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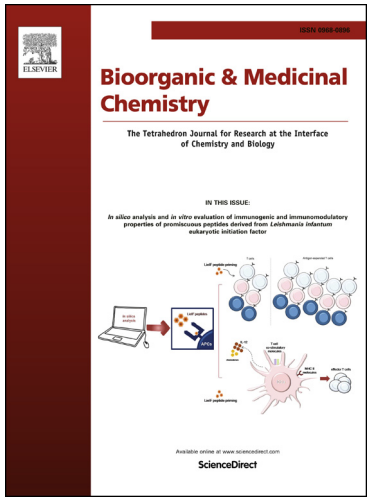

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Design, synthesis and biological mechanisms research on 1,2,3-triazole derivatives of Jiyuan Oridonin A

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ABSTRACT: Two series of derivatives with 1,2,3-triazole as heterocyclic moiety of Jiyuan Oridonin A, a new *ent*-kaurene diterpenoid which was isolated from genus *Isodon rubescens,* were synthesized and biologically evaluated. All the derivatives possessed good anti-proliferative activities. Among them, compound **8g** was found to significantly induce cell apoptosis and cell cycle arrest in MGC-803 via a series of signals activated by the increased intracellular ROS levels.

Keywords: *ent*-kaurene diterpenoid; 1,2,3-triazole; MGC-803; ROS

1. Introduction

Over the past century, natural products have played a major source of therapeutic agents for cancers. *Isodon* species are widely distributed plants, and more than 600 diterpenoids have been isolated from this species over the past 30 years[1]. Some of them showed potent anti-proliferative activities and low toxicity, especially those with an *ent*-kaurane skeleton such as Oridonin, Ponicidin, Longikaurin A, and so on[2-4] (Fig. 1). The unique skeleton as well as potent anticancer activity make them promising candidates as anticancer agents[5].

However, the development of *ent*-kaurene diterpenoids for cancer therapy was hampered largely by their moderate activity and structural complexity[6]. Therefore, it is highly desirable to develop novel derivatives of these diterpenoids to improve their potency without reducing the safety profile[7]. Previously, some researchers reported that the modified of 14-hydroxy group[8-10] and heterocycle-fused A-ring[11, 12] could significantly increase the anti-proliferative efficacy of Oridonin and its analogues (Fig. 1). Among these heterocycles, 1,2,3-triazole is a very well-recognized pharmacophore appeared in many molecular structures of drugs[13-15]. It is stable to metabolic degradation, oxidative/reductive conditions and actively participates in binding to molecular targets and improves their solubility[16]. It has various kinds of biological activities such as anticancer[17], anti-HIV[18], anti-tuberculosis[19], anti-malaria[20], antibacterial[21] and so on. In recently years, many evidences[22-25] have demonstrated that leading 1,2,3-triazole ring into lead compounds is a good strategy of anticancer drug design.

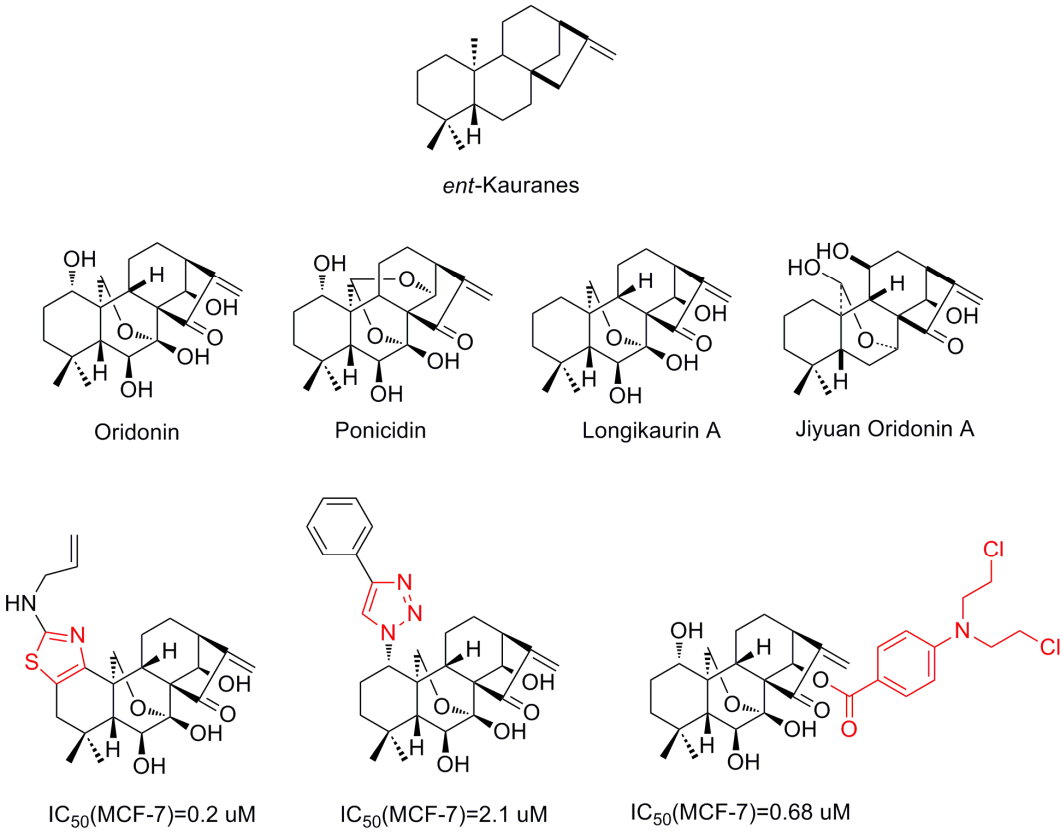


Fig. 1.Structures of *ent*-kaurene diterpenoids from *Isodon* and some Oridonin incorporated with heterocycle derivatives.

Reactive oxygen species (ROS) is a family including molecules, ions and free radicals with the ability of oxidation. ROS can be induced by Cytokine, growth factor, radiation, and inflammatory stimulation and so on[26]. When cells generate excessive ROS, it will cause oxidative stress, which is recognized as an adverse event for promoting tumorigenesis and progression[27]. In recent years, it was found that many anticancer drugs could generate ROS to change mitochondrial permeability, decrease membrane potential, release cytochrome C, initiate mitochondrial apoptosis pathway and result in inducing apoptosis of cancer cells in the end[28-31]. Meanwhile, some researchers reported that Oridonin could increase ROS level in cancer cells, which should be an important anticancer mechanism for *ent*-kaurene diterpenoids[1].

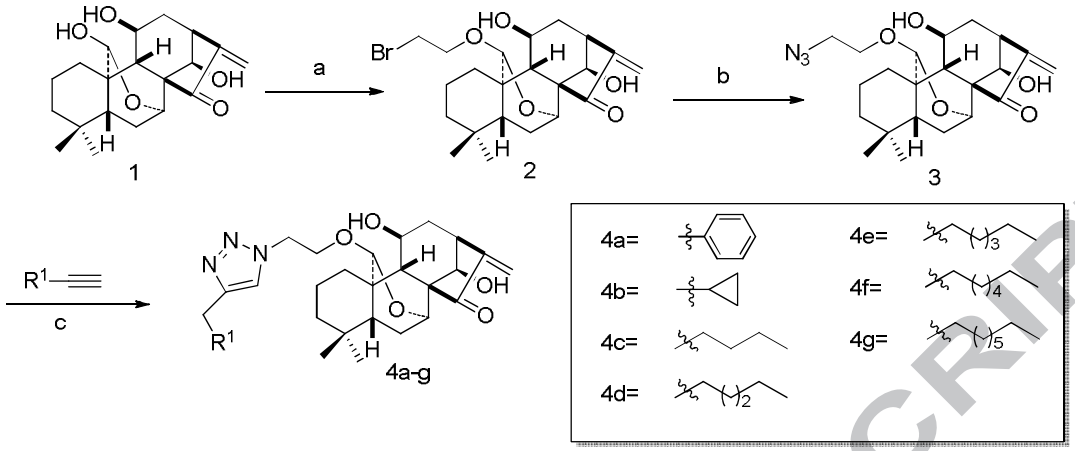
In our previous work, we have isolated a new kind of *ent*-kaurene diterpenoid, Jiyuan Oridonin A[32], with the different quantity and position of hydroxyl groups from Oridonin and Ponicidin (Fig. 1). Also, some derivatives of Jiyuan Oridonin A have been verified to exhibit good anticancer activity without obvious toxicity by our group[33-36]. Herein, we describe the design and synthesis of two series of derivatives with 1,2,3-triazole as heterocyclic moiety of Jiyuan Oridonin A. It aimed at a higher anti-proliferative efficacy and a lower systemic toxicity. The biological activities and anticancer mechanism of these derivatives were investigated as well.

2. Results and discussion

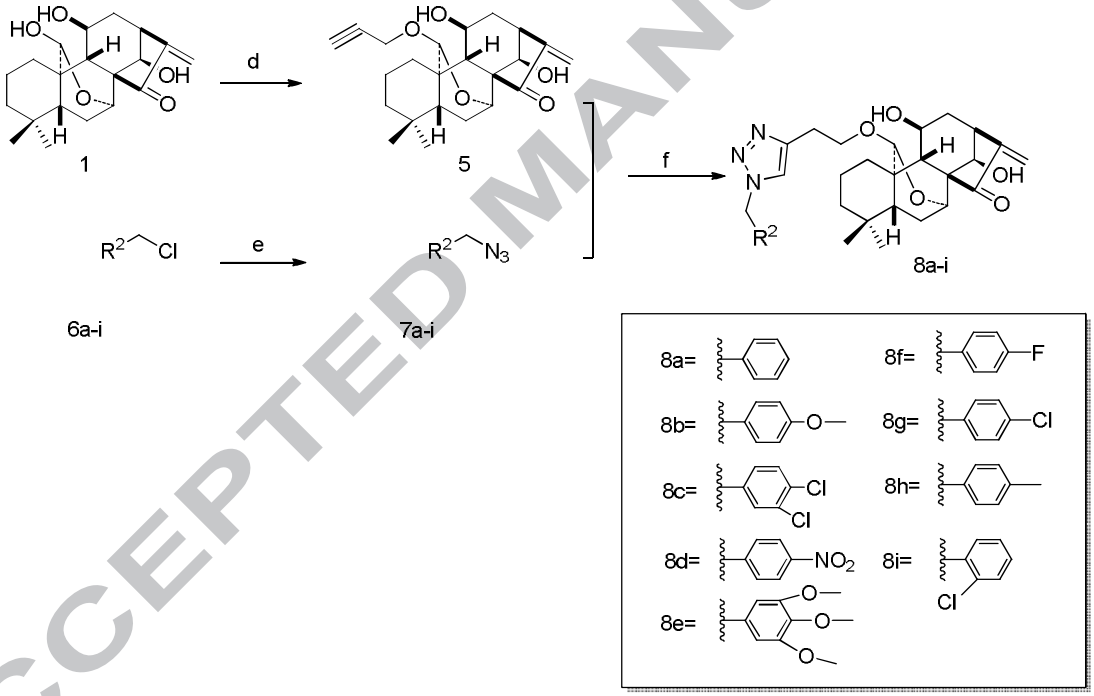
2.1. Chemistry

The synthetic route of series **1** was shown in Scheme 1. Jiyuan Oridonin A mixed with 2-bromoethanol catalyzed by toluene-p-sulfonic acid (p-TsOH) in tetrahydrofuran (THF) at room temperature to obtain compound **2**, and then stirred with NaN_3 in the system of ethyl acetate (EtOAc) and H_2O (4:1) to afford compound **3**. Compound **4a-g** were obtained through click reaction[37] from compound **3** and appropriate alkyne.

Compound **4a** showed better anti-proliferative activities than other compounds as the anti-proliferative activities of series **1** were tested. It was supposed that aromatic groups may improve the anticancer activities of compounds. On this basis, another series of compounds was designed and synthesized (Scheme 2). Similarly to the first route, Jiyuan Oridonin A were mixed with propargylbromide in THF to obtain compound **5**. Compound **6a-i** were stirred with NaN_3 in acetone to obtain compound **7a-i**. Compound **8a-i** were obtained through click reaction from compound **5** and compound **7a-i**.



Scheme 1. Synthetic route of series 1. Reagents and conditions: (a) 2-bromoethanol, THF, p-TsOH, rt, 3 h; (b) EtOAc/H_2O, NaN_3, reflux, 7 h; (c) appropriate alkyne, $CuSO_4$/Cu, THF/H_2O, rt, 8.5 h.



Scheme 2. Synthetic route of series 2. Reagents and conditions: (d) propargylbromide, THF, p-TsOH, rt, 3 h; (e) Acetone, NaN_3, reflux, 7 h; (f) $CuSO_4$/Cu, THF /H_2O, rt, 6-12 h.

2.2. MTT assay and SAR study

The anti-proliferative activities of all the prepared compounds were determined in four human cancer cell lines by MTT (3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-

phenytetrazoliumromide) assay, with Oridonin was used as control. As shown in Table 1, most of compounds exhibited stronger anti-proliferative activities than Oridonin. Almost all the compounds showed the strongest anti-proliferative activities in Eca109 cell line (human esophageal cancer cell line) of the four cell lines. The above results demonstrated that 1,2,3-triazole could significantly improve the anti-proliferative activities of lead compounds. It would be helpful to the research of structure modification for *ent*-kaurene diterpenoids. Furthermore, the SAR showed the length of carbon chain had little change on IC_{50} values for those compounds with aliphatic substituent (compound **4a-g**). It could be concluded that the cytotoxicity activity of compounds with aromatic groups (compound **8a-i**) was more potent than compounds with aliphatic substituent (compound **4a-g**). It was speculated that the lipophilicity of aromatic groups may enhance the ability of compounds across the cell membrane. Meanwhile, the position and type of substituents had little effect on anticancer activity of the benzene ring.

Table 1. The anticancer activity of two series of Jiyuan Oridonin A derivatives against four cancer cell lines. [a]

Compound	IC_{50} (μM)			
	Eca109	EC9706	SMMC7721	MCF-7
Oridonin	31.78±3.05	38.42±2.64	53.95±1.08	74.18±2.84
1(Jiyuan Oridonin A)	20.68±2.12	22.52±2.38	20.25±2.13	22.92±2.52
3	6.08±0.59	8.71±0.79	10.01±0.92	1.60±0.08
4a	4.26±0.39	5.32±0.42	6.07±0.51	8.95±0.78
4b	4.30±0.42	11.38±1.19	9.66±1.01	10.97±1.03
4c	4.45±0.40	8.29±0.70	11.02±1.09	13.42±1.32
4d	4.81±0.43	7.89±0.72	5.71±0.45	13.58±1.42
4e	4.72±0.41	>50	>50	>50
4f	3.21±0.23	5.99±0.50	5.73±0.42	8.66±0.75
4g	4.70±0.41	5.85±0.50	5.62±0.47	>50
5	10.79±1.08	7.87±0.75	15.27±2.01	14.64±1.92
8a	3.51±0.29	7.54±0.68	5.79±0.51	9.45±1,03
8b	2.57±0.20	4.67±0.41	4.81±0.40	7.81±0.71
8c	2.58±0.18	4.21±0.38	3.76±0.34	6.85±0.61
8d	4.13±0.39	7.78±0.73	3.80±0.37	6.45±0.52
8e	4.51±0.42	9.50±1.26	9.03±1.03	8.79±0.85
8f	3.68±0.26	4.50±0.42	5.88±0.49	6.62±0.52
8g	2.70±0.18	5.04±0.35	4.44±0.28	4.76±0.29

8h	2.12±0.19	6.10±0.49	5.68±0.51	7.86±0.59
8i	2.73±0.22	4.69±0.43	4.67±0.42	4.92±0.51

[a] MTT methods, cell lines were treated with target compounds for 48h, The average value of the three independent experiments was expressed by mean±SD.

As shown in Table 2, some compounds with better growth inhibitory effects were chosen to carry out further studies in PC-3 (human prostate cancer cell line) and MGC-803 (human gastric cancer cell line). Compound **8g** was found to possess the best anti-proliferative activity, and was chosen to test forward cytotoxic experiment on a number of cancer cell lines including MGC-803, HGC-27 (human gastric cancer cell line), Eca109, EC9706 (human esophageal cancer cell line), SMMC-7721 (hepatoma carcinoma cell line), MCF-7 (human breast cancer cell line) and PC-3. Based on the results (Table 3), compound **8g** was generally observed for stronger anticancer activities in all tested cell lines compared with Oridonin, suggesting that 1,2,3-triazole as heterocyclic moiety was beneficial for the cytotoxicity of derivatives. At the same time, GES-1 (human normal gastric epithelial cells) was used for comparison in Fig. 2A, and the inhibitory rate of compound **8g** on GES-1 cells (IC_{50} = 28.71±1.24 μM) was significantly less than that of MGC-803 (IC_{50} = 2.53±0.10 μM) at the same concentration. It indicated the selectivity of compound **8g** to cancer cells and normal cells. As the result, compound **8g** was selected for further pharmacological study on MGC-803.

Table 2. IC_{50} Values of part of compounds for PC-3 and MGC-803 cell line.[a]

Compd	IC_{50} (μM)	
	PC-3	MGC-803
Oridonin	27.55±3.01	33.10±3.47
1	14.99±2.09	14.70±1.91
3	12.30±1.80	11.91±1.67
5	11.22±1.36	9.62±1.27
4a	3.79±0.34	4.58±0.23
4b	5.32±0.39	5.42±0.42
4f	3.92±0.30	3.77±0.31
8b	4.95±0.46	4.79±0.43

8g	4.02±0.29	2.53±0.10
8h	4.90±0.30	3.89±0.06
8i	4.37±0.31	4.27±0.41

[a] MTT methods, cell lines were treated with target compounds for 48h, The average value of the three independent experiments was expressed by mean±SD.

Table 3. Cytotoxicity data of compound **8g** [a]

Cell Lines	IC_{50} (μM)	
	8g	Oridonin
MGC-803	2.53±0.10	33.10±3.47
HGC-27	2.97±0.14	32.03±3.01
ECa109	2.70±0.48	31.78±3.03
EC9706	5.04±0.83	38.42±3.14
SMMC-7721	4.44±0.40	53.95±4.28
MCF-7	4.76±0.43	74.18±6.41
PC-3	4.02±0.29	27.55±3.01

[a] MTT methods, cell lines were treated with target compounds for 48h, The average value of the three independent experiments was expressed by mean±SD.

2.3. Effect of compound 8g on the proliferation of MGC-803 cells

As shown in Fig. 2B, with the increase of drug concentration and the extension of time, the inhibitory effect was enhanced, which indicated that compound **8g** could inhibit cell proliferation in a time-dependent and dose-dependent manner. With higher concentration treatments (16, 32 μg/ml), the inhibition rate exceeded 90%, but the effect of acting time on inhibition rate was not obvious.

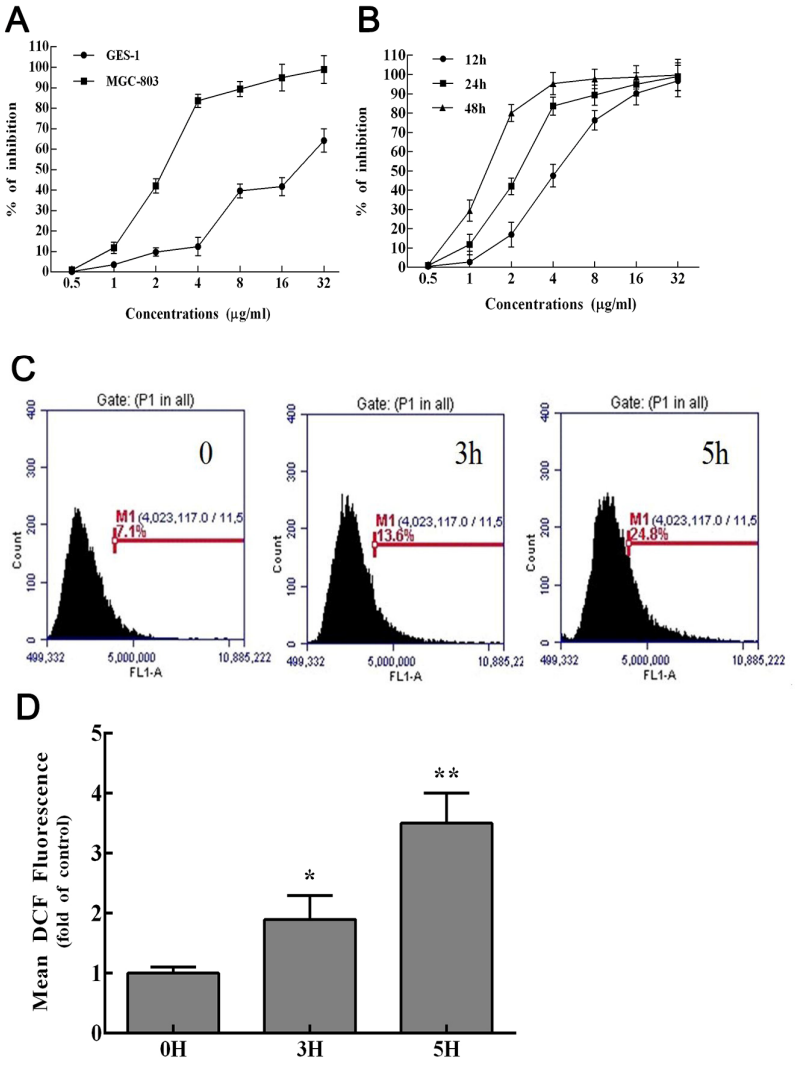


Fig. 2. (A) Effects of compound **8g** on cancer cell line and normal cell line. (B) Inhibitory activity of compound **8g** on MGC-803 cells at different time. (C, D) Level of ROS in MGC-803 cells induced by compound **8g**. *P<0.05, compared with the control group; **P<0.01, compared with the control group.

2.4. Measurement of ROS levels

As shown in Fig. 2C and Fig. 2D, the level of ROS in MGC-803 cells increased significantly with the prolongation of the acting time by compound **8g**, suggesting that compound **8g** induced the increase of ROS in MGC-803 cells, which tested by flow cytometry analysis via the DCFH-DA staining.

2.5. Effects of NAC on MGC-803 cells

Previous experiments confirmed that compound **8g** could induce an increase in intracellular ROS. Then we used ROS scavenger N-acetly cysteine (NAC) to antagonize the increase of ROS and to further verify the cell death mechanism action of compound **8g**. The results showed that (Fig. 3) compared to the only dosing group, the cell proliferation inhibition rate of the adding NAC (5 mmol) group had evidently decreased. We also found that NAC could reduce the cytotoxic of compound **8g** on MGC-803, especially at low concentration. Apparently, ROS played an important role in MGC-803 cells death caused by compound **8g**.

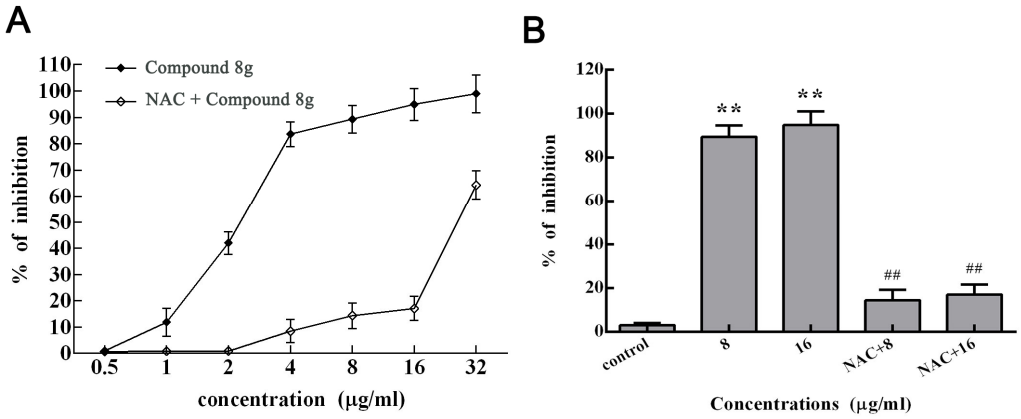


Fig. 3. The proliferation effect of NAC on compound **8g**. (A) the proliferation inhibition effect of compound **8g** on MGC-803 after NAC pretreatment; (B) the proliferation inhibition effect of compound **8g** on MGC-803 at 8 μg/ml and 16 μg/mL after NAC pretreatment. **P<0.01, compared with the control group; $^{\#\#}$P<0.01, compared with single compound **8g** treatment group.

We used JC-1 staining to observe the effect of NAC on compound **8g** induced the mitochondrial membrane potential (ΔΨm) reduced. The result was shown in Fig. 4. When 1.5 and 3 μg/mL of compound **8g** affected on MGC-803 cell for 24 h, JC-1 in monomer form showed green fluorescence. It showed that the ΔΨm of MGC-803 cells decreased with adding compound **8g** (Fig. 4A, 4B, 4C). 5 mM NAC alone did not affect

the ΔΨm, but it could significantly inhibit the decrease of ΔΨm induced by compound **8g** (Fig. 4a, 4b, 4c). The above results demonstrated that NAC had obvious protective effect on the decrease of ΔΨm induced by compound **8g** and the increase of ROS could be a prerequisite for mitochondrial membrane potential collapse.

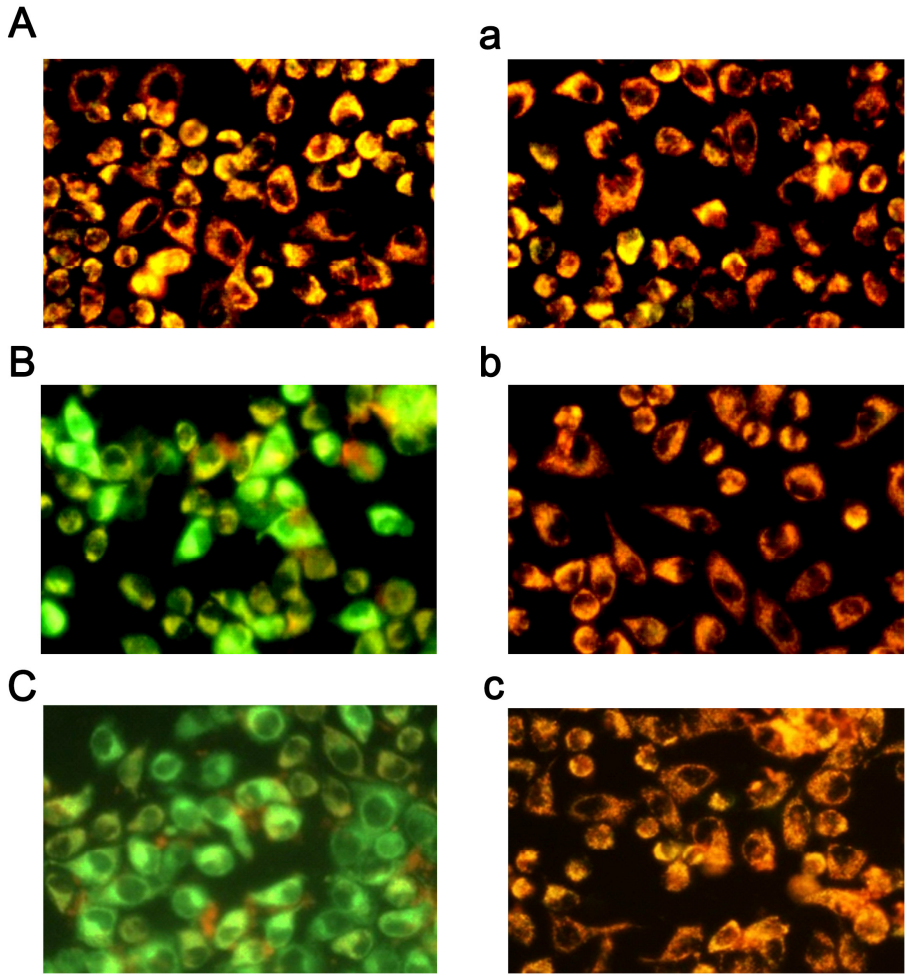


Fig. 4. Protection of compound **8g** induced decrease in membrane potential induced by NAC. (A) control group; (B) 1.5 μg/ml compound **8g**; (C) 3 μg/ml compound **8g**; (a) 5 mM NAC; (b) NAC + 1.5 μg/ml compound **8g**; (c) NAC + 3 μg/ml compound **8g**.

The decrease of mitochondrial membrane potential could lead to the release of cytochrome C in mitochondria. From Fig. 5 we could see, in the presence of compound **8g**, Caspase-9 proenzyme protein was activated followed by increased levels of

Cytochrome C in the cytoplasm. The expression of Cleaved-Caspase-9 increased with the increase of compound **8g** concentration, activation of Caspase-9 continued initiating downstream of Caspase cascade reaction, finally causing cells apoptosis.

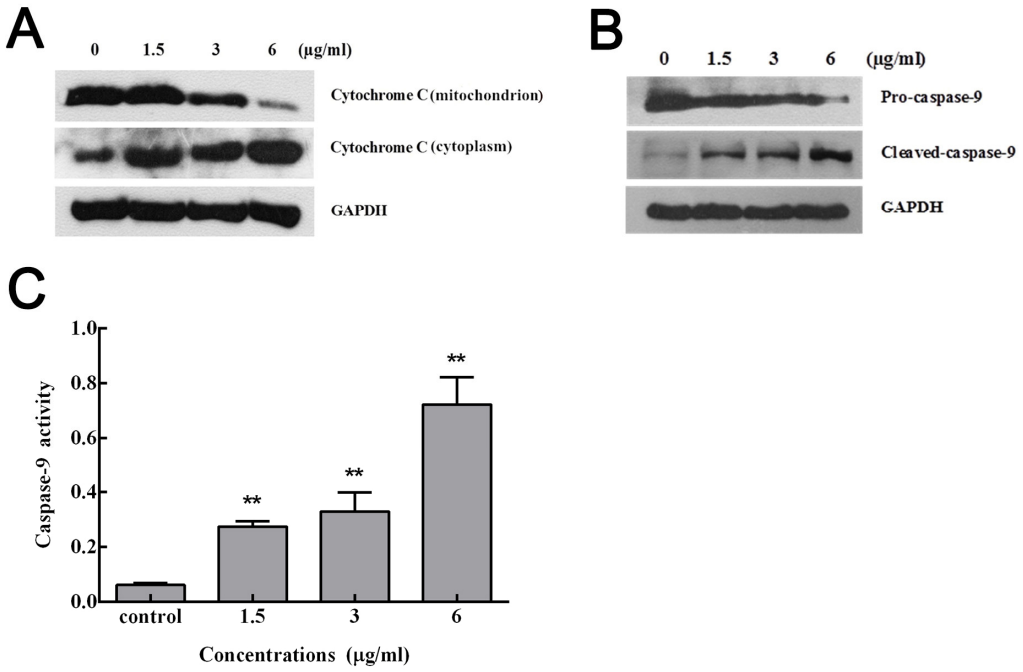


Fig. 5. Release of Cytochrome C and Caspase-9 activation by compound **8g**; (A) Cytochrome C; (B, C) Caspase-9. **P<0.01, Compared with the control group.

*2.6. Effect of NAC on cell cycle and apoptosis by compound **8g***

To obtain further insight into the mechanism of compound **8g**, the percentage of apoptotic MGC-803 cells were determined by flow cytometry and the results showed NAC could inhibit cell apoptosis induced by compound **8g** (Fig. 6). Meanwhile, it is worthwhile to discuss the morphological changes of apoptotic cells (Fig. 7) and the blocking of compound **8g** induced cell cycle arrest induced by NAC (Fig. 8). In the control group, the cell wall growth was good (Fig. 7, 1A), however, with the increase of drug concentration, the number of cell was significantly decreased and cell debris

increased, compared with the control group (Fig. 7, 1B-1D). By Hoechst 33258 staining, apoptotic bodies could be observed more clearly (Fig. 7, 2B-2D).

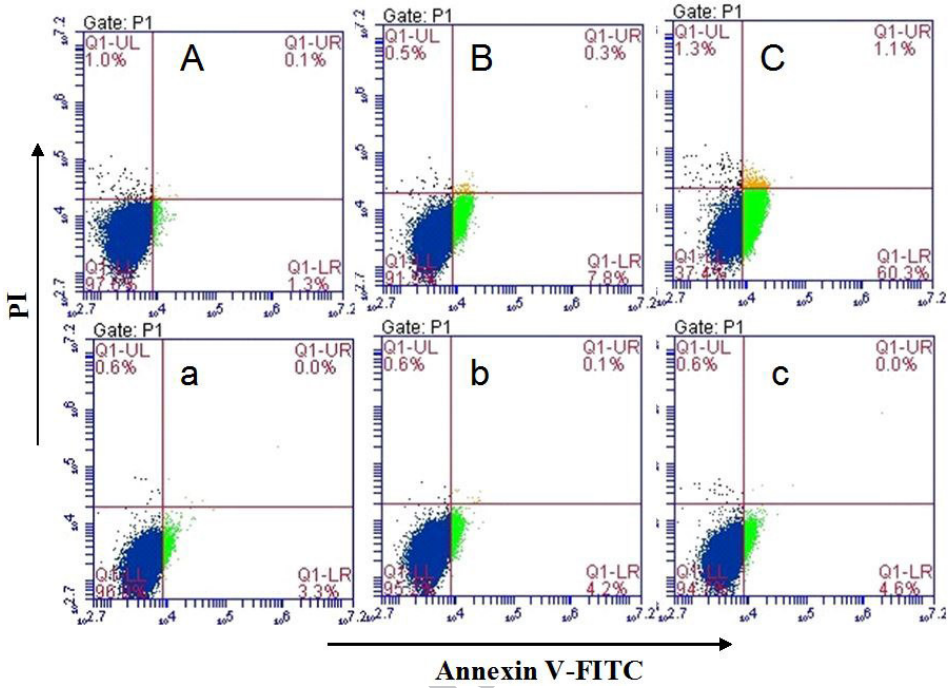


Fig. 6. Inhibition of compound **8g** induced apoptosis by NAC. (A) control group; (B) 1.5 μg/mL compound **8g**; (C) 3 μg/mL compound **8g**; (a) 5 mM NAC; (b) NAC + 1.5 μg/mL compound **8g**; (c) NAC + 3 μg/mL compound **8g**.

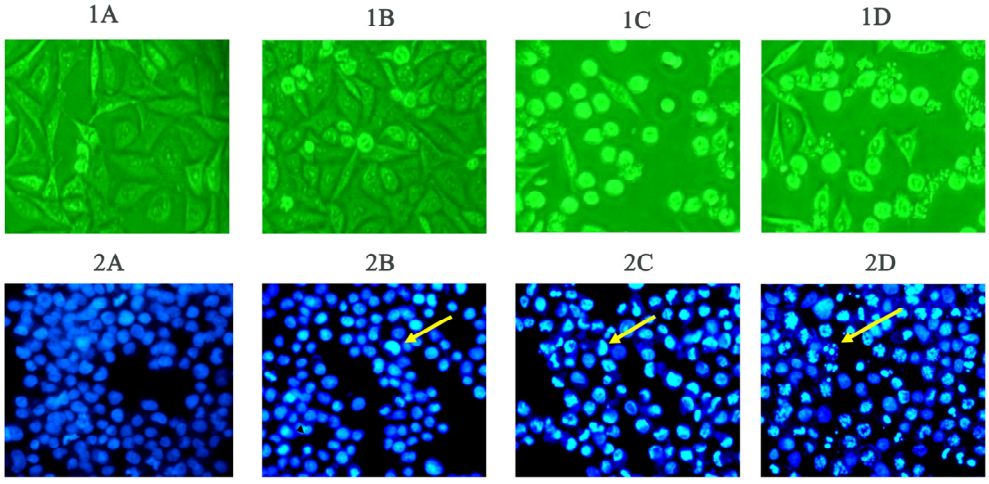


Fig. 7. Hoechst 33258 fluorescent staining method was used to treat the cells with different

concentrations of 24 h. Apoptotic cells morphology were observed under inverted microscope and fluorescence microscope (×200). (A) control group; (B) 1.5 μg/mL compound **8g**; (C) 3 μg/mL compound **8g**; (D) 6 μg/mL compound **8g**.

As shown in the Fig. 8 and Table 4, compound **8g** could increase the proportion of G2/M phase cells in MGC-803 cells in a dose-dependent manner. On the other hand, NAC almost completely reversed the cell cycle arrest induced by compound **8g** (Fig. 8, b-c, Table 4).

Table 4. Growth inhibitory effects of different reagents on MGC-803 cells.

Groups	G_0/G_1(%)	S(%)	G_2/M(%)
A	52.5±3.71	32.5±2.25	17.2±1.35
B	46.3±2.65*	29.2±1.78*	26.7±1.97*
C	30.5±3.26*	29.6±2.06*	40.3±2.16*
a	51.3±4.34	33.1±2.63	14.3±0.52
b	55.4±3.94$^{\#}$	29.1±1.56	16.1±1.31$^{\#}$
c	50.4±4.04$^{\#}$	33.2±2.26	14.8±1.01$^{\#}$

(A) control group; (B) 1.5 μg/mL compound **8g**; (C) 3 μg/mL compound **8g**; (a) 5 mM NAC; (b) NAC + 1.5 μg/mL compound **8g**; (c) NAC + 3 μg/mL compound **8g**. *P<0.05, compared with the control group; $^{\#}$P<0.05, compared with single compound **8g** treatment group.

2.7. Effect on the expression of apoptosis-related proteins

To elucidate the potential mechanisms contributed to apoptosis of MGC-803 cells induced by compound **8g**, several proteins related to apoptosis were determined by Western blotting. As shown in Fig. 9, it is clearly to find the up-regulation of proapoptotic protein p53 and p21, the down-regulation of anti-apoptotic protein surviving and the ratio of Bcl-2/Bax. It can prove that compound **8g** induced apoptosis of MGC-803 cells through the mitochondrial pathway.

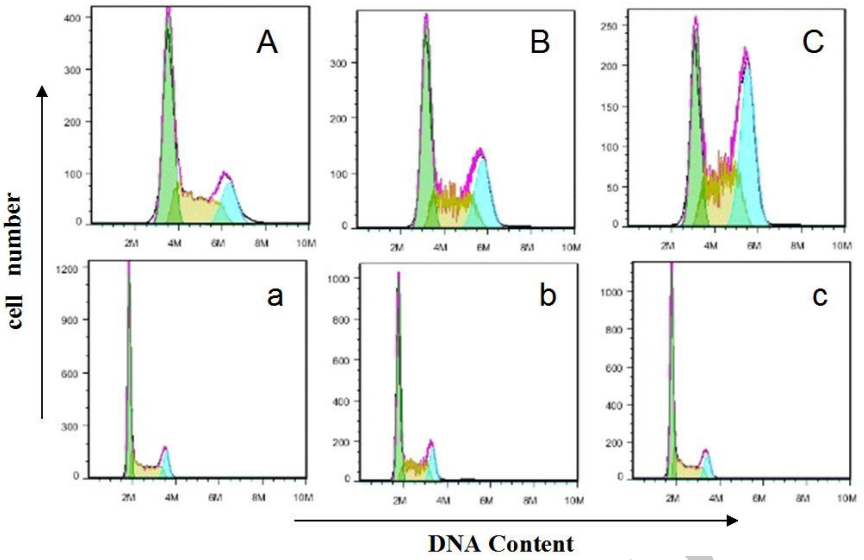


Fig. 8. The growth inhibitory effect of compound **8g** in MGC-803 cells was blocked by NAC after 24h. (A) control group; (B) 1.5 μg/mL compound **8g**; (C) 3 μg/mL compound **8g**; (a) 5 mM NAC; (b) NAC + 1.5 μg/mL compound **8g**; (c) NAC + 3 μg/mL compound **8g.**

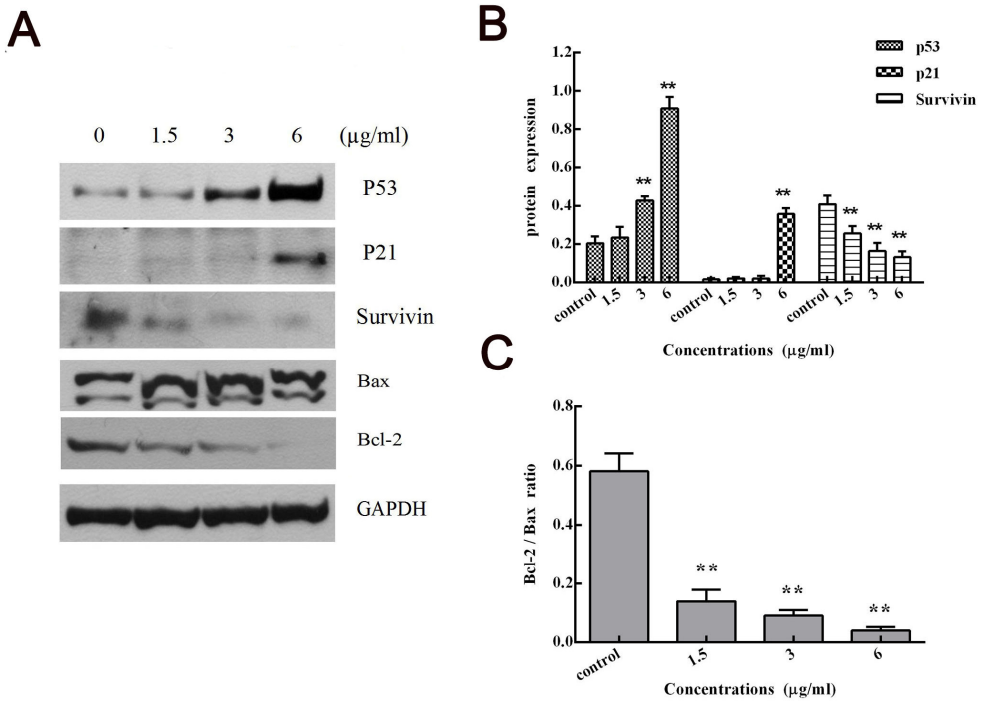


Fig. 9. Expression changes of apoptosis related proteins. (A) compound **8g** induced expression changes of apoptosis related proteins in MGC-803 cells; (B, C) Statistical analysis of protein

expression levels. The results shown were representative of three independent experiments. **P<0.01, Compared with the control group.

3. Conclusion

To summarize, we designed two series of new derivatives with 1,2,3-triazole as heterocyclic moiety of Jiyuan Oridonin A. These compounds displayed moderate to good anti-proliferative activity against the tested cancer cells and might serve as bioactive fragments and lead compounds for developing more potent cytotoxic agents. The preliminary SAR illustrated that 1,2,3-triazole could significantly improve the anti-proliferative activities of the *ent*-kaurene diterpenoids.

Compound **8g** was found to possess the best anti-proliferative activity among above compounds. The further mechanism investigation showed that it increased ROS level in cancer cells, leading to the decrease of mitochondrial membrane potential and the release of Cytochrome C into the cytoplasm, which was then cut and activated Caspase-9 to induce apoptosis. Meanwhile, it halted cell cycle progression at the G2/M phase and altered the expression of cell cycle-related proteins. More mechanism studies are underway and will be reported in due course.

4. Experimental

4.1. Chemistry

All commercially available starting materials and solvents were reagent grade and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silicagel plates (GF254) and visualized under UV light. Infrared spectra (IR) were recorded on PE-1710 type instrument of PE Company (KBr pellet). Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer, respectively. High resolution mass spectra (HRMS) of all

derivatives were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

4.2. General procedure to synthesize all compounds

*4.2.1. synthesis of compound **2** and compound **3***

Jiyuan Oridonin A (150 mg) was dissolved in THF (8 mL). 2-bromoethanol (150 μL) and p-TsOH (10 mg) were added to this solution. The mixture was stirred for 3 h at room temperature. Then the mixture was treated by heric-vacuum distillation and diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, evaporated and the crude product was purified by column chromatography to obtain compound **2**. After that, compound **2** was put in the system of EtOAc/H_2O (5:1) with NaN_3 and stirred for 7 h at 70 ℃ to obtain compound **3**.

*4.2.1.1. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-bromoethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **2**)*

White solid, 78% yield; Mp: 121-122 ℃; IR (KBr, cm^{-1}): 3608, 3362, 2951, 2869, 1723, 1643, 1431, 1362, 1258, 1103, 1037, 654; ^{1}H NMR (400 MHz, DMSO-d^6) δ 5.79, 5.38 (each 1H, s, H-17), 5.11 (1H, s, H-20), 5.10 (1H, d, *J* = 2.9 Hz, H-14-OH), 4.51 (1H, s, H-11-OH), 4.45 (1H, d, *J* = 8.0 Hz, H-11α), 4.26 (1H, d, *J* = 7.0 Hz, H-7β), 3.98-3.81 (2H, m, H-21), 2.80 (1H ,d, *J* = 8.9 Hz, H-13α), 2.68 (1H, d, *J* = 12.8 Hz, H-6β), 2.64 (1H, dd, *J* = 6.7 Hz, H-12α), 1.99 (1H, d, *J* = 12.4 Hz, H-1α), 1.86-1.72 (2H, m, H-22), 1.61 (1H, d, *J* = 4.7 Hz, H-6α), 1.49-1.32 (3H, m, H-3α, H-2), 1.29-1.26 (2H, m, H-1β, H-12β), 1.25-1.15 (2H, m, H-5β, H-9β), 1.11-1.05 (1H, m, H-3β), 0.92 (3H, s, H-19), 0.82 (3H, s, H-18). ^{13}C NMR (101 MHz, DMSO-d^6) δ 206.20, 152.73, 119.69,

116.61, 99.94, 70.15, 65.85, 63.56, 62.88, 56.84, 48.90, 39.80, 39.59, 39.38, 34.17, 33.14, 30.31, 24.71, 21.19, 18.89, 18.23. ESI-HRMS: m/z cacld. For $C_{22}H_{31}BrNaO_5$, $[M+Na]^+$: 476.1247, found 476.1242.

*4.2.1.2. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-azidoethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **3**)*

White solid, 94% yield; Mp: 130-132 ℃; IR (KBr, cm^{-1}): 3648, 3612, 3421, 3136, 2950, 2868, 1723, 1646, 1362, 1252, 1108, 1078, 1023, 766; ^{1}H NMR (400 MHz, DMSO-d^6) δ 5.80, 5.38 (each 1H, s, H-17), 5.11 (1H, d, *J* = 2.9 Hz, H-14-OH), 5.10 (1H, s, H-20), 4.51 (1H, s, H-11-OH), 4.45 (1H, d, *J* = 7.8 Hz, H-11α), 4.26 (1H, d, *J* = 7.2Hz, H-7β), 3.99-3.82 (2H, m, H-21), 2.81 (1H, d, *J* = 9.1 Hz, H-13α), 2.68 (1H, d, *J* = 12.9 Hz, H-6β), 2.60 (1H, s, H-12α), 2.00 (1H, d, *J* = 11.9 Hz, H-1α), 1.87-1.74 (2H, m, H-22), 1.63-1.44 (1H, m, H-6α), 1.39 (2H, dd, *J* = 29.4, 11.2 Hz, H-2), 1.36-1.27 (2H, m, H-1β, H-12β), 1.25-1.14 (2H, m, H-5β, H-9β), 1.09-1.01 (1H, m, H-3β), 0.92 (3H, s, H-19), 0.84 (3H, s, H-18). ^{13}C NMR (101 MHz, DMSO-d^6) δ 206.25, 152.77, 116.54, 99.99, 70.13, 66.53, 65.78, 63.59, 57.77, 56.82, 51.24, 48.97, 39.99, 39.78, 39.57, 39.36, 34.17, 33.17, 30.38, 24.75, 21.20, 18.25. ESI-HRMS: m/z cacld. For $C_{22}H_{31}N_3NaO_5$, $[M+Na]^+$: 439.2156, found 439.2152.

*4.2.2. General procedure to synthesize Compound **4a-g***

Coumpound **3** (150 mg) and appropriate alkynes were dissolved in THF (4 mL) , then added 1 mL H_2O to this solution. Finally, the mixture was stirred at room temperature for 8.5 h by using $CuSO_4$ (57 mg) and Cu (107 mg) as catalytic agents and then was filtered and condensed. The residue was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, evaporated

to afford the crude product. The crude product was purified by column chromatography to give Compound **4a-g**.

4.2.2.1. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-8-methylene-12-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethoxy)decahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (*Compound **4a***)

White solid, 92% yield; Mp: 152-153 ℃; IR (KBr, cm^{-1}): 3648, 3612, 3421, 3136, 2950, 2868, 1723, 1646, 1362, 1252, 1108, 1078, 1023, 766, 695; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 8.52 (1H, s, H-23), 7.86 (2H, d, *J* = 7.3 Hz, H-26, H-30), 7.45 (2H, t, *J* = 7.6 Hz, H-27, H-29), 7.34 (1H, t, *J* = 7.4 Hz, H-28), 5.74, 5.30 (each 1H, s, H-17), 5.12 (1H, s, H-20), 5.00 (1H, d, *J* = 2.9 Hz, H-14-OH), 4.64 (2H, dd, *J* = 10.4, 6.1 Hz, H-22), 4.25-4.05 (2H, m, H-21), 4.19 (1H, s, H-14α), 4.11 (1H, s, H-11α), 4.03 (1H, d, *J* = 7.1 Hz, H-11-OH), 3.79 (1H, dd, H-5β), 2.74-2.53 (2H, m, H-6β, H-12α), 2.42 (1H, d, *J* = 13.7 Hz, H-12α), 1.99 (1H, s, H-1α), 1.87 (1H, s, H-6α), 1.63-1.46 (1H, m, H-3α), 1.42-1.33 (2H, m, H-2), 1.25-1.18 (2H, m, H-1β, H-12β), 1.15-1.10 (2H, m, H-5β, H-9β), 1.01 (1H, dd, *J* = 19.2 Hz, H-3β), 0.88 (3H, s, H-19), 0.79 (3H, s, H-18); ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.19, 152.75, 146.74, 131.45, 129.31, 128.21, 125.63, 121.99, 116.38, 100.21, 69.94, 66.48, 65.83, 63.57, 57.75, 50.50, 48.79, 42.90, 42.12, 40.82, 39.84, 34.12, 33.12, 30.38, 24.68, 21.18, 18.06. ESI-HRMS: m/z cacld. For $C_{30}H_{37}N_3NaO_5$, $[M+Na]^+$: 542.2631, found 542.2619.

*4.2.2.2. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-cyclopropyl-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **4b**)*

White solid, 95% yield; Mp: 140-141 ℃; IR (KBr, cm^{-1}): 3647, 3613, 3445, 2950, 2870, 1716, 1646, 1557, 1394, 1362, 1254, 1107, 1024, 668; ^{1}H-NMR (400 MHz,

DMSO-d^6) δ 7.76 (1H, s, H-23), 5.77, 5.36 (each 1H, s, H-17), 5.07 (1H, s, H-20), 4.95 (1H, d, *J* = 3.0 Hz, H-14-OH), 4.59-4.39 (2H, m, H-22), 4.27-4.18 (2H, m, H-28), 4.15 (1H, s, H-14α), 4.06 (1H, d, *J* = 7.2 Hz, H-11-OH), 3.96 (1H, d, *J* = 2.9 Hz, H-11α), 3.71 (1H, d, *J* = 4.5 Hz, H-5β), 2.71 (1H, d, *J* = 9.1 Hz, H-6β), 2.64 (1H, s, H-12α), 2.00-1.90 (1H, m, H-1α), 1.84 (1H, s, H-6α), 1.61-1.49 (1H, m, H-3α), 1.42-1.35 (2H, m, H-2), 1.26-1.20 (2H, m, H-1β, H-12β), 1.18-1.10 (2H, m, H-5β, H-9β), 1.08-0.99 (1H, m, H-3β), 0.91-0.87 (5H, m, H-19, H-26, H-27), 0.80 (3H, s, H-18), 0.76-0.68 (2H, m, H-26, H-27). ^{13}C-NMR (101 MHz, DMSO-d^6) δ206.18, 152.80, 149.33, 121.17, 116.40, 100.17, 69.93, 66.60, 65.81, 63.53, 57.77, 56.71, 50.19, 48.83, 42.99, 42.18, 40.87, 39.74, 34.12, 33.13, 30.34, 24.70, 21.19, 18.14, 18.06, 17.86, 17.03. ESI-HRMS: m/z cacld. For $C_{27}H_{37}N_3NaO_5$, $[M+Na]^+$: 506.2631, found 506.2622.

*4.2.2.3. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-butyl-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **4c**)*

White solid, 92% yield; Mp: 148-150 ℃; IR (KBr, cm^{-1}): 3419, 3131, 2954, 2930, 2866, 1723, 1646, 1557, 1457, 1146, 1108, 1021, 931, 726, 667; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.80 (1H, s, H-23), 5.77, 5.36 (each 1H, s, H-17), 5.07 (1H, s, H-20), 4.98 (1H, d, *J* = 3.0 Hz, H-14-OH), 4.54-4.43 (2H, m, H-22), 4.25-4.07 (3H, m, H-21, H-14α, H-11α), 3.96 (1H, d, *J* = 3.1 Hz, H-7β), 2.69 (1H, t, *J* = 8.0 Hz, H-13α), 2.64 (2H, d, *J* = 7.4 Hz, H-25), 2.61 (1H, s, H-6β), 2.53 (1H, s, H-12α), 1.84 (1H, d, *J* = 8.1 Hz, H-1α), 1.65-1.57 (2H, m, H-26), 1.40 (1H, d, *J* = 4.9 Hz, H-6α), 1.36 (2H, d, *J* = 7.5 Hz, H-27), 1.34 (1H, s, H-3α), 1.31 (1H, d, *J* = 7.2 Hz, H-1β), 1.25 (3H, d, *J* = 5.9 Hz, H-2, H-12β), 1.21 (1H, d, *J* = 6.2 Hz, H-5β), 1.03 (1H, d, *J* = 8.8 Hz, H-3β), 0.96-0.91 (3H, m, H-28), 0.89 (3H, s, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.20,

152.77, 147.29, 122.24, 116.45, 100.13, 69.95, 66.68, 65.79, 63.53, 57.76, 56.68, 50.14, 48.82, 42.98, 42.20, 40.85, 39.69, 34.12, 33.13, 31.60, 30.33, 25.22, 24.69, 22.18, 21.17, 18.13, 14.20. ESI-HRMS: m/z cacld. For $C_{28}H_{41}N_3NaO_5$, $[M+Na]^+$: 529.2944, found 529.2940.

*4.2.2.4. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-8-methylene-12-(2-(4-pentyl-1H-1,2,3-triazol-1-yl)ethoxy)decahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **4d**)*

White solid, 91% yield; Mp: 159-160 ℃; IR (KBr, cm^{-1}): 3419, 2952, 2936, 2868, 1723, 1698, 1646, 1455, 1363, 1264, 1108, 1023, 934, 791; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.80 (1H, s, H-23), 5.77, 5.36 (each 1H, s, H-17), 5.07 (1H, s, H-20), 4.97 (1H, d, *J* = 3.0 Hz, H-14-OH), 4.51 (2H, ddd, *J* = 16.9, 10.3, 7.2 Hz, H-22), 4.31-4.19 (1H, m, H-21), 4.17 (1H, d, *J* = 1.4 Hz, H-11-OH), 4.14-4.09 (1H, m, H-14α), 4.07 (1H, d, *J* = 7.2 Hz, H-11α), 3.96 (1H, d, *J* = 2.9 Hz, H-7β), 3.72 (1H, ddd, *J* = 10.7, 6.5, 4.1 Hz, H-21), 2.69 (1H, t, *J* = 8.0 Hz, H-13α), 2.64 (1H, s, H-6β), 2.61 (2H, d, *J* = 7.4 Hz, H-25), 1.84 (1H, d, *J* = 8.1 Hz, H-1α), 1.66-1.58 (2H, m, H-26), 1.39 (1H, d, *J* = 4.9 Hz, H-6α), 1.35-1.29 (4H, m, H-27, H-3α, H-1β), 1.26 (3H, d, *J* =7.2 Hz,H-2, H-12β), 1.21 (1H, d, *J* = 6.2 Hz, H-5β), 1.14 (2H, t, *J* = 6.4 Hz, H-28), 1.04 (1H, d, *J* =8.8 Hz, H-3β), 0.91-0.85 (6H, m, H-29, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.19, 152.78, 147.34, 122.22, 116.43, 100.14, 69.95, 66.68, 65.80, 63.53, 57.77, 56.69, 50.14, 48.81, 42.99, 42.20, 40.86, 39.68, 34.12, 33.13, 31.34, 30.33, 29.14, 25.54, 24.69, 22.37, 21.17, 18.14, 14.32. ESI-HRMS: m/z cacld. For $C_{29}H_{43}N_3NaO_5$, $[M+Na]^+$: 536.3100, found 536.3094.

*4.2.2.5. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-hexyl-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **4e**)*

White solid, 90% yield; Mp: 163-164℃; IR (KBr, cm^{-1}): 3420, 2952, 2928, 2868, 1723, 1646, 1456, 1363, 1265, 1219, 1108, 1079, 1026, 933, 721, 667; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.80 (1H, s, H-23), 5.77, 5.35 (each 1H, s, H-17), 5.07 (1H, s, H-20), 4.99 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.64-4.42 (2H, m, H-22), 4.30-4.23 (1H, m, H-21), 4.15 (1H, d, *J* = 1.4 Hz, H-11-OH), 4.11 (1H, t, *J* = 6.6 Hz, H-14α), 4.09-4.00 (1H, m, H-11α), 3.95 (1H, d, *J* = 2.9 Hz, H-7β), 2.70 (1H, t, *J* = 8.0 Hz, H-13α), 2.65-2.57 (3H, m, H-6β, H-25), 2.52 (1H, s, H-12α), 1.84 (1H, d, *J* = 8.1 Hz, H-1α), 1.65-1.50 (3H, m, H-26, H-6α), 1.43-1.18 (9H, m, H-27, H-28, H-2, H-1β, H-12β, H-9β), 1.16-1.08 (2H, m, H-29), 1.07-0.99 (1H, m, H-3β), 0.92-0.84 (6H, m, H-30, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.21, 152.78, 147.35, 122.21, 116.43, 100.13, 69.94, 66.67, 65.79, 63.51, 57.75, 56.66, 50.14, 48.81, 42.97, 42.18, 40.84, 39.66, 33.13, 31.55, 30.33, 29.45, 28.82, 25.59, 24.69, 22.48, 21.16, 18.13, 14.43. ESI-HRMS: m/z cacld. For $C_{30}H_{45}N_3NaO_5$, $[M+Na]^+$: 550.3257, found 550.3253.

*4.2.2.6. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-heptyl-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **4f**)*

White solid, 92% yield; Mp: 164-165℃; IR (KBr, cm^{-1}): 3420, 2927, 2867, 1723, 1646, 1455, 1363, 1267, 1211, 1108, 1048, 1026, 932, 666; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.80 (1H, s, H-23), 5.77, 5.35 (each 1H, s, H-17), 5.07 (1H, s, H-20), 4.98 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.60-4.42 (2H, m, H-22), 4.32-4.25 (1H, m, H-21), 4.15 (1H, d, *J* = 1.4 Hz, H-11-OH), 4.10-4.02 (2H, m, H-14α, H-11α), 3.98 (1H, d, *J* = 2.9

Hz, H-7β), 3.80-3.58 (1H, m, H-21), 2.68 (1H, t, *J* = 8.0 Hz, H-13α), 2.65-2.58 (3H, m, H-6β, H-25), 1.84 (1H, d, *J* = 8.1 Hz, H-1α), 1.65-1.50 (3H, m, H-26, H-6α), 1.44-1.19 (11H, m, H-27, H-28, H-29, H-2, H-1β, H-12β, H-9β), 1.18-1.10 (2H, m, H-30), 1.03 (1H, d, *J* = 8.8 Hz, H-3β), 0.93-0.83 (6H, m, H-30, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.21, 152.78, 147.35, 122.21, 116.42, 100.13, 69.94, 66.68, 65.78, 63.51, 57.76, 56.66, 50.14, 48.81, 42.98, 42.19, 40.84, 39.67, 34.12, 33.13, 31.65, 30.33, 29.48, 29.10, 28.97, 25.59, 24.69, 22.58, 21.16, 18.14, 14.43. ESI-HRMS: m/z cacld. For $C_{31}H_{47}N_3NaO_5$, $[M+Na]^+$: 564.3413, found 564.3408.

*4.2.2.7. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-8-methylene-12-(2-(4-octyl-1H-1,2,3-triazol-1-yl)ethoxy)decahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **4g**)*

White solid, 92% yield; Mp: 171-172℃; IR (KBr, cm^{-1}): 3419, 2926, 2856, 1723, 1645, 1550, 1457, 1364, 1265, 1219, 1108, 1079, 1026, 933, 724, 655; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.80 (1H, s, H-23), 5.77, 5.35 (each 1H, s, H-17), 5.07 (1H, s, H-20), 4.98 (1H, d, *J* = 2.9 Hz, H-14-OH), 4.62-4.41 (2H, m, H-22), 4.31-4.19 (1H, m, H-21), 4.16 (1H, s, H-11-OH), 4.14-4.07 (2H, m, H-14α, H-11α), 3.96 (1H, d, *J* = 3.1 Hz, H-7β), 3.82-3.62 (1H, m, H-21), 2.68 (1H, t, *J* = 7.5 Hz, H-13α), 2.65-2.57 (3H, m, H-6β, H-25), 1.84 (1H, d, *J* = 9.0 Hz, H-1α), 1.56-1.40 (3H, m, H-26, H-6α), 1.38-1.24 (13H, m, H-27, H-28, H-29, H-30, H-2, H-1β, H-12β, H-9β), 1.17-1.09 (2H, m, H-31), 1.03 (1H, d, *J* = 15.0 Hz, H-3β), 0.90-0.84 (6H, m, H-30, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.20, 152.79, 147.35, 122.21, 116.41, 100.13, 69.94, 66.68, 65.79, 63.51, 57.76, 56.66, 50.14, 48.81, 42.98, 42.19, 40.84, 39.68, 34.11, 33.13, 31.77, 30.33, 29.47, 29.28, 29.15, 29.08, 25.59, 24.69, 22.57, 21.16, 18.14, 14.42. ESI-HRMS: m/z cacld. For $C_{32}H_{49}N_3NaO_5$, $[M+Na]^+$: 578.3570, found 578.3572.

4.2.3. synthesis of compound ***5***

Jiyuan Oridonin A (150 mg) was dissolved in THF (8 mL). Propargylbromide (150 μL) and p-TsOH (10 mg) were added to this solution. The mixture was stirred for 3 h at room temperature. Then the mixture was treated by heric-vacuum distillation and diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, evaporated and the crude product was purified by column chromatography to give compound **5**.

4.2.3.1. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-8-methylene-12-(prop-2-yn-1-yloxy)decahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound ***5)***

White solid, 80% yield; Mp: 125-126℃; IR (KBr, cm^{-1}): 3608, 3365, 3291, 2594, 2875, 1688, 1337, 1255, 1110, 1037, 990, 939, 952, 668; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 5.79, 5.36 (each 1H, s, H-17), 5.17 (1H, s, H-20), 5.03 (1H, d, *J* = 2.5 Hz, H-14-OH), 4.62 (1H, s, H-14α), 4.50 (1H, d, *J* = 16.3 Hz, H-11α), 4.14 (1H, d, *J* = 7.1 Hz, H-11-OH), 3.98 (1H, d, *J* = 2.9 Hz, H-7β), 3.81-3.73 (2H, m, H-21), 2.84 (1H, d, H-24), 2.79 (1H, d, *J* = 8.9 Hz, H-13α), 2.68 (1H, dd, *J* = 16.1, 6.7 Hz, H-6β), 2.62 (1H, dd, *J* = 16.1, 6.7 Hz, H-12α), 2.44 (2H, dd, *J* = 6.1, 4.1 Hz, H-22), 2.02 (1H, d, *J* = 12.9 Hz, H-1α), 1.56 (1H, dd, *J* = 13.8, 4.6 Hz, H-6α), 1.50-1.42 (2H, m, H-2), 1.37-1.29 (3H, m, H-3α, H-1β, H-12β), 1.27-1.15 (2H, m, H-5β, H-9β), 1.06 (1H, dd, *J* = 19.2 Hz, H-3β), 0.93 (3H, s, H-19), 0.82 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.39, 152.92, 116.36, 99.64, 82.49, 72.24, 70.15, 65.88, 65.71, 63.56, 57.80, 56.86, 48.94, 43.07, 42.39, 40.92, 39.89, 34.18, 33.15, 30.40, 24.74, 21.18, 20.02, 18.29. ESI-HRMS: m/z cacld. For $C_{24}H_{32}NaO_5$, $[M+Na]^+$: 423.2147, found 423.2143.

4.2.4. General procedure for the synthesis of Compound ***7a-i***

Compound **6a-i** was put in the system of acetone with NaN_3. The reaction mixture was stirred for 7 h at 65 ℃ to obtain compound **7a-i**. The structure confirmation of compound **7a-i**. were described in the Supplementary data.

*4.2.5. General procedure for the synthesis of Compound **8a-i***

Coumpound **5** (150 mg) and compound **7a-i** were dissolved in THF (6 mL), then put 3 mL H_2O in the system. Finally, the mixture was stirred at room temperature for 6-12 h by using $CuSO_4$ (53 mg) and Cu (103 mg) as catalytic agents and then was filtered and condensed. The residue was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, evaporated to afford the crude product. The crude product was purified by column chromatography to give Compound **8a-i**.

*4.2.5.1. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-benzyl-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (compound **8a**)*

White solid, 90% yield; Mp: 165-167 ℃; IR (KBr, cm^{-1}): 3521, 3397, 3159, 2953, 2922, 2868, 1716, 1643, 1497, 1358, 1265, 1225, 1101, 1024, 799, 736; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.91 (1H, s, H-24), 7.42-7.27 (5H, m, H-Ph), 5.78, 5.36 (each 1H, s, H-17), 5.64-5.46 (2H, s, H-25), 5.12 (1H, s, H-20), 4.99 (1H, d, *J* = 2.6 Hz, H-14-OH), 4.49-4.24 (2H, m, H-14α, H-11α), 4.10 (1H, d, *J* = 7.0 Hz, H-11-OH), 3.98 (2H, q, *J* = 6.4 Hz, H-21), 3.59 (1H, dd, *J* = 16.2, 6.8 Hz, H-7β), 2.91 (2H, t, *J* = 6.5 Hz, H-22), 2.73 (1H, d, *J* = 9.0 Hz, H-13α), 2.65 (1H, t, *J* = 12.7 Hz, H-6β), 1.90 (1H, d, *J* = 6.9 Hz, H-1α), 1.64-1.49 (1H, m, H-6α), 1.39 (1H, d, *J* = 12.6 Hz, H-3α), 1.26 (3H, t, *J* = 13.6 Hz, H-2, H-1β), 1.22-1.18 (1H, m, H-12β), 1.16 (2H, d, *J* = 9.3 Hz, H-5β, H-9β), 1.05 (1H, d, *J* = 11.4 Hz, H-3β), 0.90 (3H, s, H-19), 0.81 (3H, s, H-18). ^{13}C-NMR (101 MHz,

DMSO-d^6) δ 206.26, 152.87, 144.99, 136.64, 129.18, 128.48, 128.29, 122.92, 116.35, 99.77, 70.06, 67.19, 65.62, 63.58, 57.83, 56.78, 53.20, 48.90, 43.01, 42.32, 40.91, 39.76, 34.14, 33.16, 30.47, 26.79, 24.76, 21.19, 18.15. ESI-HRMS: m/z cacld. For $C_{31}H_{39}N_3NaO_5$, $[M+Na]^+$: 556.2787, found 556.2783.

4.2.5.2. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-12-(2-(4-(4-methoxybenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound ***8b)***

White solid, 92% yield; Mp: 203-204 ℃; IR (KBr, cm^{-1}): 3421, 2932, 1748, 1715, 1646, 1515, 1362, 1250, 1102, 1030, 667; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.85 (1H, s, H-24), 7.28 (2H, d, *J* = 8.4 Hz, H-27, H-31), 6.93 (2H, d, *J* = 8.4 Hz, H-28, H-30), 5.78, 5.35 (each 1H, s, H-17), 5.47 (2H, s, H-25), 5.11 (1H, s, H-20), 5.00 (1H, d, J = 2.4 Hz, H-14-OH), 4.40-4.31 (2H, m, H-14α, H-11α), 4.12 (1H, d, *J* = 7.0 Hz, H-11-OH), 4.00-3.92 (2H, m, H-21), 3.74 (3H, s, H-32), 3.56 (1H, dd, *J* = 15.7, 6.6 Hz, H-7β), 2.89 (2H, t, *J* = 6.5 Hz, H-22), 2.75-2.68 (1H, m, H-13α), 2.64 (1H, t, *J* = 12.6 Hz, H-6β), 1.89 (1H, d, *J* = 7.5 Hz, H-1α), 1.58-1.45 (1H, m, H-6α), 1.39 (1H, d, *J* = 12.4 Hz, H-3α), 1.34-1.22 (4H, m, H-2, H-1β, H-12β), 1.19-1.12 (2H, m, H-5β, H-9β), 1.05 (1H, d, *J* = 7.6 Hz, H-3β), 0.89 (3H, s, H-19), 0.81 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.29, 159.56, 152.87, 144.94, 129.97, 128.53, 122.56, 116.36, 114.56, 99.76, 70.04, 67.20, 65.64, 63.56, 57.82, 56.75, 55.60, 52.76, 48.88, 42.99, 42.30, 40.88, 39.51, 34.14, 33.15, 30.49, 26.79, 24.75, 21.17, 18.13. ESI-HRMS: m/z cacld. For $C_{32}H_{41}N_3NaO_6$, $[M+Na]^+$: 586.2893, found 586.2883.

4.2.5.3. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-(3,4-dichlorobenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-

*6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8c**)*

White solid, 93% yield; Mp: 170-171 ℃; IR (KBr, cm^{-1}): 3355, 3147, 2948, 2865, 2101, 1720, 1643, 1473, 1361, 1264, 1223, 1104, 1033, 938, 778; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.95 (1H, s, H-24), 7.64-7.42 (2H, m, H-27, H-31), 7.29 (1H, dd, *J* = 8.3 Hz, H-28), 5.78, 5.35 (each 1H, s, H-17), 5.59 (2H, s, H-25), 5.10 (1H, s, H-20), 4.99 (1H, d, *J* = 2.9 Hz, H-14-OH), 4.40-4.25 (2H, m, H-14α, H-11α), 4.10 (1H, d, *J* = 7.1 Hz, H-11-OH), 4.02-3.91 (2H, m, H-21), 3.58 (1H, dt, *J* = 9.5, 6.7 Hz, H-7β), 2.90-2.84 (2H, m, H-22), 2.76-2.66 (2H, m, H-6β, H-12α), 1.85 (1H, d, *J* = 7.5 Hz, H-1α), 1.60-1.52 (1H, m, H-6α), 1.37 (1H, d, *J* = 12.7 Hz, H-3α), 1.24 (2H, dd, *J* = 16.6 Hz, H-2), 1.23-1.19 (3H, m, H-1β, H-12β, H-5β), 1.16-1.11 (1H, m, H-9β), 1.10-0.97 (1H, m, H-3β), 0.88 (3H, s, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.29, 161.72, 159.27, 152.87, 144.99, 131.09, 131.06, 123.37, 123.02, 116.38, 116.14, 115.94, 99.75, 70.05, 67.15, 65.64, 63.56, 57.82, 56.75, 48.89, 40.02, 39.81, 39.60, 39.39, 34.14, 33.16, 30.47, 26.75, 24.75, 21.17, 18.12. ESI-HRMS: m/z cacld. For $C_{31}H_{37}Cl_2N_3NaO_5$, $[M+Na]^+$: 624.2008, found 624.2001.

*4.2.5.4. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-8-methylene-12-(2-(4-(4-nitrobenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)decahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8d**)*

White solid, 91% yield; Mp: 205-206 ℃; IR (KBr, cm^{-1}): 3421, 2949, 2867, 1722, 1646, 1608, 1523, 1347, 1223, 1105, 734, 667; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 8.23 (2H, d, *J* = 8.7 Hz, H-Ph), 7.98 (1H, s, H-24), 7.51 (2H, d, *J* = 8.7 Hz, H-Ph), 5.77, 5.34 (each 1H, s, H-17), 5.75 (2H, s, H-25), 5.10 (1H, s, H-20), 5.00 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.45-4.24 (2H, m, H-14α, H-11α), 4.11 (1H, d, *J* = 7.0 Hz, H-11-OH), 4.04-

3.93 (2H, m, H-21), 3.59 (1H, dt, *J* = 9.4, 6.6 Hz, H-7β), 2.98-2.86 (2H, m, H-22), 2.70 (1H, d, *J* = 9.0 Hz, H-6β), 2.64 (1H, t, *J* = 12.7 Hz, H-12α), 1.86 (1H, d, *J* = 7.5 Hz, H-1α), 1.62-1.48 (1H, m, H-6α), 1.37 (1H, d, *J* = 12.4 Hz, H-3α), 1.25 (3H, dd, *J* = 16.6, 6.2 Hz, H-2, H-1β), 1.16-1.12 (3H, m, H-12β, H-5β, H-9β), 1.01 (1H, d, *J* = 8.7 Hz, H-3β), 0.89 (3H, s, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.25, 152.85, 147.66, 145.26, 144.14, 129.39, 124.34, 123.38, 116.37, 99.78, 70.03, 67.16, 65.65, 63.60, 57.82, 56.73, 52.30, 48.86, 43.00, 42.28, 40.87, 39.71, 34.13, 33.14, 30.49, 26.77, 24.75, 21.17, 18.14. ESI-HRMS: m/z cacld. For $C_{31}H_{38}N_4NaO_7$, $[M+Na]^+$: 601.2632, found 601.2631.

*4.2.5.5. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-8-methylene-12-(2-(4-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)decahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8e**)*

White solid, 90% yield; Mp: 228-229 ℃; IR (KBr, cm^{-1}): 3446, 2939, 1722, 1646, 1593, 1507, 1463, 1423, 1362, 1242, 1126, 1020, 717; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.90 (1H, s, H-24), 6.70 (2H, s, H-Ph), 5.78, 5.35 (each 1H, s, H-17), 5.45 (H, s, H-25), 5.11 (1H, s, H-20), 4.99 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.47-4.25 (2H, m, H-14α, H-11α), 4.11 (1H, d, *J* = 7.1Hz, H-11-OH), 4.07-3.90 (2H, m, H-21), 3.76 (6H, s, H-32, H-34), 3.64 (3H, s, H-33), 2.99-2.80 (2H, m, H-22), 2.69 (1H, d, *J* = 9.1Hz, H-6β), 2.62 (1H, t, *J* = 12.6 Hz, H-12α), 1.87 (1H, d, *J* = 9.1 Hz, H-1α), 1.60-1.47 (1H, m, H-6α), 1.37 (1H, d, *J* = 12.4 Hz, H-3α), 1.31-1.10 (6H, m, H-2, H-1β, H-12β, H-5β, H-9β), 1.02 (1H, d, *J* = 8.8 Hz, H-3β), 0.89 (3H, s, H-19), 0.80 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d$_6$) δ 206.30, 153.48, 152.87, 144.98, 137.82, 131.85, 122.66, 116.34, 106.31, 99.73, 70.03, 67.13, 65.64, 63.56, 60.42, 57.80, 56.72, 56.38, 53.53, 48.87,

42.98, 42.26, 40.88, 39.91, 34.13, 33.15, 30.50, 26.80, 24.73, 21.16, 18.06. ESI-HRMS: m/z cacld. For $C_{34}H_{45}N_3O_8$, $[M+Na]^+$: 646.3099, found 646.3098.

*4.2.5.6. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-(4-fluorobenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8f**)*

White solid, 89% yield; Mp: 178-180 ℃; IR (KBr, cm^{-1}): 3420, 2950, 2868, 1722, 1645, 1607, 1512, 1393, 1362, 1224, 1103, 1019, 934, 775; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.91 (1H, s, H-24), 7.37 (2H, dd, J = 8.2, 5.7 Hz, H-Ph), 7.20 (2H, t, *J* = 8.8 Hz, H-Ph), 5.78, 5.36 (each 1H, s, H-17), 5.55 (2H, s, H-25), 5.11 (1H, s, H-20), 5.01 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.45-4.26 (2H, m, H-14α, H-11α), 4.12 (1H, d, *J* = 7.1Hz, H-11-OH), 4.01-3.90 (2H, m, H-21), 3.58 (1H, dd, *J* = 16.0, 6.6 Hz, H-7β), 2.90 (2H, t, *J* = 6.3 Hz, H-22), 2.71 (1H, d, *J* = 9.1Hz, H-6β), 2.64 (1H, t, *J* = 12.6 Hz, H-12α), 1.87 (1H, d, *J* = 9.1 Hz, H-1α), 1.55 (1H, dd, *J* = 8.6, 5.7 Hz, H-6α), 1.39 (1H, d, *J* = 12.4 Hz, H-3α), 1.22-1.17 (6H, m, H-2, H-1β, H-12β, H-5β, H-9β), 1.05 (1H, d, *J* = 9.2 Hz, H-3β), 0.89 (3H, s, H-19), 0.81 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.28, 152.86, 145.05, 122.85, 116.38, 99.76, 70.04, 67.16, 65.64, 63.57, 57.81, 56.74, 52.39 48.87, 42.99, 42.29, 40.88, 39.68, 34.14, 33.15, 30.48, 26.77, 24.74, 21.17, 18.13. ESI-HRMS: m/z cacld. For $C_{31}H_{38}FN_3NaO_5$, $[M+Na]^+$: 574.2687, found 574.2683.

*4.2.5.7. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-(4-chlorobenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8g**)*

White solid, 94% yield; Mp: 180-181 ℃; IR (KBr, cm^{-1}): 3419, 2949, 2867, 1722, 1644, 1493, 1362, 1266, 1222, 1103, 1078, 1016, 934, 769; ^{1}H-NMR (400 MHz,

DMSO-d^6) δ 7.91 (1H, s, H-24), 7.44 (2H, d, *J* = 8.2 Hz, H-Ph), 7.32 (2H, d, *J* = 8.2 Hz, H-Ph), 5.78, 5.36 (each 1H, s, H-17), 5.57 (2H, s, H-25), 5.10 (1H, s, H-20), 5.00 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.43-4.27 (2H, m, H-14α, H-11α), 4.12 (1H, d, *J* = 7.1Hz, H-11-OH), 4.06-3.89 (2H, m, H-21), 3.58 (1H, dt, *J* = 9.4, 6.6 Hz, H-7β), 2.97-2.83 (2H, m, H-22), 2.69 (1H, d, *J* =7.8 Hz, H-6β), 2.62 (1H, t, *J* = 12.5 Hz, H-12α), 1.87 (1H, d, *J* = 9.1 Hz, H-1α), 1.61-1.49 (1H, m, H-6α), 1.38 (1H, d, *J* = 12.4 Hz, H-3α), 1.30-1.11 (6H, m, H-2, H-1β, H-12β, H-5β, H-9β), 1.09 – 0.99 (1H, m, H-3β), 0.89 (3H, s, H-19), 0.81 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.28, 152.86, 145.11, 135.65, 133.22, 130.26, 129.18, 122.96, 116.38, 99.78, 70.03, 67.17, 65.64, 63.57, 57.81, 56.74, 52.39, 48.87, 42.99, 42.29, 40.88, 39.70, 34.14, 33.15, 30.49, 26.77, 24.74, 21.16, 18.12. ESI-HRMS: m/z cacld. For $C_{31}H_{38}ClN_3NaO_5$, $[M+Na]^+$: 590.2392, found 590.2391

*4.2.5.8. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-11,14-dihydroxy-4,4-dimethyl-12-(2-(4-(4-methylbenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8h**)*

White solid, 90% yield; Mp: 172-173 ℃; IR (KBr, cm^{-1}): 3422, 2949, 2867, 1722, 1646, 1516, 1454, 1362, 1220, 1103, 1020, 760; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.87 (1H, s, H-24), 7.19 (4H, q, *J* = 8.3 Hz, H-Ph), 5.78, 5.36 (each 1H, s, H-17), 5.60-5.42 (2H, m, H-25), 5.11 (1H, s, H-20), 5.01 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.40-4.32 (2H, m, H-14α, H-11α), 4.13 (1H, d, *J* = 7.1 Hz, H-11-OH), 4.01-3.93 (2H, m, H-21), 3.57 (1H, dt, *J* = 9.5, 6.7 Hz, H-7β), 2.98-2.83 (2H, m, H-22), 2.72 (1H, d, J =7.8Hz, H-6β), 2.64 (1H, t, *J* = 12.5 Hz, H-12α), 2.29 (3H, s, H-32), 1.89 (1H, d, *J* = 9.1 Hz, H-1α), 1.60-1.51 (1H, m, H-6α), 1.43-1.35 (1H, m, H-3α), 1.33-1.11 (6H, m, H-2, H-1β, H-12β, H-5β, H-9β), 1.10-0.99 (1H, m, H-3β), 0.89 (3H, s, H-19), 0.81 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.29, 152.87, 144.96, 137.81, 133.62, 129.71, 128.38,

122.74, 116.37, 99.77, 70.04, 67.20, 65.64, 63.56, 57.82, 56.74, 53.01, 48.88, 43.00, 42.30, 40.89, 39.72, 34.14, 33.16, 31.15, 30.49, 26.78, 24.75, 21.16, 18.14. ESI-HRMS: m/z cacld. For $C_{32}H_{41}N_3NaO_5$, $[M+Na]^+$: 570.2938, found 570.2940.

*4.2.5.9. (4aR,6R,6aS,9S,11S,11aS,11bS,12S,14R)-12-(2-(4-(2-chlorobenzyl)-1H-1,2,3-triazol-1-yl)ethoxy)-11,14-dihydroxy-4,4-dimethyl-8-methylenedecahydro-1H-6,11b-(epoxymethano)-6a,9-methanocyclohepta[a]naphthalen-7(8H)-one (Compound **8i**)*

White solid, 92% yield; Mp: 186-187 ℃; IR (KBr, cm^{-1}): 3526, 2932, 2870, 1714, 1646, 1447, 1362, 1266, 1224, 1101, 1040, 1022, 940, 761; ^{1}H-NMR (400 MHz, DMSO-d^6) δ 7.90 (1H, s, H-24), 7.53 (1H, d, *J* = 7.6 Hz, H-Ph), 7.38 (2H, ddd, *J* = 15.1, 7.4, 6.1 Hz, H-Ph), 7.22-7.14 (1H, m, H-Ph), 5.79, 5.36 (each 1H, s, H-17), 5.67 (2H, s, H-25), 5.13 (1H, s, H-20), 5.01 (1H, d, *J* = 2.8 Hz, H-14-OH), 4.42-4.32 (2H, m, H-14α, H-11α), 4.11 (1H, d, *J* = 7.1 Hz, H-11-OH), 4.03-3.94 (2H, m, H-21), 3.65-3.56 (1H, m, H-7β), 2.97-2.88 (2H, m, H-22), 2.74 (1H, d, *J* = 8.9 Hz, H-6β), 2.65 (1H, t, *J* = 12.5 Hz, H-12α), 1.90 (1H, d, *J* = 9.1 Hz, H-1α), 1.61-1.48 (1H, m, H-6α), 1.40 (1H, d, *J* = 12.4 Hz, H-3α), 1.32-1.13 (6H, m, H-2, H-1β, H-12β, H-5β, H-9β), 1.05-0.98 (1H, m, H-3β), 0.90 (3H, s, H-19), 0.81 (3H, s, H-18). ^{13}C-NMR (101 MHz, DMSO-d^6) δ 206.19, 152.78, 144.13, 139.18, 129.29, 128.58, 127.35, 124.22, 116.43, 100.14, 69.92, 66.81, 65.81, 63.53, 61.07, 57.72, 56.67, 48.84, 40.85, 40.65, 39.81, 39.60, 39.39, 34.11, 33.13, 30.35, 24.96, 24.70, 21.18, 18.14, 14.40. ESI-HRMS: m/z cacld. For $C_{31}H_{38}ClN_3NaO_5$, $[M+Na]^+$: 590.2392, found 590.2393.

4.3. In vitro determination of effects of synthesized compounds on cancer cell proliferation.

All cancer cells were performed in 96-well plates at a density of 1×10^4 cells/well with 100 μL/well culture medium for 24 h. After that, added 200 μL culture medium

with appropriate drug concentration into wells to incubated for 48 h in incubator, then 20 μL of MTT solution (5 mg/mL) per well was added to each cultured medium, which was incubated for another 4 – 6 h. Finally, DMSO was added to each well (150 mL/well) after pouring out the liquid and absorbance of all wells was determined by measuring OD at 490 nm.

4.4. Measurement of ROS levels

MGC-803 cells at the log phase of their growth cycle (1×10^5 cell/mL) were added to each well and treated with compound **8g** of 3 μg/mL for 0, 3, 5 hours, which tested by flow cytometry analysis via DCFH-DA staining.

4.5. Study of NAC on MGC-803 cells

The MGC-803 cells were incubated respectively with the compound **8g** of different concentrations and pretreatment with NAC incubated for 2 h as described above, then stained with JC-1 and observed under a fluorescence microscope.

*4.6. Study on apoptosis induction by compound **8g***

After incubated with compound **8g** and NAC, MGC-803 cells adopted with shortened time of trypsin digestion, then separate the cells with 1000 r/min for 5 min to discard supernatant. Lastly, cells were stained by Annexin V-FITC/PI isolated light source for 5 min, and measured using a flow cytometer. At the same time, another group's old culture medium in the 6-well was sucked and added Hoechst 33258 to incubate for 20 min, finally observed under a fluorescence microscope.

4.7. The test of cell cycle

The cell cycle experiment steps were similar to the apoptosis trial. After 48 h treatment, cells were fixed with 70% ethanol in 4 ℃ for a whole night. Through a series of physical and mechanical treatment, cells were stained by dyeing liquid formed with

1% triton, 50 μg/mL RnaseA and 50 μg/mL PI, which detected by flow cytometry analysis and analyzed by FlowJo software.

4.8. Western blot analysis

MGC-803 cells were treated with different doses of compound **8g**, after 48 h of incubation, cells were harvested and lysed, and the determined concentrations protein was extracted by electrophoresis on SDS-polyacrylamide gels and transferred to PVDF membranes. After being blocked with 5% nonfat milk, desired primary antibodies (anti-Bax, anti-Bcl-2, anti-P21, anti-P53, anti-caspase 9, anti-survivin, anti-cyto C) were added to the membranes with target proteins incubated overnight. Subsequently, the membrane was incubated with appropriate secondary antibody. The relative levels of each signaling event to control GAPDH were determined by chemiluminescence.

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Appendix A. Supplementary data

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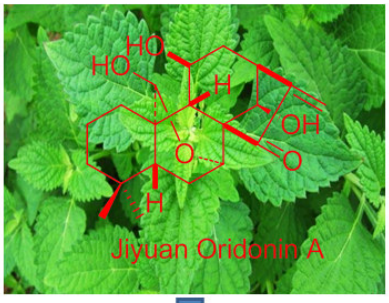

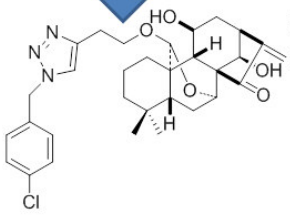

Compound **8g**
2.53±0.10 (MGC-803)

increase of ROS in cells
induce apoptosis

proliferation
inhibition

selectivity to cancer
cells

Two series of derivatives with 1,2,3-triazole as heterocyclic moiety of Jiyuan Oridonin A exhibited better anti-proliferative activities than Oridonin. Among them compound **8g** was found to possess the best anti-proliferative activity.

The SAR showed 1,2,3-triazole could significantly improve the anti-proliferative activities of the lead compound.

Compound **8g** was related to the increase of ROS in cancer cells, leading to the decrease of mitochondrial membrane potential and the release of Cytochrome C into the cytoplasm.

Compound **8g** halted cell cycle progression at the G2/M phase and altered the expression of cell cycle-related proteins.