Full Paper

Efficient Total Synthesis and Biological Activities of 6-Deoxyisojacareubin

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6-Deoxyisojacareubin was directly synthesized in a six-step route with an overall yield of about 20%. In this route, the excellent site selectivity of this Claisen rearrangement-cyclization reaction cascade was achieved by inserting a bulky *p*-tosyl group into the free 1-OH, and in the last step, some efficient demethylation methods were explored. Furthermore, all synthesized intermediates including 6-deoxyisojacareubin were evaluated for their inhibitory activity against the QGY-7703 cell line. Of these, compound **1** and 6-deoxyisojacareubin showed moderate activities with IC_{50} values of 39.61 and 9.65 μM, respectively, when compared to the positive control 5-fluorouracil with an IC_{50} value of 11.24 μM. Further investigation using non-radioactive detection of protein kinase C (PKC) suggested that these two compounds possessed potency in the inhibition of PKC.

Keywords: Antitumor / 6-Deoxyisojacareubin / Protein kinase C / Xanthone

Received: November 21, 2012; Revised: December 27, 2012; Accepted: January 18, 2013

DOI 10.1002/ardp.201200440

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Introduction

Recently, some studies have reported that natural and synthetic xanthones mediate several important biological activities such as antitumor [1], anti-inflammatory [2], antithrombotic [3], and neuropharmacological effects [4]. Furthermore, it has been suggested that these compounds may interact with protein kinase C (PKC) and cause effects compatible not only with PKC activation [5] but also with PKC inhibition [6]. PKC is a family of serine-threonine kinases with significant roles in carcinogenesis and the maintenance of a malignant phenotype, and it is one of the key elements of signal transduction and intimately involved in cell growth regulation and tumor promotion [7]. Therefore, PKC has become an attractive target for anticancer treatment.

6-Deoxyisojacareubin (**6**) is a natural product with a xanthone scaffold. Fu et al. [8] isolated this compound from *Hypericum japonicum* Thunb. ex Murray. Niu et al. [9] measured this compound against the human leukemic cell line HL-60 with GI_{50} values of 27.3 ± 1.4 μM *in vitro*. Up to now, the synthesis of 6-deoxyisojacareubin has been reported only a few times. The first total synthesis of 6-deoxyisojacareubin was accomplished by Gujral and Gupta [10]. 1,3,5-Trihydroxyxanthone was treated with 2-methylbut-3-en-2-ol in the presence of a catalytic amount of BF_3-etherate to yield a mixture of prenylated xanthone derivatives in poor yield, and one of these prenylated derivatives along with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in dry toluene afforded 6-deoxyisojacareubin. Helesbeux et al. [11] prepared

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6-deoxyisojacareubin in a different way in 2004. They applied the photooxygenation-reduction sequence to synthesize several natural secondary allylic alcohol derivatives (three in the coumarin series and two novel compounds in the xanthone series); our target compound 6-deoxyisojacareubin was isolated in the reduction step, and the total yield to synthesize 6-deoxyisojacareubin was just about 1.68%. In the above two synthesis schemes, no regioselectivity was achieved when introducing a prenyl side chain on the xanthone skeleton, and many other byproducts were produced, leading to a low yield. In the present study, we sought to develop a chemical strategy that would allow an efficient synthetic access to 6-deoxyisojacareubin and thus facilitate its biological evaluation regarding its cytotoxicity and inhibitory activity on PKC.

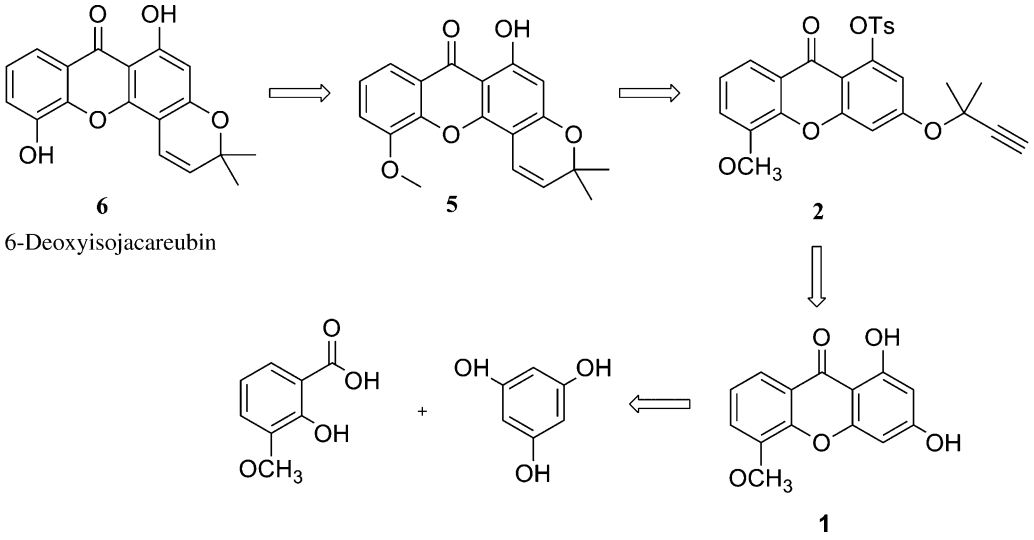
Result and discussion

Chemistry

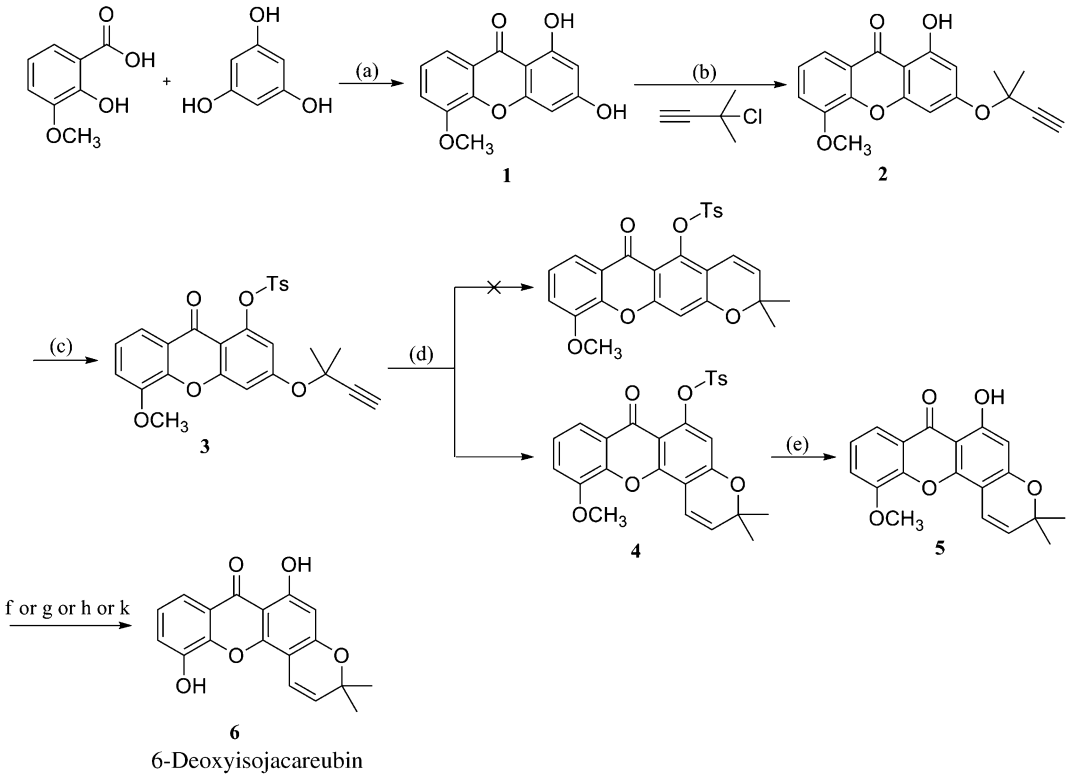
The retrosynthetic analysis is outlined in Scheme 1. In this route, the important intermediate methyl ether of 6-deoxyisojacareubin (**5**) was firstly synthesized by just five steps, from the commercially available starting materials 3-methoxysalicylic acid and phloroglucinol, in around 30% yield. Then an efficient demethylation method was explored to complete the total synthesis of 6-deoxyisojacareubin with about 20% overall yield.

The synthesis approach for the construction of the xanthone nucleus was based on the method of Grover et al. [12] and involves the condensation between phloroglucinol and 2-hydroxy-3-methoxybenzoic acid. The use of Eaton's reagent (phosphorus pentoxide and methanesulfonic acid: P_2O_5/CH_3SO_3H) [13] as the coupling agent afforded the oxygenated xanthone nucleus **1** in high yield (90%) without further purification. As the 1-OH can form a hydrogen bond with the adjacent carbonyl group, by controlling the reaction time and temperature, we can selectively get xanthone **2** without the 1-OH substitute product. So propargylic ether **2** was easily prepared in the presence of KI and K_2CO_3 with a catalytic amount of CuI, with a yield of 63%.

According to the reports, the following Claisen rearrangement-cyclization may have two cyclization directions [14, 15], which could lead to the angular or the linear isomer. Almost equal amounts of the two isomers were prepared if the 1-OH was exposed. Then, we observed that methylation of the 1-OH group prior to ring closure improves the percentage of the angular isomer, presumably due to steric hindrance, but a small amount of linear isomer was still monitored in our reaction. Then, we inserted the bulky *p*-tosyl group into the free 1-OH of propargylic ether **2** to provide the ester **3**, which was subjected to thermal cyclization to furnish selectively the angular isomer **4**; this was easily hydrolyzed to the required methylated derivative **5** (Scheme 2). Treatment of **2** with 1.5 equiv. of TsCl in acetone at room temperature for 1.5 h and purification via a simple chromatography on silica gel afforded a white powdery product **3** in 83% yield. Then, the xanthone sulfonate **3** was heated in DMF at 120°C via Claisen-cyclization reaction to form the angular isomer **4** in 85% yield. Just one spot in TLC was monitored. ^{1}H NMR, ^{13}C NMR, and HMBC analysis were used to determine its structure and confirm that it was the angular isomer (Scheme 3). The key signals were outlined to confirm the right cyclization direction. The carbon–carbon double bond proton [H-1] correlated with the oxygenated aromatic



Scheme 1. Retrosynthetic analysis of 6-deoxyisojacareubin.

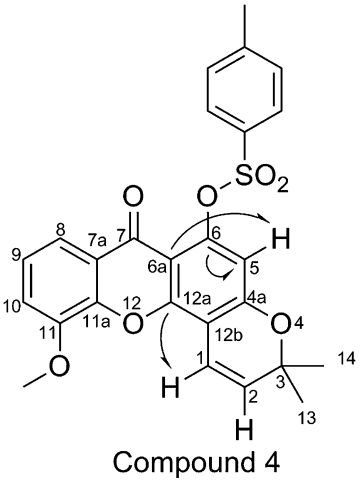


Reagents and conditions: (a) P_2O_5, CH_3SO_3H, 80°C, reflux, 30 min, 90%; (b) CuI, KI, K_2CO_3, acetone, reflux, 2 h, 63%; (c) TsCl, K_2CO_3, reflux, 90 min, 83%; (d) DMF, reflux, 2 h, 85%; (e) EtOH-H_2O, KOH, reflux, 1 h, 72%; (f) BBr_3 in CH_2Cl_2, −78°C to 0°C, 25%; (g) $AlCl_3$ in CH_2Cl_2 overnight, rt, 16%; (h) pyridine hydrochloride, 175°C 30 min, 14%; (k) $AlBr_3$ in CH_2Br_2, EtSH, 0°C 1 h, 65%.

Scheme 2. Synthetic route of 6-deoxyisojacareubin.

carbons [C-12a] and [C-4a]; the aromatic proton [H-5] correlated with [C-6] and [C-6a] (Scheme 3 and Table 1). Then, the subsequent hydrogenolysis of the Ts group led to the methylated derivative **5** in 72% yield.

With our methylated derivative **5** in hand, we directed our efforts toward the demethylation. According to the literature, a Lewis acid could be selected as our demethylation condition [16, 17]. Treating methylated derivative **5** with BBr_3 in CH_2Cl_2 at −78 to 0°C for 4 h, 6-deoxyisojacareubin **6** was obtained in a low yield (about 25%). Furthermore, more than one kind of demethylation conditions were tested. $AlCl_3$ and pyridine hydrochloride were employed by us and the results were the same as those with BBr_3. At last, the generation of 6-deoxyisojacareubin from its methylated derivative was effected by exposure to $AlBr_3$–EtSH in methylene chloride at 0°C (65% yield).



Scheme 3. Long-range correlation in the HMBC spectrum of compound **4**.

Biological activity

Aimed at discovering potential antitumor agents, 6-deoxyisojacareubin and another five intermediates were employed

Table 1. HMBC spectroscopic data of compound **4**.

Position	δ_H (ppm)	δ_C (ppm)	HMBC
1	6.80 d	113.85	C-12a, C-3, C-4a
2	5.99 d	128.52	C-13, C-14, C-3
3		79.01	
4			
4a		156.62	
5	6.42	108.22	C-6a, C-6, C-4a
6		148.03	
6a		109.46	
7		172.62	
7a		128.89	
8	7.53 dd	122.33	C-9, C-10, C-7
9	7.37–7.31 m	124.26	C-10, C-8, C-11
10	7.45 dd	116.23	C-11a, C-9, C-11
11		147.05	
11a		145.76	
12			
12a		151.78	
12b		108.55	
13, 14	3.96, 2.35	27.61, 27.3	C-13(14), C-3, C-2

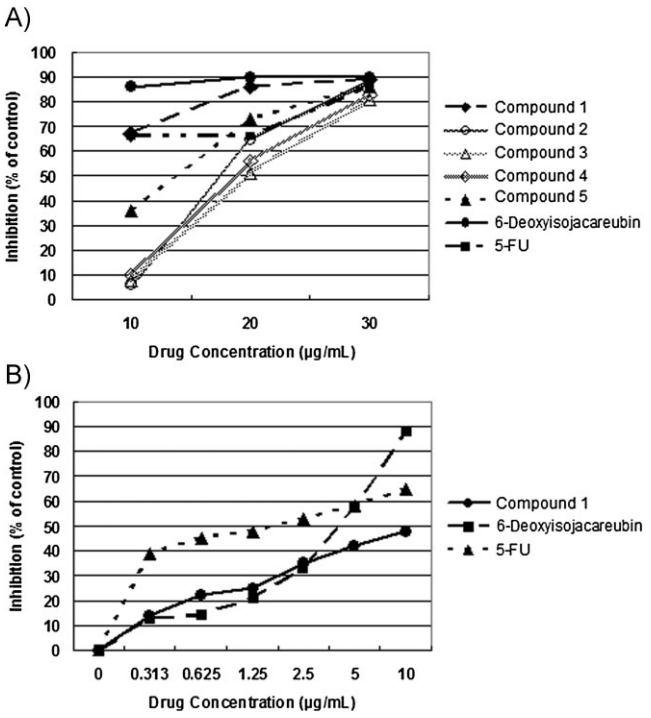


Figure 1. Cytotoxicity against the QGY-7703 cell line. The plotted data were averaged from three independent experiments. (A) Preliminary cytotoxicity screen of 6-deoxyisojacareubin and five intermediates. (B) Inhibition rates of compound **1** and 6-deoxyisojacareubin against the QGY-7703 cell line.

in the preliminary screen to identify cytotoxicity against the QGY-7703 cell line. Compared with the positive control agent 5-fluorouracil (5-FU; Sigma), compound **1** and 6-deoxyisojacareubin showed the highest activities in the preliminary cytotoxicity screen (Fig. 1A); therefore, these two compounds were evaluated further. The inhibition rates for the QGY-7703 cells pretreated with compound **1**, 6-deoxyisojacareubin and 5-FU at different concentrations are shown in Fig. 1B. Both compound **1** and 6-deoxyisojacareubin inhibited the proliferation of QGY-7703 cells in a concentration-dependent manner, as compared with the positive control 5-FU. The IC_{50} values of compound **1**, 6-deoxyisojacareubin, and 5-FU were 39.61, 9.65, and 11.24 μM, respectively. Furthermore, 6-deoxyisojacareubin presented a remarkable inhibition proliferation ability in comparison with 5-FU in the concentration range of 5–10 μg/mL.

The activity of PKC in QGY-7703 cells was assessed 3 h after the addition of compound **1**, 6-deoxyisojacareubin, and the PKC inhibitor GF109203X (GFX; Tocris Bioscience). Compared to the basal control (in the absence of any compound), the PKC activity, as measured by the phosphorylation state of a PKC-specific target peptide, was inhibited by compound **1** and 6-deoxyisojacareubin. Both compound **1** and 6-deoxyisojacareubin presented better inhibition potency at the high concentration of 20 μg/mL, as compared with 10 μg/mL, especially compound **1** at 20 μg/mL (Fig. 2A, lane 5). Compared with the basal control, the inhibition rate of GFX was 63.4%, and those of compound **1** and 6-deoxyisojacareubin (20 μg/mL) were 59.3% and 32.4%, respectively (Fig. 2B).

Interestingly, our study showed that 6-deoxyisojacareubin had better proliferation inhibition ability, while compound **1** possessed better inhibitory potency regarding the PKC activity. Based on this result, other target proteins besides PKC may be the acting substrates of 6-deoxyisojacareubin and its synthetic intermediates, which requires further research.

Conclusion

We showed a concise methodology for the preparation of 6-deoxyisojacareubin in about 20% overall yield and explored an efficient way to control the Claisen-cyclization direction. In the last step, several demethylation methods were screened and EtSH–$AlBr_3$ provided a satisfactory yield to complete this total synthesis. Our exploration of these effects could be helpful in the synthesis of other natural products or their designed analogs containing an analogous structural constitution in a more efficient way. Furthermore, the cytotoxicities of 6-deoxyisojacareubin and another five intermediates were evaluated and the results revealed that the derivatives of polyoxygenated xanthone gave a positive response against QGY-7703 cells. The PKC activity assay

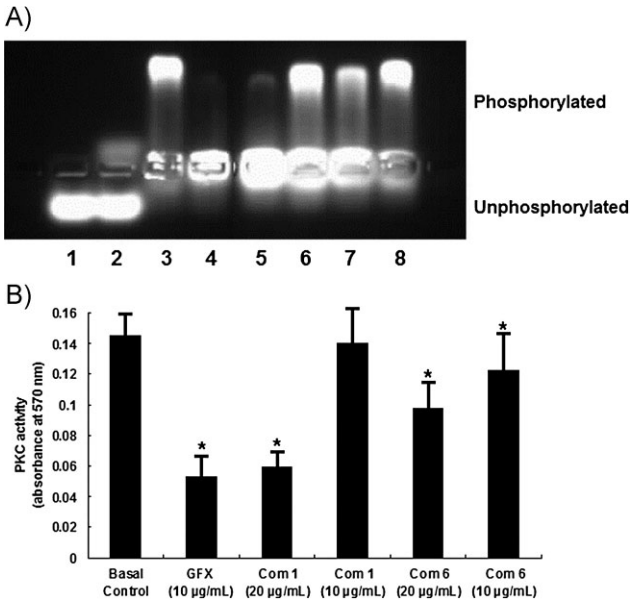


Figure 2. Effects of compound **1** and 6-deoxyisojacareubin (Com **6**) on PKC activity in QGY-7703 cells. Protein cell lysates were prepared from QGY-7703 cells and measured for the ability to phosphorylate a PKC-specific peptide substrate (PLSRTLSVAAK) in a non-radioactive assay. (A) Lanes 1 and 2 show the negative (water only) and positive controls (with 20 ng purified PKC). Supernatants from QGY-7703 cells were subjected to the PKC activity assay in the absence (basal control, lane 3) or presence (lanes 4–8) of compounds. The PKC inhibitor GFX (10 μg/mL, lane 4) or compound **1** (lanes 5 and 6) and 6-deoxyisojacareubin (lanes 7 and 8) at 20 and 10 μg/mL were added separately. (B) Quantitation of (A); $^{*}p < 0.05$ versus the basal control. Data are shown as mean ± SD of three independent experiments.

suggested that compound **1** and 6-deoxyisojacareubin possessed potency in the inhibition of PKC. The present study shows that **1** and 6-deoxyisojacareubin are of interest as prospective antitumor agents, which requires further research.

Experimental

Chemistry

Column chromatography: silica gel (200–300 mesh). ^{1}H, ^{13}C, and 2D-NMR spectra (see also Supporting Information): Bruker DRX-500 spectrometer; δ in ppm rel. to Me_4Si, J in Hz. MS: Agilent-1100-LC/MSD-Trap (ESI-MS) and Agilent Micro-Q-Tof (HR-ESI-MS) spectrometer; in m/z. Melting points (mp) were recorded on a WRS-1B digital melting point apparatus (Shenguan, Shanghai, China).

*1,3-Dihydroxy-5-methoxy-9H-xanthen-9-one (**1**, $C_{14}H_{10}O_5$)*

To a mixture containing phloroglucinol dehydrate (7.56 g, 60 mmol, dried at 120°C overnight) and 2-hydroxy-3-methoxybenzoic acid (10.08 g, 60 mmol) was added slowly 100 mL of Eaton's reagent (P_2O_5/CH_3SO_3H, Aldrich). The mixture was warmed up to 80°C for 20 min, under stirring. After cooling to room temperature, the reaction mixture was poured into ice (300 g) and stirred for 2 h. The resulting solid was collected by filtration, washed with water until pH 6, and dried at 60°C to give a reddish brown solid **1** (14.09 g, 91%) without further purification. $R_f = 0.71$ (hexane/ethyl acetate 2:1); mp 304–305°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.81 (s, 1H), 11.11 (s, 1H), 7.66 (d, $J = 8.0$ Hz, 1H), 7.50 (d, $J = 8.0$ Hz, 1H), 7.38 (t, $J = 8.0$ Hz, 1H), 6.42 (d, $J = 2.0$ Hz, 1H), 6.22 (d, $J = 2.0$ Hz, 1H), 4.03–3.93 (m, 3H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.30, 168.11, 165.02, 159.43, 150.19, 147.64, 126.32, 122.83, 118.93, 117.71, 104.44, 100.49, 96.37, 58.27 ppm; HRMS: m/z ($M+H^+$) calcd. for $C_{14}H_{10}O_5$ 259.0601, found 259.0606.

*1-Hydroxy-5-methoxy-3-(2-methylbut-3-yn-2-yloxy)-9H-xanthen-9-one (**2**, $C_{19}H_{16}O_5$)*

To a round-bottom flask containing xanthone **1** (377 mg, 1.46 mmol), KI (400 mg, 2.41 mmol), K_2CO_3 (220 mg, 1.59 mmol) and CuI (14.0 mg, 73.8 μmol) were added dry acetone (8.00 mL) and 2-chloro-2-methylbut-3-yne (164 mg, 1.61 mmol). The reaction vessel was then equipped with a reflux condenser and the reaction was stirred under argon while heating at 45°C. The reaction was monitored by TLC until complete. After 2 h, the reaction mixture was allowed to cool to room temperature and acidified with AcOH. The reaction mixture was then partitioned between EtOAc and water. The water layer was back-extracted once and the combined EtOAc layers were dried over $MgSO_4$, filtered, and concentrated. The crude material was column chromatographed (hexane/ethyl acetate 4:1) to yield a yellow solid **2** (300 mg, 63.3%); $R_f = 0.68$ (hexane/ethyl acetate 4:1); mp 167.9–169°C; IR (KBr, film): ν (cm^{-1}) 3246, 3008, 2983, 2961, 2934, 2843, 2665, 2105, 1837, 1674, 1607, 1565, 1505, 1494, 1458, 1437, 1351, 1315, 1269, 1233, 1102, 1017, 1004, 967, 881, 834, 805, 762, 722, 688, 656, 583, 544, 523, 464. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.69 (s, 1H), 7.68 (d, $J = 8.0$ Hz, 1H), 7.53 (d, $J = 8.0$ Hz, 1H), 7.41 (t, $J = 8.0$ Hz, 1H), 6.88 (d, $J = 2.1$ Hz, 1H), 6.55 (d, $J = 2.1$ Hz, 1H), 3.98 (s, 3H), 3.94 (s, 1H), 1.72 (s, 6H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.16, 164.52, 163.77, 158.25, 149.80, 147.37, 126.09, 122.41, 118.73, 117.52, 105.48, 102.86, 98.61, 86.40, 80.27, 74.77, 58.07 ppm; HRMS m/z ($M+H^+$) calcd. for $C_{19}H_{16}O_5$ 325.1071, found 325.1074.

*5-Methoxy-3-(2-methylbut-3-yn-2-yloxy)-9-oxo-9H-xanthen-1-yl-4-methylbenzenesulfonate (**3**, $C_{26}H_{22}O_7S$)*

To a solution of the xanthone **2** (410 mg, 1.26 mmol) in dry acetone (10 mL) were added *p*-toluenesulfonyl chloride (777 mg, 4.08 mmol) and potassium carbonate (938 mg, 6.8 mmol), and the resulting mixture was heated at reflux under argon for 90 min. The inorganic precipitate was then filtered off and the filtrate was evaporated to dryness. Flash chromatography on silica gel using a mixture of hexane/ethyl acetate 80:20 as the eluent provided the white solid **3** (500 mg, 83%). $R_f = 0.21$ (hexane/ethyl acetate 4:1); mp 140.9–141.2°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.81 (d, $J = 8.3$ Hz, 2H), 7.58–7.54 (m, 1H), 7.47 (d, $J = 8.0$ Hz, 1H), 7.43 (d, $J = 8.3$ Hz, 2H), 7.36 (t, $J = 8.0$ Hz, 1H), 7.32 (d, $J = 2.3$ Hz, 1H), 6.85 (d, $J = 2.3$ Hz, 1H), 3.97 (s, 3H), 3.94 (s, 1H), 2.35 (s, 3H), 1.67 (s, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.84, 161.79, 159.42, 150.12, 149.91, 148.04, 146.80, 133.99, 132.21, 130.70, 126.48, 124.76, 118.50,

113.06, 112.26, 107.83, 86.21, 81.12, 75.87, 58.50, 31.20, 21.33 ppm; HRMS *m/z* ($M+H^+$) calcd. for $C_{26}H_{22}O_7S$ 479.1159, found 479.1180.

*11-Methoxy-3,3-dimethyl-7-oxo-3,7-dihydropyrano[2,3-c]-xanthen-6-yl-4-methylbenzenesulfonate (**4**, $C_{26}H_{22}O_7S$)*

A solution of compound **3** (220 mg, 0.46 mmol) in dry DMF (20 mL) was heated at reflux for 2 h. The solvent was then vacuum-evaporated and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 8:2) to provide compound **4** (187 mg, 85%), a yellow solid. $R_f = 0.31$ (hexane/ethyl acetate 4:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, $J = 8.2$, 2H), 7.53 (dd, $J = 8.0$, 1.4 Hz, 1H), 7.45 (dd, $J = 8.1$, 1.4 Hz, 1H), 7.42 (d, $J = 8.2$ Hz, 2H), 7.37–7.31 (m, 1H), 6.80 (d, $J = 10.1$ Hz, 1H), 6.42 (s, 1H), 5.99 (d, $J = 10.1$ Hz, 1H), 3.99–3.94 (m, 3H), 2.35 (s, 3H), 1.46 (s, 6H) ppm; mp 166.8–168.2°C; ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.62 156.62, 151.78, 148.03, 147.05, 145.76, 144.57–143.35, 131.73, 131.41, 129.89, 128.52, 124.26, 122.33, 116.23, 113.85, 109.46, 108.55, 108.22, 79.01, 56.48, 27.67, 21.12 ppm; HRMS: *m/z* ($M+H^+$) calcd. for $C_{26}H_{22}O_7S$ 479.1159, found 479.1163.

*6-Hydroxy-11-methoxy-3,3-dimethylpyrano[2,3-c]-xanthen-7(3H)-one (**5**, $C_{19}H_{16}O_5$)*

A solution of potassium hydroxide (600 mg) in water (10 mL) and ethanol (10 mL) was prepared. The alkaline solution (20 mL) was added to compound **4** (80 mg, 0.17 mmol) in three 5-mL portions at 15-min intervals. After refluxing for 1 h, the solution was cooled, neutralized with acetic acid, and concentrated under diminished pressure. The mixture was extracted with ether; the combined organic layer was washed successively with aqueous sodium hydrogen carbonate and 3% aqueous sodium hydroxide solution and finally dried over magnesium sulfate. After removal of the solvent, the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 4:1). At last, we obtained a white solid **5** (41 mg, 72.69%). $R_f = 0.75$ (hexane/ethyl acetate 4:1); mp 180–182°C; IR (KBr, film): ν (cm^{-1}) 3443, 3420, 2024, 1650, 1613, 1578, 1498, 1482, 1437, 1410, 1383, 1355, 1321, 1293, 1273, 1226, 1192, 1172, 1151, 1118, 1096, 1076, 973, 891, 825, 794, 752, 722, 701, 626, 422. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.90 (s, 1H), 7.67 (dd, $J = 8.0$, 1.4 Hz, 1H), 7.55–7.53 (m, 1H), 7.41 (dd, $J = 8.0$ Hz, 1.4 Hz, 1H), 6.75 (d, $J = 10.0$ Hz, 1H), 6.27 (d, $J = 0.6$ Hz, 1H), 5.84 (d, $J = 10.0$ Hz, 1H), 4.00 (s, 3H), 1.46 (s, 6H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.10, 163.98, 162.04, 152.79, 149.97, 147.16, 129.79, 126.15, 122.30, 118.82, 117.52, 115.87, 104.86, 102.69, 101.34, 100.43, 80.36, 58.31, 29.66 ppm; HRMS: *m/z* ($M+H^+$) calcd. for $C_{19}H_{16}O_5$ 325.1071, found 325.1067.

*6,11-Dihydroxy-3,3-dimethylpyrano[2,3-c]xanthen-7(3H)-one (**6**, $C_{18}H_{14}O_5$)*

Method I: Compound **5** (324 mg, 1 mmol) was dissolved in DCM (16 mL) and stirred under argon. BBr_3 (4 mL, 1 M in 100 mmol DCM) was added dropwise to the solution and the mixture was allowed to stir at room temperature for 4 h. The reaction mixture was poured slowly into a stirred solution of saturated aqueous sodium bicarbonate (400 mL) and allowed to stir for 1 h. The resulting colorless precipitate was recovered by filtration. The filtrate was neutralized with HCl (1 M) to pH 7, to yield further product, which was also recovered by filtration. Both solids were combined and stirred in water (100 mL) for 1 h. Recovery by filtration and drying under vacuum yielded the product as a yellow solid (77 mg, 0.25 mmol, 25%). $R_f = 0.51$ (hexane/ethyl acetate 4:1); mp 222.5–223.2°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.00 (s, 1H), 10.40 (s, 1H), 7.56 (dd, $J = 7.9$, 1.5 Hz, 1H), 7.34 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.28 (t, $J = 7.9$ Hz, 1H), 6.99 (d, $J = 10.0$ Hz, 1H), 6.24 (s, 1H), 5.80 (d, $J = 10.0$ Hz, 1H), 3.30 (s, 3H), 1.45 (s, 6H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 182.42, 164.07, 162.05, 152.95, 148.02, 146.64, 129.43, 126.27, 123.03, 122.65, 116.53, 116.43, 104.80, 102.78, 100.35, 80.26), 29.70 ppm; HRMS *m/z* ($M+H^+$) calcd. for $C_{18}H_{14}O_5$ 311.0914, found 311.0915.

Method II (EtSH–$AlBr_3$): In a dry 10-mL round-bottom flask with a stir bar under Ar was added $AlBr_3$ solution (1 M in CH_2Br_2, 3 mL, 3 mmol). The flask was cooled to 0°C and EtSH (5.0 mL) was added dropwise with stirring. After 10 min, compound **5** (81 mg, 0.25 mmol) was added. After 1 h of stirring, the reaction mixture was cooled to −30°C, quenched with sat. aq. $NaHCO_3$ (15 mL) and allowed to warm to ambient temperature. Concentration of the reaction mixture was followed by filtration through a short plug of alumina (EtOAc and CH_2Cl_2 as eluent). Purification of the crude product obtained after evaporation by column chromatography afforded a yellow solid at 50 mg (65% yield).

Biology

Cell culture

The QGY-7703 cell line was obtained from the Cell Resource Center, Institute of Life Sciences, Chinese Academy of Medical Science. QGY-7703 cells were maintained in 1640 medium (Gibco) supplemented with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin at 37°C, 5% CO_2.

Cell proliferation/viability assay

QGY-7703 cells were seeded in 96-well microplates (2×10^5 cells/well in 100 μL medium) for 24 h. For the viability assay, varying concentrations of compounds or 1% DMSO vehicle control were added to the wells; the cells were then maintained at 37°C for 48 h. After incubation, the cell viability was measured by 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt (WST-8) using the Cell Counting Kit-8 (Dojindo, Tokyo, Japan), which reflects mitochondrial succinate dehydrogenase function [18, 19]. In brief, 10 μL of CCK-8 solution was added to each well of the plate. Then, the plate was incubated at 37°C for an additional 3 h. The absorbance of the WST-8 formazan dye was measured at 450 nm by an Epoch microplate spectrophotometer. The relative cell viability was presented as % inhibition calculated using the formula:

$$\%\,\text{Inhibition} = \left[1 - \frac{\text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}}\right] \times 100$$

PKC activity assay

The PKC activity assay was carried out according to the instructions of the PepTag non-radioactive PKC assay kit (Promega, Madison, WI, USA) [20]. Briefly, test compounds were added to the serum-free medium, and the cells with or without the compounds were incubated at 37°C for 3 h. The medium was removed; then, the cells were homogenized in lysis buffer (25 mM Tris–HCl, 0.5 mM EDTA, 0.5 mM EGTA, 0.1% Triton

X-100, 10 mM β-mercaptoethanol, 1 μg/L leupeptin, 1 mM phenylmethanesulfonyl fluoride). Reactions were then performed at 30°C in a final volume of 25 μL and terminated after 30 min by incubation of the reaction mixture at 95°C for 10 min. After adding 1 μL of 80% glycerol, each sample was loaded onto an agarose gel (0.8% agarose in 50 mM Tris–HCl, pH 8.0) and separated at 120 V for 15 min. For quantification, bands were photographed by the Syngene GBOX-iCHEMI-XR system and the phosphorylated bands were cut out for measurement at 570 nm. The results are expressed in OD units and differences between two groups were analyzed by Student's *t*-test.

The work was supported by program NCET Foundation, NSFC (81230090), partially supported by the Global Research Network for Medicinal Plants (GRNMP) and King Saud University, Shanghai Leading Academic Discipline Project (B906), FP7-PEOPLE-IRSES-2008 (TCMCANCER Project 230232), the Key Laboratory of Drug Research for Special Environments, PLA, the Shanghai Engineering Research Center for the Preparation of Bioactive Natural Products (10DZ2251300) and the Scientific Foundation of Shanghai China (09DZ1975700, 09DZ1971500, 10DZ1971700). National Major Project of China (2011ZX09307-002-03). National Key Technology R&D Program of China (2012BAI29B06).

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