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Cytotoxic compounds from *Goniothalamus repevensis* Pierre ex Finet & Gagnep

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ABSTRACT: Phytochemical investigation of the methanol extract of the leaves and twigs of *Goniothalamus repevensis* led to the isolation of five known compounds including two styryl lactones; (–)-goniodiol-7-monoacetate (1) and (–)-goniodiol diacetate (2) as well as three aristolactam alkaloids; piperolactam C (3), aristolactam A III (4), and aristolactam B III (5). The structure determination was established by various spectroscopic methods and by comparison with the literature data. Cytotoxic activity against a panel of mammalian cancerous cell lines of compounds 1–4 was investigated. Piperolactam C (3) was found to be active in all tested cell lines with IC_{50} values in the range of 3.30–64.6 μM. (–)-Goniodiol-7-monoacetate (1) exhibited cytotoxic activity only against ASK cells with IC_{50} value of 10.28 μM. Having been isolated as a major component, compound 1 can be considered chemotaxonomic significance for *G. repevensis*.

KEYWORDS: *Goniothalamus repevensis*, cytotoxicity, styryl lactone, goniodiol, aristolactam

INTRODUCTION

Plants in the *Goniothalamus* genus belong to the Annonaceae family with greater than 130 species recorded worldwide [1], of which about 25 species grow in Thailand [2]. Various plants in the *Goniothalamus* genus have long been used in Thai traditional medicines. In Narathiwat Province, Thailand, the flowers of *G. giganteus* and *G. malayanus* have been used as cardiotonic. In the Northeastern part of Thailand including Yasothon, Ubon Ratchathani, and Chaiyaphum Provinces, the flowers of *G. marcanii* and wood of *G. laoticus* have been used for treatment of infectious diseases in children [3].

Various *Goniothalamus* species are known to produce structurally diverse metabolites, comprising acetogenins [4–10], styryl lactones [11–17], alkaloids [14, 16–19], and flavonoids [20]. Several acetogenins and styryl lactones exhibited potent cytotoxic activity against various cancerous cell lines [21]. Some of the alkaloids and flavonoids displayed a broad spectrum of biological activities such as antifungal, antiplasmodial, antimycobacterial, insecticidal, antimalarial, anti-inflammatory, and immunosuppressive activities as well as inhibitory activity toward platelet-activating factor (PAF) receptor binding activity [22]. According to a vast biologically active compounds having been reported, plants in the *Goniothalamus* genus have drawn attention and been considered important natural resources to be searched for their potential metabolites for further development of anti-cancer drug candidates [23].

Goniothalamus repevensis Pierre Finet & Gagnep, called in Thai "Sad-Siam", is an endemic species and distributes in evergreen forest, 600–900 m above mean sea level in Chanthaburi Province, located in the Southeastern part of Thailand. Notably, small, and pink flowers are the key characteristics of *G. repevensis*, which is distinguishable from other plants in this genus. To the best of our knowledge, no phytochemical investigation or biological study on *G. repevensis* has been reported. Thus, the methanol extract of leaves and twigs of *G. repevensis* was preliminarily screened for cytotoxic activity. The results indicated that it exhibited significant cytotoxic activity against a panel of mammalian cancerous cell lines. In this work, the isolation and structural determination of chemical constituents from the methanol extract of the leaves and twigs of *G. repevensis* were firstly reported. Some isolated compounds were evaluated for their cytotoxic

activity.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter (Japan). UV absorption spectra were measured in MeOH on a Shimadzu 2800 UV-visible spectrophotometer (Japan); principal bands (λ_{max}) were recorded as wavelengths (nm) and $\log \varepsilon$. IR spectra were recorded on a Perkin-Elmer Frontier FT-IR spectrometer (USA); major bands (ν_{max}) were recorded in wave number (cm^{-1}). High resolution mass spectra (HRMS) were recorded on a Micromass model VQ-TOF spectrometer (Waters, USA). ^{1}H NMR and 13^{1}3 C NMR spectra were recorded on a Bruker DPX-400 or a Bruker Avance-500 spectrometer (Switzerland) in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) or residual non-deuterated solvent peak as an internal reference. Chromatographic methods were performed by using silica gel 60 (70–230 mesh, Merck, Germany) for column chromatography, silica gel plates (Silica gel 60 PF_{254}, Merck) for preparative TLC, and Sephadex LH 20 (GE Healthcare, USA) for gel filtration. All solvents used for extraction and isolation were distilled at their boiling point ranges prior to use.

Plant materials

The leaves and twigs of *G. repevensis* Pierre ex Finet & Gagnep were collected from Khao Khitchakut National Park, Chanthaburi Province, Thailand in October 2012. The plant was identified by Narong Nuntasaen, and the voucher specimen (BKF 192543) was deposited at the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand.

Extraction and isolation

The air-dried and finely powdered leaves and twigs of *G. repevensis* (1.88 kg) were macerated with MeOH (6 times × 4 l) at room temperature. After filtration and removal of the solvent under reduced pressure by a rotary evaporator, vacuum desiccation, and freeze-drying, the crude MeOH extract (181.5 g, 9.63%) was obtained. The crude MeOH extract was screened for cytotoxic activity against five mammalian cancerous cell lines, and the results were shown in Table 1. A part of the MeOH extract (135.0 g) was subjected to vacuum column chromatography over silica gel (400 g) and eluted with EtOAc-hexanes and MeOH-EtOAc gradients. Elution was initially conducted with EtOAc-hexanes gradient (0:1, 1:9, 1:4, 3:7, 2:3, 1:1, 7:3, and 1:0 v/v, 2 l each), followed by MeOH-EtOAc gradient (0:1, 1:9, 1:4, 1:1, and 1:0, 2 l each). Fractions (500 ml each) were collected and combined on the basis of their TLC characteristics to afford six fractions (F1–F6). Fraction F2 (14.3 g, eluted with EtOAc-hexanes 1:9, 1:4, and 3:7 v/v) provided goniodiol-7-monoacetate (1) (530.0 mg, 0.39%) after crystallization from EtOAc-hexanes. The mother liquor of fraction F2 (14.2 g) was subjected to silica gel column chromatography and eluted with EtOAc-hexanes gradient hexanes (5:95, 1:9, 1:4, 3:7, 2:3, and 1:1 v/v, 2 l each) to give 10 subfractions (F2-1 to F2-10). Piperolactam C (3) (40.0 mg, 0.03%), goniodiol diacetate (2) (20.0 mg, 0.01%), and aristolactam B III (5) (21.6 mg, 0.02%) were obtained from subfractions F2-4, F2-7, and F2-8, respectively, after Sephadex LH-20 column chromatography (1:1 v/v CH_2Cl_2/MeOH as eluent). Fraction F3 (15.8 g, eluted with EtOAc-hexanes 2:3 v/v) was subjected to silica gel column chromatography and eluted with EtOAc-hexanes gradient (1:4, 3:7, 2:3, 1:1, and 3:2 v/v, 2 l each) to give six subfractions (F3-1 to F3-6). Aristolactam A III (4) (20.0 mg, 0.01%) was isolated from subfraction F3-6. See Supplementary data for ^{1}H NMR and ^{13}C NMR data of compounds 1–5 (Tables S1–S5).

Cytotoxic assays

The crude MeOH extract and isolated compounds were evaluated for cytotoxic activity using the standard *in vitro* sulforhodamine B (SRB) assay. This method measures the cellular protein content of adherent and suspension cultures in 96 well microtiter plates. [24, 25]. Ellipticine, a potent cytotoxic plant alkaloid, was used as a positive control. Five cancerous cell lines were employed, including KB (human epidermoid carcinoma in the mouth), HT-29 (human colorectal adenocarcinoma), MCF-7 (human breast carcinoma), A549 (human lung carcinoma), and ASK (rat glioma cells). For noncancerous cell line, Hek293 (noncancerous human embryonic kidney cells) was employed for screening of the MeOH extract while CL (human normal liver hepatic cells) was employed for screening of the pure compounds. The potency of cytotoxic activity is expressed as 50% inhibitory dose (IC_{50}).

RESULTS AND DISCUSSION

The MeOH extract of the leaves and twigs of *G. repevensis* was obtained by maceration extraction. Preliminary screening indicated that the crude MeOH extract showed significant cytotoxic activity against a panel of mammalian cancerous cell lines including KB, HT29, MCF7, A549, ASK, and Hek293 cell lines with IC_{50} values in the range of 1.58–3.89 μg/ml. The isolation and purification of chemical compounds were performed by chromatographic techniques and crystallization to yield five known compounds 1–5. The structures of the isolated compounds were elucidated by spectroscopic techniques including UV, IR, NMR, and MS analysis. By comparison of the spectroscopic data of compounds 1–5 with those previously reported in the literature; see Tables S1–S5, compounds 1–5 were identified to be (–)-goniodiol-7-monoacetate (1) [26], (–)-goniodiol diacetate (2) [26], piperolactam C (3)

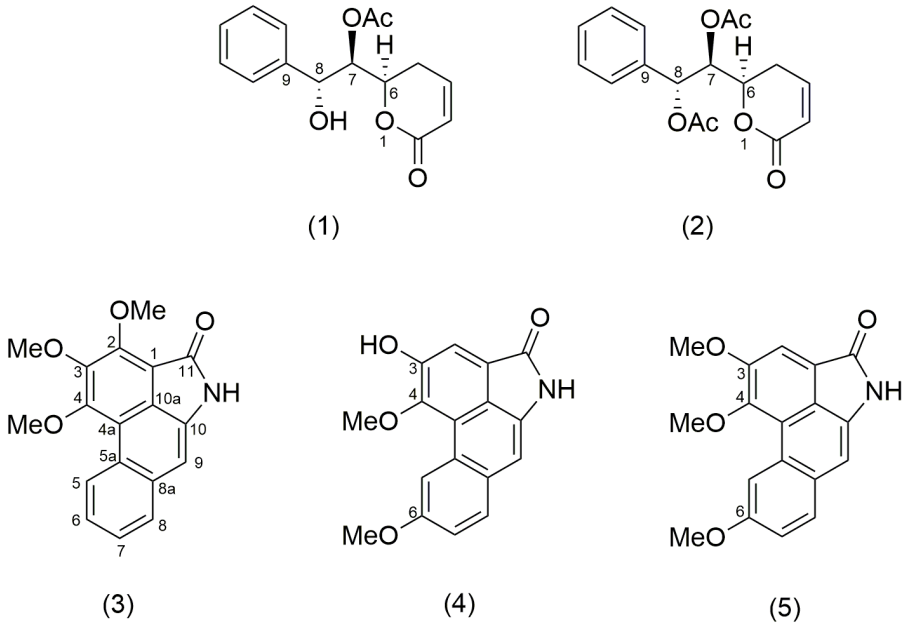

Fig. 1 Isolated compounds 1–5 from leaves and twigs of *G. repevensis*.

Table 1 Cytotoxicity of MeOH extract (IC_{50}, μg/ml) and isolated compounds 1–4 (IC_{50}, μM).

Extract/Compound	Cell line (IC_{50})[a]					
	KB	HT-29	MCF 7	A549	ASK	CL[d]
MeOH extract[b]	1.58	3.81	3.89	3.16	3.17	2.01
1	24.00	35.11	55.59	37.64	10.28	32.39
2	29.06	27.68	35.31	28.30	13.29	26.14
3	8.47	> 64.66	31.20	11.90	3.30	8.21
4	10.23	45.72	63.63	46.06	7.38	41.11
Ellipthicine[c]	2.03	2.31	1.95	1.99	1.95	2.03

[a] IC_{50} value presented in μM was obtained from three independent experiments for pure compounds. KB, human nasopharyngeal carcinoma; HT-29, human colorectal adenocarcinoma; MCF-7, human breast carcinoma; A549, human lung carcinoma; ASK, rat glioma cells; and CL, human normal liver hepatic cells.
[b] IC_{50} presented in μg/ml for extract. [c] Ellipticine was used as a positive control for cytotoxicity assay. [d] Using Hek293, noncancerous human embryonic kidney cells, in testing for the extract.

[27], aristolactam AIII (4), and aristolactam BIII (5) [28, 29]; see Fig. 1. Compounds 1–4 were evaluated for their cytotoxic activity against a panel of mammalian cancerous cell lines as well as noncancerous human liver hepatic cells (CL); see Table 1. All tested compounds were found active against ASK cancer cells with IC_{50} in the range of 3.30–13.29 μM with compound 3 being the most active compound (IC_{50} 3.30 μM). Compound 3 was also active against KB and A549 cancer cells with IC_{50} values of 8.47 and 11.90 μM, respectively. However, compound 3 was toxic to CL cells with IC_{50} value of 8.21 μM. (–)-Goniodiol-7-monoacetate (1), the major component isolated (0.39%), exhibited selective cytotoxic activity against ASK cell line with IC_{50} value of 10.28 μM while it was less toxic to CL (IC_{50} 32.39 μM). Based on these results, compound 1 can be considered a potential candidate for further in-depth study of its cytotoxic effects.

For the chemotaxonomic significance, styryl lactones are the major types of compounds found in plants of *Gonithalamus genus*. In particular, (–)-goniodiol-7-monoacetate (1), first isolated from the leaves and stems of *G. amuyon*, is commonly found in various plant species belonging to the *Goniothalamus genus* [26, 30, 31]. Recently, it was reported to be isolated from the barks of *G. lanceolatus* [32]. (–)-Goniodiol-7-monoacetate (1) showed potent cytotoxic activity against human epidermoid carcinoma of the nasopharynx (KB), murine lymphocytic leukemia (P-388), human melanoma (RPMI), and central nervous system carcinoma (TE671) with ED_{50} values of < 0.1 μg/ml [31]. Therefore, (–)-goniodiol-7-monoacetate (1) was a potential candidate obtained

from natural resources and deserves comprehensive study toward its cytotoxic effects for further development of anti-cancer drug.

CONCLUSION

The present work described the first phytochemical investigation of *Goniothalamus repevensis*, leading to the isolation of five known compounds including (–)-goniodiol-7-monoacetate (1), (–)-goniodiol diacetate (2), piperolactam C (3), aristolactam AIII (4), and aristolactam BIII (5) from the cytotoxic MeOH extract of the leaves and twigs. The structure determination of the isolated compounds was established by means of spectroscopic methods and by comparison with the literature data. Compounds 1–4 showed cytotoxic activity against a panel of mammalian cancer cell lines with IC_{50} values in the range of 3.30–64.6 μM with compound 3 displaying significant cytotoxic activity against ASK and KB cell lines with IC_{50} values of 3.30 and 8.47 μM, respectively. Compound 1 exhibited cytotoxic activity only against ASK cell line with an IC_{50} value of 10.28 μM while it was less toxic to CL cell lines (human normal liver hepatic cells) with an IC_{50} value of 32.39 μM. Compound 1, being isolated as a major component, can be potentially considered the chemotaxonomic significance for *G. repevensis* species.

Appendix A. Supplementary data

Supplementary data (Tables S1–S5) associated with this article is available upon request from the corresponding author.

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