PAPER

Check for updates

Design, synthesis and biological evaluation of valepotriate derivatives as novel antitumor agents†

Bo Zhang,‡[ab] Ruiying Guo,‡[c] Yongzhou Hu,[c] Xiaowu Dong, [ID] [c] Nengming Lin,[b] Xiaoyang Dai,[a] Honghai Wu,[a] Shenglin Ma*[b] and Bo Yang*[a]

Natural products remain the largest resources of lead compounds that can be used to develop novel anticancer drug candidates. Based on deacetylisovaltratum, a natural product with promising anticancer activity, herein we designed and synthesized of a series of valepotriate derivatives with a novel skeleton from commercially available genipin. In addition, a structure–activity relationship study demonstrated the importance of an epoxy group on the C1-position and the preferable size of the sidechain ((5-methylhexanoyl)oxy) on the C-7 position of valepotriates for their cytotoxic activities. The most potent compound **1e** showed moderate to good IC_{50} values against various cancer cells, ranging from 10.7 to 50.2 μM, which are comparable to that of deacetylisovaltratum. Additionally, we demonstrate that mitochondrion-mediated apoptosis would be its mechanism of action, thus enlightening the further development of novel valepotriate derivatives.

1 Introduction

Natural product research continues to explore a variety of lead compounds, which are potential templates for the development of new anticancer drug candidates.[1–3] However, challenges remain unsolved in either the isolation or purification of bioactive natural products; thus semisynthesis becomes a preferable method to obtain bioactive compounds.[4] The semisynthetic strategy is widely used when the precursor molecules are structurally complex, or difficult produce by total synthesis, such as anticancer drugs (*i.e.* paclitaxel, etoposide, and irinotecan), antimalarial drugs (*i.e.* roxithromycin) and antibiotics (*i.e.* cefixime) (Fig. 1).

Valerian is a very important genus of plants used as a medicinal herb in many areas of the world, the roots and rhizomes of which have been used for the treatment of epilepsy, hysteria, nervous disorders, neurasthenia and emotional stress.[5–9] In particular, *Patrinia heterophylla Bunge*, belonging to the genus Valerian, showed anticancer activity against

metrocarcinoma and cervical cancer in ancient China, whereas the biological components responsible for its anticancer activity are not fully recognized.[10,11] Indeed, a variety of pharmacologically active components (*e.g.* monoterpenes, valepotriates, and sesquiterpenes) have been isolated and characterized.[12,13] Among them, valepotriates, belonging to the family of iridoids with a 10-carbon basic skeleton, showed their significant activity against cancer cells.[1–3,14] Recently, we found that deacetylisovaltratum, a compound belonging to the class of valepotriates, could effectively cause G2/M-phase arrest in gastric cancer cells by disrupting tubulin polymerization, and inducing mitochondrion-dependent apoptosis.[15,16] Considering the unique structure of epoxy group in deacetylisovaltratum, the alkylating properties could be responsible for its potent anti-
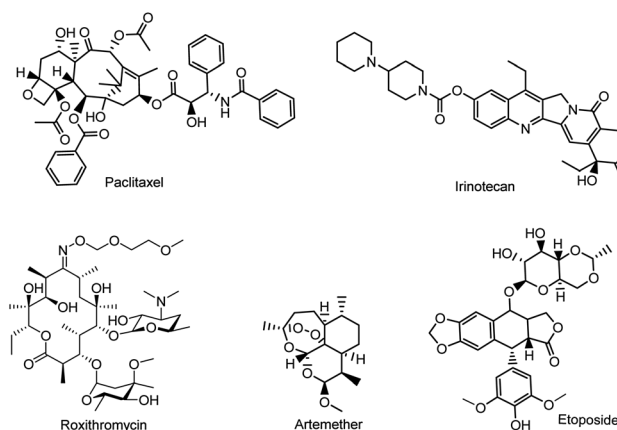
[a]Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, Institute of Pharmacology and Toxicology, School of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, P. R. China. E-mail: yang924@zju.edu.cn; Tel: +86-57188208400

[b]Translational Medicine Research Center, Nanjing Medical University, Affiliated Hangzhou Hospital, Hangzhou First People's Hospital, Hangzhou, Zhejiang 310006, P. R. China. E-mail: mashenglin@medmail.com.cn; Tel: +86-57156007908

[c]ZJU-ENS Joint Laboratory of Medicinal Chemistry, Zhejiang Province Key Laboratory of Anti-Cancer Drug Research, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, P. R. China

† Electronic supplementary information (ESI) available: NMR spectra of the synthesized compounds. See DOI: 10.1039/c6ra27478a

‡ These authors contributed equally to this work.

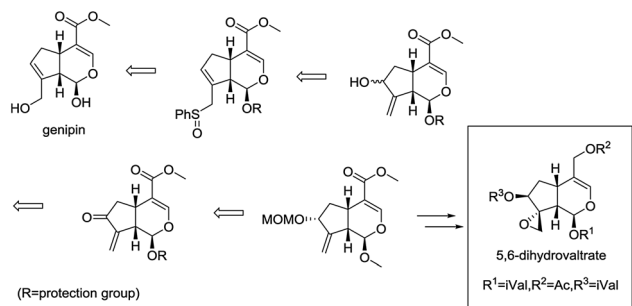Fig. 1 Examples of semisynthetic drugs.

Fig. 2 Murakami's synthetic strategy.

cancer activity. Moreover, the structural diversity might change its physicochemical properties and also its intracellular activities. However, due to the lack of structural diversity in natural valepotriates, as well as the difficulty in synthetic procedures, the structural–activity relationship (SAR) studies of valepotriate analogues is very rare, which hampers the development of valepotriate as novel anti-cancer agents. Therefore, the exploration of the crucial structural requirement of valepotriates for anticancer activities is particularly needed. In this study, we intend to acquire series of valepotriate derivatives via semisynthesis and to study the biological activity of these compounds.
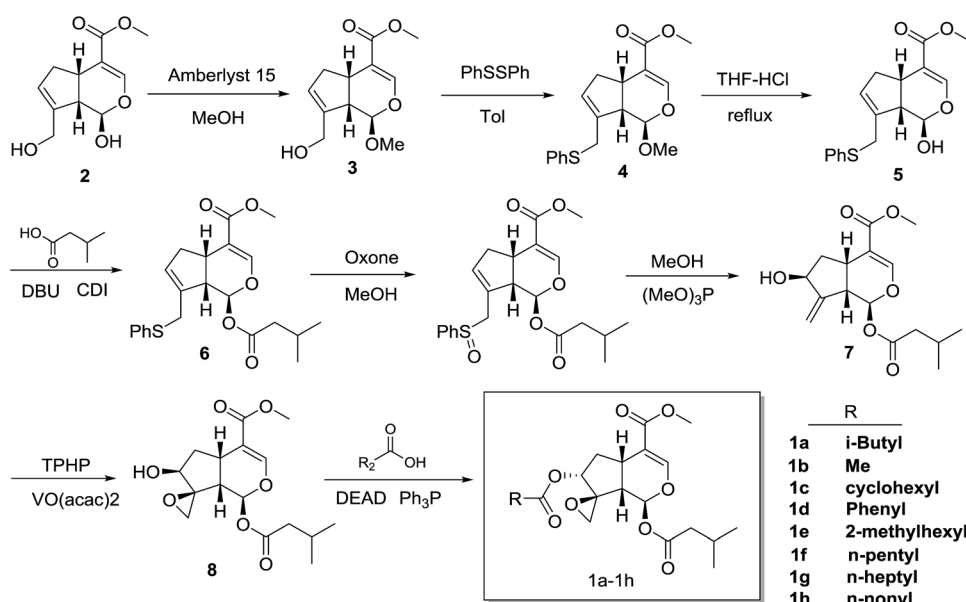
Recently, Murakami et al. reported the concise synthesis of 5,6-dihydrovaltrate from commercially available iridoid genipin, providing a novel semisynthetic route in structurally optimization of valepotriates (Fig. 2).[17,18] As part of our continued interest in the area of chemical modification of natural products,[19,20] herein, we designed, synthesized a series of valepotriate derivatives with novel skeleton from genipin using slightly modified Murakami's method. Noteworthy, some crucial SAR clues were found after investigating the effect of epoxy and

length of aliphatic side chain on cytotoxicity against a variety of cancer cells. More significantly, some compounds showed superior cytotoxic activities than that of deacetylisovaltratum. In addition, mitochondrion-mediated apoptosis was significantly observed, which would be the exact action mechanism of its cytotoxic activities, thus enlightening the further development of novel valepotriate derivatives.

2 Results and discussion

2.1 The synthesis of valepotriate derivatives

The synthesis of compounds **1a–h** started with genipin is illustrated in Scheme 1. At first, genipin **2** was treated by Amberlyst-15 resins in methanol to afford methyl acetal **3**, which was submitted to phenylsulfidation using diphenyldisulfide and tributylphosphane in toluene to give phenylsulfide **4**. After acidic hydrolysis of the methyl acetal with 10% HCl–THF, the resulting hemiacetal **5** was coupled with isovaleric acid in the presence of isovaleric acid, 1,1′-carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to yield isovaleryl acetal **6**. After oxidation of **6** with oxone in methanol, the resulting sulfoxide was subjected to Mislow–Evans rearrangement with trimethyl phosphite in MeOH to afford allyl alcohol **7**. In this rearrangement procedure, the reaction time was quite crucial for the conversion, which should be carefully monitored by TLC analysis. Then, stereoselective epoxidation associated with the adjacent hydroxyl group using tert-butyl hydroperoxide (TBHP) and vanadium oxyacetylacetonate [VO(acac)$_2$][17,18,21,22] afforded epoxyalcohols **8**.[18,22] Finally, desired compounds **1a–h** was obtained by introduction of the appropriate aliphatic acid group to C-7 of compound **7** by Mitsunobu inversion using diethyl azodicarboxylate (DEAD) and triphenylphosphine (Fig. 3).

Scheme 1 Synthesis of valepotriate derivatives. Reagents and conditions: (a) Amberlyst-15, MeOH; (b) PhSSPh, Tol; (c) 10%HCl, THF; (d) isovaleric acid, DBU, CDI; (e) oxone, MeOH; (f) (MeO)$_3$P, MeOH; (g) TPHP, VO(acac)$_2$, Tol; (h) appropriate acid, DEAD, Ph$_3$P, Tol.
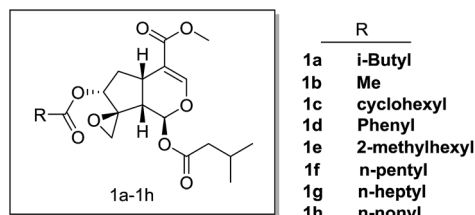
Fig. 3 The structures of valepotriate derivatives **1a–1h** in this study.

2.2 Cytotoxicity of compounds 1a–h against various cancer cells

To investigate whether these compounds could suppress the growth of cancer cells, anti-proliferative effect was tested against various cancer cell lines, including pancreatic cancer cells (SW1990, BXPC3, CAPAN2, CFPAC, PANC1), breast cancer cells (BT474, MCF7, MDA-MB-231), gastric cancer cells (AGC, HGC-27, KATOIII) and lung cancer cells (H1795) (Table 1). As expected, some of these compounds (*i.e.* **1c**, **1d**, **1e**) showed comparable cytotoxic activities as the parental compound deacetylisovaltratum, suggesting that the novel skeleton with methoxycarbonyl on C4-position of valepotriate remained the anticancer activities. Moreover, we found epoxy group on C1-position of valepotriate was very crucial by comparing with the anticancer activities of **7a** and deacetylisovaltratum, which was in consistent with the work of Murakami *et al.*[22] In another study, R. Bos *et al.* reported the cytotoxicity of valepotriates analogues against GL4 and COLO 320 cells with IC$_{50}$ values ranging from 1 to 6 μM.[12] Additionally, three valepotriate isomers isolated by S. Lin *et al.* displayed moderate cytotoxicity against various cancer cell lines with IC$_{50}$ values varied from 2.8 to 8.3 μM.[23] In this study, **1e** showed comparable cytotoxic activity as previously reported valepotriate analogues. In further SAR analysis, either shortening or prolonging the sidechain (*i.e.* **1a**, **1b**, **1f**, **1g**, **1h**) undermined its biological activities, revealing that (5-methylhexanoyl)oxy group on C-7 position would be preferable lipophilicity towards cancer cells. Meanwhile, the

replacement of (5-methylhexanoyl)oxy group with aromatic (**1d**) and cyclohexyl (**1c**) substituents reduced its cytotoxic activity. Based on our efforts in structural optimization of valepotriate analogues, we successfully discovered a potent semisynthetic compound **1e** bearing unique skeleton distinguished from deacetylisovaltratum which was previously identified as potent natural anti-cancer agent.

2.3 Cytotoxicity of 1e in human lung cancer H1975 cells

Human lung cancer H1975 cell harbours both L858R and T790M mutation in epidermal growth factor receptor (EGFR), thus it is resistant to first-generation EGFR tyrosine inhibitor such as gefitinib or erlotinib. As recently reported, several plants-derived compounds possessed anti-proliferation activity against H1975, and the IC$_{50}$ value varied from 3 to 15 μM.[24–26] In our study, the cytotoxicity of **1e** was determined by CCK-8 assay in human lung cancer H1975 cells (Fig. 4). **1e** exhibited both time- and concentration-dependent cytotoxicity against H1975 cells. The IC$_{50}$ values of 48 and 72 h treatment with **1e** were 27.4 and 13.1 μM respectively.

2.4 Compound 1e triggered apoptosis in H1975 cells

Apoptosis was usually involved in the proliferation inhibitory effect caused by natural compound.[27] Annexin V/PI staining was used to characterize the early and late apoptotic cells after cells were treated with indicated concentrations of **1e** for 48 h.[28] In H1975 cells, treatment with 20 μM of **1e** yielded a 35% apoptosis rate, which was in consistent with the IC$_{50}$ value of 48 h treatment (Fig. 5A). By comparing the apoptosis induction activity of **1e** with other natural derived agents in H1975 cells, **1e** exhibited superior anti-cancer activity than cordycepin, but slightly less effective than shikonin.[24,25] Meanwhile, depolarized mitochondria membrane potential was detected after 24 h treatment with 20 μM of **1e** (Fig. 5B). In addition, DAPI stain was used to verify the occurrence of apoptosis. After 48 h treatment with 20 μM of **1e**, nucleus with shrunk size and intensified fluorescence were seen under fluorescence microscopy. Moreover, apoptotic

Table 1 Cytotoxic activities of valepotriate derivatives **1a–h** against various cancer cells[a]

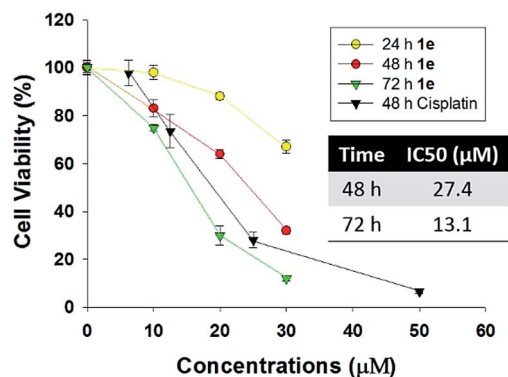| Cell lines | Cancer type | Cytotoxic activities, IC$_{50}$ (μM) | | | | | | | | | |
		DI	7a	1a	1b	1c	1d	1e	1f	1g	1h
SW1990	Pancreatic cancer	34.9	85.2	59.1	>100	40.2	54.9	26.1	N.T.	N.T.	N.T.
BXPC3		14.4	24.6	48.2	>100	28.1	37.1	25.1	N.T.	N.T.	N.T.
CAPAN2		17.5	63.7	37.4	53.8	33.9	68.0	31.6	40.7	>100	>100
CFPAC		13.0	87.7	47.4	52.0	29.5	60.9	27.9	54.3	32.0	53.9
PANC1		14.8	>100	86.9	N.T.	48.0	66.9	20.6	N.T.	N.T.	N.T.
BT474	Breast cancer	24.3	N.T.	97.8	>100	21.4	54.0	50.2	N.T.	N.T.	N.T.
MCF7		27.5	51.2	77.4	>100	18.1	19.8	10.7	N.T.	N.T.	N.T.
MDA-MB-231		23.1	51.0	42.8	>100	12.7	27.3	13.2	N.T.	N.T.	N.T.
AGS	Gastric cancer	7.7	53.2	26.3	>100	28.9	36.9	22.9	55.7	59.5	>100
HGC-27		14.8	25.7	47.5	>100	20.9	28.2	21.5	>100	54.0	26.6
KATOIII		32.2	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	48.8	51.2	92.5
H1975	Lung cancer	14.3	21.0	25.8	93.0	28.2	15.9	11.3	N.T.	N.T.	N.T.

[a] N.T. indicates not detectable.

Fig. 4 The inhibitory effect of **1e** on human lung cancer cell line H1975. H1975 cells were seeded in 96-well plates and then treated with **1e** as indicated. Treatment was washed off after 24, 48, 72 or 96 h and cell viability was calculated using CCK-8 assay.
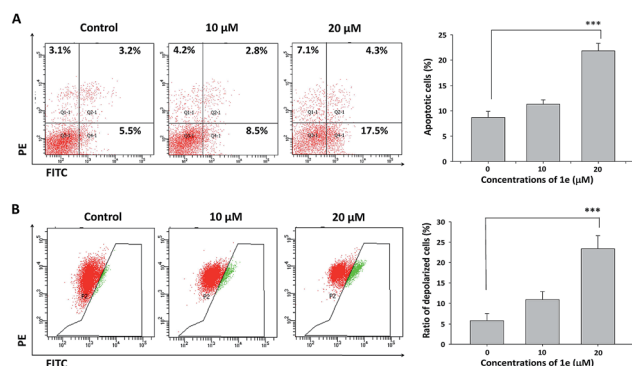

Fig. 5 **1e** triggered mitochondrion-dependent apoptosis in H1975 cells. (A) H1975 cells were seeded in 6-well plate and cultured for 24 h. Cells were treated with **1e** for 48 h before staining and followed by flow cytometric analysis. (B) H1975 cells were seeded in 6-well plate and cultured for 24 h. Cells were treated with **1e** for 12 h before JC-1 stain and flow cytometric analysis. Three independent experiments were quantitatively analyzed. Each bar represented the mean ± SD. ***$P < 0.001$.

bodies could be observed at 20 μM of **1e**, suggesting that H1975 cells were undergoing apoptosis (Fig. 6).

2.5 Compound 1e induced caspase-dependent apoptosis

Mitochondrion plays a critical role in the apoptosis caused by natural compounds. In H1975 cells, reduced expression of Bcl-
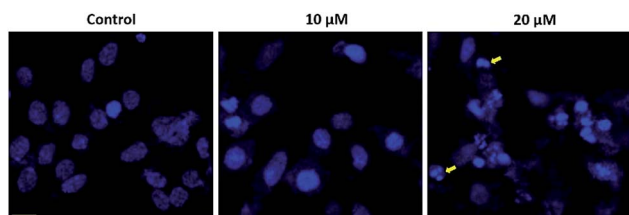

Fig. 6 H1975 cells were seeded in 6-well plate and cultured for 24 h. Cells were treated with **1e** for 48 h before DAPI stain and observed under fluorescence microscopy. Yellow arrows indicates apoptotic bodies. Scale bar = 20 μm.
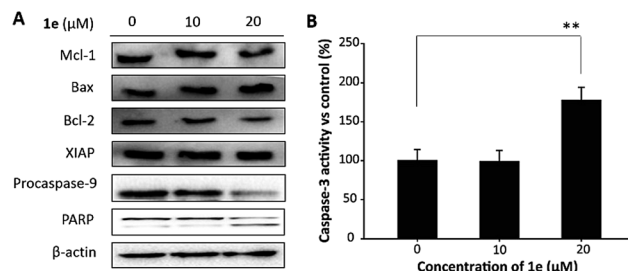

Fig. 7 **1e** triggered apoptosis in H1975 cells. (A) After treated with **1e** for 48 h, cells were lysed and proteins were analysed by western blot. (B) After treated with **1e** for 48 h, cells were lysed and caspase-3 activity was analysed using Caspase-3 Colorimetric Assay Kit (Biovision, Milpitas, CA, US).

2, activation of caspase-3 and cleavage of poly-(ADP-ribose)-polymerase (PARP) was closely associated with the apoptosis induced by isoliquiritigenin.[29] In this study, to verify the role of mitochondrion in **1e** triggered apoptosis, we performed western blots to analyze apoptosis-related proteins. As shown in Fig. 7A, 20 μM of **1e** induced an elevated level of Bax, and meanwhile the expression of Bcl-2 was decreased. In addition, decreased protein level of procaspase-9 and cleaved PARP were found after treatment with 20 μM of **1e**, indicating that **1e** could trigger mitochondrion-dependent apoptosis. In addition, 20 μM of **1e** significantly induced an increase in caspase-3 activity (Fig. 7B), which was not found in 10 μM of **1e** treatment. Meanwhile, the protein expression of XIAP was found unchanged after treatment with **1e**, suggesting that XIAP was not involved in **1e** activated caspase-3. The anti-cancer activity of **1e** was comparable with other plants derived compounds. For instance, Wang and *et al.* reported mitochondrion-dependent apoptosis induced by cordycepin at the concentration of 15 μM in H1975 cells.[25] Lu and *et al.* found that treatment with 150 μg mL^{-1} of sulfated galactoglucan could activate caspase-3 in H1975 cells.[30] These results indicated that **1e** as a derivative of deacetyliso-valtratum obtained from semi-synthesis, achieved superior anticancer activity than the parental compound. Moreover, the anti-proliferation ability was at a similar level of other plants derived compounds.

3 Conclusion

In this study, we designed and synthesized of a series of vale-potriate derivatives from genipin using slightly modified Murakami's method. Among these derivatives, **1e** which contained a novel skeleton with methoxycarbonyl on C4-position and (5-methylhexanoyl)oxy group on C-7 position possessed stronger anticancer activity than its parental compound deacetylisoval-tratum. Noteworthy, specific SAR has been found: (1) natural derived group at 1st position is better than others; (2) different substituents at the 7th position can be critical for its bioactivity; (3) the length of aliphatic side chain is crucial to its anticancer activity. Finally, **1e** has been found with superior potency than lead compound deacetylisovaltratum. In addition, **1e** induced mitochondrion-mediated apoptosis was significantly observed,

which might be the action mechanism of its cytotoxic activities, thus enlightening the further development of novel valepotriate derivatives.

4 Experimental section

4.1 Chemistry

Proton NMR spectra were obtained on a Bruker AVII 500 or 400 NMR spectrometer (500 or 400 MHz) by use of $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Carbon-13 NMR spectra were obtained a Bruker spectrometer (125 MHz) by use of $CDCl_3$ CD_3OD or DMSO-d6 as solvent. Carbon-13 chemical shifts are referenced to the central peak of $CDCl_3$ (d 77.0 ppm) or DMSO-d6 (d 39.5 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, double; t, triplet; q, quartet; m, multiplet; J, coupling constant (Hz). ESI-MS spectra were obtained from Shimadzu LCMS-2020 mass spectrometer. Unless otherwise noted, reagents and solvent were obtained from commercial suppliers and without further purification.

4.1.1 Conversion from genipin (2) to methyl acetal (3).
To a solution of genipin (3.57 g, 16.14 mmol) in MeOH (30 mL) was added Amberlyst-15 (10.51 g). The mixture was stirred rt overnight, and the resulting mixture was filtered and washed by EtOAc, and the combined filtrates were concentrated under reduced pressure to give the light yellow oil. After being purified by silica gel column chromatography (PE/EtOAc = 2 : 1) to afford 3 (3.2 g, 84%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (s, 1H), 5.81 (s, 1H), 4.46 (d, J = 8.1 Hz, 1H), 4.22 (s, 2H), 3.70 (s, 3H), 3.56 (s, 3H), 3.17 (q, J = 8.4 Hz, 1H), 2.85 (ddd, J = 16.5, 8.5, 1.3 Hz, 1H), 2.58 (t, J = 8.0 Hz, 1H), 2.09–2.02 (m, 1H). LC-MS: 241 (M + H).

4.1.2 Conversion from methyl acetal (3) to phenylsulfide (4).
To a solution of 2 (10.6 g, 44.1 mmol) in Tol (80 mL) was added PhSSPh (8.47 g, 55.2 mmol) and tributylphosphine (13.2 mL, 53.1 mmol). The mixture was stirred at rt for 3 h, the mixture was concentrated under reduced pressure to give a residue, which was purified by silica gel column chromatography (from PE to PE/EtOAc = 10 : 1) to afford 4 (13.88 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 0.9 Hz, 1H), 7.33–7.30 (m, 2H), 7.29–7.26 (m, 2H), 7.19 (d, J = 7.3 Hz, 1H), 5.68 (s, 1H), 4.51 (d, J = 7.7 Hz, 1H), 3.71 (s, 3H), 3.62–3.58 (m, 1H), 3.56 (s, 3H), 3.12 (td, J = 8.2, 0.9 Hz, 1H), 2.77 (dd, J = 16.4, 8.5 Hz, 2H), 2.05 (dd, J = 8.2, 1.4 Hz, 1H). LC-MS: 333 (M + H).

4.1.3 Conversion from phenylsulfide (4) to hemiacetal (5).
To a solution of 4 (5.0 g, 15.1 mmol) in THF (80 mL) was added aqueous 10% HCl (39 mL). The mixture was stirred at 60 °C for 16 h. The mixture was cooled to rt and poured into aqueous saturated $NaHCO_3$, then the whole was extract with EtOAc (50 mL × 3). The combined organic layer was washed by aqueous saturated NaCl and dried over Na_2SO_4. Then the organic layer was concentrated under reduced pressure to give a residue, which was purified by silica gel column chromatography (from PE/EtOAc = 10 : 1 to PE/EtOAc = 2 : 1) to afford 5 (1.98 g, 41.3%, 40% recovery of 4). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.32 (d, J = 7.5 Hz, 2H), 7.29–7.24 (m, 2H), 7.18 (t, J = 7.3 Hz, 1H), 5.71 (s, 1H), 4.85 (d, J = 8.3 Hz, 1H), 3.87 (d, J = 14.4 Hz, 1H), 3.71 (s, 3H), 3.58 (t, J = 6.6 Hz, 1H), 3.12 (q, J = 8.2 Hz, 1H),

2.79 (dd, J = 16.5, 8.6 Hz, 1H), 2.69 (t, J = 7.5 Hz, 1H), 2.03–1.97 (m, 1H), 1.87 (dd, J = 14.8, 6.8 Hz, 1H), 1.76–1.68 (m, 1H). LC-MS: 319 (M + H).

4.1.4 Conversion from hemiacetal (5) to hemiester (6).
A solution of CDI (14.4 g, 88 mmol) in CH_2Cl_2 (70 mL) was treated with isovaleric acid (14.0 mL, 88 mmol) and DBU (264 μL, 1.76 mmol) at 0 °C for 10 min. The mixture was added to a solution of the 5 (2.8 g, 8.8 mmol) in CH_2Cl_2 (10 mL), then the whole was stirred at rt overnight. After the reaction mixture was poured into aqueous saturated NaCl, the whole was extracted with EtOAc. The organic layer was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and aqueous saturated NaCl and dried over Na_2SO_4. Concentrate the organic layer under reduced pressure to give a residue, which was purified by silica gel column chromatography (PE/EtOAc = 10 : 1) to afford 6a (2.8 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 1.1 Hz, 1H), 7.33–7.26 (m, 4H), 7.20 (ddd, J = 7.2, 3.9, 1.3 Hz, 1H), 5.95 (d, J = 6.9 Hz, 1H), 5.67 (s, 1H), 3.76 (d, J = 14.2 Hz, 1H), 3.71 (s, 3H), 3.49–3.44 (m, 1H), 3.19 (dd, J = 11.4, 4.5 Hz, 1H), 2.97 (t, J = 7.3 Hz, 1H), 2.76 (dd, J = 16.7, 8.2 Hz, 1H), 2.31–2.07 (m, 4H), 0.96 (dd, J = 6.6, 4.2 Hz, 6H). LC-MS: 425 (M + Na$^+$).

4.1.5 Conversion from 6 to alcohol (7).
To a solution of 6 (750 mg, 1.87 mmol) in MeOH (30 mL) was added oxone (687 mg, 1.11 mmol) at 0 °C for 1 h. Then the reaction was quenched by aqueous saturated Na_2SO_3 for 10 min, and the mixture was extracted with EtOAc. The organic layer was washed by aqueous saturated NaCl and dried over Na_2SO_4. Concentration of the organic layer under reduced pressure gave the crude sulfoxide. To a solution of crude sulfoxide in MeOH (20 mL) was added $(MeO)_3P$ (331 μL, 2.8 mmol) and the mixture was heated under reflux for 5 h. After it's fully reacted, the mixture was cooled to rt and poured into aqueous saturated NaCl, then the mixture was extracted by EtOAc, and the organic layer was washed by aqueous saturated NaCl and dried over Na_2SO_4. Concentrate the organic layer under reduced pressure to give a residue, which was purified by silica gel column chromatography (PE/EtOAc = 2 : 1) to afford 7 (430 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56–7.53 (m, 1H), 7.42 (d, J = 1.2 Hz, 1H), 7.35 (dd, J = 4.3, 3.1 Hz, 1H), 5.89 (d, J = 6.7 Hz, 1H), 5.43–5.40 (m, 1H), 5.31 (t, J = 1.8 Hz, 1H), 4.48 (t, J = 4.2 Hz, 1H), 4.10 (q, J = 7.1 Hz, 1H), 3.81 (d, J = 12.7 Hz, 3H), 3.72 (s, 3H), 3.25 (d, J = 7.4 Hz, 1H), 2.95–2.89 (m, 1H), 2.31–2.21 (m, 2H), 2.18 (ddd, J = 13.5, 6.7, 4.4 Hz, 1H), 2.12 (dt, J = 13.7, 6.8 Hz, 1H), 2.03 (s, 2H), 1.89 (ddd, J = 13.7, 8.0, 6.0 Hz, 1H), 1.24 (t, J = 7.1 Hz, 2H), 0.96 (dd, J = 6.6, 2.6 Hz, 7H), 0.90–0.78 (m, 1H). LC-MS: 311 (M + H).

4.1.6 Conversion from alcohol (7a) to target compound 1a to 1h.
To a solution of 7 (46.5 mg, 0.15 mmol) in toluene (10 mL) was added $VO(acac)_2$ (3.9 mg, 0.015 mmol) and 70% TBHP (62 μL, 0.45 mmol) until 7 was fully consumed. Then, the reaction was quenched by aqueous saturated $NaHCO_3$ for 15 min, and the mixture was extracted with EtOAc. The organic layer was washed by aqueous saturated NaCl and dried over Na_2SO_4. The crude epoxide was given by the concentration of organic layer under reduced pressure. Under the protection of N_2, the solution of crude epoxide in toluene was added DEAD (56 μL, 0.15 mmol) at the presence of Ph_3P and acid at 0 °C. Then, the mixture was stirred at rt for 2 h. The whole was concentrated under reduced

pressure and directly purified by silica gel column chromatography (PE/EtOAc = 10 : 1) to afford the target compounds.

Compound **1a** (26.6 mg, 43.2%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42 (d, J = 1.0 Hz, 1H), 6.11 (d, J = 5.6 Hz, 1H), 5.03 (dd, J = 7.7, 4.1 Hz, 1H), 3.73 (d, J = 3.1 Hz, 3H), 3.25 (d, J = 7.4 Hz, 1H), 3.06 (d, J = 4.6 Hz, 1H), 2.95 (d, J = 4.6 Hz, 1H), 2.86–2.75 (m, 1H), 2.37 (dd, J = 7.7, 5.6 Hz, 1H), 2.24 (t, J = 6.8 Hz, 2H), 2.10 (t, J = 6.1 Hz, 3H), 2.04–1.98 (m, 1H), 1.89 (dd, J = 14.8, 1.4 Hz, 1H), 0.96 (dd, J = 6.6, 1.8 Hz, 6H), 0.91 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 170.92, 170.04, 165.64, 150.33, 109.57, 87.30, 76.21, 75.26, 64.40, 50.41, 47.01, 42.25, 42.04, 41.74, 36.07, 29.85, 24.61, 24.59, 21.30, 21.24. LC-MS: 411 (M + H).

Compound **1b** (28.5 mg, 51.6%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.41 (d, J = 1.3 Hz, 1H), 6.12 (d, J = 5.8 Hz, 1H), 5.00 (dd, J = 7.7, 4.4 Hz, 1H), 3.74 (s, 3H), 3.25 (d, J = 7.7 Hz, 1H), 3.05 (d, J = 4.6 Hz, 1H), 2.97 (d, J = 4.6 Hz, 1H), 2.82 (dd, J = 7.8, 7.0 Hz, 1H), 2.59 (s, 3H), 2.34 (dd, J = 7.6, 5.9 Hz, 1H), 2.24 (dd, J = 7.1, 5.9 Hz, 2H), 2.15–2.06 (m, 1H), 1.90–1.83 (m, 1H), 0.96 (dd, J = 6.6, 1.9 Hz, 6H). LC-MS: 369 (M + H).

Compound **1c** (27 mg, 41.2%): ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42 (d, J = 1.2 Hz, 1H), 6.10 (d, J = 5.3 Hz, 1H), 5.01 (dd, J = 7.6, 3.8 Hz, 1H), 3.73 (s, 3H), 3.25 (dd, J = 13.5, 6.6 Hz, 1H), 3.06 (d, J = 4.6 Hz, 1H), 2.93 (d, J = 4.6 Hz, 1H), 2.80–2.73 (m, 1H), 2.39 (dd, J = 7.6, 5.3 Hz, 1H), 2.23 (dd, J = 15.4, 8.6 Hz, 3H), 2.10 (m, 1H), 1.91 (ddd, J = 14.9, 5.3, 3.9 Hz, 1H), 1.79 (s, 2H), 1.70 (s, 2H), 1.62 (d, J = 9.5 Hz, 1H), 1.57 (d, J = 4.1 Hz, 3H), 1.40–1.31 (m, 2H), 0.96 (dd, J = 6.6, 1.9 Hz, 6H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 174.83, 171.08, 166.65, 151.35, 110.59, 88.27, 77.23, 76.24, 65.55, 51.41, 48.01, 43.06, 42.85, 37.02, 30.93, 28.79, 28.75, 25.66, 25.62, 25.35, 25.31, 22.26. LC-MS: 437 (M + H).

Compound **1d** (24 mg, 37.2%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.94 (dd, J = 8.3, 1.2 Hz, 2H), 7.56 (s, 1H), 7.49 (d, J = 1.3 Hz, 1H), 7.43 (t, J = 7.8 Hz, 2H), 6.20 (d, J = 5.1 Hz, 1H), 5.30 (s, 1H), 3.67 (s, 3H), 3.33 (dt, J = 7.7, 6.4 Hz, 1H), 3.12 (d, J = 4.5 Hz, 1H), 3.04 (d, J = 4.5 Hz, 1H), 2.90–2.81 (m, 1H), 2.48 (dd, J = 7.6, 5.2 Hz, 1H), 2.25 (t, J = 6.9 Hz, 2H), 2.18–2.07 (m, 2H), 0.96 (dd, J = 6.6, 3.5 Hz, 6H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 171.14, 166.60, 165.41, 151.32, 133.31, 129.67, 128.43, 110.70, 88.25, 77.43, 77.23, 65.72, 51.40, 48.20, 43.07, 37.02, 31.17, 25.65, 22.26. LC-MS: 431 (M + H).

Compound **1e** (30 mg, 44.2%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42 (s, 1H), 6.10 (d, J = 5.3 Hz, 1H), 5.05 (dt, J = 7.5, 3.8 Hz, 1H), 3.73 (d, J = 2.4 Hz, 3H), 3.26 (dd, J = 13.7, 7.3 Hz, 1H), 3.06 (dd, J = 4.5, 2.2 Hz, 1H), 2.91 (d, J = 4.6 Hz, 1H), 2.78 (dtd, J = 10.6, 7.8, 2.9 Hz, 1H), 2.41–2.36 (m, 1H), 2.28–2.20 (m, 2H), 2.20–2.14 (m, 1H), 2.10 (dt, J = 13.6, 6.8 Hz, 1H), 1.93–1.86 (m, 1H), 1.56–1.36 (m, 4H), 1.30–1.25 (m, 2H), 1.21–1.12 (m, 2H), 0.99–0.94 (m, 6H), 0.89–0.78 (m, 6H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 175.15, 175.07, 171.07, 166.64, 151.38, 110.60, 88.29, 88.28, 77.25, 76.28, 76.22, 65.62, 51.42, 48.08, 48.05, 47.35, 47.19, 43.06, 42.88, 37.17, 31.58, 31.51, 31.05, 29.53, 29.52, 25.61, 25.32, 25.27, 22.57, 22.51, 22.27. LC-MS: 453 (M + H).

Compound **1f** (30 mg, 47.2%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42 (d, J = 1.3 Hz, 1H), 6.11 (d, J = 5.6 Hz, 1H), 5.02 (dd, J = 7.7, 4.2 Hz, 1H), 3.74 (s, 3H), 3.25 (d, J = 7.6 Hz, 1H), 3.05 (d, J = 4.6 Hz, 1H), 2.95 (dd, J = 9.8, 5.2 Hz, 2H), 2.85–2.75 (m, 1H), 2.36 (dd, J = 7.7, 5.7 Hz, 1H), 2.26–2.19 (m, 4H), 2.14–2.06 (m, 1H), 1.87 (ddd, J = 14.8, 5.9, 4.3 Hz, 1H), 1.68 (s, 1H), 1.57–1.52 (m, 2H), 1.34 (dd, J = 7.4, 3.6 Hz, 2H), 0.96 (dd, J = 6.6, 2.0 Hz, 6H), 0.89–0.86 (m, 3H). LC-MS: 425 (M + H).

Compound **1g** (15 mg, 22.1%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42 (d, J = 1.1 Hz, 1H), 6.11 (d, J = 5.6 Hz, 1H), 5.01 (dd, J = 7.7, 4.2 Hz, 1H), 3.74 (s, 3H), 3.25 (d, J = 7.5 Hz, 1H), 3.05 (d, J = 4.6 Hz, 1H), 2.95 (d, J = 4.7 Hz, 2H), 2.85–2.76 (m, 1H), 2.36 (dd, J = 7.6, 5.8 Hz, 1H), 2.28–2.18 (m, 4H), 2.14–2.07 (m, 1H), 1.87 (ddd, J = 14.8, 5.8, 4.4 Hz, 1H), 1.67 (td, J = 14.5, 7.0 Hz, 1H), 1.58–1.50 (m, 2H), 1.25 (d, J = 4.0 Hz, 7H), 0.96 (dd, J = 6.6, 2.0 Hz, 6H), 0.87 (t, J = 6.9 Hz, 3H). LC-MS: 453 (M + H).

Compound **1h** (18 mg, 25.0%): colorless oil ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.41 (d, J = 1.1 Hz, 1H), 6.11 (d, J = 5.7 Hz, 1H), 5.01 (dd, J = 7.7, 4.2 Hz, 1H), 3.73 (s, 3H), 3.29–3.19 (m, 1H), 3.05 (d, J = 4.6 Hz, 1H), 2.95 (d, J = 4.6 Hz, 1H), 2.81 (dt, J = 15.4, 7.9 Hz, 1H), 2.36 (dd, J = 7.6, 5.8 Hz, 1H), 2.26–2.19 (m, 4H), 2.10 (dt, J = 13.6, 6.8 Hz, 1H), 1.87 (ddd, J = 14.8, 5.8, 4.4 Hz, 1H), 1.57–1.50 (m, 2H), 1.25 (s, 12H), 0.96 (dd, J = 6.6, 2.0 Hz, 6H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CDCl$_3$) δ 172.67, 171.06, 166.68, 151.33, 110.64, 88.36, 77.23, 76.28, 65.38, 51.45, 47.97, 43.08, 42.74, 37.10, 34.25, 31.85, 30.82, 29.40, 29.25, 29.22, 29.09, 25.62, 24.79, 22.66, 22.27, 22.25, 14.10. LC-MS: 481 (M + H).

4.2 Pharmacological assay

RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco, BRL (Grand Island, NY, USA). Human pancreatic cancer cell lines (SW1990, BXPC3, CAPAN2, CFPAC, PANC1), human breast cancer cell lines (BT474, MCF7, MDA-MB-231), human lung cancer cell line (H1975) and human gastric cancer cell lines (AGC, HGC-27, KATOIII) were purchased from Shanghai Institutes for Biological Sciences, CAS (Shanghai, China). DAPI was purchased from Sigma-Aldrich (St Louis, MO, US). The Annexin V-FITC Apoptosis Kit was purchased from BD (Franklin Lakes, NJ, US). The primary antibodies against Bax, Bcl-2, Mcl-1, procaspase-9, poly-ADP-ribose polymerase (PARP), XIAP and β-actin were purchased from Abcam Inc. (Cambridge, MA, US). Caspase-3 Colorimetric Assay Kit was purchased from Biovision (Milpitas, CA, US). Human lung cancer cell line H1975 was purchased from Shanghai Institutes for Biological Sciences, CAS (Shanghai, China). Cells were cultured with RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C, 5% CO$_2$ humidified atmosphere. **1e** was dissolved in DMSO at the concentration of 100 mM.

4.2.1 Cell viability assay. H1975 cells were counted in logarithmic phase and 5000 cells were placed in 96-well plates. After treatment with **1e** (10, 20 and 30 μM), cells were incubated for an additional 2 h with CCK-8 reagent (100 μL per mL medium) and the absorbance was read at 450 nm using a microplate reader (Sunnyvale, CA, USA). Cell proliferation inhibition rates were calculated according to the following formula: the proliferation inhibition ratio (%) = 1 − [(A_1 − A_3)/(A_2 − A_3)] × 100, where, A_1 is the OD value of drug experimental group, A_2 is the OD value of blank control group, A_3 is the OD

value of the RPMI1640 medium without cells. The IC_{50} (50% inhibitory concentration) value, which represents the concentration of the drug that demonstrates 50% of cell growth inhibition, was calculated by nonlinear regression analysis using GraphPad Prism software (San Diego, CA, USA). Assays were performed on three independent experiments.

4.2.2 DAPI stain. H1975 cells (2×10^4 cells per well) were cultured in 24-well plates. After treatment with **1e** for 48 h, cells were fixed with 4% paraformaldehyde for 20 min, and stained with DAPI for 20 min at 37 °C. After washing with PBS, cells were observed under a fluorescence microscope (Nikon, Ti-E, Japan).

4.2.3 Apoptosis assay by flow cytometry. Exponentially growing cells were seeded in 6-well plates (5×10^4 per well) and cultured overnight in a 5% CO_2 atmosphere at 37 °C. After treatment with **1e** for 24 h, cells were harvested and washed with PBS. Then cells were stained with Annexin V-FITC Apoptosis Kit according to the manufacturer's instructions and analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, US). Assays were performed on three independent experiments.

4.2.4 Caspase-3 activity assay. Caspase-3 Colorimetric Assay Kit was used to evaluate caspase-3 activity. In brief, cell lysates were prepared after treatment with **1e** (10 or 20 μM) for 48 h. Equal amounts of protein (200 μg) were incubated with the supplied reaction buffer and the colorimetric substrates at 37 °C for 2 h in the dark. Chromophore pNA released from the cleavage of peptide can be quantified using Molecular Devices M2^e (Sunnyvale, CA, US) at the wavelength of 405 nm. Caspase-3 activity was expressed by value of OD405 relative to control.

4.2.5 Western blot analysis. After treated with **1e**, total proteins were extracted using RIPA Lysing Buffer. An amount of 40 μg proteins were subjected to 12% SDS-PAGE and transferred to PVDF Membrane (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% non-fat milk at room temperature for 1 h, and then incubated with specific primary antibodies overnight at 4 °C. After washing with TBST, the membranes were incubated with secondary antibodies at room temperature for another 1 h. The protein bands were visualized by adding ECL system WBKLS0050 (EMD Millipore, Billerica, MA, USA) and analyzed using Bio-Rad Laboratories Quantity One software (Bio-Rad, Hercules, CA, USA).

Acknowledgements

References

1 Y. Fukuyama, Y. Minoshima, Y. Ishimoto, I. S. Chen, H. Takahashi and T. Esumi, *J. Nat. Prod.*, 2004, **67**, 1833–1838.

2 W. Hui-lian, Z. Dong-fang, L. Zhao-feng, L. Yang, L. i. Qian-rong and W. Yu-zhen, *Toxicol. Appl. Pharmacol.*, 2003, **188**, 36–41.

3 C. Bounthanh, C. Bergmann, J. P. Beck, M. Haagberrurier and R. Anton, *Planta Med.*, 1981, **41**, 21–28.

4 N. T. Doan, F. Crestey, C. E. Olsen and S. B. Christensen, *J. Nat. Prod.*, 2015, **78**, 1406–1414.

5 L. D. Kapoor, *Handbook of Ayurvedic medicinal plants*, CRC Press, Boca Raton, 2001.

6 B. P. Mikhova, N. V. Handjieva, S. S. Popov and S. L. Spassov, *J. Nat. Prod.*, 1987, **50**, 1141–1145.

7 H. Becker, S. Chavadej, P. W. Thies and E. Finner, *Planta Med.*, 1984, **50**, 245–248.

8 W. Kucaba, P. W. Thies and E. Finner, *Phytochemistry*, 1980, **19**, 575–577.

9 N. Handjieva and V. G. Zaikin, *Planta Med.*, 1978, **34**, 203–206.

10 B. M. Dietz, A. Hajirahimkhan, T. L. Dunlap and J. L. Bolton, *Pharmacol. Rev.*, 2016, **68**, 1026–1073.

11 X. Li, T. Chen, S. Lin, J. Zhao, P. Chen, Q. Ba, Y. Liu, J. Li, R. Chu, L. Shan, W. Zhang and H. Wang, *Curr. Cancer Drug Targets*, 2013, **13**, 472–483.

12 R. Bos, H. Hendriks, J. J. C. Scheffer and H. J. Woerdenbag, *Phytomedicine*, 1998, **5**, 219–225.

13 S. Lin, T. Chen, P. Fu, J. Ye, X.-W. Yang, L. Shan, H. L. Li, R. H. Liu, Y. H. Shen, X. K. Xu and W. D. Zhang, *J. Asian Nat. Prod. Res.*, 2015, **17**, 455–461.

14 O. Kelber, T. Wegener, B. Steinhoff, C. Staiger, J. Wiesner, W. Knoess and K. Kraft, *Phytomedicine*, 2014, **21**, 1124–1129.

15 D. Zhang, B. Zhang, L. X. Zhou, J. Zhao, Y. Y. Yan, Y. L. Li, J. M. Zeng, L. L. Wang, B. Yang and N. M. Lin, *Acta Pharmacol. Sin.*, 2016, **37**, 1597–1605.

16 B. Yang, N. Li, Y. Q. Wang, J. Chen and R. S. Zhang, *Rev. Bras. Farmacogn.*, 2011, **21**, 471–476.

17 S. Tamura, K. Fujiwara, N. Shimizu, S. Todo and N. Murakami, *Bioorg. Med. Chem.*, 2010, **18**, 5975–5980.

18 H. Hussain, I. R. Green and I. Ahmed, *Chem. Rev.*, 2013, **113**, 3329–3371.

19 X. W. Dong, J. Chen, C. Y. Jiang, T. Liu and Y. Z. Hu, *Arch. Pharm.*, 2009, **342**, 428–432.

20 H. Jing, X. L. Zhou, X. W. Dong, J. Cao, H. Zhu, J. S. Lou, Y. Z. Hu, Q. J. He and B. Yang, *Cancer Lett.*, 2010, **294**, 167–177.

21 K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, 1973, **95**, 2697–2699.

22 S. Tamura, K. Fujiwara, N. Shimizu, S. Todo and N. Murakami, *Bioorg. Med. Chem.*, 2010, **18**, 5975–5980.

23 S. Lin, P. Fu, T. Chen, J. Ye, X. W. Yang and W. D. Zhang, *J. Asian Nat. Prod. Res.*, 2017, **19**, 15–21.

24 X. Li, X. X. Fan, Z. B. Jiang, W. T. Loo, X. J. Yao, E. L. Leung, L. W. Chow and L. Liu, *Pharmacol. Res.*, 2017, **115**, 45–55.

25 Z. Wang, X. Wu, Y. N. Liang, L. Wang, Z. X. Song, J. L. Liu and Z. S. Tang, *Molecules*, 2016, **21**(10), 1267.

26 L. H. Li, P. Wu, J. Y. Lee, P. R. Li, W. Y. Hsieh, C. C. Ho, C. L. Ho, W. J. Chen, C. C. Wang, M. Y. Yen, S. M. Yang and H. W. Chen, *PLoS One*, 2014, **9**, e104203.

27 N. Khan, F. Jajeh, M. I. Khan, E. Mukhtar, S. M. Shabana and H. Mukhtar, *Carcinogenesis*, 2017, **38**, 184–195.

28 Y. Du, W. Chen, X. Fu, H. Deng and J. Deng, *RSC Adv.*, 2016, **6**, 109718–109725.

29 S. K. Jung, M. H. Lee, D. Y. Lim, J. E. Kim, P. Singh, S. Y. Lee, C. H. Jeong, T. G. Lim, H. Chen, Y. I. Chi, J. K. Kundu, N. H. Lee, C. C. Lee, Y. Y. Cho, A. M. Bode, K. W. Lee and Z. Dong, *J. Biol. Chem.*, 2014, **289**, 35839–35848.

30 M. K. Lu, T. Y. Lin, C. H. Hu, C. H. Chao, C. C. Chang and H. Y. Hsu, *Carbohydr. Polym.*, 2017, **167**, 229–239.