CHAPTER II

LITERATURE REVIEW

1. The nudibranch *Jorunna funebris*

Jorunna funebris is a nudibranch belonging to phylum Mollusca, class Gastropoda, subclass Opisthobranchia, order Nudibranchia, suborder Doridacea, family Kentrodorididae, and genus Jorunna. The shell of the nudibranch *J. funebris* is discarded at metamorphosis and the adult lacks a shell, ctenidia, and mantle cavity. It has evolved secondary gills, arranging in a circular tuft around the anus and its true cerata, containing extensions of the digestive system, is lacking (Pechenik, 1996). The rhinophores are black with a white base. The body is white with irregular black rings and its mantle is covered in small spiculate papillae (caryophyllidia) (Bidgrain, 2005). This nudibranch is a marine hermaphrodite. It is carnivorous and feeds mainly on specific sponges such as *Xestospongia* sp., *Haliclona* sp., *Euplacella cf. australis*, (Bidgrain, 2005) and *Oceanapia* sp. (Fontana *et al.*, 2000).

Figure 1 Picture of a group of the nudibranch *Jorunna funebris* feeding on the blue sponge *Xestospongia* sp.

2. The sponge *Xestospongia* sp. (Amnuoypol, 2004)

Sponge identifications are primarily based on morphology. Major characters are shape, size, color, texture, mucus production, surface ornamentation, organic and inorganic skeletons (spongin fiber and spicule), skeletal structure, larvae and reproductive strategy.

According to "Sponguide" version August 2000 by Dr. John A. Hooper from Queensland Museum, Australia, all the sponges are classified in phylum Porifera which contains 4 classes (one completely extinct), 5 subclasses, 28 orders, 232 families and 977 valid genera. Two subphyla are now recognized: subphylum Cellularia including classes Demospongiae and Calcarea, and subphylum Symplasia including class Hexactinellida. *Xestospongia* de Laubenfels, 1932 (Syn. *Neopetrosia* de Laubenfels, 1949, *Prianos* Gray, 1867) belongs to class Demospongiae contains about 95 % of living species the genus *Xestospongia*. The taxa of this genus is given by Hooper (2000) as follows: phylum Porifera, subphylum Cellularia, class Demospongiae, order Haplosclerida, and family Petrosiidae van Soest, 1980.

Hooper (2000) described the characters of sponges belonging to the genus *Xestospongia* as follows: "Ectosomal skeleton indistinct; choanosomal skeleton confused isotropic reticulation of multispicular tracts, generally lacking spongin and sometimes with single spicule scattered throughout mesohyl between major spicule tracts, stony texture; oxeote spicules in one size category only".

The bluish sponge was collected in December 1992 and later was identified by Hooper as *Xestospongia* sp. #2133 (sample code QMG 306998). The voucher specimens have been deposited at Queensland Museum, Australia and at Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. This sponge exhibits thick, encrusted, lobate growth. Its texture is rough, although it is brittle and easily crumbles. It is light bluish-gray when alive, and pinkish in ethanol. Osculal are numerous and of moderate size, and are found on apices of surface lobes, with a slightly raised lip. The surface has prominent bulbous surface lobes with some that are nearly digitate in size. The surface is translucent, membranous, optically smooth, macroscopically bulbous, microscopically even, with choanosomal drainage canals that are slightly visible below the surface. Ectosomal skeleton membranes have no specialized speculation or structure. Choanosomal

skeleton with isotropic reticulation of paucispicular tracts of oxeas forms tight oval meshes. Many free oxeas are scattered between tracts. Small to moderately sized subdermal cavities are observed throughout skeleton. There are no visible fibers, and only small amounts of collagen in the mesohyl. The oxeas are robust, straight or slightly curved at center, sharply pointed, hastate (190-210 x 12-18 micrometers).

Figure 2 Picture of the blue sponge *Xestospongia* sp.

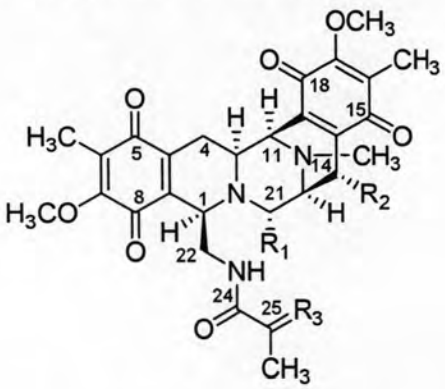
3. Chemistry and biological activity of tetrahydroisoquinoline alkaloids

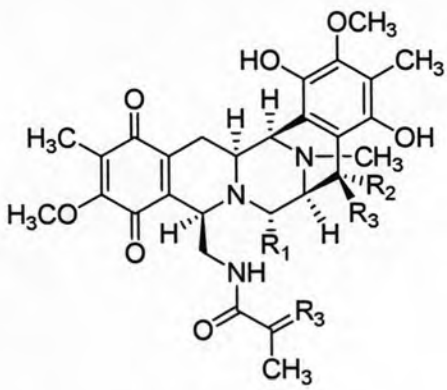
Tetrahydroisoquinoline alkaloids from natural sources have been studied over the past 25 years starting with the isolation of naphthyridinomycins in 1974. These alkaloids are classified into saframycins, renieramycins, naphthyridinomycins, bioxalomycins, quinocarcins, and ecteinascidins (Scott and Williams, 2002). Most of them showed antitumor activity and antimicrobial activity. Especially, ecteinascidin 743 displayed potent antitumor activity and has been studied in phase II/III clinical trials for ovarian, breast, endometrial, prostate and pediatric cancers (Henriquez *et al.*, 2005; Rinehart, 2000). The biological activity mechanism of ecteinascidin 743 involved on its binding to the minor groove of DNA to interfere with cell division, transcription, and DNA repair (Pommier *et al.*, 1996; Martinez *et al.*, 2001). Since the structures of renieramycins, saframycins, and ecteinascidins are closely related, this section will emphasize on the relationships between their structures and biological activities.

3.1 Saframycins

3.1.1 Structures of saframycins

Saframycins are a family of antitumor antibiotics isolated from the *Streptomyces lavendulae* No. 314. Structurally, saframycins are composed of two isoquinoline-quinone moiety fusing with a piperazine ring to form a pentacyclic system. In some cases, one isoquinolinequinone is converted to a dihydroisoquinoline rings. The side chains at C-22 are mainly pyruvamide derivatives. Saframycins A, B, C, D, E, and S were the first members of this group (Arai *et al.*, 1977; Arai *et al.*, 1980). Saframycin R, containing 2' hydroxyacetyl substituted at the dihydroisoquinoline ring, was also isolated (Asaoka *et al.*, 1982).





saframycins A; R_1= CN, R_2= H, R_3 = O

B; R_1= R_2 = H, R_3 = O

C; R_1=H, R_2 = OCH_3, R_3 = O

G; R_1= CN, R_2= OH, R_3 = O

H; R_1= CN, R_2= H, R_3 = OH, CH_2OCH_3

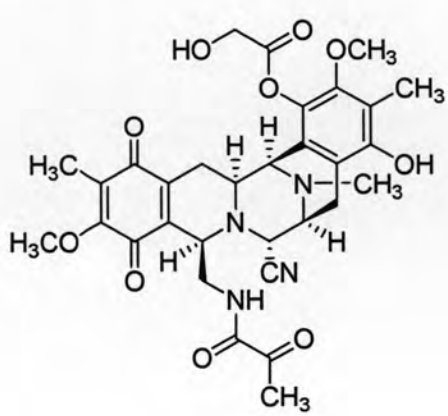
S; R_1= OH, R_2= H, R_3 = O

saframycins D; R_1= H, R_2, R_3 = O

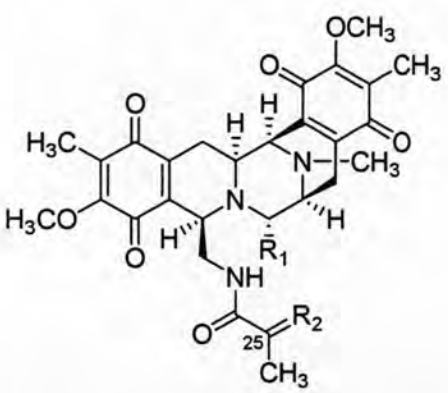
E; R_1= R_3 = H, R_2 = OH

F; R_1= CN, R_2 = R_3 = O

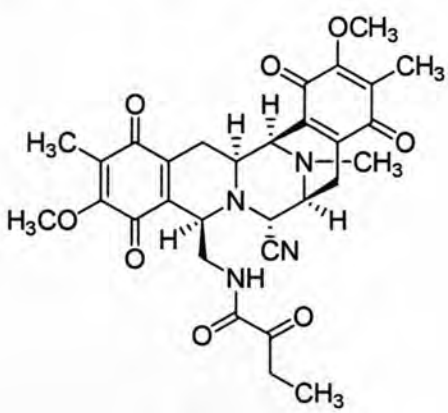
saframycin R



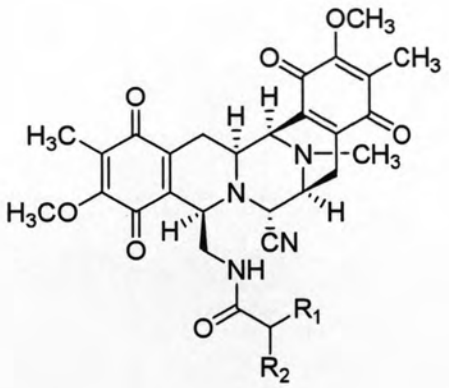
Bioconversion of saframycin A using *Rhodococcus Amidophilus* IFM 144 afforded three products including saframycins AR_1, AR_2 (saframycin B) and AR_3 (Takahashi *et al.*, 1982). The C-25 of saframycins AR_1 and AR_3 was reduced from ketone to hydroxy (Yazawa *et al.*, 1982). The other reduced products at C-25 including mixture of diastereomeric alcohols AH_1 and AH_2 (same as AR_1) were obtained by treatment saframycin A with sodium borohydride. The reduced diastereomers of AH_1 and AH_2 were then converted to their acetate forming AH_1Ac and AH_2Ac (kishi *et al.*, 1984). In the search for more biologically active saframycins, six saframycins including Y3, Yd-1, Yd-2, Ad-1, Y2b, and Y2b-d were produced by directed biosynthesis, employing *Streptomyces lavendulae* No. 314. (Yazawa *et al.*, 1986). The supplementation of alanine and glycine or alanylglycine yielded saframycin Y3 and the dimer Y2b. The addition of 2-amino-*n*-butyric acid and glycine or 2-amino-*n*-butyrylglycine produced saframycins Yd-1 and Ad-1, and the dimer Y2b-d. Saframycin Yd-2 was produced by the supplementation of glycyl-glycine. Later in 1988, three minor compounds, saframycins F, G, and H were isolated (Mikami *et al.*, 1988). The same year, saframycins Mx1, Mx2, and Mx3 were also isolated from *Myxococcus xanthus* (Irschik *et al.*, 1988; Rao and Lown, 1992). Like saframycin F, one of the aromatic rings was in the hydroquinone form.



saframycins AR_1 = AH_2; R_1 = CN, R_2 = H, OH
AH_1; R_1 = CN, R_2 = OH, H
AR_3; R_1 = H, R_2 = H, OH
AH_1Ac; R_1 = CN, R_2 = OAc, H
AH_2Ac; R_1 = CN, R_2 = H, OAc



saframycin Ad-1

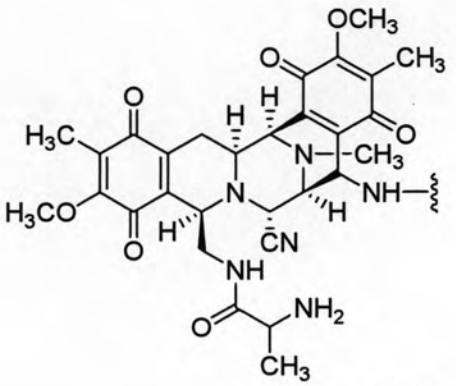


saframycins Y3; $R_1 = NH_2$, $R_2 = CH_3$

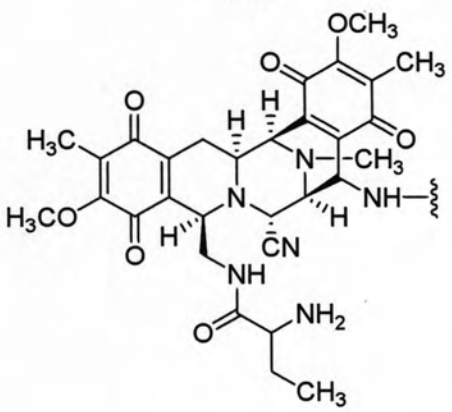
Yd-1; $R_1 = NH_2$, $R_2 = CH_2CH_3$

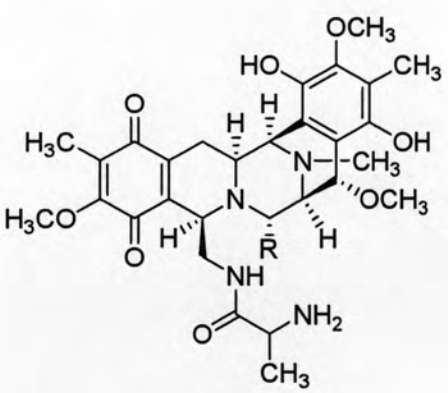
Yd-2; $R_1 = NH_2$, $R_2 = H$

saframycin Y2b; $R_1 = CH_3$, R_2 =



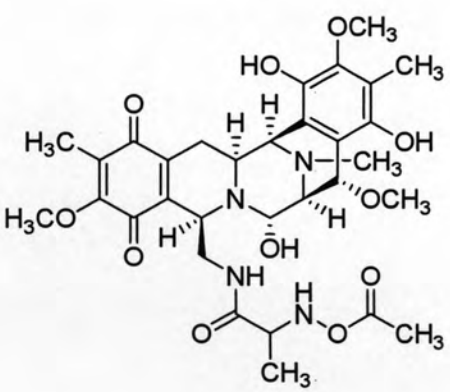
saframycin Y2b-d; $R_1 = CH_2CH_3$, R_2 =





saframycins Mx1; R = OH

Mx2; R = H



saframycin Mx3

3.1.2 Cytotoxic activity of saframycins

All of saframycins displayed antitumor and antibacterial activities. Saframycins A and S showed high antimicrobial activity as shown in Table 1 (Scott and Williams, 2002).

Table 1 Antimicrobial activity of saframycins A and S.

Tested organism	Saframycin A, MIC (μg/mL)	Saframycin S, MIC (μg/mL)
Staphylococcus aureus FDA 209P	0.1	0.025
Streptococcus faecalis	12.4	3.12
Bacillus subtilis PCI 219	0.1	0.025
Corynebacterium diphtheriae	0.003	0.004
Sarcina lutea	0.05	0.025

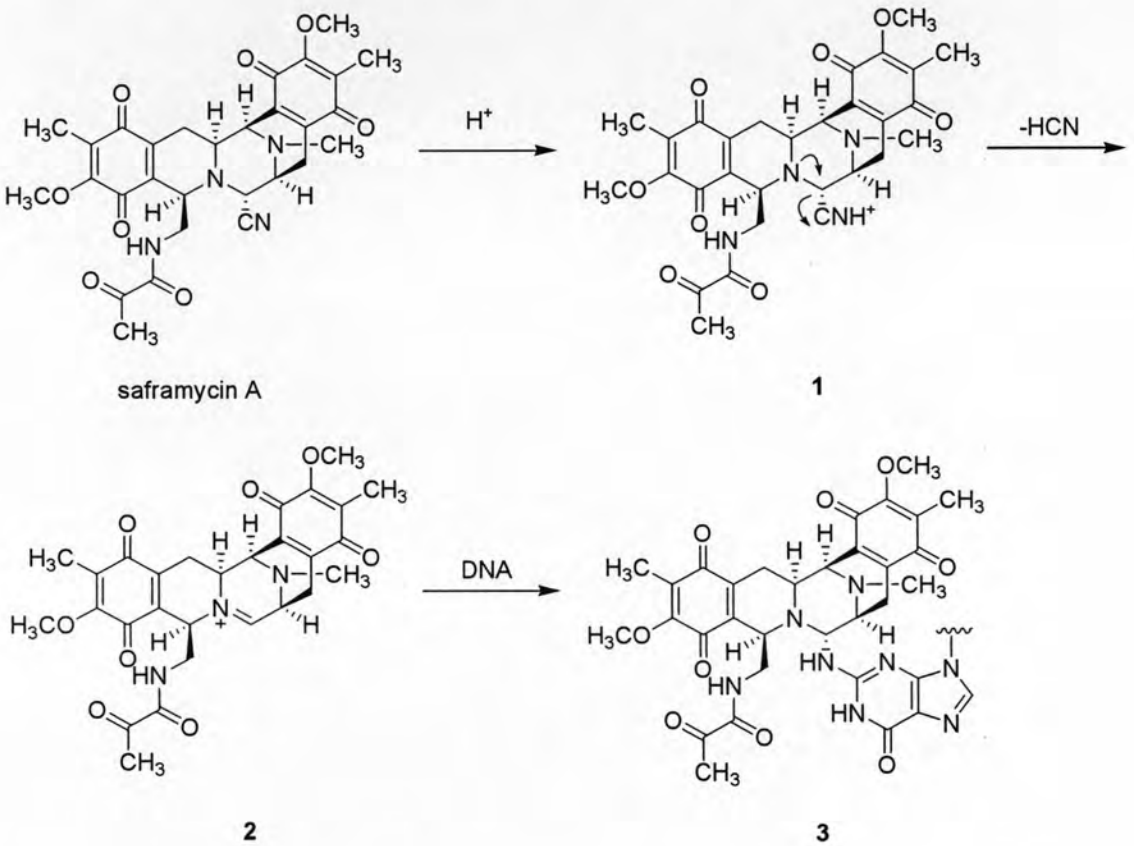
Several saframycins also displayed antitumor activity against L1210 leukemia as shown in Table 2 (Kishi *et al.*, 1984; Scott and Williams, 2002).

Table 2 Antitumor activity of saframycins and analogs against L1210 leukemia.

Compound	ID_{50} (nM)	Compound	ID_{50} (nM)
Saframycin A	5.6	Saframycin AH_1Ac	25.0
Saframycin S	5.3	Saframycin AH_2Ac	27.0
Saframycin $AR_1(AH_2)$	6.1	Saframycin B	800.0
Saframycin AH_1	8.0	Saframycin C	3,900.0
Saframycin G	30.0	Saframycin D	4,800.0
Saframycin H	33.0	Saframycin AR_3	650.0
Saframycin F	590.0		

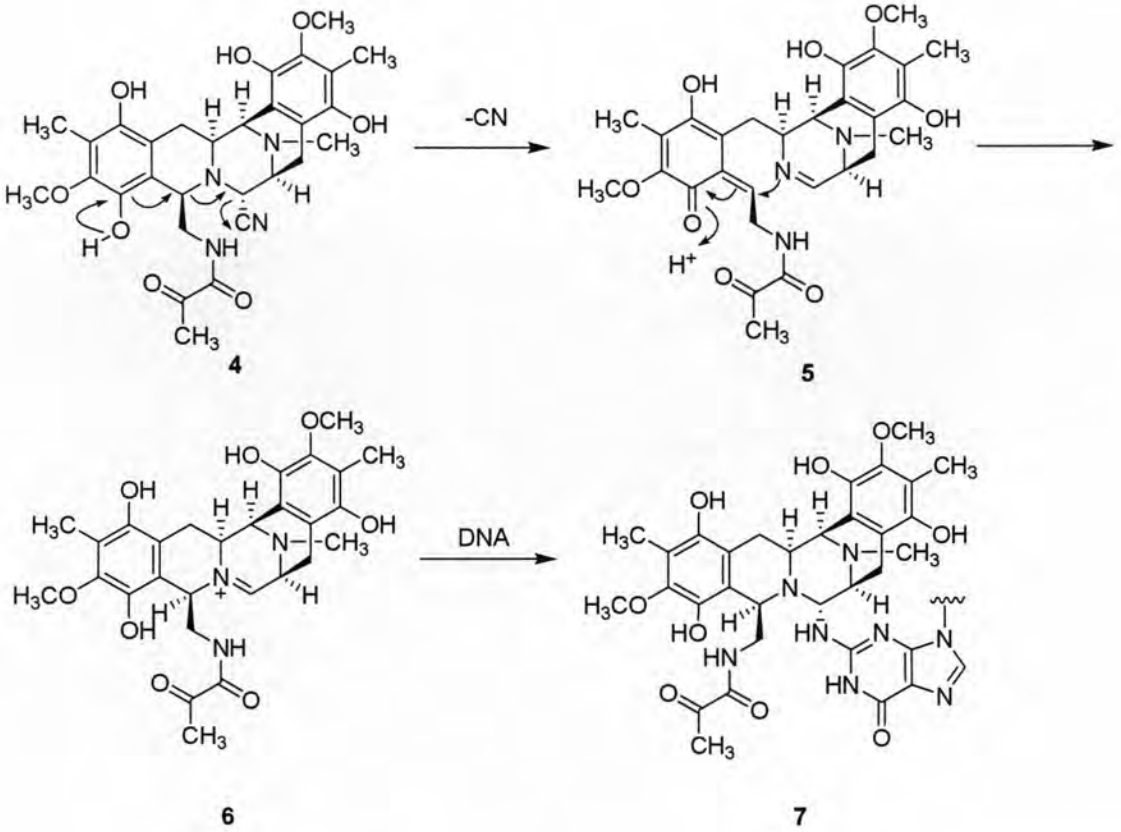
Saframycins A, AH_1, and AH_2 containing a cyanide group at C-21 and saframycin S containing a hydroxyl group at C-21 showed the highest activity. Either a cyanide or hydroxyl group at C-21 of these saframycins allows for the formation of

an electrophilic iminium species that alkylates DNA in the minor groove. The mechanism of covalent adduct formation was proposed as shown in Scheme 1 (Lown *et al.*, 1982; Scott and Williams, 2002). The alkylation invokes protonation of the cyanide with expulsion of HCN (**1**) to form the iminium ion species (**2**). The N-2 of guanine subsequently formed a covalent bond at this position (**3**).



Scheme 1 Mechanism of DNA alkylation by saframycin A through the HCN elimination.

Another mechanism (Scheme 2) based on the fact that saframycin A does not alkylate DNA unless it was converted into corresponding the hydroquinone form (**4**) (Hill and Remers, 1991; Scott and Williams, 2002). The phenol facilitates scission of the B-ring C-N bond which, in turn leads to the expulsion of cyanide. The resulting imine subsequently re-attacks the *o*-quinone methide to form the iminium species (**6**), which subsequently alkylates DNA to form the adduct (**7**).



Scheme 2 Another mechanism of DNA alkylation.

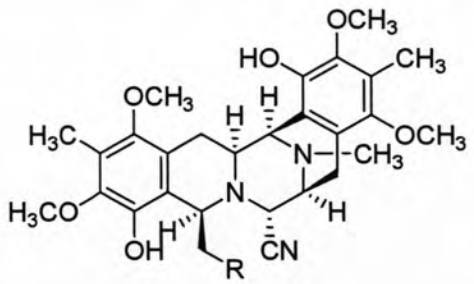
Moreover, saframycins A and S were found to be modestly sequence specific with respect to DNA alkylation, exhibiting a preference for 5'-GGG, and 5'-GGC by the use of MPE (methidium propyl EDTA) Fe (II) footprinting study (Rao and Lown, 1990). Saframycin S recognized 5'-CGG sequence while saframycin A did not. Saframycins Mx1 and Mx3, of which both contain the hydroxyl group at C-21, exhibited the same selectivity as saframycin S (Rao and Lown, 1990; Rao and Lown, 1992). Cytotoxicity of saframycins was not only due to DNA alkylation but also DNA cleavage (Lown *et al.*, 1982). Saframycin A in the hydroquinone form expressed DNA cleavage while the quinone form did not. Saframycin R containing an acyl group on the phenol, caused much less DNA cleavage than saframycin A, making it much less toxic without any loss biological activity (Lown *et al.*, 1983). However, saframycin A also inhibited RNA synthesis at 0.2 μg/ml (Ishiguro *et al.*, 1978).

Saframycins G, H, F, AH_1Ac, and AH_2Ac had a leaving group at C-21 but the activities were low because steric effect of the side chain blocked iminium ion to

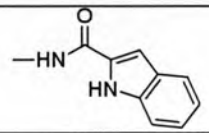
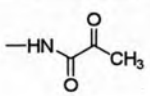
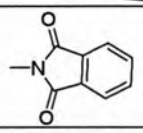
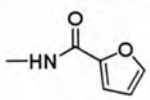
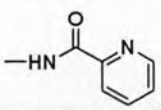
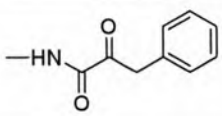
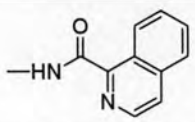
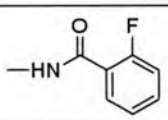
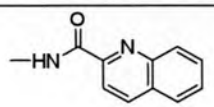
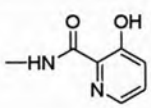
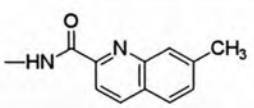
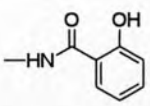
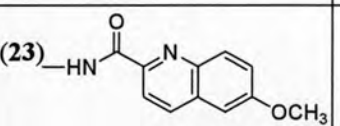
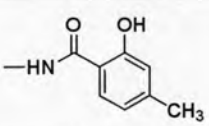
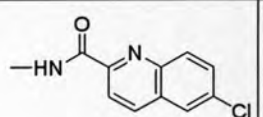
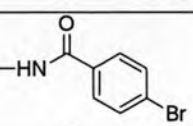
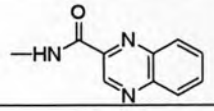
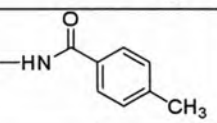
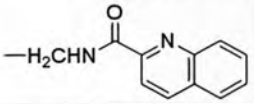
alkylate with DNA. Saframycins B, C, D and AR_3 lacked a leaving group at C-21 had lower activities. Saframycins Y3, Yd-1, Yd-2, and Ad-1 containing a amino group or a carbonyl group at C-25 had no difference in activity (Kaneda *et al.*, 1987). The dimers Y2b and Y2b-d had similar activities as the corresponding monomers. In a study to examine side chain effects on biological activity of saframycin analogs including 15 acyl, 9 akyl, and 3 carbamoyl derivatives of the C-25 amino group of saframycin Y3 which was prepared by Arai *et al.* (Kaneda, *et al.*, 1987), it was found that acyl derivatives had lower activity while the alkyl derivatives had similar activities to the natural product. If the side chain was bulky, the activity decreased. Reduction of the ketone at C-25 had no impact on antitumor activity, but antimicrobial activity could be lost (Scott and Williams, 2002).

Saframycins with a variety of the side chain at C-22 were evaluated for the cytotoxicity in order to search the new antitumor agents. Therefore, the bishydroquinone analogs of saframycin A were synthesized and investigated for cytotoxic activity. Approximately half of these analogs displayed very high activity against the A375 melanoma and A549 lung carcinoma cell lines and some analogs (**21**-**24**) showed about 20 fold greater activity in A549 cell line than saframycin A as shown in Table 3 (Myers and Plowright, 2001).

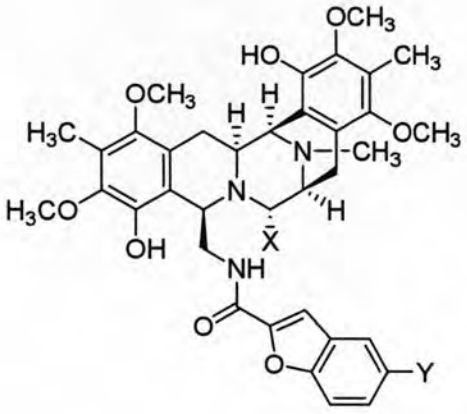
Table 3 Antiproliferative activity of the bishydroquinone analogs of saframycin A.



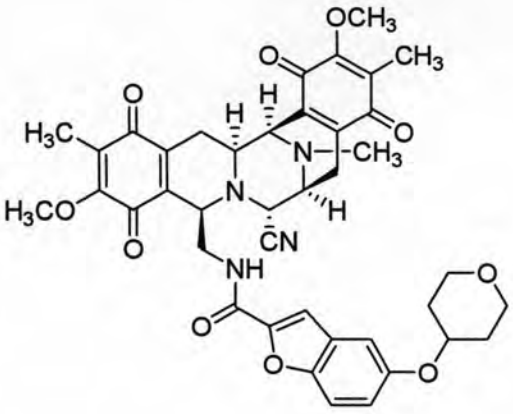
saframycin analogs

IC$_{50}$(nM)			IC$_{50}$(nM)		
R=	**A375**	**A549**	**R=**	**A375**	**A549**
Saframycin A	5.3	133	**(17)** 	2.7	31
(8) 	4.5	160	**(18)** 	1.7	9.2
(9) 	13	290	**(19)** 	3.3	40
(10) 	2.4	39	**(20)** 	2.5	32
(11) 	2.5	37	**(21)** 	1.3	4.4
(12) 	1.2	11	**(22)** 	1.4	4.6
(13) 	1.4	14	**(23)** 	2.0	3.5
(14) 	1.2	6.5	**(24)** 	1.5	4.1
(15) 	1.7	25	**(25)** 	1.2	4.7
(16) 	1.9	37	**(26)** 	3.6	78

Rencently, Spencer and co-workers found QAD saframycin analog (**21**), inhibiting the growth of A549 lung tumor cells (GI_{50} value 4.4 nM) and A375 melanoma cells (1.3 nM). The compound was used as a typify for synthesizing other potent saframycin analogs as shown in Table 4 (Spencer *et al.*, 2006).



27-31



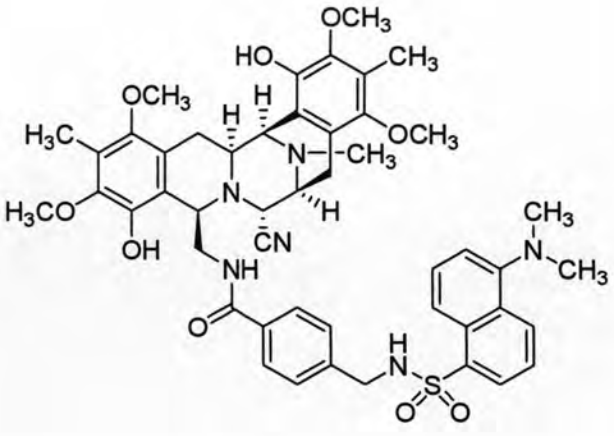
32

Table 4 GI_{50} values for the growth inhibitory effects of QAD saframycin analogs in tumor cells.

Analog	X	Y	GI_{50} (nM)			
			HCT116	DLD1	PC-3	A549
27	CN	H	5.5	ND	ND	ND
28	CN	Tetrahydropyran-4-yloxy	3.2	8.3	2.7	18.9
29	CN	2-(*N*-morpholino)-ethoxy	8.1	22.4	11.7	57.5
30	OH	Tetrahydropyran-4-yloxy	5.5	10.4	5.0	20.6
31	H	Tetrahydropyran-4-yloxy	>300	ND	ND	ND
32			17.3	26.5	16.5	ND

ND= not determined

Analogs **28** and **30** showed potent growth inhibition in the four cell lines. In a DNA alkylation study, both of them alkylated a GC-rich dsDNA oligonucleotide 21-mer sequence [5'-GGAACCGGGCTCGGGCCAAGG-3'] while analog **31** lacking a leaving group to form iminium ion did not show DNA alkylation in the gel-shift assay. Analog **32** was a bisquinone derivative of analog **28**, displaying poorer activity than analog **28**.



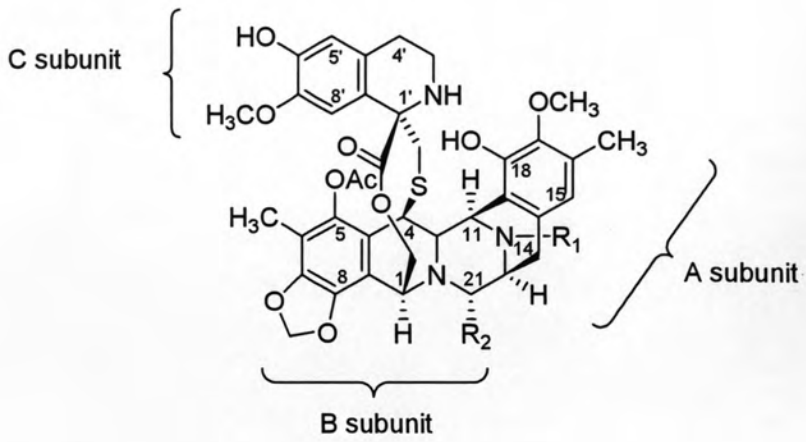
33

Analog **33** had less potent cytotoxic activity when compared to analogs **27-31**. Analog **33** (GI_{50} value 201 nM) accumulated within HCT116 cell relative to control cell. Fluorescently labled analog **33** was prepared to assist in visualizing intracellular localization of the drug. This analog was apparent in cell in metaphase, where highly structured chromatin appeared as brighter bodies. The apparent weaker staining of other cellular compartments suggested that DNA is a preferred target, independent of cell cycle. Compounds that are capable of alkylating DNA in cells may in turn activate DNA damage response pathways that lead to cell death (Spencer *et al.*, 2006).

3.2 Ecteinascidins

3.2.1 Structures of ecteinascidins

Ecteinascidins are either bis- or tris-tetrahydroisoquinoline alkaloids and were isolated from the tunicate genus Ecteinascidia, especially *Ecteinascidia turbinata*. Ecteinascidins displayed remarkable antitumor activity (Rinehart *et al.*, 1990b; Wright *et al.*, 1990; Sakai *et al.*, 1996b). The structures of ecteinascidins generally consist of two condensed tetrahydroisoquinoline units (A and B subunits) linking to C subunit which is either tetrahydroisoquinoline or tetrahydro-β-carboline by a ten membered sulfide-containing lactone ring. Ecteinascidins 729, 743, 745, 759A, 759B, and 770 containing three tetrahydroisoquinoline subunits (A-C) were the first isolated members (Rinehart *et al.*, 1990a).



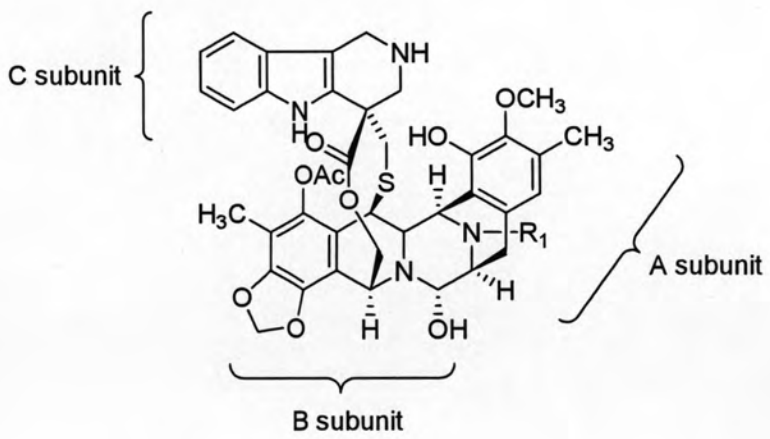
ecteinascidins 729; R_1 = H, R_2 = OH

743; R_1 = CH_3, R_2 = OH

745; R_1 = CH_3, R_2 = H

770; R_1 = CH_3, R_2 = CN

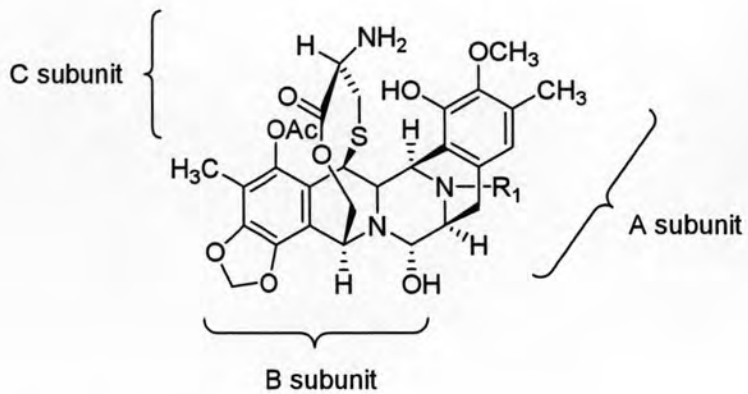
Ecteinascidins 722, 736 and 734 N^{12}-oxide containing two tetrahydroisoquinoline units (A and B subunits) plus one tetrahydro-β-carboline unit (C subunit) were reported later (Sakai *et al.*, 1992).



ecteinascidins 722; R_1 = H

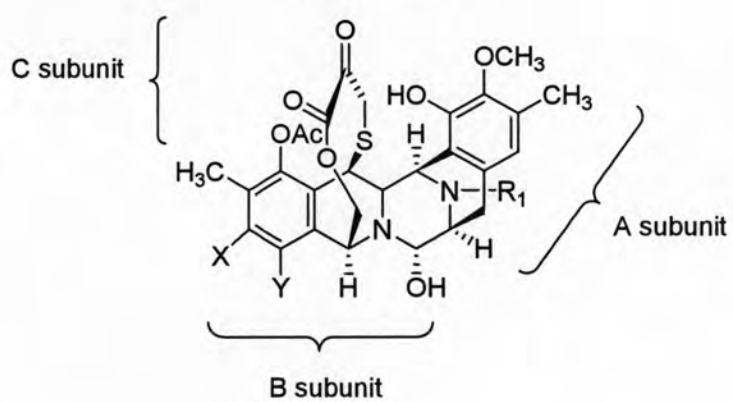
736; R_1 = CH_3

Four putative biosynthetic precursors, ecteinascidins 583, 594, 596 and 597 containing two tetrahydroisoquinoline units (A and B subunits) plus a chain of carbon (C subunit) were later isolated (Sakai *et al.*, 1996a).



ecteinascidins 583; $R_1 = H$

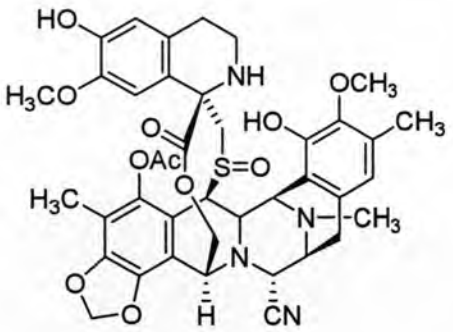
597; $R_1 = CH_3$



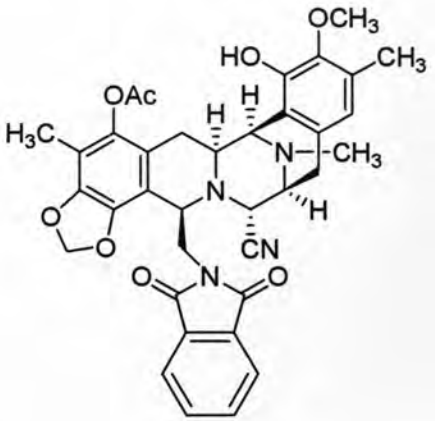
ecteinascidins 594; X, Y = -O-CH_2-O

596; X= OCH_3, Y = OH

However, most of ecteinascidins contain the unstable carbinolamine function at C-21, causing the compound to be available in minute quantity for further bioactivity evaluation. Several groups studied total synthesis of ecteinascidins including Corey group (Corey *et al.*, 1996), Fukuyama group (Endo *et al.*, 2002), Chen group (Chen *et al.*, 2005) and Zheng group (Zheng *et al.*, 2006). Moreover, preparation of ecteinascidin analogs for finding potent antitumor activity was performed. Phthalascidin (Pt 650), an analog of ecteinascidin 743, showed potent biological activity (Martinez *et al.*, 1999; Martinez and Corey, 2000).



ecteinascidin 786



phthalascidin (Pt 650)

Recently, the more stabilized ecteinascidins 770 and 786 were isolated from KCN-pretreated Thai tunicate *Ecteinascidia thurstoni* in high quantity (Suwanborirux *et al.*, 2002). Consequently, a number of acylated analogs derived at the C subunit were prepared as shown in Table 6 (Puthongking *et al.*, 2006).

3.2.2 Cytotoxic activity of ecteinascidins

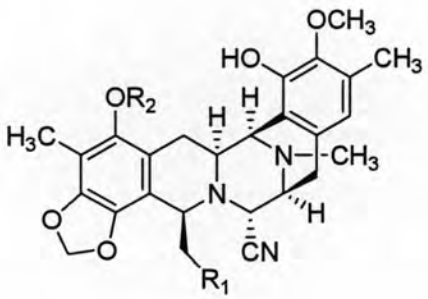
Ecteinascidins 729, 743, 583, 594, and 597 exhibited several biological activities, including cytotoxicity, antimetabolic activities, and enzyme inhibitions. Ecteinascidins 583, 594, and 597, which lacked the aromatic C subunit, showed less cytotoxicity against the MEL28 and CV-1 about 5-25 times than ecteinascidins 729 and 743. The C subunit may be essential for enhancing biological activities of ecteinascidins. Ecteinascidins 729, 743, 583, 594, and 597 showed potent inhibition of DNA and RNA syntheses. For enzyme inhibition, they inhibited DNA and RNA polymerase activities. Ecteinascidins 583, 594 and 597 showed some inhibition of protein synthesis, while ecteinascidins 729 and 743 were less active (Sakai *et al.*, 1992; Sakai *et al.*, 1996a).

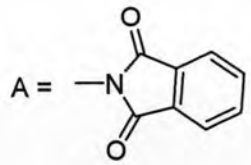
Ecteinascidin 743 has been currently under investigation in clinical trial phase II/III (Zewail-Foote and Hurley, 1999; Rinehart, 2000; Jimeno *et al.*, 2004; Henriquez *et al.*, 2005). Ecteinascidin 743 showed cytotoxic activity against a variety of tumor cell lines (P388, L1210, A549, HT29, and MEL28), and freshly explanted human tumors such as breast, colon, renal, non-small cell lung carcinoma (NSCLC), melanoma, and ovarian at low concentration *in vitro* (Rinehart, 2000). This

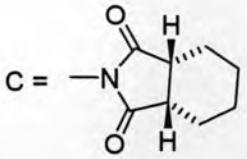
compound was also tested for advanced stage and early stage MX-1 mammary tumor xenografts (Rinehart, 2000) and showed tumor growth reduction of breast, melanoma, nonsmall cell lung cancer, and ovarian cancers (Rinehart, 2000). Additional *in vivo* studies, ecteinascidin 743 expressed complete regressions in xenografts of ovarian carcinoma HOC22, non-small cell lung carcinoma LXFL 529, and melanoma MEXF 989 (Valoti *et al.*, 1998; Hendriks *et al.*, 1999).

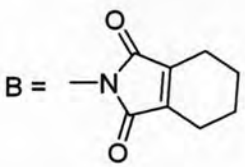
In a study of ecteinascidin derivatives, phthalascidin was found to be very similar to ecteinascidin 743 with regard to *in vitro* potency and mode of action across of variety of cell types. Phthalascidin was synthesized and more stable than ecteinascidin 743. Additional, antiproliferative activity of several phthalascidin analogs was shown in Table 5 (Martinez *et al.*,1999).

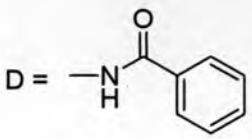
Table 5 Structures and antiproliferative activity of phthalascidin-related compounds.







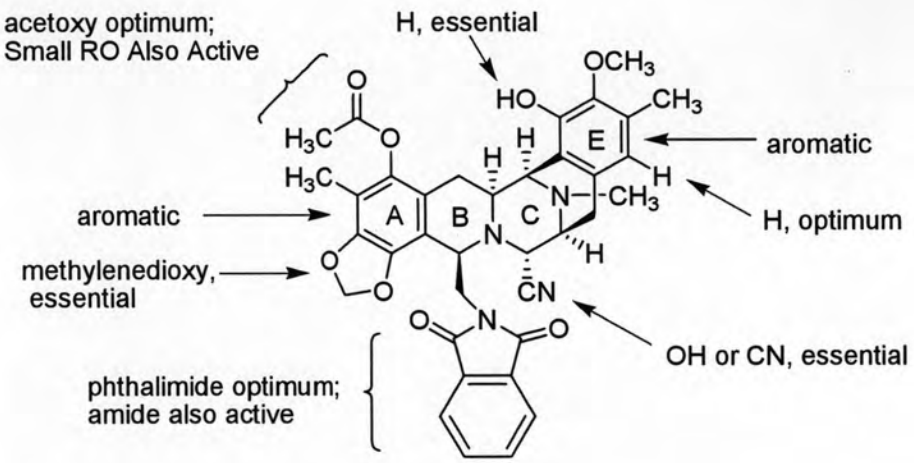




Analog	R_1	R_2	IC_{50} (nM)			
			A549	**HCT116**	**A375**	**PC-3**
Pt 650	A	$CH_3C(O)$	0.95	0.38	0.17	0.55
34	D	$CH_3C(O)$	3.2	0.59	0.35	0.64
35	C	$CH_3C(O)$	1.5	0.85	0.27	1.1
36	B	$CH_3C(O)$	1.2	0.61	0.35	0.75
37	A	$CH_3OCH_2C(O)$	1.6	0.87	0.31	0.90
38	A	$CH_3S(O)_2$	1.7	0.58	0.29	0.86
39	A	$CH_3CH_2C(O)$	2.1	1.2	0.51	2.9
40	A	CH_3	3.1	1.4	0.55	3.1
41	A	CH_3CH_2C	3.9	1.7	0.97	2.4
C15-Chloro-Pt 650			2.8	1.0	0.35	1.1
C15-, C18-*p*-quinone-Pt 650			1.7	0.80	0.45	0.68
N12-[^{14}C]-CH_3-Pt 650			0.91	-	0.22	-
C21-OH-Pt 650			1.0	0.61	0.20	0.53
C21-H-Pt 650			2200	1100	610	850
C18-OMOM-Pt 650			230	74	66	98
Et 743			1.0	0.50	0.15	0.70

Structure-activity relationships between phthalascidin and analogs could be summarized as follows. Phthalascidin exhibited the best antiproliferative activity in cell-based assay. The phenolic hydroxyl group on the A subunit must be protected by

a small group such as acetyl, propionyl, methoxyacetyl, methansulfonyl, methyl, or ethyl, acetyl being optimum. The protection of the other phenolic hydroxyl group on the E subunit resulted in diminishing antitumor activity. The leaving group substituted at C-21, such as a cyano or a hydroxyl group is essential for cytotoxicity.



The biological activities of the synthesized ester derivatives of ecteinascidins 770, 786 and 743 were concluded in Table 6 (Puthongking *et al.*, 2006).

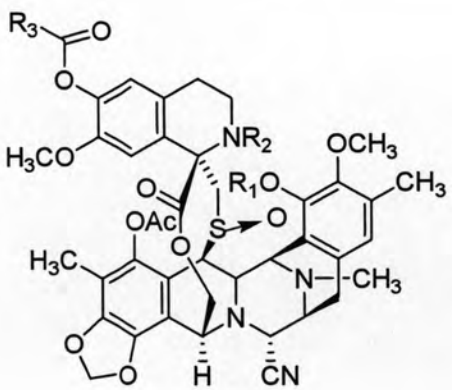
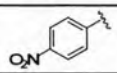
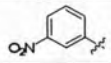
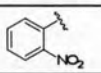
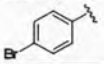
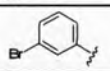
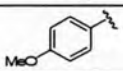
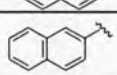
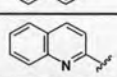
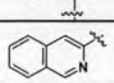
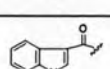


Table 6 Cytotoxicity of ecteinascidin 770 acylated compounds to various cancer cell Lines[a].

Cpds	R_1	R_2	R_3	IC_{50} (nM)		
				HCT116	QG56	DU145
Et 770	H	H	H	0.04	1.8	0.66
Diacetate Et 770	Ac	H	CH_3	1.4	8.3	4.4
Diacetate Et 786	Ac	H	CH_3	160	220	200
Diacetate Et 743	Ac	H	CH_3	1.3	9.5	4.4
42	H	H		0.68	3.2	1.1
43	H	H		0.26	0.96	0.37
44	H	H		0.49	3.3	1.6
45	H	H		1.9	7.1	2.7
46	H	H		15	36	16
47	H	H		7.1	39	11
48	H	H		4.2	19	7.6
49	H	H		0.31	1.7	0.62
50	H	H		0.62	3.6	1.1
51	H	H		0.20	1.0	0.51
52	H	H		0.15	0.87	0.48
53	H	H		13	36	24
54	H	H		6.3	24	12
55	H	H		0.41	2.4	0.95
56	H	H		2.1	9.5	4.5
57	H	H		0.64	1.9	0.83
58	H	H		0.62	2.1	1.5
59	H		H	0.07	0.53	0.37

[a]HCT116 = human colon carcinoma; QG56 = human lung carcinoma; DU145 = human prostate carcinoma

The cytotoxicities of derivatives **43-45** containing a nitro group on the benzene ring and derivatives **49** and **50** possessing a methoxy group on the benzene ring were similar to that of ecteinascidin 770. On the other hand, analogs **46-48** and **53-54** containing a bromo group on the benzene ring and the naphthoyl ring, respectively, showed significantly decreased cytotoxic activity. This reduction of cytotoxicity may relate to steric hindrance. Derivatives **51**, **52**, and **55-58** with nitrogen-containing heterocyclic had high cytotoxicity comparable to ecteinascidin 770. Interestingly, analog **59**, the only *N*-indoleacyl derivatives, displayed the highest cytotoxicity and exhibited selectivity for human colon carcinoma cell line; HCT116 (Puthongking *et al.*, 2006).

3.2.3 Mechanism of action of ecteinascidin 743

3.2.3.1. Effect on DNA:

Ecteinascidin 743 binds to the minor groove of DNA forming adducts at N2 position of guanine (Pommier *et al.*, 1996; Erba *et al.*, 1998) with DNA sequence preference for 5′-AGC, 5′-GGC and 5′-GGC (Zewail-Foote and Hurley, 1999). Furthermore, an NMR-based model of ecteinascidin 743 with duplex DNA indicates that the A- and B-subunits are responsible for DNA recognition and bonding, while the C-subunit is projected out of the minor groove (Moore *et al.*, 1998; Seaman and Hurley, 1998; Zewail-Foote and Hurley, 1999). It also induces topoisomerase I - mediated protein-linked DNA breaks (Takebayashi *et al.*, 1999). Moreover, ecteinascidin 743 halts the DNA excision repair (NER) system in cells (Takebayashi *et al.*, 2001).

3.2.3.2 Inhibition of transcription mechanism:

The inhibition of the transcription of multidrug resistance gene type 1 (mdr-1 gene) by ecteinascidin 743 was reported by Jin *et al.*, 1999. The effect appeared to be due to the ability of ecteinascidin 743 to inhibit the binding of the nuclear transcription factor Y (NFY) to a CCAAT BOX present in the mdr-1 gene promoter. These data confirm previous *in vitro* finding on the ability of ecteinascidin 743 to prevent the formation of the NFY complex with its consensus DNA sequence (Mantonvani *et al.*, 1998; Bonfanti *et al.*, 1999).

3.2.3.3 Effect on microtubules:

Ecteinascidin 743 disorganises the microtubule network but it does not seem to interact directly with tubulin (Garcia-Rocha, Garcia-Gravolos and Avila, 1996).

3.2.3.4. Cell cycle perturbations:

In the human tumor cell lines examined, ecteinascidin 743 was found to decrease the rate of progression of cell in late S phase towards G_2 phase and cause a prolonged blockade in G_2-M phase (Ghielmini *et al.*, 1998; Erba *et al.*, 2001) as assessed by biparametric Brd Urd/DNA flow cytometry. The cell cycle block is p53 independent and eventually leads to a strong apoptotic response (Henriquez *et al.*, 2005).

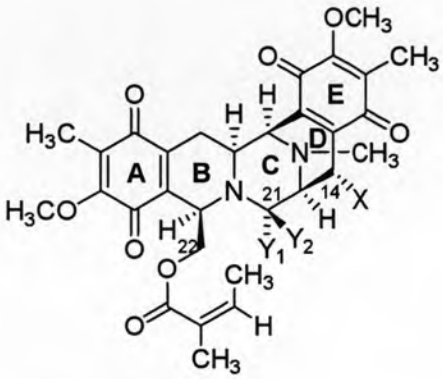
3.3 Renieramycins

3.3.1 Structures of renieramycins

Renieramycins are bistetrahydroisoquinoline marine natural products which were isolated mainly from marine sponges belonging to *Reniera* (Frincke and Faulkner, 1982; He and Faulkner, 1989), *Xestospongia* (Davidson, 1992; Suwanborirux *et al.*, 2003; Amnuoypol *et al.*, 2004), *Haliclona* (Parameswaran *et al.*, 1998), *Cribrochalina* (Pettit *et al.*, 2000; Saito *et al.*, 2001) and *Neopetrosia* (Oku *et al.*, 2003). Renieramycins could be divided into 2 groups based on their A and E rings as follows.

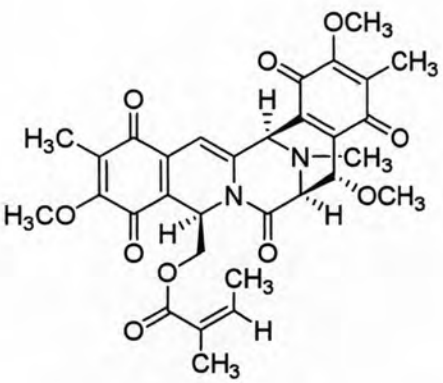
3.3.1.1 Renieramycins with bisquinone (rings A and E)

These renieramycins are composed of two quinone rings at both rings A and E. The members of this group are renieramycins A-G, I, J-K, M, O, and R-S.

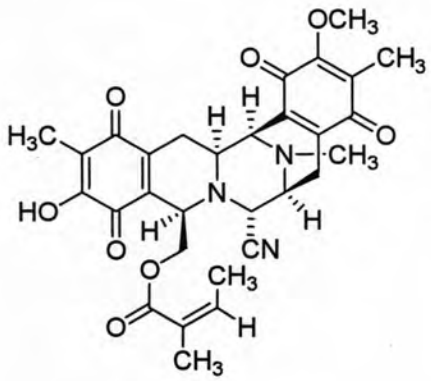


renieramycins

A : X = OH, Y_1, Y_2 = H_2
B : X = OC_2H_5, Y_1, Y_2 = H_2
C : X = OH, Y_1, Y_2 = O
D : X = OC_2H_5, Y_1, Y_2 = O
E : X = Y_2 = H, Y_1 = OH
F : X = OCH_3, Y_1 = OH, Y_2 = H
G : X = H, Y_1, Y_2 = O
J : X = Y_2 = H, Y_1 = CH_2COCH_3
K : X = OCH_3, Y_1 = CH_2COCH_3, Y_2 = H
M : X = Y_2 = H, Y_1 = CN
O : X = OH, Y_1 = CN, Y_2 = H
R : X = OCH_3, Y_1 = CN, Y_2 = H



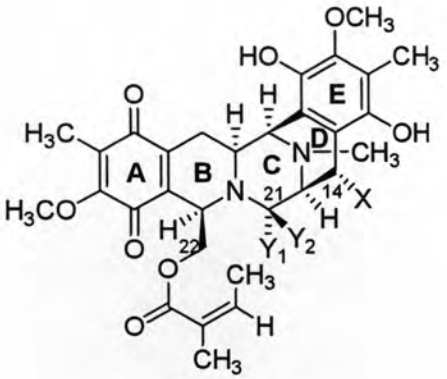
renieramycin I



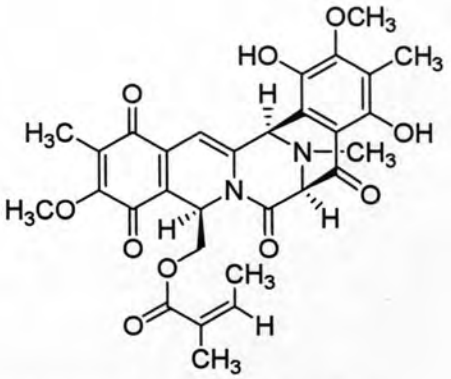
renieramycin S

3.3.1.2 Renieramycins with a quinone (ring A) and a dihydroquinone (ring E)

These renieramycins are composed of a quinone at ring A and a dihydroquinone at ring E. The members of this group are renieramycins H (cribrostatin 4), L, N, P, and Q.



renieramycins



renieramycin H (cribrostatin 4)

L: X = O, Y_1 = CH_2COCH_3, Y_2 = H

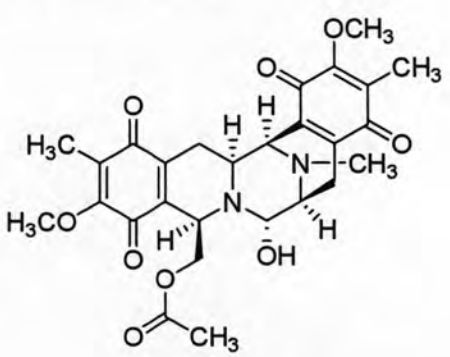
N: X = OH, Y_1 =CN, Y_2 = H

P: X = OH, Y_1 =OH, Y_2 = H

Q: X = O, Y_1 =CN, Y_2 = H

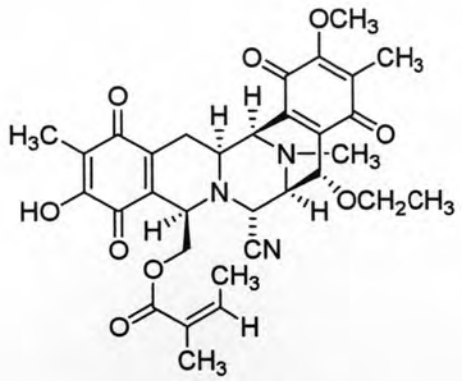
Another related compound, jorumycin was reported from the nudibranch, *Jorunna funebris* feeding on the blue sponge *Oceanapia* sp. (Fontana *et al.*, 2000).

The structure of jorumycin only differs from that of renieramycin E with an acetate ester instead of an angelate ester.

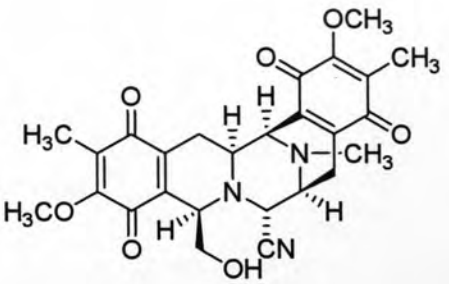


jorumycin

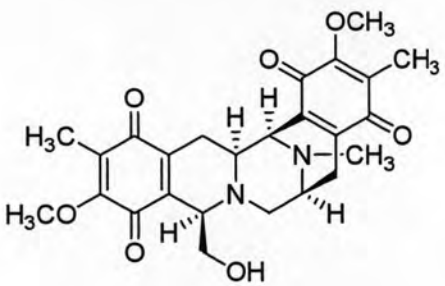
Since the isolated renieramycin M was available in high yield (Suwanborirux *et al.*, 2003), additional renieramycin derivatives were prepared for the evaluation of antitumor activity, including renieramycins E, J, and R'; jorumycin; deangeloyl-renieramycin M; decyanodeangeloylrenieramycin M; 21-cyanojorumycin; 5,8-*O*-diacetylhydroquinonerenieramycin M; 5,8,15,18-*O*-tetraacetylbishydroquinone-renieramycin M; 14-*O*-acetylrenieramycin O; and 14,15,18-*O*-triacetylrenieramycin N (Amnuoypol, 2004; Saito *et al.*, 2004).



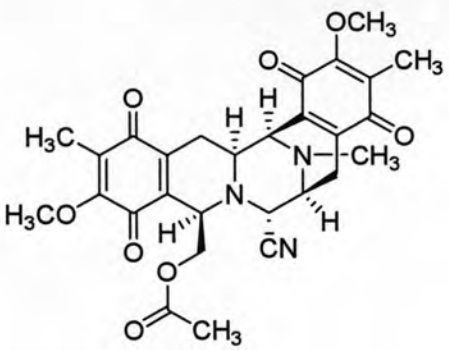
renieramycin R'



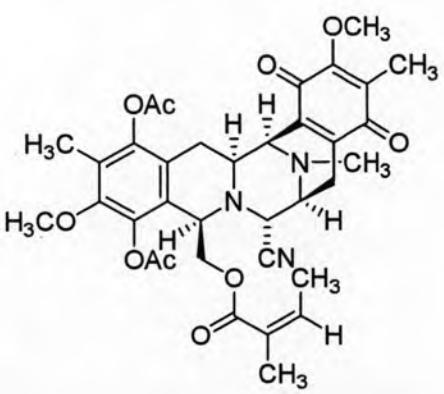
deangeloylrenieramycin M



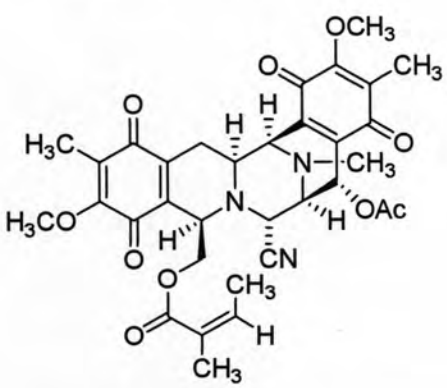
decyanodeangeloylrenieramycin M



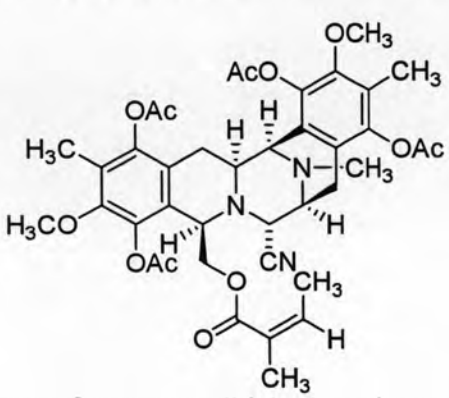
21-cyanojorumycin



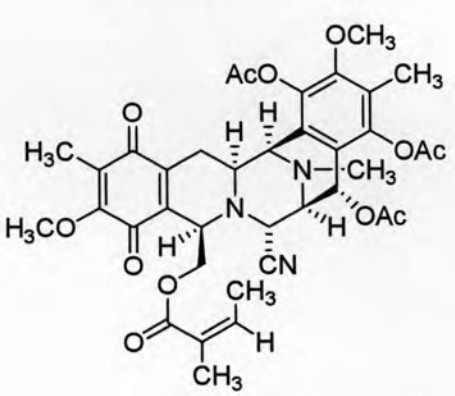
5,8-*O*-diacetylhydroquinonerenieramycin M



14-*O*-acetylrenieramycin O



5,8,15,18-*O*-tetraacetylbishydroquinone-renieramycinM



14,15,18-*O*-triacetylrenieramycin N

3.3.2 Cytotoxic activity of renieramycins

Reineramycins A-D, H, and I (Frincke and Faulkner, 1982; Parameswaran *et al.*, 1998) had moderate antimicrobial activities, while renieramycin G showed moderate activity against KB and LoVo cell lines with MIC of 0.5 and 1.0 μg/mL, respectively (Davidson, 1992). Renieramycin P displayed cytotoxicity against 3Y1, HeLa and P388 cells with IC_{50} 5.3, 12.3, and 0.53 nM, respectively (Oku *et al.*, 2003).

Moreover, cytotoxicities of other renieramycin derivatives against human carcinoma cell lines including HCT116 (human colon carcinoma), NCI-H460 (human lung carcinoma), DLD1 (human colon carcinoma), QG56 (human lung carcinoma), and DU145 (human prostate carcinoma) are summarized in Table 7 (Amnuoypol, 2004).

Table 7 Cytotoxicity of renieramycins and derivatives against human tumor cell lines.

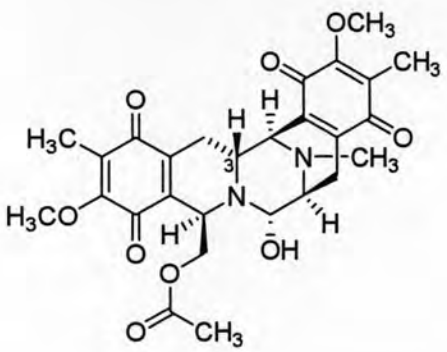
Compound	IC_{50} (nM)				
	HCT116	**QG56**	**NCI-H460**	**DLD1**	**DU145**
5-Fluorouracil	2.0	2.6	NT	NT	3.5
Saframycin A	0.4	5.5	NT	NT	NT
Ecteinascidin 770	1.2	3.9	NT	NT	NT
Renieramycin E	< 0.38	1.0	NT	NT	< 0.38
Renieramycin J	730.0	510.0	NT	NT	370.0
Renieramycin M	7.9	19.0	59.0	96.0	NT
Renieramycin N	5.6	11.0	67.0	57.0	NT
Renieramycin O	28.0	40.0	NT	NT	NT
Renieramycin Q	59.0	71.0	NT	NT	NT
Renieramycin R	23.0	29.0	NT	NT	NT
Renieramycin R'	33.0	99.0	NT	NT	NT
Renieramycin S	15.0	26.0	NT	NT	NT
Jorumycin	0.57	0.76	NT	NT	0.49
Deangeloylrenieramycin M	< 0.38	2.9	NT	NT	2.6
Decyanodeangeloylrenie-ramycin M	32.0	130.0	NT	NT	10.0
21-Cyanojorumycin	< 0.38	0.68	NT	NT	< 0.38
5,8-*O*-Diacetylhydro quinone renieramycin M	1.9	3.9	NT	NT	NT
5,8,15,18-*O*-Tetraacetyl-bishydroquinone-renieramycin M	3.0	7.9	NT	NT	NT
14-*O*-Acetylrenieramycin O	120.0	300.0	NT	NT	NT
14,15,18-*O*-Triacetyl-renieramycin N	630.0	1,600.0	NT	NT	NT

NT = not tested

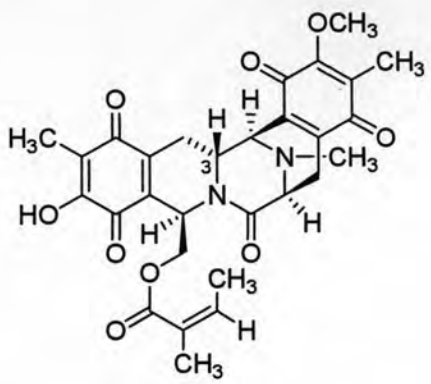
The above cytotoxic profile suggested the following observations. The leaving group (OH or CN) at C-21 was essential for cytotoxic activity. The oxygenated substituents at C-14 decreased the cytotoxicity. Renieramycin E, jorumycin,

deangeloylrenieramycin M, and 21-cyanojorumycin showed cytotoxicity with similar potency.

Other renieramycin analogs, 3-*epi*-jorumycin and 3-*epi*-renieramycin G, in addition to their respective parent natural products, jorumycin and renieramycin G were evaluated against both human colon (HCT116) and human lung (A549) cancer cell lines as shown in Table 8 (Lane *et al.*, 2006).



3-*epi*-jorumycin



3-*epi*-renieramycin G

Table 8 Antiproliferative activity of tetrahydroisoquinoline.

Compound	**GI_{50} in μM**	
	A594	**HCT116**
Jorumycin	0.0192 (± 0.0009)	0.0019 (±0.0002)
Renieramycin G	12.9	3.87 (±0.28)
3-*epi*-jorumycin	4.64 (±1.73)	0.61 (±0.04)
3-*epi*-renieramycin G	10.10 (±1.60)	1.40 (±0.46)

Jorumycin displayed a nanomolar growth inhibition profile while the corresponding epimer, 3-*epi*-jorumycin, possessed substantially less cytotoxicity. Both renieramycin G and the corresponding epimer, 3-*epi*-renieramycin G, containing and amide carbonyl group at C-21 also revealed less cytotoxicity at micromolar growth inhibition. Comparison of cytotoxicity between renieramycin G and 3-*epi*-renieramycin G showed that configuration at C-3 was not an influence. On the other hand, the difference of conformation between jorumycin and 3-*epi*-jorumycin affected to capability of alkylating ds-DNA (Lane *et al.*, 2006).

4. DNA microarrays

Microarray technology was introduced in 1995 (Manning *et al.*, 2007). DNA microarray is a revolutionary tool for high-throughput fashion. It is capable of simultaneous analysing the genomic expression levels of thousands of genes on a single chip (Celis *et al.*, 2000; Kuo *et al.*, 2002; Lamartine *et al.*, 2006). The concept behind DNA chips or microarrays technology relies on the complementary of the DNA duplex for example, two strands will always reassemble with base paring A to T and C to G. This reaction occurs with high specificity. Microarrays are usually made by deposit of DNA spots on the solid support like a coated glass surface, that differs in several ways from conventional filter-based supports such as charged nylon and nitrocellulose. Two main procedures have been used to produce DNA chips: photolithography as developed and marketed primarily by Affymetrix Inc. and mechanical gridding (Celis *et al.*, 2000). DNA microarrays have several types depending on the kind of DNA which is immobilized on a solid support for example, oligonucleotide microarray, cDNA microarray, and genomic microarray. This technique can be also used to compare gene expression levels at the messenger RNA (mRNA). The principle of DNA microarray technique for gene expression starts with isolation of RNA from a tissue which comprises a complex of mixture of different mRNA transcripts. The abundance of individual transcripts in the mixture is reflection of the expression levels of corresponding genes. A complementary DNA mixture copied from the mRNA is labelled with fluorophores and hybridized to microarray. The strength signal of fluorescence is produced in order to show the relative expression levels of the corresponding gene (Haviv and Campbell, 2002; Manning *et al.*, 2007).

At present, microarray has been proven to be useful in cancer research. This technology has become the most powerful tool for succession in cancer treatment (Manning *et al.*, 2007). Ecteinascidin 743 and pthalascidin (Pt 650), which displayed high cytotoxicity *in vitro*, were studied for gene expression by using oligonucleotide microarray. Only two compounds of tetrahydroisoquinoline alkaloids were studied in this technique. Array-based gene expression monitoring also demonstrated that ecteinascidin 743 and Pt 650 profiles were highly similar in two distinct cancer cell lines, HCT116 colon and MDA-MB-435 breast. Characteristic changed were observed in subsets of genes involved in DNA damage response, transcription and

signal transduction. In HCT116 carrying the wild-typ *p53* tumor suppressor gene, the up-regulation of several *p53*-responsive genes was evident. Furthermore, a subset of genes encoding DNA-binding proteins to specific promoter regions (e.g. the CCAAT box) was down-regulated in both cell lines, suggesting one potential mode of action of this series of antitumor agents (Martinez *et al.*, 2001).