

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 , ^{13}C , 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 IC_{50} values in the range of 0.66–7.16 $\mu\text{g}/\text{mL}$.

Key words

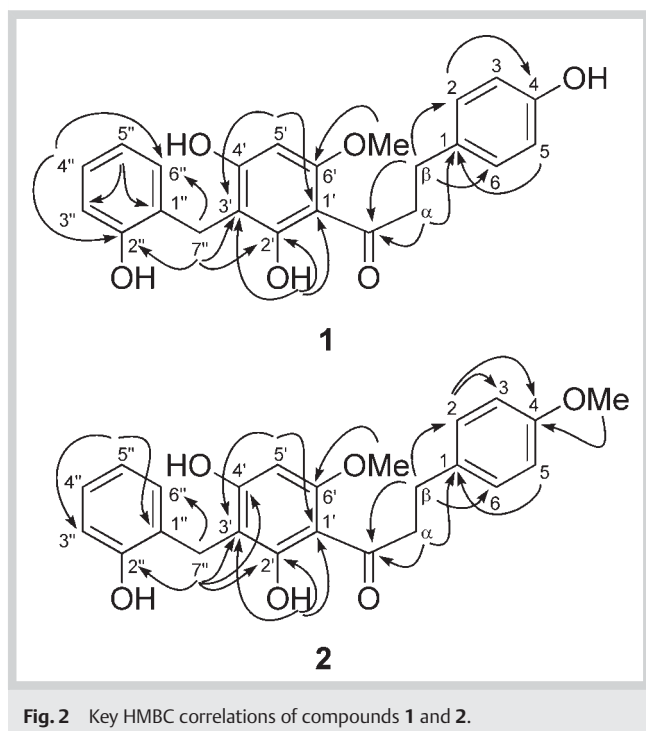
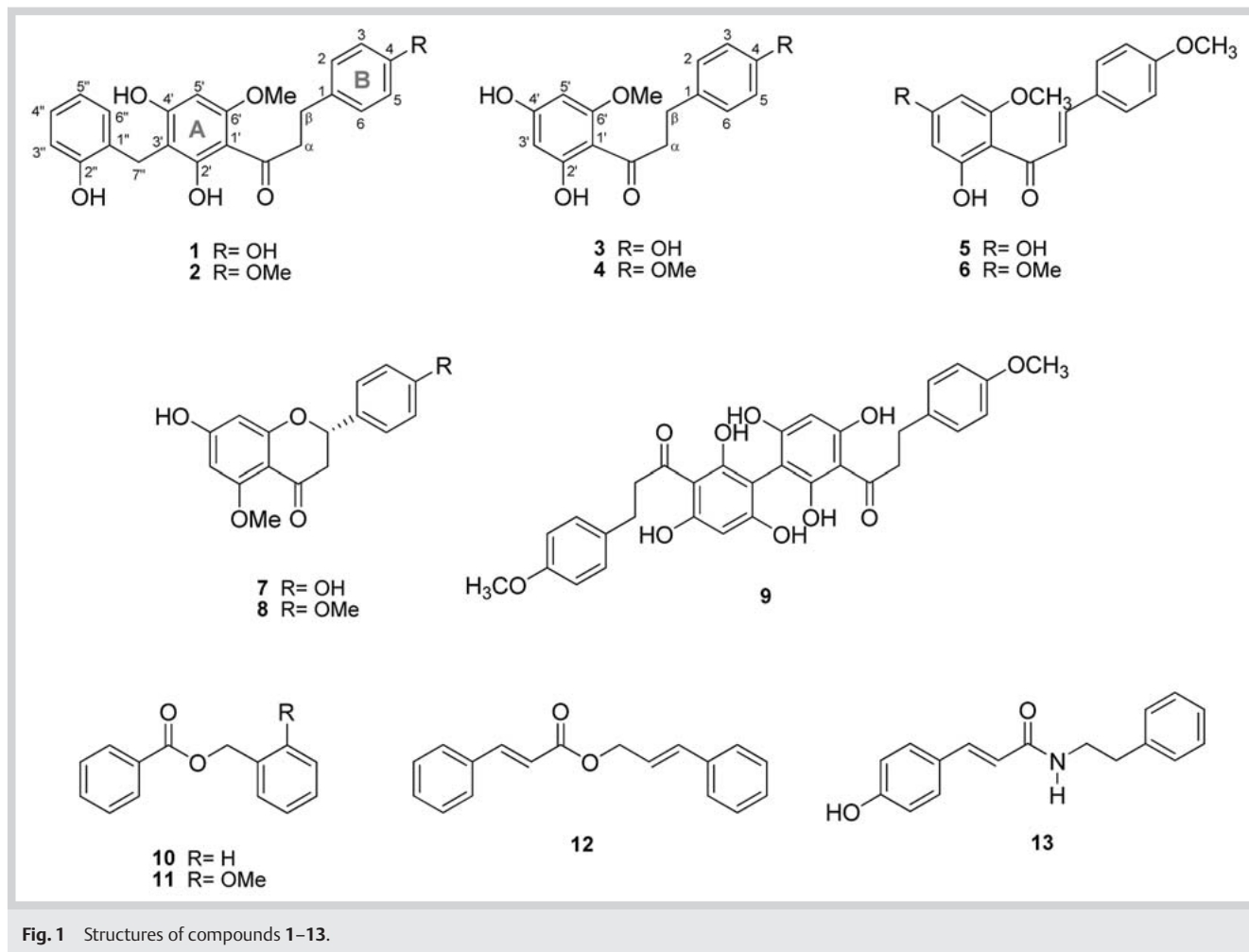
Melodorum siamensis · Annonaceae · antimycobacterial activity · antimalarial activity · cytotoxic activity

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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. fruticosum* [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 IC_{50} values of 1.7, 2.4, and 6.42 $\mu\text{g}/\text{mL}$, respectively, antimalarial activity against *Plasmodium falciparum* with an IC_{50} value of 9.7 $\mu\text{g}/\text{mL}$, and antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra with an MIC value of 200 $\mu\text{g}/\text{mL}$. Chemical and biological studies of this species have not been reported. This paper describes the isolation and structure elucidation of 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**), 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-methoxyflavanone (**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-phenylallylate (**12**) [12], and a known aromatic amide, *p*-coumaroyl- β -phenethylamine (**13**) [13] from the leaves of *M. siamensis* (● Fig. 1). Structure elucidation was performed using UV, IR, 1D and 2D NMR (^1H , ^{13}C , COSY, HMQC and HMBC) and HR-TOF-MS spectroscopic 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 $\text{C}_{23}\text{H}_{22}\text{O}_6$ by HR-TOF-MS (m/z 395.1498 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{23}\text{H}_{22}\text{O}_6$, 395.1495). The UV spectrum showed absorption bands at λ_{max} 223, 289, and 334 nm and the IR spectrum indicated hydroxyl (ν_{max} 3173 cm^{-1}), carbonyl (ν_{max} 1631 cm^{-1}), and aromatic (ν_{max} 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 ^{13}C 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 ^{13}C 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 ^{13}C 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''-CH₂ (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 $\text{C}_{24}\text{H}_{24}\text{O}_6$ by HR-TOF-MS (m/z 409.1649 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{24}\text{H}_{24}\text{O}_6$, 409.1651). The structure of **2** was closely related to **1** based on ^1H NMR, ^{13}C 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 ^{13}C NMR spectra of **2**, respectively, suggested an addi-



tional methoxy group. 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 μ g/mL. Compound **1** exhibited strong cytotoxicity in the NCI-H187 cell line with an IC₅₀ value of 3.66 μ g/mL and moderate activity in KB and MCF7 cell lines with IC₅₀ values of 7.16 and 14.86 μ g/mL, respectively. The dihydrochalcones **3** and **4** showed moderate activity in all cell lines, with IC₅₀s in the range of 5.18–14.26 μ g/mL while the flavanones **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 μ g/mL, respectively. Compounds **1–4**, **7–11**, and **13** were found to be inactive for antimalarial activity

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		133.3		134.8
2	7.09 d (8.3)	130.3	7.18 d (8.5)	130.5
3	6.75 d (8.3)	116.1	6.83 d (8.5)	114.9
4		156.6		159.2
5	6.75 d (8.3)	116.1	6.83 d (8.5)	114.9
6	7.09 d (8.3)	130.3	7.18 d (8.5)	130.5
1'		105.7		106.0
2'		165.7		165.8
3'		107.8		108.1
4'		155.2		163.4
5'	6.14 s	91.9	6.13 s	92.1
6'		162.8		163.0
α	3.27 t (7.7)	47.0	3.28 t (7.9)	47.1
β	2.86 t (7.7)	30.8	2.89 t (7.9)	30.9
CO		205.8		206.0
4-OCH ₃			3.74 s	55.7
2'-OH	14.76 s		14.77 s	
4'-OH				
6'-OCH ₃	3.86 s	56.2	3.84 s	56.3
1''		127.9		128.1
2''		155.2		155.3
3''	6.83 dd (7.6, 1.6)	116.0	6.84 dd (7.6, 1.6)	116.2
4''	7.00 td (7.6, 1.6)	127.9	7.01 td (7.6, 1.6)	128.2
5''	6.73 td (7.6, 1.6)	120.7	6.74 td (7.6, 1.6)	121.0
6''	7.21 dd (7.6, 1.6)	131.2	7.22 dd (7.6, 1.6)	131.6
7''	3.89 s	22.8	3.89 s	23.0

Table 1 NMR spectroscopic data of **1** and **2** in acetone-*d*₆ (*J* in Hz in parentheses).

against the parasite *Plasmodium falciparum* [16, 17] and for anti-mycobacterial activity against *Mycobacterium tuberculosis* (H37Ra) [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-*d*₆ 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. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2007001) 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).

4,2',4'-trihydroxy-6'-methoxy-3'(2''-hydroxybenzyl)dihydrochalcone (1): Pale yellow solid, mp 187–189 °C; UV (MeOH) λ_{max} (log ϵ): 223 (4.25), 289 (4.12), 334 (3.45) nm; IR (UATR-solid) ν_{max} : 3173, 2925, 2853, 1631, 1514, 1441, 1365, 1306, 1214, 1196, 1107, 950, 800, 754 cm⁻¹; ¹H NMR and ¹³C NMR (Table 1); HR-TOF-MS *m/z*: 395.1498 [M + H] (calcd. for C₂₂H₂₃O₆, 395.1495).

2',4'-dihydroxy-4,6'-dimethoxy-3'(2''-hydroxybenzyl)dihydrochalcone (2): Pale yellow crystals, mp 176–179 (dec.) °C; UV (MeOH) λ_{max} (log ϵ): 226 (4.11), 292 (3.93), 336 (3.42) nm; IR (UATR-solid) ν_{max} : 3303, 2933, 2800, 1612, 1512, 1454, 1423, 1296, 1244, 1196, 1138, 1105, 1036, 827, 756 cm⁻¹; ¹H NMR and ¹³C NMR (Table 1); HR-TOF-MS *m/z*: 409.1649 [M + H]⁺ (calcd. for C₂₄H₂₅O₆, 409.1651).

Supporting information

Detailed protocols for the extraction and isolation, *in vitro* cytotoxicity assay, *in vitro* antimalarial assay, and *in vitro* antibacterial assay, as well as 1D and 2D NMR spectra of compounds **1** and **2** are available as Supporting Information.

Compounds ^a	IC ₅₀ µg/mL		
	KB	MCF7	NCI-H187
1	7.16	14.86	3.66
2	2.02	20.03	2.73
3	9.09	16.72	14.26
4	5.18	10.92	8.82
5	NT	NT	NT
6	NT	NT	NT
7	17.45	NA	16.97
8	20.29	NA	17.74
9	NA	NA	NA
10	17.83	NA	NA
11	17.37	NA	NA
12	NT	NT	NT
13	4.09	NA	0.66
Ellipticine ^b	0.224		2.390
Doxorubicin ^b	0.176	1.290	0.029

^a Purity (%) of tested compounds were > 98%. ^b This compound was used as a positive control (95%); not active (NA) = IC₅₀ > 20 µg/mL; not tested (NT)

Table 2 Effects of 1–4, 7–11, and 13 against tumor cell lines replication.

Acknowledgements

We are indebted to the Office of the National Research Council of Thailand and Phuket Rajabhat University for financial support. The Bioassay Research Facility of BIOTEC/NASTDA Bangkok, Thailand is gratefully acknowledged for the bioactivity tests. We are grateful to Miss Kittiporn Trisupphakant and Mrs. Nitirat Chimnoi, Chulabhorn Research Institute, Bangkok, Thailand, for recording the UV, IR, MS, and HRMS spectra, and also to Miss Nareerat Thongtip, Phuket Rajabhat University, Phuket, Thailand, for recording the NMR spectra. We also thank Dr. Piya Chalermglin (Institute of Scientific and Technological Research, Thailand) for the identification of the plant.

Conflict of Interest

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

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received May 25, 2012
revised October 1, 2012
accepted October 18, 2012

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DOI <http://dx.doi.org/10.1055/s-0032-1327950>
Published online November 23, 2012
Planta Med 2013; 79: 83–86
© Georg Thieme Verlag KG Stuttgart · New York ·
ISSN 0032-0943

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