Synthesis and SAR Study of Prenylated Xanthone Analogues as HeLa and MDA-MB-231 Cancer Cell Inhibitors

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Abstract: In order to explore structure-activity relationship (SAR) associated with xanthone framework, a series of prenylated xanthone derivatives **2-9** was synthesized from the key building block 1,3,6,8-tetrahydroxyxanthone **1** and evaluated for their *in vitro* growth inhibitory activities against HeLa and MDA-MB-231 human cancer cell lines. The *in vitro* evidence indicated that the inhibitory activity was significantly influenced by the position and number of linked group on the xanthone skeleton, and the presence of chroman-4-one moiety in the xanthone scaffold was found to be critically important for strong cytotoxic activity. The novel 2*H*-xanthene-3,9-dione analogues **3** and **4** were reported to elicit potent activities comparable to those of standard drugs doxorubicin and cisplatin. This preliminary investigation has highlighted the therapeutic potential of 2*H*-xanthene-3,9-dione derivatives to be exploitable as lead compound for the development of potent HeLa and MDA-MB-231 cancer cell inhibitors.

Keywords: Cytotoxicity, 2*H*-xanthene-3,9-dione scaffold, 2*H*-xanthene-3,6,9(7*H*)-trione scaffold, Inhibitory activity, Synthesis, Xanthone derivatives.

INTRODUCTION

Natural or synthetic xanthones, in view of their unique structures, have been reported to exhibit a wide range of biological profiles including cytotoxic, anti-inflammatory, antioxidant, antimalarial, antibacterial and antifungal activities [1-5]. The interesting structural chromophore and biological efficacy of xanthones have attracted many researchers to isolate or synthesize these compounds for development of potential new drug leads for treatment of cancer diseases. In the past, many xanthones isolated from plants were found to bear prenyl moieties, and many of which were reported to exhibit a significant inhibitory activity against a panel of human cancer cell lines [6-9]. Previous prenylation studies on the precursors 1,3-dihydroxyxanthone and 1,3,5-trihydroxyxanthones have highlighted the cytotoxic activity to be substantially influenced by the degree of substitution and the substitution pattern of prenyl group at the xanthone ring [10,11]. Besides that, the presence of hydroxyl group was also found to be essential since etherification led to a decrease in the growth inhibitory activity [12]. More interestingly, the presence of prenyl groups in different key-positions on xanthonic nucleus was found to give selective effect on the human cancer cell lines [10,11], and this prompted us to conduct a detailed SAR study on a series of prenylated xanthone analogues derived from the key building block 1,3,6,8-tetrahydroxyxanthone **1** to clarify the structure-activity correlation underlying the compounds that can guide us to the discovery of potent inhibitors for therapeutic use. In our study, reaction of precursor **1** with prenyl bromide in alkaline medium yielded three novel xanthone derivatives **2**-**4**. Prenylated derivatives **3** and **4** with a distorted nucleus are reported to elicit a higher cytotoxic activity than the typical xanthones **5** to **9**, and a comparable activity with those of doxorubicin and cisplatin. We described herein the synthesis of xanthone analogues **1** to **9** with the aim to provide comprehensive insight into the correlation between structures and inhibitory activities of xanthones.

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RESULTS AND DISCUSSION

Chemistry

The synthetic route toward xanthone derivatives **1**-**9** is outlined in Scheme **1**. In previous study, Liu *et al.* reported the synthesis of compound **1** in low yield by using polyphosphoric acid as a catalyst [13]. In this study, the key building block **1** was synthesized by condensation of 2,4,6-trihydroxybenzoic acid with phloroglucinol in the presence of phosphorus pentoxide and methanesulfonic acid according to the method described in the literature [14]. This afforded a higher yield compared to the former synthetic method. The prenylated xanthones **2**-**9** were obtained from the reaction of their corresponding building block **1** with 3-methylbut-2-enyl bromide in 10% aqueous KOH. The use of aqueous KOH in the synthesis

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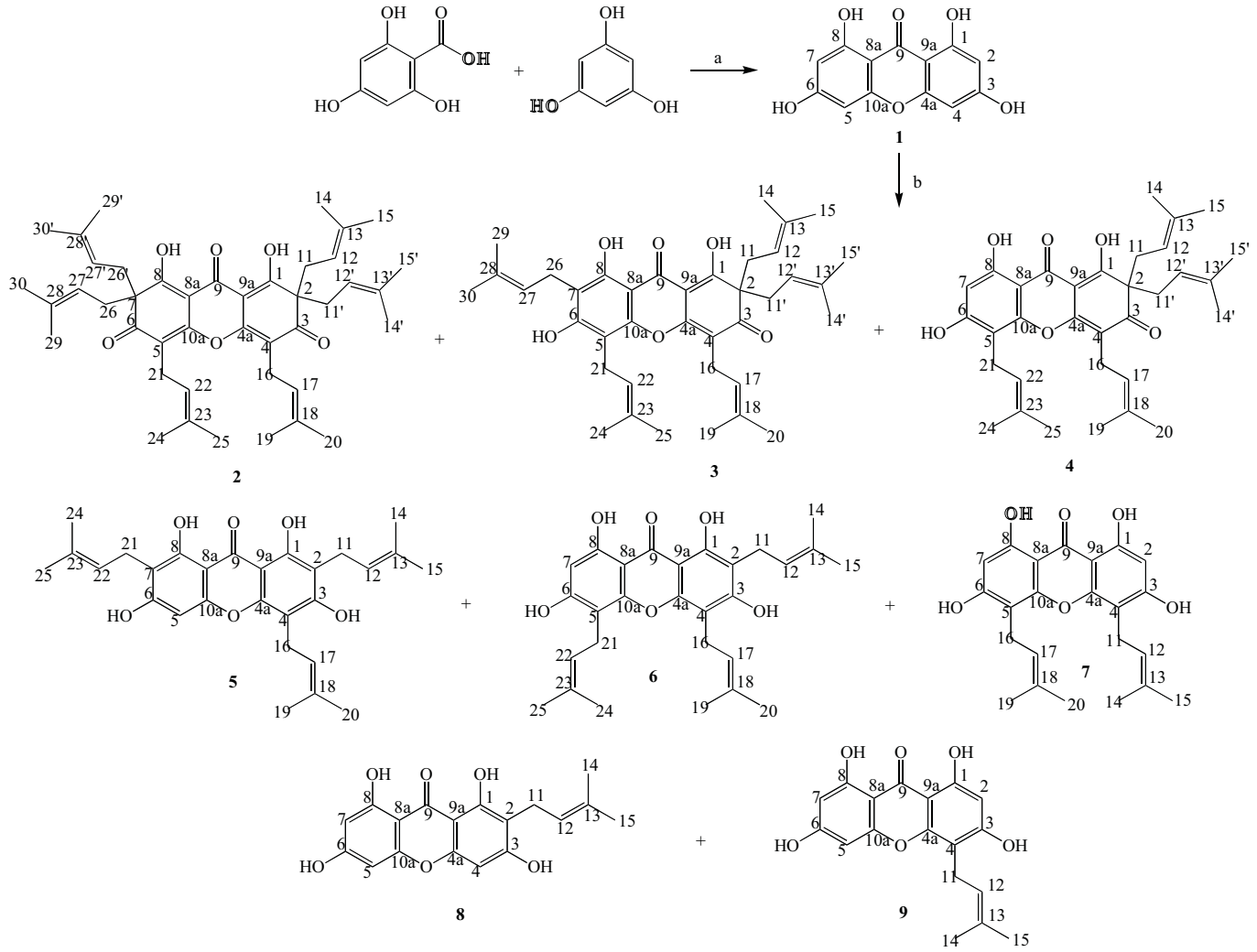

Scheme 1. Synthetic route for xanthone derivatives **1**-**9**. Reagents and conditions: (a) P_2O_5-CH_3SO_3H, 80 ^oC, 0.5 h, 62%; (b) 3-methylbut-2-enyl bromide, 10% aqueous KOH, rt, overnight, 2% for **2**, 1% for **3**, 2% for **4**, 3% for **5**, 4% for **6**, 4% for **7**, 4% for **8**, 5% for **9**.

was found to produce *C*-prenylated derivatives by suppressing the formation of *O*-prenylated derivatives [15]. The suppression of *O*-prenylation is critically important to maintain the phenolic hydroxyl groups in xanthones which were found to be crucial to the cytotoxic activities [12]. Prenylation of building block **1** was reported to give *gem*-diprenylated xanthones **2**-**4** and this is in agreement with the previous study on acylphloroglucinols which reacted under the same conditions to give *gem*-diprenylated poducts [16].

Growth Inhibition

The inhibitory activities of the parent xanthone **1** and its prenylated derivatives **2**-**9** towards HeLa (cervical carcinoma) and MDA-MB-231 (human estrogen receptor negative breast cancer) cancer cell lines were evaluated by the method described in the literature [17] with some modifications. The obtained IC_{50} values for the compounds are shown in Table **1**.

Under our assay conditions, prenylated xanthones **2**-**9** showed much higher inhibitory activities than the parent compound **1** suggesting that the cytotoxicity of xanthones can be largely amplified by the presence of prenyl functionality, and this result was in agreement with the previous reports that prenyl group plays a significant role in biological activities [10,11]. From the structural point of view, the prenylated derivatives obtained in this study can be classified into three main groups based on the variation in their xanthonic core, that are, compound **2** with 2*H*-xanthene-3,6,9(7*H*)-trione scaffold; compounds **3**-**4** with 2*H*-xanthene-3,9-dione scaffold; compound **5**-**9** with the typical xanthonic nucleus. The cytotoxic assay data of 2*H*-xanthene-3,9-dione analogues indicated that the presence of prenyl substituent at the C7 position was found to induce a highly potent growth inhibitory activity against the two cell lines tested. Compound **3** bearing the C-7 prenyl group as compared with compound **4** without the group, was found to exert a more potent antiproliferative activity towards MDA-MB-231 cells and, however, a less cytotoxic potency for HeLa cells. In comparison with doxorubicin and cisplatin as positive control, compound **4** was found to exhibit at least 3-fold much higher activity than both the standard drugs used in the HeLa assay, while for MDA-MB-231 cells, compound **3**

was found to give a comparable activity with the standard drugs.

Table 1. ***In Vitro*** **Inhibitory Activities of Synthesized Compounds Against HeLa and MDA-MB-231 Human Cancer Cell Lines**

Compound	IC_{50} (μM)	
	HeLa	MDA-MB-231
1	> 60	> 60
2	52.4	46.4
3	11.7	10.0
4	3.8	60.1
5	30.2	32.3
6	28.0	40.9
7	12.6	20.2
8	115.8	73.1
9	47.2	54.9
Doxorubicin	12.9	11.0
Cisplatin	13.3	16.7

[a]Values reported are an average of at least two independent experiments in triplicate.

For the group of compounds **5-9** with typical xanthonic nucleus, some apparent trends were observed for their inhibitory activities against the two cell lines. The results clearly indicated that the position of prenyl groups imparts determinant effect on inhibitory activities of the typical xanthone derivatives. In general, di-prenyl substituted xanthones were found to display more potent activity than mono- and tri-prenyl substituted xanthones against HeLa and MDA-MB-231 cells. Compound **7** bearing prenyl groups at C4 and C5 showed the highest activity as compared to other members in the group. The attempt to remove C4 or C5 prenyl group from compound **7** (as exemplified by compounds **8** and **9**), or introduce C2 or C7 prenyl group to compound **7** (as exemplified by compound **6**), both were found to decrease the inhibitory activity towards the two cell lines suggesting that appropriate introduction of a prenyl group to the xanthonic nucleus plays a substantial role in antiproliferative activities.

Compound **2** with 2*H*-xanthene-3,6,9(7*H*)-trione framework showed the highest number of attached prenyl groups which principally is expected to give a better activity than the former two groups of compounds but it is the other way round. This finding helps to explain that the presence of chroman-4-one moiety in xanthone nucleus is important for its cytotoxic activity as this was evident by a much higher inhibitory activity elicited by the analogues **3**-**7** bearing an intact chroman-4-one moiety in their nuclei. The impairment of conjugated π-system on both benzene rings in the nucleus as exemplified by compound **2** was found to destroy the chroman-4-one moiety and hence weaken the inhibitory activity. Apart from the discussion above, compounds **8** and **9** were found to be much less potent suggesting that a minimum of two prenyl groups have to be present together with chroman-4-one moiety in order to elicit a potent activity. Among all the test compounds, compounds **3**-**4** were found to exhibit outstanding activity suggesting that the novel 2*H*-xanthene-3,9-dione scaffold could serve as a potential drug lead in the future drug development. However, there is one drawback in this study due to the low yields of compounds obtained, and currently our team is working on a modified synthetic approach to improve the yield of compounds **3** and **4**. At the meantime, compounds **3** and **4** are aimed for apoptosis signalling pathways study to provide insights into the mechanism of action underlying the two cancer cell lines, which will be reported in due course.

EXPERIMENTAL

Melting points were determined on the Stuart SMP10 melting point apparatus and are uncorrected. UV and IR spectra were recorded on Perkin-Elmer Lambda 35 UV/VIS and Perkin-Elmer Spectrum RXI FTIR spectrometers, respectively. NMR spectra were measured on a Bruker AV-III 500 MHz NMR or JEOL JNM-ECX 400 MHz FTNMR spectrometer in $CDCl_3$ or acetone-d_6 with TMS as the internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) are expressed in Hertz. EIMS and HREIMS were measured on Agilent 5975C MSD mass spectrometer and Thermo Finnigan MAT95XL mass spectrometers, respectively. All reagents were of analytical quality and used without further purification unless otherwise specified. Column chromatography (CC) was performed with silica gel 60 (230-300 mesh, Silicycle) and Sephadex LH-20. Analytical TLC was performed on precoated silica gel 60 F_{254} (Merck; Germany).

Synthesis of Building Block 1,3,6,8-Tetrahydroxy-9H-xanthen-9-one (1)

A mixture of 2,4,6-trihydroxybenzoic acid monohydrate (9.40 g, 50 mmol) and phloroglucinol (6.30 g, 50 mmol) was added slowly to 120 mL of Eaton's reagent (P_2O_5/CH_3SO_3H, Acros). The mixture was heated at 80 ^oC for 30 minutes, under stirring. After cooling to room temperature, the reaction mixture was poured onto crushed ice and stirred for 1 hour. The resulting solid was collected by filtration and dried in the oven at 50 ^oC overnight. The solid was purified on a silica gel (40-63 μm) column with a mixture of hexane-ethyl acetate (9:1) as eluent to give **1**.

1,3,6,8-Tetrahydroxy-9H-xanthen-9-one (1)

62% (8.1 g) as a yellow solid, mp 210-212 ^oC ; UV λ_{max} nm (EtOH) (log ε): 233, 246, 334; IR υ_{max} (KBr) cm^{-1} : 3468, 3414, 2930, 2815, 2731, 2650, 1637, 1618, 1522, 1458, 1385, 1321, 1278, 1183, 1159, 1069, 833, 759; ^1H NMR (500 MHz, acetone-d_6): 11.93 (2H, s, 1- OH & 8- OH), 6.31 (2H, d, J = 1.8 Hz, H-4 & H-5), 6.19 (2H, d, J = 1.8 Hz, H-2 & H-7); ^{13}C NMR (125 MHz, acetone-d_6): 183.1 (C-9), 165.8 (C-3 & C-6), 163.0 (C-1 & C-8), 157.8 (C-4a & C-10a), 101.1 (C-8a & C-9a), 98.5 (C-2 & C-7), 94.3 (C-4 & C-5); EIMS *m/z* (rel. int.): 260 ($[M]^{+\cdot}$ 100), 231 (10), 203 (11), 69 (10).

General Procedure for the Synthesis of Prenylated Xanthones 2 - 9

A mixture of 1,3,6,8-tetrahydroxy-9*H*-xanthen-9-one **(1)** (1.02 g; 3.92 mmol) and prenyl bromide (2.09 g; 14.03 mmol) in 50 mL of 10% aqueous KOH was stirred for 16 hours at

room temperature. The mixture was then acidified with 10% HCl and extracted with AcOEt (3 × 40 mL). The combined organic layers were dried over Na_2SO_4. After removal of the solvent under reduced pressure, purification of the crude product using silica gel column eluted with a mixture of hexane-ethyl acetate in increasing polarity, followed by Sephadex LH-20 column eluted with methanol yielded successively **2-9**.

1,8-Dihydroxy-2,2,4,5,7,7-hexakis(3-methylbut-2-enyl)-2H-xanthene-3,6,9 (7H)-trione (2)

2% (43.2 mg) as a yellow liquid, UV λ_{max} nm (EtOH) (log ε): 209, 235, 245, 274; IR υ_{max} (KBr) cm^{-1} : 3436, 2973, 2927, 2856, 1638, 1438, 1381, 1278, 1174, 818, 776; ^{1}H NMR (400 MHz, $CDCl_3$): 11.73 (2H, s, 1- OH & 8- OH), 5.01 (2H, t, *J* = 6.7 Hz, H-17 & H-22), 4.72 (4H, t, *J* = 7.3 Hz, H-12, H-12', H-27 & H-27'), 3.01 (4H, d, *J* = 6.7 Hz, H-16 & H-21), 2.94 (4H, dd, *J* = 14.0, 7.3 Hz, H-11, H-11', H-26 & H-26'), 2.68 (4H, dd, *J* = 14.0, 7.3 Hz, H-11, H-11', H-26 & H-26'), 1.69 (6H, s, C-19 & C-24), 1.60 (6H, s, H-20 & H-25), 1.50 (12H, s, H-14, H-14', H-29 & H-29'), 1.49 (12H, s, H-15, H-15', H-30 & H-30'); ^{13}C NMR (100 MHz, $CDCl_3$): 193.8 (C-3 & C-6), 179.7 (C-9), 173.8 (C-1 & C-8), 161.5 (C-4a & C-10a), 135.7 (C-13, C-13', C-28 & C-28'), 130.9 (C-18 & C-23), 121.8 (C-17 & C-22), 116.9 (C-12, C-12', C-27 & C-27'), 115.7 (C-4 & C-5), 114.1 (C-8a & C-9a), 55.6 (C-2 & C-7), 38.5 (C-11, C11', C-26 & C-26'), 25.2 (C-20 & C-25), 25.0 (C-15, C-15', C-30 & C-30'), 20.6 (C-16 & C-21), 17.3 (C-14, C-14', C-29 & C-29'), 17.1 (C-19 & C-24); EIMS *m/z* (rel. int.): 668 ($[M]^{+\cdot}$ 5), 599 (31), 531 (85), 475 (88), 419 (82), 363 (33), 149 (35), 69 (100); EIHRMS: 668.4054 ($[M]^{+\cdot}$, $C_{43}H_{56}O_6^{+\cdot}$; calcd 668.4077).

1,6,8-Trihydroxy-2,2,4,5,7-pentakis(3-methylbut-2-enyl)-2H-xanthene-3,9-dione (3)

1% (18.8 mg) as a yellow liquid, UV λ_{max} nm (EtOH) (log ε): 209, 229, 262, 353; IR υ_{max} (KBr) cm^{-1} : 3608, 3524, 3412, 3004, 2967, 2924, 1722, 1421, 1367, 1220, 1092, 902, 785; ^{1}H NMR (400 MHz, acetone-d_6): 12.35 (1H, s, 1-OH), 11.80 (1H, s, 8-OH), 5.19 (1H, t, *J* = 7.0 Hz, H-27), 5.11 (1H, t, *J* = 6.2 Hz, H-22), 5.07 (1H, t, *J* = 7.0 Hz, H-17), 4.76 (2H, t, *J* = 7.4 Hz, H-12 & H-12'), 3.61 (2H, d, *J* = 6.2 Hz, H-21), 3.44 (2H, d, *J* = 7.0 Hz, H-26), 3.01 (2H, d, *J* = 7.0 Hz, H-16), 2.82 (2H, dd, *J* = 13.6, 7.4 Hz, H-11 & H-11'), 2.72 (2H, dd, *J* = 13.6, 7.4 Hz, H-11 & H-11'), 1.82 (3H, s, H-24), 1.76 (3H, s, H-29), 1.70 (3H, s, H-19), 1.64 (6H, s, H-25 & H-30), 1.61 (3H, s, H-20), 1.45 (6H, s, H-14 & H-14'), 1.42 (6H, s, H-15 & H-15'); ^{13}C NMR (100 MHz, acetone-d_6): 194.2 (C-3), 181.7 (C-9), 174.4 (C-1), 163.7 (C-4a), 161.0 (C-6), 157.0 (C-8), 152.5 (C-10a), 135.1 (C-13 & C-13'), 132.4 (C-23), 132.3 (C-28), 130.5 (C-18), 122.4 (C-17), 122.2 (C-22), 121.3 (C-27), 117.5 (C-12 & C-12'), 113.8 (C-4), 113.3 (C-7), 108.3 (C-9a), 107.3 (C-5), 101.9 (C-8a), 57.1 (C-2), 38.8 (C-11 & C11'), 25.1 (C-25 & 30), 25.0 (C-15 & C-15'), 24.9 (C-20), 21.7 (C-26), 21.5 (C-21), 20.5 (C-16), 15.5 (C-29), 17.2 (C-24), 17.1 (C-19), 17.0 (C-14 & C-14'); EIMS *m/z* (rel. int.): 600 ($[M]^{+\cdot}$ 15), 531 (100), 475 (72), 419 (76), 363 (29), 309 (23), 69 (38), 43 (21); EIHRMS: 600.3440 ($[M]^{+\cdot}$, $C_{38}H_{48}O_6^{+\cdot}$; calcd 600.3451).

1,6,8-Trihydroxy-2,2,4,5-tetrakis(3-methylbut-2-enyl)-2H-xanthene-3,9-dione (4)

2% (35.5 mg) as yellow crystals, mp 182-185 °C; UV λ_{max} nm (EtOH) (log ε): 204, 218, 266, 351; IR υ_{max} (KBr) cm^{-1} : 3415, 3035, 2972, 2914, 1672, 1614, 1550, 1455, 1413, 1293, 1132, 1194, 1172, 1110, 858, 784, 728; ^{1}H NMR (500 MHz, acetone-d_6): 12.37 (1H, s, 1-OH), 11.49 (1H, s, 8- OH), 6.51 (1H, s, H-7), 5.23 (1H, t, *J* = 6.7 Hz, H-22), 5.12 (1H, t, *J* = 6.5 Hz, H-17), 4.82 (2H, t, *J* = 7.6 Hz, H-12 & H-12'), 3.57 (2H, d, *J* = 6.7 Hz, H-21), 3.06 (2H, d, *J* = 6.5 Hz, H-16), 2.89 (2H, dd, *J* = 13.6, 7.6 Hz, H-11 & H-11'), 2.79 (2H, dd, *J* = 13.6, 7.6 Hz, H-11 & H-11'), 1.86 (3H, s, H-24), 1.74 (3H, s, H-19), 1.74 (3H, s, H-25), 1.66 (3H, s, H-20), 1.51 (6H, s, H-14 & H-14'), 1.48 (6H, s, H-15 & H-15'); ^{13}C NMR (125 MHz, acetone-d_6): 194.0 (C-3), 181.5 (C-9), 174.4 (C-1), 163.9 (C-6), 163.6 (C-4a), 160.1 (C-8), 154.4 (C-10a), 135.0 (C-13 & C-13'), 131.7 (C-23), 130.3 (C-18), 122.4 (C-17), 122.2 (C-22), 117.4 (C-12 & C-12'), 113.8 (C-4), 108.2 (C-9a), 107.5 (C-5), 103.0 (C-8a), 100.0 (C-7), 57.1 (C-2), 38.7 (C-11 & C-11'), 25.0 (C-20), 24.9 (C-15, C-15' & C-25), 21.3 (C-21), 20.4 (C-16), 17.3 (C-24), 17.0 (C-14, C-14' & C-19); EIMS *m/z* (rel. int.): 532 ($[M]^{+\cdot}$ 7), 463 (61), 407 (100), 393 (15), 365 (13), 353 (18), 69 (15); EIHRMS: 532.2825 ($[M]^{+\cdot}$, $C_{33}H_{40}O_6^{+\cdot}$; calcd 532.2825).

1,3,6,8-Tetrahydroxy-2,4,7-tris(3-methylbut-2-enyl)-9H-xanthen-9-one (5)

3% (50.9 mg) as yellow crystals, mp 179-181 °C; UV λ_{max} nm (EtOH) (log ε): 216, 237, 261, 297, 341; IR υ_{max} (KBr) cm^{-1} : 3474, 3414, 2966, 2913, 1618, 1437, 1317, 1275, 1236, 1176, 1120, 1099, 812, 787; ^{1}H NMR (500 MHz, acetone-d_6): 12.34 (1H, s, 1-OH), 12.33 (1H, s, 8- OH), 6.54 (1H, s, H-5), 5.28 (1H, t, *J* = 7.2 Hz, H-22), 5.22 (1H, t, *J* = 7.1 Hz, H-12), 5.21 (1H, t, *J* = 7.3 Hz, H-17), 3.53 (2H, d, *J* = 7.2 Hz, H-21), 3.43 (2H, d, *J* = 7.3 Hz, H-16), 3.36 (2H, d, *J* = 7.1 Hz, H-11), 1.86 (3H, s, H-24), 1.79 (6H, s, H-14 & H-19), 1.66 (3H, s, H-20), 1.65 (6H, s, H-15 & H-25); ^{13}C NMR (125 MHz, acetone-d_6): 183.5 (C-9), 163.2 (C-6), 160.4 (C-3), 159.8 (C-8), 157.7 (C-1), 155.6 (C-10a), 152.7 (C-4a), 131.7 (C-13), 131.5 (C-18), 130.8 (C-23), 122.3 (C-22), 122.0 (C-12 & C-17), 110.8 (C-7), 110.5 (C-2), 106.3 (C-4), 101.3 (C-8a), 100.9 (C-9a), 93.5 (C-5), 25.0 (C-15, C-20 & C-25), 21.6 (C-21), 21.2 (C-11), 21.0 (C-16), 17.2 (C-24), 17.1 (C-14), 17.0 (C-19); EIMS *m/z* (rel. int.): 464 ($[M]^{+\cdot}$ 100), 421 (28), 409 (91), 393 (73), 365 (62), 353 (71), 337 (41), 309 (57), 297 (72), 69 (16), 43 (24); EIHRMS: 464.2200 ($[M]^{+\cdot}$, $C_{28}H_{32}O_6^{+\cdot}$; calcd 464.2199).

1,3,6,8-Tetrahydroxy-2,4,5-tris(3-methylbut-2-enyl)-9H-xanthen-9-one (6)

4% (65.5 mg) as yellow crystals, mp 179-181 °C; UV λ_{max} nm (EtOH) (log ε): 216, 237, 261, 341; IR υ_{max} (KBr) cm^{-1} : 3420, 2966, 2921, 1618, 1466, 1313, 1232, 1170, 1085, 815, 753; ^{1}H NMR (500 MHz, acetone-d_6): 12.34 (1H, s, 1-OH), 12.01 (1H, s, 8- OH), 6.32 (1H, s, H-7), 5.28 (1H, t, *J* = 7.2 Hz, H-22), 5.22 (2H, t, *J* = 7.1 Hz, H-12 & H-17), 3.59 (2H, d, *J* = 6.7 Hz, H-21), 3.49 (2H, d, *J* = 6.9 Hz, H-16), 3.43 (2H, d, *J* = 6.9 Hz, H-11), 1.79 (9H, s, H-14, H-19 & H-24), 1.68 (3H, s, H-20), 1.66 (6H, s, H-15 & H-25); ^{13}C NMR (125 MHz, acetone-d_6): 183.7 (C-9), 163.0 (C-8), 160.7 (C-6), 160.6 (C-3), 157.7 (C-1), 154.8 (C-10a), 152.8 (C-4a), 131.9 (C-13), 131.7 (C-18), 131.1 (C-23), 122.6 (C-12 & C-17), 122.0 (C-

22), 110.6 (C-2), 106.8 (C-4), 106.7 (C-5), 101.0 (C-8a & C-9a), 97.8 (C-7), 25.0 (C-20), 24.9 (C-15 & C-25), 21.6 (C-21), 21.4 (C-11), 21.2 (C-16), 17.2 (C-24), 17.1 (C-14 & C-19); EIMS *m/z* (rel. int.): 464 ($[M]^{+\cdot}$ 100), 421 (52), 409 (62), 393 (58), 365 (43), 353 (84), 309 (22), 297 (18), 79 (17), 43 (18); EIHRMS: 464.2199 ($[M]^{+\cdot}$, $C_{28}H_{32}O_6^{+\cdot}$; calcd 464.2199).

1,3,6,8-Tetrahydroxy-4,5-bis(3-methylbut-2-en-1-yl)-9H-xanthen-9-one (7)

4% (57.4 mg) as yellow crystals, mp 250-253 °C; UV λ_{max} nm (EtOH) (log ε): 234, 260, 338; IR υ_{max} (KBr) cm^{-1} : 3468, 2966, 2914, 1617, 1508, 1431, 1312, 1273, 1213, 1173, 1090, 835, 813, 778; ^{1}H NMR (500 MHz, acetone-d_6): 12.00 (2H, s, 1-OH & 8-OH), 6.31 (2H, s, H-2 & H-7), 5.30 (2H, t, *J* = 6.8 Hz, H-12 & H-17), 3.53 (4H, d, *J* = 6.8 Hz, H-11 & H-16), 1.79 (6H, s, H-14 & H-19), 1.67 (6H, s, H-15 & H-20); ^{13}C NMR (125 MHz, acetone-d_6): 183.7 (C-9), 163.1 (C-1 & C-8), 160.7 (C-3 & C-6), 154.9 (C-4a & C-10a), 131.2 (C-13 & C-18), 122.6 (C-12 & C-17), 107.0 (C-4 & C-5), 100.9 (C-8a & C-9a), 97.9 (C-2 & C-7), 24.9 (C-15 & C-20), 21.4 (C-11 & C-16), 17.1 (C-14 & C-19); EIMS *m/z* (rel. int.): 396 ($[M]^{+\cdot}$ 100), 381 (67), 353 (18), 341 (40), 325 (73), 313 (55), 297 (25), 273 (27), 260 (20), 143 (15), 69 (14), 43 (9); EIHRMS: 396.1555 ($[M]^{+\cdot}$, $C_{23}H_{24}O_6^{+\cdot}$; calcd 396.1573).

1,3,6,8-Tetrahydroxy-2-(3-methylbut-2-enyl)-9H-xanthen-9-one (8)

4% (46.9 mg) as yellow crystals, mp 184-186 °C; UV λ_{max} nm (EtOH) (log ε): 203, 253, 334; IR υ_{max} (KBr) cm^{-1} : 3436, 2925, 2855, 1630, 1618, 1612, 1467, 1299, 1280, 1188, 1168, 1073, 816, 762; ^{1}H NMR (400 MHz, acetone-d_6): 12.22 (H, s, 1-OH), 12.03 (H, s, 8-OH), 6.43 (1H, s, H-4), 6.33 (1H, s, H-5), 6.21 (1H, s, H-7), 5.24 (1H, t, *J* = 7.6 Hz, H-12), 3.31 (2H, d, *J* = 7.6 Hz, H-11), 1.75 (3H, s, H-14), 1.62 (3H, s, H-15); ^{13}C NMR (100 MHz, acetone-d_6): 183.2 (C-9), 165.6 (C-6), 163.3 (C-3), 163.1 (C-8), 159.8 (C-1), 157.8 (C-10a), 155.6 (C-4a), 130.9 (C-13), 122.2 (C-12), 111.0 (C-2), 101.2 (C-8a), 101.0 (C-9a), 98.4 (C-7), 94.1 (C-5), 93.6 (C-4), 25.1 (C-15), 21.1 (C-11), 17.1 (C-14); EIMS *m/z* (rel. int.): 328 ($[M]^{+\cdot}$ 42), 313 (29), 285 (68), 273 (100), 260 (10), 153 (7), 69 (7).

1,3,6,8-Tetrahydroxy-4-(3-methylbut-2-enyl)-9H-xanthen-9-one (9)

5% (60.4 mg) as yellow crystals, mp 204-205 °C; UV λ_{max} nm (EtOH) (log ε): 212, 234, 254, 333; IR υ_{max} (KBr) cm^{-1} : 3370, 2967, 2928, 1654, 1630, 1618, 1511, 1305, 1271, 1163, 1096, 825, 750; ^{1}H NMR (500 MHz, acetone-d_6): 12.13 (H, s, 1-OH), 12.01 (H, s, 8-OH), 6.46 (1H, d, *J* = 2.2 Hz, H-5), 6.34 (1H, s, H-2), 6.25 (1H, d, *J* = 2.2 Hz, H-7), 5.27 (1H, t, *J* = 7.2 Hz, H-12), 3.47 (2H, d, *J* = 7.2 Hz, H-11), 1.86 (3H, s, H-14), 1.66 (3H, s, H-15); ^{13}C NMR (125 MHz, acetone-d_6): 183.4 (C-9), 165.7 (C-6), 163.2 (C-1), 163.1 (C-8), 160.7 (C-3), 157.9 (C-10a), 154.8 (C-4a), 131.0 (C-13), 122.2 (C-12), 106.9 (C-4), 101.2 (C-8a), 100.9 (C-9a), 98.3 (C-7), 98.0 (C-2), 94.2 (C-5), 24.9 (C-15), 21.3 (C-11), 17.1 (C-14); EIMS *m/z* (rel. int.): 328 ($[M]^{+\cdot}$ 70), 313 (100), 273 (49), 260 (47), 191 (30), 167 (13), 149 (45), 69 (19), 57 (23), 43 (34).

Stock Solution and Cell Culture

Stock solution of the target compounds was prepared at concentration of 10 mg/mL in absolute ethanol. The human cancer cell lines HeLa (cervical carcinoma) and MDA-MB-231 (human estrogen receptor negative breast cancer) were obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in RPMI-1640 with 5% Fetal Bovine Serum (FBS), 100 IU mL^{-1} penicillin and 100g/mL streptomycin by using 25 mL flask in a 37 °C incubator with 5% CO_2.

Cytotoxic Assay

Cytotoxic activities of the test compounds **1**-**9** against human HeLa and MDA-MB-231 cancer cell lines were evaluated using the protocol described in the literature by Rahmat *et al.* [17] with some modifications. The exponential growth HeLa and MDA-MB-231 cells were seeded in 96-well plate at concentration of 7500 and 35000 cells per well, respectively in RPMI 1460 medium. After being incubated for 24 hours at 37 °C under a 5% CO_2 atmosphere, cells were treated with six concentrations of tested compounds at 1.6, 3.1, 6.3, 12.5, 25.0 and 50.0 μg/mL and incubated for another 72 hours. Then, 20 μL of MTT stock solution (5 mg in 1 mL PBS) was added to each well and the plate was further incubated for 3 hours at 37 °C. 100 μL of ethanol was added to each well and after 30 minutes, the absorbance (OD) of the samples and the reference were measured by using ELISA spectrophotometer microplate reader at wavelength 550 nm. The concentration of substance required for 50% growth inhibition, IC_{50} was determined from the absorbance (OD) against concentration curve. Anticancer drugs, doxorubicin and cisplatin, purchased from Calbiochem were used as positive controls.

CONCLUSION

In summary, a series of xanthone derivatives with typical or modified nucleus have been synthesized and most of them were found to display significant inhibitory activity against HeLa and MDA-MB-231 cancer cell lines. It is notable that the presence of chroman-4-one and prenyl moieties is essential for cytotoxicity of xanthones. In particular, compounds **3** and **4** bearing a novel skeleton were found to show comparable activities with the standard drugs used in the assay, and this can provide useful information for synthesizing new xanthone derivatives with enhanced activity in the future.

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REFERENCES

[1] Lee, B.W.; Lee, J.H.; Lee, S.T.; Lee, H.S.; Lee, W.S.; Jeong, T.S.; Park, K.H. Antioxidant and cytotoxic activities of xanthones from *Cudrania tricuspidata*. *Bioorg. Med. Chem. Lett.,* **2005**, *15*, 5548-5552.

[2] Mahabusarakam, W.; Rattanaburi, S.; Phongpaichit, S.; Kanjana-Opas, A. Antibacterial and cytotoxic xanthones from *Cratoxylum cochinchinense*. *Phytochem. Lett*., **2008**, *1*, 211-214.

[3] Dua, V.K.; Ojha, V.P.; Roy, R.; Joshi, B.C.; Valecha, N.; Devi, C.U.; Bhatnagar, M.C.; Sharma, V.P.; Subbarao, S.K. Anti-malarial activity of some xanthones isolated from the roots of *Andrographis paniculata*. *J. Ethnopharm.*, **2004**, *95*, 247-251.

[4] Woo, S.; Jung, J.; Lee, C.; Kwon, Y.; Na, Y. Synthesis of new xanthone analogues and their biological activity test—Cytotoxicity, topoisomerase II inhibition, and DNA cross-linking study. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1163-1166.

[5] Boonnak, N.; Karalai, C.; Chantrapromma, S.; Ponglimanont, C.; Fun, H.K.; Kanjana-Opas, A.; Chantrapromma, K.; Kato, S. Anti-*Pseudomonas aeruginosa* xanthones from the resin and green fruits of *Cratoxylum cochinchinense*. *Tetrahedron*, **2009**, *65*, 3003-3013.

[6] Zhang, Y.; Song, Z.; Hao, J.; Qiu, S.; Xu, Z. Two new prenylated xanthones and a new prenylated tetrahydroxanthone from the pericarp of *Garcinia mangostana*. *Fitoterapia*, **2010**, *81*, 595-599.

[7] Boonnak, N.; Karalai, C.; Chantrapromma, S.; Ponglimanont, C.; Fun, H.K.; Kanjana-Opas, A.; Laphookhieo, S. Bioactive prenylated xanthones and anthraquinones from *Cratoxylum formosum* ssp. *pruniflorum*. *Tetrahedron*, **2006**, *62*, 8850-8859.

[8] Han, Q.B.; Yang, N.Y.; Tian, H.L.; Qiao, C.F.; Song, J.Z.; Chang, D.C.; Chen, S.L.; Luo, K.Q.; Xu, H.X. Xanthones with growth inhibition against HeLa cells from *Garcinia xipshuanbannaensis*. *Phytochemistry*, **2008**, 69, 2187-2192.

[9] Shadid, K.A.; Shaari, K.; Abas, F.; Israf, D.A.; Hamzah, A.S.; Syakroni, N.; Saha, K.; Lajis, N.H. Cytotoxic caged-polyprenylated xanthonoids and a xanthone from *Garcinia cantleyana*. *Phytochemistry*, **2007**, *68*, 2537-2544.

[10] Castanheiro, R.A.P.; Pinto, M.M.M.; Silva, A.M.S.; Cravo, S.M.M.; , Gales, L.; Damas, A.M.; Nazareth, N.; Nascimento, M.S.J.; Eaton, G. Dihydroxyxanthones prenylated derivatives: Synthesis, structure elucidation, and growth inhibitory activity on human tumor cell lines with improvement of selectivity for MCF-7. *Bioorg. Med. Chem.*, **2007**, *15*, 6080-6088.

[11] Li, N.G.; You, Q.D.; Huang, X.F.; Wang, J.X.; Guo, Q.L.; Chen, X.G.; Li, Y.; Li, H.Y. Synthesis and antitumor activities of structure-related small molecular compounds of gambogic acid. *Chin. Chem. Lett.*, **2007**, *18*, 659-662.

[12] Li, N.G.; You, Q.D.; Huang, X.F.; Wang, J.X.; Guo, Q.L.; Chen, X.G.; Li, Y.; Li, H.Y. Structure-activity Relationship of Gambogic Acid. *Chin. J. Nat. Med.*, **2008**, *6*, 37- 42.

[13] Liu, Y.; Zou, L.; Ma, L.; Chen, W.H.; Wang, B.; Xu, Z.L. Synthesis and pharmacological activities of xanthone derivatives as α-glucosidase inhibitors. *Bioorg. Med. Chem.*, **2006**, *14*, 5683-5690.

[14] Varache-Lembege, M.; Moreau, S.; Larrouture, S.; Montaudon, D. ; Robert, J. ; Nuhrich, A. Synthesis and antiproliferative activity of aryl- and heteroaryl-hydrazones derived from xanthone carbaldehydes. *Eur. J. Med. Chem.*, **2008**, *43*, 1336-1343.

[15] Helesbeux, J.-J. ; Duval, O.; Dartiguelongue, C. ; Séraphin, D ; Oger, J.-M.; Richomme, P. Synthesis of 2-hydroxy-3-methylbut-3-enyl substituted coumarins and xanthones as natural products. Application of the Schenck ene reaction of singlet oxygen with *ortho*-prenylphenol precursors. *Tetrahedron*, **2004**, *60*, 2293-2300.

[16] George, J.H.; Hesse, M.D.; Baldwin, J.E.; Adlington, R.M. Biomimetic synthesis of polycyclic polyprenylated acylphloroglucinol natural products isolated from *Hypericum papuanum*. *Org. Lett.*, **2010**, *12* (15), 3532-3535.

[17] Rahmat, A.; Rosli, R.; Zain, W.N.I.W.M.; Endrini, S.; Sani, H.A. Antiproliferative activity of pure lycopene compared to both extracted lycopene and juices from watermelon (*Citrullus vulgaris*) and papaya (*Carica papaya*) on human breast and liver cancer cell lines. *J. Med. Sci.*, **2002**, *2*, 55-58.