약학석사학위논문

Lumazine Peptides from the Marine-Derived Fungus *Aspergillus terreus*

해양 유래 진균에서 분리한 신규 Lumazine Peptides

2015년 2월

서울대학교 대학원
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유 민 정

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Abstract

Lumazine Peptides from the Marine-Derived Fungus *Aspergillus terreus*

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The marine-derived fungi are the prolific source of the novel compounds among the natural products. A number of fungal strains were isolated from the marine-related samples, and evaluated by combined spectroscopic analyses of their metabolites.

A marine-derived fungal strain, *Aspergillus terreus*, was isolated from the off-shore sediment in Jeju, Korea. It was cultivated for 6 weeks in YMM static condition, and extracted with organic solvent. The organic extracts were separated with various chromatographic methods and analyzed with combined

spectroscopic methods.

In total, 2 new compounds and 17 known compounds were structurally determined and identified as 3 peptides, 1 pyridione containing compound, 2 alkaloids, 5 terpenoids, 7 amino acid derived compounds, 1 polyene compound. The new compounds were structurally elucidated to be linear assemblies of 1-methyllumazine-6-carboxylic acid, an amino acid residue and anthranilic acid methyl ester connected by peptide bonds. The absolute stereochemistry were determined by advanced Marfey's analysis.

The new compounds have a pharmacological activity to improve the insulin sensitivity, which was evaluated in the adipogenesis model in human bone marrow mesenchymal stem cells.

Key word : lumazine, peptide, *Aspergillus terreus*, marine fungi, insulin sensitivity

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Introduction

Microorganisms from marine environments are widely recognized as emerging sources of biologically active and structurally unique secondary metabolites.[1] Although studies on these organisms began considerably later than those on their counterparts from terrestrial environments, significant numbers of metabolites have been found annually since the late 1990s.[2] 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.[3]

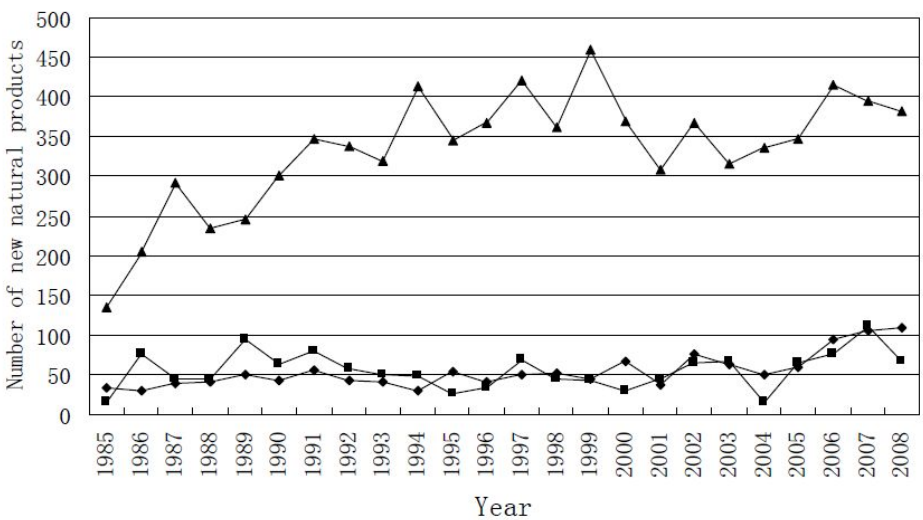


Figure 1. Temporal trends in the number of novel compounds isolated

from different marine organisms 1985 and 2008. ▲, marine invertebrate; ■, marine algae; ◆, marine microorganisms including phytoplankton.[3]

Among the marine microorganisms, fungi have been proven to be a prolific source of novel compounds as one of the dominant group and more than 400 novel natural products was isolated from the marine-fungus.[2,3] Therefore, along with bacteria originating from the same environments, marine-derived fungi are considered to be a new frontier for natural products research.

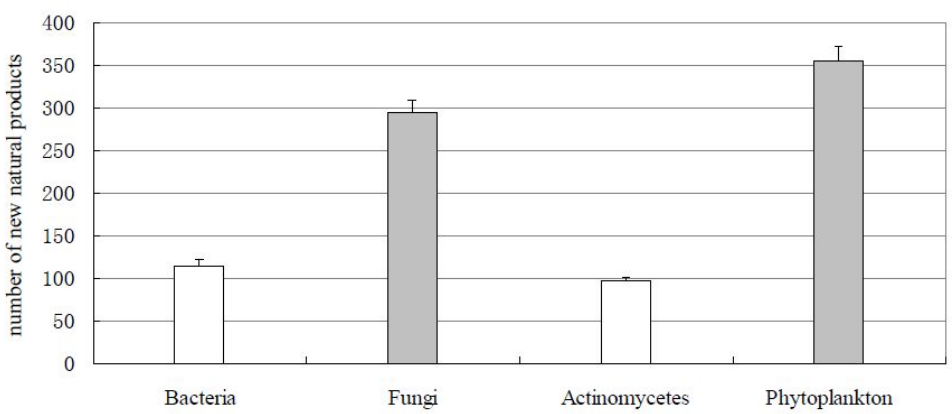


Figure 2. The number of novel compounds isolated from marine microorganisms between 1985 and 2008.[3]

In our search for novel bioactive compounds from marine fungi, a strain of *Aspergillus terreus* was collected from marine sediment from Jeju Island, Korea, and the organic extract of this fungi exhibited mild

cytotoxicity (IC_{50} 370 μg/mL) against the human leukemia K562 cell line. More importantly, the ESI-LC/MS profile of the extract revealed the presence of novel constituents which motivated us to investigate its metabolites in detail. A large-scale solid-substrate culture of the strain, followed by extraction and chromatographic separation, led to the isolation of two novel metabolites, in addition to 19 known metabolites, such as the alkaloids acetylaszonalenin and asterrelenin,[4] the meroterpenoids territrems A and B,[5] the sesterterpenoids terretonin B-C and terretonin,[4b] the pyridine-containing dihydroisoflavipucine,[6] the polyene compounds citreoviridine, the butenolide compounds butyrolactone Ⅰ-Ⅲ, 2-furancarboxylic acid, and aspernolides B,[7] the amino acid derived compounds asterriquinone SU5500 and neoasterriquinone,[8] and the small peptide methyl 3,4,5-trimethoxy-2-(2-(nicotinamido)benzamido)benzoate.[9] Here, we report the structural determinations of terrelumamides A (**1**) and B (**2**), which are novel linear lumazine peptides. These compounds are structurally related to penilumamides A-D, which were recently isolated from marine-derived *Penicillium* sp. and *Aspergillus* sp. fungi.[10,11] However, to the best of our knowledge, the 1-methyllumazine-6-carboxylic acid moiety of these terrelumamide compounds is the first such example among natural

products. These compounds exhibited the pharmacological activity to improve the insulin sensitivity, which was evaluated in a cell-based model for adipogenesis using human bone marrow mesenchymal stem cells (hBM-MSCs).

Experimental Section

1. General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. IR spectra were recorded on a JASCO 4200 FT-IR spectrometer using a ZnSe cell. UV spectra were acquired using a Hitachi U-3010 spectrophotometer. NMR spectra were recorded using Varian 300 and Bruker Avance 500 spectrometers. Proton and carbon NMR spectra were measured in DMSO-d_6 solution at 300 and 75 MHz (**1**) or at 500 and 125 MHz (**2**). High-resolution fast-atom bombardment mass spectrometry (HRFABMS) data were acquired using Jeol JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol (NBA) as the matrix at the Korea Basic Science Institute (Daegu, Korea). Low-resolution ESI-LC/MS data were recorded on an Agilent Technologies 6130 quadrupole mass spectrometer with an Agilent Technologies 1200 series HPLC. The ESI-Q-TOF-MS/MS measurements were performed on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS spectrometer with an Agilent Technologies 1260 series HPLC. Semi-preparative HPLC was performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150) and an YMC ODS column (250 × 10 mm). All solvents used were of

spectroscopic grade or were distilled from glass prior to use.

2. Isolation and Identification of Fungal Strain

The fungal strain *Aspergillus terreus* (strain number FA009) was isolated from marine sediments collected offshore of Jeju Island, Korea, in October, 2012. FA009 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 FA009 was deposited in the GenBank database under accession number KF146985. The 18S rDNA sequence of this strain exhibited 100% identity with that of *Aspergillus terreus* KAML04 (GenBank accession number KF146985).

1 tccgtaggtg aacctgcgga aggatcatta ccgagtgcgg gtctttatgg cccaacctcc
61 cacccgtgac tattgtacct tgttgcttcg gcgggcccgc cagcgttgct ggccgccggg
121 gggcgactcg ccccgggcc cgtgcccgcc ggagacccca acatgaaccc tgttctgaaa
181 gcttgcagtc tgagtgtgat tctttgcaat cagttaaaac tttcaacaat ggatctcttg
241 gttccggcat cgatgaagaa cgcagcgaaa tgcgataact aatgtgaatt gcagaattca
301 gtgaatcatc gagtctttga acgcacattg cgccccctgg tattccgggg ggcatgcctg
361 tccgagcgtc attgctgccc tcaagcccgg cttgtgtgtt gggccctcgt cccccggctc
421 ccgggggacg ggcccgaaag gcagcggcgg caccgcgtcc ggtcctcgag cgtatggggc
481 ttcgtcttcc gctccgtagg cccggccggc gcccgccgac gcatttattt gcaacttgtt
541 tttttccagg ttgacctcgg atcaggtagg gatacccgct gaacttaagc atatcaataa
601 gcggagga

Figure 3. The 18S rDNA sequence of FA009.

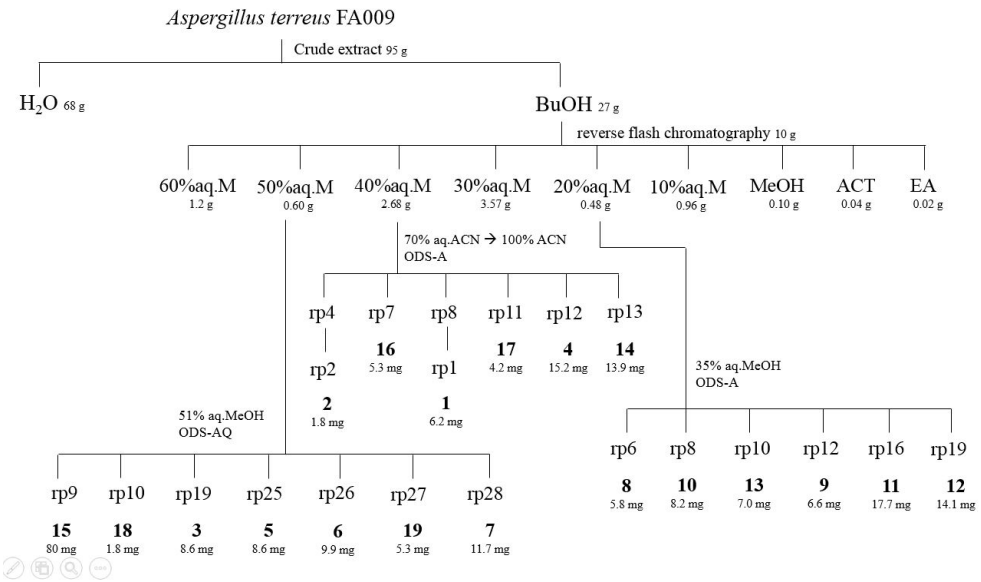
3. Fermentation of Fungal Strain

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, 600 g of rice media was prepared and cultivated for 40 days at 28 °C,

with stirring once a week.

4. Extraction and Isolation

The entire culture was macerated and repeatedly extracted with MeOH (2 L × 2) and CH_2Cl_2 (2 L × 2), and then the solvent was evaporated *in vacuo* to yield 95 g of extract. The extracts were partitioned between H_2O (68 g) and *n*-BuOH (27 g), and the latter fraction 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 H_2O–MeOH, gradient from 60:40 to 0:100), acetone, and finally EtOAc. Based on the ^1H NMR analysis results, the fractions eluted with H_2O–MeOH (40:60) (2.68 g) were separated by semi-preparative reversed-phase HPLC (H_2O–MeCN, gradient from 70:30 to 0:100 over 40 min, 2.0 mL/min) to afford 18 peaks. Peaks 4 and 8 were further purified by reversed-phase HPLC (H_2O–MeOH, 53:47, 2.0 mL/min), affording compounds **2** (1.8 mg) and **1** (6.2 mg), respectively.



Scheme 1. Isolation of compounds from *Aspergillus terreus* FA009.

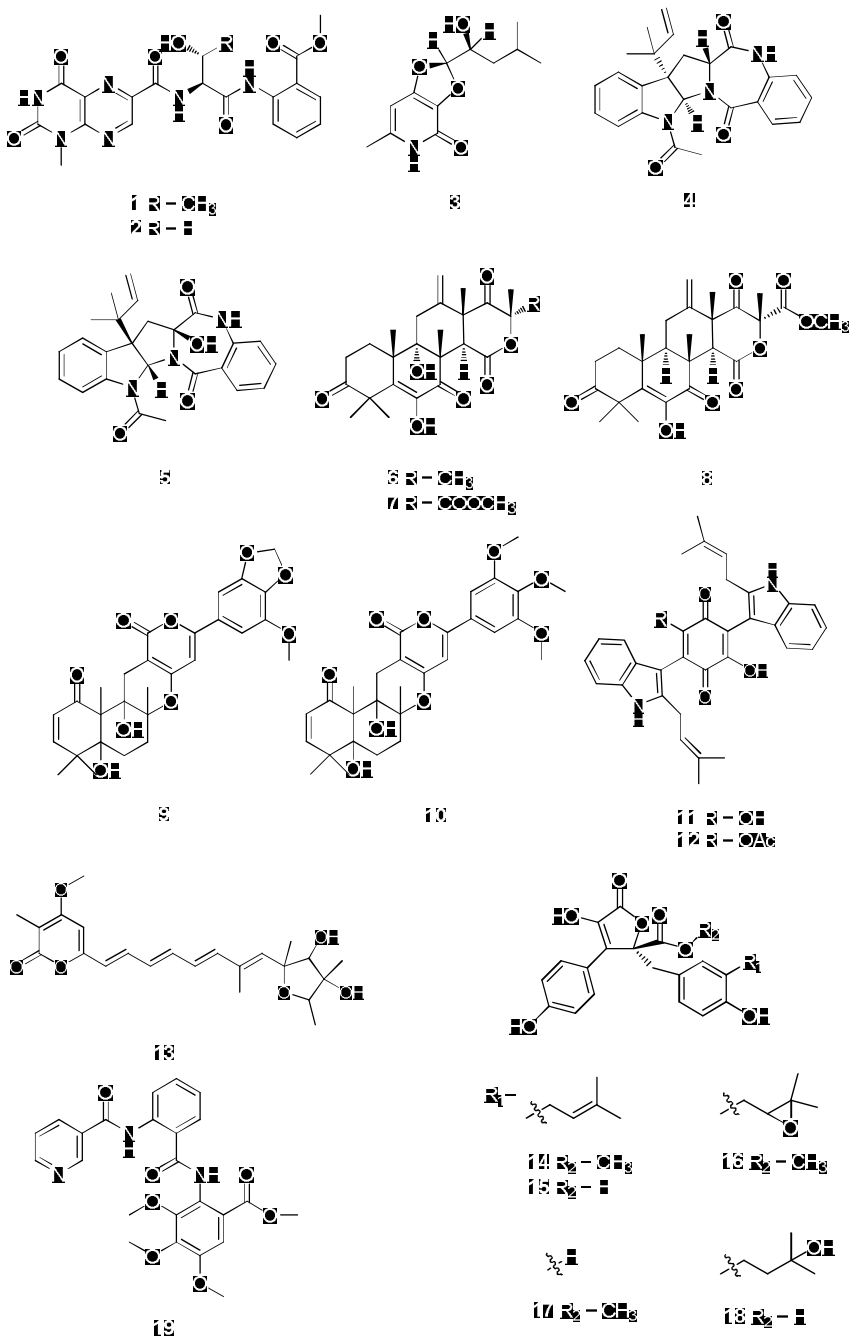


Figure 4. Structures of isolated compounds.

5. Determination of the Amino Acid Absolute Configurations[12]

Compound **1** (0.5 mg) was dissolved in 0.5 mL of 6 N HCl and hydrolyzed at 110 °C for 2 h. The solvent and traces of HCl were removed by the addition of 0.5 mL of distilled water and evaporation *in vacuo*. To the divided hydrolysate (0.25 mg each), 100 μL of 1 N $NaHCO_3$ and 50 μL of 1% L- and D-FDAA (1-fluoro-2,4-dinitrophenyl-5-L(or D)-alanine amide) in acetone were added. The solution was heated at 80 °C for 3 min. After the reaction, 50 μL of 2 N HCl was added to the mixture to neutralize the mixture, followed by the addition of 300 μL of a 50% aqueous MeCN solution. The FDAA derivatives of threonine were analyzed by ESI-LC/MS (H_2O–MeCN, from 90:10 to 30:70 containing 0.1% formic acid over 40 min using a C_{18} reversed-phase column 100 × 4.6 mm, 0.7 mL/min). The retention times of the L- and D-FDAA-derivatized hydrolysates were 14.9 and 17.3 min, respectively, indicating the L-configuration of the α-stereogenic center of the Thr residue of **1**.

To assign a configuration to the additional β-stereogenic center of Thr, the authentic samples of L-Thr and *allo*-L-Thr were derivatized with L-FDAA and their retention times were compared to those of L-FDAA-derivatized hydrolysates of **1**. The retention times of each FDAA-

derivative of L-Thr, *allo*-L-Thr, and the hydrolysates were 15.1, 19.0, and 15.2 min, respectively, as determined by ESI-LC/MS analysis (H_2O–MeOH, 60:40 containing 0.1% formic acid, over 35 min using a C_{18} reversed-phase column 250 × 4.6 mm, 1.0 mL/min). Thus, the absolute configuration of threonine was determined to be L-Thr.

The Ser residue of **2** was also analyzed using an advanced Marfey's method with the same protocol as that used for **1**. The HPLC retention times of the L- and D-FDAA-derivatized hydrolysates were 14.0 and 14.7 min, respectively, indicating an L-Ser configuration for **2**.

5. Biological Assays

The cytotoxicity assays were performed in accordance with protocols reported in the literature.[13] Isocitrate lyase, sortase A, Na^+/K^+-ATPase, and antimicrobial assays were performed according to previously reproted methods.[14] The insulin sensitivity test based on an adipogenesis model using hBM-MSCs was performed as previously reported.[15]

Results

1. Compound 1

The molecular formula of compound **1** was determined to be $C_{20}H_{20}O_7N_6$ based on HRFABMS analysis. The highly unsaturated nature of this compound was indicated by the fourteen degree of unsaturation present in the mass data, and by the presence of signals from fifteen carbons in the aromatic and carbonyl regions in the ^{13}C NMR data. This interpretation was consistent with the strong absorption bands at 1708 and 1690 cm^{-1} in the IR spectrum. The presence of several absorption maxima at 220-340 nm (E band) in the UV spectrum also supported the presence of an aromatic moiety. The remaining carbons in the ^{13}C NMR data were two methines and three methyl carbons in the upfield region (Table 1).

Given this information, the structure of compound **1** was determined through combined 2-D NMR experiments. First, the HSQC data revealed the direct attachment of an isolated methine proton at δ_H 9.30 to the carbon at δ_C 146.2. The long-range carbon-proton correlations of this proton with the quaternary carbons at δ_C 151.1, 138.2 and 127.2 in the HMBC data placed these carbons at the neighboring positions. Among these carbons, the one at δ_C 127.2 exhibited an additional correlation with

an exchangeable proton at δ_H 12.18, which also correlated with a quaternary carbon at δ_C 159.2. The chemical shifts of a methyl carbon at δ_C 28.6 and its protons at δ_H 3.52 revealed the presence of an *N*-methyl group of a heteroaromatic moiety. The neighboring positions of this methyl group were occupied by the quaternary carbons at δ_C 151.1 and 150.0 deduced from the HMBC data. Taken together, both the chemical shifts of the protons and carbons and the HMBC correlations among them revealed the presence of a 1-methyllumazine moiety, which was confirmed by comparing of the spectroscopic data with those of lumazine analogs in the literature (Figure 5).[16,17] Based on the combined HMBC data, the *N*-methyl group was placed at the N-1 position.

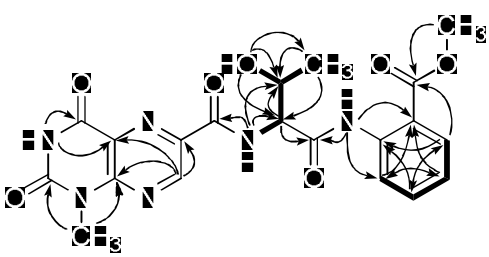


Figure 5. The COSY (bold line) and selected HMBC (arrows) correlations of compound **1**.

The ^{1}H-^{1}H COSY data revealed a spin system that consisted of the

protons at δ_H 8.51 (1 H, d, J = 8.0 Hz, exchangeable), 5.58 (1 H, d, J = 4.8 Hz, exchangeable), 4.56 (1 H, dd, J = 8.0, 2.8 Hz), 4.42 (1 H, m), and 1.19 (3 H, d, J = 6.3 Hz). Aided by the HSQC and HMBC analyses, these protons and their attached carbons were readily assigned to a threonine (Thr) amino acid residue. The carbonyl carbons of this residue and neighboring unit were found at δ_C 168.8 and 162.7 based on their HMBC correlations with the NH and α-carbonyl protons at δ_H 5.58 and 4.56, respectively (Figure 5). However, the exact assignments of these carbonyl carbons were not accomplished at this stage due to the lack of additional HMBC correlations with the Thr protons.

The ^{13}C NMR data of **1** indicated that six carbons were present in the δ_C 139.2-117.1 region, which suggested the presence of a benzene moiety (Table 1). The chemical shifts of the attached protons at δ_H 8.44-7.21 and the mutual spin couplings among these protons, as well as the large (J = 8.0 Hz) vicinal coupling constants, were indicative of a 1,2-disubstituted benzene, which was confirmed by the HMBC data (Figure 1). Additional correlations of the aromatic proton at δ_H 7.92 (H-4″) and a methyl proton at δ_H 3.70 (H-9″) with a carbonyl carbon at δ_C 167.3 (C-8″) confirmed the attachment of a methyl carboxylate group as a substituent of the benzene. Similarly the correlations of a NH proton at δ_H 11.11 (1″-NH) with the

aromatic carbons at δ_C 120.7 (C-7″) and 117.1 (C-3″) placed the amine group as the adjacent substituent, thus constructing an anthranilic acid methyl ester moiety.

The connectivity among the partial structures was also determined through an HMBC analysis. The two carbonyl carbons at δ_C 168.8 and 162.7 correlated to the Thr protons, and the additional correlation between the former carbon and the 1″-NH of anthranilic ester not only linked these moieties but also assigned this carbon as the carbonyl (C-3′) of the Thr residue. Accordingly, the remaining carbonyl carbon at δ_C 162.7 (C-10) must belong to the neighboring unit, 1-methyllumazine carboxylic acid, despite its lack of long-range correlations with the H-7 or other protons of the lumazine moiety. This interpretation was supported by the ESI-Q-TOF-MS/MS analysis in which fragments consisting of the lumazine carboxylic acid-Thr were analyzed with high-resolution (Figure 6).

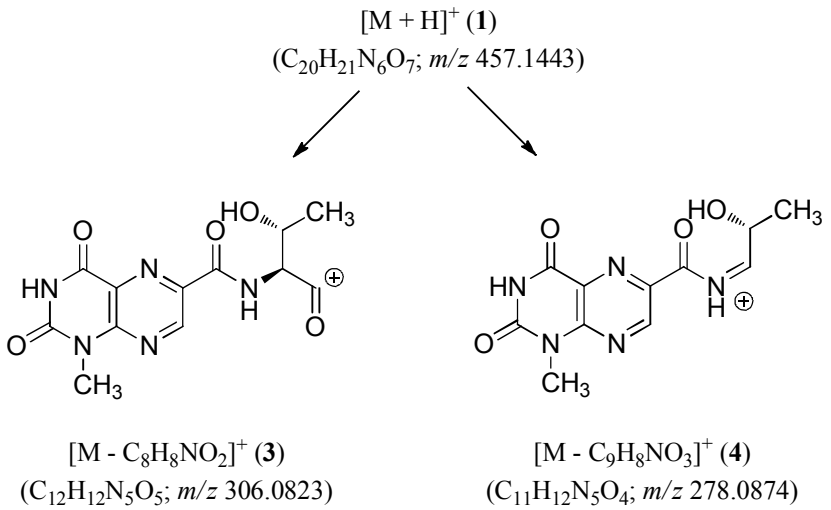


Figure 6. ESI-Q-TOF-MS/MS fragmentations of compound **1**.

Compound **1** possesses two stereogenic centers at its Thr residue. The L-configuration at the α-stereogenic center was assigned based on an advanced Marfey's analysis, in which the L-FDAA adduct of the hydrolysate presented a shorter HPLC retention time than the D-FDAA adduct (Experimental Section).[12] Similarly, the *R*-configuration was also assigned for the β-stereogenic center on the basis of Marfey's analysis, in which the L-FDAA adduct of the hydrolysate presented a retention time that were identical to those of the adduct derived from the authentic L-Thr, whereas a significant difference was found with respect to the *allo*-L-Thr adduct. Thus, the structure of compound **1** was determined to be a new

lumazine peptide.

2. Compound 2

The molecular formula of compound **2** was established to be $C_{19}H_{18}O_7N_6$ based on an HRFABMS analysis. The ^{13}C and ^{1}H NMR data of this compound were very similar to those of **1**. A detailed examination of the ^{13}C NMR data revealed that the 1-methyllumazine-6-carboxylic acid and anthranilic acid methyl ester moieties of **1** were also present in **2**. The most noticeable difference occurred at the amino acid unit, in which the C-4′ and C-5′ of Thr were replaced with a methylene carbon at δ_C 61.0. Corresponding differences were also observed in the ^{1}H NMR data; the signals of the H-4′ methine and H-5′ of **1** were replaced with those of methylene protons at δ_H 4.01 and 3.89 (Table 2). These spectroscopic changes were readily explained by the replacement of Thr of **1** with a serine (Ser) residue in **2**, which was confirmed by combined 2-D NMR analyses. For **2**, the absolute configuration of the α-stereogenic center of Ser was assigned to be L through an advanced Marfey's analysis, in which the L-FDAA adduct of the hydrolysate presented a clearly shorter HPLC retention time than the D-FDAA adduct. Thus, the structure of compound

2 was determined to be a lumazine peptide containing an L-Ser residue.

3. Biological assays

The lumazine monomer and its synthetic *N*-alkylated derivatives have been reported to inhibit the growth of methanogens and formantion of methane,[18] and the production of tumor necrosis factor-α,[19] respectively. Despite remarkable structural variations, however, more complex compounds that contain lumazine moieties do not possess significant bioactivity, and the only reported bioactivity was a weak inhibitory activity against *E. coli* at 50 μg/disk in a disk diffusion assay.[20] The recently reported penilumamides A-D obtained from marine-derived fungi were also inactive in various bioactivity assays such as cytotoxicity, antimicrobial and antiviral assays, and cellular Ca^{++} signaling activity tests.[10,11] These findings are consistent with our measurements, in which our novel compounds were inactive against the K652 and A549 cell lines (LC_{50} > 100 μM) and selected strains of Gram-positive and Gram-negative bacteria and pathogenic fungi (MIC > 100 μg/mL). These compounds were also inactive (IC_{50} > 100 μM) against various enzymes, such as sortase A, isocitrate lyase and Na^{+}/K^{+}-ATPase.

In contrast, using a cell-based assay to evaluate anti-diabetic compounds, we observed that the compound **1** and **2** increased the production of adiponectin during adipogenesis in hBM-MSCs (Figure 7). The level of adiponectin production in the adipogenesis model of hBM-MSCs has been used as a measure of insulin sensitivity.[15] In this study, glibenclamide and aspirin were used as dual positive controls because their pharmacological mechanisms for improving insulin sensitivity are different.[21] Glibenclamide achieves antidiabetic activity by binding with sulfonylurea receptor 1 (SUR1), which inhibits the conductance of the adenosine triphosphate (ATP)-dependent potassium (K_{ATP}) channel whereas aspirin inhibits the serine kinase IKKβ.[21] The pharmacological activity of glibenclamide reached a plateau at concentrations greater than 5 μM (Figure 7). Although aspirin significantly increased the production of adiponectin, the maximum effect achieved by aspirin in the hBM-MSCs was less than that of glibenclamide (Figure 7). Based on the maximum pharmacological activity of glibenclamide, the median effective concentration (EC_{50}) values of glibenclamide and aspirin were 3.47 and 145.6 μM, respectively. The EC_{50} values of compound **1** and **2** were 37.1 and 91.9 μM, respectively. In addition, the maximum increase in adiponectin levels induced by compound **1** was 56.9% relative to that

generated by glibenclamide. Therefore, compounds **1** and **2** in this study were more potent than aspirin but less potent than glibenclamide.

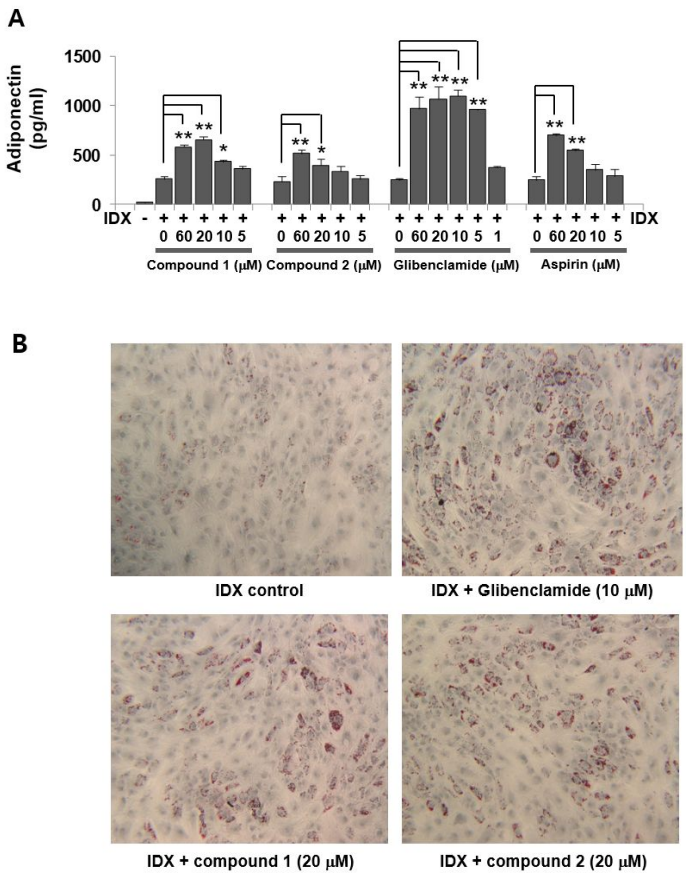


Figure 7. (A) Effects of compounds **1** and **2** on the production of adiponectin during adipogenesis in hBM-MSCs. After the induction of adipogenesis, the culture medium was changed every two days. On the 8th day of culture, the cell culture supernatants were harvested. ELISAs were performed to measure the levels of adiponectin that accumulated in the

cell culture supernatants over the 48 hours after the last medium exchange. Glibenclamide was used as the positive control. The values represent the mean ± SD (n = 3). * $P \leq 0.05$ and ** $P \leq 0.01$. (B) Phenotypic changes in the hBT-MSCs. Eight days after adipogenic stimulation with IDX, the lipid droplets in the adipocytes were stained with Oil Red O (ORO).

Compound 1: White amorphous solid; $[\alpha]_D^{25}$ +94.5 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.43), 248 (4.28), 274 (4.11), 316 (3.87), 336 (3.82) nm; IR (ZnSe) ν_{max} 3726, 3600, 1708, 1690, 1516 cm^{-1}; HRFABMS, *m/z* 457.1475 $[M+H]^+$ (calcd for $C_{20}H_{21}O_7N_6$, 457.1472).

Compound 2: White amorphous solid; $[\alpha]_D^{25}$ +103.7 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.43), 248 (4.24), 274 (4.06), 316 (3.85), 336 (3.79) nm; IR (ZnSe) ν_{max} 3728, 3341, 2963, 1708, 1690, 1523 cm^{-1}; HRFABMS, *m/z* 443.1313 $[M+H]^+$ (calcd for $C_{19}H_{19}O_7N_6$, 443.1315).

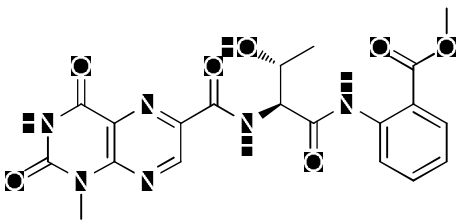


Table 1. ^{13}C and ^{1}H NMR Assignment for compound **1** in DMSO-d_6

No.	δ_C	δ_H	No.	δ_C	δ_H
2	150.0, C		1''-NH		11.11, s
3-NH		12.18, s	2''	139.2, C	
4	159.3, C		3''	117.1, C	
4a	127.2, C		4''	130.6, CH	7.92, d (8.0)
6	138.2, C		5''	123.4, CH	7.21, dd (8.0, 8.0)
7	146.2, CH	9.30, s	6''	134.1, CH	7.64, dd (8.0, 8.0)
8a	151.1, C		7''	120.7, CH	8.44, d (8.0)
9	28.6, CH_3	3.52, s	8''	167.3, C	
10	162.7, C		9''	52.4, CH_3	3.70, s
1'-NH		8.51, d (8.0)			
2'	59.7, CH				
3'	168.8, C				
4'	65.9, CH	4.42, m			
4'-OH		5.58, d (4.8)			
5'	20.5, CH_3	1.19, d (6.3)			

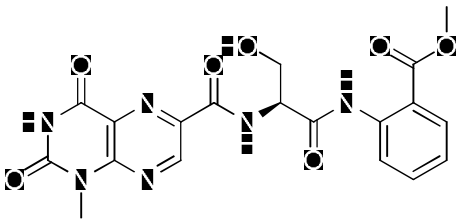


Table 2. ^{13}C and ^{1}H NMR Assignment for compound **2** in DMSO-d_6

No.	δ_C	δ_H	No.	δ_C	δ_H
2	150.0, C		1''-NH		11.11, s
3-NH		12.15, br s	2''	139.3, C	
4	159.3, C		3''	117.0, C	
4a	127.1, C		4''	130.6, CH	7.92, d (8.0)
6	138.5, C		5''	123.3, CH	7.21, dd (8.0, 8.0)
7	146.3, CH	9.28, s	6''	134.1, CH	7.63, dd (8.0, 8.0)
8a	151.0, C		7''	120.7, CH	8.44, d (8.0)
9	28.5, CH_3	3.52, s	8''	167.3, C	
10	162.6, C		9''	52.3, CH_3	3.70, s
1'-NH		8.75, d (7.5)			
2'	56.6, CH	4.66, ddd (7.5, 5.0, 5.0)			
3'	168.7, C				
4'	61.0, CH_2	4.01, ddd (10.6, 5.0, 5.0)			
		3.89, ddd (10.6, 5.0, 5.0)			
4'-OH		5.44, dd (5.0, 5.0)			

Discussion

A review of the literature revealed that alkylated lumazine derivatives have been found from a number of terrestrial and marine animals. Simple lumazine-containing compounds from marine animals include those from the sponges *Leucetta microraphis*,[22] *Corallistes fulvodesmus*,[23] *Corallistes undulatus*,[11] and *Clathria* sp.,[20] the polychaete *Odontosyllis undecimdonta*,[24] and the ascidian *Leptoclinides durust*,[25] whereas those containing the lumazine-6-carboxylic acid moiety were also found in the freshwater leech *Limnatis nilotica*.[17] Unlike the alkylated lumazines of animal origin, the recently reported penilumamides A-D from marine-derived *Penicillium* sp. and *Aspergillus* sp. fungi are structurally distinct, which is primarily due to their peptide nature and anthranilic methyl ester.[10,11] Because compound **1** contains a lumazine moiety, an amino acid residue and a methyl anthranilic ester, it is structurally related to these fungi-derived lumazine peptides. However, the 1-methyllumazine-6-carboxylic acid and the Thr unit both provide structural novelty to **1**. Furthermore, the 1-methyllumazine carboxylic acid either as a monomer or as a partial structural moiety of a compound has not been found in

natural products. Our discovery of an additional lumazine peptide may provide further evidence for the biogenetic distinction between marine-derived fungi and animals.

In the previous research of penilumamides A-D, there was no active bioactivity in spite of many trials to find a bioactivity.[10,11] Meanwhile, our novel compounds exhibited more potent than aspirin but less potent than glibenclamide on the production of adiponectin during adipogenesis in hBM-MSCs. Given the possibility of producing diverse structural variations through chemical synthesis, our findings may contribute to the development of a novel platform of insulin-related therapeutic agents.

In conclusion, these novel compounds possessed structural novelty at their 1-methyllumazine-6-carboxylic acid units, which is unprecedented as a component of natural products. The L-Thr (**1**) and L-Ser (**2**) units further distinguished these compounds from penilumamides from other marine-derived fungi. Importantly, this study is the first discovery of the enhancement effect of lumazine-containing natural products on insulin sensitivity.

References

1. (a) Fenical, W. *Chem. Rev.* **1993**, *93*, 1673-1683. (b) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143-163. (c) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666-673. (d) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012-3043. (e) Bhatnagar, I.; Kim, S. K. *Mar. Drugs* **2010**, *8*, 2673-2701.

2. Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160-258, and earlier reports in the series.

3. 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.

4. (a) Ellestad, G. A.; Mirando, P.; Kunstmann, M. P. *J. Org. Chem.* **1973**, *38*, 4204-4205. (b) Li, G.-Y.; Li, B.-G.; Yang, T.; Yin, J.-H.; Qi, H.-Y.; Liu, G.-Y.; Zhang, G.-L. *J. Nat. Prod.* **2005**, *68*, 1243-1246.

5. Ling, K. H.; Yang, C.-K.; Peng, F.-T. *Appl. Environ. Microbiol.* **1979**, *37*, 355-357.

6. Loesgen, S.; Bruhn, T.; Meindl, K.; Dix, I.; Schulz, B.; Zeeck, A.; Bringmann, G. *Eur. J. Org. Chem.* **2011**, *2011*, 5156-5162.

7. (a) Rao, K. V.; Sadhukhan, A. K. M.; Veerender, V. R.; Mohan, E. V. S.; Dhanvantri, S. D.; Siraramkumar, M. *Chem. Pharm. Bull.* **2000**, *48*, 557-560. (b) Parvatkar, R. R.; D'Souza, C.; Tripathi, A.; Naik, C. G. *Phytochedzmistry* **2009**, *70*, 128-132.

8. (a) Kaji, A.; Iwata, T.; Kiriyama, N.; Wakusawa, S.; Miyamoto, K.-i. *Chem. Pharm. Bull.* **1994**, *42*, 1682-1684. (b) Alvi, K. A.; Pu, H. *J. Antibiotics* **1999**, *52*, 215-223.

9. Arai, K.; Shimizu, S.; Yamamoto, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1005-1012.

10. Meyer, S. W.; Mordhorst, T. F.; Lee, C.; Jensen, P. R.; Fenical, W.; Köck, M. *Org. Biomol. Chem.* **2010**, *8*, 2158-2163.

11. Chen, M.; Shao, C.-L.; Fu, X.-M.; Kong, C.-J.; She, Z.-G.; Wang, C.-Y. *J. Nat. Prod.* **2014**, *77*, 1601-1606.

12. (a) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-i. *Anal. Chem.* **1997**, *69*, 3346-3352. (b) Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K.-i. *Anal. Chem.* **1997**, *69*, 5146-5151.

13. (a) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55-63. (b) Ulukaya, E.; Ozdikicioglu, F.; Oral, A. Y.; Demirci, M. *Toxicology* **2008**, *22*, 232-239.

14. (a) Lee, H.-S.; Lee, T.-H.; Yang, S. H.; Shin, H. J.; Shin J.; Oh, K.-B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2483-2486. (b) Oh, K.-B.; Kim, S.-H.; Lee, J.; Cho, W.-J.; Lee, T.; Kim, S. *J. Med. Chem.* **2004**, *47*, 2418-2421. (c) Johansson, M.; Karlsson, L.; Wennergren, M.; Jansson, T.; Powell, T. L. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 2831-2837. (d) 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.

15. Shin, D. W.; Kim, S. N.; Lee, S. M.; Lee, W.; Song, M. J.; Park, S. M.;

Lee, T. R.; Baik, J.-H.; Kim, H. K.; Hong, J.-H.; Noh, M. *Biochem. Pharmacol.* **2009**, *77*, 125-133.

16. Guerriero, A.; D'Ambrosio, M.; Pietra, F.; Debitus, C.; Ribes, O. *J. Nat. Prod.* **1993**, *56*, 1962-1970.

17. Voerman, G.; Cavalli, S.; van der Marel, G. A.; Pfleiderer, W.; van Boom, J. H.; Filippov, D. V. *J. Nat. Prod.* **2005**, *68*, 938-941.

18. Nagar-Anthal, K. R.; Worrell, V. E.; Teal, R.; Nagle, D. P. *Arch. Microbiol*. **1996**, *166*, 136-140.

19. Cottam, H. B.; Shih, H.; Tehrani, L. R.; Wasson, D. B.; Carson, D. A. *J. Med. Chem.* **1996**, *39*, 2-9.

20. Zuleta, I. A.; Vitelli, M. L.; Baggio, R.; Garland, M. T.; Seldes, A. M.; Palermo, J. A. *Tetrahedron* **2002**, *58*, 4481-4486.

21. Hundal, R. S.; Petersen, K. F.; Mayerson, A. B.; Randhawa, P. S.; Inzucchi, S.; Shoelson, S. E.; Shulman, G. I. *J. Clin. Invest.* **2002**, *109*, 1321-1326.

22. Cardellina II, J. H.; Meinwald, J. *J. Org. Chem.* **1981**, *46*, 4782-4784.

23. Debitus, C.; Cesario, M.; Guilhem, J.; Pascard, C.; Pais, M. *Tetrahedron Lett.* **1989**, *30*, 1535-1538.

24. (a) Inoue, S.; Okada, K.; Tanino, H.; Kakoi, H.; Horii, N. *Chem. Lett.* **1990**, 367-368. (b) Inoue, S.; Okada, K.; Tanino, H.; Kakoi, H.; Ohnishi, Y.; Horii, N. *Chem. Lett.* **1991**, 563-564. (c) Tanino, H.; Takakura, H.; Kakoi, H.; Okada, K.; Inoue, S. *Heterocycles* **1996**, *42*, 125-128.

25. Rudolph, K. E.; Liberio, M. S.; Davis, R. A.; Carroll, A. R. *Org. Biomol. Chem.* **2013**, *11*, 261-270.

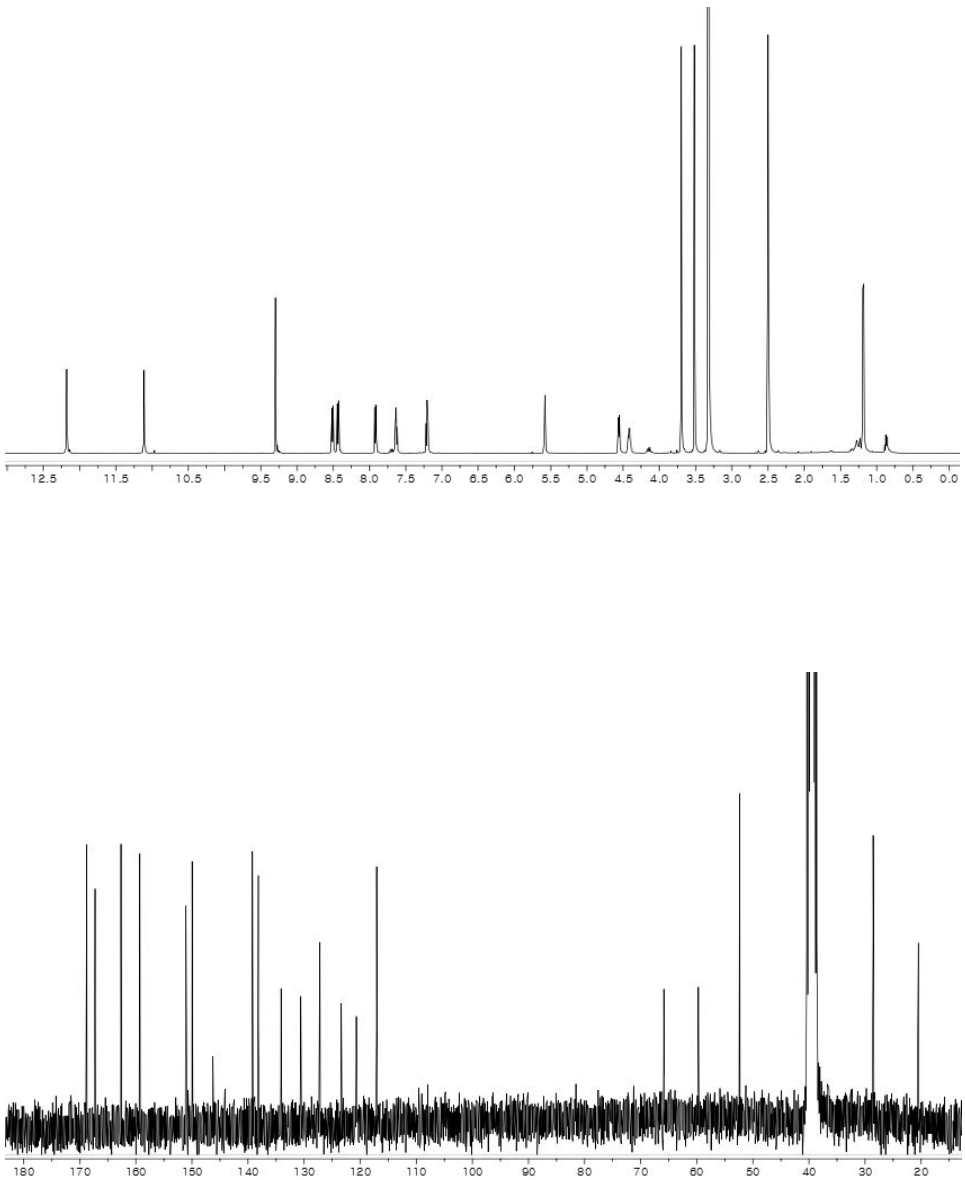


Figure 8. ^{1}H and ^{13}C NMR (600, 150 MHz, DMSO-d_6) spectrum of compound **1**.

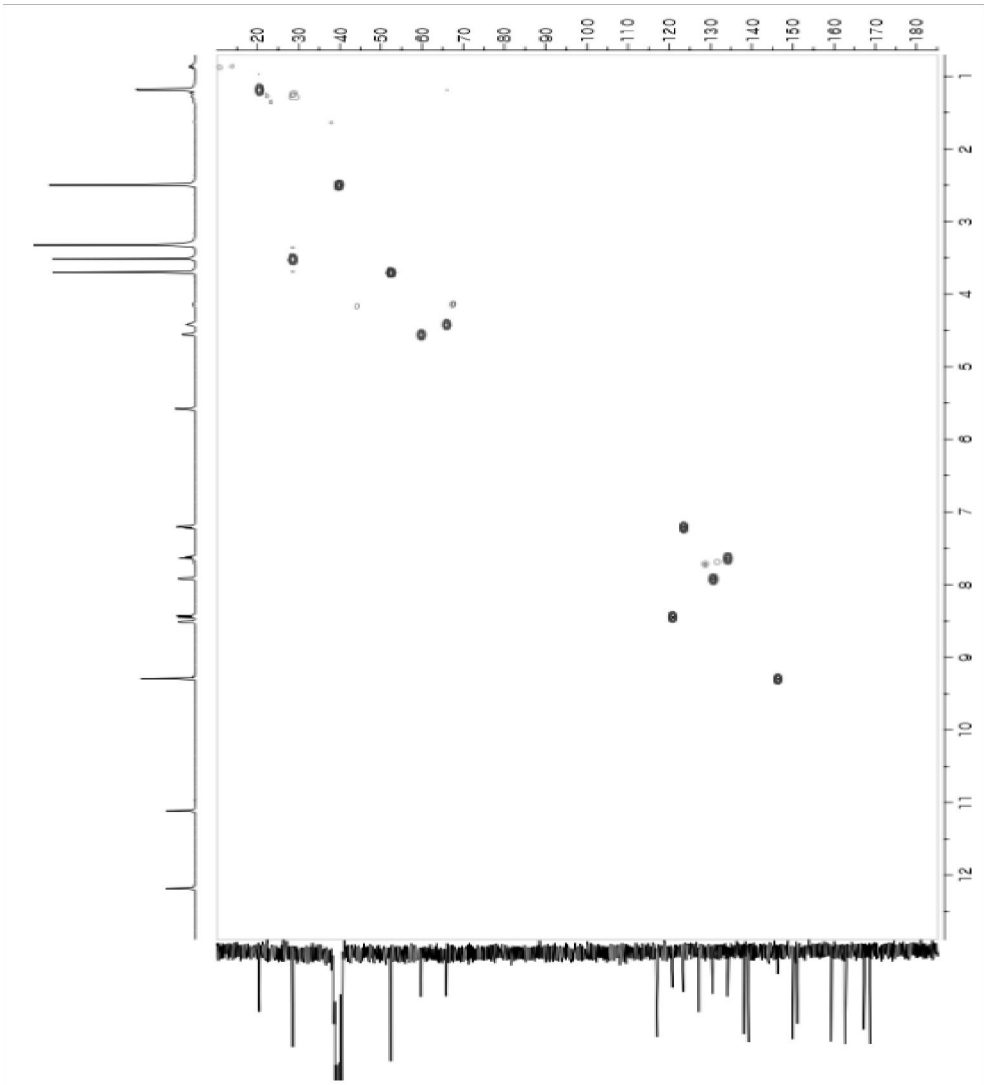


Figure 9. The HSQC (500 MHz, DMSO-d_6) spectrum of compound **1**.

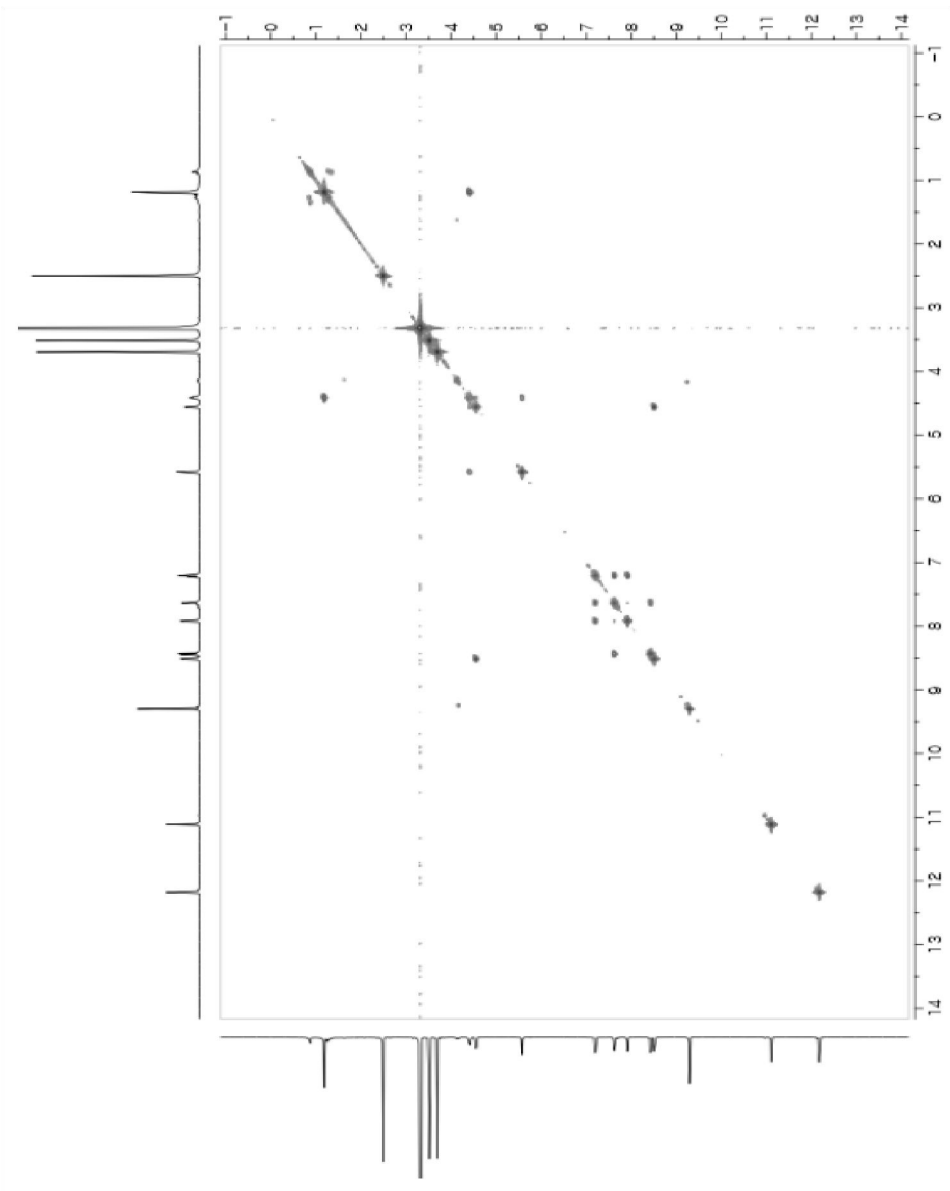


Figure 10. The COSY (500 MHz, DMSO-d_6) spectrum of compound **1**.

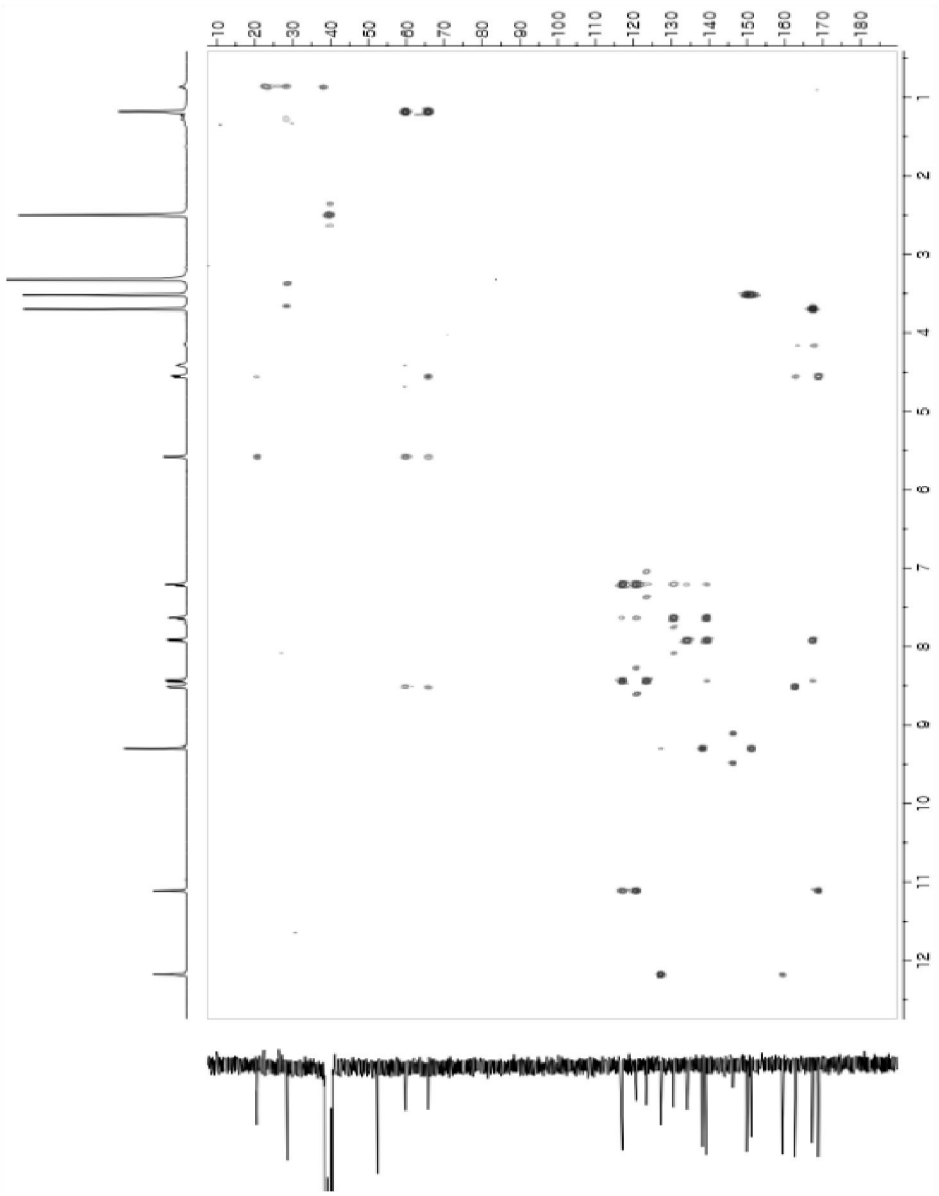


Figure 11. The HMBC (500 MHz, DMSO-d_6) spectrum of compound **1**

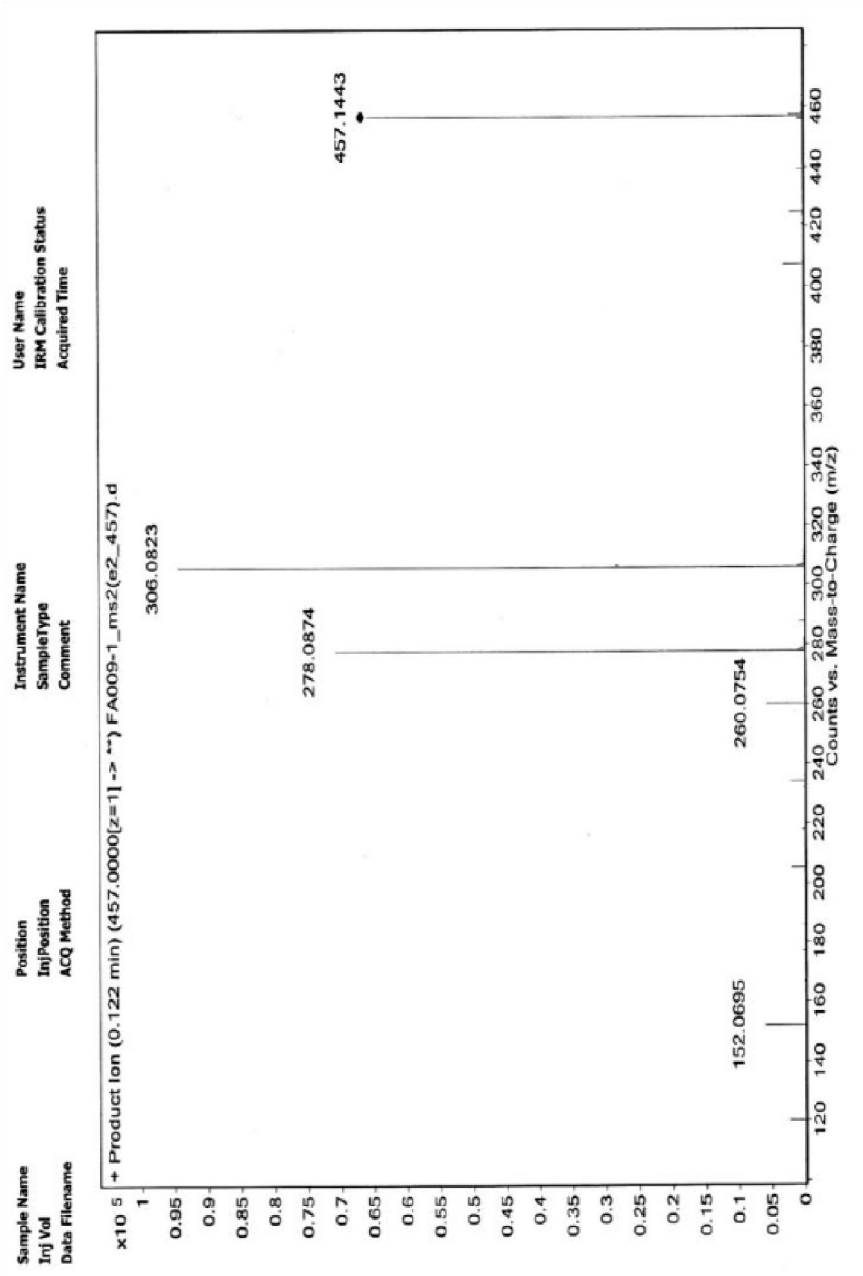


Figure 12. ESI-Q-TOF-MS/MS spectrum of compound **1**.

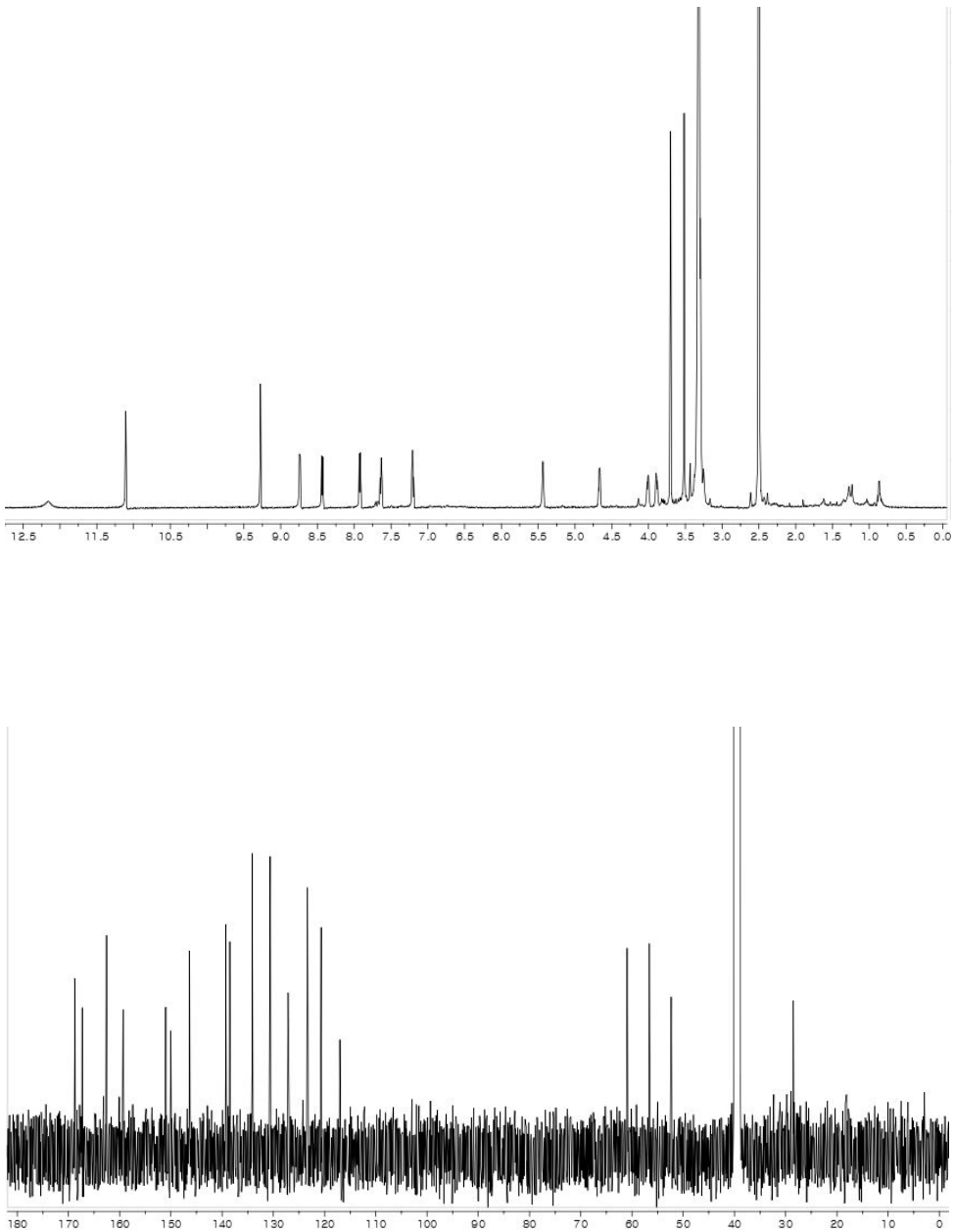


Figure 13. ^{1}H and ^{13}C NMR (500, 125 MHz, DMSO-d_6) spectrum of compound **2**.

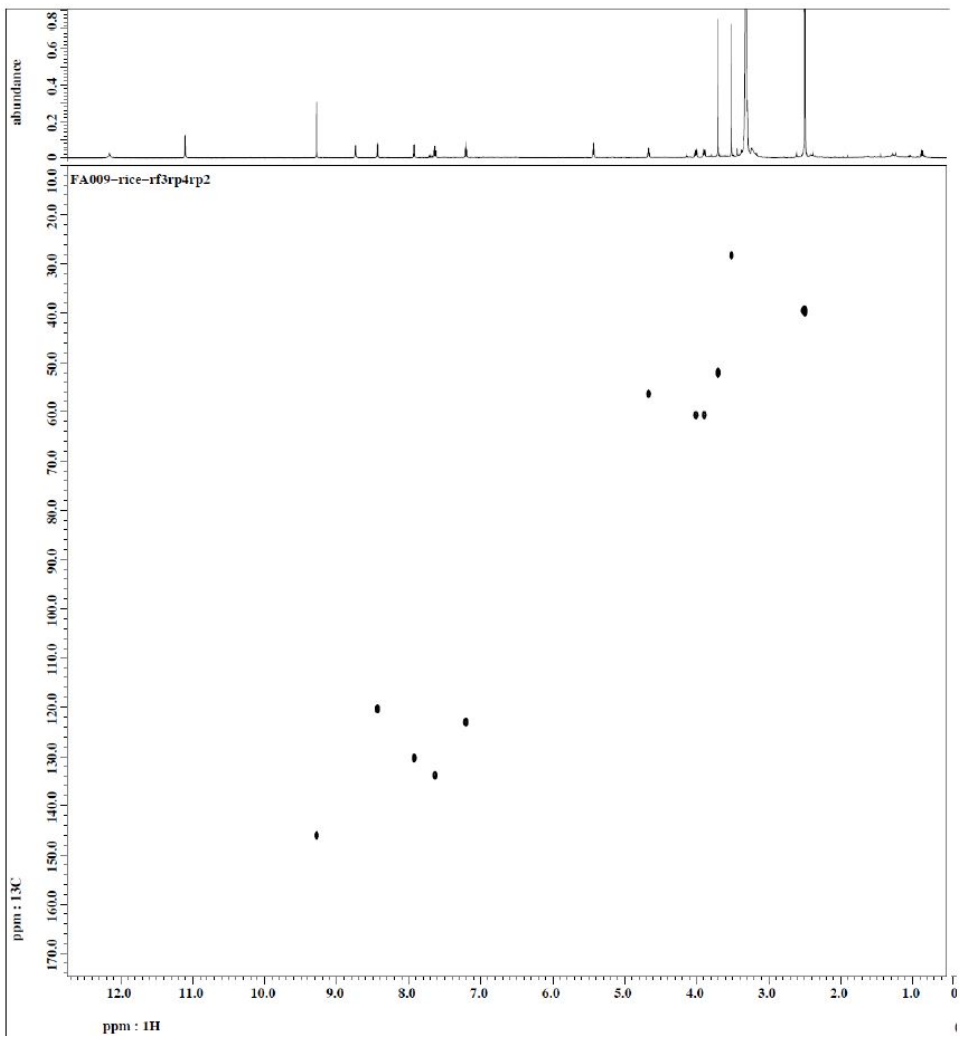


Figure 14. The HSQC (600 MHz, DMSO-d_6) spectrum of compound **2**.

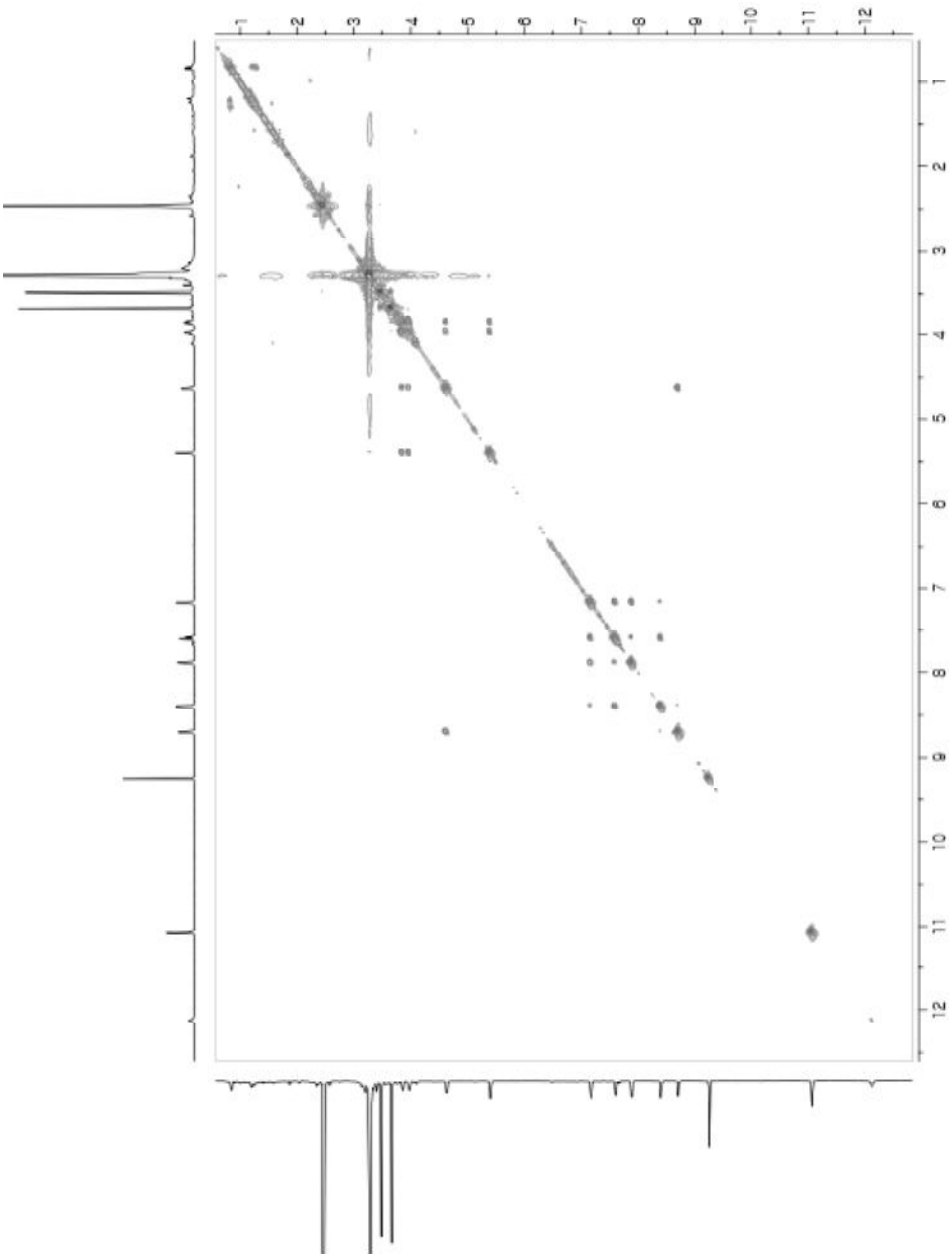


Figure 15. The COSY (600 MHz, DMSO-d_6) spectrum of compound **2**.

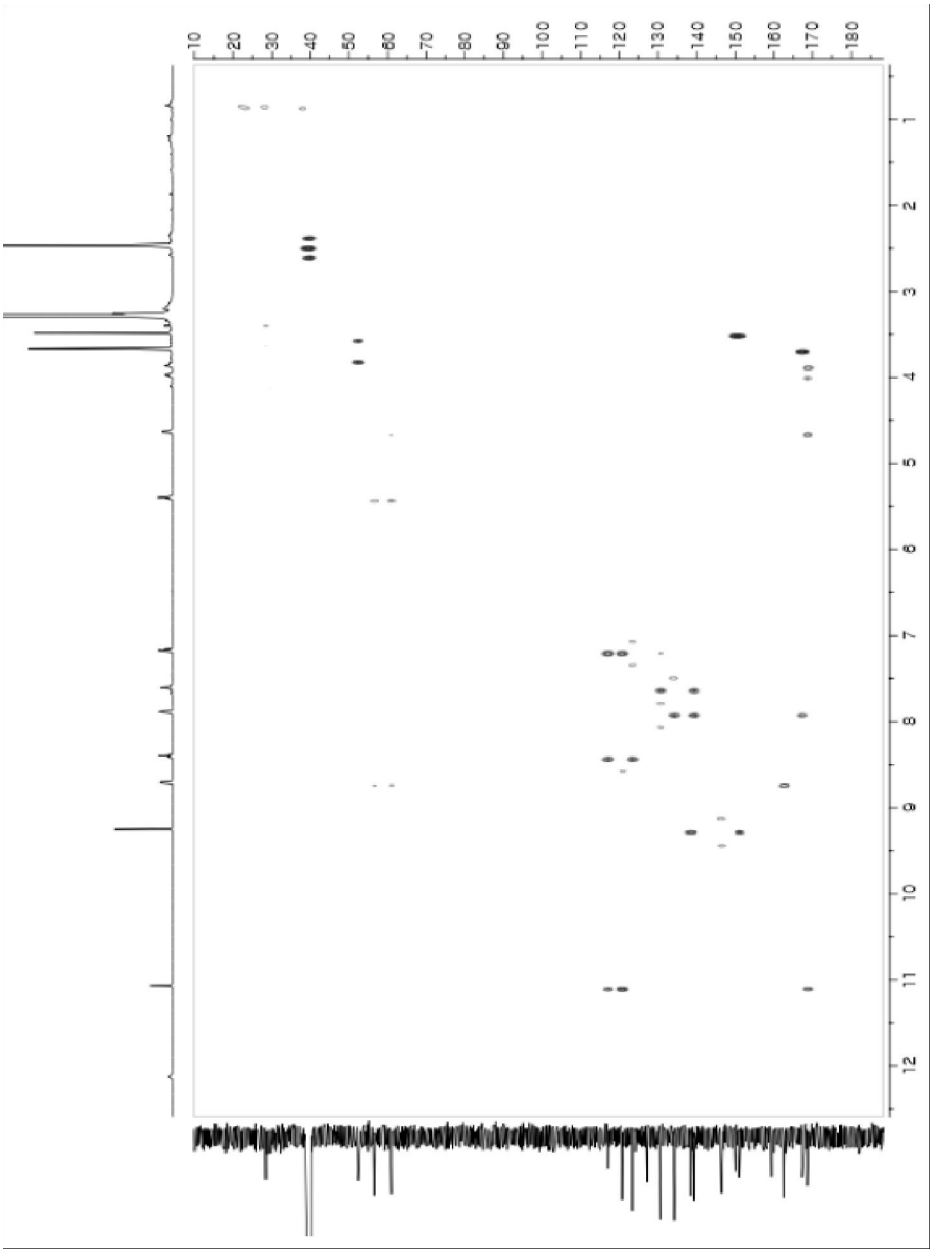


Figure 16. The HMBC (600 MHz, DMSO-d_6) spectrum of compound **2**.

국문초록

해양 유래 진균에서 분리한 신규 Lumazine Peptides

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2012년 제주도에서 채집된 해양 퇴적물로부터 분리된 균주인 *Aspergillus terreus*의 배양물을 추출 후, 정제하여 2개의 신규 peptides와 19개의 기지 물질들을 분리하였다.

다양한 분광학적 기법과 화학적 분석법을 이용하여 신규 peptides의 구조는 1-methyllumazine-6-carboxylic acid, 아미노산, 그리고 anthranilic acid methyl ester가 선형의 아미드 결합으로 연결된 것임을 규명하였다. 신규 물질들의 입체 구조는 Advanced Marfey's method를 이용하여 각각 L-Threonine과 L-Serine임을 결정하였다.

다양한 생리 활성을 검정해본 결과, 신규 물질은 인간 골수

중간엽 줄기세포의 adipogenesis model에서 인슐린 감수성을 개선시키는 약리 효과를 가지고 있음을 확인하였고, 그 효과는 glibenclamide보다 약하고 aspirin보다 강하게 나타났다.

주요어 : lumazine, peptide, *Aspergillus terreus*, 해양 진균, 인슐린 감수성

학번 : 2013-21598

감사의 글

학부 졸업과 동시에 새로운 전공에 대한 막연한 기대감과 열정만을 가지고 대학원 생활을 시작한 제가 이렇게 졸업에 이를 수 있었던 것은 많은 가르침과 도움을 주신 분들이 있었기 때문에 가능했습니다.

먼저 천연물 전공에 관한 아무런 배경 지식이 없었던 저에게, 많은 가르침과 아낌 없는 지원을 해주시고, '프로' 의식을 갖는 것의 중요성을 일깨워주신 신종헌 교수님 정말 감사드립니다. 더불어 지금의 자리로 인도해주시고 공동 연구로 많은 도움을 주신 오기봉 교수님과, 바쁘신 와중에도 항상 찾아뵐때마다 아낌없는 조언을 해주신 오동찬 교수님, 그리고 공동 연구와 개인적인 상담도 흔쾌히 해주신 노민수 교수님께도 감사드립니다.

고등학교 이후로 가장 오랜기간 함께 동고동락했던 우리 실험실 식구들 감사합니다. 부족한 것이 많았던 신입 때 실험과 실험실 생활에 있어서 많은 도움을 주신 전주은, 원태형, 이정호, 김창권, Lijuan, 우정균, 송인혜, Riswanto 선배님 감사드립니다. 함께 동기로서 의지하며 도움을 준 신보라 언니와 김형준 오빠, 후배였지만 동기처럼 의지한 조현주 언니, 그리고 힘든 내색도 없이 모든 열심히 해주시는 류연화, 황지연 언니에게도 감사드립니다. 또한 실험적으로도, 개인적으로도 많은 도움 주신 오미영 조교님께도 감사드립니다.

마지막으로 제가 하고자 하는 일을 지지해주시고 응원해주시며, 힘이 되어주신 부모님과 오빠, 감사하며 사랑합니다.