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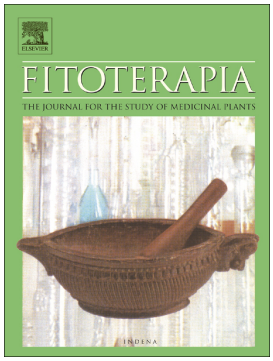

Novel hybrids of natural β-elemene bearing isopropanolamine moieties: Synthesis, enhanced anticancer profile, and improved aqueous solubility

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Novel hybrids of natural *β*-elemene bearing isopropanolamine moieties: Synthesis, enhanced anticancer profile, and improved aqueous solubility

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Absract: A series of novel *β*-elemene isopropanolamine derivatives were synthesized and evaluated for their antitumor activity. The results indicated that all of the compounds showed stronger antiproliferative activities than *β*-elemene as well as improved aqueous solubility. In particular dimer **6q** showed the strongest cytotoxicity against four tumor cell lines (SGC-7901, HeLa, U87 and A549) with IC_{50} values ranging from 4.37 to 10.20 μM. Moreover, combination of **6q** with cisplatin exhibited a synergistic effect on these cell lines with IC_{50} values ranging from 1.21 to 2.94 μM, and reversed the resistance of A549/DPP cells with an IC_{50} value of 2.52 μM. The mechanism study revealed that **6q** caused cell cycle arrest at the G2 phase and induced apoptosis of SGC-7901 cells through a mitochondrial-dependent apoptotic pathway. Further *in vivo* study in H22 liver cancer xenograft mouse model validated the antitumor activity of **6q** with a tumor inhibitory ratio (TIR) of 60.3%, which was higher than that of *β*-elemene (TIR, 49.1%) at a dose of 60 mg/kg. Altogether, the potent antitumor activity of **6q** *in vitro* and *in vivo* warranted further preclinical investigation for potential anticancer chemotherapy.

Key words: *β*-elemene; isopropanolamine; dimer; antitumor activity; aqueous solubility

1. Introduction

Over the past decade, there is a markedly increasing trend in the incidence and mortality of malignant tumors around the world [1] and cancer has become the first leading cause of death in China, beyond cerebrovascular and heart diseases [2]. The extensive prescription of synthetic antitumor drugs is being ruled out owing to their toxicity, resistance and unwanted side effects [3,4]. This led to the search for new antineoplastic agents, particularly those obtained from natural sources such as animals, plants, microbes and marine organisms [5,6]. In recent years, a large number of natural products, especially terpenes, have been discovered with marked anticancer activity *in vitro* and *in vivo*, some of which have been successfully developed for clinical use to treat human neoplastic diseases [7-9].

Curcuma wenyujin is a popular group of traditional Chinese medicine plants whose essential oils are widely used in cancer treatment in China [10]. *β*-elemene (**1**, Scheme 1), a sesquiterpene compound extracted from the essential oils of *Curcuma wenyujin*, accounts for 60-72% of elemene including *α*, *β*, *γ* and *δ* forms [10]. As the major active antitumor component in the elemene mixture, *β*-elemene has been isolated and approved by the Chinese Food and Drug Administration for the treatment of human cancers [11]. The major advantages of *β*-elemene as an anticancer drug are [12-14]: i) broad-spectrum antitumor effects in various types of cancers, including drug-resistant tumors; ii) not inducing any multidrug resistance and reversing the resistance of other antitumor drugs in tumor cells; and iii) low toxicity without bone marrow suppression. Despite these striking antitumor properties, the poor water solubility and moderate activity of *β*-elemene hampers its wide applications in clinic.

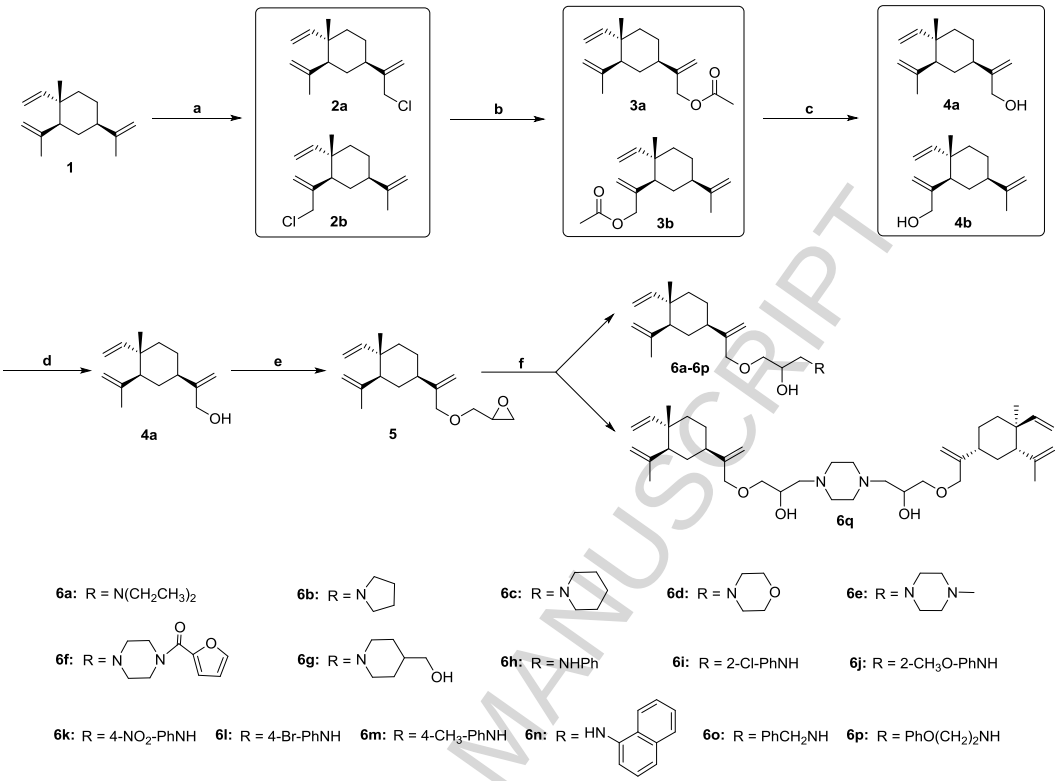
Structural modifications are an effective approach to improve the druggability of natural compounds [15]. And it has been reported that introduction of oxygen or nitrogen-containing polar group into the skeleton of *β*-elemene could favorably impact its water solubility and antitumor activity [16,17]. Enlightened by these findings, we designed a series of novel *β*-elemene isopropanolamine derivatives by introducing both amine and hydroxyl groups to increase the water solubility and antitumor activity of natural *β*-elemene. Herein, we report synthesis, *in vitro* and *in vivo* antitumor activity, and anticancer mechanism for a new class of *β*-elemene isopropanolamine derivatives with improved aqueous solubility.

2. Results and discussions

2.1. Chemistry

The synthesis of *β*-elemene isopropanolamine derivatives **6a-6q** was shown in Scheme 1. Chlorination of *β*-elemene (**1**) with NaClO produced the chlorinated mixture of **2a** and **2b**, followed by treatment with AcONa to give the acylated compounds **3a** and **3b**. The resulting products were subjected to alkaline hydrolysis to produce a mixture of 13-*β*-elemol (**4a**) and 14-*β*-elemol (**4b**), which was separated by HPLC to provide the main component **4a** [18]. Subsequent alkylation of **4a** with

epibromohydrin in the presence of NaH gave the epoxide intermediate **5**, which was further reacted with different amines in the presence of a catalytic amount of $Zn(ClO_4)_2 \cdot 6H_2O$ to obtain the target compounds **6a-6q** [19].



Scheme 1. Synthetic routes of the title compounds **6a**-**6q**. Reagents and conditions: (a) NaClO, $HOAc/CH_2Cl_2$, 0~5 ^oC, 6 h, 55%; (b) DMF, NaOAc, 120 ^oC, 8 h, 75%; (c) $MeOH/CHCl_3$, KOH, reflux, 2 h, 85%; (d) Separated by HPLC, Hexane/EtOH = 98/2 (V/V), UV = 214 nm; (e) epibromohydrin, NaH, anhydrous DMF, rt, 3 h, 72%; (f) amines, *Cat.* $Zn(ClO_4)_2 \cdot 6H_2O$, 80 ^oC, 1 h, 58-84%.

2.2. Pharmacology

2.2.1. *In vitro* antiproliferative activity

Initially, *β*-elemene isopropanolamine derivatives **6a-6q** were examined for their antiproliferative activities against three cancer cell lines (SGC-7901: human gastric carcinoma; HeLa: human cervical adenocarcinoma; U87: human glioblastoma). As shown in Table 1, all of the derivatives exhibited stronger activities than parent compound *β*-elemene, and some of them even showed preferable activities than positive control cisplatin, suggesting that introduction of an isopropanolamine moiety was beneficial for the antitumor activity of *β*-elemene. Aliphatic (**6a**), naphthenic (**6b-6g**) and aryl (**6h-6n**) amines showed nearly the same activity, approximately 3- to -10 fold more potent than *β*-elemene. When R was benzylamine (**6o**) or phenoxyethylamine (**6p**), the activity was markedly improved with IC_{50} values of 10~20 μM on all tested cell lines. It is surprising that among these compounds, the dimer derivative **6q** exhibited the strongest activity with IC_{50} values of 4.37, 7.56 and

10.20 μM against SGC-7901, HeLa and U87 cell lines, respectively, which was superior to cisplatin with IC_{50} values of 9.09, 16.28 and 21.39 μM, respectively.

Table 1. Antiproliferative activities of *β*-elemene isopropanolamine derivatives against three cancer cell lines

Compd	Cell lines (IC_{50}[a], μM)		
	SGC-7901	HeLa	U87
β-Elemene	236.27 ± 18.41	213.51 ± 15.23	179.72 ± 15.37
6a	43.32 ± 3.78	36.26 ± 3.86	49.00 ± 5.44
6b	42.86 ± 4.43	29.96 ± 1.89	35.02 ± 3.92
6c	24.33 ± 2.67	29.45 ± 3.95	37.93 ± 3.08
6d	44.87 ± 5.54	52.29 ± 4.72	39.07 ± 2.58
6e	31.25 ± 2.47	26.42 ± 0.87	23.99 ± 1.69
6f	37.75 ± 1.55	31.81 ± 2.98	38.20 ± 5.14
6g	49.23 ± 1.82	20.79 ± 3.43	22.12 ± 0.96
6h	56.76 ± 4.29	55.77 ± 6.17	56.36 ± 3.63
6i	34.42 ± 2.86	41.19 ± 3.74	38.92 ± 4.51
6j	51.89 ± 5.89	37.95 ± 3.28	66.05 ± 5.43
6k	26.41 ± 1.09	22.67 ± 1.65	28.50 ± 2.83
6l	65.16 ± 5.13	39.88 ± 4.78	54.86 ± 3.62
6m	50.27 ± 4.42	66.53 ± 3.21	61.47 ± 6.46
6n	36.49 ± 3.63	35.49 ± 1.03	57.41 ± 5.86
6o	21.40 ± 1.22	10.04 ± 0.52	12.63 ± 1.04
6p	15.40 ± 1.67	9.42 ± 0.73	10.50 ± 0.91
6q	4.37 ± 0.51	7.56 ± 0.65	10.20 ± 0.76
Cisplatin	9.09 ± 0.83	16.28 ± 1.06	21.39 ± 1.91

[a] IC_{50}: concentration of the test compound that inhibits 50% of cell growth. Results are expressed as the mean ± SD (n = 3).

2.2.2. *In vitro* antiproliferative activity of 6q in combination with cisplatin

It was reported that *β*-elemene increased the sensitivity of several cancer cells and even reversed the resistance to cisplatin [20-24]. In order to investigate whether **6q** could induce sensitization to cisplatin, an equimolar combination of **6q** with cisplatin was examined for the antiproliferative activity against SGC-7901, HeLa, U87, A549 (human lung adenocarcinoma) and A549/DDP (cisplatin-resistant human lung adenocarcinoma) cells. As presented in Table 2, combination of **6q** and cisplatin exhibited a synergistic cytotoxicity effect on SGC-7901, HeLa, U87 and A549 cells with IC_{50} values of 1.21, 2.94, 1.68 and 2.37 μM, respectively. Particularly on U87 cells, this combination was 6-fold or 13-fold more potent than **6q** or cisplatin, respectively. In addition, **6q** showed potent activity against A549/DDP cells with an IC_{50} value of 10.91 μM and the combination led to a reversal of resistance of A549/DPP cells with an IC_{50} value of 2.52 μM.

Table 2. Antiproliferative activity of **6q** and DDP alone or in combination against five cancer cell lines

Compd	Cell lines (IC_{50}[a], μM)				
	SGC-7901	HeLa	U87	A549	A549/DDP
6q	4.37 ± 0.51	7.56 ± 0.65	10.20 ± 0.76	9.28 ± 0.68	10.91 ± 0.84
DDP[b]	9.09 ± 0.83	16.28 ± 1.06	21.39 ± 1.91	9.54 ± 0.87	47.90 ± 5.63
6q + DDP	1.21 ± 0.13	2.94 ± 0.24	1.68 ± 0.09	2.37 ± 0.21	2.52 ± 0.18

[a] IC_{50}: concentration of the test compound that inhibits 50% of cell growth. Results are expressed as the mean ± SD (n =3).

[b] DDP: cisplatin.

2.2.3. Compound 6q induces cell cycle arrest

To determine whether the suppression of cell growth by **6q** is caused by a cell-cycle effect, the DNA content of cell nuclei was analyzed by flow cytometry (Figure 1). SGC-7901 cells were treated with **6q** at concentrations of 1.0, 2.0 and 4.0 μM, which resulted in accumulation of 33.12%, 44.04%, and 59.93% of cells at the G2 phase, respectively. Meanwhile, the percentage of cells at the G1 phase decreased to 38.77%, 32.09%, and 19.17%, respectively. These results indicated that **6q** caused G2-phase arrest of the cell cycle in inhibition of cell growth.

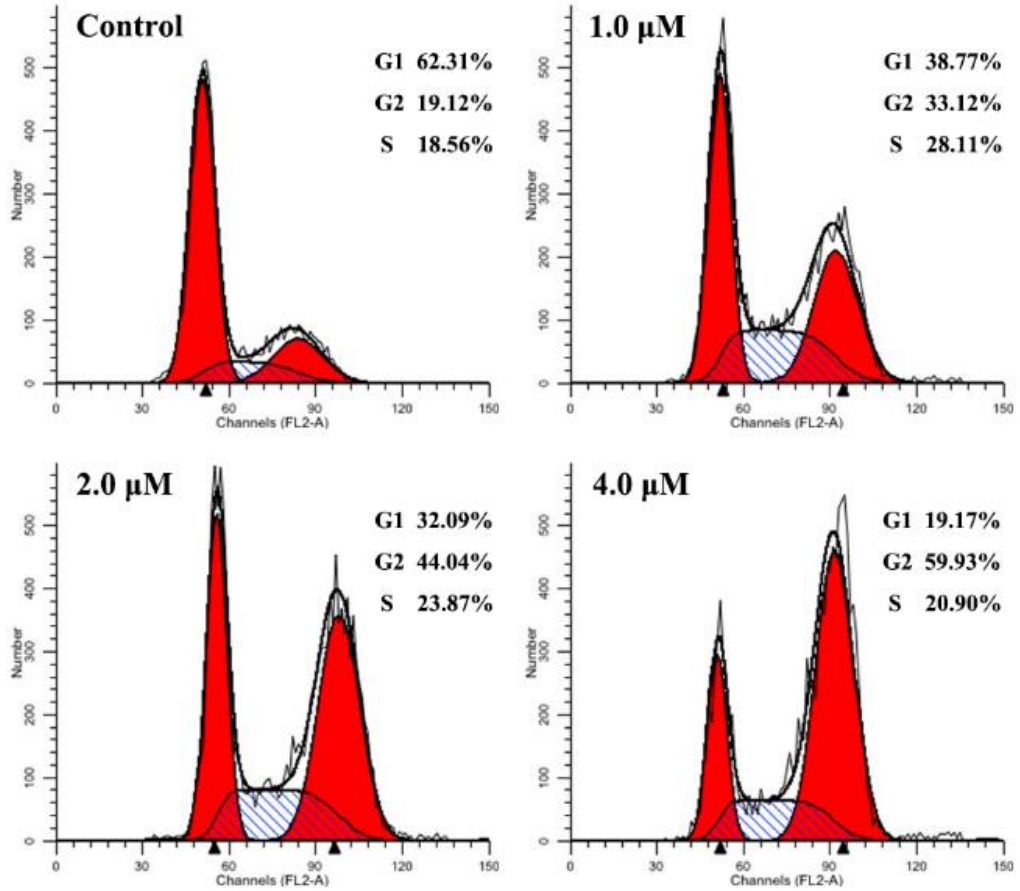


Figure 1. Compound **6q** induces G2-phase arrest in SGC-7901 cells. SGC-7901 cells were treated with varying concentrations of **6q** (0, 1.0, 2.0, 4.0 μM) for 72 h and stained with PI. The cell cycle

distributions were analyzed by flow cytometry.

2.2.4. Compound 6q induces apoptosis

To clarify whether the loss of cancer cell viability promoted by **6q** is associated with apoptosis, an Annexin V-APC/7-AAD binding assay was performed. As shown in Figure 2, **6q** significantly induced apoptosis in a dose-dependent manner. Treatment of SGC-7901 cells with **6q** at 1.0, 2.0 and 4.0 μM for 72 h resulted in 22.13%, 39.37% and 59.08% apoptotic cells (Q2 + Q4), as compared with 6.46% in an untreated vehicle control, suggesting that **6q** exerted its antitumor effect possibly by inducing apoptosis.

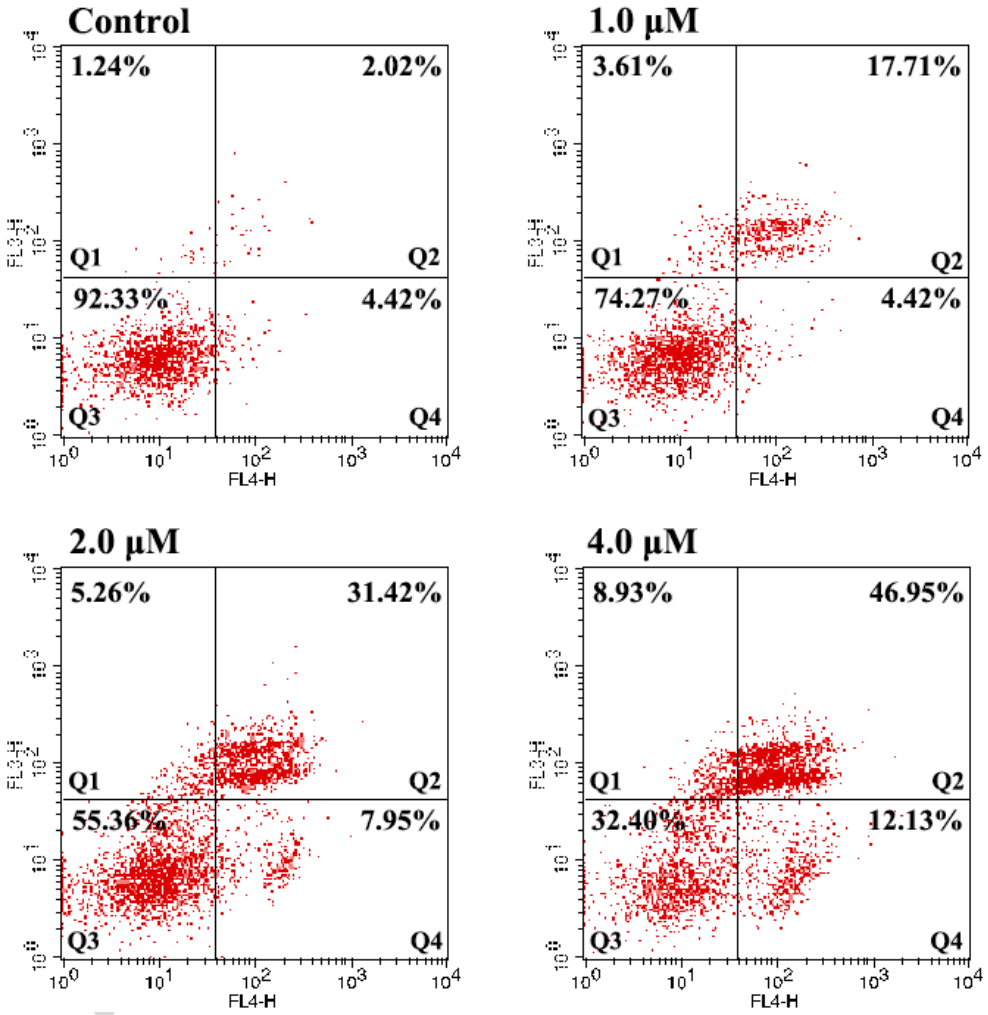


Figure 2. Compound **6q** induces apoptosis of SGC-7901 cells. Treatment with **6q** (0, 1.0, 2.0, 4.0 μM) for 72 h, SGC-7901 cells were collected and stained with Annexin V-APC/7-AAD, followed by flow cytometric analysis.

2.2.5. Compound 6q induces mitochondrial depolarization

To examine whether cell apoptosis induced by **6q** is related to the decline of mitochondrial membrane potential, the lipophilic mitochondrial probe JC-1 staining assay was carried out. When SGC-7901 cells were treated with **6q** at concentrations of 1.0, 2.0 and 4.0 μM, the number of cells with collapsed mitochondrial membrane potentials increased to 8.56%, 19.07%, and 33.94%, respectively (Figure 3). These

results demonstrated that **6q** caused mitochondrial depolarization of SGC-7901 cells in the process of apoptosis.

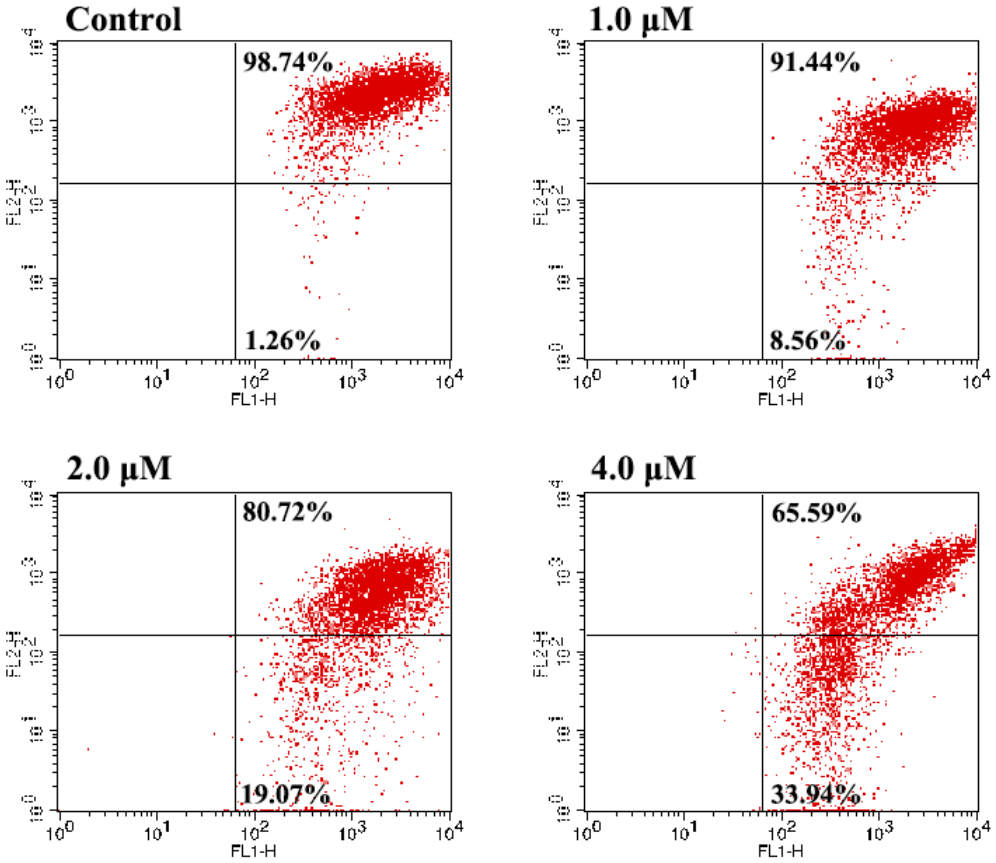


Figure 3. Compound **6q** induces mitochondrial depolarization in SGC-7901 cells. Incubation with different concentrations (0, 1.0, 2.0, 4.0 μM) of **6q** in SGC-7901 cells for 72h prior to staining with JC-1 dye, the number of cells with collapsed mitochondrial membrane potentials was determined by flow cytometry analysis.

2.2.6. Effect of 6q on apoptosis-related proteins

A decline in mitochondrial membrane potential is associated with cytochrome C. When the structure of the membrane is damaged, mitochondrial membrane permeability increases, cytochrome C releases into the cytoplasm across the membrane, which leads to mitochondrial depolarization [20]. Meanwhile, the release of cytochrome C promotes the formation of a caspase activating complex that contains cytochrome C, Apaf-1 and procaspase-9. This apoptosome complex triggers the activation of caspase-9, which leads to a proteolytic cascade that activates downstream effector caspases, including caspase-3, -6 and -7 [25]. These caspases are the final executors of the apoptotic process, and the apoptotic pathway is activated through the cleavage of PARP [26,27]. Bcl-2 plays a pivotal protective role by preserving mitochondrial structure and function. Bax, a dominant-negative inhibitor of Bcl-2, induces a mitochondrial permeability transition and promotes apoptosis [28]. As shown in Figure 4, treatment with **6q** in SGC-7901 cells led to enhancement of cytochrome C release, activation of caspase-3 and -9, cleavage of PARP, downregulation of Bcl-2 expression and upregulation of Bax expression in a concentration-dependent manner. The results indicate that the antitumor effect of **6q**

may be mediated via a mitochondrial-dependent apoptotic pathway.

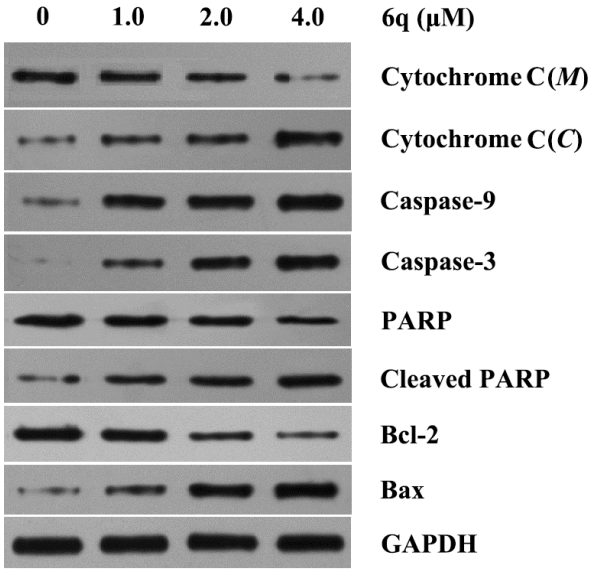


Figure 4. Effects of **6q** on apoptosis-related proteins in SGC-7901 cells. SGC-7901 cells were exposed to **6q** at 0, 1.0, 2.0 and 4.0 μM for 72 h. A total of 25 μg of cellular proteins extracted from **6q-**treated cells were separated by SDS-PAGE, transferred to a nitrocellulose membrane and immunoblotted with antibodies against Bcl-2, Bax, cytochrome C, caspase-9, caspase-3 and PARP. GAPDH antibody was used as a loading control. Cytochrome C (*M*): mitochondrial cytochrome C; cytochrome C (*C*): cytosolic cytochrome C.

2.2.7. Determination of water solubility

The poor aqueous solubility of *β*-elemene is one of the main factors that limit its clinical application. In order to verify whether the aqueous solubility of *β*-elemene was improved, representative compounds **6a** and **6q** were selected to determine their water solubility by the HPLC method [29]. As expected, incorporating an isopropanolamine moiety into *β*-elemene not only enhanced the antiproliferative activity but also significantly improved their aqueous solubility. The water solubility of **6a** and **6q** was 1.18 and 0.27 mg/mL, respectively, which was markedly higher than that of *β*-elemene (not detected).

2.2.8. *In vivo* antitumor activity of 6q

Based on the above results, we further tested the *in vivo* antitumor activity of **6q** in the mice bearing with H22 liver cancer. 32 mice were randomly assigned to four groups and intravenously administered with 60 mg/kg *β*-elemene, 30 or 60 mg/kg **6q** in a vehicle of 10% DMF/2% Tween 80/88% saline once a day, respectively. The mice were sacrificed after 21 days, and the tumors were excised and weighed. As illustrated in Table 3, **6q** showed potent antitumor activity *in vivo* in a dose-dependent manner. At a dose of 60 mg/kg, **6q** possessed a tumor inhibitory rate (TIR) of 60.3%, which was significantly superior to *β*-elemene with a TIR of 49.1%. Thus, **6q** is worthy of further investigation as a potential anticancer drug candidate.

Table 3. *In vivo* antitumor activity of **6q** against mice bearing H22 liver cancer

Drugs	Dose mg/kg	Number of mice		Weight of mice (g)		Weight of tumor X ± SD (g)	Ratio of inhibition (%)
		Start	End	Start	End		
Control[a]	-	8	8	18.2 ± 0.3	26.0 ± 0.6	4.38 ± 0.16	/
β-Elemene	60	8	8	18.4 ± 0.2	25.4 ± 0.5	2.23 ± 0.18**	49.1%
6q	30	8	8	18.2 ± 0.1	25.7 ± 0.5	2.34 ± 0.17**	46.6%
	60	8	8	18.3 ± 0.3	25.3 ± 0.4	1.74 ± 0.13**	60.3%

[a]vehicle of 10% DMF/2% Tween 80/88% saline. **$P < 0.01$ *vs.* control group.

3. Conclusion

In summary, a series of novel *β*-elemene derivatives hybridizing isopropanolamine moieties were synthesized with improved aqueous solubility. The bioassay results indicated that all of the derivatives exhibited more potent antiproliferative activities than parent compound *β*-elemene. Among them, the dimer compound **6q** showed the strongest activity against SGC-7901, HeLa, U87 and A549 cell lines, which were superior to that of positive control cisplatin. Interestingly, combination of **6q** with cisplatin exhibited a synergistic effect on these cell lines and reversed the resistance of A549/DPP cells. Further mechanism studies revealed that **6q** caused cell cycle arrest at the G2 phase and induced apoptosis of SGC-7901 cells by a mitochondrial-dependent apoptotic pathway. Moreover, the *in vivo* antitumor activity of **6q** was validated in H22 liver cancer xenograft mouse model. Collectively, the current study may provide a new insight for the design of natural product-like drug candidates to enhance the efficacy of chemotherapy.

4. Experimental

4.1. Chemistry

Most chemicals and solvents were purchased from commercial sources. Further purification and drying by standard methods were employed when necessary. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker-300 spectrometers in the indicated solvents (TMS as internal standard). Data are reported as follows: chemical shift in ppm (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet, m = multiplet), coupling constant (Hz), and integration. High Resolution Mass measurement was performed on Agilent QTOF 6520 mass spectrometer with electron spray ionization (ESI) as the ion source. Purity of all tested compounds was ≥ 95%, as estimated by HPLC analysis. Flash column chromatography was carried out using commercially available silica gel (200-300 mesh) under pressure.

4.1.1. Synthesis of intermediate 4a [18]

To a solution of *β*-elemene (4.08 g, 20.0 mmol) in glacial acetic acid, 1.60 N sodium hypochlorite (16.9 mL, 27.0 mmol) was added over 2 h period under stirring at 0~5 ^{o}C, then the mixture was kept stirring for another 4 h and extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated, and the residue was purified by flash column chromatography using petroleum ether as an eluent to give the mixture of **2a** and **2b** as yellowish liquid, yield 55%.

A mixture of **2a** and **2b** (2.38 g, 10.0 mmol), and anhydrous AcONa (2.46 g, 20 mmol) were added in anhydrous DMF, and stirred under 120 ^{o}C for 8 h. The solution was filtered and the filtrate was diluted with CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4, concentrated successively. The residue was purified by flash column chromatography using petroleum ether/ethyl acetate (30/1, V/V) as an eluent to provide the mixture of **3a** and **3b** as colorless liquid, yield 75%.

A mixture of **3a** and **3b** (1.83 g, 7.0 mmol), and KOH (785.4 mg, 14 mmol) were added in a mixture solution of methanol and chloroform (1:1, V/V), and stirred under reflux for 2 h. The solution was filtered and concentrated, and the residue was purified by flash column chromatography using petroleum ether/ethyl acetate (10/1, V/V) as an eluent to give the mixture of **4a** and **4b** (1.31 g, 85%), which was further separated by HPLC (CHIRALPAK AD-H, Hexane : EtOH = 98 : 2, 1.0 mL·min^{-1}, UV = 214 nm) to afford pure **4a** as colorless liquid, yield 66.4%.

13-*β*-elemol (**4a**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.81 (dd, J_1 = 17.8 Hz, J_2 = 10.5 Hz, 1H), 5.05 (d, J = 1.3 Hz, 1H), 4.91-4.94 (m, 2H), 4.88 (s, 1H), 4.82 (t, J = 1.7 Hz, 1H), 4.59 (s, 1H), 4.13 (s, 2H), 1.97-2.05 (m, 2H), 1.71 (s, 3H), 1.41-1.67 (m, 6H), 1.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7, 150.0, 147.4, 112.1, 109.9, 107.9, 65.1, 52.7, 41.4, 39.8, 39.7, 33.2, 27.2, 24.7, 16.5; HRMS (ESI) calcd for $C_{15}H_{25}O$ $[M+H]^+$: 221.1900, found 221.1901.

4.1.2. General procedure for synthesis of compounds 6a-6q

To a solution of **4a** (88 mg, 0.4 mmol) in anhydrous DMF (5 mL) was added NaH (60%, 0.6 mmol, 25 mg) at room temperature for 30 min, epibromohydrin (66 mg, 0.48 mmol) was then added and stirred for another 2.5 h. The reaction mixture was quenched with 5 mL water and extracted with ethyl acetate (10 mL × 3). The combined organic extracts were washed with water (5 mL × 2), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated and purified by flash column chromatography using petroleum ether/ethyl acetate (30/1, V/V) as an eluent to give intermediate **5** as colorless liquid, yield 72%.

2-(((2-((1R,3S,4S)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)methyl)oxirane (**5**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (dd, J = 17.8, 10.5 Hz, 1H), 5.05 (d, J = 1.0 Hz, 1H), 4.98 (s, 1H), 4.92 (dd, J = 5.4, 1.2 Hz, 1H), 4.88 (s, 1H), 4.82 (s, 1H), 4.59 (s, 1H), 4.11 – 3.96 (m, 2H), 3.71 (dd, J = 11.5, 3.0 Hz, 1H), 3.38 (dd, J = 11.5, 5.8 Hz, 1H), 3.21 – 3.11 (m, 1H), 2.85 – 2.78 (m, 1H), 2.62 (dd, J = 5.0, 2.7 Hz, 1H), 2.12 – 1.96 (m, 2H), 1.71 (s, 3H), 1.69 – 1.55 (m, 3H), 1.53 – 1.42 (m, 3H), 1.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.73, 149.67, 147.08, 111.65, 109.98, 109.43, 73.08, 70.09, 52.22, 50.35, 43.82, 40.80, 39.41, 39.31, 32.64, 26.57, 24.33, 16.08.

5 (55 mg, 0.2 mmol), corresponding amines (0.6 mmol for **6a-6p**, 0.1 mmol for **6q**) and $Zn(ClO_4)_2 \cdot 6H_2O$ (1 mg) were added in a 10 mL sealed tube and vigorously stirred at 80 °C for 1 h. Afterward it was cooled to ambient temperature, 5 mL water and 10 mL dichloromethane were added. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated successively. The obtained residue was purified with flash column chromatography using dichloromethane/methanol (100/1~40/1, V/V) as an eluent to give the title compounds as yellowish liquid, yield 58-84%.

1-(Diethylamino)-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol (**6a**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.94 (s, 1H), 4.91 (s, 1H), 4.85 (d, *J* = 3.8 Hz, 1H), 4.81 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 4.25 – 4.12 (m, 2H), 4.02 – 3.86 (m, 2H), 3.58 – 3.41 (m, 2H), 3.38 – 3.21 (m, 4H), 3.20 – 3.14 (m, 2H), 2.02 – 1.84 (m, 2H), 1.64 (s, 3H), 1.63 – 1.49 (m, 3H), 1.48 – 1.28 (m, 9H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.55, 149.30, 147.06, 111.67, 110.06, 109.52, 73.29, 70.93, 64.45, 55.21, 52.13, 48.32, 40.92, 39.34, 39.27, 32.68, 26.59, 24.35, 16.06, 8.31; HRMS (ESI) calculated for $C_{22}H_{40}NO_2$ $[M + H]^+$ 350.3054, found 350.3052.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-(pyrrolidin-1-yl)propan-2-ol (**6b**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (dd, *J* = 17.8, 10.5 Hz, 1H), 5.02 (s, 1H), 4.98 (s, 1H), 4.92 (d, *J* = 3.9 Hz, 1H), 4.88 (s, 1H), 4.82 (s, 1H), 4.58 (s, 1H), 4.38 (brs, 1H), 4.25 – 4.12 (m, 1H), 4.07 – 3.93 (m, 2H), 3.75 – 3.12 (m, 8H), 2.27 – 2.09 (m, 4H), 2.06 – 1.87 (m, 2H), 1.71 (s, 3H), 1.68 – 1.50 (m, 3H), 1.52 – 1.30 (m, 3H), 1.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.56, 149.34, 147.09, 111.67, 109.97, 109.53, 73.22, 70.94, 65.52, 58.18, 54.98, 52.11, 40.88, 39.34, 39.28, 32.66, 26.58, 24.36, 22.49, 16.06; HRMS (ESI) calculated for $C_{22}H_{38}NO_2$ $[M + H]^+$ 348.2897, found 348.2899.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-(piperidin-1-yl)propan-2-ol (**6c**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (dd, *J* = 17.8, 10.5 Hz, 1H), 5.02 (s, 1H), 4.98 (s, 1H), 4.92 (d, *J* = 4.0 Hz, 1H), 4.88 (s, 1H), 4.82 (s, 1H), 4.58 (s, 1H), 4.38 (s, 1H), 4.30 – 4.20 (m, 1H), 4.08 – 3.94 (m, 2H), 3.57 – 3.44 (m, 2H), 3.33 – 3.08 (m, 6H), 2.08 – 1.81 (m, 6H), 1.71 (s, 3H), 1.69 – 1.54 (m, 5H), 1.53 – 1.41 (m, 3H), 1.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.58, 149.41, 147.08, 111.66, 110.01, 109.50, 73.24, 71.25, 64.48, 60.21, 54.25, 52.14, 40.86, 39.35, 39.29, 32.65, 26.56, 24.35, 22.99, 21.60, 16.06; HRMS (ESI) calculated for $C_{23}H_{40}NO_2$ $[M + H]^+$ 362.3054, found 362.3057.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-morpholinopropan-2-ol (**6d**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.96 (s, 1H), 4.90 (s, 1H), 4.85 (d, *J* = 3.9 Hz, 1H), 4.81 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 4.04 – 3.89 (m, 2H), 3.88 – 3.80 (m, 1H), 3.73 – 3.60 (m, 4H), 3.42 – 3.31 (m, 2H), 2.63 – 2.49 (m, 2H), 2.47 – 2.31 (m, 4H), 2.05 – 1.88 (m, 2H), 1.64 (s, 3H), 1.62 – 1.47 (m, 3H), 1.46 – 1.34 (m, 3H), 0.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.79, 149.62, 147.03, 111.66, 109.89, 109.44, 73.30, 71.80, 66.41, 65.49, 60.88, 53.26, 52.28, 40.89, 39.41, 39.28, 32.66, 26.56, 24.30, 16.11; HRMS (ESI) calculated for $C_{22}H_{38}NO_3$ $[M + H]^+$ 364.2846, found 364.2843.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-(4-methylpiperazin-1-yl)propan-2-ol (**6e**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.74 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.95 (s, 1H), 4.90 (s, 1H), 4.85 (d, *J* = 4.0 Hz, 1H), 4.80 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 4.04 – 3.89 (m, 2H), 3.88 – 3.80 (m, 1H), 3.44 – 3.27 (m, 2H), 3.10 (s, 1H), 2.65 (s, 2H), 2.55 – 2.32 (m, 8H), 2.26 (s, 3H), 2.02 – 1.88 (m, 2H), 1.63 (s, 3H), 1.61 – 1.50 (m, 3H), 1.46 – 1.35 (m, 3H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.80, 149.63, 147.04, 111.65, 109.86, 109.42, 73.27, 71.86, 65.65, 60.16, 54.43, 52.26, 52.26, 45.26, 40.86, 39.41, 39.28, 32.65, 26.55, 24.30, 16.10; HRMS (ESI) calculated for $C_{23}H_{41}N_2O_2$ $[M + H]^+$ 377.3163, found 377.3167.

Furan-2-yl(4-(2-hydroxy-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propyl)piperazin-1-yl)methanone (**6f**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, 1H), 6.94 (d, *J* = 3.4 Hz, 1H), 6.42 (dd, *J* = 3.3, 1.7 Hz, 1H), 5.74 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.96 (s, 1H), 4.91 (s, 1H), 4.86 (d, *J* = 3.5 Hz, 1H), 4.81 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 4.02 – 3.85 (m, 3H), 3.80 (s, 4H), 3.44 – 3.32 (m, 2H), 2.74 – 2.63 (m, 2H), 2.59 – 2.39 (m, 4H), 2.02 – 1.86 (m, 2H), 1.64 (s, 3H), 1.62 – 1.46 (m, 3H), 1.45 – 1.33 (m, 3H), 0.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.51, 149.71, 149.61, 147.06, 143.26, 116.15, 111.68, 110.85, 109.97, 109.49, 73.32, 71.64, 65.68, 60.45, 52.95, 52.26, 40.88, 39.41, 39.30, 32.66, 26.56, 24.33, 16.10; HRMS (ESI) calculated for $C_{27}H_{41}N_2O_4$ $[M + H]^+$ 457.3061, found 457.3060.

1-(4-(Hydroxymethyl)piperidin-1-yl)-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol (**6g**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (dd, *J* = 17.7, 10.6 Hz, 1H), 5.02 (s, 1H), 4.97 (s, 1H), 4.92 (d, *J* = 3.9 Hz, 1H), 4.88 (s, 1H), 4.82 (s, 1H), 4.58 (s, 1H), 4.15 – 3.93 (m, 3H), 3.63 (brs, 2H), 3.55 – 3.32 (m, 4H), 3.23 (d, *J* = 11.1 Hz, 1H), 3.12 (d, *J* = 11.4 Hz, 1H), 2.75 – 2.55 (m, 2H), 2.45 (t, *J* = 10.9 Hz, 1H), 2.22 (t, *J* = 10.9 Hz, 1H), 2.06 – 1.92 (m, 2H), 1.81 (d, *J* = 12.4 Hz, 2H), 1.71 (s, 3H), 1.68 – 1.54 (m, 4H), 1.53 – 1.34 (m, 5H), 1.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.74, 149.63, 147.05, 111.66, 109.80, 109.44, 73.24, 71.82, 66.52, 65.24, 60.73, 54.54, 52.25, 52.09, 40.88, 39.41, 39.29, 37.21, 32.67, 27.58, 27.39, 26.56, 24.31, 16.10; HRMS (ESI) calculated for $C_{24}H_{42}NO_3$ $[M + H]^+$ 392.3159, found 392.3164.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-(phenylamino)propan-2-ol (**6h**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (t, *J* = 7.8 Hz, 2H), 6.66 (t, *J* = 7.3 Hz, 1H), 6.59 (d, *J* = 7.9 Hz, 2H), 5.73 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.96 (s, 1H), 4.92 (s, 1H), 4.85 (d, *J* = 3.6 Hz, 1H), 4.80 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 4.06 – 3.87 (m, 3H), 3.50 – 3.31 (m, 2H), 3.24 (dd, J = 12.7, 4.2 Hz, 1H), 3.09 (dd, J = 12.7, 7.2 Hz, 1H), 2.02 – 1.92 (m, 2H), 1.63 (s, 3H), 1.62 – 1.45 (m, 3H), 1.44 – 1.33 (m, 3H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.09, 148.00, 147.54, 129.30, 118.05, 113.40, 112.23, 110.58, 110.36, 110.02, 73.77, 72.28, 69.04, 52.74, 47.01, 41.41, 39.90, 39.81, 33.19, 27.11, 24.85, 16.61; HRMS (ESI) calculated for $C_{24}H_{36}NO_2$ $[M + H]^+$ 370.2741, found 370.2743.

1-((2-Chlorophenyl)amino)-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol (**6i**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 – 7.15 (m, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 6.65 – 6.51 (m, 2H), 5.73 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.97 (s, 1H), 4.92 (s, 1H), 4.85 (d, *J* = 3.2 Hz, 1H), 4.80 (s, 1H), 4.75 (s, 1H), 4.51 (s,

1H), 4.07 – 3.85 (m, 3H), 3.53 – 3.38 (m, 2H), 3.29 (dd, *J* = 12.8, 4.6 Hz, 1H), 3.16 (dd, *J* = 12.8, 6.8 Hz, 1H), 2.06 – 1.84 (m, 2H), 1.63 (s, 3H), 1.60 – 1.45 (m, 3H), 1.44 – 1.27 (m, 3H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.59, 147.04, 143.48, 136.87, 128.74, 127.31, 119.15, 117.16, 111.72, 111.00, 110.22, 109.51, 73.29, 71.55, 68.42, 52.22, 46.03, 40.87, 39.40, 39.31, 32.67, 26.60, 24.37, 16.10; HRMS (ESI) calculated for $C_{24}H_{35}ClNO_2$ $[M + H]^+$ 404.2351, found 404.2349.

1-((2-Methoxyphenyl)amino)-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol (**6j**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89 (t, *J* = 7.6 Hz, 1H), 6.80 (d, *J* = 7.4 Hz, 1H), 6.70 (dd, *J* = 15.9, 7.7 Hz, 2H), 5.84 (dd, *J* = 17.7, 10.5 Hz, 1H), 5.08 (s, 1H), 5.02 (s, 1H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.91 (s, 1H), 4.86 (s, 1H), 4.62 (s, 1H), 4.22 – 3.97 (m, 3H), 3.88 (s, 3H), 3.63 – 3.47 (m, 2H), 3.47 – 3.29 (m, 1H), 3.22 (dd, *J* = 12.8, 7.1 Hz, 1H), 2.65 (brs, 1H), 2.12 – 1.94 (m, 2H), 1.74 (s, 3H), 1.73 – 1.57 (m, 3H), 1.56 – 1.40 (m, 3H), 1.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.23, 150.14, 147.55, 147.12, 138.08, 121.25, 116.97, 112.22, 110.48, 110.19, 110.00, 109.53, 73.72, 72.28, 69.17, 55.41, 52.73, 46.66, 41.39, 39.92, 39.82, 33.18, 27.11, 24.87, 16.61; HRMS (ESI) calculated for $C_{25}H_{38}NO_3$ $[M + H]^+$ 400.2846, found 400.2841.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-((4-nitrophenyl)amino)propan-2-ol (**6k**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.9 Hz, 2H), 6.51 (d, *J* = 9.0 Hz, 2H), 5.74 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.97 (s, 1H), 4.95 (s, 1H), 4.86 (d, *J* = 2.2 Hz, 1H), 4.81 (d, *J* = 2.7 Hz, 1H), 4.76 (s, 1H), 4.51 (s, 1H), 4.10 – 3.91 (m, 3H), 3.56 – 3.38 (m, 2H), 3.37 – 3.27 (m, 1H), 3.20 (dd, *J* = 12.8, 7.1 Hz, 1H), 2.05 – 1.86 (m, 2H), 1.64 (s, 3H), 1.62 – 1.44 (m, 3H), 1.43 – 1.32 (m, 3H), 0.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.23, 149.94, 149.90, 147.49, 134.90, 126.41, 112.25, 111.45, 110.86, 110.11, 73.89, 71.82, 68.74, 52.75, 46.09, 41.49, 39.86, 39.78, 33.22, 27.11, 24.84, 16.60; HRMS (ESI) calculated for $C_{24}H_{35}N_2O_4$ $[M + H]^+$ 415.2591, found 415.2594.

1-((4-Bromophenyl)amino)-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol (**6l**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, *J* = 8.5 Hz, 2H), 6.47 (d, *J* = 8.7 Hz, 2H), 5.74 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.96 (s, 1H), 4.92 (s, 1H), 4.86 (d, *J* = 2.6 Hz, 1H), 4.81 (d, *J* = 2.0 Hz, 1H), 4.76 (s, 1H), 4.51 (s, 1H), 3.94 (s, 3H), 3.51 – 3.34 (m, 2H), 3.30 – 3.11 (m, 2H), 3.06 (dd, *J* = 12.6, 7.2 Hz, 1H), 2.02 – 1.88 (m, 2H), 1.63 (s, 3H), 1.61 – 1.46 (m, 3H), 1.45 – 1.33 (m, 3H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.58, 149.53, 147.01, 146.37, 131.49, 116.41, 114.54, 111.74, 110.15, 109.55, 73.29, 71.66, 68.39, 52.26, 46.57, 40.95, 39.39, 39.28, 32.70, 26.62, 24.33, 16.12; HRMS (ESI) calculated for $C_{24}H_{35}BrNO_2$ $[M + H]^+$ 448.1846, found 448.1843.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-(p-tolylamino)propan-2-ol (**6m**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (d, *J* = 8.2 Hz, 2H), 6.61 (d, *J* = 8.2 Hz, 2H), 5.84 (dd, *J* = 17.7, 10.6 Hz, 1H), 5.07 (s, 1H), 5.02 (s, 1H), 4.96 (d, *J* = 3.5 Hz, 1H), 4.91 (s, 1H), 4.86 (s, 1H), 4.62 (s, 1H), 4.16 – 3.98 (m, 3H), 3.61 – 3.39 (m, 2H), 3.38 – 3.26 (m, 1H), 3.16 (dd, *J* = 12.7, 7.3 Hz, 1H), 2.27 (s, 3H), 2.12 – 1.96 (m, 2H), 1.74 (s, 3H), 1.73 – 1.55 (m, 3H), 1.56 – 1.43 (m, 3H), 1.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.70, 149.61, 147.05, 145.29, 129.29, 126.75,

113.07, 111.74, 110.05, 109.51, 73.24, 71.83, 68.56, 52.23, 46.87, 40.88, 39.41, 39.32, 32.69, 26.61, 24.37, 19.92, 16.11; HRMS (ESI) calculated for $C_{25}H_{38}NO_2$ $[M + H]^+$ 384.2897, found 384.2899.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-(naphthalen-1-ylamino)propan-2-ol (**6n**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 – 7.66 (m, 2H), 7.51 – 7.31 (m, 2H), 7.30 – 7.12 (m, 2H), 6.56 (d, *J* = 7.3 Hz, 1H), 5.71 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.98 (s, 1H), 4.93 (s, 1H), 4.84 (d, *J* = 2.0 Hz, 1H), 4.79 (d, *J* = 2.7 Hz, 1H), 4.74 (s, 1H), 4.50 (s, 1H), 4.19 – 4.07 (m, 1H), 4.03 – 3.91 (m, 2H), 3.58 – 3.45 (m, 2H), 3.39 (dd, *J* = 12.4, 4.1 Hz, 1H), 3.24 (dd, *J* = 12.4, 7.0 Hz, 1H), 2.03 – 1.84 (m, 2H), 1.62 (s, 3H), 1.62 – 1.45 (m, 3H), 1.44 – 1.29 (m, 3H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.65, 149.59, 147.05, 142.84, 133.83, 128.14, 126.01, 125.33, 124.36, 123.36, 119.64, 117.55, 111.73, 110.29, 109.51, 104.45, 73.36, 72.06, 68.42, 52.22, 46.75, 40.90, 39.38, 39.30, 32.70, 29.23, 26.62, 24.37, 16.10; HRMS (ESI) calculated for $C_{28}H_{38}NO_2$ $[M + H]^+$ 420.2897, found 420.2893.

1-(Benzylamino)-3-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol (**6o**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 – 7.11 (m, 5H), 5.74 (dd, *J* = 17.7, 10.5 Hz, 1H), 4.94 (s, 1H), 4.89 (s, 1H), 4.85 (d, *J* = 3.3 Hz, 1H), 4.81 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 3.90 (s, 2H), 3.90 – 3.80 (m, 1H), 3.79 – 3.69 (m, 2H), 3.46 – 3.24 (m, 2H), 2.79 – 2.57 (m, 2H), 2.48 (s, 2H), 2.01 – 1.85 (m, 2H), 1.63 (s, 3H), 1.62 – 1.46 (m, 3H), 1.45 – 1.30 (m, 3H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.29, 150.15, 147.56, 139.56, 128.50, 128.21, 127.20, 112.18, 110.36, 109.96, 73.70, 72.64, 68.81, 53.72, 52.74, 51.44, 41.35, 39.91, 39.81, 33.14, 27.07, 24.83, 16.60; HRMS (ESI) calculated for $C_{25}H_{38}NO_2$ $[M + H]^+$ 384.2897, found 384.2895.

1-((2-((*1R,3S,4S*)-4-Methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)-3-((2-phenoxyethyl)amino)propan-2-ol (**6p**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.15 (m, 2H), 6.92 – 6.78 (m, 3H), 5.74 (dd, *J* = 17.8, 10.5 Hz, 1H), 4.95 (s, 1H), 4.90 (s, 1H), 4.85 (d, *J* = 3.6 Hz, 1H), 4.80 (s, 1H), 4.75 (s, 1H), 4.51 (s, 1H), 4.01 (t, *J* = 5.1 Hz, 2H), 3.93 (s, 2H), 3.90 – 3.80 (m, 1H), 3.44 – 3.30 (m, 2H), 2.98 (t, *J* = 5.1 Hz, 2H), 2.83 – 2.61 (m, 4H), 2.02 – 1.88 (m, 2H), 1.63 (s, 3H), 1.62 – 1.46 (m, 3H), 1.45 – 1.33 (m, 3H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.18, 149.77, 149.65, 147.07, 128.99, 120.45, 114.01, 111.68, 109.86, 109.46, 73.21, 72.06, 68.23, 66.44, 52.24, 51.44, 48.21, 40.87, 39.42, 39.31, 32.65, 26.57, 24.34, 16.09; HRMS (ESI) calculated for $C_{26}H_{40}NO_3$ $[M + H]^+$ 414.3003, found 414.3005.

3,3'-(Piperazine-1,4-diyl)-bis(1-((2-((*1R,3S,4S*)-4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)allyl)oxy)propan-2-ol) (**6q**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (dd, *J* = 17.8, 10.5 Hz, 2H), 5.03 (s, 2H), 4.97 (s, 2H), 4.93 (d, *J* = 3.7 Hz, 2H), 4.88 (s, 2H), 4.82 (s, 2H), 4.58 (s, 2H), 4.09 – 3.97 (m, 4H), 3.96 – 3.85 (m, 2H), 3.52 – 3.37 (m, 4H), 3.21 (brs, 2H), 2.69 (s, 4H), 2.57 – 2.36 (m, 8H), 2.08 – 1.98 (m, 4H), 1.71 (s, 6H), 1.69 – 1.58 (m, 6H), 1.53 – 1.41 (m, 6H), 1.01 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.81, 149.65, 147.05, 111.67, 109.88, 109.45, 73.29, 71.83, 65.58, 60.21, 52.72, 52.26, 40.85, 39.42, 39.30, 32.64, 26.55, 24.34, 16.10; HRMS (ESI) calculated for $C_{40}H_{67}N_2O_4$ $[M + H]^+$ 639.5095, found 639.5090.

4.2. Pharmacology

4.2.1. *In vitro* antiproliferative assay

SGC-7901, HeLa, U87, A549, and A549/DDP cells were purchased from Nanjing Key Gen Biotech Co. Ltd. (Nanjing, China). The cytotoxicity of the compounds was determined using MTT assay. Briefly, test cell lines were plated on 96-well plates at the density of 5×10^4/well and incubated for 24 h at 37^{o}C under an atomosphere of 5% CO_2. The test compounds were dissolved in the culture medium with 0.5% DMSO at different concentrations and treated to the cells for another 72 h. The MTT (5 mg/mL in PBS) was added and incubated for another 4 h, the optical density was detected with a microplate reader at 490 nm. The IC_{50} values were calculated according to the dose-dependent curves. All the tests were repeated in at least three independent experiments.

4.2.2. Cell cycle analysis

SGC-7901 cells were incubated with **6q** at indicated concentrations. After incubating for 72 h, the treated cells were trypsinized, washed with PBS and centrifuged. The collected cells were fixed by adding 70% ethanol at 4 ^{o}C overnight and incubated for 30 min in PBS containing 100 μL RNase A and 400 μL of propidium iodide. Analysis of the cell DNA content was performed with the system software (Cell Quest, BD Biosciences, USA).

4.2.3. Apoptosis analysis

SGC-7901 cells were seeded into 6-well plates and incubated at 37 ^{o}C for 24 h, and then treated with or without **6q** at indicated concentrations for another 72 h. Cells were washed twice in PBS and resuspended in 500 μL Annexin V binding buffer. Then 5 μL of Annexin V-APC and 7-AAD were added successively and the mixture was incubated for 15 min under dark conditions at 25 °C. Apoptosis was analyzed using a FACS Calibur flow cytometer (BectoneDickinson, San Jose, CA, USA).

4.2.4. Mitochondrial membrane potential determination

The mitochondrial membrane potential assay was performed according to the manufacturer's instruction (KGA601, KeyGEN Biotech, Nanjing, China). Briefly, SGC-7901 cells were seeded into 6-well plates and incubated at 37 °C for 24 h, and then treated with or without **6q** at indicated concentrations for another 72 h. Cells were washed in PBS, centrifuged, and then stained with JC-1 dye under dark conditions. The percentage of cells with healthy or collapsed mitochondrial membrane potentials was monitored by flow cytometry analysis (BectoneDickinson, San Jose, CA, USA).

4.2.5. Western blotting

SGC-7901 cells were incubated with different doses of **6q** for 72 h. The cells were harvested and lysed using lysis buffer, and the solution was centrifuged. Then the protein concentrations were determined, and individual cell lysates (25 μg per lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% gel, SDS-PAGE) and transferred onto nitrocellulose membranes. After being blocked with 5% fat-free milk, the target proteins in the membranes were probed with

monoclonal anti-Bax, anti-Bcl 2, anti-caspase 3, anti-caspase 9, anti-cytochrome C, anti-PARP and anti-GAPDH antibodies (KeyGEN Biotech, Nanjing, China), respectively. The bound antibodies were detected by horseradish peroxidase (HRP) conjugated second antibodies and visualized using an enhanced chemiluminescent reagent. The relative levels of each signaling event to control GAPDH were determined by densimetric scanning.

4.2.6. Aqueous solubility assay

The water solubility of **6a**, **6q** and *β*-elemene was determined by HPLC according to a previously published protocol [29]. Firstly, 10 mg of **6a**, **6q** or *β*-elemene was weighed and added to 1 mL of water, respectively. The suspensions were shaken at 25 °C for 24 h and then centrifuged, and the supernatants were filtered. Aliquots (10 μL) of the supernatants were injected into the HPLC system equipped with a C18 reverse-phase column (No. 03050804, 0.46 cm × 25 cm, Hanbang Tech., Huai'an, China). One-point calibration [30] was done by injecting 10 μL aliquots of the corresponding buffer solutions of **6a**, **6q**, or *β*-elemene with known concentrations.

4.2.7. *In vivo* antitumor assay

Antitumor activity against mice bearing H22 liver cancer was performed as described by Shi *et al*. [31] with a slight modification. Mice from Institute of Cancer Research with body weight 18-22 g were subcutaneously transplanted with H22 cells on the right oxter according to protocols of tumor transplant research. Incubated after one day, mice were weighted and divided randomly into 4 groups (8 mice/group). The groups were intravenously administered with *β*-elemene (60 mg/kg) or **6q** (30 or 60 mg/kg) in a vehicle of 10% DMF/2% Tween 80/88% saline once per day for 3 weeks. The mice were sacrificed after the treatments and the tumors were excised and weighed. The inhibition rate was calculated as follows: Tumor inhibitory ratio (%) = (1-average tumor weight of treated group/average tumor weight of control group) × 100%. All procedures were performed following institutional approval in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

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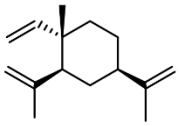
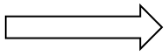
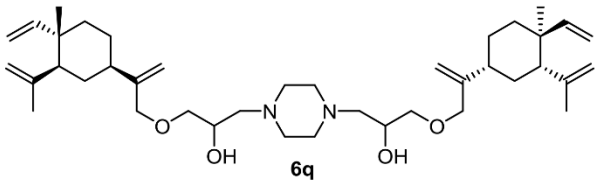
Graphic Abstract



β–elemene





6q

- Improved aqueous solubility
- Enhanced antiproliferative activity
- Inducing sensitization of tumor cells to cisplatin
- Significantly suppressing liver cancer xenograft tumor growth *in vivo*