

Supplementary information

**An epipolythiodioxopiperazine alkaloid and diversified aromatic polyketides with cytotoxicity from the Beibu Gulf coral-derived fungus *Emericella nidulans* GXIMD 02509**

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## **Materials and methods**

### **General Experimental Procedures**

UV spectra were measured on an UV Thermo Fisher scientific Evolution 350 spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA, USA). ECD spectra were recorded on a JASCO J-1500 polarimeter (JASCO Corporation, Tokyo, Japan). The NMR spectra were obtained on a Bruker Avance spectrometer (Bruker BioSpin, Fällanden, Switzerland) operating at 500 MHz for  $^1\text{H}$  NMR, 125 MHz  $^{13}\text{C}$  NMR, using TMS as an internal standard. HR-ESIMS spectra were collected on a Waters Xevo G2-S TOF mass spectrometer (Waters Corporation, USA). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF<sub>254</sub> (10–40  $\mu\text{m}$ ) and over silica gel (200–300 mesh) (Qingdao Marine Chemical Factory, China), respectively. All solvents employed were of analytical grade (Shanghai Youshi Chemical Co., Ltd., China) and were distilled prior to use. Semi-preparative high-performance liquid chromatography (Semi-pre HPLC) was performed on a Shimadzu Prominence-I LC 2030 system (Shimadzu, Tokyo, Japan), equipping with an ODS column (YMC-pack ODS-A, YMC Co. Ltd., Japan, 10  $\times$  250 mm, 5  $\mu\text{m}$ , 2.5 mL/min).

### **Fungal Material**

The strain GXIMD 02509 was isolated from fresh tissue of the coral *Pocillopora damicornis* collected in the Weizhou Islands coral reefs in July 2020. It was taxonomically identified as *Emericella nidulans* GXIMD 02509 based on sequence analysis of the 18S rDNA (GenBank accession no. OP686588), which revealed 99% similarity to that of *Emericella nidulans* DAOM 222012 (accession no. JN939001.1). A voucher specimen was deposited in Guangxi Key Laboratory of Marine Drugs.

### **Fermentation, Extraction and Isolation**

The strain GXIMD 02509 was grown on Müller Hinton broth (MB) agar plates (malt extract 15 g, artificial sea salt 15 g, and agar 20 g) at 25 °C for 7 days. Then, it was inoculated in the seed medium (malt extract 15 g and artificial sea salt 15 g in 1.0 L tap distilled H<sub>2</sub>O, pH 7.4–7.8) at 25 °C on a rotary platform shaker at 180 rpm for 48 h. Subsequently, a large-scale fermentation of *E. nidulans* GXIMD 02509 was carried out in rice solid medium (120 g rice, 2.4 g artificial sea salt, and 140 mL H<sub>2</sub>O) at room temperature for 52 days. The whole fermented cultures (63 flasks, 7.6 Kg) were then extracted with EtOAc three times to provide a brown crude extract (180 g). The EtOAc

crude extract was fractionated by medium pressure liquid chromatography (MPLC) (silica gel, 200–300 mesh) using a step gradient elution with petroleum ether/CH<sub>2</sub>Cl<sub>2</sub>/MeOH (petroleum ether/CH<sub>2</sub>Cl<sub>2</sub>, 1:0–0:1; CH<sub>2</sub>Cl<sub>2</sub>/methanol, 1:0–1:1, v/v), which afforded 10 fractions (Frs.1~10) based on TLC analysis.

Fr.9 was separated into 13 subfractions (Frs.9-1~9-13) via reversed-phase MPLC (octadecylsilyl, ODS, 5 μm) with MeOH/H<sub>2</sub>O (10~100%) and then Fr.9-5 was then divided into 4 subfractions (Frs.9-5-1~9-5-4) by semipreparative high performance liquid chromatography (HPLC) with MeCN/H<sub>2</sub>O (71:29, v/v, 5.0 mL/min). Fr.9-5-1 was further purified by semiprep-HPLC on a YMC ODS column (ODS-A, 10 × 250 mm, 5 μm) eluting with MeCN/H<sub>2</sub>O (68:32, v/v, 2.0 mL/min) to afford compounds **5** (*t<sub>R</sub>* = 22 min, 5 mg) and **10** (*t<sub>R</sub>* = 26 min, 8 mg). Fr.9-5-2 was further separated by semiprep-HPLC on a YMC ODS column eluting with MeCN/H<sub>2</sub>O (58:42, v/v, 2.0 mL/min) to obtain compounds **4** (*t<sub>R</sub>* = 32.5 min, 44 mg) and **7** (*t<sub>R</sub>* = 41 min, 2.5 mg). Fr.9-6 was purified by semiprep-HPLC on a YMC ODS column eluting with MeCN/H<sub>2</sub>O (80:20, v/v, 5.0 mL/min) to yield compound **2** (*t<sub>R</sub>* = 28 min, 15 mg). Fr.9-9 was further purified by semiprep-HPLC on a YMC ODS column eluting with MeCN/H<sub>2</sub>O (95:5, v/v, 5.0 mL/min) to provide compounds **1** (*t<sub>R</sub>* = 20 min, 8.4 mg), **11** (*t<sub>R</sub>* = 26 min, 18 mg), and **3** (*t<sub>R</sub>* = 34 min, 30 mg). Moreover, compounds **6** (10 mg), **8** (98 mg), and **9** (17 mg) were obtained from Fr.2, Fr.3, and Fr.7 via reversed-phase MPLC coupled with semiprep-HPLC, respectively.

4a-*O*-methoxyarugosin H (**1**). bright yellow solid; UV (MeOH) λ<sub>max</sub> (log ε) 377 (2.90), 321 (2.98), 255 (3.39), 203 (3.89) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, **Table 1**; HR-ESIMS *m/z* 371.1499 [M + H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>23</sub>O<sub>6</sub>, 371.1495).

## Biological Activity Assays

### Cytotoxic activity assay

These obtained compounds were evaluated for their cytotoxic activities against three human cancer cell lines, 786-O (human renal carcinoma cell), SW1990 (human pancreatic cancer cell), and SW480 (human colorectal cancer cell), along with the normal human liver cell line LO2 (Shanghai Cell Bank, Chinese Academy of Sciences) by the CCK-8 (Dojindo) method. Briefly, cells were cultured in RPMI1640, L15, and DMEM media supplemented with 10% fetal bovine serum (FBS), respectively. The cells were seeded at a density of 1000 to 5000 cells/well in

96 well plates and then incubated with the compounds in a gradient concentration (2.5, 5, 10, 20, and 40  $\mu\text{M}$ ) or with a solvent control for 72 h, followed by the addition of CCK reagent and the OD value of each well was measured at 450 nm using SpectraMax M5 Microplate Reader (Molecular Devices). Cisplatin functioned as the positive control. Dose response curves were plotted to determine  $\text{IC}_{50}$  values based upon the average values of three parallel experiments using Prism 5.0.

#### Wound-Healing assay

Cells in the exponential growth phase were seeded at an appropriate concentration in 6-well plates and grown to 90% confluence. Then a “wound” was scratched with 200  $\mu\text{L}$  pipette tip in each well, after that, cells were incubated with compounds in fresh medium (containing 3% FBS) for another 24 h. Photos of the wounds were taken every 6 h. The widths of the wounds were measured at three positions for each replicate using Leica Application Suite (Leica Microsystems GmbH, Wetzlar, Germany). The migratory distances were measured by Image J (National Institutes of Health, Bethesda, MD, USA) and the wound closure rates were calculated.

#### Cell colony formation assay

For cell colony formation assay, cells were seeded in 6-well plates, after 12 h incubation for attachment and growth, cells were treated with compounds (0–4  $\mu\text{M}$ ) for another 24 h. After 12–14 days’ culture, cell colonies were washed with PBS buffer, then fixed with methanol and stained with 0.5% crystal violet. the cell colony formation ability was analyzed using the Image J software.

#### Cell cycle and apoptosis assay

Cell cycle was analyzed by propidium iodide (PI) DNA staining using flow cytometry (Luo et al., 2018). Briefly, exponentially growing cells were incubated with different concentrations of compound **10** for 24 h. After treatment, cells were collected and fixed with 70% ethanol overnight. The fixed cells were then washed, resuspended, and finally stained with PI (BD). Cellular DNA was measured using LSRFotessa (BD) flow cytometer for the cell cycle distribution analysis. For

apoptosis assay, cells were incubated with compound **10** for 24 h, then cells were harvested and finally were stained with Annexin V-FITC/PI according to the manufacturer's protocol (BD). Stained cells were examined and analyzed quantitatively by flow cytometer.

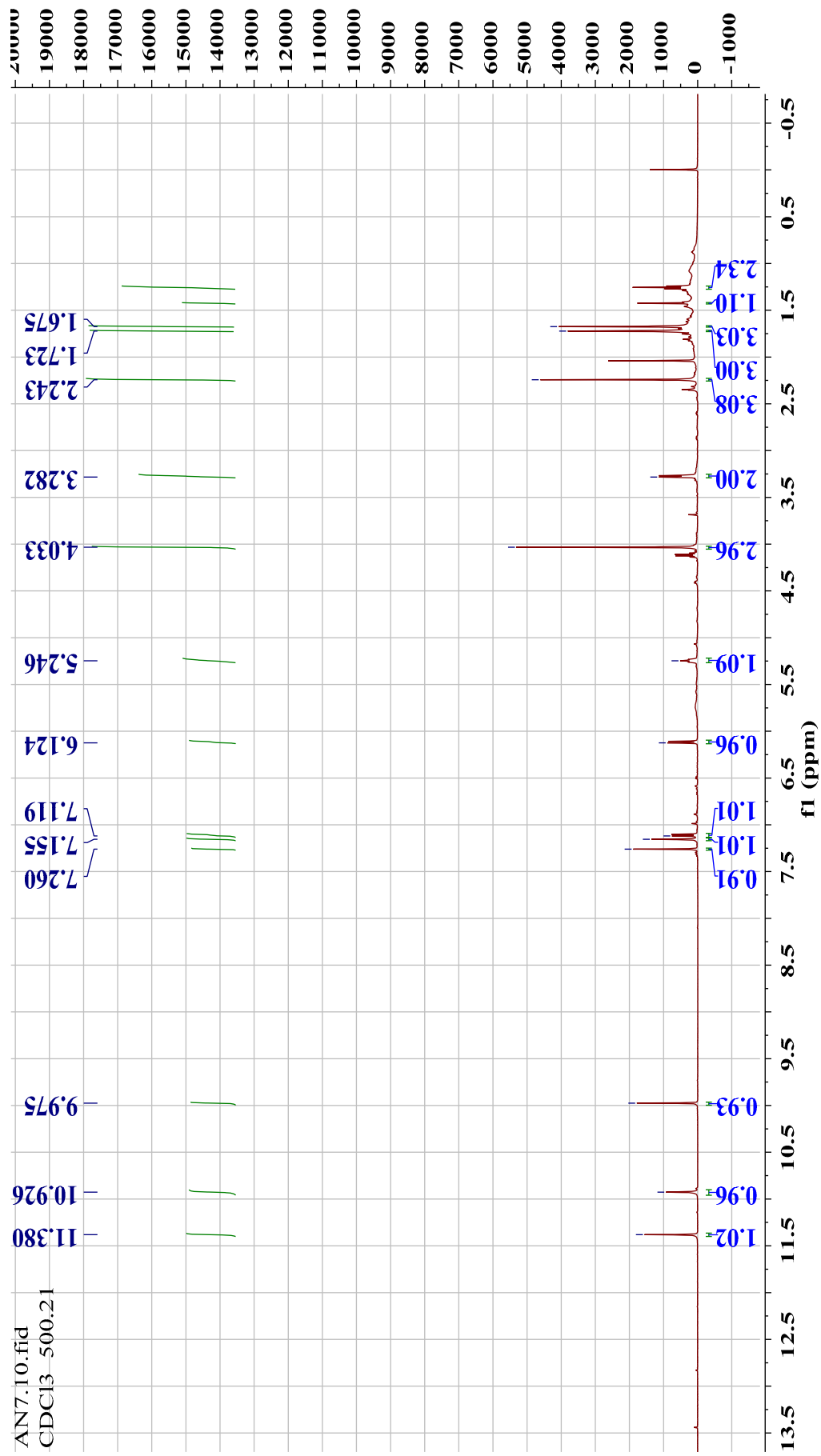


Fig. S1 <sup>1</sup>H NMR spectrum of 4a-O-methoxyarugosin H (1) (CDCl<sub>3</sub>, 500 MHz)

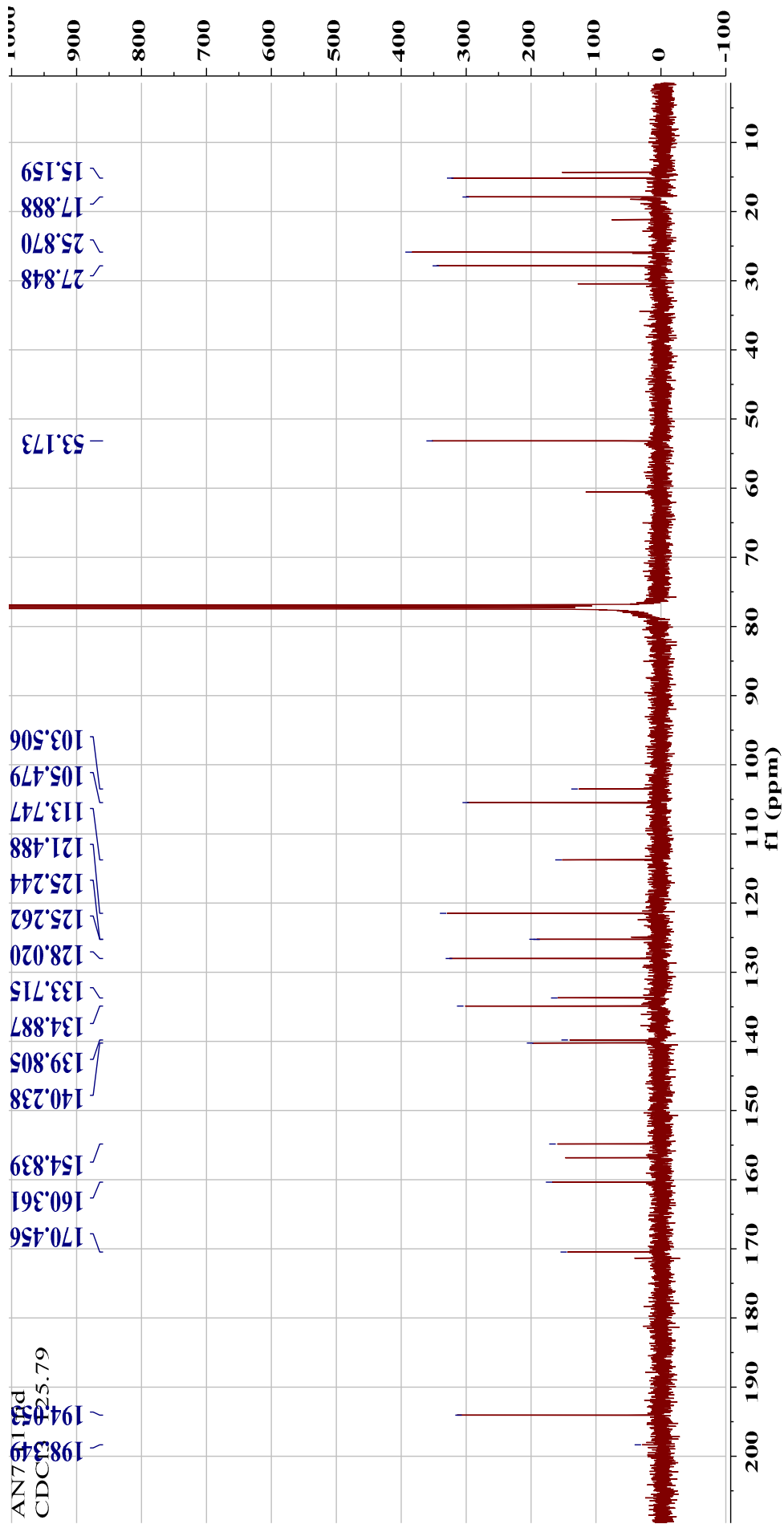


Fig. S2 <sup>13</sup>C NMR spectrum of 4a-O-methoxyarugosin H (1) (CDCl<sub>3</sub>, 125 MHz)

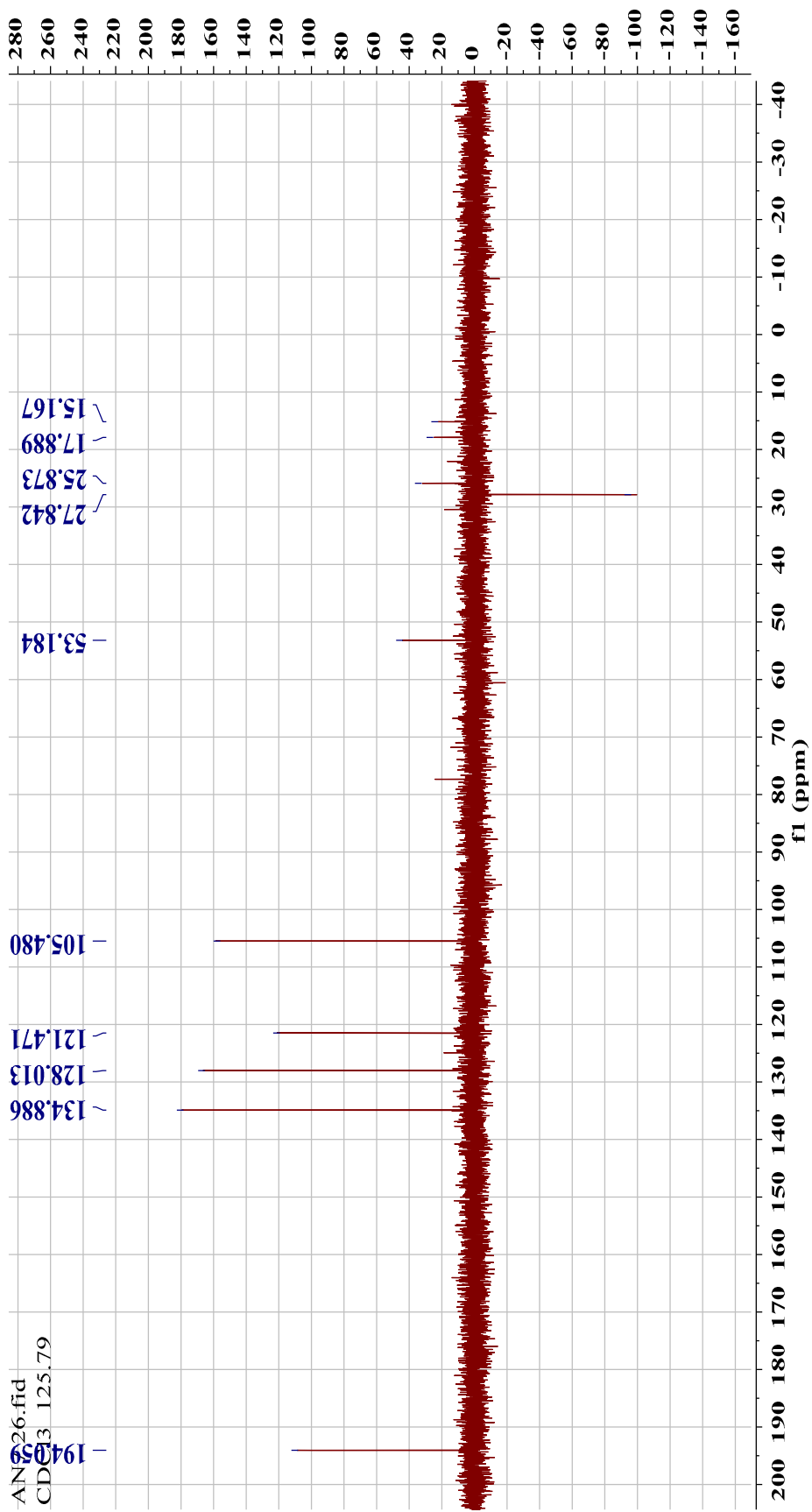


Fig. S3 DEPT 135 NMR spectrum of 4a-O-methoxyarugosin H (1) (CDCl<sub>3</sub>, 125 MHz)



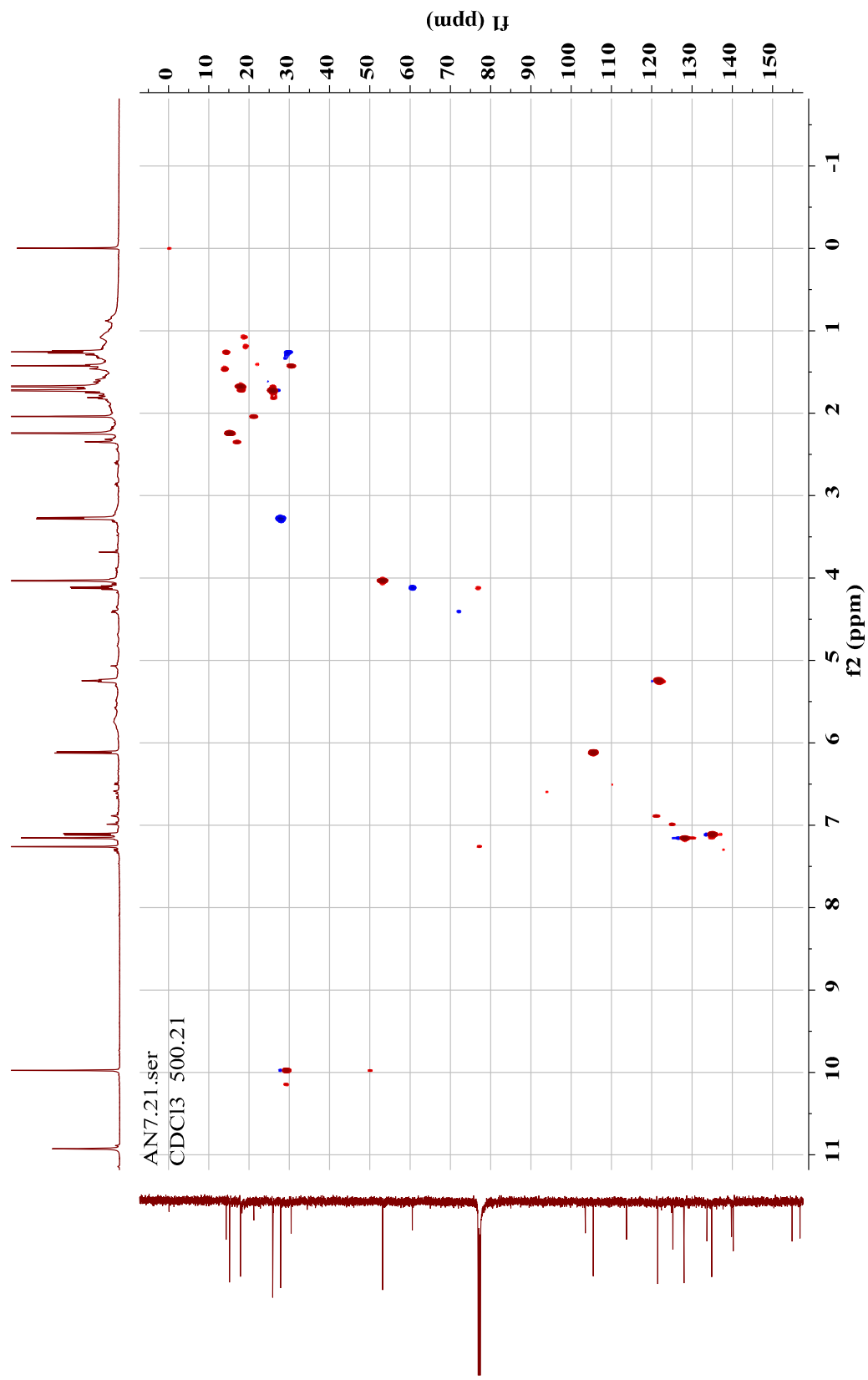


Fig. S4 HSQC spectrum of 4a-O-methoxyyarugosin H (1) (CDCl<sub>3</sub>)

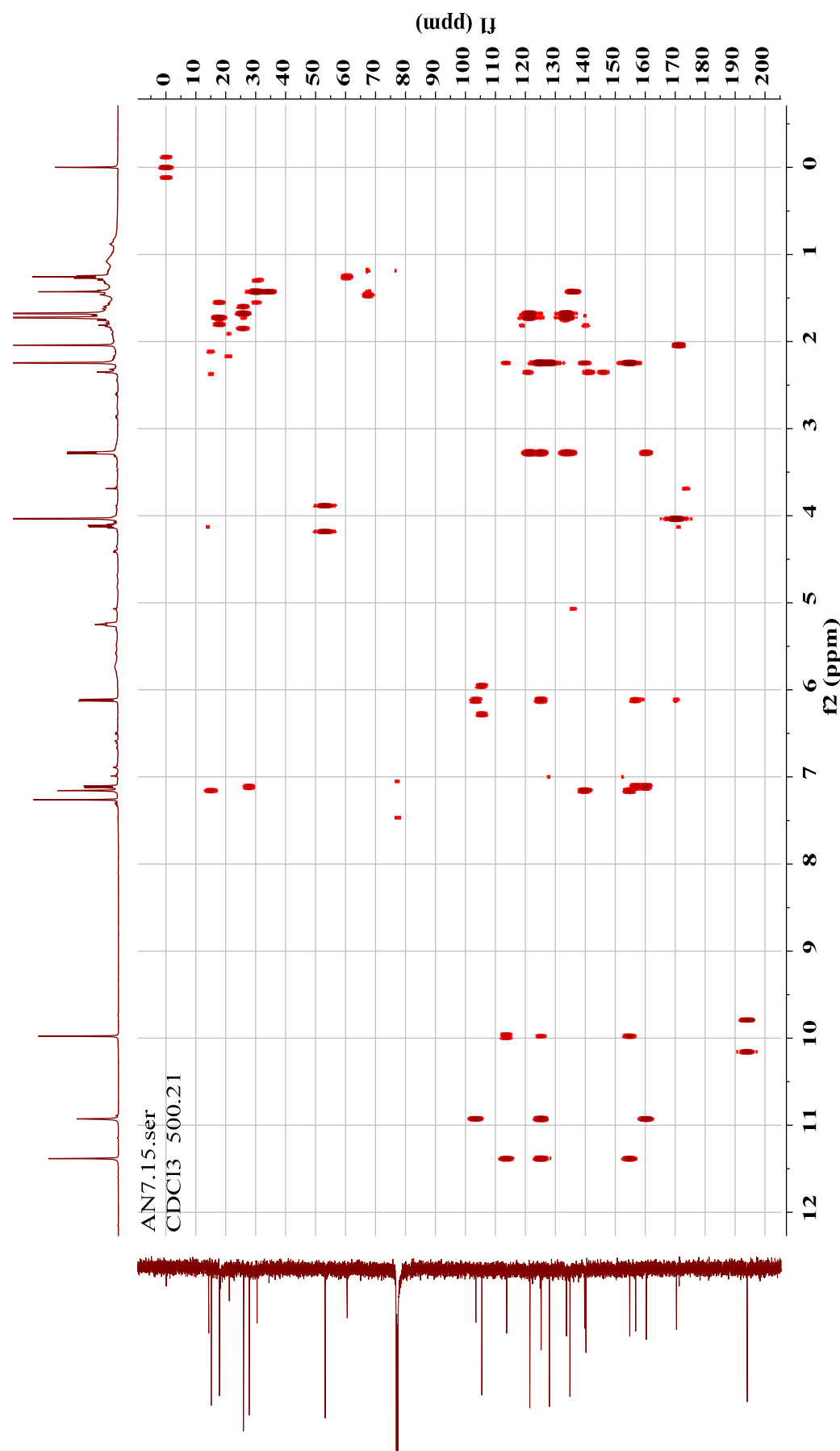


Fig. S5 HMBC spectrum of 4a-O-methoxyarugosin H (I) (CDCl<sub>3</sub>)

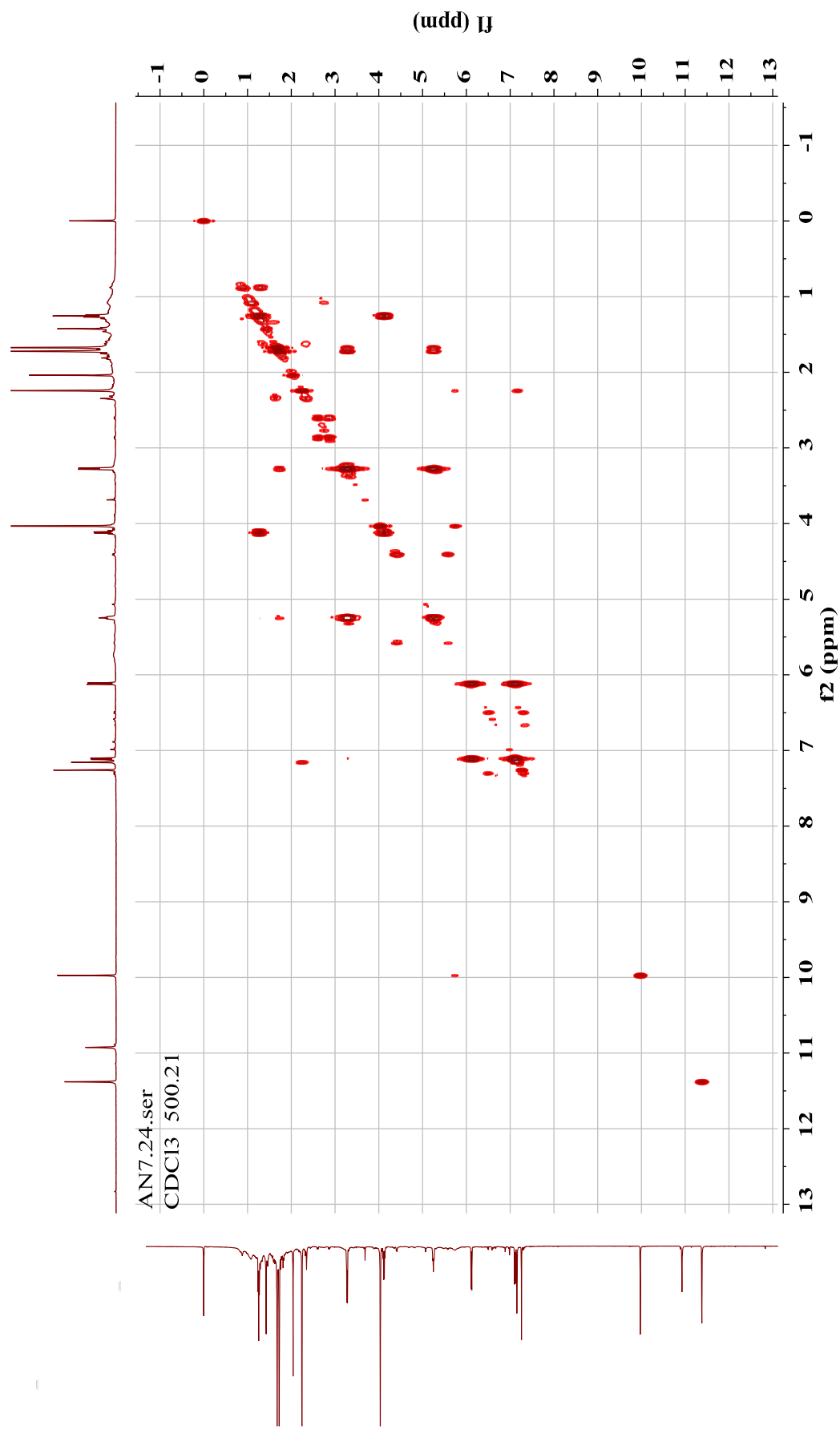


Fig. S6 <sup>1</sup>H-<sup>1</sup>H COSY spectrum of 4a-O-methoxyarugosin H (1) (CDCl<sub>3</sub>).

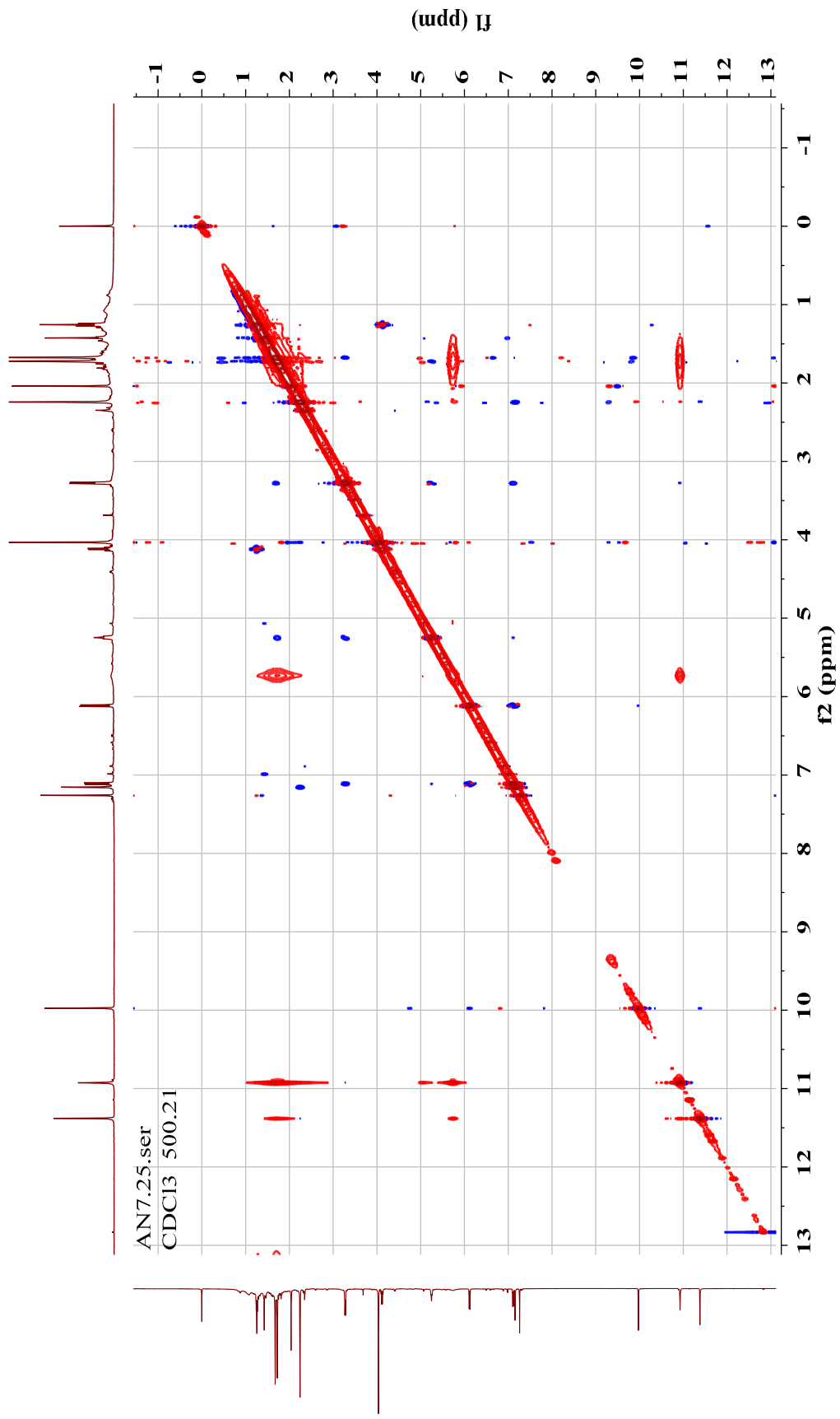


Fig. S7 NOESY spectrum of 4a-O-methoxyarugosin H (1) (CDCl<sub>3</sub>).

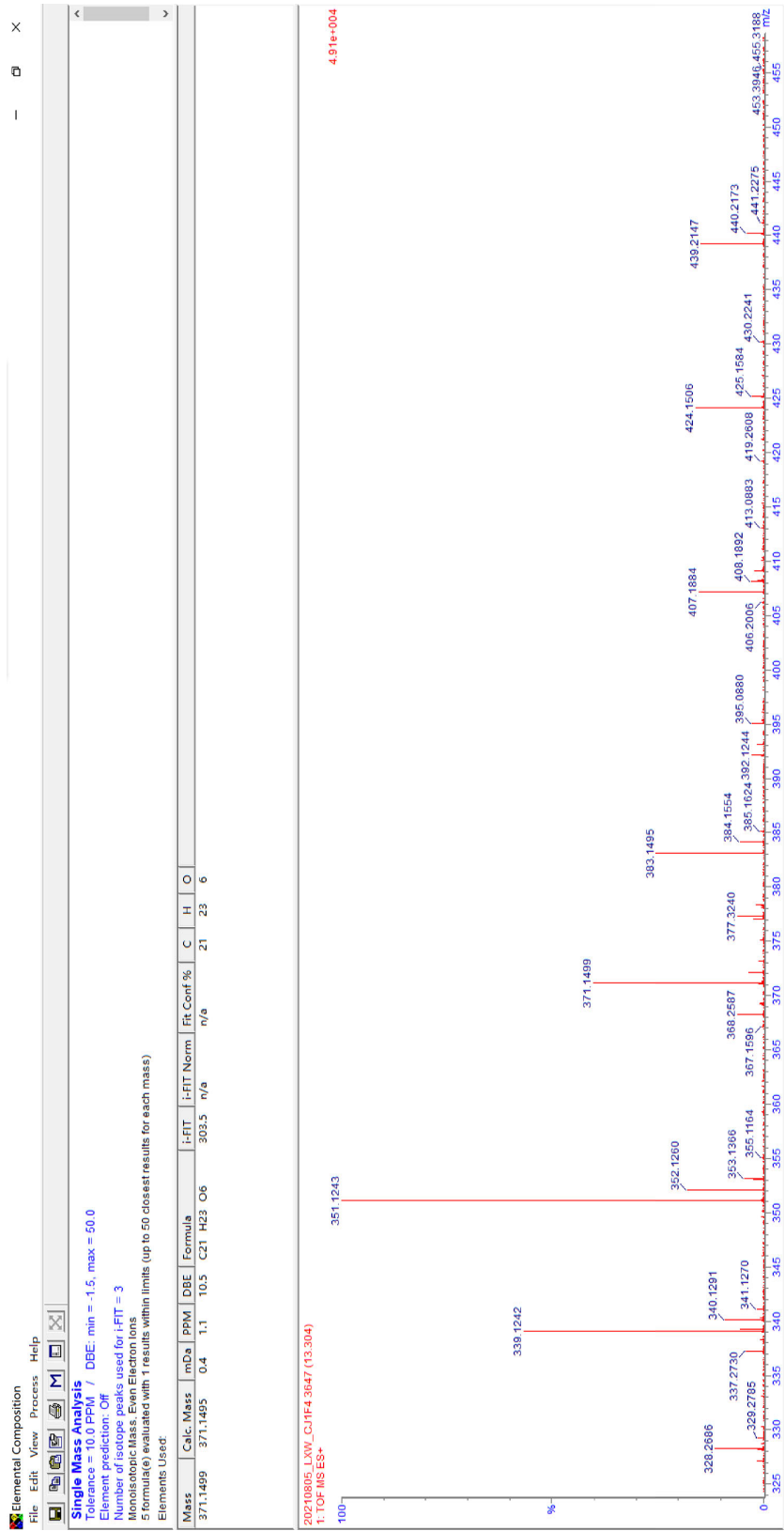
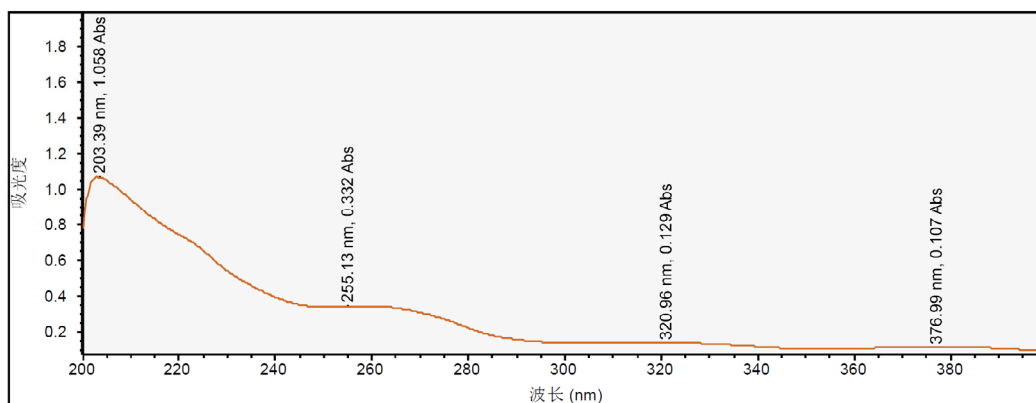


Fig. S8 HR-ESIMS spectrum of 4a-O-methoxyarugosin H (1).

# 样品ID	用户名	日期和时间
3 AN7	Evolution 350	2022/6/30 10:33:55

峰:

nm	Abs
203.394	1.058
255.129	0.332
320.963	0.129
376.989	0.107



**Fig. S9** UV spectrum of 4a-*O*-methoxyarugosin H (**1**).

### Physicochemical data of known compounds 2–11

Pre-shamixanthone (**2**): pale-yellow solid. ECD (0.2 mg/mL, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 207 (+13.6), 239 (+0.90), 279 (+3.18), 310 (−1.12) nm;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  7.20 (1H, d,  $J = 8.4$  Hz, H-3), 6.62 (1H, s, H-5), 6.36 (1H, d,  $J = 8.4$  Hz, H-2), 5.26 (1H, m, H-15), 5.03 (2H, br s, H<sub>2</sub>-21), 4.85 (1H, d,  $J = 2.6$  Hz, H-23a), 4.69 (1H, d,  $J = 2.6$  Hz, H-23b), 4.21 (1H, overlapped, H-19), 3.24 (2H, d,  $J = 7.3$  Hz, H<sub>2</sub>-14), 2.63 (1H, m, H-20), 2.16 (3H, s, H<sub>3</sub>-25), 1.83 (3H, s, H<sub>3</sub>-24), 1.73 (3H, s, H<sub>3</sub>-18), 1.69 (3H, s, H<sub>3</sub>-17);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  199.4 (qC, C-13), 158.9 (qC, C-10), 158.3 (qC, C-1), 147.2 (qC, C-11), 145.2 (qC, C-7), 141.4 (qC, C-22), 138.0 (CH, C-3), 133.3 (qC, C-16), 131.2 (qC, C-12), 125.9 (qC, C-6), 122.2 (CH, C-15), 121.4 (qC, C-8), 120.2 (qC, C-4), 119.7 (CH, C-5), 113.5 (CH<sub>2</sub>, C-23), 112.8 (qC, C-9), 63.6 (CH, C-19), 44.1 (CH, C-20), 27.5 (CH<sub>2</sub>, C-14), 25.9 (CH<sub>3</sub>, C-17), 23.1 (CH<sub>3</sub>, C-24), 17.9 (CH<sub>3</sub>, C-25), 16.4 (CH<sub>3</sub>, C-18). HR-ESIMS  $m/z$  423.1800 [ $\text{M} - \text{H}$ ]<sup>−</sup> (calcd for C<sub>25</sub>H<sub>27</sub>O<sub>6</sub>, 423.1808).

Cycloisoemicellin (**3**): yellow solid.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  7.23 (1H, d,  $J = 8.4$  Hz, H-3), 7.18 (1H, s, H-5), 6.85 (1H, d,  $J = 8.4$  Hz, H-4), 6.31 (1H, d,  $J = 9.8$  Hz, H-1'), 5.64 (1H,

d,  $J = 9.8$  Hz, H-2'), 5.62 (1H, m, H-2''), 5.03 (2H, s, H<sub>2</sub>-11), 5.00 (1H, s, 11-OH), 4.44 (2H, d,  $J = 7.2$  Hz, H<sub>2</sub>-1''), 2.41 (3H, s, 6-Me), 1.80 (1H, s, H<sub>3</sub>-4''), 1.71 (1H, s, H<sub>3</sub>-5''), 1.56 (3H, s, H<sub>3</sub>-4'), 1.56 (1H, s, H-5'); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_C$  179.3 (qC, C-9), 156.5 (qC, C-4a), 154.4 (qC, C-1), 152.9 (qC, C-10a), 152.4 (qC, C-7), 140.7 (qC, C-6), 138.8 (qC, C-3''), 134.5 (qC, C-8), 131.8 (CH, C-3), 129.5 (CH, C-2'), 121.7 (CH, C-1'), 120.7 (qC, C-8a), 120.0 (CH, C-2''), 119.0 (CH, C-5), 116.9 (qC, C-2), 112.9 (qC, C-9a), 108.3 (CH, C-4), 78.0 (qC, C-3'), 72.1 (CH<sub>2</sub>, C-1''), 57.3 (CH<sub>2</sub>, C-11), 28.1 (CH<sub>3</sub>, C-4'), 26.0 (CH<sub>3</sub>, C-5''), 18.2 (CH<sub>3</sub>, C-4''), 17.6 (CH<sub>3</sub>, 6-Me). HR-ESIMS  $m/z$  407.1855 [M + H]<sup>+</sup> (calcd for C<sub>25</sub>H<sub>27</sub>O<sub>5</sub>, 407.1858),

Sterigmatocystin (**4**): yellow oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_H$  13.20 (1H, s, 3-OH), 7.47 (1H, t,  $J = 8.3$  Hz, H-5), 6.80 (1H, d,  $J = 8.3$  Hz, H-4), 6.80 (1H, d,  $J = 7.2$  Hz, H-14), 6.79 (1H, d,  $J = 8.3$  Hz, H-6), 6.49 (1H, dd,  $J = 2.8, 2.1$  Hz, H-17), 6.41 (1H, s, H-11), 5.43 (1H, t,  $J = 2.6$  Hz, H-16), 4.77 (1H, dt,  $J = 7.1, 2.2$  Hz, H-15); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_C$  181.4 (qC, C-1), 164.7 (qC, C-10), 163.4 (qC, C-12), 162.4 (qC, C-3), 155.0 (qC, C-7), 154.0 (qC, C-8), 145.5 (CH, C-17), 135.8 (CH, C-5), 113.4 (CH, C-14), 111.3 (CH, C-4), 109.1 (qC, C-2), 106.6 (qC, C-9), 106.0 (CH, C-6), 106.0 (qC, C-13), 102.6 (CH, C-16), 90.6 (CH, C-11), 56.9 (CH<sub>3</sub>, C-18), 48.2 (CH, C-15). HR-ESIMS  $m/z$  325.0713 [M + H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>13</sub>O<sub>6</sub>, 325.0712), 347.0534 [M + Na]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>12</sub>O<sub>6</sub>Na, 347.0532).

Dihydrosterigmatocystin (**5**): pale-yellow amorphous powder. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_H$  13.3 (H, s, 3-OH), 7.49 (1H, t,  $J = 8.3$  Hz, H-5), 6.82 (1H, d,  $J = 8.3$  Hz, H-6), 6.75 (1H, d,  $J = 8.3$  Hz, H-4), 6.49 (1H, d,  $J = 5.7$  Hz, H-14), 6.36 (1H, s, H-11), 4.22 (1H, dd,  $J = 9.5, 5.7$  Hz, H-15), 4.18 (1H, m, H-17a), 4.00 (3H, s, H<sub>3</sub>-18), 3.69 (1H, td,  $J = 9.0, 6.6$  Hz, H-17b), 2.31 (2H, m, H<sub>2</sub>-16); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_C$  181.5 (qC, C-1), 166.3 (qC, C-10), 163.6 (qC, C-12), 162.5 (qC, C-3), 155.1 (qC, C-7), 154.6 (qC, C-8), 135.7 (CH, C-5), 113.6 (CH, C-14), 111.4 (CH, C-4), 109.1 (qC, C-2), 106.0 (CH, C-6), 106.0 (qC, C-9), 105.5 (qC, C-13), 89.9 (CH, C-11), 68.0 (CH<sub>2</sub>, C-17), 56.9 (CH<sub>3</sub>, C-18), 44.4 (CH, C-15), 31.6 (CH<sub>2</sub>, C-16). HR-ESIMS  $m/z$  327.0871 [M + H]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>15</sub>O<sub>6</sub>, 327.0869), 349.0689 [M + Na]<sup>+</sup> (calcd for C<sub>18</sub>H<sub>14</sub>O<sub>6</sub>Na, 349.0688).

Dehydromicroperfuranone (**6**): colorless crystal. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_H$  7.60 (2H, overlapped, H-8, 10), 7.50 (3H, overlapped, H-7, 9, 11), 7.30 (2H, overlapped, H-15, 17), 7.25 (1H, overlapped, H-16), 7.19 (2H, overlapped, H-14, 18), 4.02 (2H, s, H<sub>2</sub>-12); <sup>13</sup>C NMR (125

MHz, CDCl<sub>3</sub>):  $\delta_C$  166.0 (qC, C-2), 165.0 (qC, C-5), 141.3 (qC, C-3), 140.8 (qC, C-13), 135.6 (qC, C-4), 131.3 (CH, C-9), 129.5 (CH, C-14), 129.5 (CH, C-18), 129.2 (CH, C-8), 129.2 (CH, C-10), 129.2 (CH, C-15), 129.2 (CH, C-17), 128.6 (CH, C-7), 128.6 (CH, C-11), 127.5 (CH, C-16), 127.3 (qC, C-6), 30.6 (CH<sub>2</sub>, C-12). HR-ESIMS  $m/z$  265.0869 [M + H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>13</sub>O<sub>3</sub>, 265.0865).

Varioxiranol I (**7**): yellow gum. ECD (0.2 mg/mL, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 224 (+1.12), 252 (-1.33), 286 (+0.94) nm; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_H$  7.09 (1H, s, 6-OH), 6.92 (1H, d,  $J$  = 8.2 Hz, H-10), 6.74 (1H, s, H-6), 6.74 (1H, s, H-2), 6.25 (1H, d,  $J$  = 8.2 Hz, H-9), 5.24 (2H, t,  $J$  = 7.0 Hz, H-21, H-16), 4.22 (1H, dd,  $J$  = 10.9, 7.2 Hz, H-15b), 3.80 (1H, dd,  $J$  = 10.9, 7.2 Hz, H-15a), 3.25 (1H, d,  $J$  = 7.0 Hz, H<sub>2</sub>-20), 2.28 (3H, s, H<sub>3</sub>-25), 1.71 (3H, s, H<sub>3</sub>-18), 1.77 (3H, s, H<sub>3</sub>-23), 1.77 (3H, s, H<sub>3</sub>-24), 1.52 (3H, s, H<sub>3</sub>-19); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_C$  172.8 (qC, C-13), 154.9 (qC, C-12), 154.4 (qC, C-8), 151.9 (qC, C-1), 144.8 (qC, C-4), 141.5 (qC, C-3), 141.0 (qC, C-5), 138.2 (qC, C-17), 136.6 (qC, C-22), 131.4 (CH, C-10), 121.4 (CH, C-21), 120.2 (CH, C-16), 119.2 (qC, C-11), 118.0 (CH, C-2), 111.2 (qC, C-14), 109.3 (qC, C-7), 108.3 (CH, C-9), 75.2 (CH, C-6), 69.7 (CH<sub>2</sub>, C-15), 30.2 (CH<sub>2</sub>, C-20), 26.0 (CH<sub>3</sub>, C-18), 25.9 (CH<sub>3</sub>, C-23), 18.1 (CH<sub>3</sub>, C-19), 18.0 (CH<sub>3</sub>, C-24), 17.1 (CH<sub>3</sub>, C-25). HR-ESIMS 447.1800 [M + Na]<sup>+</sup> (calcd for C<sub>25</sub>H<sub>28</sub>O<sub>6</sub>Na, 447.1784).

Arugosin G (**8**): bright yellow solid. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_H$  13.43 (H, s, 1-OH), 10.98 (H, brs, 10a-OH), 7.26 (1H, s, H-3), 6.83 (1H, s, H-5), 6.62 (1H, s, 11-OH), 5.55 (1H, m, H-2''), 5.30 (1H, m, H-2'''), 5.23 (1H, d,  $J$  = 7.2 Hz, H-2'), 4.36 (2H, d,  $J$  = 7.3 Hz, H<sub>2</sub>-1''), 2.31 (3H, s, H<sub>3</sub>-6), 3.29 (1H, d,  $J$  = 6.9 Hz, H-1'''), 2.31 (2H, d,  $J$  = 6.9 Hz, H<sub>2</sub>-1'), 1.80 (3H, s, H<sub>3</sub>-4'''), 1.75 (3H, s, H<sub>3</sub>-5''), 1.78 (3H, s, H<sub>3</sub>-5'''), 1.71 (3H, brs, H<sub>3</sub>-4''), 1.70 (3H, brs, H<sub>3</sub>-5'), 1.66 (3H, s, H<sub>3</sub>-4'), 1.52 (3H, s, H<sub>3</sub>-19); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_C$  197.7 (qC, C-9), 162.0 (qC, C-1), 157.3 (qC, C-10a), 153.1 (qC, C-4a), 145.8 (qC, C-7), 141.1 (qC, C-6), 139.9 (CH, C-3), 137.5 (qC, C-3''), 132.9 (qC, C-3'), 132.9 (CH<sub>3</sub>, C-3'''), 131.1 (qC, C-8), 123.9 (qC, C-2), 123.9 (qC, C-4), 122.0 (CH, C-2'), 122.0 (CH, C-2'''), 120.8 (CH, C-5), 120.8 (CH, C-2''), 118.2 (qC, C-8a), 113.5 (qC, C-9a), 93.0 (CH, C-11), 71.9 (CH<sub>2</sub>, C-1''), 28.7 (CH<sub>2</sub>, C-1'), 27.8 (CH<sub>2</sub>, C-1'''), 25.9 (CH<sub>3</sub>, C-4'), 25.9 (CH<sub>3</sub>, C-4''), 25.9 (CH<sub>3</sub>, C-4'''), 18.1 (CH<sub>3</sub>, C-5''), 17.8 (CH<sub>3</sub>, C-5'), 17.8 (CH<sub>3</sub>, C-5'''), 17.0 (CH<sub>3</sub>, 6-Me). HR-ESIMS  $m/z$  515.2412 [M + Na]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>36</sub>O<sub>6</sub>Na, 515.2410), 531.2144 [M + K]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>36</sub>O<sub>6</sub>K, 531.2149).

Arugosin C (**9**), yellow amorphous powder. ECD (0.2 mg/mL, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 207 (+46.1),



253 (+5.97), 284 (-0.14), 316 (+13.1) nm;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  13.87 (H, s, 12-OH), 10.70 (H, s, 3-OH), 7.27 (1H, d,  $J = 8.3$  Hz, H-10), 6.81 (1H, s, H-4), 6.40 (1H, d,  $J = 8.3$  Hz, H-9), 5.32 (1H, d,  $J = 7.3$  Hz, H-15), 5.08 (1H, d,  $J = 4.6$  Hz, H-25), 4.37 (1H, dd,  $J = 11.6, 3.8$  Hz, H-19a), 4.19 (1H, dd,  $J = 11.5, 7.0$  Hz, H-19b), 3.31 (2H, d,  $J = 7.3$  Hz, H<sub>2</sub>-14), 2.37 (1H, dt,  $J = 8.0, 4.2$  Hz, H-20), 2.23 (3H, s, H<sub>3</sub>-24), 1.76 (3H, s, H<sub>3</sub>-17), 1.72 (3H, s, H<sub>3</sub>-18), 1.32 (3H, s, H<sub>3</sub>-22), 1.26 (3H, s, H<sub>3</sub>-23);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  197.3 (qC, C-1), 163.6 (qC, C-12), 159.2 (qC, C-8), 155.8 (qC, C-3), 145.4 (qC, C-6), 137.6 (CH, C-10), 136.6 (qC, C-5), 133.4 (qC, C-16), 124.2 (qC, C-11), 122.0 (CH, C-15), 120.7 (qC, C-7), 120.3 (CH, C-4), 119.8 (qC, C-2), 112.7 (qC, C-13), 109.0 (CH, C-9), 74.3 (CH, C-25), 71.3 (qC, C-21), 65.3 (CH<sub>2</sub>, C-19), 49.5 (CH, C-20), 29.1 (CH<sub>3</sub>, C-22), 28.4 (CH<sub>3</sub>, C-23), 27.8 (CH<sub>2</sub>, C-14), 26.0 (CH<sub>3</sub>, C-17), 17.9 (CH<sub>3</sub>, C-18), 16.7 (CH<sub>3</sub>, C-24). HR-ESIMS  $m/z$  425.1970 [ $\text{M} + \text{H}$ ]<sup>+</sup> (calcd for C<sub>25</sub>H<sub>29</sub>O<sub>6</sub>, 425.1964).

Emestrin J (**10**): white powder. ECD (0.3 mg/mL, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ) 204 (+35.5), 233 (-60.2), 264 (+14.6) nm;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  7.72 (1H, d,  $J = 8.5, 2.0$  Hz, H-6"), 7.68 (1H, d,  $J = 2.1$  Hz, H-2"), 7.23 (2H, d,  $J = 7.4$  Hz, H-2', 6'), 7.17 (1H, t,  $J = 7.4$  Hz, H-4'), 7.08 (2H, t,  $J = 7.4$  Hz, H-3', 5'), 6.89 (1H, d,  $J = 8.5$  Hz, H-5"), 6.67 (1H, d,  $J = 2.0$  Hz, H-10), 6.32 (1H, dd,  $J = 8.5, 2.0$  Hz, H-8), 5.85 (1H, dt,  $J = 8.5, 1.6$  Hz, H-6), 5.81 (1H, br s, 3"-OH), 5.34 (1H, dd,  $J = 8.7, 1.8$  Hz, H-5a), 4.69 (1H, dd,  $J = 8.5, 1.6$  Hz, H-7), 4.12 (1H, d,  $J = 18.4$  Hz, H-7'a), 3.96 (3H, s, 4"-OCH<sub>3</sub>), 3.67 (2H, s, H<sub>2</sub>-11), 3.13 (1H, dt,  $J = 18.4, 1.7$  Hz, H-7'b), 2.99 (3H, s, 2-NCH<sub>3</sub>);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  166.4 (qC, C-7"), 165.5 (qC, C-1), 162.1 (qC, C-4), 150.8 (CH, C-4"), 145.3 (qC, C-3"), 141.2 (CH, C-8), 139.4 (CH, C-10), 134.0 (qC, C-1'), 130.0 (CH, C-2', 6'), 128.5 (CH, C-3', 5'), 127.5 (CH<sub>2</sub>, C-4'), 123.8 (CH, C-6"), 123.1 (qC, C-1"), 116.5 (qC, C-2"), 113.6 (qC, C-10a), 109.9 (CH, C-5"), 106.0 (CH, C-7), 78.8 (qC, C-3), 73.5 (qC, C-11a), 70.7 (CH, C-6), 63.1 (CH, C-5a), 36.6 (CH<sub>2</sub>, C-11), 35.6 (CH<sub>2</sub>, C-7'), 29.5 (CH<sub>3</sub>, C-2), 29.5 (CH<sub>3</sub>, 4"-OCH<sub>3</sub>). HR-ESIMS  $m/z$  575.0920 [ $\text{M} + \text{Na}$ ]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>24</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub>Na, 575.0923).

Farnesylenefuranone D (**11**): colorless oil.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  7.1 (1H, s, H-7), 5.47 (1H, t,  $J = 7.0$  Hz, H-3'), 5.25 (2H, s, H<sub>2</sub>-3), 5.08 (2H, t,  $J = 7.0$  Hz, H-7', H-11'), 4.71 (2H, d,  $J = 7.0$  Hz, H<sub>2</sub>-2'), 3.96 (3H, s, 6-OMe), 2.03-2.10 (6H, m, H<sub>2</sub>-5', 6', 10'), 1.96 (2H, d,  $J = 7.9$  Hz, H<sub>2</sub>-9'), 1.68 (3H, s, H<sub>3</sub>-13'), 1.68 (3H, s, H<sub>3</sub>-16'), 1.60 (3H, s, H<sub>3</sub>-14'), 1.59 (3H, s, H<sub>3</sub>-15');  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  171.5 (qC, C-1), 149.5 (qC, C-6), 143.6 (qC, C-4'), 143.5 (qC, C-4),

139.5 (qC, C-5), 135.7 (qC, C-8'), 132.9 (qC, C-12'), 124.4 (qC, C-3a), 123.6 (CH, C-7'), 123.6 (CH, C-11'), 119.3 (qC, C-7a), 119.3 (CH, C-3'), 102.0 (CH, C-7), 69 (CH<sub>2</sub>, C-2'), 68 (CH<sub>2</sub>, C-3), 56.8 (CH<sub>3</sub>, 6'-OMe), 39.8 (CH<sub>2</sub>, C-5'), 39.7 (CH<sub>2</sub>, C-9'), 26.8 (CH<sub>2</sub>, C-6'), 26.3 (CH<sub>2</sub>, C-10'), 25.8 (CH<sub>3</sub>, C-13'), 17.8 (CH<sub>3</sub>, C-14'), 16.6 (CH<sub>3</sub>, C-16'), 16.1 (CH<sub>3</sub>, C-15'). HR-ESIMS *m/z* 401.2328 [M + H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>33</sub>O<sub>5</sub>, 401.2328), 423.2147 [M + Na]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>32</sub>O<sub>5</sub>Na, 423.2147).

**The 18S rDNA sequence of *Emericella nidulans* GXIMD 02509**

>Seq1

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