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Bioactive Goniothalamin from *Goniothalamus tapis* with Cytotoxic Potential

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Abstract: Goniothalamus (Annonaceae) is well known for the rich of styryllactone contents and these plants are famous for the potential to be applied as alternative biological activities. The chemical and biological investigations of *Goniothalamus tapis* is tested for anticancer activities. The research was processioned to extract, isolate, purify and elucidate structure from the leaves and twigs of *G. tapis*. The isolated compound was evaluated with cytotoxic activity. Goniothalamin was isolated from ethyl acetate extract of *G. tapis* and the spectroscopic techniques were used for structure elucidation. In addition, goniothalamin was the most powerful to cytotoxic activity which was the first reported for this specie. The goniothalamin was possessed a potent cytotoxicity. Therefore, it is possible to use this compound as a pharmacological agent.

Keywords: Annonaceae, *Goniothalamus tapis*, Goniothalamin, Cytotoxicity

Introduction

The genus *Goniothalamus* comprises about 160 species (Wiart, 2007), growing in Asia and many of them are used as the traditional medicine in several countries (Surivet and Vatele, 1999; Wiart, 2007) such as Vallay fever Typhoid fever (root of *G. tapis*), Scabies (leaves of *G. macrophyllus*), Rheumatism, Tympanites (seeds of *G. amuyon* Merr) and stomachic (Efdi *et al*., 2010; Surivet and Vatele, 1999; Wiart, 2007). In Thailand, found nine species (Tip-Pyang *et al*., 2010). Phytochemical studies on some species of *Goniothalamus* have found several of this genus are discovered throughout the country such as acetogenins (Fujimoto *et al*., 1988; Gu *et al*., 1994; Jiang *et al*., 1997), alkaloids (Cao *et al*., 1998; Omar *et al*., 1992; Soonthornchareonnon *et al*., 1999), flavonoids (Deepralard *et al*., 2007; Likhitwitayawuid *et al*., 2006), styryllactone derivatives (Bermejo *et al*., 1997; 1998; 1999; Cao *et al*., 1998; Hisham *et al*., 2000; 2003; Jiang *et al*., 2008; Lekphrom *et al*., 2009), furanopyrones (Bermejo *et al*., 1997; 1998; 1999; Fang *et al*., 1990; 1991), arvensin, stigmasterol, (+)-aromadendrene, γ-gurjunene, goniothalamin, liriodenine and oxostaphanine (Ahmad *et al*., 1991; Hasan *et al*., 1994; Jiang *et al*., 1997). Therefore, *Goniothalamus* family could represent potentials sources of drugs for the treatment of cancers. In addition, we herein report the isolation, purification and structure elucidation of this plant.

Materials and Methods

General Procedures

IR spectrum was recorded on Shimadzu 8900 FTIR spectrophotometer by using KBr pellets technique. The ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D NMR (COSY, DEPT, HMBC, HMQC) spectra were reccord by using a DPX on a BrÜker DPX 400 spectrometer in $CDCl_3$ as an internal standard. The EIMS was obtained by using a Finnigan LC-Q. Silica gel (Merck grade 7734, 70-230 mesh, 60 A) was used for column chromatography and TLC analysis manage with

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silica gel GF_{254} precoated plates with detection using UV detector.

Plant Material

Leaves and twigs of *G. tapis* were collected from Trang province, Thailand in January 2011. The plant was identified by Mr. Narong Nutasaen. A voucher specimen (BKF no. 130978) has been deposited at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

Extraction and Isolation

Leaves and twigs air-dried powdered of *G. tapis* (2.115 kg) was fermented with hexane 7 litres for 5 days. Filtration was used for separated hexane extracted solution and residue. The residue was re-extracted for 6 times with this solvent. Hexane extracted solution was combined and evaporated to dryness under reduced pressure to receive 25.33 g. The residue of hexane extraction was sequential extracted with ethyl acetate (4 litres x 5 days x 6 times) with same process of hexane to give 79.22 g, then acetone (3.8 litres x 5 days x 4 times) to obtain 54.22 g and methanol (3.63 litres x 5 days x 4 times) to gain 59.25 g of extracted, respectively. The ethyl acetate extracted (79.22 g) of *G. tapis* was separated on the silica gel column and eluted with gradient elution system such as hexane:EtOAc (0:100%) to EtOAc:MeOH (0:100%) to afford seven fractions (F_1-F_7). Fraction F_4 (11.59 g) was rechromatographed (silica gel) followed by crystallization with ethanol to obtain a goniothalamin (1.80 g).

Evaluation of Cytotoxic Activity

The cytotoxic activities of goniothalamin from *G. tapis* were performed by using the standard *in vitro* sulforhodamine B (SRB) assay. The cancer cell lines were grown in a 96-well plate (Vichai and Kirtikara, 2006). Ellipticine was used as a positive control. The cancer cell lines used were P-388 (murine lymphocytic leukemia), KB (human oralnasopharyngal), Col-2 (human colon cancer), MCF-7 (human breast cancer), Lu-l (human lung cancer), A549 (adenocarcinomic human alveolar basal epithelial cells), T24 (human urinary bladder cancer cells) and ASK (rat glioma cell). The normal cell line employed was HEK-293 (human embryonic kidney). The cytotoxic activity is expressed as 50% effective dose (ED_{50}).

Results

In the present work, the defatted ethyl acetate was subjected to phytochemical investigation leading to the isolation of goniothalamin (Fig. 1). The compound was performed on the basis of spectral and chemical evidence from spectroscopic techniques data (^1H, ^{13}C and 2D NMR) in Table 1 and also by comparison with closely related literature (Ahmad *et al*., 1991). Additionally, goniothalamin was assessed for cytotoxicities against nine cell lines (Table 2).

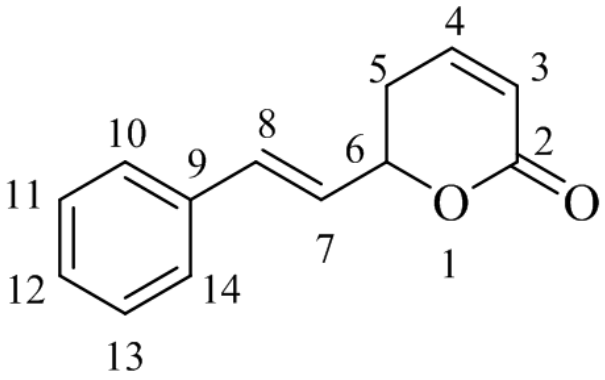

Fig. 1. Goniothalamin structure

Table 1. ^1H (400 MHz, $CDCl_3$), ^{13}C (100 MHz, $CDCl_3$), DEPT, HMBC and COSY spectral data of goniothalamin.[a]

Position	$\delta^{13}C$ (DEPT)	δ^1H (*J* Hz)	HMBC correlation	COSY correlation
2	163.83 (C)	-	-	-
3	121.57 (CH)	6.08 (*tt,* 1.83,1.83)	C-2, C-5	H-4
4	144.61 (CH)	6.92 (*m*)	C-2, C-5,C-6	H-3,H-5
5	29.80 (CH_2)	2.53 (*m*)	C-6,C-7	H-4,H-6
6	77.87 (CH)	5.09 (*ddd*, 6.32, 6.36,8.87)	C-7,C-8	H-5,H-7
7	125.61 (CH)	6.27 (*dd,* 15.97,6.34)	C-8	H-6,H-8
8	133.05 (CH)	6.73 (*dd*, 15.97, 0.73)	-	H-7
9	135.71 (C)	-	-	-
10,14	126.63 (CH)	7.24-7.43 (*m*)	-	-
11,13	128.62 (CH)	7.24-7.43 (*m*)	-	-
12	128.28 (CH)	7.24-7.43 (*m*)	-	-

[a]Chemical shift values in ppm and *J* values (in Hz) are presented in parentheses

Table 2. Cytotoxicity of goniothalamin from *G. tapis*

	Cytotoxicity (ED_{50}, $\mu g.mL^{-1}$)																	
	Cancer cells																Normal cell	
	P-388		KB		Col-2		MCF-7		Lu-1		A549		T24		ASK		HEK-293	
Compounds	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Goniothalamin	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
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: $ED_{50} < 20$ $\mu g.mL^{-1}$ was considered active for extracts and < 4 $\mu g.mL^{-1}$ for pure compounds. NR: No response ($ED_{50} \geq 20$ $\mu g.mL^{-1}$)

Discussion

Chemical Structure Elucidation

Goniothalamin obtained as a white crystal, mp 80-82°C. The UV spectra (EtOH) showed the presence of absorption at λ_{max} 254.5 nm. The IR (KBr) showed the absorption band at 1720, 1704, 1662 and 1247 cm^{-1}. The EIMS showed an ion peak $[M+H]^+$ at *m/z* 201 (17), 200(9), 184(16) and 183(100), corresponding to $C_{13}H_{12}O_2$. The ^1H NMR displayed two olefinic protons at δ 6.08 and 6.73 for H-3 and H-7, which also showed that they are in *a trans* configuration. It also showed aromatic protons at δ 7.43-7.24 (*m*, 5H). The ^{13}C NMR spectrum showed a carbonyl group signal at δ 163.83. Goniothalamin was first isolated from *Goniothalamus* species and found several times from the same genus (Ahmad *et al*., 1991; Hasan *et al*., 1994; Jewers *et al*., 1972; Jiang *et al*., 1997).

This compound is known natural product. However, the accurate structure was insensitively established by spectroscopic means (Table 1) and further confirmed by comparison with the spectral and physical data of literature (Ahmad *et al*., 1991).

Cytotoxic Activity

To investigate whether goniothalamin inhibited effect of cytotoxicity. It was showed that the goniothalamin exhibited cytotoxicity against eight cancer cell lines which were P-388, KB, Col-2, MCF-7, Lu-1, A549, T24, ASK and normal cell lines HEK-293. Ellipticine was used as positive control. Goniothalamin displayed highly potent cytotoxicity against P-388 (ED_{50} values of 0.19 $\mu g.mL^{-1}$) more than positive control ellipticine (ED_{50} values of 0.42 $\mu g.mL^{-1}$) and secondary inhibited Col-2, T24, HEK-293, Lu^{-1}, KB, MCF-7, A549 and ASK cell lines with ED_{50} values of 0.36, 0.39, 0.50, 0.54, 0.56, 0.56, 0.67 and 0.67 $\mu g.mL^{-1}$, respectively (Table 2).

Conclusion

This study focused on the phytochemical of Thai medicinal plant together with biochemical evaluation. The results presented herein reveal the goniothalamin from the *G. tapis* and its cytotoxic activity. Therefore, further intensive studies on the structure-anticancer activity relationships of this class of compound is highly recommended.

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Author's Contributions

Kallaya Sangrueng: Designed the research plan and participated in all experiments and contributed to the writing of the manuscript.

Saksri Sanyacharernkul and Sirinapa Nantapap: Coordinated the data analysis and contributed to the writing of the manuscript.

Narong Nantasaen: Collected the plant and identified voucher specimen (BKF no.).

Wilart Pompimon: Coordinated the data analysis and contributed to the writing of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of other authors have read and approved the manuscript.

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