Master's Thesis of Natural Sciences

A Novel Cytotoxic Secondary Metabolite from *Streptomyces* sp. CMDD20H95 through Bioactivity-Guided Fractionation: Its Isolation and Structure Elucidation

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Laboratory of Marine Drugs

Graduate School of Earth and Environmental Sciences

Seoul National University

Marine Natural Products Chemistry Major

Juni Lee

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지도 교수 강 헌 중

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서울대학교 대학원
지구환경과학부 해양천연물화학
이 준 이

이준이의 이학석사 학위논문을 인준함

2022년 2월

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부위원장 _____(인)

위 원 _____(인)

Abstract

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Juni Lee
School of Earth and Environmental Sciences
Marine Natural Products Chemistry Major
The Graduate School
Seoul National University

Cancer is among the leading causes of death worldwide and characterized by spreading uncontrollably growing cells over each different part of the body. Natural products have been a great source of anti-cancer medicinal agents. Many anti-cancer drugs were originated from natural sources. Due to the wide range of structural diversity of natural products, we can discover innovative candidates for the therapeutic application of cancer. In this study, we mainly focused on the discovery of anti-cancer compounds from the secondary metabolites of *Streptomyces cavourensis*. The strain was isolated from the leaves of the plant *Cinnamomum cassia* in Hoa Binh province of northern Vietnam. Experiments including cultivation of *S. cavourensis*, and isolation, purification, structure elucidation of the compound were conducted and bioactive-guided fractionation was carried out according to MTT assay results which show cell viability. First, the crude extract was examined whether it contains anti-cancer compounds through MTT assay. Then, further separation and purification of the active compound using various chromatographic methods, and its biological activity investigation were performed. The structure of the compound was elucidated and confirmed using spectroscopic methods such as Nuclear Magnetic Resonance (NMR) spectroscopy (^{1}H, ^{13}C, COSY, HSQC, HMBC, and ROESY), mass spectrometry, and UV spectrometry. From these data, we obtained the full structure of the compound and named it cavomycin A. In the MTT assay, five cancer

cell lines were tested including lung carcinoma cells (A549), prostate carcinoma cells (DU145), colorectal carcinoma cells (HCT116), breast carcinoma cells (MCF7), and pancreatic carcinoma cells (PANC-1). The cells were treated with cavomycin A at different doses (from 50 nM to 100 μM). As a result, cavomycin A showed potent inhibitory activity against the proliferation of all tested cancer cells with IC_{50} values of around 0.1 μM.

Keywords: Natural products, Secondary metabolites, Actinobacteria, *Streptomyces*, Cancer, Bioactivity-guided fractionation

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Chapter 1

General Introduction of Natural Products

1.1 Natural Products

A well-known journal, nature portfolio defines natural products as below.

"Natural products are small molecules produced naturally by any organism including primary and secondary metabolites. They include very small molecules, such as urea, and complex structures, such as Taxol. As they may only be isolable in small quantities, have interesting biological activity and chemical structures, natural product synthesis poses an interesting challenge in organic chemistry."

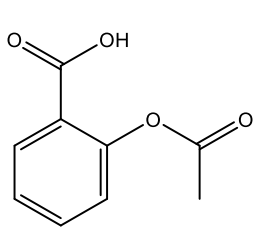
World Health Organization announced that about 80% of the world's population uses traditional medicine for their health care. Looking at ancient history, our ancestors used natural products as healing agents[1,2]. Traditional medicinal practices have been the foundation of pharmaceutical studies. Approximately 1000 plants and plant-derived products including the oils of *Cedrus* species (cedar), *Cupressus sempevirens* (cypress) and, *Papaver somniferum* (poppy juice) are still used for the treatment of various diseases. Also, Ebers Papyrus, the historical record includes over 700 natural substances originated drugs. In Greek, hundreds of natural products and plant-derived medicines were used by physicians and pharmacists. The Chinese, the major country in the use of natural products in the pharmaceutical field, possess the collection of Chinese herbs, Shen-nung Pen-ts'ao which contains 385 substances. Until 1979, 5967 medicinal herbs had been discovered in China. Ginseng root *Panax ginseng* is the most famous Chinese traditional herb. It is used for various purposes including health maintenance. The United States also has a long history in

the development of drugs based on natural products. Although natural product-based drugs have long been used, the proportion of their usage is still very low compared to the modern medical methods, particularly for the medications of currently emerging diseases[1].

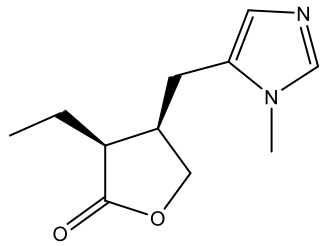
Therefore, natural products are the significant key in drug discovery and are also a milestone of the drug development industry. Natural products are the starting point for many drugs on the market today. For example, morphine, a pain medicine was isolated from opium. After repeated research and trials, it was finally commercialized in 1826 as the first drug and is still clinically used.

As mentioned before, natural products originating from all types of living cells (plants, animals, microorganisms) are great resources of pharmaceutical agents[4]. They provide us with diverse chemical structural substances. For many living organisms, this chemical diversity is the result of evolution as their survival strategy to protect themselves from their predators. Due to a wide range of novel structures of natural products, these give us various biological activities, such as antibiotic, antifungal, and antitumor effects[5]. Such biodiversity provides an expandable range of novel chemical entities or secondary metabolites with various bioactivities. Based on the practices to discover drugs from natural products, over 1602 new chemical entities have been discovered from 1981 to 2019[6].

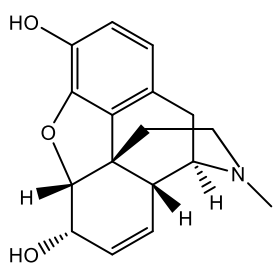
From the past until now, investigators have been researching on discovering drugs from natural products to treat various diseases. Natural products are the most abundant source of potential drug candidates so it's worthy to study more about natural products. Since only about 10% of the world's biodiversity including terrestrial organisms and marine organisms has been used for their potential biological activities, more and more valuable sources from natural products are ready to be examined to apply this natural compound to therapeutic agents[1].



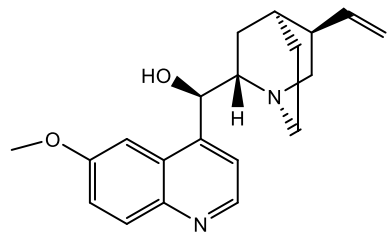
Acetylsalicylic acid



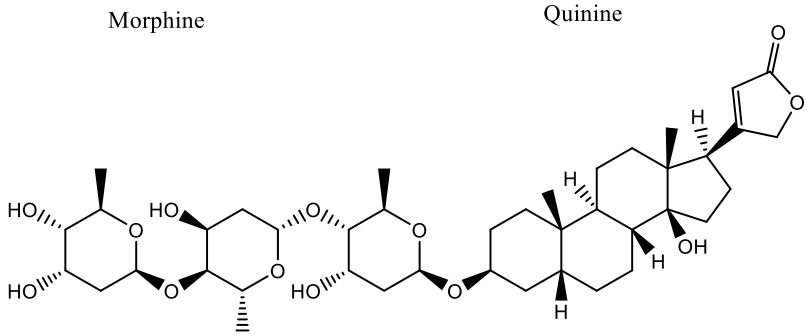
Pilocarpine



Morphine



Quinine



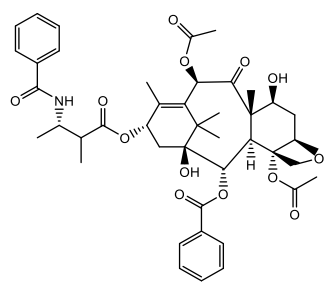
Digitoxin

Figure 1-1 Several historically important natural products. Acetylsalicylic acid (aspirin) isolated from white willow (*Salix alba*), pilocarpine isolated from *Pilocarpus jaborandi*, morphine isolated from the poppy plant (*Papaver somniferum*), quinine isolated from the bark of a cinchona tree, and digitoxin isolated from *Digitalis purpurea L*[1].

1.2 Anticancer Drugs Derived from Natural Products

Cancer is among the leading causes of death worldwide. According to National Cancer Institute, cancer is defined as "It is a disease in which some of the body's cells grow uncontrollably spread to other parts of the body." The number of cancer patients is still increasing and this causes a heavy national loss. According to International Agency for Research on Cancer, by 2040, the number of new cancer cases per year is expected to rise to 29.5 million and the number of cancer-related deaths to 16.4 million. To overcome this disease, people have been using various remedies such as cancer therapy including surgery, chemotherapy, and/or radiotherapy. Although technology continues to advance, traditional medicine is still widely used in many cases. Due to the limitations of treatments available so far and the expectation for structural diversity of natural products, interest in natural medicines is growing again.

Historically, natural products in cancer research have achieved remarkable results, and over 60% of clinical uses for cancer treatments are derived from natural products[7]. Since 1961, several natural products have been approved for use as therapeutic cancer drugs in the USA. Taxol (paclitaxel), a diterpene alkaloid is a very successful anti-cancer molecule. It was derived from the bark of the Pacific yew, *Taxus brevifolia*. Besides Taxol, there are some more approved anti-cancer drugs in Table 1-1[8].



Paclitaxel

Figure 1-2 Structure of paclitaxel.

Table 1-1 Approved anti-cancer drugs derived from natural sources

Compound	Cancer use	Derived from
Vincristine	Leukemia, lymphoma	Plant
Vinblastine	Breast cancer, lymphoma	Plant
Paclitaxel	Breast, lung, ovarian cancer	Plant
Docetaxel	Breast, lung cancer	Plant
Topotecan	Ovarian, lung cancer	Plant
Irinotecan	Colorectal cancer	Plant
Bleomycin	Lymphoma, cervix, head and neck cancer	Microbe
Daunomycin	Leukemia	Microbe
Doxorubicin	Lymphoma, breast, lung, ovarian cancer	Microbe
Epirubicin	Breast cancer	Microbe
Idarubicin	Leukemia	Microbe
Mitomycin	Gastric, pancreatic cancer	Microbe
Cytarabine	Leukemia,	Marine-organism

Chapter 2

An Anticancer Compound from *Streptomyces cavourensis*

2.1 Introduction

Microorganisms are a rich source of novel bioactive compounds that play an important role in the drug industries and medical therapy. Discovered in 1929, penicillin was an astonishing discovery as the first antibiotic introduced by Fleming, Chain, and Florey[1]. Based on this discovery, until today, many antibiotics derived from microorganisms are being actively studied and some of them are released in the market[9].

Among many resources for natural products, bacteria are plentiful sources for bioactive natural products[10]. About 70% of the antibiotics were isolated from actinobacteria, from among these, over 10,000 of them were derived from genus *Streptomyces*[11]. And this reason has made *Streptomyces* become the major antibiotic producer used for drug discovery in the pharmaceutical industries. Over the past few decades, *Streptomyces,* the largest genus of Actinobacteria, has been extensively studied. In 1943, Waksman and Henrici discovered the genus *Streptomyces*. Actually, in 1940, Waksman and Woodruff firstly discovered *Streptomyces antibioticus* which produced an active compound designated as actinomycin[12]. In addition to inhibiting the growth of some pathogenic bacteria and fungi, actinomycin also showed anticancer activity, so it was approved by the United States Food and Drug Administrative in 1964 under the trade name Cosmegen. For this reason, studying about natural products of *Streptomyces* is continually ongoing to discover novel compounds with high efficacy and potency in the pharmaceutical industry[12].

Like the case above, natural products produced by *Streptomyces* bacteria are a great source of bioactive compounds in medicine[13]. Furthermore, genomic analysis

has shown that tens of such secondary metabolites could be produced by any one strain and numerous related biosynthetic gene sets have been revealed by metagenomic analysis. For this reason, these organisms are being researched more intensively in anticipation of novel therapeutics to overcome the emergence of global antibiotic resistance caused by pathogenic bacteria and also, provide other bioactive compounds for medical applications[14].

Based on the above, in the course of searching for new secondary metabolites from *Streptomyces cavourensis*, we obtained a novel bioactive compound named cavomycin A that showed potent inhibitory activity on the proliferation of cancer cells. The isolation, structural elucidation, and biological activity of cavomycin A were described herein.

2.2 Results & Discussion

2.2.1 Structure Elucidation

Cavomycin A showed UV absorption at 200 nm, 220 nm. Its molecular formula $C_{54}H_{92}N_6O_{19}$ was established by the combined analysis of NMR and ESI-MS with ion peak at *m/z* 1129 $[M+H]^+$. It requires twelve degrees of unsaturation.

The ^1H NMR spectrum gave nine methine protons attributable to isopropyl moieties [δ_H 2.0-2.2], six nitrogenated protons [δ_H 7.62, 7.80, 8.27, 8.30, 8.31 8.40], three methyl doublets [δ_H 1.21, (J = 6.8), δ_H 1.28, (J = 6.3, 5.4)], and a cluster of overlapped methyl signals at the highest field at around δ_H 0.85. The ^{13}C NMR spectrum indicated the presence of 54 carbons in the structure including twelve carbonyl carbons [δc 168.40-174.85].

The structure of cavomycin A was established by the detailed analysis of the COSY and HMBC data. COSY correlations were observed from methine protons at δ_H 2.0-2.2 (H-9, 21, 33, 40, 54, 68) to methyl protons of the isopropyl moiety at around δ_H 0.85 (H-10, 11, 22, 23, 34, 35, 41, 42, 55, 56, 69, 70). HMBC showed cross-peaks of protons at δ_H 1.21 (H-4) to carbons at δ_C 67.27 (C-2), δ_C 174.85 (C-3), cross-peaks of protons at δ_H 1.28 (H-16) to carbons at δ_C 69.84 (C-14), δ_C 170.23 (C-15), and cross-peaks of protons at δ_H 1.29 (H-28) to carbons at δ_C 69.89 (C-26), δ_C 170.24 (C-27). ROESY showed the correlation of the amide proton at δ_H 7.62 (H-5) to protons at δ_H 4.02 (H-2) and δ_H 2.0-2.2 (H-9), correlation of the amide proton at δ_H 8.40 (H-17) to protons at δ_H 5.03 (H-14) and δ_H 2.0-2.2 (H-21), correlation of the amide proton at δ_H 8.31 (H-29) to protons at δ_H 5.05 (H-26) and δ_H 2.0-2.2 (H-33), correlation of the amide proton at δ_H 7.80 (H-43) to protons at δ_H 4.78 (H-46) and δ_H 2.0-2.2 (H-40), correlation of the amide proton at δ_H 8.27 (H-57) to protons at δ_H 4.76 (H-60) and δ_H 2.0-2.2 (H-54), correlation of the amide proton at δ_H 8.30 (H-71) to protons at δ_H 4.77 (H-74) and δ_H 2.0-2.2 (H-68).

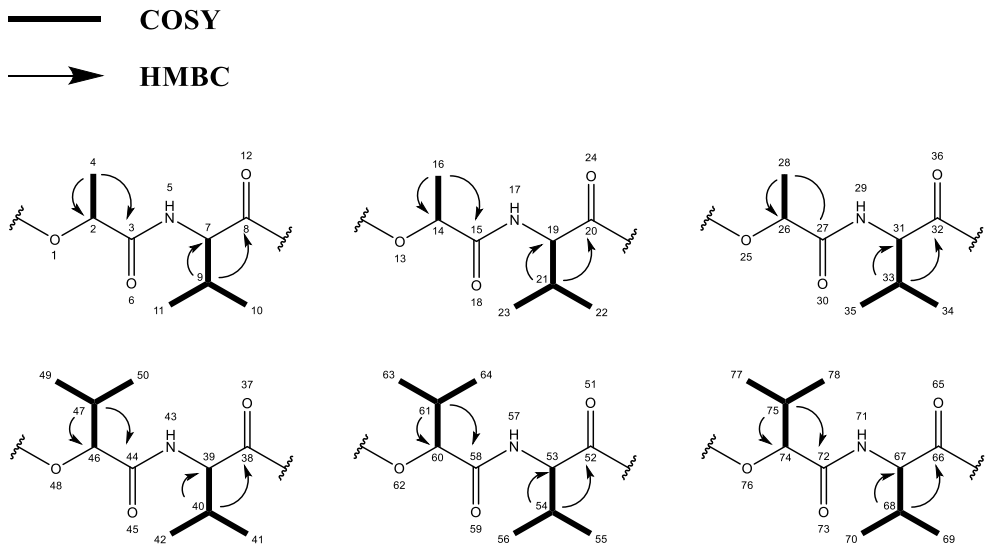
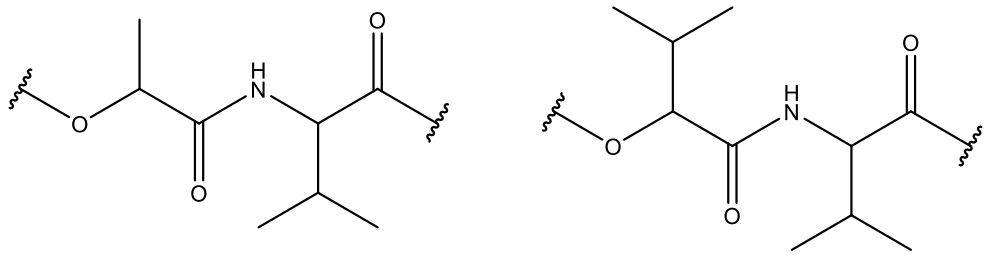


Figure 2-1 Partial structures of cavomycin A with COSY and HMBC.

From this data, we obtained two types of partial structures (Figure 2-2). According to the ratio of proton integration in the ^{1}H NMR spectrum, there are 3 units of type A and 3 units of type B. Then, we used tandem mass spectrometry to bind each unit of the partial structure.



Type A Type B

Figure 2-2 Partial structures of cavomycin A.

Tandem mass spectrometry is the branch of mass spectrometry based on the following principle. Through one or more collisions, secondary fragments ions are generated from a selected particular ion in a molecule or a mixture of molecules. In this study, 50 eV collision energy was used to fragment the compound. Shown in Fig. 2-3 is the main bean mass spectrum recorded for cavomycin A (*m/z* 1129 $[M+H]^{+}$). Signals for fragments $[M+H]^{+}$ ions appear at *m/z* 72, 144, 172, 272, 343, 371, 443, 514, 543, 642, 713, 741, 785, 884, 912, 1012. Sequences determined for cavomycin A appear in Table 2-1.

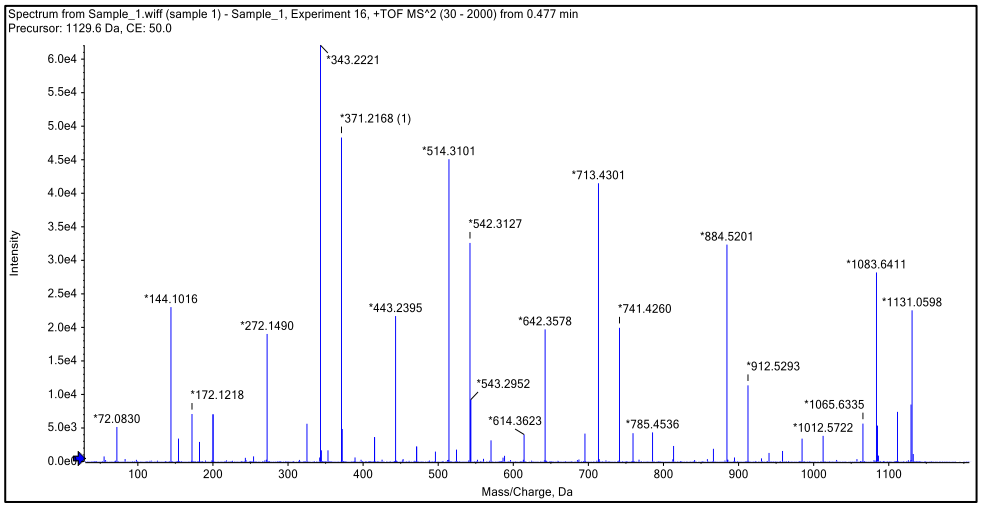


Figure 2-3 Tandem mass (MS/MS) spectrum of cavomycin A, $[M+H]^+$ ion at *m/z* 1129.

Table 2-1 Determined sequence of cavomycin A using units A and B according to $[M+H]^+$ *m/z* values

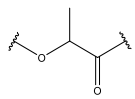
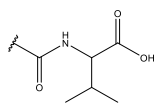
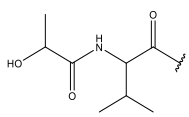
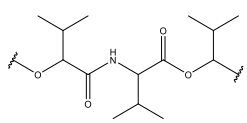
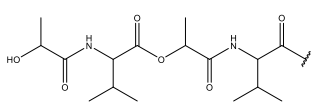
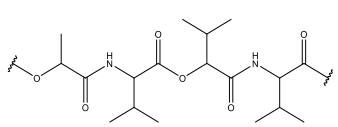
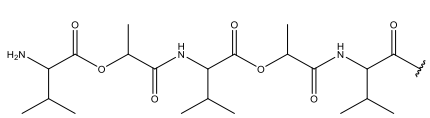
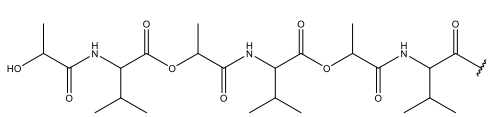
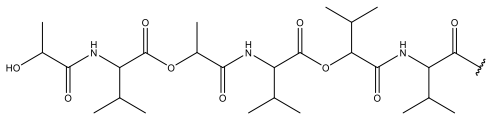
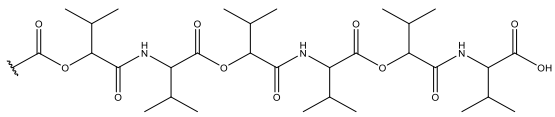
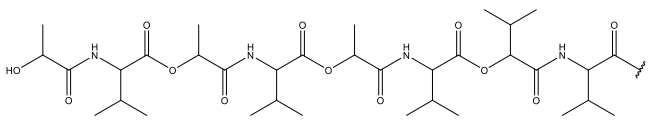
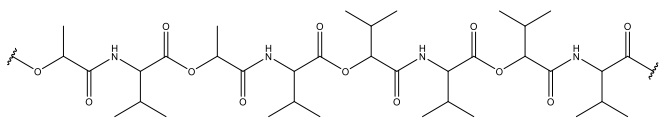
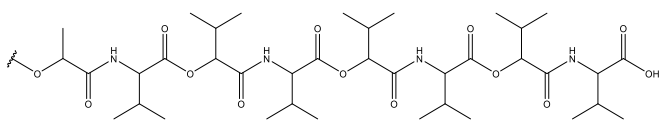
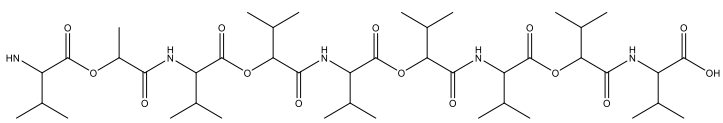
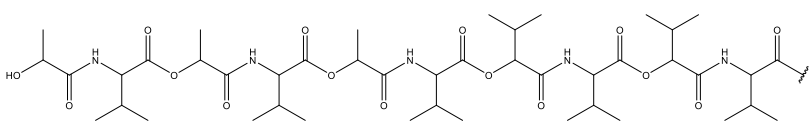
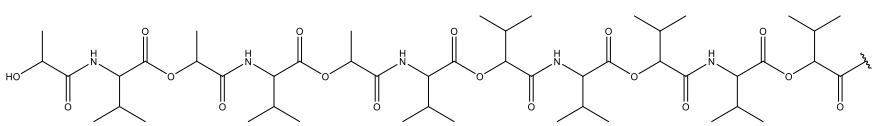
m/z	**MS Fragments of cavomycin A**
72	
144	
172	
272	
343	
371	
443	
514	

Table 2-1 continued

m/z	MS Fragments of cavomycin A
542	
642	
713	
741	
785	
884	
912	
1012	

Through the result of above, we elucidated the structure of cavomycin A and finally, the full structure of the compound was confirmed once again by MS/MS/MS spectrometry. Fig. 2-4 shows the fragment pattern of $[M+H]^+$ ion at *m/z* 884 which is also the fragment of $[M+H]^+$ ion at *m/z* 1129 in the tandem mass spectrum. Signals for fragments $[M+H]^+$ ions appear at *m/z* 371, 343, 443, 514, 542, 585, 713, 741, 769, 786, 813. Partial structures of *m/z* 884 appear in Table 2-2. Through all of these data, we can assemble the arrangement of 3 A moieties and 3 B moieties in order of AAABBB.

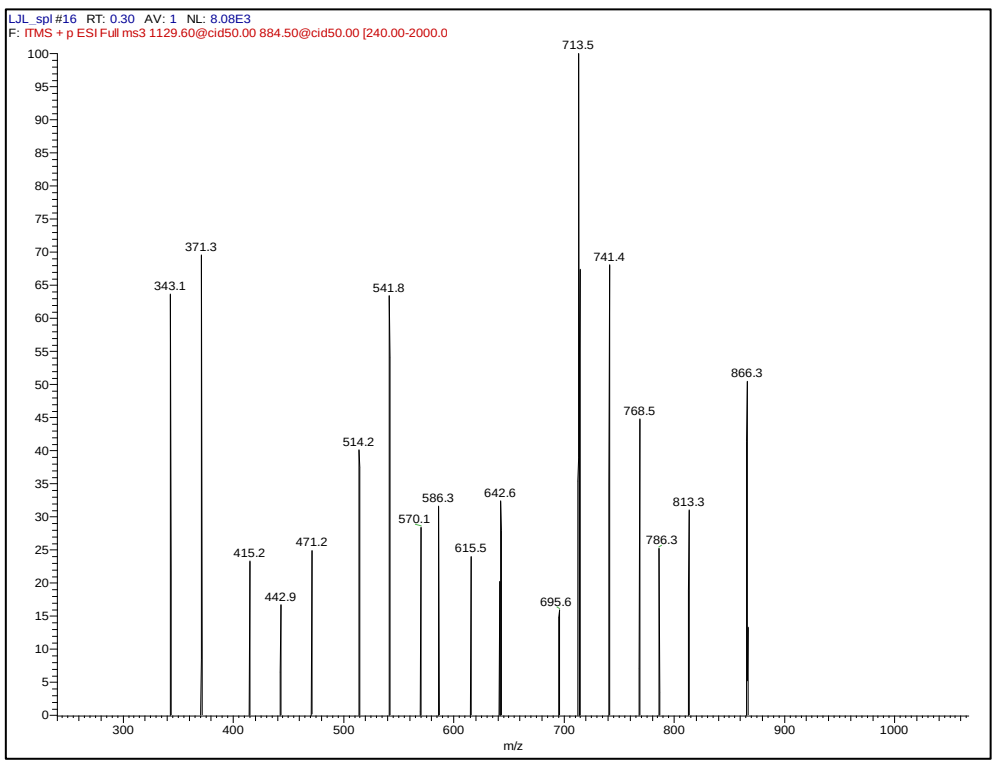


Figure 2-4 MS/MS/MS spectrum of $[M+H]^+$ ion at *m/z* 884.

Table 2-2 Some composing structures of $[M+H]^+$ ion at *m/z* 884

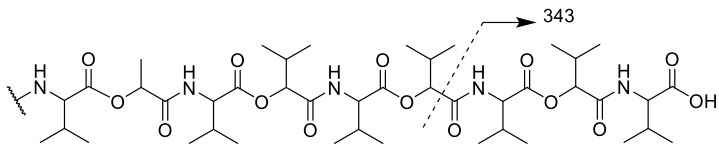
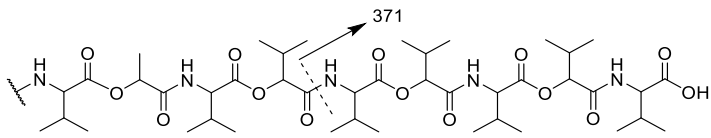
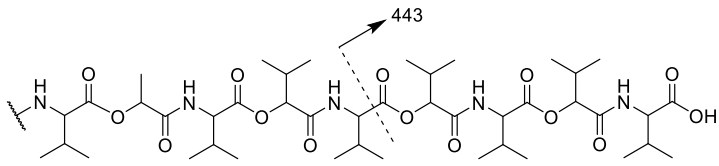
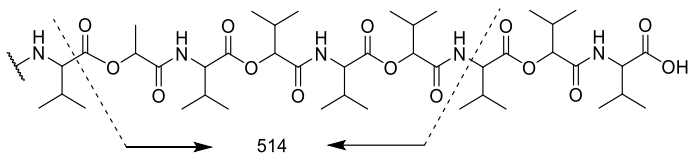
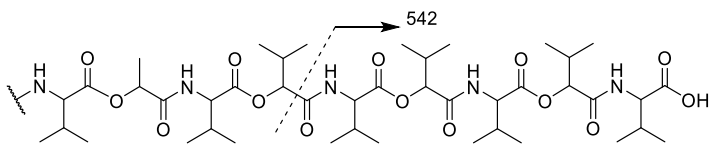
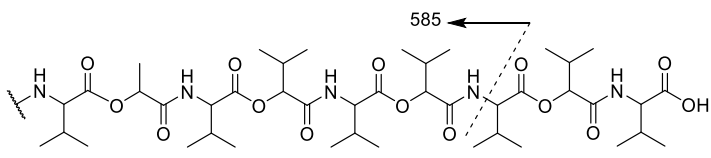
m/z	**MS Fragments of $[M+H]^+$ ion at *m/z* 884**
343	
371	
443	
514	
542	
585	

Table 2-2 continued

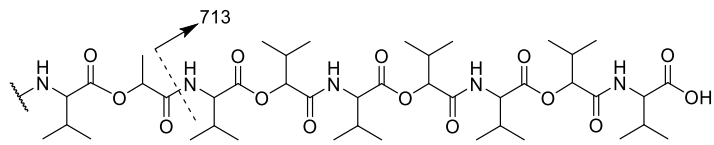
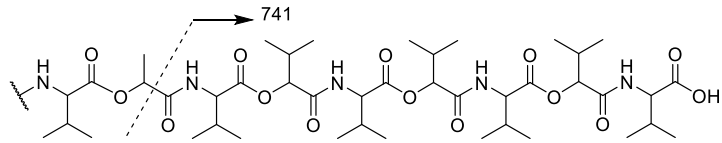
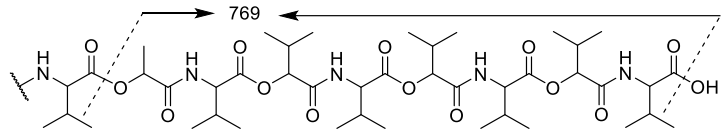
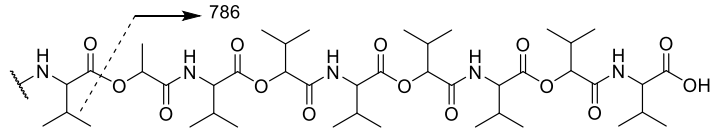
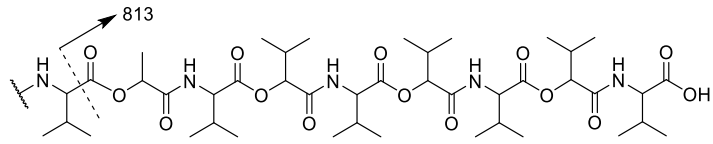
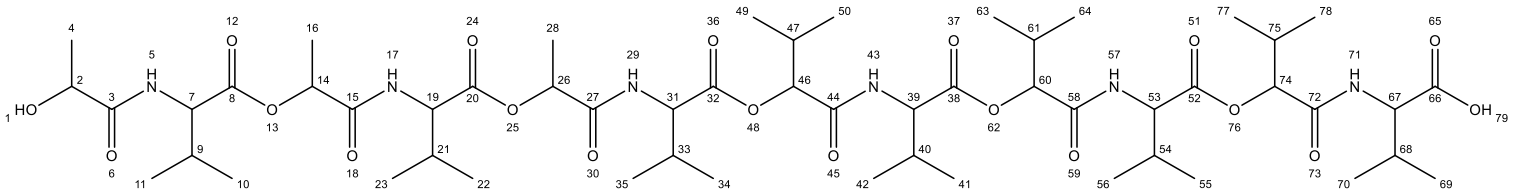
m/z	MS Fragments of $[M+H]^+$ ion at *m/z* 884
713	
741	
769	
786	
813	

Table 2-3 Physical and spectral properties of cavomycin A



Yellow amorphous solid
Molecular Formula: $C_{54}H_{92}N_6O_{19}$
LRMS: $[M+H]^+$ ion at *m/z* 1129
HRMS: $[M+H]^+$ ion at *m/z* 1129.6488 (calcd for $C_{54}H_{92}N_6O_9$, 1129.6495)
UVλ_{max}: 200, 220 nm

Table 2-3 continued

NMR data of cavomycin A in DMSO-d_6

No.	δ_C, type	δ_H, m (J in Hz)	COSY	HMBC
2	67.27, CH	4.02, m	4	
3	174.85, C			
4	21.34, CH_3	1.21, d (6.71)	2	2, 3
7	56.87, CH	4.27, dd (8.60, 5.75)		
8	170.80, C			
9	30.X[a], CH	2.0-2.2, m	7, 10, 11	7, 8
10	17-19, CH_3	0.7-0.9, overlapped	9	7, 9
11	17-19, CH_3	0.7-0.9, overlapped	9	7, 9
14	69.84, CH	5.03, d (6.75)	16	
15	170.23, C			
16	17.53, CH_3	1.28, d (6.22)	14	14, 15
19	57.53, CH	4.24, dd (7.97, 6.5)	21	
20	170.60, C			
21	30.X[a], CH	2.0-2.2, m	19, 22, 23	19, 20
22	17-19, CH_3	0.7-0.9, overlapped	21	19, 21
23	17-19, CH_3	0.7-0.9, overlapped	21	19, 21
26	69.84, CH	5.05, d (6.75)	28	
27	170.24, C			
28	17.54, CH_3	1.29, d (5.60)	26	26, 27
31	57.53, CH	4.22, dd (8.21, 6.88)	33	
32	170.X[a], C			
33	30.X[a], CH	2.0-2.2, m	31, 34, 35	31, 32
34	17-19, CH_3	0.7-0.9, overlapped	33	31, 33
35	17-19, CH_3	0.7-0.9, overlapped	33	31, 33
38	170.69, C			
39	57.91, CH	4.01, m	40	
40	30.X[a], CH	2.0-2.2, m	39, 41, 42	38, 39
41	17-19, CH_3	0.7-0.9, overlapped	40	39, 40
42	17-19, CH_3	0.7-0.9, overlapped	40	39, 40
44	168.40, C			
46	78.12, CH	4.78, d (5.07)	47	
47	30.X[a], CH	2.0-2.2, m	46, 49, 50	46, 44
49	17-19, CH_3	0.7-0.9, overlapped	47	46, 47
50	17-19, CH_3	0.7-0.9, overlapped	47	46, 47

Table 2-3 continued

52	170.46, C			
53	57.60, CH	4.18, d (7.52)	54	
54	30.X[a], CH	2.0-2.2, m	53, 55, 56	52, 53
55	17-19, CH_3	0.7-0.9, overlapped	54	53, 54
56	17-19, CH_3	0.7-0.9, overlapped	54	53, 54
58	169.03, C			
60	77.90, CH	4.76, d (5.36)	61	
61	30.X[a], CH	2.0-2.2, m	60, 63, 64	58, 60
63	17-19, CH_3	0.7-0.9, overlapped	61	60, 61
64	17-19, CH_3	0.7-0.9, overlapped	61	60, 61
66	170.46, C			
67	57.60, CH	4.16, d (7.52)	68	
68	30.X[a], CH	2.0-2.2, m	67, 69, 70	66, 67
69	17-19, CH_3	0.7-0.9, overlapped	68	67, 68
70	17-19, CH_3	0.7-0.9, overlapped	68	67, 68
72	168.99, C			
74	77.99, CH	4.77, d (5.21)	75	
75	30.X[a], CH	2.0-2.2, m	74, 77, 78	72, 74
77	17-19, CH_3	0.7-0.9, overlapped	75	74, 75
78	17-19, CH_3	0.7-0.9, overlapped	75	74, 75

[a]X indicates that it is difficult to assign the exact value.

The ^{1}H and all 2D NMR spectra were recorded at 700MHz, the ^{13}C NMR spectrum was recorded at 175 MHz.

2.2.2 Biological Activity

Cavomycin A isolation was conducted through bioactive-guided fractionation. To evaluate the cytotoxic activity of the compound isolated from the *Streptomyces cavourensis* against five cancer cell lines including lung carcinoma cells (A549), prostate carcinoma cells (DU145), colorectal carcinoma cells (HCT116), breast carcinoma cells (MCF7), and pancreatic carcinoma cells (PANC-1), the five cancer cell lines were treated with cavomycin A at different doses (from 50 nM to 100 μM). After 72 hours of incubation, cell viability was measured at 570 nm using a microplate reader. The compound induced cell cytotoxicity depending on concentration. The results of the cytotoxicity assay are presented in Fig 2-5. The compound was able to inhibit the proliferation of all cancer cell lines with a low concentration of IC_{50} values.

Table 2-4 IC_{50} values of cavomycin A with five other human cancer cell lines[a,b]

compound	IC_{50} (μM)				
	A549 (lung)	DU145 (prostate)	HCT116 (colorectal)	MCF7 (breast)	PANC-1 (pancreatic)
cavomycin A	0.09	0.06	0.1	0.07	0.08
5-fluorouracil		18	37		
cisplatin	52				

[a]IC_{50} value is the concentration of compound required to inhibit the growth of the cells by 50%.
[b]5-fluorouracil was used as a positive control for DU145, HCT116, MCF7, PANC-1, and cisplatin was used as a positive control for A549.

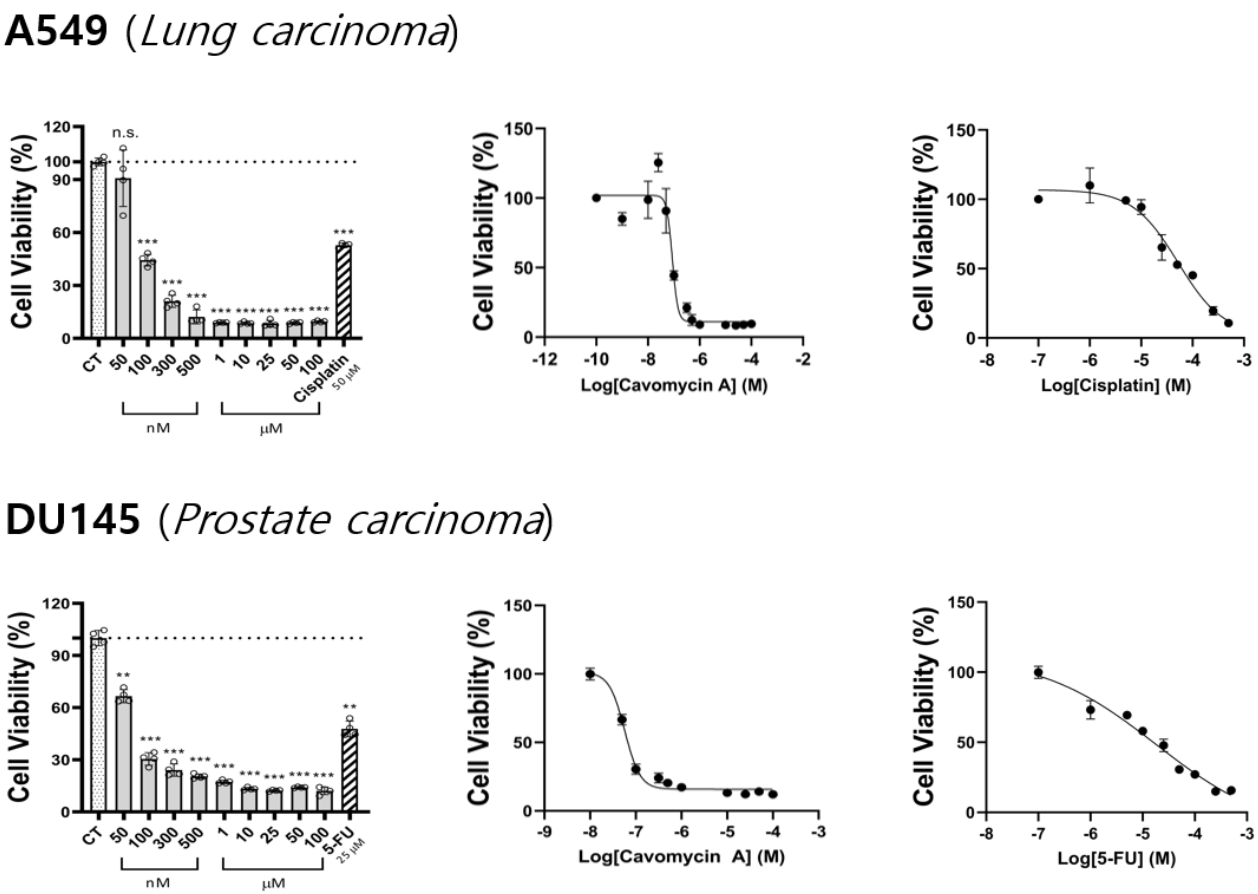
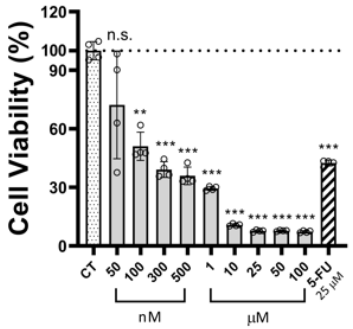
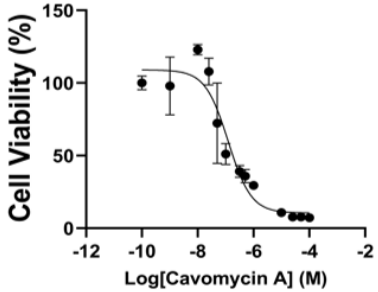
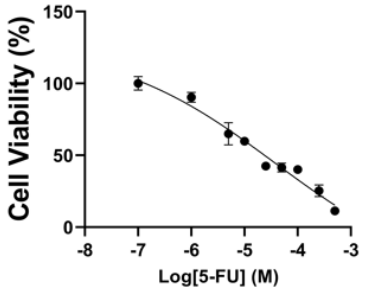


Figure 2-5 Results of MTT assay about the inhibitory activity of cancer cell proliferation with cavomycin A.

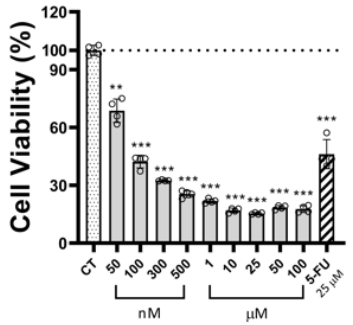
HCT116 (*Colorectal carcinoma*)

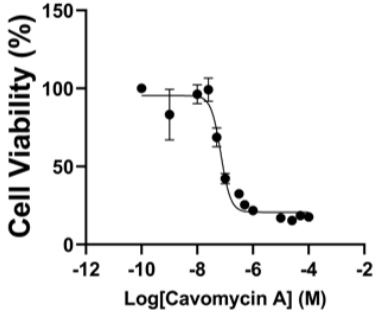






MCF7 (*Breast carcinoma*)





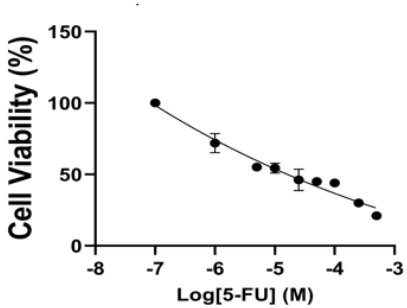


Figure 2-5 continued.

PANC-1 (*Pancreatic carcinoma*)

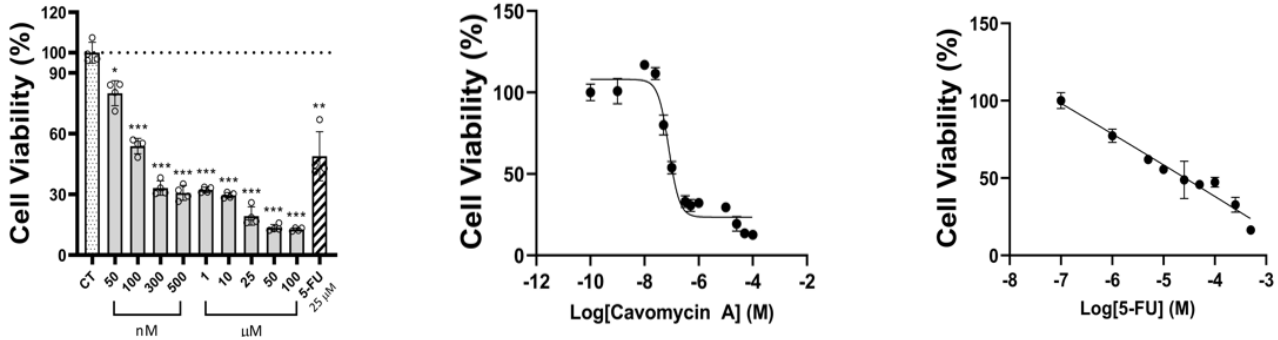


Figure 2-5 continued.

Chapter 3

Materials and Methods

3.1 Instruments and Data Collection

High-performance liquid chromatography was performed with a Waters HPLC (Waters Corporation, Massachusetts, U.S.A.) equipped with an HPLC WATERS 1525 binary HPLC pump and a WATERS 2489 UV/visible detector using Luna 5 µm C18 (250 × 10.00 mm 5 micron) at flow rate 2.0 mL/min. ESI low-resolution mass data was acquired by Agilent Technologies 6120 quadrupole mass system coupled with an Agilent Technologies 1260 series HPLC with a reversed-phase Phenomenex Luna 5 µm C18 (100 × 4.6 mm, 5 µm). All NMR spectra were recorded on a Bruker Ascend TM 700 spectrometer using DMSO-d_6 as solvents. Chemical shifts were reported with reference to the respective solvent peaks [δ_H 2.50 and δ_C 39.51 for DMSO-d_6]. First-grade solvents from Dae-Jung chemicals & Metals Co. Ltd, Korea were used for the fractionation of extracts. HPLC grade solvents from J. T. Baker and Dae-Jung chemicals & Metals Co. Ltd were used for HPLC. NMR solvents were purchased from EURISO-TOP.

3.2 Isolation and Cultivation of Bacteria

Streptomyces cavourensis was isolated from the leaves of *Cinnamomum cassia* plants in Hoa Binh province of northern Vietnam. The bacterium was identified according to its 16S rRNA gene sequence (99% similar to the sequence of *Streptomyces cavourensis* strain NRRL 2740). *Streptomyces cavourensis* was grown on YIM (4 g of glucose, malt, yeast each in 1 L of distilled water) agar then inoculated into 96 Ultra Yield flasks, each containing 1 L of YIM. The cultivation was carried out in a shaking incubator (131 rpm) at 28℃ for 3 days.

Figure 3-1 Culture plate and Ultra Yield flasks of *Streptomyces cavourensis*

3.3 Extraction and Isolation

After 3 days of incubation, the broth was extracted with ethyl acetate (EtOAc) and the EtOAc layer was evaporated in vacuo then finally obtained 2.3 g of crude extract from 96 L of the broth. The crude extract was fractioned over silica flash column chromatography using stepwise-gradient elution of methanol in dichloromethane (1%, 2%, 4%, 6%, 10%, 20%, 100%) to afford 8 fractions (Frac.1: 251 mg, Frac.2: 158 mg, Frac.3: 255 mg, Frac.4: 83 mg, Frac.5: 141 mg, Frac.6: 97 mg, Frac.7: 129.8 mg, Frac.8: 214.2 mg). After that, according to MTT assay results, fraction 5 (141 mg) was subjected to further-fractionation to 9 fractions (Frac.1: 1.3 mg, Frac.2: 39.3 mg, Frac.3: 40.3 mg, Frac.4: 20.2 mg, Frac.5: 22.8 mg, Frac.6: 6.8 mg, Frac.7: 2.4 mg, Frac.8: 3.1 mg, Frac.9: 0.9 mg) by Sephadex LH-20 column chromatography using methanol. Then, these fractions were also tested cytotoxic effect to choose the highest effective fraction. As the result, fraction 2 from Sephadex LH-20 column chromatography was subjected to reversed-phase HPLC (Phenomenex Luna C18 (2), 5 micron, 100 Å, 250 × 10.00 mm, 2.0 mL/min, UV = 200 nm, 220 nm), eluting with 80% CH_3CN in H_2O to afford three compounds to test cytotoxic activity. The yield for each compound is as follows. 4.7 mg for compound **1**, 10 mg for compound **2**, and 19.7 mg for compound **3**.

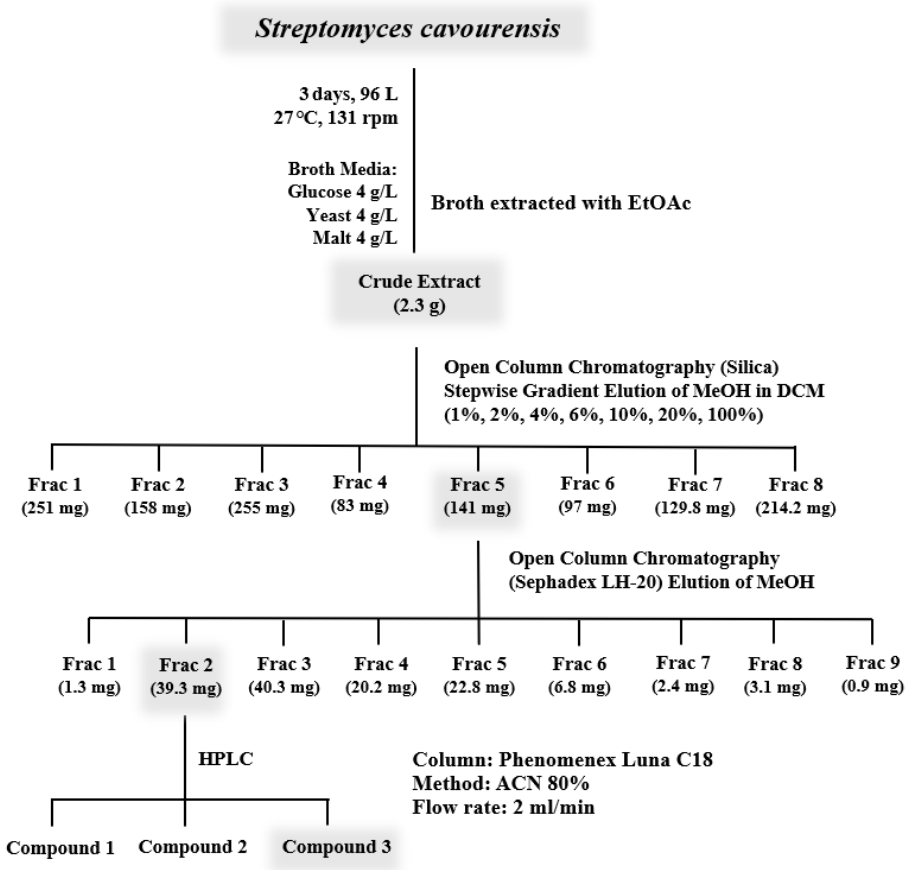


Figure 3-2 Scheme of extraction and isolation method for compound **3** (cavomycin A).

3.4 Bioassay

3.4.1 Cell Culture

A549 (lung carcinoma), DU145 (prostate carcinoma), HCT116 (colorectal carcinoma), MCF7 (breast adenocarcinoma), and PANC-1 (pancreatic carcinoma) were purchased from ATCC (American Type Culture Collection). A549, DU145, HCT116, and MCF7 were maintained in RPMI 1640 containing 10% fetal bovine serum (FBS) (Welgene, Korea) and 1% penicillin and streptomycin (P/S) (Welgene, Korea), whereas PANC-1 was maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (Welgene, Korea) and 1% penicillin and streptomycin (P/S) (Welgene, Korea). The cells were incubated at 37 ℃ in a 5% CO_2 incubator.

3.4.2 MTT Assay

The cell viability was determined by measuring the ability of cells to transform MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to a purple formazan dye. The cells were seeded in 96-well cell culture plates at 3×10^3 cells/well for 24 h. The cells were then treated with different concentrations of cavomycin A for 72 h. After incubation, 20 μL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The medium was sucked and replaced with 100 μL/well of DMSO to dissolve the formazan salt. The color intensity of the formazan solution, which reflects the cell growth conditions, was measured at 570 nm using a microplate reader. Graphical representations and the values of IC_{50} were generated using GraphPad Prism software version 9.3.0.

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Appendix

Figure S1 ^{1}H NMR spectrum of cavomycin A in DMSO-d_6·····33
Figure S2 ^{13}C NMR spectrum of cavomycin A in DMSO-d_6·····34
Figure S3 COSY spectrum of cavomycin A in DMSO-d_6·····35
Figure S4 HSQC spectrum of cavomycin A in DMSO-d_6·····36
Figure S5 HMBC spectrum of cavomycin A in DMSO-d_6·····37
Figure S6 ROESY spectrum of cavomycin A in DMSO-d_6·····38
Figure S7 High-resolution mass spectrum of cavomycin A·····39

Figure S1 ^{1}H NMR spectrum of cavomycin A in DMSO-d_6.

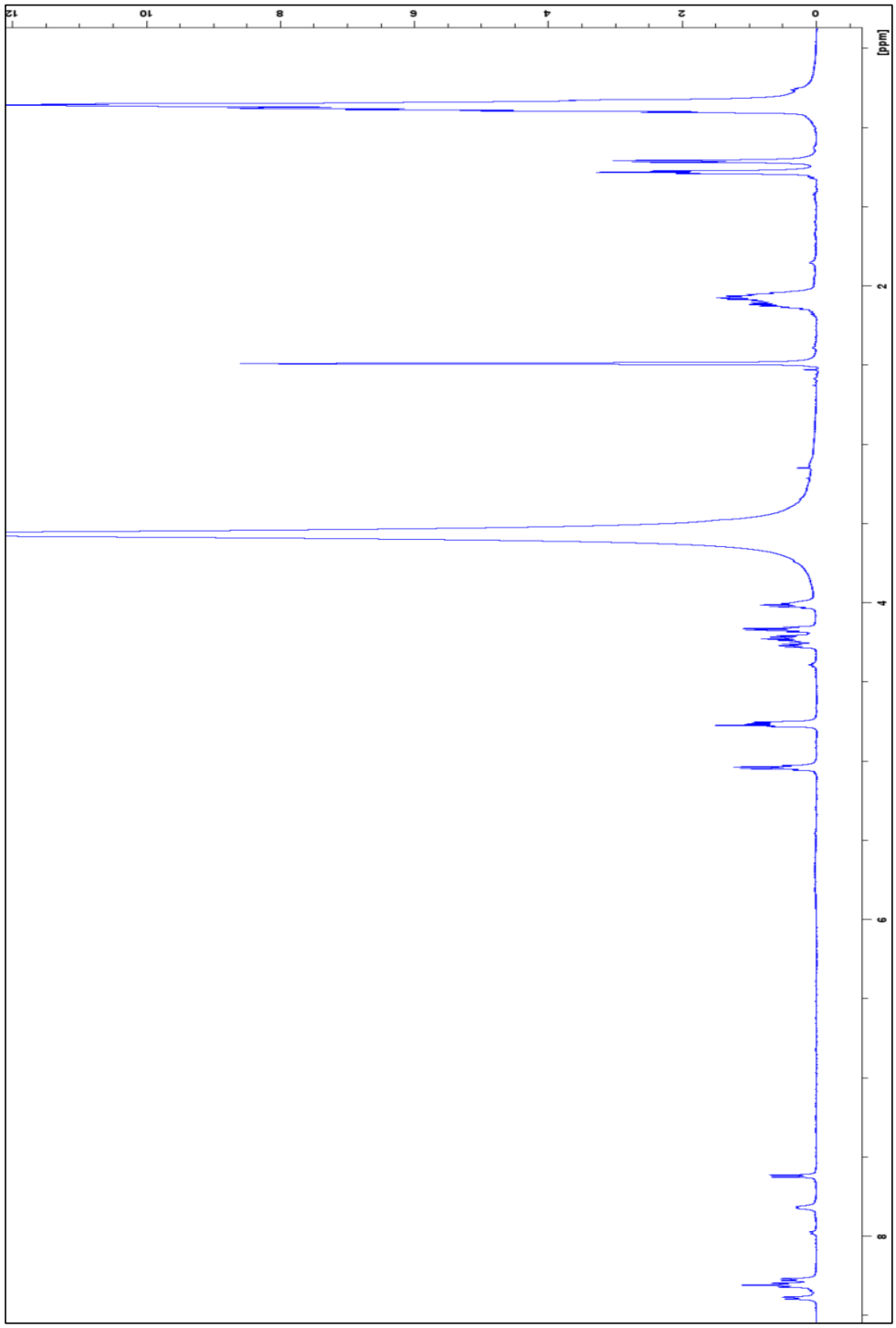


Figure S2 ^{13}C NMR spectrum of cavomycin A in DMSO-d_6.

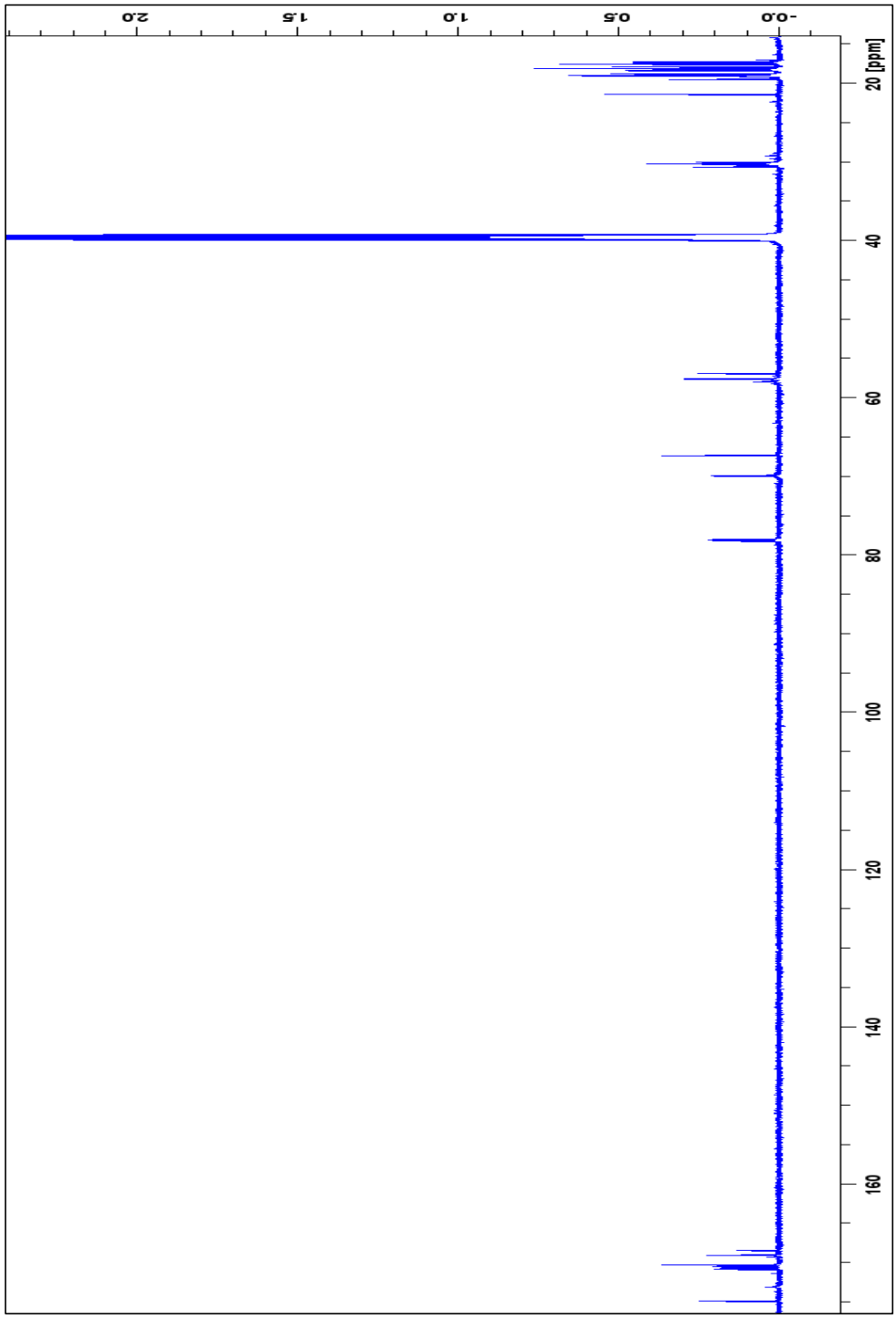


Figure S3 COSY spectrum of cavomycin A in DMSO-d_6.

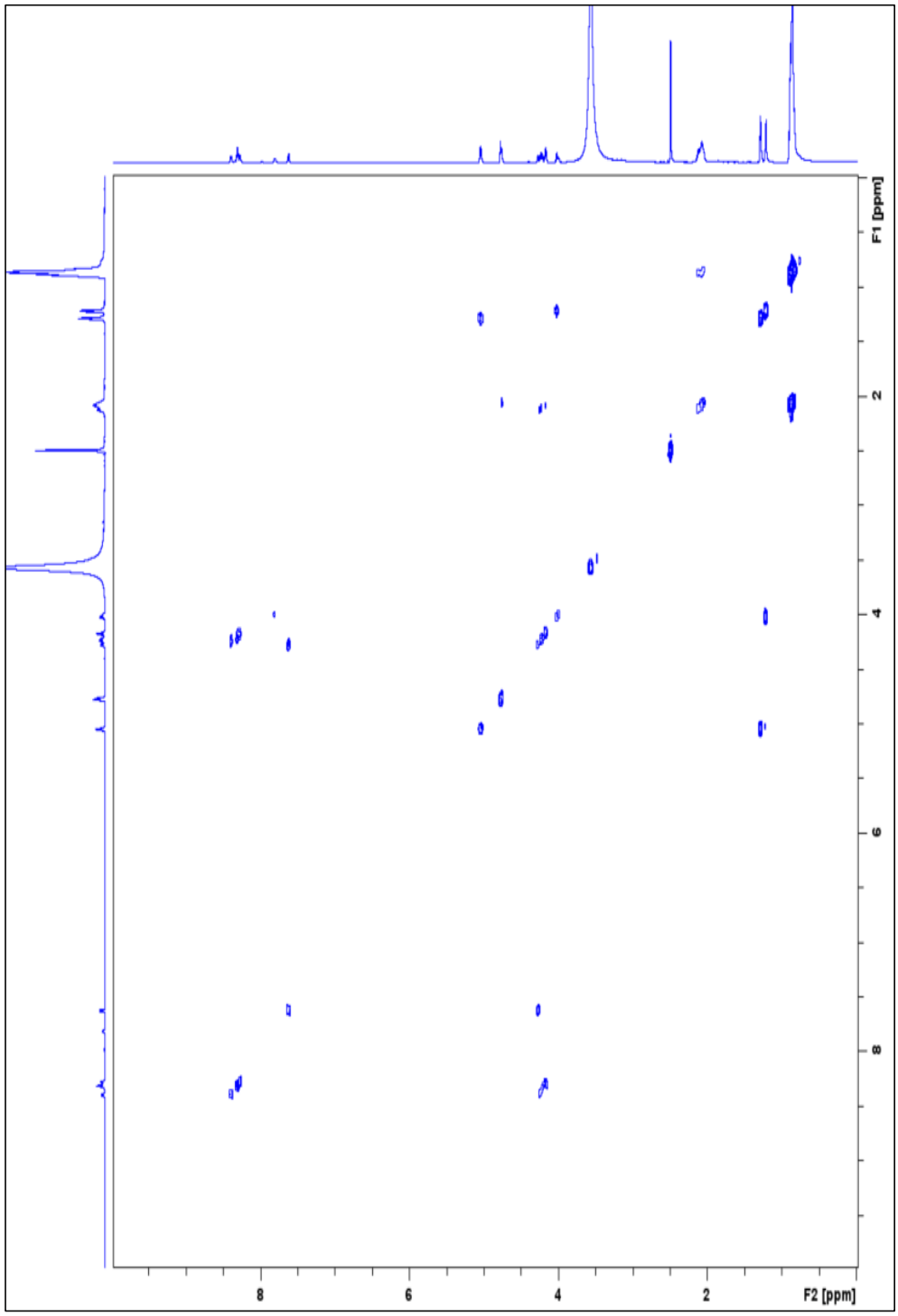


Figure S4 HSQC spectrum of cavomycin A in DMSO-d_6.

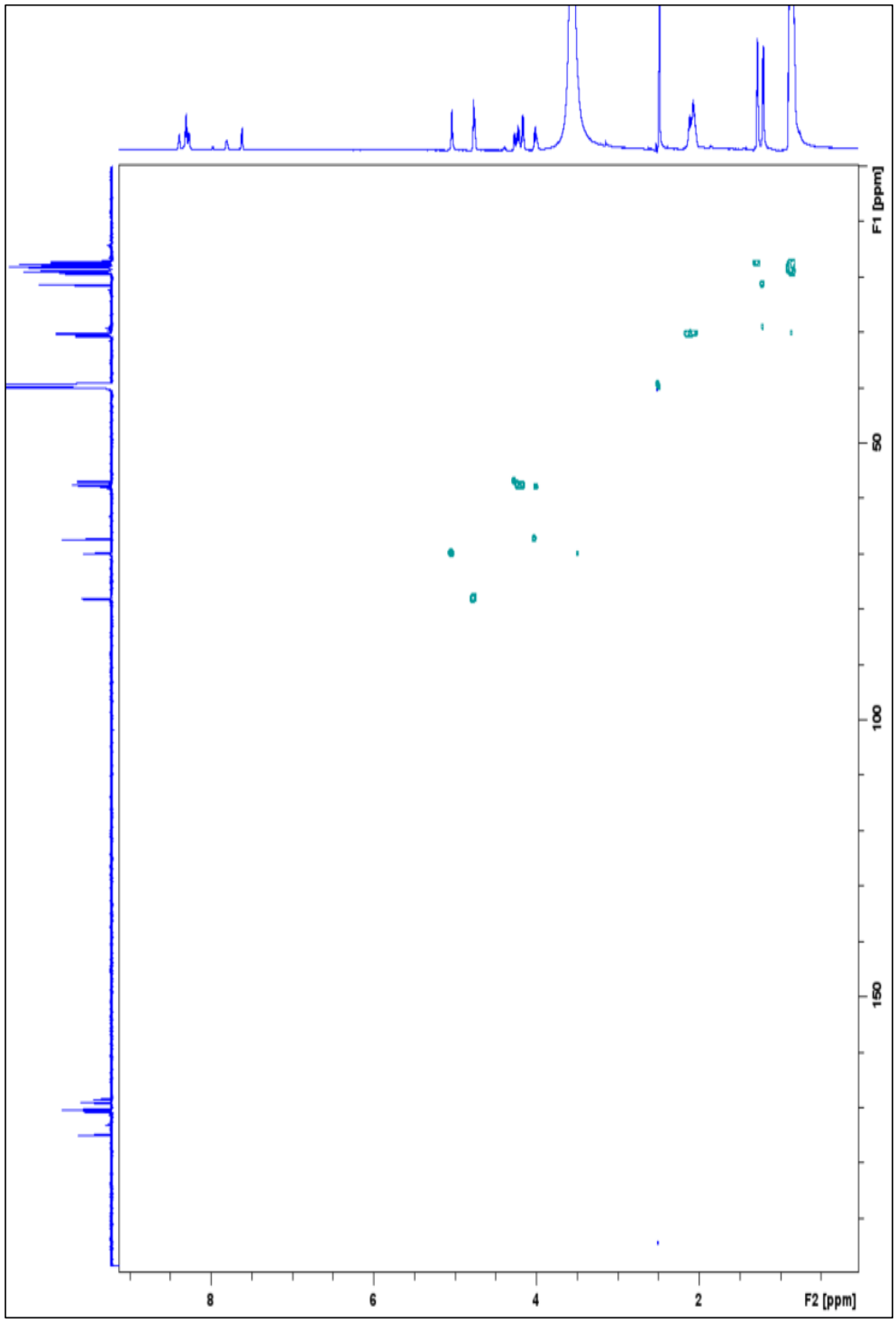


Figure S5 HMBC spectrum of cavomycin A in DMSO-d_6.

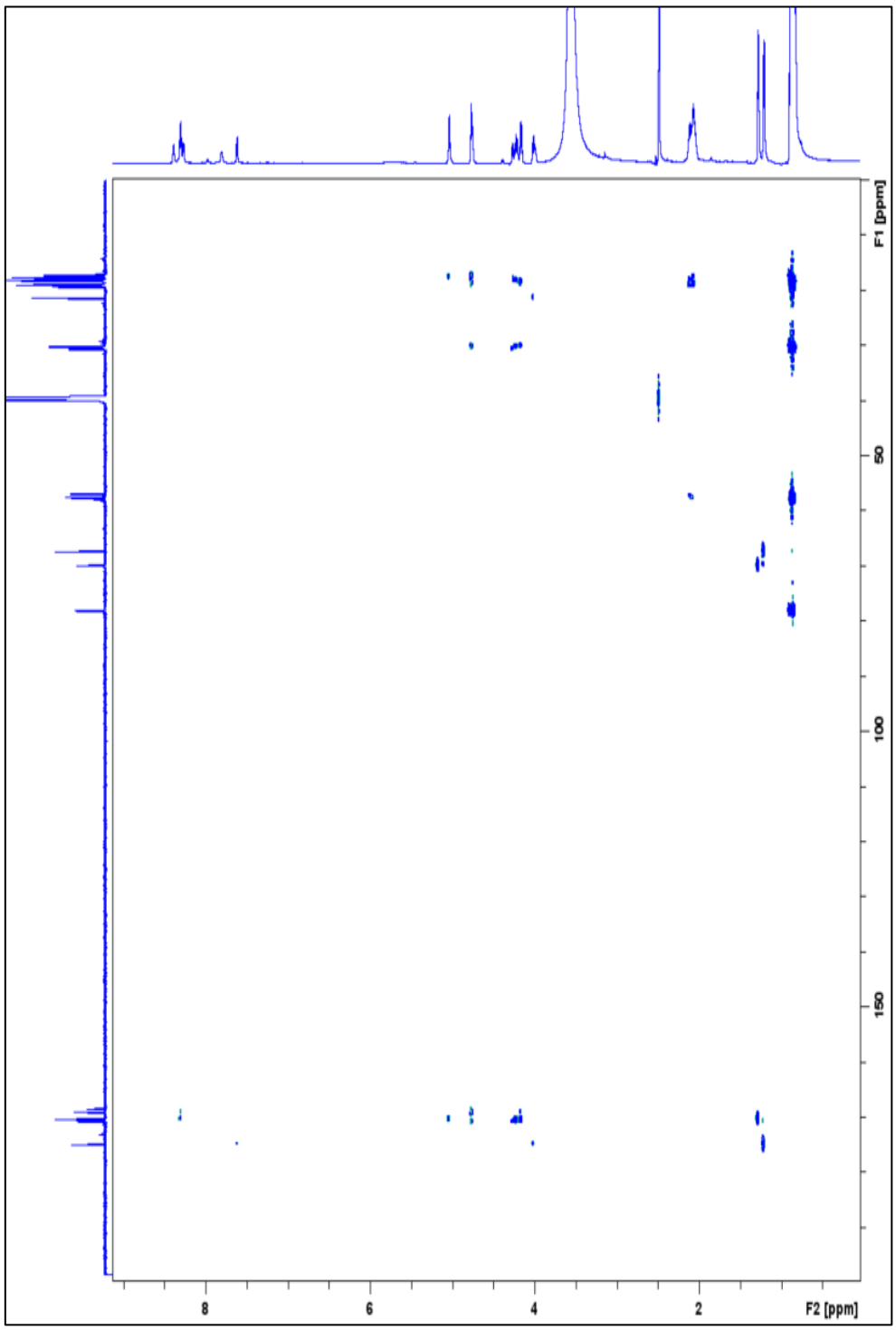


Figure S6 ROESY spectrum of cavomycin A in DMSO-d_6.

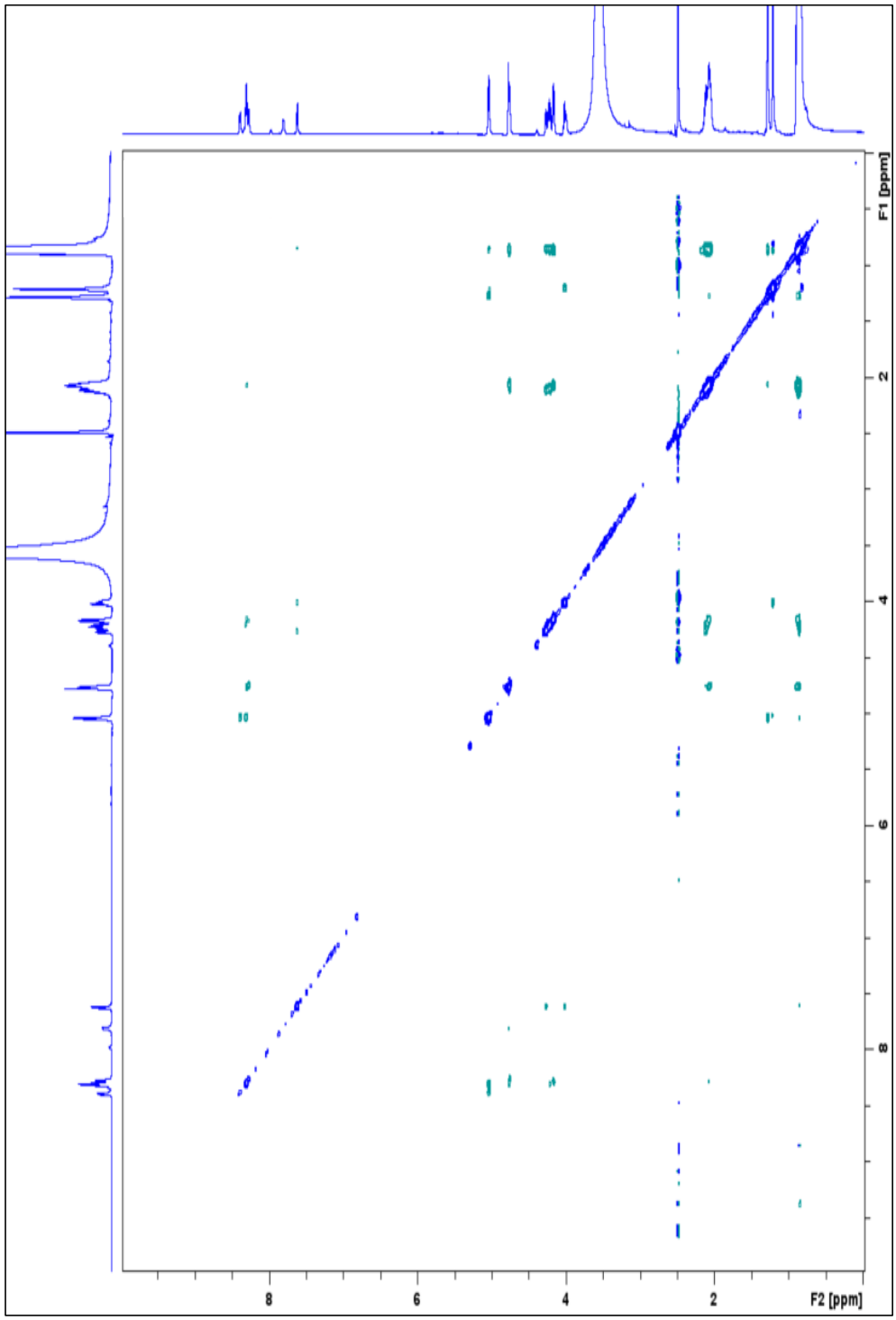
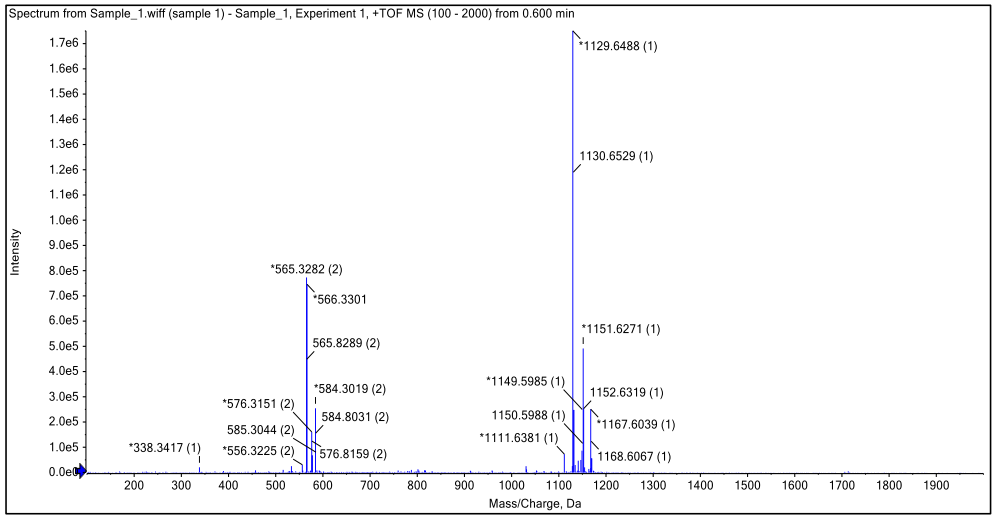


Figure S7 High-resolution mass spectrum of cavomycin A



국문초록

암은 전 세계 주요 사망 원인 중 하나로 신체의 각 부분에 걷잡을 수 없이 세포가 성장하는 것을 특징으로 하는 질병이다. 천연물은 오래전부터 암의 치료 약물의 주요자원으로 사용되었다. 천연물은 광범위한 구조적 다양성을 가지고 있어 이로부터 암 치료에 혁신적인 후보물질을 발견할 수 있다. 본 연구에서는 베트남의 호아빈 지역의 *Cinnamomum cassia*의 잎으로부터 분리한 *Streptomyces cavourensis*가 생산한 이차대사산물로부터 항암효과를 갖는 물질을 찾는 연구를 진행하였다. 다양한 크로마토그래피 기법과 화학적 방법으로 *S. cavourensis*의 배양 추출물을 분리 및 정제하였고 물질의 화학구조는 NMR 분석, 질량 분석, UV 분광법을 이용하여 결정하였다. 분리된 물질은 cavomycin A로 명명하였으며 세포 생존율을 나타내는 MTT assay를 통해 물질의 항암 활성을 확인하였다. MTT assay에는 5가지의 암세포(폐암, 전립선암, 대장암, 유방암, 췌장암)가 사용되었으며 각 세포에 cavomycin A을 다양한 농도 조건(50 nM 부터 100 μM)으로 처리해 물질의 활성을 확인하였다. 그 결과, 암세포에 대한 cavomycin A의 IC_{50} 값이 0.1 μM 이하로 나타났으며 이를 통해 cavomycin A의 잠재적인 항암활성을 확인하였다.