



(-)-7-O-ACETYLGONIODIOL AS CANCER CHEMOPREVENTIVE AGENT FROM *Goniothalamus Griffithii*

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ABSTRACT

(-)-7-O-Acetylgoniodiol (**1B**) together with goniothalamine (**2**) and pinocembrin (**3**), have been isolated from leaves and twigs of *Goniothalamus griffithii*. All compounds were identified by spectroscopic analyses and comparison with published data. **1B** can be formed by recrystallization from EtOH/acetone and its stereochemistry was further confirmed by X-ray crystallographic analysis. Goniothalamine exhibited most potent cytotoxicity against P-388, KB, Col-2, MCF-7, Lu-1, A549, T24, ASK, HEK-293 and cells with ED₅₀ values of 0.19, 0.56, 0.36, 0.56, 0.54, 0.67, 0.39, 0.67 and 0.50 µg/mL, respectively. In addition, **1B** was also showed high selective inhibitory effect on the P-388, KB and HEK-293 with ED₅₀ values of 3.31, 3.26 and 1.89 µg/mL.

Key words: *Goniothalamus griffithii*, Annonaceae, Stereomeric styryllactone, Cytotoxicity.

INTRODUCTION

Goniothalamus is one of the largest palaeotropical genera of plants in family Annonaceae with over 25 species distributed throughout all part of Thailand¹. Phytochemical studies on genus *Goniothalamus* have led to the isolation and characterization of a large number of styryllactone, which found to possess significant cytotoxic activities against several mammalian cancer cell lines²⁻⁶. In previous publications, we have described for the styryllactone from *G. maewongensis* leaves and twigs⁷. In a screen for anticancer agents from *G. griffithii*, two solvents (hexane, ethylacetate) extract of the leaves and twigs were found to be significantly cytotoxic against a number of cancer cell lines. In this paper, we

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depict the cytotoxicity of ethylacetate extract of this plant against a panel of nine mammalian cancer cell lines. Further, we herein reported the isolation and characterization of a (-)-7-*O*-acetylgoniodiol (**1B**) along with goniothalamine (**2**) and pinocembrin (**3**). To the best of our knowledge, the study of stereochemistry of **1B** has been only revealed by ^1H NMR^{8a} is not more clearly, therefore, we are further confirmed by X-ray crystallographic technique and compared the molecular geometries, absolute configuration, and strong hydrogen bonding results with (+)-7-*O*-acetylgoniodiol (**1A**).

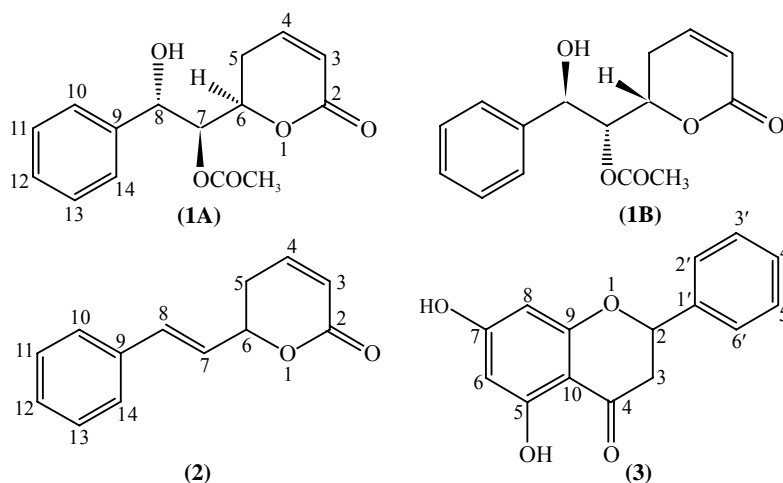


Fig. 1: The chemical structures of compounds (1-3)

EXPERIMENTAL

General

Melting points were recorded in degree Celsius ($^{\circ}\text{C}$) and were measured on a digital Electrothermal melting apparatus. UV spectra were obtained on a Shimadzu UV-1601 spectrophotometer with EtOH as solvent. Principle bands (λ_{max}) were reported as wavelengths (nm) and $\log \epsilon$. Optical rotations were determined with a JASCO DIP-370 digital polarimeter. IR spectra in KBr disk were recorded on Shimadzu 8900 FTIR spectrophotometer. Major bands (ν_{max}) were recorded in wave number (cm^{-1}). ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were determined in CDCl_3 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 signal of chloroform at δ 7.26 was used as a reference in the case of ^1H -NMR spectra and at δ 77.00 in the case of ^{13}C -NMR spectra, using a DPX on a Bruker AV 400 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. High resolution mass spectra (electrospray ionization mode, ESI-MS) were measured on a micromass Q-TOF-2Tm (Waters) spectrometer. Column chromatography was conducted on silica gel 60 (Merck 7734, 70-230 mesh). TLC was performed on aluminium backed pre-coated silica gel 60 PF₂₅₄ sheets and detection with using UV detector.

Plant material

The leaves and twigs of *G. griffithii* were collected from Chiang Mai province of Thailand in January, 2011 and identified by Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand, where a voucher specimen (BKF16447) has been deposited

Extraction and isolation

Dried and powdered leaves and twigs of *G. griffithii* (2.0 Kg) were extracted, at room temperature, with hexane and ethylacetate, successively. The cytotoxicity potential (Table 4) of ethylacetate (EtOAc) extract (54.65 g) was subjected to silica gel (Merck 7737, Mesh 70-230) column chromatography (CC), eluted in gradient system with increasing concentration of EtOAc in hexane, to give main eight fractions (F1-F8). F3 was subjected to further CC on silica gel, eluted with increasing amounts of EtOAc in hexane until EtOAc-hexane (1 : 19) and was further purified by recrystallization from MeOH-EtOAc (1 : 1), to afford pinocembrin (**3**) 150 mg. F4 was subjected to repeat silica gel column, eluted with EtOAc-hexane (1 : 1) to obtain five subfractions (A1-A2). Then A2 was rechromatographed by CC over silica gel and eluted with EtOAc-hexane (1 : 1) and was further purified by recrystallization from CHCl₂-EtOH (1 : 1), to afford goniotalamin (**2**) 600 mg. Additionally, F5 was submitted to CC on silica gel, eluted with EtOAc-hexane (3 : 17) and was further purified by recrystallization from EtOH-Acetone (10 : 2), to get hold of (-)-7-O-acetylgoniodiol (**1B**) 150 mg.

(-)-7-O-Acetylgoniodiol (**1B**)

Colorless prism crystal; m.p. 132-133°C; $[\alpha]_D^{23}$: +29.72 (0.65, MeOH); UV (EtOH) λ_{\max} (log ϵ) 333 (2.72) and 211 (5.14); IR (KBr) ν_{\max} 3467, 1738, 1709, 1558, 1506, 1458, 1261, 1232, 1035 cm⁻¹; EIMS: m/z 276 [M]⁺ (12), 170 (49), 165 (11), 110 (100), 105 (82), 97 (43), 77 (14), 72 (42), 59 (46), 58 (53), 46 (32), 32 (30); The ¹H and ¹³C NMR (Table 1).

Goniotalamin (**2**)⁹

White crystal; m.p. 80-82 °C; UV (EtOH) λ_{\max} (log ϵ) 212 (4.02) and 254.5 (4.05); IR (KBr) ν_{\max} 1720, 1704, 1662, 1247 cm⁻¹; EI-MS: m/z 201 [M+H]⁺ (17), 200 (9), 184 (16),

183 (100); ^1H NMR (CDCl_3) δ 6.08 (1H, *tt*, 1.83, 1.87, 9.84 Hz, H-3), 6.92 (1H, *m*, H-4), 2.53 (1H, *m*, H-5), 5.09 (1H, *ddd*, 6.32, 6.36, 8.87 Hz, H-6), 6.27 (1H, *dd*, 15.97, 6.34 Hz, H-7), 6.73 (1H, *dd*, 15.97, 0.73 Hz, H-8), 7.24-7.43 (5H, *m*, aromatic protons); ^{13}C NMR (CDCl_3) δ 163.83 (C-2), 121.57 (C-3), 144.61 (C-4), 29.80 (C-5), 77.87 (C-6), 125.61 (C-7), 133.05 (C-8), 135.71 (C-9), 126.63 (C-10,14), 128.62 (C-11, 13), 128.28 (C-12).

Pinoembrin (3)¹⁰

Yellow crystal; m.p. 190-192°C; UV (EtOH) λ_{max} (log ϵ) 252.6 (4.2) nm; IR (KBr) ν_{max} 3452, 3089, 1639, 1629, 1602, 1581, 1301, 1168 cm^{-1} ; EI-MS: m/z 256 $[\text{M}]^+$ (82), 238 (25), 179 (100), 152 (37), 124 (41), 96 (18) and 78 (20), 69.(16); ^1H NMR (CD_3OD) δ : 12.04 (C5-OH, C7-OH), 5.39 (1H, *dd*, 12.81, 3.08 Hz, H-2), 3.04 (1H, *dd*, 17.14, 12.83 Hz, H α -3), 2.73 (1H, *dd*, 17.13, 3.12 Hz, H β -3), 5.93 (1H, *d*, 12.84 Hz, H-6), 5.90 (1H, *d*, 8.45 Hz, H-8), 7.30-7.50 (5H, *m*, aromatic protons). ^{13}C NMR (CD_3OD) δ : 79.01 (C-2), 42.75 (C-3), 195.90 (C-4), 164.05 (C-5), 95.86 (C-6), 167.00 (C-7), 94.91 (C-8), 163.25 (C-9), 102.90 (C-10), 138.96 (C-1'), 125.95 (C-2', 6'), 128.32 (C-3', 5'), 128.25 (C-4').

X-ray crystallographic study

The colorless prism of **1B** was suitable for a single-crystal X-ray diffraction with size 0.50 x 0.50 x 0.28 mm was used. The unit cell parameters and intensity data were recorded on a X8 APEX II diffractometer equipped with a graphite-monochromator Mo- $K\alpha$ radiation at 296(2) K. Absorption corrections were made with semi-empirical from equivalents. The crystal structure was resolved by direct methods using SHELXS-97^{11a} and refined by full-matrix least-squares methods on F^2 using SHELXL-97^{11b}. All non-H atoms were refined anisotropically. The crystal data is listed in Table 2. The bond lengths, bond angles, atomic coordination, and isotropic and anisotropic displacement parameters, and strong hydrogen bonding are listed in *CIF* files. The molecular graphics were illustrated by ORTEP^{12a}, and the ring puckering parameters were calculated by CremerPopl procedure in PLATON^{12b,c}.

Evaluation of cytotoxic activity

The cytotoxic activities of the tested extracts and compounds from *G. griffithii* 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 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

$$\% \text{ Survival} = \frac{\text{OD (test sample)} - \text{OD (Day 0)}}{\text{OD (0.5\% DMSO control)} - \text{OD (Day 0)}} \times 100$$

Criteria of activity: Extracts having an ED₅₀ < 20 µg/mL and pure compounds having and ED₅₀ < 4 µg/mL = Active; No Response = ED₅₀ > 20 µg/mL

RESULTS AND DISCUSSION

1B was obtained as colourless prism crystal by recrystallization from EtOH–Acetone (10 : 2). IR spectrum result is similar to previous report, absorption bands attributable to hydroxyl (3467 cm⁻¹) and aromatics (1458, 1506 and 1558 cm⁻¹). The C=O of vibrations of cyclic esters are shifted to higher frequencies with decreasing ring size. The strained, six-membered cyclic α , β -conjugated ester and α , β , γ , δ -conjugated ester absorbed at 1709 cm⁻¹. In addition, the acetoxy groups showed typical C=O stretching vibration at 1738 cm⁻¹ and C–O stretching absorptions, which appeared in the range from 1261, 1232 to 1036 cm⁻¹ ^{8a,b}. In addition, the EIMS mass spectrum (found m/z 276, [M⁺]) showed typical styrylpyrone skeleton structure ^{7,8a}. The key fragmentation ions in the mass spectrum at 170, 165, 110, 105, 97, 77, 72, 59, 58 and 46 were useful to obtain the structure of **1B**. The ¹H NMR signals (Table 1) at δ 7.3-7.4 (5H) represented a monosubstituted phenyl moiety. Three oxygen bearing methine carbons were suggested by the ¹H NMR (δ 4.9, 5.1, 5.1 and ¹³C NMR (Table 1) (δ 71.7, 76.8, 77.0). The acetoxy group was also indicated at δ 1.8 (3H) in the ¹H NMR spectrum. Further spectral evidence was required to confirm the structure of **1B** (Fig. 1). The COSY spectrum showed coupling correlations through the sequence of H-3 to H-4 for the double bonds of conjugated system. In the other hand, the connectivities of the chain carbons skeleton e.g. C-5, C-6, C-7, C-8, were also confirmed by the COSY correlations. The HMBC spectrum showed crossed peaks between the aromatic signals (H-10, H-14), (H-6) and C-8, and between H-7 and C-9, which indicated the aromatic ring was connected to C-8. In addition, the ¹H NMR spectrum of **1B** showed olefinic protons of lactone ring as clearly splitting pattern at δ 6.0 (*ddd*, $J = 9.8, 2.3, 1.5$ Hz, H-3) and 7.0 (*ddd*, $J = 9.8, 5.3, 3.3$, Hz, H-4). The ¹H-¹³C spectrum exhibited the signals of 15 atoms and

DEPT experiments showed 12 protonated carbon signals thereby revealing three quaternary carbons in the molecule. The presence of a monosubstituted phenyl ring was evident from the signals at δ . 128.9, 129.0, 129.1, 129.2, 129.3 and 142.7. Additionally, the oxymethine carbon signals at δ 71.7, 76.8, 77.0 and the carbonyl carbon at δ 166.3 were reminiscent of a pyrone and lactone moieties, respectively. For the carbon carbonyl of acetoxy group showed the resonance at δ 171.3. The HMBC data of **1B** has shown correlations between H-7 with C-15 (δ 171.3) suggesting the location of acetoxy groups on C-7. At this point, the show of a saturated δ -lactone, a monosubstituted phenyl moiety and one hydroxyl group in **1B** is a justifiable structure. The ^1H NMR chemical shifts, together with selective homo-nuclear ^1H - ^1H and hetero-nuclear HMBC correlations, suggesting that **1B** is similar to (+)-7-*O*-acetylgoniodiol (**1A**) was reported by Wu and co-workers^{8a} that isolated from *Goniothalamus amuyon*.

Table 1: ^1H , ^{13}C and 2D NMR spectral data for **1B** in CDCl_3 *

Position	δ_{C}	δ_{H}	HMBC	COSY
1	-	-	-	-
2	166.3 (C)	-	-	-
3	121.1 (CH)	6.0 (<i>ddd</i> , 9.8, 2.3, 1.5)	5	4
4	148.6 (CH)	7.0 (<i>ddd</i> , 9.8, 5.3, 3.3)	2, 6	3, 5
5	27.3 (CH_2)	2.3 (<i>obsc.</i>), 2.4 (<i>m</i>)	3, 7	4, 6
6	71.7 (CH)	4.9 (<i>brd</i>)	4, 7, 8	5, 7
7	76.8 (CH)	5.1 (<i>m</i>)	4, 5, 9, 15	6, 7
8	77.0 (CH)	5.1 (<i>m</i>)	7, 9	7
9	142.7 (C)	-	-	-
10	129.0 (CH)	7.3-7.4 (<i>m</i>)	8, 12, 14	-
11	129.3 (CH)	7.3-7.4 (<i>m</i>)	9, 13	-
12	129.1 (CH)	7.3-7.4 (<i>m</i>)	10, 14	-
13	129.2 (CH)	7.3-7.4 (<i>m</i>)	9, 11	-
14	128.9 (CH)	7.3-7.4 (<i>m</i>)	8, 10, 12	-
15	171.3 (C)	-	-	-
16	20.4 (CH_3)	1.8 (<i>brs</i>)	15	-
OH		1.29 (<i>brs</i>)		-

*Chemical shift values are given in ppm, and *J* values in parentheses are given in Hz.

Assignments were confirmed by ^1H - ^1H COSY and HMBC experiments; *obsc.* = obscure signal

However, the evident of the physical data of **1B**: mp 132-133°C, $[\alpha]_D^{23} = +29.72$ (0.65, MeOH), and UV spectrum at 211 and 333 nm, suggested that **1B** conformer is significantly different to the **1A** conformer and/or supramolecular interactions in crystal packing compared to previously reported. To further confirm the structure of **1B**, single crystal X-ray crystal diffraction was performed with the suitable colorless prism crystal was recrystallized from EtOH–Acetone (10 : 2). **1B** crystallizes in the triclinic system space group *P1* with $a = 5.4551(4)$ Å, $b = 8.8442(7)$ Å, $c = 15.3290(13)$ Å, $\alpha = 94.393(3)^\circ$, $\beta = 91.920(2)^\circ$, $\gamma = 105.088(3)^\circ$, $Z = 2$, and $V = 710.87(10)$ Å³, $T = 296 \pm 2$ K and $R_1[I > 2\sigma(I)] = 0.0317$ and 3827 the observed reflections as listed in Table 2. It consists of two crystallographic independent molecules similar as **1A** but all atoms member almost oriented in the opposite direction, with the phenyl ring adopts in planar whereas the lactone ring represented an envelope conformation with the ring puckering parameters (Ω , θ , ϕ) for rings O1-C6 and O7-C6A: 0.452(3) and 0.434(3) Å, and 62.5(4) and 63.4(5)°, and 289.3(5) and 291.4(5)° as illustrated in Fig. 2. **1B** conformer is indicated by H-7–C-7–C-8–H-8 and H-7A–C-7A–C-8A–H-8A torsion angles, 166.35(3)°, –179.02(3)° different to **1A** conformer (*R*-form), –168(3)° and –179(3)°, suggesting that the absolute configuration of **1B** would be *S*-form.

Table 2: Crystal data and refinement for 1B

Empirical formula	C ₁₅ H ₁₆ O ₅	
Formula weight	276.28	
Crystal system	Triclinic	
Space group	<i>P1</i>	
Unit cell dimensions (Å, °)	$a = 5.4551(4)$	$\alpha = 94.393(3)$
	$b = 8.8442(7)$	$\beta = 91.920(2)$
	$c = 15.3290(13)$	$\gamma = 105.088(3)$
Volume (Å ³)	710.87(10)	
<i>Z</i>	2	
D_{calcd} (Mg/m ³)	1.291	
Absorption coefficient, μ (mm ⁻¹)	0.097	
F(000)	292	
Crystal size (mm)	0.50 x 0.50 x 0.28	
θ Range (°)	1.33-25.75°	
Index ranges	–3 ≤ <i>h</i> ≤ 6	
	–10 ≤ <i>k</i> ≤ 9	
	–18 ≤ <i>l</i> ≤ 18	

Cont...

Reflections collected	4690
Independent reflections	3827 [R(int) = 0.0199]
Max. and min. transmission	0.9733 and 0.9531
Data / restraints / parameters	3827 / 5 / 447
Goodness-of-fit on F^2	1.158
Final R indices [$I > 2 \sigma(I)$]	$R_1 = 0.0317$, $wR_2 = 0.0893$
R indices (all data)	$R_1 = 0.0391$, $wR_2 = 0.1090$
Absolute structure parameter	0.5(9)
Largest diff. peak and hole ($e. \text{\AA}^{-3}$)	0.168 and -0.195

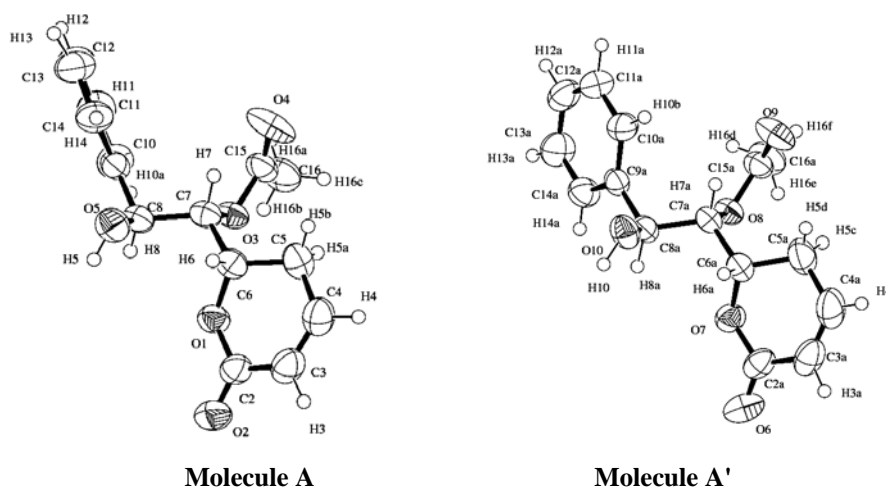


Fig. 2: ORTEP drawing and atom labeling scheme of 1B in the asymmetric crystal unit

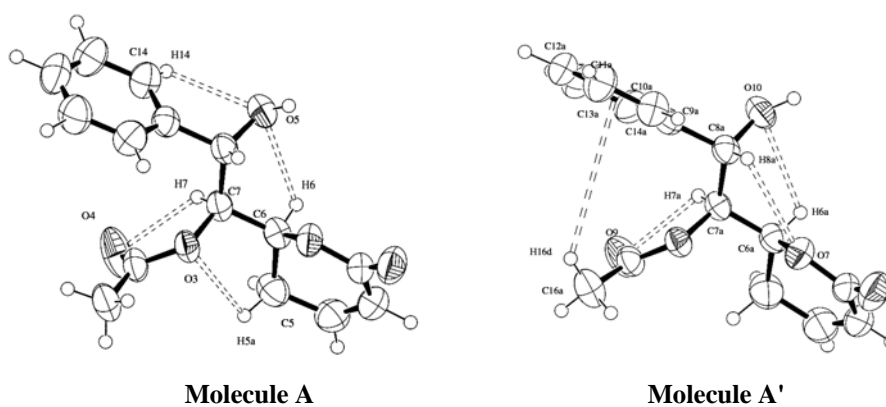


Fig. 3: The intramolecular hydrogen bonding interactions

The unsuperimposed styryllactones in asymmetric crystal unit of **1B** depend on the phenyl rings arrangement with C-7-C-8-C-9-C-12 and C-7A-C-8A-C-9A-C-12A torsion angles = -23.54 and -124.17° . Due to the different direction of hydrogen bonded attractions and the numbers of weak C-H \cdots O/ π intramolecular interactions as shown in Fig. 3 and Table 3. Fig. 4 showed the crystal packing in a 1-D, 2-D and 3-D by intermolecular interactions. The observed different value of melting point would be clearly described by hydrogen bond interactions especially strong O-H \cdots O intermolecular interactions with different value about 0.12 Å as shown in Table 4. Other properties, not only the isomeric conformation and the supramolecular interactions, but also the solvent effects possible influenced.

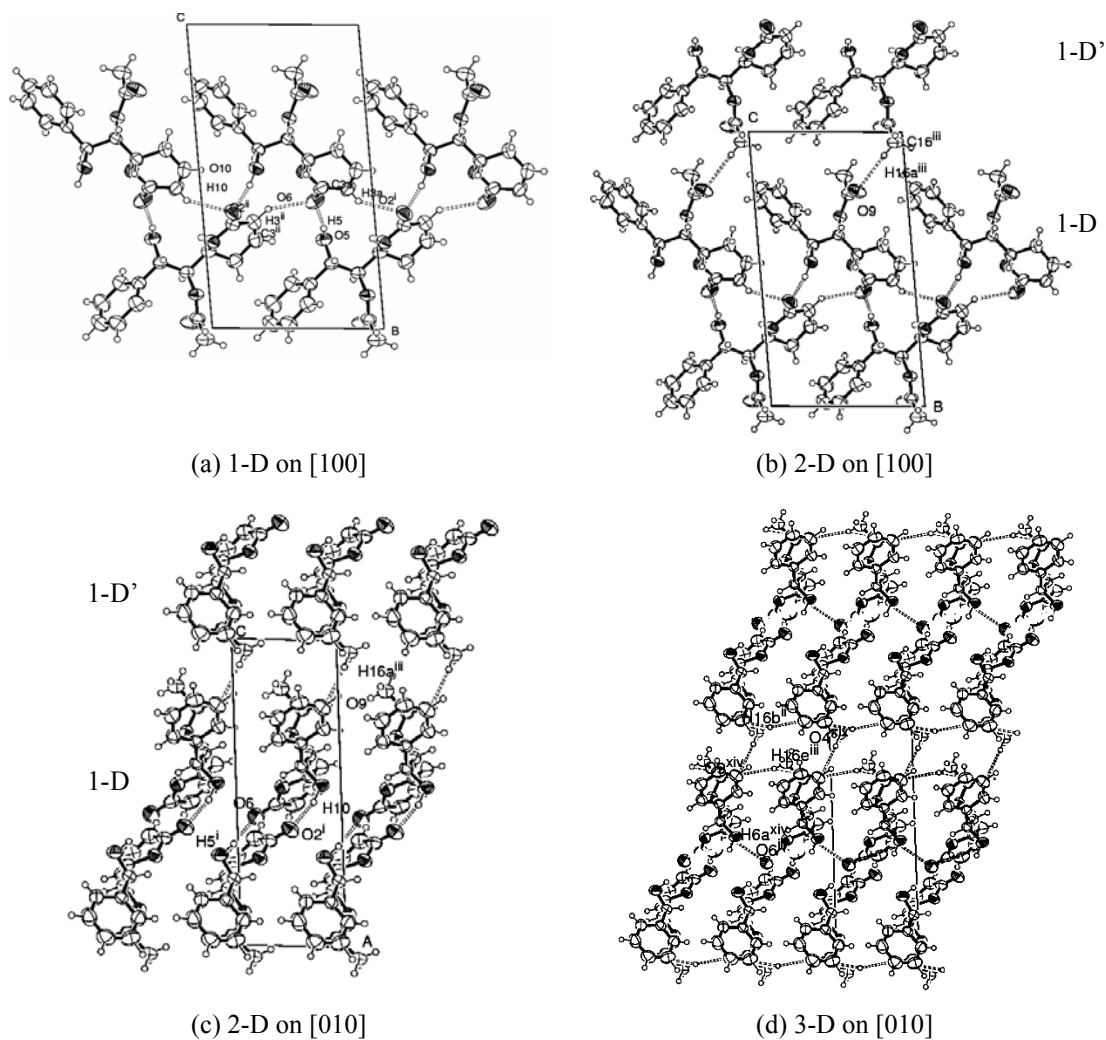


Fig. 4: The intermolecular hydrogen bonding interactions

Table 3: The selected hydrogen bond interactions in 1B

Interactions with dimensions	D–H...A	d[D–H] (Å)	d[H...A] (Å)	d[D...A] (Å)	∠[D–H...A] (°)
Intramolecular					
0-D	C(5)–H(5A)···O(3)	0.98(4)	2.56(4)	2.936(4)	103(2)
	C(6)–H(6)···O(5)	0.94(3)	2.48(3)	2.856(3)	104(2)
	C(7)–H(7)···O(4)	0.99(4)	2.26(3)	2.708(3)	106(2)
	C(14)–H(14)···O(5)	0.88(4)	2.49(3)	2.798(4)	101(3)
	C(6A)–H(6A)···O(6)	0.96(3)	2.58(3)	2.925(3)	101(2)
	C(7A)–H(7A)···O(9)	0.99(3)	2.25(3)	2.707(3)	107(2)
	C(8A)–H(8A)···O(7)	1.02(3)	2.58(3)	2.933(3)	100(2)
	C(16A)–H(16D)···π _{C9a-C14a}	0.96	3.919	4.511	122
Intermolecular					
1-D	O(5)–H(5)···O(6) ⁱ	0.90(4)	1.94(4)	2.812(3)	164(4)
	O(10)–H(10)···O(2) ⁱⁱ	0.94(3)	1.88(4)	2.794(3)	163(4)
	C(3)–H(3)···O(6) ⁱⁱⁱ	0.98(5)	2.52(4)	3.316(4)	139(3)
	C(3A)–H(3A)···O(2) ^{iv}	0.90(4)	2.46(4)	3.230(4)	143(4)
2-D	C(16)–H(16A)···O(9) ^v	0.96	2.55	3.500(4)	170
3-D	C(6A)–H(6A)···O(6) ⁱ	0.96(3)	2.46(3)	3.365(4)	157(2)
	C(16)–H(16B)···O(4) ⁱ	0.96	2.56	3.488(5)	162
	C(16A)–H(16E)···O(9) ^{iv}	0.96	2.56	3.498(5)	167
Symmetry codes (i) 1+x,y,z; (ii) -1+x,-1+y,z; (iii) 1+x,1+y,z; (iv) -1+x,y,z; (v) x,y,-1+z					

Table 4: Comparing strong O–H...O hydrogen bond interactions and melting point in 1A and 1B

Compound	Melting point (°C)	D–H...A	d[D...A] (Å)	Ref
1A	148-149	O(20)···O(15')	2.800(4)	8a
		O(20')···O(15)	2.782(3)	
1B	132-133	O(5)···O(6) ⁱ	2.812(3)	This work
		O(10)···O(2) ⁱⁱ	2.794(3)	
Symmetry codes (i) 1+x,y,z; (ii) -1+x,-1+y,z				

Table 5: Cytotoxicity of crude extracts and pure compounds from *G. griffithii*

Crude extracts/ Pure compds.	Cytotoxicity (ED ₅₀ , µg/mL)																	
	Cancer cells							Normal cells										
	P-388	KB	Col-2	MCF-7	Lu-1	A549	T24	ASK	HEK-293									
	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD				
Hexane	3.29	0.540	10.90	0.690	12.67	0.082	10.38	0.550	11.71	0.921	NR	-	12.40	0.33	18.81	0.910	<4	0.456
Ethylacetate	1.99	0.226	7.80	0.330	11.81	0.458	9.72	0.370	5.04	1.064	2.85	0.050	10.11	0.21	15.10	0.740	2.94	0.052
(-)-7-O-acetyl- goniodiol (1B)	3.31	0.086	3.26	0.148	9.64	0.193	6.24	0.328	7.74	0.295	8.95	0.157	8.55	0.476	9.41	0.754	1.89	0.768
Gonithalamin (2)	0.19	0.084	0.56	0.015	0.36	0.025	0.56	0.021	0.54	0.011	0.67	0.035	0.39	0.029	0.67	0.051	0.50	0.018
Pinocembrin (3)	8.31	0.457	NR	-	NR	-	19.57	6.660	NR	-	NR	-	NR	-	NR	-	9.48	4.110
Ellipticine	0.42	0.087	0.52	0.060	0.48	0.031	0.41	0.060	0.22	0.056	0.23	0.025	0.55	0.035	0.53	0.080	0.41	0.085

Cytotoxic assay : ED₅₀ less than 20 µg/mL was considered active for extracts and less than 4 µg/mL for pure compounds. P-388 : murine lymphocytic leukemia, KB : human oralnasopharyngeal, Col-2 : human colon cancer, MCF-7 : human breast cancer, Lu-1 : human lung cancer, A549: adenocarcinomic human alveolar basal epithelial cells, T24 : human urinary bladdercancer cells, ASK : rat glioma, HEK-293 : human embryonic kidney, NR : no response (ED₅₀> 20 µg/mL)

CONCLUSION

The present results clearly indicate that the ethylacetate extracts of *G. griffithii* possessed significant cytotoxic activity. In addition, (-)-7-*O*-acetylgoniodiol (**1B**) and goniotalamin (**2**), proved to be promising agents. To our knowledge, this is to the first report on crystal structure for proof of stereochemistry, and the cytotoxicity of extract of styryllactone derivative. Therefore, further intensive studies on the structure-anticancer activity relationships of this class of compounds are highly recommended.

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