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Synthesis and Anti-tumor Evaluation of B-ring Modified Caged Xanthone Analogues of Gambogic Acid

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Gambogic acid (GA, **1**), the most prominent member of *Garcinia* natural products, has been reported to be a promising anti-tumor agent. Previous studies have suggested that the planar B ring and the unique 4-oxa-tricyclo-[4.3.1.$0^{3,7}$]dec-2-one caged motif were essential for anti-tumor activity. To further explore the structure-activity relationship (SAR) of caged *Garcinia* xanthones, two new series of B-ring modified caged GA analogues **13a**—**13e** and **15a**—**15e** were synthesized utilizing a Claisen/Diel-Alder cascade reaction. Subsequently, these compounds were evaluated for their *in vitro* anti-tumor activities against A549, MCF-7, SMMC-7721 and BGC-823 cancer cell lines by MTT assay. Among them, **13b**—**13e** exhibited micromolar inhibition against several cancer cell lines, being approximately 2—4 fold less potent in comparison to GA. SAR analysis revealed that the peripheral gem-dimethyl groups are essential for maintaining anti-tumor activity and substituent group on C1 position of B-ring has a significant effect on potency, while modifications at C-2, C-3 and C-4 positions are relatively tolerated. These findings will enhance our understanding of the SAR of *Garcinia* xanthones and lead to the development of simplified analogues as potential anti-tumor agents.

Keywords 4-oxa-tricyclo[4.3.1.$0^{3,7}$]dec-2-one, gambogic acid, synthesis, anti-tumor activity, SAR studies

Introduction

Gamboge (tenghuang in Chinese), the resin secreted by tropical trees of the genus *Garcinia*, has been used as pigments and traditional medicine in Southeast Asia for hundreds of years.[1,2] Efforts to identify the bioactive constituents of gamboge have yielded a growing family of natural products defined as "caged xanthones", the structural features of which share an intriguing 4-oxa-tricyclo[4.3.1.$0^{3,7}$]dec-2-one scaffold merged into a common xanthone backbone.[3-5] Gambogic acid (GA, **1**), the most prominent member of these caged xanthones, has been found to selectively inhibit tumor cell proliferation and exhibit potent anti-tumor activity both *in vitro* and *in vivo*.[6-8] In addition, **1** has recently finished phase II clinical trials in cancer patients in China, which suggests the promising clinical potential of the caged *Garcinia* xanthones and designed analogues.

Biological studies revealed that the mode of action of **1** was complex, involving apoptotic induction, cell cycle arrest, cell metastasis inhibition as well as anti-angiogenesis.[9-11] Furthermore, **1** was reported to suppress telomerase activity,[12] inhibit CDK7-mediated phosphorylation of CDC2/34,[13] regulate the expressions of bcl-2 family proteins,[9] inhibit VEGFR2 activation,[14,15] induce proteasome-mediated degradation of mutant p53,[16] elevate the phosphorylation of JNK and p38,[17] inhibit Hsp90 activity[18] and interact with the transferrin receptor TfR1.[19] Recent interaction studies using biotinylated GA demonstrated that **1** covalently binded to IKKβ to inhibit NF-κB activation,[20] which established the role of 4-oxa-tricyclo[4.3.1.$0^{3,7}$]dec-2-one moiety as a bioactive Michael acceptor.

Preliminary SAR studies of GA indicated that modifications of the peripheral moieties such as 6-hydroxy group, two prenyl groups and 30-carboxyl groups were well tolerated, while reduction of the C(9)=C(10) double bond of the caged moiety led to dramatically decreased potency. In addition, some analogues (Figure 1) lacking the dihydropyran A ring still retained considerable cytotoxicity, for instance, gaudichaudione A (**2**) showed strong inhibitory activities against parental

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murine leukemic P388 cell lines, and the ability of cluvenone (**3**) to exhibit moderate to potent cytotoxicity against a panel of human cancer cell lines at micromolar concentrations has also been well documented.[21-23] However, further removal of planar B ring resulted in substantial loss of activity, for example, caged analogues **4** and **5** (Figure 1) with only C, D rings had IC_{50} values more than 80 μmol•L^{-1} against several cell lines.[22,23] These primary SAR information suggested that the D caged core and BC planar region were both essential for the anti-proliferative activity.

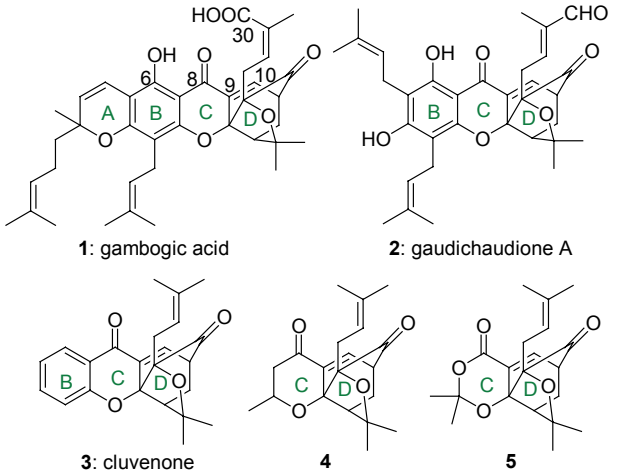


Figure 1 Chemical structures of GA and selected caged analogues.

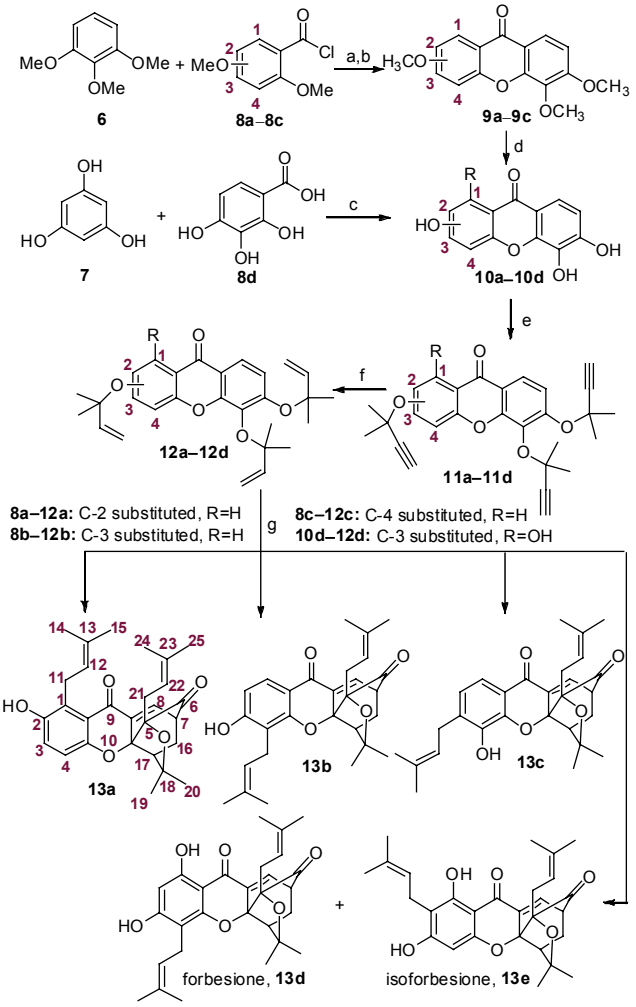
Taking into consideration the structural requirements for anti-tumor activity, two new series of B-ring modified GA analogues that bear both an electrophilic 4-oxa-tricyclo[4.3.1.$0^{3,7}$]dec-2-one caged core and a suitable planar region were designed as shown in Figure 2. Variation of the hydrophilic hydroxyl group and the hydrophobic prenyl group at different positions on B-ring produced analogues **13a**—**13e** for thoroughly exploring the SAR of *Garcinia* xanthones. Subsequently, the peripheral gem-dimethyl groups were cut off to provide more simplified analogues **15a**—**15e**. The SAR of the *in vitro* anti-tumor effects of these compounds was discussed.

Results and Discussion

Chemistry

Synthesis of caged compounds 13a—13e The synthetic routes for caged xanthones **13a**—**13e** are summarized in Scheme 1. Compounds **9a**—**9c** were produced by aluminum trichloride catalyzed Friedel-Crafts acylation of 1,2,3-trimethoxybenzene (**6**) with the corresponding dimethoxybenzoyl chloride (**8a**—**8c**) in diethyl ether followed by an intramolecular cyclization in basic conditions. Demethylation of **9a**—**9c** using 40% hydrobromic acid in acetic acid provided the hydroxyxanthone **10a**—**10c**, while hydroxyxanthone **10d** was

Scheme 1 Synthesis of compounds **13a**—**13e**



Reagents and conditions: (a) $AlCl_3$, Et_2O, r.t., 12 h; (b) 5 mol/L NaOH, CH_3OH, reflux, 36 h; (c) $ZnCl_2$, $POCl_3$, 65 °C, 3 h; (d) 40% HBr and HOAc mixed solvent, reflux, 12 h; (e) 2-chloro-2methylbutyne, K_2CO_3, KI, CuI, acetone, 45 °C, 5 h; (f) 10% Pd/$BaSO_4$, EtOH, 25 °C, 2 h; (g) DMF, 120 °C, 1 h.

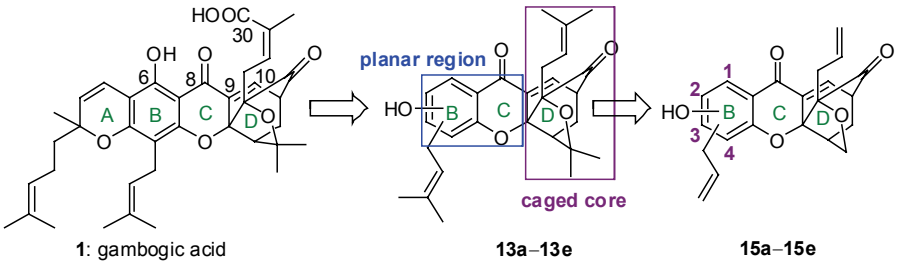


Figure 2 Design of simplified caged xanthones.

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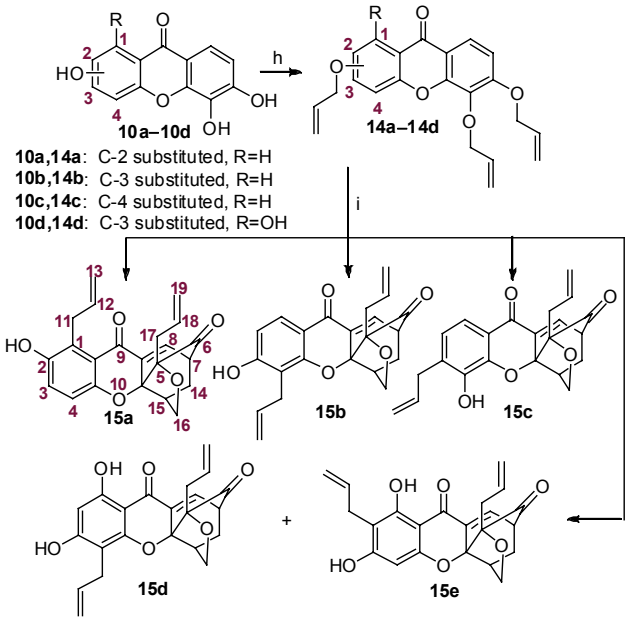
obtained by a zinc chloride mediated condensation of phloroglucinol (**7**) with 2,3,4-trihydroxybenzoic acid (**8d**) in $POCl_3$ in one step.[24]

Propargylation of phenols of **10a** — **10d** with 2-chloro-2-methyl butyne gave rise to tris(dimethylallyloxy)xanthones **11a**—**11d**. Further reduction of the alkynyl groups using Lindlar catalyst led to the precursors **12a**—**12d**. Subsequently, application of the expected Claisen/Diels-Alder cascade reaction by heating **12a**—**12c** in DMF[24-27] furnished caged xanthones **13a**—**13c**, respectively, while **12d** afforded forbesione (**13d**) and isoforbesione (**13e**) as isomers.

Synthesis of caged compounds 15a—**15e** The synthetic routes for caged xanthones **15a**—**15e** are summarized in Scheme 2. Compounds **14a**—**14d** were obtained by allylation of **10a**—**10d** using allyl chloride in the presence of potassium iodide and potassium carbonate in acetone. Subsequently, application of the expected Claisen/Diels-Alder cascade reaction by heating **14a**—**14c** in decalin[28] furnished caged xanthones **15a**—**15c**, respectively, while **14d** afforded caged xanthones **15d** and **15e** as isomers.

Scheme 2 Synthesis of compounds **15a**—**15e**



Reagents and conditions: (h) allyl chloride, K_2CO_3, KI, acetone, 45 °C; (i) decalin, reflux, 3 h.

Anti-proliferation and SAR studies

The *in vitro* anti-proliferative activities of the caged xanthones were assessed by the tetrazolium-based MTT assay using human lung adenocarcinoma A549 cell line, human breast carcinoma MCF-7 cell line, human hepatocellular carcinoma SMMC-7721 cell line and human gastric carcinoma BGC-823 cell line. Cancer cells were allowed to proliferate in presence of the respective compounds for 24 h, and then cell viability was evaluated through measurements of mitochondrial dehydrogenase activity. The results are summarized in Table 1.

Table 1 Anti-proliferative activity of caged xanthones

Compound	$IC_{50}\pm SD^a/(\mu mol\cdot L^{-1})$			
	A549	MCF-7	SMMC-7721	BGC-823
13a	35.0±1.71	11.9±1.11	21.7±0.76	22.9±0.92
13b	17.2±0.82	6.97±0.60	15.0±1.10	18.5±0.55
13c	19.1±1.75	9.06±0.91	20.0±1.04	17.0±1.20
13d	5.34±0.93	6.36±0.12	10.3±0.95	11.9±1.15
13e	5.60±0.88	8.15±0.37	9.50±0.71	6.83±0.15
15a	>100	>100	>100	>100
15b	>100	49.3±3.47	>100	>100
15c	>100	>100	>100	>100
15d	45.2±2.31	14.5±0.10	22.3±1.43	62.3±3.79
15e	52.8±3.28	19.3±1.59	23.2±2.21	39.8±1.95
GA (1)	2.64±0.68	2.19±0.20	3.20±0.36	2.35±0.07

[a] The IC_{50} values were determined from eight different concentrations of compounds at two-fold dilutions, and were the means of two separate experiments.

As shown in Table 1, compounds **13b**—**13e** exhibited moderate anti-tumor activity against the four cancer cell lines. Especially, compound **13d** with micromolar potency was found to be the most active against A549 and MCF-7cancer cell lines and compound **13e** was the most potent against SMMC-7721 and BGC-823 cancer cell lines among all the analogues, whereas they were approximately 24 fold less active than GA. On the other hand, compounds **13a** and **15d**—**15e** showed relatively decreased potency and compounds **15a** — **15c** were mostly inactive at the concentration of 100 $\mu mol\cdot L^{-1}$ against the four cancer cell lines.

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From the data of the anti-tumor activities of these synthesized caged analogues, the following SAR could be derived:

(1) In general, compounds **13a**—**13e** with peripheral gem-dimethyl groups exhibited more potent anti-tumor activities than compounds **15a** — **15e** that lack gem-dimethyl groups. It was even observed that removal of the gem-dimethyl groups of compounds **13a**—**13c** resulted in nearly loss of cytotoxicities as shown by data of **15a**—**15c**, which suggested that the gem-dimethyl groups contributed significantly to the activity of these caged analogues.

(2) When an additional hydroxyl group was intro-

duced at the C-1 position of compound **13b**, the derived corresponding compound **13d** showed enhanced inhibition against the four cancer cells. Similar activity changes were also observed between compounds **15b** and **15d**. It indicated that the presence of hydroxyl group at C-1 position of B-ring was of benefit to anti-tumor activity, which was also in agreement with earlier report from our group.[23]

(3) Among the analogues with gem-dimethyl groups, compound **13a**, possessing prenyl substitution at C-1 position of B ring, exhibited relatively less anti-tumor activity, which demonstrated that hydrophobic prenyl group at C-1 position was unfavorable for cytotoxicity.

(4) Compounds **13b** and **13c**, **13d** and **13e** as well as **15d** and **15e** exhibited similar activities against the four cancer cell lines, which revealed that a hydrophilic hydroxyl group or a hydrophobic prenyl group at relatively different positions among C-2, C-3 and C-4 of B-ring had no significant effect on the anti-tumor activity. It implicated that C-2, C-3 and C-4 sites on B-ring might be suitable for modifications to improve the activity and physical properties.

Conclusions

In conclusion, two new series of B-ring modified simple analogues of GA bearing both the 4-oxa-tricyclo[4.3.1.$0^{3,7}$]dec-2-one caged core and the planar region were synthesized and evaluated for their *in vitro* anti-tumor activities against A549, MCF-7, SMMC-721 and BGC-823 cancer cell lines. Among all the investigated analogues, compounds **13b**—**13e** exhibited micromolar inhibition against several cancer cell lines, being 24 fold less potent in comparison to GA. By analyzing the activity data, some interesting SAR considerations have been highlighted. Firstly, periphery gem-dimethyl groups are essential for maintaining anti-tumor activity. Secondly, substituent group at C-1 position of B-ring has a significant effect on potency, since hydroxyl group at C-1 position enhances the potency while prenyl group reduces it. Thirdly, the variation of hydroxyl or prenyl groups at C-2, C-3 and C-4 positions has no significant effect on the anti-tumor activity, which indicates that these sites could be used for modifications to improve the drug-like properties. These findings will enhance our understanding of the SAR of *Garcinia* xanthones and lead to the development of simplified analogues as potential anti-tumor agents.

Experimental

Chemistry

All reagents were purchased from commercial sources and used without further purification unless otherwise noted. Melting points were determined by a Melt-temp II apparatus and are reported without any correction. IR spectra were recorded on a Nicolet Impact 410 spectrometer using KBr film. The ^{1}H NMR spectra were collected on Bruker AV-300 MHz instruments using deuterated solvents with tetramethylsilane (TMS) as internal standard. EI-MS was recorded on Shimadzu GCMS-2010 apparatus. Each of the target compounds was purified by silica gel (60 Å, 70230 mesh) column chromatography. Concentration and evaporation of the solvent after reaction or extraction was carried out on a rotary evaporator (Büchi Rotavapor) operated at reduced pressure.

General procedure for the synthesis of trimethoxy-9*H*-xanthen-9-one (9a—9c)[23]

To a solution of dimethoxylbenzoic acid (2.39 g, 13.14 mmol) in dry dichloromethane (60 mL) was added oxalyl chloride (5.73 mL, 65.7 mmol) and a catalyst amount of DMF. The mixture was refluxed for 6 h and then concentrated under reduced pressure to afford dimethoxybenzoyl chloride **8a**—**8c** as colorless residue. To a solution of **8a**—**8c** and 1,2,3-trimethoxybenzene (**6**, 2.43 g, 14.45 mmol) in 20 mL anhydrous ether, aluminum trichloride (5.26 g, 39.42 mmol) was added at 0 ℃. The resulting mixture was stirred at 25 ℃ under N_2 protection for 12 h. Then a mixture of 15% hydrochloric acid and ethyl acetate (100 mL, V∶V=1∶1) was added. The ethyl acetate layer was partitioned, washed with brine (30 mL×3), dried over magnesium sulfate and concentrated. The residue was suspended in a solution containing methanol (22.4 mL), water (14.9 mL) and sodium hydrate (4.9 g, 0.12 mol) at 25 ℃. Then the reaction mixture was heated to 110 ℃ for next 36 h. After cooled to 0 ℃, the mixture was acidified with 2 mol•L^{-1} HCl solution till pH=23. The precipitate was formed, filtered, washed with cold water and dried to provide **9a**—**9c**, respectively.

2,5,6-Trimethoxy-9*H*-xanthen-9-one (9a) Yield 51%; white solid; m.p. 155—156 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.93—4.04 (m, 9H, 3×OCH_3), 7.03 (d, J=9.0 Hz, 1H, ArH), 7.32 (dd, J=9.2, 3.1 Hz, 1H, ArH), 7.52 (d, J=9.2 Hz, 1H, ArH), 7.70 (d, J=3.1 Hz, 1H, ArH), 8.11 (d, J=9.0 Hz, 1H, ArH); IR (KBr) v: 3456, 2939, 2830, 1649, 1611, 1593, 1488, 1438, 1289, 1090, 783 cm^{-1}; EI-MS m/z: 286 (M^+).

3,4,6-Trimethoxy-9*H*-xanthen-9-one (9b) Yield 58%; white solid; m.p. 120—121 ℃; ^{1}H NMR (Acetone-d_6, 300 MHz) δ: 3.98—4.03 (m, 9H, 3×OCH_3), 7.00 (dd, J=8.9, 2.4 Hz, 1H, ArH), 7.09 (d, J=2.4 Hz, 1H, ArH), 7.19 (d, J=9.0 Hz, 1H, ArH), 7.95 (d, J=9.0 Hz, 1H, ArH), 8.13 (d, J=8.9 Hz, 1H, ArH); IR (KBr) v: 3437, 2968, 2838, 1653, 1603, 1500, 1461, 1434, 1109, 774 cm^{-1}; EI-MS m/z: 286 (M^+).

3,4,5-Trimethoxy-9*H*-xanthen-9-one (9c) Yield 85%; yellow solid; m.p. 130—131 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.03—4.06 (m, 9H, 3×OCH_3), 7.22 (d, J=9.0 Hz, 1H, ArH), 7.35 (t, J=8.0 Hz, 1H, ArH), 7.45 (dd, J=8.0, 1.6 Hz, 1H, ArH), 7.78 (dd, J=8.0, 1.6 Hz, 1H, ArH), 7.91 (d, J=9.0 Hz, 1H, ArH); IR (KBr) v: 3461, 2968, 2910, 1659, 1602, 1490, 1441, 1285, 1095, 770 cm^{-1}; EI-MS m/z: 286 (M^+).

General procedure for the synthesis of trihydroxy-9*H*-xanthen-9-one (10a—10c)[23]

To a stirred mixture of 40% hydrobromic acid (17 mL) in acetic acid (34 mL) was added **9a**—**9c** (500 mg, 1.75 mmol). The reaction mixture was refluxed for 12 h under N_2 protection. After cooled to 0 ℃, the mixture was basified with 10% NaOH solution till pH=3—4, the precipitate was filtered, washed with ice water and dried to provide **10a**—**10c**, respectively.

2,5,6-Trihydroxy-9*H*-xanthen-9-one (10a) Yield 85%; white solid; m.p.>300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.91 (d, J=8.8 Hz, 1H, ArH), 7.26 (dd, J=9.0, 3.0 Hz, 1H, ArH), 7.44 (d, J=3.0 Hz, 1H, ArH), 7.50 (d, J=9.0 Hz, 1H, ArH), 7.53 (d, J=8.8 Hz, 1H, ArH), 9.82 (s, 1H, OH); IR (KBr) v: 3502, 3416, 3234, 1645, 1626, 1601, 1588, 1462, 1356, 1247, 1062, 787, 772 cm^{-1}; EI-MS m/z: 244 (M^+).

3,4,6-Trihydroxy-9*H*-xanthen-9-one (10b) Yield 75%; white solid; m.p.>300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.83—6.93 (m, 3H, ArH), 7.50 (d, J=8.8 Hz, 1H, ArH), 8.00 (d, J=8.8 Hz, 1H, ArH), 9.28 (s, 1H, OH), 10.31 (s, 1H, OH), 10.77 (s, 1H, OH); IR (KBr) v: 3342, 1607, 1460, 1400, 1339, 1252, 1115, 772, 684 cm^{-1}; EI-MS m/z: 244 (M^+).

3,4,5-Trihydroxy-9*H*-xanthen-9-one (10c) Yield 84%; white solid; m.p.>300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.93 (d, J=9.0 Hz, 1H, ArH), 7.22—7.31 (m, 2H, ArH), 7.53—7.60 (m, 2H, ArH), 9.82 (br s, 3H, 3×OH); IR (KBr) v: 3514, 3269, 3134, 1611, 1584, 1471, 1383, 1342, 1261, 1155, 1053, 763, 457 cm^{-1}; EI-MS m/z: 244 (M^+).

Synthesis of 1,3,5,6-tetrahydroxy-9*H*-xanthen-9-one (10d)[24]

To a solution of 2,3,4-trihydroxybenzoic acid (**10d**) (5.38 g, 31.65 mmol) in $POCl_3$ (95 mL), phloroglucinol (**7**) (5.98 g, 47.48 mmol) and $ZnCl_2$ (29.70 g, 219.50 mmol) were added. The reaction mixture was stirred at 65 ℃ for 3 h. After cooled to 25 ℃, the reaction mixture was poured into a beaker of ice. The dark red precipitate was filtered, washed with ice water and dried. The crude residue was purified by chromatography on a silica-gel column eluted with PE-EtOAc (V∶V=1∶4) to afford xanthone **10d** (4.11 g, 50%) as a yellow solid. m.p.>300 ℃; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 6.16 (d, J=2.1 Hz, 1H, ArH), 6.40 (d, J=2.1 Hz, 1H, ArH), 6.92 (d, J=8.7 Hz, 1H, ArH), 7.49 (d, J=8.7 Hz, 1H, ArH), 9.44 (s, 1H, OH), 10.55 (s, 1H, OH), 10.93 (s, 1H, OH), 13.12 (s, 1H, OH); IR (KBr) v: 3489, 3418, 3212, 3138, 1654, 1615, 1593, 1514, 1462, 1343, 1295, 1212, 1158, 1097, 1061, 806 cm^{-1}; EI-MS m/z: 260 (M^+).

General procedure for the synthesis of tris(2-methylbut-3-yn-2-yloxy)-9*H*-xanthen-9-one (11a—11d)

To a solution of xanthone **10a**—**10d** (4.1 mmol) in acetone (50 mL), potassium iodide (2.5 g, 16.4 mmol), potassium carbonate (2.26 g, 16.4 mmol) and CuI (0.1 g, 0.4 mmol) were added. After the reaction mixture was stirred at 25 ℃ for 10 min, 2-chloro-2-methylbut-3-yne (2.3 mL, 20.5 mmol) was added. The resulted mixture was stirred at 45 ℃ for 5 h, then cooled and filtered. The filtrate was concentrated and purified by chromatography on a silica-gel column eluted with PE-EtOAc (V∶V=8∶1) to afford **11a**—**11d**, respectively.

2,5,6-Tris(2-methylbut-3-yn-2-yloxy)-9*H*-xanthen-9-one (11a) Yield 30%; yellow solid; m.p. 104—105 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.69 (s, 6H, 2×CH_3), 1.76 (s, 6H, 2×CH_3), 1.83 (s, 6H, 2×CH_3), 2.30 (s, 1H, C≡CH), 2.63 (s, 1H, C≡CH), 2.66 (s, 1H, C≡CH), 7.45 (d, J=6.0 Hz, 1H, ArH), 7.53 (dd, J=9.0, 3.0 Hz, 1H, ArH), 7.64 (d, J=9.0 Hz, 1H, ArH), 8.05 (d, J=9.0 Hz, 1H, ArH), 8.13 (d, J=3.0 Hz, 1H, ArH); EI-MS m/z: 442 (M^+).

3,4,6-Tris(2-methylbut-3-yn-2-yloxy)-9*H*-xanthen-9-one (11b) Yield 49%; yellow solid; m.p. 117—119 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.76 (s, 12H, 4×CH_3), 1.82 (s, 6H, 2×CH_3), 2.33 (s, 1H, C≡CH), 2.65 (s, 1H, C≡CH), 2.69 (s, 1H, C≡CH), 7.16 (dd, J=8.7, 2.1 Hz, 1H, ArH), 7.40 (d, J=2.1 Hz, 1H, ArH), 7.62 (d, J=9.0 Hz, 1H, ArH), 8.04 (d, J=9.0 Hz, 1H, ArH), 8.22 (d, J=8.7 Hz, 1H, ArH); EI-MS m/z: 442 (M^+).

3,4,5-Tris(2-methylbut-3-yn-2-yloxy)-9*H*-xanthen-9-one (11c) Yield 45%; yellow solid; m.p. 121—122 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.56 (s, 12H, 4×CH_3), 1.79 (s, 6H, 2×CH_3), 2.24 (s, 1H, C≡CH), 2.62 (s, 1H, C≡CH), 2.66 (s, 1H, C≡CH), 7.27—7.34 (m, 1H, ArH), 7.67 (d, J=9.0 Hz, 1H, ArH), 7.90 (d, J=8.4 Hz, 1H, ArH), 8.02 (d, J=9.3 Hz, 1H, ArH), 8.06 (d, J=9.0 Hz, 1H, ArH); EI-MS m/z: 442 (M^+).

1-Hydroxy-3,5,6-tris(2-methylbut-3-yn-2-yloxy)-9*H*-xanthen-9-one (11d) Yield 25%; yellow solid; m.p. 101—102 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.76 (s, 6H, 2×CH_3), 1.78 (s, 6H, 2×CH_3), 1.82 (s, 6H, 2×CH_3), 2.34 (s, 1H, C≡CH), 2.68 (s, 1H, C≡CH), 2.70 (s, 1H, C≡CH), 6.72 (d, J=2.2 Hz, 1H, ArH), 6.82 (d, J=2.2 Hz, 1H, ArH), 7.64 (d, J=9.0 Hz, 1H, ArH), 7.97 (d, J=9.0 Hz, 1H, ArH), 12.85 (s, 1H, OH); EI-MS m/z: 458 (M^+).

General procedure for the synthesis of tris(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one (12a—12d)

To a solution of **11a**—**11d** (0.53 mmol) in ethanol (10 mL) was added 10% Pd/$BaSO_4$ (20 mg). The reaction mixture was stirred under an atmosphere of hydrogen at 25 ℃ for 2 h and filtered through a plug of silica gel. The filtrate was concentrated and purified by chromatography on a silica-gel column eluted with PE-EtOAc (V∶V=8∶1) to afford **12a**—**12d**, respectively.

2,5,6-Tris(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one (12a) Yield 87%; yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.49 (s, 6H, 2×CH_3), 1.56 (s, 6H, 2×CH_3), 1.58 (s, 6H, 2×CH_3), 5.02—5.24 (m, 6H, 3×CH=CH_2), 6.11—6.34 (m, 3H, 3×CH=CH_2), 7.11 (d, J=9.0 Hz, 1H, ArH), 7.34—7.41 (m, 2H, ArH), 7.86 (d, J=2.7 Hz, 1H, ArH), 7.91 (d, J=9.0 Hz, 1H, ArH);

EI-MS *m*/*z*: 448 (M^+).

3,4,6-Tris(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one (12b) Yield 85%; yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.56 (s, 12H, 4×CH_3), 1.60 (s, 6H, 2×CH_3), 5.16—5.28 (m, 6H, 3×CH=CH_2), 6.11—6.34 (m, 3H, 3×CH=CH_2), 6.95 (dd, J=9.0, 2.1 Hz, 1H, ArH), 7.05—7.10 (m, 2H, ArH), 7.89 (d, J=9.0 Hz, 1H, ArH), 8.14 (d, J=9.0 Hz, 1H, ArH); EI-MS *m*/*z*: 448 (M^+).

3,4,5-Tris(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one (12c) Yield 85%; yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.59 (s, 6H, 2×CH_3), 1.60 (s, 6H, 2×CH_3), 1.63 (s, 6H, 2×CH_3), 4.90—5.26 (m, 6H, 3×CH=CH_2), 6.15—6.33 (m, 2H, 2×CH=CH_2), 6.41—6.47 (m, 1H, CH=CH_2), 7.14 (d, J=9.0 Hz, 1H, ArH), 7.18 (d, J=8.4 Hz, 1H, ArH), 7.41 (dd, J=8.4, 1.2 Hz, 1H, ArH), 7.92 (d, J=9.0 Hz, 1H, ArH), 7.93 (dd, J=8.4, 1.2 Hz, 1H, ArH); EI-MS *m*/*z*: 448 (M^+).

1-Hydroxy-3,5,6-tris(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one (12d)[26] Yield 25%; yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.55 (s, 6H, 2×CH_3), 1.57 (s, 12H, 4×CH_3), 5.02—5.31 (m, 6H, 3×CH=CH_2), 6.12—6.31 (m, 3H, 3×CH=CH_2), 6.42 (d, J=2.1 Hz, 1H, ArH), 6.54 (d, J=2.1 Hz, 1H, ArH), 7.10 (d, J=9.0 Hz, 1H, ArH), 7.81 (d, J=8.9 Hz, 1H, ArH), 12.82 (s, 1H, OH); EI-MS *m*/*z*: 464 (M^+).

General procedure for the synthesis of caged xanthones (13a—13e)

A solution of **12a—12d** (0.45 mmol) in DMF (2.0 mL) was heated at 120 ℃ under N_2 protection for 1 h. The yellow reaction mixture was cooled to 25 ℃ and concentrated. The residue was purified by chromatography on a silica-gel column eluted with PE-EtOAc (V ∶ V=4 ∶ 1) and then crystallized from methanol-H_2O (V ∶ V=5 ∶ 1) to afford **13a—13e**, respectively.

Caged xanthone (13a) Yield 40%; yellow solid; m.p. 138—139 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.03 (s, 3H, C^{14}-H), 1.16—1.23 (m, 4H, C^{24}-H, C^{16}-H_a), 1.32 (s, 3H, C^{15}-H), 1.63 (s, 3H, C^{25}-H), 1.67 (s, 3H, C^{19}-H), 1.77 (s, 3H, C^{20}-H), 2.22 (dd, J=13.5, 4.5 Hz, 1H, C^{16}-H_b), 2.29 (d, J=9.6 Hz, 1H, C^{17}-H), 2.56 (d, J=8.7 Hz, 2H, C^{21}-H), 3.38 (dd, J=6.9, 4.5 Hz, 1H, C^7-H), 3.88 (d, J=6.6 Hz, 2H, C^{11}-H), 4.38 (t, J=8.7 Hz, 1H, C^{22}-H), 5.13 (t, J=6.6 Hz, 1H, C^{12}-H), 5.39 (s, 1H, OH), 6.81 (d, J=9.0 Hz, 1H, C^3-H), 7.00 (d, J=9.0 Hz, 1H, C^4-H), 7.18 (d, J=6.9 Hz, 1H, C^8-H); IR (KBr) *v*: 3506, 3171, 2967, 2918, 1737, 1659, 1606, 1486, 1443, 1376, 1298, 1221, 1145, 1042, 822, 790 cm^{-1}; EI-MS *m*/*z*: 448 (M^+). Anal. calcd for $C_{28}H_{32}O_5$•H_2O: C 72.08, H 7.35; found C 72.10, H 7.30.

Caged xanthone (13b) Yield 53%; yellow solid; m.p. 158—160 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.93 (s, 3H, C^{14}-H), 1.29—1.33 (m, 7H, C^{24}-H, C^{15}-H, C^{16}-H_a), 1.71 (s, 3H, C^{25}-H), 1.77 (s, 3H, C^{19}-H), 1.83 (s, 3H, C^{20}-H), 2.33 (dd, J=13.5, 4.5 Hz, 1H, C^{16}-H_b), 2.50 (d, J=9.3 Hz, 1H, C^{17}-H), 2.57 (d, J=8.7 Hz, 2H, C^{21}-H), 3.46—3.55 (m, 3H, C^{11}-H, C^7-H), 4.40—4.45 (m, 1H, C^{22}-H), 5.28 (t, J=6.6 Hz, 1H, C^{12}-H), 6.30 (s, 1H, OH), 6.58 (d, J=8.7 Hz, 1H, C^2-H), 7.43 (d, J=6.9 Hz, 1H, C^8-H), 7.80 (d, J=8.7 Hz, 1H, C^1-H); IR (KBr) *v*: 3438, 2964, 2922, 2849, 1738, 1649, 1605, 1433, 1298, 1262, 1079, 1046, 802 cm^{-1}; EI-MS *m*/*z*: 448 (M^+). Anal. calcd for $C_{28}H_{32}O_5$•H_2O: C 72.08, H 7.35; found C 72.33, H 7.36.

Caged xanthone (13c) Yield 40%; yellow solid; m.p. 157—158 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.81 (s, 3H, C^{14}-H), 1.24—1.29 (m, 7H, C^{24}-H, C^{15}-H, C^{16}-H_a), 1.64 (s, 3H, C^{25}-H), 1.67 (s, 3H, C^{19}-H), 1.68 (s, 3H, C^{20}-H), 2.27 (dd, J=13.5, 4.8 Hz, 1H, C^{16}-H_b), 2.45—2.49 (m, 3H, C^{17}-H, C^{21}-H), 3.33 (d, J=7.2 Hz, 2H, C^{11}-H), 3.43 (dd, J=6.6, 4.8 Hz, 1H, C^7-H), 4.47 (t, J=6.6 Hz, 1H, C^{22}-H), 5.20 (dd, J=7.2, 1.2 Hz, 1H, C^{12}-H), 5.41 (s, 1H, OH), 6.79 (d, J=8.1 Hz, 1H, C^2-H), 7.37 (d, J=8.1 Hz, 1H, C^1-H), 7.41 (d, J=6.6 Hz, 1H, C^8-H); IR (KBr) *v*: 3416, 2969, 2909, 1739, 1654, 1607, 1448, 1313, 1249, 1213, 1037 cm^{-1}; EI-MS *m*/*z*: 448 (M^+). Anal. calcd for $C_{28}H_{32}O_5$•CH_3OH: C 72.08, H 7.35; found C 72.02, H 7.30.

Caged xanthone (13d, forbesione)[26] Yield 50%; yellow solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.22 (s, 3H), 1.28 (dd, J=13.6, 10.5 Hz, 1H), 1.31 (s, 3H), 1.49 (s, 3H), 1.62 (s, 3H), 1.69 (d, J=1.1 Hz, 3H), 1.74 (s, 3H), 2.28 (dd, J=13.6, 4.8 Hz, 1H), 2.42 (d, J=9.3 Hz, 1H), 2.51—2.54 (m, 2H), 3.34—3.38 (m, 2H), 3.43 (dd, J=6.9, 4.3 Hz, 1H), 4.33—4.38 (m, 1H), 5.15—5.20 (m, 1H), 5.97 (s, 1H), 6.00 (br s, 1H), 7.39 (d, J=6.9 Hz, 1H), 12.53 (s, 1H); EI-MS *m*/*z*: 464 (M^+). Anal. calcd for $C_{28}H_{32}O_6$: C 72.39, H 6.94; found C 72.35, H 6.89.

Caged xanthone (13e, isoforbesione)[26] Yield 35%; yellow solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.12 (s, 3H), 1.30 (s, 3H), 1.36 (dd, J=13.6, 10.5 Hz, 1H), 1.40 (s, 3H), 1.67 (s, 3H), 1.77 (d, J=1.1 Hz, 3H), 1.82 (s, 3H), 2.33 (dd, J=13.6, 4.5 Hz, 1H), 2.39 (d, J=9.6 Hz, 1H), 2.61—2.59 (m, 2H), 3.35—3.39 (m, 2H), 3.48 (dd, J=6.8, 4.3 Hz, 1H), 4.48—4.44 (m, 1H), 5.26—5.23 (m, 1H), 6.08 (s, 1H), 6.60 (br s, 1H), 7.40 (d, J=6.8 Hz, 1H), 12.80 (s, 1H); EI-MS *m*/*z*: 464 (M^+). Anal. calcd for $C_{28}H_{32}O_6$: C 72.39, H 6.94; found C 72.32, H 6.91.

General procedure for the synthesis of tris(allyloxy)-9*H*-xanthen-9-one (14a—14d)

To a solution of xanthone **10a—10d** (0.98 mmol) in dry acetone (70 mL), potassium iodide (539 mg, 3.25 mmol), potassium carbonate (449 mg, 3.25 mmol) and allyl chloride (0.42 mL, 4.31 mmol) were added. The reaction mixture was heated at 45 ℃ under N_2 protection for 5 h, which was then allowed to cool to 25 ℃, filtered, washed with acetone and concentrated. The residue was purified by chromatography on a silica-gel column eluted with PE-EtOAc (V ∶ V=10 ∶ 1) to afford **10a—10d**, respectively.

2,5,6-Tris(allyloxy)-9*H*-xanthen-9-one (14a)

Yield 89%; white solid; m.p. 100—102 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.69—4.82 (m, 6H, 3×CH_2CH=CH_2), 5.30—5.55 (m, 6H, 3×CH_2CH=CH_2), 6.08—6.24 (m, 3H, 3×CH_2CH=CH_2), 7.18 (d, J=9.0 Hz, 1H, ArH), 7.42 (dd, J=9.1, 3.1 Hz, 1H, ArH), 7.57 (d, J=9.1 Hz, 1H, ArH), 7.62 (d, J=3.1 Hz, 1H, ArH), 7.94 (d, J=9.0 Hz, 1H, ArH); IR (KBr) ν: 3414, 3083, 2917, 1657, 1617, 1594, 1488, 1446, 1313, 1213, 1080, 825, 776 cm^{-1}; EI-MS m/z: 364 (M^+).

3,4,6-Tris(allyloxy)-9*H*-xanthen-9-one (14b) Yield 91%; white solid; m.p. 77—78 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.66—4.74 (m, 6H, 3×CH_2CH=CH_2), 5.23—5.37 (m, 6H, 3×CH_2CH=CH_2), 6.05—6.23 (m, 3H, 3×CH_2CH=CH_2), 6.95—6.99 (m, 3H, ArH), 8.03 (d, J=9.0 Hz, 1H, ArH), 8.23 (d, J=9.0 Hz, 1H, ArH); IR (KBr) ν: 2917, 1650, 1622, 1600, 1437, 1286, 1186, 1077, 844, 774, 685 cm^{-1}; EI-MS m/z: 364 (M^+).

3,4,5-Tris(allyloxy)-9*H*-xanthen-9-one (14c) Yield 87%; white solid; m.p. 113—114 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.78—4.85 (m, 6H, 3×CH_2CH=CH_2), 5.19—5.64 (m, 6H, 3×CH_2CH=CH_2), 6.12—6.30 (m, 3H, 3×CH_2CH=CH_2), 7.22 (d, J=9.0 Hz, 1H, ArH), 7.36 (t, J=8.0 Hz, 1H, ArH), 7.47 (dd, J=8.0, 1.6 Hz, 1H, ArH), 7.79 (dd, J=8.0, 1.6 Hz, 1H, ArH), 7.96 (d, J=9.0 Hz, 1H, ArH); IR (KBr) ν: 3415, 1658, 1615, 1601, 1489, 1441, 1290, 1088, 769, 739 cm^{-1}; EI-MS m/z: 364 (M^+).

1-Hydroxy-3,5,6-tris(allyloxy)-9*H*-xanthen-9-one (14d) Yield 85%; yellow solid; m.p. 77—78 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 4.62—4.75 (m, 6H, 3×CH_2CH=CH_2), 5.32—5.50 (m, 6H, 3×CH_2CH=CH_2), 6.03—6.17 (m, 3H, 3×CH_2CH=CH_2), 6.35 (d, J=2.0 Hz, 1H, ArH), 6.51 (d, J=2.0 Hz, 1H, ArH), 6.97 (d, J=9.0 Hz, 1H, ArH), 7.95 (d, J=9.0 Hz, 1H, ArH), 12.89 (s, 1H, OH); IR (KBr) ν: 3473, 1658, 1604, 1569, 1516, 1443, 1299, 1166, 814, 690 cm^{-1}; EI-MS m/z: 380 (M^+).

General procedure for the synthesis of caged xanthones (15a—15e)

A stirred solution of tri(allyloxy)-9*H*-xanthen-9-one (**10a—10d**, 0.82 mmol) in decalin (10 mL) was refluxed under N_2 protection for 3 h, then cooled to 25 ℃ and 100 mL of petroleum ether was added. The precipitate was filtrated and washed with petroleum. The crude residue was purified by chromatography on a silica-gel column eluted with PE-EtOAc (V∶V=10∶1) and then crystallized from methanol-H_2O (V∶V=5∶1) to afford caged xanthones **15a—15e**.

Caged xanthone (15a) Yield 8%; yellow solid; m.p. 85—86 ℃; ^{1}H NMR (Acetone-d_6, 300 MHz) δ: 1.79—1.94 (m, 2H, C^{14}-H), 2.43—2.65 (m, 1H, C^{17}-H), 2.67—2.76 (m, 2H, C^{15}-H, C^{17}-H), 3.48—3.52 (m, 1H, C^7-H), 3.90 (d, J=7.8 Hz, 1H, C^{16}-H), 4.44 (q, J=7.8, 3.9 Hz, 1H, C^{16}-H), 4.52—4.64 (m, 4H, C^{11}-H, C^{19}-H), 5.10—5.22 (m, 1H, C^{18}-H), 5.24—5.47 (m, 2H, C^{13}-H), 6.02—6.15 (m, 1H, C^{12}-H), 7.11 (dd, J=8.5, 0.9 Hz, 1H, C^3-H), 7.28—7.32 (m, 3H, C^2-H, C^3-H, C^8-H); IR (KBr) ν: 3414, 3061, 2917, 1640, 1614, 1481, 1439, 1290 cm^{-1}; EI-MS m/z: 364 (M^+). Anal. calcd for $C_{22}H_{20}O_5$: C 72.51, H 5.53; found C 72.12, H 5.44.

Caged xanthone (15b) Yield 7%; yellow solid; m.p. 109—111 ℃; ^{1}H NMR (Acetone-d_6, 300 MHz) δ: 1.78—1.96 (m, 2H, C^{14}-H), 2.22—2.53 (m, 2H, C^{17}-H), 2.67—2.75 (m, 1H, C^{15}-H), 3.46—3.51 (m, 3H, C^7-H, C^{11}-H), 3.93 (d, J=7.6 Hz, 1H, C^{16}-H), 4.50 (dd, J=7.6, 3.8 Hz, 1H, C^{16}-H), 4.55—4.63 (m, 2H, C^{19}-H), 4.94—5.12 (m, 2H, C^{13}-H), 5.14—5.21 (m, 1H, C^{18}-H), 5.95—6.08 (m, 1H, C^{12}-H), 6.71 (d, J=8.7 Hz, 1H, C^2-H), 7.22 (d, J=7.0 Hz, 1H, C^8-H), 7.68 (d, J=8.7 Hz, 1H, C^1-H), 9.44 (s, 1H, C^3-OH); IR (KBr) ν: 3412, 3076, 2982, 2924, 1744, 1701, 1638, 1608, 1435, 1285, 786 cm^{-1}; EI-MS m/z: 364 (M^+). Anal. calcd for $C_{22}H_{20}O_5$: C 72.51, H 5.53; found C 72.19, H 5.77.

Caged xanthone (15c) Yield 6%; yellow solid; m.p. 91—93 ℃; ^{1}H NMR (Acetone-d_6, 300 MHz) δ: 1.85—1.91 (m, 2H, C^{14}-H), 2.47—2.70 (m, 3H, C^{15}-H, C^{17}-H), 3.47—3.57 (m, 3H, C^7-H, C^{11}-H), 3.86 (d, J=7.8 Hz, 1H, C^{16}-H), 4.68 (q, J=7.8, 3.8 Hz, 1H, C^{16}-H), 5.03—5.15 (m, 2H, C^{13}-H), 5.20—5.27 (m, 1H, C^{18}-H), 5.95—6.12 (m, 1H, C^{12}-H), 6.90 (d, J=8.1 Hz, 1H, C^2-H), 7.30 (d, J=7.5 Hz, 1H, C^8-H), 7.34 (d, J=8.1 Hz, 1H, C^1-H), 8.08 (s, 1H, C^4-OH); IR (KBr) ν: 3473, 3076, 2982, 2924, 1740, 1664, 1640, 1614, 1503, 1450, 1318, 1258, 1124, 923 cm^{-1}; EI-MS m/z: 364 (M^+). Anal. calcd for $C_{22}H_{20}O_5$: C 72.51, H 5.53; found C 72.38, H 5.49.

Caged xanthone (15d) Yield 6%; yellow solid; m.p. 54—55 ℃; ^{1}H NMR (Acetone-d_6, 300 MHz) δ: 1.89—1.91 (m, 2H, C^{14}-H), 2.17—2.53 (m, 2H, C^{17}-H), 2.67—2.72 (m, 1H, C^{15}-H), 3.37—3.41 (m, 2H, C^{11}-H), 3.50—3.53 (m, 1H, C^7-H), 3.92 (d, J=3.7 Hz, 1H, C^{16}-H), 4.47 (dd, J=7.7, 3.7 Hz, 1H, C^{16}-H), 4.67—4.71 (m, 1H, C^{19}-H), 4.91—4.95 (m, 1H, C^{19}-H), 5.04—5.12 (m, 2H, C^{13}-H), 5.58—5.64 (m, 1H, C^{18}-H), 5.93—6.05 (m, 1H, C^{12}-H), 6.07 (s, 1H, C^2-H), 7.31 (d, J=6.8 Hz, 1H, C^8-H), 9.83 (s, 1H, C^3-OH), 12.50 (s, 1H, C^1-OH); IR (KBr) ν: 3449, 2922, 1748, 1634, 1600, 1582, 1500, 1430, 1271, 1131 cm^{-1}; EI-MS m/z: 380 (M^+). Anal. calcd for $C_{22}H_{20}O_6$: C 69.46, H 5.30; found C 69.52, H 5.30.

Caged xanthone (15e) Yield 10%; yellow solid; m.p. 55—57 ℃; ^{1}H NMR (Acetone-d_6, 300 MHz) δ: 1.78—1.91 (m, 2H, C^{14}-H), 2.37—2.65 (m, 2H, C^{17}-H), 2.68—2.73 (m, 1H, C^{15}-H), 3.26—3.30 (m, 2H, C^{11}-H), 3.45—3.49 (m, 1H, C^7-H), 3.85 (d, J=7.8 Hz, 1H, C^{16}-H), 4.35 (dd, J=7.8, 3.8 Hz, 1H, C^{16}-H), 4.60—4.69 (m, 2H, C^{19}-H), 4.85—4.99 (m, 2H, C^{13}-H), 5.18—5.24 (m, 1H, C^{18}-H), 5.83—5.96 (m, 1H, C^{12}-H), 6.14 (s, 1H, C^4-H), 7.30 (d, J=7.0 Hz, 1H, C^8-H), 9.77 (s, 1H, C^3-OH), 12.83 (s, 1H, C^1-OH); IR (KBr) ν: 3447, 3220, 1740, 1642, 1621, 1582, 1571, 1450, 1328, 1118 cm^{-1}; 380 (M^+). Anal. calcd for $C_{22}H_{20}O_6$: C 69.46, H 5.30;

found C 69.50, H 5.34.

Anti-proliferation activity

Cancer cell lines involved in this study were obtained from Cell Bank of Shanghai, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Cells were cultured in 90% RPMI 1640 Medium (GIBCO, Invitrogen Corporation, NY) supplemented with 10% fetal bovine serum (Sijiqing, Zhejiang, China), 100 μg/mL benzyl penicillin and 100 mg/mL streptomycin in a humidified environment with 5% CO_2 at 37 ℃. Samples containing ＞95% Gambogic acid isolated from Gamboge resin according to the protocols reported previously was used in the experiments. All the tested compounds were dissolved in DMSO to a concentration of 0.01 mol•L^{-1} and stored at －4 ℃.

Cell viabilities were measured by a colorimetric assay using 3-(4,5-dimethylthiaz-ol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT; Sigma, Ltd.) as described previously.[29] Experiments were carried out in triplicate in a parallel manner for each concentration of target compounds used and the results were presented as mean ±SE. Control cells were given only culture media. After incubation for 24 h, absorbance (A) was measured at 570 nm. Survival ratio (%) was calculated using the following equation: survival ratio (%)＝($A_{treatment}/A_{control}$) ×100%. IC_{50} was taken as the concentration that caused 50% inhibition of cell viabilities and calculated by the SigmaPlot software (Systat Software Inc., USA).

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