



Design, Synthesis and Biological Evaluation of Novel Osthole Derivatives as Potential Agents for the Treatment of Cancer

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Abstract: Aim: Osthole, an ingredient of Traditional Chinese Medicine (TCM) from natural product *Cnidium monnieri* (L.) Cusson, showed many biological activities related to the treatment of cancer. However, the comparatively weak activity hinders its further application in clinical. Therefore, the aim of the present study was design and synthesis of a series of novel osthole derivatives by introducing different secondary amine groups at 7-O position of coumarin ring to improve its anticancer activity. Method: After the demethylation of osthole, the target derivatives 4a-l were successfully synthesized through two steps in high yield. The structures of the synthesized compounds were confirmed by ¹H and ¹³C NMR. The antiproliferative activity of these compounds was evaluated against four selected cancer cell lines (SGC7901, MCF-7, HCT116 and HepG2) using MTT method, and the most potent compound was selected for Hoechst 33258 staining assay to further investigate its possible mechanism on cancer cells. Results: Twelve osthole derivatives were synthesized and their structures were identified. Biological studies showed that most of them showed moderate to good growth inhibition against all the tested cancer cells. Especially, compound 4l displayed the most potent activity with IC₅₀ values of 37.57 μM, 25.12 μM, 46.54 μM and 18.39 μM against SGC7901, HCT116, MCF-7 and HepG2, respectively, which was more potent than those of its parent compound osthole and the anticancer agent 5-Fu. The Hoechst 33258 staining assay revealed that compound 4l could induce cell apoptosis in HepG2 cells. Conclusion: The modification of osthole to improve its activity is feasible, and compound 4l can be considered as a potential agent for the treatment of cancer.

Keywords: Osthole, Anticancer Activity, Synthesis, Apoptosis, Coumarin

1. Introduction

Cancer is one of the major diseases that threatens human health characterized by uncontrolled cell proliferation and spread of malignant cells [1, 2]. According to the World health organization (WHO) report, there is nearly 7.6 million people died of cancer per year, and the figure is expecting to reach 13 million by 2030 [3]. Although much progress has been made from the identification to the treatment of cancer, the factors like poor patient compliance, drug resistance and drug induced toxicities make successful treatment of cancer

remain a challenge [4, 5]. Therefore, the search for new effective anticancer drugs is highly needed.

Natural products with their inherent ability to interact with biological targets have played an important role in drug discovery [6-8]. Especially in cancer therapy, it is roughly estimated that more than half of modern marketed drugs are natural products or their derivatives [9-10]. Osthole 1, a prenylated coumarin derivative, is extracted from *Cnidium monnieri* (L.) Cusson, which has been clinically ingested as an important component of medicinal plants and herbs in Tradition Chinese Medicine (TCM) [11]. A lot of studies

showed that osthole could inhibit cancer cell proliferation, prevent cell migration and invasion, and induce differentiation, apoptosis and cell cycle arrest [12]. Additionally, it could also prolong the survival days of tumor-bearing mice and suppressed tumor growth in vivo [13]. However, although osthole have so many promising profiles related to cancer treatment, the comparatively weak activity hampered its application in clinical development [14, 15]. Therefore, structural modification of osthole to improve its anticancer activity is highly needed. In this work a series of novel osthole derivatives by introducing diversified secondary amines to 7-O position was designed and synthesized. All synthesized compounds were evaluated for their antiproliferative activity on HepG2 (liver carcinoma), SGC7901 (gastric carcinoma), HCT-116 (colon cancer) and MCF-7 (breast carcinoma) cells. In addition, compound with the most potent activity was also selected for mechanism assay.

2. Materials and Methods

2.1. Materials

All chemical reagents used in synthesis were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). The reactions were monitored by TLC on glass-packed precoated silica gel GF₂₅₄ (Qingdao Haiyang Chemical Plant, Qingdao, China) plates. Column chromatography was performed on silica gel (90-150 μ m; Qingdao Marine Chemical Inc.). ¹H NMR spectra (600 MHz) and ¹³C NMR spectra (151 MHz) were recorded on a Bruker ACF-600 spectrometer at 25°C. Chemical shifts are reported in ppm (δ) using the TMS as internal standard, and the coupling constants are reported in hertz (Hz). The purity of all compounds for biological evaluation was confirmed to > 95% by analytical HPLC conducted on an Agilent 1200 HPLC System. The desired derivatives were synthesized according to procedure depicted in Figure 1.

2.2. General Procedure for the Synthesis of Compounds

Synthesis of compound 2

To a solution of 1 (1.00 g, 4.09 mmol) in anhydrous CH₂Cl₂ (10 mL), BBr₃ (2.00 mL) was added dropwise at -40 °C under N₂. After the addition of BBr₃, the resulting solution was warmed to r.t. and stirred for an additional 12 h. Then, it was diluted with CH₂Cl₂ (10 mL), cooled in an ice-bath and slowly quenched with 10% aq NaHCO₃. The organic phase was washed with brine, dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using EtOAc/petrol (1:4) as eluent to obtain target compound 2 as white solid.

Synthesis of compounds 3a-c.

A mixture of 2 (2.0 mmol) with suitable α , ω -dibromoalkanes (50 mmol) and anhydrous K₂CO₃ (1.4 g, 10 mmol) in acetone (15 mL) was refluxed under stirring for 4 h. After cooling, the reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The obtained

residue was purified by silica gel chromatography with EtOAc/petrol (1:7) as eluent to give compounds 3a-c as white solid.

Synthesis of compounds 4a-l.

A mixture of 3a-c (1.2 mmol) and the corresponding secondary amines (1.3 mmol) in dry acetonitrile (10 mL) was refluxed in the presence of anhydrous K₂CO₃ (1.3 mmol) for 8 h. After cooling to the room temperature, the mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel chromatography using CH₂Cl₂/MeOH (100:1) as eluent to obtain target compounds 4a-l.

2.3. Biological Evaluation

Cytotoxicity study

In all the experiments, different cell lines were plated into 96-well plates at a concentration of 5×10^3 cells per well. After overnight incubation, the culture medium was removed and replaced with fresh medium containing the test compounds in different concentrations. The cells were incubated for another 48 h. Afterward, 20 μ L of MTT solution (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Then, the supernatant was aspirated and 150 μ L of dimethyl sulfoxide (DMSO) was added to dissolve the dark blue crystals (formazan crystals). After shaking for 20 min, the absorbance was measured at a wavelength of 570 nm using a microplate reader (SpectraMax plus 384, Molecular Devices). Each concentration was analyzed in triplicate and the experiment was repeated three times. The average 50% inhibitory concentration (IC₅₀) was determined from the dose-response curves according to the inhibition ratio for each concentration.

Hoechst 33258 staining

Cells grown on a sterile cover slip in six-well tissue culture plates were treated with compounds for a certain range of time. The culture medium containing compounds was removed, and the cells were fixed in 4% paraformaldehyde for 10 min. After being washed twice with PBS, the cells were stained with 0.5 mL of Hoechst 33258 (Beyotime) for 5 min and then again washed twice with PBS. The stained nuclei were observed under an IX71SIF-3 fluorescence microscope using 350 nm excitation and 460 nm emission.

3. Results and Discussion

3.1. Chemistry

The synthetic routes for the preparation of compounds 4a-l are depicted in Figure 1. Osthole was demethylated by using boron tribromide etherate under -40 °C to room temperature to afford compound 2 in high yield. Then, reacting the compound 2 with appropriate α , ω -dibromoalkanes to give compound 3. Finally, compound 3 was coupled with the corresponding secondary amines to accomplish the target compounds 4a-l. The structures of all compounds were confirmed by NMR spectroscopy, and the detail data are shown in Table 1.

Table 1. The NMR data of compounds 2, 3a-c and 4a-l.

Compd.	Yield	¹ H and ¹³ C NMR data
2	92%	¹ H NMR (600 MHz, DMSO) δ 7.91 (d, <i>J</i> = 9.4 Hz, 1H), 7.37 (d, <i>J</i> = 8.5 Hz, 1H), 6.87 (d, <i>J</i> = 8.5 Hz, 1H), 6.19 (d, <i>J</i> = 8.7 Hz, 1H), 5.18 (m, 1H), 3.37 (d, <i>J</i> = 5.2 Hz, 2H), 1.78 (s, 3H), 1.62 (s, 3H).
3a	86%	¹ H NMR (600 MHz, DMSO) δ 7.99 (d, <i>J</i> = 9.5 Hz, 1H), 7.57 (d, <i>J</i> = 8.6 Hz, 1H), 7.07 (d, <i>J</i> = 8.7 Hz, 1H), 6.31 (d, <i>J</i> = 9.5 Hz, 1H), 5.28 – 5.15 (m, 1H), 4.51 – 4.38 (m, 2H), 3.90 – 3.80 (m, 2H), 3.47 (d, <i>J</i> = 7.4 Hz, 2H), 1.81 (s, 3H), 1.63 (s, 3H).
3b	88%	¹ H NMR (600 MHz, DMSO) δ 8.01 (d, <i>J</i> = 9.5 Hz, 1H), 7.59 (d, <i>J</i> = 8.6 Hz, 1H), 7.08 (d, <i>J</i> = 8.7 Hz, 1H), 6.32 (d, <i>J</i> = 9.5 Hz, 1H), 5.26 – 5.16 (m, 1H), 4.44 (t, <i>J</i> = 6.0 Hz, 2H), 3.84 (t, <i>J</i> = 6.4 Hz, 2H), 3.47 (d, <i>J</i> = 7.4 Hz, 2H), 2.42 – 2.36 (m, 2H), 1.82 (s, 3H), 1.62 (s, 3H).
3c	83%	¹ H NMR (600 MHz, DMSO) δ 7.98 (d, <i>J</i> = 9.5 Hz, 1H), 7.58 (d, <i>J</i> = 8.6 Hz, 1H), 7.09 (d, <i>J</i> = 8.7 Hz, 1H), 6.32 (d, <i>J</i> = 9.5 Hz, 1H), 5.28 – 5.17 (m, 1H), 4.32 (t, <i>J</i> = 6.0 Hz, 2H), 3.67 (t, <i>J</i> = 6.0 Hz, 2H), 3.47 (d, <i>J</i> = 7.4 Hz, 2H), 2.32 – 2.16 (m, 2H), 2.17 – 2.06 (m, 2H), 1.80 (s, 3H), 1.62 (s, 3H).
4a	76%	¹ H NMR (600 MHz, CDCl ₃) δ 7.61 (d, <i>J</i> = 9.4 Hz, 1H), 7.28 (s, 1H), 6.83 (d, <i>J</i> = 8.6 Hz, 1H), 6.24 (d, <i>J</i> = 9.4 Hz, 1H), 5.23 (d, <i>J</i> = 1.2 Hz, 1H), 4.18 (t, <i>J</i> = 5.9 Hz, 2H), 3.55 (d, <i>J</i> = 7.2 Hz, 2H), 2.81 (t, <i>J</i> = 5.9 Hz, 2H), 2.37 (s, 6H), 1.84 (s, 3H), 1.66 (s, 3H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.36, 159.34, 152.94, 143.72, 132.51, 126.22, 121.24, 118.18, 113.14, 108.23, 67.30, 58.12, 46.02, 25.79, 22.07, 18.07.
4b	79%	¹ H NMR (600 MHz, CDCl ₃) δ 7.63 (d, <i>J</i> = 9.1 Hz, 1H), 7.29 (d, <i>J</i> = 5.3 Hz, 1H), 6.85 (d, <i>J</i> = 8.3 Hz, 1H), 6.26 (d, <i>J</i> = 9.3 Hz, 1H), 5.26 (s, 1H), 4.16 (s, 2H), 3.57 (d, <i>J</i> = 6.6 Hz, 2H), 2.95 (s, 2H), 2.75 – 2.59 (m, 4H), 1.86 (s, 3H), 1.69 (s, 3H), 1.11 (s, 6H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.40, 159.51, 152.92, 143.75, 132.48, 128.81, 126.19, 121.27, 118.09, 113.02, 108.25, 67.74, 51.82, 47.93, 25.79, 22.04, 18.07, 11.95.
4c	82%	¹ H NMR (600 MHz, CDCl ₃) δ 7.53 (d, <i>J</i> = 9.0 Hz, 1H), 7.19 (d, <i>J</i> = 8.9 Hz, 1H), 6.75 (d, <i>J</i> = 8.5 Hz, 1H), 6.15 (d, <i>J</i> = 9.4 Hz, 1H), 5.16 (s, 1H), 4.17 – 4.06 (m, 2H), 3.47 (d, <i>J</i> = 7.1 Hz, 2H), 2.93 – 2.81 (m, 2H), 2.58 (s, 4H), 1.77 – 1.68 (m, 6H), 1.65 – 1.52 (m, 4H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.38, 159.43, 152.94, 143.75, 132.46, 128.81, 126.22, 121.31, 118.11, 113.04, 108.23, 68.16, 54.91, 54.80, 25.79, 23.59, 22.06, 18.08.
4d	78%	¹ H NMR (600 MHz, CDCl ₃) δ 7.61 (d, <i>J</i> = 9.4 Hz, 1H), 7.28 (d, <i>J</i> = 8.1 Hz, 1H), 6.83 (d, <i>J</i> = 8.6 Hz, 1H), 6.24 (d, <i>J</i> = 9.4 Hz, 1H), 5.25 – 5.20 (m, 1H), 4.22 (t, <i>J</i> = 5.9 Hz, 2H), 3.55 (d, <i>J</i> = 7.2 Hz, 2H), 2.85 (t, <i>J</i> = 5.9 Hz, 2H), 2.56 (br s, 4H), 1.85 (s, 3H), 1.67 (s, 3H), 1.66 – 1.60 (m, 4H), 1.47 (br s, 2H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.38, 159.35, 152.92, 143.74, 132.51, 126.23, 121.27, 118.10, 113.09, 110.10, 108.25, 66.99, 57.76, 55.11, 25.81, 23.98, 22.07, 18.10.
4e	84%	¹ H NMR (600 MHz, CDCl ₃) δ 7.61 (d, <i>J</i> = 9.5 Hz, 1H), 7.28 (d, <i>J</i> = 8.6 Hz, 1H), 6.82 (d, <i>J</i> = 8.6 Hz, 1H), 6.25 (d, <i>J</i> = 9.4 Hz, 1H), 5.25 – 5.16 (m, 1H), 4.22 (s, 2H), 3.81 – 3.66 (m, 4H), 3.54 (d, <i>J</i> = 7.2 Hz, 2H), 2.87 (s, 2H), 2.62 (s, 4H), 1.83 (s, 3H), 1.67 (s, 3H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.10, 159.22, 152.94, 143.68, 132.68, 126.24, 121.24, 118.17, 113.23, 108.18, 66.84, 57.54, 54.08, 25.30, 22.08, 18.11.
4f	86%	¹ H NMR (600 MHz, CDCl ₃) δ 7.54 (d, <i>J</i> = 7.8 Hz, 1H), 7.31 – 7.17 (m, 1H), 6.74 (d, <i>J</i> = 8.6 Hz, 1H), 6.16 (d, <i>J</i> = 9.4 Hz, 1H), 5.15 (d, <i>J</i> = 7.0 Hz, 1H), 4.12 (t, <i>J</i> = 5.7 Hz, 2H), 3.46 (d, <i>J</i> = 7.1 Hz, 2H), 2.80 (t, <i>J</i> = 5.7 Hz, 2H), 2.40 (br s, 8H), 2.22 (s, 3H), 1.78 (s, 3H), 1.59 (s, 3H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.34, 159.38, 152.90, 143.74, 132.46, 126.21, 121.25, 118.12, 113.05, 108.21, 67.11, 57.04, 55.09, 53.60, 45.99, 25.80, 22.05, 18.10.
4g	76%	¹ H NMR (600 MHz, CDCl ₃) δ 7.64 (d, <i>J</i> = 9.0 Hz, 1H), 7.47 (d, <i>J</i> = 8.4 Hz, 1H), 6.76 (d, <i>J</i> = 9.0 Hz, 1H), 6.17 (d, <i>J</i> = 9.4 Hz, 1H), 5.19 – 5.12 (m, 1H), 4.05 (t, <i>J</i> = 6.3 Hz, 2H), 3.47 (d, <i>J</i> = 7.2 Hz, 2H), 2.67 – 2.29 (m, 10H), 2.25 (s, 3H), 1.99 – 1.96 (m, 2H), 1.77 (s, 3H), 1.61 (s, 3H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.41, 159.57, 152.93, 143.76, 132.44, 128.83, 126.19, 121.33, 118.02, 113.00, 108.15, 68.18, 54.91, 54.78, 52.66, 45.63, 28.95, 25.84, 22.04, 18.10.
4h	74%	¹ H NMR (600 MHz, CDCl ₃) δ 7.63 (d, <i>J</i> = 7.2 Hz, 1H), 7.50 (d, <i>J</i> = 7.5 Hz, 1H), 6.82 (d, <i>J</i> = 9.0 Hz, 1H), 6.20 (d, <i>J</i> = 9.6 Hz, 1H), 5.16 (t, <i>J</i> = 7.2 Hz, 1H), 4.16 – 4.13 (m, 2H), 4.01 (m, 2H), 3.47 (d, <i>J</i> = 7.2 Hz, 2H), 2.40 (br s, 8H), 2.22 (s, 3H), 1.81 – 1.75 (m, 5H), 1.65 – 1.59 (m, 5H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.42, 159.61, 152.92, 143.76, 132.46, 128.81, 126.17, 121.29, 117.97, 112.94, 108.09, 68.16, 58.02, 54.91, 52.85, 38.73, 30.37, 28.93, 27.26, 25.81, 23.75, 22.04, 18.03.
4i	68%	¹ H NMR (600 MHz, CDCl ₃) δ 7.62 (d, <i>J</i> = 9.4 Hz, 1H), 7.55 (d, <i>J</i> = 8.4 Hz, 1H), 6.83 (d, <i>J</i> = 8.6 Hz, 1H), 6.25 (d, <i>J</i> = 9.4 Hz, 1H), 5.24 (s, 1H), 4.28 – 4.17 (m, 2H), 3.56 (d, <i>J</i> = 7.2 Hz, 2H), 2.90 (t, <i>J</i> = 5.7 Hz, 2H), 2.66 (br s, 9H), 1.85 (s, 3H), 1.71 (s, 3H), 1.07 (br s, 6H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.35, 159.38, 152.93, 143.72, 132.46, 128.81, 126.20, 121.25, 118.16, 113.10, 108.19, 68.16, 67.06, 65.20, 57.03, 54.74, 53.69, 25.81, 23.75, 22.99, 22.07, 18.12.
4j	84%	¹ H NMR (600 MHz, CDCl ₃) δ 7.62 (d, <i>J</i> = 9.6 Hz, 1H), 7.28 (d, <i>J</i> = 8.4 Hz, 1H), 6.82 (d, <i>J</i> = 8.6 Hz, 1H), 6.25 (d, <i>J</i> = 9.4 Hz, 1H), 5.30 – 5.16 (m, 1H), 4.22 (t, <i>J</i> = 5.7 Hz, 2H), 3.54 (d, <i>J</i> = 7.2 Hz, 2H), 2.89 (t, <i>J</i> = 5.7 Hz, 2H), 2.67 (br s, 9H), 1.84 (s, 3H), 1.67 (s, 3H), 0.47 – 0.43 (m, 4H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.36, 159.34, 152.93, 143.72, 132.52, 126.20, 121.25, 118.16, 113.12, 108.19, 66.25, 57.08, 53.37, 53.06, 38.44, 25.32, 21.85, 18.02, 5.51.
4k	85%	¹ H NMR (600 MHz, CDCl ₃) δ 8.25 – 8.24 (m, 2H), 7.55 (d, <i>J</i> = 9.4 Hz, 1H), 7.24 – 7.20 (m, 1H), 6.77 (d, <i>J</i> = 8.6 Hz, 1H), 6.43 (t, <i>J</i> = 4.7 Hz, 1H), 6.18 (d, <i>J</i> = 9.4 Hz, 1H), 5.16 (s, 1H), 4.20 (br s, 2H), 3.80 (br s, 4H), 3.48 (d, <i>J</i> = 7.2 Hz, 2H), 2.88 (br s, 2H), 2.62 (br s, 4H), 1.77 (s, 3H), 1.60 (s, 3H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.58, 161.26, 159.15, 157.78, 152.97, 143.69, 132.64, 126.26, 121.20, 118.19, 113.26, 108.23, 66.75, 57.16, 54.75, 53.53, 25.78, 22.03, 18.09.
4l	83%	¹ H NMR (600 MHz, CDCl ₃) δ 7.66 (d, <i>J</i> = 9.0 Hz, 1H), 7.26 (d, <i>J</i> = 8.6 Hz, 1H), 6.83 (d, <i>J</i> = 8.6 Hz, 1H), 6.25 (d, <i>J</i> = 9.4 Hz, 1H), 5.26 – 5.19 (m, 1H), 4.18 (d, <i>J</i> = 5.8 Hz, 2H), 3.54 (d, <i>J</i> = 7.2 Hz, 2H), 3.11 (t, <i>J</i> = 6.0 Hz, 2H), 2.87 – 2.81 (m, 9H), 1.46 – 1.36 (m, 10H). ¹³ C NMR (151 MHz, CDCl ₃) δ 161.75, 159.49, 152.80, 143.63, 132.46, 130.90, 128.82, 121.16, 118.02, 112.80, 108.27, 68.17, 56.73, 54.74, 52.61, 38.73, 30.37, 28.93, 26.02, 25.81, 23.75, 22.03, 18.10.

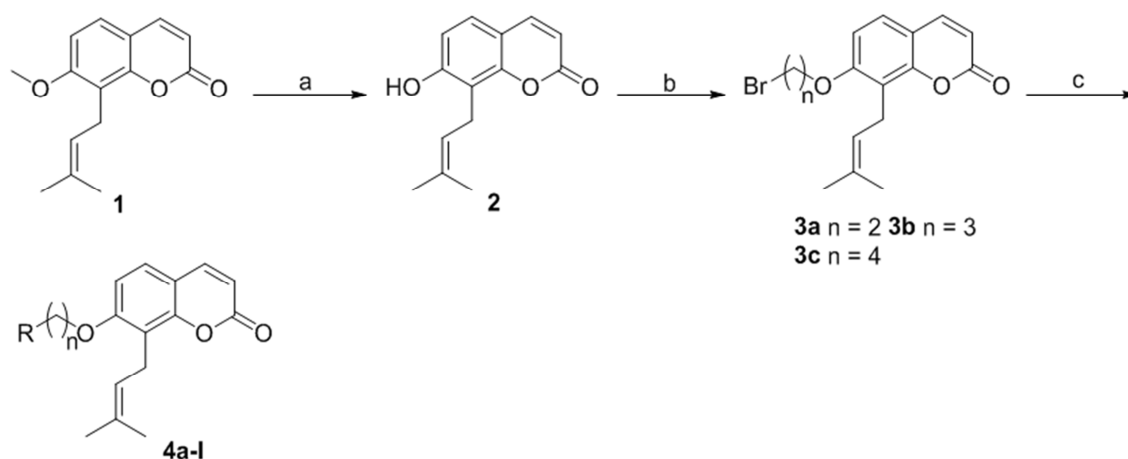


Figure 1. Synthesis of compounds 4a-l. Reagents and conditions: (a) BBr_3 , DCM, $-40^\circ C$ to r.t., 12 h; (b) $Br(CH_2)_nBr$, anhydrous K_2CO_3 , acetone, reflux, 4 h; (c) Anhydrous K_2CO_3 , corresponding secondary amines, CH_3CN , reflux, 8 h.

3.2. Biological Studies

Cytotoxic activity screening and SAR

In vitro antiproliferative activity of all the synthesized osthole derivatives was evaluated against four human cancer cell lines (MCF-7, HepG2, HCT116 and SCG7901) by using MTT method. 5-Fluorouracil (5-Fu), an effective anticancer agent, and osthole were used as positive control for comparison [16-19]. All biological results are summarized in Table 2. From the results, it can be seen that most compounds show moderate anti-proliferation activities with IC_{50} values less than 200 μM . The structure-activity relationship (SAR) analysis indicated that, with exception of compound 4l, compounds (4f-k) with substituted piperazine showed much higher anticancer activity than compounds with other

secondary amines (4a-e). The piperazine substituted with a pyrimidine group seemed more favorable for the activity, as compound 4k showed stronger inhibition against these four cell lines than its congeners 4f-j. Lengthening the linker between osthole and the secondary amine groups could not improve the anticancer activity. For example, compound 4f with short linker ($n=2$) showed more potent activity than 4g ($n=3$) and 4h ($n=4$) with long linker. Compound 4l with a 4-piperidinopiperidine group showed the most potent activity in this series with IC_{50} values of 37.57 μM , 25.12 μM , 46.54 μM and 18.39 μM against SCG7901, HCT116, MCF-7 and HepG2, respectively, which was more potent than those of 5-Fu and its lead compound osthole. These results demonstrated the rationality of our molecular design.

Table 2. Cytotoxic activity (IC_{50} , μM) of synthesized compounds 4a-l.

Compd.	R	IC_{50} (μM) ^a			
		SGC7901	HCT116	MCF-7	HepG2
4a		>200	>200	>200	109.38 \pm 10.32
4b		>200	>200	>200	>200
4c		>200	>200	>200	>200
4d		>200	>200	64.41 \pm 6.77	115.57 \pm 10.37
4e		>200	>200	150.75 \pm 13.22	>200
4f		90.00 \pm 8.11	62.74 \pm 2.67	124.46 \pm 12.19	65.01 \pm 4.15
4g		106.16 \pm 11.32	120.18 \pm 9.53	87.00 \pm 10.14	79.92 \pm 8.16
4h		120.12 \pm 9.75	146.05 \pm 14.03	120.43 \pm 12.15	137.20 \pm 14.19
4i		190.78 \pm 13.32	61.83 \pm 4.22	>200	100.61 \pm 9.12
4j		141.72 \pm 11.12	131.22 \pm 7.69	80.79 \pm 6.11	61.31 \pm 3.47

Compd.	R	IC ₅₀ (μM) ^a			
		SGC7901	HCT116	MCF-7	HepG2
4k		76.42 ± 5.24	65.82 ± 5.33	48.96 ± 2.93	53.79 ± 5.15
4l		37.57 ± 3.70	25.12 ± 2.19	46.54 ± 6.56	18.39 ± 2.10
Osthole	-	125.34 ± 9.67	>200	98.98 ± 7.91	120.01 ± 11.18
5-Fu	-	47.83 ± 4.52	65.99 ± 9.31	50.60 ± 2.02	49.84 ± 10.03

^a Data are expressed as the means ± SD of three independent experiments data.

Hoechst 33258 staining.

Apoptosis plays a central role in cancer, since its induction in cancer cells is critical to a successful therapy [20-22]. It is thus believed that apoptosis assay may provide important information to preliminary investigation of the mode of action. Therefore, in the present study, compound 4l that exhibited good cytotoxic inhibition in four cancer cell lines was selected as a representative for mechanism of growth inhibition of HepG2 cells by Hoechst 33258 staining [23-25]. Hoechst 33258 is a membrane permeable blue fluorescent dye that stains the cell nucleus. Live cells with uniformly light blue nuclei were observed under fluorescence microscope after

treatment with Hoechst 33258, whereas apoptotic cells had bright blue nuclei because of karyopyknosis and chromatin condensation; the nuclei of dead cells could not be stained [26-28]. During the assay, HepG2 cells treated with compound 4l at 15 μM from 12 to 48 h were stained with Hoechst 33258. HepG2 cells not treated with the 4l were used as control for 48 h. As shown in Figure 2, HepG2 cells not treated with compound 4l were normally blue, whereas the cells treated with 4l displayed strong blue fluorescence and indicated typical apoptotic morphology after 12, 24 and 48 h. These findings demonstrated that compound 4l could induce apoptosis against HepG2 cell line.

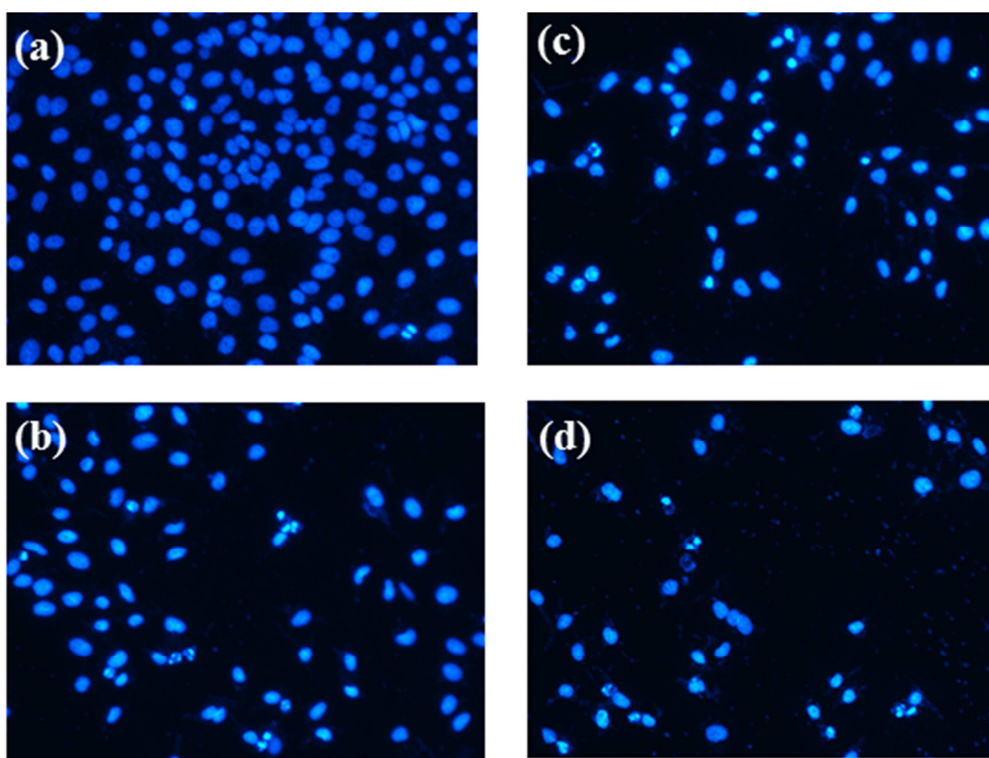


Figure 2. Hoechst 33258 staining of compound 4l in HepG2 cells. (a) Not treated with compound 4l was used as control for 48 h and (b, c and d) treatment with compound 4l (15 μM) for 12, 24 and 48 h, respectively.

4. Conclusion

Osthole is an important natural product with antitumor bioactivity. However, the comparatively weak activity hinders its further application in clinical. In present study, with aim to improve the activity of osthole, a series of novel osthole derivatives have been designed and synthesized by connecting osthole with different secondary amine groups through

polymethene linkers. All compounds were evaluated for their cell growth inhibition activities against four cell lines (SGC7901, HCT116, MCF-7 and HepG2). The results indicated that most of compounds exhibited moderate to good anti-cancer activities with IC₅₀ values ranging from 18.39 to 190.78 μM. Of these compounds, compound 4l stood out as most potent compound in this series with IC₅₀ values of 37.57 μM, 25.12 μM, 46.54 μM and 18.39 μM against SGC7901,

HCT116, MCF-7 and HepG2, respectively, which was more potent than those of 5-Fu and its parent compound osthole. The SAR analysis suggested that introduction of substituted piperazine was favorable to antiproliferative activity, and compound with short linker displayed more potent activity than compound with long linker. The Hoechst 33258 staining assay revealed that compound 4l could induce cell apoptosis in HepG2 cells. As apoptosis is important for the prevention and treatment of cancer, the further study should be performed to investigate the exact pathway of compound 4l. Overall, the above results demonstrate that modification of osthole to improve its activity is feasible, and compound 4l can be considered as a novel anticancer agent for clinical development.

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