Supporting Information

Structure Elucidation of the Spiro-polyketide Svalbardine B from the Arctic Fungal Endophyte Poaceicola sp. E1PB with Support from Extensive ESI-MS Interpretation

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Contents

S0: Fluxogram of the isolation of compounds 1-6 ........................................................................5
S1: UV spectrum of 1 (svalbardine A) in MeCN ........................................................................6
S2: IR spectrum (ATR) of 1 (svalbardine A) ...........................................................................6
S3: (-)-HR-ESI-MS spectrum of 1 (svalbardine A)...................................................................7
S4: 1H-NMR spectrum of 1 (svalbardine A) at 600 MHz in MeOD. ........................................7
S5: 13C-NMR spectrum of 1 (svalbardine A) at 150 MHz in MeOD. ......................................8
S6: Dept 90 spectrum of 1 (svalbardine A) at 150 MHz in MeOD. ...........................................8
S7: Dept 135 spectrum of 1 at (svalbardine A) 150 MHz in MeOD. ........................................9
S8: COSY spectrum of 1 (svalbardine A) at 600 MHz in MeOD. ...........................................9
S9: COSY spectrum of 1 (svalbardine A) at 600 MHz in MeOD (enlargement).......................10
S10: HMBC spectrum of 1 at (svalbardine A) 600 MHz in MeOD. ........................................10
S11: HMBC spectrum of 1 at (svalbardine A) 600 MHz in MeOD (enlargement)...................11
S12: HMBC spectrum of 1 at (svalbardine A) 600 MHz in MeOD (enlargement) .......................11
S13: HMBC spectrum of 1 at (svalbardine A) 600 MHz in MeOD (enlargement) .......................12
S14: HMQC spectrum of 1 (svalbardine A) at 600 MHz in MeOD ..............................................12
S15: NOESY spectrum of 1 (svalbardine A) at 600 MHz MeOD .................................................13
S16 UV spectrum of 2 (svalbardine B) in MeCN ..........................................................................13
S17 IR spectrum (film) of 2 (svalbardine B) .................................................................................14
S18 (+)-HR-ESI-MS spectrum of 2 (svalbardine B) .....................................................................14
S19: (-)-HR-ESI-MS spectrum of 2 (svalbardine B) ......................................................................14
S20: MS<sup>n</sup> analysis of 2 (svalbardine B) by ESI-QTRAP-MS .......................................................15
  S20.1: Full scan (+)-mode ...........................................................................................................15
  S20.2: Full scan (+)-mode (enlargement) ....................................................................................15
  S20.3: (+)-ESI-MS ER (Enhanced Resolution Scan) spectrum ...................................................16
  S20.4: (+)-MS<sup>2</sup> spectrum (EPI 627) ......................................................................................16
  S20.5: (+) MS<sup>2</sup> spectrum (EPI 605) .......................................................................................17
  S20.6: (+) MS<sup>2</sup> spectrum (EPI 179) .......................................................................................17
  S20.7: (+)-ESI-MS precursor ion spectrum (PREC 397) ...............................................................18
  S20.8: (+)-ESI-MS precursor ion spectrum (PREC 379) ...............................................................18
  S20.9: (+)-ESI-MS precursor ion spectrum (PREC 365) ...............................................................19
  S20.10: (+)-ESI-MS precursor ion spectrum (PREC 365) .............................................................19
  S20.11: (+)-ESI-MS precursor ion spectrum (PREC 323) .............................................................20
  S20.12: (+)-ESI-MS precursor ion spectrum (PREC 209) .............................................................20
  S20.13: (+)-ESI-MS precursor ion spectrum (PREC 177) .............................................................21
  S20.14: (+)-ESI-MS precursor ion spectrum (PREC 149) .............................................................21
  S20.15: (+)-ESI-MS<sup>3</sup> spectrum (605→209) ...........................................................................22
  S20.16: (+)-ESI-MS<sup>3</sup> spectrum (605→189) ...........................................................................22
  S20.17: (-)-ESI-MS ER (Enhanced Resolution Scan) spectrum ..................................................23
  S20.18: (-)-ESI-MS<sup>2</sup> spectrum (EPI 603) ...............................................................................23
  S20.19: (-)-ESI-MS<sup>3</sup> spectrum (603→363) ...........................................................................24
  S20.20: (-)-ESI-MS<sup>3</sup> spectrum (603→321) ...........................................................................24
  S20.21: (-)-ESI-MS<sup>3</sup> spectrum (603→181) ...........................................................................25
  S20.22: (-)-ESI-MS neutral loss spectrum (NL 15) .......................................................................25
S21: <sup>1</sup>H-NMR spectrum of 2 (svalbardine B) at 500 MHz in MeOD. .................................26
S22: $^1$H-NMR spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) ..................26
S23: $^1$H-NMR spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) ...................27
S24: $^1$H-NMR spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) ...................27
S25: $^{13}$C-NMR spectrum of 2 (svalbardine B) at 125 MHz in MeOD .....................................28
S26: DEPT 90-NMR spectrum of 2 (svalbardine B) at 125 MHz in MeOD ..............................28
S27: DEPT 135-NMR spectrum of 2 (svalbardine B) at 125 MHz in MeOD ..............................29
S28: COSY spectrum of 2 at (svalbardine B) 500 MHz in MeOD. ...........................................29
S29: COSY spectrum of 2 (svalbardine B) at 500 MHz (enlargement). ....................................30
S30: COSY spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement). .......................30
S31: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD. ...........................................31
S32: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement). .......................31
S33: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................32
S34: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................32
S35: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................33
S36: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................33
S37: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................34
S38: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................34
S39: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................35
S40: HMBC spectrum of 2 (svalbardine B) at 500 MHz in MeOD (enlargement) .......................35
S41: NOESY spectrum of 2 (svalbardine B) at 500 MHz in MeOD. .........................................36
S42: UV spectrum of 3 (annularin K) in MeCN ........................................................................36
S43: (+)-HR-ESI-MS spectrum of 3 (annularin K). .................................................................37
S44: $^1$H-NMR spectrum of 3 (annularin K) at 500 MHz in MeOD. .........................................37
S45: $^{13}$C-NMR spectrum of 3 (annularin K) at 125 MHz in MeOD. ........................................38
S46: DEPT 90-NMR spectrum of 3 (annularin K) at 125 MHz in MeOD .................................38
S47: DEPT 135-NMR spectrum of 3 (annularin K) at 125 MHz in MeOD .................................39
S48: COSY spectrum of 3 (annularin K) at 500 MHz in MeOD ..............................................39
S49: HMBC spectrum of 3 (annularin K) at 500 MHz in MeOD. .............................................40
S50: HMBC spectrum of 3 (annularin K) at 500 MHz in MeOD (enlargement) .......................40
S51: HMBC spectrum of 3 (annularin K) at 500 MHz in MeOD (enlargement) .......................41
S52: HMQC spectrum of 3 (annularin K) at 500 MHz in MeOD. .............................................41
S53: NOESY spectrum of 3 (annularin K) at 500 MHz in MeOD..................................................42
S54: ECD predicted spectra of (R)-conformers and experimental spectrum of 3 (annularin K) .....42
S55: Antibacterial Assay ...............................................................................................................46
S56: Antibiotic-resistance alleviating assay ..................................................................................47
S57: Antiproliferative assay...........................................................................................................49
S58: Pictures of mycelium and conidia of the mature fungus grown on sterilised carnation stem with corn meal agar (CMA) were taken using ESEM .................................................50
S0: Fluxogram of the isolation of compounds 1-6

Crude extract (638 mg)

Preparative LC

F1 179 mg
F2 85 mg
F3 58 mg
F4 14.8 mg
F5 75.4 mg
F6 7.2 mg
F7 11.2 mg
F8 92.4 mg

Preparative LC

F1-1 14 mg
F1-2 52 mg
F1-3 12 mg
F1-4 20.1 mg
F1-5 39 mg

Recycling HPLC

F1-1 6 mg
F1-2 3.5 mg

F1-3 2.7 mg
S1: UV spectrum of 1 (svalbardine A) in MeCN

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S13: HMBC spectrum of 1 at (svalbardine A) 600 MHz in MeOD (enlargement).

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S15: NOESY spectrum of 1 (svalbardine A) at 600 MHz MeOD.

S16 UV spectrum of 2 (svalbardine B) in MeCN.
S17 IR spectrum (film) of 2 (svalbardine B)

S18 (+)-HR-ESI-MS spectrum of 2 (svalbardine B)

S19:(-)-HR-ESI-MS spectrum of 2 (svalbardine B)
S20: MS<sup>n</sup> analysis of 2 (svalbardine B) by ESI-QTRAP-MS

**S20.1: Full scan (+)-mode**

**S20.2: Full scan (+)-mode (enlargement)**
S20.3: (+)-ESI-MS ER (Enhanced Resolution Scan) spectrum

S20.4: (+)-MS² spectrum (EPI 627)
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S20.21: (-)-ESI-MS$^3$ spectrum (603$\rightarrow$181)

S20.22: (-)-ESI-MS neutral loss spectrum (NL 15)
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S26: DEPT 90-NMR spectrum of 2 (svalbardine B) at 125 MHz in MeOD.
S27: DEPT 135-NMR spectrum of 2 (svalbardine B) at 125 MHz in MeOD.

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S29: COSY spectrum of 2 (svalbardine B) at 500 MHz (enlargement).

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S51: HMBC spectrum of 3 (annularin K) at 500 MHz in MeOD (enlargement).

S52: HMQC spectrum of 3 (annularin K) at 500 MHz in MeOD.
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S54: ECD predicted spectra of (R)-conformers and experimental spectrum of 3 (annularin K)

<table>
<thead>
<tr>
<th>Conformer (Boltzmann distribution, %)</th>
<th>Structure</th>
<th>Predicted and experimental ECD spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cf1 (24.4)</td>
<td>![Structure Image]</td>
<td>![ECD-Cf1 Image]</td>
</tr>
<tr>
<td>Compounds</td>
<td>Structural Image</td>
<td>ECD Spectra</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Cf2</td>
<td><img src="image1" alt="Cf2 Structure" /></td>
<td><img src="image2" alt="ECD-Cf2" /></td>
</tr>
<tr>
<td>Cf3</td>
<td><img src="image3" alt="Cf3 Structure" /></td>
<td><img src="image4" alt="ECD-Cf3" /></td>
</tr>
<tr>
<td>Cf4</td>
<td><img src="image5" alt="Cf4 Structure" /></td>
<td><img src="image6" alt="ECD-Cf4" /></td>
</tr>
<tr>
<td>Cf5</td>
<td><img src="image7" alt="Cf5 Structure" /></td>
<td><img src="image8" alt="ECD-Cf5" /></td>
</tr>
<tr>
<td>Compound</td>
<td>ECD Graph</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Cf6 (6.8)</td>
<td><img src="image1" alt="Cf6 ECD Graph" /></td>
<td></td>
</tr>
<tr>
<td>Cf7 (5.9)</td>
<td><img src="image2" alt="Cf7 ECD Graph" /></td>
<td></td>
</tr>
<tr>
<td>Cf8 (5.4)</td>
<td><img src="image3" alt="Cf8 ECD Graph" /></td>
<td></td>
</tr>
<tr>
<td>Cf9 (4.8)</td>
<td><img src="image4" alt="Cf9 ECD Graph" /></td>
<td></td>
</tr>
</tbody>
</table>
Weighted Spectra

- \( \text{calcd spectrum (R-C8)} \)
- \( \text{calcd curve x (-1) (S-C8)} \)
- \( \text{experimental curve} \)

Weighted ECD

Graph showing weighted ECD with peaks at different wavelengths.
**S55: Antibacterial Assay**

The antibacterial screening was conducted *in vitro* using resazurin as cell growth indicator against 10 test pathogens (*Staphylococcus aureus* ATCC 25923, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus cohnii* subsp. *urealyticum* (clinical strain obtained from Abdul-Aziz et al., 2015),

*Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 12020, *Enterobacter cloacae* ATCC 35030, *Salmonella typhimurium* ATCC 14028, *Yersinia enterocolitica* ATCC 23715, *Pseudomonas aeruginosa* ATCC 10145 and *Serratia marcescens* ATCC 14756). The protocol described by Low (2016)\(^2\) was implemented with slight modifications. In brief, 50 µL of adjusted inoculum (10\(^6\) cfu/mL) was added to the microplate followed by 50 µL of 3 % DMSO solution of the tested compounds (1, 2, 4, 5 and 6) to reach a final concentration of 20 µM. A similar microplate where the inoculum was substituted with sterile Mueller Hinton broth (MHB) was also prepared. This served as the blank control for the samples. The microplates were then incubated at 37 °C for 18 hours. Next, 20 µL of 0.5 mM resazurin was added to each well and the plates were re-incubated for another 4 hours. After incubation, the absorbance of each well was measured at 570 nm and 600 nm using a microplate reader (Biotek Synergy HT, USA). The percentage of cell growth inhibition was subsequently calculated. Assays were conducted in triplicates. An unreduced resazurin (blue color) and a percent inhibition of more than 85 % were considered as a positive inhibition. Each microplate contained a set of suitable controls: antibiotics (20 µM of chloramphenicol, nalidixic acid and erythromycin) served as positive controls, untreated cells as the negative control whereas MHB alone served as the sterility control. The results are presented in Table 1 below.


\(^2\) Low A.M.L. *Antimicrobial activities and enhancers of DNA gyrase inhibitors from manure compost actinomycetes*. PhD thesis, 2016, Universiti Teknologi MARA.
Table 1: Antibacterial screening of five compounds expressed in % inhibition

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Compounds (final concentration: 20 µM)</th>
<th>+ve control</th>
<th>-ve control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>-8.0 ±6.9</td>
<td>-5.2 ±7.3</td>
<td>-9.4 ±5.5</td>
</tr>
<tr>
<td>S. haemolyticus ATCC 29970</td>
<td>-20.7 ±3.1</td>
<td>-16.0 ±1.4</td>
<td>-0.3 ±16.0</td>
</tr>
<tr>
<td>S. cohnii subsp. urealyticum#</td>
<td>-15.5 ±7.0</td>
<td>-41.8 ±1.4</td>
<td>8.8 ±2.2</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>-23.3 ±1.5</td>
<td>-7.6 ±3.7</td>
<td>-1.9 ±4.5</td>
</tr>
<tr>
<td>S. flexneri ATCC 12022</td>
<td>-10.3 ±11.4</td>
<td>2.8 ±15.3</td>
<td>7.3 ±12.5</td>
</tr>
<tr>
<td>E. cloacae ATCC 35030</td>
<td>-7.2 ±11.4</td>
<td>-29.6 ±0.9</td>
<td>0.1 ±13.6</td>
</tr>
<tr>
<td>S. typhimurium ATCC 14028</td>
<td>-77.8 ±18.6</td>
<td>-60.8 ±8.4</td>
<td>-39.8 ±13.9</td>
</tr>
<tr>
<td>Y. enterocolitica ATCC 23715</td>
<td>-1.8 ±1.6</td>
<td>-2.6 ±1.1</td>
<td>2.6 ±3.9</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 10145</td>
<td>2.8 ±10.7</td>
<td>2.3 ±5.2</td>
<td>1.2 ±5.1</td>
</tr>
<tr>
<td>S. marcescens ATCC 14756</td>
<td>-1.3 ±2.5</td>
<td>-1.6 ±3.2</td>
<td>-2.2 ±0.7</td>
</tr>
</tbody>
</table>

Positive control = chloramphenicol, nalidixic acid, erythromycin (20 µM); negative control = 1.5 % DMSO; a: clinical isolate obtained by Abdul-Aziz et al., 2015; Not active (NA) < 85 % cell inhibition; Active (A) >85 % cell inhibition.

S56: Antibiotic-resistance alleviating assay

Three antibiotic-resistant strains (in-house trained strains of streptomycin-resistant *S. aureus*, chloramphenicol-resistant *S. aureus* and streptomycin-resistant *P. aeruginosa*) were used for the screening of resistance alleviating activities. The minimum inhibitory concentration (MIC) of streptomycin as well as chloramphenicol was initially pre-determined for each of the above strains and a sub-inhibitory concentration of $\frac{1}{2}$ MIC (1715 µM, 99 µM and 1715 µM, respectively) was used for this purposed. The assay was conducted in the presence and absence of the antibiotics. Briefly, 25 µL of solution of each tested compound (1, 2, 4, 5 and 6) (with final concentration of 20 µM in each well) and 25 µL of antibiotic at sub-inhibitory concentration were introduced into the microplate together with 50 µL suspension of antibiotic-resistant strain (adjusted at ca 10⁶ cfu/mL). A similar microplate without the addition of antibiotic was also prepared concomitantly. Incubation conditions, addition of resazurin and absorbance measurements were implemented according to the methods.
described above (S55). A cell-free microplate was likewise prepared to serve as the blank control. A growth percent inhibition of more than 85% in the presence of antibiotics of sub-inhibitory concentration combined with normal growth without the antibiotics would be considered as positive inhibition. The results are presented in Table 2 below.

Table 2: Antimicrobial activities and anti-resistance potential of 5 compounds against antibiotic resistant S. aureus and P. aeruginosa

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Streptomycin</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td></td>
<td>+C</td>
<td>+C+Ab</td>
</tr>
<tr>
<td>1</td>
<td>-18.3 ±4.8 (NA)</td>
<td>-1.0 ±1.2 (NA)</td>
</tr>
<tr>
<td></td>
<td>-3.7 ±20.7 (NA)</td>
<td>-11.2 ±13.7 (NA)</td>
</tr>
<tr>
<td>2</td>
<td>-17.6 ±9.4 (NA)</td>
<td>-0.4 ±1.7 (NA)</td>
</tr>
<tr>
<td></td>
<td>-4.7 ±9.0 (NA)</td>
<td>8.5 ±14.9 (NA)</td>
</tr>
<tr>
<td>4</td>
<td>-15.4 ±9.8 (NA)</td>
<td>3.0 ±0.9 (NA)</td>
</tr>
<tr>
<td></td>
<td>0.1 ±12.8 (NA)</td>
<td>2.1 ±19.4 (NA)</td>
</tr>
<tr>
<td>5</td>
<td>-12.6 ±6.8 (NA)</td>
<td>6.2 ±2.9 (NA)</td>
</tr>
<tr>
<td></td>
<td>1.1 ±11.6 (NA)</td>
<td>13.7 ±8.9 (NA)</td>
</tr>
<tr>
<td>6</td>
<td>-19.3 ±4.2 (NA)</td>
<td>1.2 ±2.3 (NA)</td>
</tr>
<tr>
<td></td>
<td>98.5 ±2.3 (NA)</td>
<td>97.4 ±1.1 (NA)</td>
</tr>
<tr>
<td></td>
<td>-12.8 ±6.7 (NA)</td>
<td>3.4 ±4.4 (NA)</td>
</tr>
</tbody>
</table>

+ve control | 98.5 ±2.3 | 97.4 ±1.1 | 98.5 ±0.1 | 97.9 ±1.7 | 99.3 ±0.7 | 99.0 ±0.8 |

-ve control | -12.8 ±6.7 | 3.4 ±4.4 | -12.8 ±6.7 | 3.4 ±4.4 | -0.2 ±10.3 | 2.9 ±17.3 |

+C: Mueller Hinton broth with compound; +C+Ab: Mueller Hinton broth with compound and antibiotic. Not active (NA) <85 % growth inhibition.
S57: Antiproliferative assay

Human neuroblastoma cells, SH-SY5Y (ATCC CRL-2266) were cultured according to standard protocols as mentioned by Kovalevich and Langford (2013). The cells were seeded into 96-well microplates with $1 \times 10^3$ cells/well. The cells were treated with a single concentration (20 µM) of each tested compound (1, 2, 4, 5 and 6) and incubated for 24 hours. The MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (prepared in 5 mg/ml of PBS), was added to SH-SY5Y cells in each well and the plates were incubated for 3 hours. Resulting formazan was solubilized with 100 µL DMSO. The plates were shaken for 15 minutes and the absorbance of each well was determined at 540 nm using the above microplate reader. The results are presented in Table 3 below.

Table 3: Preliminary screening of antiproliferative assay of five compounds (20 µM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antiproliferative assay (% cell viability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NT (161.98)</td>
</tr>
<tr>
<td>2</td>
<td>NT (84.61)</td>
</tr>
<tr>
<td>4</td>
<td>NT (155.82)</td>
</tr>
<tr>
<td>5</td>
<td>NT (103.61)</td>
</tr>
<tr>
<td>6</td>
<td>NT (158.63)</td>
</tr>
<tr>
<td>Control (cells only)</td>
<td>NT (100.00)</td>
</tr>
<tr>
<td>Blank</td>
<td>0</td>
</tr>
<tr>
<td>LPS100</td>
<td>-</td>
</tr>
<tr>
<td>NC</td>
<td></td>
</tr>
</tbody>
</table>

Not toxic towards cells (NT) > 50 % cell viability; NC= media and cell only.

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S58: Pictures of mycelium and conidia of the mature fungus grown on sterilised carnation stem with corn meal agar (CMA) were taken using ESEM.

Scanning electron micrographs of E1PB:
(a) asci and mycelium; (b,c,d) mycelium and conidia

References


(2) Low A.M.L. Antimicrobial activities and enhancers of DNA gyrase inhibitors from manure compost actinomycetes. PhD thesis, 2016, Universiti Teknologi MARA.

(3) Kovalevich, J.; Langford, D., Methods in Molecular Neurobiology, 2013, 1078, 9-21