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Original article

Synthesis and biological evaluation of phenyl substituted polyoxygenated xanthone derivatives as anti-hepatoma agents

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ABSTRACT

A series of novel derivatives of phenyl substituted tetramethoxy xanthone were synthesized and evaluated for their *in vitro* cytotoxicity against human hepatocellular carcinoma (HCC) and non-tumor hepatic cells. Among these derivatives, compound **6** was more potent than positive control 5-fluorouracil (5-Fu) on QGY-7703 and SMMC-7721 cells with IC_{50} values of 6.27 μM, 7.50 μM and 15.56 μM, 14.55 μM, respectively. Furthermore, compounds **6**, **14**, **16**, and **29** exhibited much better selectivity toward the normal hepatic cell line QSG-7701 than 5-Fu. Additionally, compound **6** significantly induced cell apoptosis in QGY-7703 cells. Our findings suggested that these phenylxanthone derivatives may hold promise as chemotherapeutic agents for the treatment of human HCC.

1. Introduction

Human hepatocellular carcinoma (HCC) is the sixth-most common cancer and the third leading cause of cancer-related mortality worldwide [1,2]. Chemotherapy is one of the commonly used treatment options, especially for patients with unresectable tumors. Currently, there is no effective chemotherapy for HCC in humans, and the use of conventional cytotoxic drugs, including cyclophosphamide, cisplatin, and fluorouracil, has not shown any improvement in survival. Additionally, severe adverse effects are commonly observed in patients with these treatments [3,4]. Therefore, development of new chemotherapeutic agents will be of great significance.

Xanthones are a class of oxygen-containing heterocyclic compounds that are widely distributed in nature [5] and exhibit a variety of biological activities [6–8]. During the past several years, natural xanthone has attracted increasing interest in the search for new antitumor agents, such as monodictyxanthone analogs [9], α-mangostin [10,11], psorospermin [12]. Another naturally abundant type of xanthones in plants, prenylated xanthones [13–26] have also been described to exhibit antitumor activity. Among these polyprenylated derivatives, some caged xanthone compounds were identified as a potent anticancer agent, such as 7-hydroxy-forbesione, cantleyanone B, cantleyanone C [25] and gambogic acid [26].

Phomoxanthones A and B (Fig. 1) [27], two novel xanthone dimers, which were isolated from the endophytic fungus *Phomopsis* sp. BCC 1323, exhibited significant *in vitro* cytotoxicity. Furthermore, another type of compounds containing a biphenyl moiety, such as honokiol and magnolol (Fig. 1), a biphenolic neolignan isolated from *Magnolia officinalis*, has been reported to possess cytotoxic activities against A459 cell line (human nonsmall lung cell cancer), SK-MEL-2 (human melanoma), SK-OV-3 (ovarian cancer), XF498 (CNS cell line), HCT-15 (colon cancer), and Hep-G2 (liver cancer) [28–31]. Professor Chen, designed and synthesized a series of honokiol analogs and derivatives to investigate their antiangiogenic activity by application of the transgenic zebrafish screening model, antiproliferative and cytotoxic activity against HUVECs, and three tumor cell lines by MTT assay [32]. Further structure–activity relationship (SAR) studies in his research suggested that the orientation of the two allyl groups of the biphenyl scaffold was responsible for the antiproliferative activity. Based on these results, a series of phenyl substituted tetramethoxy xanthone derivatives were synthesized and their potential cytotoxic activity

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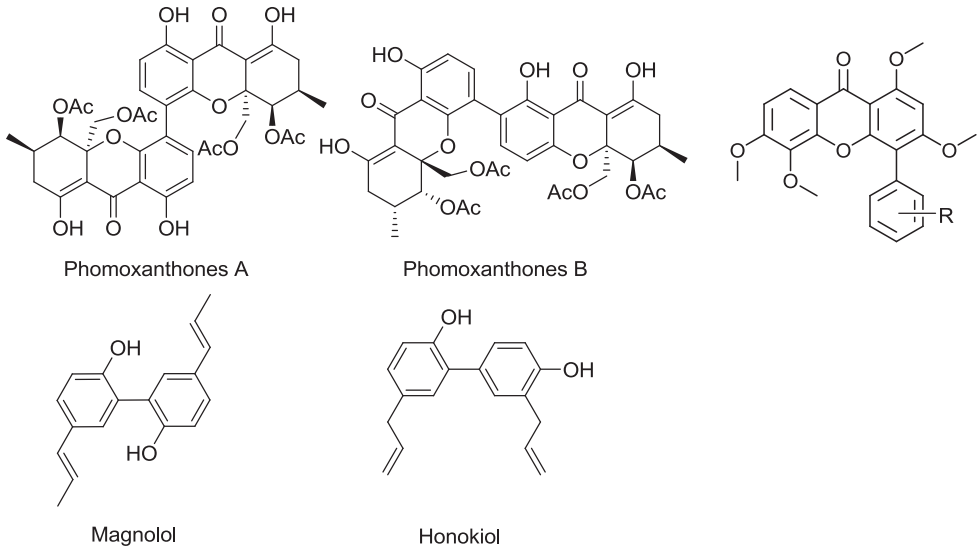


Fig. 1. Biphenyl moiety with promising antitumor activity.

against HCC cells was investigated. The effects of the prepared compounds on HCC cell apoptosis were further evaluated. Herein, the synthesis and biological evaluation of these derivatives are reported.

2. Chemistry

The initial synthesis of the xanthone nucleus is illustrated in Scheme 1. First, 2,3,4-thrimethoxybenzoyl chloride **2** was prepared from the corresponding acid **1** in the presence of thionyl chloride, and then reacted with 1,3,5-trimethoxybenzene to afford 2-hydroxy-2′,3,4,4′,6′-pentamethoxybenzophenone **3** in 65% yield. As already described by Quillinan [33], a mono-demethylation occurred on the ring ortho to the carbonyl function provided by the acid moiety. Subsequent base-catalyzed cyclization of **3** led to 1,3,5,6-tetramethoxy xanthone **4** in 83% yield along with methanol elimination. Treatment of tetramethoxy xanthone **4** with 1.50 equiv of iodine in DMF at room temperature provided xanthone **5** in satisfactory yield (approximately 85% yield). Due to the long reaction time and complicated operation, iodine was replaced with NIS to prepare compound **5** without a loss in yield. With this key compound in hand, title compounds **6**–**34** were accessed by Suzuki reaction using palladium tetrakis(triphenylphosphine) as catalyst. The structures of the synthesized target compounds were elucidated by ^{1}H NMR, ^{13}C NMR and MS. All spectral data were in accordance with assumed structures.

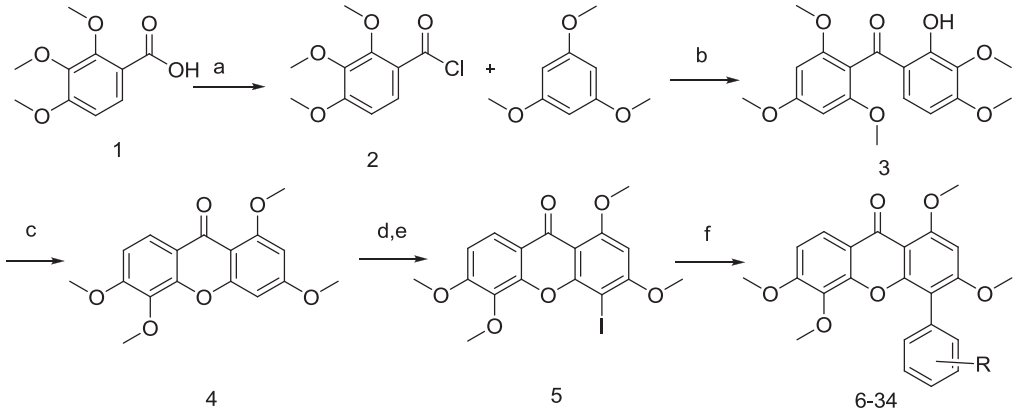
3. Results and discussion

All newly synthesized compounds were tested *in vitro* and quantified as the percentage inhibition of treated QGY-7703 cells at a single dose of 10 μM in comparison to untreated control cells (Table 1). Of the 28 compounds tested, compounds **6**, **12**, **13**, **14**, **16**, **22**, **24**, **26**, **29** and **32** exhibited greater than 50% inhibition of QGY-7703 cell growth at 10 μM. Compounds contained no group or electron donating groups on substituted phenyl ring, such as alkyl and methoxy showed more potent cytotoxic activity against QGY-7703 cells. Decreased electron density on the aromatic ring therefore appeared to diminish activity, especially for the strongly electron-withdrawing group at *para* position of substituted phenyl ring, such as compounds **10**, **15**, **19**, **20**. Introduction of a weak electron-withdrawing fluoro group on the phenyl ring (compound **22**) at the ortho position was well tolerated, whereas the *para*-, *meta*-substituted analogs (compounds **11**, **31**) and two fluoro substituted analogs (compounds **9**, **30**, **33**) led to almost total loss of activity. It appears that the substitution with phenyl ring at 4 position of xanthone nuclear is essential for activity, replacement of the phenyl ring with heterocyclic ring (compound **27**) or no substitution (compound **4**) led to dramatic loss of activity. To further study the cytotoxic profile, the hit compounds (**6**, **12**, **13**, **14**, **16**, **22**, **24**, **26**, **29** and **32**) were selected for further cytotoxicity investigation at different concentrations against three different types of HCCs. In these tests, 5-fluorouracil (5-Fu, Sigma) was used as the positive control. The IC_{50} values of individual compounds for the 5-dose test against each HCC cell line are presented in Table 2. Most of these compounds showed more potent activity against QGY-7703 and SMMC-7721 than HepG-2.

Among the 10 hit compounds, phenyl-substituted xanthone analogs containing electron-withdrawing substituents on the phenyl group (compounds **22**) showed weaker cell growth inhibition than alkyl-substituted phenyl (compounds **12**, **13**, **14**, **16**, **29** and **32**) or methoxy substituted phenyl (compounds **24** and **26**) analogs. This conclusion was consistent with the preliminary screen results. Furthermore, no conclusive relationship was found between the cytotoxic activity and the substituted position.

Among the derivatives tested, phenylxanthone **6** displayed the most potent cytotoxicity against QGY-7703, Hep-G2 and SMMC-7721 with IC_{50} values of 6.27 μM, 19.18 μM and 7.5 μM. For comparison, the positive control 5-Fu had IC_{50} values of 15.66 μM, 12.48 μM and 14.55 μM, respectively. Adding alkyl groups on the phenyl ring of compound **6** did not affect the cytotoxicity against HCC (e.g., compounds **12**, **13**, and **29**). However, the introduction of a bulky substituent had a detrimental effect on inhibition potency, as seen with isopropyl substituted **14** and *tert*-butyl substituted **16**. Methoxy is a strong electronic-donating group, but compared with phenylxanthone **6**, methoxy substituted phenylxanthones **24** and **26** displayed moderate to weak cytotoxicity toward all three HCCs, presumably due to steric hindrance.

Cytotoxic selectivity against HCC cells is the most important characteristic of the newly synthesized derivatives. Thus, the inhibitory effect of active compounds **6**, **12**, **13**, **14**, **16**, **22**, **24**, **26**, **29** and **32** on the proliferation of non-tumor hepatic cells (QSG-7701) was further examined. According to the data shown in Table 2, alkyl substituted analogs (compounds **6**, **14**, **16**, **29**) exhibited better



Scheme 1. Reagents and conditions: (a) thionyl chloride, rt, 2 h; (b) $AlCl_3$/ether, rt, 8 h; (c) tetramethylammonium hydroxide, pyridine, water, reflux, 36 h; (d) I_2 in DMF, rt, 2 days; (e) NIS in CH_2Cl_2, rt, overnight; (f) phenylboronic acid, Na_2CO_3, DME–H_2O, 85 °C, tetrakis(triphenylphosphine) palladium(0).

cytotoxic selectivity against the tumor cells and normal cells than methoxy substituted analogs (**24**). However, fluoro substituted compounds (**22**) and dimethoxy substituted analogs (**26**) had no cytotoxic selectivity. Compared to 5-Fu, all these target compounds presented a weak cytotoxic activity toward the non-tumor hepatic cells.

To further explore the potential mechanisms of antiproliferative effect induced by compound **6**, annexin V-FITC binding analysis and PI staining were performed to quantify cell apoptosis (Fig. 2). HCC QGY-7703 cells were cultured for 48 h in the absence or presence of compound **6** at different concentrations (3.75, 7.5, 15 and 30 μM). Treatment with a low concentration (3.75 μM) of compound **6** did not alter the number of apoptotic cells compared to the control group. In contrast, the apoptosis percentage increased significantly after treatment with higher concentrations of compound **6**. The results demonstrated that compound **6**-induced apoptosis of HCC at least partially contributed to its antiproliferative effect.

Table 1
Inhibition rates of compounds **4**, **6**–**33** against the QGY-7703 cell line at 10 μM.

Compd	R	Yield	Inhibitory ratio (%)
4	–	–	14.13
6	H	91%	66.45
7	3′,5′-Di-CF_3	62%	14.16
8	4′-CHO	55%	19.73
9	3′,5′-Di-F	70%	11.22
10	4′-F_3CO	75%	0.53
11	4′-F	72%	13.94
12	4′-CH_3CH_2	85%	77.14
13	4′-CH_3	87%	68.11
14	4′-$CH_3(CH)CH_3$	88%	52.67
15	4′-CF_3	76%	5.23
16	4′-$C(CH_3)_3$	88%	56.31
17	4′-CH_3CO	70%	13.18
18	4′-CH_3OCO	72%	−5.48
19	4′-CH_3SO_2	65%	1.59
20	4′-CN	60%	−1.84
21	3′-CHO	65%	12.97
22	2′-F	65%	52.38
23	3′-CN	67%	−4.17
24	3′-OMe	80%	55.25
25	3′- CF_3	75%	30.00
26	3′,5′-Di-OMe	88%	57.99
27	3′-Pydidine	75%	18.02
28	2′-CF_3O	50%	33.04
29	3′-CH_3	82%	51.36
30	3′,4′-Di-F	72%	5.85
31	3′-F	75%	35.16
32	2′,5′-Di-CH_3	92%	54.26
33	2′,5′-Di-F	78%	17.40

4. Conclusions

In summary, a novel series of phenyl substituted tetramethoxy xanthone derivatives were synthesized to survey the SAR of substituents on the phenyl ring. The SAR analysis indicated that: (i) electronic effects in the phenyl ring played a crucial role in the antiproliferative activity; and (ii) bulky substituents had a detrimental effect on the inhibition. Among the new compounds, **6**, **14**, **16**, and **29** had selective inhibitory activity against HCC cells. Furthermore, compound **6** induced a high frequency of apoptosis in human HCC QGY-7703 cells. These derivatives, especially **6**, presented better cytotoxic selectivity for HCC cells, suggesting their potential in targeted chemotherapy for HCC. Further lead optimization and mechanism studies are thus worth pursuing.

5. Experimental section

5.1. Chemistry

5.1.1. Instruments

Nuclear magnetic resonance (NMR) spectra were recorded using TMS as the internal standard in DMSO-d_6 or $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 300 MHz and 600 MHz. When peak multiplicities are reported, the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broadened, dd = doublet of doublets, dt = doublet of triplets; ESI-MS was recorded on Agilent 1100 LC/MSD (70 eV) spectrometers. HRMS was recorded on a Waters Q-Tof micro. THF was distilled from sodium/benzophenone ketyl. Dichloromethane was distilled over CaH_2. All other reagents and starting materials were purchased and

Table 2
Effects of the target compounds on proliferation of human HCC cells and normal hepatic cells.

Compd	IC_{50} (μM)			
	QGY-7703	HepG-2	SMMC-7721	QSG-7701
6	6.27	19.18	7.50	>100
12	22.55	27.99	16.35	69.21
13	14.05	24.60	3.15	66.32
14	26.14	32.46	20.11	>100
16	33.13	39,21	18.15	>100
22	39.56	48.85	26.76	20.74
24	11.94	28.69	10.56	48.10
26	31.90	45.00	31.47	33.12
29	13.26	5.00	14.82	>100
32	24.58	28.62	16.99	90.68
5-Fu	15.66	12.48	14.55	0.60

used as received (Aldrich, TCI, Adamas). BL-GHX-V was purchased from Shanghai Bilang Instrument Co., Ltd. Reactions were monitored by analytical TLC using silica gel 60 F_{254} plates and spots were visualized by UV light irradiation (254 nm). Flash column chromatography was performed by using silica gel (200–300 mesh). All tested compounds showed ≥95% purity as determined by combustion analysis or by high-performance liquid chromatography (HPLC). HPLC conditions were as follows: CHIRALPAK ADH, 4.6 mm × 250 mm, 5% → 90% CH_3CN/MeOH/0.1% acetic acid, 15 min run, flow rate 0.7 mL/min, UV detection ($\lambda = 220$ nm).

*5.1.2. Synthesis of compound **4***

*5.1.2.1. 1,3,5,6-Tetramethoxy-9H-xanthen-9-one (**4**, $C_{17}H_{16}O_6$).* 2,3,4-Trimethoxybenzoic acid (2.78 g, 13.14 mmol) in anhydrous benzene (60 mL) was treated with 5.0 mL of thionyl chloride and thoroughly stirred at room temperature. After 2 h, the solvent and the excess reagent were removed under reduced pressure. The residue, 2,3,4-trimethoxybenzoyl chloride was dissolved in anhydrous ether (80 mL), 1,3,5-trimethoxybenzene (2.20 g, 13.03 mmol) and $AlCl_3$ (5.0 g) were added. After stirring for 8 h at room temperature, the mixture was hydrolyzed with ice-cold water (500 mL) containing concentrated HCl (45 mL) and extracted with CH_2Cl_2. Solvent removal and purification with gel-column chromatography ($CHCl_3$) gave yellow solid 2-hydroxy-2′,3,4,4′,6′-pentamethoxybenzophenone (2.97 g, 8.53 mmol, 65%). The yellow solid (2.97 g, 8.53 mmol) was treated with pyridine (100 mL), H_2O (50 mL) and aqueous 10% tetramethylammonium hydroxide (45 mL). The mixture was refluxed for 36 h, poured into ice, acidified with HCl,

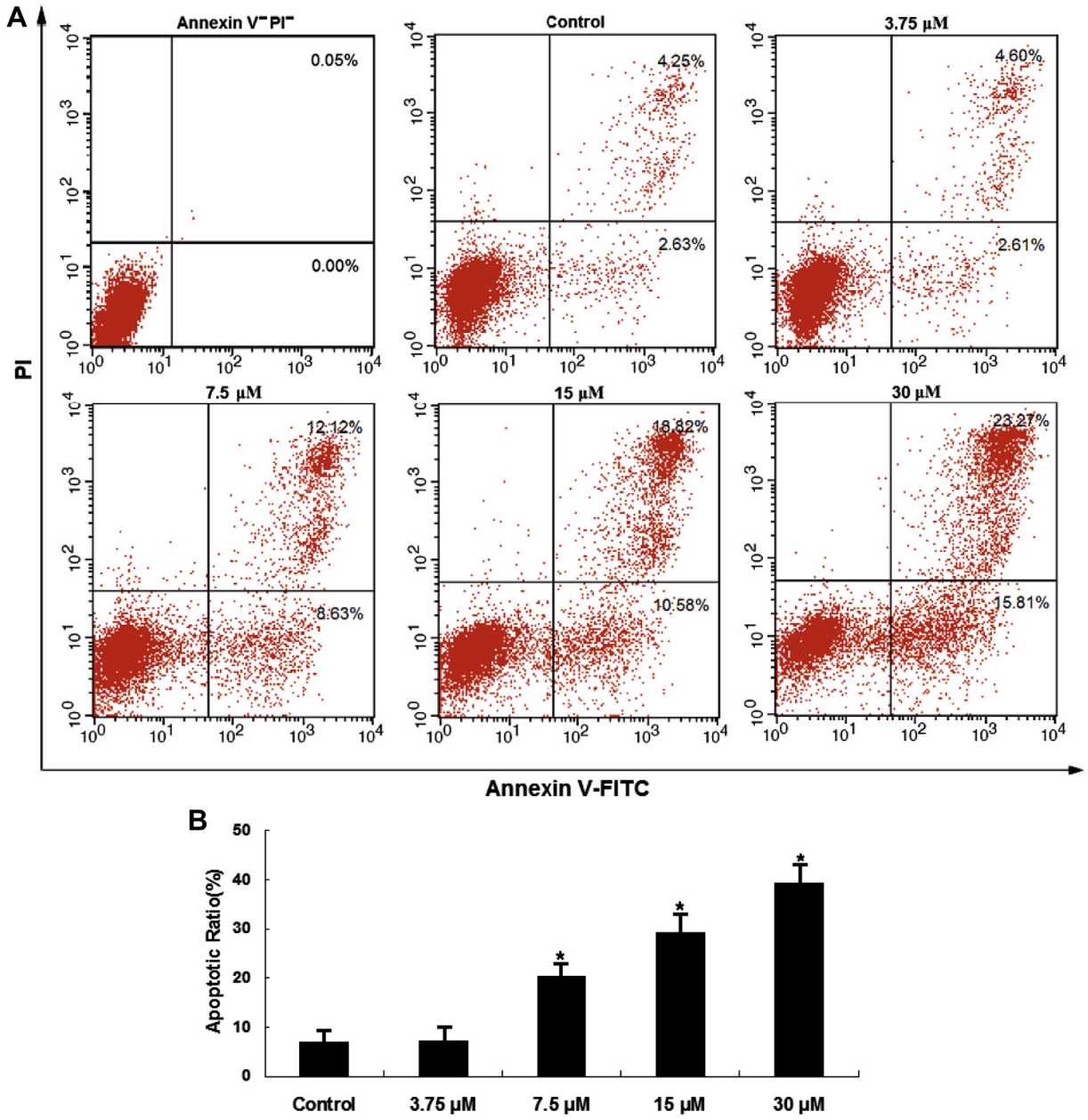


Fig. 2. Effects of compound **6** on QGY-7703 cell apoptosis. QGY-7703 cells were treated with DMSO (control) or compound **6** at various concentrations for 48 h and apoptosis was quantified using flow cytometry after staining with annexin V/PI. A: Flow cytometry analysis of apoptotic QGY-7703 cells; B: Quantitation of A. Data were presented as the mean ± SD for three independent experiments. $^*P < 0.05$ versus control cells.

and extracted with ether. Purification with gel-chromatography ($CHCl_3$) and recrystallization ($CHCl_3$) gave colorless needle crystals, 1,3,5,6-tetramethoxy xanthone (2.24 g, 7.08 mmol, 83%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm), 7.78 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H), 6.71 (d, J = 2.4 Hz, 1H), 6.49 (d, J = 2.4 Hz, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 174.9, 166.5, 163.3, 161.0, 158.3, 150.4, 137.3, 123.0, 119.0, 111.0, 107.7, 97.4, 95.2, 62.8, 58.2, 58.0, 57.9; HR-ESI-MS calcd for $C_{17}H_{16}O_6$ $[M + H]^+$ 316.0947, found 316.0949.

5.1.3. Synthesis of compound ***5***

*5.1.3.1. 4-Iodo-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**5**, $C_{17}H_{15}IO_6$).* Method I: A solution of compound **4** (1.11 g, 3.5 mmol), iodine (1.9 g, 7.5 mmol) in DMF (5 mL) was stirred under argon at room temperature for 2 days, the reaction mixture was poured into water (15 mL), followed by filtration to give a brown powder. The brown powder was stirred in cyclohexene (100 mL) for 3 h to give a light tan color powder **5** (1.31 g, 2.98 mmol, 85%) after filtration; Method II: A solution of compound **4** (1.11 g, 3.5 mmol), NIS (1.18 g, 5.25 mmol) in CH_2Cl_2 (5 mL) was stirred under argon at room temperature overnight, the reaction mixture was quenched with 1 M aqueous sodium hydroxide and extracted with CH_2Cl_2, the combined organic layer was washed successively with brine and dried over $MgSO_4$, filtered and concentrated. The crude material was purified by column chromatographed to yield light tan color powder (1.31 g, 2.98 mmol, 85%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.78 (d, J = 9.0 Hz, 1H), 7.17 (d, J = 9.0 Hz, 1H), 6.69 (s, 1H), 4.02 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H), 3.94–3.93 (m, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 174.9, 165.3, 164.2, 158.7, 158.7, 150.3, 137.3, 123.4, 118.4, 111.4, 108.6, 94.3, 67.8, 63.0, 59.1, 58.4, 58.3; HR-ESI-MS calcd for $C_{17}H_{15}IO_6$ $[M + H]^+$ 442.9986, found 442.9985.

5.1.4. Synthesis of biaryls (general procedure to ***6*****–****33*)

To a suspension of $Pd(PPh_3)_4$ (0.03 equiv) in anhydrous DME was added the aryl bromide and the mixture was stirred for 10 min at rt. To this solution were added sequentially the arylboronic acid (1.5 equiv) in aqueous Na_2CO_3 (2 M solution, 2.0 equiv), and the mixture was refluxed for 18 h, cooled, and subjected to filtration. The filtrate was evaporated to dryness and the residue was treated with saturated NaCl solution. Standard workup followed by column chromatography gave the biaryl product.

5.1.5. ^{1}H and ^{13}C NMR data of compounds ***6*****–****33***

*5.1.5.1. 1,3,5,6-Tetramethoxy-4-phenyl-9H-xanthen-9-one (**6**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.43 (dd, J = 9.4, 5.4 Hz, 2H), 7.40–7.31 (m, 3H), 7.08 (d, J = 9.1 Hz, 1H), 6.71 (s, 1H), 3.97 (s, 3H), 3.86 (s, 3H), 3.84 (d, 3H), 3.36 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 175.4, 163.3, 163.0, 158.3, 156.9, 150.5, 137.2, 134.1, 133.1, 129.6, 128.9, 122.9, 118.7, 112.5, 110.9, 107.4, 93.9, 62.3, 58.1, 56.5, 56.2; HR-ESI-MS calcd for $C_{23}H_{20}O_6$ $[M + H]^+$ 393.1338, found 393.1269.

*5.1.5.2. 4-(3,5-Bis(trifluoromethyl)phenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**7**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 8.17 (s, 2H), 8.12 (s, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 6.74 (s, 1H), 3.99 (s, 2H), 3.90 (s, 2H), 3.86 (s, 1H), 3.37 (s, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 177.1, 164.0, 161.0, 155.8, 155.7, 148.6, 137.9, 136.9, 133.0, 133.0, 128.8, 128.8, 122.4, 122.4, 119.5, 118.8, 116.7, 113.6, 108.9, 107.5, 96.3, 61.0, 56.7, 56.6, 56.2; HR-ESI-MS calcd for $C_{25}H_{18}F_6O_6$ $[M + H]^+$ 529.1080, found 529.1086.

*5.1.5.3. 4-(1,3,5,6-Tetramethoxy-9-oxo-9H-xanthen-4-yl)benzaldehyde (**8**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 10.07 (s, 1H), 7.98 (d, J = 8.2 Hz, 2H), 7.76 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 8.2 Hz, 2H), 6.82 (d, J = 9.0 Hz, 1H), 6.74 (s, 1H), 3.99 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.40 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 194.8, 175.3, 163.6, 158.5, 157.9, 156.7, 150.5, 144.8, 140.8, 137.2, 136.9, 134.0, 130.7, 129.3, 123.0, 118.7, 115.8, 111.2, 111.0, 107.5, 94.0, 62.4, 61.6, 58.2, 51.6; HR-ESI-MS calcd for $C_{24}H_{20}O_7$ $[M + H]^+$ 421.1282, found 421.1276.

*5.1.5.4. 4-(3,5-Difluorophenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**9**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.75 (d, J = 8.9 Hz, 1H), 7.24 (t, J = 9.4 Hz, 1H), 7.20–7.17 (m, 2H), 7.11 (d, J = 9.2 Hz, 1H), 6.72 (s, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H), 3.50 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 177.1, 163.7, 162.1, 162.1, 161.0, 155.7, 155.2, 148.6, 140.2, 136.9, 118.8, 116.7, 113.4, 109.4, 109.4, 108.9, 107.5, 100.8, 90.2, 61.0, 56.7, 56.6, 56.2; HR-ESI-MS calcd for $C_{23}H_{18}O_6F_2$ $[M + H]^+$ 428.1071, found 428.1053.

*5.1.5.5. 1,3,5,6-Tetramethoxy-4-(4-(trifluoromethoxy)phenyl)-9H-xanthen-9-one (**10**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.79–7.69 (m, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.08 (d, J = 9.1 Hz, 1H), 6.71 (s, 1H), 3.97 (s, 3H), 3.89–3.88 (m, 3H), 3.85 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.3, 163.3, 163.2, 158.4, 157.3, 156.8, 150.4, 149.3, 137.1, 136.8, 133.6, 123.0, 122.9, 122.3, 121.7, 121.2, 118.7, 110.9, 107.4, 93.9, 62.1 (2C), 58.2 (2C); HR-ESI-MS calcd for $C_{24}H_{19}F_3O_7$ $[M + H]^+$ 477.1156, found 477.1170.

*5.1.5.6. 4-(4-Fluorophenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**11**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.73 (d, J = 8.9 Hz, 1H), 7.46–7.38 (m, 2H), 7.30–7.21 (m, 2H), 7.07 (d, J = 9.1 Hz, 1H), 6.69 (s, 1H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.42 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.3, 164.1, 163.3, 163.1, 162.5, 158.4, 156.9, 150.5, 137.2, 135.1, 135.1, 130.3, 123.0, 116.5, 116.4, 111.3, 110.9, 107.4, 93.9, 62.4 (2C), 58.2 (2C); HR-ESI-MS calcd for $C_{23}H_{19}FO_6$ $[M + H]^+$ 411.1238, found 411.1246.

*5.1.5.7. 4-(4-Ethylphenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**12**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.62–7.58 (m, 2H), 7.27 (d, J = 1.4 Hz, 2H), 7.07 (d, J = 9.1 Hz, 1H), 6.70 (s, 1H), 3.97 (s, 3H), 3.87 (s, 3H), 3.85–3.84 (s, 3H), 3.37 (br. s., 3H), 2.66 (q, J = 7.7 Hz, 2H), 1.25–1.21 (t, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 175.4, 163.4, 162.9, 158.3, 156.9, 150.5, 144.5, 137.3, 133.9, 133.4, 133.3, 133.0, 130.7, 130.6, 129.0, 122.9, 112.5, 110.8, 107.4, 94.0, 62.3, 58.2, 58.2, 58.1, 29.9, 17.7; HR-ESI-MS calcd for $C_{25}H_{24}O_6$ $[M + H]^+$ 421.1646, found 421.1657.

*5.1.5.8. 1,3,5,6-Tetramethoxy-4-p-tolyl-9H-xanthen-9-one (**13**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.26–7.22 (m, 4H), 7.07 (d, J = 8.9 Hz, 1H), 6.70 (s, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.39 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 175.3, 163.4, 162.9, 158.3, 156.9, 150.5, 144.3, 138.1, 133.9, 137.3, 132.9, 131.0, 130.2, 122.9, 118.7, 112.4, 110.8, 107.4, 94.0, 62.4, 58.2, 58.2, 58.1, 22.8; HR-ESI-MS calcd for $C_{24}H_{22}O_6$ $[M + H]^+$ 406.1416, found 406.1428.

*5.1.5.9. 4-(4-Isopropylphenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**14**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.73 (d, J = 8.9 Hz, 1H), 7.30–7.26 (m, 4H), 7.07 (d, J = 9.1 Hz, 1H), 6.70 (s, 1H), 3.97–3.96 (m, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.32 (s, 3H), 2.97–2.91 (m, 1H), 1.26–1.24 (m, 6H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 175.4, 163.4, 162.9, 158.3, 156.9, 150.5, 149.1, 137.2, 133.9, 133.3, 132.9, 130.7, 130.6, 127.5, 122.9, 118.7, 112.5, 110.9, 107.4, 93.9, 62.2, 58.2, 58.2, 58.1, 35.2, 25.9; HR-ESI-MS calcd for $C_{26}H_{26}O_6$ $[M + H]^+$ 435.1802, found 435.1834.

*5.1.5.10. 1,3,5,6-Tetramethoxy-4-(4-(trifluoromethyl)phenyl)-9H-xanthen-9-one (**15**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.83 (d, J = 8.9 Hz, 1H), 7.76 (m, 2H), 7.74 (m, 2H), 7.04 (d, J = 8.9 Hz, 1H), 6.50 (s, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.84 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 177.1, 163.3, 161.4, 155.7, 154.1, 148.6, 140.5, 136.9, 129.9, 129.9, 129.0, 123.2, 123.2, 124.9, 118.8, 116.7, 114.0, 108.9, 107.6, 94.9, 61.0, 56.7, 56.6, 56.3; HR-ESI-MS calcd for $C_{24}H_{19}F_3O_6$ $[M + H]^+$ 461.1206, found 461.1209.

*5.1.5.11. 4-(4-tert-Butylphenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**16**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.73 (d, J = 8.9 Hz, 1H), 7.46–7.43 (m, 2H), 7.30–7.27 (m, 2H), 7.07 (d, J = 9.1 Hz, 1H), 6.71 (s, 1H), 3.97 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 3.36 (br. s., 3H), 1.33 (s, 9H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 175.4, 163.4, 162.9, 158.3, 157.0, 151.3, 150.5, 137.2, 132.7, 131.1 (2C), 126.3 (2C), 122.9, 118.7, 112.4, 110.8, 107.4, 93.9, 62.1, 58.2, 58.2, 58.1, 36.2, 33.1 (3C); HR-ESI-MS calcd for $C_{27}H_{28}O_6$ $[M + H]^+$ 449.1959, found 449.1937.

*5.1.5.12. 4-(4-Acetylphenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**17**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 8.01 (s, 2H), 7.75 (d, J = 8.9 Hz, 1H), 7.59–7.55 (m, 2H), 7.09 (d, J = 8.9 Hz, 1H), 6.74 (s, 1H), 3.98 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.40 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 199.6, 175.3, 163.5, 163.2, 158.5, 156.7, 150.5, 137.4, 133.5, 133.4, 129.4, 123.0, 118.7, 111.5, 111.3, 111.0, 107.4, 94.0, 93.9, 66.9, 62.4, 61.6, 58.3, 58.2, 28.7; HR-ESI-MS calcd for $C_{25}H_{22}O_7$ $[M + H]^+$ 435.1438, found 435.1428.

*5.1.5.13. Methyl 4-(1,3,5,6-tetramethoxy-9-oxo-9H-xanthen-4-yl) benzoate (**18**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 8.03–8.01 (m, 2H), 7.75 (d, J = 8.9 Hz, 1H), 7.57–7.55 (m, 2H), 7.09 (d, J = 9.1 Hz, 1H), 6.73 (s, 1H), 3.98 (s, 3H), 3.88 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.38 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm), 175.3, 168.1, 163.5, 163.2, 158.4, 156.7, 150.4, 139.4, 137.2, 133.6, 130.7, 130.6, 130.4, 130.2, 123.0, 118.7, 111.2, 111.0, 107.4, 94.0, 62.4, 58.4,58.2, 58.2, 54.1; HR-ESI-MS calcd for $C_{25}H_{22}O_8$ $[M + H]^+$ 450.1314, found 450.1322.

*5.1.5.14. 1,3,5,6-Tetramethoxy-4-(4-(methylsulfonyl)phenyl)-9H-xanthen-9-one (**19**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.99 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 9.1 Hz, 1H), 6.75 (s, 1H), 3.99 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.39 (s, 3H), 3.28 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.3, 163.7, 163.1, 158.4, 156.9, 150.5, 141.4, 139.8, 137.2, 134.2, 128.2, 123.0, 118.6, 111.1, 110.7, 107.4, 94.0, 62.3, 58.3, 58.2, 58.1, 45.5; HR-ESI-MS calcd for $C_{24}H_{22}O_8S$ $[M + H]^+$ 471.1108, found 471.1105.

*5.1.5.15. 4-(1,3,5,6-Tetramethoxy-9-oxo-9H-xanthen-4-yl)benzonitrile (**20**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.92–7.89 (m, 2H), 7.75 (d, J = 8.9 Hz, 1H), 7.65–7.63 (m, 2H), 7.10 (d, J = 9.1 Hz, 1H), 6.73 (s, 1H), 3.98 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.44 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.2, 163.7, 163.1, 158.5, 156.7, 150.4, 139.5, 137.2, 134.4, 134.3, 133.4, 133.4, 123.0, 120.9, 118.7, 111.7, 111.0, 110.6, 107.4, 94.0, 62.4, 58.3, 58.3, 58.2; HR-ESI-MS calcd for $C_{24}H_{19}NO_6$ $[M + H]^+$ 418.1285, found 418.1283.

*5.1.5.16. 3-(1,3,5,6-Tetramethoxy-9-oxo-9H-xanthen-4-yl)benzaldehyde (**21**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 10.05 (s, 1H), 7.96–7.95 (m, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.77–7.74 (m, 2H), 7.70–7.66 (m, 1H), 7.09 (d, J = 9.1 Hz, 1H), 6.74 (s, 1H), 3.99 (s, 4H), 3.89 (s, 3H), 3.85 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 195.0, 175.3, 163.5, 163.2, 158.4, 156.8, 150.5, 139.5, 137.9, 137.1, 135.1, 134.5, 130.6, 130.0, 123.0, 118.7, 111.0, 107.4, 94.0, 62.3, 58.3, 58.2, 58.2; HR-ESI-MS calcd for $C_{24}H_{20}O_7$ $[M + H]^+$ 421.1282, found 421.1280.

*5.1.5.17. 4-(2-Fluorophenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**22**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.75 (d, J = 9.0 Hz, 1H), 7.50–7.39 (m, 2H), 7.32–7.25 (m, 2H), 7.09 (d, J = 9.1 Hz, 1H), 6.73 (s, 1H), 3.99 (s, 3H), 3.89 (s, 3H), 3.86–3.83 (m, 3H), 3.38 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.2, 163.8, 163.7, 158.4, 157.1, 150.4, 137.2, 135.3, 131.7, 131.7, 125.8, 123.0, 118.7, 117.2, 117.0, 111.0, 107.3, 105.9, 94.0, 62.2, 58.3, 58.2, 58.2; HR-ESI-MS calcd for $C_{23}H_{19}FO_6$ $[M + H]^+$ 411.1238, found 411.1242.

*5.1.5.18. 3-(1,3,5,6-Tetramethoxy-9-oxo-9H-xanthen-4-yl)benzonitrile (**23**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.95 (m, 1H), 7.84 (m, 1H), 7.75 (d, J = 9.2 Hz, 1H), 7.66 (m, 2H), 7.11 (d, J = 9.2 Hz, 1H), 6.74 (s, 1H), 3.99 (s, 1H), 3.90 (s, 1H), 3.87 (s, 1H), 3.47 (s, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.2, 164.1, 162.1, 158.7, 157.1, 150.5, 138.6, 136.9, 132.6, 131.6, 130.0, 128.3, 118.8, 117.4, 116.7, 116.5, 114.7, 108.9, 108.1, 94.0, 62.0, 58.7, 58.6, 58.3; HR-ESI-MS calcd for $C_{24}H_{19}NO_6$ $[M + H]^+$ 418.1285, found 418.1283.

*5.1.5.19. 1,3,5,6-Tetramethoxy-4-(3-methoxyphenyl)-9H-xanthen-9-one (**24**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.36–7.32 (m, 1H), 7.08 (d, J = 9.1 Hz, 1H), 6.95 (m, 1H), 6.94–6.93 (m, 2H), 6.70 (s, 1H), 3.97 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H), 3.39–3.38 (m, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.5, 163.3, 163.0, 160.7, 158.3, 156.8, 150.5, 137.2, 135.3, 130.6, 125.4, 122.9, 118.8, 118.7, 114.5, 112.4, 110.8, 107.4, 94.0, 62.3, 58.2, 58.2, 58.1, 56.9; HR-ESI-MS calcd for $C_{24}H_{22}O_7$ $[M + H]^+$ 423.1438, found 423.1427.

*5.1.5.20. 1,3,5,6-Tetramethoxy-4-(3-(trifluoromethyl)phenyl)-9H-xanthen-9-one (**25**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.77 (m, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.70–7.69 (m, 1H), 7.68–7.67 (m, 1H), 7.08 (d, J = 9.1 Hz, 1H), 6.84–6.81 (m, 1H), 6.72 (s, 1H), 3.98 (s, 1H), 3.89 (s, 1H), 3.85 (s, 1H), 3.47 (s, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 177.1, 163.8, 161.1, 155.7, 154.7, 148.5, 138.1, 136.9, 131.0, 130.4, 129.7, 126.5, 125.6, 122.3, 118.8, 116.7, 113.7, 108.9, 107.6, 95.7, 61.0, 56.7, 56.6, 56.3; HR-ESI-MS calcd for $C_{24}H_{19}F_3O_6$ $[M + H]^+$ 461.1206, found 461.1194.

*5.1.5.21. 4-(3,5-Dimethoxyphenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**26**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 9.1 Hz, 1H), 7.11 (d, J = 9.2 Hz, 1H), 6.83–6.81 (m, 1H), 6.70 (s, 1H), 6.52 (m, 2H), 3.97–3.95 (m, 3H), 3.89–3.87 (m, 3H), 3.85 (s, 3H), 3.73 (s, 6H), 3.42 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.4, 163.3, 163.0, 161.8, 158.3, 157.9, 156.8, 150.5, 144.8, 137.3, 135.9, 129.3, 122.9, 115.8, 111.2, 110.9, 107.3, 101.1, 93.9, 70.8, 62.2, 58.2, 58.1, 57.0, 57.0; HR-ESI-MS calcd for $C_{25}H_{24}O_8$ $[M + H]^+$ 453.1544, found 453.1508.

*5.1.5.22. 1,3,5,6-Tetramethoxy-4-(pyridin-3-yl)-9H-xanthen-9-one (**27**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 8.60–8.60 (m, 1H), 8.60–8.58 (m, 1H), 8.56 (dd, J = 1.5, 4.8 Hz, 1H), 7.74 (d, J = 9.1 Hz, 1H), 7.50–7.46 (m, 1H), 7.08 (d, J = 8.9 Hz, 1H), 6.72 (s, 1H), 3.97 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.42 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.3, 163.6, 163.4, 158.5, 157.0, 153.4, 150.4, 149.9, 140.6, 137.2, 130.1, 124.9, 123.0, 118.7, 111.0, 108.9, 107.4, 93.9, 62.4, 58.3, 58.2, 58.2; HR-ESI-MS calcd for $C_{22}H_{19}O_6N$ $[M + H]^+$ 393.1212, found 393.1224.

*5.1.5.23. 1,3,5,6-Tetramethoxy-4-(2-(trifluoromethoxy)phenyl)-9H-xanthen-9-one (**28**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz):

δ (ppm), 7.77 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.9 Hz, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.14 (m, 2H), 7.09 (d, J = 9.2 Hz, 1H), 6.74 (s, 1H), 3.99 (s, 3H), 3.88 (s, 3H), 3.85–3.84 (m, 3H), 3.33 (br. s., 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.1, 166.5, 163.8, 163.6, 158.4, 157.1, 150.4, 149.0, 135.6, 133.9, 133.3, 131.6, 130.7, 130.6, 129.0, 123.0, 122.6, 118.6, 111.0, 93.7, 62.8, 62.0, 58.2, 58.2; HR-ESI-MS calcd for $C_{24}H_{19}F_3O_7$ $[M + H]^+$ 477.1156, found 477.1170.

*5.1.5.24. 1,3,5,6-Tetramethoxy-4-m-tolyl-9H-xanthen-9-one (**29**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.33–7.29 (m, 1H), 7.20–7.16 (m, 2H), 7.14 (d, J = 7.7 Hz, 1H), 7.07 (d, J = 9.1 Hz, 1H), 6.70 (s, 1H), 3.97 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.38 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.4, 163.3, 162.9, 158.3, 156.8, 150.5, 138.6, 137.3, 134.0, 133.6, 130.1, 129.6, 129.5, 122.9, 118.7, 112.6, 110.9, 107.4, 93.9, 62.3, 58.2, 58.1, 58.0, 22.9; HR-ESI-MS calcd for $C_{24}H_{22}O_6$ $[M + H]^+$ 406.1416, found 406.1428.

*5.1.5.25. 4-(3,4-Difluorophenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**30**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.56–7.45 (m, 2H), 7.27–7.22 (m, 1H), 7.09 (d, J = 9.1 Hz, 1H), 6.69 (s, 1H), 3.96 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 3.49 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.2, 163.4, 163.2, 158.5, 156.8, 150.5, 137.2, 131.4, 130.3, 123.0, 122.3, 122.2, 118.7, 118.6, 118.5, 111.0, 110.2, 107.4, 93.9, 62.3, 58.3, 58.2, 58.1; HR-ESI-MS calcd for $C_{23}H_{18}F_2O_6$ $[M + H]^+$ 429.1144, found 429.1131.

*5.1.5.26. 4-(3-Fluorophenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**31**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.75 (d, J = 8.9 Hz, 1H), 7.49–7.45 (m, 1H), 7.28–7.24 (m, 1H), 7.24–7.22 (m, 1H), 7.21–7.18 (m, 1H), 7.09 (d, J = 9.1 Hz, 1H), 6.71 (s, 1H), 3.97 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.43 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.3, 163.4, 163.2, 158.5, 156.8, 150.5, 137.2, 131.4, 131.3, 129.4, 123.0, 120.0, 119.9, 118.7, 115.7, 111.1, 111.0, 107.4, 93.9, 62.3, 58.2, 58.2 (2C); HR-ESI-MS calcd for $C_{23}H_{19}FO_6$ $[M + H]^+$ 411.1238, found 411.1249.

*5.1.5.27. 4-(2,5-Dimethylphenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**32**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.55–7.50 (m, 1H), 7.43 (d, J = 8.1 Hz, 2H), 7.09 (d, J = 9.1 Hz, 1H), 6.72 (s, 1H), 3.98 (s, 3H), 3.91–3.89 (m, 3H), 3.85 (s, 3H), 3.37 (s, 3H), 2.35 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 177.5, 161.4, 160.2, 155.7, 155.1, 149.3, 137.6, 136.5, 135.7, 134.7, 130.1, 127.8, 126.7, 118.8, 116.7, 114.1, 108.9, 107.4, 94.6, 61.0, 56.7, 56.6, 56.3, 21.3, 20.1; HR-ESI-MS calcd for $C_{25}H_{24}O_6$ $[M + H]^+$ 420.1573, found 420.1586.

*5.1.5.28. 4-(2,5-Difluorophenyl)-1,3,5,6-tetramethoxy-9H-xanthen-9-one (**33**).* White solid; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm), 7.74 (d, J = 8.9 Hz, 1H), 7.35 (dd, J = 6.8, 11.4 Hz, 1H), 7.17–7.12 (m, 2H), 7.10 (d, J = 9.1 Hz, 1H), 6.72 (s, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H), 3.46 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm), 175.1, 164.1, 163.5, 159.1, 158.5, 157.5, 157.0, 150.3, 137.1, 123.1, 118.7, 118.5, 118.5, 118.4, 118.3, 111.1, 107.3, 104.8, 93.9, 62.2, 58.3, 58.3, 58.2; HR-ESI-MS calcd for $C_{23}H_{18}F_2O_6$ $[M + H]^+$ 429.1144, found 429.1167.

5.2. Cell culture

Human hepatocellular carcinoma (HCC) cell lines QGY-7703, HepG-2 and SMMC-7721 and normal hepatic cell lines QSG-7701 were obtained from Cell Resource Center, Institute of Life Sciences, Chinese Academy of Medical Science. QGY-7703, SMMC-7721 and QSG-7701 cells were maintained in 1640 medium (GBICO) and HepG-2 cells were maintained in MEM medium (GBICO), both of the medium were supplemented with 10% fetal bovine serum, 100 Units/mL penicillin/streptomycin at 37 °C, 5% CO_2.

5.3. Cell proliferation/viability assay

Cell proliferation was measured using a Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. In brief, the cells (2×10^5 cells/well in 100 μL medium) were cultivated for 24 h in 96-well microplates in a humidified 5% CO_2 incubator at 37 °C, then treated with varying concentrations of compounds or 1% DMSO vehicle control. After incubation for 48 h, 10 μL of CCK8 solution was added to each well, then the samples were incubated for additional 3 h before the absorbance was measured at 450 nm by an epoch microplate spectrophotometer.

5.4. Flow cytometry analysis

For measuring apoptosis, QGY-7703 cells were seeded in six-plates (5×10^5 cells/mL) for 24 h and then treated with compound **6** at 3.75, 7.5, 15 and 30 μM. After 48 h, the cells floating in the medium were collected and the adherent cells were detached with 0.05% trypsin, washed twice with cold PBS, and centrifuged at $1000 \times g$ for 5 min at 4 °C. Subsequently, QGY-7703 cells were gently resuspended in the binding buffer. Thereafter, the cells were stained using Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, San Jose, CA, USA). After incubation at room temperature for 15 min in the dark, the apoptotic cells were immediately analyzed by flow cytometry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.08.020. These data include MOL files and InChiKeys of the most important compounds described in this article.

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