

การตัดแปลงโครงสร้างทางเคมีของสารต้านมะเร็ง อัลคาลอยด์เอคเตนาสซิดิน
จากเฟรียงหัวหอมไทย *Ecteinascidia thurstoni*

นางสาวเพลินทิพย์ ภูทองกิ่ง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเภสัชเคมีและผลิตภัณฑ์ธรรมชาติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2548
ISBN 974-14-2340-3
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHEMICAL STRUCTURE MODIFICATION OF ANTICANCER
ECTEINASCIDIN ALKALOIDS FROM THE THAI TUNICATE
ECTEINASCIDIA THURSTONI

Miss Ploenthip Puthongking

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy Program in Pharmaceutical Chemistry and Natural Products
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2005
ISBN 974-14-2340-3

481696

Thesis Title CHEMICAL STRUCTURE MODIFICATION OF
ANTICANCER ECTEINASCIDIN ALKALOIDS FROM
THE THAI TUNICATE ECTEINASCIDIA THURSTONI

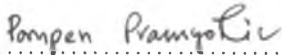
By Miss Ploenthip Puthongking

Field of Study Pharmaceutical Chemistry and Natural Products

Thesis Advisor Assistant Professor Chamnan Patarapanich, Ph.D.

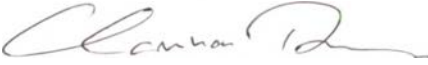
Thesis Co-advisor Khanit Suwanborirux, Ph.D.


Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn
University in Partial Fulfillment of the Requirements for the Doctor's Degree

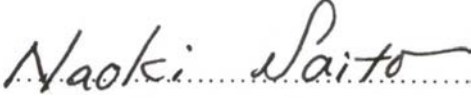

..... Dean of Faculty of
Pharmaceutical Sciences
(Associate Professor Pompen Pramyothin, Ph.D.)


THESIS COMMITTEE

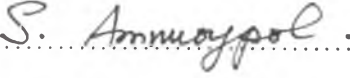

..... Chairman
(Assistant Professor Mitr Pathipvanich, Ph.D.)


..... Thesis Advisor
(Assistant Professor Chamnan Patarapanich, Ph.D.)


..... Thesis Co-advisor
(Khanit Suwanborirux, Ph.D.)


..... Member
(Professor Naoki Saito, Ph.D.)


..... Member
(Associate Professor Sunibhond Pummangura, Ph.D.)


..... Member
(Associate Professor Surattana Amnuoypol, Ph.D.)

เพลินทิพย์ ภูทองกิ่ง: การดัดแปลงโครงสร้างทางเคมีของสารต้านมะเร็ง อัลคาลอยด์เอคเตนาสซิดินจากเพรียงหัวหอมไทย *Ecteinascidia thurstoni* (CHEMICAL STRUCTURE MODIFICATION OF ANTICANCER ECTEINASCIDIN ALKALOIDS FROM THE THAI TUNICATE *ECTEINASCIDIA THURSTONI*) อ. ที่ปรึกษา: ผศ.ดร. ชำนาญ ภัทรพานิช, อ. ที่ปรึกษาร่วม: ดร. คณิต สุวรรณบริรักษ์, 169 หน้า, ISBN 974-14-2340-3

เอคเตนาสซิดิน 743 (Et 743: 1) เป็นสารกลุ่มทริสเตรราไฮโดรไอโซควินโนลินอัลคาลอยด์ที่แยกได้จากเพรียงหัวหอม *Ecteinascidia turbinata* เป็นสารที่มีประสิทธิภาพสูงในการต้านมะเร็ง และในปัจจุบันกำลังศึกษาสารต้านมะเร็งชนิดนี้ทางคลินิกขั้นที่ II/III ต่อมาได้มีการแยกสาร Et 770 (2) และ Et 786 (4) ในปริมาณสูงจากเพรียงหัวหอมไทย *E. thurstoni* ที่ผ่านกระบวนการแช่สกัดด้วยสารละลาย KCN จึงได้ทำการสังเคราะห์อนุพันธ์ ของเอคเตนาสซิดินที่แยกได้ทั้งสิ้น 21 ชนิด ได้แก่ อนุพันธ์ diacetate ของสาร Et 743, Et 770 และ Et 786 (18-20) โดยเป็นการแทนที่หมู่ฟังก์ชัน phenolic hydroxyl ที่ตำแหน่ง C-18 และ C-6' ของ A-subunit และ C-subunit ตามลำดับ และ อนุพันธ์ monoacyl ของสาร Et 770 (21-37) โดยเป็นการแทนที่หมู่ฟังก์ชัน phenolic hydroxyl ที่ตำแหน่ง C-6' ของ C-subunit โดยใช้ acid anhydrides, acid chlorides หรือ acids ร่วมกับ *N,N*-dicyclohexylcarbodiimide (DCC) ในทางตรงกันข้ามเมื่อสาร Et 770 ทำปฏิกิริยากับ indole-3-carboxylic acid ร่วมกับ DCC จะได้อนุพันธ์ amide 39 ซึ่งเป็นการแทนที่ตำแหน่ง N-2' ของ C-subunit

ได้นำอนุพันธ์ที่เตรียมได้ทั้งหมดมาทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง 3 ชนิด ได้แก่ HCT116 (เซลล์มะเร็งลำไส้) QG56 (เซลล์มะเร็งปอด) และ DU145 (เซลล์มะเร็งต่อมลูกหมาก) พบว่า หมู่ฟังก์ชัน cyano และ hydroxyl ที่ตำแหน่งคาร์บอนที่ 21 มีความจำเป็นต่อฤทธิ์ความเป็นพิษต่อเซลล์ นอกจากรอกไซไคส์หมู่ซัลไฟด์มีผลทำให้ฤทธิ์ความเป็นพิษต่อเซลล์ลดลงอย่างมาก อนุพันธ์ ester ของ benzoyl ส่วนใหญ่มีฤทธิ์ความเป็นพิษต่อเซลล์น้อยกว่าสารตั้งต้น (2) ในขณะที่อนุพันธ์ ester ของ 4"-nitrobenzoyl (22) และ 4"-methoxybenzoyl (28) แสดงฤทธิ์ความเป็นพิษต่อเซลล์ใกล้เคียงกับสารตั้งต้น (2) เช่นเดียวกับอนุพันธ์ ester ที่มีอะตอมไนโตรเจนอยู่ในวงแหวน (30-31 และ 34-37) และพบว่าอนุพันธ์ amide ของ indole-3-carbonyl (39) เป็นอนุพันธ์ชนิดเดียวที่แสดงฤทธิ์ความเป็นพิษต่อเซลล์ได้ดีกว่าสารตั้งต้น (2)

เมื่อศึกษาวิธีการ *N*-demethylation ของโมเลกุลจำลอง ซึ่งประกอบด้วยวงแหวน ABC ที่คล้ายคลึงกับ A-subunit ของสารเอคเตนาสซิดิน โดยใช้สาร cerium (IV) ammonium nitrate (CAN) พบสภาวะที่เหมาะสมคือ สารตั้งต้นทำปฏิกิริยากับ CAN จำนวน 5 เท่าในสารละลายของ acetonitrile อย่างไรก็ตามพบว่า มีข้อจำกัดคือ ต้องเปลี่ยนหมู่ cyanoamine เป็นหมู่ amide carbonyl และปกป้องหมู่ฟังก์ชัน phenolic hydroxyl ก่อนจึงจะทำให้การสังเคราะห์ตามวิธีดังกล่าวได้ผล

สาขาวิชา เกษษเคมีและผลิตภัณฑ์ธรรมชาติ

ปีการศึกษา 2548

ลายมือชื่อนิสิต..... พ.จ.ศ.ก. ภูทองกิ่ง

ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4576965833: MAJOR PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS

KEYWORD: ECTEINASCIDIN 770 / ACYL DERIVATIVES / CYTOTOXICITY / ABC RING SYSTEM

PLOENTHIP PUTHONGKING: CHEMICAL STRUCTURE MODIFICATION OF ANTICANCER ECTEINASCIDIN ALKALOIDS FROM THE THAI TUNICATE *ECTEINASCIDIA THURSTONI*: THESIS ADVISOR: ASST. PROF. CHAMNAN PATARAPANICH, Ph.D., THESIS CO-ADVISOR: KHANIT SUWANBORIRUX, Ph.D., 169 pp. ISBN 974-14-2340-3

Ecteinascidin 743 (Et 743; **1**), a member of the tristetrahydroisoquinoline alkaloids previously isolated from the tunicate, *Ecteinascidia turbinata*, is a potent anticancer agent and is currently undergoing in phase II/III clinical trials. Et 770 (**2**) and Et 786 (**4**), two anticancer agents from the Thai tunicate, *E. thurstoni*, were efficiently isolated with the KCN pretreated process. Three diacetate esters of Ets 743, 770, and 786 (**18-20**) were prepared and the acetylation was reacted to the phenolic hydroxyls at C-18 and C-6' of the ecteinascidins. Seventeen acyl esters were prepared on the phenolic hydroxyl at C-6' of Et 770 with the corresponding acid anhydrides or acid chlorides or acids in the presence of *N,N*-dicyclohexylcarbodiimide (DCC) as the coupling reagent to yield the monoacyl derivatives (**21-37**). In contrast, the reaction of indole-3-carboxylic acid with Et 770 in the presence of DCC provided the amide derivative **39**, which was substituted at N-2' of the C'-subunit.

The synthesized derivatives were evaluated for cytotoxicity against HCT116 (human colon carcinoma), QG56 (human lung carcinoma), and DU145 (human prostate carcinoma) and exhibited excellent cytotoxic activities at nanomolar concentration. The cytotoxic data supported that the cyano or the hydroxy groups at C-21 position are essential for the cytotoxic activity. Moreover, oxidation of the sulfide bridge of ecteinascidins resulted in dramatically diminished activity. Most benzoyl ester derivatives exhibited dramatically decreased cytotoxicity while 4"-nitrobenzoyl (**22**) and 4"-methoxybenzoyl (**28**) ester derivatives as well as the *N*-containing heterocyclic ester derivatives (**30-31** and **34-37**) possessed cytotoxicity similar to the precursor (**2**). Interestingly, only the indole-3-carbonyl amide derivative (**39**) exhibited higher cytotoxicity than the parent compound (**2**).

The selective *N*-demethylation with cerium (IV) ammonium nitrate (CAN) was worked on the structural models containing the ABC ring system, which included the *A*-subunit of Ets. The optimal condition was achieved in 5 equimolars of CAN in the presence of aqueous-acetonitrile. However, this investigation revealed that the protection at the phenolic hydroxyl and the transformation of cyanoamine to amide carbonyl group are the key steps for the oxidative *N*-demethylation using CAN for those model compounds.

Field of Study Pharmaceutical Chemistry
and Natural Products

Academic Year 2005

Student's signature..... *Ploenthip Puthongking*

Advisor's signature..... *Chamnana Patrapanich*

Co-advisor's signature..... *Khanit Suwanborirux*

ACKNOWLEDGEMENTS

I would like to express my appreciation to my thesis advisors, Assistant Professor Chamnan Patarapanich and Dr. Khanit Suwanborirux and my Japanese teachers, Professor Naoki Saito and Professor Akinori Kubo, of Meiji Pharmaceutical University, than whom no-one could have been more helpful and courteous, for their valuable advice, continual guidance, kindness, and understanding throughout this research study.

I would like to thank the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences for providing a 300 MHz NMR spectrometer and other scientific equipments, Ms. T. Kozeki and S. Kubota of the Analytical Center of Meiji Pharmaceutical University, for recording 500 MHz NMR and mass spectra, and Dr. N. Shimma, Chugai Pharmaceutical Company Research Center, Japan for cytotoxic assay.

I would like to thank Mr. Sombat Poovachiranon (Phuket Marine Biological Center) for supporting the collection of the Thai tunicate.

Financial supports for this work were provided by a Grant (BRT R646004) from the Biodiversity Research and Training Program (BRT), National Science and Technology Development Agency (NSTDA), Thailand, and a Grant-in-Aid for Scientific Research (B) (No. 1437025) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The Bioactive Marine Natural Product Chemistry Research Unit (BMNCU) is supported by a Grant for Centers of Excellence, Chulalongkorn University. Japan Society for the Promotion of Sciences (JSPS) and the National Research Cooperation of Thailand (NRCT) supported the collaboration between Thai and Japanese researchers. The partial supports by Onda Foundation, Meiji Pharmaceutical University, Japan, the Graduate School of Chulalongkorn University and Khon Kaen University were acknowledged.

I would like to thank all staff of the Department of Pharmaceutical Chemistry for their supporting during my study.

My sincere appreciation is conveyed to Associate Professor Surattana Amnuoypol, Miss Emi Saito and all my friends at Chulalongkorn University, Khon Kaen University and Meiji Pharmaceutical University who have shared happy time together.

Finally, I would like to express my special and deepest appreciation to my family, for their love, understanding and encouragement.

CONTENTS

	Page
THAI ABSTRACT.....	iv
ENGLISH ABSTRACT.....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF FIGURES.....	x
LIST OF SCHEMES.....	xix
LIST OF TABLES.....	xxi
ABBREVIATIONS.....	xxiii
CHAPTER	
I INTRODUCTION.....	1
II LITERATURE REVIEW.....	7
1. Characteristics of tunicates.....	7
2. The tetrahydroisoquinoline antitumor agents.....	8
3. Isolation and biosynthesis of ecteinascidin alkaloids.....	9
4. Structure determination of Et 743.....	14
5. Synthetic studies of ecteinascidin.....	16
6. Biological activity of ecteinascidins.....	22
7. N-demethylation the ABC ring model compounds.....	31
III EXPERIMENTAL.....	39
1. Animal material.....	39
2. General experimental procedures.....	39
2.1. Thin-layer chromatography (TLC).....	39
2.2. Column Chromatography.....	39
2.2.1. Flash column chromatography.....	39
2.2.2. Gel filtration chromatography.....	40

CHAPTER	Page
3. Physical constants and spectroscopy.....	40
3.1. Proton and carbon nuclear magnetic resonance (¹ H- and ¹³ C-NMR) spectroscopy.....	40
3.2. Mass spectroscopy.....	40
3.3. Infrared (IR) spectroscopy.....	41
3.4. Optical rotation.....	41
4. Chemical reagents.....	41
5. Solvents.....	42
6. Extraction and isolation of ecteinascidins from the Thai tunicate, <i>Ecteinascidia thurstoni</i>	42
7. Structural modification of ecteinascidins.....	45
7.1. Transformation of ecteinascidins.....	45
7.1.1. Transformation of Et 770 to Et 743.....	45
7.1.2. Transformation of Et 770 to Et 786.....	45
7.1.3. Transformation of Et 786 to Et 759B.....	46
7.2. Preparation of ecteinascidins analogs.....	46
7.3. Selective N-demethylation on ABC ring system with CAN.....	63
8. Biological assay.....	66
IV RESULTS AND DISCUSSION.....	67
1. Extraction and isolation of the ecteinascidins from the Thai tunicate, <i>Ecteinascidia thurstoni</i>	67
2. Structural modification.....	70
2.1. Transformations of Et 770 to Et 743 and Et 786 to Et 759B.....	70
2.2. Preparation of ecteinascidin analogs.....	72

CHAPTER	Page
2.2.1. Preparation of acetate ester analogs.....	72
2.2.2. Preparation of aromatic ester analogs.....	78
3. Cytotoxic activity of ecteinascidin analogs.....	95
4. Selective <i>N</i> -demethylation on ABC ring system with CAN.....	98
V CONCLUSION.....	107
REFERENCES.....	109
APPENDICES.....	117
VITA.....	169

LIST OF FIGURES

Figure	Page
1. Structure of the natural ecteinascidins.....	2
2. Representative examples of the α -carbinolamine-containing and the α -cyanoamine containing antitumor agents.....	3
3. Chemical structures of ABC model ring system.....	6
4. Structures of some other representative tetrahydroisoquinoline antitumor antibiotics.....	9
5. The picture of <i>Ecteinascidia turbinata</i> from the Caribbean tunicate.....	10
6. The picture of <i>Ecteinascidia thurstoni</i> collected from Phuket island, Thailand.....	10
7. The proposed biosynthetic precursors of saframycin A.....	11
8. The partial fragmentation in FABMS of Et 743.....	14
9. The partial long-range proton-carbon correlations in the HMBC spectra of Et 743.....	15
10. Stereoscopic X-ray crystallographic structure of 21-(<i>O</i>)-methyl- <i>N</i> ¹² -formyl Et 729 and Et 734 <i>N</i> ¹² -oxides.....	15
11. A model of the Et-DNA adduct produced by a computer modeling study (The DNA sequence is d(TTGGGAA), with the middle G as the target base for the alkylation reaction of Et).....	27
12. Partial model of Et-743 bound to the 12-mer oligonucleotide.....	28
13. Structure of Ets 743, 729, 736 and 722.....	32
14. Structure of Et 743, safracin A and the ABC ring model of ecteinascidins...	36
15. Partial ¹ H and ¹³ C-NMR assignment data of the <i>A</i> - and <i>C</i> '-subunits of Et 770 (2) and Et 770 18, 6'-diacetyl [18].....	76
16. 3D-structure of ecteinascidin molecule.....	79

Figure	Page
17. The proposed FABMS fragmentat pattern of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	85
18. Partial ¹³ C-NMR assignment data in the <i>C</i> -subunit of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	88
19. Partial correlations observed in the NOESY spectrum of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22) in the <i>A</i> -subunit.....	89
20. 300 MHz ¹ H-NMR spectra of 2'- <i>N</i> -3"-Indolecarbonyl ecteinascidin 770....	93
21. Structural of the acylation of eceinascidins.....	96
22. ¹ H-NMR spectra data of 40 , 40a , and 40b	102
23. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of Et 770 (2).....	117
24. The 125 MHz ¹³ C-NMR, DEPT-135 spectra (in CDCl ₃) of Et 770 (2).....	117
25. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of Et 786 (4).....	118
26. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of Et 759B (3).....	118
27. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of Et 743 (1).....	119
28. The FAB-mass spectrum of Et 743 (1).....	119
29. The IR spectrum 18,6'-diacetylecteinascidin 770 (18).....	120
30. The FAB-mass spectrum of 18,6'-diacetyl-ecteinascidin 770 (18).....	120
31. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 18,6'-diacetyl- ecteinascidin 770 (18).....	121
32. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of 18,6'-diacetyl- ecteinascidin 770 (18).....	121
33. The 125 MHz ¹³ C-NMR and DEPT-135 spectra (in CDCl ₃) of 18,6'-diacetylecteinascidin 770 (18).....	122
34. The IR spectrum 18,6'-diacetylecteinascidin 786 (19).....	122
35. The FAB-mass spectrum of 18,6'-diacetyl-ecteinascidin 786 (19).....	123

Figure	Page
36. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 18,6'-diacetyl- ecteinascidin 786 (19).....	123
37. The IR spectrum 18,6'-diacetylecteinascidin 743 (20).....	124
38. The FAB-mass spectrum of 18,6'-diacetyl-ecteinascidin 743 (20).....	124
39. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 18,6'-diacetyl- ecteinascidin 743 (20).....	125
40. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -benzoate (21).....	125
41. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -benzoate (21).....	126
42. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -benzoate (21).....	126
43. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	127
44. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	127
45. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	128
46. The 500 MHz NOESY spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	128
47. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	129
48. The 500 MHz ¹³ C-NMR and DEPT spectra (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	129
49. The 500 MHz HMQC spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	130
50. The 500 MHz HMBC spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate (22).....	130
51. The 500 MHz HMBC spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"- nitrobenzoate (22) (expanded from δ _H 1.8-4.7 ppm and δ _C 115-173 ppm)..	131

Figure	Page
52. The 500 MHz HMBC spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -4"-nitrobenzoate (22) (expanded from δ_H 4.9-8.5 ppm and δ_C 105-172 ppm)..	131
53. The FAB-mass spectrum of Ecteinasidin 770 6'- <i>O</i> -3"-nitrobenzoate (23).	132
54. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -3"-nitrobenzoate (23).....	132
55. The FAB-mass spectrum of Ecteinasidin 770 6'- <i>O</i> -2"-nitrobenzoate (24).	133
56. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -2"-nitrobenzoate (24).....	133
57. The IR spectrum of Ecteinasidin 770 6'- <i>O</i> -4"-bromobenzoate (25).....	134
58. The FAB-mass spectrum of Ecteinasidin 770 6'- <i>O</i> -4"-bromobenzoate(25).	134
59. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -4"-bromobenzoate (25).....	135
60. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -4"-bromobenzoate (25).....	135
61. The IR spectrum of Ecteinasidin 770 6'- <i>O</i> -3"-bromobenzoate (26).....	136
62. The FAB-mass spectrum of Ecteinasidin 770 6'- <i>O</i> -3"-bromobenzoate (26)	136
63. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -3"-bromobenzoate (26).....	137
64. The IR spectrum of Ecteinasidin 770 6'- <i>O</i> -2"-bromobenzoate (26).....	137
65. The FAB-mass spectrum of Ecteinasidin 770 6'- <i>O</i> -2"-bromobenzoate(27).	138
66. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinasidin 770 6'- <i>O</i> -2"-bromobenzoate (27).....	138
67. The IR spectrum of Ecteinasidin 770 6'- <i>O</i> -4"-methoxybenzoate (27).....	139
68. The FAB-mass spectrum of Ecteinasidin 770 6'- <i>O</i> -4"-methoxybenzoate (27).....	139

Figure	Page
69. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-methoxybenzoate (28).....	140
70. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -2"-methoxybenzoate (29).....	140
71. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -2"-methoxybenzoate (29).....	141
72. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -2"-methoxybenzoate (29).....	141
73. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -2"-methoxybenzoate (29).....	142
74. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -nicotinate (30).....	142
75. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -nicotinate (30).....	143
76. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -nicotinate (30).....	143
77. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -nicotinate (30).....	144
78. The 500 MHz ¹³ C-NMR and DEPT spectra (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -nicotinate (30).....	144
79. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -isonicotinate (31).....	145
80. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -isonicotinate (31)...	145
81. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -isonicotinate (31).....	146
82. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -isonicotinate (31).....	146
83. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -1"-naphthoate (32).....	147
84. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -1"-naphthoate (32).....	147

Figure	Page
85. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -1"-naphthoate (32).....	148
86. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -2"-naphthoate (33).....	148
87. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -2"-naphthoate (33)....	149
88. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -2"-naphthoate (33).....	149
89. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -2"-naphthoate (33).....	150
90. The 500 MHz ¹³ C-NMR and DEPT spectra (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -2"-naphthoate (33).....	150
91. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -2"-quinolinecarboxylate (28)..	151
92. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -2"-quinoline- carboxylate (34).....	151
93. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -2"-quinolinecarboxylate (34).....	152
94. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -4"-quinolinecarboxylate (35)..	152
95. The FAB-mass spectrum of Ecteinascidin 770 6'- <i>O</i> -4"-quinoline- carboxylate (35).....	153
96. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) Ecteinascidin 770 6'- <i>O</i> -4"-quinolinecarboxylate (35).....	153
97. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-quinolinecarboxylate (35).....	154
98. The 500 MHz ¹³ C-NMR and DEPT spectra (in CDCl ₃) of Ecteinascidin 770 6'- <i>O</i> -4"-quinolinecarboxylate (35).....	154
99. The IR spectrum of Ecteinascidin 770 6'- <i>O</i> -1"- isoquinoline- carboxylate (36).....	155

Figure	Page
100. The FAB-mass spectrum of Ecteinascidin 770 6'-O-1"- isoquinoline-carboxylate (36).....	155
101. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'-O-1"- isoquinolinecarboxylate (36).....	156
102. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'-O-1"- isoquinolinecarboxylate (36).....	156
103. The IR spectrum of Ecteinascidin 770 6'-O-3"- isoquinolinecarboxylate (37)	157
104. The FAB-mass spectrum of Ecteinascidin 770 6'-O-3"- isoquinoline-carboxylate (37).....	157
105. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'-O-3"- isoquinolinecarboxylate (37).....	158
106. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of Ecteinascidin 770 6'-O-3"- isoquinolinecarboxylate (37).....	158
107. The IR spectrum of 2'-N-3"-indolecarboxylecteinascidin 770 (39).....	159
108. The FAB-mass spectrum of 2'-N-3"-indolecarboxylecteinascidin 770 (39)	159
109. The 500 MHz ¹ H-NMR spectrum (in CDCl ₃) of 2'-N-3"-indolecarbonyl ecteinascidin 770 (39).....	160
110. The 500 MHz ¹ H- ¹ H COSY spectrum (in CDCl ₃) of 2'-N-3"-indolecarbonyl ecteinascidin 770 (39) (expanded from δ ₁₁ 1.9 to 4.7 ppm).....	160
111. The 125 MHz ¹³ C-NMR spectrum (in CDCl ₃) of 2'-N-3"-indolecarboxylecteinascidin 770 (39).....	161
112. The 500 MHz ¹³ C-NMR and DEPT spectra (in CDCl ₃) of 2'-N-3"-indolecarboxylecteinascidin 770 (39).....	161
113. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-7,9,10-trimethoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin (40)..	162

Figure	Page
114. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-7,9,10-trimethoxy-3,8-dimethyl-4-oxo-1,5-imino-3-benzazocin (40a).....	162
115. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) 1,2,3,4,5,6-Hexahydro-7,9,10-trimethoxy-3,8-dimethyl-4-oxo-1,5-imino-11-carbonyl-3-benzazocin (40b).....	163
116. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-9-methoxy-3,8-dimethyl-4-oxo-1,5-imino-3-benzazocin (41a).....	163
117. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-9-methoxy-8,11-dimethyl-3-{4-methoxy-1-phenylmethyl}-4-oxo-1,5-imino-3-benzazocin (42).....	164
118. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-9-methoxy-8-methyl-3-{4-methoxy-1-phenylmethyl}-4-oxo-1,5-imino-3-benzazocin (42a).....	164
119. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-9-methoxy-8-methyl-3-{4-methoxy-1-phenylmethyl}-4-oxo-1,5-imino-11-carbonyl-3-benzazocin (42b).....	165
120. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-9-methoxy-3,8-dimethyl-7,10-quinone-4-oxo-1,5-imino-3-benzazocin (43a).....	165
121. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-hexahydro-10-hydroxy-9-methoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin (44).....	166
122. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of Bis-1,2,3,4,5,6-hexahydro-10-hydroxy-9-methoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin (44b).....	166

Figure	Page
123. The FAB-mass spectrum of Bis-1,2,3,4,5,6-hexahydro-10-hydroxy-9-methoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin (44b).....	167
124. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-10-acetate-9-methoxy-3,8,11-trimethyl-4-oxo-1,5-imino-3-benzazocin (45).....	167
125. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-10-acetate-9-methoxy-3,8-dimethyl-4-oxo-1,5-imino-3-benzazocin (45a).....	168
126. The 300 MHz ¹ H-NMR spectrum (in CDCl ₃) of 1,2,3,4,5,6-Hexahydro-10-acetate-9-methoxy-3,8-dimethyl-4-oxo-1,5-imino-11-carbonyl-3-benzazocin (45b).....	168

LIST OF SCHEMES

Scheme	Page
1. Proposed biosynthetic pathway of saframycin A.....	12
2. Proposed biosynthetic pathway of ecteinascidins.....	13
3. Corey's total synthesis of Et 743.....	17
4. Corey's synthesis of Phthalascidin-650.....	18
5. Preparation of Phthalascidin analogs.....	18
6. Cuevas' s semi-synthetic route of Et 743.....	20
7. Cuevas' s synthetic route of Phthalascidin-650.....	20
8. Fukuyama's total synthetic route of Et 743.....	21
9. To proposed mechanism reaction of an iminium intermediate with the guanine 2-amino group.....	27
10. Synthetic pathway of Et 729.....	34
11. The oxidative <i>N</i> -demethylation of amines using the ruthenium- catalyzed reaction with a) <i>tert</i> -butyl hydroperoxide. b) hydrogen peroxide.....	35
12. The oxidative <i>N</i> -demethylation of amines methyl chloroformate and hydrazine.....	35
13. Kubo's synthesis of the ABC ring model of ecteinascidins.....	37
14. Synthetic rout of the useful intermediate 129	38
15. Practical synthesis of the ABC ring models of ecteinascidins.....	38
16. Extraction of <i>Ecteinascidia thurstoni</i> collected in October 2002 and January 2003.....	43
17. Isolation of ecteinascidin compounds from the tunicate collected in October 2002 and January 2003.....	44
18. The extraction and isolation methods of Et 770 and Et 786 from <i>Ecteinascidia thurstoni</i>	69

Scheme	Page
19. Transformation routes of Et 770 to Et 743, and Et 786 to Et 759B.....	70
20. The proposed mechanism of hydroxylation reaction <i>via</i> an iminium ion intermediate.....	71
21. The mechanism of the S-oxidation of Et 770.....	72
22. Preparative scheme of diacetate ester analogs of the ecteinascidins.....	73
23. The proposed reaction mechanism of esterification reaction.....	74
24. The proposed mechanism of acylation of isoquinolinoyl derivatives <i>via</i> DCC coupling reagent	83
25. Preparation of isoquinolinoyl derivatives <i>via</i> anhydride reagent.....	84
26. The reaction of Et 770 and indole-3-carboxylic acid bearing acid chloride, acid anhydride and the acid with DCC.....	91
27. The Polonovsky reaction of 99	98
28. The oxidative <i>N</i> -demethylation of 40 with CAN.....	99
29. The oxidative <i>N</i> -demethylation of 41 with CAN	100
30. The oxidative <i>N</i> -demethylation of 42 , 46 and 47 with CAN	101
31. The oxidative <i>N</i> -demethylation of 43 containing the quinone moiety with CAN.....	102
32. The oxidative <i>N</i> -demethylation of 48 and 49 , containing the cyanoamine group with CAN	103
33. The oxidative <i>N</i> -demethylation of 44 with CAN	104
34. The acetylation reaction and the oxidative <i>N</i> -demethylation of 50 with CAN.....	105
35. The acetylation reaction and the oxidative <i>N</i> -demethylation of 44 with CAN.....	106

LIST OF TABLES

Table	Page
1. Chemical structures of ecteinascidin derivatives prepared in this study.....	5
2. The <i>in vitro</i> activity of Et 743 against several tumor cell lines.....	22
3. The <i>in vitro</i> antitumor activity of Et 743.....	23
4. The <i>in vitro</i> activity of Et 743 against Human tumor Xenografted in Nude Mice ^a	23
5. Structure and antiproliferative of phthalascidin-related compounds.....	24
6. Antiproliferative activity against mutant P388 of Et 743 and Pt 60.....	25
7. The mode of action of Et 743 ^a	25
8. The <i>in vitro</i> biochemistry profile of Et 743 ^a	26
9. The activity of Et 722 against several tumor cell lines.....	32
10. The activity of Ets 729, 743, and 745 against P 388 leukemia.....	33
11. Isolation data of Et 770 and Et 786 of each tunicate collection	68
12. ¹ H and ¹³ C-NMR spectra data of Et 770 [2] and Et 770 18,6'-diacetyl [18] recorded in CDCl ₃	77
13. Some of the most potent bishydroquinone derivatives of saframycin A and their antiproliferative activity.....	78
14. Summarized yields, High-resolution FABMS data and methods for prepared monoacyl derivatives of Et 770.....	81
15. ¹ H-and ¹³ C-NMR spectra data of the acyl moiety of the aroyl substituted Et 770 derivatives (21-37).....	86
16. Partial ¹ H and ¹³ C-NMR spectra data of core structure of Et 770 [2] and Ecteinascidin 770 6'- <i>O</i> -4"-nitrobenzoate [22] recorded in CDCl ₃	90
17. Partial ¹ H and ¹³ C-NMR spectra data of core structure of Et 770 [2] and 2'- <i>N</i> -3"-Indolecarboxyl ecteinascidin 770 [32] recorded in CDCl ₃	94

Table	Page
18. Cytotoxicity of ecteinascidin 770 acylated compounds to various cancer cell lines (IC_{50} nM) ^a	97
19. The different conditions of oxidative <i>N</i> -demethylation of 40 with CAN...	100
20. The different conditions of oxidative <i>N</i> -demethylation of 41 with CAN...	100

ABBREVIATIONS

%	=	percent
μg	=	microgram
μl	=	microliter
μM	=	micromolar
$[\alpha]_D^{25}$	=	specific rotation at 25 °C and sodium D line (589 nm)
Å	=	angstrom
°C	=	degree Celsius
¹ H-NMR	=	proton nuclear magnetic resonance
¹³ C-NMR	=	carbon-13-nuclear magnetic resonance
2D NMR	=	two dimensional nuclear magnetic resonance
A357	=	malignant melanoma cell line
B16	=	melanoma cell line
br s	=	broad singlet
c	=	concentration
cm	=	centrimeter
calcd	=	calculated
d	=	doublet (for NMR spectra)
dd	=	doublet of doublets (for NMR spectra)
dt	=	doublet of triplets (for NMR spectra)
DEPT	=	Distortionless Enhancement by Polarization Transfer
DU145	=	human prostate cancer cell line
eq.	=	equation or equivalent
ESI	=	Electrospray Ionization
g	=	gram
h	=	hour
HCT116	=	human colon cancer cell line
¹ H- ¹ H COSY	=	¹ H- ¹ H correlation spectroscopy
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
HT29	=	colon cancer cell line
Hz	=	hertz

IC ₅₀	=	50% inhibition concentration
IR	=	infrared spectrometry
<i>J</i>	=	coupling constant (for NMR spectra)
Kg	=	kilogram
L, l	=	liter
L1210	=	leukemia cell line
m	=	multiplet (for NMR spectra)
mg	=	milligram
min	=	minute
ml	=	milliliter
<i>m/z</i>	=	mass to charge
M	=	molar
M ⁺	=	molecular ion
MEL-28	=	melanoma cell line
MHz	=	megahertz
nM	=	nanomolar
NMR	=	Nuclear Magnetic Resonance
NOE	=	Nuclear Overhauser Effect
ppm	=	part(s) per million
P388	=	murine leukemia cell
PC-3	=	prostrate carcinomar
q	=	quartet
QC56	=	human carcinomar cell line
rt	=	room temperature
s	=	singlet (for NMR spectra)
t	=	triplet (for NMR spectra)
w, wt	=	weight