Two New C-benzylated Dihydrochalcone Derivatives from the Leaves of Melodorum siamensis

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Abstract

Two new C-benzylated dihydrochalcone derivatives, 4,2,′,4-trihydroxy-6′-methoxy-3′(2′-hydroxybenzyl)dihydrochalcone (1) and 2,′4-dihydroxy-4,′,6′-dimethoxy-3′(2′-hydroxybenzyl)dihydrochalcone (2), along with six known flavonoid derivatives (3-8), a known dihydrochalcone dimer (9), three known aromatic esters (10-12), and one known aromatic amide (13), were isolated from the leaves of Melodorum siamensis. The structures of the compounds were elucidated by spectroscopic analysis, mainly 1D and 2D NMR techniques (1H, 13C, COSY, HMQC, and HMBC), as well as by comparison with literature data. The isolated compounds with a sufficient amount for biological assays were evaluated for their antimalarial, antimycobacterial, and cytotoxic activities. Compounds 1, 2, and 13 exhibited strong cytotoxicity against human tumor cell lines KB and NCI-H187, with IC50 values in the range of 0.66-7.16 µg/mL.

Key words

Melodorum siamensis · Annonaceae · antimycobacterial activity · antimalarial activity · cytotoxic activity

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The genus Melodorum (Annonaceae) comprises 55 species, which grow in tropical Asia [1]. Two species have been phytochemically investigated. The aporphine alkaloids were isolated from a mixed sample of M. punctulatum leaves and bark [2]. Several cytotoxic butenolides were isolated from the leaves of M. fructicosum [3, 4] and two oxidized heptanes were found in the flowers of the same plant [5]. In the course of our continuing search for bioactive constituents from Thai medicinal plants, a preliminary screening of the ethyl acetate extract of the leaves of Melodorum siamensis (Scheff.) Ban revealed cytotoxicity against human epidermoid carcinoma (KB), human breast cancer (MCF7), and human small cell lung cancer (NCI-H187) cell lines with IC50 values of 1.7, 2.4, and 6.42 µg/mL, respectively, antimalarial activity against Plasmodium falciparum with an IC50 value of 9.7 µg/mL, and antimycobacterial activity against Mycobacterium tuberculosis H37Ra with an MIC value of 200 µg/mL. Chemical and biological studies of this species have not been reported. This paper describes the isolation and structure elucidation of two new C-benzylnated dihydrochalcone derivatives, 4,2,′,4-trihydroxy-6′-methoxy-3′(2′-hydroxybenzyl)dihydrochalcone (1) and 2,′4-dihydroxy-4,′,6′-dimethoxy-3′(2′-hydroxybenzyl)dihydrochalcone (2), six known flavonoid derivatives, 4,2,′,4-trihydroxy-6′-methoxydihydrochalcone (3), 2,′4-dihydroxy-4,′,6′-dimethoxydihydrochalcone (4), 2,′4-dihydroxy-4,′,6′-dimethoxychalcone (5), 2′-hydroxy-4,4′,6′-trimethoxychalcone (6) [6], 7,4′-dihydroxy-5-methoxyflavone (7) [7, 8], and 7-hydroxy-5,4′-dimethoxyflavanone (8) [6, 9], a known dihydrochalcone dimer, 3′3′′-bis-2,′4,′,6′-trihydroxy-4-methoxydihydrochalcone (9) [10], three known aromatic esters, benzyl benzoate (10), 2-methoxybenzyl benzoate (11) [11], and 3-phenylpropenyl 3-phenylallylamine (12) [12], and a known aromatic amide, p-coumaroyl-b-phenethylamine (13) [13] from the leaves of M. siamensis (Fig. 1). Structure elucidation was performed using UV, IR, 1D and 2D NMR techniques, as well as comparison with literature data. The biological activities of compounds 1-4, 7-11, and 13 are also reported. Compound 1 was obtained as a pale yellow solid and had the molecular formula C23H23O6 by HR-TOF-MS. The UV spectrum showed absorbance bands at 223, 289, and 334 nm and the IR spectrum indicated hydroxyl (νmax 3173 cm⁻¹), carbonyl (νmax 1631 cm⁻¹), and aromatic (νmax 1514 cm⁻¹) groups. The 1H NMR spectrum of 1 showed two triplets at δ 2.86 (J = 7.7 Hz) and 3.27 (J = 7.7 Hz), which are typical of a dihydrochalcone moiety. This was consistent with the 13C NMR data of 1, which contained signals from two methylene carbons (δ 30.8 and 47.0) and a carbonyl carbon (δ 205.8). The 1H NMR spectrum of 1 also showed two doublets at δ 6.75 (2H, J = 8.3 Hz) and δ 7.09 (2H, J = 8.3 Hz) of a p-substituted aromatic ring (ring B) and a singlet at δ 6.14 (1H) at a pentasubstituted aromatic ring (ring A) of the dihydrochalcone. In addition, signals of one methoxy group at δ 3.86 (3H, s), one hydrogen bonded phenolic hydroxyl group at δ 14.76 (1H, s), and two phenolic hydroxyl groups were also observed in the 1H NMR spectrum of 1. This was consistent with the 13C NMR data of 1 which exhibited signals of one p-substituted aromatic ring (ring B) at δ 133.3 (C-1), 130.3 (C-2 and C-6), 116.1 (C-3 and C-5), and 156.6 (C-4), and one pentasubstituted aromatic ring (ring A) at δ 105.7 (C-1′), 165.7 (C-2′), 107.8 (C-3′), 155.2 (C-4′), 91.9 (C-5′), and 162.8 (C-6′). The 1H NMR of 1 also indicated the presence of four adjacent aromatic protons at δ 6.83 (dd, J = 7.6, 1.6 Hz, H-3′), 7.00 (td, J = 7.6, 1.6 Hz, H-4′), 6.73 (td, J = 7.6, 1.6 Hz, H-5′), and 7.21 (dd, J = 7.6, 1.6 Hz, H-6′), and a phenolic hydroxyl group and a singlet of two methylene protons at δ 3.89, which were assigned to an o-hydroxybenzyl moiety [14]. This was consistent with the 13C NMR spectrum, which exhibited signals of four aromatic methine carbons at δ 116.0 (C-3′), 127.9 (C-4′), 120.7 (C-5′), and 131.2 (C-6′), one quaternary aromatic carbon at δ 127.9 (C-1′), one oxyquaternary aromatic carbon at δ 155.2 (C-2′), and one methylene carbon at δ 22.8 (C-7′). The 2D HMBC data (Fig. 2) revealed correlations between the proton signal of 6′-OCH3 (δ 3.86) and C-6′ (δ 162.8), between 2′-OH (δ 14.76) and C-2′ (δ 165.7), C-1′ (δ 105.7) and C-3′ (δ 107.8), and between 7′-CH3 (δ 3.89) and C-3′ (δ 107.8), C-2′ (δ 165.7), and C-4′ (δ 155.2). These results indicated that the methoxy group, the hydrogen bonded phenolic hydroxyl group, and the o-hydroxybenzyl moiety were attached to C-6′, C-2′, and C-3′, respectively. Compound 1 was, therefore, assigned as 4,2,′,4-trihydroxy-6′-methoxy-3′(2′-hydroxybenzyl)dihydrochalcone.

Compound 2 was isolated as pale yellow crystals and had the molecular formula C24H24O6 by HR-TOF-MS (m/z 409.1649 [M + H]+), calc. for C24H25O6, 409.1651. The structure of 2 was closely related to 1 based on 1H NMR, 13C NMR (Table 1), IR, and UV spectroscopic data (Materials and Methods). However, 2 had one carbon and two hydrogen atoms more than 1. The appearance of a three-proton singlet at δ 3.74 and a methoxy carbon at δ 55.7 in the 1H and 13C NMR spectra of 2, respectively, suggested an addi-
The 2D HMBC correlations of the methoxy group, 4-OCH₃ (δ 3.74) to C-4 (δ 159.2), and H-2 and H-6 (δ 7.18) to C-3 (δ 114.9), C-5 (δ 114.9), and C-4 (δ 159.2), confirmed the substitution of the methoxy group at C-4. Compound 2 was, therefore, deduced as 2′,4′-dihydroxy-4,6′-dimethoxy-3′(2″-hydroxybenzyl)dihydrochalcone.

Most of the isolated compounds, except compounds 5, 6, and 12, which were isolated with insufficient amounts for the biological assay, were evaluated for their cytotoxicity against three human cancer cell lines [15] as summarized in Table 2. Compounds 2 and 13 showed strong cytotoxicity in KB and NCI-H187 cell lines, with the IC₅₀ in the range of 0.66–4.09 µg/mL. Compound 1 exhibited strong cytotoxicity in the NCI-H187 cell line with an IC₅₀ value of 3.66 µg/mL and moderate activity in KB and MCF7 cell lines with IC₅₀ values of 7.16 and 14.86 µg/mL, respectively. The dihydrochalcones 3 and 4 showed moderate activity in all cell lines, with IC₅₀ in the range of 5.18–14.26 µg/mL while the flavonones 7 and 8 were less active. The dimeric dihydrochalcone 9 was inactive in all cell lines. Bioactivity results in Table 2 showed that the presence of C-benzylated substituent on ring A of 1 and 2 appear to be an important moiety for cytotoxic activity, while the appearance of methoxy on ring B of 2 is essential for cytotoxicity against KB and NCI-H187 cells. The benzyl esters 10–11 were only moderately active in KB cell lines with IC₅₀ values of 17.83 and 17.37 µg/mL, respectively. Compounds 1–4, 7–11, and 13 were found to be inactive for antimalarial activity.
spectra were measured in CDCl₃ and acetone-given in Hz. The signals in the ¹H and ¹³C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC, and HMBC. EIMS were recorded on an MS Finnigan P...</p>...against the parasite Plasmodium falciparum [16, 17] and for antimycobacterial activity against Mycobacterium tuberculosis (H37Rv) [18].

Materials and Methods

General: Melting points were determined on the Fisher-John melting point apparatus and the Buchi melting point B-540 apparatus, and are reported without correction. Optical rotations [α]D were measured in CHCl₃ solution at the sodium D line (590 nm) with a JASCO DIP-370 digital polarimeter. UV spectra were recorded with a Shimadzu UV-VIS 2001S spectrophotometer. IR spectra were recorded with a Perkin Elmer Spectrum One FT-IR spectrophotometer using the UATR technique. ¹H and ¹³C NMR spectra were measured in CDCl₃ and acetone-d₆ on a Bruker AVANCE 400 (400 MHz for ¹H NMR and 100 MHz for ¹³C NMR) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. The signals in the ¹H and ¹³C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, HMQC, and HMBC. EIMS were recorded on an MS Finnigan Polaris spectrometer. HRMS were recorded on a Bruker MicroTOF mass spectrometer. HPLC was performed using a system comprised of Thermo Separation Product instruments (P4000 pump, UV6000LP for analysis, UV2000 for preparative). A reverse-phase column (SunFire Prep C8 250 × 21 mm, 10 mm; Waters) was used for preparative HPLC. Column chromatography (CC) and vacuum liquid chromatography (VLC) were carried out on silica gel 60 (Scharlau, 230–400 mesh) and RP-18 and silica gel 60H (Scharlau, 200–300 mesh), respectively. TLC was performed on precoated silica gel 60 F₂₅₄ plates (Merck); spots were detected by UV or spraying with 1% Ce(SO₄)₂ in 10% aq. H₂SO₄ followed by heating. All commercial grade solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

Plant material: The leaves of M. siamensis were collected in Songkhla Province, Thailand in 2007 and were identified by Dr. Piyta Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU007001) is deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

Extraction and isolation: The fresh leaves of M. siamensis (1.7 kg) were exhaustively extracted with EtOAc (3 × 8 L) at room temperature, filtered, and concentrated to give a green crude extract (150 g). The EtOAc extract (145 g) was adsorbed onto 250 g of silica gel and fractionated by vacuum liquid chromatography (VLC) over a sintered glass filter column of silica gel to isolate pure compounds 1–13 (see Supporting Information).

Table 1 NMR spectroscopic data of 1 and 2 in acetone-d₆ (δ in Hz in parentheses).
Table 2  Effects of 1–4, 7–11, and 13 against tumor cell lines replication.

<table>
<thead>
<tr>
<th>Compounds*</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; µg/mL</th>
<th>KB</th>
<th>MCF7</th>
<th>NCI-H187</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.16</td>
<td>14.86</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.02</td>
<td>20.03</td>
<td>2.73</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.09</td>
<td>16.72</td>
<td>14.26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.18</td>
<td>10.92</td>
<td>8.82</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17.45</td>
<td>NA</td>
<td>16.97</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>20.29</td>
<td>NA</td>
<td>17.74</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17.83</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>17.37</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>NT</td>
<td>NT</td>
<td>0.66</td>
<td></td>
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<td>13</td>
<td>4.09</td>
<td>NA</td>
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<td>0.224</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>0.176</td>
<td>1.290</td>
<td>0.029</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Purity (%) of tested compounds were > 98%. <sup>b</sup> This compound was used as a positive control (95%); not active (NA) = IC<sub>50</sub> > 20 µg/mL; not tested (NT)

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Conflict of Interest

There are no conflicts of interest of all authors with respect to this work.

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