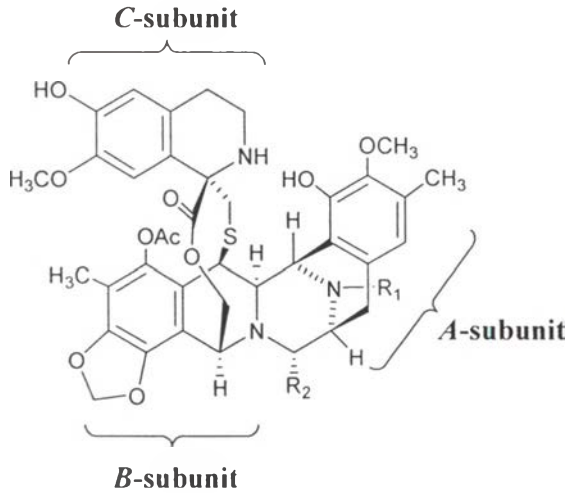
CHAPTER I

INTRODUCTION

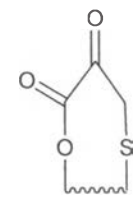
In the past several decades, marine organisms have been extensively explored as the potential new sources of natural products and developing number of new anticancer candidates have been disclosed. Among them, the ecteinascidins (**1-11**) with the novel tetrahydroisoquinoline entity have been isolated from the Caribbean tunicate *Ecteinascidia turbinata* (Rinehart *et al.*, 1990; Wright *et al.*, 1990) and received much attention as the promising candidate and the lead compound for drug optimization. The most abundant ecteinascidin, ecteinascidin 743 [Et 743; (**1**)] was originally selected and further developed as an anticancer agent due to its extremely potent cytotoxic activity in nanomolar concentration against varieties of tumor cell lines (Sakai *et al.*, 1992; 1996). Currently, Et 743 is undergoing the phase II/III clinical trials and has shown good activity against soft tissue sarcomas (STS), breast and ovarian cancers (Valoti *et al.*, 1998; Ryan *et al.*, 2002; Villalona-Calero *et al.*, 2002; Meco *et al.*, 2003; Twelves *et al.*, 2003).

Structurally, Et 743 bears significant structural similarity to the well known carbinolamine-containing natural antitumor agents such as saframycins (**13**) (Arai *et al.*, ; 1977; 1980; Yazawa *et al.*, 1982; 1986; Lown *et al.*, 1984; Saito *et al.*, 2000), safracins (**14-15**) (Ikeda *et al.*, 1983), renieramycins (**16**) (Frincke *et al.*, 1982; Davidson *et al.*, 1992; Suwanborirux *et al.*, 2003; Saito *et al.*, 2004; Amnuoypol *et al.*, 2004) and jorumycins (**17**) (Fontana *et al.*, 2000) as shown in Figure 2. The primary different structure is that the Et 743 contains an extra tetrahydroisoquinoline ring, namely C-subunit, through a spiro ten-membered lactone linkage, which is absent in the structure of the other carbinolamine-containing antitumor agents. While both *A*-and *B*-subunits provide the scaffold for DNA recognition and bonding, the C-subunit is projected out of the minor groove and make limited contacts with the target DNA (Pommier *et al.*, 1996; Moore *et al.*, 1997; 1998; Takebayashi *et al.*, 1999; Zewail-Foote *et al.*, 1999). Those carbinolamine-containing antitumor antibiotics without C-subunit moiety are less active

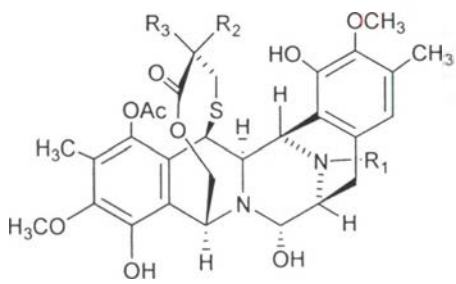
than Et 743 (Sakai *et al.*, 1992; 1996), therefore, it is interesting that the paucity of *C*-subunit might significantly interact with DNA or other functionality for cytotoxicity. Although, it remains questions how the minor groove of DNA recognizes the molecules and how the chemical modification on the *C*-subunit would affect the biological activity possess by the compounds.



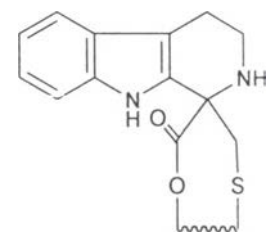
Et 743 (**1**); $R_1 = CH_3$, $R_2 = OH$
Et 770 (**2**); $R_1 = CH_3$, $R_2 = CN$
Et 759B (**3**); $R_1 = CH_3$, $R_2 = OH$, S-oxide
Et 786 (**4**); $R_1 = CH_3$, $R_2 = CN$, S-oxide
Et 729 (**5**); $R_1 = H$, $R_2 = OH$



Et 759 (**9**); $R_1 = CH_3$, $R_2 = OH$

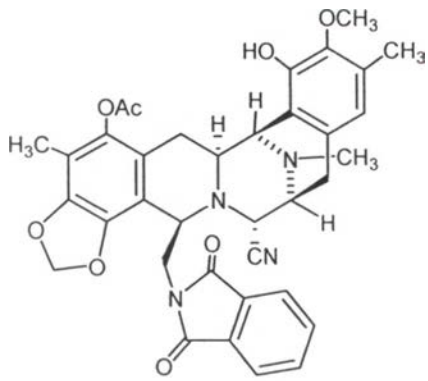


Et 597 (**6**); $R_1 = CH_3$, $R_2 = NH_2$, $R_3 = H$
Et 583 (**7**); $R_1 = H$, $R_2 = NH_2$, $R_3 = H$
Et 596 (**8**); $R_1 = CH_3$, R_2, $R_3 = O$

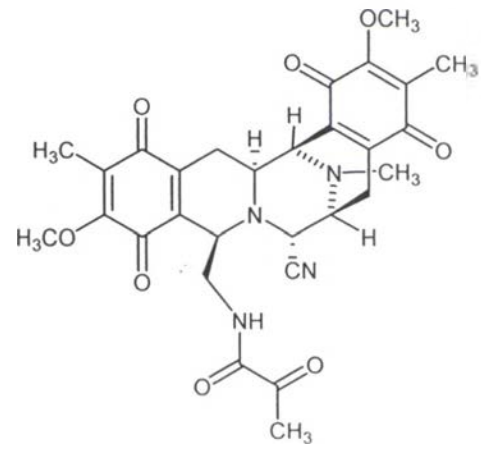


Et 736 (**10**); $R_1 = CH_3$, $R_2 = OH$
Et 722 (**11**); $R_1 = H$, $R_2 = OH$

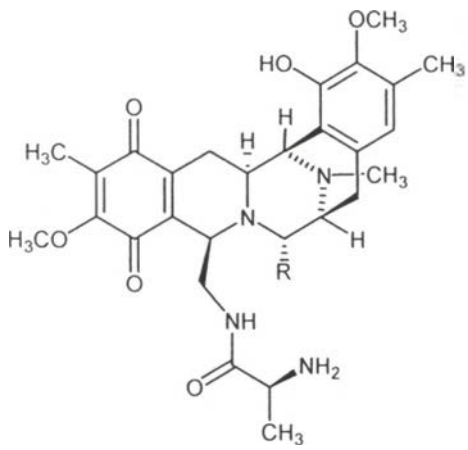
Figure 1. Structures of the natural ecteinascidins



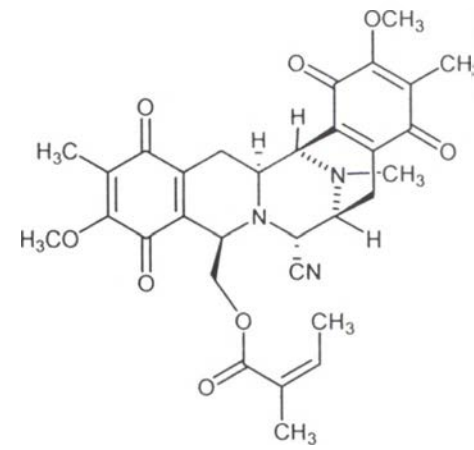
Phthalascidin (**12**)



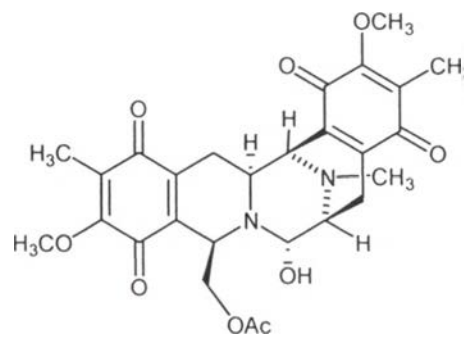
Saframycin A (**13**)



Safracin A (**14**), R = H
Safracin B (**15**), R = OH



Renieramycin M (**16**)



Jorumycin (**17**)

Figure 2. Representative examples of the α-carbinolamine-containing and the α-cyano-amine containing antitumor agents

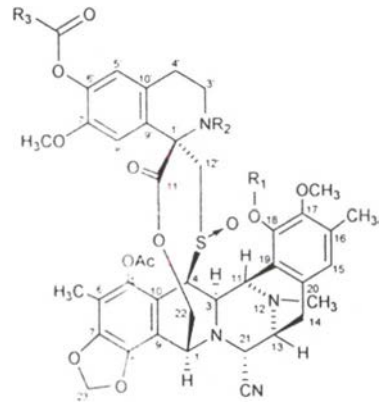
Due to the meager availability of natural source (one gram of Et 743 require huge amount of approximately one ton of the natural tunicate source) and the unique mechanism of action exhibited by this molecule made the drug a very attractive synthetic target (Zewail-Foote *et al.*, 2001; D'Incalci *et al.*, 2002). The first total synthesis of Et 743 was accomplished by Corey and co-worker (1996). Later, Corey, Schreiber and co-worker discovered a synthetic analogue of Et 743, phthalascidin (Pt-650, **12**) which was virtually the same in vitro activity (Martinez *et al.*, 1999; 2000). In 2000, the research scientist of the PharmaMar Laboratories developed a semi-synthetic route of Et 743 from cyanosafracin B (Cuevas *et al.*, 2000, Menchaca *et al.*, 2003), which was available through fermentation of the bacterial *Pseudomonas fluorescens* (Ikeda *et al.*, 1983). In addition, other total syntheses of Et 743 were described by Fukuyama (Endo *et al.*, 2002), Chen (2006), and Zheng (2006) groups.

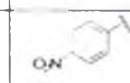
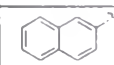
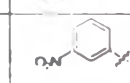
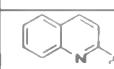
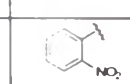
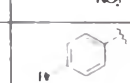
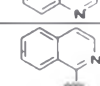
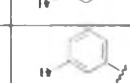
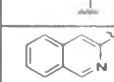
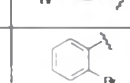
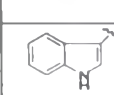
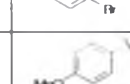
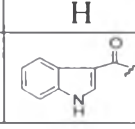
Herein, two ecteinascidins, Et 770 and Et 786 were isolated in high yield by the KCN pretreated method from the Thai tunicate *Ecteinascidia thurstoni* collected from Phuket Island of Thailand. Both Et 770 and Et 786 were chemically stabilized by converting the labile α-carbinolamine group to the more stable α-cyanoamine group. Both compounds also exhibited significant cytotoxic activities against breast cancer cell (BC) and nasopharynx carcinoma cell (KB) (Suwanborirux *et al.*, 2002). Previous studies indicated that the presence of either the nitrile or the hydroxyl groups at C-21 in close proximity to the nitrogen atom is essential for the biological activities especially the alkylating effect. Mechanistically, the elimination of these two moieties under the physiological condition resulted in the formation of the reactive iminium ion which on subsequence covalently bound to target DNA.

As described above, the Thai tunicate is the worthy source for the discovery of new anticancer agents. In this research, the KCN-pretreated strategy will be used to prepare Ets 770 and 786 from the Thai tunicate, which will serve as the starting materials for further structural modification. Herein, the effects of acetyl and aroyl moieties at the phenolic hydroxyl of the *A*- and *C*-subunits on cytotoxic activity would be our interests. The chemical structures of the desired compounds are shown in Table 1. The ester

derivatives of Ets 770, 786 and 743 synthesized would be studied on the antiproliferative assay, using three human cancer cell lines including colon carcinoma (HCT116), lung carcinoma (QG56) and prostate cancer (DU145).

Table 1. Chemical structures of ecteinascidin derivatives prepared in this study



Cpds	R_1	R_2	R_3	Cpds	R_1	R_2	R_3
18 (Et 770)	Ac	H	CH_3	**29**	H	H	
19 (Et 786)	Ac	H	CH_3	**30**	H	H	
20 (Et 743)	Ac	H	CH_3	**31**	H	H	
21	H	H		**32**	H	H	
22	H	H		**33**	H	H	
23	H	H		**34**	H	H	
24	H	H		**35**	H	H	
25	H	H		**36**	H	H	
26	H	H		**37**	H	H	
27	H	H		**38**	H	H	
28	H	H		**39**	H		H

Interestingly, Et 729 (**5**), the 12-*N*-demethyl derivative of Et 743, exhibited potent *in vitro* inhibitor of P388 murine leukemia with IC_{50} 0.93 nM, which was approximately equal potent to **1** (0.5 nM) (Rinehart *et al.*, 1990; Wright, *et al.*, 1990). In addition, the preclinical studies of **5** was found to be the active metabolite of **1** in rat (Reid *et al.*, 2002; Beumer *et el.*, 2005). Hence, *N*-demethylation of Et 770 (**2**) is an attractive target for new anticancer agent. The method for *N*-demethylation of **2** will be studied based on some simplified structures of ectienascidin molecule. Several types of the model ABC ring system representing the *A*-subunit of **1** will be used for the investigation as shown in Figure 3.

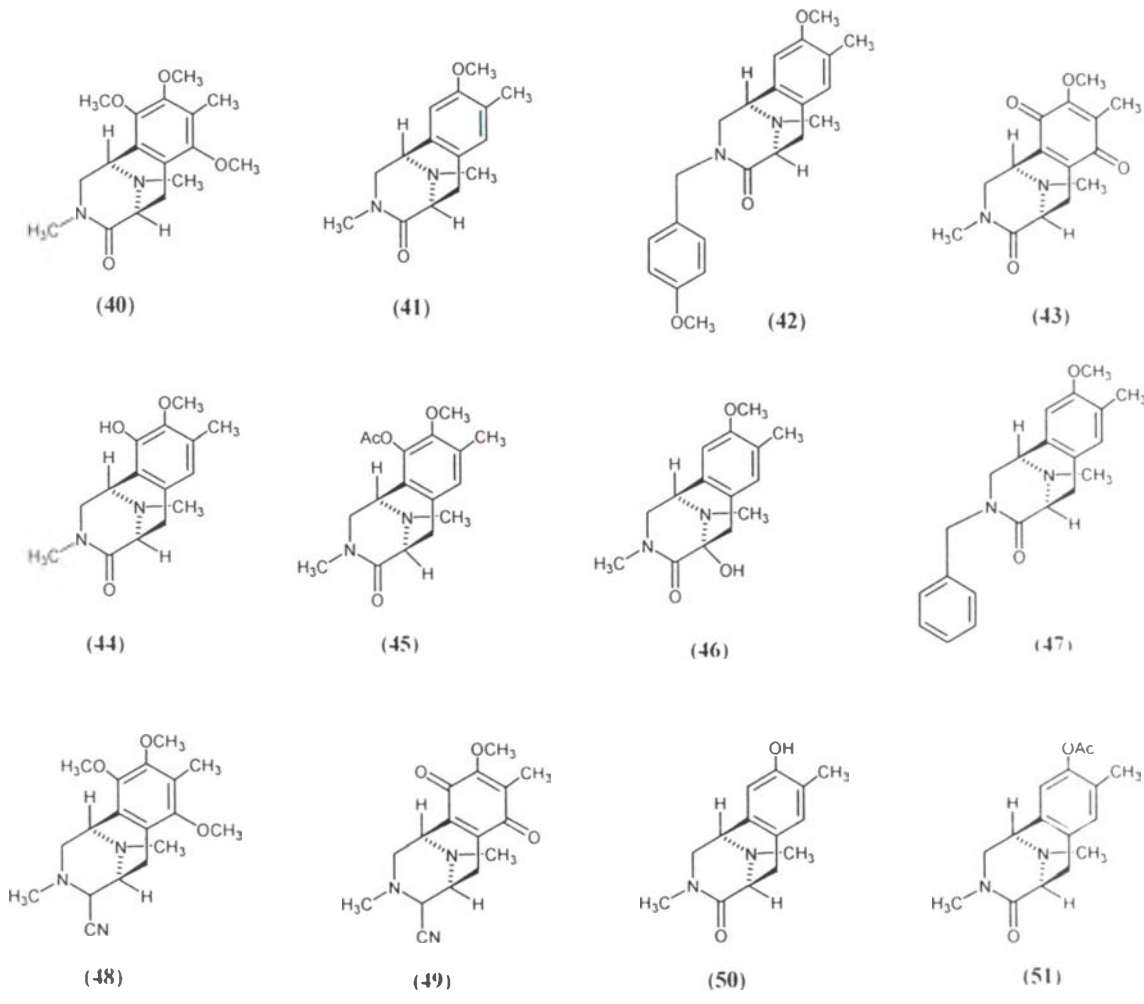


Figure 3. Chemical structures of the ABC model ring system