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Kitasatodine and Kitasatopenoid from *Kitasatospora* sp. H6549, a New Strain from Malaysia

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Abstract: A new pyridine-containing natural product, kitasatodine (**1**), and a new sesquiterpene, kitasatopenoid (**2**), together with a known cycloheximide (**3**) were isolated from the ethyl acetate extract of the strain H6549 (*Kitasatospora* sp.), which was isolated from dipterocarp forest of Kepong Kuala Lumpur in Malaysia. Their structures were established by spectroscopic methods including 1D and 2D-NMR experiments and HR-Q-TOF-MS. In addition, compounds **1** and **2** showed moderate cytotoxicity against HeLa and HepG-2 cell lines.

Keywords: *Kitasatospora* sp.; **k**itasatodine; kitasatopenoid; spectroscopic; cytotoxicity.

1. Introduction

Actinomycetes are valuable economical and biotechnological prokaryotes. They have been known for the production of bioactive secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents and enzymes [1]. Studies demonstrate that actinomycetes offer some promises for the discovery of novel metabolites with pharmacological and agricultural potentials.

In an ongoing search for novel inhibitors of eukaryotic cellular functions, large numbers of putatively rare and novel actinomycetes were isolated from Malaysia [2–5]. Among those the genus *kitasatospora* was a potentially rich source of metabolic diversity, which mainly inhabited the soil [6]. For example, antibiotic Sch 725424 from *Kitasatospora* sp. showed inhibitory activity against *Staphylococcus aureus* [7], bafilomycin C1-amide from *Kitasatospora cheerisanensis* showed cytotoxic activity against a series of cancer cell lines [8].

The strain H6549 isolated from dipterocarp forest in Malaysia was a novel *Kitasatospora* species and its crude extract can inhibit luciferase activity in the screening for (TGF-β) signal transduction [9].

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To explore active ingredient of *Kitasatospora* sp. H6549, this strain was cultivated on solid state ISP3 media. Two novel compounds, kitasatodine (**1**) and kitasatopenoid (**2**), along with cycloheximide (**3**) were isolated.

2. Materials and Methods

2.1. Microorganism Material

Kitasatospora sp. H6549 isolated from dipterocarp forest of Kepong Kuala Lumpur in Malaysia was provided kindly by Professor Coy Choke Ho.

2.2. Fermentation and Isolation

The fermentation of *Kitasatospora* sp. H6549 was performed on ISP3 (20 L) agar media for 9 d at 28°C. The culture was diced and extracted with EtOAc/MeOH/AcOH (80 : 15 : 5, v/v/v). The crude extract was partitioned between EtOAc and H_2O (1 : 1) until the EtOAc layer was colorless. The EtOAc-soluble fraction was concentrated in *vacuo* to afford a crude extract (12.7 g). The crude extract was subjected to MPLC [*RP-18* (170 g), MeOH/H_2O (1 : 1 and 3 : 2)] to afford *Fr.1* and *Fr.2*.

Fr.1 (896.9 mg) was subjected to *Sephadex LH-20* column (in MeOH) and then further subjected to MPLC [*RP-18* (30 g), MeOH/H_2O (9 : 11)] to obtain *Fr.1.1* (181.6 mg). *Fr.1.1* was further subjected to MPLC [*RP-18* (30 g), MeOH/H_2O (7 : 13 and 2 : 3)] to afford two fractions *Fr.1.1.1* - *Fr.1.1.2*. *Fr.1.1.1* (45.3 mg) was subjected to *Sephadex LH-20* column (in acetone), then subjected to silica gel chromatography (petroleum ether/acetone 15 : 1) to yield **2** (1.0 mg).

Fr.1.1.2 (73 mg) was subjected to *Sephadex LH-20* column (in acetone) to yield **1** (20.0 mg).

Fr.2 (896.9 mg) was subjected to *Sephadex LH-20* column (in MeOH) to produce *Fr.2.1*. *Fr.2.1* was subjected to *Sephadex LH-20* column (in acetone), then further subjected to silica gel chromatography (petroleum ether/EtOAc 10 : 1, 7 : 1 and 6 : 1) to yield **3** (13.0 mg).

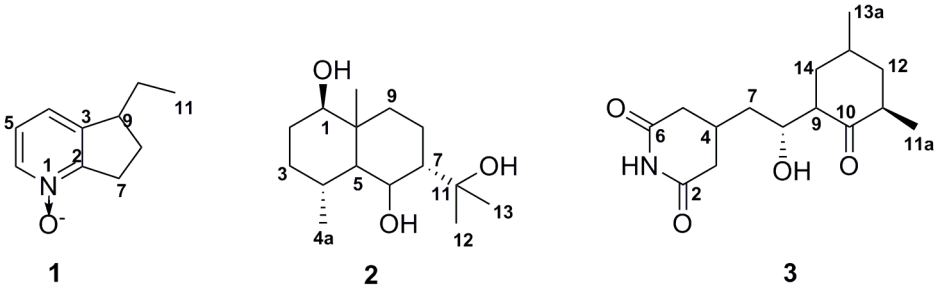


Figure 1. The structures of compounds **1**-**3**

3. Results and Discussion

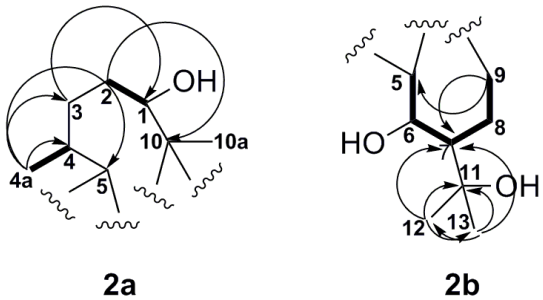
3.1. Structure elucidation

Compound **1** was obtained as colourless oil. HR-Q-TOF-MS gave its molecule ions peaks at *m/z* 164.1135 $[M + H]^+$ and 186.0967 $[M + Na]^+$. The IR absorption at 2922 cm^{-1} indicated the presence of CH_3 and CH_2 groups. The ^{13}C-NMR and DEPT spectra showed ten carbon signals including one Me, three CH_2, four CH, and two quaternary carbons (Table 1). The HSQC and HMBC spectra facilitated the assignments of all ^{1}H- and ^{13}C-NMR signals, indicating a bicyclic structure. The HMBC correlations from H-C(4) to C(2) and C(6), and from H-C(5) to C(3), as well as H-C(6) to C(2), C(4) and C(5), along with the coupling constants $J_{H\text{-}4,5}$, $J_{H\text{-}5,4}$, $J_{H\text{-}5,6}$ suggested the presence of di-substituted

pyridine ring. Additionaly, the HMBC correlations from H-C(7) to C(2), C(3), C(8) and C(9), and ^{1}H-^{1}H COSY indicated five-membered ring, which linked to the pyridine ring via C(2) and C(3). A ethyl group substituent at C(9) was indicated by the HMBC from H-C(10) to C(3) and C(9), and from H-C(11) to C(9) and C(10). Finally, N(1) was oxidized supporting by the molecular $C_{10}H_{13}NONa^{+}$ derived by HR-Q-TOF-MS (*m/z* 186.0967, calcd: 186.0900).

Compound **2** was isolated as amorphous. HR-Q-TOF-MS gave its molecule ions peaks at *m/z* 257.2474 $[M + H]^{+}$ and 279.1966 $[M + Na]^{+}$. The IR absorption at 3441 cm^{-1} and 2924 cm^{-1} indicated the presence of OH and CH_3 groups. The ^{13}C-NMR and DEPT spectra showed fifteen carbon signals including four Me, four CH_2, five CH (two being oxygenated), and two quaternary carbons (one being oxygenated) (Table 2). The HMBC correlations from Me(4a) to C(3), C(4) and C(5), and from H-C(2) to C(10), and from H-C(3) to C(1), along with ^{1}H-^{1}H COSY correlations, established fragment **2a** (Figure 2). In combination with the HMBC correlations from Me(12) to C(7), C(11), and C(13), and from Me(13) to C(7), C(11), and C(12), from H-C(9) to C(5) and C(7), along with ^{1}H-^{1}H COSY correlations, established fragment **2b** (Figure 2). Finally, the HMBC correlations from Me(10a) to C(5) and C(9), connected fragment **2a** and **2b**.

The relative configuration of **2** was determined by the analysis of the NOESY spectrum. The presence of NOESY correlations between H-C(1) and H-C(8), between H-C(4) and H-C(5), and between H-C(7) and H-C(5) indicated that H-C(4) and H-C(7) were on the same orientation.



2a **2b**

Figure 2. Fragments **2a** and **2b**, and selected HMBC (H→C) correlations and ^{1}H-^{1}H COSY (▬) correlations

Compound **3** was isolated as colourless oil. ESI-MS gave its molecule ions at *m/z* 304.1 $[M + Na]^{+}$ and 320.1 $[M + K]^{+}$. The ^{13}C-NMR and DEPT spectra showed fifteen carbon signals including two Me, five CH_2, five CH (one being oxygenated), and three quaternary carbons, at δ 172.3, 172.1 and 216.5. On the basis of the NMR data and comparision with those previously published [10], the structure of compound **3** was identified as cycloheximide.

Cycloheximide is an antibiotic produced by *Streptomyces griseus*. It has been an inhibitor of protein synthesis for decades. Bradbury reported that cycloheximide inhibited TGF-β-induced COX-2 protein expression in pulmonary artery smooth muscle cells [11]. And Aranzazu reported that cycloheximide blocked the synthesis of a protein required for TGF-β induced cell death in fetal rat hepatocytes [12]. So we proposed that cycloheximide might inhibited luciferase activity in the screen for (TGF-β) signal transduction. Further research was required.

3.2. Cytotoxicity activity

HeLa cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cells were maintained at 37°C in a humidified atmosphere at 95% air and 5% CO_2. Cell viability was measured by MTT assay [13].

Compound **1** showed moderate activity against HeLa and HepG-2 cell lines in the concentration of 20 μM (69.6%, 62.4%, respectively). Compound **2** showed moderate activity against HepG-2 cell line in the concentration of 20 μM (68.2%).

Table 1. The NMR data for compound **1** (at 600 MHz in C_3D_6O, δ in ppm, *J* in Hz).

No.	δ_H	δ_C	HMBC	COSY
2	/	152.1s	/	/
3	/	145.5s	/	/
4	7.20 (1H, *d*, *J* = 8.3)	120.8d	C-2, C-6, C-9	/
5	7.22 (1H, *t*, *J* = 7.2)	124.3d	C-3	H-6
6	7.99 (1H, *d*, *J* = 6.1)	137.1d	C-2, C-4, C-5	H-5
7	2.36 (1H, *m*)	28.0t	C-2, C-3, C-8, C-9	H-8
	1.80 (1H, *ddd*, *J* = 7.0, 13.4, 16.1)			
8	3.05 (1H, *ddd*, *J* = 4.8, 9.3, 14.2)	27.9t	C-2, C-3, C-7, C-9	H-7
	2.93 (1H, *ddd*, *J* = 8.0, 16.6)			
9	3.21 (1H, *m*)	45.3d	C-2, C-3, C-8, C-11	H-10, H-8
10	1.90 (1H, *m*)	27.3t	C-3, C-9	H-9, H-11
	1.53 (1H, *m*)			
11	0.97 (3H, *t*, *J* = 7.4)	10.8q	C-9, C-10	H-10

Table 2. The NMR data for compound **2** (at 600 MHz in $CDCl_3$, δ in ppm, *J* in Hz).

No.	δ_H	δ_C	HMBC	COSY
1	3.26 (1H, *dd*, *J* = 4.1, 11.5)	80.2d	/	H-2
2	1.69 (1H, overlapped)	26.1t	C-10	H-1
	1.61 (1H, overlapped)			
3	1.63 (2H, overlapped)	30.9t	C-1	/
4	2.25 (1H, *m*)	25.8d	/	H-4a
4a	0.99 (3H, *d*, *J* = 7.6)	14.5q	C-3, C-4, C-5	H-4
5	1.25 (1H, *dd*, *J* = 4.5, 10.7)	52.0d	C-9	H-4a, H-7
6	3.98 (1H, *t*, *J* = 10.3)	69.6d	/	H-5, H-7
7	1.54 (1H, *ddd*, *J* = 4.0, 9.7, 13.7)	54.3d	/	H-6
8	1.62 (1H, overlapped)	22.7t	/	H-7, H-9
	1.19 (1H, *m*)			
9	1.84 (1H, *dt*, *J* = 2.7, 12.1)	39.5t	C-5, C-7, C-10	/
	1.14 (1H, *m*)			
10	/	39.9s	/	/
10a	0.92 (3H, *s*)	14.9q	C-1, C-5, C-9, C-10	/
11	/	75.5s	/	/
12	1.31 (3H, *s*)	23.8q	C-7, C-11, C-13	/
13	1.26 (3H, *s*)	30.5q	C-7, C-11, C-12	/

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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