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A Thesis for the Degree of Master of Science in Pharmacy

Structure Determination of Bianthraquinones and Meroterpenoids from Marine-Derived Fungi

해양 유래 진균에서 분리한 신규 Bianthraquinones와 Meroterpenoids

February 2017

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Abstract

Structure Determination of Bianthraquinones and Meroterpenoids from Marine-Derived Fungi

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Three new meroterpenoids (**1-3**), along with eleven known compounds (**4-14**) were isolated from the rice fermentation of the marine-derived fungus *Penicillium brasilianum* (strain number FCH061) obtained from the sponge collected off the coast of Chuja-do, Korea. Three new bianthraquinone metabolites (**1-3),** along with twelve known compounds (**4-15**), were isolated from the rice fermentation of the fungus *Stemphylium lycopersici* (strain number FJJ006) obtained from the sponge collected from Jeju island, Korea. Their structures were elucidated using comprehensive spectroscopic methods. Structures of these new compounds were determined on the basis of NMR and HR-MS analyses, as well as comparison with literatures.

Keywords : meroterpenoid, *Penicillium brasilianum*, bianthraquinone, *Stemphylium lycopersici*

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Introduction

In recent years, secondary metabolites obtained from marine-derived fungi have gained considerable attention, as many of them are structurally unique and possess interesting biological and pharmacological properties.[1-6] Although studies on these organisms began considerably later than those on their counterparts from terrestrial environments, more than a hundred novel compounds have been found annually since the late 1990s.[7] This trend has accelerated in recent years due to both the demand for the production of mass bioactive compounds and the technical progress in related fields, such as microbial genetics and bioinformatics.[8] Consequently, fungi, along with bacteria from the marine environments, are considered to be a new frontier for research into natural products.

In our search for novel bioactive metabolites from the fungi of marine environments, we selected two marine-derived fungi (FCH061 and FJJ006). The first strain (FCH061) was isolated from the marine sponge and was identified to be *Penicillium brasilianum.* The second strain (FJJ006) was isolated from the marine sponge and was identified as *Stemphylium lycopersici.* The large scale cultivation, extraction and separation using diverse chromatographic methods were conducted to yield 6 new compounds and 23 known compounds from *P. brasilianum* and *S. lycopersici* in total.[16-20,21-30] Structure elucidation was performed mainly using 1D and 2D NMR techniques and chemical characterization of isolated metabolites. The biological activity assays were performed for nitrite assay, cytotoxicity assays, antimicrobial assays, Isocitrate lyase, sortase A, and Na^+/K^+–ATPase inhibition assays. [9-14] Compounds of *P. brasilianum* were not significantly active against all biological activity assays. Compounds of *S. lycopersici* exhibited anti-inflammatory activities in LPS-stimulated RAW 264.7 cells.

Experimental Section

1. General Experimental Procedures

Optical rotations were measured on a JASCO P1020 polarimeter (Jasco, Tokyo, Japan) using a 1 cm cell. UV spectra were acquired with a Hitachi U-3010 spectrophotometer (Hitachi High-Technologies, Tokyo, Japan). IR spectra were recorded on a JASCO 4200 FT-IR spectrometer (Jasco, Tokyo, Japan) using a ZnSe cell. NMR spectra were recorded on Bruker Avance 600 and 500 spectrometers (Bruker, Massachusetts, USA). ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ and CD_3OD solutions at 800 and 200 MHz, 600 and 150 MHz, 500 and 125 MHz, 400 and 100 MHz or 300 and 75 MHz, respectively. High resolution FAB mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and were acquired using a JEOL JMS 700 mass spectrometer (Jeol, Tokyo, Japan) with *meta*-nitrobenzyl alcohol (NBA) as a matrix for the FABMS. Low-resolution ESIMS data were recorded on an Agilent Technologies 6130 quadrupole mass spectrometer (Santa Clara, CA, USA) coupled to an Agilent Technologies 1200 series HPLC (Santa Clara, CA, USA). Semi-preparative HPLC was performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150) and GILSON 321 equipped with a UV-Vis detector (GILSON UV-Vis-151). All solvents used were spectroscopic grade or distilled from glass prior to use.

2. Isolation and Identification of Fungal Strain

The fungal strain *Penicillium brasilianum* (strain number FCH061) was isolated from sponge collected off the coast of Chuja-do, Korea, in October, 2012. FCH061 was identified using standard molecular biological protocols by DNA amplification and sequencing of the ITS region. Genomic DNA extraction was performed using Intron's i-genomic BYF DNA Extraction

Mini Kit according to the manufacturer's protocol. The nucleotide sequence of FCH061 was deposited in the GenBank database under accession number KU519426. The 18S rDNA sequence of this strain exhibited 100% identity with that of *Penicillium sp.* TR052 (GenBank accession number HQ608086).

```
   1 tccgtaggtg aacctgcgga aggatcatta ctgagtgagg gccctctggg tccaacctcc
  61 cacccgtgtt tattgtacct tgttgcttcg gcgcgcccgc ctcacggccg ccgggggggca
 121 cccgcccccg ggcccgcgcc cgccgaagac accattgaac tcttgtctga agattgcagt
 181 ctgagtagat tagctaaatc agttaaaact ttcaacaacg gatctcttgg ttccggcatc
 241 gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag tgaatcatcg
 301 agtctttgaa cgcacattgc gccccctggt attccggggg gcatgcctgt ccgagcgtca
 361 ttgctgccct caagcacggc ttgtgtgttg ggcttcgccc cccgttcgtc gggggggcggg
 421 cccgaaaggc agcggcggca ccgcgtccgg tcctcgagcg tatggggctt tgtcacccgc
 481 tctgtaggcc cggccggcgc ccgccggcga cacccaaatc aatctatcca ggttgacctc
 541 ggatcaggta gggatacccg ctgaacttaa gcatatcaat aagcggagga
```

Figure 1. The 18S rRNA sequence of FCH061

The fungal strain *Stemphylium lycopersici* (strain number FJJ006) was isolated from sponge collected from Jeju-do, Korea, in September, 2014. FJJ006 was identified using standard molecular biological protocols by DNA amplification and sequencing of the ITS region. Genomic DNA extraction was performed using Intron's i-genomic BYF DNA Extraction Mini Kit according to the manufacturer's protocol. The nucleotide sequence of FJJ006 was deposited in the GenBank database under accession number KU519425. The 18S rDNA sequence of this strain exhibited 100% identity with that of Uncultured fungus clone F3-O12 (GenBank accession number JX984724).

1 tccgtaggtg aacctgcgga gggatcatta cacaatatga aagcgggctg ggaccttact
61 tcggtgaggg ctccagcttg tctgaattat tcacccatgt cttttgcgca cttcttgttt
121 cctgggcggg ttcgcccgcc accaggacca aaccataaac ctttttttgta attgcaatca
181 gcgtcagtaa acaatgtaat tattacaact ttcaacaacg gatctcttgg ttctggcatc
241 gatgaagaac gcagcgaaat gcgatacgta gtgtgaattg cagaattcag tgaatcatcg
301 aatctttgaa cgcacattgc gccctttggt attccaaagg gcatgcctgt tcgagcgtca
361 tttgtaccct caagctttgc ttggtgttgg gcgtcttgtc tctcacgaga ctcgccttaa
421 aatcattggc agccgaccta ctggtttcgg agcgcagcac aattcttgca ctttgaatca
481 gccttggttg agcatccatc aagaccctat tttcttaact tttgacctcg gatcaggtag
541 ggatacccgc tgaacttaag catatcaata agcggagga

Figure 2. The 18S rRNA sequence of FJJ006

3. Fermentation of Fungal Strain

I. *Penicillium brasilianum* (strain number FCH061)

The fungal strain was cultured on solid YPG media (5 g of yeast extract, 5 g of peptone, 10 g of glucose, 16 g of agar, and 24.8 g of Instant Ocean in 1 L of distilled water) for 7 days. An agar plug (1 cm × 1 cm) was inoculated in a 250 mL flask that contained 100 mL of YPG media for 7 days. Then, 10 mL of each culture was transferred to a 2.8 L Fernbach flask that contained rice media (200 g of rice, 0.5 g of yeast extract, 0.5 g of peptone, and 12.4 g of Instant Ocean in 500 mL of distilled water). In total, 400 g of rice media was prepared and cultivated for 40 days at 28 ℃, with stirring once a week.

II. *Stemphylium lycopersici* (strain number FJJ006)

The fungal strain was cultured on solid YPG media (5 g of yeast extract, 5 g of peptone, 10 g of glucose, 16 g of agar, and 24.8 g of Instant Ocean in 1 L of distilled water) for 7 days. An agar plug (1 cm × 1 cm) was inoculated in a 250 mL flask that contained 100 mL of YPG media for 7

days. Then, 10 mL of each culture was transferred to a 2.8 L Fernbach flask that contained rice media (200 g of rice, 1 g of yeast extract, 1 g of peptone, 2 g of glucose, and 5 g of Instant Ocean in 200 mL of distilled water). In total, 2000 g of rice media was prepared and cultivated for 35 days at 28 ℃, with stirring once a week.

4. Extraction and Isolation

I. *Penicillium brasilianum* (strain number FCH061)

The entire culture was macerated and extracted by EtOAc (1 L × 3). The solvent was evaporated *in vacuo* to obtain a brown organic extract (8.2g). The extract was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of H_2O and MeOH as the eluents (six fractions in a H_2O–MeOH, gradient from 50:50 to 0:100), acetone, and finally EtOAc. Based on the ^1H NMR analysis results, the fractions eluted with H_2O–MeOH (20:80) (670 mg), and (10:90) (290 mg) were chosen for the separation. The fraction that eluted with H_2O–MeOH (20:80) was separated by semi-preparative reversed-phase HPLC (H_2O–MeCN, 58:42, 2.0 mL/min), yielding eleven peaks rich with secondary metabolites. Further purification of ninth peak by reversed-phase HPLC (YMC-ODS column, 4.6 × 250 nm; H_2O–MeOH, 42:58, 0.7 mL/min) provided compound **2** (6.9 mg) and compound **3** (0.8 mg), respectively. The fraction that eluted with H_2O–MeOH (10:90) was separated by semi-preparative reversed-phase HPLC (H_2O–MeOH, 35:65, 2.0 mL/min), affording compounds **1** (3.0 mg).

compound (**1**): yellow amorphous solid, $[\alpha]_D^{25}$ +36.6(*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.02), 284 (3.16) nm; IR (ZnSe) ν_{max} 3720, 3483, 2981, 2312, 1736 cm^{-1}; HRFABMS, *m/z* 515.2620 $[M+Na]^+$ (calcd for $C_{27}H_{40}O_8Na$, 515.2621)

compound (**2**): white amorphous solid, $[\alpha]_D^{25}$ -26.9 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (3.49) nm; IR (ZnSe) ν_{max} 3412, 2973, 2938, 1758, 1694 cm^{-1}; HRFABMS, *m/z* 429.2275 $[M+H]^+$ (calcd for $C_{25}H_{33}O_6$, 429.2277).

compound (**3**): white amorphous solid, $[\alpha]_D^{25}$ -50.9 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (3.83) nm; IR (ZnSe) ν_{max} 3120 2973, 2931, 2307, 1755, 1687 cm^{-1}; HRFABMS, *m/z* 429.2279 $[M+H]^+$ (calcd for $C_{25}H_{33}O_6$, 429.2277).

II. *Stemphylium lycopersici* (strain number FJJ006)

The entire culture was macerated and extracted by EtOAc (1 L × 3). The solvent was evaporated *in vacuo* to obtain a brown organic extract (5.8g). The extract was separated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of H_2O and MeOH as the eluents (seven fractions in a H_2O–MeOH, gradient from 60:40 to 0:100), acetone, and finally EtOAc. On the basis of the results of LC-MS analysis, the fractions eluted with H_2O–MeOH (40:60) (125 mg), and (20:80) (670 mg) were chosen for the separation. The fraction that eluted with H_2O–MeOH (40:60) was separated by semi-preparative reversed-phase HPLC (YMC ODS column, 10 mm × 250 mm; 1.8 ml/min, gradient from H_2O–MeCN (65:35) to (40:60)) to afford compound **1** (7.0 mg) and compound **2** (3.0 mg), respectively. The fraction that eluted with H_2O–MeOH (20:80) was separated by semi-preparative reversed-phase HPLC (YMC ODS column, 10 mm × 250 mm; 1.8 ml/min, gradient from H_2O–MeCN (65:35) to (25:75)) to afford 20 peaks. Peak 18 was further purified by reversed-phase HPLC (YMC-ODS column, 4.6 × 250 nm; H_2O–MeCN, 45:55, 0.7 mL/min), affording compounds **3** (2.0 mg).

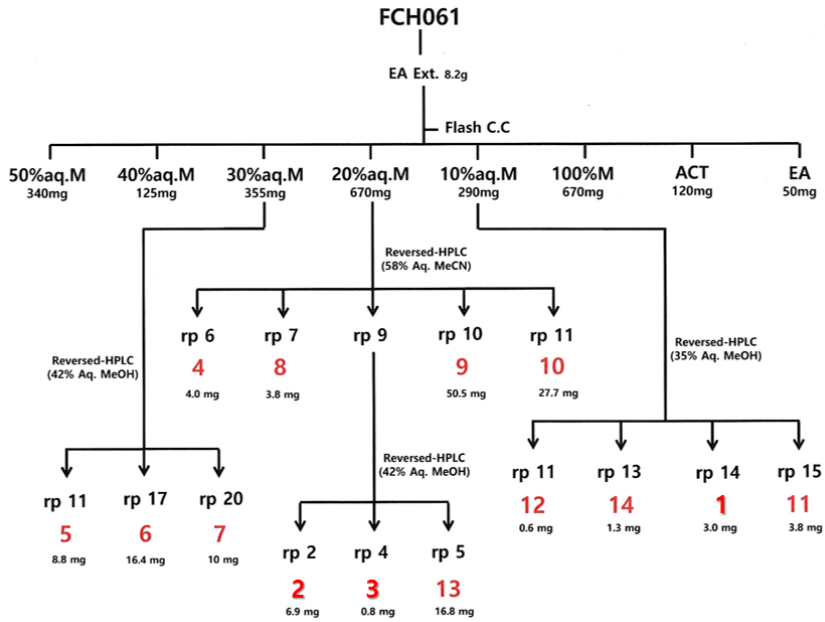
compound (**1**): orange amorphous powder, $[\alpha]_D^{25}$ +1.77(*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.97), 275 (3.86), 430 (3.36) nm; IR (ZnSe) ν_{max} 3544, 2970, 1622, 1372, 1054 cm^{-1}; HRFABMS, *m/z* 619.1449 $[M+H]^+$ (calcd for $C_{32}H_{27}O_{13}$, 619.1452)

compound (**2**): orange amorphous powder, $[\alpha]_D^{25}$ -2.45 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.79), 274 (3.68), 432 (3.09) nm; IR (ZnSe) ν_{max} 3412, 2973, 2938, 1758, 1694 cm^{-1}; HRFABMS, *m/z* 619.1454 $[M+H]^+$ (calcd for $C_{32}H_{27}O_{13,}$ 619.1452).

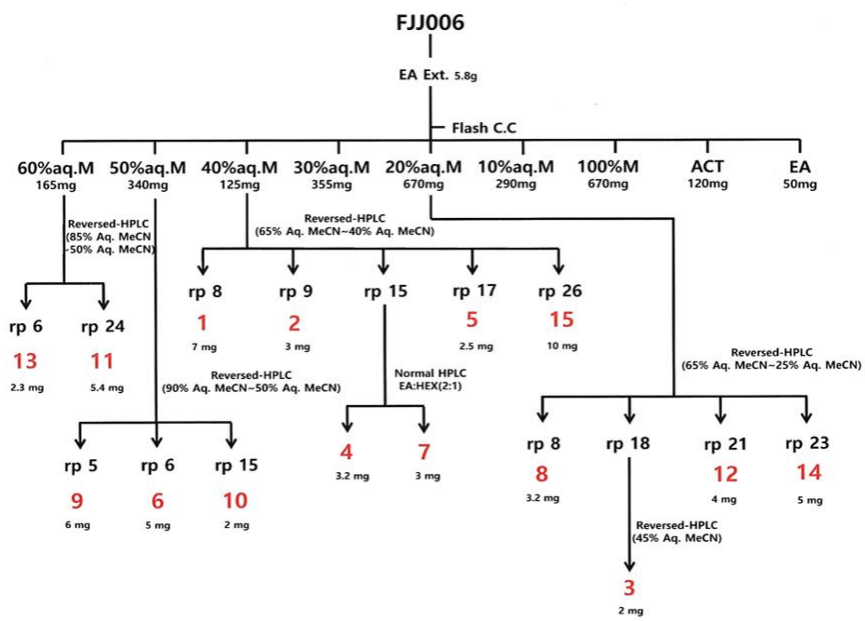
compound (**3**): dark red amorphous powder, $[\alpha]_D^{25}$ +13.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 212 (3.83) nm; IR (ZnSe) ν_{max} 3120 2973, 2931, 2307, 1755, 1687 cm^{-1}; HRFABMS, *m/z* 655.1430 $[M+Na]^+$ (calcd for $C_{33}H_{28}O_{13}Na_{,}$ 655.1428).

5. Biological Assays

To evaluate the effect of compounds and its analogues on LPS-induced NO production, the nitrite assay was performed as described previously.[15] Briefly, RAW264.7 cells in 24-well plates were treated with 1 mg/ml LPS in the presence or absence of test compounds diluted in the medium. After 20 h of incubation, the media were collected and analyzed by the Griess reaction. In addition, to determine the cytotoxic effect of the tested compounds under the assay conditions, after the Griess reaction, MTT solution (final concentration 500 mg/ml) was added to each well and further incubated for 3 h at 378. Media were discarded, and DMSO was added to each well to dissolve generated formazan. The absorbance was measured at 570 nm, and the percentage of cell survival was determined by comparison with the control group.[14]



Scheme 1. Isolation of meroterpenoids compounds (**1-3**) from *Penicillium brasilianum*



Scheme 2. Isolation of bianthraquinone compounds (**1-3**) from *Stemphylium lycopersici*

RESULTS

I. *Penicillium brasilianum* (strain number FCH061)

Compound 1

The molecular formula $C_{27}H_{40}O_8$ for compound **1** was deduced on the basis of the LC-MS data which detected $[M+Na]^+$ at *m/z* = 515 and $[M–H]^-$ at *m/z* = 491. The HRFABMS spectrum gave *m/z* = 515.2620 (calcd for 515.2621 $[M+Na]^+$) confirming this molecular formula. The IR spectrum showed absorptions at 3483 cm^{-1} (hydroxyl group), 1736 cm^{-1} (carbonyl groups). A comparison of the ^1H and ^{13}C NMR data of compound **1** (Table 1) with those of preaustinoid A[16] indicated that these two meroterpenes have identical B, C and D rings. However, the presence of one methoxyl group at δ_H 3.66 was observed in the ^1H NMR spectrum of compound **1**. The correlation of these methoxyl hydrogens with a carbonyl carbon at δ_C 175.3 in the HMBC (Figure 3**)** suggested the presence of a methyl ester in the molecule. In addition, ^{13}C NMR data showed signal of oxygenated quaternary carbon at δ_C 75.3. According to the data obtained from the NOESY, compound **1** shows the same relative stereochemistry at the stereogenic centers of austin.[17] Based on the results of combined spectroscopic analyses, the structure of compound **1** was determined to be the ring-opened derivative of preaustinoid A.[16]

Compound 2

Compound **2** was isolated as a white powder. The molecular formula $C_{25}H_{33}O_6$ for compound **2** was deduced on the basis of the LC-MS data which detected $[M+H]^+$ at *m/z* = 429 and $[M–H]^-$ at *m/z* = 427. The HRFABMS spectrum gave *m/z* = 429.2275 (calcd for 429.2277 $[M+H]^+$) confirming this molecular formula. The IR spectrum showed absorptions at 3412 cm^{-1} (hydroxyl group), 1758 cm^{-1} (acetyl group), 1694 cm^{-1} (carbonyl

group). Compound **2** showed similar ^{1}H and ^{13}C NMR spectra to those of preaustinoid A2 indicated that these two meroterpenes have identical A, B and C rings. However, the expected ^{1}H (δ_H = 3.74) and ^{13}C (δ_C = 52.6) NMR signals that characterize the carbomethoxyl group in preaustinoid A2 are not present in the spectra of compound **2**.[18] Furthermore, the remaining keto group detected at δ_C = 213.9 (C-4′) and a carbonyl group at δ_C = 171.7 (C-8′) The doublet at δ_H = 1.29 was attributed to methyl group Me-10′ (d, *J* = 6.4 Hz) which resulted from the coupling with a deshielded H-5′ at δ_H = 4.23 (q, *J* = 6.4 Hz) This suggested the presence of a γ -lactone forming the ring E in compound **2**. According to the data obtained from the NOESY, compound **2** shows the different relative stereochemistry at the C-7′ of austin. A correlation only between hydroxyl group δ_H 3.05 and methyl group δ_H = 1.29. Other correlations of these protons are not present in the NOESY (Figure 6).

Compound 3

The LC-MS data of compound **3** showed the ions $[M+H]^+$ detected at *m/z* = 429 and $[M–H]^-$ at *m/z* = 427, from which the molecular formula $C_{25}H_{33}O_6$ was deduced and confirmed by HRFABMS *m/z* = 429.2279; calcd. 429.2277, $[M+H]^+$). The IR spectrum showed absorptions at 3120 (hydroxyl group) and 1755, 1687 cm^{-1} (carbonyl groups). A comparison of the ^{1}H and ^{13}C NMR data of compound **3** with those of compound **2** indicated that these two meroterpenes were very similar. Planar structure was almost same. However, C-5′ and H-5′ of Compound **2** were changed from δ_C = 75.6 and δ_H = 4.23 to δ_C = 84.3 and δ_H = 4.42. ^{1}H and ^{13}C Chemical shifts of methyl group Me-10′ were changed from δ_C = 12.5 and δ_H = 1.29 to δ_C = 18.0 and δ_H = 1.13.[20] According to the data obtained from the NOESY, compound **3** shows the different relative stereochemistry at the C-5′ of compound **2**(Figure 6)**.**

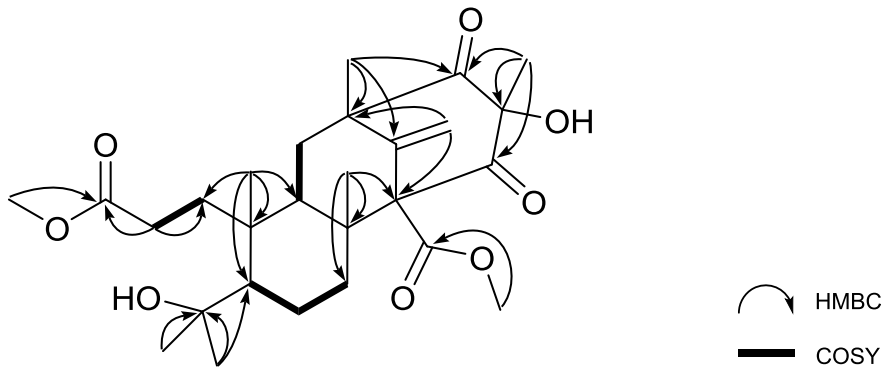


Figure 3. COSY and HMBC correlations of Compound **1**(FCH061)

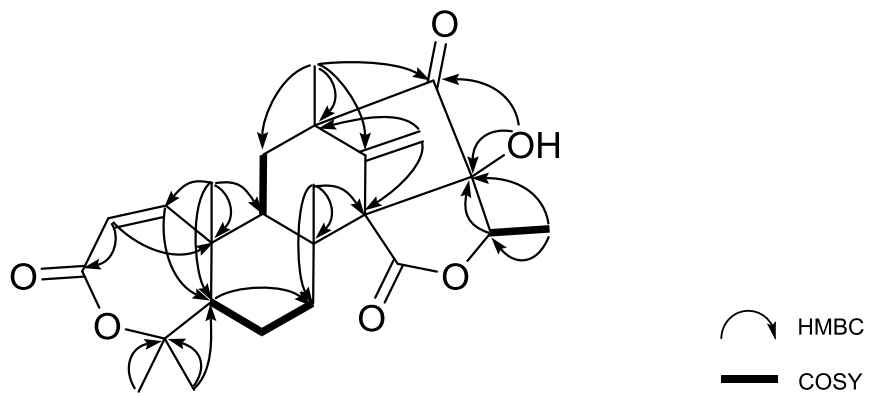


Figure 4. COSY and HMBC correlations of Compound **2**(FCH061)

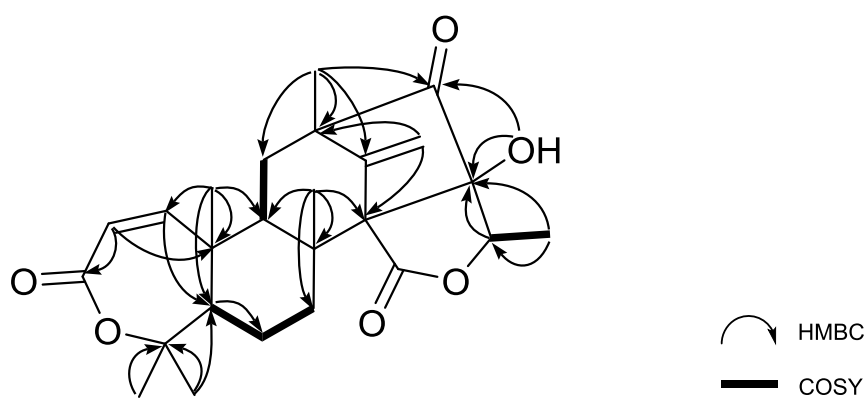


Figure 5. COSY and HMBC correlations of Compound **3**(FCH061)

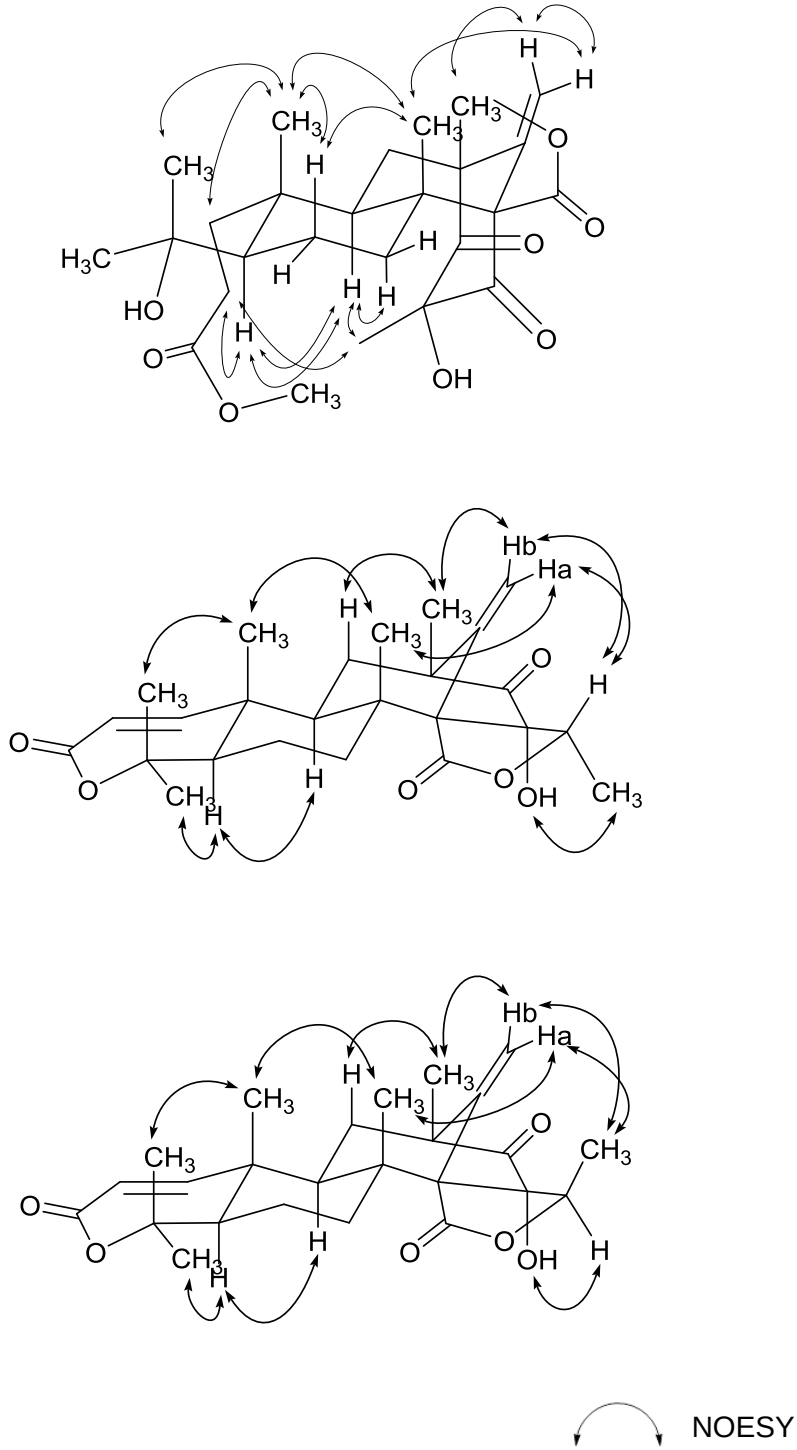


Figure 6. NOESY correlations the ring of compound **1-3** (FCH061)

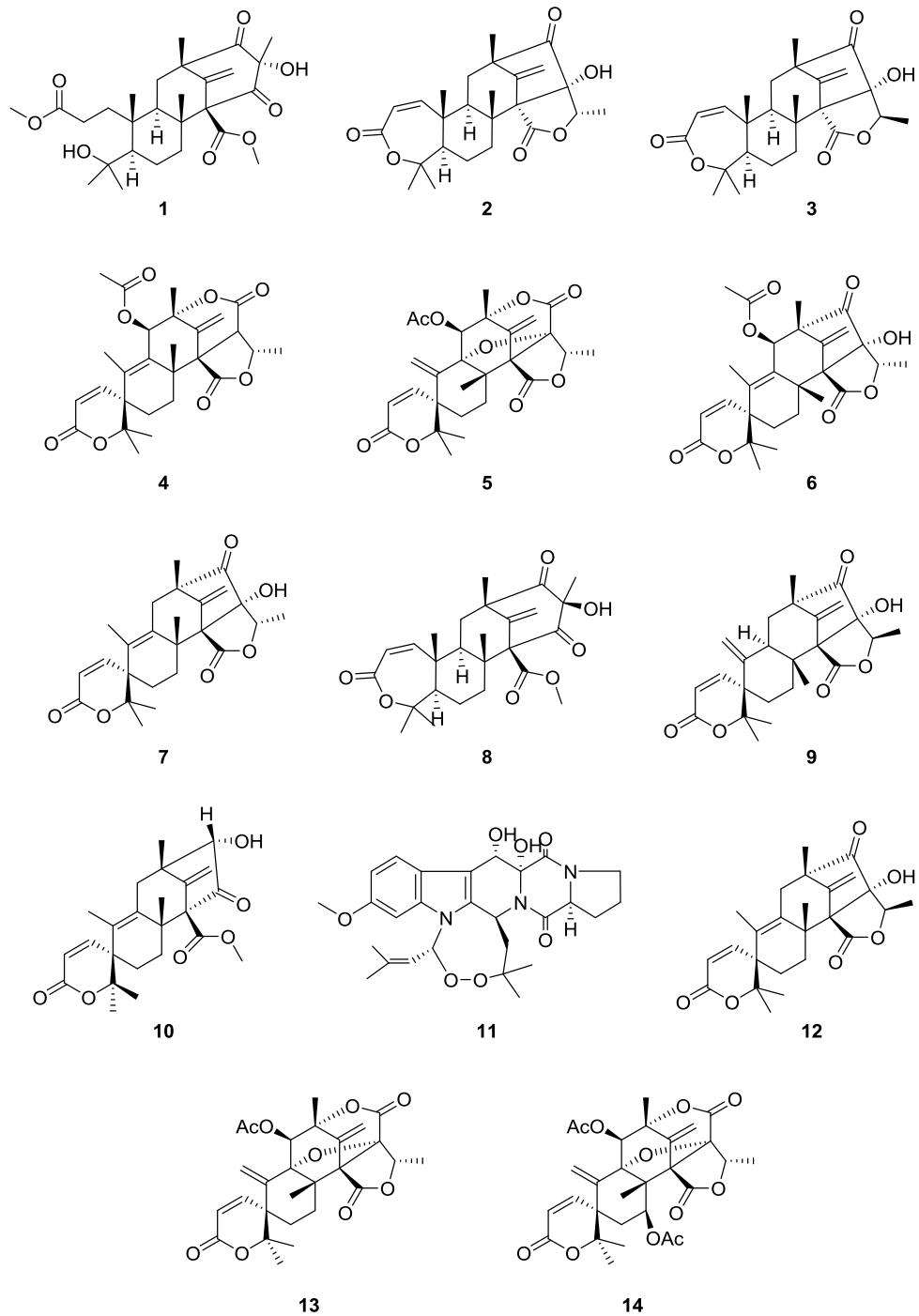


Figure 7. Structures of isolated compounds from FCH061

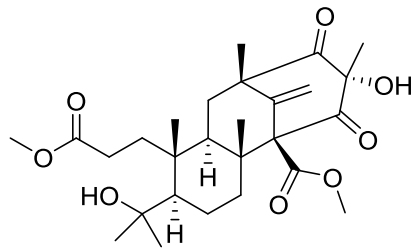


Table 1. ^{13}C and ^{1}H NMR Assignment for **1** (FCH061) in $CDCl_3$

Position	δ_C	δ_H
1α	33.4, CH_2	1.53, m
1β		2.55, ddd (15.2, 9.7, 5.4)
2α	27.8, CH_2	1.78, m
2β		2.05, ddd (9.7, 8.9, 5.4)
3	175.3, C	
4	75.3, C	
5α	50.4, CH	1.13, dd (11.6, 3.2)
6α	22.7, CH_2	1.50, m
6β		1.50, m
7α	32.3, CH_2	1.88, td (13.2, 4.1)
7β		2.15, dt (13.5, 3.4)
8	47.8, C	
9α	44.3, CH	0.67, dd (13.6, 2.7)
10	42.1, C	
11α	38.9, CH_2	1.56, m
11β		1.96, dd (13.1, 2.7)
12	16.9, CH_3	1.25, s
13	20.2, CH_3	0.96, s
14	26.8, CH_3	1.18, s
15	34.2, CH_3	1.24, s
OCH_3	51.8, CH_3	3.66, s
1′a	112.5, CH_2	4.87, s
1′b		5.39, s
2′	145.8, C	
3′	50.8, C	
4′	207.9, C	
5′	80.1, C	
6′	203.7, C	
7′	72.6, C	
8′	168.5, C	
9′	22.2, CH_3	1.47, s
10′	14.9, CH_3	1.38, s

OCH_3	52.5, CH_3	3.72, s
OH		3.25, s

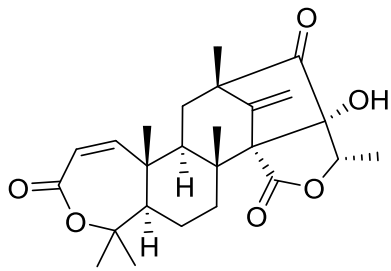


Table 2. ^{13}C and ^{1}H NMR Assignment for **2** (FCH061) in $CDCl_3$

Position	δ_C	δ_H
1	155.3, CH	6.22, d (12.2)
2	120.9, CH	5.83, d (12.2)
3	167.6, C	
4	85.5, C	
5	56.0, CH	2.05, dd (12.9, 3.4)
6α	23.4, CH_2	1.61, m
6β		1.73, qd (13.3, 3.7)
7α	32.3, CH_2	1.90, dt (13.0, 3.4)
7β		2.16, td (13.0, 4.0)
8	41.3, C	
9	47.8, CH	2.15, dt (13.6, 3.5)
10	43.7, C	
11α	39.8, CH_2	1.92, m
11β		1.80, m
12	18.2, CH_3	1.29, s
13	15.2, CH_3	1.16, s
14	32.3, CH_3	1.40, s
15	26.6, CH_3	1.42, s
1′a	107.6, CH_2	5.12, s
1′b		5.14, s
2′	146.6, C	
3′	55.1, C	
4′	213.9, C	
5′	75.6, CH	4.23, q (6.4)
6′	90.6, C	
7′	66.4, C	
8′	171.7, C	
9′	16.0, CH_3	1.29, s
10′	12.5, CH_3	1.29, s
OH		3.05, s

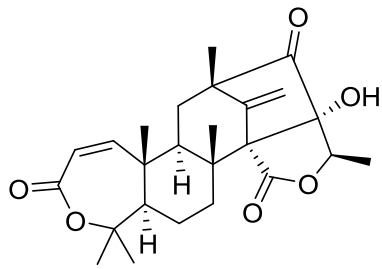


Table 3. ^{13}C and ^{1}H NMR Assignment for **3** (FCH061) in $CDCl_3$

Position	δ_C	δ_H
1	155.1, CH	6.20, d (12.2)
2	120.8, CH	5.82, d (12.2)
3	167.5, C	
4	85.5, C	
5	55.9, CH	2.07, dd (12.8, 3.4)
6α	23.5, CH_2	1.61, ddd (13.8, 7.2, 3.6)
6β		1.72, td (13.3, 3.7)
7α	32.4, CH_2	1.85, dt (13.1, 3.4)
7β		2.21, td (13.0, 4.0)
8	42.1, C	
9	47.5, CH	2.03, dt (13.6, 3.5)
10	43.8, C	
11α	40.3, CH_2	1.92, dd (12.3, 3.8)
11β		1.78, dd (13.9, 12.4)
12	18.2, CH_3	1.29, s
13	15.4, CH_3	1.15, s
14	32.3, CH_3	1.39, s
15	26.6, CH_3	1.42, s
1′a	108.5, CH_2	5.15, s
1′b		5.18, s
2′	147.7, C	
3′	55.4, C	
4′	215.5, C	
5′	84.3, CH	4.42, q (7.2)
6′	90.5, C	
7′	64.9, C	
8′	171.8, C	
9′	15.6, CH_3	1.25, s
10′	18.0, CH_3	1.13, s
OH		3.47, s

II. *Stemphylium lycopersici* (strain number FJJ006)

Compound 1

Compound **1** was a orange amorphous powder. The HRFABMS exhibited a peak at *m/z* = 619.1449 $[M+H]^+$ indicating a molecular formula of $C_{32}H_{27}O_{13}$ (calcd. For $C_{32}H_{27}O_{13}$, 619.1452). Comparison of the ^1H and ^{13}C NMR spectral data of compound **1** (Table 4) with that of Alterporriol A showed a close structural relationship between both compounds, except for the difference of carbon-carbon linkage.[21-22] The presence of a bond connecting C-7 and C-6′ was suggested by the absence of two sets of *ortho*-coupled doublets and the presence of two singlets H-6 (δH = 6.78 ppm, s) and H-7′ (δH = 6.81 ppm, s). Moreover, HMBC correlations of H-6 with C-5, C-7, C-10a, C-6′ and C-10, and of H-7′ with C-6′, C-8′, C-8a′, C-9′, and C-7, respectively (Figure 8) provided evidence for C-7–C-6′ linkage of compound **1**. The relative configuration of the aliphatic ring in **1** was determined by interpretation of the NOESY spectrum (Figure 11) and analysis of the coupling constants. This relative configuration is also known for other structurally related natural products, for instance altersolanol F.[23,30] Several alterporriols have been isolated as atropisomers. It has been reported that some biphenyl-like compounds with *ortho*-substituted methoxy groups exhibit atropisomerism with different degrees of stability, because the steric size of the methoxy groups is large enough to restrict rotation.[24-25] Compound **1** have *ortho* methoxy groups and therefore might be expected to exist as atropisomerism. In order to assign axial chirality of compound **1**, CD spectra was compared with known compound Alterporriol A, and determined an a*R* configuration.[26] The new compound **1** exhibited anti-inflammatory activities in LPS-stimulated RAW 264.7 cells. The compounds did not induce cytotoxicity and showed concentration-dependent inhibitory effect (Figure 15).

Compound 2

Compound **2** was a orange amorphous powder. The HRFABMS exhibited a peak at *m/z* = 619.1454 $[M+H]^+$ indicating a molecular formula of $C_{32}H_{27}O_{13}$ (calcd. For $C_{32}H_{27}O_{13}$, 619.1452). A comparison of the ^1H and ^{13}C NMR data of compound **2** with those of compound **1** indicated that these two bianthraquinones were very similar. Planar structure and relative configuration of the aliphatic ring were almost same. However, CD spectra of compound **1**/**2** recorded in MeOH showed a near-quasi-mirror images pattern (Figure 12). Compound **2** has different axial chirality of compound 1. CD spectra were compared with known compound Alterporriol B and compound **1**. Compound **2** was determined an a*S* configuration.[26,30] Compound **2** also exhibited anti-inflammatory activities in LPS-stimulated RAW 264.7 cells (Figure 15).

Compound 3

Compound **3** was a orange amorphous powder. The HRFABMS exhibited a peak at *m/z* = 655.1430 $[M+Na]^+$ indicating a molecular formula of $C_{33}H_{28}O_{13}$ (calcd. For $C_{33}H_{28}O_{13}Na$, 655.1428). Comparison of the ^1H and ^{13}C NMR spectral data of compound **3** (Table 6) with that of Alterporriol N[27] showed a close structural relationship between both compounds, except for the presence of one methoxyl group at δ_H 3.77 was observed in the ^1H NMR spectrum of compound **3**. It also has same relative configuration of the aliphatic ring other new compounds. Compound **3** was determined that hydroxyl group of Alterporriol N was changed to methoxyl group. CD spectra were compared with new compound **1** and compound **2**. Compound 3 was determined an a*R* configuration.[28-29]

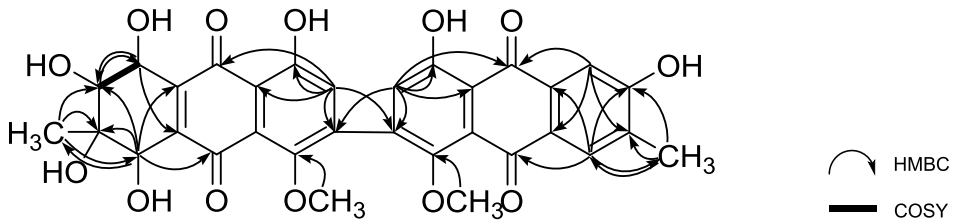


Figure 8. COSY and HMBC correlations of Compound **1**(FJJ006)

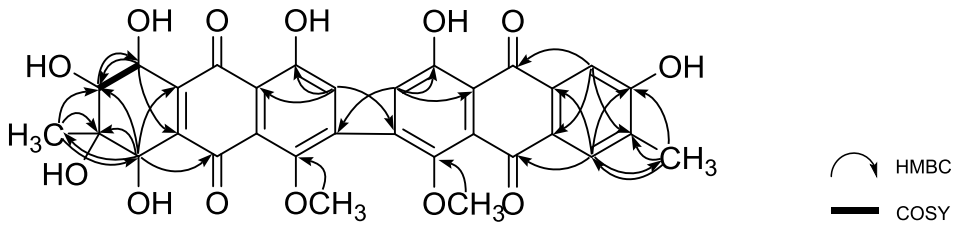


Figure 9. COSY and HMBC correlations of Compound **2**(FJJ006)

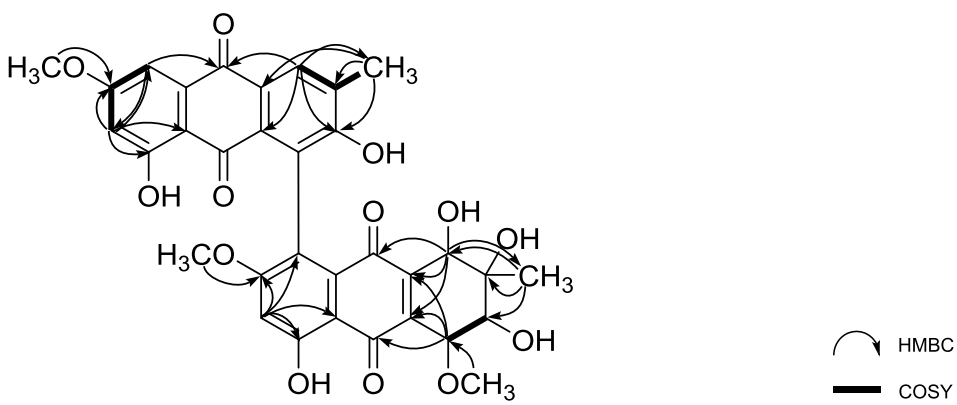


Figure 10. COSY and HMBC correlations of Compound **3**(FJJ006)

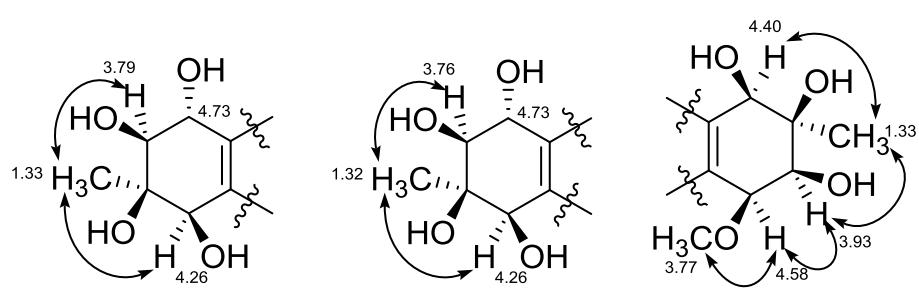
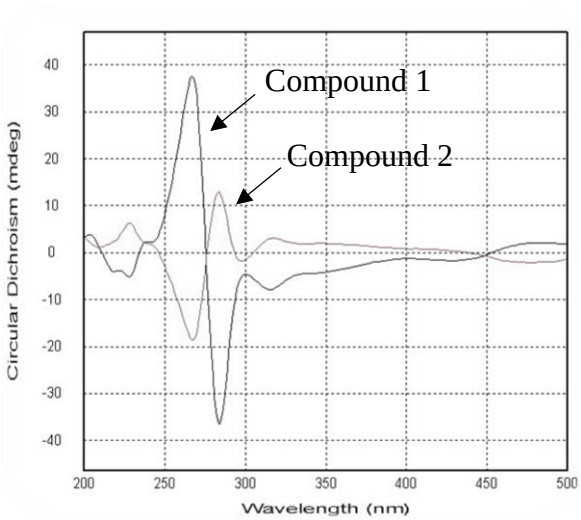
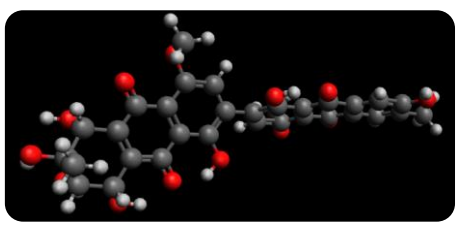
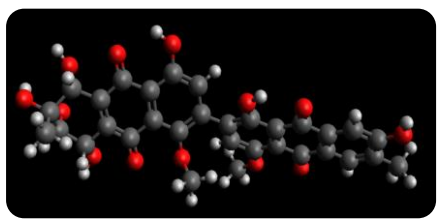


Figure 11. NOESY correlations the ring of compound **1-3** (FJJ006)



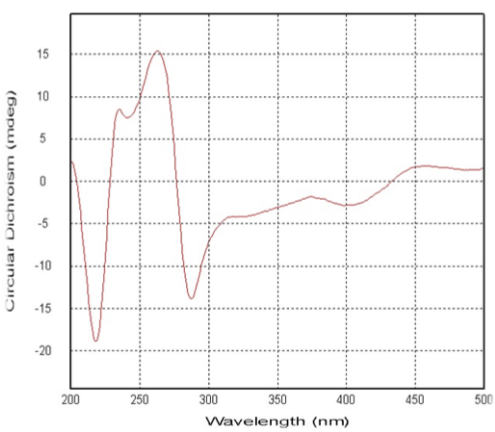


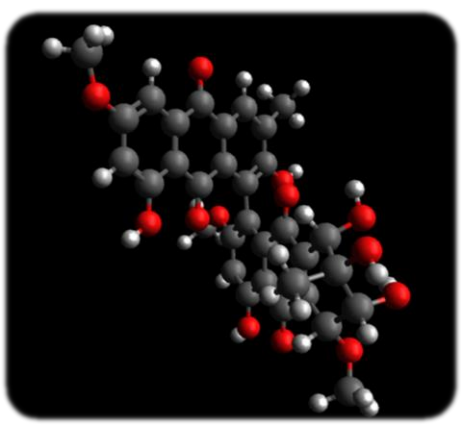
a*R*



a*S*

Figure 12. CD spectrum of compound **1** and compound **2** (FJJ006)





a*R*

Figure 13. CD spectrum of compound **3** (FJJ006)

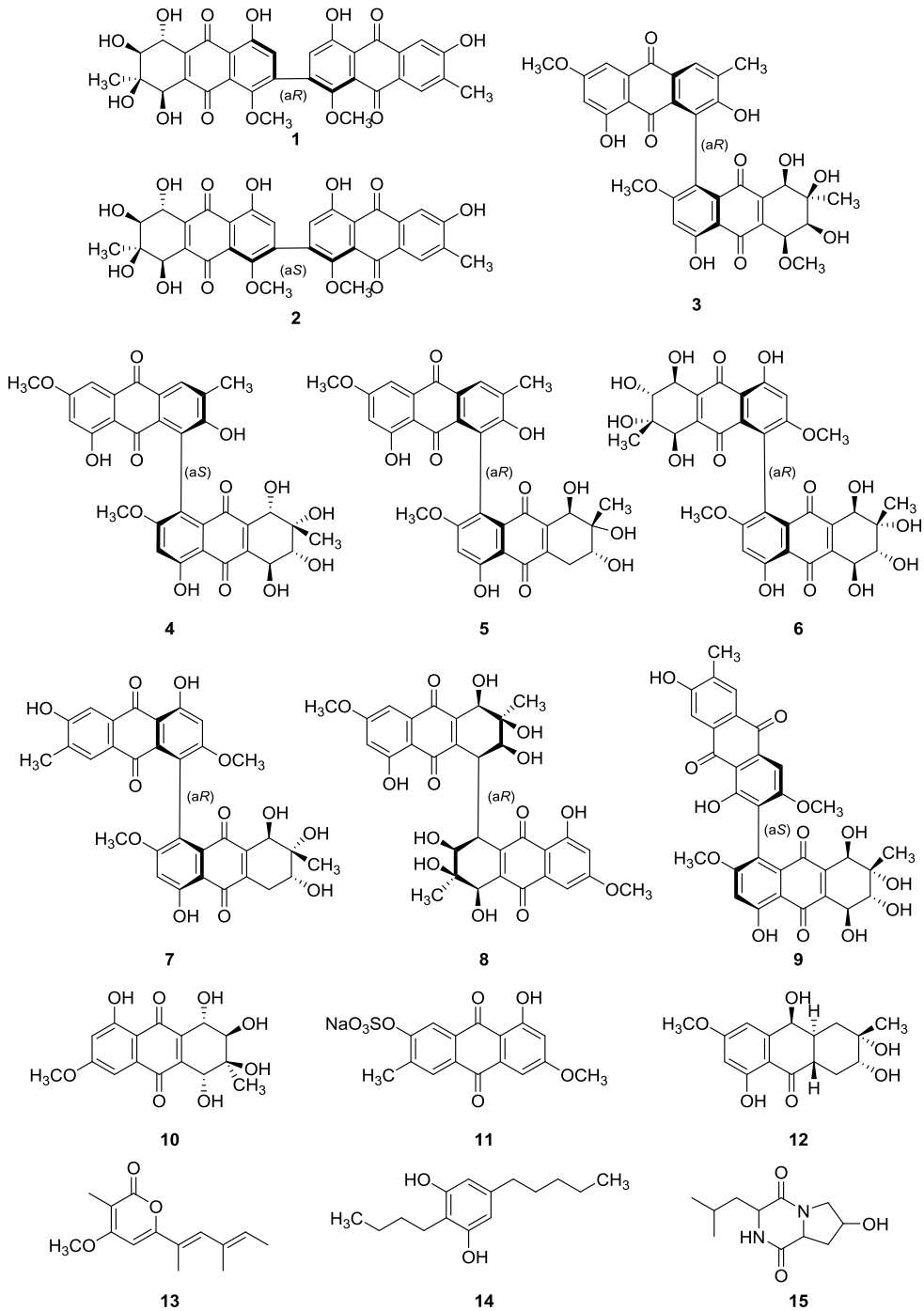


Figure 14. Structures of isolated compounds from FJJ006

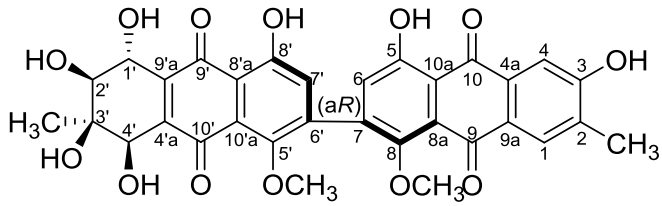


Table 4. ^{13}C and ^{1}H NMR Assignment for **1** (FJJ006) in CD_3OD

Position	δ_C	δ_H
1	131.2, CH	7.65, s
2	133.8, C	
3	164.0, C	
4	111.7, CH	7.51, s
4a	134.7, C	
5	166.3, C	
6	104.6, CH	6.78, s
7	125.3, C	
8	166.9, C	
8a	133.4, C	
9	183.5, C	
9a	126.8, C	
10	188.7, C	
10a	111.9, C	
11	16.6, CH_3	2.23, s
12	57.0, CH_3	3.69, s
1′	70.6, CH	4.73, d (7.5)
2′	75.2, CH	3.79, d (7.5)
3′	74.6, C	
4′	70.1, CH	4.26, s
4′a	143.8, C	
5′	165.8, C	
6′	123.4, C	
7′	104.6, CH	6.81, s
8′	166.1, C	
8′a	111.0, C	
9′	190.5, C	
9′a	143.9, C	
10′	185.7, C	
10′a	130.8, C	
11′	22.3, CH_3	1.33, s
12′	56.9, CH_3	3.70, s

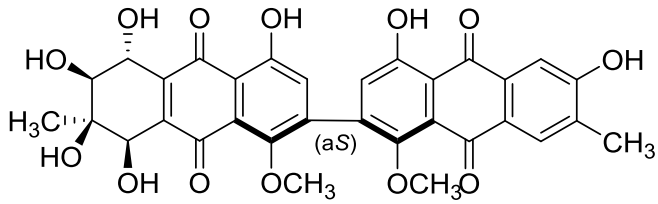


Table 5. ^{13}C and ^{1}H NMR Assignment for **2** (FJJ006) in CD_3OD

Position	δ_C	δ_H
1	131.3, CH	7.65, s
2	133.8, C	
3	163.7, C	
4	111.8, CH	7.51, s
4a	127.1, C	
5	166.4, C	
6	104.3, CH	6.80, s
7	125.7, C	
8	167.1, C	
8a	132.8, C	
9	188.6, C	
9a	134.6, C	
10	183.5, C	
10a	111.7, C	
11	16.6, CH_3	2.23, s
12	56.9, CH_3	3.70, s
1′	70.6, CH	4.73, d (7.4)
2′	75.2, CH	3.76, d (7.4)
3′	74.7, C	
4′	70.2, CH	4.26, s
4′a	143.9, C	
5′	166.5, C	
6′	123.9, C	
7′	104.6, CH	6.82, s
8′	166.2, C	
8′a	111.0, C	
9′	190.6, C	
9′a	143.7, C	
10′	185.5, C	
10′a	130.7, C	
11′	22.3, CH_3	1.32, s
12′	57.0, CH_3	3.71, s

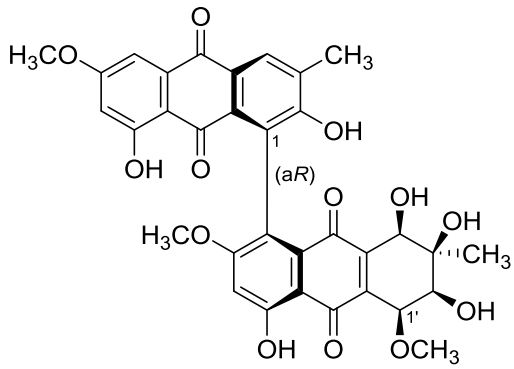
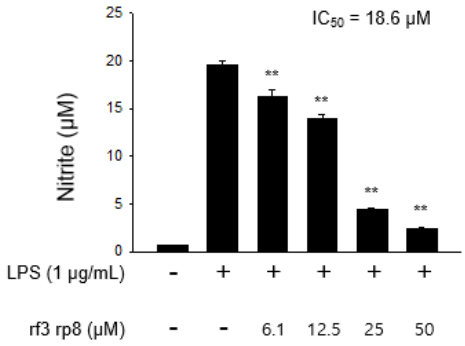


Table 6. ^{13}C and ^{1}H NMR Assignment for **3** (FJJ006) in CD_3OD

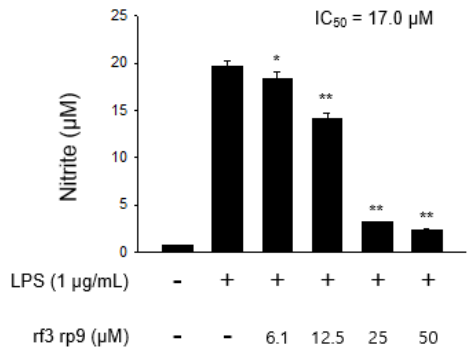
Position	δ_C	δ_H
1	129.0, C	
2	165.5, C	
3	134.5, C	
4	131.2, CH	8.00, s
4a	n.d.	
5	106.9, CH	7.20, d (2.5)
6	167.1, C	
7	105.9, CH	6.52, d (2.5)
8	166.0, C	
8a	112.1, C	
9	191.0, C	
9a	132.8, C	
10	182.3, C	
10a	137.8, C	
11	17.9, CH_3	2.21, s
12	56.3, CH_3	3.88, s
1′	75.2, CH	4.58, d (4.7)
2′	70.7, CH	3.93, d (4.7)
3′	75.4, C	
4′	70.6, CH	4.39, s
4′a	144.7, C	
5′	130.7, C	
6′	166.8, C	
7′	104.5, CH	6.79, s
8′	165.9, C	
8′a	110.9, C	
9′	189.6, C	
9′a	141.0, C	
10′	185.7, C	

10′a	n.d.	
11′	62.8, CH_3	3.77, s
12′	56.8, CH_3	3.71, s
13′	56.8, CH_3	3.71, s

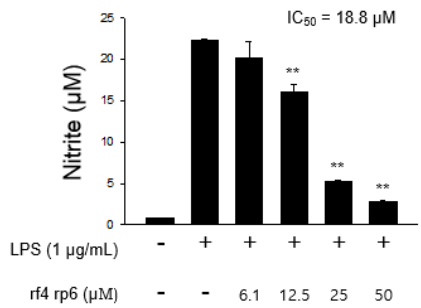
Compound **1**(FJJ006)



Compound **2**(FJJ006)



Alterporriol G



Alterporriol N

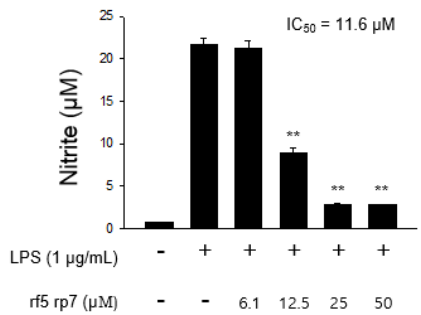


Figure 15. Results of Bioactivity Tests

Conclusion

The purpose of this work is the research of new bioactive secondary metabolites from marine-derived fungi.

Marine-derived fungi were investigated by performing small scale cultivation and extraction for screenings. Strain selection was conducted by LC-ESIMS analysis. Two fungal strains were chosen for a large cultivation and isolation of new compounds. Three new meroterpenoids were isolated from the marine-derived fungus *Penicillium brasilianum* (strain number FCH061). Three new bianthraquinone derivatives were isolated from the sponge-derived fungus *Stemphylium lycopersici* (strain number FJJ006).

Diverse bioassay tests have been performed. The new compounds **1**-**2** (FJJ006) exhibited anti-inflammatory activities in LPS-stimulated RAW 264.7 cells. The compounds did not induce cytotoxicity. Three new meroterpenoid compounds are currently under biological evaluation.

References

1. Mostafa, E.R.; Rainer, E. *Nat. Prod. Rep.* **2011**, *28*, 290-344.
2. Fenical, W. *Chem. Rev.* **1993**, *93*, 1673-1683.
3. Bugni, T.S.; Ireland, C.M. *Nat. Prod. Rep.* **2004**, *21*, 143-163.
4. Fenical, W.; Jensen, P.R. *Nat. Chem. Biol.* **2006**, *2*, 666-673.
5. Cragg, G.M.; Grothaus, P.G.; Newman, D.J. *Chem. Rev.* **2009**, *109*, 3012-3043.
6. Bhatnagar, I.; Kim, S.K. *Mar. Drugs* **2010**, *8*, 2673-2701.
7. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. *Nat. Prod. Rep.* **2016**, *33*, 382-431.
8. Hu, G.-P.; Yuan, J.; Sun, L.; She Z.-G.; Wu, J.-H.; Lan, X.-J.; Zhu, X.; Lin, Y.-C.; Chen, S.-P. *Mar. Drugs* **2011**, *9*, 514-525.
9. Oh, K.-B.; Lee, J. H.; Chung, S.-C.; Shin, J.; Shin, H. J.; Kim, H.-K.; Lee, H.-S. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 104-108.
10. Van le, T. K.; Hung, T. M.; Thuong, P. T.; Ngoc, T. M.; Kim, J. C.; Jang, H. S.; Cai, X. F.; Oh, S. R.; Min, B. S.; Woo, M. H.; Choi, J. S.; Lee, H. K.; Bea, K. *J. Nat. Prod*. **2009**, *72*, 1419−1423.
11. Johansson, M.; Karlsson, L.; Wennergren, M.; Jansson, T.; Powell, T. L. *J. Clin. Endocrinol. Metab*. **2003**, *88*, 2831−2837.
12. Oh, K.-B.; Kim, S. H.; Lee, J.; Cho, W. J.; Lee, T.; Kim, S. *J. Med. Chem*. **2004**, *47*, 2418−2421.
13. Chung, S. C.; Jang, K. H.; Park, J.; Ahn, C. H.; Shin, J.; Oh, K.-B. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 1958−1961.
14. Min, H.Y.; Song, S.H.; Lee, B.; Kim, S.H.; Lee, S.K. *Chem. Biodivers.* **2010**, *7*, 409–414.

15. Y. Fu, S. K. Lee, H.-Y. Min, T. Lee, J. Lee, M. Cheng, S. Kim, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 97-100.
16. Geris dos Santos, R. M.; Rodrigues-Fo, E. *Phytochemistry* **2002**, *61*, 907-912.
17. Chexal, K.K.; Springer, J.P.; Clardy, J.; Cole, R.J.; Kirksey, J.W.; Domer, J.W.; Cutler, H.G.; Sterawter, B.J. *J. Am. Chem. Soc.* **1976**, *98*, 6748–6750.
18. Santos, R. M. G.; Rodrigues–Filho, E.; *Z. Naturforsch.* **2003**, *58*, 663-669.
19. Hayashi, H.; Mukaihara, M.; Murao, S.; Arai, M.; Lee, A. Y.; Clardy, J. *Biosci. Biotechnol. Biochem*. **1994**, *58*, 334-338.
20. Fill, T. P.; Pereira, G. K.; Geris dos Santos, R. M.; Rodrigues-Fo, E. Z. *Naturforsch.* **2007**, *62b*, 1035-1044.
21. Stoessl, A. *Can. J. Chem.* **1969**, *47*, 777−784.
22. Suemitsu, R ; Sane. T.; Yamamoto, M.; Arimoto, Y.; Morimatsu, F.; Nabeshima, T. *Agric. Biol. Chem*. **1984**, *48*, 2611-2613.
23. Höller, U.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod*. **2002**, *65*, 876–882.
24. Suemitsu, R.; Yamamoto, T.; Miyai, T.; Ueshima, T. *Phytochemistry* **1987**, *26*, 3221−3224.
25. Suemitsu, R.; Sakurai, Y.; Nakachi, K.; Miyoshi, I.; Kubota, M.; Ohnishi, K. *Agric. Biol. Chem*. **1989**, *53*, 1301−1304.
26. Zhou, X.-M.; Zheng, C.-J.; Chen, G.-Y.; Song, X.-P.; Han, C.-R.; Li, G.-N.; Fu, Y.-H.; Chen, W.-H.; Niu, Z.-G. *J. Nat. Prod*. **2014**, *77*, 2021–2028.

27. Debbab, A.; Aly, A. H.; Edrada-Ebel, R.; Wray, V.; Pretsch, A.; Pescitelli, G.; Kurtan, T.; Proksch, P. *Eur. J. Org. Chem.* **2012**, 1351–1359.
28. Zheng, C.-J.; Shao, C.-L.; Guo, Z.-Y.; Chen, J.-F.; Deng, D.-S.; Yang, K.-L.; Chen, Y.- Y.; Fu, X.-M.; She, Z.-G.; Lin, Y.-C.; Wang, C.-Y. *J. Nat. Prod.* **2012**, *75*, 189–197.
29. Huang, C.H.; Pan, J.H.; Chen, B.; Yu, M.; Huang, H.B.; Zhu, X.; Lu, Y.J.; She, Z.G.; Lin, Y.C. *Mar. Drugs* **2011**, *9*, 832–843.
30. Kanamaru, S.; Honma, M.; Murakami, T.; Tsushima, T.; Kudo, S.; Tanaka, K.; Nihei, K.-I.; Nehira, T.; Hashimoto, M. *Chirality* **2012**, *24*, 137–146.

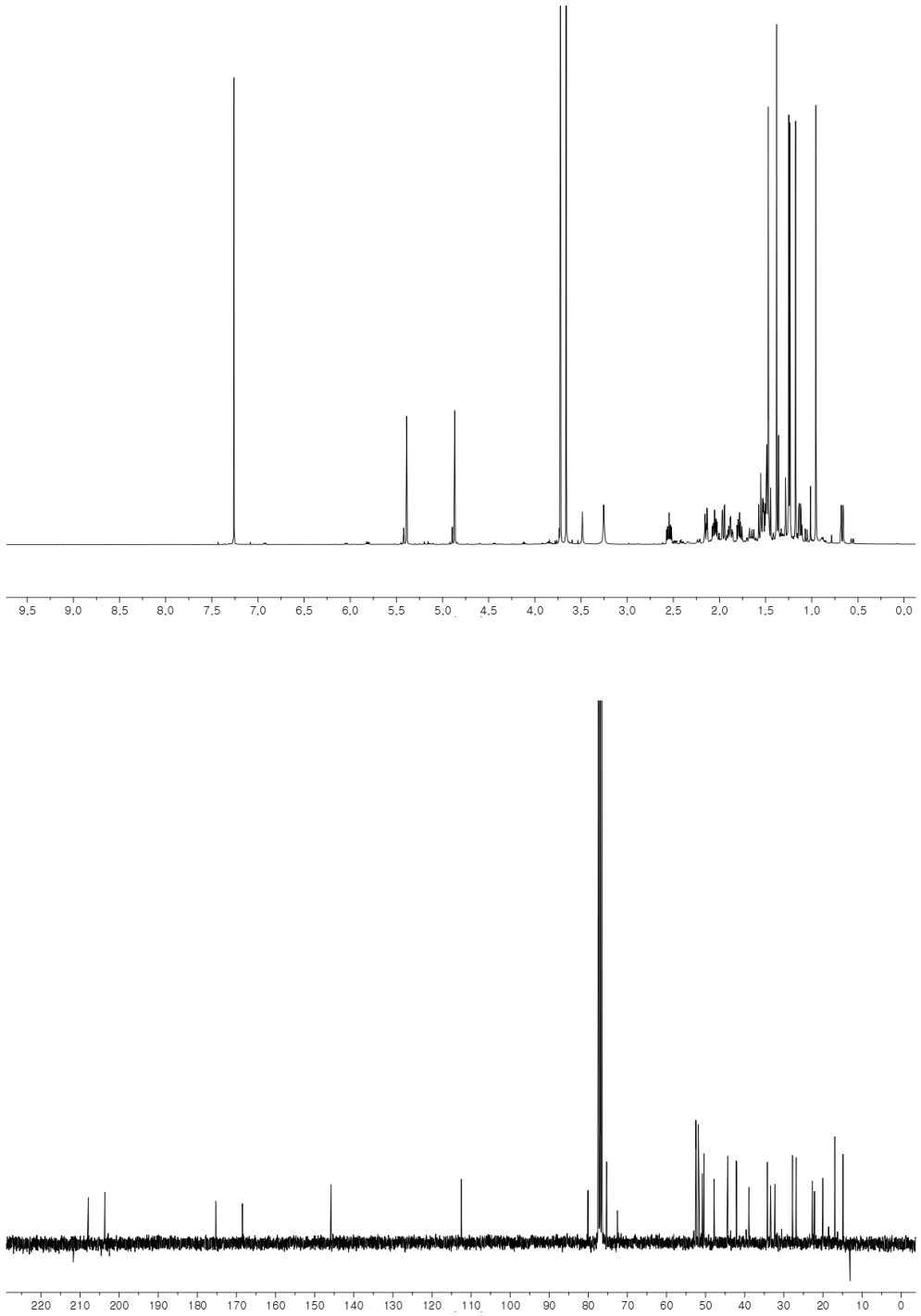


Figure 16. ^{1}H and ^{13}C NMR spectrum (600 and 150 MHz, respectively) of **1**(FCH061) in $CDCl_3$

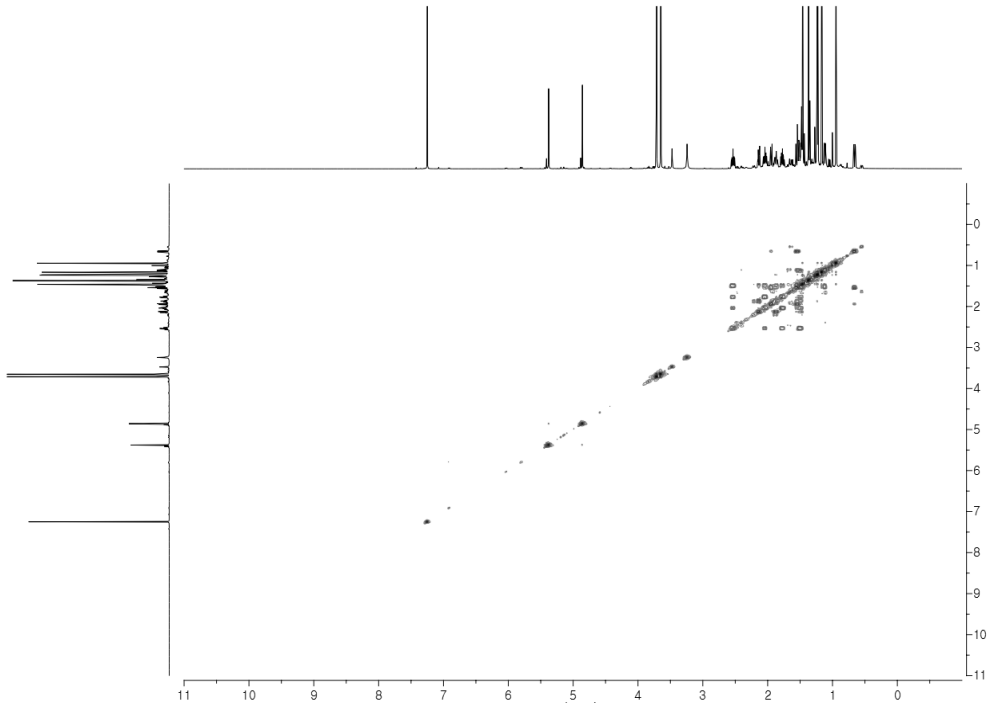


Figure 17. COSY spectrum (600 MHz) of **1**(FCH061) in $CDCl_3$

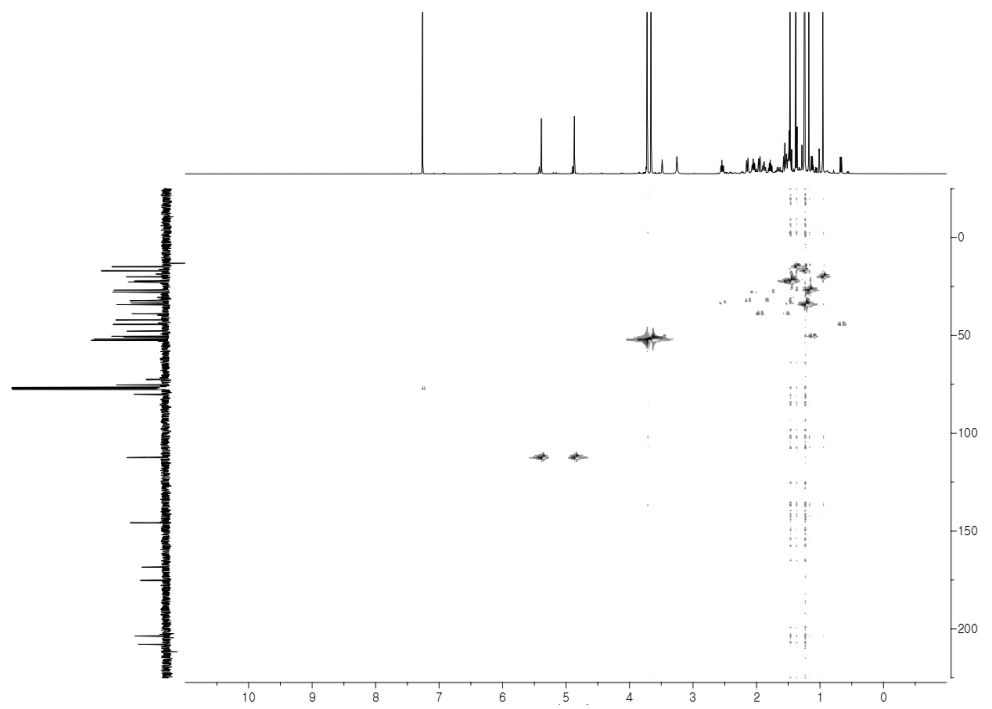


Figure 18. HSQC spectrum (600 MHz) of **1**(FCH061) in $CDCl_3$

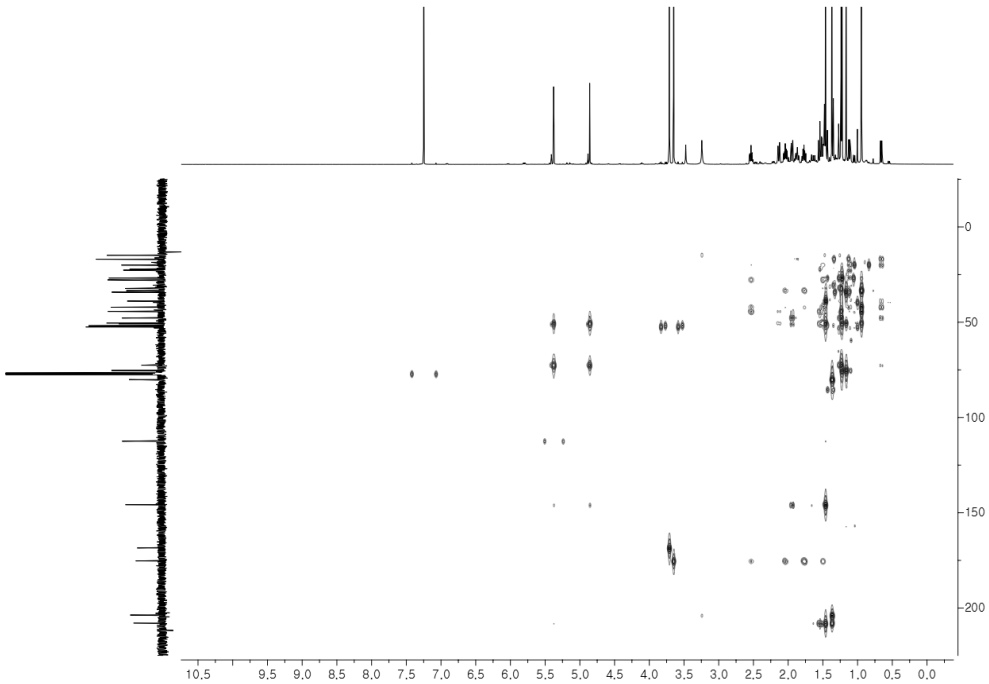


Figure 19. HMBC spectrum (600 MHz) of **1**(FCH061) in $CDCl_3$

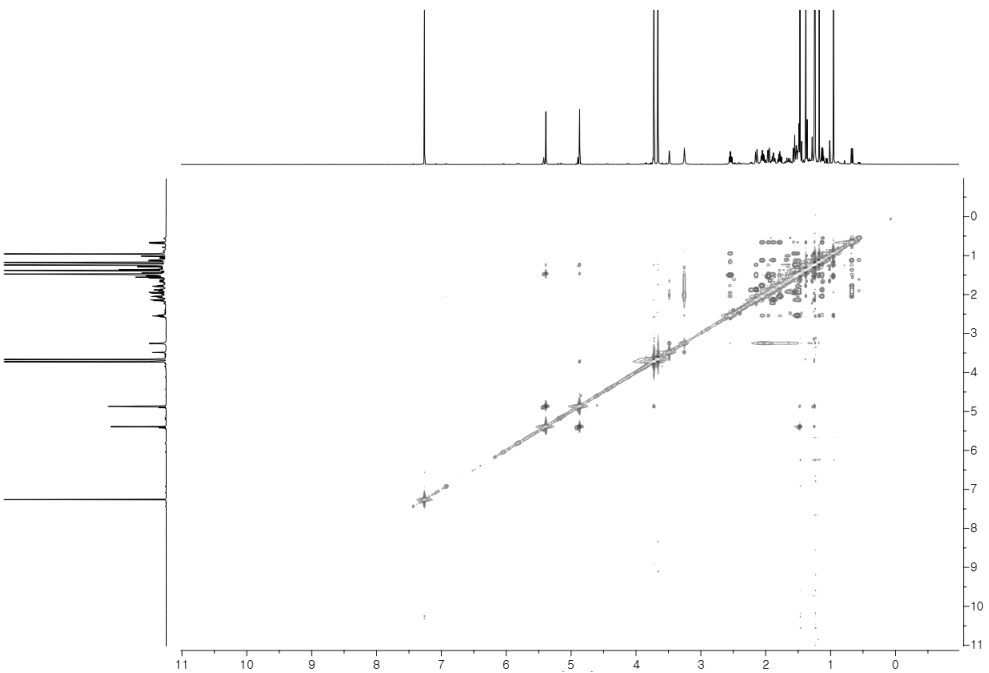


Figure 20. NOESY spectrum (600 MHz) of **1**(FCH061) in $CDCl_3$

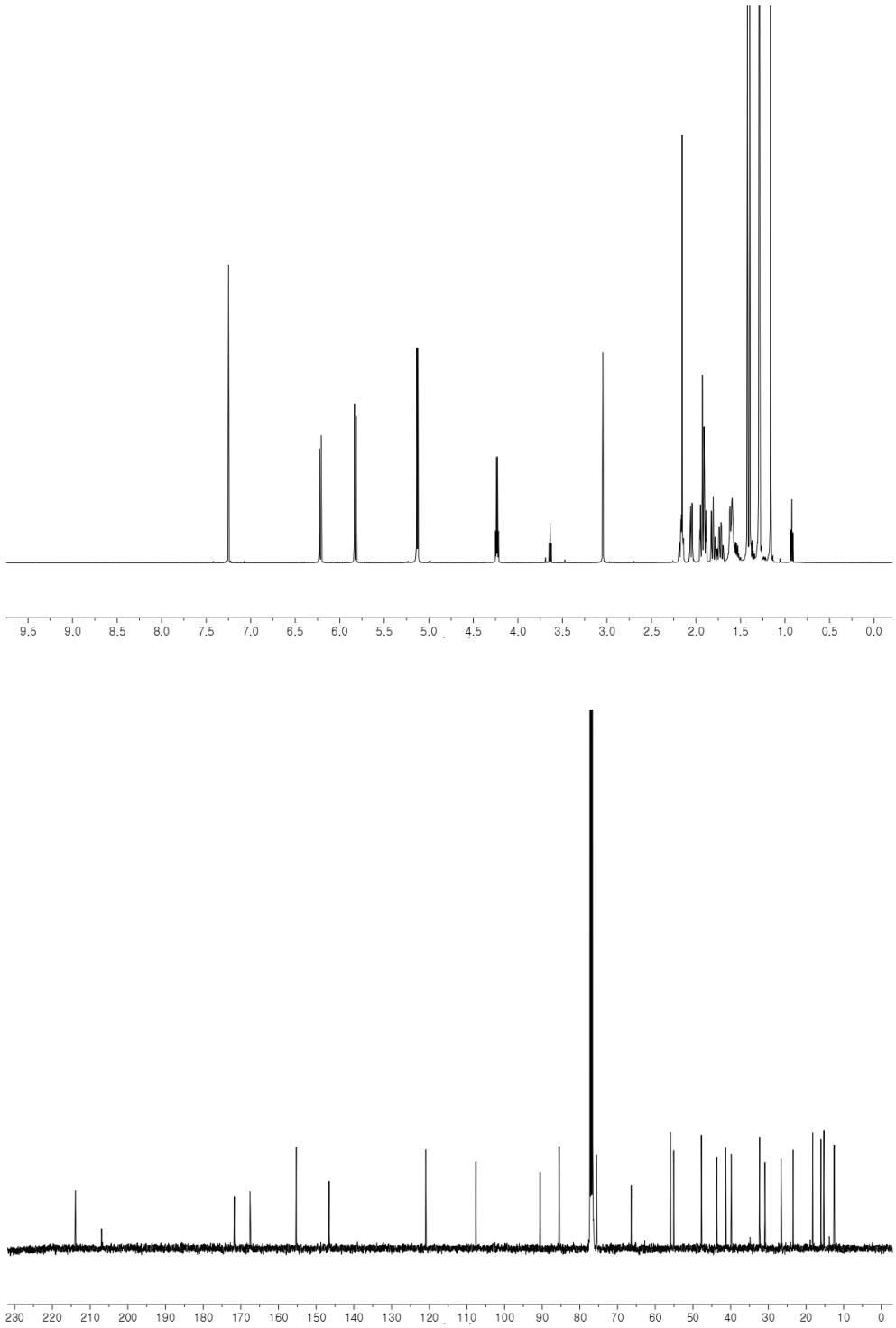


Figure 21. ^{1}H and ^{13}C NMR spectrum (600 and 100 MHz, respectively) of **2**(FCH061) in $CDCl_3$

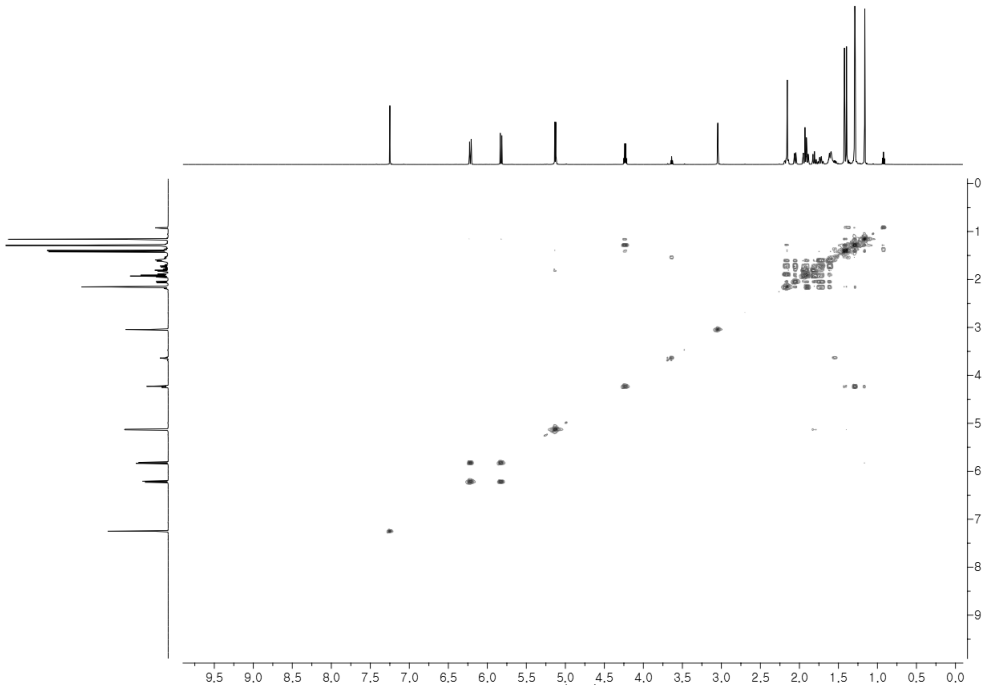


Figure 22. COSY spectrum (600 MHz) of **2**(FCH061) in $CDCl_3$

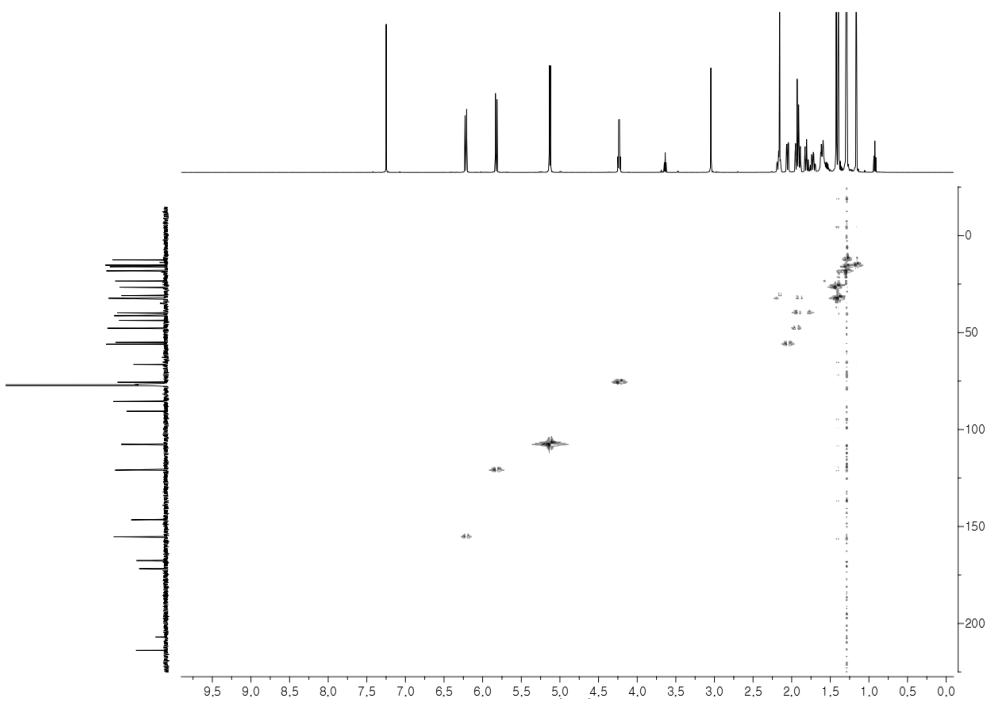


Figure 23. HSQC spectrum (600 MHz) of **2**(FCH061) in $CDCl_3$

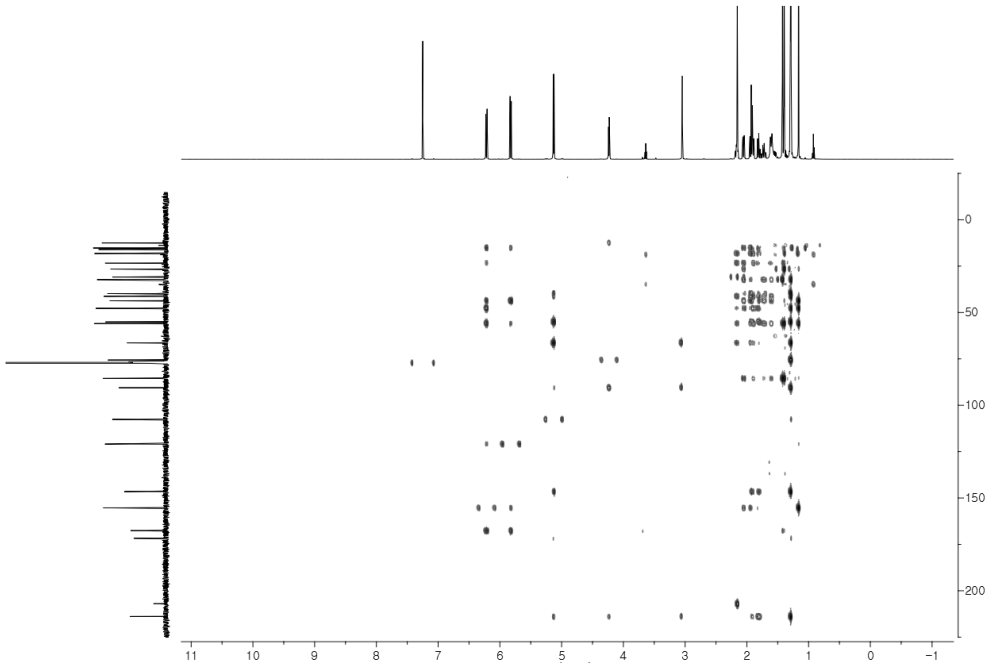


Figure 24. HMBC spectrum (600 MHz) of **2**(FCH061) in $CDCl_3$

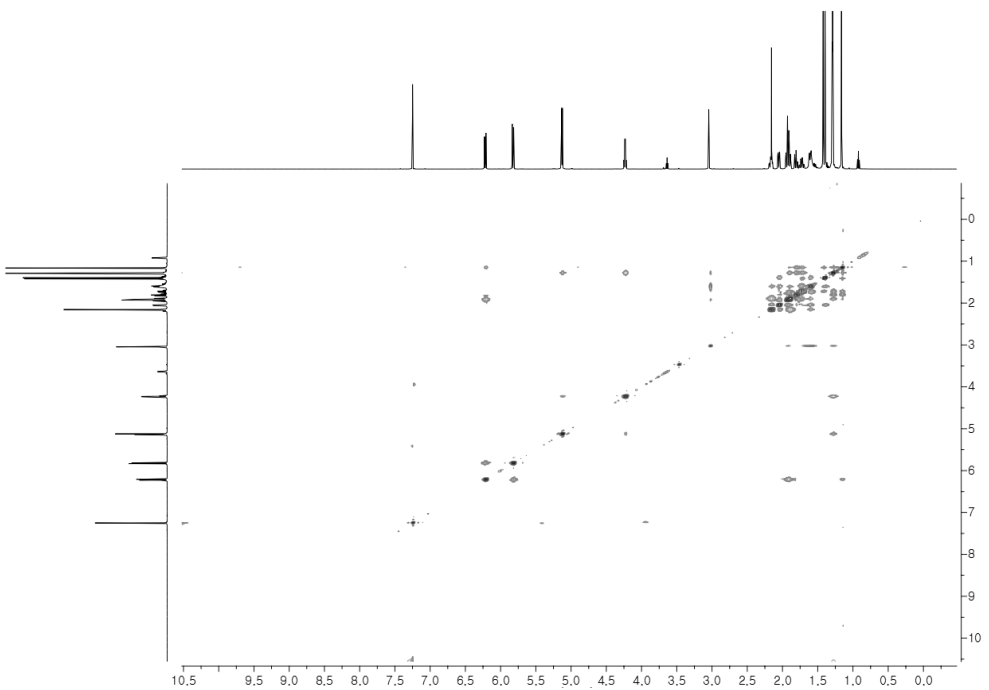


Figure 25. NOESY spectrum (500 MHz) of **2**(FCH061) in $CDCl_3$

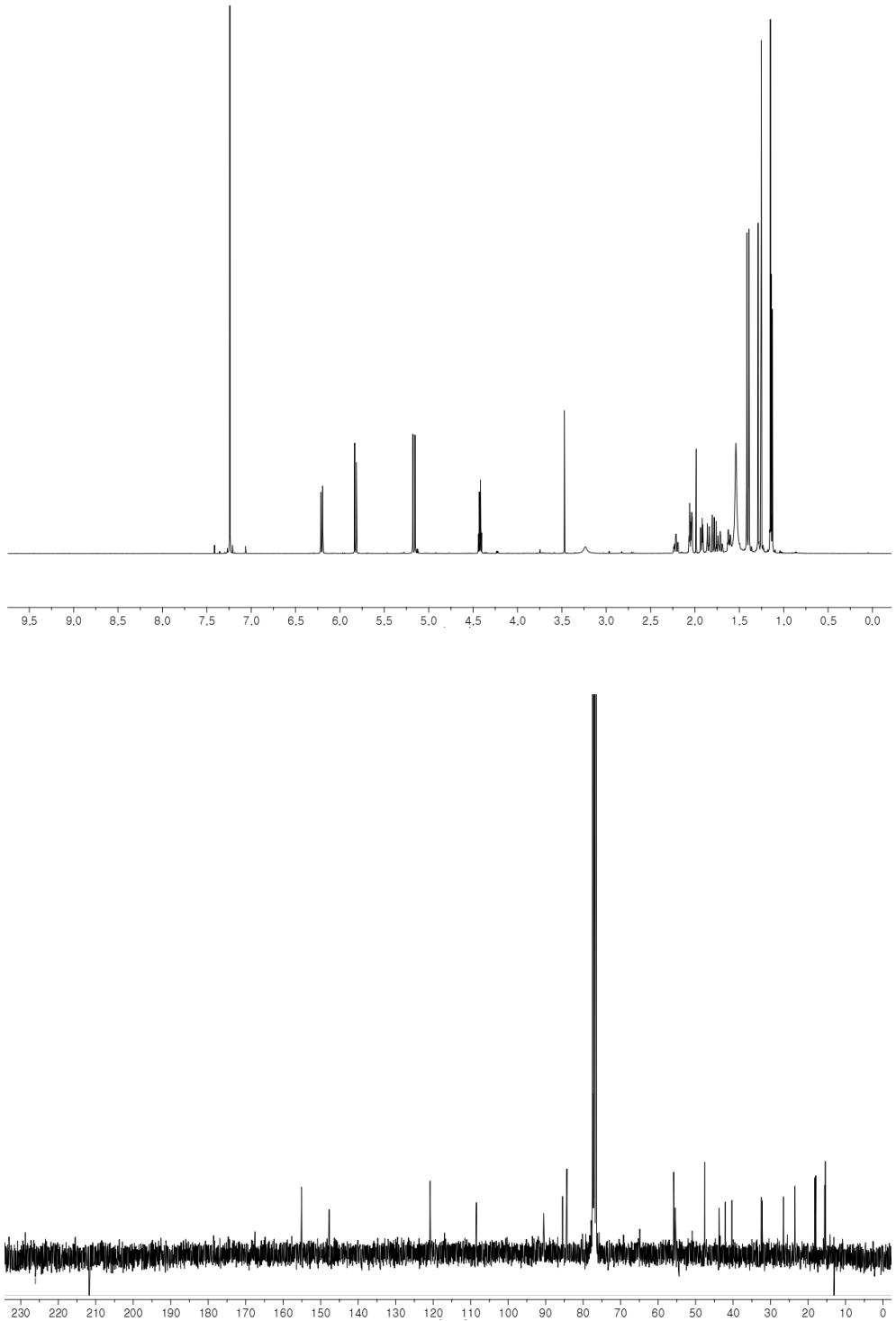


Figure 26. ^{1}H and ^{13}C NMR spectrum (600 and 75 MHz, respectively) of **3**(FCH061) in $CDCl_3$

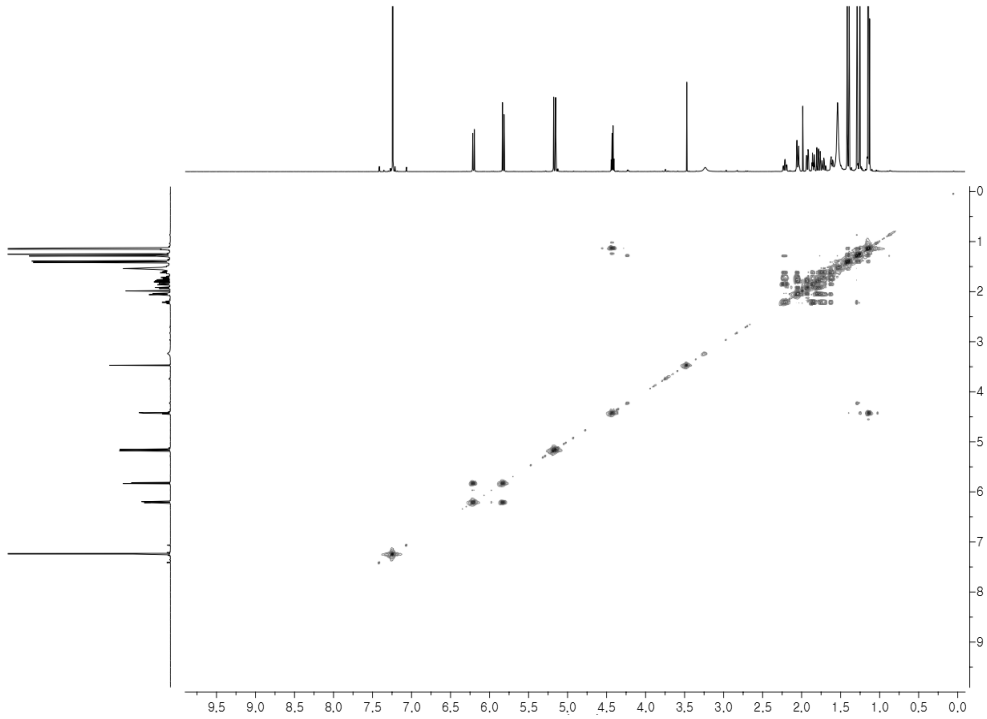


Figure 27. COSY spectrum (600 MHz) of **3**(FCH061) in $CDCl_3$

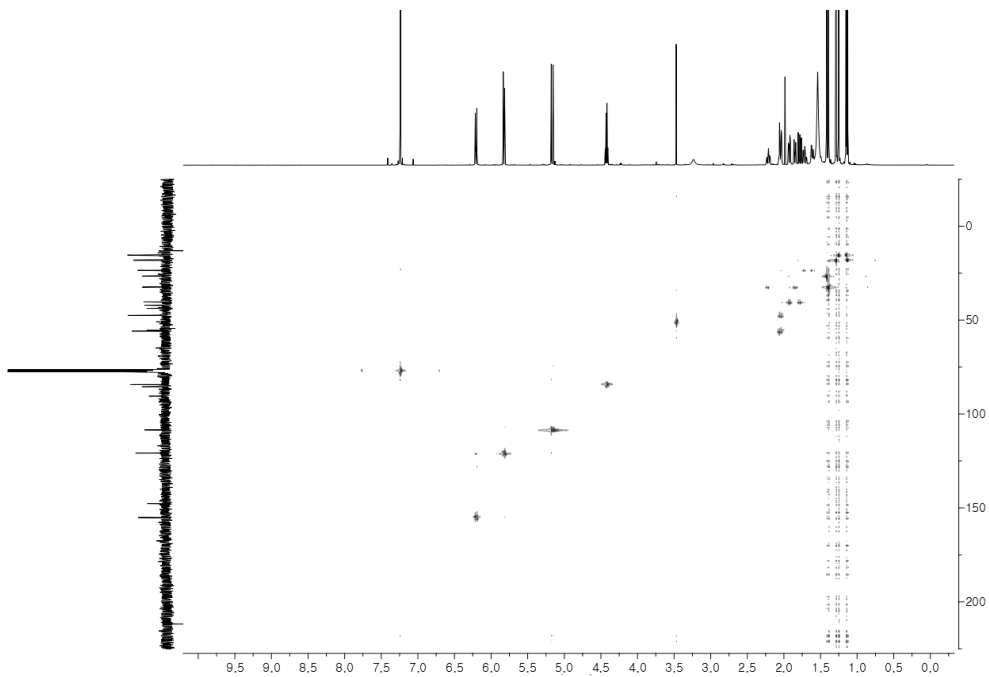


Figure 28. HSQC spectrum (600 MHz) of **3**(FCH061) in $CDCl_3$

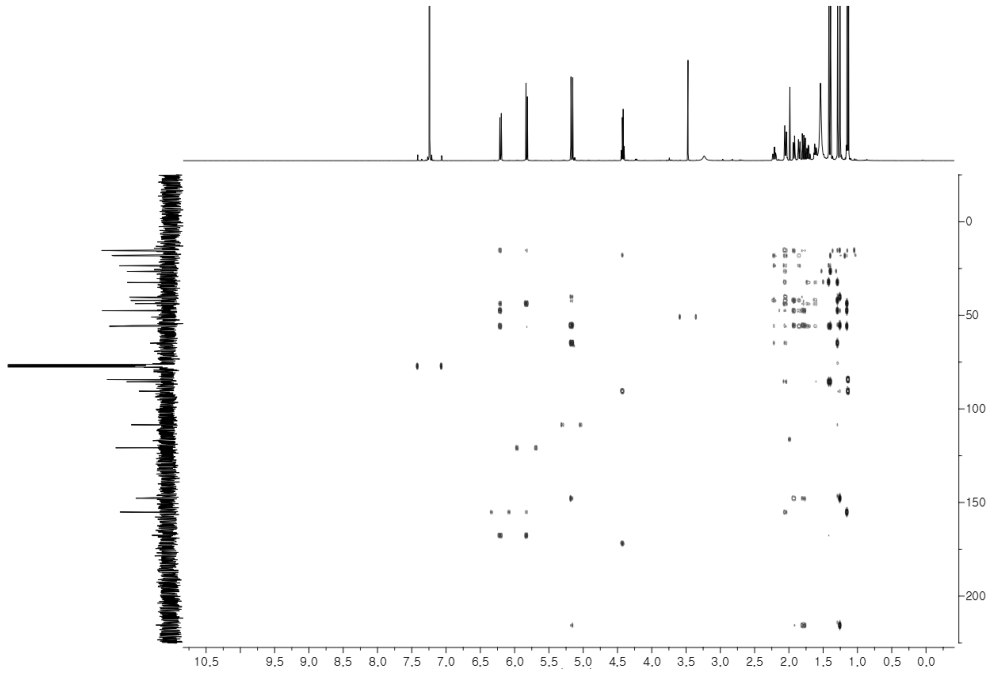


Figure 29. HMBC spectrum (600 MHz) of **3**(FCH061) in $CDCl_3$

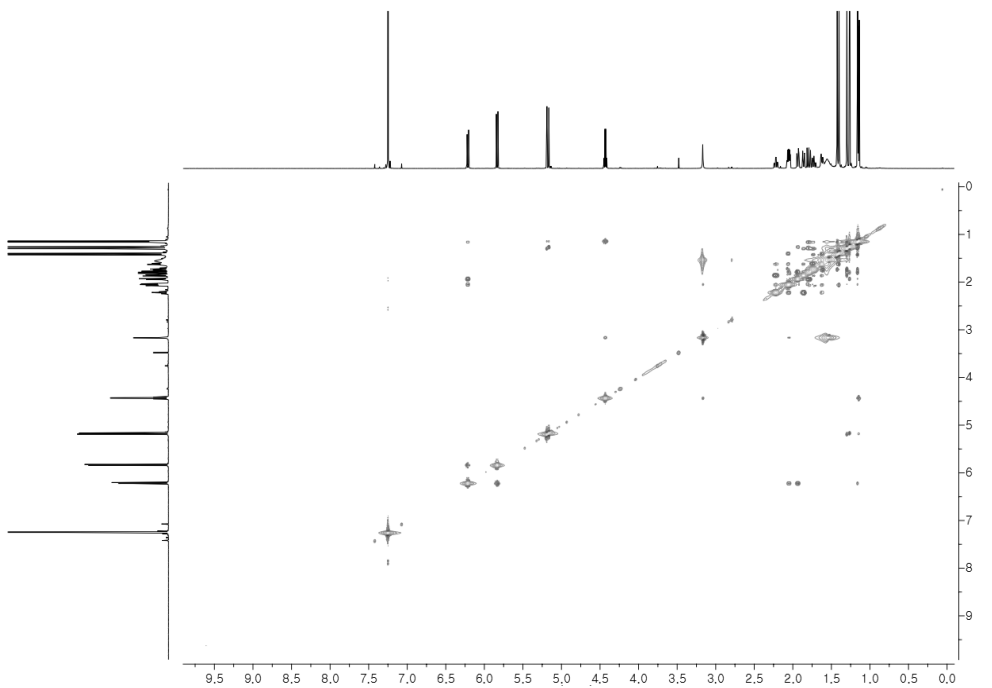


Figure 30. NOESY spectrum (600 MHz) of **3**(FCH061) in $CDCl_3$

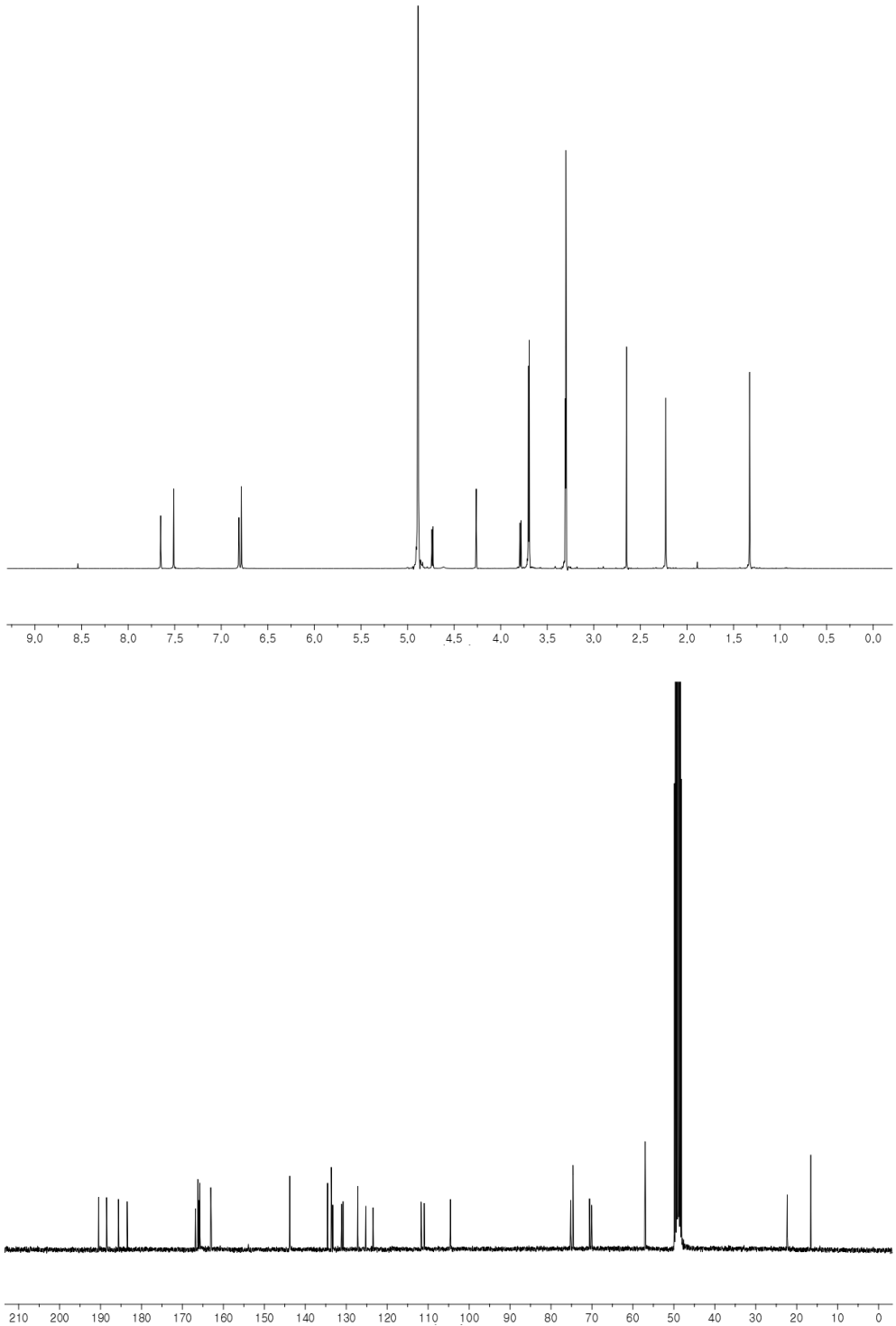


Figure 31. ^{1}H and ^{13}C NMR spectrum (600 and 150MHz, respectively) of **1**(FJJ006) in CD_3OD

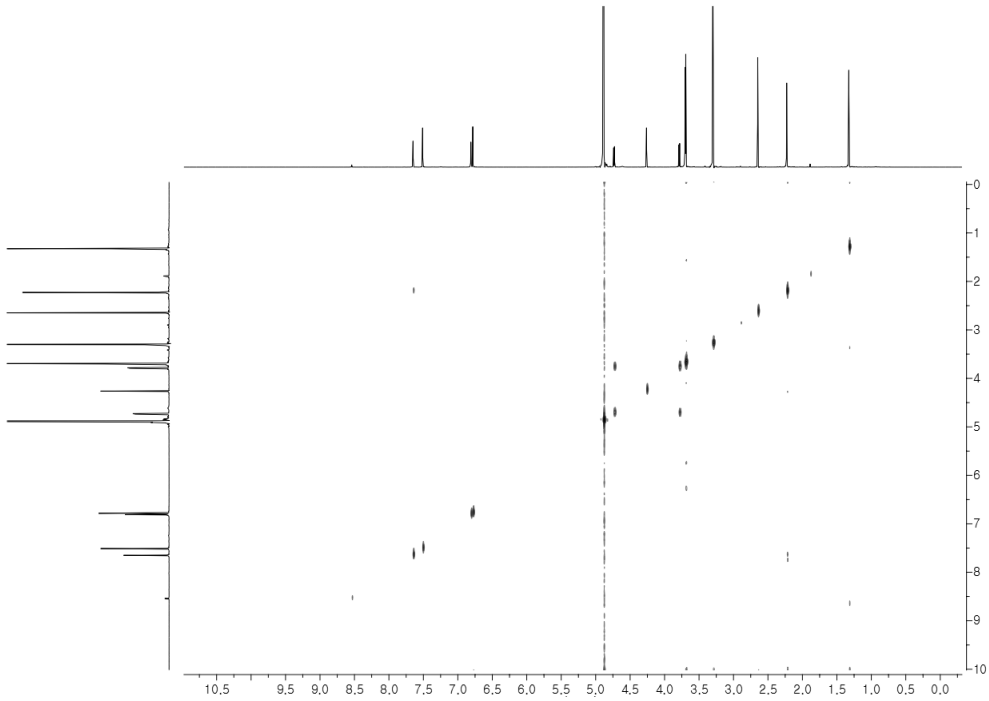


Figure 32. COSY spectrum (600 MHz) of **1**(FJJ006) in CD_3OD

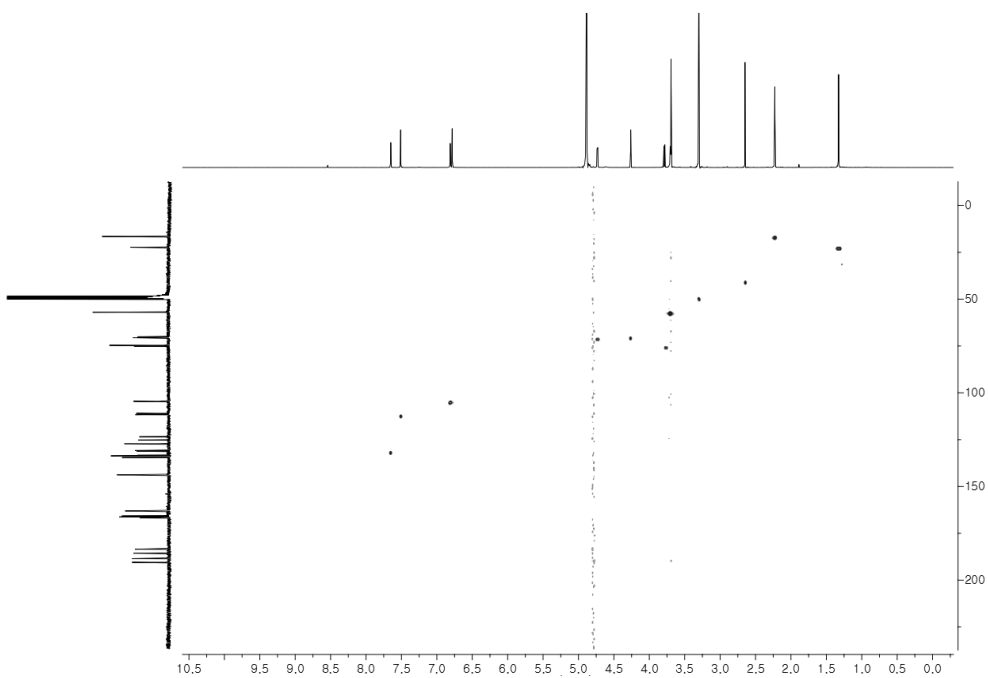


Figure 33. HSQC spectrum (600 MHz) of **1**(FJJ006) in CD_3OD

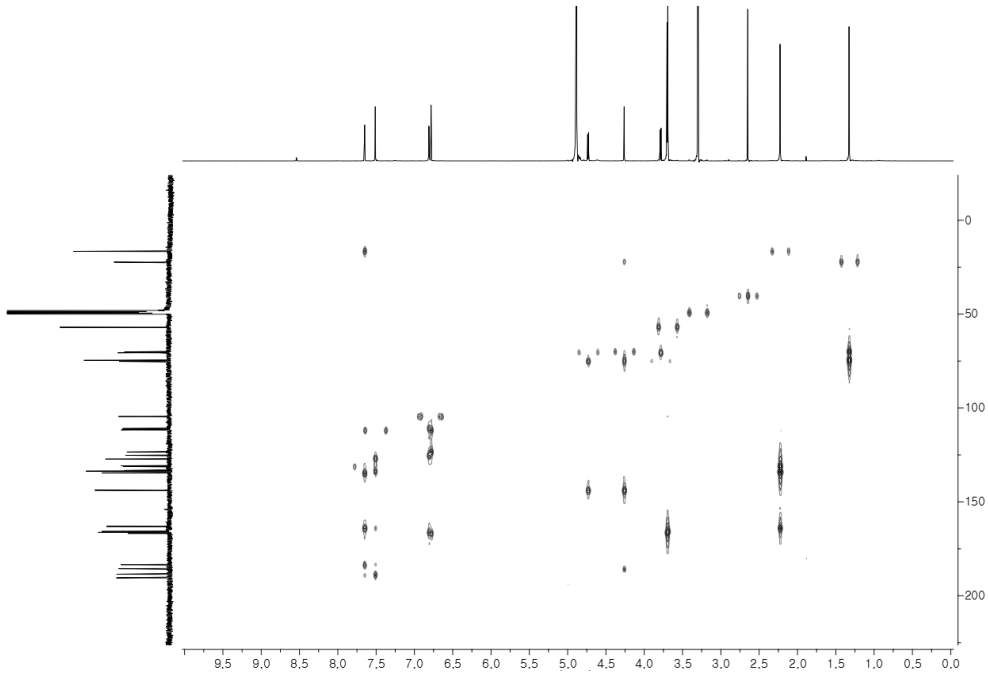


Figure 34. HMBC spectrum (600 MHz) of **1**(FJJ006) in CD_3OD

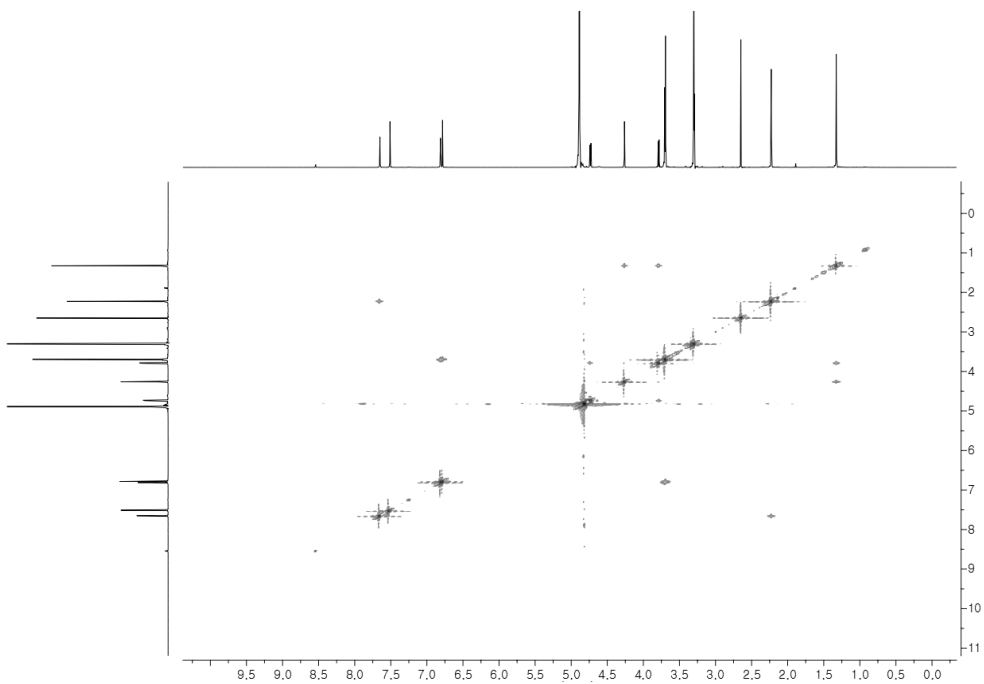


Figure 35. NOESY spectrum (600 MHz) of **1**(FJJ006) in CD_3OD

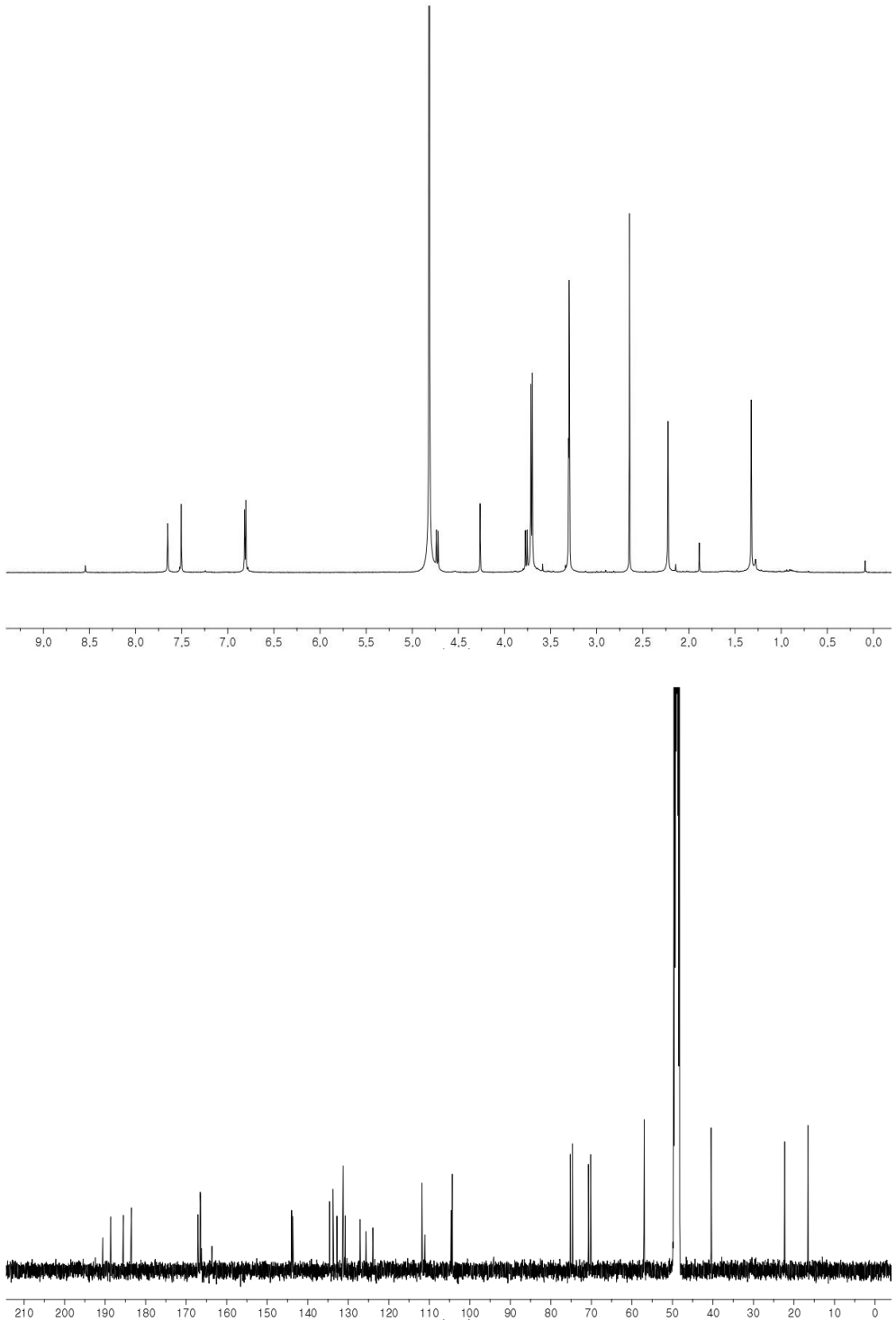


Figure 36. ^{1}H and ^{13}C NMR spectrum (400 and 100MHz, respectively) of **2**(FJJ006) in CD_3OD

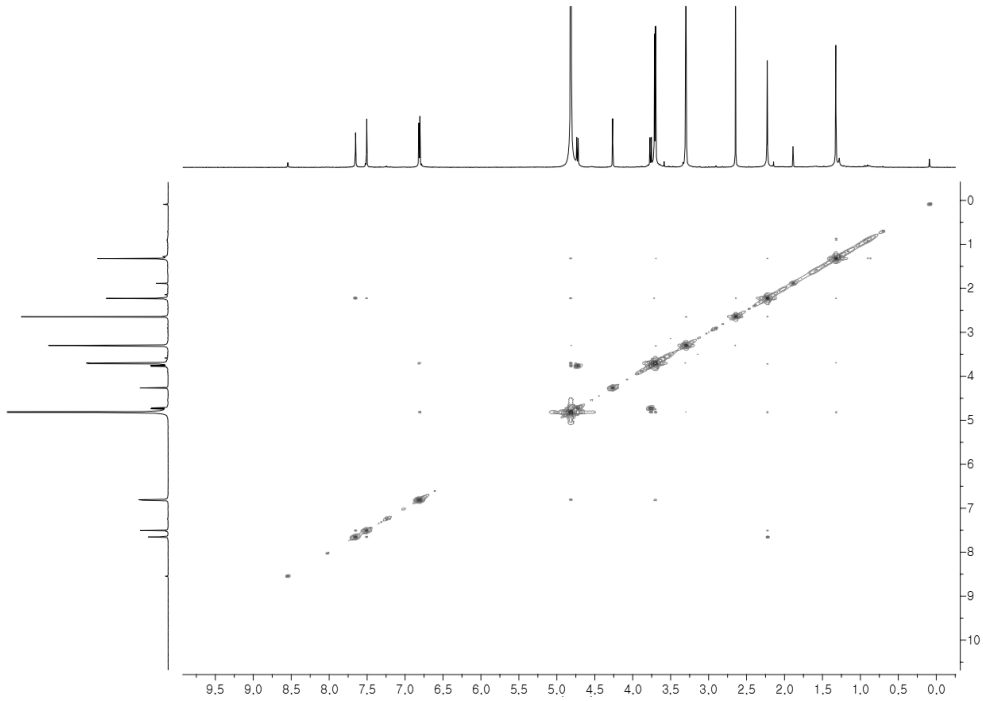


Figure 37. COSY spectrum (400 MHz) of **2**(FJJ006) in CD_3OD

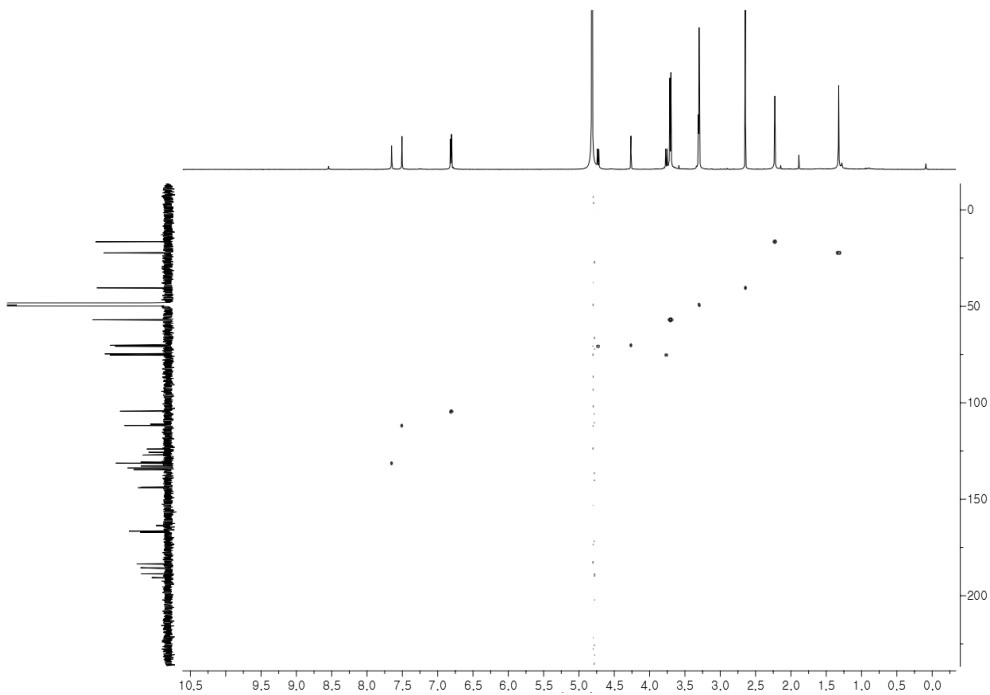


Figure 38. HSQC spectrum (400 MHz) of **2**(FJJ006) in CD_3OD

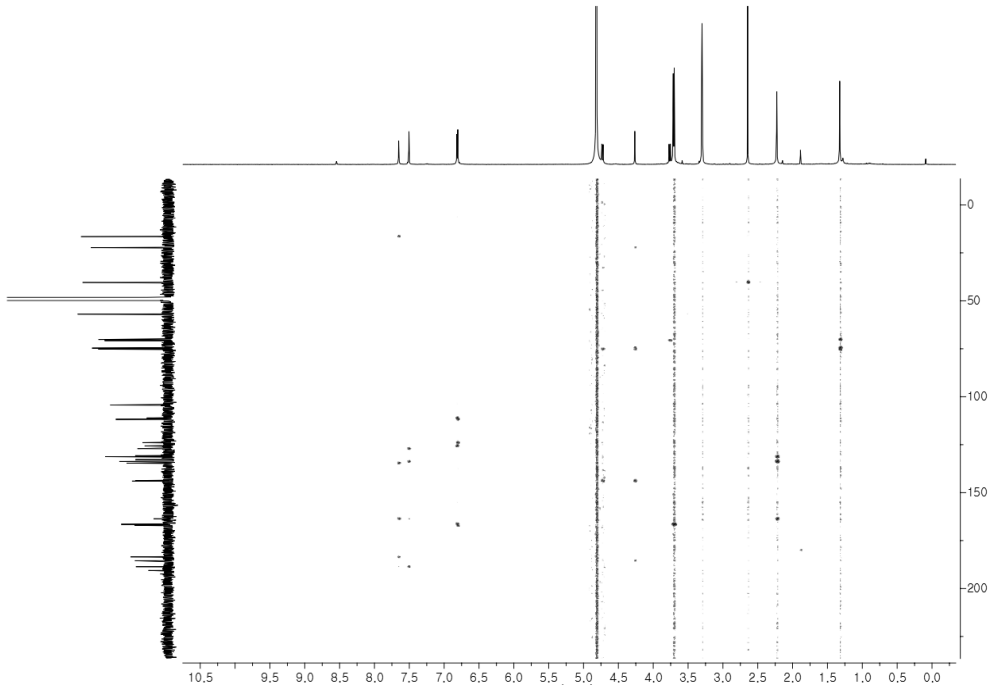


Figure 39. HMBC spectrum (600 MHz) of **2**(FJJ006) in CD_3OD

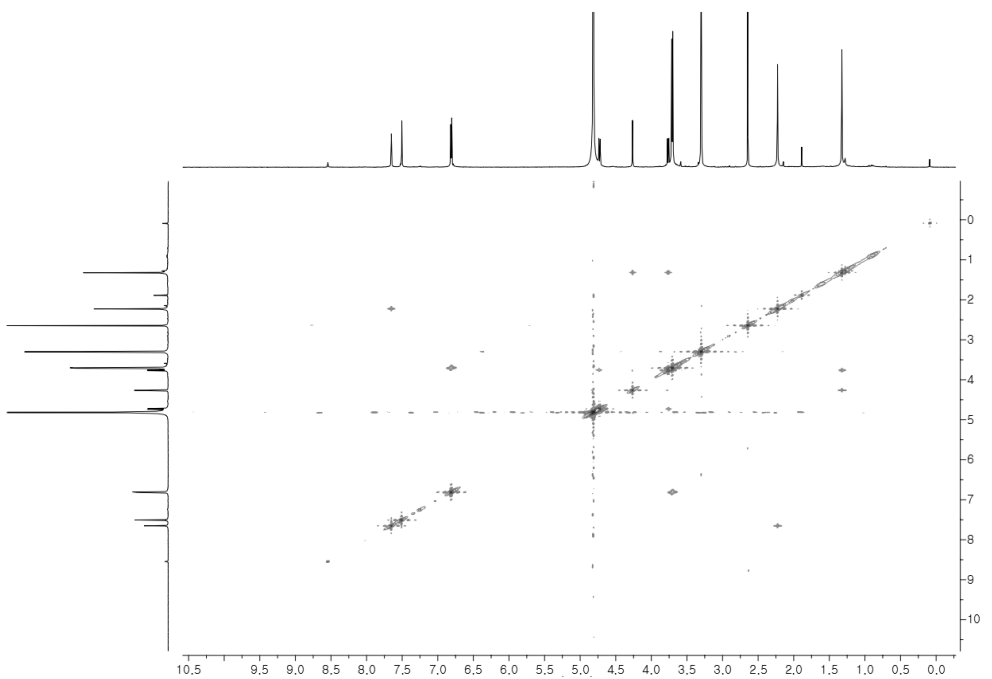


Figure 40. NOESY spectrum (600 MHz) of **2**(FJJ006) in CD_3OD

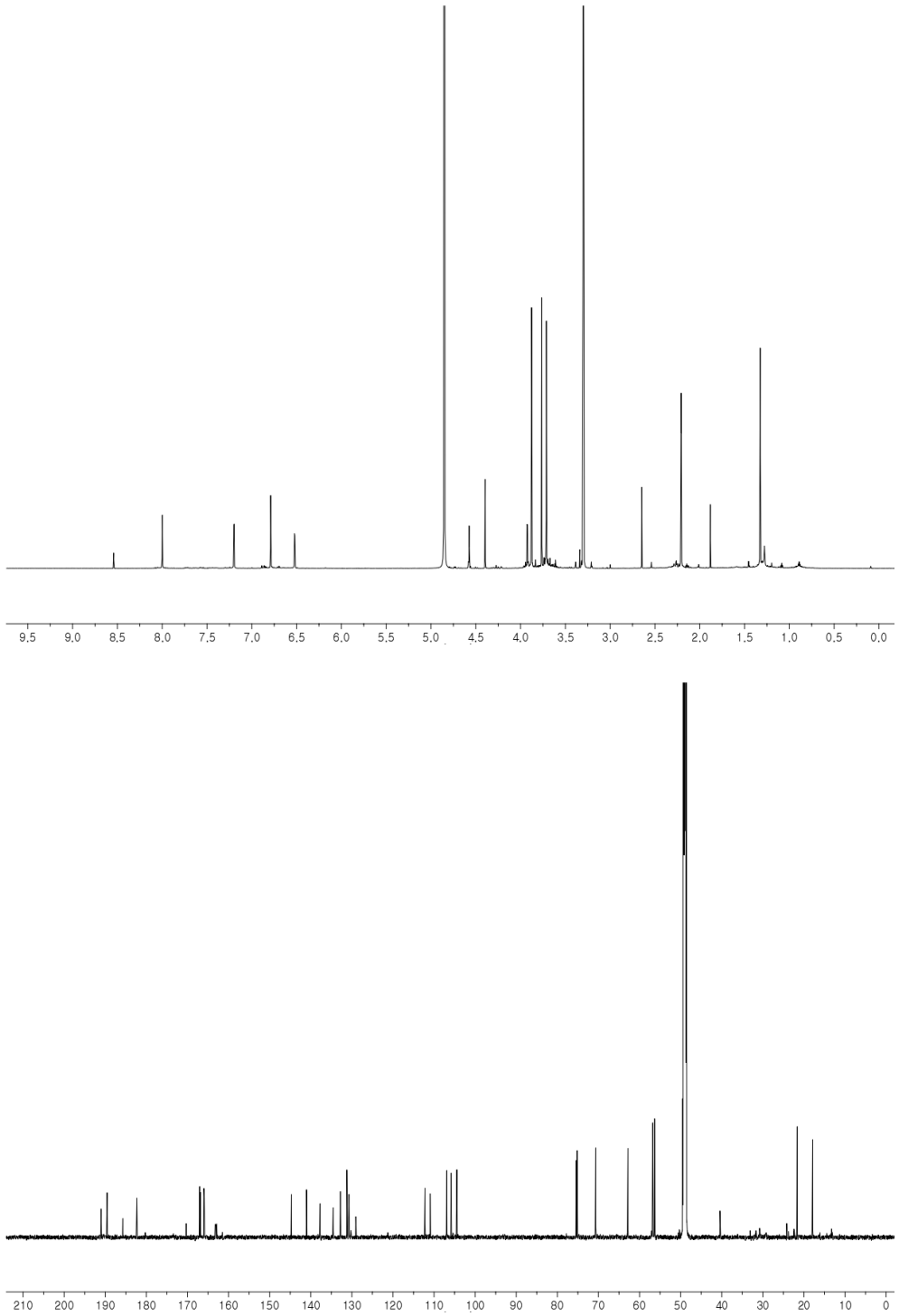


Figure 41. ^{1}H and ^{13}C NMR spectrum (800 and 200MHz, respectively) of **3**(FJJ006) in CD_3OD

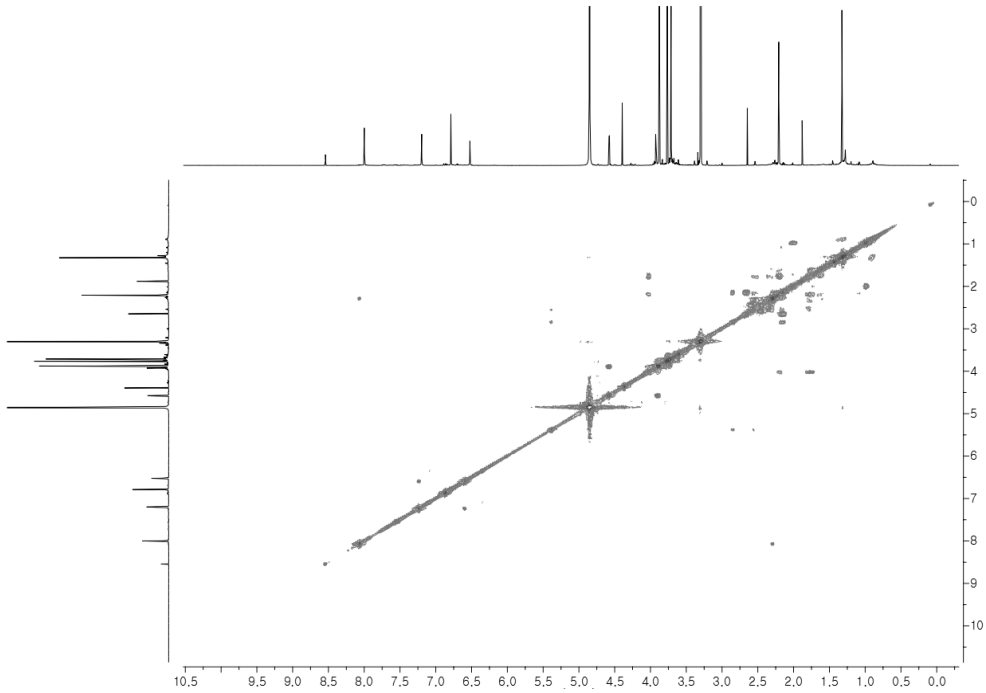


Figure 42. COSY spectrum (500 MHz) of **3**(FJJ006) in CD_3OD

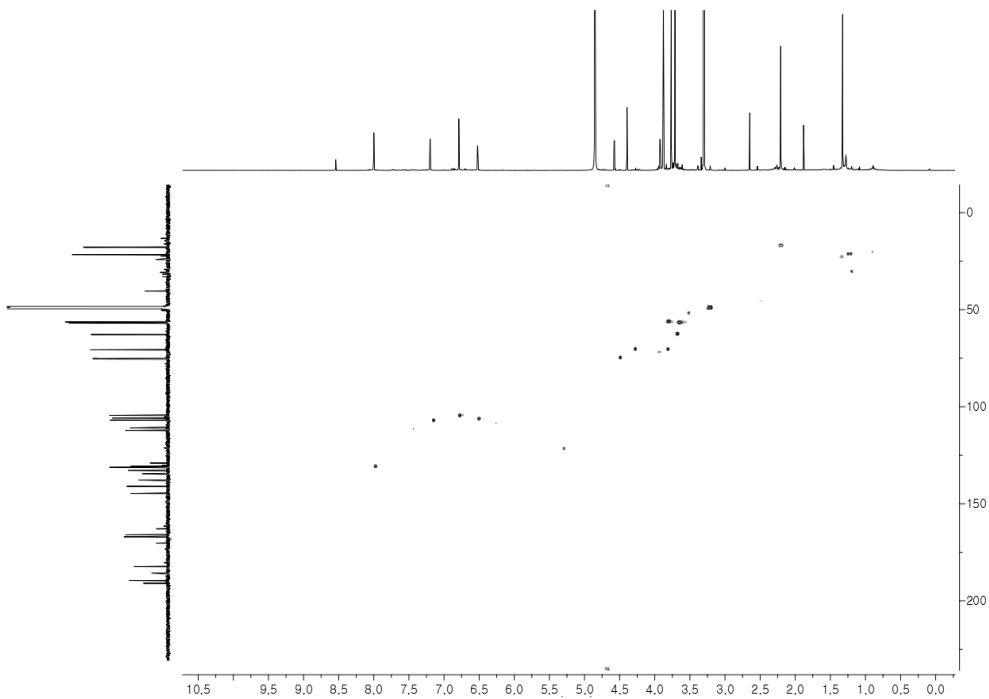


Figure 43. HSQC spectrum (500 MHz) of **3**(FJJ006) in CD_3OD

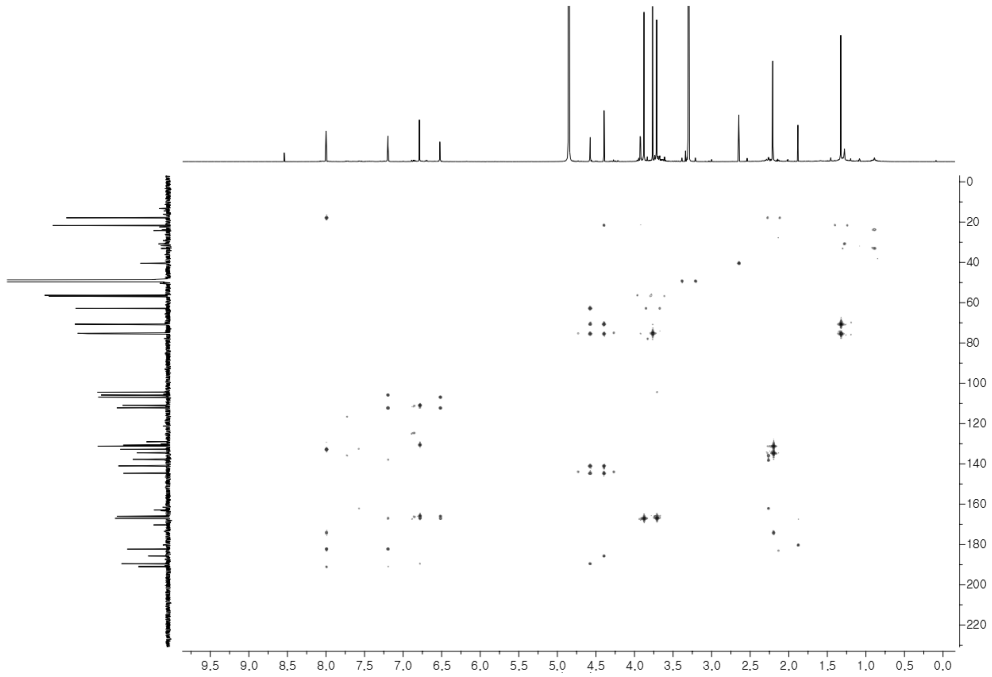


Figure 44. HMBC spectrum (800 MHz) of **3**(FJJ006) in CD_3OD

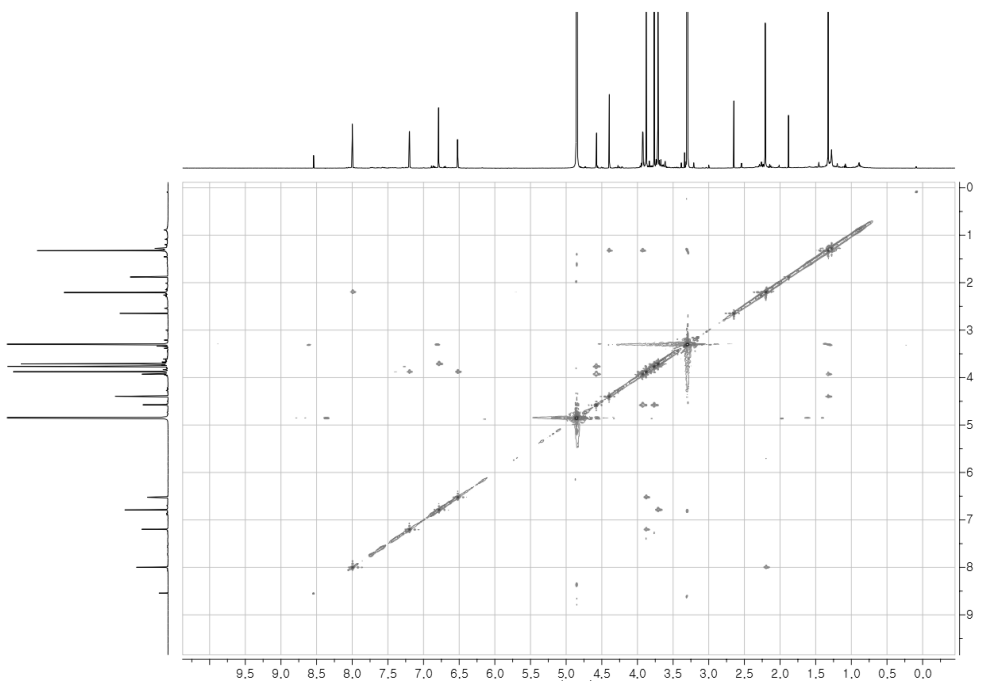


Figure 45. NOESY spectrum (500 MHz) of **3**(FJJ006) in CD_3OD

국문초록

해양 유래 진균에서 분리한 신규 Bianthraquinones와 Meroterpenoids

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2012년 추자도에서 채집된 해면으로부터 분리된 균주인 *Penicillium brasilianum*과, 2014년 제주도에서 채집된 해면으로부터 분리된 균주인 *Stemphylium lycopersici*의 배양물을 추출 후 정제하여, *P. brasilianum* 균주로부터 3개의 신규 Meroterpenoids와 11개의 기지 물질들을, *S. lycopersici* 균주로부터는 3개의 신규 Bianthraquinones와 12개의 기지 물질들을 분리하였다.

여러 분광학적 기법과 화학적 분석법을 이용하여, *P. brasilianum* 균주유래 신물질 1의 구조는 기지 물질인 preaustinoid A에서 A ring이 열린 물질로, 신물질 2와 3은 기지 물질인 preaustinoid A2에서 C와 D ring 사이에 α-ketol

rearrangement가 일어난 것으로 두 물질의 평면구조는 같고 C-10′ 메틸기의 입체가 다른 것으로 구조를 규명하였다. 또한 *S. lycopersici* 균주의 신물질 1과 2의 구조는 기지 물질들과 다르게 C-7에서 C-6′ 으로 연결된 물질로 평면구조는 같고, 서로 atropisomer 관계이며 신물질 3은 기지 물질인 alterporriol N에서 하이드록시기가 메톡시기로 바뀐 구조로 규명하였음

다양한 생리 활성을 검정해본 결과, 신규 Bianthraquinones가 항염 활성검사에서 NO의 생성을 억제시키는 것을 확인할 수 있었고, 강한 항염효과를 나타내는 것을 알 수 있었다.

주요어: 해양 진균, *Penicillium brasilianum, Stemphylium lycopersici*, Meroterpenoid, Bianthraquinone, 항염효과

학번 : 2015-21910