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Synthesis and anticancer potential of novel xanthone derivatives with 3,6-substituted chains

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Abstract

In an effort to develop new drug candidates with enhanced anticancer activity, our team synthesized and assessed the cytotoxicity of a series of novel xanthone derivatives with two longer 3,6-disubstituted amine carbonyl methoxy side chains on either benzene ring in selected human cancer cell lines. An MTT assay revealed that a set of compounds with lower IC_{50} values than the positive control, **5-FU**, exhibited greater anticancer effects. The most potent derivative (**XD8**) exhibited anticancer activity in MDA-MB-231, PC-3, A549, AsPC-1, and HCT116 cells lines with IC_{50} values of 8.06, 6.18, 4.59, 4.76, and 6.09 μM, respectively. Cell cycle analysis and apoptosis activation suggested that the mechanism of action of these derivatives includes cell cycle regulation and apoptosis induction.

1. Introduction

With their diverse structures and bioactivity, natural products (NP) have been an exceptional source for drug discovery. Of the drugs in use today, more than 50% of them developed over the past 30 years are from NP or their structural scaffolds.[1, 2] For anticancer drugs, 74.8% of these drugs approved worldwide between 1940 and 2010 owe their origins to NP. Anticancer agents derived from plants and their derivatives have been proven to be effective for cancer prevention and therapeutics.[3] More recently, considerable attention has been given to NP due to the increased understanding of their broad spectrum of antitumor activity.[1, 4]

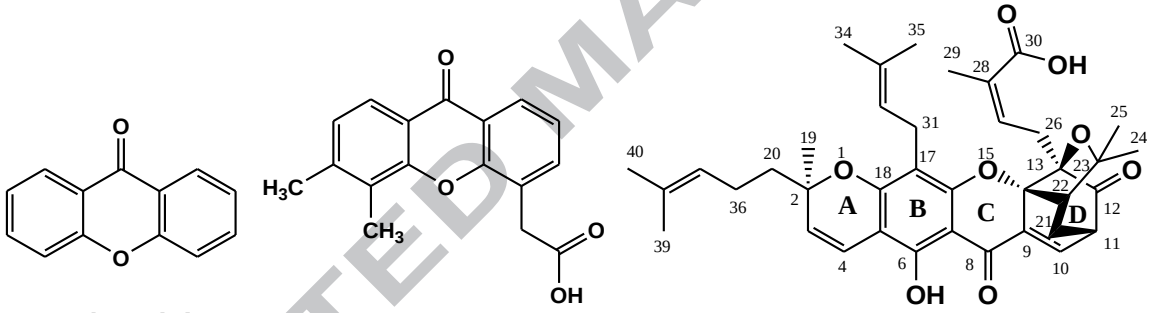


Fig.1. Structures of xanthone skeleton, DMXAA and gambogic acid

Xanthone (9*H*-xanthen-9-one or 9-xanthone) has a planar and oxygenated tricyclic skeleton in which one pyranone ring is fused with two benzene rings on both sides (Fig. 1). Natural xanthones are mainly isolated from higher plants, fungi, and lichens as secondary metabolites. They have diverse biological profiles, including antioxidative,[5, 6] anticancer,[7-13] antibacterial, antifungal,[14, 15] anti-inflammatory,[16] and antihypertensive activities,[17] depending on their diverse structures modified by substituents on the ring

system.[18] Their interesting structural scaffolds and significant biological activities have prompted many scientists to isolate and synthesize xanthones and their derivatives for the development of novel drug candidates, particularly for anticancer drug candidates.[19] For example, one synthetic xanthone compound, 5,6-dimethylxanthone-4-acetic acid (DMXAA, Fig. 1), has been shown to interact with diverse biological targets providing its anticancer activity.[20, 21] Gambogic acid (Fig.1) is a naturally caged xanthone compound with potent anticancer activities. Multiple mechanisms, including apoptosis induction, cell cycle regulation, angiogenesis inhibition, tumor cell adhesion inhibition, and metastasis prevention, have been proposed as the basis of the ability of gambogic acid to inhibit the growth of a broad panel of cancer cell lines both *in vitro* and *in vivo*. Gambogic acid has recently passed phase IIa clinical trials in China.[22, 23] Although the primary molecular targets of these xanthone drug candidates are still debatable, their anticancer activities might be related to their interaction with DNA and can be significantly altered by ring substituents with different positions because of their planar pharmacophoric structure involving a three-ring skeleton.[24] After a careful analysis of the structure of many natural and synthetic xanthone compounds, we found that most have simple small substituents on both benzene rings in different positions, such as the hydroxyl, methoxyl, or methyl groups. In addition to these common substituents, some natural xanthones are also substituted with isoprene, which binds to the outer benzene ring carbon atom, as with mangostins.[13] On occasion, the isoprenyl substituent groups form a bridge-like structure with the benzene ring to form caged xanthone compounds, such as gambogic acid.[8, 22, 24] Of the synthetic xanthone derivatives, studies have shown

that one longer side-chain substituent on one of the benzene rings has much more potent anticancer activity.[7, 9, 10, 12]

In order to search for new xanthone drug candidates with potential antitumor properties, we synthesized a series of novel xanthone derivatives (**XD**) with two longer side chain substituents on either benzene ring (e.g., 3,6-disubstituted carbonyl methoxy groups) and screened their anticancer activities *in vitro*. A mechanism of action was suggested through cell cycle analysis and apoptosis induction.

2. Material and methods

Solvents and reactants were of the highest commercial grade available and were used without further purification unless otherwise noted. Thin layer chromatography (TLC) plates (Partisil K6 thin layer chromatography plates, silica gel 60, Whatman, Maidstone, United Kingdom) and column chromatography (Purasil 60 A Silica gel [230-400 mesh], Whatman) were used to purify intermediates and final derivatives. ^{1}HNMR spectra were recorded on a Mercury 300 MHz broadband (mbb) or Mercury 300 MHz 4-nuc (m4n) spectrometer (Oxford Instruments, Oxfordshire, United Kingdom) in either $CDCl_3$ with tetramethylsilane (TMS) used as an internal standard or dimethyl sulfoxide (DMSO)-d_6 without TMS and trace DMSO used as an internal standard. ^{13}CNMR spectra were recorded on an Inova 500 MHz 2 RF channels (i2C) spectrometer (Oxford) in DMSO-d_6 without TMS and trace DMSO used as an internal standard. Chemical shifts (δ) were in parts per million (ppm) relative to tetramethylsilane or trace DMSO as an internal standard, and coupling constants (*J* values) were in Hertz. Mass spectra were measured on a DSQ low-resolution mass spectrometer or on a Shimadzu LCMS-2010A liquid

chromatography mass spectrometer (Shimadzu Corporation, Kyoto, Japan). Melting points were determined on an electrothermal digital melting point apparatus and were uncorrected.

2.1. Synthesis of 3,6-dihydroxy-4-methylxanthone

2.1.1. Synthesis of 2-hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone or 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone and 3-methyl-2,2',4,4'-tetramethoxybenzophenone

2,4-Dimethoxybenzoic acid (18.2 g, 100mmol), benzene (190 mL), and thionyl chloride (50 mL) were combined in a 500-mL, 3-necked flask. The mixture was refluxed for 3 h. Then the solvent and excess thionyl chloride were removed by distillation. The residual was used in the next step without any further purification.

To the above acyl chloride, 2,6-dimethoxytoluene (15.2 g, 100mmol) and dry ethyl ether (200 mL) were added. The mixture was cooled to 5-10°C with ice. Then $AlCl_3$ (34.2 g, 256mmol) was added in small portions while stirring to keep the temperature of the reaction mixture below 10°C. After $AlCl_3$ was added, the mixture was left overnight. An analysis of the post-reaction mixture revealed a combination of 2-hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone or 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone and 3-methyl-2,2',4,4'-tetramethoxybenzophenone; it weighed 29.7 g and yielded 98.3%. There were clearly two spots on TLC plate (eluent, ethyl acetate/petroleum ether, 1/4). The mixture was separated by silica gel column chromatography (eluent, ethyl acetate/petroleum ether, 1/5) to yield two products:

1. 2-hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone and 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone; a light yellow solid; m.p. 113-115°C; ^{1}H NMR (300 MHz, δ, ppm, $CDCl_3$), 12.76 (s, 1 H, OH), 7.20-7.28 (m, 2 H, aromatic H), 6.60-6.50 (m, 2 H, aromatic H), 6.36 (d, *J* = 9.0 Hz, 1 H, aromatic H), 3.87 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 200.45, 163.91, 161.91, 160.42, 156.67, 133.79, 127.67, 124.94, 119.48, 114.40, 112.28, 106.31, 103.16, 62.17, 56.47, 56.34, 9.33, 8.51
2. 3-methyl-2,2',4,4'-tetramethoxybenzophenone; a white solid; m.p. 75-77°C; ^{1}H NMR (300 MHz, δ, ppm, $CDCl_3$), 7.49 (d, *J*=8.4 Hz, 1 H, aromatic H), 7.33 (dd, *J*= 8.4 Hz, 0.6 Hz, 1 H, aromatic H), 6.63 (d, *J*= 8.7 Hz, 1 H, aromatic H), 6.51-6.45 (m, 2 H, aromatic H), 3.87 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 3.56 (s, 3 H, OCH_3), 2.14 (s, 3 H, CH_3).

2.1.2. Transformation of 3-methyl-2,2',4,4'-tetramethoxybenzophenone to 2-hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone or 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone

A CH_2Cl_2 (50 ml) solution of 3-methyl-2,2',4,4'-tetramethoxybenzophenone (3.16 g, 10mmol), and BBr_3 (2.51 g, 10mmol) was stirred at -5°C for 2 h. Some cold water (30 mL) was added and the mixture was stirred for further 10 min. The CH_2Cl_2 layer was separated and water layer was extracted with CH_2Cl_2 (30 mL x 2). The combined CH_2Cl_2 solution was washed with water (20 mL x 2) and brine (30 mL), and then dried over Na_2SO_4. After the removal of the solvent, the residual was recrystallized in methanol

giving a light yellow solid; weighed 2.7g and yielded 89.4%. The sample was purified by dry-column flash chromatography to afford a light yellow solid; m.p. 113-114°C. ^{1}HNMR confirmed that this product is the same as 2-hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone or 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone.

2.1.3. Synthesis of 3,6-dimethoxy-4-methylxanthone

2-Hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone or 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone (20.6 g, 68.2mmol), methanol (150 mL), potassium carbonate (34.5g, 250mmol), and water (300 mL) were combined in a 1000-mL, 3-necked flask. The mixture was stirred under reflux for 28 h. After cooling to room temperature, the white solid was filtered out and washed with water until a pH of approximately 7 was obtained. The solid was recrystallized with methanol to afford 3,6-dimethoxy-4-methylxanthone; white solid; m.p. 175-177°C; weight of 17.0 g and yield of 92.4%; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.03 (d, J=9.0 Hz, 1 H, aromatic H), 8.01 (d, J=8.7 Hz, 1 H, aromatic H), 7.14 (d, J= 9.3 Hz, 1 H, aromatic H), 7.12 (d, J= 2.4 Hz, 1 H, aromatic H), 7.00 (dd, J= 9.0 Hz, 2.4 Hz, 1 H, aromatic H), 3.94 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 2.30 (s, 3 H, CH_3); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.97, 164.98, 162.18, 158.02, 154.86, 127.76, 125.16, 115.52, 114.95, 113.74, 113.01, 108.41, 100.91, 56.68, 56.52, 8.51.

2.1.4. Synthesis of 3,6-dihydroxy-4-methylxanthone

3,6-Dimethoxy-4-methylxanthone (13.5 g, 50mmol), conc. hydrobromic acid (120 mL), and glacial acetic acid (120 mL) were combined in a 500-mL, 3-necked flask. After the

mixture was stirred under reflux for 48 h, the solid was filtered out, washed with water, dried, and recrystallized in methanol to afford a white solid, m.p. >260°C. It weighed 8.1 g and yielded 66.9%; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 10.89 (s, 1 H, OH), 10.75 (s, 1 H, OH), 8.08 (d, *J*=9.3 Hz, 1 H, aromatic H), 7.95 (d, *J*= 8.7 Hz, 1 H, aromatic H), 7.03 (d, *J*= 8.7 Hz, 1 H, aromatic H), 6.98-6.94 (m, 2 H, aromatic H), 2.37 (s, 3 H, CH_3); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 179.59, 168.51, 166.07, 162.73, 160.57, 132.93, 129.49, 119.20, 118.90, 118.84, 117.62, 115.85, 107.35, 13.32.

2.2. Synthesis of 3,6-dihydroxyxanthone

3,6-Dihydroxyxanthone was synthesized in the same way as 3,6-dihydroxy-4-methylxanthone. It also produced a white solid, m.p. >260°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO- d_6), 10.91 (s, 2 H, OH x 2), 8.08 (d, *J* = 8.7 Hz, 2 H, aromatic H), 6.92-6.98 (m, 4 H, aromatic H); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d6), 179.15, 168.59, 162.69, 133.00, 119.22, 118.88, 107.31.

2.3. Synthesis of xanthone derivatives (**XD**) **1-10**

K_2CO_3 (4.14 g, 30mmol) and *N*-substituted or *N*,*N*-disubstituted-α-chloroacetamide (2mmol) were added to a solution of 3,6-dihydroxy-4-methylxanthone (484 mg, 2mmol) or 3.6-dihydroxyxanthone (456 mg, 2mmol) in acetone (60 mL). The mixture was stirred under reflux for 3h, after which more *N*-substituted or *N*,*N*-disubstituted-α-chloroacetamide (2mmol) were added. Then, the mixture was stirred under reflux overnight. The solid was filtered out and washed with acetone. The residual left after the

removal of the acetone by a rotavapor was purified by dry-column flash chromatography to afford **XD1-10**.

2.3.1. 3,6-Di-[(2,6-dimethylpiperidinyl)-carbonylmethoxy]xanthone (**XD1**).

White solid; yield 68.4%; formed from 3,6-dihydroxyxanthone and α-chloro-(2,6-dimethylpiperidinyl)acetamide; m.p. 179-180°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.23 (d, J = 9.0 Hz, 2 H, aromatic H), 7.00-6.94 (m, 4 H, aromatic H), 4.84 (s, 4 H, OCH_2 x 2), 1.95-1.20 (m, 28 H, piperidine ring 8H x 2, CH_3 x 4); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.47, 165.84, 164.09, 157.76, 127.82, 115.56, 114.11, 102.00, 66.92, 46.49, 43.80, 30.32, 29.91, 21.93, 13.98; ESIMS (M/Z): 535.28 $[M+H]^+$, 557.26 $[M+Na]^+$.

2.3.2. 3,6-Di-(morpholinyl-carbonylmethoxy)xanthone (**XD2**).

White solid; yield 89.6%; formed from 3,6-dihydroxyxanthone and α-chloro-morpholinylacetamide; m.p. 251-253°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.06 (d, J = 8.7 Hz, 2H, aromatic H), 7.09-7.03 (m, 4 H, aromatic H), 5.08 (s, 4 H, OCH_2 x 2), 3.64-3.36 (m, 16 H, morpholine ring CH_2 x 8); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.47, 165.74, 163.92, 157.75, 127.85, 115.65, 114.10, 102.03, 66.55, 66.44, 45.06, 42.10; ESIMS (M/Z): 483.18 $[M+H]^+$, 505.16 $[M+Na]^+$.

2.3.3. 3,6-Di-(*N*-tetrahydrofurfurylamino-carbonylmethoxy)xanthone (**XD3**).

White solid; yield 30.6%; formed from 3,6-dihydroxyxanthone and α-chloro-*N*-tetrahydrofurfurylacetamide; m.p. 183-184°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6),

8.24 (br. s, 2 H, NH x 2), 8.09 (d, *J* = 8.4 Hz, 2 H, aromatic H), 7.10-7.07 (m, 4 H, aromatic H), 4.71 (s, 4 H, OCH_2 x 2), 3.88 (m, 2 H, tetrahydrofuran ring OCH x 2), 3.74 (q, *J* = 6.6 Hz, 2 H, tetrahydrofuran ring OCH_2), 3.61 (q, *J* = 6.9 Hz, 2 H, tetrahydrofuran ring OCH_2), 3.21 (t, *J* = 5.7 Hz, 4 H, $=NCH_2$- x 2), 1.82-1.70 (m, 6 H, tetrahydrofuran ring CH x 2 and CH_2 x 2), 1.58-1.46 (m, 2 H, tetrahydrofuran ring CH x 2); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.48, 167.43, 163.47, 157.73, 127.99, 115.82, 114.23, 101.98, 77.41, 67.69, 67.62, 42.88, 28.89, 25.59; ESIMS (M/Z): 511.21 $[M+H]^+$, 533.19 $[M+Na]^+$.

2.3.4. 3,6-Di-[(2,6-dimethylpiperidinyl)-carbonylmethoxy]-4-methylxanthone (**XD4**).

White solid; yield 87.2%; formed from 3,6-dihydroxy-4-methylxanthone and α-chloro-(2,6-dimethylpiperidinyl)acetamide; m.p. 245-247°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.04 (d, *J* = 9.0 Hz, 1 H, aromatic H), 7.97 (d, *J* = 9.0 Hz, 1 H, aromatic H), 7.11 (d, *J* = 2.1 Hz, 1 H, aromatic H), 7.02 (dd, *J* = 9.0 Hz, 2.4 Hz, 2 H, aromatic H), 5.18 (br. s, 2 H, OCH_2), 4.91 (br. s, 2 H, OCH_2), 4.53 (br. s, 2 H, =NCH= x 2), 4.09 (br. s, 2 H, =NCH= x 2), 2.37 (s, 3 H, CH_3), 1.90-1.10 (m, 24 H, piperidine ring H, CH_3 x 4); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.93, 166.06, 165.84, 164.06, 161.49, 157.87, 155.06, 127.74, 124.80, 115.59, 115.17, 114.17, 113.38, 109.70, 102.02, 67.03, 66.87, 46.45, 43.68, 30.34, 21.96, 20.68, 13.97, 8.82; ESIMS (M/Z): 549.30 $[M+H]^+$, 1097.57 $[2M+H]^+$.

2.3.5. 3,6- Di-(morpholinyl-carbonylmethoxy)-4-methylxanthone (**XD5**).

White solid; yield 91.3%; formed from 3,6-dihydroxy-4-methylxanthone and α-chloro-morpholinylacetamide; m.p. 249-251°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.13 (d, J = 9.0 Hz, 1 H, aromatic H), 8.05 (d, J = 9.0 Hz, 1 H, aromatic H), 7.24 (d, J = 2.4 Hz, 1 H, aromatic H), 7.14 (d, J = 9.0 Hz, 1 H, aromatic H), 7.13-7.10 (m, 1 H, aromatic H), 5.17 (s, 2 H, OCH_2), 5.15 (s, 2 H, OCH_2), 3.71-3.55 (m, 16 H, morpholine ring CH_2 x 8), 2.45 (s, 3 H, CH_3); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.95, 165.99, 165.76, 163.91, 161.26, 157.89, 155.06, 127.77, 124.80, 115.70, 115.26, 114.20, 113.46, 109.72, 102.03, 66.72, 66.50, 45.09, 42.10, 8.79; ESIMS (M/Z): 497.19 $[M+H]^+$, 519.16 $[M+Na]^+$, 1015.41 $[2M+Na]^+$.

2.3.6. 3,6-Di-(*N*-tetrahydrofurfurylamino-carbonylmethoxy)-4-methylxanthone (**XD6**).

White solid; yield 48.3%; formed from 3,6-dihydroxy-4-methylxanthone and α-chloro-*N*-tetrahydrofurfurylacetamide; m.p. 202-203°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.31 (m, 1 H, NH), 8.16 (m, 1 H, NH), 8.15 (d, J = 8.7 Hz, 1 H, aromatic H), 8.07 (d, J = 8.7 Hz, 1 H, aromatic H), 7.22 (d, J = 2.4 Hz, 1 H, aromatic H), 7.17-7.10 (m, 2 H, aromatic H), 4.80 (s, 4 H, OCH_2 x 2), 3.99-3.94 (m, 2 H, tetrahydrofuran ring OCH_2), 3.85-3.79 (m, 2 H, tetrahydrofuran ring OCH_2), 3.72-3.65 (m, 2 H, tetrahydrofuran ring OCH x 2), 3.30 (t, J = 5.4Hz, 4H, $=NCH_2$- x 2), 2.47 (s, 3 H, CH_3), 1.95-1.82 (m, 6 H, tetrahydrofuran ring CH x 2, CH_2 x 2), 1.63-1.55 (m, 2 H, tetrahydrofuran ring CH x 2);

^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.94, 167.65, 167.45, 163.46, 160.90, 157.82, 155.00, 127.89, 125.01, 115.91, 115.39, 114.25, 113.80, 110.00, 102.00, 77.42, 67.66, 67.64, 42.87, 42.81, 28.89, 28.84, 25.63, 25.60, 8.76; ESIMS (M/Z): 525.22 $[M+H]^+$, 547.21 $[M+Na]^+$, 1071.42 $[2M+Na]^+$.

2.3.7. 3,6- Di-[(3,5-dimethylmorpholinyl)-carbonylmethoxy]-4-methylxanthone (**XD7**).

White solid; yield 73.7%; formed from 3,6-dihydroxy-4-methylxanthone and α-chloro-(3,5-dimethylmorpholinyl)acetamide; m.p. 170-171°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.13 (d, J = 9.0 Hz, 1H, aromatic H), 8.05 (d, J = 9.0 Hz, 1 H, aromatic H), 7.22 (d, J = 3.6 Hz, 1 H, aromatic H), 7.16-7.10 (m, 2 H, aromatic H), 5.27-5.18 (m, 2 H, OCH_2), 5.12-5.05 (m, 2 H, OCH_2), 4.30-3.60 (m, 4 H, morpholine ring =CHO- x 4), 3.60-3.19 (m, 4 H, morpholine ring =NCH= x 4), 2.88-2.35 (m, 4 H, morpholine ring =NCH= x 4), 2.45 (s, 3 H, CH_3), 1.24-1.14 (m, 12 H, CH_3 x 4); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.94, 166.26, 166.01, 165.68, 165.45, 163.92, 161.26, 157.89, 155.06, 127.76, 124.80, 115.69, 115.24, 114.21, 113.43, 109.72, 101.99, 71.74, 71.60, 66.79, 66.52, 66.10, 65.32, 49.92, 49.71, 46.99, 46.27, 19.12, 18.86, 17.80, 17.53, 8.81; ESIMS (M/Z): 553.26 $[M+H]^+$, 575.24 $[M+Na]^+$, 1127.48 $[2M+Na]^+$.

2.3.8. 3,6- Di-[(3,5-dimethylpiperidinyl)-carbonylmethoxy]-4-methylxanthone (**XD8**).

White solid; yield 30.1%; formed from 3,6-dihydroxy-4-methylxanthone and α-chloro-(3,5-dimethylpiperidinyl)acetamide; m.p. 188-190°C; ^{1}H NMR (300 MHz, δ, ppm,

DMSO-d_6), 8.03 (d, J = 9.0 Hz, 1 H, aromatic H), 7.96 (d, J = 8.7 Hz, 1 H, aromatic H), 7.14 (d, J = 2.4 Hz, 1 H, aromatic H), 7.04 (d, J = 9.0 Hz, 1 H, aromatic H), 7.01 (dd, J = 8.7 Hz, 2.4 Hz, 1 H, aromatic H), 5.16-4.92 (m, 4 H, OCH_2 x 2), 4.36-4.23 (m, 2 H, piperidine ring =NCH= x 2), 3.80-3.70 (m, 2 H, piperidine ring =NCH= x 2), 3.55-3.43 (m, 1 H, piperidine ring =NCH=), 3.06-2.92 (m, 1 H, piperidine ring =NCH=), 2.58-2.42 (m, 2 H, piperidine ring =NCH= x 2), 2.36 (s, 3 H, CH_3), 1.98-1.32 (m, 6 H, C_3, C_4 and C_5 positions of two piperidine rings CH x 6), 0.96—0.68 (m, 12 H, CH_3 x 4); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.96, 165.22, 164.98, 163.97, 161.31, 157.87, 155.05, 127.76, 124.83, 115.65, 115.19, 114.19,113.40, 110.00, 109.66, 101.97, 67.03, 66.71, 51.40, 48.67, 42.14, 32.00, 31.96, 31.20, 19.42, 19.21, 14.34, 8.77; ESIMS (M/Z): 549.29 $[M+H]^+$, 571.28 $[M+Na]^+$, 1119.56 $[2M+Na]^+$.

2.3.9. 3,6- Di-[(*N*-2-methylcyclohexyl)amino-carbonylmethoxy]-4-methylxanthone (**XD9**).

White solid; yield 42.4%; formed from 3,6-dihydroxy-4-methylxanthone and *N*-(α-chloroacetyl)-2-methylcyclohexylamine; m.p. 242-243°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.14 (d, J = 9.0, 1 H, aromatic H), 8.13 (d, J = 9.0 Hz, 1 H, NH), 8.06 (d, J = 9.0 Hz, 1 H, aromatic H), 7.94 (d, J = 9.0 Hz, 1 H, NH), 7.21-7.12 (m, 3 H, aromatic H), 4.86-4.85 (m, 2 H, OCH_2), 4.78-4.77 (m, 2 H, OCH_2), 3.95-4.04 (m, 2 H, cyclohexane ring =CHN= x 2), 2.45 (s, 3 H, CH_3), 1.81-1.25 (m, 18 H, cyclohexane ring H), 0.92-0.87 (m, 6 H, CH_3 x 2); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.93, 166.94, 166.75, 166.51, 163.54, 161.08, 157.79, 155.00, 127.85, 124.96, 115.84, 115.32, 114.25, 113.79,

109.46, 101.96, 68.19, 67.75, 53.85, 53.80, 37.35, 34.45, 33.24, 25.87, 25.67, 19.57, 8.72; ESIMS (M/Z): 549.29 $[M+H]^+$, 1135.53 $[2M+K]^+$.

2.3.10. 3,6- Di-[(4-ethoxycarbonylpiperazinyl)-carbonylmethoxy]-4-methylxanthone (**XD10**).

White solid; yield 70.4%; formed from 3,6-dihydroxy-4-methylxanthone and ethyl 4-(α-chloroacetyl)piperazine-1-carboxylate; m.p. 170-171°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.08 (d, J = 9.0 Hz, 1 H, aromatic H), 8.01 (dd, J = 9.0 Hz, 0.6 Hz, 1 H, aromatic H), 7.21 (d, J = 2.1 Hz, 1 H, aromatic H), 7.10 (d, J = 9.3 Hz, 1 H, aromatic H), 7.07 (dd, J = 8.7 Hz, 2.4 Hz, 1 H, aromatic H), 5.14 (s, 2 H, OCH_2), 5.13 (s, 2 H, OCH_2), 4.14-4.05 (m, 4 H, ethoxy group OCH_2 x 2), 3.51-3.42 (m, 16 H, piperazine ring H), 2.41 (s, 3 H, CH_3), 1.26-1.18 (m, 6 H, ethoxy group CH_3 x 2); ^{13}CNMR (500MHz, δ, ppm, DMSO-d_6), 174.95, 166.02, 165.79, 163.88, 161.24, 157.89, 155.08, 155.06, 127.77, 124.81, 115.71, 115.26, 114.18, 113.46, 102.06, 66.81, 66.60, 61.43, 44.27, 41.49, 15.03, 8.82; ESIMS (M/Z): 639.26 $[M+H]^+$, 1299.50 $[2M+Na]^+$.

2.4. Synthesis of **XD11** and **XD12**

2,2'-[(xanthone-3,6-diyl)bis(oxy)]bis-acetic acid (344 mg, 1mmol) or 2,2'-[(xanthone-4-methyl-3,6-diyl)bis(oxy)]bis-acetic acid (358 mg, 1mmol) was dissolved in DMF (20 mL). *N*,*N*'-dicyclohexylcarbodiimide (DCC, 247 mg, 1.2mmol) and 4-dimethylaminopyridine (DMAP, 2.4 mg, 0.02mmol) were added to the solution. After stirring for 1 h at room temperature, 1-methyl piperazine (300 mg, 3mmol) was added, and the mixture was stirred for 6 h at 70°C. After the mixture was cooled to room

temperature, ice water (40 mL) was added while the mixture was stirred. The solid was filtered out, washed with water, and dried. **XD11** and **XD12** were purified by dry-column flash chromatography.

2.4.1. 3,6- Di-[(4-methylpiperazinyl)-carbonylmethoxy]xanthone (**XD11**). White solid; yield 63.6%; formed from 2,2'-[(xanthone-3,6-diyl)bis(oxy)]bis-acetic acid and 1-methyl piperazine; m.p. 193-195°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.06 (dd, J = 8.7 Hz, 0.9 Hz, 2 H, aromatic H), 7.07-7.02 (m, 4 H, aromatic H), 5.06 (s, 4 H, OCH_2 x 2), 3.47-3.44 (m, 8 H, piperazine ring CH_2 x 4), 2.36-2.27 (m, 8 H, piperazine ring CH_2 x 4), 2.19 (s, 6 H, NCH_3 x 2); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.47, 165.47, 163.92, 157.73, 135.79, 127.85, 115.62, 114.11, 102.00, 66.66, 55.05, 54.71, 46.15, 44.40, 41.68; ESIMS (M/Z): 509.24 $[M+H]^+$, 1039.45 $[2M+Na]^+$.

2.4.2. 3,6- Di-[(4-methylpiperazinyl)-carbonylmethoxy]-4-methylxanthone (**XD12**). White solid; yield 49.1%; formed from 2,2'-[(xanthone-4-methyl-3,6-diyl)bis(oxy)]bis-acetic acid and 1-methyl piperazine; m.p. 175-176°C; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6), 8.13 (d, J = 9.3 Hz, 1 H, aromatic H), 8.05 (d, J = 9.0 Hz, 1 H, aromatic H), 7.24 (d, J = 2.4 Hz, 1 H, aromatic H), 7.14-7.08 (m, 2 H, aromatic H), 5.16 (s, 2 H, OCH_2), 5.14 (s, 2 H, OCH_2), 3.50-3.57 (m, 8 H, piperazine ring CH_2 x 4), 2.51-2.58 (m, 8 H, piperazine ring CH_2 x 4), 2.37 (s, 3 H, CH_3), 2.29 (s, 3 H, NCH_3), 2.28 (s, 3 H, NCH_3); ^{13}CNMR (500 MHz, δ, ppm, DMSO-d_6), 174.94, 165.74, 165.50, 163.91, 161.27, 157.87, 155.05, 127.76, 124.80, 115.67, 115.23, 114.20, 113.43, 110.00, 102.00, 66.83, 66.58,

55.04, 54.66, 46.09, 44.40, 41.64, 8.79; ESIMS (M/Z): 523.25 $[M+H]^+$, 1045.49 $[2M+H]^+$.

2.5. Cell culture

Five human cancer cell lines, breast adenocarcinoma (adenocarcinoma MDA-MB-231), prostate adenocarcinoma (PC-3), lung carcinoma (A549), pancreas adenocarcinoma (AsPC-1), and colorectal carcinoma (HCT116) were obtained from ATCC (Manassas, VA). All cell lines were maintained at 37°C in 5% CO_2 in culture flasks in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO), supplemented with 10% fetal calf serum (Life Technologies, Grand Island, NY), 100-U/ml penicillin, and 0.1-mg/mL streptomycin. Upon reaching confluence, the cells were trypsinized with 0.25% trypsin containing 0.01% EDTA for 5 minutes at 37°C and then stopped by the addition of complete medium. About 5 X 10^5 of the viable cells were then resuspended in complete medium.

2.6. *In vitro* cell viability assay

MDA-MB-231 (5×10^3 cells), PC-3 (8×10^3 cells), A549 (5×10^3 cells), AsPC-1 (5×10^3 cells), and HCT116 (1×10^4 cells) were seeded in 96-well plates and incubated for 24 h to allow the cells to attach. A stock solution of 400 mM of **XD** was prepared in DMSO (Sigma, St. Louis, MO, USA) and stored at −20°C. The stock solution was diluted to the appropriate concentrations with culture medium. The final concentration of DMSO was less than 0.1% (vol/vol). The same amount of DMSO was used as the vehicle control throughout this study. Xanthone derivatives were serial diluted (final concentration 0 - 400μM) and added to the microtiter plate. 5-Fluorouracil (**5-FU**) was added as a positive

control. The cells were incubated with compounds for 48 h. MTT (3-(4,5-dimethy-1-thiazol-2-yl)-2,5-diphenyltetrazolium bromide), a tetrazole assay that uses mitochondrial metabolic enzyme activity as an indicator of cell viability, was conducted following the protocol described previously.[25] The cell viability was determined at 560-nm absorbance using a Spectra Max M2 plate reader (Molecular Devices, Sunnyvale, CA). Experiments were performed in triplicate. Cell viability was calculated as the ratio of the mean of OD obtained for the treated cell to that of the vehicle control.

2.7. Cell cycle analysis

After exposure to **XD** for 48 h, A549 cells were collected and immediately fixed with 70% ethanol overnight. Samples were stained with propidium iodide staining solution containing 50-µg/mL propidium iodide, 200-µg/ml RNase A, and 0.3% Triton-X100 for 30 min at room temperature. The cells were then measured by Accuri C6 flow cytometry to measure the fractions of cell populations in the sub-G1 (fractional DNA content), G0/1 (quiescent state/growth phase), S (initiation of DNA replication), and G2/M (biosynthesis/mitosis) phases. Data were analyzed using the C6 software (BD Biosciences, San Jose, CA).

2.8. Cell apoptosis assay

A549 cells (2×10^5) were seeded in 12-well plates and treated with 25 µM of **XD**. 48 h after treatment, cells were trypsinized, harvested and washed with ice cold PBS. Apoptosis was assayed using the Annexin V: FITC Apoptosis Detection Kit (BD Biosciences) according to the manufacturer's instructions. 1×10^5 cells were incubated

with 100 µl of binding buffer containing Annexin V - FITC and propidium iodide at room temperature for 15 min. After incubation, 400 µl of binding buffer was added to each sample, and cells were kept on ice. Samples were analyzed with an Accuri C6 cytometer (BD Biosciences).

2.9. Caspase-3/7 activity assay

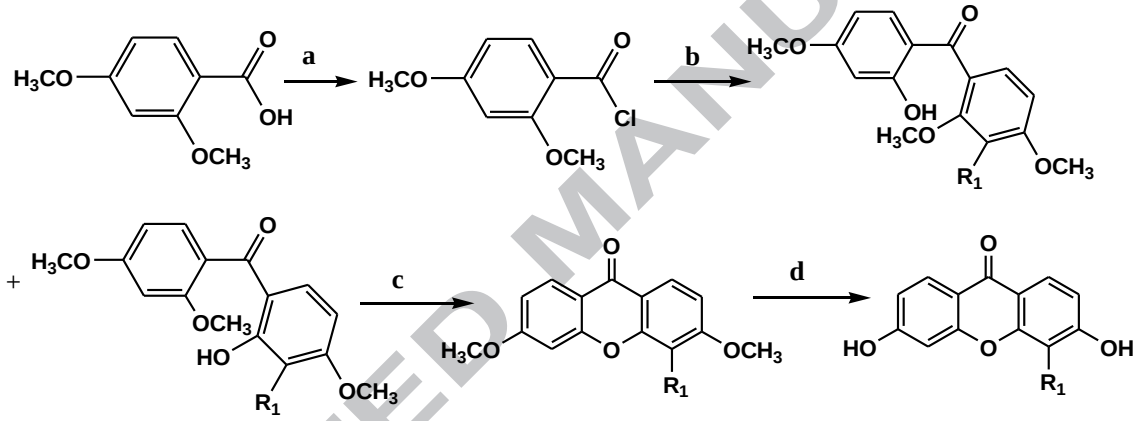
To investigate the mechanism underlying the apoptotic activity of the **XD**, the activation of caspase-3 and caspase-7 was detected using the Caspase-Glo 3/7 Assay (Promega, Madison, WI). A549 cells were plated in duplicate in 96-well plates and treated with **XD**. 48 hours after exposure, the caspase 3/7 activities were measured according to the manufacturer's instructions. Samples were read after 1 h of incubation with the caspase substrate. The luminescence was detected using the VICTOR 3V Multilabel Plate Reader (PerkinElmer, Waltham, MA). The background luminescence associated with the cell culture and assay reagent (blank reaction) was subtracted from the experimental value. The relative fold increase of caspase 3/7 was calculated by dividing the luminescence reading from the compound treatment to that of control.

3. Results and discussion

3.1. Chemistry

Scheme 1 shows the synthetic route used to produce the lead xanthone compounds. 3,6-Dihydroxy-4-methylxanthone was synthesized from 2,4-dimethoxybenzoic acid in a 54.3% general yield. The key factor ensuring a high yield of the desired xanthone derivative was the hydrolysis of 2-hydroxy or 2’-hydroxy during or after the Friedel-

Crafts reaction in step "b" in an acidic condition, which yielded 2-hydroxy-3-methyl-2',4,4'-trimethoxybenzophenone or 2'-hydroxy-3-methyl-2,4,4'-trimethoxybenzophenone. If there is no free hydroxyl group in the 2- or 2'-position, there is no cyclization in the next step to yield 3,6-dimethoxy-4-methylxanthone.[26] The last step "c" yielded 66.9% in the hydrolysis of 3,6-dimethoxy using a mixture of conc. hydrobromic acid and glacial acetic acid. The yield for this step might have been higher if a more costly demethylation agent, such as boron tribromide, was used. 3,6-Dihydroxyxanthone was synthesized in the same way, with a general yield of 58.3%.

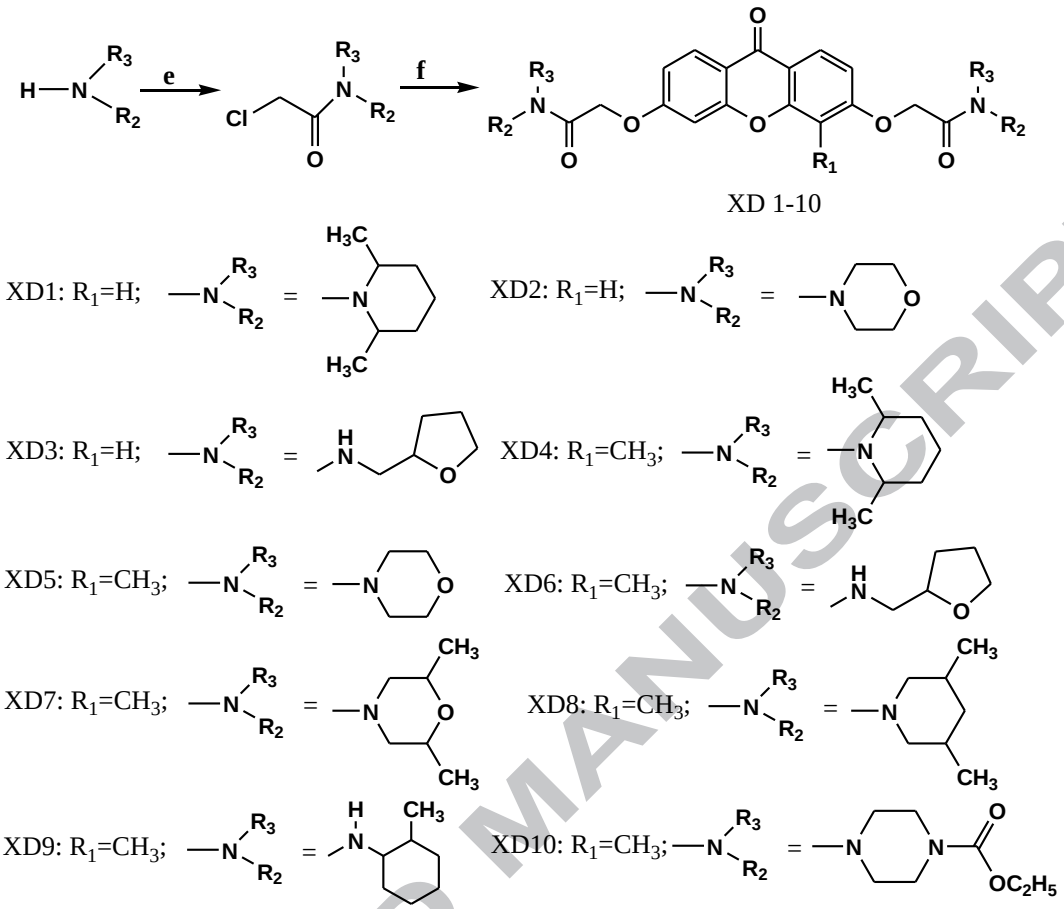


3,6-dihydroxy-4-methylxanthone, R_1=CH_3; 3,6-dihydroxyxanthone, R_1=H

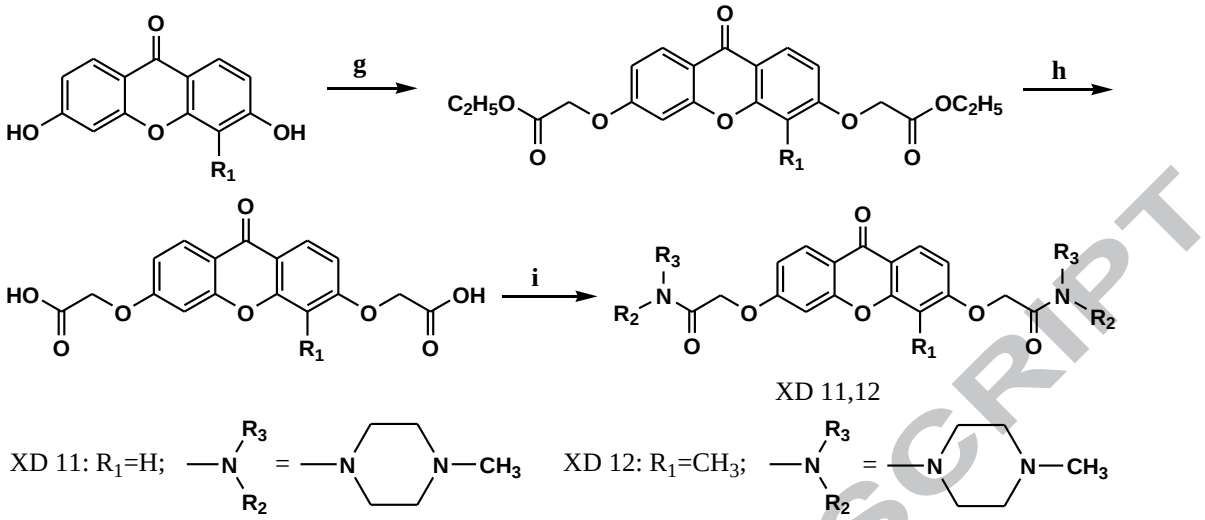
Scheme 1. Synthetic route of 3,6-dihydroxylxanthone with or without a 4-methyl group. Reagents and conditions: (a) Benzene, $SOCl_2$, refluxed for 3h; (b) 2,6-dimethoxytoluene or 2,6-dimethoxybenzene, $AlCl_3$, diethyl ether, 0-10° C, 10h; (c) CH_3OH, K_2CO_3, H_2O, 28h; (d) conc. hydrobromic acid, glacial acetic acid, refluxed for 48 h.

The target xanthone derivative compounds (**XD**), 3,6-*N*-substituted or 3,6-*N*,*N*-disubstituted aminocarbonylmethoxy xanthones with or without a 4-methyl group, were synthesized in two ways. Scheme 2 illustrates the synthesis of **XD 1-10**, in which single-

or double-substituted amines react with alpha-chloroacetic chloride under conditions of 1,2-dichloroethane as a solvent and sodium hydroxide as a catalyst to yield alpha-chloroacetamide (step "e"). These alpha-chloroacetamides were then introduced into 3,6-dihydroxy-4-methylxanthone or 3,6-dihydroxyxanthone molecule by etherification with potassium carbonate as a catalyst and acetone as a solvent (step "f") to achieve the final products. The second approach is represented in scheme 3: it illustrates the synthesis of **XD11** and **12** in which 3,6-dihydroxy-4-methylxanthone or 3,6-dihydroxyxanthone were first etherified with ethyl alpha-bromoacetate in the catalysis of K_2CO_3 (step "g") to give the diethyl carboxylate derivatives. These derivatives were then hydrolyzed with sodium hydroxide solution in ethanol to yield the dicarboxylic acid product (step "h"). Finally, this dicarboxylic acid compound was condensed with 1-methylpiperazine under the catalysis of DCC and DMAP (step "i"), thus yielding the target compounds.



Scheme 2. Synthetic route of 3,6-*N*-substituted, or 3,6-*N*,*N*-disubstituted aminocarbonylmethoxyxanthone with or without a 4-methyl group by direct etherification. Reagents and conditions: (e) $ClCH_2COCl$, $ClCH_2CH_2Cl$, 20% NaOH solution, 0°C; (f) 3,6-dihydroxylxanthone with or without a 4-methyl group, K_2CO_3, acetone, refluxed for 8-14 h.



Scheme 3. Synthetic route of 3,6- di-(4-methyl-1-piperazinyl-carbonylmethoxy)xanthone with or without 4-methyl group by amidation of dicarboxylic acid. Reagents and conditions: (g) ethyl alpha-bromoacetate, K_2CO_3, acetone, refluxed for 6h; (h) 2N NaOH solution, ethanol, refluxed for 1h; (i) DCC, DMAP, DMF, 70° C, 6 h.

3.2. Cytotoxicity on human cancer cell lines

The effect of **XD** and **5-FU** on cell growth in five human cancer cell lines (breast adenocarcinoma [MDA-MB-231], prostate adenocarcinoma [PC-3], lung carcinoma [A549], pancreatic adenocarcinoma [AsPC-1], and colorectal carcinoma [HCT116]) was determined with an MTT assay. The adherent cancer cells were treated with different concentrations of **XD** and **5-FU**, and each treatment was performed in triplicate. The fifty percent (IC_{50}) values of the derivatives were calculated by plotting the cell viability against the concentration of compounds. As Table 1 shows, some synthesized **XD** exhibited strong antiproliferative effects on cancer cell viability *in vitro*. Out of 12 derivatives, compounds **XD1**, **XD4**, and **XD8-9** were found to be potent growth

inhibitors (IC_{50} < 25 μM), whereas compounds **XD6** and **XD10** had a more moderate effect on growth inhibition with IC_{50} values close to those of **5-FU**. The IC_{50} values showed that the compounds **XD4**, **XD8**, and **XD9** exhibited similar anticancer potency against all cancer cell lines. Compounds **XD1** and **XD6** were more active against prostate cancer cells and less active against breast cancer cells. Compound **XD10** demonstrated strong growth inhibition against colorectal cancer cells.

Table 1. Growth inhibition effect (IC_{50}) of xanthone derivatives against human cancer cell lines.

Compounds	Breast Cancer MDA-MB-231	Prostate Cancer PC-3	Lung Cancer A549	Pancreatic Cancer AsPC-1	Colorectal Cancer HCT116
5-FU	35.91	72.07	39.86	98.00	15.38
XD1	24.33	6.68	17.25	13.80	15.73
XD2	>400	>400	>400	>400	>400
XD3	>400	249.20	>400	>400	>400
XD4	7.31	4.27	10.58	7.10	7.63
XD5	>400	>400	>400	>400	>400
XD6	98.44	34.50	27.53	78.74	33.81
XD7	385.23	213.50	295.00	115.54	140.88
XD8	8.06	6.18	4.59	4.76	6.09

XD9	11.03	23.30	22.32	15.41	18.33
XD10	64.49	53.32	84.85	34.13	8.16
XD11	>400	>400	>400	>400	>400
XD12	257.10	89.17	180.56	97.79	119.39

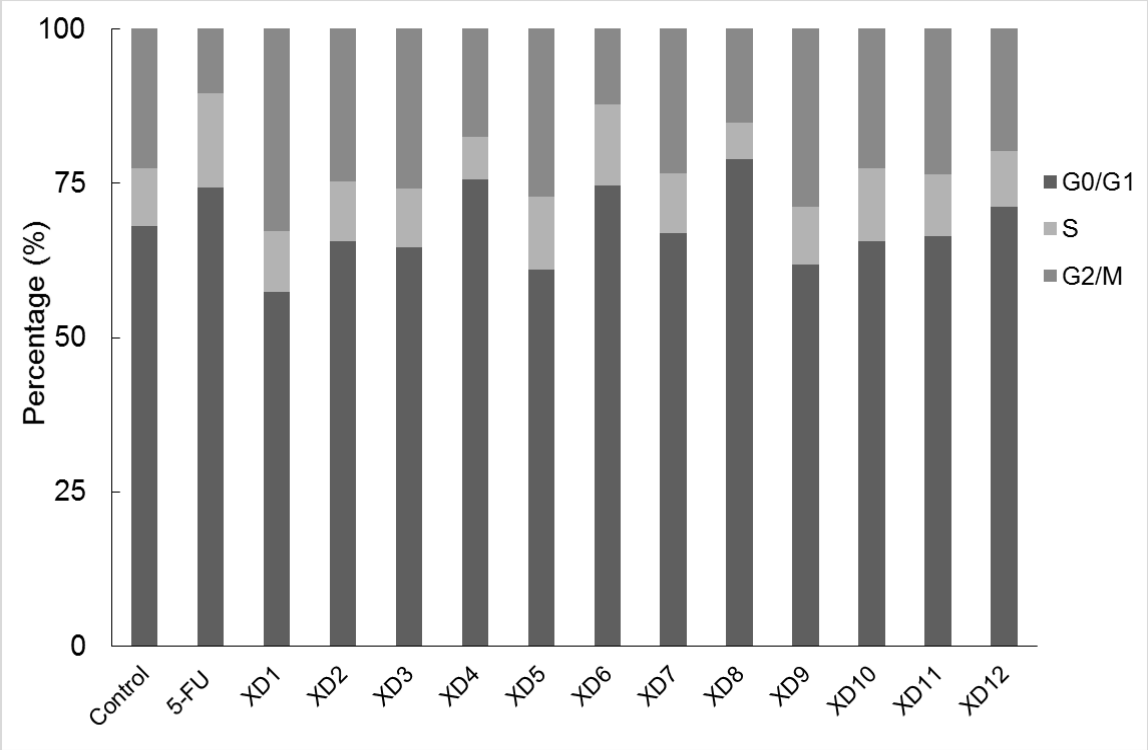
The IC_{50} values are given in μM. 5-FU was used as a positive control.

According to a structure-activity relationship analysis of the **XD** cytotoxicity results, we found that most **XD** with a 4-methyl group exhibited more potent anticancer activities than their counterparts without a 4-methyl group. Some xanthones with a 2,6-dimethyl piperidine ring, 3,5-dimethyl piperidine ring, or 2-methyl cyclohexane amine in their side chains more potently inhibited cancer cell growth than those with a morpholine ring, 3,5-dimethyl morpholine ring, or 4-methyl and ethoxycarbonyl piperazine rings in their side chains. These results suggest that electronegative atoms like oxygen, which could form hydrogen bonds with biological macromolecules in their side chains, reduce the growth inhibitory properties of the **XD**. It also appears that steric hindrance around the nitrogen atom in acetamide side chains decreases the IC_{50} of the **XD** (e.g., the compound **XD9** with the larger *N*-2-methylcyclohexyl acetamide side chain has a smaller IC_{50} than **XD6** with its *N*-tetrahydrofurfuryl acetamide side chain).

3.3. Cell cycle analysis

To establish whether the tested compounds inhibited cell growth by interrupting cells in the cell cycle progression, cellular DNA was analyzed and stained with PI and the cells were analyzed using flow cytometry. The cell cycle profile (Fig. 2) was represented

through 3 independent experiments on A549 cell lines. The results in Figure 2 indicate that a significant increased percentage of cells were in the G0/G1 phase 48 hours following **XD4**, **XD6**, **XD8**, and 5-FU treatment. **XD1** and **XD9** exposure induced cell accumulation in the G2/M phase. Consistent with the MTT results, compounds without cytotoxic properties did not affect cell cycle distribution. It has been reported that some **XD** exhibit cytotoxicity in cancer cell lines through cell cycle arrest and resulting apoptosis.[27-29] The alterations in cell cycle reported here for five of our **XD** suggest that cell cycle arrest is one of the primary mechanisms responsible for the anticancer activity of these derivatives.



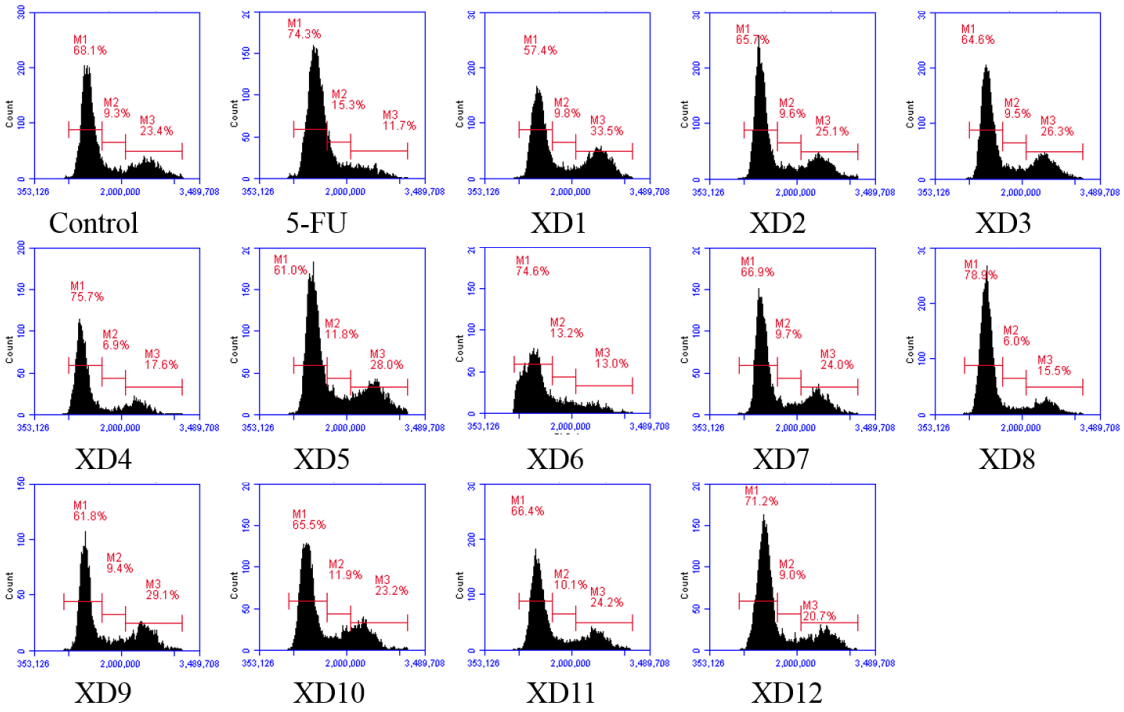


Figure 2. Effect of **XD** on cell cycle progression. A549 cells were incubated with **XD** (25 µM) for 48 hours. Cells were collected, fixed in 70% ethanol, and stained with propidium iodide solution. M1, M2, and M3 in the figure represent the G0/1 (quiescent state/growth phase), S (initiation of DNA replication), and G2/M (biosynthesis/mitosis) phases, respectively.

3.4. Cell apoptosis analysis

In addition to the cell cycle analysis, we tested the **XD** for apoptosis activity using Annexin V/propidium iodide staining. Flow cytometry analysis of stained cells distinguished between four groups: (1) viable (annexin V- PI-), (2) early apoptosis (annexin V+ PI-), (3) late apoptosis (annexin V+ PI+), and (4) necrotic (annexin V- PI+) cells. Apoptosis staining showed that treatment with **XD1**, **XD4**, and **XD8** increased

apoptosis and necrosis in A549 cells. As shown in Figure 3, there was increased late apoptotic activity in cells treated with **XD1** (control 1.2% and **XD1** treated 9.1%). XD4 was found to induce apoptotic activity in early (control 0.5% and **XD4** 7.3%) and late (control 1.2% and **XD4** 9.8%) apoptotic populations. **XD8** could inhibit the cancer cell growth at IC_{50} <10 µM with an accompanying increase in early apoptotic cell population (control 0.5% and **XD8** 37.5%), followed by a late apoptotic population (control 1.2% and **XD8** 12.4%). These results suggest that apoptosis is an anticancer mechanism for **XD1**, **XD4**, and **XD8**.

Although **XD6**, **XD9**, and **XD10** exhibited potent anticancer properties in the cytotoxicity assay, these compounds did not cause significant alterations in cell apoptosis. In contrast, the control compound, **5-FU**, was found to induce an increase in the early apoptosis population.

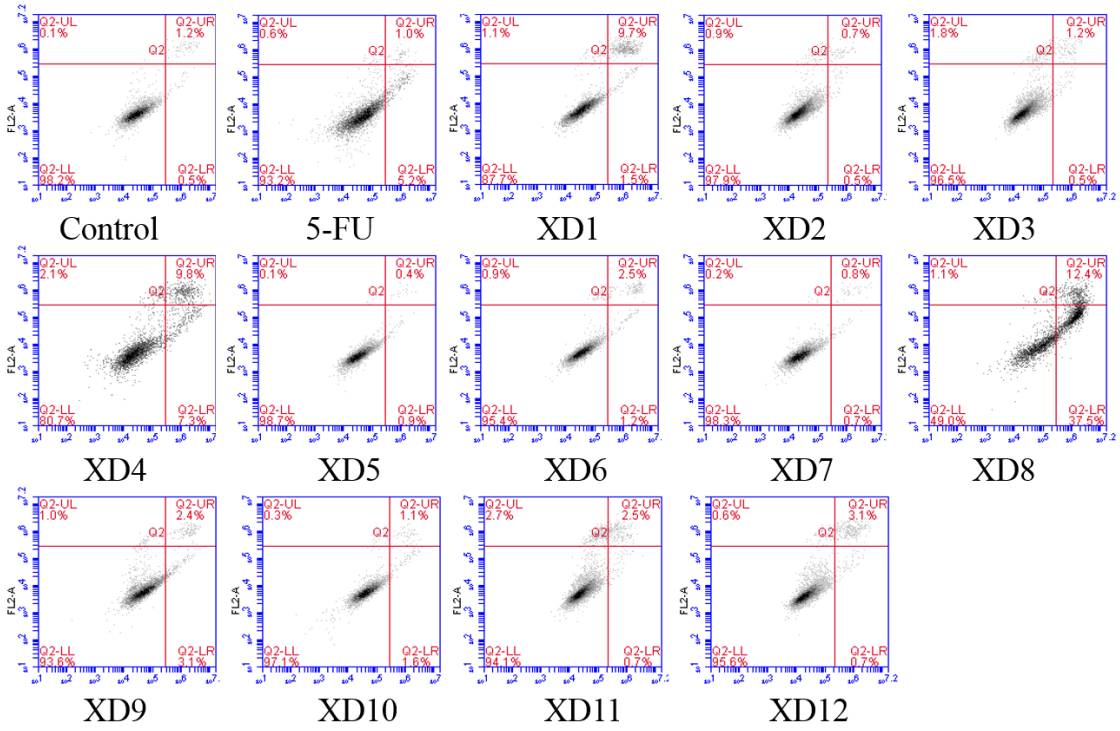


Figure 3. Cell apoptosis analysis of A549 cell exposed to xanthone derivatives at a concentration of 25 μM for 48 hours. Cells were treated with tested compounds and collected for evaluation of apoptosis via Annexin V: FITC Apoptosis Detection Kit per manufacture's protocol. The lower left quadrant shows the viable cells, the upper left shows necrotic cells, the lower right shows the early apoptotic cells while the upper right shows late apoptotic cells.

3.5. Caspase 3/7 activity

To further explore the molecular mechanism underlying the apoptotic activity of the **XD**, we tested the induction of caspase 3/7 activity. As shown in Figure 4, we observed an elevation in caspase 3/7 activity after **XD1**, **XD4**, and **XD8** treatment. Caspase 3/7 activity increased 2.31-fold, 3.45-fold, and 2.04-fold for **XD1**, **XD4**, and **XD8**,

respectively, compared to the untreated control. **5-FU** treatment induced a 4.2-fold increase in caspase 3/7 activity. Although apoptosis is a complex process that includes caspase-dependent and caspase-independent mechanisms,[30] the results shown in Figure 4 suggest that **XD1**, **XD4**, and **XD8** induce apoptosis through activation of a caspase-dependent pathway. No activation of caspase 3/7 was observed for the compounds **XD6**, **XD9**, and **XD10**, suggesting that they induce growth inhibition through a different pathway.

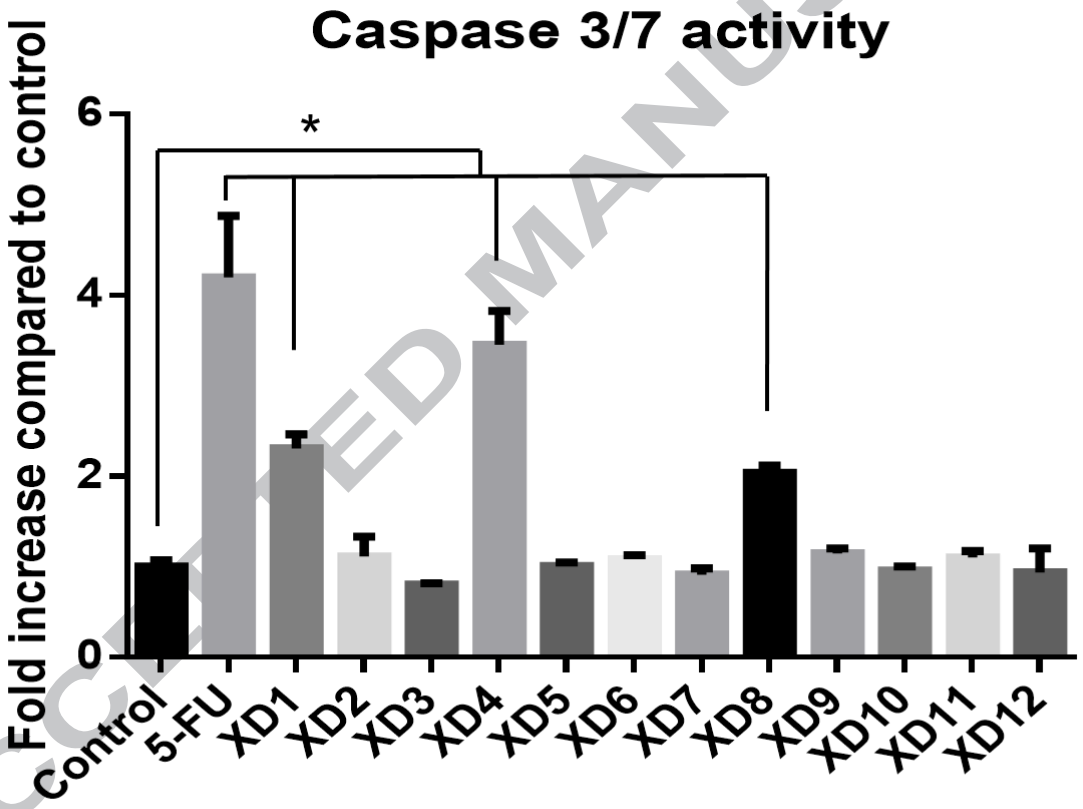


Figure 4. Effect of xanthone derivatives on caspase 3/7 activation. A549 cells (8 x 10^3) were plated in duplicate in 96-well plates and treated with xanthone derivatives (25 μM). 48 hours after exposure, caspase 3/7 activities were measured using the Caspase-Glo 3/7 Assay. Data are represented as the mean (± 1 standard deviation) in the bar graph. Analysis of variance with Dunnett's post hoc testing was used to analyze any potential difference between the treated groups and the control group. A *P* value <0.05 (labeled with asterisk) was regarded as statistically significant.

4. Conclusions

In summary, a series of novel 3,6-*N*-substituted or 3,6-*N*,*N*-disubstituted aminocarbonylmethoxyxanthones with or without a 4-methyl group have been synthesized and characterized by ^{1}HNMR, ^{13}CNMR, and MS. Most of these **XD** had very good anticancer activities *in vitro* and obvious structure-activity relationships. Further studies aiming at clarifying the mechanisms underlying the anticancer activities of these **XD** are continuing in our laboratories, which will be reported in due course. The compounds **XD1**, **XD4**, **XD6**, and **XD8-10** inhibited the growth of human breast cancer (MDA-MB-231), prostate cancer (PC-3), lung cancer (A549), pancreatic cancer (AsPC-1), and colorectal cancer (HCT116) cells. Treatment of cancer cells with **XD1** and **XD4** resulted in cell accumulation in G2/M phase, while **XD6** and **XD8** led to G0/G1 accumulation. Furthermore, the results from the cell apoptosis staining and caspase 3/7 activation testing suggested that the anticancer mechanism of compounds **XD1**, **XD4**, and **XD8** are mediated through apoptosis via a caspase-dependent pathway.

Acknowledgments

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