

## Supporting Information

*Rec. Nat. Prod.* X:X (202X) XX-XX

### A New Indole Glucoside and Other Constituents from the Sea Cucumber-Derived *Aspergillus fumigatus* M580 and Their Biological Activities

Cao Duc Tuan<sup>1\*</sup>, Nguyen Van Hung<sup>1\*</sup>, Le Thi Hong Minh<sup>2</sup>, Hoang Thi Hong Lien<sup>3</sup>, Jung-Woo Chae<sup>4</sup>, Hwi-yeol Yun<sup>4</sup>, Young-Ho Kim<sup>4</sup>, Pham Van Cuong<sup>2</sup>, and Doan Thi Mai Huong<sup>2</sup>

<sup>1</sup>*Hai Phong University of Medicine and Pharmacy, 72A Nguyen Binh Khiem, Haiphong, Vietnam*

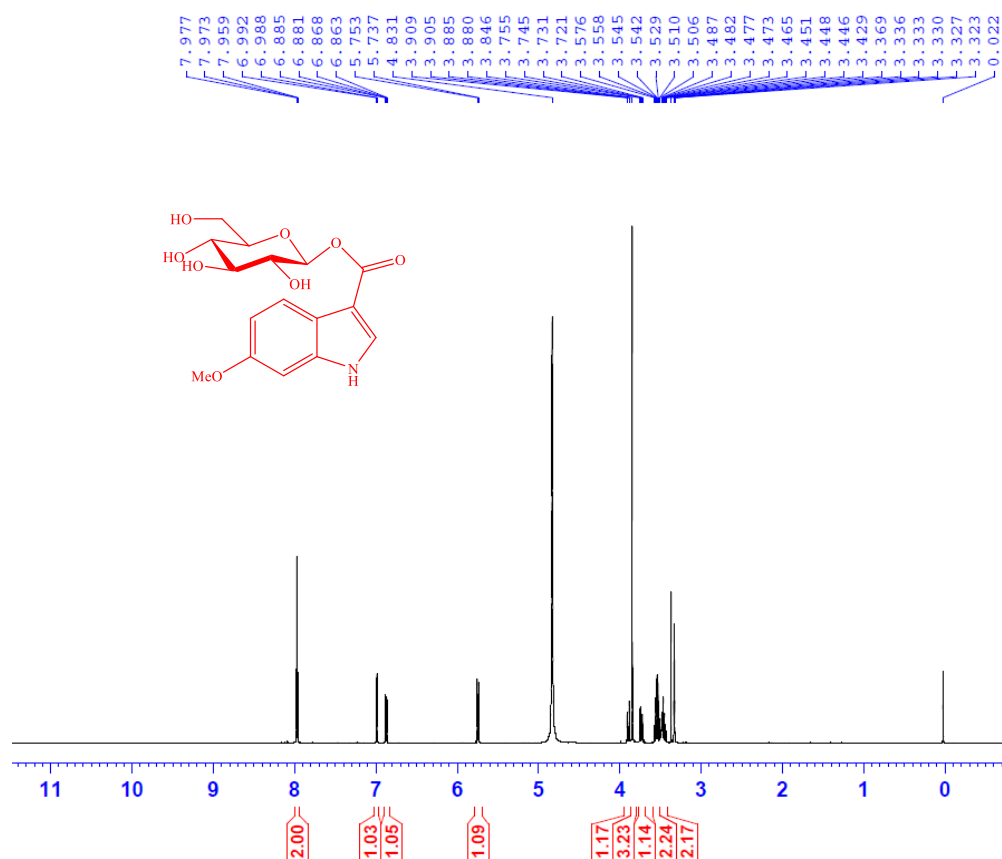
<sup>2</sup>*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

<sup>3</sup>*Buon Ma Thuot University, 298 Ha Huy Tap, Buon Ma Thuot, Daklak, Vietnam*

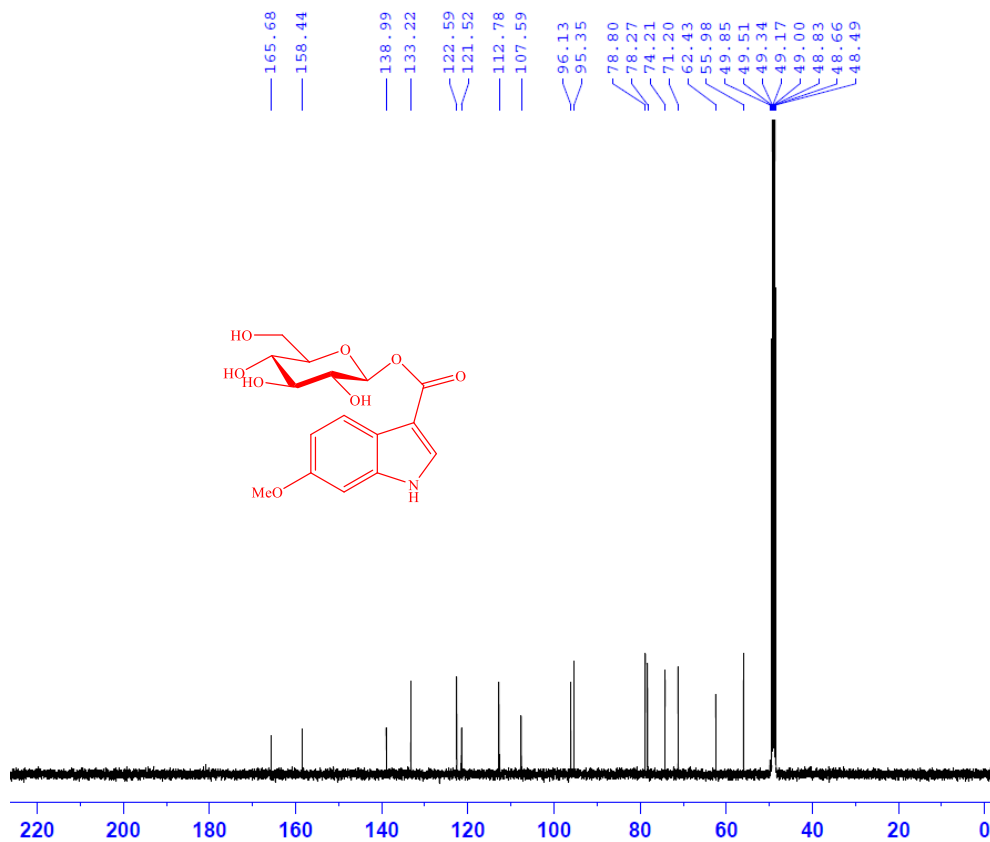
<sup>4</sup>*College of Pharmacy, Chungnam National University, Daejeon, Republic of Korea*

Table of Contents	Page
Figure S1: <sup>1</sup> H-NMR spectrum of compound 1	3
Figure S2: <sup>13</sup> C-NMR spectrum of compound 1	3
Figure S3: HSQC spectrum of compound 1	4
Figure S4: HMBC spectrum of compound 1	4
Figure S5: COSY spectrum of compound 1	5
Figure S6: <sup>1</sup> H-NMR spectrum of compound 2	5
Figure S7: <sup>13</sup> C-NMR spectrum of compound 2	6
Figure S8: <sup>1</sup> H-NMR spectrum of compound 3	6
Figure S9: <sup>13</sup> C-NMR spectrum of compound 3	7
Figure S10: <sup>1</sup> H-NMR spectrum of compound 4	7
Figure S11: <sup>13</sup> C-NMR spectrum of compound 4	8
Figure S12: <sup>1</sup> H-NMR spectrum of compound 5	8
Figure S13: <sup>13</sup> C-NMR spectrum of compound 5	9
Figure S14: <sup>1</sup> H-NMR spectrum of compound 6	9
Figure S15: <sup>13</sup> C-NMR spectrum of compound 6	10
Figure S16: <sup>1</sup> H-NMR spectrum of compound 7	10
Figure S17: <sup>13</sup> C-NMR spectrum of compound 7	11
Figure S18: <sup>1</sup> H-NMR spectrum of compound 8	11
Figure S19: <sup>13</sup> C-NMR spectrum of compound 8	12
Figure S20: <sup>1</sup> H-NMR spectrum of compound 9	12

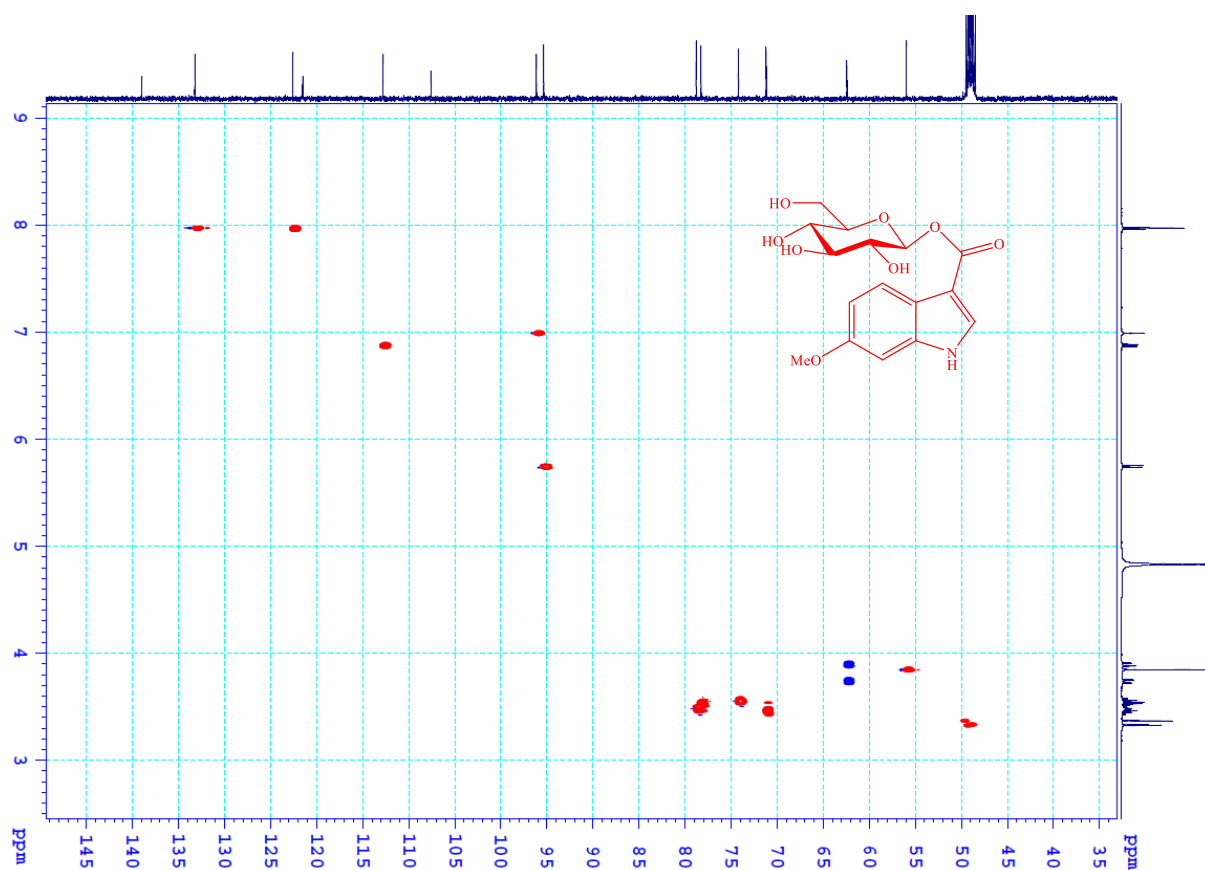
<b>Figure S21:</b> <sup>13</sup> C-NMR spectrum of compound <b>9</b>	13
<b>Figure S22:</b> <sup>1</sup> H-NMR spectrum of compound <b>10</b>	13
<b>Figure S23:</b> <sup>13</sup> C-NMR spectrum of compound <b>10</b>	14
<b>Figure S24:</b> <sup>1</sup> H-NMR spectrum of compound <b>11</b>	14
<b>Figure S25:</b> <sup>13</sup> C-NMR spectrum of compound <b>11</b>	15
<b>Figure S26:</b> $\alpha$ -Glucosidase inhibitory activity of compounds <b>1–10</b> .	15
<b>Figure S27:</b> Photos of collected sea cucumber	16
<b>Figure S28:</b> Figure: Popular bond types of the collected sea cucumber (10X)	17
<b>Figure S29:</b> Bowls and ellipsoids bonds of collected sea cucumber (20X)	17
<b>Figure S30:</b> Figure: Ellipsoids, Rosettes and Rods bonds of collected sea cucumber (20X)	18
<b>Figure S31:</b> The results of SciFinder searching for new compound	18
<b>Table S1:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>2</b> and Fumiquinazoline D	19
<b>Table S2:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>3</b> and Fumiquinazoline C	19
<b>Table S3:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>4</b> and Fumiquinazoline J	20
<b>Table S4:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>5</b> and bisdethiobis(methylthio)gliotoxin	20
<b>Table S5:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>6</b> and cyclo(L-prolyl-L-tryptophane)	21
<b>Table S6:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>7</b> and tryprostatin B	21
<b>Table S7:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>8</b> and 12,13-dihydroxy-fumitremorgin C	22
<b>Table S8:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>9</b> and 6-methoxyspirotryprostatin B	22
<b>Table S9:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>10</b> and cyclo(L-prolinyl-L-phenylalanine)	23
<b>Table S10:</b> <sup>1</sup> H and <sup>13</sup> C-NMR data of compound <b>11</b> and cyclo(L-prolinyl-L-valine)	23
<b>Table S11:</b> Cytotoxic activity of compounds <b>1-11</b>	23
<b>S1.</b> Sea Cucumber Identification	24
<b>S2.</b> <i>Aspegillus fumigatus</i> M580 isolation and characterization: Method and Data	24
Colony morphological characteristics of the strain M580 grown on PDA medium for 10 days at 28°C	24
<b>S3.</b> Fungal identification	25
<b>S4.</b> M580 strain's 18S rRNA gene sequence comparison results on GenBank	25
<b>S5.</b> The 18S rRNA gene sequence of strain M580 is displayed on GenBank	26
<b>S6.</b> Physical properties of compounds <b>2-10</b>	26
<b>References</b>	27



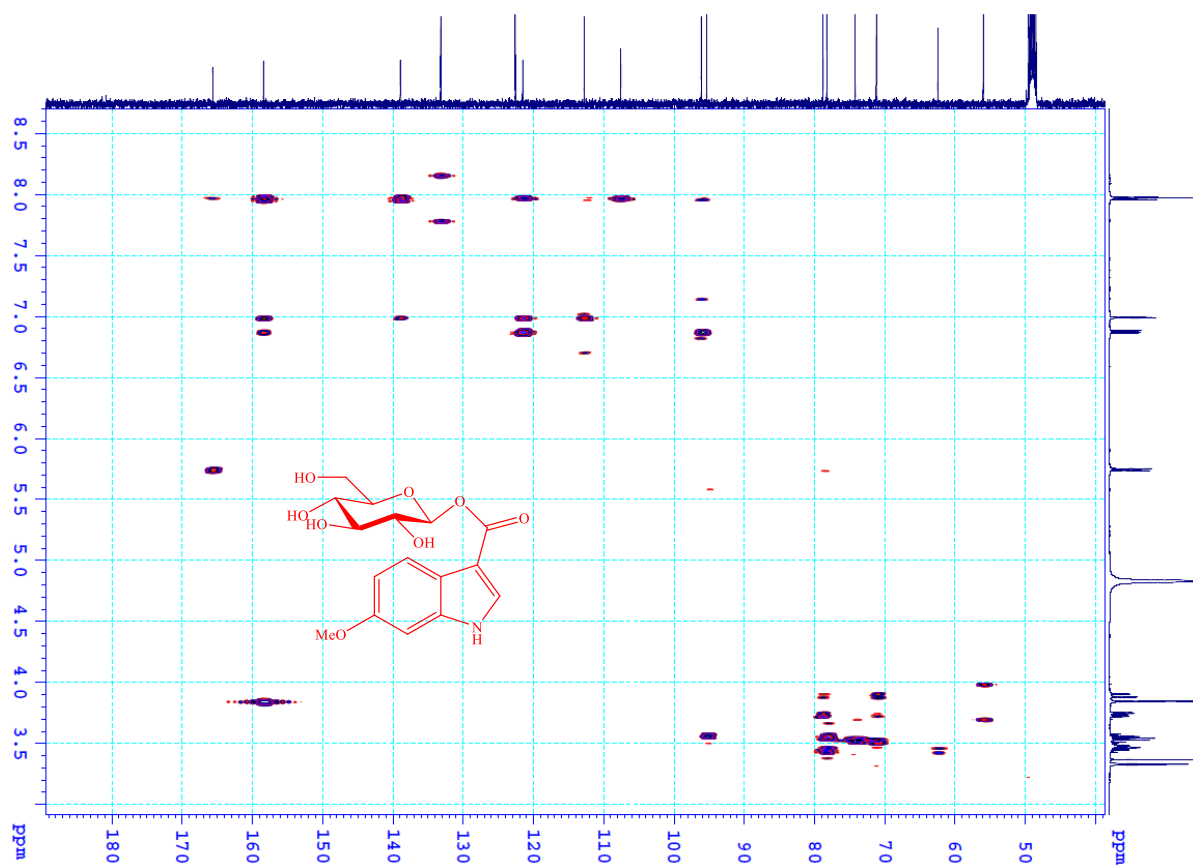
**Figure S1:** <sup>1</sup>H-NMR spectrum of compound 1



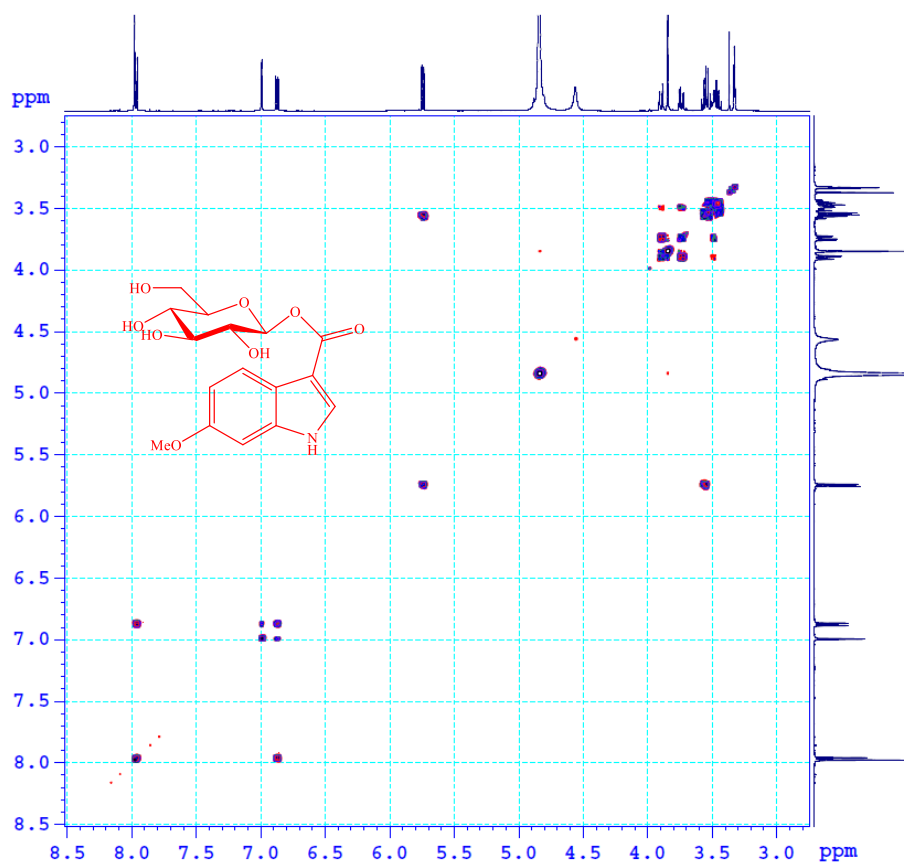
**Figure S2:** <sup>13</sup>C-NMR spectrum of compound 1



**Figure S3:** HSQC spectrum of compound 1

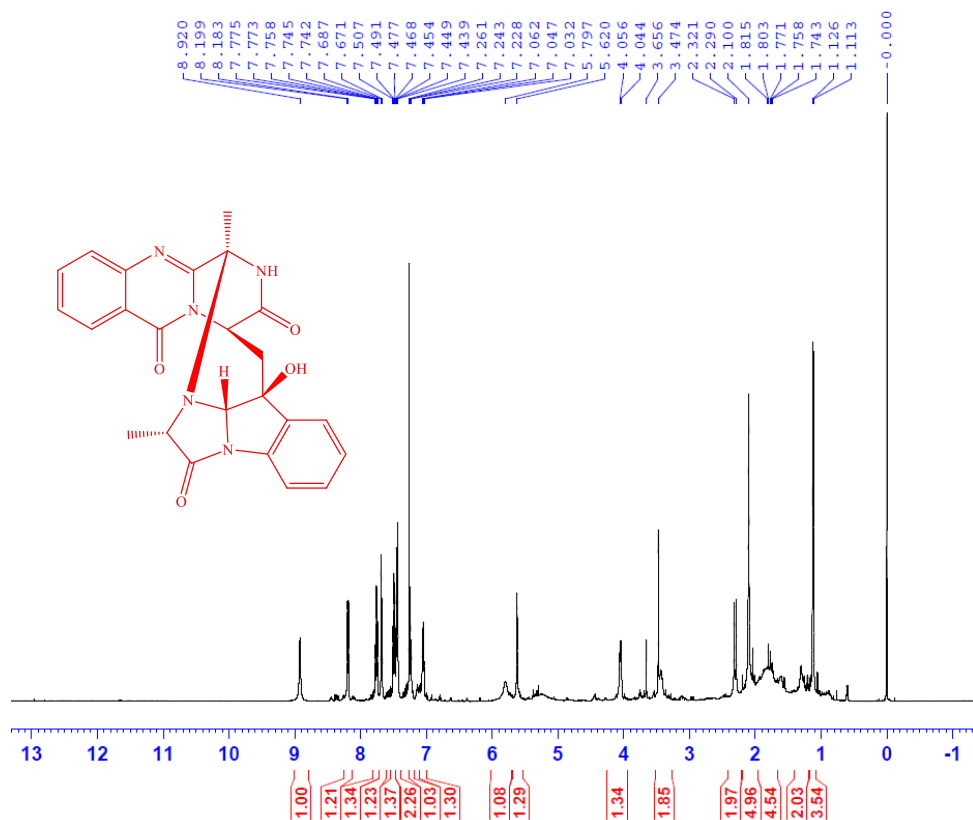


**Figure S4:** HMBC spectrum of compound 1



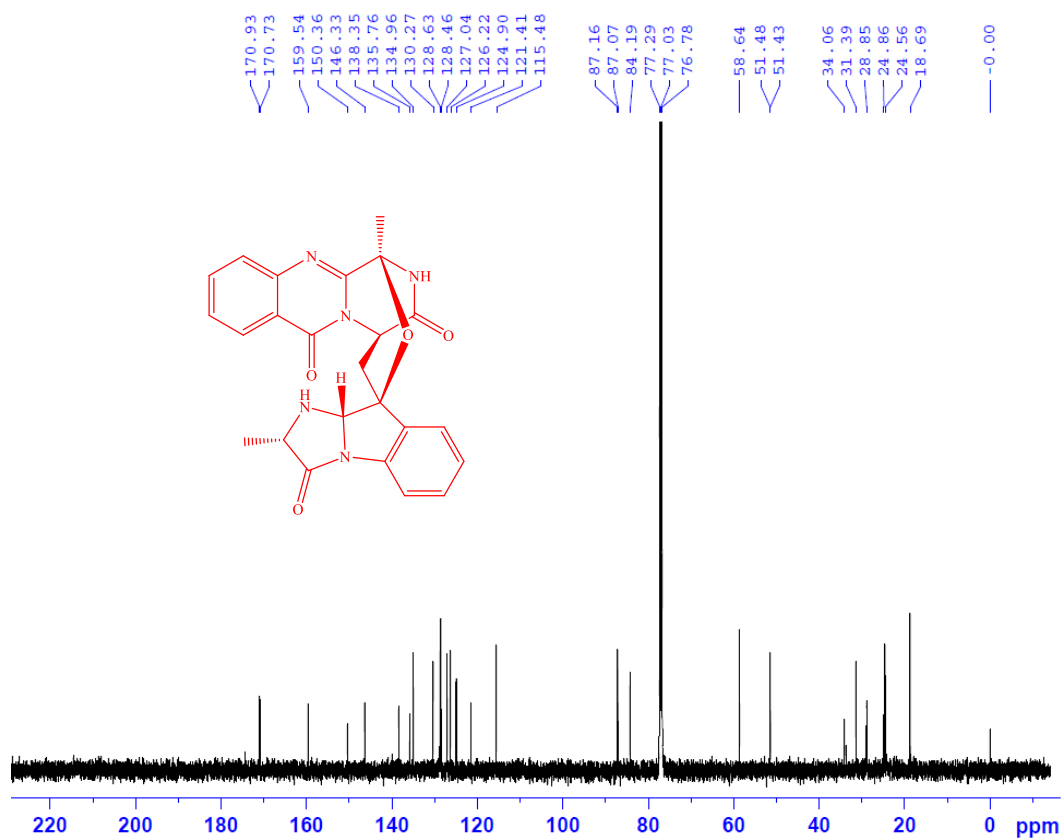
**Figure S5:** COSY spectrum of compound 1

CMR3C3 - CDC13 - 1H



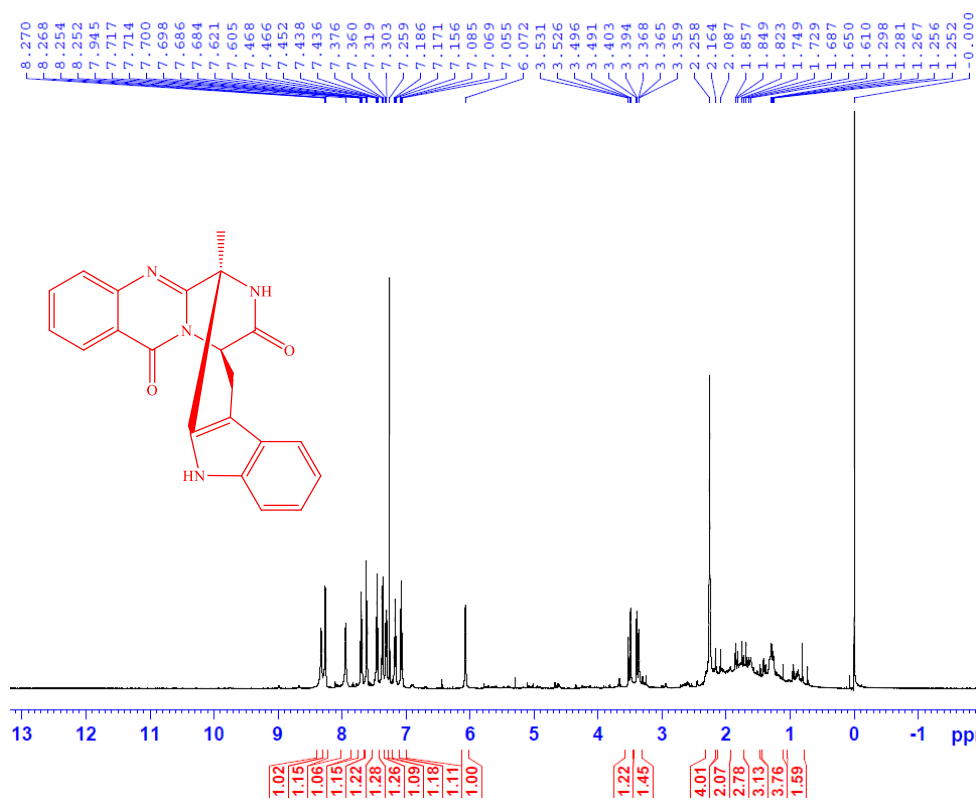
**Figure S6:**  $^1\text{H}$ -NMR spectrum of compound 2



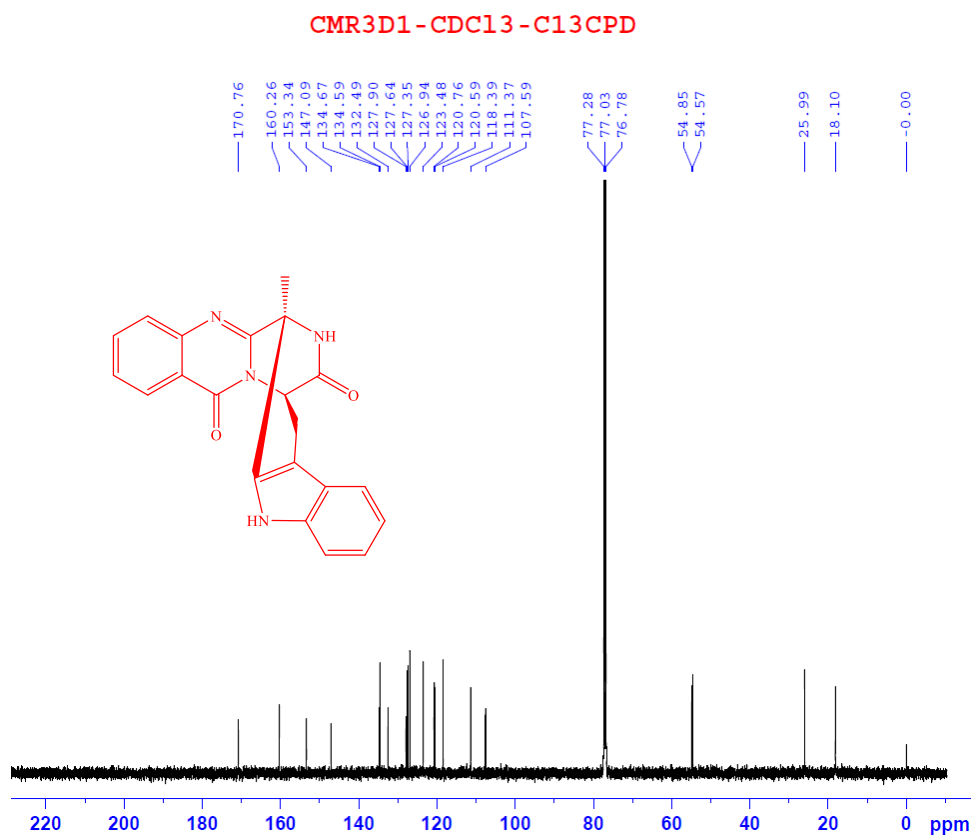


**Figure S9:** <sup>13</sup>C-NMR spectrum of compound 3

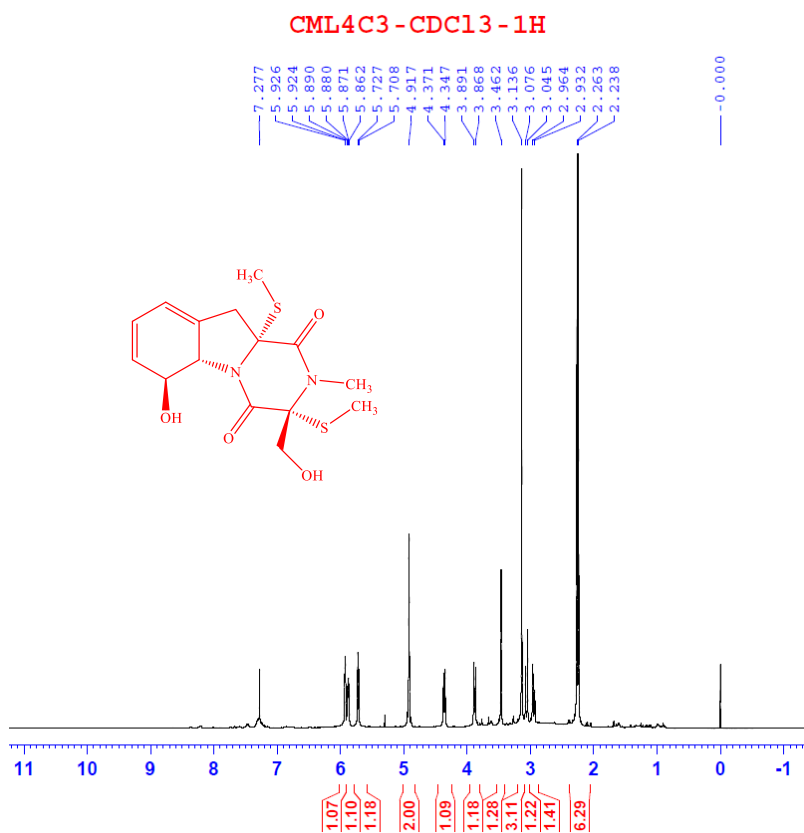
CMR3D1-CDCl<sub>3</sub>-1H



**Figure S10:** <sup>1</sup>H-NMR spectrum of compound 4



**Figure S11:** <sup>13</sup>C-NMR spectrum of compound 4



**Figure S12:** <sup>1</sup>H-NMR spectrum of compound 5

CML4C3-CDC13-C13CPD

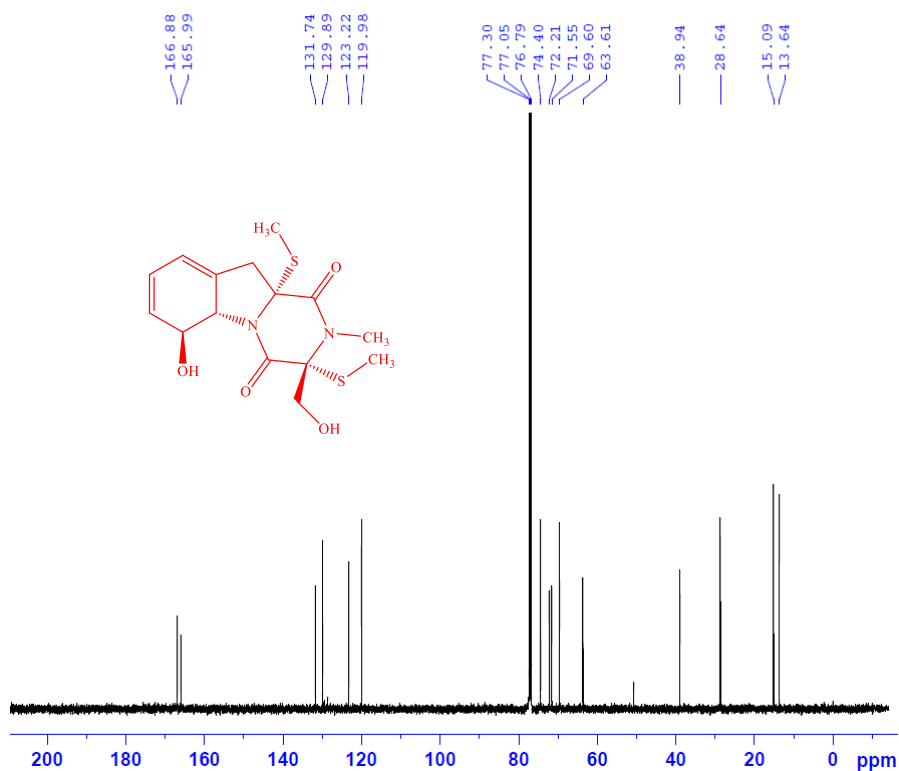


Figure S13: <sup>13</sup>C-NMR spectrum of compound 5

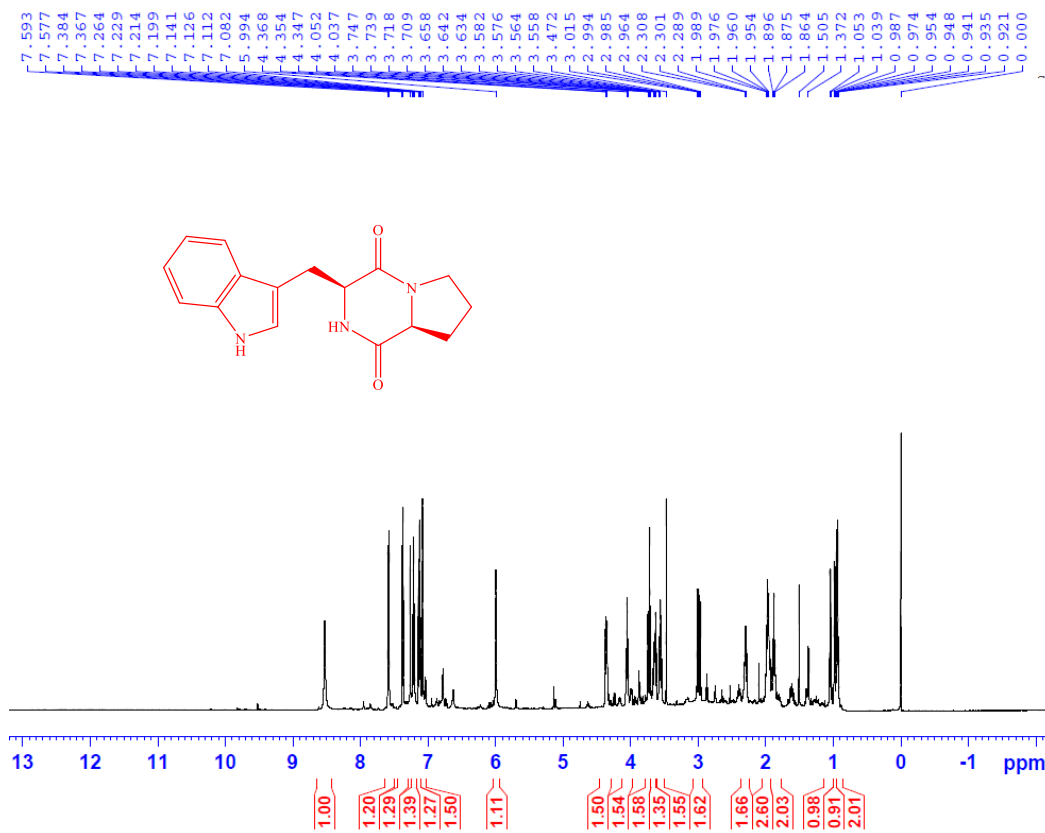


Figure S14: <sup>1</sup>H-NMR spectrum of compound 6

CML4B4 - CDCl<sub>3</sub> - C13CPD

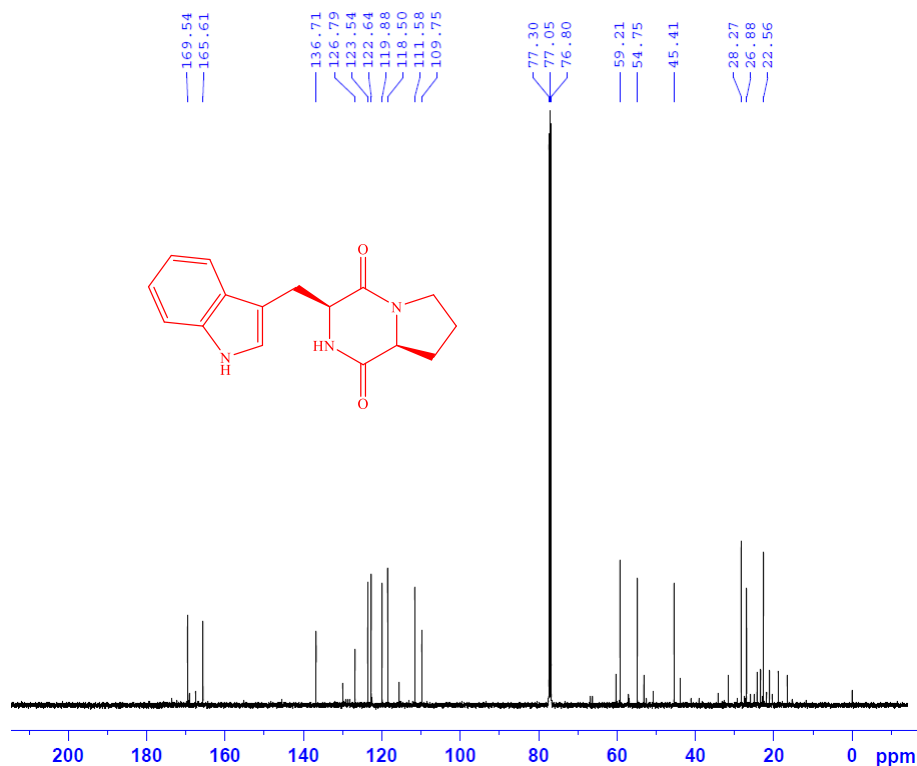


Figure S15: <sup>13</sup>C-NMR spectrum of compound 6

CML4E4A - CDCl<sub>3</sub> - 1H

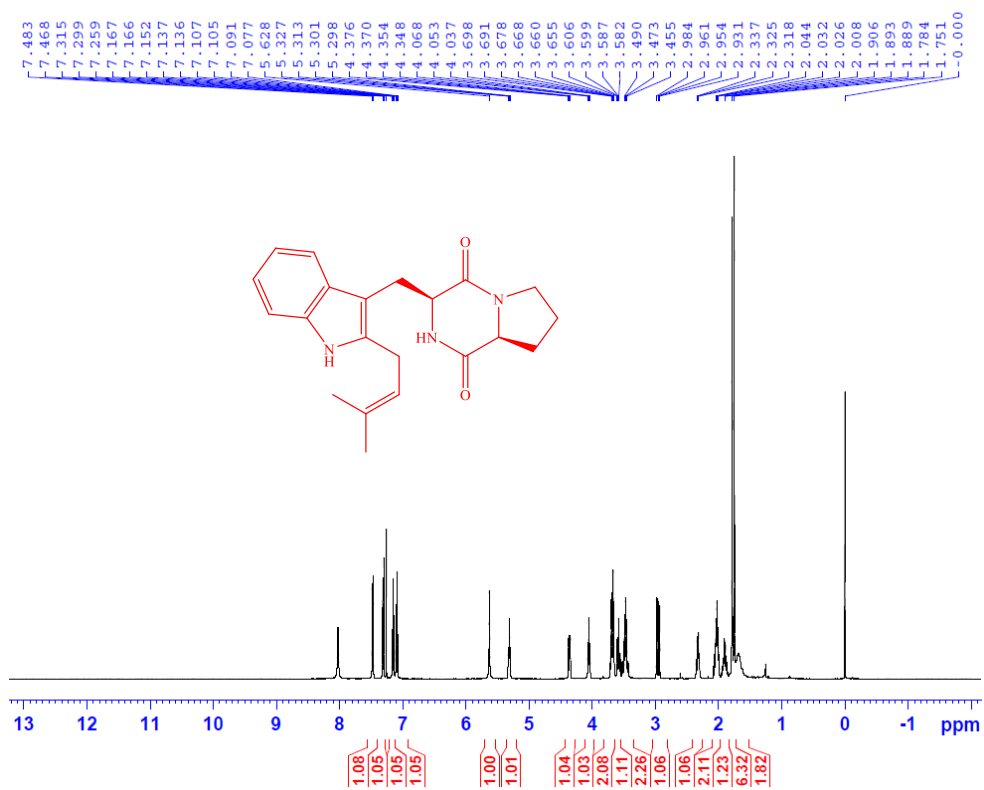


Figure S16: <sup>1</sup>H-NMR spectrum of compound 7

CML4E4A-CDC13-C13CPD

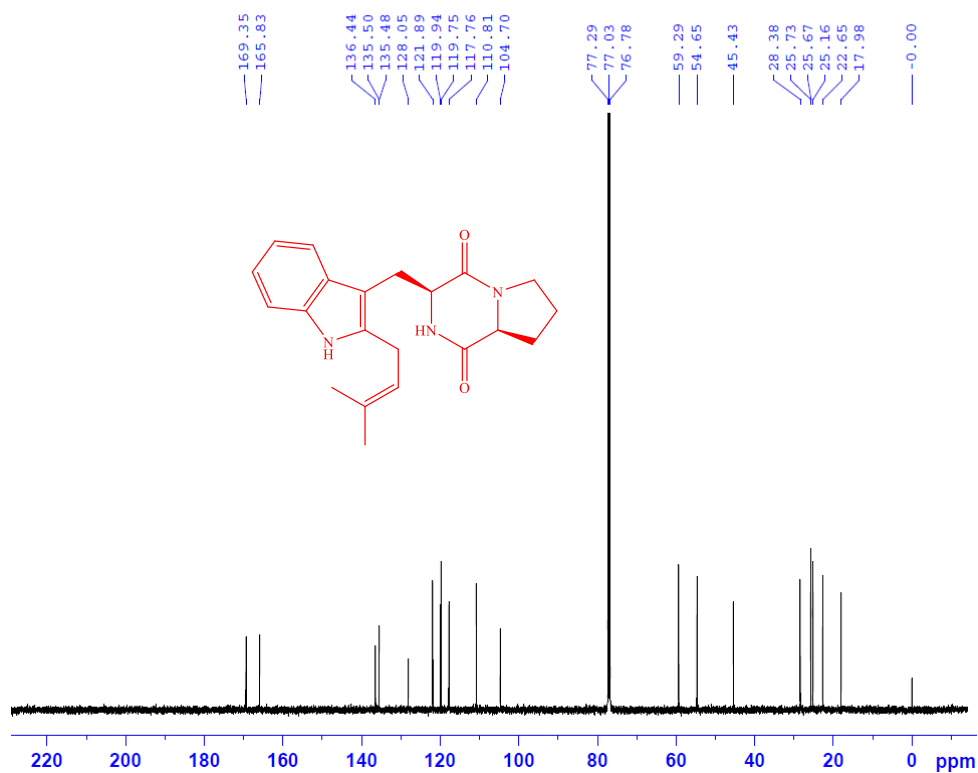


Figure S17: <sup>13</sup>C-NMR spectrum of compound 7

CML4E3-CDC13-1H

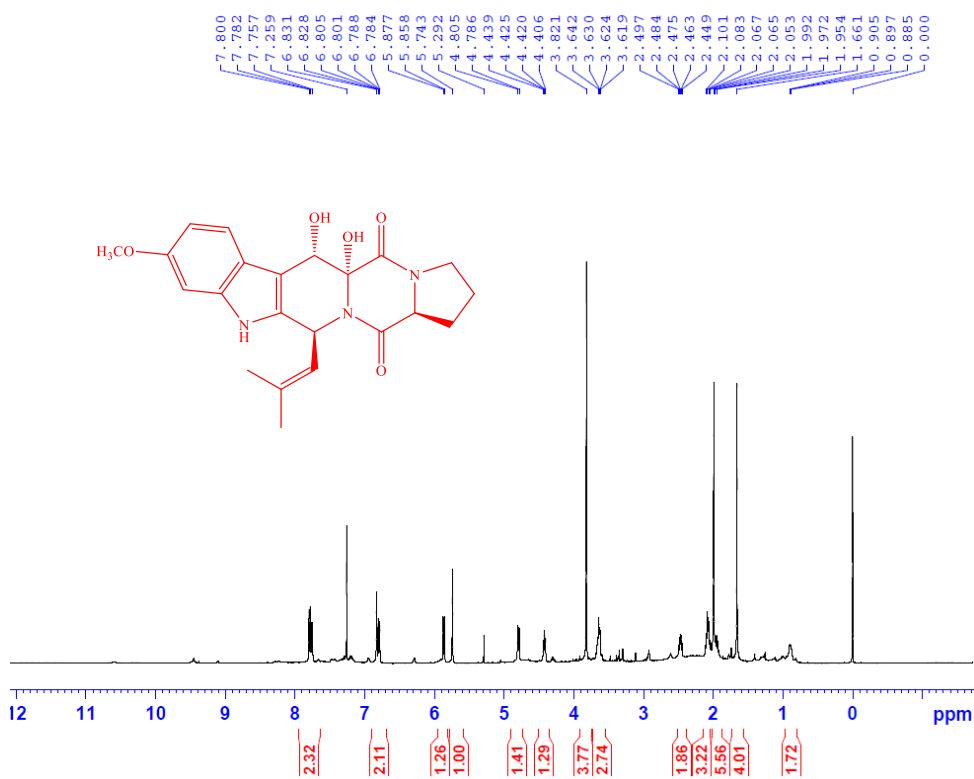


Figure S18: <sup>1</sup>H-NMR spectrum of compound 8

CML4E3-CDC13-C13CPD

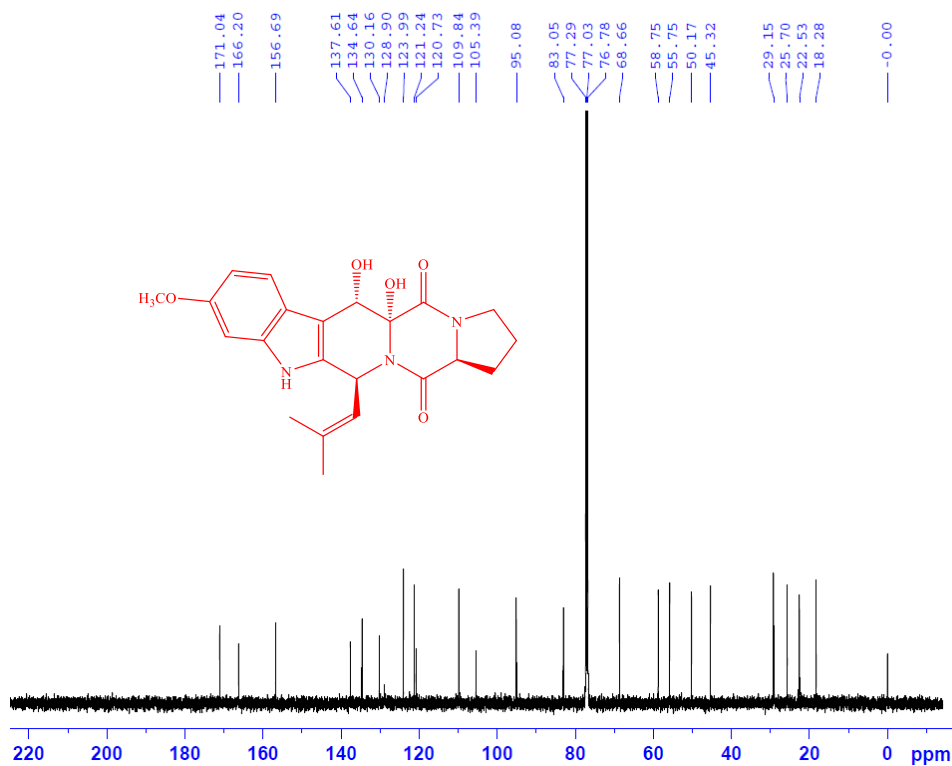


Figure S19:  $^{13}\text{C}$ -NMR spectrum of compound 8

CML4C2B-CDC13-1H

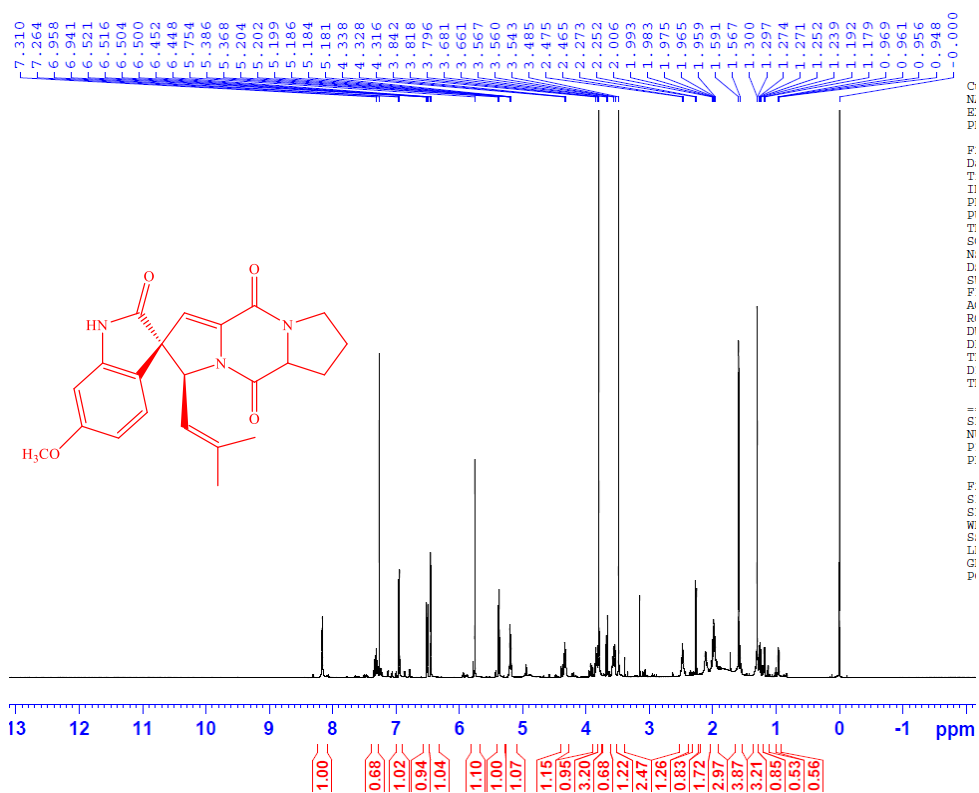
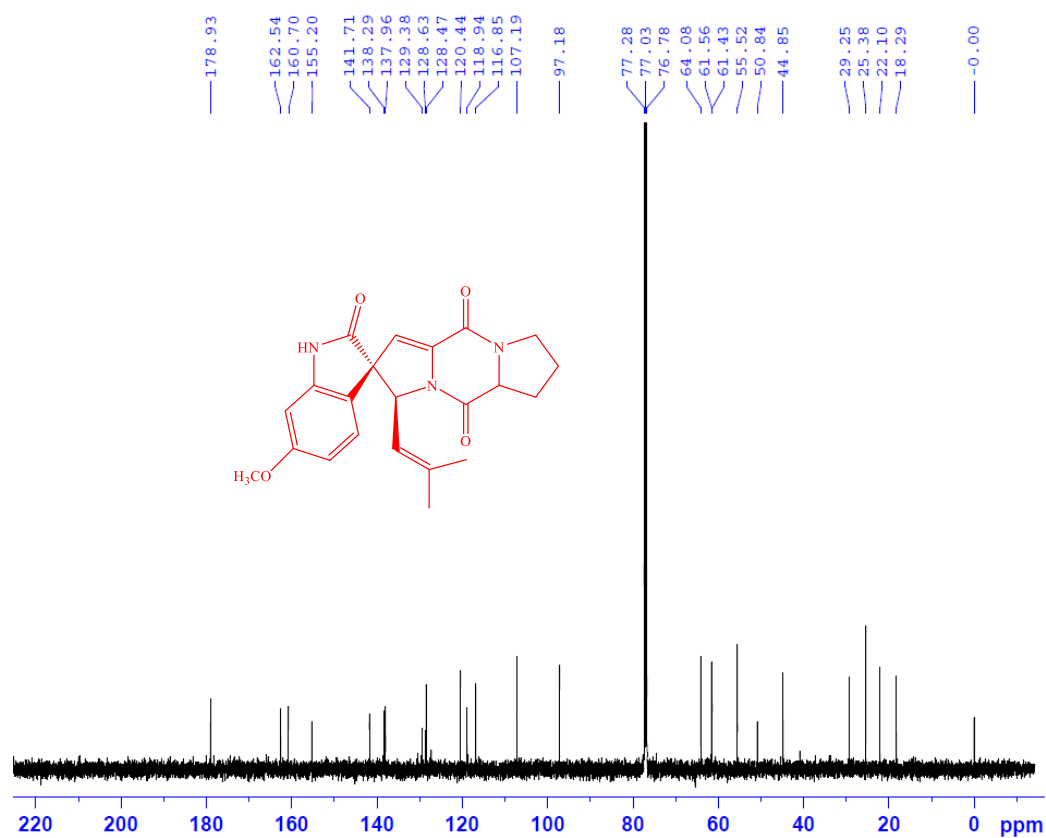
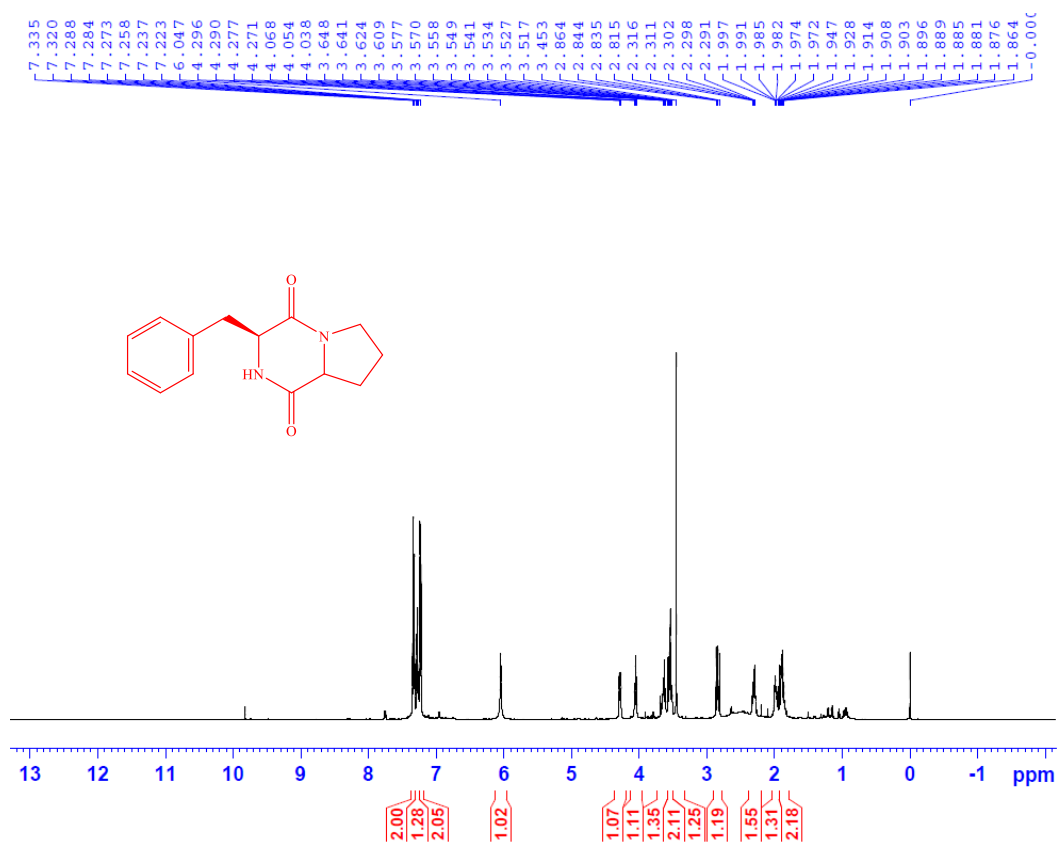


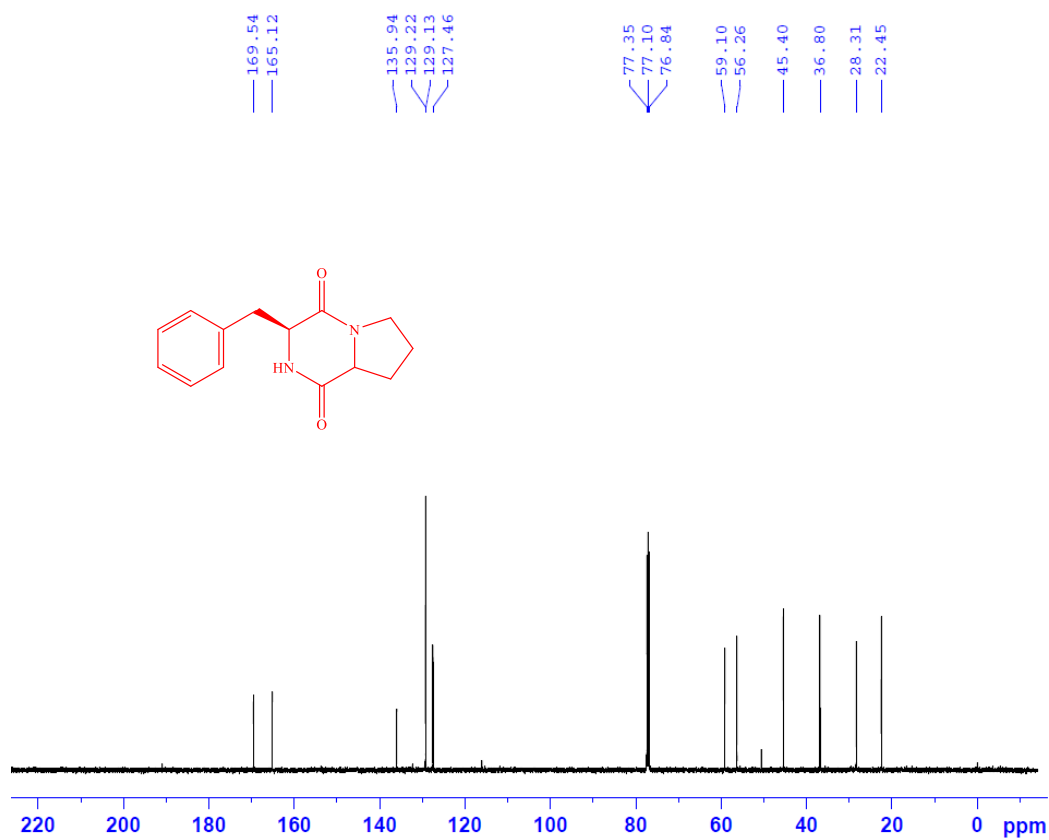
Figure S20:  $^1\text{H}$ -NMR spectrum of compound 9



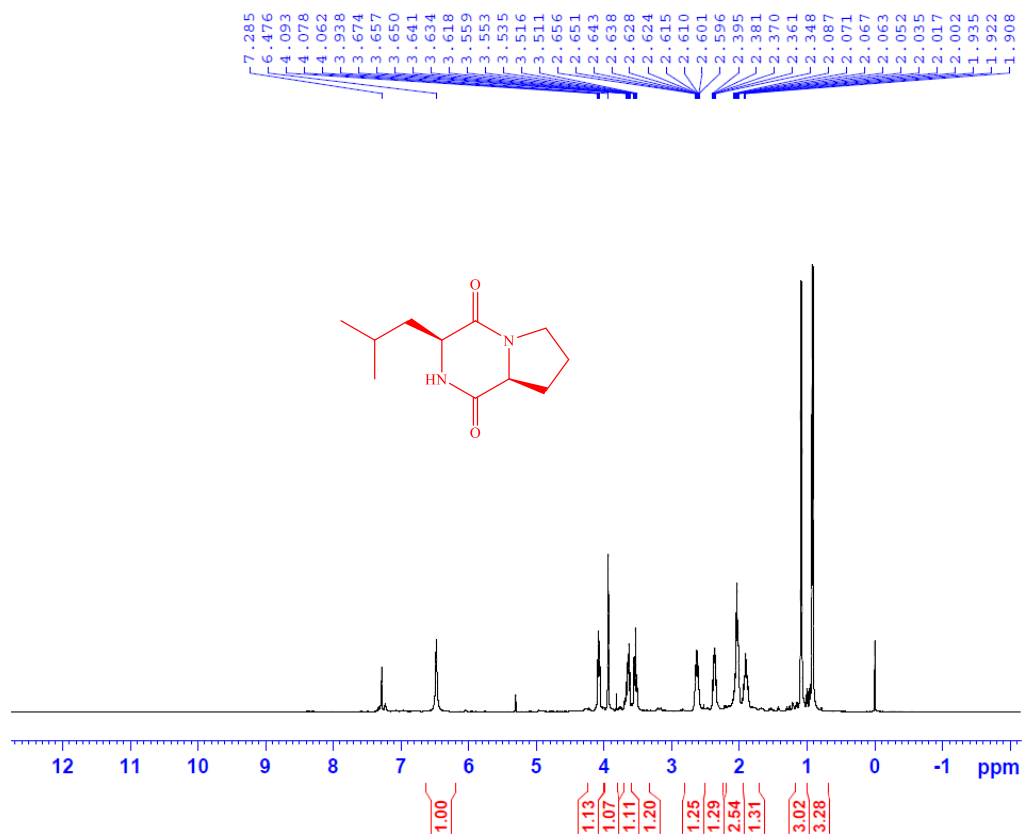
**Figure S21:** <sup>13</sup>C-NMR spectrum of compound 9



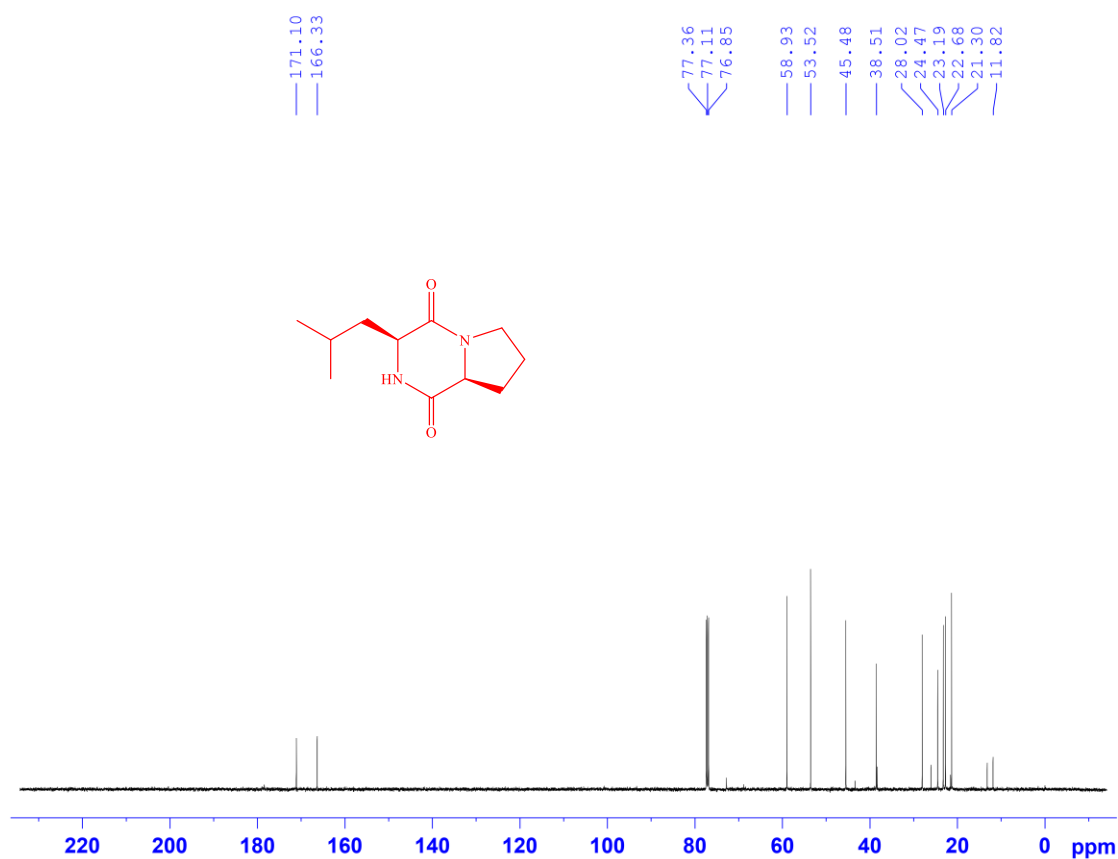
**Figure S22:** <sup>1</sup>H-NMR spectrum of compound 10



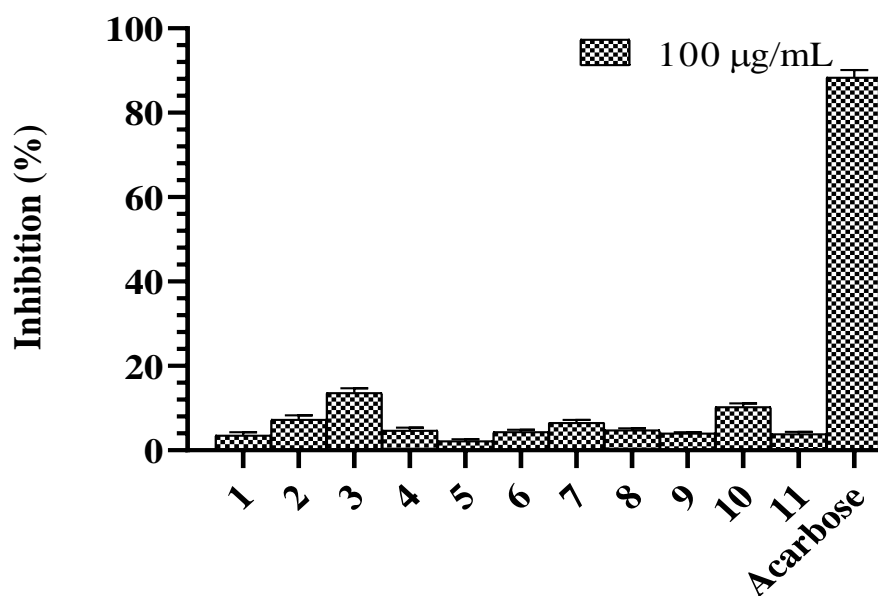
**Figure S23:** <sup>13</sup>C-NMR spectrum of compound 10



**Figure S24:** <sup>1</sup>H-NMR spectrum of compound 11



**Figure S25:** <sup>13</sup>C-NMR spectrum of compound 11



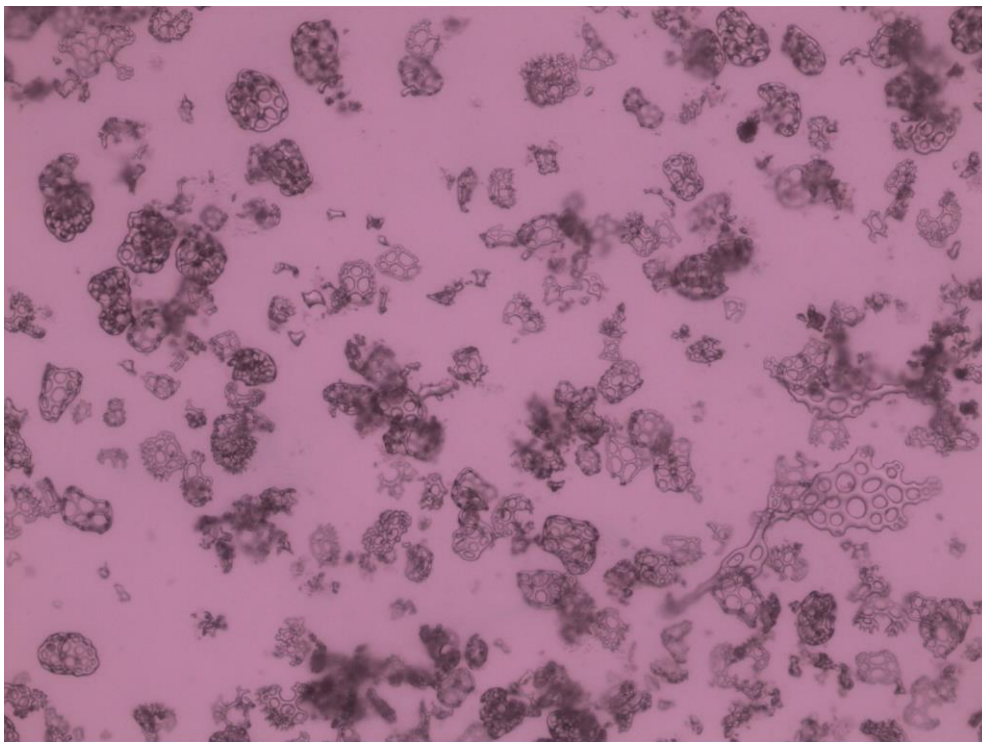
**Figure S26:**  $\alpha$ -Glucosidase inhibitory activity of compounds 1–10.

***Colochirus quadrangularis* Troschel, 1846 identification: Method and Data**

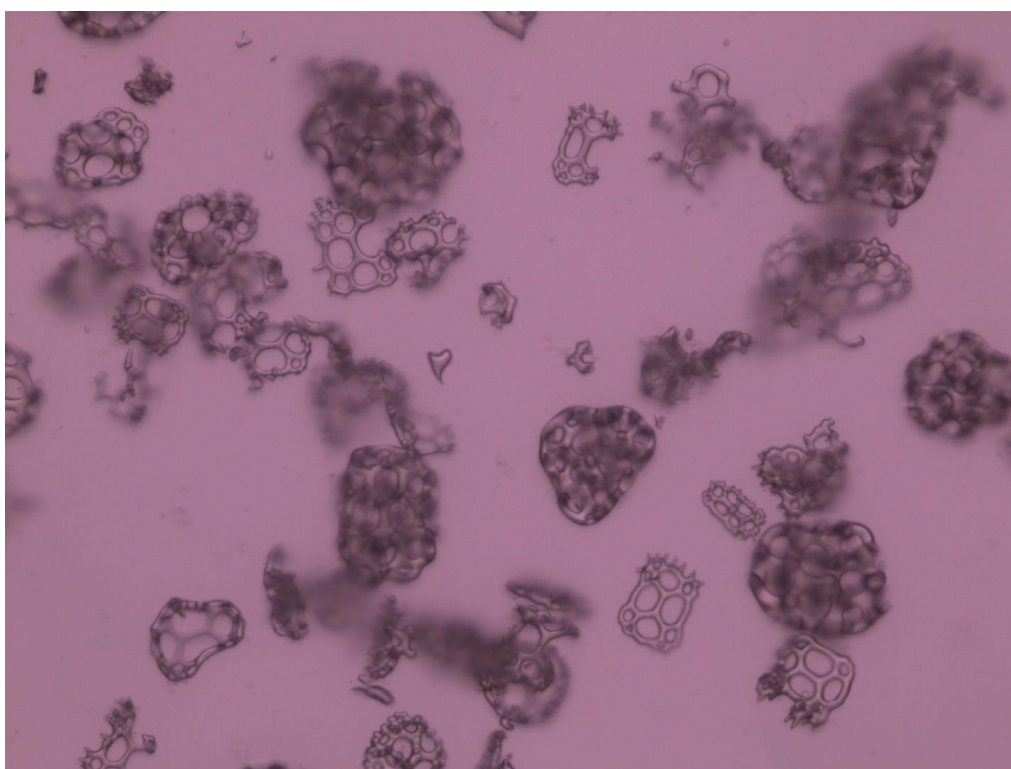


**Figure S27:** Photos of collected sea cucumber

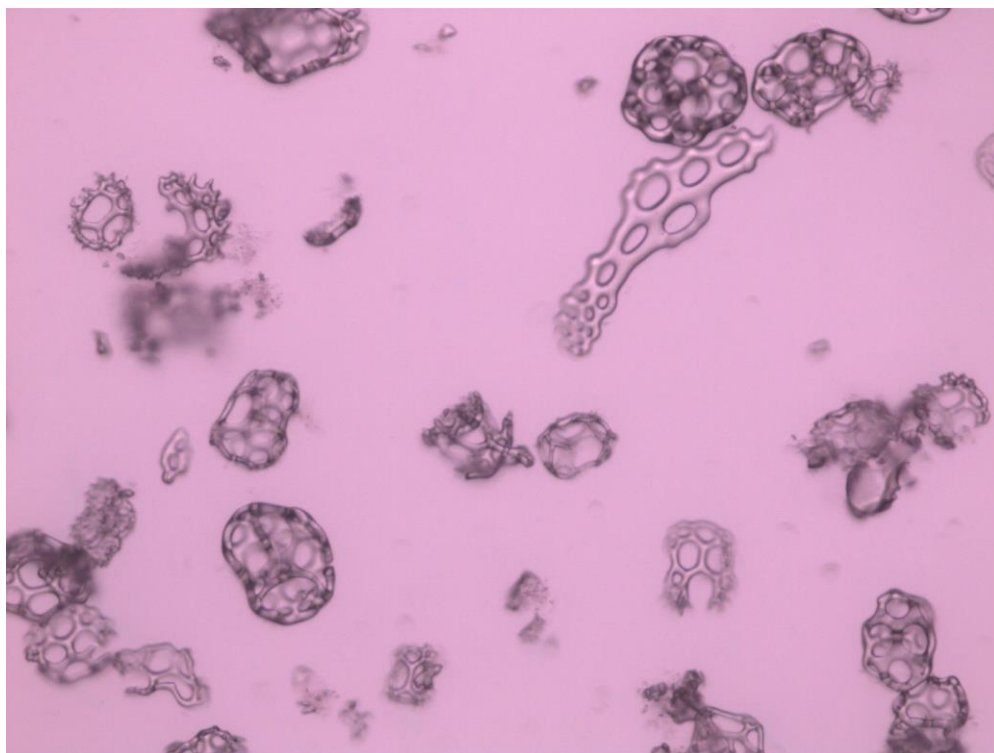
The identification of collected sea cucumber was done base on the analysis of its spicule morphology and skeleton structure followed published methods (Conand 1990; Hooper and Van Soest 2002). Briefly, thin slides at various body parts of the sea cucumber were prepared. The slides were then soak with sodium hypochlorite for 10-15 minutes to remove all contained tissues. The spicule and skeleton structure was observed under microscope (Figure S28-S30).



**Figure S28:** Figure: Popular bond types of the collected sea cucumber (10X)



**Figure S29:** Bowls and ellipsoids bonds of collected sea cucumber (20X)



**Figure S30:** Figure: Ellipsoids, Rosettes and Rods bonds of collected sea cucumber (20X)

As Drawn (0) | Substructure (1) | **Similarity (29K)**

Chemscape Analysis  
Visually explore structure similarity with a powerful new tool.  
[Learn more about Chemscape.](#)  
**Create Chemscape Analysis**

Filter Behavior  
**Filter by** | Exclude

Similarity  
☒ 95-98 (2)  
☐ 90-94 (6)  
☐ 85-89 (31)  
☐ 80-84 (117)  
☐ 75-79 (172)  
[View All](#)

Commercial Availability  
☐ Available (1)  
☐ Not Available (1)

References | Reactions | Suppliers | | | Save And Alerts

Filtering: Similarity: 95-98 | Number of Components: 1 | [Clear All Filters](#)

1

**2640210-38-2**

Absolute stereochemistry shown

**C<sub>16</sub>H<sub>19</sub>NO<sub>8</sub>**

1 Reference | 0 Reactions | 0 Suppliers

Key Physical Properties	Value	Condition
Molecular Weight	353.32	-
Boiling Point (Predicted)	648.7±55.0 °C	Press: 760 Torr
Density (Predicted)	1.58±0.1 g/cm <sup>3</sup>	Temp: 20 °C; Press: 760 Torr
pKa (Predicted)	12.49±0.70	Most Acidic Temp: 25 °C

2

**863507-77-1**

Absolute stereochemistry shown

**C<sub>15</sub>H<sub>17</sub>NO<sub>8</sub>**  
β-D-Glucopyranose, 1-(6-hydroxy-1*H*-indole-3-carboxylate)

7 References | 0 Reactions | 2 Suppliers

Key Physical Properties	Value	Condition
Molecular Weight	339.30	-
Boiling Point (Predicted)	688.9±55.0 °C	Press: 760 Torr
Density (Predicted)	1.70±0.1 g/cm <sup>3</sup>	Temp: 20 °C; Press: 760 Torr
pKa (Predicted)	9.58±0.40	Most Acidic Temp: 25 °C

[Spectra](#)

**Figure S31:** The results of SciFinder searching for new compound

**Table S1:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **2** and Fumiquinazoline D

Position	<b>2</b>	<b>Fumiquinazoline D [1]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1	-	172.4	172.4
3	-	70.9	70.9
4	-	152.3	152.3
6	-	146.4	146.4
7	7.68 d (8.5)	127.8	127.8
8	7.76 t (8.5)	134.9	134.9
9	7.49 t (8.5)	127.7	127.7
10	8.19 d (8.5)	126.9	126.9
11	-	120.4	120.5
12	-	161.0	161.0
14	5.80 m	52.8	52.8
15	2.30 d (15.5)/3.45 d (15.5)	43.5	43.5
16	2.10 s	19.0	19.0
17	-	84.0	84.1
18	5.62 s	85.6	85.7
20	4.05 q (6.5)	59.2	59.2
21	-	171.5	171.5
23	-	137.6	137.6
24	7.45 d (8.0)	115.4	115.5
25	7.24 t (8.0)	130.0	130.1
26	7.05 t (8.0)	125.8	125.8
27	7.45 d (8.0)	124.3	124.3
28	-	137.6	137.6
29	1.12 d (6.5)	17.5	17.5

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S2:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **3** and Fumiquinazoline C

Position	<b>3</b>	<b>Fumiquinazoline C [1]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1	-	170.9	170.8
3	-	84.2	84.2
4	-	150.4	150.4
6	-	146.3	146.3
7	7.80 d (8.0)	128.5	128.4
8	7.85 t (8.0)	135.0	134.9
9	7.62 t (8.0)	128.6	128.6
10	8.36 d (8.0)	127.0	127.0
11	-	121.4	121.4
12	-	159.5	159.5
14	5.72 d (7.5)	51.4	51.5
15	2.13 d (15.5)/2.97 dd (15.5, 7.5)	31.4	31.5
16	2.06 s	24.6	24.6
17	-	87.2	87.2
18	5.34 s	87.1	87.0
20	3.70 d (7.0)	58.6	58.6
21	-	170.7	170.0
23	-	135.8	135.8
24	7.45 d (8.0)	115.5	115.4
25	7.33 t (8.0)	130.3	130.2
26	7.20 t (8.0)	126.2	126.2
27	7.35 d (8.0)	124.9	124.8
28	-	138.4	138.3
29	1.07 d (7.0)	18.7	18.7

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S3:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **4** and Fumiquinazoline J

Position	<b>4</b>	<b>Fumiquinazoline J [2]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1		170.8	170.8
3		54.9	54.8
4		153.3	153.3
6		147.1	147.0
7	7.61 d (8.0)	127.6	127.6
8	7.70 d (8.0)	134.6	134.4
9	7.45 t (8.0)	172.4	172.4
10	8.26 d (8.0)	126.9	126.9
11		120.8	120.7
12		160.3	160.3
14	6.07 br s	54.6	54.5
15	3.37 dd (17.5, 4.5) 3.52 dd (17.5, 2.5)	26.0	25.9
16	2.26 s	18.1	18.0
17		107.6	107.5
18		132.5	132.4
20		134.7	134.6
21	7.31 d (8.0)	111.4	111.3
22	7.17 t (8.0)	123.5	123.5
23	7.07 t (8.0)	120.6	120.6
24	7.37 d (8.0)	118.4	118.4
25		127.9	127.8

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> recorded in

**Table S4:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **5** and bisdethiobis(methylthio)gliotoxin

Position	<b>5</b>	<b>bisdethiobis(methylthio)gliotoxin [1]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1		166.0	165.9
3		72.2	71.9
4		166.9	166.8
5a	4.92 (m)	69.6	69.6
6	4.92 m	74.4	74.4
7	5.88 d (9.5)	123.2	123.1
8	5.72 dd (9.5, 1.0)	129.9	130.1
9	5.92 d (1.0)	120.0	120.0
9a		131.7	131.6
10	2.95 d (16.0) 3.06 d (16.0)	38.9	38.9
11		71.6	71.5
12	2.24 s	15.1	14.6
13	3.14 s	28.6	28.7
14	2.26 s	13.6	13.7
15	3.88 d (12.0) 4.36 d (12.0)	63.6	63.6

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S5:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **6** and cyclo(L-prolyl-L-tryptophane)

Position	<b>6</b>	<b>cyclo(L-prolyl-L-tryptophane) [3]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1	8.60 s	-	-
2	-	165.6	168.23
3	4.35 dd (4.0, 10.5)	54.8	59.18
5	-	169.5	171.89
6	4.05 t (7.5)	59.2	59.51
7	1.98 m	28.3	29.74
	2.30 m		
8	1.88 m	22.6	22.26
	1.94 m		
9	3.55 m	45.4	46.04
	3.63 m		
10	2.99 dd (10.5, 15.0)	28.3	31.19
	3.72 dd (4.0, 15.0)		
11	-	109.8	109.31
12	-	126.8	128.66
13	7.58 d (8.0)	118.5	119.55
14	7.13 t (8.0)	119.9	120.04
15	7.21 t (8.0)	122.6	122.73
16	7.38 d (8.0)	111.6	112.37
17	-	136.7	138.01
18	5.99 s	-	-
19	7.08 s	123.5	126.21

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CD<sub>3</sub>OD

**Table S6:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **7** and tryprostatin B

Position	<b>7</b>	<b>Tryprostatin B [4]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1(NH)	8.02 br s		-
2	-	136.4	136.4
3	-	104.7	104.6
3a	-	128.1	128.0
4	7.47 d (7.5)	117.8	117.7
5	7.09 t (7.5)	119.9	119.9
6	7.16 t (7.5)	121.9	121.9
7	7.30 d (7.5)	110.8	110.8
7a	-	135.5	135.4
8	2.99 dd (10.5, 15.0)/3.72 dd (4.0, 15.0)	25.7	25.6
9	4.36 dd (1.5, 11.0)	54.7	54.6
10 (NH)	5.62 s	-	-
11	-	169.4	169.4
12	4.05 t (7.5)	59.3	59.3
13	2.02 m	28.4	28.3
	2.33 m		
14	1.91 m	22.7	22.6
	2.03 m		
15	3.60 m	45.4	45.4
	3.68m		
17	-	165.8	165.8
18	3.49 m	25.2	25.1
19	5.30 t (8.0)	119.8	119.7
20	-	135.5	135.5
21	1.78 s	25.7	25.7
22	1.75 s	18.0	18.0

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S7:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **8** and 12,13-dihydroxy-fumitremorgin C

Position	<b>8</b>	<b>12,13-Dihydroxy-fumitremorgin C [5]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
2	-	130.2	130.3
3	5.87 d (9.5)	58.8	58.8
5	-	166.2	166.3
6	4.42 dd (2.0, 9.5)	50.2	50.2
7	2.09 m/2.48 m	29.2	29.2
8	1.95 m/2.10 m	22.5	22.6
9	3.64 m	45.3	45.3
11	-	171.0	171.1
12	-	83.1	83.1
13	5.74 m	68.7	68.8
14	-	105.4	105.6
15	-	120.7	120.9
16	6.79 d (9.5)	121.2	121.4
17	7.79 d (9.5)	109.8	109.9
18	-	156.7	156.9
19	6.83 s	95.1	95.3
20	-	137.6	137.7
21	1.99 m	124.0	124.1
22	-	134.6	134.6
23	4.79 d (9.5)	25.7	25.7
24	1.66 m	18.3	18.3
25	3.82 s	55.8	55.8

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S8:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **9** and 6-methoxyspirotryprostatin B

Position	<b>9</b>	<b>6-methoxyspirotryprostatin B [6]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1(NH)	8.16 s	-	-
2	-	178.9	179.0
3	-	61.4	61.4
3a	-	118.9	118.8
4	6.95 d (8.5)	128.5	128.4
5	6.51 dd (8.5, 2.0)	107.2	107.1
6	-	160.7	160.6
7	6.45 d (2.0)	97.2	97.1
7a	-	141.7	141.7
8	5.75 s	116.9	116.9
9	-	138.0	137.9
11	-	162.5	162.5
12	4.33 dd (11.0, 5.5)	61.6	61.5
13	1.99 m/2.48 m	29.3	29.2
14	1.99 m/2.11 m	22.1	22.1
15	3.58 m/3.83 m	44.9	44.8
17	-	155.2	155.1
18	5.38 d (9.0)	64.1	64.0
19	5.20 d (9.0)	120.4	120.4
20	-	138.3	138.3
21	1.59 s	25.4	25.4
22	1.30 s	18.3	18.3
6-OCH <sub>3</sub>	3.80 s	55.5	55.5

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S9:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **10** and cyclo(L-prolinyl-L-phenylalanine)

Position	<b>10</b>	<b>cyclo(L-prolinyl-L-phenylalanine) [7]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1	-	169.5	169.4
3	3.55 m/3.64 m	45.4	45.2
4	1.88 m/1.97 m	22.5	22.2
5	1.99 m/2.31 m	28.3	28.3
6	4.29 dd (9.5, 3.0)	59.1	58.9
7	-	165.1	164.9
9	4.27 d (3.0)	56.3	56.1
10	2.84 dd (14.5, 10.0)/3.54 m	36.8	36.6
1'	-	135.9	135.8
2'	7.33 m	129.1	128.9
3'	7.24 m	127.5	127.3
4'	7.28 m	129.2	129.1

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S10:** <sup>1</sup>H and <sup>13</sup>C-NMR data of compound **11** and cyclo(L-prolinyl-L-valine)

Position	<b>11</b>	<b>cyclo(L-prolinyl-L-valine) [8]</b>	
	$\delta_{\text{H}}^{\text{a,b}}$ mult. (J in Hz)	$\delta_{\text{C}}^{\text{a,c}}$	$\delta_{\text{C}}^{\text{#}}$
1	-	171.1	170.7
3	3.51 m/3.60 m	45.5	45.9
4	1.90 m/2.09 m	22.7	23.1
5	2.13 m/2.34 m	28.0	28.5
6	4.13 t (8.5)	58.9	59.4
7	-	166.3	166.6
9	4.02 d (9.0)	53.5	53.8
10	1.56 m/2.02 m	38.5	39.0
11	1.85 m	24.5	25.1
12	0.95 d (6.5)	23.2	23.7
13	1.00 d (6.5)	21.3	21.6

<sup>a)</sup> recorded in CDCl<sub>3</sub>, <sup>b)</sup> 500 MHz, <sup>c)</sup> 125 MHz, <sup>#)</sup> CDCl<sub>3</sub>

**Table S11:** Cytotoxic activity of compounds **1-11**

Compounds	<b>IC<sub>50</sub> (μg/mL)</b>	
	<b>Huh-7</b>	<b>HT-29</b>
<b>2</b>	70.9±2.1	60.9±6.2
<b>3</b>	66.2±3.3	61.7±2.4
<b>4</b>	9.7±0.9	10.3±0.9
<b>Ellipticine</b>	0.4±0.1	0.4±0.1

Compounds **1**, **4-10** did not showed cytotoxic activity ( $\text{IC}_{50} > 100$  (μg/mL)). Ellipticine was used as a positive control. Data are presented as mean ± SD of experiments performed in triplicate.

## S1. Sea cucumber Identification

The obtained spicule morphology and skeleton structure were compared with reference data (Mark O'Loughlin 2016) revealed that the collected sea cucumber belongs to *Colochirus quadrangularis* Troschel, 1846, with detail classification as follow:

Phylum: Echinodermata

Class: Holothuroidea

Order: Dendrochirotida

Family: Cucumariidae

Genus: *Colochirus*

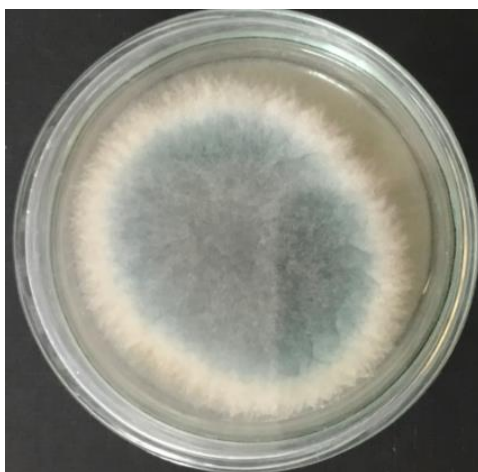
Species: *Colochirus quadrangularis* Troschel, 1846

Reference source:

O'Loughlin, P.M., Harding, C. & Paulay, G., 2016. The sea cucumbers of Camden Sound in northwest Australia, including four new species (Echinodermata: Holothuroidea). *Memoirs of Museum Victoria* 75: 7-52. <http://doi.org/10.24199/j.mmv.2016.75.02>.

## S2. *Aspegillus fumigatus* M580 Isolation and Characterization: Method and Data

0.5 g of *Colochirus quadrangularis* Troschel, 1846 was suspended in 4.5 ml of sterile distilled water, homogenized by vortexing for 1 min, and the suspension was treated at 60 °C for 6 min. Next, 0.5 ml of the heat-treated suspension was used for serial dilution in sterile distilled water to  $10^{-3}$ . 50 µl of the final dilution were spread on the petri dishes containing solid media, PDA - potato dextrose agar (30 g/L potato extract, 20g/L dextrose 5g/L soluble starch, 30g/L instant ocean, 15g/L agar). Plates were incubated at 28 °C for 7 days. Single colonies of fungi were transferred onto new petri dishes of PDA medium for further purification steps (Figure).



Colony morphological characteristics of the strain M580 grown on PDA medium for 10 days at 28°C

### S3. Fungal Identification

Genomic DNA of strain M580 was extracted by Wizard® Genomic DNA Purification Kit (Promega, USA). Sequences of 18S rRNA was used for taxonomical identification of the fungal strain. Gene amplifications were performed in a 25.0 µl mixture containing 10 µl of sdH<sub>2</sub>O, 12.5 µl of 2× PCR Master mix (Thermo Scientific), 1.0 µl of 0.05 mM for both primers NS3F (5'-GCAAGTCTGGTGCCAGCAGCC-3') and NS8R (5'-TCCGCAGGTTACCTACGGA-3') and 0.5 µl of genomic DNA. The thermocycling was performed on MJ Thermal cycler (Bio - Rad), with a preheating step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30s and extension at 72 °C for 45s before a final extension of 72 °C for 10 minus. The PCR product size was about 1300 bp. PCR products were purified by DNA purification kit (Invitrogen) and sequenced by DNA Analyzer (ABI PRISM 3100, Applied Bioscience). Gene sequences were handled by BioEdit v.2.7.5. and compared with fungal 18S rDNA sequences available in GenBank database using NCBI Blast program. The alignment was manually verified and adjusted prior to the reconstruction of a phylogenetic neighbour-joining tree by using the MEGA program version 4.1. The results showed that strain M580 belonged to species *Aspergillus fumigatus* (Table S1). Strain M580 was registered with GenBank code: **MW015802**.

### S4. M580 strain's 18S rRNA gene sequence comparison results on GenBank

Descriptions	Graphic Summary	Alignments	Taxonomy				
Sequences producing significant alignments							
Download <span>▼</span> Manage Columns <span>▼</span> Show <span>100</span> <span>▼</span> <span>?</span>							
<input checked="" type="checkbox"/> Select all 100 sequences selected							
<a href="#">GenBank</a> <a href="#">Graphics</a> <a href="#">Distance tree of results</a>							
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain TMS-26 18S ribosomal RNA gene, partial sequence</a>	2130	2130	98%	0.0	100.00%	<a href="#">KJ746594.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain YuZhu2 18S ribosomal RNA gene, partial sequence</a>	2128	2128	98%	0.0	100.00%	<a href="#">KU512836.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus ATCC 13073 gene for 18S ribosomal RNA, partial sequence</a>	2124	2124	98%	0.0	99.91%	<a href="#">LC485158.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain UPSC 1771 18S ribosomal RNA gene, partial sequence</a>	2124	2124	98%	0.0	99.91%	<a href="#">AF548061.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus gene for 18S rRNA, partial sequence</a>	2124	2124	98%	0.0	99.91%	<a href="#">AB008401.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus small subunit ribosomal RNA</a>	2124	2124	98%	0.0	99.91%	<a href="#">M60300.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus small subunit ribosomal RNA</a>	2124	2124	98%	0.0	99.91%	<a href="#">M55626.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain MJ-X6 18S ribosomal RNA gene, complete sequence</a>	2122	2122	98%	0.0	99.91%	<a href="#">HM590663.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus sp. ISSFT-021 18S ribosomal RNA gene, partial sequence</a>	2121	2121	97%	0.0	100.00%	<a href="#">KT832787.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain CY018 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene</a>	2119	2119	98%	0.0	99.83%	<a href="#">KJ809565.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain WL002 18S ribosomal RNA gene, partial sequence</a>	2119	2119	98%	0.0	99.83%	<a href="#">KJ528402.1</a>
<input checked="" type="checkbox"/>	<a href="#">Aspergillus fumigatus strain FS160 18S ribosomal RNA gene, partial sequence</a>	2119	2119	98%	0.0	99.83%	<a href="#">FJ840490.1</a>

## S5. The 18S rRNA gene sequence of strain M580 is displayed on GenBank

GenBank: MW015802.1

[FASTA](#) [Graphics](#)

[Go to:](#) ☒

```
LOCUS      MW015802          1175 bp    DNA        linear    PLN 23-SEP-2020
DEFINITION Aspergillus fumigatus isolate M580 small subunit ribosomal RNA
            gene, partial sequence.
ACCESSION  MW015802
VERSION    MW015802.1
KEYWORDS   .
SOURCE     Aspergillus fumigatus
  ORGANISM Aspergillus fumigatus
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Eurotiomycetes; Eurotiomycetidae; Eurotiales; Aspergillaceae;
            Aspergillus; Aspergillus subgen. Fumigati.
REFERENCE  1 (bases 1 to 1175)
  AUTHORS  Tuan,C.D., Khai,N.V., Hung,N.V., Anh,N.M., Minh,L.T., Huong,D.T.,
            Cuong,P.V. and Lien,H.T.
  TITLE    18s rRNA of fungi
  JOURNAL  Unpublished
REFERENCE  2 (bases 1 to 1175)
  AUTHORS  Tuan,C.D., Khai,N.V., Hung,N.V., Anh,N.M., Minh,L.T., Huong,D.T.,
            Cuong,P.V. and Lien,H.T.
  TITLE    Direct Submission
  JOURNAL  Submitted (18-SEP-2020) biotechnology, Institute of marine
            biochemistry, hoang quoc viet, ha noi, 0243 084, Viet Nam
COMMENT    ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES   Location/Qualifiers
```

## S6. Physical properties of compounds 2-10

*Fumiquinazoline D (2)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} +62.5$  (c 0.1, CHCl<sub>3</sub>); C<sub>24</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S1.

*Fumiquinazoline C (3)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} -150.0$  (c 0.1, CHCl<sub>3</sub>); C<sub>24</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S2.

*Fumiquinazoline J (4)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} -72.4$  (c 0.1, CHCl<sub>3</sub>); C<sub>21</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S3.

*Bisdethiobis(methylthio)gliotoxin (5)*: White amorphous powder;  $[\alpha]_{\text{D}}^{25} -42.0$  (c 0.1, CHCl<sub>3</sub>); C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S4.

*Cyclo(L-Pro-L-Trp) (6)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} -58.3$  (c 0.1, CHCl<sub>3</sub>); C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>2</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S5.

*Tryprostatin B (7)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} -55.9$  (c 0.1, CHCl<sub>3</sub>); C<sub>21</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S6.

*12,13-dihydroxy-fumitremorgin C (8)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} +20.5$  (c 0.1, CHCl<sub>3</sub>); C<sub>22</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S7.

*6-Methoxyspirotryprostatin B (9)*: Pale yellow amorphous powder;  $[\alpha]_{\text{D}}^{25} -37.0$  (c 0.1, CHCl<sub>3</sub>); C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S8.

*Cyclo(D-Pro-D-Phe) (10)*: White amorphous powder;  $[\alpha]_{\text{D}}^{25} +75.0$  (c 0.1, CHCl<sub>3</sub>); C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S9.

*Cyclo(S-Pro-S-Leu) (11)*: White amorphous powder;  $[\alpha]_{\text{D}}^{25} +75.0$  (c 0.1, CHCl<sub>3</sub>); C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>; <sup>1</sup>H NMR (CDCl<sub>3</sub>) and <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: see Table S10.

## References

- [1] S. S. Afiyatullo, A. I. Kalinovskii, M. V. Pivkin, P. S. Dmitrenok, and T. A. Kuznetsova (2005). Alkaloids from the marine isolate of the fungus *Aspergillus fumigatus*, *Chem. Nat. Comp.* **41**, 236-238.
- [2] R. Liu, H. Li, J. Yang, and Z. An (2018). Quinazolinones isolated from *Aspergillus* sp., an endophytic fungus of *Astragalus membranaceus*, *Chem. Nat. Compd.* **54**, 808-810.
- [3] V. Ivanova, U. Graefe, R. Schlegel, B. Schlegel, A. Gusterova, M. Kolarova, and K. Aleksieva (2003). Isolation and structure elucidation of tyramine and indole alkaloids from antarctic strain *Microbispora aerata* IMBAS-11A, *Biotechnol. Biotechnol. Equip.* **17**, 128-133.
- [4] C. B. Cui, H. Kakeya, and H. Osada (1996). Novel mammalian cell cycle inhibitors, tryprostatins A, B and other diketopiperazines produced by *Aspergillus fumigatus*. II. Physico-chemical properties and structures, *J. Antibiot.* **49**, 534-540.
- [5] W.-R. Abraham and H.-A. Arfmann (1990). 12,13-Dihydroxy-fumitremorgin C from *Aspergillus fumigatus*, *Phytochemistry* **29**, 1025-1026.
- [6] M. Zhang, W.-L. Wang, Y.-C. Fang, T.-J. Zhu, Q.-Q. Gu, and W.-M. Zhu (2008). Cytotoxic alkaloids and antibiotic nordammarane triterpenoids from the marine-derived fungus *Aspergillus sydowi*, *J. Nat. Prod.* **71**, 985-989.
- [7] F. Fdhila, V. Vázquez, J. L. Sánchez, and R. Riguera (2003). Diketopiperazines: antibiotics active against *Vibrio anguillarum* isolated from marine bacteria associated with cultures of *Pecten maximus*, *J. Nat. Prod.* **66**, 1299-1301.
- [8] M. S. C. Pedras, Y. Yu, J. Liu, and Y. A. Tandron-Moya (2005). Metabolites produced by the phytopathogenic fungus *Rhizoctonia solani*: isolation, chemical structure determination, syntheses and bioactivity, *Z. Naturforsch.* **60c**, 717-722.