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Structure-Activity Relationship of Xanthones from *Mesua daphnifolia* and *Garcinia nitida* towards Human Estrogen Receptor Negative Breast Cancer Cell Line

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Abstract – Extensive chemical studies on the stem bark extracts of two Guttifereous plants namely *Mesua daphnifolia* and *Garcinia nitida* have led to the isolation of eight xanthones. *Mesua daphnifolia* gave cudraxanthone G (**1**), ananixanthone (**2**), 1,3,5-trihydroxy-4-methoxyxanthone (**3**) and euxanthone (**4**) while *Garcinia nitida* gave inophyllin B (**5**), 1,3,7-trihydroxy-2,4-*bis* (3-methylbut-2-enyl)xanthone (**6**), 3-isomangostin (**7**) and rubraxanthone (**8**). All these compounds were assayed against the MDA-MB-231 (human estrogen receptor negative breast cancer) cells. A structure-activity relationship study showed that structurally, all the 1, 3-oxygenated xanthones which carried unsaturated prenyl side chains (either 3-methylbut-2-enyl or 1,1-dimethyl-2-propenyl) at carbons C-2 and C-4 in the xanthone ring A are essential for the outstanding activities in the assay.
Keywords – Xanthones, *Mesua daphnifolia*, *Garcinia nitida*, Cytotoxic activities, MDA-MB-231

Introduction

Xanthones are polyphenolic secondary metabolites which are found to be widely distributed in higher plant families such as Clusiaceae, Gentianaceae, Guttiferae and Polygalaceae. Previous studies on xanthones have revealed it to have a wide range of pharmacological activities such as antitumour, cytotoxic, antimicrobial, antiviral, antiulcer, anti-inflammatory, antihepatotoxic, antifungal, antioxidant and nerve growth factor potentiating activities (Chanmahasathien *et al.*, 2003; Minami *et al.*, 1994; Peres *et al.*, 2000). Guttiferae species have been reported to produce a wide variety of simple and prenylated xanthones (Bennett *et al.*, 1989). Our recent study on two Guttiferous plants, namely *Mesua daphnifolia* and *Garcinia nitida* have successfully led to the isolation of five prenylated xanthones (**1**, **2**, **5-7**), one geranylated xanthone (**8**) and two simple oxygenated xanthones (**3**, **4**). These compounds were subjected to cytotoxic assay towards the MDA-MB-231 cell line. So far, there is little or no information available on pharmacological activities of xanthones (**1-8**). This paper reports the isolation and structure-activity relationships of the compounds.

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Experimental

Plant Material – The stem bark of *Mesua daphnifolia* and *Garcinia nitida* were collected from Pahang and Sarawak, Malaysia. The plant materials were identified by Mr. Shamsul from the Institute of Bioscience, Universiti Putra Malaysia and Miss Runi Sylvester from the Herbarium of Sarawak Forestry Department, Kuching, Sarawak.

General – Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500MHz NMR/JEOL 400 MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard. Ultra violet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.

Extraction and Isolation – The air-dried and powdered stem bark of *Mesua daphnifolia* (2.0 kg) was extracted successively with n-hexane, chloroform and acetone at room temperature. The extracts were evaporated to dryness under reduced pressure to yield 53.7 g of crude n-hexane extract, 60.3 g of crude chloroform extract and 150.3 g of crude acetone extract. Meanwhile, solvent

extractions on the powdered stem bark of *Garcinia nitida* (2.0 kg) yielded 102.0 g of crude n-hexane extract, 101.5 g of crude chloroform extract and 85.3 g of crude acetone extract. The crude n-hexane extract of *Mesua daphnifolia* (16.0 g) was chromatographed on a silica gel column using a stepwise gradient system (hexane/$CHCl_3$ and $CHCl_3$/Me_2CO) to give 50 fractions (*Frs.*). *Frs. 23-26* were combined and purified by CC (SiO_2; n-hexane/$CHCl_3$ gradient) to furnish 20 subfractions. Subfractions 9-10 were combined and further purified by CC (*Sephadex LH-20*; MeOH) to afford cudraxanthone G (**1**) (10 mg). *Frs. 31-32* were combined and subjected to CC (*Sephadex LH-20*; MeOH) to yield ananixanthone (**2**) (12 mg). Fractionation of crude acetone extract of *Mesua daphnifolia* (16.0 g) over a silica gel column (n-hexane/$CHCl_3$, $CHCl_3$/EtOAc and EtOAc/MeOH gradient) provided 40 fractions. *Frs. 8-10* were combined and further purified by silica gel column eluted with the same solvent system as above to give euxanthone (**3**) (8 mg). *Frs. 14-15* were combined and separated over a silica gel column (n-hexane/$CHCl_3$ and $CHCl_3$/Me_2CO gradient) to give daphnifolin (**4**) (5 mg). On the other hand, vacuum CC (SiO_2; n-hexane/$CHCl_3$, $CHCl_3$/Me_2CO and Me_2CO/MeOH gradient) on the crude n-hexane extract of *Garcinia nitida* (20.0 g) afforded 25 fractions. *Fr. 11* was purified by repeated CC (SiO_2; n-hexane/EtOAc and $CHCl_3$/Me_2CO gradient) to give inophyllin B (8 mg) (**5**). The crude chloroform extract of *Garcinia nitida* (20.0 g) was fractionated by vacuum CC (SiO_2; n-hexane-EtOAc and $CHCl_3$-Me_2CO gradient) to yield 15 fractions. *Frs. 2-3* were combined and purified by repeated CC (SiO_2; n-hexane/EtOAc and $CHCl_3$/Me_2CO gradient) and finally by CC (*Sephadex LH-20*; MeOH) to yield 1,3,7-trihydroxy-2,4-*bis* (3-methylbut-2-enyl) xanthone (**6**). The crude acetone extract of *Garcinia nitida* (20.0 g) was subjected to CC (SiO_2; n-hexane-$CHCl_3$, $CHCl_3$-EtOAc and EtOAc-MeOH gradient) to give 25 fractions. *Frs. 11* afforded rubraxanthone (**8**). *Fr. 8* was rechromatographed on a silica gel column (n-hexane-$CHCl_3$, $CHCl_3$-EtOAc and EtOAc-MeOH gradient) to give 20 subfractions. Subfraction 9 yielded 3-isomangostin (**7**).

Cudraxanthone G (**1**) – Yellow needle, mp 130-132°C. UV (EtOH) λ_{max} nm (log ε): 215.0 (1.00), 259.0 (1.58), 315.0 (0.47), 379.5 (0.47). IR ν_{max} cm^{-1} (NaCl): 3184, 2968, 1636, 1562, 1126. EI-MS m/z (rel. int.): 394 (37), 351 (100), 339 (90), 323 (29), 295 (23), 281 (18), 269 (40), 69 (13), 43 (14), 41 (36). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.90 (1H, s, 1-OH), 7.78 (1H, d, J = 9.2 Hz, H-8), 7.32 (1H, d, J = 9.2 Hz, H-6), 7.26 (1H, t, J = 9.2 Hz, H-7), 5.75 (1H, s, 5-OH), 5.27 (2H, t, J = 6.4 Hz, H-12 & H-17), 3.81 (3H, s, 3-OCH_3), 3.58 (2H, d, J = 6.4 Hz, H-16), 3.43 (2H, d, J = 6.4 Hz, H-11), 1.89 (3H, s, H-20), 1.82 (3H, s, H-15), 1.75 (3H, s, H-19), 1.71 (3H, s, H-14). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.7 (C-9), 163.6 (C-3), 159.2 (C-1), 152.5 (C-4a), 144.6 (C-5), 144.4 (C-10a), 132.1 (C-13), 132.1 (C-18), 124.0 (C-7), 123.4 (C-17), 122.3 (C-12), 120.8 (C-9a), 120.0 (C-6), 119.8 (C-8a), 117.8 (C-2), 116.8 (C-8), 112.5 (C-4), 62.1 (3-OCH_3), 25.7 (C-14), 25.5 (C-19), 23.1 (C-16), 22.5 (C-11), 18.0 (C-20), 17.9 (C-15).

Ananixanthone (**2**) – Yellow needle, mp 172-173°C. UV (EtOH) λ_{max} nm (log ε): 253.5 (1.04), 272.0 (0.98), 331.5 (0.38), 383.5 (0.09). IR ν_{max} cm^{-1} (KBr): 3382, 2920, 1716, 1578. EI-MS m/z (rel. int.): 378 (37), 364 (23), 363 (100), 336 (26), 323 (31), 307 (29). ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.23 (1H, s, 1-OH), 7.81 (1H, d, J = 7.5 Hz, H-8), 7.33 (1H, d, J = 7.5 Hz, H-6), 7.26 (1H, t, J = 7.5 Hz, H-7), 6.81 (1H, d, J = 10.0 Hz, H-11), 5.68 (1H, s, 5-OH), 5.67 (1H, d, J = 10.0 Hz, H-12), 5.28 (1H, t, J = 7.5 Hz, H-17), 3.39 (2H, d, J = 7.5 Hz, H-16), 1.85 (3H, s, H-19), 1.72 (3H, s, H-20), 1.52 (3H, s, H-14), 1.52 (3H, s, H-15). ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.0 (C-9), 160 (C-1), 158.9 (C-3), 149.5 (C-4a), 144.5 (C-5), 144.3 (C-10a), 132.0 (C-18), 127.7 (C-12), 124.2 (C-7), 122.1 (C-17), 121.4 (C-8a), 120.4 (C-6), 117.4 (C-8), 115.2 (C-11), 112.5 (C-2), 103.4 (C-9a), 100.9 (C-4), 78.4 (C-13), 28.4 (C-14), 28.3 (C-15), 26.1 (C-20), 21.5 (C-16), 18.2 (C-19).

Euxanthone (**3**) – Yellow needle, mp 237-238°C. UV (EtOH) λ_{max} nm (log ε): 205.0 (0.44), 235.5 (0.84), 260.0 (1.05), 286.5 (0.21), 386.5 (0.21). IR ν_{max} cm^{-1} (KBr): 3302, 2924, 1638, 1478, 1232. EI-MS m/z (rel. int.): 228 (100), 200 (17), 171 (4), 144 (7), 136 (3), 115 (18), 107 (7), 89 (6), 72 (5), 63 (23), 53 (4), 43 (5). ^{1}H NMR (400 MHz, acetone-d_6): δ 12.71 (1H, s, 1-OH), 9.25 (1H, s, 7-OH), 7.68 (1H, t, J = 8.2 Hz, H-3), 7.57 (1H, d, J = 2.8 Hz, H-8), 7.50 (1H, d, J = 9.2 Hz, H-5), 7.41 (1H, dd, J = 9.2, 2.8 Hz, H-6), 6.97 (1H, d, J = 8.2 Hz, H-4), 6.75 (1H, d, J = 8.2 Hz, H-2). ^{13}C NMR (100 MHz, acetone-d_6): δ 182.2 (C-9), 161.9 (C-1), 156.5 (C-4a), 154.3 (C-7), 150.2 (C-10a), 137.0 (C-3), 125.4 (C-6), 121.0 (C-8a), 119.4 (C-5), 109.7 (C-9a), 109.7 (C-2), 108.3 (C-8), 107.0 (C-4).

Daphnifolin (**4**) – Yellow solid, mp 240-242°C. UV (EtOH) λ_{max} nm (log ε): 207.0 (0.65), 222.0 (0.69), 244.5 (0.90), 313.0 (0.49). IR ν_{max} cm^{-1} (KBr): 3470, 2948, 1658, 1582, 1218. EI-MS m/z (rel. int.): 274 (100), 259 (90), 231 (66), 202 (14), 136 (23), 51 (15). ^{1}H NMR (400MHz, acetone-d_6): δ 13.09 (1H, s, 1-OH), 7.63 (1H, dd, J = 7.4,1.8 Hz, H-8), 7.30 (1H, dd, J = 7.4, 1.8 Hz, H-

6), 7.24 (1H, t, J = 7.4 Hz, H-7), 6.51 (1H, s, H-2), 3.83 (3H, s, 4-OCH_3). ^{13}C NMR (100 MHz, acetone-d_6): δ 182.1 (C-9), 159.4 (C-1), 155.3 (C-4a), 153.8 (C-3), 146.9 (C-5), 146.1 (C-10a), 131.5 (C-4), 124.8 (C-7), 121.7 (C-8a), 121.3 (C-6), 116.0 (C-8), 103.9 (C-9a), 94.8 (C-2), 60.7 (4-OCH_3).

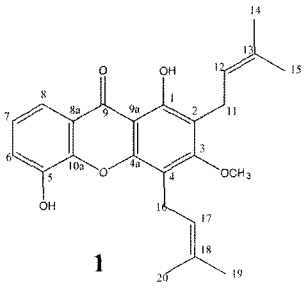
Inophyllin B (**5**) – Yellow needle, mp 174-175°C. UV (EtOH) λ_{max} nm (log ε): 213.0 (0.89), 241.0 (0.98), 281.0 (1.99), 336.5 (0.93). IR ν_{max} cm^{-1} (KBr): 3444, 2968, 1632, 1464, 1290, 1264, 1186, 1128. EI-MS m/z (rel. int.): 394 (100), 380 (93), 365 (30), 353 (42), 339 (10), 325 (9), 309 (8), 182 (32), 168 (12), 162 (42), 153 (10), 139 (8), 115 (9), 53 (9), 41 (9). ^1H NMR (400 MHz, acetone-d_6): δ 13.85 (1H, s, 1-OH), 7.50 (1H, d, J = 8.2, H-8), 6.79 (1H, d, J = 8.2 Hz, H-7), 6.64 (1H, d, J = 10.1 Hz, H-11), 6.52 (1H, dd, J = 18.3, 11.0 Hz, H-19), 5.51 (1H, d, J = 10.1 Hz, H-12), 5.04 (1H, d, J = 18.3 Hz, H_a-20), 4.89 (1H, d, J = 11.0 Hz, H_b-20), 1.56 (3H, s, H-17), 1.56 (3H, s, H-18), 1.39 (3H,s, H-14), 1.39 (3H,s, H-15). ^{13}C NMR (100 MHz, acetone-d_6): δ 180.1 (C-9), 157.8 (C-3), 155.6 (C-1), 154.3 (C-4a), 151.0 (C-19), 150.1 (C-6), 145.0 (C-10a), 132.0 (C-5), 126.6 (C-12), 115.4 (C-8), 114.6 (C-11), 112.6 (C-2), 112.5 (C-8a), 112.0 (C-7), 105.6 (C-20), 103.9 (C-4), 101.9 (C-9a), 77.3 (C-13), 40.1 (C-16), 28.2 (C-17), 28.2 (C-18), 26.2 (C-14), 26.2 (C-15).

1,3,7-trihydroxy-2,4-*bis*(3-methylbut-2-enyl)xanthone (**6**) – Yellow needle, mp 128-129°C. UV (EtOH) λ_{max} nm (log ε): 217.5 (0.85), 234.0 (1.14), 270.0 (1.23), 285.0 (1.07), 386.5 (0.23). IR ν_{max} cm^{-1} (KBr): 3378, 1644, 1480, 1230. EI-MS m/z (rel. int.): 380 (39), 363 (34), 337 (22), 325 (31), 309 (68), 295 (18), 281 (62), 269 (100), 257 (9), 41 (11). ^1H NMR (400 MHz, acetone-d_6): δ 13.25 (1H, s, 1-OH), 7.52 (1H, d, J = 3.7 Hz, H-8), 7.40 (1H, d, J = 9.2 Hz, H-5), 7.29 (1H, dd, J = 9.2, 3.7 Hz, H-6), 5.20 (2H, t, J = 7.3 Hz, H-12 & H-17), 3.53 (2H, d, J = 7.3 Hz, H-16), 3.39 (2H, d, J = 7.3 Hz, H-11), 1.83 (3H, s, H-20), 1.74 (3H, s, H-15), 1.60 (6H, s, H-14 & H-19). ^{13}C NMR (100 MHz, acetone-d_6): δ 181.6 (C-9), 161.1 (C-3), 159.2 (C-1), 154.6 (C-7), 153.9 (C-4a), 150.8 (C-10a), 132.5 (C-13), 132.3 (C-18), 125.0 (C-6), 123.0 (C-12), 123.0 (C-17), 121.6 (C-8a), 119.8 (C-5), 110.8 (C-2), 109.2 (C-8), 106.7 (C-4), 103.6 (C-9a), 25.8 (C-14), 25.8 (C-19), 22.4 (C-16), 22.1 (C-11), 18.1 (C-20), 18.0 (C-15).

3-Isomangostin (**7**) – Yellow solid, mp 154-156°C. UV (EtOH) λ_{max} nm (log ε): 212.5 (1.10), 242.5 (1.04), 289.5 (1.42). IR ν_{max} cm^{-1} (KBr): 3440, 1602, 1464, 1286. EI-MS m/z (rel. int.): 408 (43), 393 (100), 365 (53), 335 (27), 295 (18), 201 (11), 175 (20), 115 (26), 69 (10). ^1H NMR (300 MHz, $CDCl_3$): δ 13.72 (1H, s, 1-OH), 6.85 (1H, s, H-5), 6.75 (1H, d, J = 9.9 Hz, H-4'), 6.26 (1H, s, H-4), 5.59 (1H, d, J = 9.9 Hz, H-5'), 5.27 (1H, t, J = 5.1 Hz, H-5"), 4.10 (2H, d, J = 5.1 Hz, H-4"), 3.82 (3H, s, 7-OCH_3), 1.85 (3H, s, H-7"), 1.71 (3H, s, H-8"), 1.49 (3H, s, H-7'), 1.49 (3H, s, H-8'). ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.2 (C-9), 159.9 (C-3), 157.9 (C-1), 156.3 (C-4a), 155.7 (C-10a), 154.6 (C-6), 142.6 (C-7), 137.0 (C-8), 132.2 (C-6"), 127.2 (C-5'), 123.1 (C-5"), 115.7 (C-4'), 112.1 (C-8a), 104.5 (C-2), 103.7 (C-9a), 101.7 (C-5), 94.2 (C-4), 77.9 (C-6'), 62.1 (7-OCH_3), 28.3 (C-7'), 28.3 (C-8'), 26.5 (C-4"), 25.9 (C-8"), 18.3 (C-7").

Rubraxanthone (**8**) – Orange crystals, mp 201-202°C. UV (EtOH) λ_{max} nm (log ε): 211.0 (0.97), 241.5 (1.12), 312.0 (0.76). IR ν_{max} cm^{-1} (KBr): 3428, 1608, 1466, 1162. EI-MS m/z (rel. int.): 410 (16), 341 (100), 311 (24), 299 (30), 288 (14), 271 (10), 69 (29), 41 (39). ^1H NMR (400 MHz, acetone-d_6): δ 13.48 (1H, s, 1-OH), 6.82 (1H, s, H-5), 6.28 (1H, d, J = 1.8 Hz, H-4), 6.17 (1H, d, J = 1.8 Hz, H-2), 5.26 (1H, t, J = 6.4 Hz, H-12), 5.02 (1H, t, J = 6.9 Hz, H-16), 4.09 (2H, d, J = 6.4 Hz, H-11), 3.78 (3H, s, 7-OCH_3), 2.03 (2H, m, H-15), 1.96 (2H, m, H-14), 1.81 (3H, s, H-18), 1.54 (3H, s, H-19), 1.50 (3H, s, H-20). ^{13}C NMR (100 MHz, acetone-d_6): δ 182.7 (C-9), 165.3 (C-3), 164.8 (C-1), 157.9 (C-6), 157.5 (C-10a), 156.2 (C-4a), 144.5 (C-7), 138.2 (C-8), 135.1 (C-13), 131.5 (C-17), 125.1 (C-16), 124.7 (C-12), 111.9 (C-8a), 103.7 (C-9a), 102.8 (C-5), 98.7 (C-2), 93.8 (C-4), 61.4 (7-OCH_3), 40.4 (C-14), 27.2 (C-11), 26.7 (C-15), 25.7 (C-19), 17.6 (C-20), 16.5 (C-18).

Cytotoxicity Assay – The MDA-MB-231 cell line (human estrogen receptor negative breast cancer) was obtained from American Type Culture Collection, USA. The cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5% Fetal Bovine Serum (FBS), 100 $IUml^{-1}$ penicillin and 100 g/ml streptomycin by using 25 cm^2 flask in a 37°C incubator with 5% CO_2. The stock solution was prepared at a concentration of 1 mg/ml by dissolving 1 mg of sample (compound) in 1 ml of dimethylsulfoxide (DMSO). Serial dilution of the stock solution in the growth medium provided seven sample solutions at concentrations of 2.5, 5.0, 7.5, 10.0, 20.0, 30.0 and 40.0 μg/ml. Cells were grown in a 96 well microliter plate by filling each well with 100 μl of stock culture (1×10^5 cells/ml) and incubated at 37°C for 24 hours. Growth medium was removed from the wells and each well was then treated with 100 μl of varying concentrations of sample solution. Controls were made containing only untreated cell population in 100 μl of growth medium. The assay for each concentration of sample was performed in triplicate and the culture plate was incubated for 3 days at 37°C, 5% CO_2 and 90%



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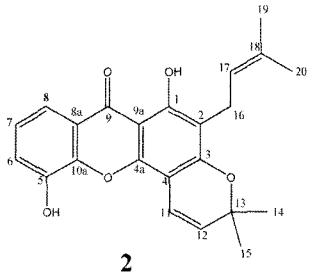

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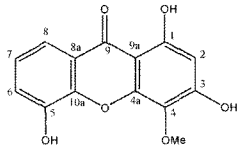

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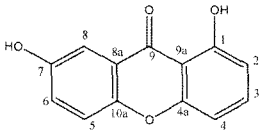

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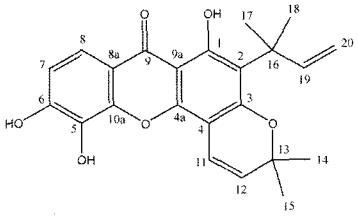

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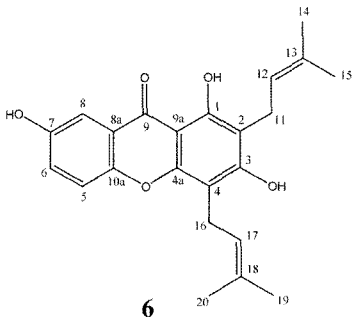

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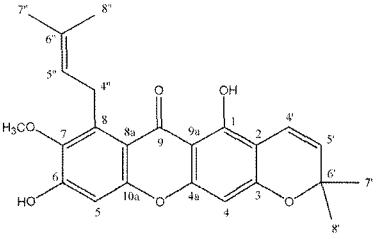

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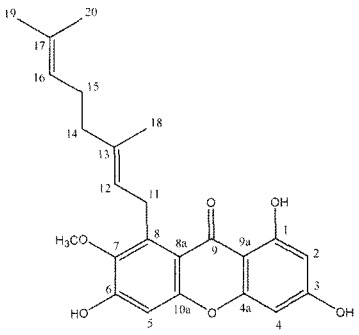

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humidity. After 3 days, 10 μl of the MTT labeling reagent (0.5 mg/ml) (Roche Diagnostics, USA) was added to each well. The plate was then incubated for a further 4 hours at 37°C with 5% CO_2. After that, 100 μl of the solubilization solution was added to each well and the plate was allowed to stand overnight in the incubator at 37°C with 5% CO_2. Cell viability was measured using ELISA spectrophoto-meter (EL_x 800) at a wavelength of 550 nm (Rahmat *et al.*, 2002).

Results and Discussion

Xanthones are unique compounds with a symmetrical xanthone nucleus. A series of substitutions by various groups such as hydroxyl, methoxyl, prenyl and geranyl to the xanthone nucleus has led to the existence of a great variety of xanthones. This huge variety of structures in xanthones offers a good opportunity for studying their structure-activity relationship since many of these compounds were found to show various bioactivities in previous pharmacological studies. In this study, biological results were obtained in terms of inhibitory activity towards the MDA-MB-231 cell line. All xanthones tested produced a cytotoxic effect on the cell line with IC_{50} values ranging between 1.0 and 40.0 μg/ml. However, these cytotoxic results obtained showed significant differences in inhibitory activity between two groups of xanthone, prenylated xanthones (**1**, **2**, **5-7**) and the simple oxygenated xanthones (**3**, **4**). The screening results of these compounds are summarized in Table 1.

A structure-activity relationship study was carried out. Of the compounds tested, cudraxanthone G (**1**) exhibited the strongest inhibitory activity against the cell line with IC_{50} value of 1.3 μg/ml. Structurally, it was found that compounds **1** and **6** with 1,3-dioxygenated and 2,4-*bis*(3-methylbut-2-enyl) groups in xanthone ring A gave strong cytotoxic activities with IC_{50} values of 1.3 and 2.2 μg/ml,

Table 1. Cytotoxic activities of xanthones **1-8** against MDA-MB-231 cell line.

Compound	IC_{50} (μg/ml)
1	3.3×10^{-3}
2	1.2×10^{-2}
3	1.6×10^{-1}
4	1.2×10^{-1}
5	3.6×10^{-3}
6	5.7×10^{-3}
7	1.8×10^{-2}
8	3.3×10^{-2}

respectively. Cyclization of one of the two units of 3-methylbut-2-enyl side chains at C-4 in xanthone ring A give rise to compound **2** which led to a decrease in activity (IC_{50} = 4.6 μg/ml). The removal of both the prenyl substituents from carbons C-2 and C-4 which is shown by compounds **3** and **4** had led to an almost complete loss of inhibitory activity (IC_{50} > 30.0 μg/ml). Besides that, comparison of activity was also made between the two types of prenyl groups, 3-methylbut-2-enyl and 1,1-dimethyl-2-propenyl groups. It was found that pyranoxanthone **5** which carried a 1,1-dimethyl-2-propenyl group at C-2 indicated stronger inhibitory activity (IC_{50} = 1.4 μg/ml) if compared to that of pyranoxanthone **2** (IC_{50} = 4.6 μg/ml) which carried a 3-methylbut-2-enyl group at C-2. Therefore, it is suggested that xanthone with 1,3-dioxygenated and 2,4-*bis* (1,1-dimethyl-2-propenyl) groups in the ring A tend to exhibit a more prominent inhibitory activity than that of compound **1** which gave the strongest activity in the assay. It was also found that the potent inhibitory activity shown by these xanthones was not much affected by the location of hydroxyl groups either at C-5 or C-7 in xanthone ring B as shown by compound **1** and **6**. Compound **8** which is without the presence of prenyl groups at C-2 and C-4 but with a geranyl group at C-5 gave only a moderate cytotoxic effect (IC_{50} = 13.6 μg/ml).

In summary, all 1, 3-oxygenated xanthones which carried unsaturated prenyl side chains (either 3-methylbut-2-enyl or 1,1-dimethyl-2-propenyl) at carbons C-2 and C-4 in the ring A are essential for the outstanding activities in the suppression of proliferation of the MDA-MB-231 cell line. Therefore, it was concluded that xanthones with 1,3-dioxygenated and 2, 4-prenylated skeleton might be a lead compound for the MDA-MB-231 cell line.

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