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Structural modification of isoalantolactone and biological activity against the hepatoma cell lines

Abstract: Structural modifications were performed on isoalantolactone in an effort to find compounds with potential anticancer activity. Seven previously unknown adducts of active methylene compounds with isoalantolactone were synthesized by the Michael reaction. Moreover, four derivatives of aryl-substituted isoalantolactone were prepared by the Heck reaction. All synthetic products were evaluated for toxic activities against three different hepatoma cell lines, Bel-7402, SMMC-7721, and Hep G2. Products prepared through the Heck reaction and **3a,b** show potential antiproliferative activity against the Hep G2 cell.

Keywords: antiproliferative activity; Heck reaction; isoalantolactone; Michael reaction; structural modification.

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Introduction

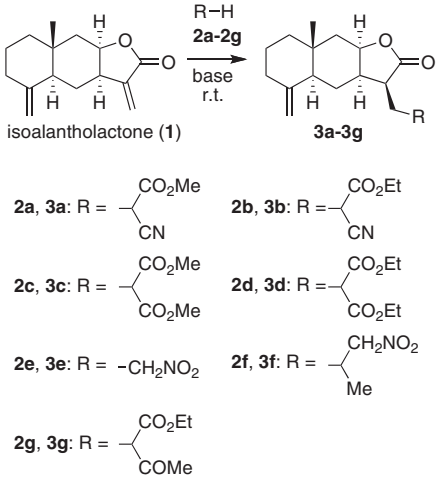
As part of a search for anticancer agents from medicinal herbs, we focused our attention on one such species, *Inula helenium*, a traditional Chinese medicinal herb [1]. It grows wild in Europe, North America, and the eastern part of Asia. In addition, it is distributed in northeast, northwest, and central regions of China [2]. Sesquiterpene lactones are components of *I. helenium* [3]. Among them, isoalantolactone (**1**) is the major and important bioactive constituent. It possesses toxicity for leukocytes *in vitro* cultures, significant anti-inflammatory and hepatoprotective activity similar to that of silymarin, anticancer, and antifungal activities [4, 5]. Spiridonov et al. [6] found that **1** possesses marked cytotoxicity, suppressing the growth of cultured human lymphoblastoid Raji cells. The sesquiterpene lactone **1** exhibits significant antiproliferative activity to human tumor cells cultivated *in vitro*, the U251SP, HLE, and MM1-CB cells [7, 8].

In this paper, we describe a modification of **1** by the Heck reaction and the Michael reaction. Active methylene compounds react well with **1**, adding to the α-methylene lactone subunit in a conjugative manner. This reaction therefore presents a potentially efficient method for the preparation of a range of isoalantolactone derivatives for screening. The goal of such modifications is to enhance the biological activity of the natural compound or to impart it to new types of activity. This article also discusses the relationship between the structure and activity of these derivatives. The results provide some useful information for further research on isoalantolactone **1**.

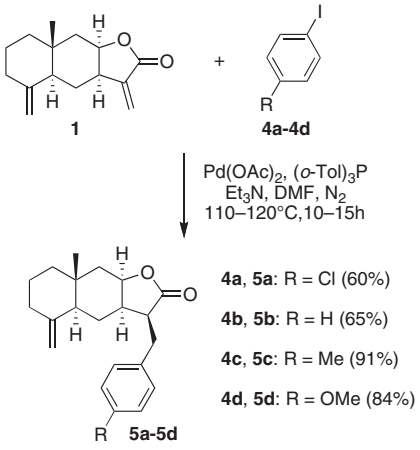
Results and discussion

It is widely believed that α,β-unsaturated carbonyl compounds, and particularly α-methylene lactones, exert their biological effects by acting as alkylating agents. The lactones can form covalent adducts *in vivo* with proteins and other nucleophilic biomolecules, via a Michael-type addition of a free sulfhydryl or amino group. The retention of activity upon addition of the amine can be explained by the reversible nature of the Michael-type reaction.

Michael addition reactions using the active methylene compounds and proper base were performed on isoalantolactone **1** (Scheme 1). Compound **1** was allowed to react with active methylene compounds **2a–g** under mild conditions, that is, stirring the reagents in absolute alcohol at room temperature for 0.5–3 h. Some products, **3a** and **3b**, crystallized directly from the reaction mixture. The reaction rates and product yields depend on the nature of the starting compounds. The methylene compounds **2a** and **2b** are the most reactive. In the presence of potassium hydroxide, additions of **1** to both **2a** and **2b** produced



Scheme 1 Synthesis of compounds **3a–g** through the Michael reaction.



Scheme 2 Synthesis of compounds **5a–d** through the Heck reaction.

the desired products **3a** and **3b** in yields of 95% and 93%, respectively. However, the yields of the diethyl malonate adduct **3d** and ethyl acetoacetate adduct **3g** were low. The explanation could be that reaction yields are reduced with the increase of steric hindrance. Besides, as electron-withdrawing ability of compounds becomes strong, the reactivity becomes higher.

Reaction of isoalantolactone **1** with aryl iodides **4a–d** is catalyzed by the system $Pd(OAc)_2$-$(o\text{-}Tol)_3P$ in DMF in the presence of triethylamine (Scheme 2). The main reaction products were isolated in 60–91% yield by column chromatography on silica gel. In agreement with the general rule of metal-catalyzed coupling reactions, the coupling of the aromatic iodide with an electron-donating substituent was efficient.

The synthesized compounds were screened *in vitro* for their anticancer potential against three different hepatoma cell lines Bel-7402, SMMC-7721, and Hep G2, with three anticancer agents, cisplatin, jifitinib, and taxol, used as controls. The concentration of these compounds was 100 mM. The results, shown in Figure 1, indicate that none of these compounds exhibits any significant or selective hepatoma cell growth inhibitory properties. However, it was encouraging to see that the compounds are able to inhibit Hep G2 cell growth in the submicromolar concentration range. Most derivatives show more potent inhibitory activity than cisplatin to the Hep G2 cell but weaker than taxol. The compounds obtained by Heck reaction inhibit hepatoma cell growth, especially the growth of Hep G2. In addition, the Michael reaction products **3a** and **3b** can significantly inhibit the proliferation of the Hep G2 cell.

Conclusion

In total, 14 isoalantolactone derivatives were synthesized by using the Michael reaction or the Heck reaction, of which seven are new compounds. The reactions proceed in high yields. All products were tested *in vitro* for anticancer

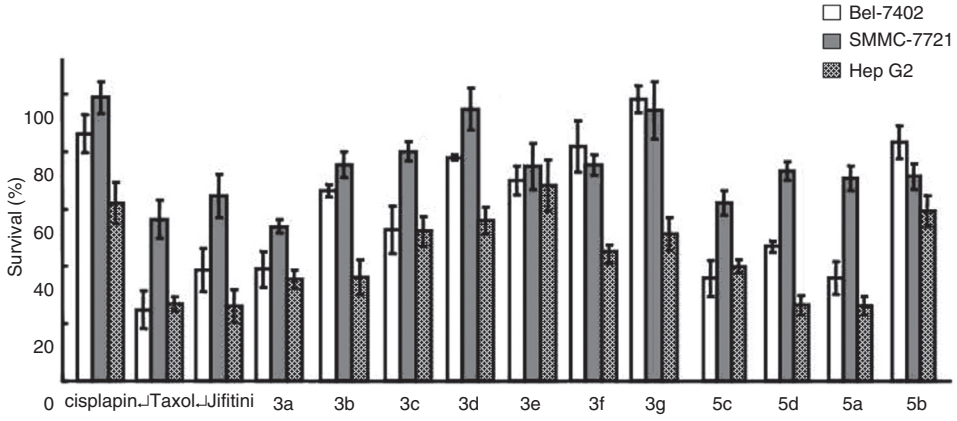


Figure 1 Cell growth inhibition properties of isoalantolactone derivatives against hepatoma cells.

activity on three different hepatoma cell lines: Bel-7402, SMMC-7721, and Hep G2. Several compounds show potential antiproliferative activity against the Hep G2 cell.

Experimental

General

Melting points were obtained on a Laboratory Devices SGW X-4B melting apparatus and are uncorrected. NMR spectra were recorded on a Bruker AV-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$. HR mass spectra (MS) were acquired with an Agilent Accurate-Mass-Q-TOF MS 6520 system equipped with an electrospray ionization (ESI) source. All MS experiments were conducted in the positive ionization mode. Isoalantolactone (**1**) was isolated from *I. helenium* by our laboratory and its purity was determined by analytical HPLC (purity > 95%).

Extraction and isolation of isoalantolactone (1)

Dried crushed roots of *I. helenium* (3 kg) were extracted three times with 99% ethanol at refluxing temperature for 2 h. The residue was scattered in saturated brine (1 g/mL) and then extracted with petroleum ether, dichloromethane, and ethyl acetate. The dichloromethane extract (58 g) was subjected to purification on a silica gel column, eluting with petroleum ether and ethyl acetate (9:1–1:1) gradient. Compound **1** was visualized using HPLC detection. Further separation of the mixture was achieved by column chromatography using an SiO_2-$AgNO_3$ (90:10) adsorbent and a benzene-petroleum ether-ethyl acetate (3:15:3) as an eluent. White needles of isoalantolactone (**1**) were obtained. EI-MS (acetone): m/z 232 $[M]^+$; ^{1}H NMR: δ 6.13 (1H, br, s, H-13a), 5.59 (1H, br, s, H-13b), 4.77 (1H, br, s, H-15a), 4.45 (1H, br, s, H-15b), 4.51 (1H, br, s, H-8), 2.99 (1H, m, H-7), 2.34 (1H, d, *J* = 12.0 Hz, H-3b), 2.21 (1H, d, *J* = 16.0 Hz, H-9a), 2.01 (1H, m, H-3a), 1.85 (1H, *J* = 12.5 Hz, H-5), 1.75 (1H, m, H-6a), 1.71 (1H, m, H-9b), 1.60 (2H, m, H-2), 1.54 (1H, m, H-1a), 1.40 (1H, m, H-6b), 1.25 (1H, m, H-1b), 0.83 (3H, s, 14-CH_3); ^{13}C NMR: δ 41.3 (C-1), 22.7 (C-2), 36.8 (C-3), 148.9 (C-4), 46.2 (C-5), 27. 5 (C-6), 40.5 (C-7), 76.8 (C-8), 42.2 (C-9), 34.3 (C-10), 142.2 (C-11), 170.7 (C-12), 120.2 (C-13), 17.7 (C-14), 106.6 (C-15). The data above are virtually identical to literature data [9].

General procedure for the synthesis of 3a,b

Methyl cyanoacetate or ethyl cyanoacetate (0.2 mmol) and potassium hydroxide (11 mg, 0.2 mmol) were added to a solution of **1** (46.4 mg, 0.200 mmol) in 5 mL of 100% anhydrous EtOH. The mixture was stirred at room temperature for 0.5 h and the resulting white precipitate was crystallized from acetone.

Methyl (3a*R*,4a*S*,8a*R*,9a*R*)-2-cyano-3-[(8a-methyl-5-methylene-2-oxododecahydronaphtho[2,3-*b*](3-furyl)]propanoate (3a) White solid; yield 94%; mp 194–195°C; ^{1}H NMR: δ 0.79 (3H, s, H-14), 1.23 (1H, m, H-1b), 1.34 (2H, m, H-13), 1.47 (1H, m, H-6b), 1.54–1.59 (4H, m, H-1a, 2, 2, 9b), 1.77 (1H, d, *J* = 12.0 Hz, H-6a), 1.85 (1H, m, H-5), 1.99 (1H, m, H-3b), 2.18 (1H, dd, *J* = 15.5, 4 Hz, H-9a), 2.32 (1H, m, H-3a), 2.65 (1H, m, H-11), 2.75 (1H, m, H-7), 2.95 (1H, br, t, H-16), 4.29 (3H, m, H-19), 4.47–4.51 (2H, m, H-8, 15b), 4.80 (1H, d, *J* = 8.5 Hz, H-15a); ^{13}C NMR: δ 17.7 (C-14), 21.6 (C-13), 22.6 (C-2), 28.6 (C-6), 32.1 (C-16), 34.8 (C-10), 36.7 (C-3), 39.4 (C-7), 41.4 (C-1), 42.1 (C-9), 46.5 (C-5), 48.6 (C-11), 63.7 (C-19), 78.2 (C-8), 106.7 (C-15), 118.2 (C-17), 148.8 (C-4), 168.0 (C-18), 176.5 (C-12); HR-ESI-MS. Calcd for $C_{19}H_{25}NNaO_4$ $([M+Na]^+)$: m/z 354.1675, found: m/z 354.1676.

Ethyl (3a*R*,4a*S*,8a*R*,9a*R*)-2-cyano-3-[(8a-methyl-5-methylene-2-oxododecahydronaphtho[2,3-*b*](3-furyl)]propanoate (3b) White solid; yield 93%; mp 193–194°C; ^{1}H NMR: δ 0.79 (3H, s, H-14), 1.09 (3H, m, H-20), 1.23 (1H, m, H-1b), 1.34 (2H, m, H-13), 1.48 (1H, m, H-6b), 1.53–1.62 (4H, m, H-1a, 2, 2, 9b), 1.77 (1H, m, H-6a), 1.85 (1H, d, *J* = 12.0 Hz, H-5), 1.99 (1H, m, H-3b), 2.15 (1H, m, H-9a), 2.31 (1H, m, H-3a), 2.66 (1H, m, H-11), 2.75 (1H, m, H-7), 2.95 (1H, br, t, H-16), 4.28 (2H, m, H-19), 4.47–4.51 (2H, m, H-8, 15b), 4.80 (1H, d, *J* = 2.0 Hz, H-15a); ^{13}C NMR: δ 13.89 (C-20), 17.73 (C-14), 21.63 (C-13), 22.63 (C-2), 28.56 (C-6), 32.04 (C-16), 34.76 (C-10), 36.67 (C-3), 39.44 (C-7), 41.35 (C-1), 42.12 (C-9), 46.45 (C-5), 48.58 (C-11), 63.68 (C-19), 78.17 (C-8), 106.70 (C-15), 118.19 (C-17), 148.84 (C-4), 167.94 (C-18), 176.54 (C-12); HR-ESI-MS. Calcd for $C_{20}H_{27}NNaO_4$ $([M+Na]^+)$: m/z 368.1832, found: m/z 368.1834.

General procedure for the synthesis of 3c–g

Active methylene compound **2c–g** (0.24 mmol) and triethylamine (0.24 mmol) were added to a solution of **1** (46.4 mg, 0.002 mmol) in 5 mL of 100% anhydrous EtOH. The mixture was stirred at room temperature for 2 h and then quenched with deionized H_2O. After extraction with ethyl acetate (3 × 15 mL), the combined organic phases were washed successively with 0.1% hydrochloric acid (10 mL), a saturated NaCl solution (2 × 20 mL), and dried over Na_2SO_4. After concentration, the residue was purified by silica gel column chromatography eluting with petroleum ether-ethyl acetate.

Dimethyl (3a*R*,4a*S*,8a*R*,9a*R*)-2-[(8a-methyl-5-methylene-2-oxododecahydronaphtho[2,3-*b*](3-furylmethyl)]malonate (3c) White needles; yield 83%; mp 105–107°C; ^{1}H NMR: δ 0.80 (3H, s, H-14), 1.23 (1H, m, H-1b), 1.45 (1H, dd, *J* = 15.5, 4.5 Hz, H-6b), 1.52–1.61 (4H, H-1a, 2, 2, 9b), 1.79 (1H, d, *J* = 15.5 Hz, H-6a), 2.00 (1H, td, *J* = 12.5, 6.5 Hz, H-3b), 2.11–2.17 (3H, m, H-3a, 5, 9a), 2.34 (2H, m, H-13), 2.45 (1H, m, H-11), 2.74 (1H, m, H-7), 3.76 (6H, s, H-18, 18′), 3.86 (1H, dd, *J* = 8.5, 6.5 Hz, H-16), 4.45 (1H, td, *J* = 4.0, 2.0 Hz, H-8), 4.48 (1H, d, *J* = 1.0 Hz, H-15b), 4.78 (1H, d, *J* = 1.0 Hz, H-15a); ^{13}C NMR: δ 17.8 (C-14), 21.1 (C-13), 22.7 (C-2), 24.2 (C-6), 34.8 (C-10), 36.7 (C-3), 39.7 (C-7), 41.5 (C-1), 42.2 (C-9), 44.5 (C-11), 46.5 (C-5), 49.3 (C-16), 52.7 (C-18), 52.7 (C-18′), 77.9 (C-8), 106.6 (C-15), 149.1 (C-4), 169.5 (C-17), 169.4 (C-17′), 177.7 (C-12); HR-ESI-MS. Calcd for $C_{20}H_{29}O_6$ $([M+H]^+)$: m/z 365.1959, found: m/z 365.1954.

Diethyl (3a*R*,4a*S*,8a*R*,9a*R*)-2-[(8a-methyl-5-methylene-2-oxododecahydronaphtho[2,3-*b*](3-furylmethyl)]malonate (3d) Colorless liquid; yield 76%; ^{1}H NMR: δ 0.80 (3H, s, H-14), 1.19 (1H, m, H-1b), 1.27 (6H, td, *J* = 7.5, 3.5 Hz, H-19, 19′), 1.45 (1H, dd, *J* = 15.5 Hz, 4.0 Hz, H-6b), 1.52–1.63 (4H, m, H-1a, 2, 2, 9b), 1.78 (1H, d, *J* = 12 Hz, H-6a), 1.98 (1H, m, H-3b), 2.10–2.17 (3H, m, H-3a, 5, 9a),

2.32 (2H, m, H-13), 2.45 (1H, m, H-11), 2.75 (1H, m, H-7), 3.76 (1H, m, H-16), 4.22 (4H, m, H-18, 18′), 4.45 (1H, m, H-8), 4.48 (1H, d, *J* = 1.0 Hz, H-15b), 4.78 (1H, d, *J* = 1.0 Hz, H-15a); ^{13}C NMR: δ 14.0 (C-19), 14.1 (C-19′), 17.7 (C-14), 21.1 (C-13), 22.6 (C-2), 24.0 (C-6), 34.8 (C-10), 36.7 (C-3), 39.6 (C-7), 41.5 (C-1), 42.2 (C-9), 44.5 (C-11), 46.5 (C-5), 49.6 (C-16), 61.6 (C-18), 61.6 (C-18′), 77.9 (C-8), 106.5 (C-15), 149.1 (C-4), 168.9 (C-17), 169.1 (C-17′), 177.7 (C-12); HR-ESI-MS. Calcd for $C_{22}H_{32}NaO_6$ ([M+Na]$^+$): m/z 415.2091, found: m/z 415.2094.

(3a*R*,4a*S*,8a*R*,9a*R*)-8a-Methyl-5-methylene-3-(2-nitroethyl) decahydronaphtho[2,3-*b*]furan-2-one (3e) White solid; yield 80%; mp 113–114°C; ^{1}H NMR: δ 0.81 (3H, s, H-14), 1.21 (1H, m, H-1b), 1.48 (1H, dd, *J* = 15.5, 4 Hz, 6b), 1.54–1.61 (4H, m, H-1a, 2, 2, 9b), 1.74 (1H, m, H-6a), 1.81 (1H, m, H-5), 2.00 (1H, m, H-3b), 2.18 (1H, dd, *J* = 15.5, 2.0 Hz, H-9a), 2.27 (1H, m, H-11), 2.35 (1H, m, H-3a), 2.41 (1H, m, H-13), 2.49 (1H, m, H-13), 2.78 (1H, m, H-7), 4.46 (1H, d, *J* = 1.5 Hz, H-15b), 4.51 (1H, td, *J* = 4.5, 2.0 Hz, H-8), 4.69 (2H, m, H-16), 4.79 (1H, d, *J* = 1.5 Hz, H-15a); ^{13}C NMR: δ 17.8 (C-14), 21.3 (C-13), 22.6 (C-2), 23.1 (C-6), 34.8 (C-10), 36.7 (C-3), 39.6 (C-7), 41.4 (C-1), 42.2 (C-9), 44.0 (C-11), 46.4 (C-5), 73.3 (C-16), 78.1 (C-8), 106.6 (C-15), 149.0 (C-4), 177.3 (C-12); HR-ESI-MS. Calcd for $C_{16}H_{23}NNaO_4$ ([M+Na]$^+$): m/z 316.1519, found: m/z 316.1520.

(3a*R*,4a*S*,8a*R*,9a*R*)-8a-Methyl-5-methylene-3-(2-nitropropyl)-decahydronaphtho[2,3-*b*]furan-2-one (3f) White needles; yield 80%; mp 134–136°C; ^{1}H NMR: δ 0.80 (3H, s, H-14), 1.25 (1H, m, H-1b), 1.47 (1H, m, H-6b), 1.53–1.59 (4H, m, H-1a, 2, 2, 9b), 1.62 (3H, dd, *J* = 6.5, 1.5 Hz, H-17), 1.81 (1H, m, H-5), 1.97 (1H, m, H-3b), 2.06–2.27 (2H, m, H-6a, 9a), 2.34 (1H, m, H-3a), 2.68 (1H, m, H-7), 2.53 (2H, m, H-13), 2.68 (1H, m, H-11), 4.44 (1H, d, *J* = 1.0 Hz, H-15b), 4.47 (1H, m, H-8), 4.77 (1H, s, H-15a), 5.05 (1H, m, H-16); ^{13}C NMR: δ 17.7 (C-14), 19.2 (C-17), 22.6 (C-2), 23.0 (C-6), 30.6 (C-13), 34.7 (C-10), 36.7 (C-3), 39.8 (C-7), 41.4 (C-1), 42.1 (C-9), 44.2 (C-11), 46.4 (C-5), 78.1 (C-8), 82.1 (C-16), 106.5 (C-15), 149.0 (C-4), 177.6 (C-12); HR-ESI-MS. Calcd for $C_{17}H_{25}NNaO_4$ ([M+Na]$^+$): m/z 330.1676, found: m/z 330.1677.

Ethyl(3a*R*,4a*S*,8a*R*,9a*R*)-2-[(8a-methyl-5-methylene-2-oxododecahydronaphtho[2,3-*b*](3-furylmethyl)]-3-oxobutanoate (3g) Colorless liquid; yield 71%. ^{1}H NMR: δ 0.79 (3H, s, H-14), 1.19 (1H, m, H-1b), 1.29 (3H, m, H-19), 1.45 (1H, dd, *J* = 15.5, 4.0 Hz, H-6b), 1.52–1.61 (4H, m, H-1a, 2, 2, 9b), 1.78 (1H, d, *J* = 12.5 Hz, H-6a), 1.98 (1H, m, H-3b), 2.11–2.19 (4H, m, H-5, 9a, 13), 2.31 (4H, m, H-21, 3a), 2.44 (1H, m, H-11), 2.67 (1H, m, H-7), 4.05 (1H, m, H-16), 4.22 (2H, m, H-18), 4.44 (1H, m, H-15b), 4.48 (1H, dd, *J* = 4.0, 1.5 Hz, H-8), 4.78 (1H, s, H-15a); ^{13}C NMR: δ 14.0 (C-21), 16.8 (C-18), 17.8 (C-14), 21.2 (C-13), 22.6 (C-2), 29.2 (C-6), 34.8 (C-10), 36.7 (C-3), 39.8 (C-7), 41.5 (C-1), 42.2 (C-9), 44.5 (C-11), 46.5 (C-5), 56.5 (C-16), 61.6 (C-20), 78.0 (C-8), 106.6 (C-15), 149.1 (C-4), 169.1 (C-19), 178.2 (C-12), 202.7 (C-17); HR-ESI-MS. Calcd for $C_{21}H_{31}O_5$ ([M+H]$^+$): m/z 363.2166, found: m/z 363.2163.

General procedure for synthesis of 5a–d by the Heck reaction

Compounds **5a**–**d** were synthesized by modification of the literature methods [10, 11]. Briefly, a three-necked glass ampule, filled with isoalantolactone (**1**, 70 mg, 0.3 mmol), aromatic iodide (0.33 mmol), $Pd(OAc)_2$ (0.015 mmol, 5 mol%), tris(*o*-tolyl)phosphine (0.051 mmol, 17 mol%), DMF (10 mL) and Et_3N (61 mg, 0.6 mmol), and 3A molecular sieves was sealed under argon. The ampule was heated for 10–15 h at 120°C, then cooled and opened. The mixture was poured into water and extracted with ethyl acetate. After concentration, the residue was purified by silica gel column chromatography eluting with petroleum ether-ethyl acetate.

(3a*R*,4a*S*,8a*R*,9a*R*,*E*)-3-(4-Chlorobenzyl)-8a-methyl-5-methylenedecahydronaphtho[2,3-*b*]furan-2-one (5a) White needles; yield 60%; mp 219–220°C, lit. [10] mp 207–209°C; ^{1}H NMR: δ 0.88 (3H, s, H-14), 1.27 (1H, m, H-1b), 1.43 (1H, m, H-6b), 1.54–1.63 (4H, m, H-1, 2, 2, 9), 1.93–2.04 (3H, m, H-3b, 5, 6a), 2.27 (1H, dd, *J* = 15.5, 1.5 Hz, H-9a), 2.36 (1H, br, d, *J* = 13.5 Hz, H-3a), 3.40 (1H, m, H-7), 4.42 (1H, d, *J* = 1.0 Hz, H-15b), 4.51 (1H, td, *J* = 4.5, 1.5 Hz, H-8), 4.78 (1H, d, *J* = 1.5 Hz, H-15a), 7.39 (3H, m, H-3′, 5′, 13), 7.46 (2H, d, *J* = 8.5, H-2′, 6′).

(3a*R*,4a*S*,8a*R*,9a*R*,*E*)-3-Benzyl-8a-methyl-5-methylenedecahydronaphtho[2,3-*b*]furan-2-one (5b) White needles; yield 65%; mp 220–222°C, lit. [10] mp 202–204°C; ^{1}H NMR: δ 0.89 (3H, s, H-14), 1.28 (1H, m, H-1b), 1.44 (1H, m, H-6b), 1.52–1.63 (4H, m, H-1, 2, 2, 9), 1.95 (1H, d, *J* = 12.5 Hz, H-6a), 2.03 (2H, m, H-3b, 5), 2.27 (1H, dd, *J* = 15.5, 1.5 Hz, H-9a), 2.36 (1H, m, H-3a), 3.44 (1H, m, H-7), 4.43 (1H, d, *J* = 1.0 Hz, H-15b), 4.50 (1H, td, *J* = 5.0, 1.5 Hz, H-8), 4.78 (1H, d, *J* = 1.0 Hz, H-15a), 7.40–7.44 (4H, m, H-3′, 4′, 5′, 13), 7.53 (2H, d, *J* = 8.5, H-2′, 6′).

(3a*R*,4a*S*,8a*R*,9a*R*,*E*)-8a-Methyl-3-(4-methylbenzyl)-5-methylenedecahydronaphtho[2,3-*b*]furan-2-one (5c) White needles; yield 91%; mp 239–240°C, lit. [10] mp 220–222°C; ^{1}H NMR: δ 0.88 (3H, s, H-14), 1.28 (1H, m, H-1b), 1.43 (1H, m, H-6b), 1.52–1.63 (4H, m, H-1, 2, 2, 9), 1.95 (1H, d, *J* = 12.5 Hz, H-6a), 2.03 (2H, m, H-3b, 5), 2.27 (1H, dd, *J* = 15.5, 1.5 Hz, H-9a), 2.35 (1H, m, H-3a), 2.38 (3H, s, H-CH_3), 3.43 (1H, m, H-7), 4.43 (1H, d, *J* = 1.5 Hz, H-15b), 4.49 (1H, td, *J* = 4.5, 1.5 Hz, H-8), 4.77 (1H, d, *J* = 1.5 Hz, H-15a), 7.22 (2H, d, *J* = 8.0 Hz, H-3′, 5′), 7.42 (3H, m, H-2′, 6′, 13).

(3a*R*,4a*S*,8a*R*,9a*R*,*E*)-3-(4-Methoxybenzyl)-8a-methyl-5-methylenedecahydronaphtho[2,3-*b*]furan-2-one (5d) White solid; yield 84%; mp 202–204°C, lit. [11] mp 199–201°C; ^{1}H NMR: δ 0.88 (3H, s, H-14), 1.27 (1H, m, H-1b), 1.41 (1H, m, H-6b), 1.53–1.63 (4H, m, H-1, 2, 2, 9), 1.94 (1H, d, *J* = 12.5 Hz, H-6a), 2.01–2.06 (2H, m, H-3b, 5), 2.26 (1H, dd, *J* = 15.5, 1.5 Hz, H-9a), 2.35 (1H, m, H-3a), 3.41 (1H, m, H-7), 3.85 (3H, s, OCH_3), 4.43 (1H, d, *J* = 1.5 Hz, H-15b), 4.49 (1H, m, H-8), 4.77 (1H, d, *J* = 1.0 Hz, H-15a), 6.94 (2H, d, *J* = 9.0 Hz, H-3′, 5′), 7.39 (1H, s, H-13), 7.49 (2H, d, *J* = 9.0 Hz, H-2′, 6′).

In vitro anticancer activity

For comparison of cell viability in human hepatoma carcinoma, cells were treated with compounds, cisplatin, taxol, and jifitinib (reference agents), for 2 days, followed by estimation of cell viability by the MTT assay, as described below. Data are presented as means ± SD of three independent experiments. The compounds were dissolved in DMSO (SERVA, Germany) and immediately after dissolving used for the test. Anticancer drugs, cisplatin, taxol, jifitinib (Wako Pure Chemicals Industries, Ltd.), and Methotrexate (F6627, Sigma, St. Louis, MO, USA), were also dissolved in DMSO. The following human hepatoma carcinoma cells were used: Bel-7402, SMMC-7721, and Hep G2. Cells were purchased from ShangHai MEIXUAN Biological Science and Technology Ltd. (China). Hep G2 cells was cultured in DMEM (GIBCO,

USA), containing 10% (v/v) calf serum (Biological Industries, BioInd) and antibiotics, 100 μg/mL of streptomycin (Nalgene, China) and 100 units/mL of penicillin G (Nalgene, China), at 37°C in a humidified atmosphere containing 5% CO_2. Instead, RPMI 1640 (GIBCO, USA) and fetal calf serum (Biological Industries, BioInd), respectively, were used for culture of Bel-7402, SMMC-7721 cells.Cell viability was estimated by the MTT assay as described elsewhere [12]. Briefly, logarithmically proliferating cells were plated onto 96-well plates (JETBIOFIL, China) (1×10^4 cells/well) with the medium containing test compounds at 100 μmol/L doses, followed by culture for 2 days. After the culture, the activity of mitochondrial succinic dehydrogenase was measured by further incubation of the cells with 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (TOKYO KASEI KOGYO, Japan) for 4 h, followed by measurement of absorbance at 570 nm with a reference wavelength at 655 nm. Cell survival was calculated from absorbance and presented as a percentage of the surviving cells.

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