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Prenylated Benzoylphloroglucinols and Biphenyl Derivatives from the Leaves of *Garcinia multiflora* Champ.

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Wenwei Fu,[a,b] Man Wu,[a,b] Lunlun Zhu,[a,b] Yuanzhi Lao,[a,b] Liping Wang,[a,b] Hongsheng Tan,[a,b] Qinghong Yuan[d] and Hongxi Xu*[a,b]

Garcimultiflorones H-J (**1-3**), three new polyprenylated benzoylphloroglucinols, multiflorabiphenyls B-D (**4-6**), three new biphenyl derivatives, and six known compounds, were isolated from an acetone extract of the leaves of *Garcinia multiflora* by bioassay-guided fractionation. The structures of the new compounds were elucidated by extensive 1D and 2D NMR spectroscopic analyses and mass spectrometry, and the absolute configurations of compounds **1-3** were determined by the comparison of experimental and calculated electronic circular dichroism (ECD) spectra. All of the isolates were evaluated for cytotoxic activities against several human cancer cell lines. Garcimultiflorone I (**2**) exhibited significant inhibitory activity against the SGC7901 and HCT116 cell lines, with IC_{50} values of 4.20 and 5.96 μM, respectively. A cell cycle analysis using flow cytometry showed that the compound arrests the cell cycle at the G1 phase and induces cell death. Moreover, Bcl-2, caspase-3, caspase-9, and PARP western blotting suggested that compound **2** can induce apoptosis. Taken together, these results suggest that compound **2** has anticancer activity that targets the cell cycle through apoptosis signalling pathways.

Introduction

Garcinia L. (Clusiaceae) is a large genus of polygamous trees or shrubs that is distributed in the tropical areas of Asia, Africa, and Polynesia. It consists of 450 species, of which 21 are spread across China[1]. Our research group has reported many novel bioactive xanthone derivatives and prenylated benzoylphloroglucinols from Garcinia plants in China.[2-11] Through continuous efforts in searching for anticancer agents from natural sources, the acetone extract of the leaves of *Garcinia multiflora* Champ. (Guttiferae) was found to possess moderate cytotoxic activities against HeLa, SGC 7901, TE1, HCT116, and caspase2 (Table S1, Supporting Information). *G. multiflora*, a small evergreen tree that is distributed throughout South China and Hong Kong, contains xanthones,[12] benzophenone derivatives[11-13], and biflavonoids[14, 15] as the main components, and it exhibits a variety of bioactivities, including cytotoxic,[11] anti-inflammatory,[13] anti-HIV,[14] antioxidant,[13,16] and antituberculosis activities.[15] These findings prompted us to systematically investigate the cytotoxic compounds from the leaves of *G. multiflora*.

As a result, three new polyprenylated benzoylphloroglucinols garcimultiflorones H-J (**1-3**), three new biphenyl derivatives multiflorabiphenyls B-D (**4-6**), and six known compounds (**7-12**), were isolated from the acetone extract of the leaves of *G. multiflora* by bioassay-directed fractionation (Fig. 1). Their structures were determined by spectroscopic analyses. Garcimultiflorones H-I (**1-2**) are the most simple polyprenylated benzoylphloroglucinols, which features a bicyclo[3.3.1] nonane- 2,4,9-trione core that is only decorated with prenyl side chains. All of the isolates were evaluated for cytotoxic activities against several human cancer cell lines. Garcimultiflorones I (**2**) exhibited significant inhibitory activity against the SGC7901 and HCT116 cell lines. The cell cycle analysis using flow cytometry and bcl-2, caspase-3, caspase-9, and PARP western blotting showed that the compound has anticancer activity that targets the cell cycle through apoptosis signalling pathways. In this report, the isolation and structural elucidation of these compounds and their inhibitory effects on the proliferation of cancer cells are described.

Results and discussion

Garcimultiflorone H (**1**) was obtained as a light yellow amorphous powder and showed a deprotonated molecular ion peak at *m/z* 533.2899 $[M - H]^-$ in HRESIMS, corresponding to the formula $C_{33}H_{41}O_6$ (calcd. 533.2903). The infrared spectroscopy (IR) spectra showed absorption bands that were

[a] School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China. E-mail: xuhongxi88@gmail.com; Fax: +86-21-51323089; Tel: +86-21-51323089
[b] Engineering Research Center of Shanghai Colleges for TCM New Drug Discovery, Shanghai, People's Republic of China
[c] Department of Physics, East China Normal University, Dongchuan Road 500, Shanghai 200241, People's Republic of China
† Electronic Supplementary Information (ESI) available: The spectra including 1D, 2D-NMR, HRESIMS of compounds 1 – 6 as well as related original ECD calculation data for garcimultiflorones H-J (1-3). See DOI: 10.1039/x0xx00000x

indicative of hydroxyl (3408 cm^{-1}) and carbonyl (1730 and 1664 cm^{-1}) groups. The ^{1}H NMR of **1** revealed the presence of one 1,3,4-trisubstituted benzene ring [δ_H 6.69 (1H, d, *J*=8.2 Hz), 6.97 (1H, br d, *J*=8.2 Hz), and 7.17 (1H, br s)], together with two ortho-dihydroxy aromatic carbon signals (δ_C 146.2 and 152.6), and a conjugated carbonyl signal (δ_C 196.0) suggesting the presence of a 3,4-dihydroxybenzoyl group (Table 1). Moreover, the ^{13}C NMR spectrum showed signals for a nonconjugated ketone (δ_C 209.9), two quaternary carbons (δ_C 67.7 and 62.4), and an enolized 1, 3-diketone (δ_C 195.2, 117.9, and 194.7), which along with the quaternary (δ_C 49.3), methine (δ_C 47.8), and methylene (δ_C 40.8) carbons allowed us to identify the bicyclo[3.3.1] nonane ring system typical of the polyprenylated benzoylphloroglucinols. The NMR data of **1** also revealed the presence of two tertiary methyl groups (δ_H 1.01/ δ_C 27.3 and δ_H 1.24/ δ_C 23.2), three isoprenyl groups [three one-proton signals at δ_H 4.87 (1H, overlap), 4.93 (1H, br s) and 5.16 (1H, br s)] (Table 1). The positions of the side chains were assigned by the HMBC and HSQC-TOCSY experiments: three isoprenyl groups were located at C-1, C-5, and C-7 (Fig. 2).

The relative configuration of **1** was revealed by the ^{1}H and ^{13}C NMR data and NOE correlations. The axial orientation of the isoprenyl group at C-7 can be deduced from the chemical shifts of the C-7 at δ_C 47.8 and gem-dimethyl at δ_C 27.3 and 23.2 (Me-22 and Me-23, respectively) observed in the ^{13}C NMR spectra.[17-19] $J_{H\text{-}7,H\text{-}8ax}$ (ca. 6.6 Hz) also favoured the axial position in the ^{1}H NMR spectrum.[17] In the NOESY spectrum, NOE interactions between H-17, Me-22$_{ax}$ and H-7 (δ_H 2.47, 1.01 and 1.51, respectively) (Fig. 2) indicated that the CH_2-17, CH_3-22, and H-7 were on the same side of the cyclohexanone ring. Thus, the *trans* relationship of the isoprenyl group at C-7 with the bridgehead substituents at C-5 was determined.

For the relative configuration of polyprenylated benzoylphloroglucinols, it should be noted that the bridged bicyclic system requires that the side chains at C-1 and C-5 are equatorial.[17, 19] The possible isomers (1*R*, 5*R*, 7*R*)-**1**, and (1*S*, 5*S*, 7*S*)-**1**, were then considered. The absolute configuration of **1** was finally determined by comparison of its experimentally measured electronic circular dichroism (ECD) curve with that predicted using the TDDFT theory. The calculated ECD spectrum of (1*S*, 5*S*, 7*S*)-**1** matched well with the experimental ECD spectrum of **1** (Fig. 5a). Thus, the absolute configurations of **1** were finally determined to be 1*S*, 5*S*, 7*S* (Fig. 1).

Garcimultiflorone I (**2**) was obtained as a light yellow, amorphous powder and showed a deprotonated molecular ion peak at *m/z* 531.2735 $[M-H]^-$ (calcd. for $C_{33}H_{39}O_6$ 531.2747), suggesting a molecular formula of $C_{33}H_{40}O_6$. This elemental composition indicated 15 degrees of unsaturation, which showed **2** to have one more unit of unsaturation than **1**. The IR spectrum showed absorptions attributable to hydroxyl (3429 cm^{-1}) and carbonyl groups (1738 and 1680 cm^{-1}). The ^{1}H NMR spectrum of **2** revealed the presence of two aromatic protons [δ_H 6.87 (1H, s) and 7.23 (1H, s)], three isoprenyl groups [three one-proton signals at δ_H 4.49 (1H, t, *J*=6.4Hz), 4.81 (1H, t-like) and 5.15 (1H, br s)], six singlet methyl groups [δ_H 1.68 (3H, s), 1.63 (3H, s), 1.60 (3H, s)×2, 1.40 (3H, s), and 1.34 (3H, s)], and two tertiary methyl groups on a saturated carbon [δ_H 1.14 (3H, s) and 1.02 (3H, s)] (Table 1). The ^{1}H NMR spectrum of **2** was comparable to that of garcimultiflorone H, except for the loss of one aromatic proton. The ^{13}C NMR spectrum exhibited the presence of a 2, 2-dimethylbicyclo [3.3.1] nonane ring, as in compound **1** (Table 1). Consequently it was clear that the carbonyl at C-4 in **1** was enolized and that the oxygen was attached to C-16.[5, 20, 21] The gross structure of **2** was further elucidated by careful interpretation of the 2D NMR experiments.

The C-7 isoprenyl group was axial, which was also deduced from the chemical shifts of the C-7 at δ_C 45.8 and gem-dimethyl at δ_C 25.6 and 20.6 (Me-22 and Me-23, respectively) observed in the ^{13}C NMR spectra, as in compound **1**. The *trans* relationship of the isoprenyl group at C-7 with the bridgehead substituents at C-5 was also determined by the cross peak of H-17/Me-22ax, and Me-22ax/H-7 in the NOESY spectrum (Fig. 3). The two possible isomers, (1*R*, 5*R*, 7*S*)-**2**, and (1*S*, 5*S*, 7*R*)-**2**, were then considered. The calculated ECD spectrum of (1*R*, 5*R*, 7*S*)-**2** matched well with the experimental ECD spectrum of **2** (Fig. 5b) in the two possible isomers. Thus, the absolute configurations of **2** were finally determined to be 1*R*, 5*R*, 7*S* (Fig. 1). From a biogenetics point of view, **2** could be formed by an oxidative cyclization of **1** catalysed by a xanthone synthase.[22]

Garcimultiflorone J (**3**) was obtained as a light yellow, amorphous powder. The molecular formula $C_{38}H_{48}O_6$ for compound **3** was calculated from the HREIMS of the ion peak $[M+H]^+$ *m/z* 601.3521 (calc. 601.3529). Its IR spectrum showed absorption bands that were consistent with hydroxyl (3421 cm^{-1}) and carbonyl groups (1732 and 1685 cm^{-1}). The ^{1}H NMR spectrum showed two unique singlet aromatic proton signals at δ_H 6.91 (1H, s) and 7.43 (1H, s) assignable to a 1, 2, 4, 5-tetrasubstituted benzene ring. The ^{13}C NMR spectrum also revealed a 2,2-dimethylbicyclo [3.3.1] nonane ring (Table 1). The aforementioned data implied that **3** possessed a polycyclic xanthone core derived from an appropriate polyprenylated benzoylphloroglucinol, as in compound **2** (Table 1). In addition, resonances for seven methyl at δ_H 1.34 (3 H, s, Me-27), 1.45 (3 H, s, Me-21), 1.55 (3 H, s, Me-33), 1.60 (3 H×2, s, Me-28, 38), 1.65 (3 H, s, Me-37) and 1.77 (3H, s, Me-20); three trisubstituted double bonds at δ_H 4.69 (1 H, t-like, H-18), 4.81(1H, t-like, H-25) and 5.00 (1 H, t-like, H-35); one olefinic exomethylenes at δ_H 4.37 (2H, d, H-32); eight methylene protons at δ_H 1.8-2.9 (8 H, m); and one methine proton at δ_H 2.7 (1 H, m), were observed (Table 1). These data, associated with the correlations observed in the COSY and the HMBC spectra, suggested the presence of a lavandulyl and two isoprenyl side chains (Fig. 4). The key HMBC correlations from H-29 [δ_H 1.81 (1H, dd, *J*=14.0, 4.5 Hz), 2.18 (1H, dd, *J*=13.5, 11.2 Hz)] to C-2, C-8 and C-9 (δ_C 194.8, 44.8 and 209.1, respectively) and from H-17 [δ_H 2.90 (2H, br s)] to C-4 (δ_C 176.6) confirmed that an isoprenyl group and the lavandulyl side chain were located at position C-5 and C-1 respectively, and also suggested that the oxygenated bridge was located between C-4 and C-16. The other isoprenyl group was also located at position C-7 by the HMBC experiments (Fig. 4).

The location of the other isoprenyl side chain, axial oriented, at position C-7 was deduced from the chemical shifts of the gem-dimethyl at δ_C 23.6 and 27.0 (Me-22 and Me-23, respectively) observed in the ^{13}C NMR spectra, as in the above polyprenylated benzoylphloroglucinols. The *trans* relationship of the isoprenyl group at C-7 with the bridgehead substituents was also determined by the cross peak of H-17/Me-22ax, Me-23eq/H-24, H-8ax/H-29 and H-8ax/Me-23ax in the NOESY spectrum (Fig. 4). To determine the absolute

configuration of **3**, ECD calculations were conducted. The calculated ECD spectrum of (1*S*, 5*S*, 7*R*, 30*S*/*R*)-**3** matched well with the experimental ECD spectrum of **3** (Fig. 5c) in the two possible isomers, (1*R*, 5*R*, 7*S*, 30*S*/*R*)-**3** and (1*S*, 5*S*, 7*R*, 30*S*/*R*)-**3**. However, the ECD calculation could be not used to determine the absolute configuration of the methine C-30.[10]

From a biogenetics point of view, **3** could be formed by an oxidative cyclization of guttiferone F, a major polyprenylated benzoylphloroglucinols from *Garcinia multiflora*. The absolute configuration of the methine C-30 should be assigned by comparison with samples obtained through a biomimetic synthesis. By the modified oxidation reaction with DPPH, GF-ox1, the main reaction product obtained from the oxidative cyclization of guttiferone F, was elucidated as **3** by the mass spectrometry analysis and superimposed NMR spectra. Thus, the absolute configuration of methine C-30 was determined as *R*, as in guttiferone F. This conclusion was also supported by comparing the $J_{H\text{-}H}$ value at C-29 [δ_H 1.81(1H, dd, *J*=14.0, 4.5 Hz) and 2.16 (1H, dd, *J*=13.5, 11.2 Hz] in **3** with that at C-29 [δ_H 1.92 (1H, dd, *J*=13.5, 4.5 Hz), and 1.98 (1H, m)] in guttiferone F[23] and at C-29 [δ_H 1.79 (1H, m) and 2.28 (1H, m)] in GDPPH-1.[24] Thus, the absolute configurations of compound **3** were established as 1*S*, 5*S*, 7*R*, 30*R* (Fig. 1).

Multiflorabiphenyl B (**4**) was isolated as a colourless amorphous power. The HRESIMS gave an $[M+H]^-$ ion peak at *m/z* = 283.1322, consistent with a molecular formula of $C_{18}H_{20}O_3$. The UV absorptions at 205, 227 sh (shoulder), and 262 nm was similar to those of garcibiphenyl B.[25] The presence of hydroxy groups in the molecule was revealed by a band at 3315 cm^{-1} in the IR spectrum. The ^{1}H-NMR spectrum of **4** showed the signals of an A_2B_2 spin system at δ_H 6.79 and 7.32 (each 2H, d, *J*= 8.6 Hz); two meta-coupled signals at δ_H 6.85 (1H, d, *J*= 2.2 Hz) and 6.77 (1H, t, *J*= 2.2Hz); a 3-methyl-2-butenyl group [δ_H 1.72 (3H, s, H-4'), 1.73 (3H, s, H-5'), 3.33 (2H, d, *J*= 7.2 Hz, H-1'), and 5.28 (1H, ddd, *J*=7.2, 4.3, 1.3 Hz, H-2')] at C-4; and a methoxy group at δ_H 3.76 (3H,s). In agreement with the above data, the structure of 3 was elucidated as 10-methoxy-11-(3' -methyl-2' -butenyl)- [1,7-biphenyl]-4,9-diol, which was further confirmed by the DEPT, HSQC and HMBC data (Fig. 6).

Multiflorabiphenyl C (**5**) was shown to have the elemental formula $C_{18}H_{17}O_4$ from HREIMS, which exhibited a molecular ion peak at *m/z* 297.1130. Comparison of the ^{1}H and ^{13}C NMR spectra with those of compounds **4** suggested that **5** was also a biphenyl compound but with different substituents. The ^{1}H and ^{13}C NMR spectra of **5** displayed signals for a dimethylpyran ring at δ_H 6.57/δ_C 117.2 (C-1'), 5.76/131.1 (C-2'), 1.32/27.0 (C-4' and C-5'), and 74.8 (C-3'). These results suggested that compound **5** was formed from the cyclization of the isoprenyl group with an adjacent hydroxyl group. The ^{1}H NMR spectrum of **5** showed a methoxy group at δ_H 3.73 (3H, s) and a set of ortho-coupled doublets at δ_H 7.26 (2H, d, *J* = 8.6 Hz) and 6.76 (2H, d, *J* = 8.6 Hz) integrated as two protons for each, which also appeared in the spectra of **4**, indicating the presence of a similarly para-substituted phenyl ring in **5**. Signals of the ^{1}H and ^{13}C NMR spectra of **5** were assigned unambiguously by detailed analysis of the DEPT, HSQC, and HMBC spectra (Table 2/ Fig. 6). The data indicated that compound **5** was a new biphenyl compound, multiflorabiphenyl C.

Multiflorabiphenyl D (**6**) gave a molecular ion peak at *m/z* 297.1130 from the HREIMS, corresponding to an elemental formula of $C_{18}H_{17}O_4$. Comparison of the ^{1}H and ^{13}C NMR spectra with the spectra of compound **5** suggested that **6** was also a biphenyl compound, but with different substituents. Compound **6** was formed from the cyclization of the isoprenyl group at C-9 with an adjacent hydroxyl group at C-10, which is different compared to compound **5**. The signals of the ^{1}H and ^{13}C NMR spectra of **6** were assigned unambiguously by a detailed analysis of DEPT, HSQC, and HMBC spectra (Table 2/ Fig. 6), and the structure of **6** was thus established.

The structures of the known compounds were identified as guttiferone E (**7**),[26] guttiferone F (**8**),[23] 30-epicambogin (**9**),[23] garcicowin C (**10**),[7] toxyloxanthone B (**11**) [27] and oblongifolia-garcinine A (**12**) [28] by comparison of their spectroscopic data with published values.

In this study, all of the isolated compounds (**1-12**) were evaluated for cytotoxicity against five human cancer cell lines (HeLa, SGC7901, TE1, HCT116, and Capan 2) and selectivity was determined by using immortalized HL7702 normal human liver cells. The anticancer drug paclitaxel was used as a positive control. Compound **2** showed a significant cytotoxicity (IC_{50} < 10 μM, Table 3) against four human cancer cell lines (HeLa, SGC7901, TE1, and HCT116). Based on its potency and selectivity for the cancer cells, compound **2** was selected as a potential chemotherapeutic compound, and further investigation of its mechanism of action was undertaken. We first evaluated effects of compound **2** on apoptosis and cell cycle arrest by flow cytometry. We found that compound **2** induced cell apoptosis in a dose-dependent fashion (Fig. 7a) and induced cell cycle arrest at the G1 phase (Fig. 7b). Next, we performed western blot analysis of the key proteins mediating apoptosis, including Bcl-2, Bcl-XL, caspase-3, caspase-9, and PARP (Fig. 8). Compound 2 can activate PARP, caspase-3 and caspase-9 cleavage and can inhibit the expression of Bcl-2, suggesting that compound **2** can activate apoptosis.

Conclusions

Taken together, we isolated six new and six known analogues by bioassay-guided isolation from the leaves of *G. multiflora*. These compounds were fully characterized by extensive NMR spectroscopic analyses and by mass spectrometry or by comparison of experimental and calculated electronic circular dichroism (ECD) spectra. Among a multitude of bioactive findings, compound **2** has strong activity in arresting the cell cycle and inducing apoptosis. Our findings indicate that the leaves of *G. multiflora* have anticancer potential. In addition, garcimultiflorone I (**2**) can be used as a lead compound for the development of anticancer drugs. The detailed mechanism of action of the compound will be of interest for further exploration.

Experimental section

General experimental procedures

Optical rotations were measured using an Autopol VI polarimeter. Ultraviolet absorption spectra were recorded on a Varian CARY 50 UV/Vis Spectrophotometer. ECD spectra were recorded on a Chirascan Circular Dichroism Spectrometer. IR spectra were obtained from a Perkin-Elmer 577 spectrometer. The NMR spectra were measured on a Bruker AV-400 or Bruker 600MHz spectrometer and calibrated based on the

solvent peak used. Mass spectrometry was performed on a Waters Q-TOF Premier spectrometer (Micromass MS Technologies, Manchester, UK) with an electrospray ion source (Waters, Milford, MA, USA) connected to a lock-mass apparatus, which performed a real-time calibration correction. Column chromatography was performed using CHP20P MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (100-200, or 200-300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden), and reversed-phase C_{18} silica gel (50 μm, YMC, Kyoto, Japan). Precoated TLC sheets of silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) were used. A Waters 2535 series machine equipped with an Xbridge C_{18} column (4.6 × 250 mm, 5 μm) was used for HPLC analysis, and a preparative Xbridge Prep C_{18} OBD column (19 × 250 mm, 5 μm) was used for the sample preparation.

Plant Material

The leaves of *G. multiflora* were collected on Diaoluo Mountain, Hainan Province, People's Republic of China, in May 2006. The sample was authenticated by Dr. Chunfeng Qiao (Institute of Chinese Medical Sciences, The University of Macau), and a voucher specimen (Herbarium no. 2013003) was deposited at the Innovative Research Laboratory of TCM, Shanghai University of Traditional Chinese Medicine.

Extraction and Isolation.

The air-dried and powdered leaves of the plant (3.10 kg) were extracted with acetone (3 × 20 L, each two days) at room temperature. The extracted solution was evaporated under reduced pressure to yield a dark green residue (194.2 g). The 190-gresidue was chromatographed on silica gel and eluted sequentially by CH_2Cl_2, CH_2Cl_2,-MeOH (9:1), CH_2Cl_2,-MeOH (1:1), and acetone. The CH_2Cl_2 fraction was determined to be the active fraction (Table S1, Supporting Information) and evaporated *in vacuum* to give a residue (35.1 g), part of which (33.0 g) was subjected to passage over a chromatography column (CC) on MCI, and successively eluted with H_2O, 30% EtOH, 60% EtOH, 90% EtOH, 95% EtOH, and EtOAc to fractions (1000 ml/fraction). These fractions were combined by their different TLC profiles to give Fractions A-H, respectively. Fraction B was collected from the 60% EtOH eluent, and Fractions D-G were collected from the 90% EtOH and 95% EtOH eluent.

Fraction G (2.7 g) was subjected to a reversed-phase C_{18} silica gel CC and eluted in a step-gradient manner with MeOH-H_2O (60:40 to 100:0) to obtain five subfractions Ga – Ge. Subfraction Gc was purified by preparative HPLC (MeOH-H_2O, 83:27, with 0.1% formic acid in H_2O, 16 mL/min) to yield the compound guttiferone F (200 mg), and subfraction Ge was purified by preparative HPLC (MeCN-H_2O, 80-100, with 0.1% formic acid in H_2O, 16 mL/min) to yield the compound guttiferone E (t_R=33.97 min, 9.0 mg).

Fraction E (1.1 g) was subjected to reversed-phase C_{18} silica gel CC and eluted in a step-gradient manner with MeOH-H_2O (60:40 to 100:0), to obtain ten subfractions, Ha – Hj. Subfraction Hi was purified by preparative HPLC (MeOH-H_2O, 80:20, with 0.1% formic acid in H_2O, 16 mL/min) to yield compound 30-epicambogin (t_R=31 min, 4.90 mg) and garcicowin C (t_R, 12.50 mg). Subfraction Hf was purified by preparative HPLC (MeOH-H2O, 83:17, with 0.1% formic acid in H_2O, 16 mL/min) to yield compound **3** (t_R=27.72 min, 6.66 mg).

Fraction D (3.2 g) was subjected to reversed-phase C_{18} silica gel CC and eluted in a step-gradient manner with MeOH-H_2O (60:40 to 100:0) to obtain six subfractions Da – Df. Subfraction De was purified by preparative HPLC (MeCN-H_2O, 82:18, with 0.1% formic acid in H_2O, 16 mL/min) to yield compound **1** (t_R=17.30 min, 300 mg). Subfraction Db was further separated by preparative HPLC (MeCN-H_2O, 65:35, with 0.1% formic acid in H_2O, 16 mL/min) to give compound **2** (t_R=27.41 min, 7.61 mg)

Fraction B (0.8 g) was subjected to reversed-phase C_{18} silica gel CC and eluted in a step-gradient manner with MeOH-H_2O (55:45 to 100:0) to obtain subfractions Ba–Bo. Subfraction Bj was further purified by preparative HPLC (MeCN-H_2O, 55:45, with 0.1% formic acid in H_2O, 16 mL/min) to give compound toxyloxanthone B (t_R=8.65 min, 1.18 mg) and compound [illegible] (t_R=12.45 min, 8.44 mg). Subfraction Bg was further separated by preparative HPLC (MeOH-H_2O, 57:43, with 0.1% formic acid in H_2O, 16 mL/min) to give compound **5** (t_R=15.71 min, 4.6 mg). Subfraction Bd was further separated by preparative HPLC (MeOH-H_2O, 60:40, with 0.1% formic acid in H_2O, 16 mL/min) to give compound **6** (t_R=12.2 min, 6.32 mg) and oblongifoliagarcinine A (t_R=13.2 min, 6.53 mg).

Conversion of guttiferone F to 3.

Guttiferone F (30.0 mg, 1.67 mmol) was allowed to react with DPPH (39.0 mg, 3.34 mmol) in toluene at room temperature under darkness for 24 h. After evaporation of the solvent *in vacuo*, the residue was applied to a silica gel column and eluted with chloroform first to remove the DPPH, and then with chloroform-acetone (10:1) to obtain a mixture of reaction products. The mixture of reaction products was applied to a RP-C18 silica gel column and eluted by 90% methanol-water solvent system to give 9.0 mg of GF-ox1(compound **3**).

GF-ox1: light yellow, amorphous powder; Positive MS *m/z* 601.40 $[M+H]^+$; ^1H and ^{13}C NMR, see Table 1. The HPLC, LC-MS analysis and the superimposed NMR spectrums are available (Fig. S57-61, Supporting Information).

Structure characterization

Garcimultiflorone H (**1**): light yellow, amorphous powder; $[\alpha]^{20}_D$ + 29.8 (*c* 0.62, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.02), 209 (3.95), 243 (3.75), 282 (4.00) nm; ECD (*c* 5.76 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 224 (+ 4.39), 258 (-2.99), 363 (+ 0.74); IR (KBr) ν_{max} 3408, 2974, 2825, 1730, 1664, 1601, 1522, 1442, 1377, 1392, 1194, 1119, 773 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) data, see Table 1; ^{13}C NMR (CD_3OD, 151 MHz) data, see Table 1; HRESIMS *m/z* 533.2899 $[M-H]^+$ (calcd. for $C_{33}H_{41}O_6$, 533.2903).

Garcimultiflorone I (2): light yellow, amorphous powder; $[\alpha]^{20}_D$ 42.1 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.30), 250 (4.12), 265 (4.13), 332 (3.79) nm; ECD (*c* 6.39 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 205 (- 11.74), 226 (+ 16.54), 256 (- 16.66), 310 (– 4.32), 352 (+2.32); IR (KBr) ν_{max} 3429, 2960, 2924, 2850, 1738, 1680, 1612, 1466, 1383, 1294, 1144, 837 cm^{-1}; ^1H NMR (DMSO-d_6,

400 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 151 MHz) data, see Table 1; HRESIMS *m/z* 531.2735 [M-H]$^-$ (calcd. for $C_{33}H_{39}O_6$, 531.2747).

Garcimultiflorone J (3): light yellow, amorphous powder; $[\alpha]^{20}_D$ +11.11 (*c* 0.44, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.12), 266 (3.88), 330 (3.54) nm; ECD (*c* 5.02 × 10^{-4} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 203 (+6.84), 227 (-2.95), 259 (+8.39), 310 (-1.29); IR (KBr) ν_{max} 3421, 2960, 2924, 1732, 1685, 1616, 1554, 1516, 1466, 1385, 1292, 1144, 891 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) data, see Table 1; ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; HRESIMS *m/z* 601. 3521 [M+H]$^+$ (calcd. for $C_{38}H_{49}O_6$, 601.3529).

Multiflorabiphenyl B (4): colourless amorphous power; UV (MeOH) λ_{max} (log ε) 204 (4.55), 227 (4.31), 262 (4.18) nm; IR (KBr) ν_{max} 3315, 3020, 2974, 2916, 2530, 2463, 1730, 1599, 1579, 1518, 1481, 1439, 1308, 1215, 1169, 993, 835, 526 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) data, see Table 2; ^{13}C NMR (CD_3OD, 101 MHz) data, see Table 3; HRESIMS *m/z* 283.1322 [M-H]$^-$ (calcd. for $C_{18}H_{19}O_3$, 283.1334).

Multiflorabiphenyl C (5): colourless amorphous power; UV (MeOH) λ_{max} (log ε) 204 (4.16), 252 (4.10), 338 (3.24) nm; IR (KBr) ν_{max} 3444, 2918, 2850, 1714, 1647, 1456, 1385, 1223, 1157, 1070; ^{1}H NMR (DMSO-d_6, 400 MHz) data, see Table 2; ^{13}C NMR (DMSO-d_6, 101 MHz) data, see Table 3; HRESIMS *m/z* 297.1130 [M-H]$^-$ (calcd. for $C_{33}H_{41}O_5$, 297.1127).

Multiflorabiphenyl D (6): colourless amorphous power; UV (MeOH) λ_{max} (logε) 203 (3.82), 256 (3.82), 330 (2.78) nm; IR (KBr) ν_{max} 3419, 2972, 1612, 1518, 1473, 1377, 1360, 1363, 1217, 1171, 1124, 1045, 984, 889, 841, 592 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) data, see Table 2; ^{13}C NMR (CD_3OD, 151 MHz) data, see Table 3; HRESIMS *m/z* 297.1130 [M-H]$^-$ (calcd. for $C_{33}H_{41}O_5$, 297.1127).

Cytotoxicity Assay

Test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and were further diluted in culture media for assays. Paclitaxel was used as a positive control. HeLa, Capan 2, SGC-9701, HCT116, TE1 and KB cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). HeLa and Capan 2 cells were maintained in DMEM (Gibco/ Invitrogen, 12800-017). HCT116, TE1 and KB cells were maintained in 1640 (Hyclone, SH0809.01B), supplemented with 10% fetal bovine serum (Gibco/Invitrogen, St. Louis, MO, USA) and 10U/ml penicillin-streptomycin (Gibco/Invitrogen, 15140-122) at 37°C in a humidified 5% CO_2 incubator. Cell viability was assessed by an MTT assay. The cells were seeded in 96-well plates and treated with compounds at different concentrations. The viability was measured at 72 h after drug treatment. The cells were incubated with 100 µl of fresh medium containing 10 µl of 3-(4, 5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO, USA) and the subsequent dissolving of formazan crystals in DMSO. The absorbance was measured at 490 nm. The absorbance of the untreated cells in the media was determined to be 100% survival.

Flow Cytometry

HeLa cancer cells were treated with vehicle (0.1% DMSO) or test compounds at the indicated concentrations and times. The cells were fixed in 70% ethanol in PBS overnight. For cell cycle distribution, the cells were treated with propidium iodide (Sigma) and analyzed for their DNA content using BD FACSCalibur flow cytometry.

Western Blot Analysis

The cells were lysed in ice-cold whole cell extract buffer (50 mM pH8.0 Tris-HCl, 4 M urea and 1% TritonX-100), supplemented with a complete protease inhibitor mixture. The cell extracts were resolved by SDS-PAGE gel electrophoresis and then transferred to a polyvinylidene fluoride membrane. After blocking with 5% non-fat milk in Tris-buffered saline containing 0.2% Tween-20, the membranes were probed with the following antibodies: caspase-3 (Cell signalling, #9662), Bcl-2 (Cell signalling, #2870), Bcl-XL (Cell signalling, #2764), caspase-9 (Cell signalling, #9502), PARP (Cell signalling, #9542) or GAPDH (abcam, 2251-1). Following incubation with horseradish peroxidase coupled with secondary anti-rabbit antibodies (KPL, 474-1506), the protein bands were visualized using an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA). GAPDH was used to ensure an equal loading of protein.

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1

Figure Legends:

Figure 1. New compounds (**1**- **6**) obtained from the leaves of *Garcinia multiflora*.

Figure 2. Selected key correlations observed in the HMBC, TOCSY, and NOESY NMR spectra of **1**.

Figure 3. Selected key correlations observed in the HMBC, TOCSY, and NOESY NMR spectra of **2**.

Figure 4. Key correlations observed in the HMBC, TOCSY, and NOESY NMR spectra of **3**

Figure 5. Calculated ECD spectra of **1**-**3** and their experimental curves.

Figure 6. Key correlations observed in the HMBC NMR spectra of **4** - **6**.

Figure 7. Effects of compound **2** on cell apoptosis and cycle arrest.

Figure 8. Effects of compound **2** on apoptosis related proteins.

Table 1. NMR spectroscopic data for compounds **1**-**3**[a]

	1		**2**		**3**	
Position	δ_C [b]	δ_H [b]	δ_C [c]	δ_H [c]	δ_C [b]	δ_H [b]
1	62.4		65.7		63.4	
2	195.2		190.9		194.8	
3	117.9		116.5		120.9	
4	194.7		175.7		176.6	
5	67.7		62.1		65.1	
6	49.3		47.6		50.6	
7	47.8	1.51, m	45.8	1.28, m	47.8	2.68, m
8eq	40.8	2.15, d (13.7)	37.4	2.20, dd (13.5);	44.8	2.04, dd (14.1, 6.8)
8ax		2.08, dd (13.7, 6.6)		1.82, dd (14.2, 4.2)		2.20, d (14.0)
9	209.9		206.0		209.1	
10	196.0		171.2		173.8	
11	129.7		115.3		117.8	
12	117.4	7.17, s	107.5	7.23, s	109.5	7.43, s
13	146.2		146.0		147.0	
14	152.6		154.4		155.0	
15	115.2	6.69, d (8.2)	102.6	6.87, s	104.0	6.91, s
16	125.1	6.97, d (8.2)	149.3		151.5	
17	27.1	2.72, t-like	26.4	2.82, dd (13.7, 9.7)	27.5	2.90, br s
		2.58, d-like		2.66, d-like (12.5)		
18	120.8	4.93, br s	118.4	4.48, t-like	120.3	4.69, t-like
19	135.6		134.2		135.9	
20	26.3	1.71, s	18.1	1.63, s	18.6	1.77, s
21	18.2*	1.68, s	25.6	1.34, s	26.1	1.45, s
22	27.3	1.01, s	25.6	1.02, s	23.6	1.27, s
23	23.2	1.24, s	20.6	1.14, s	27.0	1.11, s
24	30.2	2.15, d-like	28.5	1.93, br d(13.1)	30.7	2.02, overlap
		2.06, m		1.69, overlap		1.80, overlap
25	125.4	4.87, overlap	123.1	4.81, t-like	125.2	4.81, t-like
26	133.7		132.3		133.8	
27	26.0	1.67, s	17.5	1.40, s	18.0	1.34, s
28	18.1	1.50, s	25.8	1.60, s	25.9	1.60, s
29	32.1	2.50, t-like	28.0	2.39, dd (14.3, 8.2)	38.2	2.16, dd (13.5, 11.2)
		2.47, d-like		2.33, dd (14.4, 6.8)		1.81, dd (14.0, 4.5)
30	120.9	5.16, br s	119.6	5.15, t (6.8)	44.7	2.70, m
31	135.5		133.6		150.2	
32	18.3*	1.68, s	25.8	1.68, s	113.9	4.37, d (43.3)
33	26.3	1.71, s	17.6	1.60, s	18.3	1.55, s
34					33.4	1.97, t-like
35					124.1	5.00, t-like
36					132.8	
37					25.9	1.65, s
38					18.0	1.60, s

[a]Recorded at 600 MHz (^{1}H) and 151 MHz (^{13}C). [b]In MeOD. [c]In DMSO-d_6.

Table 2. NMR spectroscopic data for compounds **4**–**6**[a]

	4		**5**		**6**	
Position	δ_C[b]	δ_H[b]	δ_C[c]	δ_H[c]	δ_C[b]	δ_H[b]
1	133.8		128.2		131	
2 (6)	128.8	7.32, d (8.6)	129.9	7.26, d (8.6)	131	7.33, d (8.4)
3 (5)	116.4	6.79, d (8.6)	114.8	6.76, d (8.6)	116	6.80, d (8.4)
4	157.8		156.2		157.5	
7	138.7		124.3		128	
8	113.4	6.85, d (2.2)	143.4		147.1	
9	151.2		141.4		116.8	
10	145.9		115.4		140.7	
11	136.6		141.8		142.9	
12	119.7	6.77, d (2.2)	116.8	6.62, s	117.7	6.64, s
1′	29.6	3.33, br d (7.2)	117.2	6.57, d (9.9)	118.6	6.63, d (9.9)
2′	124.5	5.28, ddd (7.2, 4.2, 1.3)	131.1	5.76, d (9.9)	131.9	5.73, d (9.9)
3′	132.9		74.8		77.1	
4′	25.9	1.73, s	27	1.32, s	27.7	1.45, s
5′	17.9	1.72, s	27	1.32, s	27.7	1.45, s
8-Me					61.5	3.33, s
10-Me	60.9	3.76, s				
11-Me			60.5	3.73, s		

[a]Recorded at 600 MHz (^{1}H) and 151 MHz (^{13}C). [b]In MeOD. [c]In DMSO-d_6.

Table 3. Cytotoxicity of Isolated Compounds against Cancer Cell Lines [a,b]

	Cytotoxicity IC_{50} (μM)					
Compounds	HeLa	SGC7901	TE1	HCT116	Capan 2	HL-7702 [d]
2	7.65±0.18	4.20±0.33	8.14±1.74	5.96±0.13	> 10	5.44±0.067
9	> 10	> 10	9.01±0.61	9.95±0.75	> 10	8.61±0.67
Paclitaxel [c]	0.54±0.35	0.34±0.02	0.25±0.025	0.16±0.021	3.50±0.18	0.24±0.06

[a] Results are expressed as IC_{50} (mean values ± SD, n = 3) in μM. [b] Compounds **1, 3-8** and **10–12** were inactive in these human cancer cell lines (IC_{50} > 10 μM). [c] Positive control. [d] Human normal hepatic cells.

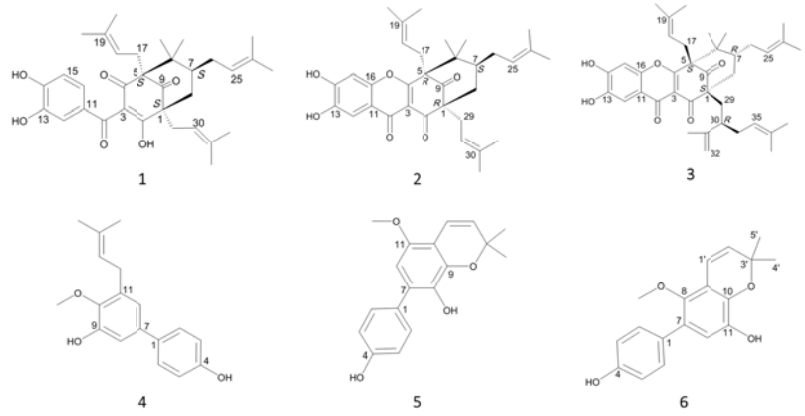

Fig. 1 New compounds (**1** - **6**) obtained from the leaves of *Garcinia multiflora*.

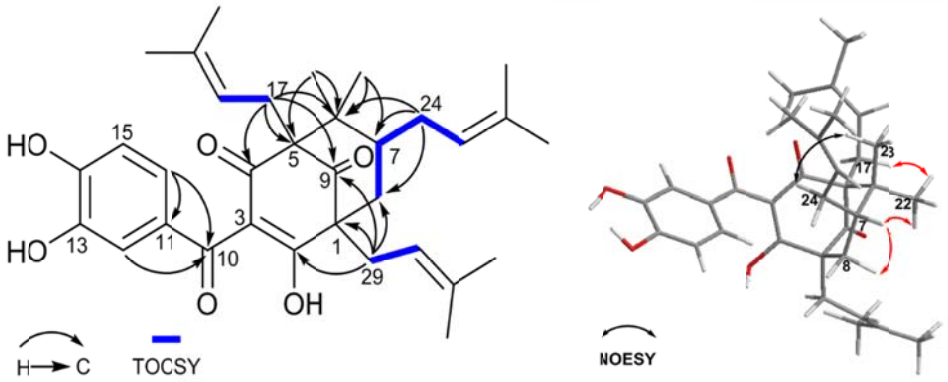

Fig. 2 The key correlations observed in the HMBC, TOCSY, and NOESY NMR spectra of **1**

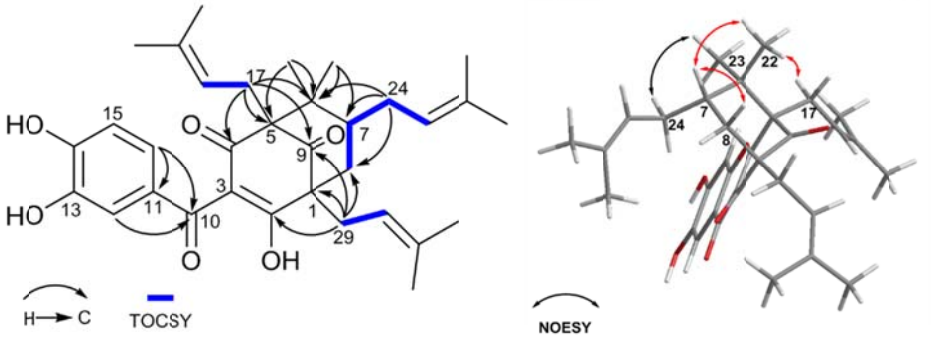

Fig. 3 The key correlations observed in the HMBC, TOCSY, and NOESY NMR spectra of **2**.

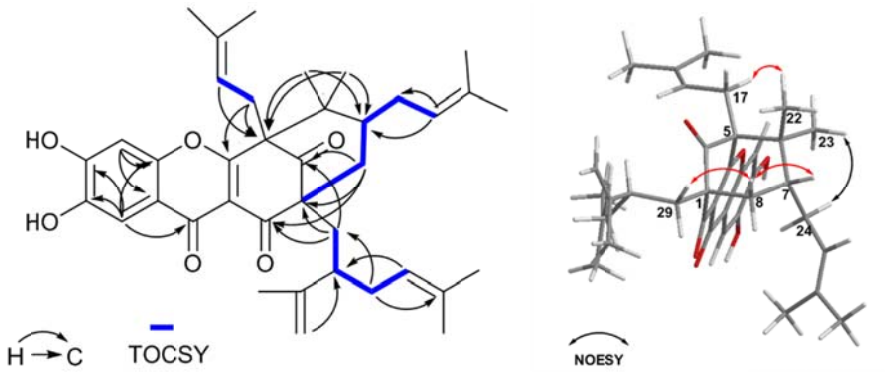

Fig. 4 Key correlations observed in the HMBC, TOCSY, and NOESY NMR spectra of 3.

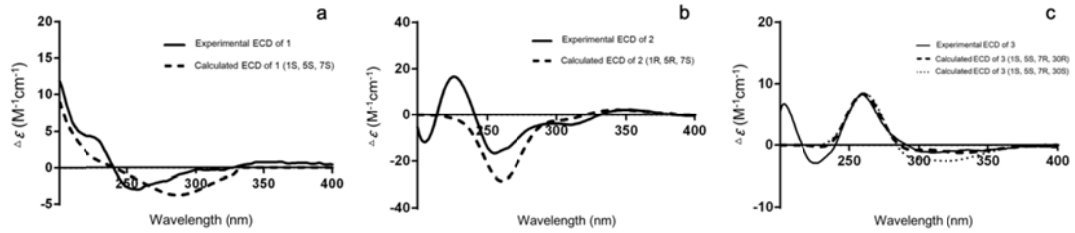

Fig. 5 Calculated ECD spectras of **1**-**3** and their experimental curves.

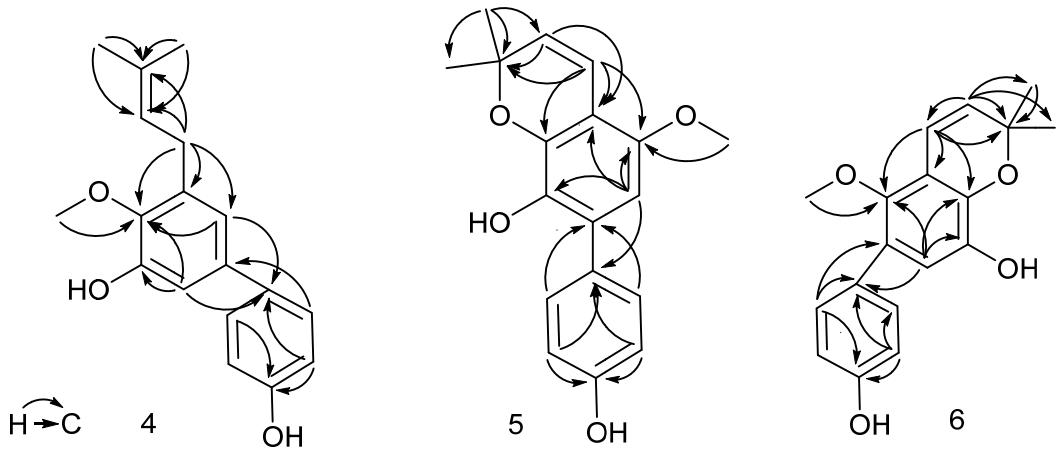

Fig. 6 Key correlations observed in the HMBC and H-H COSY NMR spectra of **4** - **6**.

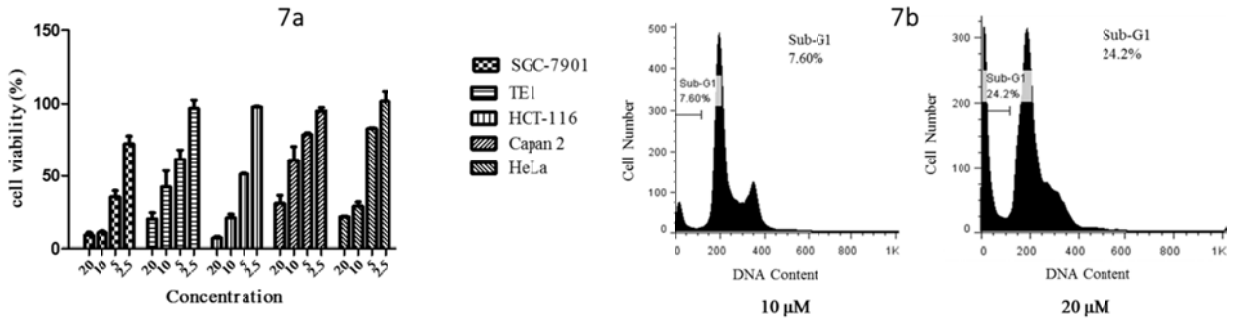

Fig. 7 Effects of compound **2** on cell apoptosis and cycle arrest. HeLa cells were treated with indicated chemicals. After 24 h of treatment, the cells were harvested, fixed in 70% EtOH, and stained with PI. The cell cycle and apoptosis rate were detected by FACS. (a). Cell apoptosis in a dose-dependent fashion. (b). Cell cycle arrest was induced at the G1 phase (compound **2** at 10 μ M and 20 μ M).

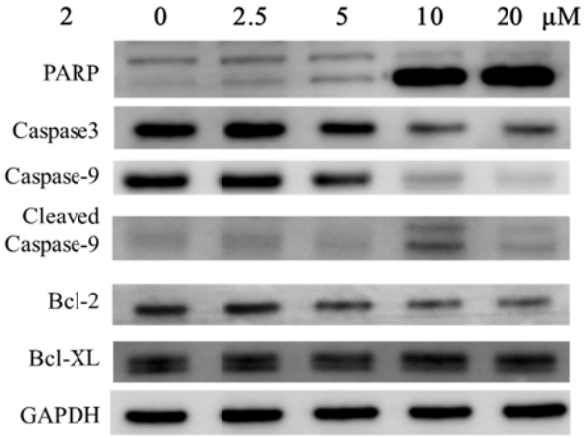

Fig. 8 Effects of compound **2** on apoptosis-related proteins. Western blots of Caspase 3, Caspase 9, cleaved Caspase 9, PARP, Bcl-2, Bcl-XL and GAPDH of HeLa cells after 24 h treatment with compound **2**.