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新型蓝萼甲素-噻唑类衍生物的设计、合成与生物学评价

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摘要 蓝萼甲素(Glaucocalyxin A, GLA)是从蓝萼香茶菜中分离得到的四环二萜类活性天然产物. 设计并合成了一系列基于蓝萼甲素的噻唑类衍生物, 评估了它们对六种肿瘤细胞(HepG2, NCI-H460, JEG-3, K562, HL-60 和 Hela)的增殖抑制活性. 结果表明在 GLA 的 A 环上引入氨基噻唑结构可以显著提高其细胞增殖抑制活性, 尤其是 *N*-烷基氨基噻唑类衍生物对六种肿瘤细胞都表现出较好的抑制活性. 其中, 化合物 **6** 和 **8** 对 HL-60 和 Hela 细胞的抑制活性都优于阳性药物阿霉素, IC_{50} 低至 0.51 μmol•L^{-1}. 形态学和流式细胞仪的测定表明蓝萼甲素-噻唑类化合物可以诱导肿瘤细胞凋亡.
关键词 蓝萼甲素; 噻唑衍生物; 抗肿瘤

Design, Synthesis and Biological Evaluation of Novel Thiazole-Fused Glaucocalyxin A Derivatives

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Abstract Glaucocalyxin A (GLA) is an active natural tetracyclic diterpenoid isolated from the traditional Chinese herb Isodon glaucocalyx (maxin) Hara. In this work, a series of thiazole type derivatives based on GLA were designed and prepared. Their antiproliferative activities against six tumor cell lines (HepG2, NCI-H460, JEG-3, K562, HL-60 and Hela) were evaluated *in-vitro*. The results revealed that the introduction of aminothiazole substructures into A-ring of the GLA could improve their antiproliferative effects significantly. Among them, *N*-alkyl thiazole derivatives showed remarkably activities to the six tumor cell lines. Especially, compounds **6** and **8** presented significant antitumor activities against HL-60 and Hela cell lines with IC_{50} as low as 0.51 μmol•L^{-1}, which were better than the positive drug adriamycin. The apoptosis morphology and flow cytometry studies indicated that the thiazole-fused GLA derivatives could induce apoptosis of tumor cells.
Keywords glaucocalyxin A; thiazole; antitumor

1 Introduction

Effective anticancer agents with novel scaffolds or new target are urgently needed as cancer is a fatal disease to humans.[1-2] Natural products play a pivotal role in drug discovery and many natural compounds show great anti-cancer activities.[3-4]. More and more natural compounds with low toxicity and high therapeutic efficacy have been reported.[5] Among the natural products, tetracyclic diterpenes with ent-kaurane skeleton, such as oridonin[6-7] and stevioside,[8] have attracted much attention due to their good bioactivities.

Glaucocalyxin A (GLA), a complex *ent*-kaurane diterpenoid isolated from the traditional Chinese herb Isodon glaucocalyx (maxin) Hara, has attracted wide attention due to its unique and broad spectrum of bioactivities.[9-12]

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Early studies have indicated that the α,β-unsaturated ketone on D-ring is an essential pharmacophore to bioactivities, so the structural modification of GLA is mainly focused on 7-OH and 14-OH groups.[13-14] Yang and coworkers[15] have found that when the double bond GLA is transformed by amines Michael addition, the antitumor activities of the most derivatives are less than GLA. Nevertheless, glaucocalyxin H, acetalization product of 7,14-hydroxyl of GLA with protocatechualdehyde, has been proved to have noteworthy anti-tumor activities superior to GLA in our previous work.[16]

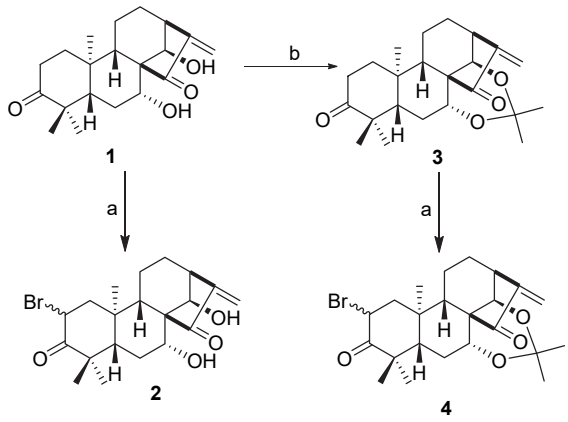
Although some progress has been made, the application of GLA in cancer therapy is limited by its low bioactivity and bioavailability, which is a common phenomenon for natural products.[17] Therefore, the design and synthesis of novel GLA derivatives with strong anti-tumor activity and low toxicity have become the focus of the current research.

Thiazole ring, a significant pharmacophore of many bioactive natural products,[18-19] can improve the bioactivities and reduce toxicity of parent compounds.[20-22] Thus, the introduction of thiazole ring is an effective strategy for optimization of bioactive compounds. To this regard, our efforts have been devoted to introducing thiazole heterocycle to GLA without altering the pharmacophore of α,β-unsaturated ketone on D-ring and evaluating its bioactivity so as to discover new antitumor agents.

2 Results and discussion

2.1 Chemistry

Hantzsch thiazole synthesis, with α-haloketone and thiourea (or thioamide) as reactants, has been proved to be an efficient way for the formation of thiazole compounds.[23] Herein, thiazole fragment was introduced to the A-ring of GLA via Hantzsch reaction in this work. As shown in Scheme 1, α-haloketone derivatives of GLA were synthesized initially with 1.1 equiv. of $PyHBr_3$ as bromination reagent under 0 ℃ in 56% yield (Table 1, Entries 1～5). The attempts of changing bromination reagents with *N*-bromo-succinimide (NBS) would reduce the yield greatly due to the formation of too many by-products (Table 1, Entry 6). In addition, protection of **7**, 14-hydroxyl groups of GLA by isopropylidene[24] (compound **3**) still could not improve the bromination yield of compound **4** (Table 1, Entry 7). From the ^{13}C NMR of compound **2**, it can be seen that the carbon signals all exist in pairs, which suggests that the bromine at the C-2 position has two configurations of α-substituted and β-substituted.



Reagents and conditions: (a) $PyHBr_3$, THF, 0 ℃, 0.5 h; (b) $Me_2C(OMe)_2$, TsOH, Acetone, r.t., 1 h

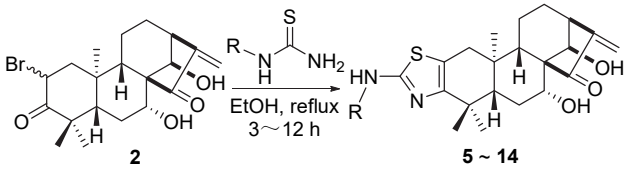
Scheme 1 Synthesis of α-haloketone derivatives of GLA

Table 1 Optimization of bromide reaction conditions

Entry	Compd.	Reaction conditions[a]	Yield/%
1	**1**	$PyHBr_3$ (1.2 equiv.), THF, 0 ℃, 1 h	51
2	**1**	$PyHBr_3$ (1.2 equiv.), THF, −20 ℃, 12 h	36
3	**1**	$PyHBr_3$ (1.2 equiv.), THF, 20 ℃, 1 h	41
4	**1**	$PyHBr_3$ (1.5 equiv.), THF, 0 ℃, 1 h	30
5	**1**	$PyHBr_3$ (1.1 equiv.), THF, 0 ℃, 0.5 h	56
6	**1**	NBS (1.2 equiv.), THF, 0 ℃, 12 h	<10
7	**3**	$PyHBr_3$ (1.1 equiv.), THF, 0 ℃, 12 h	40
8	**3**	NBS (1.2 equiv.), THF, 0 ℃, Et_3N, 12 h	<10
9	**3**	$PyHBr_3$ (1.0 equiv.), THF, 20 ℃, 6 h	32

[a] The reaction progress was monitored by TLC.

Then compound **5** was obtained through Hantzsch thiazole synthesis reaction with compound **2** and thiourea as reactants in EtOH under reflux condition. Compared with compound **2**, the C-3 carbonyl group signals at δ 216.92 was not observed in the ^{13}C NMR of compound **5**. The newly resonances at δ 114.13, 152.91 and 167.19 belong to the $C_2{=}C_3$ double bond and C=N of thiazole ring, respectively. HRMS peak at *m/z* 389.1895 (calcd for 389.1893) demonstrates the formation of compound **5**. Subsequently, compounds **6**～**15** were synthesized readily by the reaction of compound **2** and *N*-substituted thiourea in refluxing ethanol (Scheme 2). But attempts of the reaction between compound **2** and thioamide failed to meet our expectation.



5 R = H, **6** R = CH_3, **7** R = C_2H_5, **8** R = $CH_2CH_2CH_3$, **9** R = $(CH_2)_3CH_3$, **10** R = $(CH_2)_5CH_3$, **11** R = $CH_2CH{=}CH_2$, **12** R = Cyclohexy, **13** R = C_6H_5, **14** R = $CH_2C_6H_5$

Scheme 2 Construction of aminothiazole derivatives

2.2 Biological evaluation

2.2.1 Antiproliferative activity

The antiproliferative activities of the thiazole type derivatives were determined by methyl thiazolyl tetrazolium (MTT) assay *in vitro* against HepG2 (human liver carcinoma), NCI-H460 (human lung carcinoma), JEG-3 (Human choriocarcinoma), K562 (Human Chronic myeloid leukemia), HL-60 (human promyelocytic leukemia), and Hela (Human cervical cancer) cell lines with adriamycin as the positive control drug in each panel. The IC_{50} values

were summarized in Table 2.

From the observed cytotoxic activity data, most of the novel derivatives of GLA showed significant cytotoxic activities. Notably, all the eleven new synthesized compounds exhibited better antiproliferative activity than GLA to HL-60 and Hela cell lines, indicating that the aminothiazole derivatives have better bioactivity and selectivity for HL-60 and Hela cell lines. Especially, compounds **6** and **8** showed prominent activity with IC_{50} values of 0.56 and 0.51 μmol•L^{-1} against HeLa cell line, respectively. Meanwhile, compounds **6**, **7**, **8**, **9**, and **10** with IC_{50} values 0.82, 0.84, 0.90, 1.19, and 1.50 μmol•L^{-1} against HL-60 cell line, were superior to adriamycin. Moreover, compounds **7**, **8** and **9** also showed better activity than adriamycin against K562 cell line with IC_{50} values of 4.00, 3.25, and 4.08 μmol•L^{-1}. In addition, the inhibitory activities of *N*-cyclohexyl aminothiazole derivative **12** were superior than GLA, but were inferior than that of linear alkane substituted derivatives (**12** vs **6**～**8**). The results revealed that linear alkyl with 1～3 carbon atom were more prominent than branched alkyl and cycloalkyl on thiazole derivatives. Meanwhile, when alkyl substitution was replaced by allyl or aromatic groups, the anti-tumor activities also decreased in varying degrees (**6**, **7**, **8**, **9**, **10** vs **11**, **13**, **14**). For example, compound **11** against JEG-3 cell line and compound **14** against K562 cell line just gave poor cytotoxic activities with IC_{50} values of 18.81 and 22.49

Table 2 IC_{50} values (μmol•L^{-1}) of GLA derivatives against cancer cells

Compd.	IC_{50}[a] (μmol•L^{-1})					
	Hep G2	NCI-H460	JEG-3	K562	HL-60	HeLa
5	3.97±0.09	5.27±0.21	5.19±0.06	10.12±0.20	2.40±0.15	2.33±0.11
6	3.08±0.06	4.07±0.15	1.13±0.14	5.63±0.48	0.82±0.03	0.56±0.02
7	3.20±0.30	3.31±0.24	2.13±0.37	4.00±0.21	0.84±0.06	2.17±0.06
8	1.81±0.01	2.47±0.25	3.04±0.09	3.25±0.09	0.90±0.06	0.51±0.05
9	2.53±0.13	3.35±0.18	3.70±0.12	4.08±0.29	1.19±0.04	2.19±0.04
10	4.14±0.37	3.95±0.16	6.03±0.67	7.33±0.72	1.50±0.10	2.02±0.08
11	6.66±0.53	8.07±0.34	18.81±1.92	6.54±0.41	3.31±0.15	4.47±0.03
12	3.15±0.08	3.18±0.26	4.97±0.46	6.03±0.30	2.02±0.09	2.12±0.11
13	10.70±0.29	9.69±0.40	9.71±0.21	9.72±0.54	3.09±0.19	5.28±0.03
14	5.17±0.34	4.83±0.04	6.28±0.10	22.49±0.75	2.42±0.11	2.49±0.05
GLA	3.94±0.07	8.99±0.18	4.25±0.08	7.20±0.17	6.46±0.05	8.19±1.96
Adriamycin	0.50±0.01	1.73±0.02	0.52±0.02	5.15±0.11	1.73±0.02	0.69±0.01

[a] Inhibitory activity was assayed by exposure for 48 h; values were the mean of triplicate of three independent experiments; mean±SD.

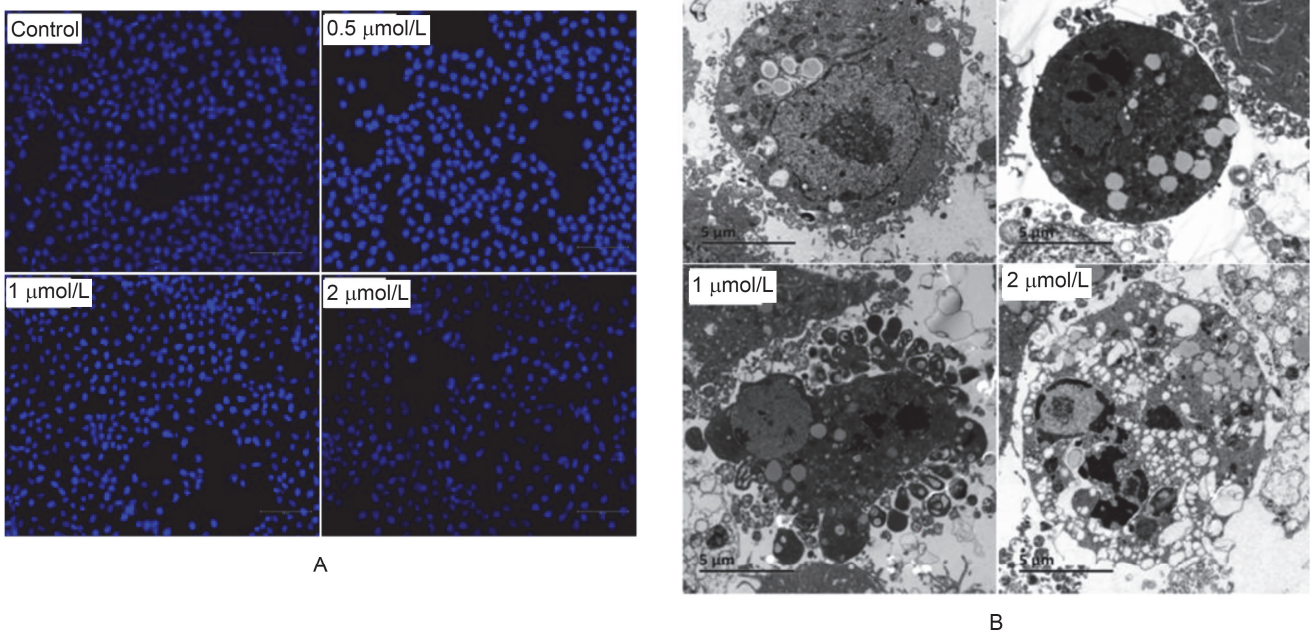


Figure 1 Effects of compound **8** on nuclear morphological and cell morphological changes of HepG2 cells

After treated with indicated concentrations of compound **8** for 48 h, (A) the HepG2 cells were stained with Hochest33342 for 10 min and examined by fluorescence microscopy (×200); (B) the morphological changes of HepG2 cells were detected by electron microscope (×5000)

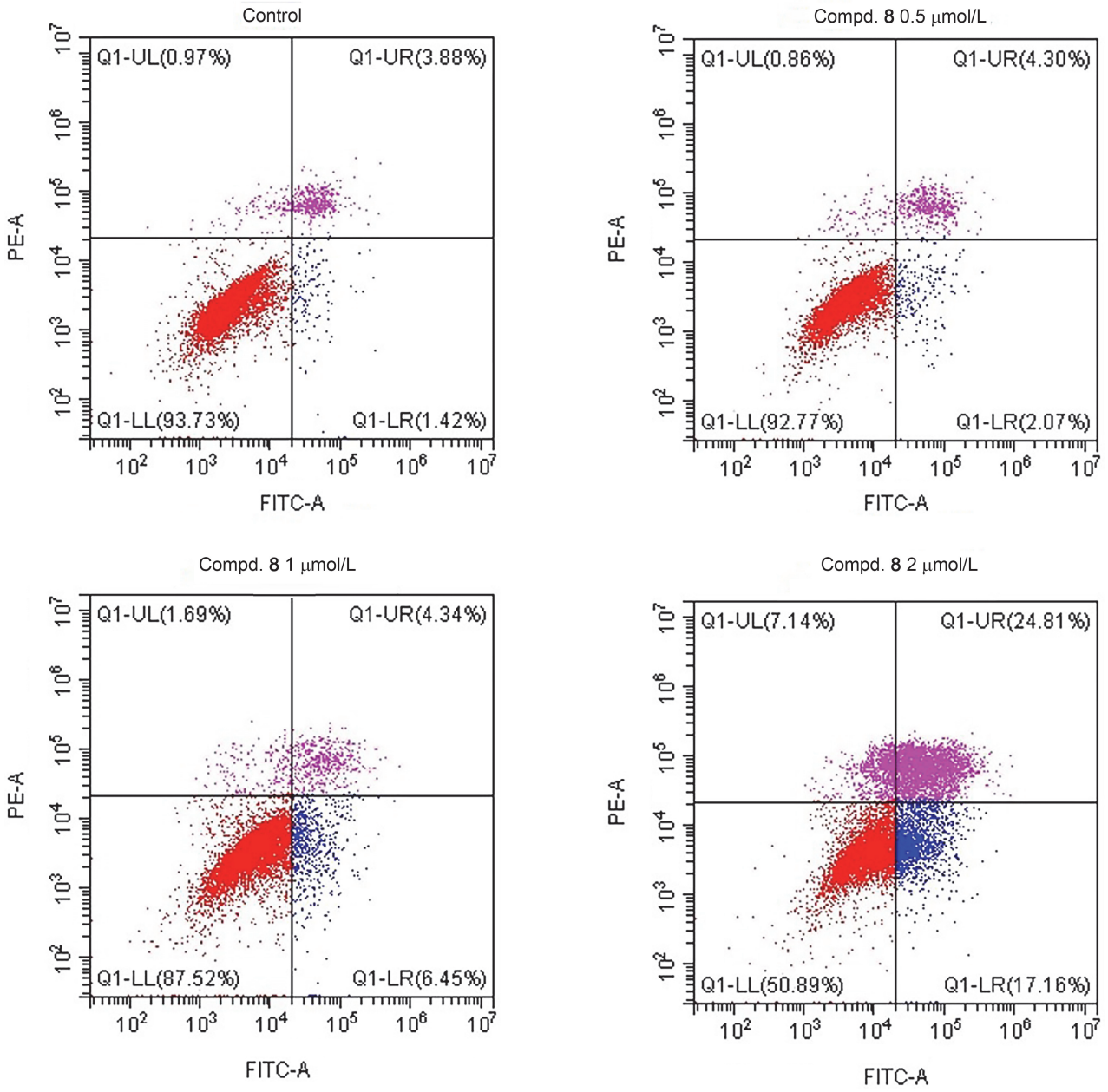


Figure 2 Effects of compound **8** on apoptosis of HepG2 cells detected by flow cytometry

After HepG2 cells were treated with compound **8** at the indicated concentrations for 48 h, the cells were stained with Annexin V-FITC/PI and then analyzed by flow cytometry

μmol•L^{-1}, respectively. However, the inhibitory activity of unsubstituted aminothiazole derivative was better than that of aromatic and allyl substituted derivatives (**5** vs **11**, **13**, **14**), but was worse than alkyl substituted derivatives (**5** vs **6**～**10**).

Therefore, the results reveal that the aminothiazole structure on C-2/C-3 of GLA plays an important role in improving the bioactivity of GLA, and the aminothiazole structure with *N*-alkyl substitution will significantly increase its antitumor activity.

2.2.2 Morphological research

The effects of compound **8** on nuclear morphological and cell morphological changes of HepG2 cells were shown in Figure 1. As shown in Figure 1A, $HepG_2$ cells were dyed by hochest33258. The cell nuclei of $HepG_2$ in control group were evenly dyed blue. The nuclei were oval and full. Some nuclei of HepG2 in 0.5 μmol•L^{-1} group were stained unevenly and some nuclei had irregular shapes of nuclear film indentations. In 1 μmol•L^{-1} group and 2 μmol•L^{-1} group, there was a significant increase in nuclear membrane invagination in the nuclei. Additionally, the apoptotic bodies were clearly visible in the two groups. As shown in Figure 1B, $HepG_2$ cells and nuclei were round and plump, with nucleoli centered in control group. In 0.5 μmol•L^{-1} group the cell condensed and became smaller than that in control group. The nucleus shifted and the nucleus showed crescent

shape. The cell disintegrated with no normal cell morphology in 1 μmol•L^{-1} group and 2 μmol•L^{-1} group. The cells disintegrated into fragments of different sizes and the apoptotic bodies were clearly visible.

Furthermore, the percentages of apoptotic cells with control and compound **8** treatments were further detected by flow cytometric analysis, following Annexin V-FITC/PI staining. As shown in Figure 2, the treatment of HepG2 cells with 0.5, 1 and 2 μmol•L^{-1} of compound **8** for 48 h resulted in a significant increase of apoptosis cell population up to 6.37%, 10.79%, and 41.97%, respectively, compared to control cells (5.30%). These data indicate that the morphological damage effect of compound **8** to HepG2 cells is due to apoptosis.

3 Conclusions

In this study, ten thiazole-fused GLA derivatives (**5**～**14**) were designed and synthesized via Hantzsch reaction. Biological evaluation against six cancer cell lines showed that the introduction of thiazole ring could improve its antitumor activity significantly. Especially, the *N*-alkyl substituted thiazole derivatives, such as compounds **6**～**10**, exhibited remarkably activities which were superior to positive drug adriamycin against K562, HL-60 or HeLa cell line. The apoptosis morphology and flow cytometry studies of compound **8** to HepG2 cell line indicated that the inhibitory effect of these compounds on tumor cells was achieved by inducing apoptosis. Overall, the results suggested that the introduction of thiazole into A-ring was an effective strategy for the modification of natural product GLA. Further studies on *in vivo* antitumor activity and mechanism of these compounds are ongoing and will be reported in due course.

4 Experimental section

4.1 Materials and reagents

All commercial reagents were purchased from commercial suppliers and used without further purification unless specified. NMR spectra were obtained with a Bruker Avance 400 spectrometer operating respectively at 400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR. The peaks were internally referenced to TMS (δ 0.00) or residual undeuterated solvent signal. High resolution mass spectra were recorded on a Bruker micro TOF-Q III spectrometer. The melting points were determined using a WRS-1B apparatus and are uncorrected. Optical rotations were measured on an Anton paar MCP5100 Polarimeter at 20 ℃ (g/100 mL). The nuclear morphological was examined by Leica DMi8 fluorescence microscopy. The morphological changes were detected by JOEL JEM1230 transmission electron microscope. The apoptosis was detected by Beckman A00-1-1102 flow cytometry. GLA was obtained according to the method reported in our previous work[25-26].

4.2 General methods

Synthesis of compound **2**: GLA (166 mg, 0.5 mmol) was dissolved in 5 mL of tetrahydrofuran (THF) and the solution was cooled to 0 ℃ in an ice bath. Then the solution of $PyHBr_3$ (160 mg 0.5 mmol) in 1 mL of THF was added dropwise under stirring; The resulting mixture was stirred at 0 ℃ for 30 min, and then poured into water and extracted with CH_2Cl_2 (30 mL×3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a yellow oily residue. The crude product was further purified by silica gel column chromatography with petroleum ether/ethyl acetate (V∶V=2∶1) to afford the title compound **2** (116.7 mg, 66.8%) as a colorless amorphous gel and a mixture of two isomers. Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.15 (s, 1H), 5.42 (s, 1H), 4.90 (dd, J=12.2, 6.8 Hz, 1H), 4.84 (s, 1H), 4.33 (dd, J=11.9, 3.9 Hz, 1H), 3.07 (brs, 1H), 2.65 (dd, J=13.4, 6.8 Hz, 1H), 2.09 (dd, J=13.4, 9.3 Hz, 1H), 1.98～1.86 (m, 2H), 1.83～1.66 (m, 2H), 1.60～1.38 (m, 4H), 1.27 (s, 3H), 1.16 (s, 3H), 1.12 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 207.9, 207.2, 147.1, 119.0, 75.1, 74.2, 61.5, 52.9, 52.5, 50.7, 49.8, 48.6, 45.9, 41.5, 30.8, 29.5, 28.9, , 22.2, 21.3, 18.2. Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.15 (s, 1H), 5.42 (s, 1H), 5.04 (dd, J=10.9, 9.5 Hz, 1H), 4.71 (s, 1H), 4.33 (dd, J=11.9, 3.9 Hz, 1H), 3.07 (s, 1H), 2.39～2.29 (m, 1H), 2.09 (dd, J=13.6, 9.3 Hz, 1H), 1.98～1.86 (m, 2H), 1.83～1.66 (m, 2H), 1.60～1.38 (m, 3H), 1.25 (s, 3H), 1.17 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 207.3, 206.7, 147.1, 119.0, 74.8, 73.6, 61.3, 52.5, 52.4, 50.2, 49.7, 47.3, 45.9, 40.8, 30.6, 29.2, 27.8, 20.3, 18.5, 17.8. HRMS (ESI) calcd for $C_{20}H_{27}BrO_4Na$ $[M+Na]^+$ 433.0985, found 433.0991.

Synthesis of compound **3**: Compound **3** has been obtained by Fujita group in 1981 and its ^{1}H NMR data was reported.[24] In this work, compound **3** was prepared via the following way, and the ^{1}H NMR data are consistent with those reported in the literature. In a 50 mL dry round-bottom flask, $Me_2C(OMe)_2$ (156 mg, 1.5 mmol) and GLA (332 mg, 1.0 mmol) were dissolved in 5 mL of dry acetone under stirring. Then 10 mg of TsOH was added. The reaction was completed in 1 h at room temperature monitored by thin-layer chromatography (TLC). After addition of 30 mL of dichloromethane, the system was washed with distilled water, saturated brine, and saturated sodium bicarbonate solution in turn. The organic phase was separated and dried with anhydrous $NaSO_4$. Then the organic phase was filtered, concentrated, and separated by silica gel column chromatography (petroleum ether/ethyl acetate, V∶V=4∶1). Compound **3** was obtained as a white powder in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.19 (s, 1H), 5.41 (s, 1H), 4.59 (d, J=1.8 Hz, 1H), 4.22 (dd, J=12.6, 5.4 Hz, 1H), 3.08 (s, 1H), 2.56 (ddd, J=15.9, 12.0, 6.7 Hz, 1H), 2.39 (ddd, J=15.9, 6.1, 3.8 Hz, 1H), 2.17～2.04 (m, 1H), 2.04～1.92 (m, 3H), 1.92～1.83 (m, 1H), 1.60 (s, 3H), 1.59～1.50 (m, 2H), 1.38 (dd, J=12.3, 2.4 Hz, 1H), 1.34～1.27 (m, 2H), 1.25 (s, 3H), 1.20 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 215.6, 205.5, 146.4, 117.4, 97.4, 71.0, 70.5, 54.6, 51.3, 51.2, 47.1, 43.1, 38.2, 37.8, 34.0, 31.1, 30.4, 28.3, 25.9, 25.5, 21.5, 18.1, 16.9.

HRMS (ESI) calcd for $C_{23}H_{32}O_4Na$ $[M+Na]^+$ 395.2193, found 395.2190.

Synthesis of compound **4**: Same to the synthetic route of compound **2**, compound **4** was obtained as a white solid in 40% yield. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.19 (s, 1H), 5.43 (s, 1H), 5.06 (dd, J=13.8, 5.7 Hz, 1H), 4.60 (d, J=1.8 Hz, 1H), 4.21 (dd, J=12.5, 5.6 Hz, 1H), 3.13～3.04 (m, 1H), 2.65 (dd, J=13.1, 5.7 Hz, 1H), 2.12 (q, J=12.5 Hz, 1H), 2.06～1.96 (m, 2H), 1.96～1.82 (m, 2H), 1.69 (t, J=13.4 Hz, 1H), 1.59 (s, 5H), 1.37 (s, 3H), 1.36～1.26 (m, 3H), 1.25 (s, 3H), 1.23 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 205.2, 205.0, 145.9, 117.8, 97.4, 71.0, 70.3, 54.3, 52.3, 51.3, 51.0, 50.5, 48.6, 42.9, 41.2, 30.9, 30.3, 28.1, 26.0, 25.3, 21.9, 18.1, 17.0. HRMS (ESI) calcd for $C_{23}H_{31}BrO_4Na$ $[M+Na]^+$ 473.1298, 475.1277; found 473.1294, 475.1277.

Synthesis of compound **5**: To a solution of **2** (82 mg, 0.2 mmol) in ethanol (3 mL) was added thiourea (15 mg, 0.2 mmol) at room temperature. The reaction mixture was heated under reflux for 3 h. After cooling and basifying with saturated $NaHCO_3$ aqueous solution, the mixture was concentrated *in vacuo* to give an oily residue. The crude product was purified by silica gel column chromatography with petroleum ether/ethyl acetate (V∶V=2∶1) to afford the title compound **5** (28.6 mg, 36.8%) as a white solid. m.p. 254～256 ℃; $[\alpha]_D^{20}$ −155.5 (c 0.479, MeOH). ^1H NMR (400 MHz, Pyridine-d_5) δ: 8,12 (d, J=4.92 Hz, 1H), 7.59 (s, 1H), 7.26 (s, 1H), 6.36 (s, 1H), 5.42 (s, 1H), 5.15 (s, 1H), 4.90 (s, 1H), 4.82 (dt, J=11.9, 4.0Hz, 1H), 3.29 (s, 1H), 2.61 (d, J=15.2 Hz , 1H), 2.30 (d, J=12.6 Hz, 1H), 2.18～2.00 (m, 2H), 1.94～1.97 (m, 1H), 1.72 (dd, J=9.3, 4.4 Hz, 1H), 1.64 (dd, J=17.1, 4.8 Hz, 2H), 1.56～1.47 (m, 2H), 1.43 (s, 3H), 1.35 (s, 3H), 1.12 (s, 3H); ^{13}C NMR (101 MHz, Pyridine-d_5) δ: 208.1, 167.2, 152.9, 150.2, 116.7, 114.1, 75.9, 74.1, 62.2, 53.3, 51.0, 47.3, 41.1, 39.0, 37.8, 31.8, 31.2, 31.2, 22.7, 18.7, 18.6. HRMS (ESI) calcd for $C_{21}H_{29}N_2O_3S$ $[M+H]^+$ 389.1893, found 389.1895.

Same to compound **5**, compounds **6**～**15** were synthesized through Hantzsch reaction with compound **2** and different substituted thiourea compounds as reactants.

Synthesis of compound **6**: Compound **6** was obtained as white solid in 87.7% yield. m.p. 255 ℃ (dec.); $[\alpha]_D^{20}$ −168.5 (c 0.292, MeOH); ^1H NMR (400 MHz, Pyridine-d_5) δ: 8.12 (s,1H), 7.85 (d, J=4.5 Hz, 1H), 7.61～7.55 (m, 1H), 7.22 (q, J=7.9 Hz, 3H), 6.34 (s, 1H), 5.41 (s, 1H), 5.14 (s, 1H), 4.80 (d, J=9.0 Hz, 1H), 3.28 (s, 1H), 3.05 (d, J=4.0 Hz, 3H), 2.63 (d, J=15.2 Hz, 1H), 2.17～2.01 (m, 2H), 1.50 (dd, J=13.5, 8.1 Hz, 2H), 1.43 (s, 4H), 1.34 (d, J=6.5 Hz, 4H), 1.11 (s, 3H); ^{13}C NMR (101 MHz, Pyridine-d_5) δ: 208.1, 168.6, 153.3, 150.2, 116.7, 113.0, 75.9, 74.1, 62.2, 53.3, 51.1, 47.3, 41.1, 39.0, 37.9, 32.0, 31.8, 31.2, 31.2, 22.7, 18.7, 18.6. HRMS (ESI) calcd for $C_{22}H_{31}N_2O_3S$ $[M+H]^+$ 403.2050, found 403.2059.

Synthesis of compound **7**: Compound **7** was obtained as an white solid in 61.7% yield. m.p. 196～198 ℃; $[\alpha]_D^{20}$ −168.8 (c 0.337, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.17 (s, 2H), 5.86 (s, 1H), 5.45 (s, 1H), 4.89 (s, 1H), 4.41～3.45 (dd, J=12.0, 3.2 Hz, 1H), 3.22 (m, 1H), 3.16 (s, 1H), 3.11 (m, 1H), 2.60～2.64 (d, J=16 Hz, 1H), 2.15 (d, J=13.4 Hz, 1H), 2.01～2.10 (m, 2H), 1.91～1.98 (m, 1H), 1.81～1.84 (m, 1H), 1.56～1.63 (m, 1H), 1.44～1.54 (m, 3H), 1.36 (t, J=7.2 Hz, 3H), 1.28 (s, 3H), 1.26～1.27 (m, 1H), 1.24 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.6, 169.5, 151.2, 148.3, 118.1, 112.1, 75.1, 72.6, 62.0, 52.8, 50.9, 46.2, 41.5, 40.6, 38.9, 37.0, 31.8, 31.0, 30.4, 21.9, 18.4, 18.3, 14.5. HRMS (ESI) calcd for $C_{23}H_{33}N_2O_3S$ $[M+H]^+$ 417.2206, found 417.2210.

Synthesis of compound **8**: Compound **8** was obtained as white solid in 44.2% yield. m.p. 186～188 ℃; $[\alpha]_D^{20}$ −208.4 (c 0.285, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.17 (s, 1H), 5.44 (s, 1H), 4.88 (s, 1H), 4.42 (dd, J=12.0, 3.6 Hz, 1H), 3.20～3.15 (m, 2H), 3.07～3.02 (m, 1H), 2.61 (d, J=15.3 Hz, 1H), 2.14 (d, J=12.5 Hz, 1H), 2.06 (d, J=15.4 Hz, 2H), 1.94 (d, J=12.6 Hz, 1H), 1.91～1.77 (m, 2H), 1.77～1.72 (q, J=7.2 Hz, 2H), 1.64～1.50 (m, 2H), 1.46 (d, J=8.3 Hz, 2H), 1.29 (s, 3H), 1.23 (s, 3H), 1.14 (m, 4H), 1.00 (t, J=7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.1, 169.5, 150.4, 148.0, 118.0, 111.8, 74.9, 72.8, 61.7, 52.5, 50.7, 48.7, 46.0, 40.5, 38.7, 36.8, 31.4, 30.8, 29.9, 22.4, 21.7, 18.2, 18.1, 11.7. HRMS (ESI) calcd for $C_{24}H_{35}N_2O_3S$ $[M+H]^+$ 431.2363, found 431.2365.

Synthesis of compound **9**: Compound **9** was obtained as white solid in 72.8% yield. m.p. 212～214 ℃; $[\alpha]_D^{20}$ −200.0 (c 0.065, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.15 (s, 1H), 5.42 (s, 1H), 4.87 (s, 1H), 4.40～4.44 (dd, J=12.0, 3.2 Hz, 1H), 3.17～3.20 (m, 1H), 3.13 (s, 1H), 3.02～3.06 (m, 1H), 2.58～2.62 (d, J=15.3 Hz, 1H), 2.16～1.92 (m, 4H), 1.82～1.57 (m, 5H), 1.50～1.37 (m, 5H), 1.28 (s, 3H), 1.23 (s, 3H), 1.13 (s, 3H), 1.08 (s, 1H), 0.97～0.91 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.2, 169.5, 151.1, 148.1, 117.8, 111.8, 74.9, 72.6, 61.8, 52.6, 50.8, 46.6, 46.0, 40.5, 38.8, 36.9, 31.6, 31.2, 30.9, 30.1, 21.7, 20.1, 18.2, 18.1, 13.8. HRMS (ESI) calcd for $C_{25}H_{37}N_2O_3S$ $[M+H]^+$ 455.2519, found 455.2515.

Synthesis of compound **10**: Compound **10** was obtained as white solid in 38.7% yield; m.p. 161～163 ℃; $[\alpha]_D^{20}$ −264.9 (c 0.114, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ: 6.16 (s, 1H), 5.42 (s, 1H), 4.87 (s, 1H), 4.40～4.44 (dd, J=12.0, 3.3 Hz, 1H), 3.21～3.13 (m, 2H), 3.08～3.01 (m, 1H), 2.59～2.62 (d, J=15.4 Hz, 1H), 2.15～2.13 (d, J=12.3 Hz, 1H), 2.07～2.04 (d, J=13.5 Hz, 2H), 1.95～1.92 (d, J=12.5 Hz, 1H), 1.83～1.79 (m, 1H), 1.75～1.66 (dd, J=14.2, 7.1 Hz, 2H), 1.62～1.50 (m, 2H), 1.46～1.44 (m, 2H), 1.41～1.37 (m, 2H), 1.32～1.31 (m, 2H), 1.28 (s, 3H), 1.25 (s, 1H), 1.23 (s, 3H), 1.13 (s, 3H), 1.02～0.93 (m, 1H), 0.91～0.87 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.2, 169.5, 151.2, 148.2, 117.9, 112.0, 75.0, 72.9, 62.0, 52.7, 50.9, 47.1, 46.2, 40.6, 38.9, 37.0, 31.6, 31.0, 30.2, 29.3, 26.8, 22.7, 21.8, 18.3, 18.3, 14.2. HRMS (ESI) calcd for $C_{27}H_{41}N_2O_3S$ $[M+H]^+$ 473.2832, found 473.2835.

Synthesis of compound **11**: Compound **11** was obtained as white solid in 55.4% yield; m.p. 162～164 ℃; $[\alpha]_D^{20}$ −193.1 (c 0.349, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ:

6.16 (s, 1H), 5.94 (ddt, J=17.2, 10.3, 5.6 Hz, 1H), 5.43 (s, 1H), 5.32 (dd, J=17.2, 1.4 Hz, 1H), 5.21 (dd, J=10.2, 1.3 Hz, 1H), 4.87 (s, 1H), 4.41 (dd, J=12.0, 3.4 Hz, 1H), 3.88～3.71 (m, 2H), 3.14 (s, 1H), 2.60 (d, J=15.3 Hz, 1H), 2.13 (d, J=12.6 Hz, 1H), 2.08～1.99 (m, 2H), 1.98～1.89 (m, 1H), 1.85～1.77 (m, 1H), 1.64～1.48 (m, 2H), 1.44 (d, J=8.1 Hz, 3H), 1.27 (s, 3H), 1.21 (m, 4H), 1.12 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.5, 169.2, 151.2, 148.2, 133.8, 118.5, 117.8, 112.7, 75.2, 73.1, 62.1, 52.8, 51.0, 49.4, 46.3, 40.8, 39.0, 37.2, 31.6, 31.1, 30.2, 22.0, 18.6, 18.4. HRMS (ESI) calcd for $C_{24}H_{33}N_2O_3S$ $[M+H]^+$ 429.2206, found 429.2205.

Synthesis of compound **12**: Compound **12** was obtained as white solid in 87.0% yield; m.p. 195～196 ℃; $[\alpha]_D^{20}$ −146.6 (c 0.305, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.17 (s, 1H), 5.92 (s, 1H), 5.45 (s, 1H), 4.88 (s, 1H), 4.44 (dd, J=12.1, 3.7 Hz, 1H), 3.15 (s, 1H), 2.98 (s, 1H), 2.59 (d, J=15.3 Hz, 1H), 2.17 (t, J=13.0 Hz, 2H), 2.05 (d, J=15.1 Hz, 2H), 1.94 (m, 2H), 1.80 (m, 3H), 1.68～1.57 (m, 3H), 1.54～1.42 (m, 4H), 1.31 (m, 3H), 1.29 (s, 4H), 1.26 (d, J=4.0 Hz, 1H), 1.23 (s, 3H), 1.13 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.0, 168.3, 150.3, 148.1, 117.9, 111.4, 75.0, 72.6, 61.7, 56.3, 52.6, 50.7, 46.0, 40.5, 38.6, 36.8, 33.0, 32.7, 31.4, 30.8, 30.1, 25.6, 25.1, 21.7, 18.2, 18.1. HRMS (ESI) calcd for $C_{27}H_{39}N_2O_3S$ $[M+H]^+$ 471.2676, found 471.2669.

Synthesis of compound **13**: Compound **13** was obtained as white solid in 29.7% yield; m.p. 166～168 ℃; $[\alpha]_D^{20}$ −128.8 (c 0.212, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46～7.37 (m, 2H), 7.34 (dd, J=8.6, 7.1 Hz, 2H), 7.13～6.98 (m, 1H), 6.20 (s, 1H), 5.91 (s, 1H), 5.46 (s, 1H), 4.98～4.81 (m, 1H), 4.51 (dd, J=12.2, 3.7 Hz, 1H), 3.16 (s, 1H), 2.67 (d, J=15.4 Hz, 1H), 2.19 (d, J=12.8 Hz, 1H), 2.11 (d, J=15.6 Hz, 1H), 2.04～1.94 (m, 2H), 1.88～1.70 (m, 2H), 1.65～1.54 (m, 2H), 1.51 (d, J=4.9 Hz, 2H), 1.35 (s, 3H), 1.30 (s, 3H), 1.27～1.20 (m, 1H), 1.17 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.3, 163.7, 151.1, 147.7, 140.7, 129.3, 123.0, 118.6, 118.5, 113.6, 75.0, 73.2, 61.9, 52.5, 50.8, 46.0, 40.5, 38.5, 36.9, 31.3, 30.8, 29.7, 21.8, 18.2, 18.7. HRMS (ESI) calcd for $C_{27}H_{33}N_2O_3S$ $[M+H]^+$ 487.2026, found 487.2025.

Synthesis of compound **14**: Compound **14** was obtained as white solid in 61.1% yield; m.p. 160～162 ℃; $[\alpha]_D^{20}$ −178.5 (c 0.712, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, J=7.1 Hz, 2H), 7.35 (t, J=7.3 Hz, 2H), 7.32～7.27 (m, 1H), 5.97 (s, 1H), 5.36 (s, 1H), 4.89～4.82 (m, 1H), 4.45～4.30 (m, 3H), 3.11 (s, 1H), 2.57 (d, J=15.4 Hz, 1H), 2.17～2.07 (m, 1H), 2.03 (d, J=15.4 Hz, 2H), 1.95 (d, J=12.6 Hz, 1H), 1.90～1.74 (m, 2H), 1.56～1.51 (m, 2H), 1.47～1.37 (m, 2H), 1.28 (s, 3H), 1.22 (s, 3H), 1.19～1.15 (m, 1H), 1.11 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.0, 169.0, 150.6, 147.6, 137.4, 128.7, 127.8, 127.7, 118.4, 112.4, 77.4, 77.1, 76.7, 74.9, 73.1, 61.8, 52.4, 50.7, 50.5, 45.9, 40.5, 38.6, 36.9, 31.1, 30.8, 29.6, 21.7, 18.3, 18.1. HRMS (ESI) calcd for $C_{28}H_{35}N_2O_3S$ $[M+H]^+$ 479.2363, found 479.2361.

4.3 MTT assay *in vitro*

In this paper, cancer cells included HepG2 (human liver carcinoma), NCI-H460 (human lung carcinoma), JEG-3 (Human choriocarcinoma), K562 (Human Chronic myeloid leukemia), HL-60 (human promyelocytic leukemia), Hela (Human cervical cancer). Specifically, the logarithmic cells were collected and suspended in complete DMEM (dulbecco's modified eagle medium) medium. The cell suspension concentration was adjusted to 3×10^4 cells/mL, and 96-well cell culture plates were inoculated with 100 μL/well. Set the supernatant at 37 ℃ and 5% CO_2 incubator for 24 h. Add fresh supernatant and fresh complete DMEM medium (90 μL/well) and add different concentrations of test solution (10 μL/well), and then set at 37 ℃, 5% CO_2 for 48 h. Add 100 μL of MTT solution (0.5 mg/mL, incomplete DMEM medium) to each well and incubate the incubator for 48 h. After 48 hours, the culture was terminated and the supernatant was discarded. 150 μL of DMSO was added to each well. The shaker was shaken at low speed for 5 min to dissolve the crystals. The absorbance of each well was measured at 570 nm at the enzyme-linked immunosorbent assay.

4.4 Morphological changes of HepG2 cells under fluorescence microscopy

$HepG_2$ cells were cultured in DMEM medium with high glucose. $HepG_2$ cells in the logarithmic phase were digested with trypsin and spread in the 6-well plate. The number of cells per pore is 1×10^5. The cells were divided into control group, 0.5 μmol•L^{-1} group, 1 μmol•L^{-1} group and 2 μmol•L^{-1} group. After the cells were attached to the wall, the cells of control group were replaced with new culture medium of DMEM. While for the drug groups, the cells were also replaced with new culture medium and compound **8** were dropped into the plate. The final concentrations of compound **8** were 0.5, 1 and 2 μmol•L^{-1}. After 48 h, the medium was poured out and the cells were cleaned with 4 ℃ PBS for 3 times. Then 1 mL of 4% paraformaldehyde was dropped into the plate. 10 min later, paraformaldehyde was poured out and 4 ℃ PBS was used to clean the cells 3 times. Finally, Hoest33258 staining solution was added and dyed in dark for 10 min and the cells were observed under fluorescence microscope.

4.5 Morphological changes of HepG2 cells under electron microscope

The logarithmic $HepG_2$ cells were laid out in sterile 10-centimeter petri dishes. There were 4 petri dishes and 1×10^6 cells in each petri dish. The cells were divided into control group, 0.5 μmol•L^{-1} group of compound **8**, 1 μmol•L^{-1} group of compound **8** and 2 μmol•L^{-1} group of compound **8** according to different dish. After the cells were attached to the wall, the cells in the control group were washed with PBS twice and then 5 mL of DMEM medium was added in the petri dish. The cells in 0.5 μmol•L^{-1} group of compound **8** were washed with PBS twice and compound **8** solution was dropped in with DMEM medium. The final concentration of compound **8**

was 0.5 μmol•L^{-1}. The cells in 1 μmol•L^{-1} group of compound **8** and 2 μmol•L^{-1} group of compound **8** were treated in the same way with the cells in c 0.5 μmol•L^{-1} group of compound **8**. The final concentrations of compound **8** were 1 and 2 μmol•L^{-1}, respectively. The cells were gathered and fixed with Gluta (Solarbio, p1126, Beijing, China) 48 h later. Then they were mailed to Wuhan Biomisp Biotechnology Co., LTD for electron microscopy.

4.6 Apoptosis of HepG2 cells detected by flow cytometry

The HepG2 cells were cultured in a 6-well plate with 1×10^5 cells in each well. The cell processing method and grouping were the same to those of the fluorescence staining method and grouping. After 48 h, the DMEM culture medium was sucked out of the cells with a pipette and the floating cells were collected after 1000 revolutions for 3 min. Then the cells in the culture plate were rinsed three times with 4 ℃ PBS and digested with trypsin for 1 min before cells were collected. The cells collected twice were suspended in 100 μL of 1×Binding Buffer. Then 5 μL of Annexin V-FITC and 10 μL of PI Staining Solution were dropped into the cells for 15 min avoiding light. Finally, 400 μL of 1×Binding Buffer was dropped into the solution and the solution was mixed before detected by flow cytometry.

Supporting Information NMR spectra of the synthesized compounds. The Supporting Information is available free of charge via the Internet at http://siocjournal.cn/.

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