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 (^{1}H, ^{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, antimycobactirial, 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 µg/mL.

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

Supporting information available online at
http://www.thieme-connect.de/ejournals/toc/plantamedica

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 epimermoid 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 µg/mL, respectively, antimalarial activity against *Plasmodium falciparum* with an IC_{50} 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-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 (^{1}H, ^{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 $C_{23}H_{22}O_6$ by HR-TOF-MS (*m/z* 395.1498 $[M + H]^+$, calcd. for $C_{23}H_{23}O_6$, 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 ^{1}H 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 ^{1}H 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 ^{1}H 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 ^{1}H 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′-OCH_3 (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″-CH_2 (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 $C_{24}H_{24}O_6$ by HR-TOF-MS (*m/z* 409.1649 $[M + H]^+$, calcd. for $C_{24}H_{25}O_6$, 409.1651). The structure of **2** was closely related to **1** based on ^{1}H 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 ^{1}H and ^{13}C NMR spectra of **2**, respectively, suggested an addi-

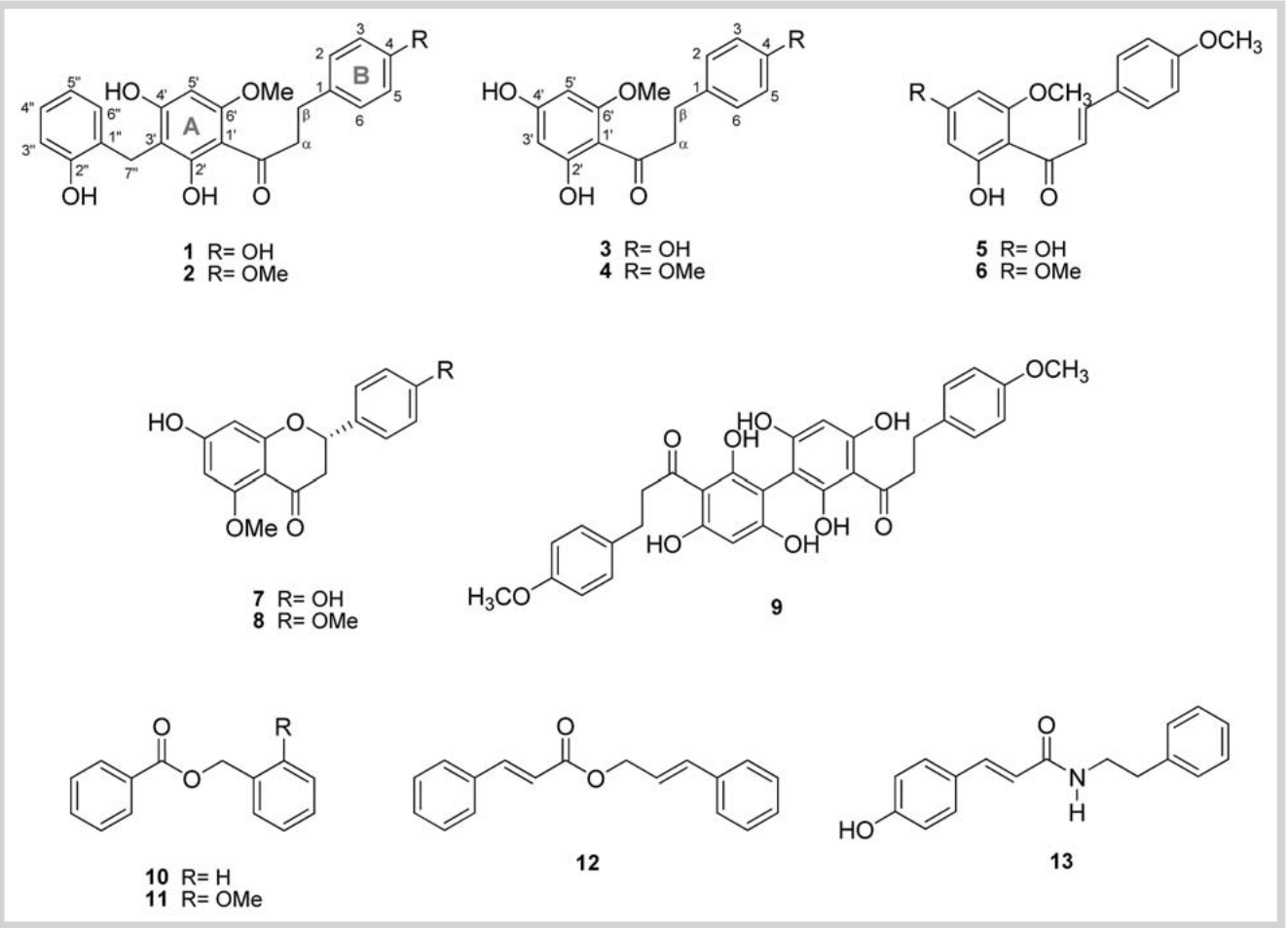

Fig. 1 Structures of compounds **1**–**13**.

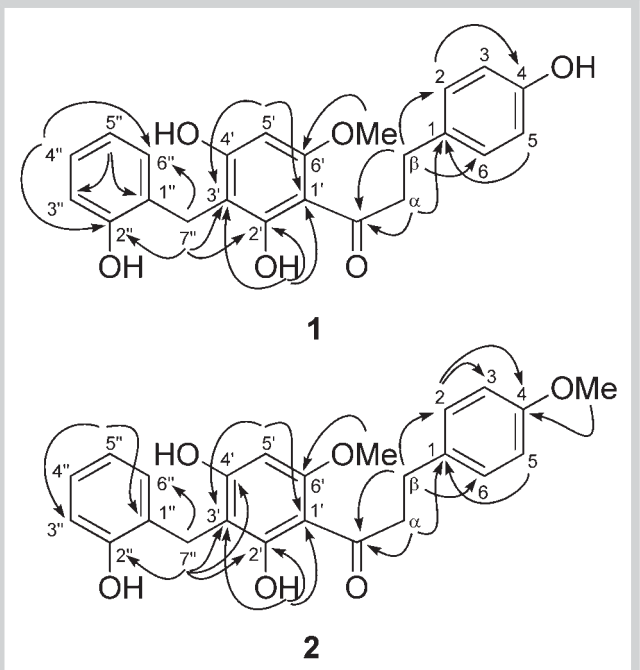

Fig. 2 Key HMBC correlations of compounds **1** and **2**.

tional methoxy group. The 2D HMBC correlations of the methoxy group, 4-OCH_3 (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 IC_{50} in the range of 0.66–4.09 µg/mL. Compound **1** exhibited strong cytotoxicity in the NCI-H187 cell line with an IC_{50} value of 3.66 µg/mL and moderate activity in KB and MCF7 cell lines with IC_{50} values of 7.16 and 14.86 µg/mL, respectively. The dihydrochalcones **3** and **4** showed moderate activity in all cell lines, with IC_{50}s in the range of 5.18–14.26 µg/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 IC_{50} 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

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 antimycobacterial 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 $[\alpha]_D$ were measured in $CHCl_3$ 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. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ and acetone-d_6 on a Bruker AVANCE 400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C 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 ^{1}H and ^{13}C 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 F_{254} plates (Merck); spots were detected by UV or spraying with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 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}; ^{1}H NMR and ^{13}C NMR (**Table 1**); HR-TOF-MS *m/z*: 395.1498 [M + H] (calcd. for $C_{22}H_{23}O_6$, 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}; ^{1}H NMR and ^{13}C NMR (**Table 1**); HR-TOF-MS *m/z*: 409.1649 [M + H]$^+$ (calcd. for $C_{24}H_{25}O_6$, 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

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

[a] Purity (%) of tested compounds were > 98%. [b] This compound was used as a positive control (95%); not active (NA) = IC_{50} > 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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