

Antioxidant and antimicrobial alkaloids isolated from twigs of *Uvaria grandiflora* Roxb.

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ABSTRACT: Phytochemical investigation of the MeOH-EtOAc (1:1 v/v) soluble fraction partitioned from the MeOH extract of twigs of *Uvaria grandiflora* Roxb. led to the isolation of six known compounds including aristolactam AII (1), aristolactam BI (2), velutinam (3), griffithinam (4), isoursuline (5), and sinactine (6). Their structures were determined on the basis of spectroscopic analysis and comparison with the literature data. Described in this work is the first report of isoursuline (5), an azafluorene derivative, and sinactine (6), a tetrahydroepiberberine, being isolated from plants in the *Uvaria* genus. Antioxidant and antimicrobial activities of some isolated compounds were evaluated. Among screened compounds, velutinam (3) showed antioxidant activity in the DPPH radical scavenging assay.

KEYWORDS: *Uvaria grandiflora*, alkaloids, aristolactams, antioxidant activity, antimicrobial activity

INTRODUCTION

Uvaria genus belongs to the family Annonaceae. Plants in the *Uvaria* genus are distributed throughout tropical areas of Asia, Australia, and Africa, of which 15 species were reported to be found in various parts of Thailand [1]. Most of the *Uvaria* plants grown in Thailand are climbing shrubs and small trees. The fruits have deep orange, yellow, or rich red color, are usually aggregated, and are edible in some species [2]. Based on ethnopharmacological investigation, several species are used as folk medicine for treatment of several diseases. For example, the ethanolic extract of the roots of *U. chamae* was used as an antidiabetic agent and for the treatment of infections [3]. The decoction of the roots of *U. cherrevensis* was used for treatment of urinary disorders and being used as tonic for blood system and kidney [4].

Uvaria grandiflora Roxb. is a long climbing tree and is distributed in Southeast Asian countries including Vietnam, Malaysia, and Thailand. Various

parts of *U. grandiflora* have long been used in traditional medicines for curing contusion and body tonic therapies and as a cardiogenic [5]. Previous phytochemical studies on the *U. grandiflora* resulted in the isolation of various types of compounds including polyoxygenated cyclohexene oxides [6], terpenoids [6], aromatic derivatives [7, 8] as well as an alkaloid, velutinam [6], and some of which exhibited promising biological activities. For example, zeylenone, a polyoxygenated cyclohexenone derivative isolated from the leaves and roots of *U. grandiflora*, displayed significant cytotoxic activity in chronic myelogenous leukemia-derived K562 cell line in both *in vitro* and *in vivo* assays [9]. Inspired by the ethnomedicinal use of various parts of *U. grandiflora* as folk medicines and interesting biologically active components reported to be found in its leaves and roots, it is of high interest to further examine other plant parts of the *U. grandiflora*. In the course of our continuing efforts to search for biologically active substances from this plant [6–9], we reported herein the isolation and structural iden-

tification of phytochemical components from the MeOH extract of twigs of *U. grandiflora*. It is worth mentioning that there was no prior phytochemical investigation on the twigs of *U. grandiflora* found in the literatures. Thus, the MeOH-EtOAc (1:1 v/v) soluble fraction partitioned from the MeOH extract of twigs of *U. grandiflora* Roxb. was investigated and led to the isolation of six known compounds comprising aristolactam AII (1), aristolactam BI (2), velutinam (3), griffithinam (4), isoursuline (5), and sinactine (6). Antioxidant and antimicrobial activities of some isolated compounds were evaluated, and the results were described and discussed in this manuscript.

MATERIALS AND METHODS

General experimental procedures

^1H , ^{13}C , and 2D NMR spectra were recorded on either a Bruker Ascend™ 400 (Germany) spectrometer and/or a Jeol 400 YH (Japan) spectrometer in deuterated chloroform (CDCl_3 , Merck, Germany) or deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$, Merck, Germany) solutions using TMS or residual non-deuterated solvent peak as an internal standard. Solvents for extraction, chromatography, and crystallization were distilled at their boiling point ranges prior to use. Pre-coated TLC aluminium sheets of silica gel 60 PF₂₅₄ (20 × 20 cm, layer thickness 0.2 mm, Merck, Germany) were used for analytical purposes. The chemical compositions were visualized under ultraviolet light (at 254 and/or 365 nm) and/or spraying with 12% H_2SO_4 in ethanol, anisaldehyde solution, and/or Dragendoff's reagents. Plates of silica gel PF₂₅₄ (5–40 μm , Merck, Germany) were activated at 120 °C for 2 h and utilized for preparative TLC separation. Column chromatography was performed by using silica gel 60 (60–200 mm or 70–230 mesh ASTM, Merck, Germany) and Sephadex LH-20 (Merck, Germany).

Plant materials

The twigs of *Uvaria grandiflora* Roxb. were collected from Trang Province, Thailand in May 2019. The plant was identified by Dr. Sakchai Hongthong, and a voucher specimen (RRU-SH-008) was deposited at the Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand.

Extraction and isolation

The air-dried and powdered twigs of *U. grandiflora* (1.2 kg) were extracted with EtOAc (3 l × 5 times) and MeOH (3 l × 5 times) at room temperature.

After filtration and removal of the solvents under reduced pressure, the EtOAc extract (46.3 g) and MeOH extract (154.8 g) were obtained. The MeOH extract was dissolved in MeOH-EtOAc (1:1 v/v, 2 l) to provide MeOH-EtOAc (1:1 v/v) soluble fraction (92.0 g) and insoluble residue (49.1 g) after removal of solvents under reduced pressure and freeze-drying. The MeOH-EtOAc (1:1 v/v) fraction was subjected to screening for its antioxidant activity using DPPH assay. Guided by the results of biological activity and the fraction with sufficient amount, the MeOH-EtOAc (1:1 v/v) fraction was first separated by vacuum column chromatography (VCC) technique using a gradient solvent system of EtOAc-hexanes (0–100%) and MeOH-EtOAc (0–100%) to give six subfractions (A1–A6).

Subfraction A2 (6.4 g) was purified using silica gel column chromatography (Si-gel CC) eluted with a gradient solvent system of EtOAc-hexanes (0–100%) and MeOH-EtOAc (0–100%) to afford subfractions A2.1–A2.4. Compound 6 (66.9 mg) was obtained after A2.2 was purified on preparative thin layer chromatography (prep. TLC) using 5% acetone-hexanes as eluent. Subfraction A2.3 was purified on Sephadex LH-20 CC (MeOH as eluent) and prep. TLC with 5% EtOAc-hexanes as eluent to give compound 2 (17.1 mg).

After purification of A3 (7.3 g) on Si-gel CC with MeOH- CH_2Cl_2 (0–100%) as eluent, subfractions A3.1–A3.4 were obtained. Subfractions A3.2 and A3.3 were combined and purified on Sephadex LH-20 CC eluted with MeOH followed by crystallization (MeOH- CH_2Cl_2) to provide compound 2 (21.9 mg) and compound 3 (64.0 mg).

Subfraction A4 (18.4 g) were fractionated by Si-gel CC using MeOH- CH_2Cl_2 (0–100%) as eluent to afford subfractions A4.1–A4.7. Subfraction A4.3 was chromatographed on Sephadex LH-20 CC eluted with MeOH to yield compound 1 (18.2 mg). Subfraction A4.4 was isolated by Si-gel CC eluted with a gradient solvent system of MeOH- CH_2Cl_2 (0–100%) followed by Sephadex LH-20 column eluted with MeOH to give compound 5 (9.4 mg). Subfraction A4.5 was purified by Si-gel CC eluted with MeOH- CH_2Cl_2 (0–100%) to give subfractions A4.5.1–A4.5.3. Purification of subfraction A4.5.1 on Sephadex LH-20 CC eluted with MeOH and prep. TLC using 5% MeOH- CH_2Cl_2 yielded compound 4 (25.0 mg) (for details of the ^1H , ^{13}C NMR and HR-ESI-MS data of each compound, see Tables S1–S3; and their ^1H and ^{13}C NMR spectra, see Figs. S1–S12).

Antioxidant activity

Antioxidant activity of fractions and isolated compounds was examined on the basis of the scavenging effect on the stable DPPH free radical activity. The samples were prepared in various concentrations by dissolution and dilution in MeOH (analytical grade). Two ml of methanol was added to 1 ml of pure compound solution with different concentrations (31.25, 62.50, 125, 250, and 500 $\mu\text{g/ml}$) and the fraction solution (93.75, 187.50, 375, 750, and 1500 $\mu\text{g/ml}$). Four ml of methanolic solution of 0.3 mM DPPH was added, and the mixture was shaken vigorously. The mixtures were immediately incubated at room temperature in the absence of light for 30 min before the absorbance at 517 nm was measured. The assay was performed in triplicate. The percentage of inhibition of DPPH free radical was evaluated following the below equation.

$$(\%) \text{inhibition} = \frac{AB - AA}{AB} \times 100$$

where AA and AB are the absorbance values of the test and of the blank sample, respectively. IC_{50} value indicating the concentration at which a sample would inhibit free radicals by 50% was also calculated [10].

Antimicrobial activity

The antibacterial activity against Gram-positive bacteria: *Staphylococcus aureus* (ATCC29213), Gram-negative bacteria: *Escherichia coli* (ATCC25922), and fungal pathogen: *Candida albicans* (ATCC 10231) was examined by disc diffusion method and was performed at Scientific Instruments Centre, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Maximum tested concentration was done at 10 $\mu\text{g/ml}$ for antibacterial activity. The disc diffusion test was performed. The inoculum suspension of each microbial strain was swabbed on the entire surface of Mueller-Hinton agar (MHA, Difco) for bacteria and SDA for yeasts. Sterile 6 mm filter paper discs were aseptically placed on MHA and SDA surfaces. Twenty μl of sample was immediately added to discs. A 20 μl aliquot of 10% DMSO was also added to a sterile paper disc as a negative control. Penicillin G (10 unit/ml), gentamicin (10 $\mu\text{g/ml}$), and nystatin (10 $\mu\text{g/ml}$) were used as positive controls for *S. aureus*, *E. coli*, and *C. albicans* test, respectively. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation at 37 $^{\circ}\text{C}$ for 24 h for bacteria and

at 30 $^{\circ}\text{C}$ for 72 h for fungus. Diameters of inhibition zones were measured [11].

RESULTS AND DISCUSSION

Chromatographic purification of the MeOH-EtOAc (1:1 v/v) soluble fraction partitioned from the MeOH extract of twigs of *U. grandiflora* Roxb. yielded the known compounds 1–6. By comparison of their spectroscopic data with those previously reported in the literature, the structures of the isolated compounds were identified as four aristolactam derivatives: aristolactam AII (1) [12], aristolactam BI (2) [12], velutinam (3) [12], and griffithinam (4) [13]; an azafluorene, isoursuline (5) [14], and a tetrahydroepiberberine, sinactine (6) [15]; see Fig. 1. The spectroscopic data including ^1H , ^{13}C NMR, and HR-ESI-MS data were summarized and mentioned in Supplementary data, Tables S1–S3, respectively.

To date, many plant species of the *Uvaria* genus have been thoroughly investigated, and a number of biologically active substances were characterized and reported. Among those, polyoxygenated cyclohexene derivatives are a large group found in the plants of the *Uvaria* genus. Zeylenone, a natural cyclohexene oxide first isolated from the EtOH extract of the leaves of *U. grandiflora*, exhibited strong suppressive activity in several cancer cells [9]. Aristolactams were reported as a common type of alkaloids found in the *Uvaria* genus. Piperolactam C was isolated as a major component from the MeOH extract of stem bark of *U. hamiltonii* [16] together with aristolactam AIII, aristolactam BII, goniopedaline, and griffithinam [17]. A few aporphine alkaloids consisting of crotspatine, crotonosine, and zenkerine were isolated from the stems of *U. klaineana* [18]. In addition, indole derivatives, 3-farnesylindole, and its derivatives were isolated from the MeOH extract of the root bark of *U. pandensis* [19]. Recent study of *U. grandiflora* revealed that an aristolactam, velutinam (3), was isolated from the aerial parts of *U. grandiflora* [20]. The present work expanded the library of aristolactam alkaloids including aristolactam AII (1) [12], aristolactam BI (2) [12], and griffithinam (4) [13] to be isolated from *U. grandiflora*. Additionally, an azafluorene, isoursuline (5) [14], and a tetrahydroepiberberine, sinactine (6) [15], were isolated for the first time from plants in the *Uvaria* genus. Moreover, velutinam (3) and griffithinam (4) were considered potential chemotaxonomic markers for plants in the *Uvaria* genus. On the basis of an extensive review on alkaloids derived from plants in the *Uvaria*

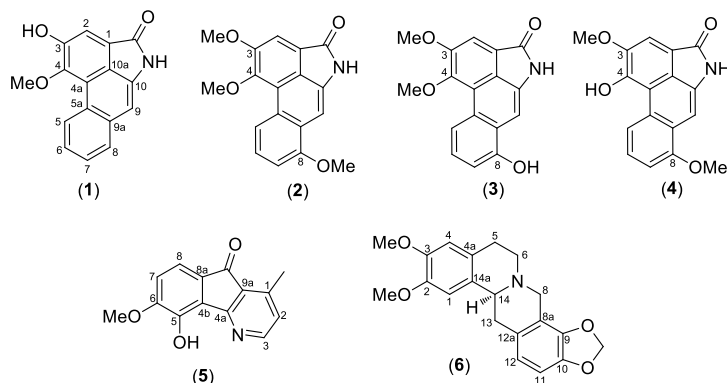


Fig. 1 Structure of compounds 1–6.

genus along with our present research results, the aristolactam derivatives can be considered powerful evidences to support the taxonomic location of the *Uvaria* plants. The MeOH-EtOAc (1:1 v/v) soluble fraction and the isolated compounds 1–4 and 6 were evaluated for their antioxidant activity using DPPH free radical scavenging assay. The MeOH-EtOAc (1:1 v/v) soluble fraction and velutinam (3) exhibited free radical scavenging activity with an IC_{50} (50% inhibitory concentration) values of 310.0 ± 7.6 and 240.1 ± 6.5 $\mu\text{g/ml}$, respectively. Compounds 1–2, 4, and 6 were inactive in the DPPH free radical scavenging assay (Table 1). The antimicrobial activity against tested microbes including *Staphylococcus aureus* (ATCC29213), *Escherichia coli* (ATCC25922), and *Candida albicans* (ATCC 10231) at 10 $\mu\text{g/ml}$ concentration revealed that all the compounds screened did not show inhibitory activity (for information detail; see Table S4). According to literature reports, the crude extracts and pure compounds isolated from various parts of *U. grandiflora* were examined for several biological activities such as cytotoxic, anti-inflammatory, antioxidant, and antimicrobial activities [6–9, 20–22]. To the best of our search from SciFinder database, compounds 1–6 have never been investigated for their antioxidant activity. Therefore, described in this work was the first study on antioxidant activity of compounds 1–4 and 6. Aristolactam AII (1) and velutinam (3) isolated from *Goniothalamus velutinus* were previously examined for their antimicrobial activity against *Bacillus subtilis*, *Bacillus spizizenii*, and *Staphylococcus aureus* and found to be inactive [23]. In the present work, velutinam (3) isolated from *U. grandiflora* also did not exhibit antimicrobial activity to all tested bacterial strains. Thus, our observation on antimicrobial activity of velutinam (3) agreed well with the previous report [23].

Table 1 Antioxidant activity of MeOH-EtOAc (1:1 v/v) soluble fraction, isolated compounds 1–4, and 6 isolated from the twigs of *U. grandiflora* on DPPH free radical.

Sample	IC_{50} ($\mu\text{g/ml}$)
MeOH-EtOAc (1:1 v/v) fraction	310.0 ± 7.6
Aristolactam AII (1)	Inactive
Aristolactam BI (2)	Inactive
Velutinam (3)	240.1 ± 6.5
Griffithinam (4)	Inactive
Sinactine (6)	Inactive
Quercetin	40.9 ± 0.8

Results are average of three independent experiments \pm standard deviation. Quercetin was used as a positive control. Inactive = >500 $\mu\text{g/ml}$.

CONCLUSION

The present study described phytochemical investigation on the MeOH-EtOAc (1:1 v/v) soluble fraction partitioned from the MeOH extract of twigs of *U. grandiflora* Roxb. and led to the isolation of four aristolactams 1–4, azafluorene 5, and tetrahydroepiberberine 6. Except for velutinam (3), aristolactam AII (1), aristolactam BI (2), and griffithinam (4) were isolated for the first time from *U. grandiflora*. It is also worth emphasizing that isoursuline (5) and sinectine (6) were isolated for the first time from the *Uvaria* genus. In addition, velutinam (3) and griffithinam (4) are potentially being used as chemotaxonomic markers for plants in the *Uvaria* genus. For biological activity aspects, except for compound 5, there was no prior report on antioxidant activity of other isolated compounds examined in this work. Among compounds screened for their antioxidant and antimicrobial activities, only velutinam (3) exhibited antioxidant activity in the DPPH radical scavenging assay.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at <http://dx.doi.org/10.2306/scienceasia1513-1874.2021.S002>.

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Appendix A. Supplementary data

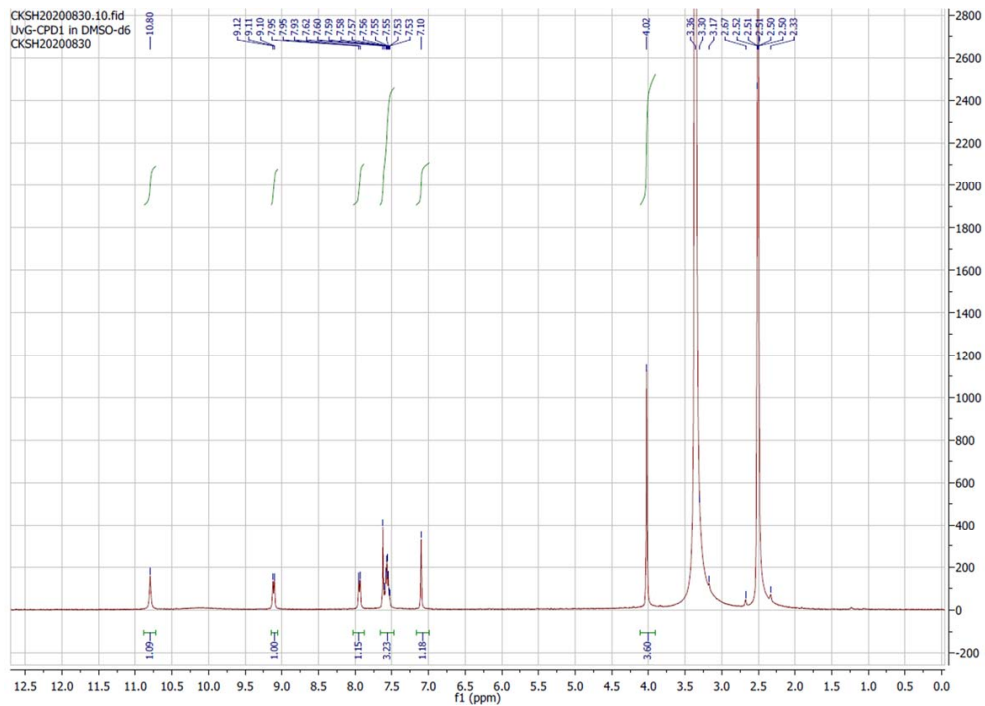


Fig. S1 The ¹H NMR spectrum of aristolactam AII (1) in DMSO-d₆.

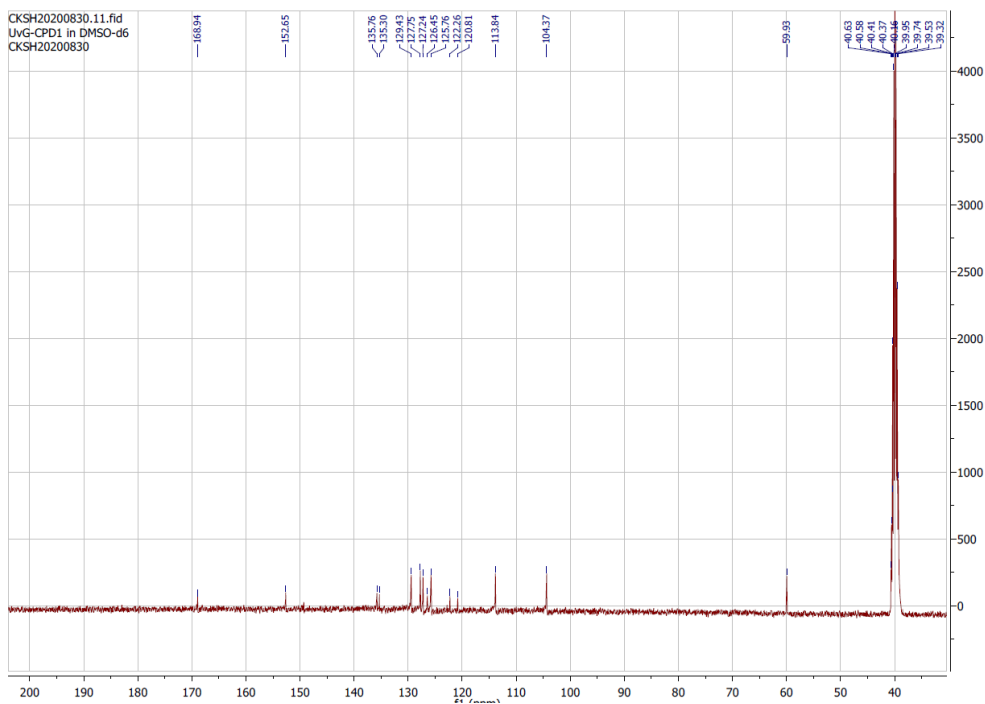


Fig. S2 The ¹³C NMR spectrum of aristolactam AII (1) in DMSO-d₆.

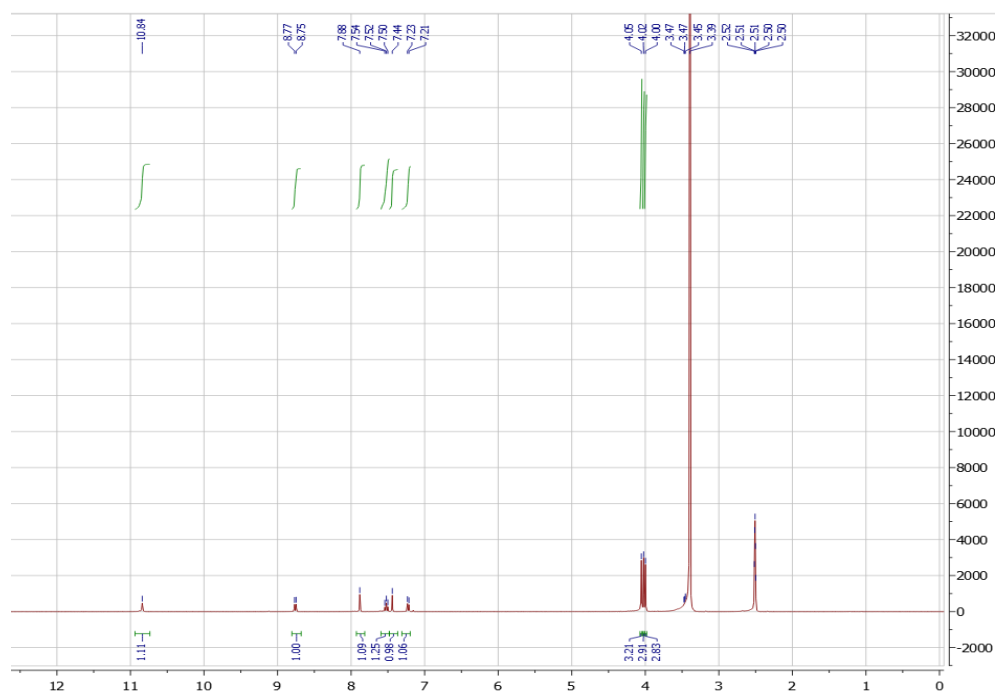


Fig. S3 The ^1H NMR spectrum of aristolactam BI (2) in $\text{DMSO}-d_6$.

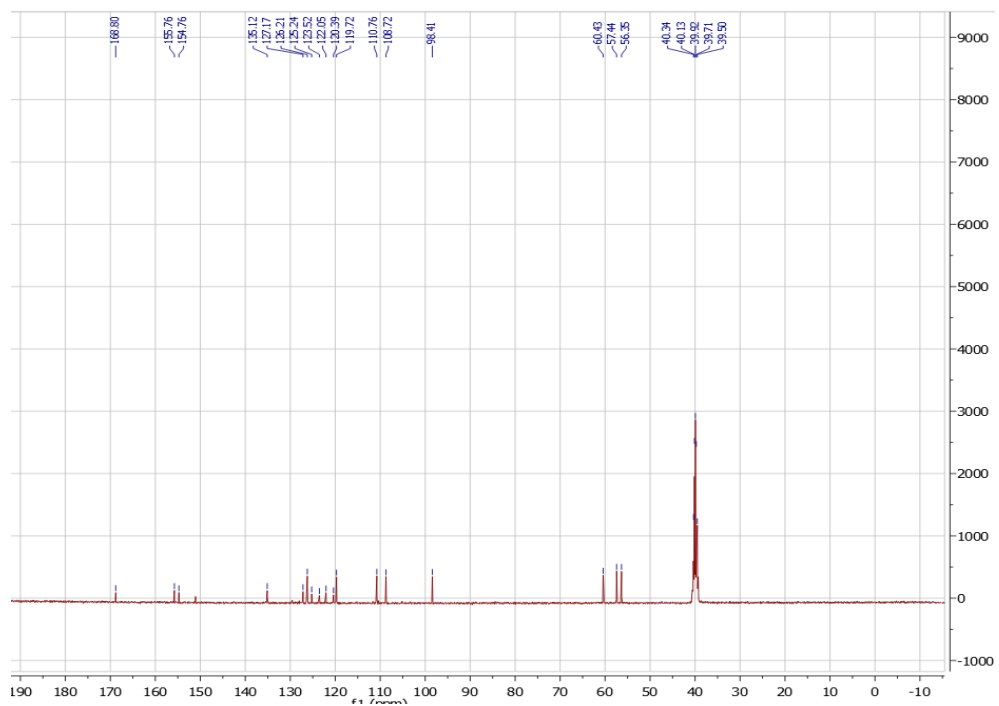


Fig. S4 The ^{13}C NMR spectrum of aristolactam BI (2) in $\text{DMSO}-d_6$.

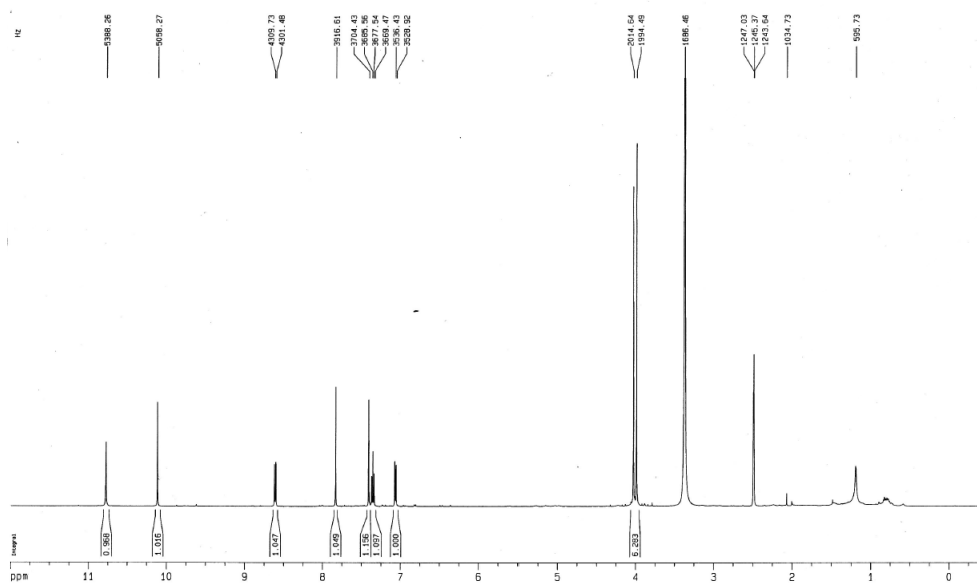


Fig. S5 The ¹H NMR spectrum of velutinam (3) in DMSO-*d*₆.

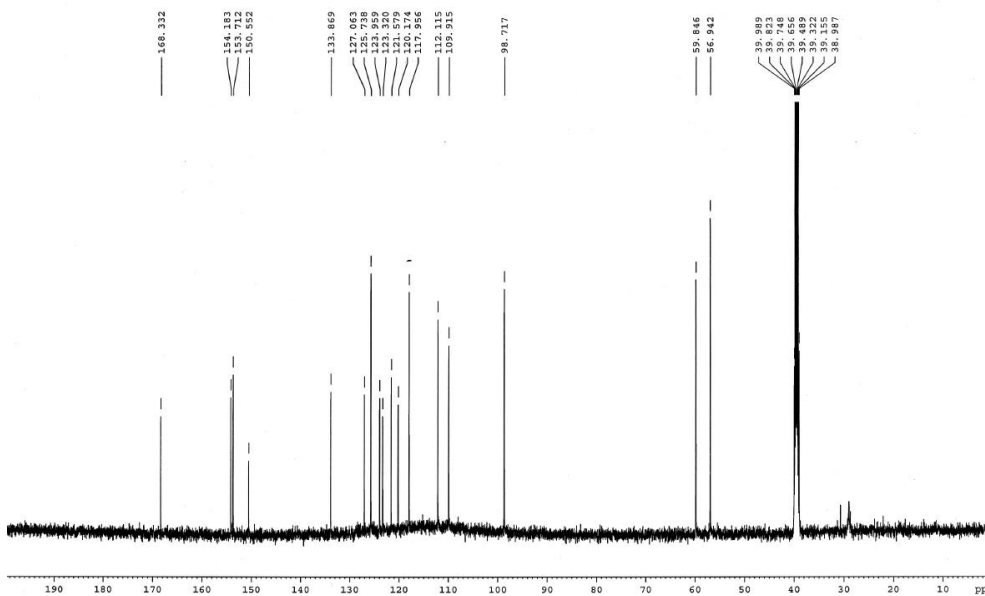


Fig. S6 The ¹³C NMR spectrum of velutinam (3) in DMSO-*d*₆.

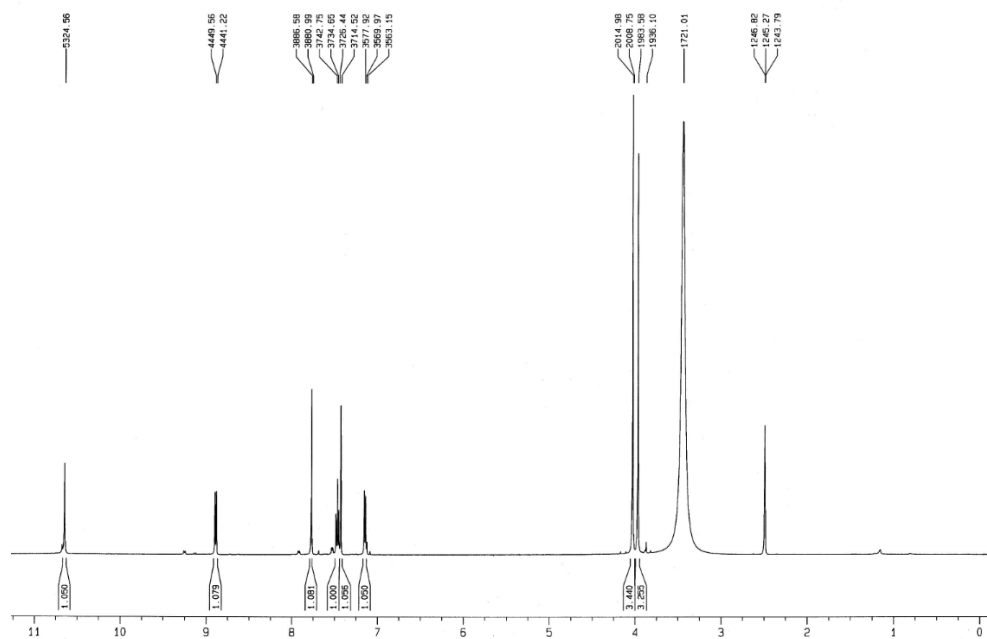


Fig. S7 The ^1H NMR spectrum of griffithinam (4) in DMSO-d_6 .

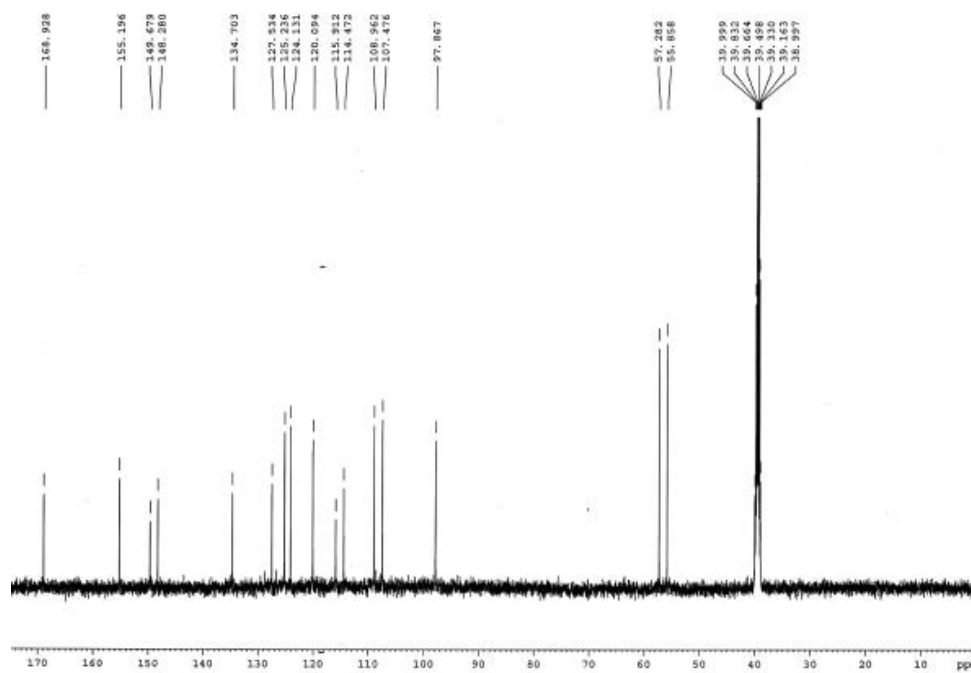


Fig. S8 The ^{13}C NMR spectrum of griffithinam (4) in DMSO-d_6 .

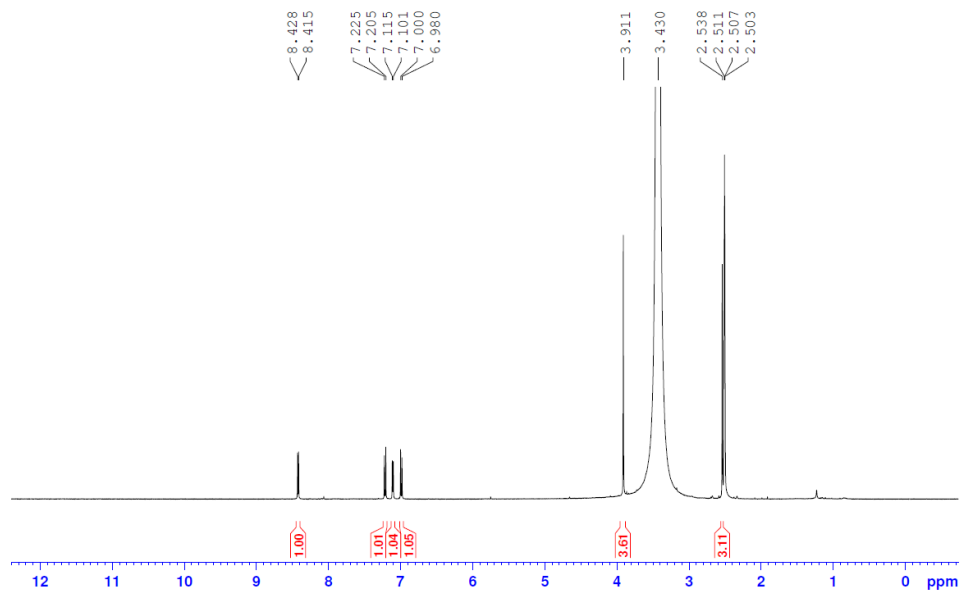


Fig. S9 The ^1H NMR spectrum of isoursuline (5) in $\text{DMSO-}d_6$.

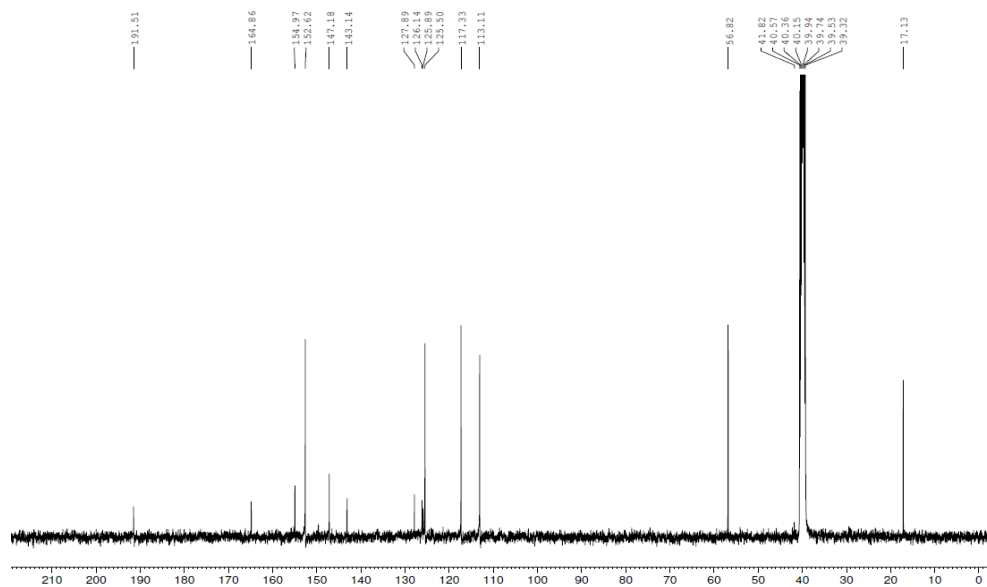


Fig. S10 The ^{13}C NMR spectrum of isoursuline (5) in $\text{DMSO-}d_6$.

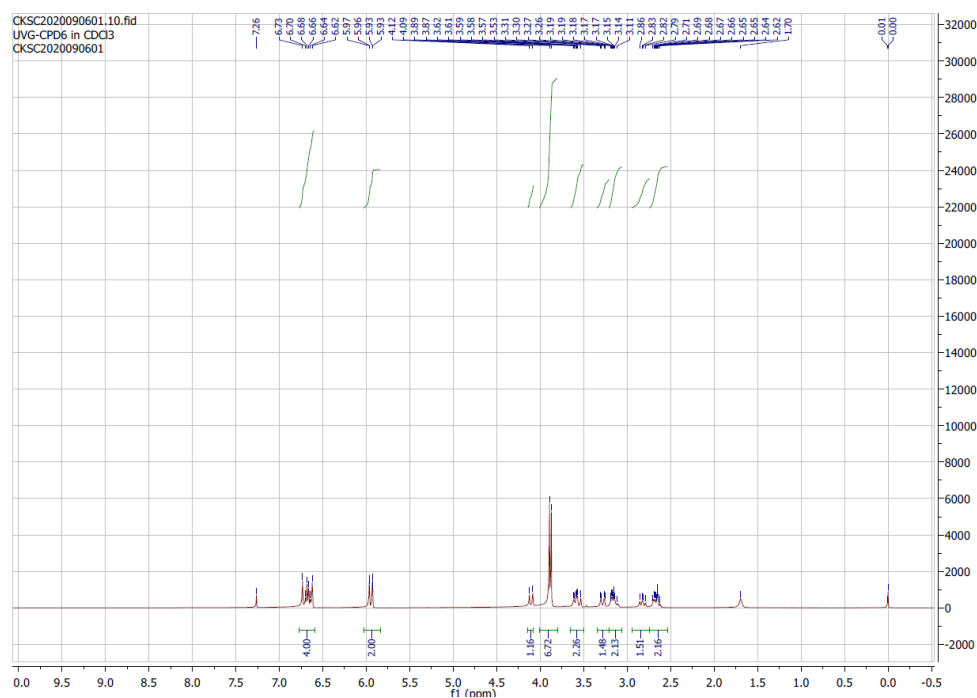


Fig. S11 The ^1H NMR spectrum of sinactine (6) in CDCl_3 .

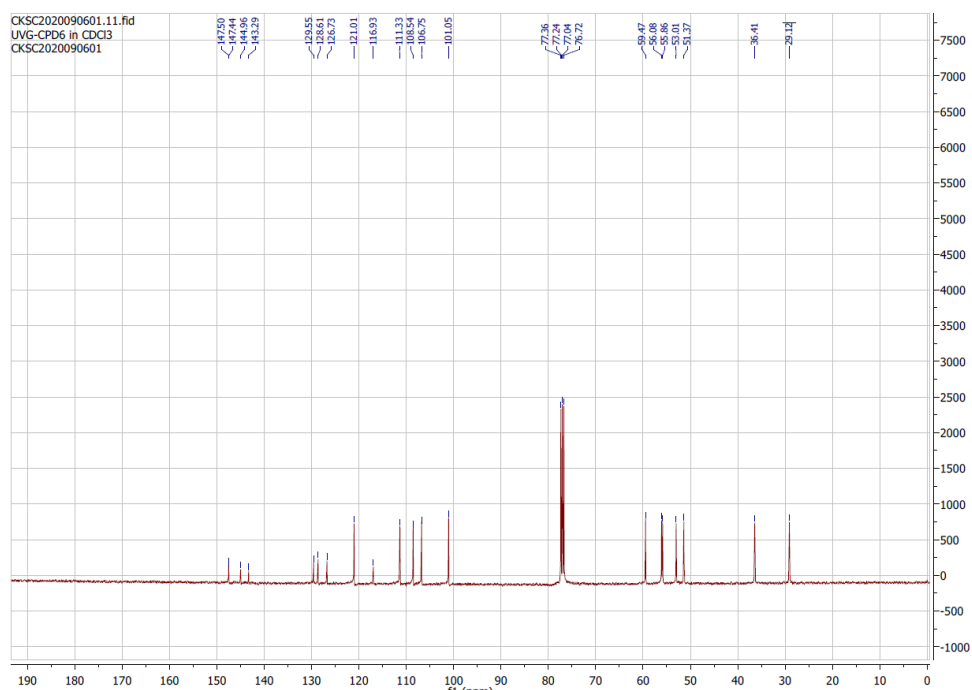


Fig. S12 The ^{13}C NMR spectrum of sinactine (6) in CDCl_3 .

Table S1 ¹H NMR data of compounds 1–6.

position	1 in DMSO- <i>d</i> ₆ δ _H , mult, <i>J</i> (Hz) ^a	2 in DMSO- <i>d</i> ₆ δ _H , mult, <i>J</i> (Hz) ^a	3 in DMSO- <i>d</i> ₆ δ _H , mult, <i>J</i> (Hz) ^b	4 in DMSO- <i>d</i> ₆ δ _H , mult, <i>J</i> (Hz) ^b	5 in DMSO- <i>d</i> ₆ δ _H , mult, <i>J</i> (Hz) ^a	6 in CDCl ₃ δ _H , mult, <i>J</i> (Hz) ^a
1	–	–	–	–	–	6.73–6.62, m
2	7.62, s	7.88, s	7.83, s	7.61, s	7.11, d (5.3)	–
3	–	–	–	–	8.42, d (5.3)	–
4	–	–	–	–	–	6.73–6.62, m
5	9.11, d (8.1)	8.76, d (8.3)	8.61, d (8.0)	8.89, d (8.3)	–	a) 3.25–3.11, m b) 2.77–2.61, m
6	7.60–7.53, m	7.52, t (8.2)	7.35, t (8.0)	7.46, d, (8.2)	–	a) 3.28, dd (15.9, 3.7) b) 2.77–2.61, m
7	7.60–7.53, m	7.22, d (8.0)	7.06, d (8.0)	7.14, d, (8.0)	6.99, d (8.0)	–
8	7.94, d (8.7)	–	–	–	7.22, d (8.0)	4.11, d (15.3) 3.70–3.46, m
9	7.10, s	7.44, s	7.41, s	7.43, s	–	–
11	–	–	–	–	–	6.73–6.62, m
12	–	–	–	–	–	6.73–6.62, m
13	–	–	–	–	–	a) 3.25–3.11, m b) 2.92–2.74, m 3.70–3.46, m
14	–	–	–	–	–	–
NH	10.80, s	10.84, s	10.78, s	10.65, s	–	–
1–CH ₃	–	–	–	–	2.50, s	–
2–OCH ₃	–	–	–	–	–	3.87, s
3–OCH ₃	–	4.05, s	4.03, s	4.03, s	–	3.89, s
4–OCH ₃	4.02, s	4.02, s	3.99, s	–	–	–
6–OCH ₃	–	–	–	–	3.91, s	–
8–OCH ₃	–	4.00, s	–	3.96, s	–	–
O–CH ₂ –O	–	–	–	–	–	5.95, dd (14.6, 1.6)

^a Data were recorded at 400 MHz.^b Data were recorded at 500 MHz.**Table S2** ¹³C NMR data of compounds 1–6.

position	1 in DMSO- <i>d</i> ₆ δ _C (ppm) ^a	2 in DMSO- <i>d</i> ₆ δ _C (ppm) ^a	3 in DMSO- <i>d</i> ₆ δ _C (ppm) ^b	4 in DMSO- <i>d</i> ₆ δ _C (ppm) ^b	5 in DMSO- <i>d</i> ₆ δ _C (ppm) ^a	6 in CDCl ₃ δ _C (ppm) ^a
1	122.3	125.2	121.6	115.9	147.2	108.5
2	113.8	108.7	109.9	109.0	125.5	147.4
3	152.7	154.8	154.2	148.3	152.6	147.5
4	152.7	151.1	150.6	149.7	–	111.3
4a	122.3	122.1	120.2	114.5	164.8	126.7
4b	–	–	–	–	125.9	–
5	126.4	119.7	118.0	120.1	143.1	29.1
5a	127.2	120.4	127.1	124.1	–	–
6	127.8	123.5	125.7	125.2	154.9	51.4
7	125.8	110.8	112.1	107.5	113.1	–
8	129.4	155.8	153.7	155.2	117.3	53.0
8a	–	–	–	–	127.9	116.9
9	104.3	98.4	98.7	97.9	191.5	143.3
9a	135.8	126.2	124.0	127.5	126.1	–
10	135.3	135.1	133.9	134.7	–	145.0
10a	120.8	127.2	123.3	124.1	–	–
11	–	–	–	–	–	106.8
12	–	–	–	–	–	121.0
12a	–	–	–	–	–	128.6
13	–	–	–	–	–	36.4
14	–	–	–	–	–	59.5
14a	–	–	–	–	–	129.6
C=O	168.9	168.8	168.3	168.9	–	–
1–CH ₃	–	–	–	–	17.1	–
2–OCH ₃	–	–	–	–	–	55.9
3–OCH ₃	–	60.4	56.9	57.3	–	56.0
4–OCH ₃	59.9	57.4	59.9	–	–	–
6–OCH ₃	–	–	–	–	56.8	–
8–OCH ₃	–	56.4	–	55.9	–	–
O–CH ₂ –O	–	–	–	–	–	101.1

^a Data were recorded at 100 MHz.^b Data were recorded at 125 MHz.

Table S3 HR-ESI-MS of compounds 1–6.

Compound	HRMS-ESI-TOF (<i>m/z</i> , Mol)		
	Molecular formula	Found	Calculated
1	C ₁₆ H ₁₂ NO ₃ [M + H] ⁺	266.0818	266.0817
2	C ₁₈ H ₁₅ NO ₄ Na [M + Na] ⁺	332.0841	332.0899
3	C ₁₇ H ₁₄ NO ₄ [M + H] ⁺	296.1050	296.0923
4	C ₁₇ H ₁₃ NO ₄ Na [M + Na] ⁺	318.0760	318.0742
5	C ₁₄ H ₁₁ NO ₃ Na [M + Na] ⁺	264.2356	264.0637
6	C ₂₀ H ₂₁ NO ₄ [M] ⁺	339.1497	339.1471

Table S4 Antimicrobial activity of compounds 2, 3, 4, and 6.

Sample code	Replicate	Zone diameter (mm)		
		<i>Escherichia coli</i> (ATCC25922)	<i>Staphylococcus aureus</i> (ATCC29213)	<i>Candida albicans</i> (ATCC 10231)
aristolactam BI (2)	1	–	–	–
velutinam (3)	1	–	–	–
griffithinam (4)	1	–	–	–
sinectine (6)	1	–	–	–
gentamicin *		22.54		
penicillin *			30.51	
nystatin *				23.72

* Positive control.