus, it was

proposed that production of ROS likely occurs through reduction of the iminoquinone moiety. This hypothesis was supported by the experiments that used cyclic voltammetry to measure reductive potential of these compounds, which showed that the three cleaving analogues, i.e., ascididemin (**40**), **88** and **89**, were marginally more easily reduced than the two non-cleaving compounds (**90** and **91**). As the reductive potentials of ascididemin (**40**), **88** and **89** are comparable to that of NADPH (−280 mV), it is consistent with bioreduction in the cell. They have also found that the capacity of these compounds did not correlate with the DNA cleavage activity since ascididemin (**40**) and **91** were found to completely displace ethidium bromide at 10 and 20 μM concentrations, whereas **88**, **89** and **90** did not at 20 μM concentrations. Consequently, the authors proposed that the ability of ascididemin (**40**) and its cleaving analogues (**88**, **89**) to interact closely with DNA increases the likelihood that ROS formed as a result of their reduction would directly affect DNA. Although the analogues **90** and **91** can intercalate DNA, they cannot produce ROS, thus fail to cleave the DNA. The ROS-mediated mechanism was also corroborated by the fact that antioxidants and antioxidant enzymes were able to protect DNA against ascididemin-induced DNA cleavage. Mechanism-based cytotoxicity screening revealed the capacity of ascididemin (**40**), **88** and **89** to predominantly cause single strand DNA breaks since the mutant of Chinese hamster ovary (CHO) cell line EM9 was particularly sensitive to the lethality of these molecules as compared to the wild-type AA8 cell line. Since the EM9 cell has a defective XRCC1 protein, which plays a critical role in DNA ligase III activity, DNA polymerization and repaired of DNA single strand breaks, this deficiency makes it hypersensitive to compounds that cause ROS, or single strand breaks *via* topoisomerase I. Finally, they have found also that glutathione not only afforded strong protection of DNA cleavage induced by ascididemin (**40**), **88** and **89** but also from the cytotoxicity of these compounds to AA8 cells. Interestingly, they have shown that heme oygenase-1 (OH-1), an oxidative stress protein, was induced in the AA8 cells by ascididemin (**40**), **88** and **89,** thus linking cellular oxidative stress with cell damage by these compounds.

Following the work of Dassonneville et al. [55] which demonstrated that ascididemin (**40**) was a potent inducer of apoptosis in the HL-60 leukemia cells, probably associated with caspase-3 activation, Dirsch et al. [57] have attempted to investigate the molecular pathway utilized by this pentacyclic pyridoacridine alkaloid to trigger cell death. They have found that treating the leukemia Jurkat T cells with ascididemin (**40**) produced all signs of apoptosis, including in cell shrinkage, membrane blebbing, chromatin condensation and fragmented nuclei. They have also shown that the ascididemin-triggered apoptosis was concentration and time-dependent. That this apoptosis did not involve protein synthesis was substantiated by their finding that pre-incubation of Jurkat cells with cycloheximide had no influence on ascididemin-mediated cell death. Also, the anti-CD95 antibody ZB4 was found to be unable to reduce ascididemin-triggered apoptosis, thus ruling out the involvement of CD5 death receptor. Additionally, ascididemin (**40**) was found to induce outer (cytochrome *c* release) and inner (loss of electrochemical gradient) mitochondrial membrane permeabilization; and the release of cytochrome *c* was found to be kinetically parallel a decrease of mitochondrial potential. These results led to the

conclusion that ascididemin (**40**) was able to induce mitochondrial dysfuntion. By treating Jurkat cells overexpressing mitochondria-protecting Bcl-2 or Bcl-x_L with 5 μM ascididemin (**40**), it was found that these cells were protected against ascididemin (**40**) compared to control. The results suggested that ascididemin (**40**) induces a mitochondria-dependent pathway of apoptosis. They also compared the kinetics by which the initiator caspase-2, -9 and -8 and the effector caspase-3 are activated by ascididemin (**40**). It was found that the activation of caspase-8 correlated well with activation of the effector caspase-3, which corroborated the findings that ascididemin-triggered apoptosis occurs independent of death receptor. Interestingly, caspase-2 processing was found to be more pronounced at 4 h after treatment with ascididemin (**40**) than caspase-9, and pre-treatment of Jurkat T cells with the specific caspase-2 inhibitor zVDVADfmk was found to completely prevent ascididemin-mediated apoptosis. Thus, these data suggested an initial and relevant role for caspase-2 in ascididemin-triggered apoptosis. As caspase-2 was found to process in Jurkat/*bcl-*X_L cells incubated with ascididemin (**40**), it was concluded that caspase-2 activation occurs upstream of mitochondria events. On the other hand, caspase-8 was found to process only in Jurkat/*neo* and not in Jurkat/*bcl-*X_L cells, confirming that caspase-8 acts as effector caspase downstream of mitochondria. Moreover, they have shown that pre-treatment with the caspase-2 inhibitor zVDVADfmk resulted in strong reduction of the cleavage of caspase-9, corroborating that caspase-2 acts upstream of caspase-9. Moreover, ascididemin (**40**) was also found to induce Bid cleavage, and this cleavage is inhibited by the caspase-2 inhibitor zVDVADfmk, suggesting that caspase-2 activation lies upstream of ascididemin-induced mitochondria activation and that Bid cleavage by caspase-2 may contribute to mitochondrial perturbation. Finally, they hypothesized that ascididemin (**40**) might activate c-Jun N-terminal protein kinase (JNK), which might be involved in ascididemin-induced apoptosis signaling. In order to prove this hypothesis, they have set up experiments whose results revealed that ascididemin (**40**) activated/phosphorylated JNK after 4 h, paralleling caspase-2 activation. Since Bcl-x_L was unable to abrogate JNK phosphorylation, it was concluded that activation of JNK occurs upstream of mitochondria. That ascididemin (**40**) triggered ROS generation which contributed significantly to JNK activation was substantiated by the fact that anti-oxidant *N*-acetylcystein (NAC) almost completely abrogated JNK phosphorylation, and since caspase-2 and -9 processing was reduced by JNK inhibitor SP00125, it was suggested that JNK acts upstream of both caspase-2 and -9. It was found also that pre-treatment with the JNK inhibitor SP00125 caused a decrease of cytochrome *c* release from the mitochondria as well as ascididemin-induced DNA fragmentation. These data led Dirsch et al. to conclude that ascididemin triggers JNK activation *via* ROS generation and active JNK contributes *via* non identified target to caspase activation and cytochrome *c* release from mitochondria.

G-quadruplexes are a family of secondary DNA structures that consists of four-stranded structure stabilized by G-quartets [58]. It is well recognized that telomeres protect chromosomal ends from fusion events and provide a mean for complete replication of chromosome, and telomere repeats are added by a specialized enzyme, telomerase, which is overexpressed in most tumor cells. It is also known that

the 3'-terminal region of the G-rich strand of human telomeres is single-stranded and may adopt a G-quadruplex conformation [59, 60]. As this structure has been shown to directly inhibit telomerase elongation *in vitro* [61], ligands that selectively bind to G-quadruplex structures may interfere with telomere structure and telomere elongation and replication of cancer cells [62, 63]. Base on this concept, Guittat et al. [64]) have investigated the capacity of ascididemin (**40**) and meridine (**43**) to stabilize G-quadruplexes and to inhibit telomerase. Using several methods such as equilibrium dialysis, mass spectrometry and fluorescence melting experiments to study the interaction of ascididemin (**40**) and meridine (**43**) with unusual DNA structures, they have found that ascididemin (**40**) and meridine (**43**) were able to bind G-quadruplexes, however their binding affinities are relatively modest, especially ascididemin (**40**). Furthermore, they have demonstrated that ascididemin (**40**) and meridine (**43**) significantly preferred the human telomeric intramolecular quadruplex over the parallel one. Using TRAP (Telomeric Repeat Amplification Protocol) assay, ascididemin (**40**) was found to inhibit telomerase with an IC_{50} of 87 μM, while meridine (**43**), which has higher affinity for quadruplexes, was more potent showing an IC_{50} of 11 μM.

36.3.4 Mechanism of Antitumor Activity of Neoamphimedine and Deoxyamphimedine

Although the structures of amphimedine (**27**), neoamphimedine (**29**) and deoxyamphimedine (**31**) are closely related, their biological activities and mechanisms of action differ significantly. Amphimedine (**27**) is relatively inactive compound compared to neoamphimedine (**29**) and deoxyamphimedine (**31**). Amphimedine (**27**) does not significantly intercalate DNA while neoamphimedine (**29**) and deoxyamphimedine (**31**) were found to displace ethidium bromide from DNA, although deoxyamphimedine (**31**) was found to be more effective than neoamphimedine (**29**). Furthermore, neoamphimedine (**29**) and deoxyamphimedine (**31**) are cytotoxic, although by different mechanisms, amphimedine (**27**) is not.

De Guzman et al. [22] have shown that neoamphimedine (**29**) was cytotoxic to normal CHO AA8 cells with an IC_{50} = 2 mg/ml, and quantitative DNA cleavage assays revealed it stimulated topoisomerase II dependent cleavage 3% above control compared to etoposide (which stimulated 38% cleavage at the same concentration) while no DNA cleavage was detected with amphimedine (**27**) in the presence of topoisomerase II. They have also found that neoamphimedine (**29**) was able to stimulate topoisomerase II to catenate DNA to a high molecular weight complex. By using Rad 52+/− yeast strains that express normal or elevated topoisomerase levels to test the cytotoxicity of amphimedine (**27**) and neoamphimedine (**29**), with etoposide (a topoisomerase II poison) as positive control and topotecan (a topoisomerase I poison) as a negative control, Marshall et al. [65] have found the enhanced cytotoxicity for neoamphimedine (**29**) and etoposide but not for amphimedine (**27**) and topotecan. Furthermore, they have tested both amphimedine

(**27**) and neoamphimedine (**29**) in mammalian cell lines: HCT-116 (human colon tumor), SK-mel-5 (human melanoma), KB (human epidermoid nasopharyngeal tumor), MCF-7 (human breast cancer), A2780wt (human ovarian tumor wild type), A2780AD (human ovarian tumor multi-drug resistant), AA8 (CHO wild type), xrs-6 (CHO double strand break repair), and have found that while neoamphimedine (**29**) was cytotoxic in every of these cell lines, amphimedine (**27**) did not show any toxicity at doses tested. Interestingly, neoamphimedine (**29**) was found to retain its cytotoxicity in the MDR-expressing A2780AD cell line while doxorubicin, M-AMSA and taxol all had significantly reduced toxicity compared to the A2780wt. Since the results obtained from the yeast cell cytotoxicity assays suggested a topoisomerase II dependent mechanism, they have tested amphimedine (**27**) and neoamphimedine (**29**) in a panel of mutant CHO cell lines. In contrast to all clinical topoisomerase II drugs tested in the xrs-6 cell line, which showed significant enhanced toxicity [66], no significant enhancement of cytotoxicity was detected at IC_{50} for amphimedine (**27**) and neoamphimedine (**29**) in the xrs-6 cells, although a small increase was observed in the xrs-6 cell line treated with neoamphimedine (**29**) compared to the AA8 cell line. Since this result was inconsistent with the yeast data, Marshall et al. [65] hypothesized that neoamphimedine (**29**) is a topoisomerase II-dependent drug, whose primary mechanism of action is not the stabilization of cleavable complexes. In order to investigate the mechanism underlying its cytotoxicity, the ability of neoamphimedine (**29**) to interfere with the function of purified human topoisomerase II was investigated *in vitro*. It was found that neoamphimedine (**29**) induced minimal DNA cleavage *via* formation of cleavage complex. In experiments using 84 ng topoisomerase II, up to 8.9% DNA cleavage (50 μM neoamphimedine) was detected. They have found also that the percentage of cleavage decreased as the concentrations of neoamphimedine (**29**) increased, and cleavage was not detected with 140 ng topoisomerase II. Using gel electrophoresis, they have confirmed that the cleavage was due to single strand DNA nicking of the plasmid substrate. Interestingly, during the experiments they have found that in the reactions containing active topoisomerase II and supercoiled substrate DNA routinely appeared as a high molecular weight (HMW) complex upon electrophoresis analysis, and this activity was concentration-dependent and was apparent whether the substrate plasmid was relaxed or supercoiled. However, this HMW complex formation was not observed with amphimedine (**27**) nor any other pyridoacridines tested in their laboratory. Further experiments have allowed them to confirm that the HMW complex was not formed by a protein-DNA aggregation or chemical cross linking but, it was in fact a catenated complex of plasmid DNA. In the experiments using SDS or TE wash, neoamphimedine (**29**) was found to induce topoisomerase to catenate DNA in a time and concentration-dependent manner. Additionally, by filter-binding assay to quantify DNA aggregation, they have shown that DNA aggregation increased in proportion to the concentration of neoamphimedine (**29**). Since KB tumors respond well to topoisomerase II drugs, including doxorubicin and etoposide, neoamphimedine (**29**) was evaluated for its *in vivo* anticancer activity in nude mice bearing human KB tumors, together with amphimedine (**27**) and etoposide. The results showed that neoamphimedine (**29**) was as effective as etoposide while amphimedine (**27**),

a topoisomerase II-inactive isomer, did not exhibit any antitumor activity. In addition, neoamphimedine (**29**) was shown to have an antitumor activity in mice bearing HCT-116 cell tumors and there was no difference between neoamphimedine (**29**) and 9-aminocamptothecin, a drug effective in this system.

The structure of deoxyamphimedine (**31**) differs from that of neoamphimedine (**29**) in the substituents on C-8 and C-9 of ring C of the 8H-pyrido[4,3,2-*mn*] acridone (**2**). While deoxyamphimedine (**31**) has a positively charged 1-methylpyridinium ring attached to C-8 and C-9 of ring C, neoamphimedine (**29**) has a 1-methylpyridin (2H)-one ring on the same position. Marshall et al. [67] have shown that deoxyamphimedine (**31**) was cytotoxic to every human tumor cell lines previously tested with amphimedine (**27**) and neoamphimedine (**29**); however its IC_{50} observed values were slightly lower than those reported for neoamphimedine (**29**) [65]. Furthermore, deoxyamphimedine (**31**) was found to be almost equally toxic to the A2780wt (IC_{50} = 0.3 μM) and its paired multi-drug resistant cell line AD2780wt. Thus, they concluded that the lack of significant fold difference for deoxyamphimedine (**31**) in both of these cell lines indicated that it was not likely a substrate for multi-drug resistant pump that often impedes the efficacy of cancer drugs in chemotherapy. Furthermore, they have found also that deoxyamphimedine (**31**) was selectively more toxic to the mutant CHO cell lines (EM9, IC_{50} = 3.8 μM and xrs-6, IC_{50} = 4.2 μM), than the wild type (AA8, IC_{50} = 13.7 μM), thus confirming DNA strand breakage as a contributing mechanism for its cytotoxicity. Ethidium bromide displacement assays revealed that deoxyamphimedine (**31**) was a potent DNA intercalator, displacing 50 % EtBr at 1 μM (while neoamphimedine required approximately 100 μM to displace 50 % EtBr), suggesting that deoxyamphimedine (**31**) had a higher affinity for DNA than its analogues. They have also carried out the topoisomerase I and II DNA cleavage assays and found that deoxyamphimedine (**31**) was a potent cleaver of DNA *in vitro* and this activity was independent on the presence of topoisomerase I or II. Like ascididemin (**40**), deoxyamphimedine (**31**) could fully cleavage DNA under aerobic conditions in the experiments carried out with DDT and DNA. It was found also that the amount of cleaved DNA observed was greatly increased with the increasing amounts of deoxyamphimedine (**31**) and DTT, but the amount of induced DNA cleavage was attenuated under hypoxic conditions. Additionally, higher temperature and high salt conditions did not reverse deoxyamphimedine-induced DNA damage, suggesting that the DNA damage was ROS-induced. As addition of metals ($FeSO_4$) and chelators did not alter the DNA cleaving ability, it was concluded that the ROS generation was not a Fenton-type reaction. Extensive protection against DNA cleavage by catalase suggested H_2O_2 as a likely ROS intermediate. Additionally, since antioxidants and ROS scavengers such as glutathione, benzoic acid and NAC were found to be able to protect against DNA damage, it was suggested that the generation of the hydroxyl radical most likely occurred. Thus they hypothesized that the iminoquinone portion of deoxyamphimedine (**31**) is responsible for the redox cycling and ROS generation. For this, direct reduction of the iminoquinone portion of deoxyamphimedine (**31**) to the semi-iminoquinone species seems to likely facilitate the production of H_2O_2 and DNA damaging free radicals.

36.3.5 Mechanism of Antitumor Activity of Lissoclinidine B and Diplamine B

Lissoclinidine B (**63**) and diplamine B (**19**) were isolated together from the ascidian *Lissoclinum* cf. *dadum,* and were tested in the Hdm2 (Human double minute 2) electrochemiluminescence assay by Clement et al. [15]. The structures of these compounds are quite distinct since the pentacyclic lissoclinidine B (**63**) has an extra 1,3-oxathiolane ring fused to ring C of the 11H-pyrido[4, 3, 2-*mn*]acridine (**1**), while the tetracyclic diplamine B (**19**) contains the iminoquinone portion.

Lissoclinidine B (**63**) and diplamine B (**19**) were tested for their effects on cellular p53 and Hdm2 in the tert-immortalized human retinal pigment epithelial (RPE) cells, and the cellular p53 and Hdm2 levels were determined by immunoblotting. They have shown that lissoclinidine B (**63**) and diplamine B (**19**) were able to increase p53 and Hdm2 in a dose-dependent manner, and at 10 μM exhibited the greatest increase in p53 and Hdm2, similar to 50 μM of the proteasome inhibitor *N*-acetyl-leucyl-leucyl-norleucinal (ALLN). Furthermore, it was found that lissoclinidine B (**63**) and diplamine B (**19**) displayed more potent activity in cells (10 μM) than in free cell assay system (IC_{50} values 98.1 ± 6 μM and 101.3 ± 4 μM, respectively). They have also treated the $p53^{-/-}mdm2^{-/-}$mouse embryo fibroblasts (MEFs) transiently transfected with plasmid encoding Hdm2 under the control of a p53-independent CMV (cytomegalovirus) promotor, with 10 μM lissoclinidine B (**63**) and diplamine B (**19**). After 8 h of incubation, both compounds were found to increase Hdm2 in MEFs, demonstrating that lissoclinidine B (**63**) and diplamine B (**19**) functioned at least in part by stabilizing Hdm2. Since diplamine B (**19**) was quite unstable and degraded over time, only lissoclinidine B (**63**) was further investigated. Using U2OS (a human osteosarcoma) cells transiently co-transfected with plasmids encoding p53 and Hdm2 prior to incubation with ALLN or lissoclinidine B (**63**) to directly investigate whether lissoclinidine B (**63**) blocked Hdm2-mediated p53 ubiquitylation, they have found that ALLN caused an accumulation of p53, including higher molecular weight forms of protein indicative of ubiquitylation, while incubation with lissoclinidine B (**63**) similarly blocked p53 degradation in a dose-dependent manner. They have also confirmed the results in an additional experiment with HCT-116 colon carcinoma cells expressing wild-type p53 which also showed that lissoclinidine B (**63**) prevented the accumulation of ALLN-induced ubiquitylated p53, following treatment with both lissoclinidine B (**63**) and ALLN. Thus, they concluded that lissoclinidine B (**63**) inhibits both Hdm2-mediated ubiquitylation and degradation of p53. Furthermore, they have also used U2OS-pG13 cells that stably express endogenous wild-type p53 and p53-responsive luciferase reporter (reporter pG 13) to examine whether lissoclinidine B (**63**) activates p53-dependent transactivation. Since treatment with lissoclinidine B (**63**) produced a dose-dependent increase in luciferase activity, they suggested that stabilization of p53 resulted in activation of p53-dependent transcription. Finally, they have shown that treatment of *p53*-deficient (A9) and wild-type *p53* MEFs (C8) with lissoclinidine B (**63**) produced a marked increase in cell death in C-8 cells while *p53*-deficient A9 cells were relatively resistant. They have also observed cleavage of PARP in

response to lissoclinidine B (**63**) specifically in the p53-expressing C-8 cells but not in A-9 cells, which is consistent with apoptotic cell death. Thus, these data indicated that lissoclinidine B (**63**) induces cell apoptosis in a p53-dependent manner which is consistent with a primary role in inhibiting Hdm2 and thereby activating p53.

It is interesting to point out that despite the structural difference between lissoclinidine B (**63**) and diplamine B (**19**), both of them were found to be equally active (IC_{50} values 98.1 ± 6 and 101.3 ± 4 μM, respectively). Thus, it was suggested that the functionality required for their activity lies in the aromatic ring system and sidechain, and may not be affected by substituent differences at C-8, C-9 or N-11.

36.3.6 Anticancer Activity Evaluation of Kuanoniamines A and C

Kijjoa et al. [44] have investigated the effects of kuanoniamines A (**51**) and C (**53**), isolated from the marine sponge *Oceanapia sagittaria* collected from the Gulf of Thailand, on the growth of five human tumor cell lines: MCF-7 (breast adenocarcinoma, estrogen-dependent ER+), MDA-MB-231 (breast adenocarcinoma, estrogen-independent ER−), SF-286 (glioma), NCI-H460 (non-small cell lung cancer), UACC-62 (melanoma), and a non-tumor human cell line MRC-5 (diploid embryonic lung fibroblast), by SRB method. Kuanoniamine A (**51**) was found to potently inhibit the growth of all five human tumor and the non-tumor MRC-5 cell lines, exhibiting its GI_{50} (concentration that causes 50% of cell growth inhibition) values less than 5 μM. Although kuanoniamine C (**53**) also inhibited the growth of all these tumor cell lines, its effect was at least ten times weaker than that observed for kuanoniamine A (**51**) for MDA-MB-231, SF-268, NCI-H460, and UACC-62. Specifically, the low GI_{50} value of kuanoniamine C (**53**) on MCF-7 (0.81 ± 0.11 μM) suggested a high selectivity of this compound for this estrogen dependent (ER+) breast cancer cell line. Using [^{3}H] thymidine incorporation assay, they have found that the DNA synthesis of the MCF-7 cells was dramatically affected by treatment with kuanoniamine A (**51**), and the DNA synthesis was found to be dependent on its concentration rather than the exposure time. In contrast, kuanoniamine C (**53**) exhibited a stimulatory effect at low concentrations and inhibitory effect at high concentrations. This biphasic effect of kuanoniamine C (**53**) on DNA synthesis of the MCF-7 cells resembled that of phytoestrogens on the estrogen dependent breast cancer cells [68]. The MTT-reducing capacity assay revealed that the effect of kuanoniamine A (**51**) on cell viability of MCF-7 cells was much more potent than that of kuanoniamine C (**53**), and the loss of cell viability by kuanoniamine A (**51**) was in agreement with its drastic effect on DNA synthesis. Flow cytometric analysis of the DNA content revealed that kuanoniamine A (**51**) caused an extensive reduction of the MCF-7 cells in G2/M phase with a concomitant increase in G1 phase and a cellular fraction in S phase. In contrast, kuanoniamine C (**53**) exhibited no significant effect on these cells. The TUNEL assay showed that both kuanoniamines A (**51**) and C (**53**) caused an increase in apoptotic cells, suggesting that their antiproliferative effects on the MCF-7 cells could be in part due to the phenomenon of apoptosis.

36.4 Conclusion

The pyridoacridines represent an interesting group of marine alkaloids which exhibit interesting biological activities. However, much attention has focused on their anticancer activity since nearly most of the compounds of this class exhibit cytotoxicity in the cultured human tumor cells. Although many of the naturally occurring marine pyridoacridines tested so far are too toxic to be considered viable for development of anticancer drugs, the study on the mechanisms underlying their cytotoxicy has shed light to the relationship between the structure and the mechanism responsible for their cytotoxicity. The progress in synthetic chemistry combined with a rapid advance in bioassays, which can allow scientists to identify molecular targets of this class of compounds, will lead to the optimization of the molecules with retain or increase the activity while diminishing the toxicity.

Acknowledgements This work was partially supported by the European Regional Development Fund (ERDF) through the COMPETE—Operational Competitiveness Program and national funds through FCT—Foundation for Science and Technology, under the project PEst-C/MAR/LA0015/2013", and by the Project MARBIOTECH (reference NORTE-07–0124-FEDER-000047) within the SR&TD Integrated Program MARVALOR-Building research and innovation capacity for improved management and valorization of marine resources, supported by the Programa Operacional Regional do Norte (ON.2-O Novo Norte) and by the European Regional Development Fund.

References

1. Indumathy S, Dass CR (2013) Finding chemo: the search for marine-based pharmaceutical drugs active against cancer. J Pharm Pharmacol 65:1280–1301
2. Børrensen T, Boyen C, Dobson A et al (2010) Marine biotechnology: a new vision and strategy for Europe. Marine Board-ESF Position Paper 15, pp 1–91
3. Marshall KM, Barrows LR (2004) Biological activities of pyrodoacridines. Nat Prod Rep 21:731–751
4. Delfourne E, Bastide J (2003) Marine pyridoacridine alkaloids and synthetic analogues as antitumor agents. Med Res Rev 23:234–252
5. Molinski TF (1993) Marine pyridoacridine alkaloids: structure, synthesis, and biological chemistry. Chem Rev 93:1825–1838
6. Ding Q, Chichak K, Lown JW (1999) Pyrroloquinoline and pyridoacridine alkaloids from marine sources. Curr Med Chem 6:1–27
7. Carroll AR, Sheuer PJ (1990) Kuanoniamines A, B, C, and D: pentacyclic alkaloids from the tunicate and its prosobranch mollusk predator *Chelynotus semperi*. J Org Chem 55:4426–4431
8. Kobayashi J, Cheng JF, Wälchli MR et al (1988) Cystodytins A, B, and C, novel tetracyclic aromatic alkaloids with potent antineoplastic activity from the Okinawan tunicate *Cystodytes dellechiajei*. J Org Chem 53:1800–1804
9. Kobayashi J, Tsuda M, Tanabe A et al (1991) Cystodytins D–I, new cytotoxic tetracyclic aromatic alkaloids from the Okinawan marine tunicate Cystodytes dellechiajei. J Nat Prod 54:1634–1638
10. McDonald LA, Eldredge GS, Barrows LR et al (1994) Inhibition of topoisomerase II catalytic activity by pyridoacridine alkaloids from a *Cystodytes* sp. ascidian: a mechanism for the apparent intercalator-induced inhibition of topoisomerase II. J Med Chem 37:3819–3827

11. Appleton DR, Pearce AN, Lambert G et al (2002) Isodiplamine, cystodytin K and lissoclinidine: novel bioactive alkaloids from the New Zealand ascidian *Lissoclinum notti*. Tetrahedron 58:9779–9783
12. Kim J, Pordesimo EO, Toth SI et al (1993) Pantherinine, a cytotoxic aromatic alkaloid, and 7-deazainosine from the ascidian *Aplidium pantherinum*. J Nat Prod 56:1813–1816
13. Searle PA, Molinski TF (1994) Five new alkaloids from the tropical ascidian, *Lissoclinum* sp., lissoclinotoxin A is chiral. J Org Chem 59:6600–6605
14. Charyulu GA, McKee TC, Ireland CM (1989) Diplamine, a cytotoxic polyaromatic alkaloid from the tunicate *Diplosoma* sp. Tetrahedron Lett 30:4201–4202
15. Clement JA, Kitagaki J, Yang Y et al (2008) Discovery of new pyridoacridine alkaloids from *Lissoclinum* cf. badium that inhibit the ubiquitin ligase activity of Hdm2 and stabilize p53. Bioorg Med Chem 16:10022–10028
16. Copp BR, Jompa J, Tahir A et al (1998) Styelamines A–D: new tetracyclic pyridoacridine alkaloids from the Indonesian ascidian *Eusynstyela latericius*. J Org Chem 63:8024–8026
17. Bry D, Banaigs B, Long C et al (2011) New pyridoacridine alkaloids from the purple morph of the ascidian *Cystodytes dellechiajei*. Tetrahedron Lett 52:3041–3044
18. Molinski TF, Ireland CM (1989) Varamines A and B, new thioalkaloids from *Lissoclinum vareau*. J Org Chem 54:4256–4259
19. Rudi A, Kashman Y (1989) Six new alkaloids from the purple Red Sea tunicate *Eudistoma* sp. J Org Chem 54:3331–5337
20. Rudi A, Benayahu Y, Goldberg I et al (1988) Alkaloid metabolites from the marine tunicate *Eudistoma* sp. segoline, isosegoline and norsegoline. Tetrahedron Lett 29:3861–3862
21. Schmitz FJ, Agrawal SK, Gunasekera SP et al (1983) Amphidine, new aromatic alkaloid from a pacific sponge Amphimedon sp. carbon connectivity determination from natural abundance ^{13}C–^{13}C coupling constants. J Am Chem Soc 105:4835–4836
22. De Guzman FS, Carte B Troupe N et al (1999) Neoamphimedine: a new pyridoacridine topoisolerase II inhibitor which catenates DNA. J Org Chem 64:1400–1402
23. Tasdemir D, Marshall KM, Mangalindan GC et al (2001) Deoxyamphimedine, a new pyridoacridine alkaloid from two tropical *Xestospongia* sponges. J Org Chem 66:3246–3248
24. Thale Z, Johnson T, Tenney K et al (2002) Structures and cytotoxic properties of sponge-derived bisannulated acridines. J Org Chem 67:9384–9391
25. Wei X, Bugni TS, Harper MK et al (2010) Evaluation of pyridoacridine alkaloids in a zebrafish phenotypic assay. Mar Drugs 8:1769–1778
26. Molinski TF, Fahy E, Faulkner DJ et al (1988) Petrosamine, a novel pigment from the marine sponge *Petrosia* sp. J Org Chem 53:1340–1341
27. Nukoolkarn VS, Saen-oon S, Rungrotmongkol T et al (2008) Petrosamine, a potent anticholinesterase pyridoacridine alkaloid from a Thai marine sponge *Petrosia* n. sp. Bioorg Med Chem 16:6560–6567
28. Carroll AR, Ngo A, Quinn RJ et al (2005) Petrosamine B, an inhibitor of the Helicobacter pylori enzyme aspartyl semialdehyde dehydrogenase from the Australian sponge *Oceanapia* sp. J Nat Prod 68:804–806
29. Kobayashi J, Cheng JF, Nakamura H et al (1988) Ascididemin, a novel pentacyclic aromatic alkaloid with potent antileukemic activity from the Okinawan tunicate *Didemnum* sp. Tetrahedron Lett 29:1177–1180
30. Bonnard I, Bontemps N, Lahmy S et al (1995) Binding to DNA and cytotoxic evaluation of ascididemin, the major alkaloid from the Mediterranean ascidian *Cystodytes dellechiajei*. Anticancer Drugs Des 10:333–346
31. Smith CJ, Venables DA, Hopmann C et al (1997) Plakinidine D, a new pyrroloacridine alkaloid from two ascidians of the genus *Didemnum*. J Nat Prod 60:1048–1050
32. Nilar N, Sidebottom PJ, Carté BK et al (2002) Three new pyridoacridine type alkaloids from a Singaporean ascidian. J Nat Prod 65:1198–1200
33. Feng Y, Davis RA, Sykes ML et al (2010) Antitrypanosomal pyridoacridine alkaloids from the Australian ascidian *Polysyncraton echinatum*. Tetrahedron Lett 51:2477–2479

34. Schmitz FJ, De Guzman FS, Hossain MB et al (1991) Cytotoxic aromatic alkaloids from the ascidian *Amphicarpa meridiana* and *Leptoclinides* sp.: meridine and 11-hydroxyascididemin. J Org Chem 56:804–808
35. Barnes EC, Said NABM, Williams ED et al (2010) Ecionines A and B, two new cytotoxic pyridoacridine alkaloids from the Australian marine sponge, *Ecionemia geodides*. Tetrahedron 66:283–287
36. Bontemps N, Bonnard I, Banaigs B et al (1994) Cystodamine, a new cytotoxic fused polyaromatic alkaloid from the Mediterranean ascidian *Cystodytes dellechiajei*. Tetrahedron Lett 35:7023–7026
37. Delfourne E, Bontemps-Subielos N, Bastide J (2000) Structure revision of the marine pentacyclic aromatic alkaloid: cystodamine. Tetrahedron Lett 41:3863–3864
38. Carroll AR, Cooray NM, Poiner A et al (1989) A second shermilamine alkaloid from a tunicate *Trididemnum* sp. J Org Chem 54:4231–4232
39. Cooray NM, Scheuer PJ, Parkanyi L et al (1988) Shermilamine A: a pentacyclic alkaloid from a tunicate. J Org Chem 53:4619–4620
40. Bontemps N, Bry D, López-Legentil S et al (2010) Structures and antimicrobial activities of pyridoacridine alkaloids isolated from different chromotypes of the ascidian *Cystodytes dellechiajei*. J Nat Prod 73:1044–1048
41. Koren-Goldshlager G, Aknin M, Gaydou EM et al (1998) Three new alkaloids from the marine tunicate *Cystodytes violatinctus*. J Org Chem 63:4601–4603
42. Gunawardana GP, Koehn FE, Lee AY et al (1992) Pyridoacridine alkaloids from deep-water marine sponges of the family Pachastrellidae: structure revision of dercitin and related compounds and correlation with the kuanoniamines. J Org Chem 57:1523–1526
43. Eder C, Schupp P, Proksch P et al (1998) Bioactive pyridoacridine alkaloids from the Micronesian sponge *Oceanapia* sp. J Nat Prod 61:301–305
44. Kijjoa A, Wattanadilok R, Campos N et al (2009) Anticancer activity evaluation of kuanoniamines A and C isolated from the marine sponge *Oceanapia sagittaria*, collected from the Gulf of Thailand. Mar Drugs 5:6–22
45. Gunawardana GP, Kohmoto S, Gunasekera SP et al (1988) Dercitin, a new biologically active acridine alkaloid from a deep water marine sponge, *Dercitus* sp. J Am Chem Soc 110:4856–4858
46. Gunawardana GP, Kohmoto S, Burres NS (1989) New cytotoxic acridine alkaloids from two deep water marine sponges of the family Pachastrellidae. Tetrahedron Lett 30:4359–4362
47. Solomon CE, Faulkner DJ (1996) Sagitol, a pyridoacridine alkaloid from the sponge *Oceanapia sagittaria*. Tetrahedron Lett 37:9147–9148
48. Plubrukarn A, Davidson BS (1998) Arnoamines A and B, new cytotoxic pentacyclic pyridoacridine alkaloids from the ascidian *Cystodytes* sp. J Org Chem 63:1657–1659
49. Torres YR, Bugni TS, Berlinck RGS et al (2002) Sebastianines A and B, novel biologically active pyridoacridine alkaloids from the Brazilian ascidian *Cystodytes dellechiajei*. J Org Chem 67:5429–5432
50. Koren-Goldshlager G, Aknin M, Kashman Y (2000) Cycloshermilamine D, a new pyridoacridine from the marine tunicate *Cystodytes violatinctus*. J Nat Prod 63:830–831
51. Viracaoundin I, Faure R, Gaydou EM et al (2001) A new alkaloid from the purple Indian Ocean tunicate *Eudistoma bituminis*. Tetrahedron Lett 42:2669–2671
52. He HY, Faulkner DJ (1991) Eudistones A and B: two novel octacyclic alkaloids from a Seychelles tunicate, *Eudistoma* sp. J Org Chem 56:5369–5371
53. Fong HKH, Copp BR (2013) Synthesis, DNA binding and antitumor evaluation of styelsamine and cystodytin analogues. Mar Drugs 11:274–299
54. Lindsay B, Barrows L, Copp B (1995) Structural requirements for biological activity of marine alkaloid ascididemin. Bioorg Med Chem Lett 5:739–742
55. Dassonneville L, Wattez N, Baldeyrou B et al (2000) Inhibition of topoisomerase II by the marine alkaloid ascididemin and induction of apoptosis in leukemia cells. Biochem Pharmacol 60:527–537

56. Matsumoto SS, Biggs J, Copp BR et al (2003) Mechanism of ascididemin-induces cytotoxicity. Chem Res Toxicol 16:113–122
57. Dirsch VM, Kirschke SO, Estermeier M et al (2004) Apoptosis signaling triggered by the marine alkaloid ascididemin is routed via caspase-2 and JNK to mitochondria. Oncogene 23:1586–1593
58. Williamson JR (1994) G-quartet structures in telomeric DNA. Annu Rev Biophys Biomol Struct 23:703–730
59. Parkinson GN, Lee MPH, Neidle S (2002) Crystal structure of parallel quadruplexes from human telomeric DNA. Nature 417:876–880
60. Wang Y, Patel DJ (1993) Solution structure of the human telemetric repeat d[AG3(T2AG3)3] G-tetraplex. Structure 1:263–282
61. Zahler AM, Williamson JR, Cech TR et al (1991) Inhibition of telomerase by G-quartet DNA structures. Nature 350:718–720
62. Mergny JL, Hélène C (1998) G-quadruplex DNA: a target for drug design. Nat Med 4:1366–1367
63. Sun D, Thomson B, Cathers BE et al (1997) Inhibition of human telomerase by a G-quadruplex—interactive compound. J Med Chem 40:2113–2116
64. Guittat L, De Cian A, Rosu F et al (2005) Ascididemin and meridine stabilize G-quadruplexes and inhibit telomerase in vitro. Biochim Biophys Acta 1724:375–384
65. Marshall KM, Matsumoto SS, Holden JA et al (2003) The anti-neoplastic and novel topoisomerase II-mediated cytotoxicity of neoamphimedine, a marine pyridoacridine. Biochem Pharmacol 66:447–458
66. Jeggo PA, Caldecott K, Pidsley S et al (1989) Sensitivity of Chinese hamster ovary mutants defective in DNA double strand break repair to topoisomerase II inhibitors. Cancer Res 49:7057–7063
67. Marshall KM, Andjelic CD, Tasdemir D et al (2009) Deoxyamphimedine, a pyridoacridine alkaloid, damages DNA via the production of oxidative oxygen species. Mar Drugs 7:196–209
68. Wang C, Kurzer MS (1997) Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. Nutr Cancer 28:236–247