Chemical constituents and cytotoxic effect from the barks of *Goniothalamus chinensis* Merr. & Chun. growing in Vietnam

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**ABSTRACT**

*Goniothalamus chinensis* Merr. & Chun is a medicinal plant which has cytotoxicity, antitumor, antifungal, antimalaria and antituberculosis and antioxidant activity. The styryl-lactone, alkaloid and acetogenin are the main group compounds in species of *Goniothalamus*. In this study, we aimed to investigate phytochemical and cytotoxic effect of compounds from bark of *Goniothalamus chinensis* Merr. & Chun. The bark of *Goniothalamus chinensis* Merr. & Chun grown in Vietnam was extracted by methanol. Three compounds were isolated using on Sephadex LH-20 and preparative glass-backed TLC plates. The compound's structure were characterized on the basis of spectroscopic data and by comparing their physicochemical and spectral data with those published in literatures. The cytotoxicity of three compounds was evaluated against four cancer cell lines: epithelial cancer (KB), liver (Hep G2); breast cancer (MCF-7) and lung (LU-1). We have been isolated three compounds. Their structures were identified as Goniolthalamin (1), Aristolactam BII (2), 3-Methyl-1H-benz[f]indole-4,9-dione (3). Isolated compounds were tested cytotoxicity activity against different cancer cell lines. The compound 1 demonstrated the highest potency against the epithelial cancer cell line with an IC₅₀ of 4.18 ± 0.60µg/mL. Other compounds have weak cytotoxic activity.

**INTRODUCTION**

With the tropical climate, Vietnam has diversity of plant and animals species. In total, Vietnam has more than 12000 plant species; of which nearly 4000 species can be used in traditional medicine. The genus of *Goniothalamus* contains about 160 species in the world, mostly in Southeast Asia, among which about 19 are distributed in Vietnam (Wiart, 2007). Some species have been used as folk medicines for healing, for digestion and as a tonic plant. Main compounds in *Goniothalamus* are styryl-lactone, alkaloid and acetogenin which have cytotoxic, antitumor, insecticides, antifungal, antimalaria, antituberculosis and antioxidant activities (Blázquez et al., 1999). Orlikova et al., have showed goniolahtamin, a styryl-lactone isolated from *Goniothalamus macrophyllus*, have cytotoxic and anti-inflammatory effects. Thí compound also inhibits the tumor necrosis factor-α-induced NF-κB activation and have anti-leukemic potential (Orlikova et al., 2013). Other study demonstrated that goniolahtone C, a styryl lactone, was extracted from *Goniothalamus chelensis*, strongly inhibited platelet-derived growth factor-BB-induced vascular smooth muscle cell migration and proliferation (Sun et al., 2014). Goniolahtamin, an active compound extracted from *Goniothalamus griffithii*, also inhibits human leukemic cells undergo apoptosis via intrinsic and extrinsic pathways (Petsphantsakul et al., 2013) and cervical cancer cells (HeLa) (Alabasi et al., 2012). Furthermore, goniolahtamin also showed potent gastroprotective activity on gastric ulcers models in rats. The mechanism is goniolahtamin may induce the production of sulfhydryl compounds and prostaglandins (Vendramini-Costa et al., 2014). For now, species of *Goniothalamus* continue being interested by many researches. However, there has been no report about the studies on the chemical constituents and bioactivity of this plant growing in Vietnam.

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Therefore a study about the chemical composition of *Goniothalamus chinensis* Merr. & Chun is much needed. In this study, from barks of *Goniothalamus chinensis* Merr. & Chun grown in Vietnam, have extracted, isolated and identified three compounds: Goniothalamin (1), Aristolactam BII (2), 3-Methyl-1H-benz[f]indole-4,9-dione (3). Furthermore, we have evaluated the cytotoxic of these compounds on four cell line epithelial cancer (KB), liver (Hep G2); breast cancer (MCF-7) and lung (LU-1).

**MATERIALS AND METHODS**

**Plant material**

Barks of *Goniothalamus chinensis* Merr. & Chun. (Kingdom: Plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Magnoliidae; Family: Annonaceae) were collected in Ha Giang province, Vietnam during 2013 and authenticated by the School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam (SMP-VNU). A voucher specimen (No. SMP-2013-0018) has been deposited at the Herbarium of SMP-VNU.

**General experimental procedures**

Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB WaagetechnikRapido, Germany). The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). The NMR [1H (500 MHz), 13C (125 MHz), and DEPT-90 and 135 MHz]) spectra were recorded on an AVANCE spectrometer AV 500 (Bruker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST). Chemical shifts were reported in ppm downfield from TMS with J in Hz. Electrospray Ionization Mass Spectra (ESI-MS) were recorded on a Varian Agilent 1100 LC-MSD mass spectrometer. Analytical TLC was performed on Kieselgel 60 F254 (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F254 (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 254 and 365 nm) and by spraying with 10% H2SO4, followed by heating with a heat gun. Column chromatography was performed on silica gel (70–230 and 230–400 mesh, Merck). Organic solvents were of analytical grade.

**Extraction and isolation**

*Goniothalamus chinensis* Merr. & Chun barks of 1.7 kg was dried and extracted with methanol (5L × 24 hours × 3 times) at room temperature. The methanol extracts were combined and then evaporated to dryness in vacuo at 40°C to yield crude extract (40.3 g). This crude extract was chromatographed on Sephadex LH-20 column and eluted with gradient of CH2Cl2/MeOH to yield ten fractions F1–F10. Fraction F3 was further separated over a silica gel column and eluted with n-hexane/acetone (9:1) to yield eight subfractions F3.1–F3.8. Fraction 3.7 was further separated on Sephadex LH-20 column and eluted with MeOH/CH2Cl2 (95/5) to yield two fractions F3.7.1 and F3.7.2. Fraction F3.7.2 was applied on preparative glass-backed TLC plates, coated with silica gel 60 and eluted with gradient of n-hexane/acetone to yield compound 1 (14 mg). Fraction 3.8 was further separated on Sephadex LH-20 column and eluted with MeOH/CH2Cl2 (95/5) to yield compound 3 (17.5 mg). Fraction F4 was chromatographed over a silica gel column and eluted with n-hexane/acetone (9:1) to yield fifteen subfractions F4.1–F4.15. Fraction F4.13 was crystallized on solvents CH2Cl2-MeOH (1:1) to yield compound 2 (6.4 mg).

**Goniothalamin (1):** was obtained as a yellow light, solid powder, melting point 82-84°C; Rf = 0.44 (n-hexane-CH2Cl2 20/80, v/v); [α]D27 = +81.0° (C 0.2; EtOH). FT-IR (KBr) νmax (cm−1): 2925, 1707, 1664, 1627, 1454, 1382, 1244, 1066, 966, 822, 759, 699, 584, 512, 440. UV λmax MeOH nm (log ε): 210 (4.43); 251 (4.32); 283 (2.52); 290 (3.03). (+)-ESI-MS: m/z 223 [M+Na]+. 1H-NMR (500 MHz, CDCl3): δ (ppm) 7.40 (2H, m, H-10, H-14); 7.34 (2H, m, H-11, H-13); 7.27 (1H, m, H-12); 6.92 (1H, dd, J=4.10; 5.0; 9.5 Hz, H-4); 6.73 (1H, d, J=16.0 Hz, H-8); 6.27 (1H, dd, J=6.5, 16.0 Hz, H-7); 6.09 (1H, dt, J=2.0, 10.0 Hz, H-3); 5.10 (1H, dd, J=1.0; 6.5; 15.5 Hz, H-6); 2.54 (2H, m, H-5). 13C-NMR (125 MHz, CDCl3): δ (ppm) 163.8 (C-2); 144.5 (C-4); 135.8 (C-9); 133.1 (C-8); 128.7 (C-11, C-13); 128.3 (C-12); 126.7 (C-10, C-14); 125.7 (C-7); 121.7 (C-3); 77.9 (C-6); 29.9 (C-5).

**Aristolactam BII (2):** was obtained as a yellow, crystalline powder, melting point 248-250°C; Rf = 0.38 (n-hexane-acetone 67/33, v/v). FT-IR (KBr) νmax (cm−1): 3449, 3194, 2926, 2855, 1717, 1644, 1607, 1240, 1011, 838, 720, 624, 530, 466. UV λmax MeOH nm (log ε): 205 (3.59); 232 (3.56); 263 (3.49); 276 (3.54); 286 (3.53); 317 (2.97); 385 (2.94). (+)-ESI-MS: m/z 302 [M+Na]+; 581 [2M+Na]+. 1H-NMR (500 MHz, CDCl3 + CD3OD): δ (ppm) 9.12 (1H, m, H-5); 7.72 (1H, s, H-2); 7.72 (1H, m, H-8); 7.47 (2H, m, H-6, H-7); 7.00 (1H, s, H-9); 4.02 (3H, s, 4-OCH3); 3.98 (3H, s, 3-OCH3). 13C-NMR (125 MHz, CDCl3 + CD3OD): δ (ppm) 170.2 (C=O); 154.4 (C-3); 151.5 (C-4); 134.8 (C-8a); 134.2 (C-10); 128.9 (C-12), 127.4 (C-5), 127.4 (C-7), 126.9 (C-5a), 125.8 (C-6), 124.2 (C-10a), 121.2 (C-1), 120.8 (C-4a), 109.5 (C-2), 106.2 (C-9), 60.2 (4-OCH3), 56.8 (3-OCH3).

**3-Methyl-1H-benz[f]indole-4,9-dione (3):** was obtained as a yellow, solid powder, melting point 248-249°C; Rf = 0.44 (n-hexane-CH2Cl2 25/75, v/v). (+)-ESI-MS: m/z 210 [MH]+. (+)-ESI-MS: m/z 212 [M+H]+; (+)-HR-ESI-MS m/z 212.0710 [M+H]+, FT-IR (KBr) νmax (cm−1): 3429, 3322, 2938, 1647, 1588, 1507, 1438, 1328, 1031, 932, 714. UV (MeOH) λmax nm (log ε): 258 (4.07); 330 (3.32). 1H-NMR (500 MHz, CDCl3+ CD3OD): δ (ppm) 8.08 (1H, m, H-5); 8.01 (1H, m, H-8); 7.59 (2H, m, H-6, H-7); 6.83 (1H, m, H-2); 2.34 (3H, s, CH3-10). 1H-NMR (500 MHz, DMSO): δ (ppm) 12.65 (1H, br s, H-1); 8.18 (2H, m, H-5, H-8); 7.76 (2H, m, H-6, H-7); 7.13 (1H, m, H-2); 2.31 (3H, s, CH3-10). 13C-NMR (125 MHz, CDCl3...
Cytotoxicity Assay

Cytotoxicity of these compounds was evaluated against four types of cancer cell lines: epithelial cancer (KB), liver (Hep G2); breast cancer (MCF-7) and lung (LU-1) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell lines were cultured in Dulbecco’s Modified Eagle medium (DMEM) with 10% foetal bovine serum, at 37°C, 5% CO2 and 95% humidity. Cells were plated in 96-well flat bottom tissue culture plates and allowed to density of approximately 3x10^4/mL then begin experiment. The cells were incubated with 100 μL of compound at a range of concentration (from 1 μg/mL to 1000 μg/mL) for 72 hours. Then, 0.2 mg/mL MTT reagent was added to each well, and the plate was incubated for 4 hours at 37°C. Next, DMSO (100 μL) was added and the plates shaken for 5 min. The absorbance for each well was measured at 540 nm in a microtiter plate reader and the percentage cell viability (CV) was calculated manually using the formula:

CV = (Average abs of duplicate drug wells - Average abs of control wells) x 100

The effects of compounds were expressed by IC_{50} values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells) (Monks et al., 1991).

RESULTS AND DISCUSSION

Isolation and Chemistry

Goniothalamin (1)

Goniothalamin (1) was obtained as a light yellow, solid powder, melting point 82-84°C and the optical rotation [α]_{D}^{27} = +81.0° (c 0.2; EtOH). The ring γ-lactone α,β-unsaturated showed typical absorption bands arising from carbonyl group (1707 cm^{-1}) inIR spectrum, maximum absorption wavelength at λ_{max} 210 nm (log e: 4.43; MeOH) in UV spectrum and δ_{C} 163.8 in 13C-NMR spectrum. The molecular formula was established as C_{17}H_{17}O_{2} based on a molecular ion peak at m/z 302 [M + Na]^{+}. The 1H-NMR spectrum showed the presence of phenyl group with five signals proton at δ_{H} 7.27- 7.40 (5H, m, C_{6}H_{5}); signals of 4 proton olefinic at δ_{H} 6.92 (1H, ddd, J=4.0; 5.0; 9.5 Hz, H-4); 6.73 (1H, d, J=16.0 Hz, H-8); 6.27 (1H, dd, J=6.5; 16.0 Hz, H-7); 6.09 (1H, dt, J=2.0; 10.0 Hz, H-3). The 1H-NMR spectrum of compound 1 also exhibited the presence of a methylene signal at δ_{H} 2.54 (m, 2H, H-5) and a methinoxy signal at δ_{H} 5.10 (1H, ddd, J=1.0; 6.5; 15.5 Hz; H-6). The 13C-NMR and DEPT spectra of compound 1 indicated the presence of 13 carbon atoms in the molecule. There are a carbonyl carbon (δ_{C} 163.8), one CH_{2} group at δ_{C} 29.9, one sp^{3} methine oxygenated carbons at δ_{C} 77.9; nine methine sp^{2} group at δ_{C} 121.7-144.5 and one quaternary carbon sp^{3} at δ_{C} 135.8. The signal in COSY spectra allowed correlating from C-3 to C-8. The coupling constants J (16.0 Hz) between H-7 and H-8 indicated that two protons are in trans position. The coupling constants J (9.5 Hz) between H-3 and H-4 indicated that two protons are in cis position. Based on the above evidence and the literature data (Wattanapiromsakul et al., 2005)compound 1 was identified as goniothalamin. This compound is styryl lactone has been isolated from many Goniothalamus species. It has antitumor activity in vitro and in vivo (de Fátima et al., 2005; Merelyala and Joe, 2001; Tian et al., 2006). Furthermore, it has antifungal (Fatima et al., 2008) and antioxidant activity (Kim et al., 2012).

Aristolactam BII (2)

Compound 2 was obtained as a yellow, crystalline powder, melting point 248-250°C, gave positive result with Dragendorff agent. The IR spectrum showed typical absorption bands arising from NH (ν_{max} 3449 cm^{-1}) and C=O (ν_{max} 1717 cm^{-1}). The UV spectrum in solvent MeOH showed typical maximum absorption wavelength of phenanthrene structure at λ_{max} nm (log e): 232 (3.56), 263 (3.49), 276 (3.54), 286 (3.53), 317 (2.97) and 385 (2.94). The molecular formula was established as C_{17}H_{15}NO_{2} based on a molecular ion peak at m/z 302 [M + Na]^{+}. The 13C-NMR spectra of compound 2 indicated the presence of 17 carbon atoms in the molecule. There are a carbonyl carbon (δ_{C} 170.2), two methoxy groups (δ_{C} 60.2 and 56.8), six methine sp^{2} group at δ_{C} 106.2; 109.5; 125.8; 127.4; 127.4; 128.9 and eight quaternary carbon sp^{3}.

The 1H-NMR spectrum of compound 2 exhibited the presence of 6 signals proton of aromatic ring at δ_{H} 9.12 (1H, m, H-5); 7.72 (1H, s, H-2); 7.72 (1H, m, H-8); 7.47 (2H, m, H-6 + H-7); 7.00 (1H, s, H-9) and two methoxy groups OCH_{3} at δ_{H} 4.02 (3H, s, OCH_{3}); 3.98 (3H, s, OCH_{3}) . HMBC spectrum showed the correlation between H-9 with C-8, C-5a, C-8a, C-10a and C-10;
between H-5 with C-7, C-8a and C-5a, between H-8 with C-6, C-7, C-5a and C-9. Then it can deduced the correlation between C-8 with C-8a, C-9, C-10 and C-10a; C-5 with C-5a, C-8a and C-7. There is also the correlation between H-2 with C-3, C-4, C=O and C-1, hence C-2 correlates with C=O through C-1. The correlation between protons of methoxy group (δH 4.02) with C-3 (δC 154.4) and protons of methoxy group (δH 3.98) with C-4 (δC 151.5) indicated that two methoxy OCH₃ group linked with carbon C-3 and C-4. Based on the above evidence and the literature data (Marques et al., 2011) compound 2 was identified as Aristolactam BII. This compound has neuroprotective (Kim et al., 2004) and cytotoxicity with P-388 leukemia and A-549 lung adenocarcinoma epithelial cell line (Tsai et al., 2005).

3-Methyl-1H-benz[f]indole-4,9-dione (3)

Compound 3 was obtained as a yellow, solid powder. The molecular formula was established as C₁₃H₁₂O₃N based on a molecular ion peak at m/z 212.0710 [M+H]+. The IR spectrum showed typical absorption bands arising from NH (νₘₐₓ 3429 cm⁻¹) and carbonyl C=O (νₘₐₓ 1647 cm⁻¹).

The ¹H-NMR spectrum of compound 3 exhibited the presence of 5 signals proton of aromatic ring at δH 8.08 (1H, m, H-5); 8.01 (1H, m, H-8); 7.59 (2H, m, H-6 and H-7) and 6.83 (1H, m, H-2); and 1 methyl group at δH 2.31 (3H, s). The ¹³C-NMR spectra of compound 3 indicated the presence of 13 carbon atoms in the molecule. There are a methyl group; 5 methine sp² groups; and 2 carboxyls group at δC 182.3 (C-9) v 175.7 (C-4) and 5 quaternary carbons in aromatic ring at δC 122.8 (C-3), 125.1 (C-3a), 133.4 (C-4a), 134.5 (C-8a) and 132.7 (C-9a).

The signal in COSY spectra allowed determining the correlation from H-3 to H-8. HMBC spectrum showed the correlation between H-5 and H-8 with C-4a and C-8a, confirmed the presence of benzene A ring. Also there are correlations between 2 carboxyls C-4 and C-9 with H-5 and H-8 in HMBC spectrum respectively. It allowed confirming the C-4 and C-9 linked to ring A at position C-4a and C-8a. The pyrole C ring also is determined by HMBC spectrum: H-2 correlates with C-3, C-3a and C-9a; CH₃-10 correlates with C-2 and C-3a. The methyl group position at C-3 in pyrole C ring is also confirmed by NOE correlation between H-2 and CH₃-10. Based on the above evidence and the literature data (Efđi et al., 2010) compound 3 was identified as 3-methyl-1H-benz[f]indole-4,9-dione. This compound was first isolated from Goniothalamustapis Miq (Efđi et al., 2010).

Bioactivity

The result of cytotoxicity tests of isolated compounds against epithelial cancer (KB), liver (Hep G2), breast cancer (MCF-7) and lung (LU-1) cell lines are shown in Table 1.

Table 1: Cytotoxicity of isolated compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µg/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>KB</td>
</tr>
<tr>
<td>Compound 1</td>
<td>4.18 ± 0.60</td>
</tr>
<tr>
<td>Compound 2</td>
<td>52.32 ± 5.21</td>
</tr>
<tr>
<td>Compound 3</td>
<td>67.32 ± 3.73</td>
</tr>
</tbody>
</table>

Only compound 1 exhibited strong cytotoxic activity against epithelial cancer (KB), liver (Hep G2), breast cancer (MCF-7) and lung (LU-1) cell lines. Previous study have demonstrated Goniothalamin possess anticanancer potential activity through mechanism of induces apoptosis and inhibits TNF-α-induced NF-κB activation (Orlikova et al., 2013; Seyed et al., 2014). For the compounds 2, 3, we have found the IC₅₀ against fours tested cancer cell line were greater over than ten times as compared with compound 1.

CONCLUSION

From the bark of Goniothalamus chinensis Merr. & Chun. we have isolated three compounds, Goniothalamin (1), Aristolactam BII (2), 3-Methyl-1H-benz[f]indole-4,9-dione (3). The compound 1 showed the highest potency against the epithelial cancer (KB) cell line with an IC₅₀ of 4.18 ± 0.60 µg/mL. Others compounds have weak cytotoxic activity against all cell lines tested.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES


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