Cytotoxic Polyprenylated Benzoylphloroglucinol Derivatives from the Branches of *Garcinia schomburgkiana*

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ABSTRACT

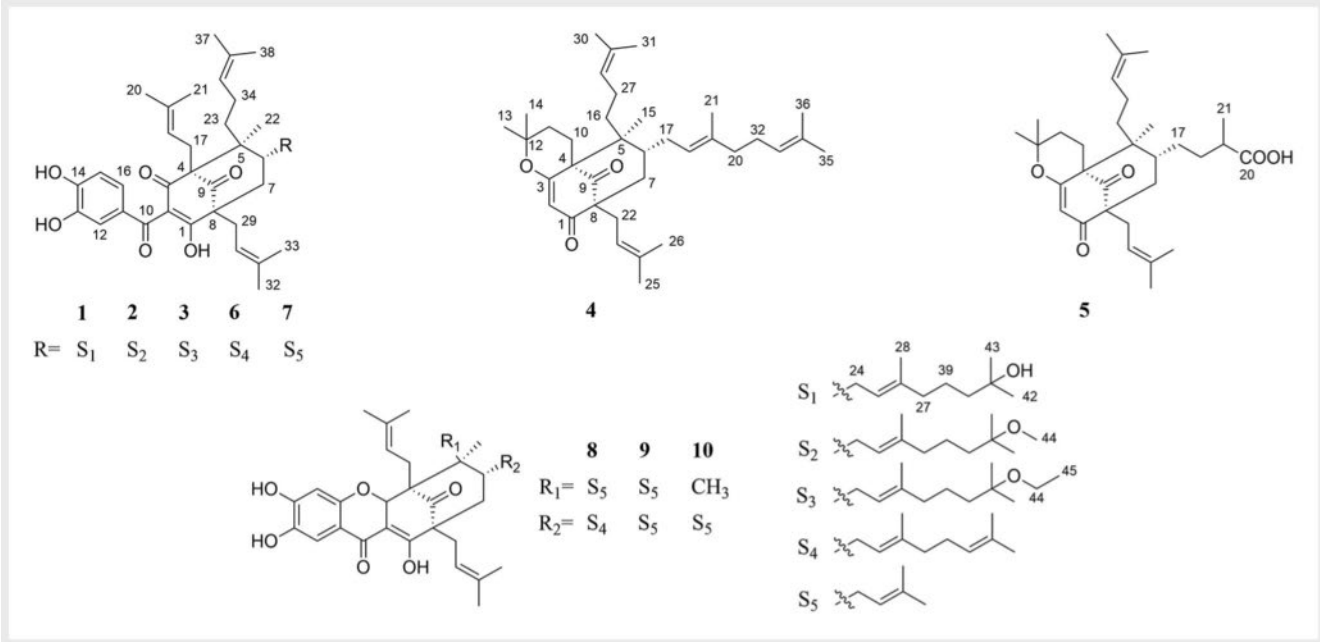
Five undescribed polyprenylated benzoylphloroglucinol derivatives (**1–5**), named garschomcinols A–E, and five known analogues (**6–10**) were isolated from the branches of *Garcinia schomburgkiana*. Their structures were determined on the basis of 1D and 2D NMR and HRESIMS analyses. The absolute configuration of the bicyclo [3.3.1]nonane core structure of the polyprenylated benzoylphloroglucinols was assigned by comparison of its experimental electronic circular dichroism data with that of related compounds. All isolated compounds were evaluated for their cytotoxicity *in vitro* against five cancer cell lines. Compound **6** showed potent cytotoxicity against five cancer cell lines including KB, HeLa S3, HT-29, MCF-7, and Hep G2 with IC_{50} values in the range of 5.05–7.03 µM.

Introduction

The genus *Garcinia*, belonging to the family Clusiaceae, has been widely studied for their chemical constituents and biological activities and comprises about 20 species in Thailand [1–5]. *Garcinia schomburgkiana* Pierre, locally named "Ma dan" in Thai, is an edible plant in Southeast Asia [6]. In Thai folk medicine, its roots, leaves, and fruits are used for the treatment of cough, menstrual disturbances, and diabetes as well as an expectorant and laxative [7]. Previous phytochemical studies of *G. schomburgkiana* showed the presence of xanthones, phloroglucinols, depsidones, biphenyls, flavonoids, and triterpenoids, some of which exhibited cytotoxic and antimalarial activity [8–12]. Herein, we report the isolation and structural elucidation of five undescribed polyprenylated benzoylphloroglucinol derivatives, named garschomcinols A–E, and five known analogues from the branches of *G. schomburgkiana*. The structures of the isolated compounds were determined by spectroscopic analysis, especially 1D and 2D NMR spectroscopy, and comparison with literature data. The configuration of the bicyclo [3.3.1]nonane core structure of the polyprenylated benzoylphloroglucinols was assigned by comparison of the experimental electronic circular dichroism (ECD) data with those of related compounds. The cytotoxicity *in vitro* of all isolated compounds against five cancer cell lines (KB, HeLa S3, HT-29, MCF-7, and Hep G2) was evaluated by the MTT colorimetric method.

Results and Discussion

Phytochemical investigation of the CH_2Cl_2 crude extract from the branches of *G. schomburgkiana* led to the isolation of five undescribed polyprenylated benzoylphloroglucinol derivatives, named garschomcinols A–E (**1–5**), and five known analogues (**6–10**)



▶ **Fig. 1** Chemical structures of **1–10**.

(▶ **Fig. 1**), including oblongifolin C (**6**) [13], guttiferone K (**7**) [14], garciyunnanin B (**8**) [15], oxyguttiferone K (**9**) [16], and oblongifolin G (**10**) [17]. The chemical structures of the known compounds were confirmed by NMR spectroscopic data and comparison with previously published data.

Garschomcinol A (**1**) was obtained as a yellow gum $[\alpha]_D^{20}$ + 12.5 (*c* 0.20, MeOH). Its molecular formula was determined as $C_{43}H_{60}O_7$ from the $[M + Na]^+$ ion peak at *m/z* 711.4233 (calcd. for $C_{43}H_{60}O_7Na$, 711.4237) in positive HRESIMS. UV absorptions maxima at λ_{max} 242, 257, and 324 nm revealed aromatic and conjugated carbonyl chromophores. The IR spectrum showed absorption bands at 3425 cm^{-1} (hydroxyl groups) and 1720 and 1665 cm^{-1} (carbonyl groups). The ^1H NMR data of **1** (▶ **Table 1**) exhibited signals for a 1,2,4-trisubstituted benzene ring at δ_H 6.69 (1H, d, *J* = 8.3 Hz, H-15), 6.95 (1H, dd, *J* = 1.4, 8.3 Hz, H-16), and 7.19 (1H, br s, H-12), a tertiary methyl proton at δ_H 0.81 (3H, s, H-22), a methylene group at δ_H 1.44 and 2.05 (2H, H-7), a methine proton at δ_H 1.74 (1H, m, H-6), and signals attributed to a 2,6-dimethyloct-6-en-2-ol and three 3-methylbut-2-enyl groups. The ^{13}C NMR data showed resonances for six aromatic carbons, a conjugated carbonyl group at δ_C 196.5 (C-10), an enolized 1,3-diketone at δ_C 119.2 (C-2), 191.6 (C-3), and 194.8 (C-1), a nonconjugated carbonyl at δ_C 208.9 (C-9), three quaternary carbons at δ_C 51.4 (C-5), 64.0 (C-8), and 69.3 (C-4), a methyl at δ_C 16.2 (C-22), a methylene at δ_C 43.0 (C-7), a methine at δ_C 42.1 (C-6), and 25 signals assignable to five isoprene units. The ^1H and ^{13}C NMR spectroscopic data (▶ **Table 1**) of **1** were similar to those of oblongifolin C (**6**) except for the geranyl group in **6**, in which the second double bond was hydrated to a 2,6-dimethyloct-6-en-2-ol group. The structure of the side chain was confirmed by the presence of an oxygenated carbon in the ^{13}C NMR spectrum at δ_C 71.4 (C-41), the COSY correlations of H-24/H-25, H-27/H-39 and H-39/H-40, and the HMBC correlations of H-25 with C-6, C-26, C-27, and C-28, H-42 and H-43 with C-40 and C-41, and H-39 with C-26, C-27, C-40, and C-41 (▶ **Fig. 2**). The relative configuration of **1** was determined using ^1H-^1H coupling constants and NOESY correlations. The coupling constant *J* = 12.8 Hz of H-6/H-7*ax* and the NOESY interactions between H-7*ax*/H-24, H-7*ax*/H-29, H-22/H-17, H-22/H-24, and H-25/H-27 suggested an axial orientation of H-6 and H-22, an equatorial orientation of H-17, H-24, and H-29, and an *E*-configuration of the $\Delta^{25,26}$ double bond (▶ **Fig. 3**). The ^{13}C NMR data at δ_C 42.1 supported an equatorial orientation of the C-6 substituent since the C-6 resonance with an axial substituent is reportedly observed at δ_C 46–48 [16]. Thus, the structure of **1** was assigned as shown in ▶ **Fig. 1**.

Garschomcinol B (**2**) was obtained as a yellow gum with $[\alpha]_D^{20}$ + 13.5 (*c* 0.28, MeOH). Its molecular formula of $C_{44}H_{62}O_7$ was established by the positive HRESIMS $[M + Na]^+$ ion peak at *m/z* 725.4382 (calcd. for $C_{44}H_{62}O_7Na$, 725.4393). The NMR data (▶ **Table 1**) of **2** and **1** were nearly identical except for the side chain of **2**, which showed a signal for a methoxy group at C-41. The presence of the methoxy group was confirmed by the ^{13}C NMR resonance at δ_C 49.4 (C-44) and the HMBC correlation of the methoxy protons at δ_H 3.13 (3H, s, H-44) with C-41 (δ_C 76.3) (▶ **Fig. 2**). Finally, the structure of **2** was determined as shown in ▶ **Fig. 1**.

Garschomcinol C (**3**) was obtained as a yellow gum with $[\alpha]_D^{20}$ + 14.7 (*c* 0.34, MeOH). Its molecular formula was determined to be $C_{45}H_{64}O_7$ by HRESIMS ($[M + Na]^+$ *m/z* 739.4524, calcd. for $C_{45}H_{64}O_7Na$, 739.4550). According to the NMR analysis (▶ **Table 1**), compound **3** possessed the same structure as **2**, except that the methoxy group of the side chain was replaced by an ethoxy group. The ^1H and ^{13}C NMR spectra showed protons at δ_H 1.11 (3H, m, H-45) and 3.36 (2H, m, H-44), which were correlated in

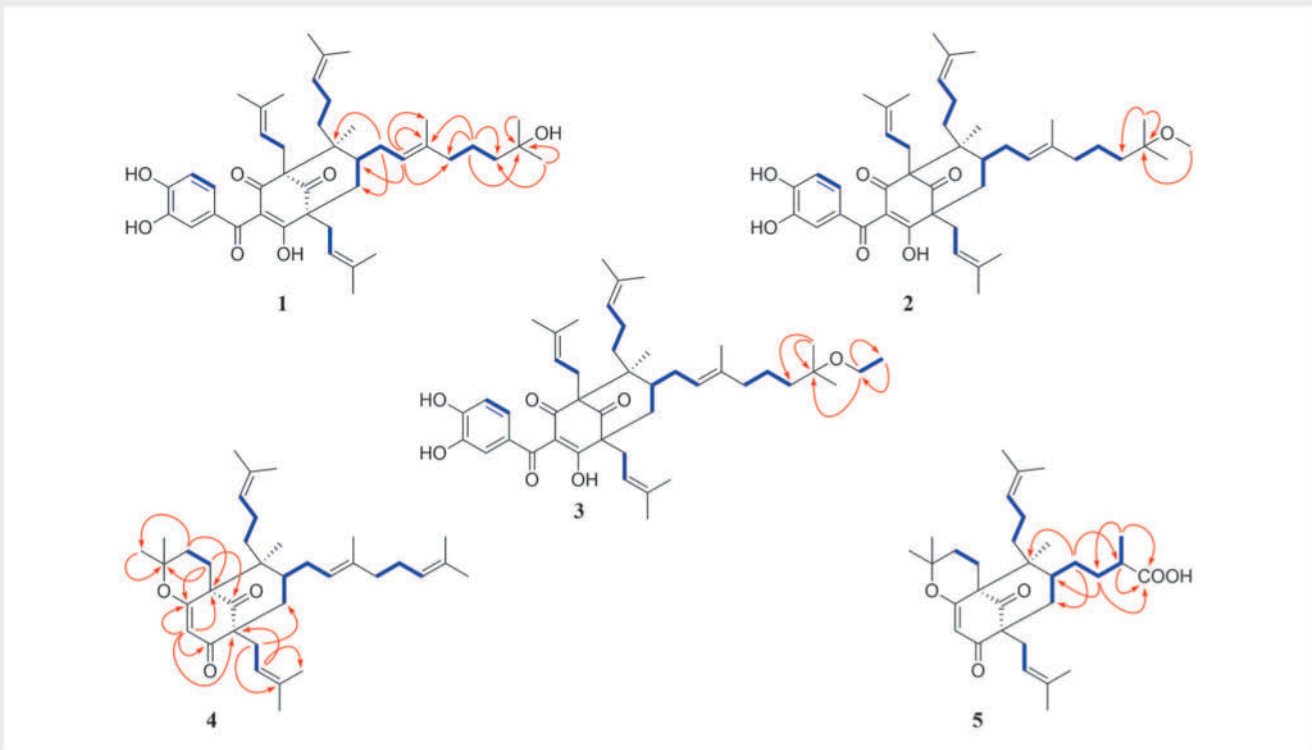
▶ **Table 1** ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **1–3** in CD_3OD.

Position	1		2		3	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		194.8		194.5		194.7
2		119.2		119.3		119.3
3		191.6		191.4		191.4
4		69.3		69.4		69.4
5		51.4		51.5		51.5
6	1.74, m	42.1	1.78, m	42.0	1.79, m	42.0
7eq	2.05, m	43.0	2.07, m	43.1	2.09, m	43.1
7ax	1.44, t (12.8)		1.47, t (12.6)		1.47, t (13.0)	
8		64.0		63.9		63.9
9		208.9		208.9		208.9
10		196.5		196.5		196.5
11		130.0		130.0		130.0
12	7.19, br s	117.4	7.21, d (1.8)	117.4	7.21, d (1.5)	117.4
13		146.1		146.1		146.2
14		152.3		152.3		152.3
15	6.69, d (8.3)	115.0	6.70, d (8.3)	115.1	6.71, d (8.3)	115.1
16	6.95, dd (1.4, 8.3)	124.9	6.97, dd (1.9, 8.3)	124.9	6.97, dd (1.5, 8.3)	124.9
17	2.70, m	26.6	2.71, m	26.6	2.72, m	26.6
18	4.87, br s	121.3	4.86, br s	121.3	4.87, br s	121.3
19		134.8		134.9		134.9
20	1.62, s	26.3	1.64, s	26.3	1.65, s	26.3
21	1.69, s	18.4	1.71, s	18.4	1.72, s	18.4
22	0.81, s	16.2	0.83, m	16.2	0.84, m	16.2
23	1.67, m	37.4	1.68, m	37.4	1.69, m	37.4
24	1.76, m, 2.07, m	29.9	1.78, m, 2.09, m	29.9	1.78, m, 2.10, m	29.9
25	5.01, br s	123.7	5.04, m	123.9	5.04, m	123.9
26		138.1		138.0		138.0
27	1.95, m	41.1	1.99, m	40.9	2.00, m	40.9
28	1.56, s	16.4	1.57, s	16.4	1.58, s	16.4
29	2.49, m	31.6	2.51, m	31.6	2.53, m	31.6
30	5.13, br s	120.9	5.13, br s	120.9	5.14, br s	120.9
31		135.4		135.4		135.4
32	1.71, s	26.3	1.72, s	26.3	1.73, s	26.3
33	1.66, s	18.3	1.68, s	18.3	1.69, s	18.3
34	1.97, m	25.2	1.99, m	25.2	1.99, m	25.2
35	5.06, br s	125.5	5.07, m	125.5	5.08, m	125.5
36		132.3		132.4		132.4
37	1.66, s	26.0	1.68, s	25.9	1.69, s	25.9
38	1.59, s	18.0	1.61, s	18.0	1.62, s	18.0

continued

▶ **Table 1** *Continued*

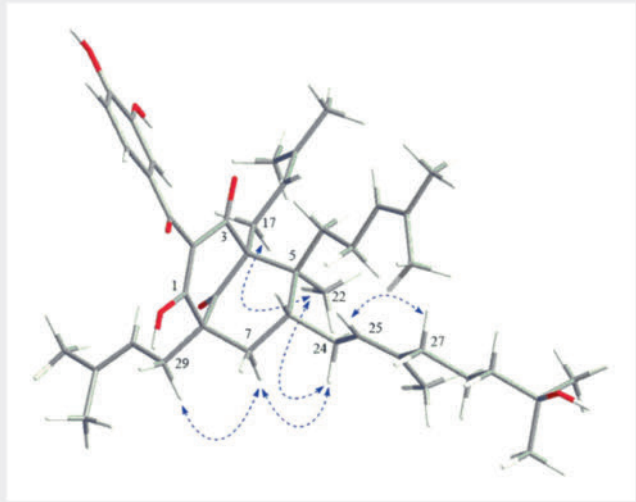
Position	1		2		3	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
39	1.44, m	23.3	1.39, m	22.7	1.40, m	22.7
40	1.34, m	44.1	1.39, m	39.6	1.40, m	40.0
41		71.4		76.3		76.0
42	1.12, s	29.2	1.11, s	25.5	1.13, s	26.2
43	1.14, s	29.2	1.12, s	25.5	1.13, s	26.2
44			3.13, s	49.4	3.36, m	57.6
45					1.11, m	16.4



▶ **Fig. 2** Key HMBC (arrow curves) and COSY (bold lines) correlations of **1**–**5**.

the HSQC spectrum with carbons at δ_C 16.4 and 57.6, respectively. The HMBC spectrum showed cross-peaks between H-44 and C-41 (δ_C 76.0) and C-45 (▶ **Fig. 2**), indicating that the ethoxy group was attached at C-41 in the side chain. Thus, the structure of **3** was characterized as shown in ▶ **Fig. 1**.

Garschomcinol D (**4**) was obtained as a yellow gum with $[\alpha]_D^{20}$ + 13.1 (*c* 0.30, MeOH). Its molecular formula of $C_{36}H_{54}O_3$ was suggested by the positive HRESIMS $[M + K]^+$ ion peak at *m*/*z* 573.3786 (calcd. for $C_{36}H_{54}O_3K$, 573.3710). The IR spectrum displayed bands at 1719, 1653, and 1642 cm^{-1} for carbonyl groups. The ^1H NMR data of **4** (▶ **Table 2**) exhibited signals for two methine protons at δ_H 2.02 (1H, m, H-6) and 5.99 (1H, s, H-2), a methylene group at δ_H 1.35 and 1.93 (2H, H-7), the tertiary methyl protons at δ_H 0.68 (3H, s, H-15), and signals attributed to a 2,2-dimethylpyran ring, a 3,7-dimethylocta-2,6-dienyl, and two 3-methylbut-2-enyl groups. The ^{13}C NMR data showed resonances for an enolized 1,3-diketone at δ_C 120.2 (C-2), 175.2 (C-3), and 198.7 (C-1), a non-conjugated carbonyl at δ_C 208.1 (C-9), three quaternary carbons at δ_C 47.4 (C-5), 62.6 (C-4), and 63.2 (C-8), a methyl at δ_C 17.1 (C-15), a methylene at δ_C 39.6 (C-7), a methine at δ_C 36.3 (C-6), and 25 signals assignable to five isoprene units. The NMR data of **4** showed close similarity to those of **6** except



▶ **Fig. 3** Key NOESY correlations of **1**.

for the absence of a benzoyl group at C-2 in **4**, which was confirmed by the HMBC correlations (▶ **Fig. 2**) from H-2 to C-1, C-3, C-4, and C-8. In addition, the prenyl group at C-4 in **6** was cyclized to build a pyran ring, which was determined by the HMBC correlations from H-10 [δ_H 1.71, 2.52 (2H, m)] to C-3, C-4, and C-12 (δ_C 83.5), and from H-11 [δ_H 1.27, 1.71 (2H, m)] to C-4, C-12, C-13 (δ_C 29.5), and C-14 (δ_C 26.2). The relative configuration of **4** was assigned using ^{1}H-^{1}H coupling constants and NOESY correlations as in **1**. The coupling constant J = 13.1 Hz of H-6/H-7*ax* and the interactions in the NOESY spectrum between H-7*ax*/H-17, H-7*ax*/H-22, H-15/H-10, H-15/H-17, and H-18/H-20 suggested an axial orientation of H-6 and H-15, an equatorial orientation of H-10, H-17, and H-22, and the *E*-configuration of the $\Delta^{18,19}$ double bond. Thus, the structure of **4** was assigned as shown in ▶ **Fig. 1**.

Garschomcinol E (**5**) was obtained as a yellow gum with $[\alpha]_D^{20}$ + 12.5 (*c* 0.20, MeOH). Its molecular formula was determined as $C_{31}H_{46}O_5$ from the positive HRESIMS [M + Na]$^+$ ion peak at *m/z* 521.3233 (calcd. for $C_{31}H_{46}O_5Na$, 521.3243). The NMR data (▶ **Table 2**) of **5** were similar with those of **4** except for the geranyl group at C-6, which was replaced by a 2-methylbutanoic acid group in **5**. The structure of the side chain was confirmed by the presence of a carboxylic acid carbon at δ_C 181.5 (C-20) in the ^{13}C NMR spectrum, the COSY correlations of H-17/H-18, H-18/H-19 and H-19/H-21, and the HMBC correlations (▶ **Fig. 2**) of H-17 [δ_H 0.89, 1.36 (2H, m)] with C-5 (δ_C 47.6), C-7 (δ_C 39.5), and C-19 (δ_C 39.9), H-18 [δ_H 1.26, 1.73 (2H, m)] with C-6 (δ_C 36.0), C-20, and C-21 (δ_C 17.3), H-19 [δ_H 2.33 (1H, m)] with C-17 (δ_C 28.1), C-20, and C-21, and H-21 [δ_H 1.13 (3H, d, J = 7.0 Hz)] with C-18 (δ_C 32.2), C-19, and C-20. Compound **5** had the same relative configuration as **4** based on the J value and NOESY analysis, while the configuration at C-19 remains undetermined. Finally, the structure of **5** was determined as shown in ▶ **Fig. 1**.

The absolute configurations of the bicyclo [3.3.1]nonane core in **1–5** were assigned by comparison of the experimental ECD data with data reported for related compounds. The experimental ECD curves of **1–3** (**Fig. 6S**, Supporting Information) showed similar-

▶ **Table 2** ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **4** and **5** in $CDCl_3$.

Position	4		5	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		198.7		198.9
2	5.99, s	120.2	5.95, s	119.9
3		175.2		175.4
4		62.6		62.5
5		47.4		47.6
6	2.02, m	36.3	1.87, m	36.0
7eq	1.93, m	39.6	1.92, m	39.5
7ax	1.35, t (13.1)		1.34, t (13.0)	
8		63.2		62.8
9		208.1		207.7
10	1.71, m, 2.52, m	18.1	2.47, m	18.0
11	1.27, m, 1.71, m	33.7	1.21, m, 1.67, m	33.5
12		83.5		83.5
13	1.23, s	29.5	1.20, s	29.5
14	1.42, s	26.2	1.39, s	26.0
15	0.68, s	17.1	0.61, s	17.1
16	1.39, m, 1.63, m	37.0	1.31, m, 1.56, m	36.8
17	1.69, m, 1.99, m	29.1	0.89, m, 1.36, m	28.1
18	5.05, m	124.2	1.26, m, 1.73, m	32.2
19		137.1	2.33, m	39.9
20	1.96, m	39.9		181.5
21	1.54, s	16.4	1.13, d (7.0)	17.3
22	2.42, d (6.7)	30.1	2.40, m	30.1
23	4.93, m	120.2	4.86, t (6.6)	119.7
24		133.6		133.7
25	1.69, s	26.2	1.62, s	25.8
26	1.66, s	18.1	1.62, s	18.0
27	1.85, m, 2.04, m	23.0	1.73, m, 1.95, m	23.0
28	4.96, m	124.4	4.92, t (6.1)	124.2
29		131.6		131.5
30	1.66, s	25.9	1.59, s	25.9
31	1.57, s	18.0	1.53, s	17.9
32	2.04, m	26.8		
33	5.00, m	122.5		
34		133.4		
35	1.63, s	26.0		
36	1.59, s	18.1		

▶ **Table 3** *In vitro* cytotoxicity of compounds **1–10** against five human cancer cell lines.

Compounds	IC_{50} (μM); 95 % CI				
	KB	HeLa S3	HT-29	MCF-7	Hep G2
1	5.93; 5.36–6.49	5.33; 5.15–5.50	7.89; 7.28–8.50	5.42; 5.00–5.84	12.03; 11.39–12.66
2	7.13; 6.06–8.20	6.24; 4.6#7–7.81	12.73; 6.58–18.87	6.72; 6.03–7.41	14.39; 13.98–14.79
3	9.37; 7.33–11.41	6.65; 5.80–7.49	14.13; 10.64–17.63	6.50; 6.30–6.70	14.12; 11.96–11.28
4	> 100	> 100	NT	NT	NT
5	> 100	> 100	NT	NT	NT
6	5.38; 5.10–5.65	5.22; 3.85–6.60	5.05; 4.77–5.33	5.90; 4.33–7.47	7.03; 6.20–7.86
7	6.39; 5.91–6.88	5.48; 5.45–5.51	6.29; 4.63–7.95	4.06; 2.90–5.22	11.46; 9.34–13.58
8	5.70; 4.79–6.61	16.09; 15.95–16.24	20.80; 20.18–21.42	18.13; 14.47–21.49	> 100
9	5.03; 4.92–5.15	11.05; 10.35–11.74	19.17; 18.19–20.14	18.29; 9.13–27.44	> 100
10	2.52; 2.42–2.63	6.39; 5.44–7.34	13.48; 9.45–17.51	12.12; 2.57–21.67	11.20; 9.40–13.01
Doxorubicin[a]	0.02; 0.00–0.02	0.15; 0.11–0.19	0.59; 0.51–0.67	1.29; 1.25–1.34	1.00; 0.57–1.43

NT = not tested; [a]doxorubicin was used as the positive control

ities to those of **6** and **7** [9] (positive Cotton effect at 200–240 nm, negative Cotton effect at 240–310 nm, and positive Cotton effect at 310–400 nm), thereby suggesting the 4*S*, 5*S*, 6*R*, 8*S* absolute configuration of **1–3**. The experimental ECD curves of **4** and **5** (**Fig. 6S**, Supporting Information) showed a similar pattern to those of 32-hydroxy-*ent*-guttiferone M [18] (two negative high-amplitude Cotton effects at 254 and 320 nm, along with a positive Cotton effect at 215 nm), which revealed the 4*R*, 5*S*, 6*R*, 8*S* absolute configuration of **4** and **5**.

Most isolated compounds showed potent cytotoxicity against cancer cell lines, while no cytotoxicity was found for **4** and **5** (IC_{50} > 100 μM) (▶ **Table 3**). It is worth noting that compound **6** showed cytotoxicity against all five cancer cell lines including KB, HeLa S3, HT-29, MCF-7, and Hep G2 with IC_{50} values in the range of 5.05–7.03 μM. Compounds **1** and **7** exhibited cytotoxicity against four cell lines (KB, HeLa S3, HT-29, and MCF-7) with IC_{50} values in the range of 4.06–7.89 μM. Compounds **2** and **3** were cytotoxic against three cell lines (KB, HeLa S3, and MCF-7) with IC_{50} values in the range of 6.24–9.37 μM. Compound **10** was cytotoxic against KB and HeLa S3 cells with IC_{50} values of 2.52 and 6.39 μM, respectively. In addition, compounds **8** and **9** showed significant cytotoxic effects against KB cells with IC_{50} values of 5.70 and 5.03 μM, respectively. These data suggest that the presence of a 3,4-dihydroxybenzoyl group at C-2 might improve the cytotoxicity of phloroglucinols.

Material and Methods

General experimental procedures

UV-visible absorption spectra were recorded on a UV-2550 UV-vis spectrometer. IR spectra were measured on a Nicolet 6700 FT-IR spectrometer using KBr discs. Optical rotations were measured with a Jasco P-1010 polarimeter. NMR spectra were recorded on a Bruker 400 AVANCE spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The HRESIMS were obtained using a Bruker MICROTOF model mass spectrometer. ECD data were recorded on a JASCO J-815 spectropolarimeter. Silica gel 60 G, silica gel 70–230 mesh, silica gel RP-C18 40–63 μm, and Sephadex LH-20 (all Merck) were used for column chromatography.

Plant material

Branches of *G. schomburgkiana* were collected in January 2020 from Pho Si Suwan district, Sisaket province, Thailand (15°16′55″ N 104°01′40″ E). The plant material was identified by Dr. Suttira Sedlak, botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University. A voucher specimen (Khumkratok no. 92–08) was deposited at Mahasarakham University.

Extraction and isolation

The air-dried branches of *G. schomburgkiana* (10.0 kg) were ground and then macerated with CH_2Cl_2 over a period of 5 days at room temperature with 2 × 15 L. Removal of the solvent under reduced pressure provided the CH_2Cl_2 crude extract (120.0 g), which was further separated by column chromatography (45 × 10 cm, i. d.) over silica gel with a gradient of *n*-hexane-EtOAc (1 : 0, 8 : 2, 6 : 4, 4 : 6, 2 : 8, each 5 L) to give 12 fractions (A–L). Fraction C (1.5 g) was purified by a silica gel RP-C18 column (55 × 3 cm, i. d.) with H_2O-MeOH (2 : 8, 1 L) to afford compound **4** (6.5 mg). Fraction D (6.2 g) was applied to a Sephadex LH-20 column (75 × 5 cm, i. d.) with CH_2Cl_2-MeOH (8 : 2, 2 L) and further purified by a silica gel RP-C18 column (55 × 3 cm, i. d.) with H_2O-MeOH (2 : 8, 1 L) to obtain compounds **8** (8.5 mg), **9** (4.2 mg), and **10** (6.0 mg). Compounds **3** (10.8 mg), **6** (15.5 mg), and **7** (16.2 mg) were obtained from fraction E (10.5 g) by chromatography on a Sephadex LH-20 column (75 × 5 cm, i. d.) with CH_2Cl_2-MeOH (8 : 2, 2 L) followed by a silica gel RP-C18 column (55 × 3 cm, i. d.) with H_2O-MeOH (2 : 8, 1 L). Compound **2**

(12.5 mg) was isolated from fraction F (3.0 g) using a silica gel RP-C18 column (55 × 3 cm, i. d.) with H_2O-MeOH (2 : 8, 1 L). Finally, Fraction G (3.5 g) was subjected to a Sephadex LH-20 column (75 × 5 cm, i. d.) using CH_2Cl_2-MeOH (8 : 2, 2 L) to provide compounds **1** (15.5 mg) and **5** (7.5 mg).

Garschomcinol A (**1**): yellow gum; $[\alpha]_D^{20}$ + 12.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 324 (0.2), 257 (0.5), and 242 (0.5) nm.; IR (KBr) ν_{max} 3425, 1720, 1665 cm^{-1}; ECD (*c* 0.05, MeOH) λ_{max} ($\Delta\varepsilon$) 330 (+ 4.9), 250 (− 21.0), 220 (+ 25.2) nm.; ^1H and ^{13}C NMR data, see ▸ **Table 1**; HRESIMS (positive ion mode) *m/z* 711.4233 $[M + Na]^+$ (calcd. for $C_{43}H_{60}O_7Na$, 711.4237).

Garschomcinol B (**2**): yellow gum; $[\alpha]_D^{20}$ + 13.5 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 326 (0.3), 253 (0.4), and 240 (0.5) nm.; IR (KBr) ν_{max} 3428, 1728, 1645 cm^{-1}; ECD (*c* 0.05, MeOH) λ_{max} ($\Delta\varepsilon$) 332 (+ 5.7), 252 (− 32.2), 221 (+ 38.0) nm.; ^1H and ^{13}C NMR data, see ▸ **Table 1**; HRESIMS (positive ion mode) *m/z* 725.4382 $[M + Na]^+$ (calcd. for $C_{44}H_{62}O_7Na$, 725.4393).

Garschomcinol C (**3**): yellow gum; $[\alpha]_D^{20}$ + 14.7 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 318 (0.1), 260 (0.3), and 238 (0.2) nm.; IR (KBr) ν_{max} 3423, 1727, 1647 cm^{-1}; ECD (*c* 0.05, MeOH) λ_{max} ($\Delta\varepsilon$) 331 (+ 5.2), 250 (− 25.8), 220 (+ 30.3) nm.; ^1H and ^{13}C NMR data, see ▸ **Table 1**; HRESIMS (positive ion mode) *m/z* 739.4524 $[M + Na]^+$ (calcd. for $C_{45}H_{64}O_7Na$, 739.4550).

Garschomcinol D (**4**): yellow gum; $[\alpha]_D^{20}$ + 13.1 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 328 (0.3), 259 (0.4), and 246 (0.2) nm.; IR (KBr) ν_{max} 1719, 1653, 1642 cm^{-1}; ECD (*c* 0.05, MeOH) λ_{max} ($\Delta\varepsilon$) 316 (− 18.7), 254 (− 39.7), 212 (+ 49.9) nm.; ^1H and ^{13}C NMR data, see ▸ **Table 2**; HRESIMS (positive ion mode) *m/z* 573.3786 $[M + K]^+$ (calcd. for $C_{36}H_{54}O_3K$, 573.3710).

Garschomcinol E (**5**): yellow gum; $[\alpha]_D^{20}$ + 12.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 318 (0.2), 245 (0.2), and 238 (0.1) nm.; IR (KBr) ν_{max} 1725, 1648, 1635 cm^{-1}; ECD (*c* 0.05, MeOH) λ_{max} ($\Delta\varepsilon$) 316 (− 22.7), 253 (− 52.5), 208 (+ 72.2) nm.; ^1H and ^{13}C NMR data, see ▸ **Table 2**; HRESIMS (positive ion mode) *m/z* 521.3233 $[M + Na]^+$ (calcd. for $C_{31}H_{46}O_5Na$, 521.3243).

Cytotoxicity assay

The cytotoxicity of compounds **1–10** was evaluated using the MTT colorimetric method against KB, HeLa S3, HT-29, MCF-7, and Hep G2 cell lines as previously reported [19], with doxorubicin as the positive control. The cancer cells were cultured in 100 µL/well of MEM containing 10% fetal bovine serum and 1% streptomycin-penicillin, seeded in a 96-well plate (3000 cells/well), and preincubated in a 5% CO_2 incubator at 37 °C for 24 h. Various concentrations of the sample, DMSO as the negative control, and the positive control (10 µL/well) were added, and then incubated for 72 h under the above conditions. The supernatant was removed and 100 µL of MTT solution (0.5 mg/mL) were added into each well and further incubated for 3 h. The supernatant was decanted and DMSO (100 µL/well) was added to dissolve Formosan, which was measured at 550 nm by a microplate reader. The tests were performed in triplicate. The IC_{50} value was calculated by curve fitting with SigmaPlot 10 (Systat Software Inc.) and the 95% confidence interval for the mean values was identified by using IBM SPSS Amos 19 (SPSS Inc.).

Supporting information

NMR, HRESIMS, and ECD spectra of compounds **1–5** and concentration-response curves of **1–3**, **6–10**, and doxorubicin are available as Supporting Information.

Contributors' Statement

Design of the work: Kaennakam, Sutin; critical revision of the manuscript: Tip-pyang, Santi and Sukandar, Edwin Risky; activity test: Siripong, Pongpun and Rassamee, Kitiya.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Kaennakam S, Siripong P, Tip-Pyang S. Kaennacowanols A–C, three new xanthones and their cytotoxicity from the roots of *Garcinia cowa*. Fitoterapia 2015; 102: 171–176

[2] Sukandar ER, Kaennakam S, Rassamee K, Ersam T, Siripong P, Tip-Pyang S. Tetrandraxanthones A–I, prenylated and geranylated xanthones from the stem bark of *Garcinia tetrandra*. J Nat Prod 2019; 82: 1312–1318

[3] Sukandar ER, Kaennakam S, Aree T, Nöst X, Rassamee K, Bauer R, Siripong P, Ersam T, Tip-Pyang S. Picrorhizones A–H, polyprenylated benzoylphloroglucinols from the stem bark of *Garcinia picrorhiza*. J Nat Prod 2020; 83: 2102–2111

[4] Sukandar ER, Kaennakam S, Raab P, Nöst X, Rassamee K, Bauer R, Siripong P, Ersam T, Tip-Pyang S, Chavasiri W. Cytotoxic and anti-inflammatory activities of dihydroisocoumarin and xanthone derivatives from *Garcinia picrorhiza*. Molecules 2021; 26: 6626

[5] Ngernsaengsaruay C, Suddee S. *Garcinia nuntasaenii* (Clusiaceae), a new species from Thailand. Thai For Bull (Bot) 2016; 44: 134–139

[6] Lim TK. *Garcinia schomburgkiana*. In: Lim TK, ed. Edible Medicinal and Non-Medicinal Plants: Volume 2, Fruits. Dordrecht, Netherlands: Springer; 2012: 123–124

[7] Lim TK. Edible Medicinal and Non-Medicinal Plants. Dordrecht: Springer; 2012

[8] Sukandar ER, Siripong P, Khumkratok S, Tip-Pyang S. New depsidones and xanthone from the roots of *Garcinia schomburgkiana*. Fitoterapia 2016; 111: 73–77

[9] Le DH, Nishimura K, Takenaka Y, Mizushina Y, Tanahashi T. Polyprenylated benzoylphloroglucinols with DNA polymerase inhibitory activity from the fruits of *Garcinia schomburgkiana*. J Nat Prod 2016; 79: 1798–1807

[10] Kaennakam S, Mudsing K, Rassamee K, Siripong P, Tip-Pyang S. Two new xanthones and cytotoxicity from the bark of *Garcinia schomburgkiana*. J Nat Med 2019; 73: 257–261

[11] Lien Do TM, Duong TH, Nguyen VK, Phuwapraisirisan P, Doungwichitrkul T, Niamnont N, Jarupinthusophon S, Sichaem J. Schomburgkixanthone, a novel bixanthone from the twigs of *Garcinia schomburgkiana*. Nat Prod Res 2021; 35: 3613–3618

[12] Kaennakam S, Sukandar E, Juntagoot T, Siripong P, Tip-Pyang S. Four new xanthones and their cytotoxicity from the stems of *Garcinia schomburgkiana*. J Nat Med 2021; 75: 871–876

[13] Hamed W, Brajeul S, Mahuteau-Betzer F, Thoison O, Mons S, Delpech B, Hung NV, Sévenet T, Marazano C. Oblongifolins A–D, polyprenylated benzoylphloroglucinol derivatives from *Garcinia oblongifolia*. J Nat Prod 2006; 69: 774–777

[14] Cao S, Brodie PJ, Miller JS, Ratovoson F, Birkinshaw C, Randrianasolo S, Rakotobe E, Rasamison VE, Kingston DGI. Guttiferones K and L, antiproliferative compounds of *Rheedia calcicola* from the Madagascar rain forest. J Nat Prod 2007; 70: 686–688

[15] Xu G, Feng C, Zhou Y, Han QB, Qiao CF, Huang SX, Chang DC, Zhao QS, Luo KQ, Xu HX. Bioassay and ultraperformance liquid chromatography/mass spectrometry guided isolation of apoptosis-inducing benzophenones and xanthone from the pericarp of *Garcinia yunnanensis* Hu. J Agric Food Chem 2008; 56: 11144–11150

[16] Cottet K, Neudörffer A, Kritsanida M, Michel S, Lallemand MC, Largeron M. Polycyclic polyprenylated xanthones from *Symphonia globulifera*: Isolation and biomimetic electrosynthesis. J Nat Prod 2015; 78: 2136–2140

[17] Huang SX, Feng C, Zhou Y, Xu G, Han QB, Qiao CF, Chang DC, Luo KQ, Xu HX. Bioassay-guided isolation of xanthones and polycyclic prenylated acylphloroglucinols from *Garcinia oblongifolia*. J Nat Prod 2009; 72: 130–135

[18] Acuña UM, Figueroa M, Kavalier A, Jancovski N, Basile MJ, Kennelly EJ. Benzophenones and biflavonoids from *Rheedia edulis*. J Nat Prod 2010; 73: 1775–1779

[19] Kongkathip N, Kongkathip B, Siripong P, Sangma C, Luangkamin S, Niyomdecha M, Pattanapa S, Piyaviriyagul S, Kongsaeree P. Potent antitumor activity of synthetic 1,2-naphthoquinones and 1,4-naphthoquinones. Bioorg Med Chem 2003; 11: 3179–3191