

Potential Cancer Chemopreventive Activity of Styryllactones from *Goniothalamus marcanii*

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Received 2012-11-26, Revised 2012-12-28; Accepted 2013-03-13

ABSTRACT

Goniothalamus plants belonging to the family Annonaceae are found in Thailand and have been used as Thai traditional medicines. Members of this family are rich in a wide variety of biological active compounds including alkaloids, acetogenins and styryllactones. The present research described phytochemical study of the leaves and twigs of *G. marcanii* together with their cytotoxicity. The *G. marcanii* was selected and percolated with hexane, ethyl acetate and methanol. The extracts were purified and elucidated chemical structures. The constituents of ethyl acetate extract of *G. marcanii* have been investigated. We isolated and identified three derivative styryllactones e.g., 5-hydroxygoniothalamine (1), 5-acetylgoniothalamine (2) and goniopyrone (3). The structures of these compounds were elucidated on the basis of spectroscopic evidence. Studies on ethyl acetate extract of *G. marcanii* have now resulted the isolation and structural characterization of three styryllactones. Their anticancer activities were evaluated using SRB assays. In this method, compound 1 and 2 showed potential activity in cell lines.

Keywords: *Goniothalamus marcanii*, *Goniothalamus*, Styryllactone

1. INTRODUCTION

The genera *Goniothalamus* (Annonaceae family) consists about 160 species (Wiert *et al.*, 2007), mostly distributed throughout the tropical and subtropical countries of Asia such as Malaysia and Thailand (Cao *et al.*, 1998; Tip-Pyang *et al.*, 2010). This genus has been studied for bioactive constituents due to their proven use in folk medicine for treatment of various diseases (Hisham *et al.*, 2000) resulting the isolation of styryllactones, alkaloids, annonaceous acetogenins, flavonoids, azaanthraquinones and naphthoquinones

(Cao *et al.*, 1998; Tip-Pyang *et al.*, 2010; Soonthornchareonnon *et al.*, 1999). These isolated compounds showed anticancer, antiplasmodial, antimycobacterial, cytotoxic, antimicrobial and antibacterial activities (Lekphrom *et al.*, 2009; Humeirah *et al.*, 2010; Abdelwahab *et al.*, 2009). *G. marcanii*, locally known as Khao Lam, grows widely in the northern, northeastern and southern parts of Thailand. It is a small tree with 1.5 to 3 m high, leaves 11 to 17 cm long, 4 to 6 cm wide and its flowers are greenish yellow (Soonthornchareonnon *et al.*, 1999). In Thailand, herbalists used *G. marcanii* for infectious

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diseases in early childhood (under 5 years old). Phytochemical investigation of this plant demonstrated the presence of 1-azaanthraquinone and naphthoquinone derivative which were possessed cytotoxicity against human tumor cell lines including lung carcinoma (A549), colonadenocarcinoma (HT-29), breast carcinoma (MCF-7), melanoma (RPMI) and brain carcinoma (U251), (Soonthornchareonnon *et al.*, 1999). Our preliminary screening for bioactivities of the crude extracts of *G. marcanii* revealed that the crude hexane extract exhibited cytotoxicity against MCF-7, murine lymphocytic leukemia (P-388), human oral nasopharyngeal carcinoma (KB), human colon cancer (Col-2), human lung cancer (Lu-1), rat glioma (ASK), noncancerous human embryonic kidney (Hek 239) and human urinary bladder (T24) cell lines with ED₅₀ in the range of 2.36-17.97 $\mu\text{g mL}^{-1}$, while crude ethyl acetate showed cytotoxicity against those cell lines with ED₅₀ in the range of 0.55-8.83 $\mu\text{g mL}^{-1}$. In the present study, we report the isolation and characterization of derivative styryllactones, 5-hydroxygoniothalamin (1), 5-acetylgoniothalamin (2) and goniopyprone (3) as well as their cytotoxicity.

2. MATERIALS AND METHODS

2.1. General Experimental Procedure

Melting points were determined on a digital Electro thermal melting apparatus and were uncorrected. IR spectra were recorded as KBr disks, using Shimadzu 8900 FTIR spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). ¹H (400 MHz) and ¹³C (125 MHz) NMR spectra were determined in CDCl₃ and CD₃OD solution, the chemical shifts were recorded in δ values which were referenced to TMS as the internal standard in ppm down field from TMS (internal standard at δ 0.00). The signals at δ 7.26 (chloroform), δ 3.31 (methanol) were used as a reference in the case of ¹H-NMR spectra and at δ 77.00 (chloroform), δ 48.99 (methanol) in the case of ¹³C-NMR spectra, using a DPX on a Bruker AV 500 spectrometer for 1D and 2D determination. Low resolution mass spectra were recorded on a Thermo Finnegan Polaris Q mass spectrometer at 70 eV (probe) for EIMS. Optical rotation was obtained using a JASCO DIP-370 digital polarimeter. CC was carried out over silica gel (0.063-0.200 mm or less than 0.063 mm, MERCK). Fractions obtained from CC were monitored by TLC on silica gel 60 F₂₅₄, aluminum sheets and the chromatograms were visualized at 254 and 366 nm and sprayed with

anisaldehyde reagent and then heated until charred. Prep. TLC was carried out on silica gel PF₂₅₄ (0.5 mm, MERCK) plates. Commercial grade solvents were distilled at their boiling point ranges prior to use for extraction and chromatographic separation (CC and preparative TLC), whereas AR solvents were used for crystallization.

2.2. Plant Material

The leaves and twigs of *G. marcanii* were collected from Nong Khai, a province in the north east of Thailand, in July, 2011 by Mr. Narong Nuntasae. The plant was identified and the specimen (BKF. No. 134592) has been deposited in the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of National Resources and Environment, Bangkok, Thailand.

2.3. Extraction and Isolation

Dried leaves and twigs of *G. marcanii* (2.6 kg) were ground into powder and then extracted successively with hexane, ethyl acetate and methanol for 5 times each (5×7 L). Removal of solvents from each extract under reduced pressure afforded crude hexane (40.5 g), crude ethyl acetate (71.5 g) and crude methanol (115.2 g) extracts, respectively.

The crude ethyl acetate extract (71.5 g) was separated by flash column chromatography (CC) over silica gel (600 g) eluted with gradient systems of acetone: hexane and methanol: acetone. Based on TLC patterns, overall 12 fractions were combined to give 9 fractions, F₁-F₉. Fraction F₄ (1.33 g), obtained from 15% acetone: hexane, was further purified by CC over silica gel, eluted with gradient systems of acetone: hexane and methanol: acetone. Fractions (100 mL each) were collected and combined based on the basis of their TLC behavior to afford 3 sub-fractions, F_{4a}-F_{4c}. The precipitate in sub-fraction F_{4b} (0.43 g) was filtered out and then recrystallized from the combination solvent between chloroform and ethanol to yield compound 3 (0.04 g). The precipitate in fraction F₅ (1.87 g), obtained from 20% acetone: hexane, was filtered out and then recrystallized from ethanol to afford compound 2 (0.18 g). Fraction F₇ (21.79 g), obtained from 30% acetone: hexane, was further purified by CC over silica gel, eluted with gradient systems of acetone: hexane and methanol: acetone. Fractions (500 mL each) were collected and combined base on the basis of their TLC behavior to afford 3 sub-fractions, F_{7a}-F_{7c}. The separation of sub-fraction F_{7b} (16.01 g) was then performed by CC over silica gel, eluted with gradient systems of acetone:

hexane and methanol: acetone to obtain TLC pattern, 3 sub-fractions, F_{7ba}- F_{7bc}. The sub-fraction F_{7bb} (1.49 g) was further purified by preparative TLC, eluted with 20% acetone: hexane to afford the white precipitate which was filtered out and then recrystallized from 20% acetone: hexane to yield compound 1 (0.06 g).

2.4. Evaluation of Cytotoxic Activity

The cytotoxic activities of the tested extracts and compounds from *G. marcanii* were carried out using the *in vitro* Sulforhodamine B (SRB) method and ellipticine was used as a positive control. Test samples were dissolved in DMSO as a stock concentration at 4 mg mL⁻¹ and were tested in triplicate with a final concentration of DMSO at 0.5%. The cancer cell lines were grown in a 96-well plate in the following media: P-388, in RPMI-1640 with 5% Fetal Bovine Serum (FBS). The P-388, KB, Col-2, MCF-7, Lu-1, ASK, Hek 293 and T24 cell lines were cultured in MEM (minimum essential medium with Earle's salt and L-glutamine) with 10% FBS, while Lu-1 was grown in MEM with 5% FBS. After drug exposure at 37°C for 72 h (48 h for P-388) with 5% CO₂ in air and 100% relative humidity, cells were fixed with a final concentration of 10% trichloroacetic acid and stained with 0.4% sulforhodamine B in 1% acetic acid. The bound and dried stain was solubilized with 10 mM. trizma base, after removal of the unbound dye by washing. The absorbance at wavelength 510 nm was read on a Fluostar optima BMG plate reader. The cytotoxic activity is expressed as 50% effective dose (ED₅₀):

Determine ED₅₀ value

$$\% \text{ Survival} = \frac{\text{OD}(\text{test sample}) - \text{OD}(\text{Day 0})}{\text{OD}(0.5\% \text{ DMSO control}) - \text{OD}(\text{Day 0})}$$

Criteria of activity:

Extracts having an ED₅₀ < 20 µg mL⁻¹ and pure compounds having an ED₅₀ < 4 µg mL⁻¹ = Active;
ED₅₀ > 20 µg mL⁻¹ = No Response

3. RESULTS

The chromatographic procedure with the ethyl acetate extract of *G. marcanii* afforded three compounds; 5-hydroxygoniothalamin (1), 5-acetyl goniothalamin (2) and goniopyrone (3) which were isolated from the leaves and twigs extract. The structures of compounds were proposed by ¹H and ¹³C-NMR spectral data

analysis and comparison with the literature data. The structures are shown in **Fig. 1**.

4. DISCUSSION

Compound 1 was obtained as white needle crystals, mp 112-113°C, [α]_D^{24.5} -44.09 (c 1.00, CHCl₃). The IR spectrum showed absorption band at 3361 (O-H), 2918 and 2849 (C-H stretching), 1705 (C = O of lactone), 1264 (C-O-C of ether group), 1087 and 1033 (C-O stretching). The UV spectrum of the compound in ethanol exhibited absorption maxima at 209 and 251 nm (log ε 5.07 and 4.93 respectively), also confirmed the presence of the π → π* of aromatic together with n → π* which belonged to the carbonyl chromophore. The molecular formula C₁₃H₁₂O₃ for 1 was established by EIMS at m/z 216 [M]⁺, indicating eight degrees of unsaturation. The structure was further elucidated by examination of the NMR techniques (**Table 1 and 2**).

Compound 2 was obtained as white crystals, mp 127-128°C. [α]_D -325 (c 0.02, CHCl₃), (Ahmad *et al.*, 1991). The IR spectrum showed the absorption bands at 2941 and 2945 (C-H stretching), 1741 and 1726 (C = O of ester and lactone, respectively), 1247 (C-O-C of ether group), 1070 and 1031 (C-O stretching). The UV spectrum of the compound in ethanol exhibited absorption maxima at 207 and 250 nm (log ε 4.78 and 4.49 respectively), also confirmed the presence of the π → π* of aromatic together with n → π* which belonged to the carbonyl chromophore. The molecular formula C₁₅H₁₄O₄ for 2 was established by EIMS at m/z 258 [M]⁺, indicating nine degrees of unsaturation. The structure was further elucidated by examination of the NMR technique (**Table 1 and 2**).

Compound 3 was obtained as colorless crystals, mp 193-194°C. The IR spectrum exhibited the broad absorption band at 3415 (O-H), 2927 and 2873 (C-H stretching), 1716 (C = O of lactone), 1276 (C-O-C stretching of ether group), 1083 and 1043 (C-O stretching). The UV spectrum of the compound in ethanol exhibited absorption maxima at 208 nm (log ε 3.91), also confirmed the presence of the π → π* of aromatic moiety. The molecular formula C₁₃H₁₄O₅ for 3 was established by EIMS at m/z 250 [M]⁺, indicating seven degrees of unsaturation. The structure was further elucidated by examination of the NMR technique (**Table 1 and 2**).

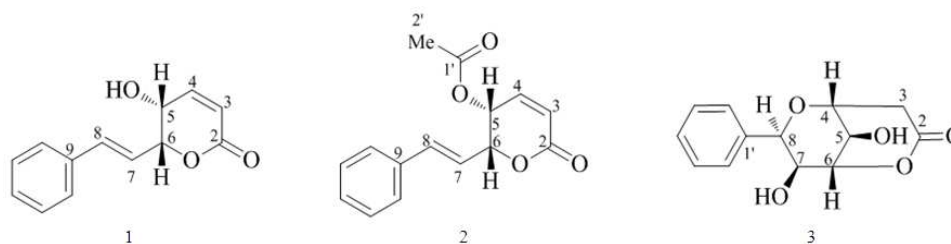


Fig 1. Structures of isolated compounds

Table 1. ¹H NMR (400 MHz), ¹³C NMR (100 MHz) data for isolated styryllactones in CDCl₃

5-hydroxygoniothalamin (1)			5-acetylgoniothalamin (2)			Goniopyrrone (3)		
Position	*δ ¹ H(J Hz)	δ ¹³ C (DEPT)	Position	*δ ¹ H(J Hz)	δ ¹³ C (DEPT)	Position	*δ ¹ H(J Hz)	δ ¹³ C (DEPT)
2	-	163.5 (C)	2	-	162.4 (C)	2	-	169.7 (C)
3	6.13 d (9.7)	122.8 (CH)	3	6.20 dd (6.4, 10.0)	121.1 (CH)	3a	3.10 dd (5.1, 19.4)	35.0 (CH ₂)
4	7.02 dd (5.4, 9.7)	144.8 (CH)	4	7.00 dd (5.5, 10.0)	140.7 (CH)	3b	2.97 dd (1.5, 19.4)	-
5	4.28 m	63.1 (CH)	5	5.37 dd (3.1, 5.5)	63.9 (CH)	4	4.22 m	69.9 (CH)
6	5.02 ddd (1.3, 3.1, 6.7)	81.3 (CH)	6	5.19 ddd (1.3, 3.1, 6.4)	79.1 (CH)	5	4.30 dd (4.6, 1.5)	65.2 (CH)
7	6.39 dd (6.7, 16.0)	121.7 (CH)	7	6.25 dd (6.4, 16.0)	124.7 (CH)	6	4.65 m	78.7 (CH)
8	6.84 d (16.0)	135.2 (CH)	8	6.83 d (16.0)	135.0 (CH)	7	4.09 dd (2.6, 9.9)	68.0 (CH)
9	-	135.7 (C)	9	-	135.7 (C)	8	4.43 d (9.9)	73.8 (CH)
10 (14)	7.29 - 7.41 m	126.9 (CH)	10 (14)	7.26 - 7.41 m	126.8 (CH)	1'	-	139.0 (C)
11 (13)	7.29 - 7.41 m	128.7 (CH)	11 (13)	7.26 - 7.41 m	128.7 (CH)	2' (6')	7.33 - 7.49 m	127.7 (CH)
12	7.29 - 7.41 m	128.5 (CH)	12	7.26 - 7.41 m	128.5 (CH)	3' (5')	7.33 - 7.49 m	127.9 (CH)
5-OH	2.80 br s	-	1'	-	170.0 (C)	4'	7.33 - 7.49 m	127.8 (CH)
			2'	2.06 s	20.6 (CH ₃)	5-OH	4.58 br s	-
						7-OH	5.48 br s	-

*δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]

Table 2. ¹H-¹³C, ¹H-¹H correlations for isolated styryllactones

5-hydroxygoniothalamin (1)			5-acetylgoniothalamin (2)			Goniopyrrone (3)		
Position	HMBC Correlation	COSY Correlation	Position	HMBC Correlation	COSY Correlation	Position	HMBC Correlation	COSY Correlation
2	-	-	2	-	-	2	-	-
3	C-2, C-4, C-5	H-4	3	C-2, C-5	H-4	3a	C-2, C-4, C-5	H-4
4	C-2, C-3, C-5, C-6	H-3, H-5	4	C-2, C-3, C-5, C-6	H-3, H-5	3b	C-2, C-4, C-5	H-4
5	C-3, C-4, C-6	H-4, H-6	5	C-3, C-4, C-6, C-7, C-1'	H-4, H-6	4	C-2, C-3, C-5, C-6, C-8	H-3, H-5
6	C-4, C-5, C-7, C-8	H-5, H-7	6	C-2, C-4, C-5, C-8	H-5, H-7	5	C-4, C-6, C-7	H-4, H-6
7	C-5, C-6, C-8, C-9	H-6, H-8	7	C-5, C-6, C-8, C-9	H-6, H-8	6	C-2, C-4, C-5, C-7, C-8	H-5, H-7
8	C-6, C-7, C-9, C-10	H-7	8	C-6, C-7, C-9, C-10	H-7	7	C-6, C-8, C-1'	H-6, H-8
9	-	-	9	-	-	8	C-6, C-7, C-1', C-2'	H-7
10 (14)	-	-	10 (14)	-	-	1'	-	-
11 (13)	-	-	11 (13)	-	-	2' (6')	-	-
12	-	-	12	-	-	3' (5')	-	-
5-OH	-	-	1'	-	-	4'	-	-
			2'	C-1'	-	5-OH	-	-
						7-OH	-	-

All pure isolates obtained in the present investigation were evaluated against a panel of mammalian cancer cell lines and the noncancerous human embryonic kidney cell Hek 293 (Table 3). Among these compounds, the styryllactones 1 and 2 showed cytotoxicity to P-388, KB, MCF-7, A549, Hek 293 and ASK cell lines; moreover, 2 also exhibited activity against Col-2, Lu-1 and T24 cell line. The styryllactone 1 exhibited high cytotoxicity to all tested cancer cell lines, but most active in the P-388, A549 and Hek 293 cell lines with the same ED₅₀ values of <0.16 μg mL⁻¹.

Compounds 3 were found inactive in all tested cell lines. The styryllactones 1 and 2 showed high selectivity toward cancer cells, thus making these compounds as attractive anticancer agents.

5. CONCLUSION

Phytochemical investigation of the crude ethyl acetate extract from *G. marcanii* had led to the isolation of three styryllactones, 5-hydroxygoniothalamin (1), 5-acetylgoniothalamin (2) and goniopyrrone (3).

Table 3. Cytotoxicity of crude extracts and pure compounds from *G. marcanii*

Crude extracts/ Pure compounds	Cytotoxicity (ED ₅₀ , µg/mL)									
	Cancer cells								Normal cells	
	P-388	KB	Col-2	MCF-7	Lu-1	A549	HT29	T24	ASK	Hek293
Hexane	2.36	10.22	11.41	10.33	11.45	NT	NT	13.03	17.97	5.65
Ethyl acetate	0.79	6.25	0.55	7.01	8.83	NT	NT	3.16	2.69	<4
Methanol	10.75	NR	NR	NR	NR	NT	NT	NR	NR	NR
5-Acetylgoniothalamin (1)	<0.16	0.77	NT	0.69	NT	<0.16	NT	0.31	0.74	<0.16
5-Hydroxygoniothalamin (2)	0.34	1.38	1.53	0.71	1.18	0.9	1.56	0.94	NT	0.49
Goniopyrone (3)	0.31	0.49	NT	0.62	NT	0.36	NT	0.41	0.56	0.24
Ellipticine (Positive control)	0.40	0.48	0.51	0.37	0.23	0.23	0.58	0.58	0.24	0.43

Cytotoxic assay: ED₅₀ less than 20 µg mL⁻¹ were considered active for extracts and less than 4 µg mL⁻¹ for pure compounds. P388: murine lymphocytic leukemia, KB: human oral nasopharyngeal carcinoma, Col-2: human colon cancer, MCF-7: human breast cancer, Lu-1: human lung cancer, A549: human lung cancer, HT29: human colon cancer, T24: human urinary bladder cancer cell, ASK: rat glioma cell, Hek293: noncancerous human embryonic kidney cell, NR: no response (ED₅₀>20 µg mL⁻¹), NT: not test.

Compound 1 and 2 were showed potential anticancer activities. Moreover, these two compounds can play an important role for solving the cancer therapy. It is noted that the worthy finding of this study could be considered as a valuable economic medicinal natural products which helpful the cancer rehabilitation to human health.

6. ACKNOWLEDGEMENTS

The authors are grateful to Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education for financial support and Dr. Kruawon Wongpaibon for English editing.

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