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Cytotoxic lactam and naphthoquinone alkaloids from roots of *Goniothalamus lanceolatus* Miq.

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ABSTRACT

Two new alkaloids, (−)-goniolanceolactam (**1**) and 2-acetyl-3-amino-1,4-naphthoquinone (**2**), along with two known naphthoquinone alkaloids, 2-acetyl-3-amino-5-hydroxy-1,4-naphthoquinone (**3**) and cleistopholine (**4**) were isolated from the cytotoxic, dichloromethane root extract of *Goniothalamus lanceolatus* (Annonaceae). The structures were elucidated by spectroscopic techniques and the absolute configuration of **1** was established by single-crystal X-ray diffraction. Alkaloid **1** showed cytotoxic activity on human colon and lung cancer cell lines with IC_{50} values ranging from 5.32 to 9.91 μM.

1. Introduction

In Malaysia, *Goniothalamus* species are widely used as a traditional remedy for abortion and postpartum treatment, as well as for fever and skin infections (Wiart, 2007). *Goniothalmus lanceolatus* is an ethomedicinal plant indigenous to Sarawak, Malaysia. Among other uses, this plant is used by the indigenous people as a treatment for cancer. Several lactam and naphthoquinone alkaloids isolated from species of this genus were reported to possess cytotoxic activity (Cao et al., 1998; Soonthornchareonnon et al., 1999; Macabeo et al., 2013; Tran et al., 2013; Nordin et al., 2016). In this study, the dichloromethane root extract of *G. lanceolatus* (100 μg/mL) exhibited promising cytotoxic activity against a panel of human colon and lung cancer cell lines with percent cell viability of less than 15%. Herein the isolation of two new alkaloids; (−)-goniolanceolactam (**1**) and 2-acetyl-3-amino-1,4-naphthoquinone (**2**), along with two known alkaloids, 2-acetyl-3-amino-5-hydroxy-1,4-naphthoquinone (**3**) and cleistopholine (**4**) are reported from the dichloromethane root extract of *G. lanceolatus* (Fig. 1). Alkaloid **1** demonstrated cytotoxicity with IC_{50} values of less than 10 μM against a panel of eight human colon and lung cancer cell lines.

2. Results and discussion

Alkaloid **1** was obtained as white crystals, $[\alpha]_D^{25}$ −31.0 (c 0.52, MeOH) with a melting point of 165–167 °C. Its molecular formula was determined to be $C_{17}H_{14}O_4N$ by LC-ESI-OBITRAP-MS (*m/z* 296.0919, $[M+H]^+$; calculated 296.0917). The IR spectrum displayed an adsorption band at 1733 cm^{-1} for the lactam carbonyl functionality, and the UV spectrum showed absorption bands at λ_{max} 211, 264, and 326 nm. The ^{13}C NMR spectrum (Table 1) showed presence of 17 carbons. Carbon signals for the methylenedioxy, methoxy, and lactam carbonyl were observed at δ_C 102.5, 65.0, and 169.6, respectively. In addition, the DEPT spectrum revealed methylene and methine carbons at δ_C 34.7 and 58.0, respectively. The ^1H NMR spectrum (Table 1) showed two doublets at δ_H 6.09 and 6.18 for the methylenedioxy protons, a methoxy group at δ_H 4.00, and five aromatic protons, one of which is an isolated proton (δ_H 7.08). The proton and carbon NMR spectra of **1** have strong resemblance to those of tapisoidin, a 9,10-dihydroarisolactam alkaloid isolated from *G. tapisoides* (Kim et al., 2013). The presence of a methylenedioxy group between C-3 and C-4 in **1** was confirmed through HMBC correlations of its protons to C-3 (δ_C 150.2) and C-4 (δ_C 147.6). The isolated aromatic proton at δ_H 7.08 (s) was assigned to H-2 of ring A, while the four adjacent aromatic protons at δ_H 7.90 (H-5, *d*, 7.4), 7.35 (H-6 and H-7, *m*), and 7.29 (H-8, *m*) were assigned to ring C (Fig. 2). Assignment of H-10 (δ_H 4.52) and H-9 (δ_H 2.77 and 3.39) were confirmed through a COSY experiment. The remaining quaternary carbons at C-1, C-4a, C-5a, C-8a, and C-11 were established through their HMBC correlations, as listed in Table 1. The

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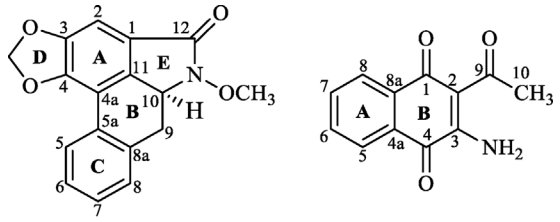

Fig. 1. Structures of alkaloids **1** and **2**.

Table 1
^{1}H NMR, ^{13}C NMR and HMBC data of **1** in $CDCl_3$ (600 MHz).

Position	δ_H (*J* in Hz)	δ_C	HMBC
1	–	136.0	–
2	7.08 (*s*)	102.5	C-1, C-3, C-4, C-4a, C-11, C-12
3	–	150.2	–
4	–	147.4	–
4a	–	113.7	–
5a	–	133.8	–
5	7.90 (d, 7.4)	127.1	C-4a, C-5a, C-7, C-8a, C-9
6	7.29 (*m*)	129.9	C-5a, C-8, C-8a
7	7.29 (*m*)	128.8	C-5, C-5a
8	7.35 (*m*)	128.1	C-5a, C-6, C-8a, C-9
8a	–	129.6	–
9a	3.39 (dd, 6.1, 13.8)	34.7	C-1, C-4a, C-5, C-5a, C-6, C-7, C-8, C-8a, C-10, C-11
9b	2.77 (*t*, 13.8)		
10	4.52 (dd, 6.1, 13.8)	58.0	C-1, C-3, C-4, C-4a, C-5a, C-9, C-11
11	–	120.9	–
12	–	169.6	–
O–CH_2–O	6.09 (d, 1.4) 6.18 (d, 1.4)	102.5	C-3, C-4
N–OCH_3	4.00 (*s*)	65.0	–

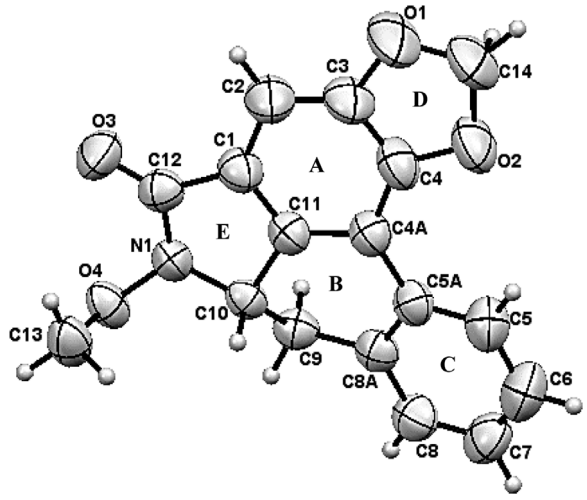

Fig. 2. ORTEP diagram of the structure **1** established by single X-ray crystallography.

IR spectrum of **1** did not reveal any N–H absorption, leading to placement of the methoxy group on the N atom, thus establishing *N*-methoxy lactam functionality in alkaloid **1**. Occurrence of *N*-methoxy aristolactam in nature is rare, have been reported only in *G. tapisoides* (Kim et al., 2013) and two *Piper* species (Wu et al., 1992; Tabopda et al., 2008). It is interesting to note that **1** and tapisoidin from *Goniothalamus* are 9,10-dihydroaristolactams, whereas piperumbellactam C, piperumbellactam D, and piperlactam S from *Piper* species are unsaturated between C-9 and C10.

The absolute stereochemistry of **1** at C-10 was established through single-crystal X-ray diffraction analysis. The ORTEP diagram of **1** (Fig. 2) showed five fused rings A, B, C, D, and E. The five-membered rings were labelled as D and E rings. E (C-1/C-11/C-10/N-1/C-12) is a lactam, whilst D (C-3/C-4/O-1/O-2/C-14) is the dioxolane ring. Ring B and ring C were in an envelope conformation at N-1 and C-14, respectively. The olefinic bond in the six-membered ring B is non-planar with maximum deviation of 0.330 (3)° for the C-9 atom from the least-squares plane. It exists as a screw-boat conformation. No intramolecular hydrogen bonds were observed in the crystal structure of the alkaloid. In the crystal packing, the molecules are linked by C-13-H-13–O-4 intermolecular hydrogen bonds to form two-dimensional chains along the *a* and *b* axes in the unit cell. It was observed that H-10 was positioned with an α-orientation, thus C-10 has an *S* configuration.

Based on the spectroscopic data, the structure of **1** was established as a new aristolactam alkaloid, 10-amino-*N*-methoxy-3,4-methylenedioxyphenyl-9,10*S*-dihydroaristolactam, or (−)-goniolanceolactam. Biosynthetically **1** could be formed through *N*-hydroxylation of aristolactam II followed by *O*-methylation and hydrogenation at C-9 and C-10.

Alkaloid **2** was obtained as a yellow powder with a molecular formula $C_{12}H_{10}O_3N$ established by LC-ESI-OBITRAP-MS ($[M+H]^+$ at *m/z* 216.0760, calculated 216.0661). The IR spectrum indicated the presence of primary amine (3266 and 3160 cm^{-1}), conjugated carbonyl (1626 cm^{-1}), and quinonoid carbonyl (1581 cm^{-1}) groups. The 1D and 2D NMR data of **2** showed strong resemblance to 2-acetyl-3-amino-5-hydroxy-1,4-naphthoquinone **3** (Soonthornchareonnon et al., 1999). However, **2** lacks the hydroxyl group at C-5. The primary amine and methyl ketone groups in **2** are vinylic in nature, hence, this new napthoquinone alkaloid is identified as 2-acetyl-3-amino-1,4-naphthoquinone (Table 2). Two known alkaloids isolated from the dichloromethane root extract of *G. lanceolatus* are 2-acetyl-3-amino-5-hydroxy-1,4-naphthoquinone **3** and cleistopholine **4** (Levrier et al., 2013), also identified based on their spectroscopic data and comparison with literature values.

Compounds **1** and **4** were evaluated for their cytotoxicity against eight human cancer cell lines namely colon (HT29, HCT116, Caco2, and SW48) and lung (A549, Calu-1, NCI-H23, and NCI-H1299) cancer cell lines, as summarized in Table 3. Alkaloid **1** exhibited cytotoxic activity on human colon cancer cells (7.03–9.91 μM) and human lung cancer cells (IC_{50} 5.32–8.46 μM) with IC_{50} values less than that of the positive control, 5-fluorouracil (12.33–35.00 μM). Meanwhile, **4** exhibited IC_{50} values closed with positive control against HT29, SW48, Caco2 and NCI-H23 cancer cell lines. Interestingly, these isolates were non-toxic to non-cancerous cell lines (ARPE19, MCF10A and MRC5) with IC_{50} values greater than 100 μM compared with 5-fluorouracil (17.87–34.86 μM). 5-Fluorouracil is a well-known conventional chemotherapy agent, exhibits cytotoxicity effects against both cancerous and non-cancerous cell (Mai et al., 2014). For human lung cancer cells,

Table 2
^{1}H NMR, ^{13}C NMR and HMBC data of **2** in $CDCl_3$ (600 MHz).

Position	δ_H (*J* in Hz)	δ_C	HMBC
1	–	181.5	–
2	–	130.0	–
3	–	152.7	–
4	–	185.0	–
4a	–	109.3	–
5	8.22 (d, 7.8)	127.5	C-2, C-7
6	7.82 (t, 7.6)	136.3	C-8, C-8a
7	7.67 (t, 7.5)	132.7	C-2, C-5
8	8.09 (d,7.7)	126.6	C-1, C-6, C-8a
8a	–	134.3	–
9	–	202.6	–
10	2.73 (*s*)	33.4	C-9
3-NH_2	7.10 (*br s*)	–	–
	10.67 (*br s*)	–	–
5-OH	–	–	–