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Bioactive polyprenylated benzophenone derivatives from the fruits extracts of *Garcinia xanthochymus*

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ABSTRACT

Two new polycyclic prenylated xanthones (**1** and **2**) and a new phenylpropanoid glycoside (**3**), along with seven known compounds (**4**–**10**) were isolated from the fruits of *Garcinia xanthochymus*. The structures were elucidated by 1D- and 2D-NMR, and HRMS experiments. The isolates were evaluated for their inhibitory effects against the viability of U251MG glioblastoma and MDA-MB-231 breast cancer cells that harbor an aberrantly active signal transducer and exhibit activation of transcription 3 (STAT3), and compared to normal NIH3T3 mouse fibroblasts. Among the isolates, compounds **1**, **2**, **5**, and **6**–**9** inhibited the viability of glioma cancer cells with IC_{50} values in the range of 1.6–6.5 μM. Furthermore, treatment of U251MG with **6** and **7** inhibited intracellular STAT3 tyrosine phosphorylation and glioma cell migration *in vitro*, respectively.

Garcinia belongs to the plant family Guttiferae (Clusiaceae), which is a large genus with 13 genera and approximately 7500 species. It is widely distributed in tropical Africa, Asia, and Oceania. In Thailand, *Garcinia xanthochymus* Hook. f. has been used in folk medicine for eradicating worms and combating food toxins.[1] Previous phytochemical studies on *G. xanthochymus* have shown that this species accumulates polyprenylated benzophenones (PPBs),[2–4] a class of natural products with complex structural features and have a wide range of biological activities, including cytotoxic,[3–5] antioxidant,[5] antiplasmodial,[6] and antibacterial effects.[7]

In a continuing search for anticancer agents of natural origin, an ethyl acetate-soluble crude extract of *G. xanthochymus* fruits exhibited inhibitory activity on the viability of U251MG glioblastoma and MDA-MB-231 breast cancer cells, but had no effect on cells that do not harbor aberrantly active STAT3, such as NIH3T3 mouse fibroblasts. Reported herein are the isolation and structure elucidation of three new compounds, garcinoxanthocins A and B (**1** and **2**), and garcinol phenylpropanic acid (**3**). Also obtained were seven known polyprenylated benzophenones, namely, spiritone (**4**),[8] 14-deoxygarcinol (**5**),[9] xanthochymol (**6**),[2] garcicowin C (**7**),[3] isogarcinol (**8**),[10] cycloxanthochymol (**9**),[10] and garcinialiptone (**10**)[4] (Fig. 1 and Supporting information). All compounds were evaluated for their inhibitory effects against the viability of two cancer cell lines (U251MG glioblastoma and MDA-MB-231 breast cancer), and the NIH3T3 normal mouse fibroblast cell line.

Compound **1** was obtained as a yellow gum. The HRESIMS exhibited a pseudomolecular ion peak at *m*/*z* 601.3500 $[M+H]^+$ (calcd 601.3524), consistent with a molecular formula of $C_{38}H_{48}O_6$. The UV spectrum showed absorption bands at λ_{max} 234, 295 and 331 nm, suggesting the presence of an aromatic ring and a conjugated carbonyl chromophore in the molecule.[10,11] The IR spectrum displayed absorption bands for the presence of a hydroxyl (3355 cm^{-1}), a ketone (1722 cm^{-1}), and α,β-unsaturated carbonyl (1625 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** showed the presence of two singlet aromatic ring protons at δ_H 7.39 (1H, s, H-12) and 6.90 (1H, s, H-15). In turn, the ^{13}C NMR spectrum of **1** (Table 1) revealed signals for three oxygenated aromatic carbons at δ_C 146.4 (C-13), 154.9 (C-14), and 152.5 (C-16), a quaternary carbon at δ_C 116.8 (C-11), two protonated aromatic carbons at δ_C 108.2 (C-12) and 103.5 (C-15), along with enolized ketone group at δ_C 179.5 (C-10), 123.3 (C-3), and 165.5 (C-4) and a carbonyl carbon at δ_C 211.4 (C-9). The NMR data were consistent with the presence of a caged polycyclic prenylated xanthone molecule where an oxygen atom of the keto-enol system is linked

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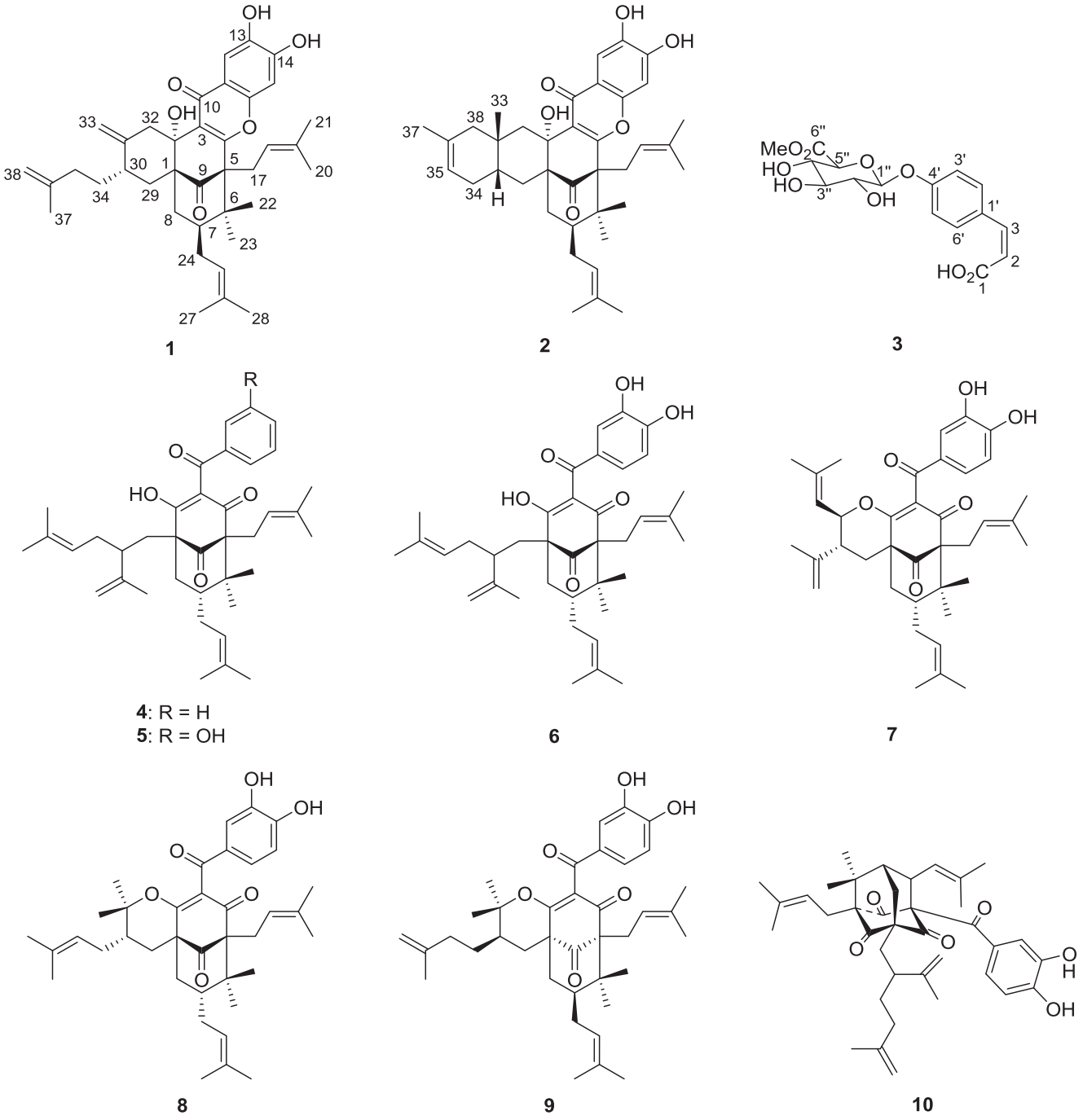


Fig. 1. Structures of compounds **1**–**10** isolated from the fruits of *G. xanthochymus*.

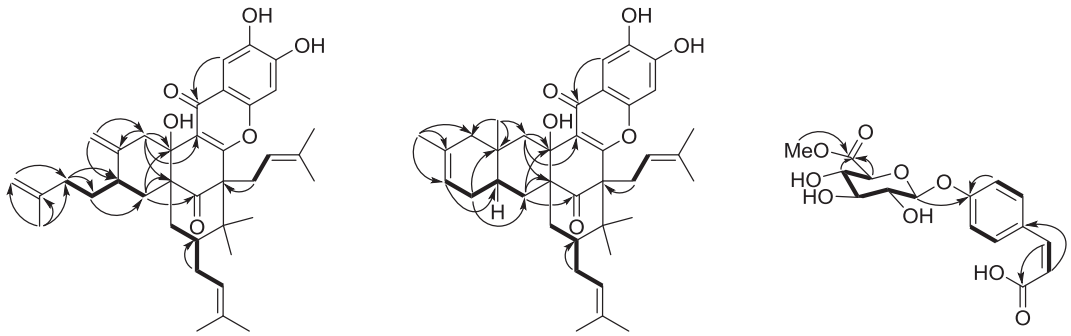
to the C-16 position. The observed ^{1}H and ^{13}C NMR signals of **1** were quite similar to those of symphonone I isolated from *Symphonia globulifera*,[6] except for the substitution of a 3-methylbut-3-enyl group [δ_H 4.75 (1H, br s, H-38a), 4.74 (1H, br s, H-38b)/δ_C 110.3 (C-38), δ_H 2.19 (2H, m, H_2-35)/δ_C 36.4, δ_H 1.84 (1H, m, H-34a), 1.42 (1H, m, H-34b)/δ_C 31.7 (C-34), and δ_H 1.79 (3H, s, Me-37)/δ_C 22.7 (C-37)], instead of resonances for a 3-methylbut-2-enyl group in symphonone I. This inference was confirmed by detailed ^{1}H-^{1}H COSY and HMBC analysis (Fig. 2).

The negative optical rotation value, $[\alpha]_D^{25}$ −33 (*c* 0.075, MeOH) of **1** indicated a β oriented C-9 carbonyl bridge, based on comparison with that of symphonone I ($[\alpha]_D^{25}$ −22 (*c* 0.2, $CHCl_3$)).[6] The relative configuration of the remaining asymmetric positions in **1** was determined to be the same as that of symphonone I through the NOESY spectrum analysis observed from H-32$_{ax}$ (δ_H 1.78, d, J = 13.6 Hz) to H-30β_{ax} (δ_H 2.18, m) and from H-7α (δ_H 1.10, m) to H-8$_{eq}$ (δ_H 2.56, m)/CH_3-23$_{eq}$ (δ_H 1.05, s) (Fig. 2), along with the comparison of their chemical shift values. Although the NOESY correlation of the hydroxyl group at C-2 with neighboring protons was undetectable, the hydroxyl group might be configured in the α form since the configurations of other groups were the same as those of simphonone I, as shown in Fig. 3. Therefore, the OH group at C-2 would be oriented in an α axial position in the chair configuration of the cyclohexane ring (Fig. 3). On the basis of the above data, the structure of the polycyclic prenylated xanthone, garcinoxanthocin A (**1**) was determined as shown.

Table 1
^{1}H and ^{13}C NMR data of Garcinoxanthocins A (**1**) and B (**2**).

Position	**1**		**2**	
	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
1		55.9		56.0
2		77.8		77.8
3		123.3		123.9
4		165.5		164.8
5		62.5		62.2
6		50.2		50.3
7	1.10, m	45.2	1.05, m[a]	45.0
8 ax	1.58, m	39.0	1.44, dd (14.0, 3.2)	40.4
eq	2.56, m		2.42, brt (14.0)	
9		211.4		211.6
10		179.5		179.6
11		116.8		116.8
12	7.39, s	108.2	7.39, s	108.1
13		146.4		146.4
14		154.9		155.0
15	6.90, s	103.5	6.89, s	103.4
16		152.5		152.4
17	2.81, m	28.1	2.79, dd (17.4, 5.6)	28.2
	2.70, m		2.65, dd (17.4, 8.4)	
18	4.50, m	120.2	4.45, m	120.5
19		135.7		135.5
20	1.37, s	26.0	1.35, s	26.1
21	1.63, s	18.4	1.62, s	18.4
22	1.05, s	20.7	1.02, s	20.4
23	1.05, s	25.5	1.02, s	25.4
24	1.99, m	29.5	1.95, m[a]	29.7
	1.63, m		1.62, m[a]	
25	4.97, m	124.2	4.96, m[a]	124.4
26		134.2		134.1
27	1.67, s	17.9	1.52, s	18.0
28	1.52, s	25.9	1.67, s	25.9
29	2.39, t (14.0)	38.0	1.75, m	33.5
	2.19, m[a]		1.40, m[a]	
30	2.18, m	36.4	2.02, m[a]	37.1
31		148.2		33.7
32ax	1.78, d (13.6)	48.5	0.81, d (14.4)	51.4
eq	2.90, d (13.6)		2.33, d (14.4)	
33	4.81, br s	108.2	1.08, s	18.9
	4.79, br s			
34	1.84, m	31.7	1.95, m[a]	30.7
	1.42, m		1.91, m[a]	
35	2.19, m[a]	36.4	5.37, br s	121.6
36		147.3		133.1
37	1.79, s	22.7	1.59, s	24.1
38	4.75, br s	110.3	1.57, m[a]	48.3
	4.74, br s			

Spectra were recorded at ^{1}H (400 MHz) and ^{13}C (100 MHz) in CD_3OD. Chemical shift (δ) are in ppm, and coupling constants (*J* in Hz) are given in parentheses.
[a] Overlapping signals.



1 2 3

Fig. 2. Key HMBC (arrow) and COSY (bold) correlations of compounds **1**–**3**.

Garcinoxanthocin B (**2**) was also obtained as a yellow gum, and its molecular formula, $C_{38}H_{49}O_6$ ([M+H]$^+$, *m*/*z* 601.3523) was deduced from the HRESIMS showing 15 degrees of unsaturation. Thus, the same molecular formula was obtained for garcinoxanthocin A (**1**). The ^{1}H and ^{13}C NMR spectra of **2** showed close resemblance to those of **1**, except for the presence of a trisubstituted

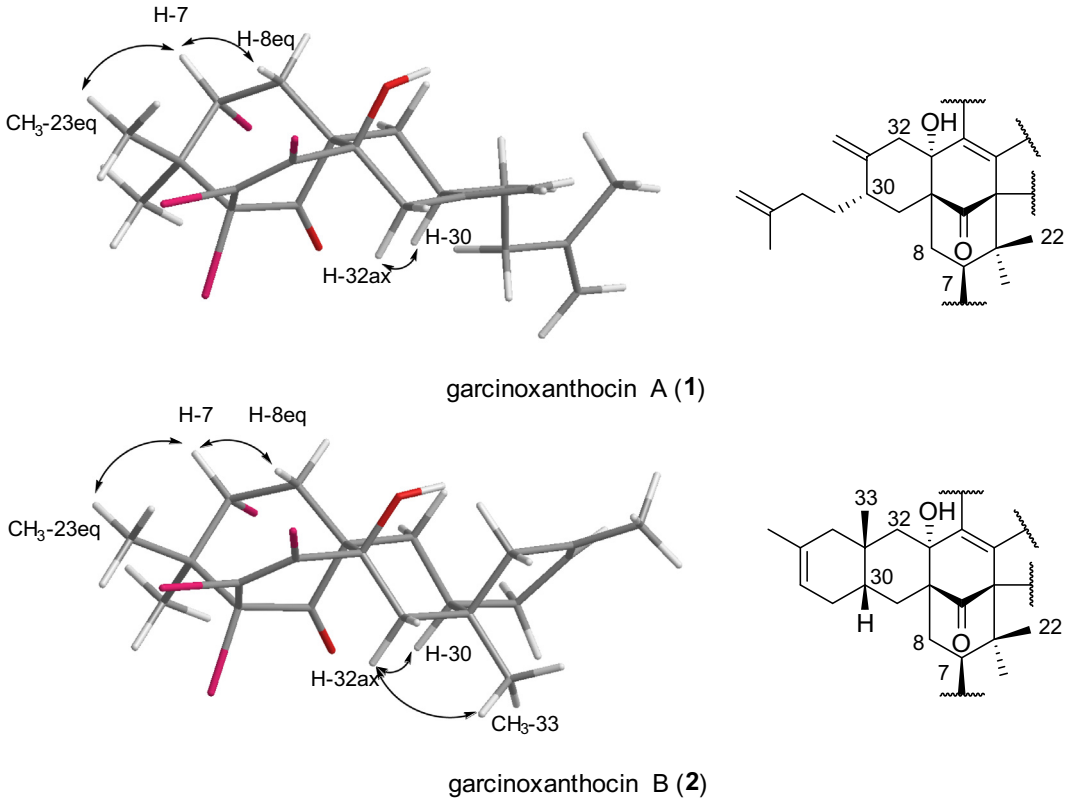


Fig. 3. Key NOESY correlations of compounds **1** and **2**.

olefinic group at δ_H 5.37 (1H, br s)/δ_C 121.6 (C-35) and δ_C 133.1 (C-36) was present, instead of the two *exo*-methylene groups in **1**. In addition, both compounds **1** and **2** possess 15 degrees of unsaturation, this implied that **2** has an additional cyclic ring system including an olefinic group. The ^{1}H NMR spectrum of **2** revealed an additional methyl proton shifted at δ_H 1.08 (3H, s, CH_3-33), which showed HMBC correlations with C-30 (δ_C 37.1), C-31 (δ_C 33.7), C-32 (δ_C 51.4), and C-38 (δ_C 48.3). In addition, the ^{1}H-^{1}H COSY correlations observed from the methine proton (H-30) to two methylene protons (H-29 and H-34) and from H-34 to H-35, and the HMBC correlations of the olefinic proton (H-35) with C-30 and C-38, clearly indicated a cyclohexene ring formed by cyclization (Supporting information, Scheme S1). The relative configuration of **2** was determined to be the same with that of **1**, based on the NMR and NOESY data analysis, along with the comparison of its optical rotation, $[\alpha]_D^{25}$ −27 (*c* 0.05, MeOH).[6] In addition, the NOESY correlations observed from H-32$_{ax}$ (δ_H 0.81, d, J = 14.4 Hz) to H-30 (δ_H 2.02, m)/CH_3-33 (δ_H 1.08, s) implied the α orientation of the 2-OH group, as in **1**. Accordingly, **2** was established as the new compound, garcinoxanthocin B.

Garcinophenylpropanic acid (**3**) was obtained as a colorless gum. The molecular formula, $C_{16}H_{18}O_9$, was deduced from the HRESIMS, which showed a protonated molecular ion peak at *m*/*z* 355.1013 $[M+H]^+$ (calcd 355.1024). The NMR and HSQC spectra showed resonances for a *para*-substituted aromatic ring at δ_H 7.58 (2H, d, J = 8.8 Hz)/δ_C 130.0 (C-2′ and C-6′), δ_H 7.34 (2H, d, J = 8.8 Hz)/δ_C 117.7 (C-3′ and C-5′), 160.0 (C-4′), and 130.5 (C-1′), an α,β-unsaturated carboxylic acid at δ_H 8.04 (1H, d, J = 12.0 Hz)/δ_C 144.1 (C-3), δ_H 6.82 (1H, d, J = 12.0 Hz)/δ_C 119.5 (C-2), and δ_C 169.8 (C-1). This indicated the presence of a phenyl propanoic acid moiety, and an HMBC correlation was observed between H-2 and C-1′. The geometry of the olefinic double bond (C-2–C-3) was determined to be in the *Z* configuration, based on the magnitude of the ^{1}H NMR coupling constant value (J = 12.0 Hz) and a NOESY correlation between H-2 and H-3 (Table 2). Furthermore, the NMR and HSQC spectra (Table 2) of **3** showed additional signals for a methoxy group at δ_H 3.69 (3H, s)/δ_C 52.6 (-$COOCH_3$) attached to a carbonyl carbon, an ester carbonyl carbon at δ_C 170.6 (C-6″), and those of a hexose sugar unit at δ_H 5.83 (1H, d, J = 7.2 Hz/δ_C 102.4 (C-1″), 4.45 (1H, m)/78.0 (C-2″), 4.40 (1H, m)/75.0 (C-3″), 4.61 (1H, t, J = 8.4 Hz)/73.3 (C-4″), 4.84 (1H, d, J = 8.4 Hz)/77.6 (C-5″), supporting the presence of β-D-glucuronic acid methyl ester unit.[12,13] The linkage of the glucuronic acid methyl ester and the phenyl propanoic acid moieties was confirmed on the basis of the HMBC correlation between H-1″ and C-4′ (Fig. 2). Thus, the structure of garcinophenylpropanoic acid (**3**) was determined as shown in Fig. 1.

Table 2
^{1}H and ^{13}C NMR data of compound **3**.

Position	δ_H, mult (J in Hz)	δ_C
1		169.8
2	6.82, d (12.0)	119.5
3	8.04, d (12.0)	144.1
1′		130.5
2′/6′	7.58, d (8.8)	130.0
3′/5′	7.34, d (8.8)	117.7
4′		160.0
1″	5.83, d (7.2)	102.4
2″	4.45, m	78.0
3″	4.40, m	75.0
4″	4.61, t (8.4)	73.3
5″	4.84, d (8.4)	77.6
6″		170.6
6″-OCH_3	3.69, s	52.6

Spectra were recorded at ^{1}H (400 MHz) and ^{13}C (100 MHz) in Pyridine-d_5. Chemical shift (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses.

Table 3
STAT3 inhibition effect of compounds isolated from *G. xanthochymus*.

Compounds	IC_{50}, μM		
	U251MG[a]	MDA-MB-231[b]	NIH3T3[c]
1	3.5 ± 0.10	NA[d]	NA
2	1.8 ± 0.43	NA	NA
3	–[e]	–	–
4	–	–	–
5	1.3 ± 0.12	NA	NA
6	2.3 ± 0.67	NA	NA
7	3.7 ± 0.37	NA	NA
8	2.8 ± 0.31	NA	NA
9	6.0 ± 0.80	NA	NA
10	NA	NA	NA

[a] U251MG: glioblastoma cell.
[b] MDA-MB-231: breast cancer cell.
[c] NIH3T3: normal cell.
[d] NA, indicates not active.
[e] –, not tested.

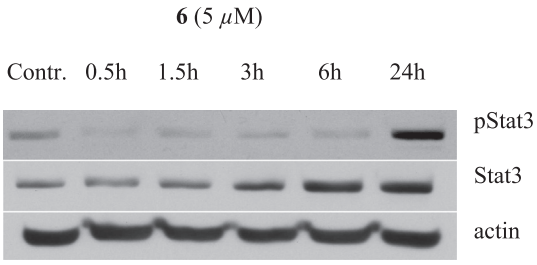


Fig. 4. The inhibitory effect of **6** against pStat3 induction in U251MG cells.

Glioblastoma multiforme (GBM) is a highly malignant and aggressive form of cancer and the most common invasive brain tumor with a poor prognosis. Current standard treatment combines surgery, chemotherapy, and radiation. Yet, the median survival period for GBM is less than two years. Additionally, invading cells are highly resistant to radiation and chemotherapy.[14,15] Signal transducer and activator of transcription (STAT) proteins are latent cytoplasmic transcription factors that mediate cellular responses to cytokines and growth factors.[15] The family consists of seven isoforms (STATs 1–4, 5a, 5b and 6).[14,15] Normal STAT3 activation is transient in keeping with cellular requirements for proliferation, development, apoptosis and inflammation.[16] In contrast, aberrant STAT3 activity dysregulates growth and survival,[17–19] promotes angiogenesis,[20] and migration and invasion[21] of tumor cells, and induces tumor immune tolerance.[22,23] Therefore, STAT3 has emerged as an appealing target for a wide variety of human tumors including GBM.

As summarized in Table 3, all isolated compounds were tested for their inhibitory activities against the growth of the U251MG glioma and MDA-MB-231 breast cancer cells that harbor aberrantly active STAT3, and compared to normal NIH3T3 mouse fibroblast cells that do not. The polyprenylated benzophenone derivatives, showed no inhibition on the viability of NIH3T3 cells. Among them, compound **5** showed the greatest inhibition of the viability of U251MG cells, with an IC_{50} value of 1.3 μM, and the remaining compounds also displayed notable activity in the range IC_{50} 1.8–6.0 μM, while compound **10** was inactive. Additionally, they had no effect on the viability of MDA-MB-231 cells (Table 3). Moreover treatment of U251MG cells with **6** inhibited intracellular phospho-tyrosine STAT3 (pY705STAT3) (Fig. 4), suggesting that its inhibition of aberrant STAT3 activity in the tumor cells contributes to the loss of U251MG viability induced by this compound. Also, compound **7** inhibited both glioma cell migration in a wound-healing assay and STAT3 DNA-binding activity in an electrophoretic

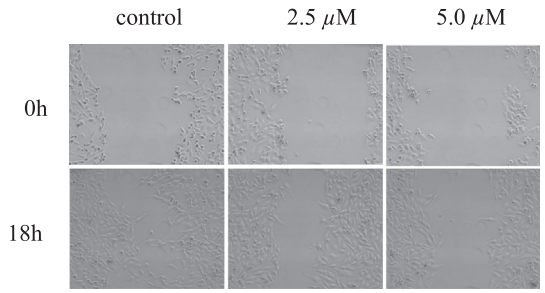


Fig. 5. Wound-healing assay showing inhibitory effects of **7** on U251MG cell migration after 18 h.

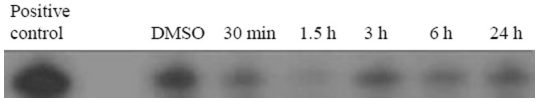


Fig. 6. Inhibition of Stat3 DNA binding activity in U251MG cells treated with 5 μM compound **7**, as measured by EMSA analysis.

mobility shift assay (EMSA), as shown in Figs 5 and 6, respectively. These results suggest **6** and **7** may have tumor-inhibitory potential by modulating aberrant STAT3 activity in tumor cells.

Garcinoxanthocin A (**1**): yellow gum; $[\alpha]_D^{25}$ −33 (*c* 0.075, MeOH); UV (MeOH) λ_{max} (log ε) 234 (6.08), 295 (7.74), 331 (7.77) nm; IR (neat) ν_{max} 3355, 2967, 2924, 1722, 1625, 1574, 1471, 1374, 1285, 1175, 1148 and 884 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 601.3500 $[M+H]^+$, calcd for $C_{38}H_{49}O_6$, 601.3524.

Garcinoxanthocin B (**2**): yellow gum; $[\alpha]_D^{25}$ −27 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 237 (6.40), 295 (6.08), 329 (6.11) nm; IR (neat) ν_{max} 3356, 2920, 1720, 1624, 1582, 1068, 881 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 601.3523 $[M+H]^+$, calcd for $C_{38}H_{49}O_6$, 601.3524.

Garcinophenylpropanic acid (**3**): colorless gum; $[\alpha]_D^{25}$ −308 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 221 (5.41), 287 (5.55) nm; IR (neat) ν_{max} 3350, 2920, 2850, 1730, 1680, 1610, 1520, 1230, 1180, 1050 and 1020 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 355.1013 $[M+H]^+$, calcd for $C_{16}H_{19}O_9$, 355.1024.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.06.073.

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