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Research paper

Antiproliferative effect of mitochondria-targeting allobetulin 1,2,3-triazolium salt derivatives and their mechanism of inducing apoptosis of cancer cells

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ABSTRACT

Herein we report the targeting effect of 1,2,3-triazolium salt derivatives of allobetulin on cancer cells mitochondria and their antiproliferative mechanism. A series of allobetulin derivatives with 1,2,3-triazolium positively charged units was designed and synthesized by multi-component triazolization reaction and alkylation. The screening of cytotoxicity showed that all the 1,2,3-triazolium salt derivatives of allobetulin displayed better cytotoxicity than the parent compound allobetulin and commercial anticancer drugs gefitinib. The most potent compound **4q** showed strong anticancer activity, especially for Eca-109 cells. Compound **4n** showed the strongest inhibitory effect on SGC-7901 cells. Further anticancer mechanism studies indicated that compounds **4n** and **4q** induced apoptosis through the mitochondrial pathway. Compounds **4n** and **4q** acted on mitochondria to cause an increase in intracellular reactive oxygen species and a change in the level of apoptosis-related protein (Bcl-2, Bcl-xL and Bax), which resulted in a decrease in membrane potential and activation of caspase family to induce cancer cells apoptosis. Meanwhile, compounds **4n** and **4q** could induce cancer cells apoptosis by arresting the cell cycle. Due to the strong cytotoxicity of compounds **4n** and **4q**, they are expected to become new anticancer agents and deserve further study.

1. Introduction

Cancer has become a major global public health problem. 1,762,450 new cancer cases and 606,880 cancer deaths were expected in the USA in 2019 [1]. In the light of the assessment of GLOBOCAN 2012, because of the global population growth and ageing, the world is expected to add 19.3 million new cases of cancer every year by 2025 [2]. At present, due to the high efficiency of chemotherapy, it is still the primary treatment for various cancers, but its significant side effects limit clinical application [3–5]. Therefore, the development of low-toxic and highly effective targeted anti-cancer drugs is a priority. Compared with normal cells, the most distinguishing feature of cancer cells is that they cannot perform normal apoptosis and can proliferate indefinitely [6]. The mitochondrion is an organelle, which is closely related to apoptosis in cells and has received extensive attention [7,8]. Some studies reported that mitochondria in cancer cells have undergone significant changes, such as DNA mutations [9,10], increased membrane potential ($\Delta\psi_m$) [11,12], changes in energy supply [13–15] and so on. According to the specificity of mitochondrial function and its changes in cancer cells, it is an effective strategy to induce apoptosis of cancer cells and cause cancer cell death by preparing mitochondrial targeting agents [16–18]. Based on the increased mitochondrial membrane potential in cancer cells, delocalized lipophilic cations (DLCs) have proven to be effective mitochondrial targeting groups [19,20]. The triphenylphosphonium cation (TPP^+) is the most widely studied DLC, and it has been used to prepare various mitochondria-targeted conjugates and antiproliferative activity study [21–24].

Pentacyclic triterpenoids (PTs) are natural products that are widely present in plants and have attracted much attention due to their remarkable biological activity [25], such as betulin, betulinic acid, glycyrrhetinic acid and oleanolic acid [26–28]. Especially in the aspect of anticancer studies, the multi-target and multi-site characteristics of PTs and their derivatives suggests that they

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could become a new generation of anticancer drugs [29]. Allobetulin is also a pentacyclic triterpene compound that could be easily obtained from the rearrangement reaction of betulin (Fig. 1) [30,31], and it also showed good biological activity [32]. Conversely, it is remarkable that there are very little literature reports on it and its derivatives [33,34]. As an important nitrogen-containing heterocyclic ring, 1,2,3-triazole has attracted much attention due to its good pharmacological activity, and today it is often used as the primary structural unit of medicines and plays an important role in the synthesis of medicines [35]. There have been many reports of 1,2,3-triazole compounds [36–39], including 1,2,3-triazole derivatives of pentacyclic triterpenoids (prepared by click reaction) [40,41]. However, 1,2,3-triazolium derivatives with potential DLC properties have been rarely reported, especially their targeting effect on mitochondria [42].

To investigate the anticancer mechanism of 1,2,3-triazolium pentacyclic terpenoid derivatives and obtain potential anticancer drugs, we used a new multicomponent triazolization reaction developed by our group to prepare a series of 1,2,3-triazole derivatives of allobetulin [43–45], and then a series of 1,2,3-triazolium salt derivatives of allobetulin was afforded by alkylation [46]. As there are few reports on the cytotoxicity of allobetulin, we selected common five cell lines (SGC-7901 (human gastric cancer cells), Eca-109 (human esophageal cancer cells), HeLa (human epithelial cervical cancer cells), HepG2 (human liver cancer cells) and HL-7702 (human normal liver cells)) for their *in vitro* cytotoxicity evaluation by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Through a series of pharmacological experiments, we conducted a preliminary anticancer mechanism research on compounds with great potential.

2. Results and discussion

2.1. Chemistry

The synthesis of 1,2,3-triazole derivatives of allobetulin and 1,2,3-triazolium salt derivatives of allobetulin was demonstrated in Scheme 1. Allobetulin (**1**) reacted with Jones reagent to give allobetulone (**2**). A multi-component triazolization reaction of allobetulone with 4-nitrophenyl azide and various amines to yielded 1,2,3-triazole derivatives of allobetulin **3a-y** (Table 1). The 1,2,3-triazolium salt derivatives **4a-x** (Table 2) were obtained by treating 1,2,3-triazole fused derivatives of allobetulin with methyl iodide in acetonitrile. As shown in Scheme 2, bisterpene compound **3z** was given by treating allobetulone with 1,4-butanediamine and 4-nitrophenyl azide.

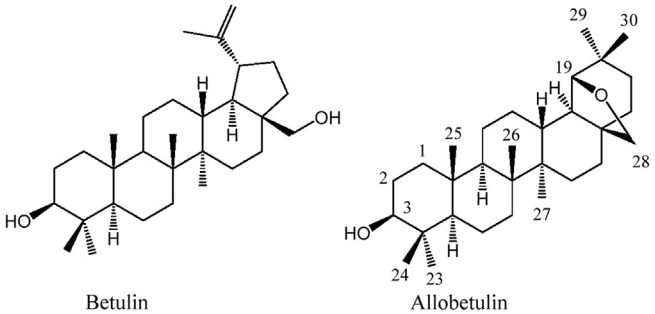


Fig. 1. Structure of betulin and allobetulin.

2.2. Biological evaluations

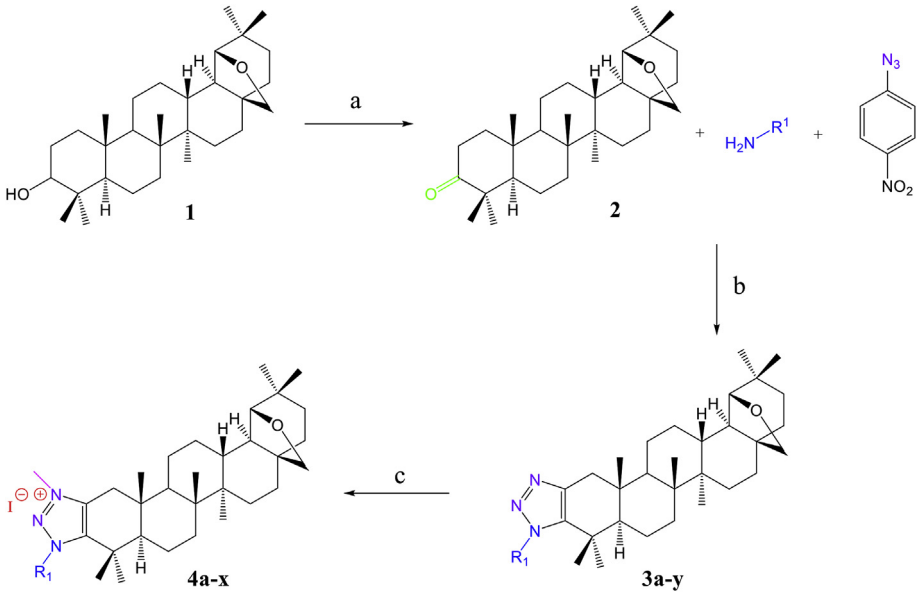
2.2.1. Cytotoxicity in vitro

The cytotoxicity of 1,2,3-triazole derivatives of allobetulin **3a-z**, 1,2,3-triazolium salt derivatives of allobetulin **4a-x** and parent compound allobetulin was evaluated by the MTT method in five different cell lines (Eca-109, HeLa, HepG2, SGC-7901 and HL-7702) and the commercially available anticancer drugs gefitinib and doxorubicin as the reference drug. The results were collected in Table 3. The parent compound allobetulin showed a weak inhibitory effect on those four cancer cells. Among 1,2,3-triazole derivatives **3a-z**, compounds **3a**, **3e**, **3g-i**, **3k**, **3n-p**, **3r-u** and **3y-z** displayed good inhibition against those four cancer cell lines as compared with allobetulin. Compounds **3a**, **3g-h**, **3o-p**, **3r** and **3y** even showed better cytotoxicity than gefitinib, and compound **3a** is the most potent one. However, compounds **3b**, **3d**, **3f**, **3j**, **3l-m** and **3q** exhibited weak antiproliferative activity, and compounds **3c** and **3v-w** even did not show any cytotoxicity. In HepG2 cells, the cytotoxicity of compound **3h** was the most potent one with IC_{50} values of 6.56 μM. For Eca-109 cells, compound **3r** exhibited strongest inhibitory activity with IC_{50} values of 6.24 μM. In contrast to compounds **3b** and **3c**, **3a** exhibited better antitumor activity, indicating that the introduction of short chains can significantly increase cytotoxicity in comparison to longer chains. Compound **3o** with 4-OCH_3 substitutions at an aromatic ring displayed better inhibitory effect on those four cancer cells lines as compared with compounds **3d-e** and **3i-j**, proposing that the electron-donating group methoxy introduced at that position could significantly enhance inhibition. This trend also applies to compounds with substituents in the ortho position (compounds **3g**, **3k** and **3p**).

As we expected, all the 1,2,3-triazolium salt derivatives of allobetulin **4a-x** displayed higher antiproliferative activities. Except for compound **4v**, all other 1,2,3-triazolium salt derivatives showed higher cytotoxicity than allobetulin, 1,2,3-triazole derivatives **3a-z** and gefitinib, and compound **4q** was the most effective one with IC_{50} values of 1.04–1.92 μM. Compound **4u** was also exhibiting significant inhibitory activities for Eca-109, HeLa, HepG2 and SGC-7901 cell lines with IC_{50} values of 1.67, 1.67, 1.55 and 1.24 μM, respectively. As compared with compounds **4o-p** and **4s-t**, compounds **4q** and **4u** showed similar or even stronger anticancer cell proliferation effects than doxorubicin and exhibited less cytotoxicity to normal cells than doxorubicin, which suggested that when the benzene ring was substituted with a 2-methoxy group, this could enhance antiproliferative activities. Additionally, some compounds showed strong inhibitory effects on certain cancer cells. Compounds **4l** and **4s** displayed strong cytotoxicity to Eca-109 cells with IC_{50} values of 1.98 and 1.97 μM, respectively. For SGC-7901 cells, compounds **4n** and **4p** displayed powerful inhibition with IC_{50} values of 1.12 and 1.81 μM, respectively. Compound **4j** showed strong cytotoxicity to HepG2 cells with IC_{50} values of 1.73 μM. As shown in Table 3, compounds with high anticancer activity also exhibited high cytotoxicity to normal cells (HL-7702), demonstrating that the target compounds exhibited low selectivity. By further evaluating the Selectivity Index (SI) of the most potent compounds **4n** and **4q** (Table 4), we found that these two compounds were safer for normal cells (HL-7702) than doxorubicin. Because compounds **4n** and **4q** showed most significant Cytotoxicity to SGC-7901 cells and Eca-109 cells respectively, these two compounds were picked for further pharmacological research in SGC-7901 cells and Eca-109 cells respectively.

2.2.2. Cell apoptosis induced by compounds *4n* and *4q*

To test if the cytotoxicity of **4n** and **4q** is related with induction of apoptotic effects, the Hoechst 33342 staining and Acridine-Orange (AO)/Propidium Iodide (PI) dual-staining method were



Scheme 1. a) Jones reagent, acetone, 0 °C; b) Toluene, CH_3COOH, 24 h, 100 °C; c) Acetonitrile, methyl iodide, 85 °C.

used to discover cell apoptosis, and Annexin V-PE/7-AAD dual-staining assay was used to detect apoptosis rate (Figs. 2–4).

SGC-7901 cells were treated with **4n** (0 μM, 1 μM, 2 μM and 4 μM) for 48 h, and Eca-109 cells were treated with **4q** (0 μM, 1 μM, 2 μM and 4 μM) for 48 h. As can be seen from Fig. 2, as the doses of compounds **4n** and **4q** increased, more and more cells with bright blue fluorescence and marked apoptosis characteristics (nucleus fragmentation and chromatin concentration) appeared. AO is a dye that can permeate the cell membrane. It can stain the nucleus of normal cells and emit green or yellow-green fluorescence. PI is a dye that cannot penetrate cell membranes, so it can only stain dead cells. It can make the early apoptotic cells emit weak red light, the late apoptotic cells show a strengthening of the red light, and the necrotic cells show strong red fluorescence. As can be seen from Fig. 3, SGC-7901 cells and Eca-109 cells with homogeneous green fluorescence and normal morphology were obtained in control cells. After treatment with compounds **4n** and **4q**, we could clearly see that as the concentration of the compound increases that the normal cells with green fluorescence gradually decrease, and the cells with red fluorescence gradually increase. Especially after treatment with 4 μM of **4n** and **4q**, most cells manifested a state of apoptosis. These results indicated that these two compounds can induce corresponding cell apoptosis. As shown in Fig. 4, compounds **4n** and **4q** led to cell apoptosis in a dose-dependent manner, particularly for late apoptosis. When treating cells with the maximum test concentration, **4n** (4 μM) led to early and late apoptosis in 2.07% and 97.3% of SGC-7901 cells, respectively, and **4q** (4 μM) led to early and late apoptosis in 2.92% and 92.2% of Eca-109 cells, respectively. These results demonstrated that compound **4n** could uncommonly induce apoptosis of SGC-7901 cells and compound **4q** could obviously induce apoptosis of Eca-109 cells.

2.2.3. *Effects of compounds **4n** and **4q** on mitochondrial membrane potential ($\Delta\psi_m$)*

In order to study the influence of compounds **4n** and **4q** on mitochondrial membrane potential ($\Delta\psi_m$), the JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide) staining assay was used. As illustrated in Fig. 5, after treating SGC-7901 cells with different concentrations of **4n** and Eca-109 cells with different concentrations of **4q**, the red fluorescence ratio gradually decreased, and the green fluorescence ratio increased significantly. This result indicated that these two compounds could reduce the mitochondrial membrane potential in a concentration-dependent manner.

2.2.4. *Role of reactive oxygen species (ROS) in apoptosis*

The ROS of most eukaryotic cells are mainly produced by mitochondria, and it has been reported in the literature that ROS have an important role in antitumor activity [47]. Therefore, in order to study the effects of compounds **4n** and **4q** on ROS production and ROS on apoptosis, DCFH-DA (2′,7′-dichlorofluorescein diacetate) staining was used to detect the production of ROS, and NAC (N-acetyl-L-cysteine) was used as a ROS scavenger to detect the effect of ROS on apoptosis. As illustrated in Fig. 6, no obvious green fluorescence was found in the control group, and as the concentration of compounds **4n** and **4q** increased, the green fluorescence gradually increased. This result demonstrated that these two compounds could induce concentration-dependent increase of ROS production in the corresponding cells. As shown in Fig. 7, after the pretreatment with ROS scavenger NAC, the percentage of apoptosis of SGC-7901 cells and Eca-109 cells was significantly reduced. This suggested that the increasing production of ROS could promote the apoptosis of these two cell types.

2.2.5. *Influence of compounds **4n** and **4q** on the cell cycle*

To confirm whether the cytotoxicity of compounds **4n** and **4q** was related to cell cycle arrest, we tested it with propidium iodide (PI) staining by flow cytometry. As illustrated in Fig. 8, the percentage of Eca-109 cells in the G2/M phase in the control group was 8.39%, after treatment with 1, 2 and 4 μM concentrations of compound **4q** for 48 h, the percentage of Eca-109 cells in G2/M phase increased to 10.81, 16.84 and 22.73%, respectively. This indicated that compound **4q** promoted cycle arrest of Eca-109 cells in G2/M phase in a dose-dependent manner. Interestingly, after SGC-7901 cells were treated with different concentrations of **4n**, their cell cycle showed different patterns. As compared with the control group, treatment of SGC-7901 cells with a low concentration of compound **4n** (1 and 2 μM) could arrest the cell cycle in the G0/G1 phase, while treatment of SGC-7901 cells with a high concentration of compound **4n** (4 μM) could arrest the cell cycle in the G2/M phase.

Table 1
1,2,3-triazole derivatives of allobetulin **3a-y**.

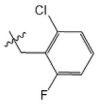
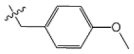
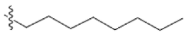
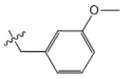
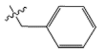
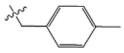
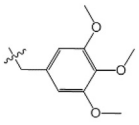
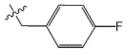
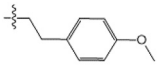
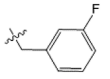
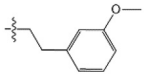
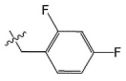
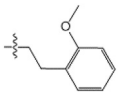
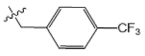
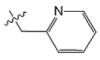
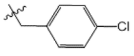
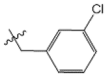
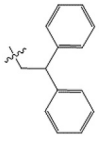
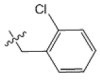
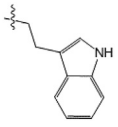
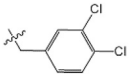
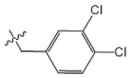
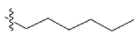
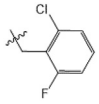
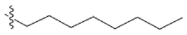
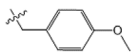
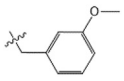
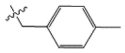
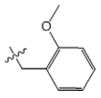
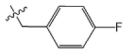
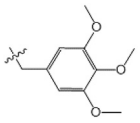
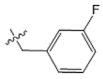
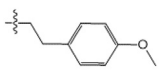
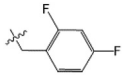
Entry	Code	R_1	Entry	Code	R_1
1	**3a**		14	**3n**	
2	**3b**		15	**3o**	
3	**3c**		16	**3p**	
4	**3d**		17	**3q**	
5	**3e**		18	**3r**	
6	**3f**		19	**3s**	
7	**3g**		20	**3t**	
8	**3h**		21	**3u**	
9	**3i**		22	**3v**	
10	**3j**		23	**3w**	
11	**3k**		24	**3x**	
12	**3l**		25	**3y**	
13	**3m**				

Table 2
1,2,3-triazolium salt derivatives of allobetulin **4a-x**.

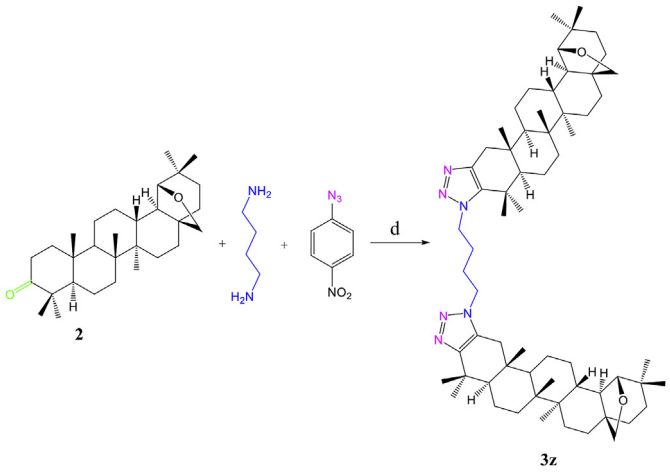
Entry	Code	R_1	Entry	Code	R_1
1	**4a**		13	**4m**	
2	**4b**		14	**4n**	
3	**4c**		15	**4o**	
4	**4d**		16	**4p**	
5	**4e**		17	**4q**	
6	**4f**		18	**4r**	
7	**4g**		19	**4s**	
8	**4h**		20	**4t**	
9	**4i**		21	**4u**	
10	**4j**		22	**4v**	
11	**4k**		23	**4w**	
12	**4l**		24	**4x**	

*2.2.6. Mitochondrial pathway-dependent apoptosis induced by compounds **4n** and **4q***

To confirm whether the apoptotic effects of compounds **4n** and **4q** was exerted via the mitochondrial pathway, we measured the expression levels of PARP, Cleaved-PARP, caspase-3, caspase-8, caspase-9, Bax, Bcl-2, and Bcl-xL using Western blotting in SGC-7901 and Eca-109 cells. As shown in Fig. 9, PARP, caspase-3, 8, and 9, the important hallmarks of mitochondrial pathway-dependent apoptosis, were dramatically decreased by compounds **4n** and **4q** in a dose-dependent manner. Meanwhile, the pro-apoptotic protein level of Bax was up-regulated, and the anti-apoptotic protein level of Bcl-2 and Bcl-xL were down-regulated by compounds **4n** and **4q** in a dose-dependent manner. This suggested that the apoptotic effects of compounds **4n** on SGC-7901 cells and **4q** on Eca-109 cells were exerted via the mitochondrial pathway.

3. Conclusions

As far as we know, this is the first report on the research of mechanism of anticancer effect of 1,2,3-triazolium salt derivatives of pentacyclic triterpenoids. In this work, a series of 1,2,3-triazolium salt derivatives of allobetulin were prepared and screened as potent antitumor agent candidates. The results



Scheme 2. d) Toluene, CH_3COOH, 24 h, 100 °C.

indicated that compound **4q** was the most potent, showing the strongest inhibitory effect on Eca-109 cells, and compound **4n** exhibited the highest cytotoxicity towards SGC-7901 cells. Through a series of studies on the mechanism of anticancer action, we have not only confirmed the targeting effect of compounds **4n** and **4q** on cancer cell mitochondria, but also confirmed that they induced cancer cell apoptosis through a mitochondrial apoptosis pathway and cell cycle arrest pathway. Because of the significant antiproliferative activity of these two compounds, they have the potential to become potential anticancer drugs and are worthy of further investigation. According to our experimental results, the 1,2,3-triazolium group was an effective mitochondrial targeting group and was worthy of extensive research.

In future work, we will carry out further structural modifications of the compounds to improve their selectivity and conduct in-depth pharmacological research on the compounds with the best antiproliferation ability and selectivity to explore other signal pathways that may be involved. Through the discovery of new targets, we aim for further improvement in our future research work.

4. Experimental section

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Avance 300 MHz or Bruker AMX 400 MHz. The Reichert Thermovar apparatus was used to test melting points (m.p.). TLC (thin-layer chromatography) was checked on silica gel 0.20 mm 60 with sulfuric acid ethanol solution (configured with 10 ml of sulfuric acid and 90 ml of ethanol). The quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA) was used to detect high-resolution mass spectra (HR-MS). Most chemical reagents and solvents were purchased from commercial sources. Allobetulin and allobetone were obtained based on the procedure reported previously.[33]

4.1.1. General procedure for the synthesis of 1,2,3-triazole derivatives of allobetulin ***3a-z***

Allobetulone, 4-nitrophenyl azide and amine were dissolved in try toluene under nitrogen atmosphere, and then a catalytic amount of acetic acid and 4 Å molecular sieves (50 mg) were added to the mixture and this was warmed to 100 °C for 24 h. When the reaction had finished, the product was purified by column chromatography (firstly dichloromethane was used to remove 4-nitroaniline, and then petroleum ether/ethyl acetate as eluent) to obtain the 1,2,3-triazole derivatives of allobetulin.

*4.1.1.1. 1′-Butyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole **3a**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 equivalent (eq.), 0.181 mmol), butylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3a**. White solid, yield 95%, m.p.: 207–208 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.30 (t, J = 7.7 Hz, 2H, H-4′), 3.79 (d, J = 7.7 Hz, 1H, H_a-28), 3.56 (s, 1H, H-19), 3.46 (d, J = 7.9 Hz, 1H, H_b-28), 2.95 (d, J = 15.2 Hz, 1H, H-1), 1.32 (s, 3H, CH_3-23), 1.21 (s, 3H, CH_3-24), 1.03 (s, 3H, CH_3-25), 0.95 (d, J = 2.6 Hz, 9H, CH_3-26, CH_3-27 and CH_3-7′), 0.81 (d, J = 2.1 Hz, 6H, CH_3-29 and CH_3-30); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.96 (C-3), 137.27 (C-2), 87.92 (C-19), 71.26 (C-28), 54.92 (C-5), 49.90 (C-9), 49.32 (C-4′), 46.78 (C-18), 41.50 (C-20), 40.74 (C-14), 40.50 (C-8), 39.04 (C-1), 38.61 (C-4), 36.75 (C-16), 36.27 (C-17), 34.30 (C-13), 33.68 (C-7), 33.01 (C-10), 32.77 (C-5′), 32.73 (C-21), 28.79 (C-30), 28.70 (C-23), 26.55 (C-15), 26.46 (C-12), 26.23 (C-22), 24.53 (C-29), 21.48 (C-24), 21.33 (C-11), 20.15 (C-6′), 18.93 (C-6), 16.33 (C-25), 15.42 (C-26), 13.64 (C-7'), 13.47 (C-27). HRMS (ESI^+) m/z calcd for $C_{34}H_{55}N_3O_1$ $[M+H]^+$: 522.4417, found 522.4414.

*4.1.1.2. 1′-Hexyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole **3b**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 1-hexylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3b**. White solid, yield 83% m.p.: 206–207 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.35–4.23 (m, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.46 (d, J = 7.8 Hz, 1H), 2.95 (d, J = 15.2 Hz, 1H), 2.16 (d, J = 15.2 Hz, 1H), 1.99 (t, J = 7.7 Hz, 2H), 1.32 (s, 3H), 1.21 (s, 3H), 1.02 (s, 3H), 0.92 (d, J = 15.5 Hz, 9H), 0.81 (d, J = 1.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 141.03, 137.27, 87.95, 71.28, 54.88, 53.43, 49.89, 49.63, 46.77, 41.51, 40.50, 39.04, 38.59, 36.75, 36.30, 34.30, 33.69, 33.01, 32.72, 31.36, 30.84, 28.81, 28.71, 26.64, 26.56, 26.48, 26.24, 24.56, 22.51, 21.48, 21.36, 18.94, 16.38, 15.44, 13.97, 13.51. HRMS (ESI^+) m/z calcd for $C_{36}H_{59}N_3O_1$ $[M+H]^+$: 550.4730, found 550.4722.

*4.1.1.3. 1′-Octyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole **3c**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 1-octylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3c**. White solid, yield 92%, m.p.: 176–178 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.29 (t, J = 7.8 Hz, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.9 Hz, 1H), 2.95 (d, J = 15.2 Hz, 1H), 2.14–2.19 (m, 1H), 1.32 (s, 3H), 1.21 (s, 3H), 1.04 (s, 3H), 0.95 (s, 9H), 0.81 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.95, 137.25, 87.92, 71.25, 54.92, 49.90, 49.59, 46.77, 41.49, 40.73, 40.50, 39.03, 38.59, 36.75, 36.27, 34.30, 33.67, 33.01, 32.73, 31.70, 30.78, 29.12, 29.07, 28.78, 28.69, 26.92, 26.54, 26.45, 26.23, 24.53, 22.57, 21.47, 21.33, 18.92, 16.33, 15.41, 14.01, 13.47. HRMS (ESI^+) m/z calcd for $C_{38}H_{63}N_3O_1$ $[M+H]^+$: 578.5043, found 578.5065.

*4.1.1.4. 1′-Benzyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole **3d**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), benzylamine (1.3 eq., 0.235 mmol),

Table 3
Cytotoxicity of allobetulin, 1,2,3-triazole derivatives of allobetulin **3a-z** and 1,2,3-triazolium salt derivatives of allobetulin **4a-x** in five different cell lines (Eca-109, HeLa, HepG2, SGC-7901 and HL-7702).

Compd.	IC_{50} (μM)[a]			HeLa	HL-7702
	HepG2	Eca-109	SGC-7901		
Allobetulin	55.91 ± 2.31	47.57 ± 1.50	62.38 ± 2.85	45.62 ± 1.42	42.73 ± 4.23
3a	10.71 ± 1.52	10.53 ± 0.80	6.61 ± 0.51	6.26 ± 0.52	7.86 ± 0.47
3b	>80	37.40 ± 0.41	40.94 ± 0.58	>80	51.85 ± 1.16
3c	>80	>80	>80	>80	>80
3d	59.44 ± 0.54	53.19 ± 0.51	55.69 ± 0.34	>80	38.52 ± 1.76
3e	26.59 ± 1.45	34.76 ± 1.42	20.29 ± 1.48	32.34 ± 2.48	22.68 ± 1.64
3f	47.67 ± 1.39	69.01 ± 0.48	>80	>80	41.95 ± 0.19
3g	21.98 ± 0.30	17.95 ± 0.92	20.06 ± 1.06	19.48 ± 1.85	18.19 ± 0.38
3h	6.56 ± 0.14	10.90 ± 0.45	9.02 ± 0.80	10.15 ± 0.20	7.47 ± 0.70
3i	28.90 ± 1.57	33.89 ± 2.74	32.23 ± 2.59	28.48 ± 1.06	25.61 ± 1.87
3j	66.02 ± 3.06	64.18 ± 2.00	61.39 ± 3.76	51.27 ± 2.28	44.93 ± 2.46
3k	30.72 ± 0.42	36.48 ± 1.19	31.47 ± 1.35	34.41 ± 1.00	26.32 ± 0.26
3l	56.15 ± 2.77	58.94 ± 2.65	49.87 ± 2.19	41.26 ± 2.59	40.50 ± 1.70
3m	53.92 ± 1.41	43.64 ± 1.29	44.48 ± 1.18	32.64 ± 1.87	35.54 ± 2.13
3n	24.33 ± 0.80	26.99 ± 1.90	26.14 ± 1.61	23.29 ± 2.36	19.48 ± 1.16
3o	13.69 ± 0.30	17.20 ± 1.86	16.93 ± 1.61	12.74 ± 1.18	11.23 ± 0.64
3p	12.65 ± 0.10	10.42 ± 0.54	11.97 ± 0.51	11.17 ± 0.34	10.54 ± 0.59
3q	46.66 ± 2.16	57.92 ± 2.89	53.04 ± 3.62	41.87 ± 3.62	38.44 ± 2.63
3r	12.12 ± 0.42	6.24 ± 0.25	10.69 ± 0.62	9.07 ± 1.86	6.65 ± 0.37
3s	36.61 ± 1.95	51.78 ± 5.54	45.22 ± 2.43	42.45 ± 3.25	39.33 ± 2.80
3t	31.94 ± 0.63	25.54 ± 1.28	39.80 ± 1.51	23.63 ± 0.32	20.89 ± 1.71
3u	32.81 ± 0.93	34.89 ± 0.31	38.56 ± 1.95	33.86 ± 1.12	31.93 ± 1.89
3v	>80	>80	>80	>80	>80
3w	>80	>80	>80	>80	>80
3x	53.88 ± 3.89	48.53 ± 2.60	47.91 ± 2.12	44.07 ± 1.84	40.68 ± 2.74
3y	14.38 ± 0.64	18.21 ± 0.28	19.77 ± 0.23	22.05 ± 0.93	10.33 ± 1.38
3z	45.47 ± 2.85	40.12 ± 1.71	38.10 ± 2.52	42.66 ± 2.94	39.61 ± 1.36
4a	3.97 ± 0.54	5.14 ± 0.17	3.25 ± 0.06	3.99 ± 0.04	2.20 ± 0.05
4b	1.93 ± 0.28	3.08 ± 0.10	1.65 ± 0.20	2.45 ± 0.08	1.33 ± 0.13
4c	2.01 ± 0.21	3.00 ± 0.15	2.18 ± 0.03	2.55 ± 0.23	1.34 ± 0.02
4d	2.89 ± 0.10	3.29 ± 0.01	2.83 ± 0.06	3.02 ± 0.01	2.00 ± 0.02
4e	2.28 ± 0.13	2.62 ± 0.17	2.16 ± 0.03	2.21 ± 0.07	1.47 ± 0.02
4f	2.89 ± 0.15	3.06 ± 0.01	3.02 ± 0.01	3.03 ± 0.01	2.20 ± 0.04
4g	2.24 ± 0.20	3.00 ± 0.14	3.05 ± 0.03	2.78 ± 0.19	1.41 ± 0.01
4h	2.99 ± 0.12	3.08 ± 0.01	2.56 ± 0.04	2.42 ± 0.07	1.68 ± 0.04
4i	2.49 ± 0.08	3.18 ± 0.04	2.84 ± 0.10	3.06 ± 0.01	2.26 ± 0.03
4j	1.73 ± 0.26	2.16 ± 0.25	2.68 ± 0.02	2.45 ± 0.15	1.62 ± 0.04
4k	2.67 ± 0.17	3.01 ± 0.11	2.41 ± 0.03	2.07 ± 0.06	1.89 ± 0.01
4l	3.03 ± 0.01	1.98 ± 0.05	2.24 ± 0.20	2.71 ± 0.10	1.30 ± 0.03
4m	2.96 ± 0.09	3.03 ± 0.07	2.99 ± 0.01	2.83 ± 0.21	2.29 ± 0.21
4n	2.62 ± 0.06	2.63 ± 0.18	1.12 ± 0.11	2.16 ± 0.11	2.33 ± 0.01
4o	2.86 ± 0.16	2.78 ± 0.10	2.01 ± 0.18	2.48 ± 0.08	2.24 ± 0.06
4p	2.18 ± 0.17	2.81 ± 0.25	1.81 ± 0.23	2.33 ± 0.05	1.72 ± 0.02
4q	1.52 ± 0.22	1.04 ± 0.24	1.92 ± 0.26	1.49 ± 0.14	1.59 ± 0.02
4r	3.04 ± 0.01	3.35 ± 0.05	5.56 ± 0.06	3.80 ± 0.22	3.80 ± 0.08
4s	2.91 ± 0.16	1.97 ± 0.02	2.39 ± 0.08	2.85 ± 0.18	2.09 ± 0.03
4t	2.72 ± 0.29	2.06 ± 0.01	2.37 ± 0.17	2.87 ± 0.16	2.21 ± 0.04
4u	1.55 ± 0.18	1.67 ± 0.03	1.24 ± 0.20	1.67 ± 0.07	1.40 ± 0.03
4v	6.33 ± 0.44	5.53 ± 0.55	6.67 ± 0.16	5.39 ± 0.38	4.10 ± 0.16
4w	2.96 ± 0.16	2.28 ± 0.05	2.18 ± 0.01	2.67 ± 0.07	1.61 ± 0.01
4x	3.17 ± 0.01	3.13 ± 0.01	3.05 ± 0.01	3.19 ± 0.08	2.90 ± 0.01
gefitinib	24.19 ± 1.22	26.47 ± 0.23	29.01 ± 0.24	23.92 ± 0.31	13.26 ± 0.17
doxorubicin	1.12 ± 0.15	2.06 ± 0.29	1.53 ± 0.06	1.07 ± 0.05	0.94 ± 0.10

[a] IC_{50} is half of the maximum inhibitory concentration, which was obtained from three independent experiments and displayed with the mean ± standard deviation (SD).

Table 4
Cytotoxic activity (IC_{50}, μM)[a] and Selectivity Index (SI) of compounds **4n** and **4q**.

Compd.	SGC-7901	HL-7702	SI[b]
4n	1.12 ± 0.11	2.33 ± 0.01	2.08
doxorubicin	1.53 ± 0.06	0.94 ± 0.10	0.61
Compd.	Eca-109	HL-7702	SI
4q	1.04 ± 0.24	1.59 ± 0.02	1.53
doxorubicin	2.06 ± 0.29	0.94 ± 0.10	0.46

[a] Values were displayed as mean ± SD from three experiments.
[b] Selectivity Index (SI) = IC_{50} value normal cell/IC_{50} value cancer cell.

acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3d**. White solid, yield 84%, m.p.: 234–237 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.32–7.25 (m, 4H), 7.05–7.03 (m, 1H), 5.64 (s, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.00 (d, J = 15.3 Hz, 1H), 1.19 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.94 (d, J = 2.6 Hz, 6H), 0.81 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.84, 138.02, 136.49, 128.71, 127.76, 126.42, 87.93, 71.26, 54.84, 52.84, 49.91, 46.78, 41.50, 40.74, 40.50,

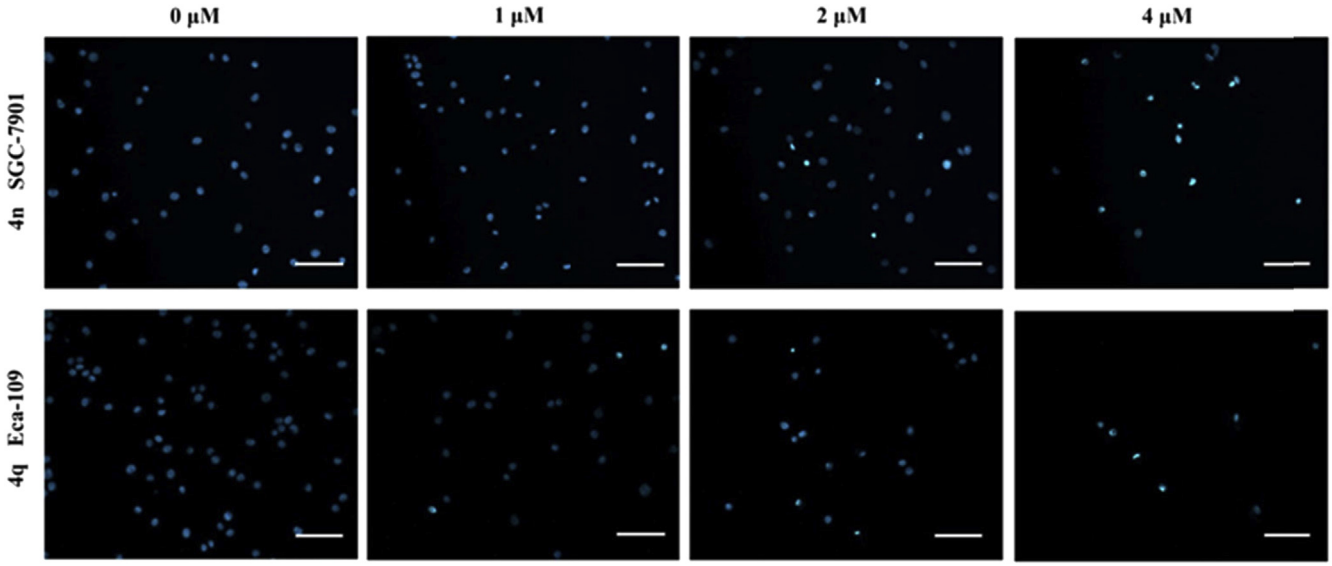


Fig. 2. Hoechst 33342 staining for apoptosis detection. SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h, and stained nuclei by Hoechst 33342 staining. Representative photomicrographs from three independent experiments. Scale bars: 30 μm.

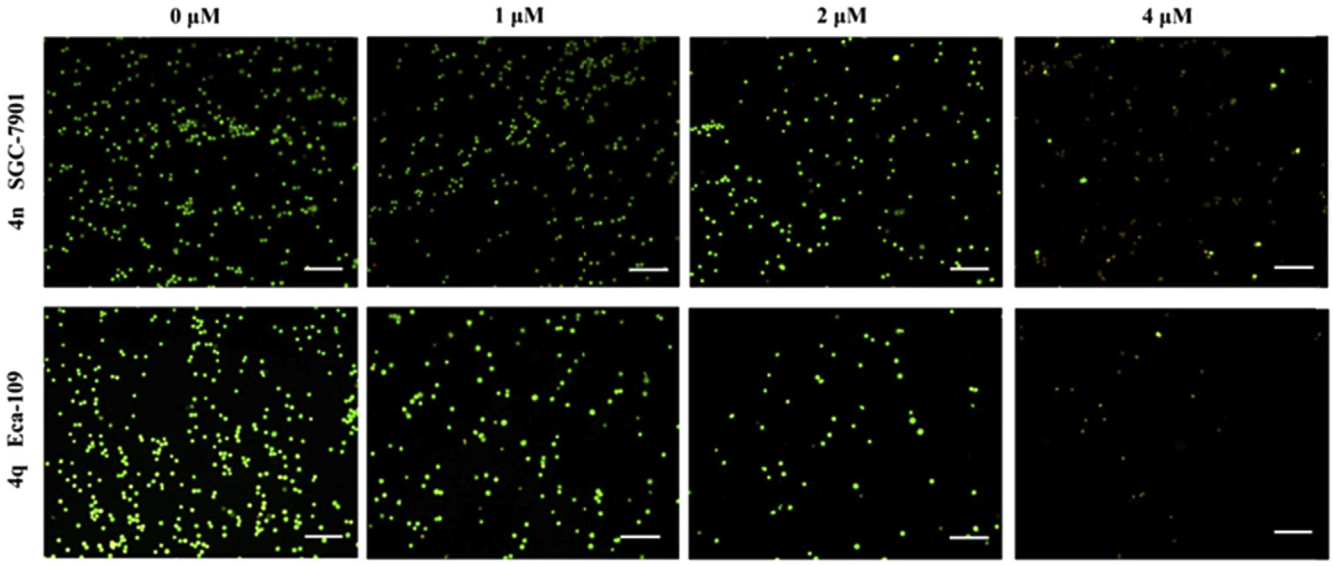


Fig. 3. Cell status were detected by Acridine Orange (AO)/Propidium Iodide (PI) double-stained cell nucleus method. SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h, and fluorescence microscope analysis after staining with AO/PI. Representative photomicrographs from three independent experiments. Scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

39.04, 38.69, 36.76, 36.29, 34.31, 33.75, 32.99, 32.74, 28.77, 26.54, 26.46, 26.23, 21.50, 21.34, 18.87, 16.38, 15.41, 13.45. HRMS (ESI^+) *m/z* calcd for $C_{37}H_{53}N_3O_1$ $[M+H]^+$: 556.4261, found 556.4267.

4.1.1.5. 1′-(4-Methylbenzyl)-19, 28- epoxy-, (19β)-1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazole ***3e****.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 4-methylbenzylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3e**. Light yellow solid, yield 35%, m.p.: 280–282 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.11 (d, J = 7.7 Hz, 2H, H-6′ and H-10′), 6.94 (d, J = 7.7 Hz, 2H, H-7′ and H-9′), 5.60 (s, 2H, H-4′), 3.79 (d, J = 7.8 Hz, 1H, H_a-28), 3.56 (s, 1H, H-19), 3.46 (d, J = 7.9 Hz, 1H, H_b-28), 3.00 (d, J = 15.3 Hz, 1H, H_a-1), 2.31 (s, 3H, H-11′), 2.21 (d, J = 15.3 Hz, 1H, H_b-1), 1.19 (s, 3H, CH_3-23), 1.07 (s, 3H, CH_3-24), 1.02 (s, 3H, CH_3-25), 0.94 (s, 6H, CH_3-26 and CH_3-27), 0.81 (d, J = 2.8 Hz, 6H, CH_3-29 and CH_3-30); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 141.81 (C-3), 137.95 (C-8′), 137.49 (C-5′), 133.44 (C-2), 129.39 (C-7′ and C-9′), 126.41 (C-6′ and C-10′), 87.95 (C-19), 71.28 (C-28), 54.81 (C-5), 52.69 (C-4′), 49.90 (C-9), 46.77 (C-18), 41.51 (C-20), 40.73 (C-14), 40.49 (C-8), 39.03 (C-1), 38.68 (C-4), 36.75 (C-16), 36.30 (C-17), 34.30 (C-13), 33.75 (C-7), 32.98 (C-10), 32.72 (C-21), 28.82 (C-30), 28.77 (C-23), 26.54 (C-15), 26.48 (C-12), 26.24 (C-22), 24.57 (C-29), 21.49 (C-24), 21.37 (C-11), 21.09 (C-11'), 18.89 (C-6), 16.41 (C-25), 15.42 (C-26), 13.49 (C-27). HRMS (ESI^+) *m/z* calcd for $C_{38}H_{55}N_3O_1$ $[M+H]^+$: 570.4417, found 570.4423.

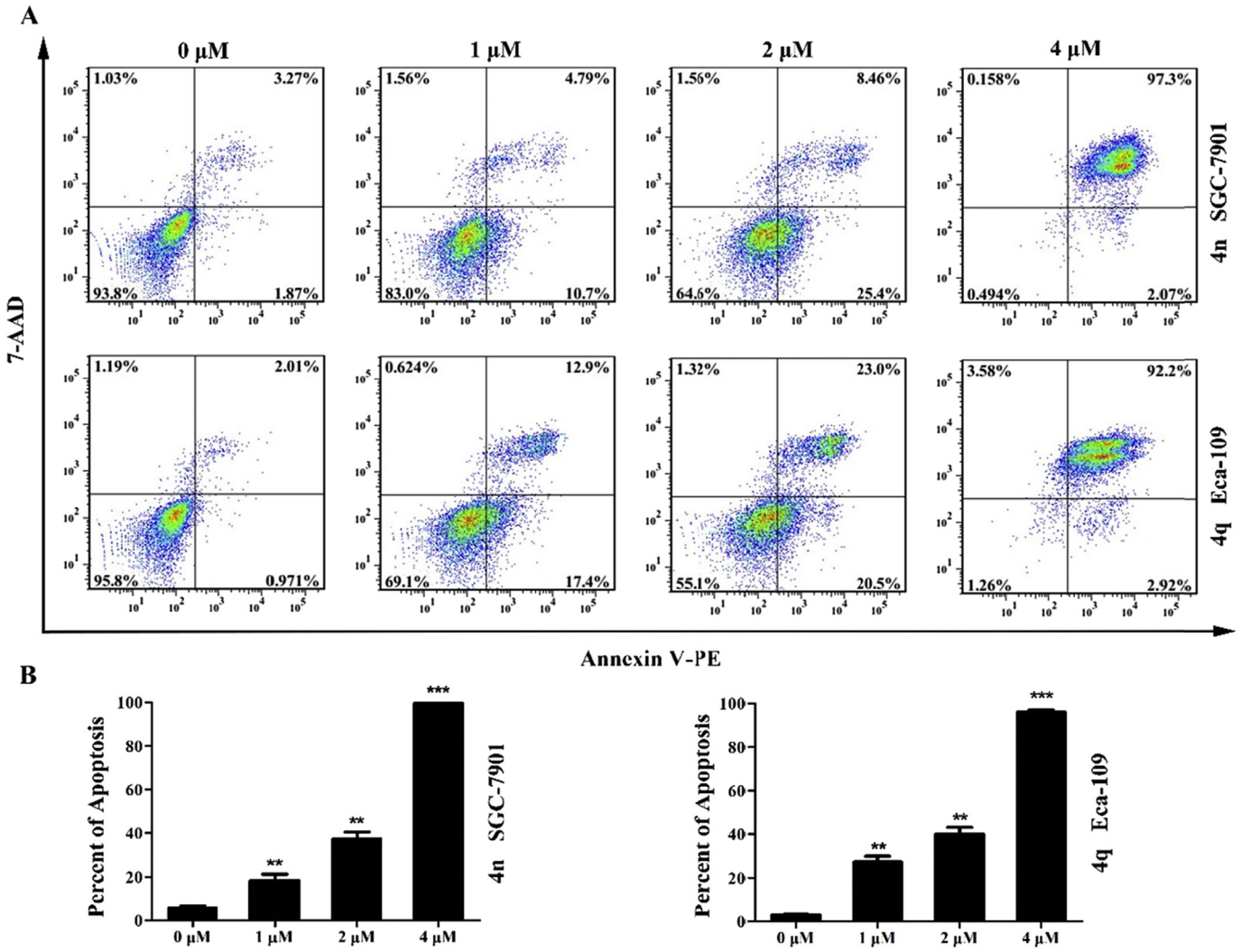


Fig. 4. The apoptosis rate of SGC-7901 cells and Eca-109 cells was discovered by Annexin V-PE/7-AAD dual-staining assay. (A) SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h, and flow cytometry analysis after staining with Annexin V-PE/7-AAD. (B) Quantitative data analysis for the number of cells (% of total) in apoptosis for different treatment groups. Data were presented as mean ± SD (n = 3), Student's *t*-test, $^{**}P < 0.01$, $^{***}P < 0.001$.

4.1.1.6. 1′-(4-Fluorobenzyl)-19, 28- epoxy-, (19β) -1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazole ***3f***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 4-fluorobenzylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were *Compound* reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3f**. White solid, yield 99%, m.p.: 268–270 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (qd, *J* = 8.9, 3.8 Hz, 4H), 5.61 (s, 2H), 3.79 (d, *J* = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, *J* = 7.7 Hz, 1H), 3.00 (d, *J* = 15.3 Hz, 1H), 2.21 (d, *J* = 15.3 Hz, 1H), 1.19 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.94 (d, *J* = 2.3 Hz, 6H), 0.81 (d, *J* = 2.6 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.54, 161.09, 141.98, 137.99, 128.28, 128.20, 115.81, 115.60, 87.94, 71.27, 54.76, 52.18, 49.89, 46.76, 41.50, 40.74, 40.48, 39.03, 38.63, 36.74, 36.30, 34.29, 33.74, 32.96, 32.71, 28.81, 26.54, 26.46, 26.23, 24.56, 21.49, 21.41, 18.87, 16.42, 15.42, 13.48. HRMS (ESI^+) *m/z* calcd for $C_{37}H_{52}F_1N_3O_1$ $[M+H]^+$: 574.4166, found 574.4165.

4.1.1.7. 1′-(3-Fluorobenzyl)-19, 28- epoxy-, (19β) -1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazole ***3g***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 3-fluorobenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3g**. White solid, yield 93%, m.p.: 245–247 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28 (td, *J* = 8.0, 5.8 Hz, 1H), 6.96 (td, *J* = 8.5, 2.6 Hz, 1H), 6.82 (dt, *J* = 7.7, 1.3 Hz, 1H), 6.74 (dt, *J* = 9.7, 2.1 Hz, 1H), 5.64 (d, *J* = 4.1 Hz, 2H), 3.79 (dd, *J* = 7.8, 1.6 Hz, 1H), 3.57 (s, 1H), 3.46 (d, *J* = 7.8 Hz, 1H), 3.01 (d, *J* = 15.3 Hz, 1H), 2.22 (d, *J* = 15.3 Hz, 1H), 1.19 (s, 3H), 1.09 (s, 3H), 1.02 (s, 3H), 0.94 (d, *J* = 2.4 Hz, 6H), 0.82 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.27, 161.81, 142.02, 139.04, 138.97, 138.12, 130.40, 130.32, 122.00, 121.97, 114.94, 114.73, 113.67, 113.44, 87.95, 71.26, 54.72, 52.27, 52.25, 49.88, 46.75, 41.50, 40.73, 40.48, 39.03, 38.58, 36.73, 36.29, 34.28, 33.73, 32.95, 32.71, 28.81, 26.53, 26.45, 26.22, 24.56, 21.49, 21.40, 18.86, 16.43, 15.42, 13.48. HRMS (ESI^+) *m/z* calcd for $C_{37}H_{52}F_1N_3O_1$ $[M+H]^+$: 574.4166, found 574.4172.

4.1.1.8. 1′-(2,4-Difluorobenzyl)-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole ***3h***. Allobetulone (80 mg, 0.181 mmol),

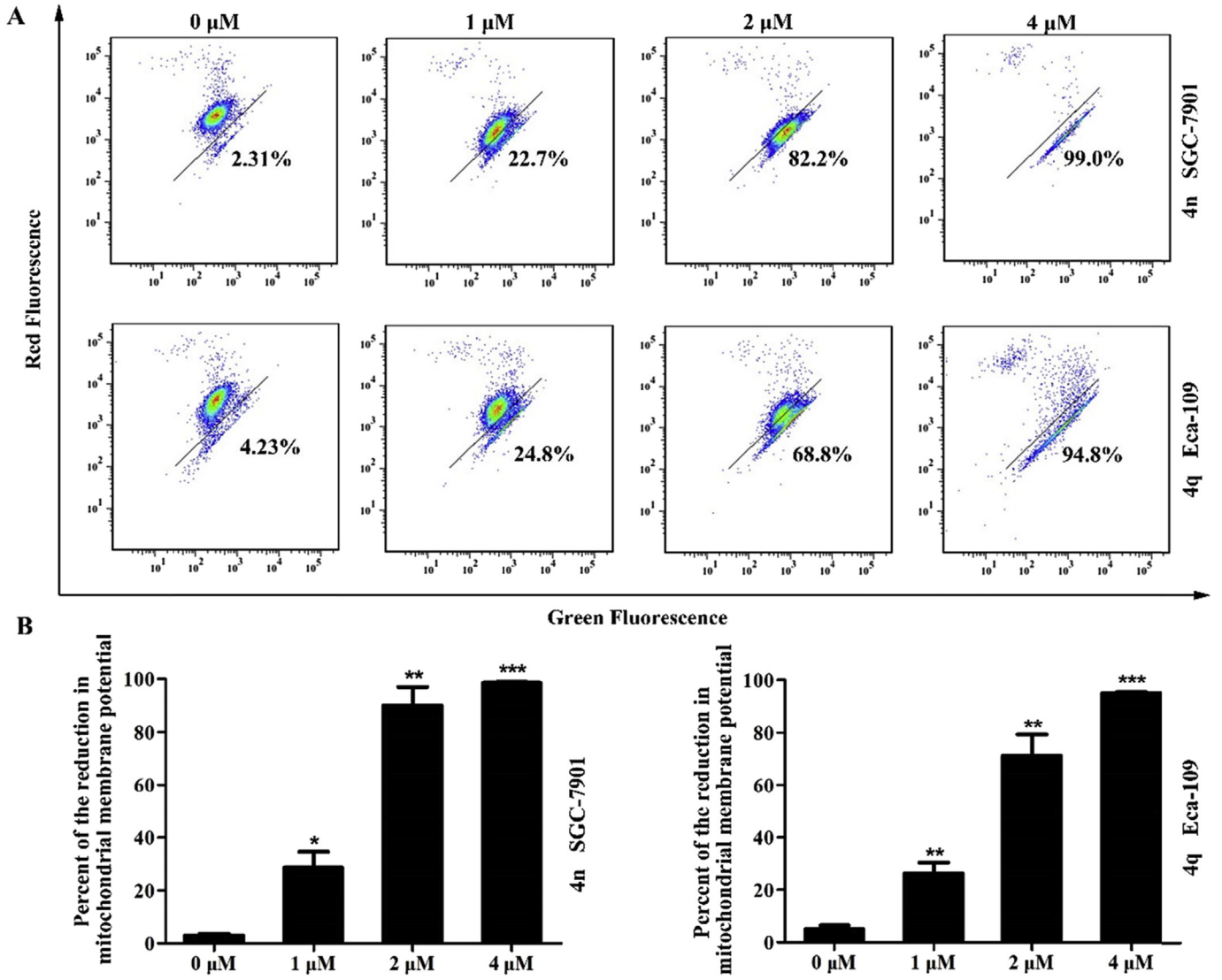


Fig. 5. JC-1 staining method was used to detect the effect of compounds **4n** and **4q** on mitochondrial membrane potential. (A) SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h, and flow cytometry analysis after staining with JC-1. (B) Quantitative data analysis for the number of cells (% of total) which lost mitochondrial membrane potential for different treatment groups. Data were presented as mean ± SD (n = 3), Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

4-nitrophenyl azide (1 eq., 0.181 mmol), 2,4-difluorobenzylamine (1.5 eq., 0.272 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3h**. White solid, yield 67%, mp: 228–230 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.82–6.67 (m, 3H), 5.55 (s, 2H), 3.72 (dd, J = 7.8, 1.5 Hz, 1H), 3.49 (s, 1H), 3.39 (d, J = 7.8 Hz, 1H), 2.93 (d, J = 15.3 Hz, 1H), 2.14 (d, J = 15.3 Hz, 1H), 1.14 (s, 3H), 1.04 (s, 3H), 0.95 (s, 3H), 0.87 (d, J = 2.0 Hz, 6H), 0.74 (d, J = 2.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.87, 162.75, 160.39, 160.27, 159.65, 159.53, 157.18, 157.06, 141.02, 137.15, 128.74, 128.69, 128.64, 128.59, 118.68, 118.64, 118.54, 118.50, 110.94, 110.90, 110.73, 110.69, 103.01, 102.76, 102.50, 86.91, 70.24, 53.71, 48.86, 45.73, 44.96, 44.91, 40.48, 39.71, 39.46, 38.02, 37.55, 35.71, 35.27, 33.26, 32.68, 31.93, 31.69, 27.79, 27.49, 25.51, 25.43, 25.20, 23.54, 22.88, 20.47, 20.16, 17.83, 15.39, 14.40, 12.46. HRMS (ESI^+) m/z calcd for $C_{37}H_{51}F_2N_3O_1$ $[M+H]^+$: 592.4072, found 592.4077.

4.1.1.9. 1′-(4-Trifluoromethylbenzyl)-19, 28- epoxy-, (19β) -1′H-olean- 2- eno[2, 3- d] [1, 2, 3] triazole ***3i***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 4-(trifluoromethyl)benzylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3i.** White solid, yield 64%, m.p.:268–270 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.57 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.0 Hz, 2H), 5.70 (d, J = 3.2 Hz, 2H), 3.79 (dd, J = 7.7, 1.5 Hz, 1H), 3.57 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.01 (d, J = 15.4 Hz, 1H), 2.23 (d, J = 15.3 Hz, 1H), 1.18 (s, 3H), 1.09 (s, 3H), 1.02 (s, 3H), 0.94 (d, J = 1.6 Hz, 6H), 0.82 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.11, 140.47, 138.20, 126.74, 125.78, 125.74, 87.93, 71.26, 54.71, 52.29, 49.88, 46.75, 41.50, 40.73, 40.48, 39.04, 38.58, 36.72, 36.29, 34.28, 33.71, 32.93, 32.70, 28.87, 28.81, 26.52, 26.45, 26.21, 24.55, 21.49, 18.84, 16.43, 15.41, 13.47. HRMS (ESI^+) m/z calcd for $C_{38}H_{52}F_3N_3O_1$ $[M+H]^+$: 624.4134, found 624.4128.

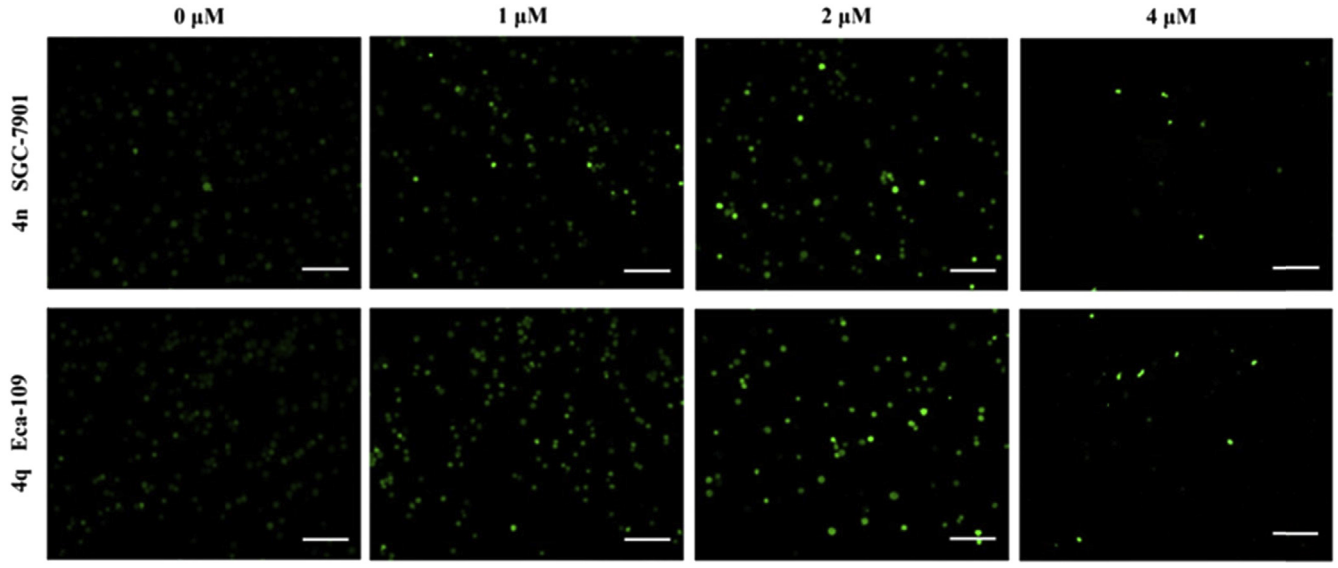


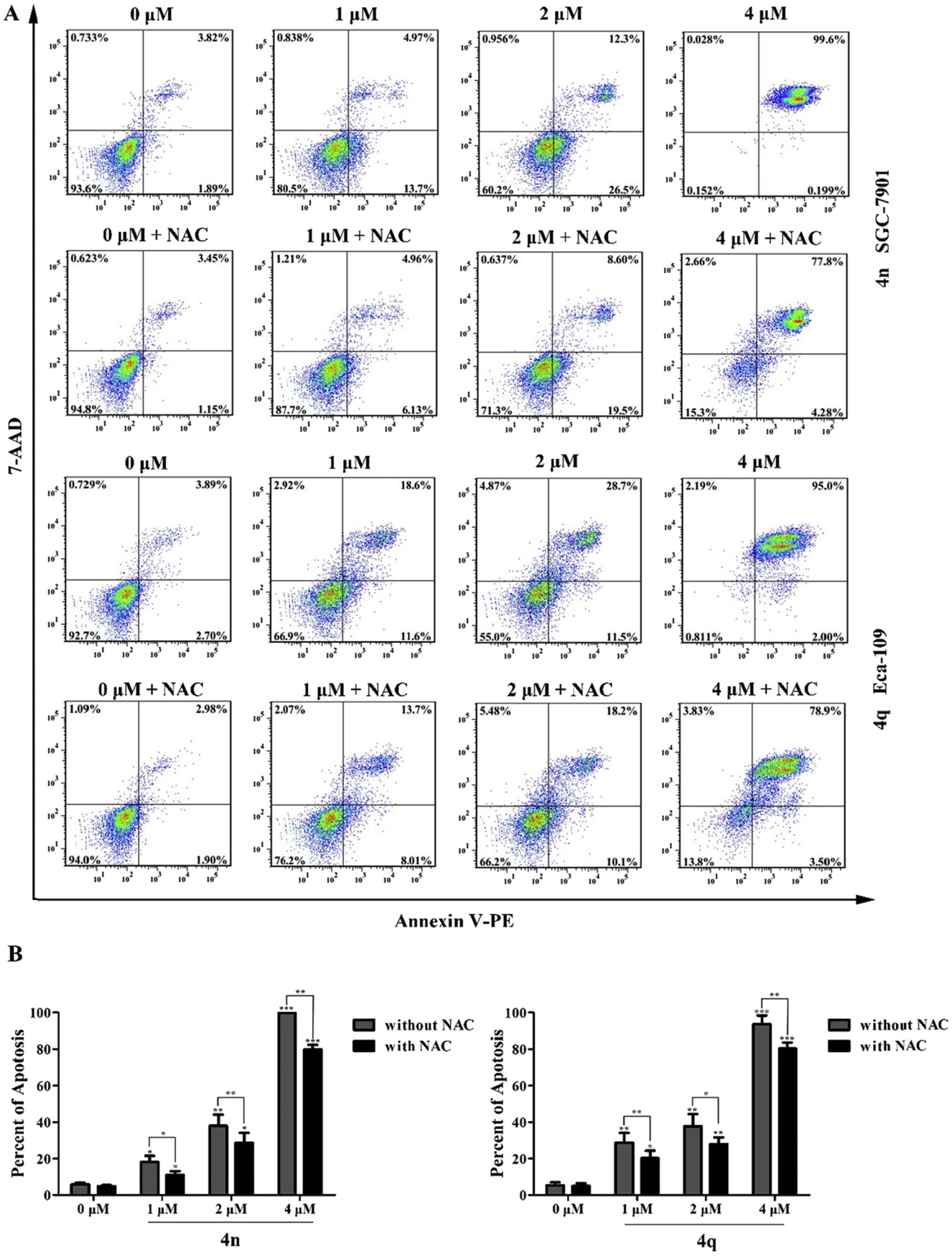
Fig. 6. DCFH-DA staining was used to detect the production of ROS. SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h, and fluorescence microscope analysis after staining with DCFH-DA. Representative photomicrographs from three independent experiments. Scale bars: 50 μm.

*4.1.1.10. 1'-(4-Chlorobenzyl)-19, 28- epoxy-, (19β) -1'H- olean- 2- eno [2, 3- d] [1, 2, 3] triazole **3j**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 4-chlorobenzylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3j**. White solid, yield 87%, m.p.: >300 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28 (d, J = 8.1 Hz, 2H), 6.99 (d, J = 8.2 Hz, 2H), 5.61 (s, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.00 (d, J = 15.3 Hz, 1H), 2.21 (d, J = 15.3 Hz, 1H), 1.18 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.94 (d, J = 2.1 Hz, 6H), 0.81 (d, J = 2.8 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 142.02, 138.05, 134.97, 133.71, 128.94, 127.83, 87.92, 71.26, 54.71, 52.17, 49.87, 46.74, 41.49, 40.72, 40.46, 39.02, 38.59, 36.72, 36.29, 34.27, 33.71, 32.93, 32.70, 28.83, 26.52, 26.45, 26.21, 24.56, 21.44, 18.85, 16.43, 15.41, 13.48. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{52}Cl_1N_3O_1$ $[M+H]^+$: 590.3871, found 590.3887.

*4.1.1.11. 1'-(3-Chlorobenzyl)-19, 28- epoxy-, (19β) -1'H- olean- 2- eno [2, 3- d] [1, 2, 3] triazole **3k**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 3-chlorobenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3k**. White solid, yield 86%, m.p.: 188–190 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.24 (d, J = 3.7 Hz, 2H), 7.06 (s, 1H), 6.92–6.88 (m, 1H), 5.61 (d, J = 2.6 Hz, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.01 (d, J = 15.3 Hz, 1H), 2.23 (d, J = 15.3 Hz, 1H), 1.19 (s, 3H), 1.09 (s, 3H), 1.02 (s, 3H), 0.98–0.91 (m, 6H), 0.82 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.04, 138.47, 138.11, 134.78, 130.04, 128.08, 126.57, 124.56, 87.93, 71.26, 54.71, 52.17, 49.88, 46.75, 41.50, 40.73, 40.48, 39.03, 38.60, 36.73, 36.29, 34.28, 33.72, 32.94, 32.71, 28.87, 28.81, 26.53, 26.45, 26.22, 24.56, 21.48, 21.45, 18.85, 16.42, 15.41, 13.47. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{52}Cl_1N_3O_1$ $[M+H]^+$: 590.3871, found 590.3886.

*4.1.1.12. 1'-(2-Chlorobenzyl)-19, 28- epoxy-, (19β) -1'H- olean- 2- eno [2, 3- d] [1, 2, 3] triazole **3l**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 2-chlorobenzylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3l**. White solid, yield 69%, m.p.: 244–246 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39 (d, J = 7.9 Hz, 1H), 7.29–7.08 (m, 2H), 6.49 (d, J = 7.7 Hz, 1H), 5.73 (s, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.02 (d, J = 15.3 Hz, 1H), 2.24 (d, J = 15.3 Hz, 1H), 1.17 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.95 (s, 6H), 0.82 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.15, 138.36, 134.39, 131.50, 129.28, 128.96, 127.61, 127.27, 87.94, 71.26, 54.70, 50.25, 49.89, 46.75, 41.50, 40.73, 40.48, 39.07, 38.60, 36.73, 36.29, 34.28, 33.72, 32.94, 32.70, 30.93, 28.81, 28.56, 26.52, 26.46, 26.22, 24.56, 21.49, 21.18, 18.84, 16.43, 15.42, 13.47. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{52}Cl_1N_3O_1$ $[M+H]^+$: 590.3871, found 590.3875.

*4.1.1.13. 1'-(3,4-Dichlorobenzyl)-19, 28- epoxy-, (19β) -1'H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole **3m**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 3,4-dichlorobenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3m**. White solid, yield 85%, m.p.: 271–274 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.38 (d, J = 8.3 Hz, 1H), 7.17 (d, J = 2.2 Hz, 1H), 6.88 (dd, J = 8.3, 2.2 Hz, 1H), 5.58 (d, J = 2.9 Hz, 2H), 3.79 (d, J = 7.8 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.00 (d, J = 15.5 Hz, 1H), 2.22 (d, J = 15.3 Hz, 1H), 1.19 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.94 (s, 6H), 0.82 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.12, 138.12, 136.62, 133.04, 132.08, 130.75, 128.48, 125.82, 87.92, 71.25, 54.67, 51.62, 49.87, 46.74, 41.49, 40.73, 40.47, 39.03, 38.55, 36.72, 36.28, 34.27, 33.70, 32.92, 32.70, 28.93, 28.80, 26.52, 26.44, 26.21, 24.55, 21.52, 21.48, 18.84, 16.41, 15.41, 13.47. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{51}Cl_2N_3O_1$ $[M+H]^+$: 624.3481, found 624.3479.


A
0 μM
1 μM
2 μM
4 μM
0.733%
3.82%
93.6%
1.89%
0.838%
4.97%
80.5%
13.7%
0.956%
12.3%
60.2%
26.5%
0.028%
99.6%
0.152%
0.199%
0 μM + NAC
1 μM + NAC
2 μM + NAC
4 μM + NAC
0.623%
3.45%
94.8%
1.15%
1.21%
4.96%
87.7%
6.13%
0.637%
8.60%
71.3%
19.5%
2.66%
77.8%
15.3%
4.28%
4n SGC-7901
0 μM
1 μM
2 μM
4 μM
0.729%
3.89%
92.7%
2.70%
2.92%
18.6%
66.9%
11.6%
4.87%
28.7%
55.0%
11.5%
2.19%
95.0%
0.811%
2.00%
0 μM + NAC
1 μM + NAC
2 μM + NAC
4 μM + NAC
1.09%
2.98%
94.0%
1.90%
2.07%
13.7%
76.2%
8.01%
5.48%
18.2%
66.2%
10.1%
3.83%
78.9%
13.8%
3.50%
4q Eca-109
7-AAD
Annexin V-PE
B
Percent of Apotosis
100
80
60
40
20
0
0 μM
1 μM
2 μM
4 μM
4n
without NAC
with NAC
Percent of Apotosis
100
80
60
40
20
0
0 μM
1 μM
2 μM
4 μM
4q
without NAC
with NAC

4.1.1.14. 1′-(2-Chloro-6-fluorobenzyl)-19, 28- epoxy-, (19β) -1′H-olean- 2- eno[2, 3- d] [1, 2, 3] triazole ***3n***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 2-chloro-6-fluorobenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3n**. Orange solid, yield 72%, m.p.:> 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36–7.22 (m, 2H), 7.05 (ddd, J = 9.5, 8.0, 1.4 Hz, 1H), 5.57 (dd, J = 5.1, 1.5 Hz, 2H), 3.80 (dd, J = 7.9, 1.7 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 2.96 (d, J = 15.3 Hz, 1H), 2.18 (d, J = 15.4 Hz, 1H), 1.44 (s, 3H), 1.32 (s, 3H), 1.04 (s, 3H), 0.95 (d, J = 3.5 Hz, 6H), 0.83 (d, J = 9.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.22, 160.72, 141.43, 137.67, 136.05, 136.00, 130.60, 130.50, 125.55, 125.51, 120.93, 120.76, 114.49, 114.26, 87.92, 71.27, 54.84, 49.90, 46.75, 41.50, 40.73, 40.51, 39.09, 38.61, 36.73, 36.29, 34.29, 33.71, 33.00, 32.71, 28.80, 28.19, 26.55, 26.45, 26.23, 24.55, 21.49, 20.85, 18.93, 16.38, 15.44, 13.51. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{51}Cl_1F_1N_3O_1$ $[M+H]^+$: 608.3777, found 608.3752.

4.1.1.15. 1′-(4-Methoxybenzyl)-19, 28- epoxy-, (19β) -1′H- olean- 2-eno[2, 3- d] [1, 2, 3] triazole ***3o***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 4-methoxybenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3o**. Yellow solid, yield 74%, m.p.: 228–230 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.01 (d, J = 8.3 Hz, 2H), 6.83 (d, J = 8.3 Hz, 2H), 5.58 (s, 2H), 3.78 (s, 3H), 3.56 (s, 1H), 3.46 (d, J = 7.7 Hz, 1H), 2.99 (d, J = 15.2 Hz, 1H), 2.22 (d, J = 16.0 Hz, 1H), 1.20 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.94 (d, J = 3.2 Hz, 6H), 0.82 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.22, 141.76, 137.85, 128.44, 127.88, 114.12, 87.93, 71.27, 55.25, 54.87, 52.42, 49.91, 46.78, 41.50, 40.74, 40.50, 39.02, 38.69, 36.76, 36.29, 34.31, 33.75, 28.78, 26.53, 26.47, 26.23, 24.54, 21.49, 21.35, 18.89, 16.37, 15.41, 13.46. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{55}N_3O_2$ $[M+H]^+$: 586.4366, found 586.4384.

4.1.1.16. 1′-(3-Methoxybenzyl)-19, 28- epoxy-, (19β) -1′H- olean- 2-eno[2, 3- d] [1, 2, 3] triazole ***3p***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 3-methoxybenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3p**. Yellow solid, yield 96%, m.p.: 207–209 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.21 (t, J = 7.9 Hz, 1H), 6.79 (dd, J = 8.3, 2.5 Hz, 1H), 6.62–6.54 (m, 2H), 5.61 (s, 2H), 3.80 (dd, J = 10.2, 7.2 Hz, 1H), 3.73 (s, 3H), 3.56 (s, 1H), 3.45 (d, J = 7.8 Hz, 1H), 3.00 (d, J = 15.3 Hz, 1H), 2.21 (d, J = 15.3 Hz, 1H), 1.20 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.94 (d, J = 2.1 Hz, 6H), 0.81 (d, J = 2.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.99, 141.81, 138.07, 138.03, 129.73, 118.69, 113.31, 112.06, 87.93, 71.25, 55.19, 54.83, 52.73, 49.90, 46.78, 41.49, 40.73, 40.49, 39.03, 38.67, 36.75, 36.28, 34.30, 33.74, 32.98, 32.73, 28.80, 28.75, 26.53, 26.46, 26.22, 24.54, 21.49, 21.33, 18.87, 16.35, 15.40, 13.45. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{55}N_3O_2$ $[M+H]^+$: 586.4366, found 586.4370.

4.1.1.17. 1′-(2-Methoxybenzyl)-19, 28- epoxy-, (19β) -1′H- olean- 2-eno[2, 3- d] [1, 2, 3] triazole ***3q***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 2-methoxybenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3q**. Yellow solid, yield 89%, m.p.: 257–259 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.30–7.17 (m, 1H), 6.84 (dd, J = 17.3, 8.1 Hz, 2H), 6.52 (d, J = 7.5 Hz, 1H), 5.63 (s, 2H), 3.87 (s, 3H), 3.79 (d, J = 7.9 Hz, 1H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.01 (d, J = 15.1 Hz, 1H), 2.29–2.12 (m, 1H), 1.18 (s, 3H), 1.08 (s, 3H), 1.02 (s, 3H), 0.94 (s, 6H), 0.82 (d, J = 2.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.75, 141.69, 138.13, 128.67, 127.33, 125.12, 120.78, 109.95, 87.93, 71.26, 55.35, 54.86, 49.91, 47.69, 46.78, 41.50, 40.74, 40.50, 39.07, 38.70, 36.76, 36.28, 34.31, 33.74, 33.00, 32.74, 28.80, 28.43, 26.54, 26.24, 24.55, 21.50, 21.01, 18.87, 16.39, 15.41, 13.46. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{55}N_3O_2$ $[M+H]^+$: 586.4366, found 586.4388.

4.1.1.18. 1′-(3,4,5-Trimethoxybenzyl)-19, 28- epoxy-, (19β) -1′H-olean- 2- eno[2, 3- d] [1, 2, 3] triazole ***3r***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 3,4,5-trimethoxybenzylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3r**. White solid, yield 95%, m.p.: 240–242 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.26 (s, 2H), 5.57 (s, 2H), 3.79 (d, J = 13.1 Hz, 10H), 3.56 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.00 (d, J = 15.3 Hz, 1H), 2.22 (d, J = 15.3 Hz, 1H), 1.23 (s, 3H), 1.10 (s, 3H), 1.02 (s, 3H), 0.94 (s, 6H), 0.82 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 153.49, 153.49, 141.89, 138.06, 137.46, 132.11, 103.49, 87.92, 71.25, 60.86, 56.09, 54.78, 52.87, 49.85, 46.74, 41.49, 40.72, 40.46, 39.02, 38.64, 36.72, 36.28, 34.27, 33.75, 32.93, 32.69, 28.82, 28.80, 26.52, 26.45, 26.21, 24.55, 21.48, 21.38, 18.89, 16.32, 15.40, 13.48. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{59}N_3O_4$ $[M+H]^+$: 646.4578, found 646.4573.

4.1.1.19. 1′-(4-Methoxyphenethyl)-19, 28- epoxy-, (19β) -1′H- olean-2- eno[2, 3- d] [1, 2, 3] triazole ***3s***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 4-methoxyphenethylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3s**. White solid, yield 90%, m.p.: 248–251 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.05 (d, J = 8.5 Hz, 2H, H-7′ and H-11′), 6.82 (d, J = 8.5 Hz, 2H, H-8′ and H-10′), 4.47 (td, J = 7.9, 7.4, 2.4 Hz, 2H, H-4′), 3.78 (s, 3H, H-12′), 3.56 (s, 1H, H-19), 3.46 (d, J = 7.8 Hz, 1H, H-28), 3.28 (p, J = 7.7, 7.0 Hz, 2H, H-5′), 2.96 (d, J = 15.2 Hz, 1H, H-1), 1.22 (s, 3H, CH_3-23), 1.05 (s, 3H, CH_3-24), 1.02 (s, 3H, CH_3-25), 0.94 (s, 6H, CH_3-26 and CH_3-27), 0.79 (d, J = 12.7 Hz, 6H, CH_3-29 and CH_3-30); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.58 (C-9′), 141.01 (C-3), 137.71 (C-2), 129.83 (C-7′ and C-11′), 129.70 (C-6′), 114.14 (C-8′ and C-10′), 87.93 (C-19), 71.26 (C-28), 55.27 (C-12′), 54.87 (C-5), 51.02 (C-4′), 49.87 (C-9), 46.77 (C-18), 41.49 (C-20), 40.73 (C-14), 40.49 (C-8), 39.00 (C-1), 38.58 (C-4), 36.75 (C-16), 36.28 (C-17), 36.21 (C-5'), 34.30 (C-13), 33.58 (C-7), 32.97 (C-10), 32.73 (C-21), 28.80 (C-30), 28.64 (C-23), 26.54 (C-15), 26.46 (C-12), 26.23 (C-22), 24.54 (C-29), 21.48 (C-24), 21.14 (C-11), 18.89 (C-6), 16.23 (C-25), 15.41 (C-26), 13.47 (C-27). HRMS (ESI$^+$) m/z calcd for $C_{39}H_{57}N_3O_2$ $[M+H]^+$: 600.4523, found 600.4530.

Fig. 7. NAC as an antioxidant to detect the effect of ROS on apoptosis of SGC-7901 cells and Eca-109 cells. (A) SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h with or without pretreatment of NAC. Flow cytometry analysis after staining with Annexin V-PE/7-AAD. (B) Quantitative data analysis for the number of cells (% of total) in apoptosis for different treatment groups. Data were presented as mean ± SD (n = 3), Student's *t*-test, *P < 0.05, **P < 0.01, ***P < 0.001.

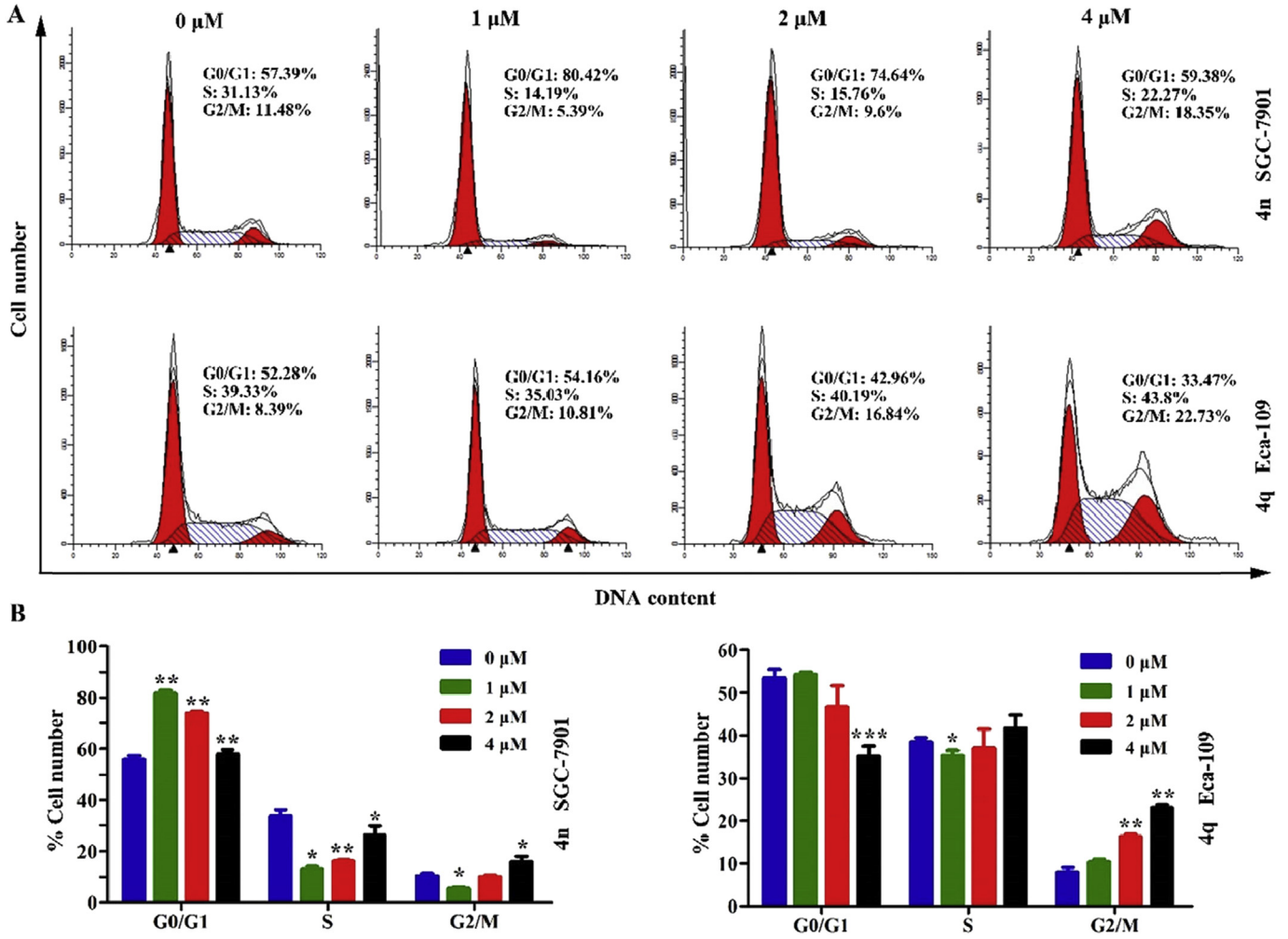


Fig. 8. Influence of compounds **4n** and **4q** on cell cycle. (A) SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h, and flow cytometry analysis after staining DNA content with PI. Representative experiments from three independent experiments. (B) Quantitative data analysis for the number of cells (% of total) in each cell phase for different treatment groups. Data were presented as mean ± SD (n = 3), Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

4.1.1.20. 1'-(3-Methoxyphenethyl)-19, 28- epoxy-, (19β) -1'H- olean-2- eno[2, 3- d] [1, 2, 3] triazole ***3t***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 3-methoxyphenethylamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3t**. White solid, yield 88%, m.p.: 225–227 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19 (t, J = 7.8 Hz, 1H), 6.81-6.72 (m, 2H), 6.65 (s, 1H), 4.51 (td, J = 8.1, 7.7, 2.6 Hz, 2H), 3.79 (d, J = 8.3 Hz, 1H), 3.75 (s, 3H), 3.56 (s, 1H), 3.31 (h, J = 6.2 Hz, 2H), 3.02–2.90 (m, 1H), 2.16 (t, J = 7.7 Hz, 1H), 1.22 (s, 3H), 1.03 (d, J = 6.5 Hz, 6H), 0.94 (s, 6H), 0.79 (d, J = 14.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.86, 141.02, 139.15, 137.78, 129.69, 121.10, 114.44, 112.39, 87.91, 71.25, 55.15, 54.87, 50.69, 49.86, 46.77, 41.49, 40.73, 40.48, 38.99, 38.59, 37.11, 36.75, 36.27, 34.30, 33.58, 32.96, 32.73, 28.80, 28.61, 26.54, 26.45, 26.23, 24.54, 21.48, 21.10, 18.89, 16.22, 15.40, 13.47. HRMS (ESI^+) m/z calcd for $C_{39}H_{57}N_3O_2$ $[M+H]^+$: 600.4523, found 600.4523.

4.1.1.21. 1'-(2-Methoxyphenethyl)-19, 28- epoxy-, (19β) -1'H- olean-2- eno[2, 3- d] [1, 2, 3] triazole ***3u***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (2 eq., 0.362 mmol), 2-methoxyphenethylamine (2.8 eq., 0.507 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3u**. White solid, yield 90%, m.p.: 297–299 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.22 (d, J = 1.8 Hz, 1H), 7.10 (dd, J = 7.4, 1.9 Hz, 1H), 6.86 (dt, J = 7.2, 3.1 Hz, 2H), 4.51 (dd, J = 9.5, 6.3 Hz, 2H), 3.85 (s, 3H), 3.56 (s, 1H), 3.46 (d, J = 7.7 Hz, 1H), 3.32 (dd, J = 9.2, 6.6 Hz, 2H), 2.96 (d, J = 15.1 Hz, 1H), 2.22–2.11 (m, 1H), 1.28 (s, 3H), 1.12 (s, 3H), 1.02 (s, 3H), 0.98 (d, J = 23.9 Hz, 6H), 0.79 (d, J = 12.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.63, 140.85, 137.65, 130.92, 128.27, 125.84, 120.74, 110.25, 87.93, 71.26, 55.08, 54.93, 49.87, 48.86, 46.78, 41.50, 40.74, 40.50, 39.01, 38.63, 36.76, 36.28, 34.32, 33.61, 33.01, 32.75, 32.64, 28.81, 28.32, 26.55, 26.24, 24.55, 21.49, 20.83, 18.93, 16.26, 15.41, 13.48. HRMS (ESI^+) m/z calcd for $C_{39}H_{57}N_3O_2$ $[M+H]^+$: 600.4523, found 600.4531.

4.1.1.22. 1'-(2-Picolyl)-19, 28- epoxy-, (19β) -1'H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole ***3v***. Allobetulone (100 mg, 0.226 mmol), 4-nitrophenyl azide (2 eq., 0.452 mmol), 2-pyridinemethanamine (2 eq., 0.452 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography

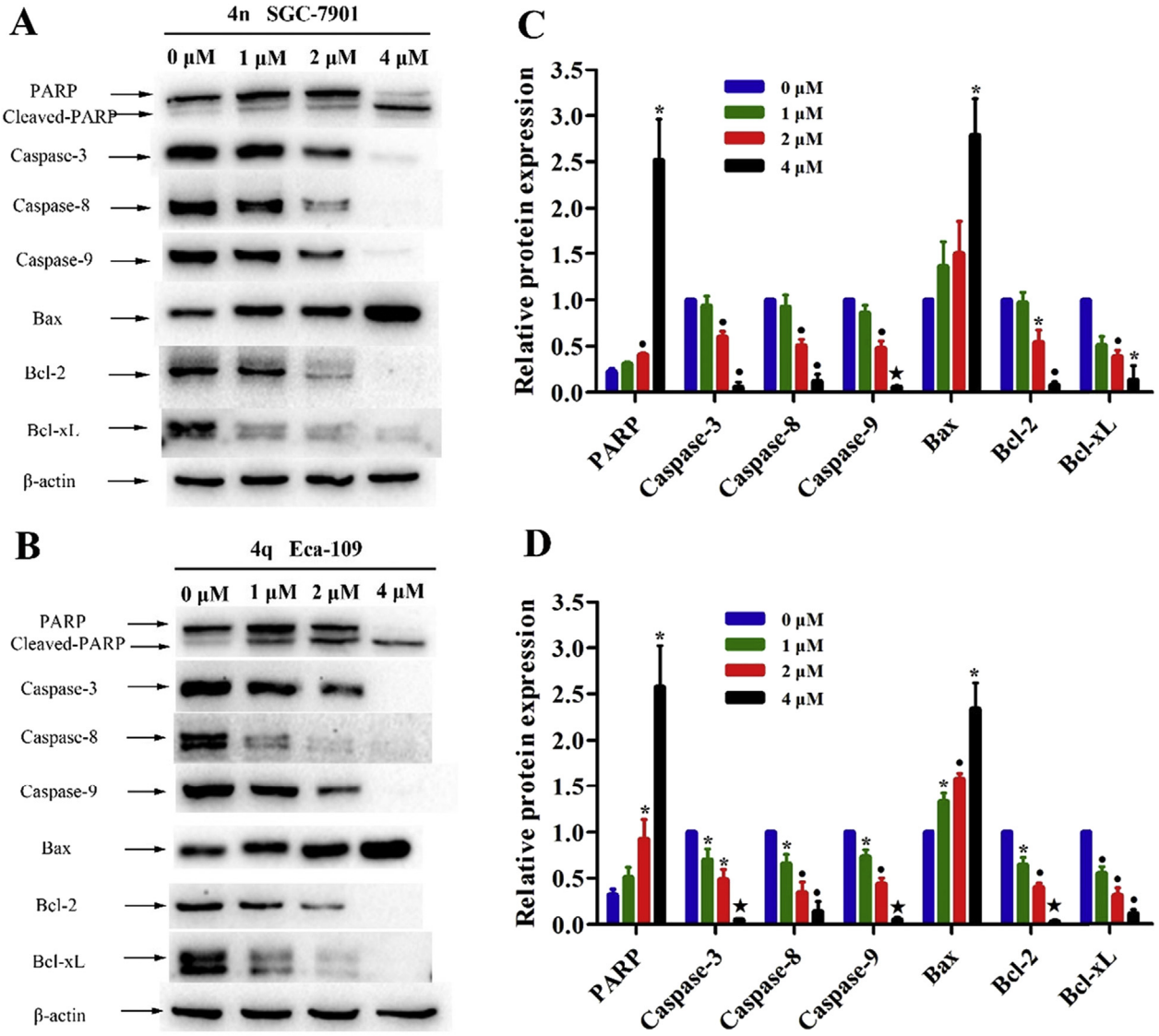


Fig. 9. Effects of **4n** and **4q** on apoptosis related protein expression. (A, B) SGC-7901 cells were treated with compound **4n** (0, 1, 2 and 4 μM) for 48 h and Eca-109 cells were treated with compound **4q** (0, 1, 2 and 4 μM) for 48 h. Cells were lysed, and the cell lysates were analyzed by Western blotting using the corresponding antibodies. Representative image of Western blotting analysis of PARP, cleaved PARP, caspase-3, caspase-8, capase-9, Bax, Bcl-2, and Bcl-xL. (C, D) Quantitative analysis of Western blotting from (A) and (B) by Image Lab program with β-actin as the internal control. Data were presented as the mean ± SD (n = 3), Student's *t*-test, **P* < 0.05, ●*P* < 0.01, ★*P* < 0.001.

(dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 1/1) to get **3v**. White solid, yield 90%, m.p.: 286–288 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.56 (dt, *J* = 4.7, 1.4 Hz, 1H), 7.60 (td, *J* = 7.7, 1.8 Hz, 1H), 7.20 (ddd, *J* = 7.5, 4.9, 1.1 Hz, 1H), 6.75 (d, *J* = 7.9 Hz, 1H), 5.79 (d, *J* = 1.8 Hz, 2H), 3.79 (dd, *J* = 7.8, 1.6 Hz, 1H), 3.56 (s, 1H), 3.46 (d, *J* = 7.8 Hz, 1H), 3.01 (d, *J* = 15.3 Hz, 1H), 2.27–2.15 (m, 1H), 1.17 (s, 3H), 1.07 (s, 3H), 1.02 (s, 3H), 0.94 (d, *J* = 3.4 Hz, 6H), 0.81 (d, *J* = 2.2 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 156.56, 149.14, 142.13, 137.15, 138.36, 122.71, 121.03, 87.93, 71.26, 54.73, 54.67, 49.88, 46.75, 41.50, 40.73, 40.48, 39.05, 38.60, 36.73, 36.29, 34.28, 33.71, 32.94, 32.70, 28.81, 28.58, 26.52, 26.46, 26.22, 24.56, 21.49, 21.21, 18.84, 16.41, 15.41, 13.47. HRMS (ESI$^+$) *m/z* calcd for $C_{36}H_{52}N_4O_1$ $[M+H]^+$: 557.4213, found 557.4222.

4.1.1.23. 1′-(2,2-Dimethoxyethyl)-19, 28- epoxy-, (19β) -1′H- olean-2- eno[2, 3- d] [1, 2, 3] triazole ***3w***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 2,2-dimethoxyethanamine (1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3w**. White solid, yield 84%, m.p.: 254–256 °C. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 5.02 (t, *J* = 5.4 Hz, 1H), 4.41 (d, *J* = 5.3 Hz, 2H), 3.79 (d, *J* = 7.9 Hz, 1H), 3.56 (s, 1H), 3.46 (d, *J* = 7.8 Hz, 1H), 3.39 (d, *J* = 9.6 Hz, 6H), 2.96 (d, *J* = 15.1 Hz, 1H), 2.18 (t, *J* = 7.7 Hz, 1H), 1.32 (s, 3H), 1.22 (s, 3H), 1.03 (s, 3H), 0.94 (s, 6H), 0.81 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 141.09, 138.57, 104.39, 87.93, 71.26, 56.10, 56.01, 54.98, 51.90, 49.86, 46.75, 41.50, 40.73, 40.49, 38.97, 38.53, 36.73, 36.28, 34.28, 33.66, 32.97, 32.71, 28.88, 28.81, 26.53, 26.46, 26.22, 24.55, 21.53, 21.47, 18.89, 16.34, 15.42, 13.49. HRMS (ESI$^+$) *m/z* calcd for $C_{34}H_{55}N_3O_3$ $[M+H]^+$: 554.4315, found 554.4314.

4.1.1.24. 1′-(2,2-Diphenylethyl)-19, 28- epoxy-, (19β) -1′H- olean- 2-eno[2, 3- d] [1, 2, 3] triazole ***3x***. Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), 2,2-diphenylethanamine

(1.3 eq., 0.235 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 6/1 to 2/1) to get **3x**. Light yellow solid, yield 79%, m.p.: 273–275 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34–7.11 (m, 6H), 7.06–6.98 (m, 4H), 5.24 (dd, J = 8.6, 6.9 Hz, 1H), 4.93–4.74 (m, 2H), 3.78 (dd, J = 7.8, 1.5 Hz, 1H), 3.55 (s, 1H), 3.45 (d, J = 7.8 Hz, 1H), 2.92 (d, J = 15.2 Hz, 1H), 2.12 (d, J = 15.2 Hz, 1H), 1.04 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.81 (s, 3H), 0.74 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.32, 141.20, 140.89, 138.37, 128.49, 128.39, 128.28, 126.83, 126.78, 87.95, 71.25, 55.12, 54.14, 50.38, 49.94, 46.89, 41.52, 40.79, 40.56, 38.91, 38.73, 36.85, 36.28, 34.42, 33.54, 33.02, 32.85, 28.74, 28.67, 26.57, 26.46, 26.27, 24.49, 21.53, 20.97, 18.90, 15.93, 15.35, 13.36. HRMS (ESI$^+$) m/z calcd for $C_{44}H_{59}N_3O_1$ $[M+H]^+$: 646.4730, found 646.4732.

*4.1.1.25. 1′-(2-(3-Indolyl)ethyl)-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazole **3y**.* Allobetulone (80 mg, 0.181 mmol), 4-nitrophenyl azide (1 eq., 0.181 mmol), tryptamine (1 eq., 0.181 mmol), acetic acid (2 μL), 4 Å molecular sieves (50 mg) and toluene (0.8 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 2/1 to 1/4) to get **3y**. Yellow solid, yield 93%, m.p.: 150–152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.77 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.15 (dt, J = 28.4, 7.6 Hz, 2H), 6.95 (s, 1H), 4.58 (td, J = 8.3, 7.7, 4.0 Hz, 2H), 3.80 (d, J = 7.9 Hz, 1H), 3.60–3.43 (m, 3H), 2.96 (d, J = 15.1 Hz, 1H), 2.21–2.09 (m, 1H), 1.01 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.93 (s, 3H), 0.82 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.99, 137.87, 136.29, 127.23, 122.52, 122.05, 119.54, 118.33, 111.75, 111.35, 87.97, 71.25, 54.90, 50.30, 49.86, 46.78, 41.51, 40.74, 40.49, 39.01, 38.58, 36.75, 36.29, 34.33, 33.62, 32.97, 32.76, 28.83, 28.60, 26.87, 26.54, 26.46, 26.23, 24.56, 21.49, 21.09, 18.88, 16.27, 15.41, 13.48. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{56}N_4O_1$ $[M+H]^+$: 609.4526, found 609.4539.

*4.1.1.26. 1,4-Bis(19,28-epoxy-, (19β)-1′H-olean-2-eno[2,3-d][1,2,3] triazole)butane **3z**.* Allobetulone (160 mg, 0.362 mmol), 4-nitrophenyl azide (2 eq., 0.724 mmol), 1, 4-butanediamine (0.45 eq., 0.163 mmol), acetic acid (4 μL), 4 Å molecular sieves (50 mg) and toluene (1.5 mL) were reacted according to the general procedure. The mixture was purified by column chromatography (dichloromethane followed by petroleum ether/ethyl acetate 2/1 to 1/4) to get **3z**. Yellow solid, yield 77%, m.p.: >300 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.80 (d, J = 7.6 Hz, 2H), 3.57 (s, 2H), 3.46 (d, J = 7.9 Hz, 2H), 2.94 (d, J = 15.3 Hz, 2H), 1.26–1.07 (m, 25H), 1.03 (s, 3H), 0.99–0.82 (m, 22H), 0.80 (d, J = 8.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 141.26, 137.66, 87.94, 71.28, 54.83, 49.89, 48.81, 46.77, 41.51, 40.74, 40.50, 39.03, 38.54, 36.74, 36.30, 34.29, 33.69, 32.98, 32.72, 28.81, 28.70, 27.73, 26.54, 26.46, 26.23, 24.56, 21.48, 21.40, 18.90, 16.38, 15.44, 13.50. HRMS (ESI$^+$) m/z calcd for $C_{64}H_{100}N_6O_2$ $[M+H]^+$: 985.7980, found 985.7983.

*4.1.2. General procedure for the synthesis of 1,2,3-triazolium salt derivatives of allobetulin **4a-x***

A solution of 1,2,3-triazole derivative of allobetulin (1 eq.) and excess methyl iodide (20 eq.) in dry acetonitrile under nitrogen atmosphere, was stirred in screw cap reaction tube at 85 °C. After the reaction finished, the product was purified by column chromatography (dichloromethane/acetonitrile 10/1 followed by dichloromethane/methanol 20/1 to 15/1 (**4a**-**w**) or 10/1 (**4x**)) to get 1,2,3-triazolium salt derivatives of allobetulin **4a**-**x**.

*4.1.2.1. 1′-Butyl-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazolium iodide **4a**.* Prepared from compound **3a** (50 mg, 0.096 mmol), methyl iodide (1.92 mmol, 120 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 72%, m.p.: 124–127 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.53 (tt, J = 14.1, 6.4 Hz, 2H), 4.27 (s, 3H), 3.78 (d, J = 7.9 Hz, 1H), 3.54 (s, 1H), 3.50–3.43 (m, 1H), 2.91 (d, J = 16.2 Hz, 1H), 2.79–2.69 (m, 1H), 2.20–2.05 (m, 3H), 1.55 (s, 3H), 1.34 (s, 3H), 1.02 (s, 3H), 0.97–0.90 (m, 12H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 144.54, 138.99, 87.94, 71.22, 52.84, 52.70, 49.16, 46.64, 41.48, 40.86, 40.52, 38.98, 38.68, 36.64, 36.35, 36.28, 34.54, 34.19, 32.68, 32.41, 31.38, 28.80, 27.69, 26.48, 26.11, 26.04, 24.58, 21.69, 21.08, 19.92, 18.54, 17.62, 15.34, 13.60, 13.53. HRMS (ESI$^+$) m/z calcd for $C_{35}H_{58}N_3O_1$ $[M]^+$: 536.4579, found 536.4580.

*4.1.2.2. 1′-Hexyl-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazolium iodide **4b**.* Prepared from compound **3b** (50 mg, 0.091 mmol), methyl iodide (1.82 mmol, 113 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 88%, m.p.: 123–125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.45 (t, J = 7.7 Hz, 2H, H-5′), 4.20 (s, 3H, CH_3-4′), 3.70 (d, J = 10 Hz, 1H, H_a-28), 3.46 (s, 1H, H-19), 3.38 (d, J = 7.7 Hz, 1H, H_b-28), 2.89 (d, J = 16.1 Hz, 1H, H-1), 1.46 (s, 3H, CH_3-23), 1.27 (s, 3H, CH_3-24), 0.95 (s, 3H, CH_3-25), 0.84 (d, J = 10.4 Hz, 12H, CH_3-26, CH_3-27, CH_3-10′ and CH_3-29), 0.75 (s, 3H, CH_3-30); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 144.63 (C-3), 138.87 (C-2), 87.82 (C-19), 71.11 (C-28), 53.19 (C-5), 52.96 (C-9), 49.25 (C-5′), 46.70 (C-18), 41.40 (C-20), 40.86 (C-14), 40.55 (C-8), 39.01 (C-1), 38.88 (C-4), 36.68 (C-16), 36.39 (C-4′), 36.17 (C-17), 34.51 (C-13), 34.24 (C-7), 32.72 (C-10), 32.46 (C-21), 30.92 (C-8′), 29.21 (C-30), 28.69 (C-23), 27.77 (C-6′), 26.45 (C-15), 26.11 (C-12), 26.08 (C-22), 26.00 (C-7′), 24.45 (C-29), 22.13 (C-9′), 21.71 (C-24), 21.03 (C-11), 18.47 (C-6), 17.54 (C-25), 15.25 (C-26), 13.65 (C-10'), 13.45 (C-27). HRMS (ESI$^+$) m/z calcd for $C_{37}H_{62}N_3O_1$ $[M]^+$: 564.4892, found 564.4886.

*4.1.2.3. 1′-Octyl-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazolium iodide **4c**.* Prepared from compound **3c** (50 mg, 0.087 mmol), methyl iodide (1.74 mmol, 108 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 59%, m.p.: 104–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.57–4.43 (m, 2H), 4.27 (s, 3H), 3.78 (d, J = 8.0 Hz, 1H), 3.54 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 2.89 (d, J = 16.2 Hz, 1H), 2.77 (d, J = 16.2 Hz, 1H), 1.55 (s, 3H), 1.33 (s, 3H), 1.03 (s, 3H), 0.94 (s, 9H), 0.91 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 144.47, 139.07, 87.97, 71.23, 53.03, 52.64, 49.12, 46.64, 41.49, 40.87, 40.53, 38.97, 38.57, 36.65, 36.36, 36.28, 34.53, 34.20, 32.69, 32.40, 31.66, 29.50, 28.99, 28.91, 28.80, 27.65, 26.61, 26.49, 26.12, 26.04, 24.58, 22.57, 21.69, 21.11, 18.56, 17.60, 15.35, 14.07, 13.60. HRMS (ESI$^+$) m/z calcd for $C_{37}H_{62}N_3O_1$ $[M]^+$: 592.5205, found 592.5215.

*4.1.2.4. 1′-Benzyl-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno [2, 3- d] [1, 2, 3] triazolium iodide **4d**.* Prepared from compound **3d** (40 mg, 0.072 mmol), methyl iodide (1.44 mmol, 90 μL) and dry acetonitrile (0.5 mL). Reaction time is 2.5 h. Yellow solid, yield 85%, mp.: 158–160 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49–7.25 (m, 5H), 5.91–5.72 (m, 2H), 4.27 (s, 3H), 3.79 (d, J = 7.8 Hz, 1H), 3.54 (s, 1H), 3.46 (d, J = 10.4 Hz, 1H), 2.98–2.74 (m, 1H), 1.55 (s, 3H), 1.28 (s, 3H), 0.93 (d, J = 5.4 Hz, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.08, 139.51, 131.80, 129.38, 129.36, 128.05, 87.94, 71.22, 56.42, 52.69, 49.19, 46.64, 41.48, 40.85, 40.52, 38.96, 38.74, 36.65, 36.46, 36.28, 34.72, 34.19, 32.68, 32.40, 30.95, 28.80, 27.79, 26.48, 26.11, 26.05, 24.58, 21.70, 21.28, 18.53, 17.67, 15.34, 13.58. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{56}N_3O_1$ $[M]^+$: 570.4423, found 570.4429.

4.1.2.5. 1'-(4-Methylbenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4e***. Prepared from compound **3e** (50 mg, 0.089 mmol), methyl iodide (1.78 mmol, 111 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 61%, m.p.: 213–215 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.28 (s, 2H), 7.22 (s, 2H), 5.84–5.65 (m, 1H), 4.24 (s, 3H), 3.77 (d, J = 10.4 Hz, 1H), 3.54 (s, 1H), 3.46 (d, J = 10.4 Hz, 1H), 2.95–2.75 (m, 1H), 2.36 (s, 3H), 1.57 (s, 3H), 1.28 (s, 3H), 0.93 (d, J = 7.7 Hz, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 144.99, 139.41, 139.36, 129.97, 128.72, 128.08, 87.91, 71.20, 56.35, 52.73, 49.20, 46.62, 41.46, 40.83, 40.50, 38.97, 38.90, 36.63, 36.47, 36.26, 34.70, 34.17, 32.66, 32.40, 28.79, 27.84, 26.46, 26.10, 26.04, 24.56, 21.70, 21.29, 21.21, 18.51, 17.71, 15.32, 13.58. HRMS (ESI^+) m/z calcd for $C_{39}H_{58}N_3O_1$ $[M]^+$: 584.4579, found 584.4556.

4.1.2.6. 1'-(4-Fluorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4f***. Prepared from compound **3f** (40 mg, 0.0698 mmol), methyl iodide (1.396 mmol, 87 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 58%, m.p.: 297–299 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.42 (dd, J = 8.5, 5.1 Hz, 2H), 7.11 (t, J = 8.5 Hz, 2H), 5.90–5.70 (m, 2H), 4.23 (s, 3H), 3.78 (d, J = 7.9 Hz, 1H), 3.65 (s, 1H), 3.46 (d, J = 10.4 Hz, 1H), 2.89 (dd, J = 16.3, 2.8 Hz, 1H), 2.02 (d, J = 2.3 Hz, 1H), 1.55 (s, 3H), 1.32 (s, 3H), 1.02 (s, 3H), 0.98–0.89 (m, 9H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.84, 161.53, 145.25, 139.57, 130.52, 130.41, 127.47, 116.42, 116.12, 87.92, 71.18, 55.92, 52.97, 49.35, 46.76, 41.48, 40.93, 40.63, 39.03, 38.71, 36.73, 36.54, 36.24, 34.75, 34.30, 32.77, 32.50, 28.71, 27.88, 26.51, 26.13, 26.05, 24.49, 21.75, 21.25, 18.51, 17.58, 15.30, 13.48. HRMS (ESI^+) m/z calcd for $C_{38}H_{55}F_1N_3O_1[M]^+$: 588.4328, found 588.4335.

4.1.2.7. 1'-(3-Fluorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4g***. Prepared from compound **3g** (50 mg, 0.087 mmol), methyl iodide (1.74 mmol, 108 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 34%, m.p.: 147–149 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (td, J = 8.1, 5.8 Hz, 1H), 7.19–7.06 (m, 2H), 7.01 (dt, J = 9.1, 2.2 Hz, 1H), 5.88 (dd, J = 37.6, 15.5 Hz, 2H), 4.29 (s, 3H), 3.78 (d, J = 7.9 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 8.1 Hz, 1H), 2.90 (d, J = 16.3 Hz, 1H), 1.54 (s, 3H), 1.29 (s, 3H), 1.02 (s, 3H), 0.99–0.89 (m, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 164.17, 161.70, 145.06, 139.62, 134.18, 134.11, 131.28, 131.20, 123.93, 123.90, 116.61, 116.40, 115.06, 114.83, 87.97, 71.23, 55.61, 52.64, 49.15, 46.64, 41.50, 40.87, 40.53, 38.91, 38.27, 36.66, 36.29, 36.25, 34.70, 34.20, 32.68, 32.41, 28.79, 27.69, 26.49, 26.12, 26.04, 24.58, 21.67, 21.30, 18.55, 17.46, 15.35, 13.54. HRMS (ESI^+) m/z calcd for $C_{38}H_{55}F_1N_3O_1$ $[M]^+$: 588.4328, found 588.4332.

4.1.2.8. 1'-(2,4-Difluorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4h***. Prepared from compound **3h** (38 mg, 0.0642 mmol), methyl iodide (1.284 mmol, 80 μL) and dry acetonitrile (0.5 mL). Reaction time is 5 h. Yellow solid, yield 59%, m.p.:103–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (td, J = 8.6, 6.1 Hz, 1H), 7.07–6.97 (m, 1H), 6.88 (ddd, J = 10.8, 8.5, 2.5 Hz, 1H), 5.89–5.75 (m, 2H), 4.17 (s, 3H), 3.78 (dd, J = 7.7, 1.4 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 7.9 Hz, 1H), 2.85 (d, J = 16.2 Hz, 1H), 2.73 (d, J = 16.2 Hz, 1H), 1.61 (s, 3H), 1.39 (s, 3H), 1.03 (s, 3H), 0.95 (s, 6H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 165.14, 165.02, 162.63, 162.51, 161.92, 161.80, 159.43, 159.31, 145.14, 139.40, 133.81, 133.76, 133.71, 133.66, 114.79, 114.75, 114.65, 114.61, 112.76, 112.72, 112.55, 112.51, 104.45, 104.20, 103.95, 87.95, 71.22, 52.66, 50.25, 50.21, 49.22, 46.64, 41.50, 40.87, 40.56, 39.00, 38.45, 36.64, 36.32, 36.28, 34.66, 34.19, 32.67, 32.41, 28.80, 27.58, 26.49, 26.11, 26.06, 24.58, 21.70, 20.82, 18.52, 17.68, 15.35, 13.58. HRMS (ESI^+) m/z calcd for $C_{38}H_{54}F_2N_3O_1$ $[M]^+$: 606.4234, found 606.4229.

4.1.2.9. 1'-(4-Trifluoromethylbenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4i***. Prepared from compound **3i** (50 mg, 0.08 mmol), methyl iodide (1.6 mmol, 80 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 35%, m.p.: 178–181 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, J = 8.0 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 5.98–5.80 (m, 2H), 4.23 (s, 3H), 3.78 (d, J = 8.0 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 7.8 Hz, 1H), 1.56 (s, 3H), 1.32 (s, 3H), 1.02 (s, 3H), 0.96–0.92 (m, 9H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.44, 139.66, 135.71, 128.61, 126.17, 87.90, 77.30, 55.79, 52.76, 49.25, 46.64, 41.46, 40.83, 40.52, 38.99, 38.79, 36.63, 36.24, 34.72, 32.66, 32.39, 28.75, 27.86, 26.46, 26.05, 24.52, 21.69, 21.25, 18.46, 17.67, 15.29, 13.51. HRMS (ESI^+) m/z calcd for $C_{39}H_{55}F_3N_3O_1[M]^+$: 638.4296, found 638.4302.

4.1.2.10. 1'-(4-Chlorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4j***. Prepared from compound **3j** (55 mg, 0.0932 mmol), methyl iodide (1.864 mmol, 116 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 30%, m.p.: 292–294 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 8.6 Hz, 2H), 5.89–5.72 (m, 2H), 4.24 (s, 3H), 3.78 (d, J = 7.7 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 7.8 Hz, 1H), 2.87 (d, J = 16.3 Hz, 1H), 2.77 (d, J = 16.3 Hz, 1H), 1.54 (s, 3H), 1.31 (s, 3H), 1.02 (s, 3H), 0.94 (d, J = 1.4 Hz, 6H), 0.92 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.10, 135.54, 139.59, 130.15, 129.76, 129.57, 87.96, 71.22, 55.71, 52.63, 49.17, 46.64, 41.49, 40.86, 40.53, 38.94, 38.47, 36.64, 36.35, 36.28, 34.70, 34.19, 32.67, 32.39, 28.79, 27.75, 26.48, 26.11, 26.04, 24.57, 21.68, 21.32, 18.53, 17.61, 15.35, 13.57. HRMS (ESI^+) m/z calcd for $C_{38}H_{55}Cl_1N_3O_1$ $[M]^+$: 604.4033, found 604.4028.

4.1.2.11. 1'-(3-Chlorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4k***. Prepared from compound **3k** (50 mg, 0.0847 mmol), methyl iodide (1.694 mmol, 106 μL) and dry acetonitrile (0.5 mL). Reaction time is 8 h. Yellow solid, yield 41%, m.p.: 125–128 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: δ 7.40–7.38 (m, 2H), 7.34–7.29 (m, 2H), 5.81 (q, J = 15.4 Hz, 2H), 4.25 (s, 3H), 3.81–3.74 (m, 1H), 3.54 (s, 1H), 3.47 (d, J = 7.9 Hz, 1H), 2.89 (d, J = 16.3 Hz, 1H), 2.77 (d, J = 16.2 Hz, 1H), 1.56 (s, 3H), 1.31 (s, 3H), 1.02 (s, 3H), 0.98–0.91 (m, 10H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.23, 139.62, 135.07, 133.61, 130.85, 129.66, 127.98, 126.78, 87.95, 71.22, 55.70, 52.63, 49.19, 46.63, 41.49, 40.86, 40.53, 38.96, 38.56, 36.64, 36.37, 36.28, 34.70, 34.18, 32.67, 32.39, 28.79, 27.80, 26.48, 26.11, 26.04, 24.58, 21.69, 21.39, 18.52, 17.65, 15.34, 13.57. HRMS (ESI^+) m/z calcd for $C_{38}H_{55}Cl_1N_3O_1$ $[M]^+$: 604.4033, found 604.4035.

4.1.2.12. 1'-(2-Chlorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4l***. Prepared from compound **3l** (50 mg, 0.0847 mmol), methyl iodide (1.694 mmol, 106 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 26%, m.p.: 132–135 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.57 (dd, J = 6.4, 2.9 Hz, 1H), 7.49–7.35 (m, 3H), 5.97–5.78 (m, 2H), 4.20 (s, 3H), 3.78 (d, J = 7.8 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 8.0 Hz, 1H), 1.56 (s, 3H), 1.37 (s, 3H), 1.03 (s, 3H), 0.95 (s, 9H), 0.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 144.99,139.65, 132.99, 131.82, 130.92, 129.63, 129.27, 128.14, 87.98, 71.24, 54.29, 52.51, 49.14, 46.64, 41.50, 40.87, 40.54, 38.96, 38.32, 36.65, 36.34, 36.29, 34.73, 34.21, 32.68, 32.40, 28.80, 27.43, 26.49, 26.14, 26.05, 24.58, 21.68, 20.66, 18.52, 17.62, 15.36, 13.57. HRMS (ESI^+) m/z calcd for $C_{38}H_{55}Cl_1N_3O_1$ $[M]^+$: 604.4033, found 604.4042.

4.1.2.13. 1'-(3,4-Dichlorobenzyl)-3'-methyl-19, 28- epoxy-, (19β) -1'H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4m***. Prepared from compound **3m** (50 mg, 0.08 mmol), methyl iodide (1.6 mmol, 100 μL) and dry acetonitrile (1 mL). Reaction time is 4 h.

Yellow solid, yield 51%, m.p.: 130–133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 2.2 Hz, 1H), 7.39 (dd, J = 8.3, 2.2 Hz, 1H), 5.87–5.68 (m, 2H), 4.21 (s, 3H), 3.81–3.74 (m, 1H), 3.54 (s, 1H), 3.47 (d, J = 7.8 Hz, 1H), 2.88–2.73 (m, 2H), 1.58 (s, 3H), 1.33 (s, 3H), 1.03 (s, 3H), 0.97–0.91 (m, 6H), 0.83 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.21, 139.60, 133.96, 133.36, 131.66, 131.55, 130.04, 128.29, 87.96, 71.23, 55.15, 52.64, 49.19, 46.64, 41.50, 40.87, 40.54, 38.93, 38.32, 36.64, 36.29, 36.23, 34.69, 34.19, 32.66, 32.40, 28.79, 27.77, 26.49, 26.11, 26.05, 24.58, 21.68, 21.38, 18.52, 17.55, 15.35, 13.57. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{54}Cl_2N_3O_1$ $[M]^+$: 638.3643, found 638.3630.

4.1.2.14. 1′-(2-Chloro-6-fluorobenzyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4n***. Prepared from compound **3n** (50 mg, 0.0821 mmol), methyl iodide (1.642 mmol, 106 μL) and dry acetonitrile (0.5 mL). Reaction time is 7 h. Yellow solid, yield 30%, m.p.: 174–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46 (td, J = 8.3, 6.0 Hz, 1H), 7.34 (dt, J = 8.3, 1.1 Hz, 1H), 7.21–7.09 (m, 1H), 5.93–5.74 (m, 2H), 4.21 (s, 3H), 3.78 (dd, J = 8.0, 1.4 Hz, 1H), 3.55 (s, 1H), 3.47 (d, J = 7.8 Hz, 1H), 1.46 (s, 3H), 1.04 (s, 3H), 0.98–0.91 (m, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 163.18, 160.66, 144.92, 139.83, 136.20, 136.25, 132.49, 132.39, 125.99, 125.96, 117.67, 117.50, 114.94, 114.72, 87.99, 71.24, 52.49, 49.08, 46.64, 41.50, 40.90, 40.54, 39.02, 38.89, 36.66, 36.56, 36.29, 34.72, 34.22, 32.70, 32.38, 28.80, 27.41, 26.52, 26.14, 26.02, 24.58, 21.71, 20.57, 18.56, 17.68, 15.37, 13.64. HRMS (ESI$^+$) m/z calcd for $C_{38}H_{54}Cl_1F_1N_3O_1$ $[M]^+$: 622.3939, found 622.3932.

4.1.2.15. 1′-(4-Methoxybenzyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4o***. Prepared from compound **3o** (40 mg, 0.0683 mmol), methyl iodide (1.366 mmol, 85 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 85%, m.p.: 235–237 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31 (d, J = 8.7 Hz, 2H, H-7′ and H-11′), 6.93 (d, J = 8.8 Hz, 2H, H-8′ and H-10′), 5.81–5.64 (m, 2H, H-5′), 4.24 (s, 3H, CH_3-4′), 3.82 (s, 3H, CH_3-12′), 3.78 (d, J = 8.0 Hz, 1H, H_a-28), 3.54 (s, 1H, H-19), 3.46 (d, J = 7.9 Hz, 1H, H_b-28), 2.88 (d, J = 16.3 Hz, 1H, H_a-1), 2.78 (d, J = 16.3 Hz, 1H, H_b-1), 1.57 (s, 3H, CH_3-23), 1.31 (s, 3H, CH_3-24), 1.02 (s, 3H, CH_3-25), 0.93 (d, J = 11.4 Hz, 9H, CH_3-26, CH_3-27 and CH_3-29), 0.82 (s, 3H, CH_3-30); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 160.35 (C-9′), 144.84 (C-3), 139.36 (C-2), 129.95 (C-7′ and C-11′), 123.49 (C-6′), 114.66 (C-8′ and C-10′), 87.95 (C-19), 71.22 (C-28), 56.16 (C-12′), 55.42 (C-5′), 52.67 (C-5), 49.17 (C-9), 46.63 (C-18), 41.49 (C-20), 40.86 (C-14), 40.52 (C-8), 38.94 (C-1), 38.65 (C-4), 36.64 (C-16), 36.44 (C-4'), 36.28 (C-17), 34.70 (C-13), 34.19 (C-7), 32.67 (C-10), 32.40 (C-21), 28.79 (C-30), 27.78 (C-23), 26.48 (C-15), 26.11 (C-12), 26.04 (C-22), 24.57 (C-29), 21.69 (C-24), 21.31 (C-11), 18.54 (C-6), 17.67 (C-25), 15.34 (C-26), 13.59 (C-27). HRMS (ESI$^+$) m/z calcd for $C_{39}H_{58}N_3O_2$ $[M]^+$: 600.4528, found 600.4529.

4.1.2.16. 1′-(3-Methoxybenzyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4p***. Prepared from compound **3p** (50 mg, 0.085 mmol), methyl iodide (1.7 mmol, 106 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 20%, m.p.: 219–221 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (t, J = 8.2 Hz, 1H), 6.98–6.81 (m, 3H), 5.87–5.66 (m, 2H), 4.25 (s, 3H), 3.83 (s, 3H), 3.78 (d, J = 7.8 Hz, 1H), 3.54 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 3.00 (d, J = 15.3 Hz, 1H), 2.21 (d, J = 15.3 Hz, 1H), 1.58 (s, 3H), 1.28 (s, 3H), 1.02 (s, 3H), 0.93 (d, J = 7.6 Hz, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 160.19, 145.13, 139.57, 133.24, 130.49, 120.10, 114.67, 113.88, 87.97, 71.24, 56.29, 55.71, 52.60, 49.15, 46.64, 41.50, 40.87, 40.53, 38.96, 38.65, 36.65, 36.52, 36.29, 34.72, 34.20, 32.68, 32.39, 28.80, 27.73, 26.49, 26.12, 26.04, 24.58, 21.70, 21.34, 18.54, 17.70, 15.35, 13.60. HRMS (ESI$^+$) m/z calcd for $C_{39}H_{58}N_3O_2$ $[M]^+$: 600.4528, found 600.4529.

4.1.2.17. 1′-(2-Methoxybenzyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H-olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4q***. Prepared from compound **3q** (50 mg, 0.085 mmol), methyl iodide (1.7 mmol, 106 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 21%, m.p.: 108–111 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (td, J = 7.9, 1.6 Hz, 1H), 7.30 (dd, J = 7.6, 1.7 Hz, 1H), 7.03 (td, J = 7.5, 1.0 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 5.78 (d, J = 14.6 Hz, 1H), 5.66 (d, J = 14.5 Hz, 1H), 4.23 (s, 3H), 3.81 (s, 3H), 3.78 (d, J = 8.1 Hz, 1H), 3.55 (s, 1H), 3.46 (d, J = 7.8 Hz, 1H), 2.87 (q, J = 16.4 Hz, 2H), 1.58 (s, 3H), 1.36 (s, 3H), 1.03 (s, 3H), 0.94 (d, J = 7.6 Hz, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 156.70, 144.71, 139.15, 131.12, 131.01, 121.18, 110.60, 87.98, 71.24, 55.46, 52.72, 52.13, 49.13, 46.64, 41.50, 40.88, 40.53, 38.98, 38.56, 36.66, 36.45, 36.29, 34.70, 34.22, 32.70, 32.42, 28.80, 27.49, 26.50, 26.14, 26.05, 24.58, 21.69, 20.50, 18.58, 17.60, 15.36, 13.60. HRMS (ESI$^+$) m/z calcd for $C_{39}H_{58}N_3O_2$ $[M]^+$: 600.4528, found 600.4529.

4.1.2.18. 1′-(3,4,5-Trimethoxybenzyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4r***. Prepared from compound **3r** (50 mg, 0.077 mmol), methyl iodide (1.54 mmol, 96 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 65%, m.p.: 145–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.61 (s, 2H), 5.78 (d, J = 15.0 Hz, 1H), 5.67 (d, J = 15.0 Hz, 1H), 4.23 (s, 3H), 3.87 (d, J = 14.6 Hz, 9H), 3.78 (d, J = 7.7 Hz, 1H), 3.54 (s, 1H), 3.47 (d, J = 7.8 Hz, 1H), 2.83 (q, J = 16.3 Hz, 2H), 1.58 (s, 3H), 1.35 (s, 3H), 1.03 (s, 3H), 0.94 (d, J = 4.4 Hz, 9H), 0.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 153.80, 145.03, 139.48, 138.83, 126.87, 105.97, 87.95, 71.22, 60.89, 56.87, 56.68, 52.69, 49.19, 46.64, 41.49, 40.86, 40.53, 38.94, 38.60, 36.64, 36.46, 36.28, 34.74, 34.19, 32.67, 32.41, 28.79, 27.78, 26.49, 26.11, 26.05, 24.57, 21.69, 21.26, 18.55, 17.68, 15.34, 13.58. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{62}N_3O_4$ $[M]^+$: 660.4740, found 660.4735.

4.1.2.19. 1′-(4-Methoxyphenethyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4s***. Prepared from compound **3s** (50 mg, 0.083 mmol), methyl iodide (1.66 mmol, 103 μL) and dry acetonitrile (0.5 mL). Reaction time is 6 h (85 °C) and 12 h (80 °C). Yellow solid, yield 68%, m.p.: 112–115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.12–7.05 (m, 2H), 6.89–6.81 (m, 2H), 4.82–4.60 (m, 2H), 4.30 (s, 3H), 3.78 (s, 3H), 3.54 (s, 1H), 2.88 (d, J = 16.3 Hz, 1H), 2.73 (d, J = 16.2 Hz, 1H), 1.48 (s, 3H), 1.14 (s, 3H), 1.00 (s, 3H), 0.94 (d, J = 5.6 Hz, 6H), 0.84 (d, J = 13.3 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 158.98, 144.93, 139.04, 129.90, 127.64, 114.46, 87.95, 71.21, 55.37, 54.40, 52.62, 49.11, 46.63, 41.48, 40.84, 40.50, 38.93, 38.74, 36.64, 36.34, 36.28, 34.87, 34.47, 34.18, 32.67, 32.35, 28.80, 27.70, 26.47, 26.10, 26.04, 24.58, 21.69, 20.88, 18.50, 17.49, 15.32, 13.58. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{60}N_3O_2$ $[M]^+$: 614.4685, found 614.4682.

4.1.2.20. 1′-(3-Methoxyphenethyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4t***. Prepared from compound **3t** (50 mg, 0.083 mmol), methyl iodide (1.66 mmol, 103 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 86%, m.p.: 93–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.23 (t, J = 8.2 Hz, 1H), 6.84–6.71 (m, 2H), 6.72 (dd, J = 2.5, 1.5 Hz, 2H), 4.86–4.64 (m, 2H), 4.86–4.64 (m, 2H), 4.29 (s, 3H), 3.79 (s, 3H), 3.76 (d, J = 1.9 Hz, 1H), 3.54 (s, 1H), 3.46 (d, J = 7.8 Hz, 3H), 2.87 (d, J = 16.2 Hz, 1H), 2.72 (d, J = 16.3 Hz, 1H), 1.50 (s, 4H), 1.14 (s, 3H), 1.00 (s, 3H), 0.94 (d, J = 5.8 Hz, 6H), 0.84 (d, J = 12.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 160.03, 144.99, 139.06, 137.27, 130.14, 120.97, 114.77, 112.65, 87.94, 71.21, 55.39, 54.05, 52.61, 49.10, 46.62, 41.47, 40.84, 40.49, 38.92, 38.70, 36.63, 36.33, 36.27, 35.69, 34.48, 34.17, 32.66, 32.34, 28.79, 27.70, 26.46, 26.10, 26.03, 24.57, 21.68, 20.83, 18.49, 17.45, 15.31, 13.58. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{60}N_3O_2$ $[M]^+$: 614.4685, found 614.4689.

4.1.2.21. 1′-(2-Methoxyphenethyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4u***. Prepared from compound **3u** (50 mg, 0.083 mmol), methyl iodide (1.66 mmol, 103 μL) and dry acetonitrile (0.5 mL). Reaction time is 6 h (85 °C) and 12 h (80 °C). Yellow solid, yield 31%, m.p.: 118−121 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 (dd, J = 15.7, 1.8 Hz, 1H), 7.08 (dd, J = 7.3, 1.7 Hz, 1H), 6.93−6.86 (m, 2H), 4.83−4.63 (m, 2H), 4.31 (s, 3H), 3.85 (s, 3H), 3.78 (d, J = 7.7 Hz, 1H), 3.55 (s, 1H), 3.45 (dd, J = 12.0, 6.7 Hz, 2H), 2.91−2.76 (m, 2H), 1.52 (s, 3H), 1.19 (s, 3H), 1.01 (s, 3H), 0.94 (d, J = 2.7 Hz, 6H), 0.83 (d, J = 6.1 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 157.51, 144.76, 139.03, 130.90, 129.20, 123.68, 121.04, 110.54, 88.00, 71.24, 55.30, 52.59, 51.99, 49.03, 46.64, 41.50, 40.88, 40.51, 38.91, 38.55, 36.66, 36.39, 36.29, 34.47, 34.22, 32.69, 32.37, 31.92, 28.80, 27.46, 26.50, 26.13, 26.03, 24.59, 21.69, 20.55, 18.57, 17.41, 15.34, 13.61. HRMS (ESI$^+$) m/z calcd for $C_{40}H_{60}N_3O_2$ [M]$^+$: 614.4685, found 614.4680.

4.1.2.22. 1′-(2,2-Dimethoxyethyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4v***. Prepared from compound **3w** (50 mg, 0.0903 mmol), methyl iodide (1.806 mmol, 112 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 58%, m.p.: 217−219 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.02 (dd, J = 6.1, 4.4 Hz, 1H, H-6′), 4.73−4.55 (m, 2H, H-5′), 4.30 (s, 3H, CH_3-4′), 3.78 (d, J = 7.8 Hz, 1H, H-28), 3.55 (s, 1H, H-19), 3.52 (s, 3H, CH_3-8′), 3.47 (s, 3H, CH_3-7′), 2.88 (d, J = 16.2 Hz, 1H, H_a-1), 2.72 (d, J = 16.3 Hz, 1H, H_b-1), 1.54 (s, 3H, CH_3-23), 1.33 (s, 3H, CH_3-24), 1.03 (s, 3H, CH_3-25), 0.94 (d, J = 3.0 Hz, 6H, CH_3-26 and CH_3-27), 0.92 (s, 3H, CH_3-29), 0.82 (s, 3H, CH_3-30); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.60 (C-3), 138.98 (C-2), 102.28 (C-6′), 87.96 (C-19), 71.23 (C-28), 56.28 (C-8′), 56.06 (C-7′), 54.24 (C-5'), 52.80 (C-5), 49.17 (C-9), 46.64 (C-18), 41.49 (C-20), 40.86 (C-14), 40.53 (C-8), 38.92 (C-1), 38.61 (C-4), 36.65 (C-16), 36.29 (C-4′), 36.27 (C-17), 34.58 (C-13), 34.19 (C-7), 32.68 (C-10), 32.41 (C-21), 28.80 (C-30), 27.87 (C-23), 26.48 (C-15), 26.12 (C-12), 26.05 (C-22), 24.58 (C-29), 21.68 (C-24), 21.27 (C-11), 18.54 (C-6), 17.50 (C-25), 15.35 (C-26), 13.56 (C-27). HRMS (ESI$^+$) m/z calcd for $C_{35}H_{58}N_3O_3$ [M]$^+$: 568.4477, found 568.4476.

4.1.2.23. 1′-(2,2-Diphenylethyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4w***. Prepared from compound **3x** (50 mg, 0.0774 mmol), methyl iodide (1.548 mmol, 96 μL) and dry acetonitrile (1 mL). Reaction time is 6 h. Yellow solid, yield 48%, m.p.: 147−150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (dd, J = 8.1, 6.6 Hz, 2H), 7.25 (ddt, J = 11.6, 7.2, 3.9 Hz, 4H), 7.16−7.09 (m, 2H), 5.18 (dd, J = 11.8, 4.9 Hz, 1H), 5.13−4.99 (m, 2H), 4.22 (s, 3H), 3.76 (d, J = 7.8 Hz, 1H), 3.53 (s, 1H), 3.45 (d, J = 7.8 Hz, 1H), 2.80 (d, J = 16.2 Hz, 1H), 2.70 (d, J = 16.2 Hz, 1H), 1.46 (s, 3H), 1.04 (s, 3H), 0.98 (s, 3H), 0.93 (d, J = 9.7 Hz, 6H), 0.82 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.35, 139.40, 139.16, 139.05, 129.11, 128.99, 128.05, 127.88, 127.74, 127.68, 87.95, 71.21, 57.14, 52.53, 49.66, 49.06, 46.61, 41.48, 40.83, 40.46, 38.76, 38.61, 36.64, 36.30, 36.27, 34.52, 34.19, 32.67, 32.30, 28.79, 27.87, 26.46, 26.10, 26.03, 24.57, 21.66, 20.91, 18.52, 17.33, 15.29, 13.56. HRMS (ESI$^+$) m/z calcd for $C_{45}H_{62}N_3O_1$ [M]$^+$: 660.4892, found 660.4894.

4.1.2.24. 1′-(2-(3-Indolyl)ethyl)-3′-methyl-19, 28- epoxy-, (19β) -1′H- olean- 2- eno[2, 3- d] [1, 2, 3] triazolium iodide ***4x***. Prepared from compound **3y** (50 mg, 0.082 mmol), methyl iodide (1.64 mmol, 102 μL) and dry acetonitrile (0.5 mL). Reaction time is 4 h. Yellow solid, yield 86%, m.p.: 125−128 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.73 (s, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.16−6.97 (m, 3H), 4.64 (td, J = 7.0, 3.5 Hz, 2H), 4.21 (s, 3H), 3.76 (d, J = 7.8 Hz, 1H), 3.56−3.41 (m, 3H), 2.82 (d, J = 16.1 Hz, 1H), 2.55 (d, J = 16.2 Hz, 1H), 1.21 (s, 3H), 0.92 (d, J = 13.4 Hz, 12H), 0.82 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 145.16, 138.52, 136.17, 126.76, 124.12, 121.78, 119.28, 117.23, 112.21, 108.23, 87.92, 71.20, 53.89, 52.87, 49.20, 46.63, 41.47, 40.80, 40.44, 38.81, 38.53, 36.64, 36.27, 36.08, 34.42, 34.16, 32.68, 32.35, 30.93, 28.80, 27.60, 26.44, 26.09, 25.86, 24.57, 21.65, 20.58, 18.42, 17.19, 15.24, 13.51. HRMS (ESI$^+$) m/z calcd for $C_{41}H_{59}N_4O_1$ [M]$^+$: 623.4688, found 623.4684.

4.2. Cell culture and cell cytotoxicity assay

Human hepatoblastoma cells HepG2, human esophageal squamous carcinoma cells Eca-109, human gastric cancer cells SGC-7901, human cervical cancer cells HeLa, and human normal liver cells HL-7702 were obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Eca-109 cells, SGC-7901 cells, and HL-7702 cells were cultured in RPMI-1640 medium (Sigma-Aldrich, Shanghai, China), while HepG2 cells and HeLa cells were cultured in DMEM medium (Sigma-Aldrich, Shanghai, China) with 10% fetal bovine serum (ScienCell Research Laboratories Inc., California, USA) at 37 °C in the presence of 5% CO_2.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used to assay the cytotoxicity *in vitro*. Cells were seeded in 96-well plated at 3.0×10^3 cells/well for human cancer cell lines HepG2 and Eca-109, 5.0×10^3 cells/well for human cancer cell lines SGC-7901 and HeLa, 4.0×10^3 cells/well for human normal liver cell line HL-7702 in a final volume of 100 μL. After 24 h, the medium was replaced by different concentrations of compounds. After incubated for 48 h, 10 μL MTT dye (5 mg/mL) was added to each well and incubation for another 4 h. After that, the medium was removed and DMSO (100 μL) was added to solubilize the MTT formazan. A microplate reader (Bio-Rad iMark™) was used to measure the OD value of each well at a wavelength of 490 nm. Each experiment was repeated at least three times and GraphPad Prism 7.0 was used to analyze the IC_{50} values.

4.3. Cell apoptosis assay

SGC-7901 cancer cells were placed into 6-well culture plates at a density of 2×10^5 cells/well and treated with different concentrations of compound **4n** (0 μM, 1 μM, 2 μM, and 4 μM) for 48 h. Eca-109 cancer cells were treated with different concentrations of compound **4q** (0 μM, 1 μM, 2 μM, and 4 μM) for 48 h using the same method. The treated cells were harvested by trypsinization and washed twice with cold PBS. Hoechst 33342 staining and Acridine Orange (AO)/Propidium Iodide (PI) dual-staining method were used to discover cell apoptosis by a fluorescence microscopy (Nikon Eclipse Ti−E, Nikon Instruments Inc., JAPAN). Annexin V-PE/7-AAD dual-staining assay was used to detect apoptosis rate by flow cytometer (FACS Aria III, BD Bioscience, USA).

4.4. Mitochondrial membrane potential assay

SGC-7901 cancer cells treated with different concentrations of compound **4n** (0 μM, 1 μM, 2 μM, and 4 μM) and Eca-109 cancer cells treated with different concentrations of compound **4q** (0 μM, 1 μM, 2 μM, and 4 μM) were harvested and washed twice with cold PBS, followed by incubated in 500 μL PBS containing 10 μg/mL JC-1 for 20 min at 37 °C in the dark. Then the cells were resuspended in PBS and analyzed by flow cytometry (FACS Aria III, BD Bioscience, USA).

4.5. Intracellular reactive oxygen species (ROS) levels assay

Cells treated with **4n** or **4q** were harvested and washed twice with cold PBS, followed by stained with 10 μM DCFH-DA for 30 min and photographed by a fluorescence microscopy (Nikon Eclipse Ti−E, Nikon Instruments Inc., JAPAN).

Cells were pretreated with 10 mM of N-acetyl-L-cysteine (NAC), as a ROS scavenger, for 1 h before addition of compound **4n** or **4q** to examine the effect of ROS on cell viability. The percentage of apoptotic cells were detected by Annexin V-PE/7-AAD staining assay after compound **4n** or **4q** treatment with or without NAC.

4.6. Cell cycle arrest analysis

Cells treated with **4n** or **4q** were harvested and fixed in 1.0 mL aqueous ethanol (70%, v/v) at −20 °C overnight. Then the cells were incubated in 500 μL PBS containing Triton X-100 (0.1%, v/v), RNase A (0.2 mg/mL), and propidium iodide (PI, 0.02 mg/mL) for 15 min. Then the cells were analyzed by flow cytometry (FACS Aria III, BD Bioscience, USA).

4.7. Western blot analysis

Cells treated with **4n** or **4q** were lysed in radio immunoprecipitation assay (RIPA) buffer with protease inhibitors, and phosphatase inhibitors. A BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China) was used to determine the protein concentration. Protein samples under denaturing conditions were electrophoresed by SDS-PAGE (8%–12%) and transferred onto the PDVF membranes (Millipore, MA, USA). Membranes were blocked with 10% skim milk (BD Difco™, USA) at RT for 1 h, followed by washed three time with TBST. Primary antibodies, including anti-PARP (CST, #9542), anti-Caspase-3 (CST, #9662), anti-Caspase-8 (CST, #9746), anti-Caspase-9 (CST, #9508), anti-Bax (CST, #2772), anti-Bcl-2 (CST, #2872), anti-Bcl-xL (CST, #2762), and anti-β-actin (CST, #4970) were used to incubate the target proteins, and detection was performed using HRP-labeled secondary antibodies by ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, Inc., California, USA).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112737.

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