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# Lansai A–D, secondary metabolites from *Streptomyces* sp. SUC1

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ABSTRACT

*Streptomyces* sp. SUC1, endophytic on the aerial roots of *Ficus benjamina* was isolated and characterized on the basis of its morphology, amino acid composition of the whole-cell extract, and genomic DNA. Four novel secondary metabolites, lansai A–D, were isolated from the culture of this endophyte. The structures were identified by spectroscopic data. The compounds were tested for anticancer and antifungal activities.

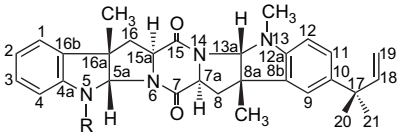


## 1. Introduction

Endophytes are likely to be rich sources of microorganisms of wide diversity, which have potential to be used in agriculture and medicine.[1] Endophytic microbes have been shown to produce novel natural products.[2,3] *Actinomyces*, specifically *Streptomyces*, are one of the most promising endophytic microorganisms able to produce important biologically active compounds.[4–7] Recently, actinomycin D has been isolated from the culture of *Streptomyces* sp. Tc022, endophytic on the roots of *Alpinia galanga*.[8] We now report the isolation and structural elucidation of four novel compounds, lansai A–D, from *Streptomyces* sp. SUC1, an endophyte on the aerial roots of *Ficus benjamina*. The EtOAc extract of culture *Streptomyces* sp. SUC1 was subjected to successive column chromatography followed by preparative TLC on silica gel to give four novel secondary metabolites, lansai A–D. The structures of lansai A–D (**1**–**4**) (Fig. 1) were elucidated on the basis of their spectroscopic data. Anticancer and antifungal activities of the compounds were tested.

## 2. Results and discussion

Lansai A (**1**) was isolated as colorless needles, mp 164–170 °C, it was optically active ($[\alpha]_D$ −497.1 (*c* 0.105)). The compound had the molecular formula $C_{30}H_{34}N_4O_2$ by HRESIMS. The UV spectrum showed bands at 249 and 303 nm. The IR spectrum showed absorption bands for NH (3356 $cm^{-1}$) and amide carbonyl (1666 $cm^{-1}$). The signals of seven aromatic protons at $\delta$ 6.56, 6.78, 7.05, 7.10, 6.28, 7.01, and 7.08 in the $^1H$ NMR spectrum of **1** (Table 1) were ascribed to two indoline units in the molecule. This was consistent with the $^{13}C$ NMR spectral data (Table 1), which exhibited seven aromatic methine carbons at $\delta$ 122.7 (C-1), 119.6 (C-2), 128.7 (C-3), 109.4 (C-4), 120.1 (C-9), 126.3 (C-11), and 105.5 (C-12) and five aromatic quaternary carbons at $\delta$ 148.3 (C-4a), 132.2 (C-16b), 132.9 (C-8b), 138.6 (C-10), and 148.1 (C-12a). In addition, signals of two ABX systems in the $^1H$ NMR spectrum of **1** at $\delta$ 2.30 (1H, dd, *J*=10.8, 12.3 Hz), 2.73 (1H, dd, *J*=6.3, 12.3 Hz), and 4.10 (1H,



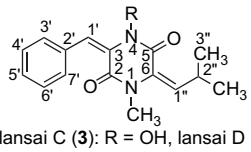




**Figure 1.** Structures of lansai A–D (**1**–**4**).

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**Table 1**
$^1$H (300 MHz) and $^{13}$C NMR (75 MHz) spectral data of lansai A (**1**) and lansai B (**2**)

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 7.10, dd (1.5, 7.8) | 122.7 | 7.06, dd (1.5, 7.8) | 122.3 |
| 2 | 6.78, dt (1.5, 7.8) | 119.6 | 6.71, dt (1.5, 7.8) | 118.1 |
| 3 | 7.05, dt (1.5, 7.8) | 128.7 | 7.11, dt (1.5, 7.8) | 128.8 |
| 4 | 6.56, dd (1.5, 7.8) | 109.4 | 6.36, dd (1.5, 7.8) | 105.8 |
| 4a | — | 148.3 | — | 150.1 |
| 5 | 5.03, s | — | — | — |
| 5-Me | — | — | 2.99, s | 32.9 |
| 5a | 5.25, s | 81.2 | 5.46, s | 86.6 |
| 7 | — | 166.6 | — | 165.6 |
| 7a | 4.10, dd (6.3, 10.8) | 60.2 | 4.16, m | 60.1 |
| 8-Ha | 2.20, dd (10.8, 12.3) | 42.2 | 2.18, dd (11.3, 12.3) | 42.7 |
| 8-Hb | 2.69, dd (6.3, 12.3) | — | 2.72, dd (5.9, 12.3) | — |
| 8a | — | 50.5 | — | 50.5 |
| 8a-Me | 1.46, s | 25.4 | 1.48, s | 25.5 |
| 8b | — | 132.9 | — | 132.8 |
| 9 | 7.01, d (1.5) | 120.1 | 7.02, d (1.5) | 120.2 |
| 10 | — | 138.6 | — | 138.7 |
| 11 | 7.08, dd (1.5, 8.1) | 126.3 | 7.08, dd (1.5, 8.1) | 126.3 |
| 12 | 6.28, d (8.1) | 105.5 | 6.29, d (8.1) | 105.4 |
| 12a | — | 148.1 | — | 148.3 |
| 13-Me | 2.96, s | 33.3 | 2.97, s | 33.1 |
| 13a | 5.39, s | 87.1 | 5.44, s | 86.9 |
| 15 | — | 166.0 | — | 165.8 |
| 15a | 4.10, dd (6.3, 10.8) | 60.5 | 4.16, m | 60.1 |
| 16-Ha | 2.30, dd (10.8, 12.3 | 40.3 | 2.17, dd (11.3, 12.3) | 42.7 |
| 16-Hb | 2.73, dd (6.3, 12.3) | — | 2.72, dd (5.9, 12.3) | — |
| 16a | | 51.8 | — | 50.3 |
| 16a-Me | 1.48, s | 24.2 | 1.49, s | 25.4 |
| 16b | — | 132.2 | — | 132.9 |
| 17 | — | 40.7 | — | 40.7 |
| 18 | 5.99, dd (10.8, 17.4) | 148.6 | 5.99,dd (10.6, 17.5) | 148.6 |
| 19 | 5.00, d (10.8) | 110.2 | 5.01, d (17.5) | 110.2 |
| | 5.01, d (17.4) | — | 5.03, d (10.6) | — |
| 20 and 21 | 1.35, s (2×) | 25.8 (2×) | 1.36, d (2×) | 28.5 (2×) |

All spectra were recorded in $CDCl_3$. *J* values in parentheses are in hertz.

dd, *J*=6.3, 10.8 Hz) and at δ 2.20 (1H, dd, *J*=10.8, 12.3 Hz), 2.69 (1H, dd, *J*=6.3, 12.3 Hz), and 4.10 (1H, dd, *J*=6.3, 10.8 Hz) were assigned to two β-methylene groups (Hab-16 and Hab-8) and two α-methine protons (H-15a and H-7a) of two α-amino acid functions, respectively. The spectrum also contained two singlets at δ 5.03 (1H) of an NH proton and 2.96 (3H) of an *N*–$CH_3$ group. Together with the $^{13}$C NMR spectral data, which contained signals for two methylene carbons at δ 40.3 and 42.2, two methine carbons at δ 60.5 and 60.2, and two amide carbonyls at δ 166.0 and 166.6, it appeared that **1** possessed two indoline units, derived from one unit of tryptophan and one unit of *N*-methyltryptophan. The $^1$H NMR spectrum of **1** also exhibited two singlets of one hydrogen each at δ 5.25 and 5.39, which were assigned to two methine protons of two –N–C*H*–N–CO systems. This was consistent with the $^{13}$C NMR spectrum, which showed peaks of two methine carbons at δ 81.2 (C-5a) and 87.1 (C-13a). Two methyl groups on C-16a and C-8a in **1** were appeared as two singlets at δ 1.48 and 1.46, respectively. The $^1$H NMR spectrum also contained a singlet of two methyl groups at δ 1.35 and an ABX system at δ 5.00 (1H, d, *J*=10.8 Hz), 5.01 (1H, d, *J*=17.4 Hz), and 5.99 (1H, dd, *J*=10.8, 17.4 Hz), which were due to the presence of a 1,1-dimethyl-2-propenyl group. This was consistent with the $^{13}$C NMR, which exhibited signals for two methyl groups at δ 25.8 (C-20 and C-21), a methylene carbon at δ 110.2 (C-19), an olefinic methine carbon at δ 148.6 (C-18), and a quaternary carbon at δ 40.7 (C-17). The $^{13}$C NMR spectrum of **1** (Table 1) was assigned by DEPT, 2D HMQC, and 2D HMBC spectra.

The position of 1,1-dimethyl-2-propenyl group at C-10 was established by 2D HMBC correlations (Fig. 2) between methyl protons (δ 1.35) and C-10 (δ 138.6). That the two methylene groups, Hab-16 at δ 2.30 and 2.73 and Hab-8 at δ 2.20 and 2.69 were

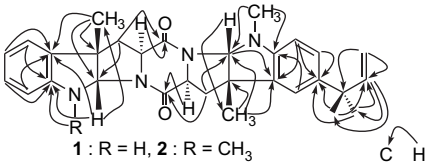


**Figure 2.** HMBC correlations of **1** and **2**.

attached to C-16a and C-8a of the two indoline nuclei, respectively, was apparent from the correlations between Hab-16 and C-5a (δ 81.2) and Hab-8 and C-13a (δ 87.1). In addition, Hab-16 and H-15a (δ 4.10) showed correlations to the amide carbonyl (C-15) at δ 166.6; similarly, Hab-8 and H-7a (δ 4.10) showed correlations to the amide carbonyl (C-7) at δ 166.6. $^2J$ and $^3J$ correlations were shown between 16a-$CH_3$ (δ 1.48) and C-16a (δ 51.8) and C-5a (δ 81.2) and also between 8a-$CH_3$ (δ 1.46) and C-8a (δ 50.5) and C-13a (δ 87.1). Other correlations were observed between H-5a (δ 5.25) and 16a-$CH_3$ (δ 24.2), C-16b (δ 132.2) and C-4a (δ 148.3) and between H-13a (δ 5.39) and 8a-$CH_3$ (δ 25.4) and C-8b (δ 132.9) and C-12a (δ 148.1). The NH proton (δ 5.03) showed correlation to C-4a (δ 148.3) while *N*–$CH_3$ (δ 2.96) showed correlations to C-12a (δ 148.1) and C-13a (δ 87.1). In the NOEDIFF spectrum of **1**, 16a-$CH_3$ gave enhancements with H-5a and one methylene proton (Ha-16) and H-15a enhanced the signal of the other methylene proton (Hb-16). This indicated that 16a-$CH_3$ and H-5a were cis to each other but trans to H-15a. Similarly, irradiation of 8a-$CH_3$ enhanced the signals of H-13a and Ha-8, while irradiation of H-7a affected Hb-8, indicating that 8a-$CH_3$ and H-13a were cis to each other but trans to H-7a in the molecule. On the basis of the above evidence, the tryptophan dimer structure **1** was identified for lansai A with the *N*–$CH_3$ group attached to N-13.

Lansai B (**2**) was obtained as colorless needles, mp 160–163 °C; it was optically active ($[\alpha]_D$ −425.4 (*c* 0.185)). Compound **2** has the molecular formula $C_{31}H_{36}N_4O_2$ by HRESIMS showing that **2** had one carbon and two protons more than compound **1**. This was confirmed by the presence of an extra *N*-methyl signal in the $^1$H and $^{13}$C NMR of **2**. Other peaks were almost identical to those of **1** (Table 1). The UV, IR, and EIMS spectra of **2** were similar to those of **1**. This data led to the conclusion that lansai B had the *N*-methyltryptophan dimer structure **2**.

Several piperazinediones derived from tryptophan dimers are known from fungi, e.g., amauromine,[9] epiamauromine, and *N*-methylepiamauromine.,[10] These compounds have 1,1-dimethylprop-2-ene substituents at C-8a and C-16a rather than methyl groups as in **1** and **2**. The large negative optical rotations found for lansai A and lansai B, similar to the value reported for amauromine, suggest that the compounds have the same absolute stereochemistry as determined for amauromine[9] that is the precursor for lansai A and lansai B is L-tryptophan. Acid hydrolysis (HCl), which was used with amauromine[9] and epiamauromine[10] was not attempted because of the limited amount of lansai A and lansaiB and also because *ipso*-demethylation to give *N*-methyltryptophan was considered to be problematical.

Lansai C (**3**) was isolated as colorless plates, mp 146–148 °C; it was optically inactive. The molecular formula was $C_{16}H_{18}N_2O_3$. The UV spectrum showed bands at 230 and 311 nm. Compound **3** had IR bands at 3210 and 1682 $cm^{-1}$ indicating the presence of hydroxyl and amide carbonyl groups, respectively. The $^1$H NMR spectrum of **3** (Table 2) showed two singlets at δ 8.02 (1H) and 3.31 (3H) from *N*–OH and *N*–$CH_3$ groups, respectively, and the $^{13}$C NMR (Table 2) contained signals for two amide carbonyls at δ 157.8 (C-2) and 157.6 (C-5) and two olefinic quaternary carbons at δ 125.7 (C-3) and 128.0 (C-6), indicating the *N*-hydroxy,*N*-methyl-3,6-dialkylidene-2,5-piperazinedione moiety. The $^1$H NMR spectrum of **3** also showed

**Table 2**
$^1$H (300 MHz) and $^{13}$C NMR (75 MHz) spectral data of lansai C (**3**) and lansai D (**4**)

| Position | **3** | | **4** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1-Me | 3.31, s | 31.1 | 3.23, s | 31.0 |
| 2 | — | 157.8 | — | 156.8 |
| 3 | — | 125.7 | — | 125.3 |
| 4 | 8.02, br s | — | 8.45, s | — |
| 5 | — | 157.6 | — | 159.1 |
| 6 | — | 128.0 | — | 128.2 |
| 1′ | 7.00, s | 115.8 | 6.40, s | 121.9 |
| 2′ | — | 133.3 | — | 133.8 |
| ArH | 7.41, m (5H) | 129.4 (2×) | 7.33, m (3H) | 129.9 (2×) |
| | | 128.5 (2×) | 7.53, d (7.2, 2H) | 127.8 (2×) |
| | | 128.6 | | 128.1 |
| 1″ | 5.51, d (9.6) | 134.8 | 5.50, d (9.6) | 134.6 |
| 2″ | 3.79, d sept (6.6, 9.6) | 26.7 | 3.82, d sept (6.7, 9.6) | 26.6 |
| 3″ and 4″ | 1.10, d (6.6) | 23.3 (2×) | 1.12, d (6.7) | 23.3 (2×) |

All spectra were recorded in $CDCl_3$. *J* values in parentheses are in hertz.

signals from five aromatic protons at $\delta$ 7.41 (m) and an olefinic methine proton at $\delta$ 7.00 (s). The $^{13}$C NMR showed signals from five aromatic methine carbons at $\delta$ 129.4 (2×), 128.5 (2×), 128.6, an aromatic quaternary carbon at $\delta$ 133.3, and an olefinic methine carbon at $\delta$ 115.8, indicating that compound **3** has a benzylidene unit. The $^1$H and $^{13}$C NMR spectra also showed signals due to the presence of an isobutylidene group. The proton signals appeared at $\delta$ 1.10 (6H, d, $J$=6.6 Hz), 3.79 (1H, d sept, $J$=6.6, 9.6 Hz), and 5.51 (1H, d, $J$=9.6 Hz) and carbon signals of two methyl carbons at $\delta$ 23.3 (C-3″ and C-4″), a methine carbon at $\delta$ 26.7 (C-2″), and an olefinic methine carbon at $\delta$ 134.8 (C-1″).

The positions of the benzylidene group at C-3, isobutylidene at C-6, and methyl group on N-1 were established by 2D HMBC correlations (Fig. 3). The olefinic hydrogen (H-1′) ($\delta$ 7.00) of the benzylidene group showed $^2J$ and $^3J$ correlations to C-3 ($\delta$ 125.7) and 2-carbonyl ($\delta$ 157.8). The aromatic carbons at $\delta$ 133.3 (C-2′) and 128.5 (C-3′ and C-7′) showed correlations to H-1′ ($\delta$ 7.00). The methyl protons (H-3″ and H-4″) ($\delta$ 1.10) and methine proton (H-2″) ($\delta$ 3.79) of the isobenzylidene group had correlations to olefinic methine carbon (C-1″) ($\delta$ 134.8) and olefinic quaternary carbon (C-6) ($\delta$ 128.0), respectively. H-1″ ($\delta$ 5.51) had a $^3J$ correlation to the C-5 amide carbonyl ($\delta$ 157.6). The *N*-methyl group ($\delta$ 3.31) had HMBC correlations to the carbonyl (C-2) ($\delta$ 157.8) and olefinic quaternary carbon (C-6) ($\delta$ 128.0). In the NOEDIFF experiments H-1′ and H-1″ gave enhancement with 4-*N*–OH and 1-*N*–$CH_3$, respectively. Thus, lansai C was characterized as 4-*N*-hydroxy-1-*N*-methyl-3-*E*-benzylidene-6-*E*-isobutylidene-2,5-piperazinedione (**3**).

Lansai D (**4**), colorless needles, mp 178–172 °C, had the molecular formula $C_{16}H_{18}N_2O_2$, which indicated that **4** had one oxygen less than **3**. The $^1$H NMR spectrum of **4** showed a singlet of NH at $\delta$ 8.45, shifted downfield compared with the signal of *N*–OH in **3** ($\delta$ 8.02). Other peaks in the $^1$H and $^{13}$C NMR spectra were almost identical to those of **3** (see Table 2) and similar HMBC correlations were observed (Fig. 3). The UV, IR, and MS spectra of **4** were similar to those of **3**. In the NOEDIFF spectra, H-1′ and H-1″ gave enhancement with 4-*N*–H and 1-*N*–$CH_3$, respectively. Lansai D was thus identified as 1-*N*-methyl-3-*E*-benzylidene-6-*E*-isobutylidene-2,5-piperazinedione (**4**).

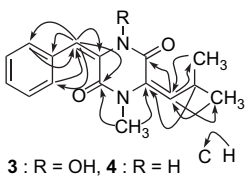


**Figure 3.** HMBC correlations of **3** and **4**.

A number of 3-ylidene- and 3,6-diylidene-piperazine-2,5-diones have been isolated from various microorganisms, involving amino acids and/or dehydroaminoacids.[11]

The crude ethyl acetate extract of the culture of *Streptomyces* sp. SUC1 showed in vitro anticancer activity (BC and KB cell lines) with $IC_{50}$ of 3.41 and >20 μg ml$^{-1}$, respectively. The crude extract also possessed antifungal activity against *Colletotrichum musae* with an MIC of 15 μg ml$^{-1}$. Unfortunately, compound **2** was only weakly active against the BC cell line ($IC_{50}$=15.03 μg ml$^{-1}$); compounds **1**, **3**, and **4** were inactive ($IC_{50}$>20 μg ml$^{-1}$). Compounds **1**–**4** were also inactive against *C. musae* (MIC>100 μg ml$^{-1}$).

Microscopic observation revealed that SUC1 had sporophores monopodially branched, flexuous, producing open spirals, spores spherical to oval-shaped (1×1–1.5 mm) with smooth surfaces, substrate mycelium was extensively branched with non-fragmenting hyphae, aerial mycelium, white, changing to cream without soluble pigment. The whole-cell extracts of SUC1 were found to contain LL-type diaminopimelic acid. Based on the results of morphological observation and the whole-cell extract analysis, SUC1 was thus assigned to be the genus *Streptomyces*.

Almost the complete 16S rDNA sequence of the endophytic *Streptomyces* sp. SUC1 (>90% of the *Escherichia coli* sequence) from position 8 to position 1523 (*E. coli* numbering system[12]) was determined. The BLAST search results generated from representative strains of the related genera showed that *Streptomyces* sp. SUC1 had high levels (99.93%) of sequence similarity to species of *Streptomyces lateritius* (accession number: AJ781326). The nucleotide sequence data of *Streptomyces* sp. SUC1 have been deposited in the GenBank, EMBL and DDBJ databases with the accession number AB246922.

## 3. Experimental section

### 3.1. General procedure

Melting points are uncorrected. Optical rotations were determined with a Jasco digital polarimeter for $CHCl_3$ solutions, unless otherwise stated. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a Jasco A-302 spectrophotometer. $^1$H and $^{13}$C NMR spectra were measured in $CDCl_3$ or $CDCl_3$/DMSO-$d_6$ on a Bruker 300 (300 MHz for $^1$H NMR and 75 MHz for $^{13}$C NMR) Avance spectrometer. Chemical shifts are given in $\delta$ (ppm) with tetramethylsilane as an internal standard. EIMS were recorded on a VG 7070 mass spectrometer operating at 70 eV with VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectra. HRESIMS were recorded on a Bruker MicrOTOF mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck, 0.063–0.200 mm or 0.015–0.040 mm). TLC and PLC were performed on precoated silica gel 60 $F_{254}$ plates (Merck); spots were detected by UV or by spraying with 1% $CeSO_4$ in 10% aq $H_2SO_4$ following by heating.

### 3.2. Fungal material

The *Streptomyces* sp. SUC1 was isolated from the aerial roots of *F. benjamina*, growing in the grounds of the Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand. This fungus was deposited as SUC1 at the Department of Microbiology, Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand.

### 3.3. Identification of *Streptomyces* sp. SUC1

The *Streptomyces* sp. SUC1 was identified on the basis of its morphology, amino acid composition of the whole-cell extract, and genomic DNA.

The SUC1 isolate was cultured on ISP-2 agar plates at 30 °C for 3 days, the cover slides were then fixed down the colony and incubated at 30 °C for a further 5 days. The SUC1 isolated was then strained with crystal violet for 1 min and the morphology observed under light microscope. For scanning electron microscopy (SEM) observation, the isolated SUC1 grown on the cover slides was air-dried in a desiccator and mounted on stubs, splutter-coated with gold and viewed on the SEM (Maxim 2000S, CamScan, UK).

Diaminopimelic acid from the whole-cell extract was analyzed and the LL-type identified.[13,14] Genomic DNA was isolated from the isolate of SUC1 using the procedure of Hopwood et al.[15] 16S rDNA was amplified by PCR using Tag DNA polymerase (Promega, USA), Primer A 7-26f (5′-CCGTCGACGAGCTCAGA GTTTGATCCTG GCTCAG-3′) and Primer B 1523-150r (5′-CCCGGGTACCAAGCT-TAAGGAGGTGATCCAGCCGCA-3′). The conditions used for thermal cycling were as follows: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min, and extension at 72 °C for 2 min. At the end of the cycles, the reaction mixture was kept at 72 °C for 10 min and then cooled to 4 °C. The 1.5 kb amplified 16S rDNA fragment was separated by agarose gel electrophoresis and purified using a Qiaquick gel extraction kit (QIAGEN, Germany). The purified fragment was cloned into pGEM-T Easy vector (Promega). 16S rDNA nucleotide sequences were determined using the dideoxy chain termination method with a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA). The primers used for nucleotide sequencing were as follows: T7 promoter, SP6 promoter, C 704-685r (5′-TCTGCGCATTTCACCGCTAC-3′), and D 115-1100r (5′-AGGGTTGCGCTCGTTG-3′). All of the products obtained were assembled and then compared with similar sequences from the reference organisms contained in the BLAST database (a genome database of the National Center for Biotechnology information).

### 3.4. Extraction and isolation

Spores of *Streptomyces* sp. SUC1 were used to inoculate 100 plates of ISP-2 and these were incubated for 14 days at 28 °C. The culture medium was then cut into small pieces. The medium pieces were extracted with EtOAc (3×300 ml). The organic solvent was pooled and then taken to dryness under rotary evaporation to give a dark brown solid (754 mg). The extract was separated by flash column chromatography using silica gel 60 (Merck, 0.015–0.040 mm, diameter×height: 4.4×3.5 cm) and the column was eluted with 30 ml each fraction with gradients of hexane/EtOAc, EtOAc, and $CH_2Cl_2$/MeOH/$H_2O$ (50:3:1, 30:3:1, 20:3:1, 10:3:1, 7:3:1, and 6:4:1) to give 13 fractions.

Fr. 3 (first column, 40% EtOAc in hexane), a pale yellow solid (10.6 mg) was a pure compound and identified as lansai C. Fr. 4 (first column, 60% EtOAc in hexane), a yellow oil (65.2 mg) was separated by column chromatography using silica gel 60 (Merck, 0.063–0.200 mm) and the column was eluted with a gradient of hexane/EtOAc to give seven fractions. Fr.1 (second column) (16.9 mg) was lansai C. Fr. 3 (second column) (3.6 mg) was identified as lansai D. Fr. 4 (second column), a colorless solid (7.4 mg) was further separated by preparative TLC (silica gel 60 $F_{254}$, 0.25 mm) using hexane/EtOAc (7:1) as the developing solvent to give lansai D (2.4 mg) and lansai B (2.8 mg). Fr. 5 (second column), a colorless solid (4.1 mg) was further purified by preparative TLC using hexane/EtOAc (5:1) as the developing solvent to give lansai B (1.2 mg). Similarly, Fr. 6 (second column) (4.4 mg) was purified by preparative TLC to give lansai A (2.5 mg). Fr. 7 (second column) (3.1 mg) was identified as lansai A.

#### 3.4.1. Lansai A (**1**)

A colorless solid crystallized from MeOH as needles, mp 164–170 °C; $[\alpha]_D^{28}$ −497.1 (*c* 0.105, MeOH); $\lambda_{max}$ (MeOH) (log $\varepsilon$) nm: 249 (4.21), 303 (3.81); $\nu_{max}$ (KBr) $cm^{-1}$: 3356, 1666, 1611, 1499, 1418, 1211, 1163, 1002, 911, 745; *m*/*z* 482 (100, $M^+$), 467 (37), 455 (6), 328 (6), 270 (6), 239 (6), 210 (43), 198 (18), 171 (6), 144 (12), 97 (6), 69 (6), 57 (12); HRESIMS: $MH^+$, found 483.2769. $C_{30}H_{35}N_4O_2$ requires 483.2760. $^1H$ and $^{13}C$ NMR: Table 1.

#### 3.4.2. Lansai B (**2**)

A colorless solid crystallized from MeOH as needles, mp 160–163 °C; $[\alpha]_D^{28}$ −425.4 (*c* 0.185, MeOH); $\lambda_{max}$ (MeOH) (log $\varepsilon$) nm: 253 (3.99), 306 (3.49); $\nu_{max}$(KBr) $cm^{-1}$: 1669, 1609, 1496, 1416, 1299, 1207, 1163, 1004, 909, 742; *m*/*z* 496 (100, $M^+$), 482 (63), 469 (6), 446 (2), 336 (25), 324 (2), 283 (6), 256 (11), 241 (6), 226 (6), 210 (77), 198 (35), 183 (14), 158 (32), 145 (37), 129 (12), 97 (8), 73 (18), 57 (28); HRESIMS: $MH^+$, found 497.2910. $C_{31}H_{37}N_4O_2$ requires 497.2916. $^1H$ and $^{13}C$ NMR: Table 1.

#### 3.4.3. Lansai C (**3**)

A colorless solid crystallized from MeOH as plates, mp 146–148 °C; $[\alpha]_D^{28}$ 0; $\lambda_{max}$ (MeOH) (log $\varepsilon$) nm: 230 (3.92), 311 (4.04); $\nu_{max}$ (KBr) $cm^{-1}$: 3210, 1682, 1614, 1410, 1377, 1098, 912, 759; *m*/*z* 270 (100, $M^+-O$), 255 (35), 241 (2), 227 (23), 201 (4), 187 (2), 177 (2), 130 (3), 124 (4), 117 (23), 110 (4), 96 (14), 90 (25), 82 (63), 68 (23), 55 (25); HRESIMS: $MH^+$, found 287.1373. $C_{16}H_{19}N_2O_3$ requires 287.1395. $^1H$ and $^{13}C$ NMR: Table 2.

#### 3.4.4. Lansai D (**4**)

A colorless solid crystallized from MeOH as needles, mp 170–176 °C; $[\alpha]_D^{28}$ 0; $\lambda_{max}$(MeOH) (log $\varepsilon$) nm: 230 (4.07), 319 (4.34); $\nu_{max}$ (KBr) $cm^{-1}$: 3183, 1682, 1630, 1604, 1420, 1395, 1357, 1231, 1106, 860, 765, 723; *m*/*z* 270 (100, $M^+$), 255 (36), 241 (1), 227 (13), 201 (9), 187 (1), 149 (13), 177 (9), 110 (8), 96 (9), 90 (9), 82 (23), 68 (10), 55 (14); HRESIMS: $MNa^+$, found 293.1272. $C_{16}H_{18}N_2O_2Na$ requires 293.1266. $^1H$ and $^{13}C$ NMR: Table 2.

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