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Bioactive styryllactones, two new naphthoquinones and one new styryllactone, and other constituents from *Goniothalamus scortechinii*

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ABSTRACT

Two new naphthoquinones, goniothalaminone A (**1**) and B (**2**), and a new styryllactone, (−)-8-*epi*-9-deoxygoniopypyrone acetate (**12**) together with one known naphthoquinone (**3**), one known indolequinone (**4**), one known 1-azaanthraquinone (**5**), six known styryllactones (**6**–**11**) and one known sesquiterpene (**13**) were isolated from the roots and leaves of *Goniothalamus scortechinii*. The structures of the new compounds were elucidated by spectroscopic analysis and of the known compounds by comparison of their physical, UV, IR, ^{1}H and ^{13}C NMR data with those of published compounds. Antiplasmodial, antimycobacterial and cytotoxic activities of the styryllactones were evaluated. Compounds **6**–**10** exhibited cytotoxic against human cancer cell lines, KB, BC and NCI-H187 with IC_{50} values ranging from 0.13 to 11.7 μg/ml.

1. Introduction

In the course of our investigation for bioactive compounds from Thai natural resources, preliminary screening of the crude EtOAc extract of the roots of *Goniothalamus scortechinii* king revealed antiplasmodial activity against *Plasmodium falciparum* with IC_{50} value of 5.69 μg/ml, showed antimycobacterial activity against *Mycobacterium tuberculosis* with MIC value of 50 μg/ml and also exhibited cytotoxicity against human tumor cell lines, KB and BC, with IC_{50} values of 2.68 and 14.57 μg/ml, respectively. The EtOAc extract of the leaves was inactive against *P. falciparum* and active against *M. tuberculosis* with MIC value of 100 μg/ml and showed cytotoxicity against cancer cell lines, KB and BC, with IC_{50} values of 14.57 and 6.46 μg/ml, respectively. Previous phytochemical investigation of plants of the genus *Goniothalamus* revealed the presence of several groups of natural chemicals, including styryllactones (Birch et al., 1961; Jewers et al., 1972; Sam et al., 1987; Fang et al., 1991, 1993; Lan et al., 2003), 1-azaanthraquinones (Soonthornchareonnon et al., 1999), aporphine alkaloids (Omar et al., 1992; Priestap, 1985; Likhitwitayawuid et al., 1997) and tetrahydrofuran acetogenins (Alkofahi et al., 1988; Jiang and Yu, 1997; Jiang et al., 1998; Alali et al., 1998, 1999). In previous chemical investigations of *G. scortechinii*, scorazanone and goniothalamin were isolated from the roots (Din et al., 1990; Zakaria et al., 1989) and pinocembrine, altholactone, goniofufurone, goniotriol and goniopypyrone were obtained from the fruit peel of this plant (Abdullah et al., 2009). We now report the investigation of the chemical constituents of the roots and leaves of *G. scortechinii*. Two new naphthoquinones, goniothalaminone A (**1**) and B (**2**) and six known compounds, 2-aceto-3-amino-5-hydroxy-1,4-naphthoquinone (**3**) (Soonthornchareonnon et al., 1999), 3-methyl-1*H*-benz[*f*]indole-4,9-dione (**4**) (Tanaka et al., 2001; Efdi et al., 2010), dielsiquinone (**5**) (Goulart et al., 1986; Soonthornchareonnon et al., 1999), goniothalamin (**6**) (Jewers et al., 1972; Sam et al., 1987), goniothalamin oxide (**7**) (Sam et al., 1987; Goh et al., 1995), and 8-chlorogoniodiol (**8**) (Lan et al., 2003) were isolated from the roots of *G. scortechinii*. A new styryllactone, (−)-8-*epi*-9-deoxygoniopypyrone acetate (**12**) was isolated together with the six known styryllactones **6**–**8** and (+)-goniodiol (**9**) (Fang et al., 1991), (+)-altholactone (**10**) (El-Zayat et al., 1985; Peris et al., 2000), (−)-8-*epi*-9-deoxygoniopypyrone (**11**) (Goh et al., 1995; Tai et al., 2010), and one known sesquiterpene, 4,5-*epi*-cryptomeridiol (**13**) (Ahmad et al., 1992) (Fig. 1) were isolated from the leaves of the same plant. The new structures were determined on the basis of spectroscopic data and the known structures were assigned by comparison of their physical, UV, IR, ^{1}H and ^{13}C NMR data with those already published. Compounds **3** and **5** were not detected in the extract of the leaves of the plant. The antiplasmodial, antimycobacterial and cytotoxic activities of selected compounds are also reported.

2. Results and discussion

Goniothalaminone A (**1**) was isolated as brown needles with a molecular formula $C_{11}H_9NO_4$ as determined by HR-TOF-MS (*m*/*z*

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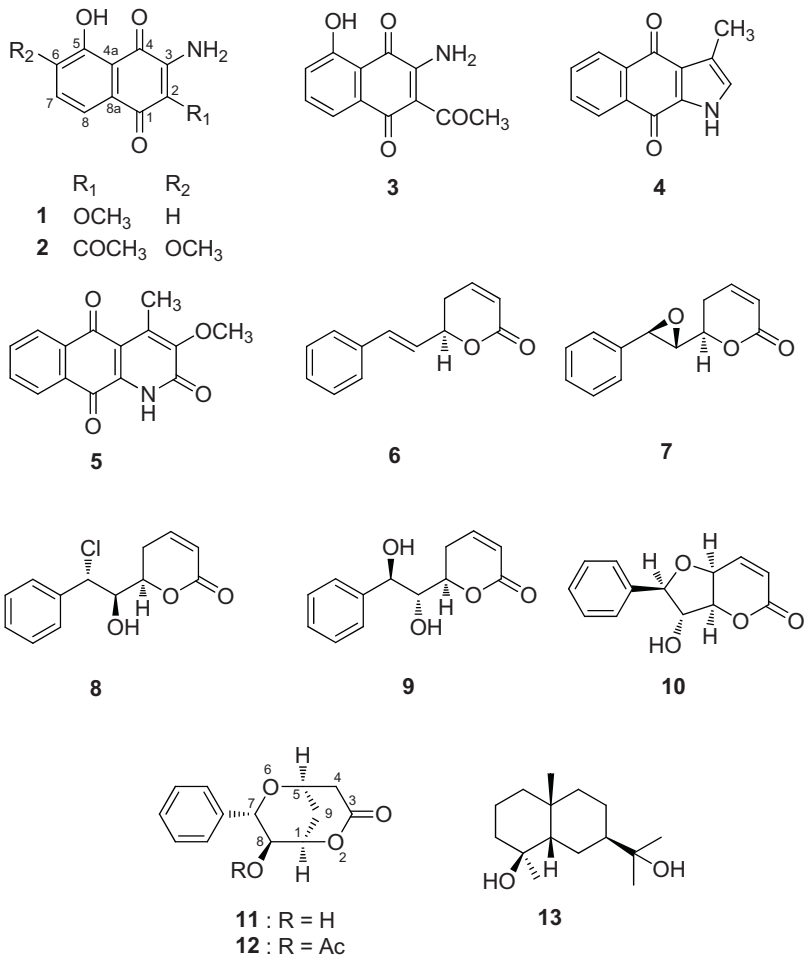

Fig. 1. Structures of compounds **1**–**13**.

220.0593 $[M+H]^+$, calcd for $C_{11}H_{10}NO_4^+$ 220.0610). The IR spectrum indicated the presence of amino and hydroxyl (ν_{max} 3490–3328 cm^{-1}), conjugated carbonyl (ν_{max} 1635 cm^{-1}), quinone carbonyl (ν_{max} 1596 cm^{-1}) and aromatic (ν_{max} 1569 cm^{-1}) groups, while the UV absorption bands (λ_{max} 224, 272, 398 nm) indicated a quinone functional group. Signals for two carbonyl groups (δ_C 177.8 and 186.4) were evident from the ^{13}C NMR spectrum. The ^1H NMR spectrum (Table 1) showed signals for three adjacent aromatic protons at δ 7.13 (dd, J = 7.8, 1.8 Hz, H-6), 7.55 (t, J = 7.8 Hz, H-7) and 7.58 (dd, J = 7.8, 1.8 Hz, H-8). The three protons signal at δ 4.04 was assigned to the methoxy group at C-2. In addition, the spectrum showed signals for two exchangeable protons at δ 4.99 (br s) which were assignable to the primary amine protons at C-3, and for one hydrogen-bonded phenolic hydroxyl group at C-5 (δ 11.57, s). The ^{13}C NMR spectrum (Table 1) exhibited signals for three aromatic methines at δ 123.0 (C-6), 136.7 (C-7)

Table 1
^1H and ^{13}C NMR spectral data of goniothalaminone A (**1**) and B (**2**) ($CDCl_3$, J in Hz).

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		177.8		180.2
2		138.5		109.4
3		160.9		152.5
4		186.4		185.5
4a		113.2		114.0
5		160.9		152.0[a]
6	7.13 dd (7.8, 1.8)	123.0		152.1[a]
7	7.55 t (7.8)	136.7	7.22 d (8.4)	117.8
8	7.58 dd (7.8, 1.8)	118.9	7.76 d (8.4)	120.7
8a		132.0		125.0
2-OCH_3	4.04 s	60.4		
2-$COCH_3$				202.4
2-$COCH_3$			2.73 s	33.2
3-NH_2	4.99 br s		7.07 br s	
10.66 br s				
5-OH	11.57 s		11.71 s	
6-OCH_3			3.99 s	56.4

[a] Assignments are interchangeable.

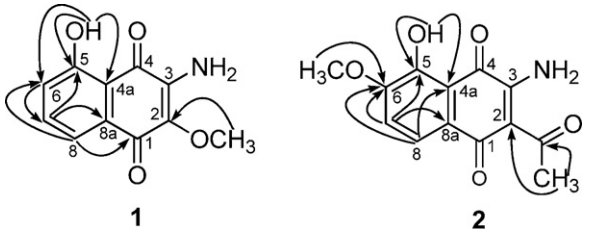

Fig. 2. HMBC correlations of **1** and **2**.

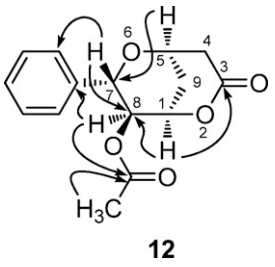

Fig. 3. HMBC correlations of **12**.

and 118.9 (C-8), five quaternary aromatic carbons at δ 138.5 (C-2), 160.9 (C-3), 113.2 (C-4a), 160.9 (C-5) and 132.0 (C-8a) and two carbonyl carbons at δ 177.8 (C-1) and 186.4 (C-4). The positions of methoxy group at C-2 and the hydroxyl group at C-5 were established by 2D HMBC correlations (Fig. 2). The methoxy proton (δ 4.04) showed correlation to C-2 (δ 138.5), hydroxyl group (δ 11.57) had correlations with C-4a (δ 113.2), C-5 (δ 160.9) and C-6 (δ 123.0) and H-8 (δ 7.58) had the long-range correlations to 1-C=O (δ 177.8) and C-6 (δ 123.0). Other correlations were also observed between H-6 (δ 7.13) and C-7 (δ 136.7), H-7 (δ 7.55) and C-5 (δ 160.9) and C-8a (δ 132.0). Goniothalaminone A (**1**) was, therefore, deduced as 3-amino-5-hydroxy-2-methoxy-1,4-naphthoquinone.

Goniothalaminone B (**2**) was obtained as orange needles. The molecular formula was established as $C_{13}H_{11}NO_5$ from HR-TOF-MS (*m*/*z* 262.0705 $[M+H]^+$, calcd for $C_{13}H_{12}NO_5^+$ 262.0715). The close similarity of the ^1H and ^{13}C NMR spectra of **2** to those of 2-aceto-3-amino-5-hydroxy-1,4-naphthoquinone (**3**) indicated the common 3-amino-5-hydroxy-1,4-naphthoquinone nucleus. The appearances of a three proton singlet at δ 3.99 and a methyl carbon at δ 56.4 in the ^1H and ^{13}C NMR spectra, respectively, suggested an additional methoxy group. The IR spectrum showed absorptions for amino and hydroxyl (ν_{max} 3421–3240 cm^{-1}), conjugated carbonyl (ν_{max} 1631 cm^{-1}), quinone carbonyl (ν_{max} 1596 cm^{-1}) and aromatic (ν_{max} 1573 cm^{-1}) groups. The UV absorption bands (λ_{max} 278, 314, 428 nm) confirmed the presence of the quinoid moiety. The ^1H NMR spectrum (Table 1) showed signals for two adjacent aromatic protons at δ 7.22 (d, J = 8.4 Hz, H-7) and 7.76 (d, J = 8.4 Hz, H-8). Signals for two methyl groups at δ 2.73 (s) and 3.99 (s) were assigned to the acetyl group at C-2 and the methoxy group at C-6, respectively. The ^1H NMR spectrum of **2** also showed signals for two exchangeable protons at δ 7.07 (br s) and 10.66 (br s) assignable to the primary amine proton at C-3, and for one hydrogen-bonded phenolic hydroxyl group at C-5 (δ 11.71, s). The ^{13}C NMR spectrum (Table 1) exhibited signals for two aromatic methine carbons at δ 117.8 (C-7) and 120.7 (C-8), six quaternary aromatic carbons at δ 109.4 (C-2), 152.5 (C-3), 114.0 (C-4a), 152.0 (C-5), 152.1 (C-6) and 125.0 (C-8a), three carbonyl carbons at δ 180.2 (C-1), 185.5 (C-4) and 202.4 (2-*CO*CH_3) and two methyl carbons at δ 33.2 (2-CO*CH*$_3$) and 56.4 (6-OCH_3). The position of the acetyl group at C-2 was established by 2D HMBC correlations (Fig. 2) between methyl protons (δ 2.73) and C-2 (δ 109.4) and aceto carbonyl (2-*CO*CH_3) (δ 202.4). The phenolic hydroxyl group at δ 11.71 and the methoxy group at δ 3.99 were attached to C-5 and C-6, respectively, were apparent from the correlations between 5-OH (δ 11.71) and C-5 (δ 152.0) and C-4a (δ 114.0) and 6-OCH_3 (δ 3.99) and C-6 (δ 152.1). Other correlations were also observed between H-7 (δ 7.22) and C-6 (δ 152.1), C-5 (δ 152.0) and C-8a (δ 125.0), H-8 (δ 7.76) and C-4a (δ 114.0), C-6 (δ 152.1) and 1-C=O (δ 180.2). Goniothalaminone B (**2**) was thus assigned as 2-aceto-3-amino-5-hydroxy-6-methoxy-1,4-naphthoquinone.

(−)-8-*Epi*-9-deoxygoniopypyrone acetate (**12**) was isolated as colorless needles with $[\alpha]^{23}_D$ −148.73 (*c* 0.32, $CHCl_3$). The molecular formula $C_{15}H_{16}O_5$ was established by HR-TOF-MS (*m*/*z* 277.1071 $[M+H]^+$, calcd for $C_{15}H_{17}O_5^+$ 277.1076). The structure of **12** was closely related to **11** based on ^1H NMR, ^{13}C NMR (Table 2), IR and UV spectral data. Compound **12** had 42 units mass more than 11, indicating the presence of one extra acetyl (CH_3CO) group and, its IR spectrum showed a strong absorption band of the ester carbonyl group (ν_{max} 1735 cm^{-1}). The ^1H NMR spectrum of **12** showed a signal for methyl protons as a singlet at δ 1.94, suggesting the presence of an acetate group in **12** with was consistent with signals for a methyl carbon at δ 20.7 and an ester carbonyl carbon at δ 169.8 in the ^{13}C NMR spectrum. The 2D HMBC experiment (Fig. 3) supported the substitution of the acetyl group at C-8 by the correlations of H-8 (δ 4.84) and ester carbonyl carbon (δ 169.8), H-7 (δ 4.67) and C-8 (δ 73.1) and H-1 (δ 5.03) and C-8 (δ 73.1). These results led to assignment of the structure of (−)-8-*epi*-9-deoxygoniopypyrone acetate for **12**. This compound was

Table 2
^1H and ^{13}C NMR spectral data of compounds **11** and **12** ($CDCl_3$, *J* in Hz).

No.	**11**		**12**	
	δ_H	δ_C	δ_H	δ_C
1	4.94–4.98 m	76.7	5.03 m	74.0
3		168.9		168.6
4	2.90 dd (19.4, 5.1) 3.01 dd (19.4, 1.5)	36.6	2.91 dd (19.4, 5.1) 3.03 dd (19.4, 1.0)	36.4
5	4.47–4.51 m	65.8	4.51 m	66.1
7	4.45 d (9.7)	74.4	4.67 d (10.1)	71.2
8	3.64 dd (9.7, 2.4)	72.6	4.84 dd (10.1, 2.4)	73.1
9	2.24–2.27 m	29.9	2.22 ddd (14.3, 4.8, 1.2) 2.34 ddd (14.3, 4.2, 2.0)	29.6
1′		137.8		137.0
2′, 6′	7.35–7.45 m	127.4	7.32–7.38 m	127.2
3′, 5′	7.35–7.45 m	128.7	7.32–7.38 m	128.5
4′	7.35–7.45 m	128.8	7.32–7.38 m	128.9
8-OH				
8-O*CO*CH_3				169.8
8-OCO*CH*$_3$			1.94 s	20.7

Table 3
Biological activities of comounds **6**–**12**.

Compounds	Antiplasmodial (IC_{50}, μg/ml)	Antimycobacterial (MIC, μg/ml)	Cytotoxicity (IC_{50}, μg/ml)		
			KB[a]	BC[b]	NCI-H187[c]
6	1.62	25	1.27	0.13	1.47
7	1.71	ND	3.22	1.55	ND
8	3.54	200	6.05	1.72	1.59
9	6.05	50	11.74	7.94	5.82
10	3.13	12.5	6.41	2.07	1.49
11	NA	NA	NA	NA	NA
12	NA	NA	NA	NA	NA
Rifampicin		0.019			
Kanamicin		1.250			
Isoniazid		0.050			
Dihydroartemisinin	0.0044				
Ellipticin			0.667	0.128	0.522
Doxorubicin			0.096	0.065	0.031

NA, not active, ND, not determined.
[a] Human epidermoid carcinoma in the mouth.
[b] Human breast cancer cells.
[c] Human lung cancer cells.

'previously synthesized by acetylation of (−)-8-*epi*-9-deoxygoniopypyrone (Surivet and Vatele, 1999). However, this is the first report of **12** from a natural source.

Among the isolated compounds, compounds **6**–**12** were evaluated for their antiplasmodial activity against *P. falciparum*, antimycobacterial activity against *M. tuberculosis* and cytotoxicity against three human cancer cell lines, KB, BC and NCl-Hl87, as summarized in Table 3. Styryllactones **6**–**10** exhibited antiplasmodial activity against *P. falciparum* with IC_{50} values of 1.62, 1.71, 3.54, 6.05 and 3.13 μg/ml, respectively and also these compounds showed antimycobacterial activity against *M. tuberculosis* with MIC values of 25, 200, 50, and 12.50 μg/ml, respectively, except for compound **7** which was not tested. In addition, compounds **6**–**10** showed cytotoxicity against the three human cancer cell lines, KB, BC, and NCl-H187, with IC_{50} values ranging from 0.13 to 11.7 μg/ml. Compound **8** has been reported to be cytotoxic against the HONE-1 (human narsopharyngeal carcinoma) cell line (IC_{50} 4.87 μg/ml) (Lan et al., 2003). Compound **10** has been reported to exhibit antiplasmodial activity against *P. falciparum* (IC_{50} 2.6 μg/ml), antimycobacterial activity against *M. tuberculosis* (MIC 6.25 μg/ml) and also showed strong activity against three human cancer cell lines (Lekphrom et al., 2009). Compound **10** was also found to cytotoxic against the leukemia cell line (L-1210) (IC_{50} 2.9 μg/ml) (Bermejo et al., 1999).

3. Experimental

3.1. General experimental procedures

Melting points were determined by Buchi melting point B-540 apparatus and are reported without correction. Optical rotations $[\alpha]_D$ were measured in $CHCl_3$ solution at the sodium D line (590 nm) on JASCO DIP-370 digital polarimeter. UV spectra were recorded with a Shimadzu UV-VIS 2001S Spectrophotometer. IR spectra were recorded with a Perkin Elmer Spectrum One FT-IR Spectrophotometer using UATR technique and Shimudzu FTIR-8900 instrument with KBr disks. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ on a Bruker AVANCE 400 (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. The signals in the ^1H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC and HMBC. EIMS were recorded on a MS Finnigan Polaris spectrometer. HRMS were recorded on a Bruker Micro TOF mass spectrometer. Column chromatography (CC) and vacuum liquid chromatography (VLC) (Coll and Bowden, 1989) were carried out on silica gel 60 (Scharlau, 70–230 mesh or 230–400 mesh). TLC was performed on precoated silica gel 60 F_{254} plates (Merck); spots were detected by UV or spraying with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 following by heating. Preparative TLC was carried out on silica gel 60 GF_{254} (Merck). All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

3.2. Plant material

The roots of *G. scortechinii* were collected in Satun Province, Thailand in 2005 and were identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2005001) is deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

3.3. Extraction and isolation

The fresh roots of *G. scortechinii* (1.8 kg) were extracted with EtOAc (3 × 6 L) at room temperature. The EtOAc extract was filtered and evaporated to give a brown residue (48.9 g). The brown residue (47.0 g) was separated by silica gel vacuum liquid chromatography (VLC) (70–230 mesh, 250 g). The column was eluted with hexane, gradient of hexane/EtOAc, EtOAc and gradient of EtOAc/MeOH to give goniothalamin (**6**) as a white solid (4.91 g) and other six fractions (f1–f6). Goniothalamin (**6**) was recrystallized from EtOAc/hexane to give colorless needles. Fraction f1 (127.0 mg) was purified by preparative TLC using hexane/CH_2Cl_2/EtOAc (2:10:1) as the developing solvent to give goniothalamin oxide (**7**) which was recrystallized from EtOAc/hexane to obtain colorless needles (5 mg). Fraction f4 (2.69 g) was separated on a column of silica gel (70–230 mesh, 80 g) using hexane/CH_2Cl_2/EtOAc (10:7:1) as the eluent to give compound **6** as a white solid (2.43 g), 2-aceto-3-amino-5-hydroxy-1,4-naphthoquinone (**3**) as a yellow solid (21 mg) and 3-methyl-1*H*-benbz[*f*]indole-4,9-dione (**4**) as a yellow solid (5 mg). Compounds **3** and **4** were recrystallized from EtOAc/hexane to give orange needles and yellow needles, respectively. Fraction f5 (3.71 g) was further chromatographed on a silica gel column (70–230 mesh, 180 g) eluting with hexane/CH_2Cl_2/EtOAc (2:10:1) to give compound **6** as a white solid (3.15 g), goniothalaminone A (**1**) as a brown solid (4 mg),

8-chlorogoniodiol (**8**) as a white solid (19 mg), goniothalaminone B (**2**) as a yellow solid (7 mg), dielsiquinone (**5**) as a yellow solid (5 mg) and a mixture of compounds **2**, **5** and **8** as a yellow solid (388 mg). The mixture was further purified by preparative TLC using hexane/CH_2Cl_2/EtOAc (2:10:1) as the developing solvent to give compound **8** as a white solid (93 mg), compound **5** as a yellow solid (7 mg) and compound **2** as a yellow solid (6 mg). Compounds **1**, **2**, **5** and **8** were recrystallized from EtOAc/hexane to provide brown needles, orange needles, yellow needles and colorless plates, respectively.

The fresh leaves of *G. scortechinii* (1.5 kg) were extracted with EtOAc (3 × 5 L) at room temperature. The EtOAc extract was filtered and evaporated to give a green solid (31.4 g). The solid (30 g) was separated by column chromatography using silica gel 60 (70–230 mesh, 1.43 kg) using hexane, gradients of hexane/EtOAc, EtOAc and gradients of EtOAc/MeOH as the eluent to give eleven fractions (fr1–fr11). Fraction fr2 was recrystallized from hexane/EtOAc to give (+)-goniothalamin (**6**) as colorless needles (0.79 g). Fraction fr5 (0.75 g) was chromatographed on a column of silica gel 60 (230–400 mesh, 70 g) using hexane/CH_2Cl_2/EtOAc (2:4:1) to give four subfractions (fr5-1–fr5-4). Subfraction fr5-2 (10.0 mg) was further purified by preparative TLC using hexane/CH_2Cl_2/EtOAc (2:6:1) to give goniothalamin oxide (**7**) (3.0 mg). Subfraction fr5-3 (184.0 mg) was recrystallized from hexane/EtOAc to give 8-chlorogoniodiol (**8**) (180.0 mg) as colorless plates. Fraction fr7 (1.41 g) was separated on a column of silica gel 60 (230–400 mesh, 108 g) using CH_2Cl_2 and gradients of CH_2Cl_2/MeOH as the developing solvent to give five subfractions (fr7-1–fr7-5). Subfraction fr7-2 (35.0 mg) was recrystallized from hexane/CH_2Cl_2 to obtain (−)-8-*epi*-9-deoxygoniopypyrone acetate (**12**) as colorless needles (25 mg). Subfraction fr7-3 (0.61 g) was chromatographed on a column of silica gel 60 (230–400 mesh, 115 g) using CH_2Cl_2, gradients of CH_2Cl_2/MeOH as the developing solvent to give (+)-altholactone (goniothalenol) (**10**) as a colorless needles (137 mg). Fraction fr8 (1.24 g) was separated on a column of silica gel 60 (Scharlau; 230–400 mesh, 126 g) using CH_2Cl_2 and a gradient of MeOH/CH_2Cl_2 as eluting solvent to give seven subfractions (fr8-1–fr8-7). Subfraction fr8-6 (178.5 mg) was chromatographed on a column on reversed-phase silica gel RP-18 eluting with H_2O/MeOH (1:9) to give 4,5-*epi*-cryptomeridiol (**13**) as a white solid (14.6 mg). Fraction fr10 (1.66 g) was separated on a column of silica gel 60 (230–400 mesh, 110 g) using hexane/CH_2Cl_2/EtOAc (2:4:1) as the developing solvent to give five fractions (fr10-1–fr10-5). Fraction fr10-4 (0.99 g) was further purified by column chromatography using silica gel 60 (230–400 mesh, 70 g) eluting with MeOH/CH_2Cl_2 (3:97) as the developing solvent to give (−)-8-*epi*-9-deoxygoniopypyrone (**11**) as colorless needles (27 mg) and (+)-goniodiol (**9**) as colorless needles (369 mg).

3.3.1. Goniothalaminone A (1)

Brown needles, mp 164 °C; UV (MeOH) λ_{max} (log ε): 224 (3.79), 272 (3.53), 398 (2.98) nm; IR (KBr) ν_{max}: 3490, 3436, 3328, 2943, 2920, 2846, 1635, 1596, 1569, 1446, 1323, 1234, 1153, 1068, 1041, 952, 875, 732 cm^{-1}; ^1H NMR and ^{13}C NMR (Table 1); MS *m*/*z* (rel. int.): 219 (100%) $[M]^+$, 218 (4), 191 (11), 190 (10), 176 (13), 173 (22), 162 (6), 149 (16), 148 (21), 145 (7), 133 (5), 121 (7), 120 (12), 105 (5), 92 (8), 76 (2), 65 (14); HR-TOF-MS *m*/*z*: 220.0593 $[M+H]^+$ (calcd for $C_{11}H_{10}NO_4$ 220.0610).

3.3.2. Goniothalaminone B (2)

Orange needles, mp 162 °C; UV (MeOH) λ_{max} (log ε): 278 (4.47), 314sh (4.11), 428 (3.85) nm; IR (KBr) ν_{max}: 3421, 3344, 3240, 2954, 2920, 2846, 1631, 1596, 1573, 1454, 1365, 1311, 1269, 1022, 756 cm^{-1}; ^1H NMR and ^{13}C NMR (Table 1); MS *m*/*z* (rel. int.): 261 (100%) $[M]^+$, 246 (51), 219 (6), 218 (14), 203 (13), 190 (10), 178 (16), 167 (34), 149 (73), 123 (7), 95 (4); HR-TOF-MS *m*/*z*: 262.0705 $[M+H]^+$ (calcd for $C_{13}H_{12}NO_5$ 262.0715).

3.3.3. (−)-8-Epi-9-deoxygoniopypyrone acetate (12)

Colorless needles, mp 144 °C; $[\alpha]_D^{23}$ −148.73 (c 0.32 $CHCl_3$) (lit. mp 140–142; $[\alpha]_D^{23}$ −170.47 (c 1.0, $CHCl_3$) (Goh et al., 1995)); UV (MeOH) λ_{max} nm (log ε): 348 (0.33), 342 (0.50), 267 (2.15), 263 (2.39), 257 (2.47), 251 (2.37), 246 (2.24), 221 (2.77), 214 (2.30), 208 (3.36) IR (UATR-solid) ν_{max} cm^{-1}: 3026, 2961, 2880, 1735, 1496, 1457, 1375, 1336, 1375, 1273, 1227, 1198, 1173, 1121, 1086, 1063, 1006, 983, 856, 759, 743, 701: ^1H NMR and ^{13}C NMR ($CDCl_3$) (Table 2); MS *m*/*z* (rel. int.): 276 $[M]^+$ (1%), 276 (1), 233 (6), 217 (1), 178 (5), 145 (3), 128 (6), 107 (34), 105 (100), 103 (4), 91 (29), 43 (27); HR-TOF-MS: *m*/*z*: 277.1071 $[M+H]^+$ (calcd for $C_{15}H_{17}O_5$ 277.1076).

3.4. Bioassays

3.4.1. Antimycobacterial assay

Antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). Standard drugs, isoniazid and rifampicin, were used as the reference compounds (Table 3).

3.4.2. Antiplasmodial assay

Antiplasmodial activity was evaluated against the parasite *P. falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen (1976). Quantitative assessments of malarial activity *in vitro* were determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al. (1979). The inhibition concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (Table 3).

3.4.3. Cytotoxicity assay

Cytotoxic assays against human epidermoid carcinoma (KB), human breast cancer (BC) and human small cell lung cencer (NCI-H187) cell lines were performed employing the colorimetric method described by Skehan et al. (1990). The reference substances were ellipticin and doxorubicin (Table 3).

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