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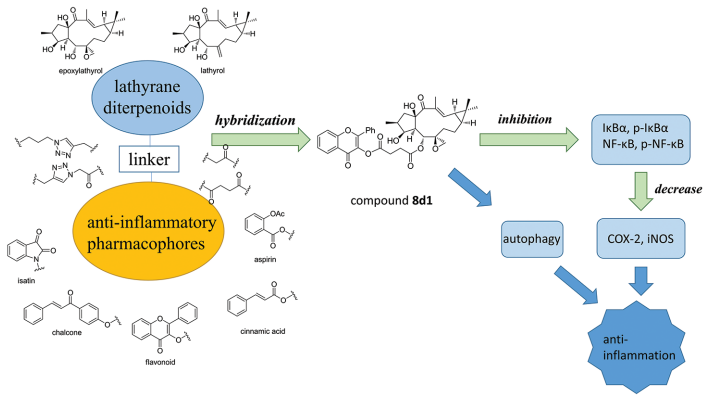
New lathyrane diterpenoid hybrids have anti-inflammatory activity through the NF-κB signaling pathway and autophagy

Graphical abstract



Highlights

- Three series of lathyrane diterpenoid hybrids were synthesized.
- Compound **8d1** showed potent anti-inflammatory activity with low cytotoxicity (IC_{50} value: 1.55 ± 0.68 μM).
- The anti-inflammatory mechanism of **8d1** was associated with the inhibition of NF-κB signaling pathways and the induction of autophagy.

Authors

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In brief

The lathyrane diterpenoid/3-hydroxyflavone hybrid **8d1** shows potent anti-inflammatory activities, which could serve as a promising anti-inflammatory agent.

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Research Article

New lathyrane diterpenoid hybrids have anti-inflammatory activity through the NF-κB signaling pathway and autophagy

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ABSTRACT

In our ongoing work on the identification of potent anti-inflammatory agents, we designed and synthesized three series of lathyrane diterpenoid hybrids in which the lathyrane diterpenoid skeleton was hybridized with other anti-inflammatory pharmacophores. Unexpectedly, lathyrane diterpenoid/3-hydroxyflavone hybrids showed more potent anti-inflammatory activity in RAW264.7 cells than did the corresponding parent compounds. Compound **8d1** exhibited potent anti-inflammatory activity with low cytotoxicity (IC_{50} = 1.55 ± 0.68 μM), and downregulated LPS-induced expression of iNOS and COX-2, as well as IκBα phosphorylation. This compound also inhibited the expression and nuclear translocation of NF-κB, and stimulated autophagy induction. Thus, **8d1**'s anti-inflammatory mechanism is associated with inhibition of the NF-κB signaling pathway and increasing autophagy. This compound may serve as a promising anti-inflammatory agent.

Keywords: lathyrane, 3-Hydroxyflavone, 1,2,3-Triazole, hybrid, anti-inflammation, structural modification

1. INTRODUCTION

Inflammation is a complex response of the body to defense harmful stimuli [1]. After immune cells are stimulated, the NF-κB pathway, together with associated pathways such as the MAPK, ERK1/2 and JNK pathways, activates target genes and releases inflammatory mediators [2, 3]. During the process, excess inflammatory mediators can lead to chronic or acute inflammatory diseases, thus posing a serious threat to human health [4].

Autophagy is a complex evolutionarily conserved process involving the degradation of damaged organelles, misfolded proteins and pathogens in cells in response to various stress reactions [5]. Autophagy can be classified as macroautophagy, microautophagy or chaperone-mediated autophagy according to how intracellular substrates enter lysosomes. Beyond its role in maintaining biological homeostasis, autophagy is involved in various diseases, such as inflammatory diseases, infectious diseases and diabetes [6, 7]. Whereas excessive autophagy may lead to a persistent inflammatory state that exacerbates disease [8], appropriate autophagy alleviates the inflammatory response by inhibiting the assembly and activation of NLRP3 inflammasomes [9, 10], and inducing the transformation of macrophages into M2-type cells with anti-inflammatory effects [11].

Active natural products are important lead-compound sources for the development of therapeutic drugs [12, 13]. The terpenes are among the most promising medicinal natural products, owing to their diverse structure and abundant sources. Terpenes are often used as lead compounds in the synthesis of drugs based on natural compounds. Some macrocyclic or polycyclic diterpenoids have various biological activities, such as anticancer [14], multidrug resistance reversal [15] and antiviral [16] effects. In recent years, some lathyrane diterpenoids have been found to display favorable anti-inflammatory activity (**Figure 1**). Researchers have explored these compounds' anti-inflammation mechanisms and performed *in vivo* experiments to verify their effectiveness [17-23].

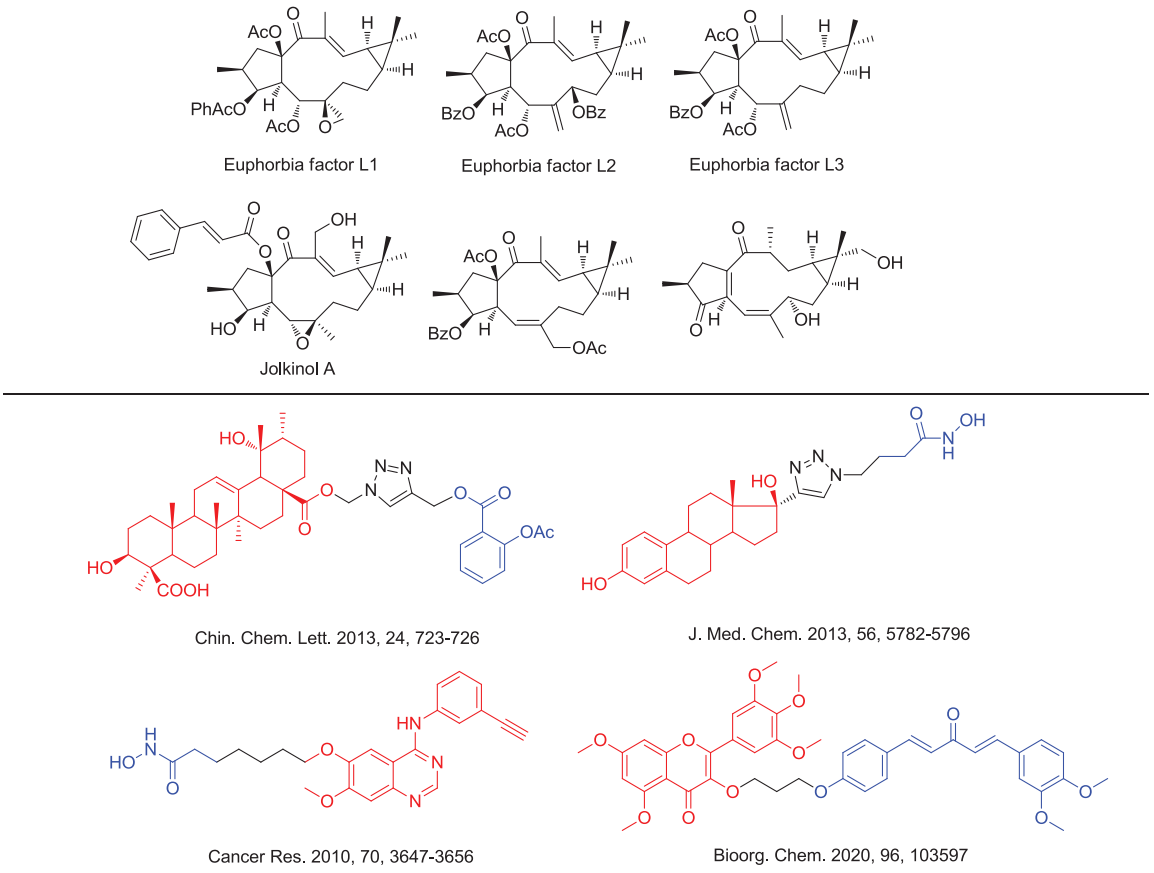

Figure 1 | Structures of several representative lathyrane-type diterpenoids and hybrid molecules with favorable bioactivity.

Hybridization of two of the same or different pharmacophore types into a new molecule can enhance bioactivity or decrease adverse effects, because hybrid molecules may have new mechanisms of action. Many hybrid molecules have been found to exhibit improved bioactivity over the original pharmacophores, thus revealing that hybridization is a useful strategy to develop novel drugs [24, 25]. Hybridizing a lathyrane diterpenoid skeleton with other anti-inflammatory pharmacophores has the potential to provide novel anti-inflammatory candidates [26].

Herein, we designed and synthesized three series of epoxylathyrol and lathyrol hybrids in which the lathyrane diterpenoid skeleton was hybridized with other anti-inflammatory pharmacophores. The inhibitory activity toward lipopolysaccharide (LPS)-induced NO production in RAW264.7 cells and the mechanisms of these hybrids were investigated. These findings encouraged us to investigate appropriate methods for modifying lathyrane diterpenoids.

2. RESULTS AND DISCUSSION

2.1 Chemistry

As important nitrogen-containing heterocycles, 1,2,3-triazole bind target proteins through non-covalent interactions such as hydrogen bonds, hydrophobic interactions dipole-dipole bonds and van der Waals forces [27]. Compounds containing 1,2,3-triazole may have multiple activities, such as anticancer [28], antimalarial [29], antibacterial [30], antiviral [31] and antifungal [32] activities. The 1,2,3-triazole moiety can be easily prepared through click chemistry, in which the azide group reacts with alkynes under Cu-catalyzed conditions [33]. Furthermore, evidence has indicated the anti-inflammatory efficacy of 1,2,3-triazole [34, 35]. Therefore, 1,2,3-triazole was first selected as the linker herein.

To maintain the high anti-inflammatory activity of derivatives, the esterification of C-5-hydroxyl was selected as the initial modification method (**Scheme 1**) on the basis of our previous work [18]. Lathyrol (and epoxylathyrol) was prepared according to a previously described method [36], and this was followed by the esterification of C-5 hydroxyl with chloroacetic acid to obtain compound **1**. The chlorine atom of **1** was substituted by an azide group via reacted with sodium azide to obtain compound **2**. We selected several anti-inflammatory pharmacophores, compounds **5a–g** (aspirin [37], isatin [26], flavonoid [38], cinnamic acid [39] and chalcone [40], respectively) with active hydrogen and reacted them with propargyl bromide to obtain

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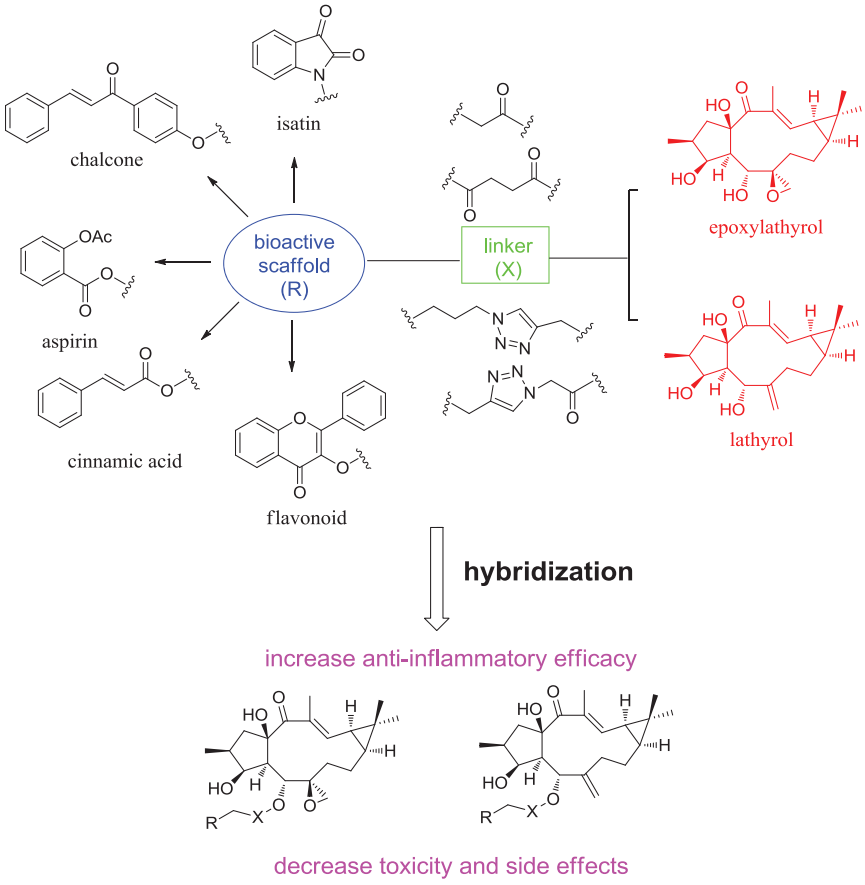


Figure 2 | Design strategy of target hybrid molecules in this work.

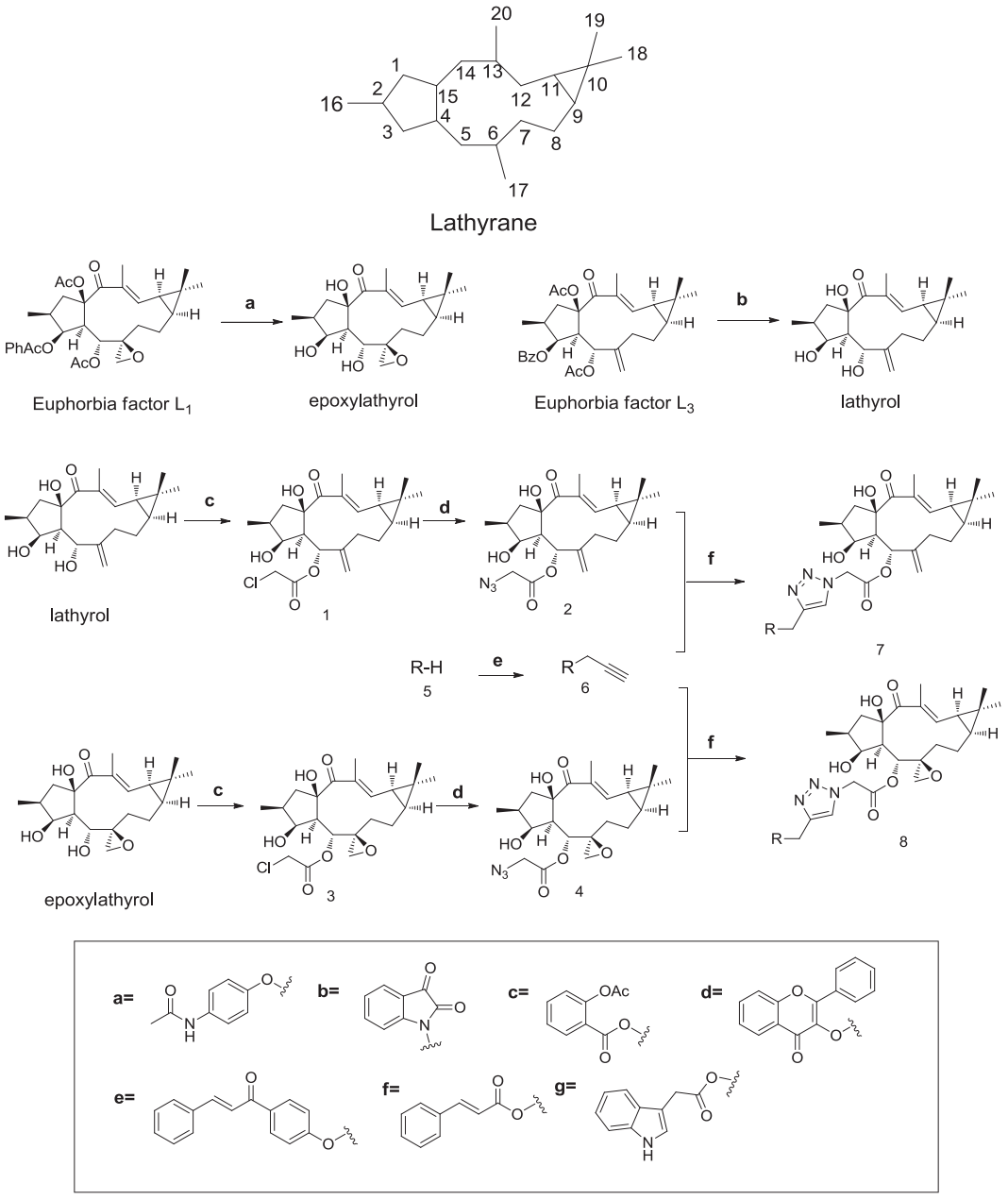
compounds **6a–g**. The synthesis of compounds **7a–g** was operationally simple, through use of Cu-catalyzed click chemistry [41] as the key step. The synthetic route of **8a–f** was similar to that of **7a–g**.

2.2 Evaluation of anti-inflammatory activity and structure activity relationships

Nitric oxide, an inflammatory mediator, is produced in response to pathogen-associated molecular patterns [42]. The excessive NO levels produced during acute or chronic inflammation are responsible for tissue injury, either directly or indirectly [43]. All prepared compounds were tested for their inhibitory activity toward LPS-induced NO production in RAW264.7 cells. The anti-inflammatory activity of series **1** is described as the IC_{50} values of NO inhibition rates in Table 1. Among the tested compounds, **8d** showed the strongest activity against LPS-stimulated NO release (IC_{50} value: 0.91 ± 1.38 μM).

The preliminary structure activity relationships (SARs) indicated that our strategy was successful: the anti-inflammatory effects were improved through combination of lathyrane diterpenoid with other anti-inflammatory pharmacophores through 1,2,3-triazole. Approximately three-quarters of the lathyrol hybrids showed better inhibitory activity than Euphorbia factor L_3. In contrast, only one-quarter of the epoxylathyrol hybrids showed more potent inhibitory activity than Euphorbia factor L_1. Meanwhile, two 3-hydroxyflavone derivatives clearly exhibited potent anti-inflammatory efficacy, in sharp contrast to aspirin and acetaminophen. However, the anti-inflammatory activity of 3-hydroxyflavone was not strong (IC_{50} >20 μM). To further improve the anti-inflammatory activity of lathyrane diterpenoid/3-hydroxyflavone hybrids, we synthesized series **2** and **3**, in which the linker and flavonoid was changed, respectively.

The general synthetic route of series **2** is provided in Scheme 2. In this series, the 1,2,3-triazole linker was replaced by a linear linker with a different length. Moreover, the effects of chemical bonds between linkers and pharmacophores or lathyrane diterpenoids on the activity of hybrids were also investigated. In series **3**, the 3-hydroxyflavone of **8d** was replaced by quercetin, kaempferol and farrerol (Scheme 3). The mono-substituted intermediates **8h–j** were obtained by controlling the equivalent of bromopropyne and potassium carbonate. The attachment position of the propynyl group was determined on the basis of heteronuclear multiple bond correlations.



Scheme 1 | Synthetic route for 7a–g and 8a–f (series 1).
Reagents and conditions: (a) K_2CO_3, methanol, room temperature, overnight; (b) KOH, methanol, room temperature, 6 h; (c) chloroacetic acid, EDCI, DMAP, DCM, room temperature, 8–10 h; (d) NaN_3, DMF, 60°C, 6 h; (e) propargyl bromide, K_2CO_3, DMF, 60°C, 5–6 h; (f) VCNa, $CuSO_4$, THF/H_2O (3/1), room temperature, 0.5 h.

The anti-inflammatory activity of series **2** and **3** is shown in Table 2. In series **2,** the activity intensity of **7d1** and **8d1** was similar to **that of 7d** and **8d**. Other compounds were clearly weaker than **7d** and **8d**. In series **3, 8h–j** were much weaker than **8d**. The above results demonstrated that 1,2,3-triazole significantly improved the anti-inflammatory effects of hybrids when the linker and lathyrane diterpenoid were linked by an ester bond. The type chemical bond between the linker and pharmacophore did not appear to be very important for activity. Meanwhile, we inferred that short linkers were more conducive to anti-inflammatory enhance activity than long linkers. From **8h–j,** we concluded that the hydroxyl groups of flavones might weaken the activity of hybrids. The above results provide ideas for further modification of lathyrane diterpenoids (Figure 3).

Table 1 | IC_{50} values for **7a–g** and **8a–f** (series **1**) inhibition of NO production in RAW264.7 cells stimulated with LPS[a]

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
Euphorbia factor L_1	9.90 ± 1.40	**7f**	19.90 ± 1.10
Euphorbia factor L_3	8.06 ± 1.40	**7g**	7.47 ± 1.09
Epoxylathyrol	25.63 ± 7.86	**8a**	> 50
Lathyrol	11.10 ± 1.14	**8b**	21.52 ± 1.05
7a	1.09 ± 0.39	**8c**	14.46 ± 1.03
7b	1.81 ± 0.64	**8d**	0.91 ± 0.44
7c	5.12 ± 1.10	**8e**	1.42 ± 0.25
7d	1.57 ± 0.38	**8f**	9.42 ± 1.07
7e	1.22 ± 0.10	**Dexamethasone**	7.90 ± 1.30

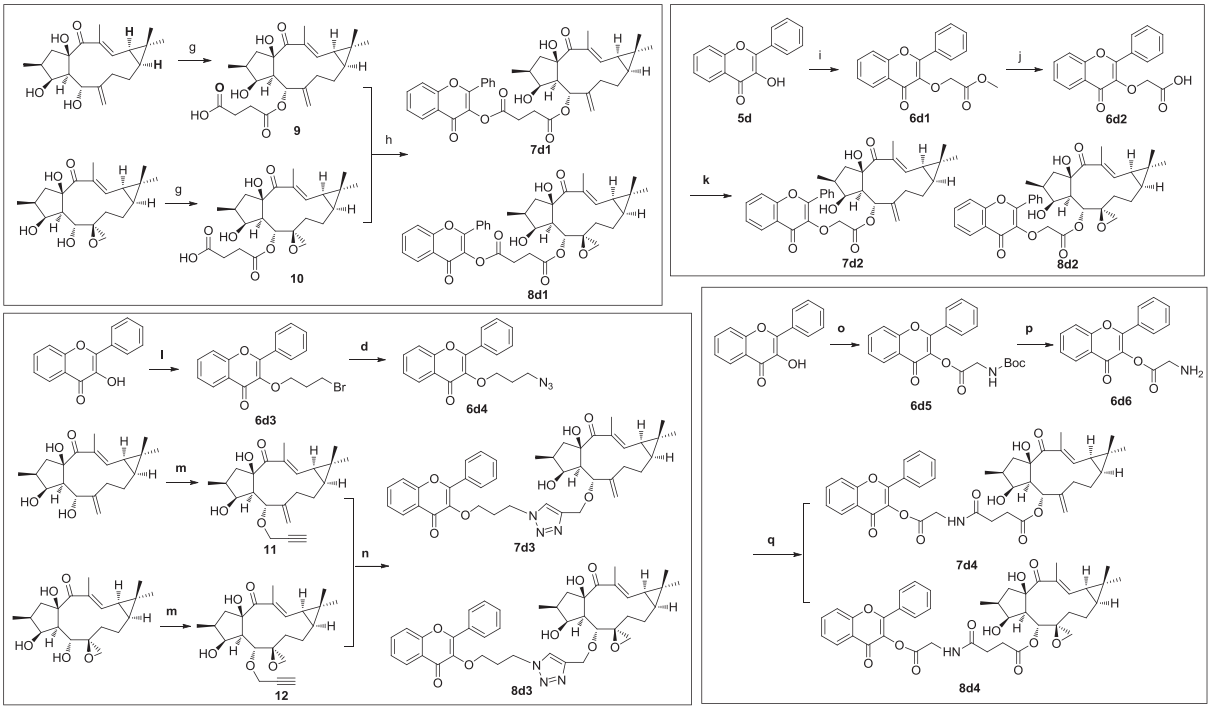
[a]The results are shown as mean ± SD of at least three independent experiments.

2.3 Toxicity

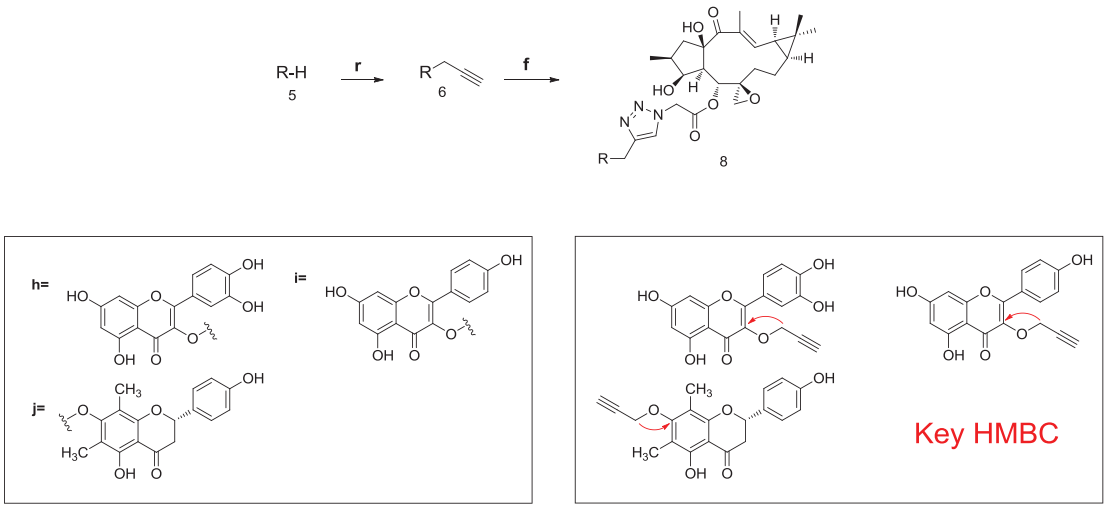
We evaluated the cytotoxicity of compounds **8d** and **8d1** toward RAW264.7 cells. As shown in **Figure 4**, compound **8d** showed clear cytotoxicity at a 12.5 mM concentration. In contrast, compound **8d1** showed lower cytotoxicity than **8d** at the same concentration. Our results revealed that 1,2,3-triazole enhanced the cytotoxicity of compounds while increasing their activity. Compound **8d1** was selected for further pharmacological study.

2.4 Compound 8d1 suppresses LPS-induced iNOS and COX-2 expression

We next explored the anti-inflammatory mechanism of compound **8d1**. In the inflammatory process, increases in iNOS and COX-2 lead to the production of various cytokines and inflammatory mediators, such as NO, TNF-α, IL-1β and IL-6. INOS, which catalyzes the expression of NO, is usually upregulated and expressed by macrophages in response to inflammatory stimuli. COX-2 was a major target for the discovery of nonsteroidal anti-inflammatory drugs, and it participates in the arachidonic acid cascade [44]. Thus, we analyzed the inhibitory effects of **8d1** on LPS-mediated expression of iNOS and COX-2 by using western blotting (**Figure 5A**). As expected, LPS stimulation markedly increased iNOS and COX-2 protein expression, and compound **8d1** significantly and completely inhibited the high expression of iNOS (**Figure 5B**) and decreased the expression of COX-2 (**Figure 5C**) induced by LPS.



Scheme 2 | Synthetic route for 7d1–d4 and 8d1–d4 (series 2).
Reagents and conditions: (g) DMAP, $(C_2H_5)_3N$, succinic anhydride, DCM, room temperature, 2–3 h; (h) **5d**, DMAP, EDCI, DCM, room temperature, overnight; (i) methyl bromoacetate, K_2CO_3, DMF, 60°C, 5–6 h; (j) $LiOH·H_2O$, THF/H_2O (3/1), room temperature, 3 h; (k) lathyrol or epoxylathyrol, EDCI, DMAP, DCM, room temperature, 7–8 h; (l) 1,3-dibromopropane, K_2CO_3, DMF, 60°C, 5–6 h; (m) NaH, propargyl bromide, DMF, ice bath to room temperature, 1–2 h; (n) **6d4**, VCNa, $CuSO_4$, THF/H_2O (3/1), room temperature, 0.5 h; (o) N-Boc-glycine, EDCI, DMAP, DCM, room temperature, overnight; (p) CF_3COOH, DCM, 0°C to room temperature, 0.5 h; (q) **9** or **10**, EDCI, HOBt, DCM, room temperature, 7–8 h.



Scheme 3 | Synthetic route of 8h–j (series 3).
Reagents and conditions: (r) propargyl bromide, K_2CO_3, DMF, room temperature, 8–9 h; (f) VCNa, $CuSO_4$, THF/H_2O (3/1), room temperature, 0.5 h.

2.5 Compound 8d1 inhibits the NF-κB pathway in LPS-induced RAW264.7 cells

NF-κB is a member of a transcription factor family that controls the expression of genes associated with inflammatory, apoptosis and immune responses [45]. NF-κB and the members of its signaling pathway play essential roles in many stages of inflammatory diseases [46]. In the cytoplasm, IκB binds NF-κB, thus masking the nuclear localization signal and inhibiting the activity of NF-κB [47, 48]. However, the phosphorylation of IκBα activates, and leads to the nuclear translocation of, NF-κB [49]. To further assess whether **8d1** exerted an anti-inflammatory effect through the NF-κB signaling pathway, we pretreated cells with different concentrations of **8d1** before induction with LPS. The ratio of p-NF-κB/NF-κB and p-IκBα/IκBα significantly decreased with **8d1** preprotection, thus indicating the inhibition of NF-κB signaling pathway (Figure 6A-C). The translocation of NF-κB in LPS-stimulated RAW264.7 cells was detected by western blotting and immunofluorescence. After treatment with LPS, NF-κB translocated into the nucleus, but NF-κB nuclear translocation was blocked by **8d1** at 10 μM (Figure 6D-F). These data demonstrated that **8d1** negatively regulated NF-κB and blocked the nuclear translocation of NF-κB in RAW264.7 cells induced with LPS.

Table 2 | IC_{50} values for series **2** and **3** inhibition of NO production in RAW264.7 cells stimulated with LPS

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
7d1	3.63 ± 0.96	**8d3**	10.48 ± 1.09
7d2	4.17 ± 1.12	**8d4**	10.54 ± 1.36
7d3	9.85 ± 0.99	**8h**	> 20
7d4	14.77 ± 1.17	**8i**	> 20
8d1	1.55 ± 0.68	**8j**	9.86 ± 1.30
8d2	4.33 ± 1.43	**Dexamethasone**	7.90 ± 1.30

2.6 Compound 8d1 activates autophagy in LPS-induced RAW264.7 cells

Autophagy is regulated by various autophagy proteins, such as LC3B, Beclin 1 and P62. P62 affects autophagy by participating in autophagy-lysosomal protein degradation [50]. LC3B plays an important role in autophagosome bilayer membrane elongation and substrate recognition [51]. Moreover, the expression of LC3B is closely associated with the number of autophagosomes; therefore, the ratio of LC3B II/LC3B I is often used to represent the level of autophagy.

To examine the effect of **8d1** on autophagy in LPS-induced RAW264.7 cells, we incubated cells with different concentrations of **8d1** (1.25, 2.5, 5 and 10 μM). The changes in autophagy proteins levels were observed through western blotting. Compound **8d1** increased the ratio of LC3B II/LC3B I, and decreased the level of P62 in a concentration-dependent manner (Figure 7A). Simultaneously, the autophagy inhibitor chloroquine (CQ) was used to block the binding of autophagosomes and lysosomes to observe the changes in autophagosomes. After treatment with 10 μM **8d1**, the number of autophagosomes significantly increased (Figure 7B). Immunofluorescence experiments also confirmed the above results (Figure 7C).

3. CONCLUSION

In summary, we designed and synthesized three series of epoxylathyrol and lathyrol hybrids, in which the

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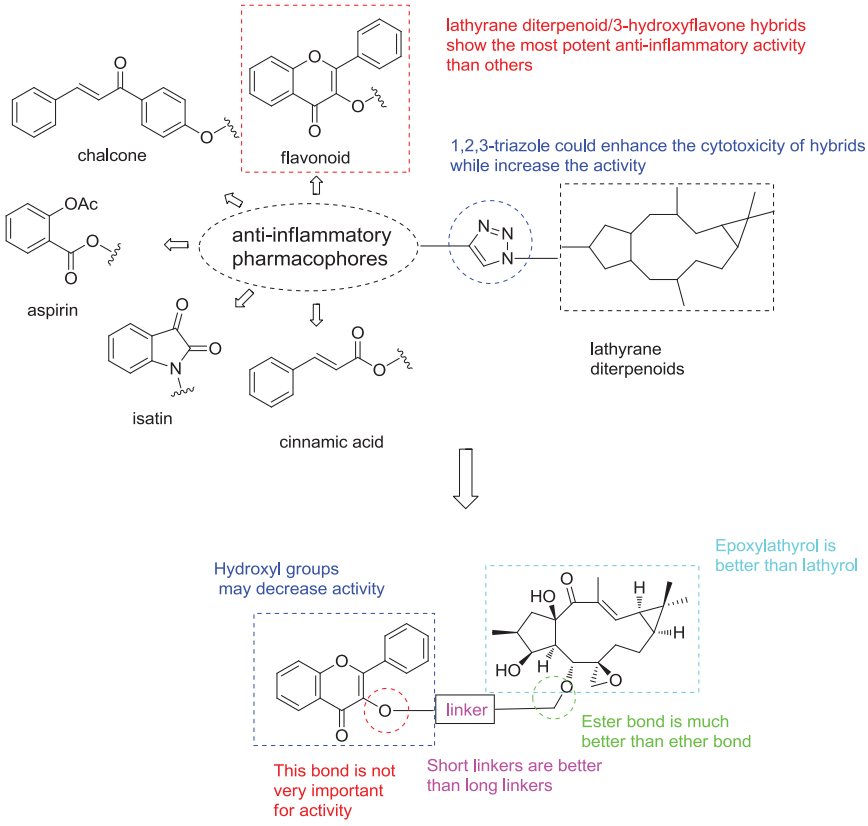

Figure 3 | SARs of lathyrane diterpenoid/anti-inflammatory pharmacophore hybrids.

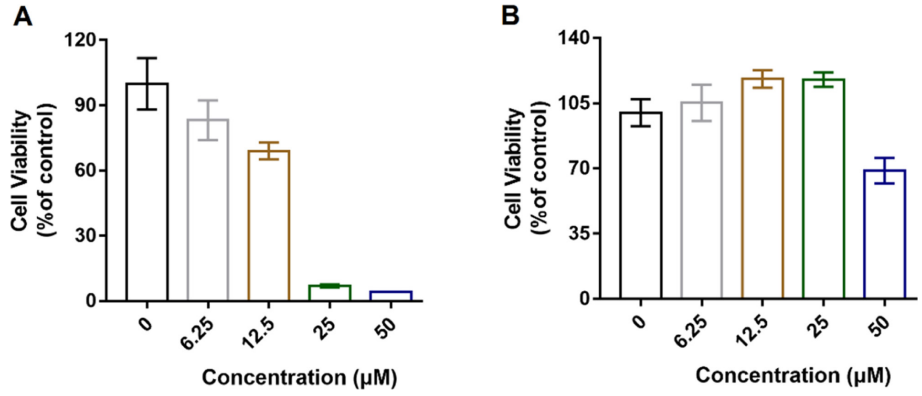

Figure 4 | Effects of compounds 8d (a) and 8d1 (b) on RAW264.7 cell viability with treatment for 24 h.

lathyrane diterpenoid skeleton was hybridized with other anti-inflammatory pharmacophores. Many compounds displayed favorable inhibitory activity toward LPS-induced NO production in RAW264.7 cells. The preliminary SARs illustrated that 3-hydroxyflavone significantly enhanced the anti-inflammatory activity of two lathyrane diterpenoids, although its own activity was not strong. Meanwhile, different linkers and the chemical bond between the linker and lathyrane diterpenoid influenced the activity of hybrids.

Among all hybrids, compound **8d1** exhibited potent anti-inflammatory activity with low cytotoxicity. Further studies revealed that **8d1** exerted anti-inflammatory effects by decreasing COX-2 and iNOS production, inhibiting the activation of NF-κB and inducing autophagy. These findings indicated that compound **8d1** may serve

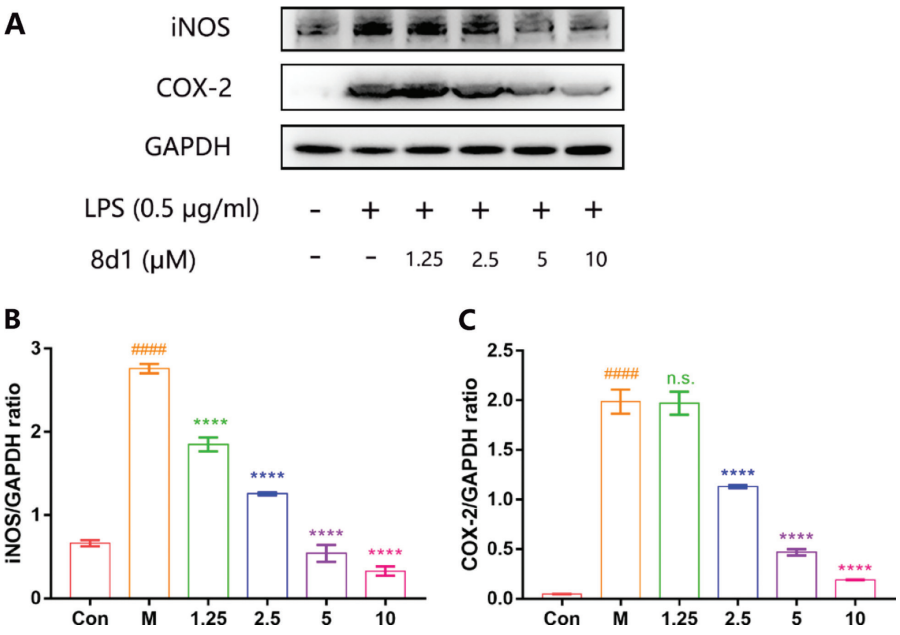

Figure 5 | Compound 8d1 decreases LPS-induced expression of iNOS and COX-2.
(a) Western blotting for iNOS and COX-2. (b,c) Relative ratio of iNOS and COX-2. The values are presented as mean ± SD of three independent experiments, n = 3. ####, $p < 0.0001$, vs. control group; ****, $p < 0.0001$, vs. LPS-induced group.

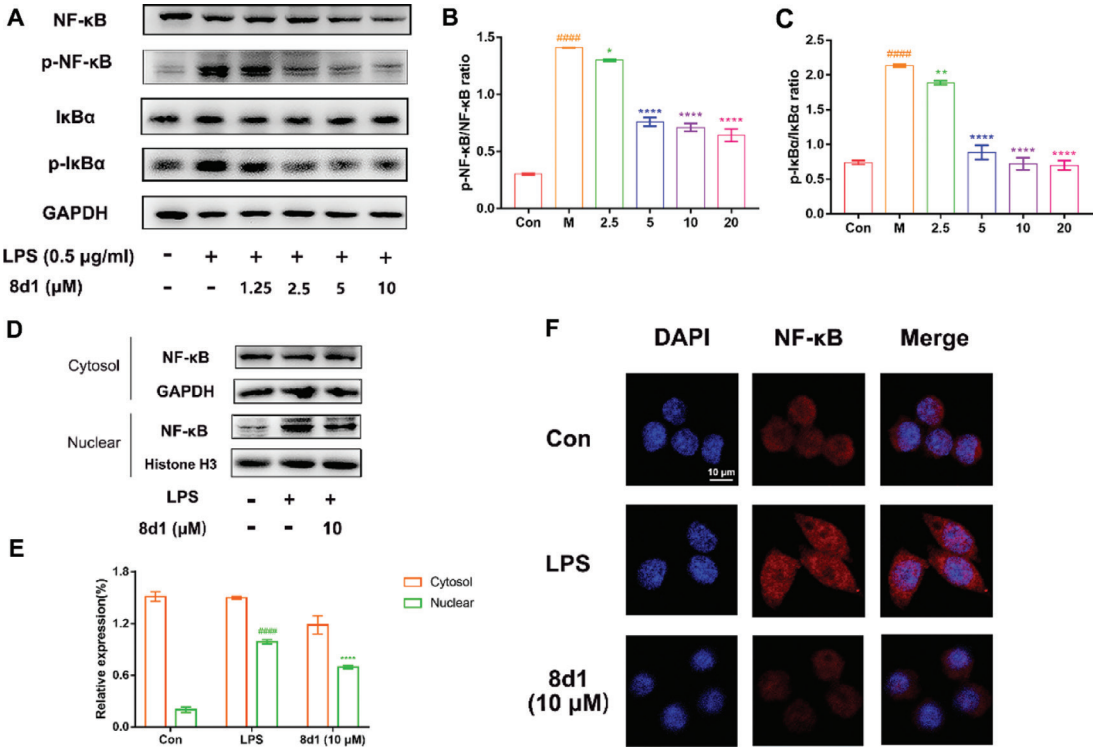

Figure 6 | Effects of 8d1 on NF-κB signaling in RAW264.7 cells stimulated with LPS.
(a–c) Expression of IκBα, p-IκBα, NF-κB and p-NF-κB in RAW264.7 cells stimulated with LPS. (d,e) Expression of NF-κB and p-NF-κB in both the cytoplasmic and nuclear fractions, determined by western blotting. (f) Immunofluorescence staining for the nuclear translocation of NF-κB. RAW264.7 cells stained for NF-κB/p65 (red) and nuclei (DAPI, blue). The values are presented as mean ± SD of three independent experiments, n = 3. ####, $p < 0.0001$, vs. control group, *, $p < 0.05$, **, $p < 0.01$, ****, $p < 0.0001$, vs. LPS-induced group.

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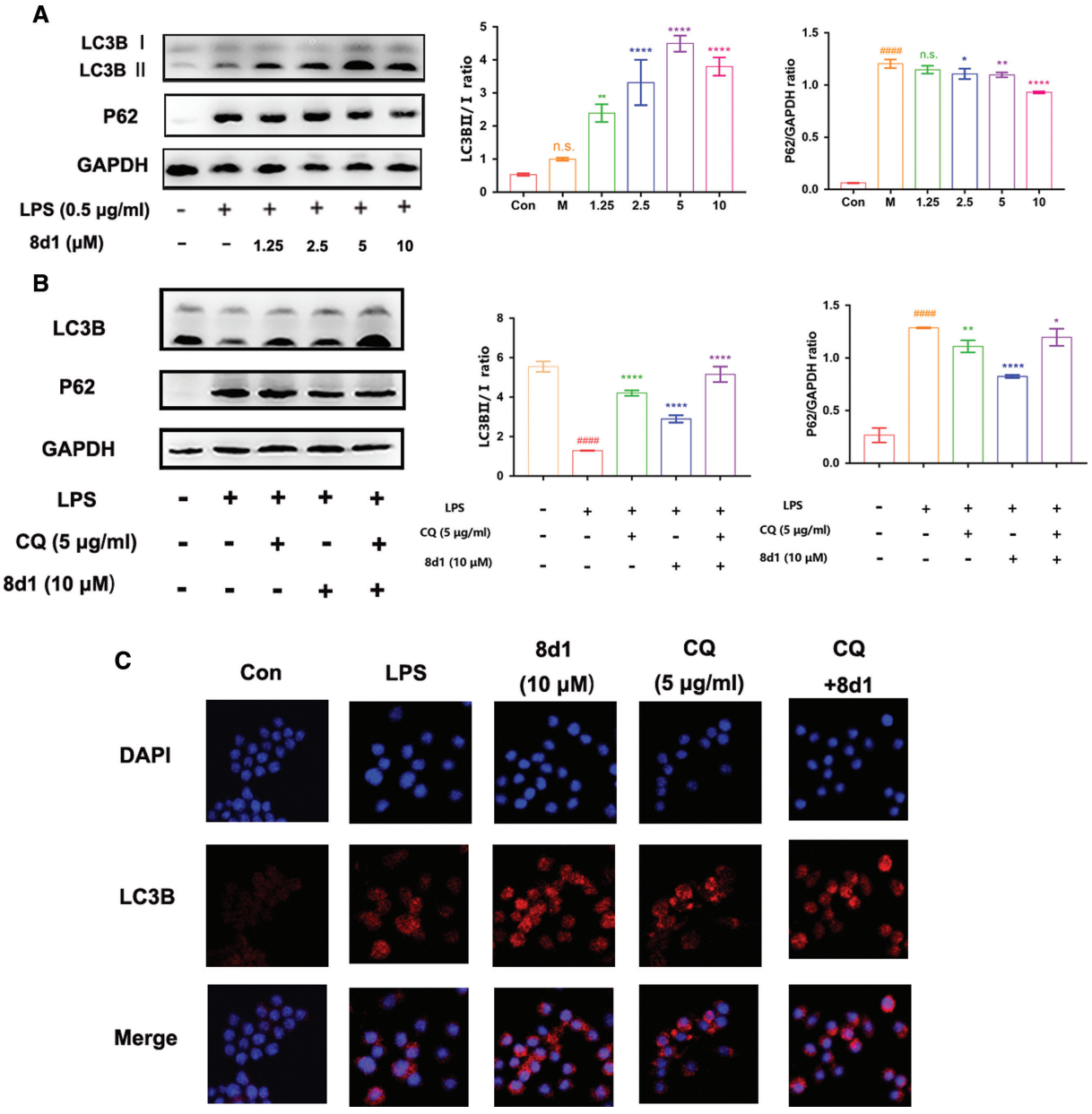

Figure 7 | Compound 8d1 activates autophagy in LPS-induced RAW264.7 cells.
(a) Immunoblot analysis of protein expression of LC3B and P62. (b) Cells were co-treated with autophagy inhibitors CQ (5 μg/mL) and **8d1** (10 mM) for 3 h before LPS treatment, and relevant proteins were detected by western blotting. (c) Immunofluorescence analysis of LC3B (red) and nuclei (blue) in LPS-induced RAW264.7 cells treated with **8d1** alone or in combination with CQ, or CQ alone. The values are presented as mean ± SD of three independent experiments, n = 3. ####, $p < 0.0001$, vs. the control group; *, $p < 0.1$, **, $p < 0.01$, ****, $p < 0.0001$, vs. LPS-stimulated group.

as a promising anti-inflammatory agent and warrants further study.

4. EXPERIMENTAL

4.1 Chemistry

All starting materials and solvents used in the synthesis were obtained from commercial sources and used without further purification. Reactions were monitored through thin-layer chromatography on silica gel plates (GF_{254}, Qingdao Haiyang Chemical Co. Ltd. China) and visualized under ultraviolet light. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AVANCE 400 or 600 spectrometer (Bruker Instruments Inc. Germany). Chemical shifts are expressed in δ values (ppm) relative to TMS, and coupling constants are reported in Hertz.

High resolution mass spectra (HR-MS) were recorded on a Bruker micrOTOF focusII or solariX mass spectrometer by electrospray ionization (ESI).

4.2 Synthetic methods for all compounds

4.2.1 Synthesis of lathyrol and epoxylathyrol. Lathyrol and epoxylathyrol were synthesized according to our previous method [36].

4.2.2 Synthesis of compounds 1 and 3. Lathyrol (0.60 mmol) was dissolved in DCM (3 mL), and then chloroacetic acid (0.72 mmol), EDCI (0.72 mmol) and DMAP (0.06 mmol) were successively added to the solution. The reaction was stirred for approximately 8–10 h at room temperature, quenched with saturated NH_4Cl solution and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and evaporated, thus yielding the crude product of compound **1** as **a** white solid (72% yield). This procedure was also applied to the preparation of compound **3** (by epoxylathyrol, white solid, 70% yield).

4.2.3 Synthesis of compounds 2 and 4. To a solution of compound **1** (1.0 eq) in DMF, NaN_3 (3 eq) was added at room temperature. The reaction was heated to 60°C and stirred for approximately 6 h. After completion of the reaction, EtOAc was added to the mixture. The organic layer was washed with brine three to five times, then dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by CC, thus yielding compound **2** as a white solid. This procedure was also applied to the preparation of compound **4** (from compound **3**).

*(1aR,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta [a]cyclopropa [f][11]annulen-8-yl 2-azidoacetate (compound **2**)*
White solid, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (s, 1H), 5.96 (d, *J* = 10.1 Hz, 1H), 4.94 (d, *J* = 12.4 Hz, 2H), 4.20 (s, 1H), 4.12 (d, *J* = 7.0 Hz, 1H), 3.87 (q, *J* = 17.0 Hz, 2H), 2.98 (dd, *J* = 14.5, 10.2 Hz, 1H), 2.58 (s, 1H), 2.53 (dd, *J* = 10.1, 3.1 Hz, 1H), 2.28–2.12 (m, 2H), 1.97–1.87 (m, 2H), 1.84 (s, 3H), 1.68–1.58 (m, 2H), 1.53 (ddd, *J* = 15.0, 7.5, 4.0 Hz, 1H), 1.43 (dd, *J* = 11.4, 8.7 Hz, 1H), 1.28–1.22 (m, 2H), 1.20 (d, *J* = 8.2 Hz, 3H), 1.17–1.11 (m, 6H).

*(1aR,2'S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11]annulene-9,2'-oxiran]-8-yl 2-azidoacetate (compound **4**)*
White solid, 72% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 1H), 5.63 (t, *J* = 3.4 Hz, 1H), 4.94 (d, *J* = 13.6 Hz, 2H), 4.20–4.07 (m, 2H), 3.97 (d, *J* = 1.0 Hz, 2H), 2.76 (dd, *J* = 10.7, 3.2 Hz, 1H), 2.37 (dd, *J* = 6.6, 3.4 Hz, 1H), 2.17 (s, 1H), 1.93 (s, 2H), 1.73 (dd, *J* = 14.4, 7.4 Hz, 2H), 1.56 (s, 5H), 1.48–1.39 (m, 2H), 1.25 (dt, *J* = 7.0, 6.4 Hz, 1H), 1.20 (s, 3H), 1.16 (s, 3H), 1.02 (d, *J* = 6.7 Hz, 3H).

4.2.4 Synthesis of compounds 6a–g. Compounds **5a–g** (0.34 mmol, 1.0 eq) were dissolved in DMF (2 mL), then K_2CO_3 (0.34 mmol, 1.0 eq) was added in the solution at room temperature. Propargyl bromide (0.38 mmol, 1.1 eq) was added to the mixture after 0.5 h. The reaction was heated to 60°C and stirred for 5–6 h at 60°C. After completion of the reaction, EtOAc was added to the mixture. The organic layer was washed with brine three to five times, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by CC, thus yielding **6a–g**.

*N-(4-(prop-2-yn-1-yloxy)phenyl)acetamide (compound **6a**)*
White solid, 90% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 9.0 Hz, 2H), 7.11 (s, 1H), 6.93 (d, *J* = 9.0 Hz, 2H), 4.67 (d, *J* = 2.4 Hz, 2H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.16 (s, 3H).

*1-(prop-2-yn-1-yl)indoline-2,3-dione (compound **6b**)*
Orange solid, 78% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (t, *J* = 7.3 Hz, 2H), 7.28–7.17 (m, 2H), 4.60 (d, *J* = 2.5 Hz, 2H), 2.37 (t, *J* = 2.5 Hz, 1H).

*prop-2-yn-1-yl 2-acetoxybenzoate (compound **6c**)*
White solid, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.70 (td, *J* = 8.0, 1.7 Hz, 1H), 7.44 (td, *J* = 7.8, 1.1 Hz, 1H), 7.23 (dd, *J* = 8.1, 0.9 Hz, 1H), 4.99 (d, *J* = 2.5 Hz, 2H), 2.64 (t, *J* = 2.5 Hz, 1H), 2.49 (s, 3H).

*2-phenyl-3-(prop-2-yn-1-yloxy)-4H-chromen-4-one (compound **6d**)*
Light yellow solid, 76% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.18–8.09 (m, 2H), 7.70 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.59–7.48 (m, 4H), 7.43 (t, *J* = 7.5 Hz, 1H), 5.00 (d, *J* = 2.4 Hz, 2H), 2.32 (t, *J* = 2.4 Hz, 1H).

*(E)-3-phenyl-1-(4-(prop-2-yn-1-yloxy)phenyl)prop-2-en-1-one (compound **6e**)*
Light yellow solid, 88% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.43 (m, 4H), 7.37–7.29 (m, 2H), 7.28–7.21 (m, 3H), 7.00–6.92 (m, 2H), 4.64 (d, *J* = 2.4 Hz, 2H), 2.40 (t, *J* = 2.4 Hz, 1H).

*prop-2-yn-1-yl cinnamate (compound **6f**)*
Colorless oil, 90% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 16.0 Hz, 1H), 7.57–7.49 (m, 2H), 7.44–7.35 (m, 3H), 6.47 (d, *J* = 16.0 Hz, 1H), 4.82 (d, *J* = 2.5 Hz, 2H), 2.52 (t, *J* = 2.4 Hz, 1H).

*prop-2-yn-1-yl 2-(3H-indol-3-yl)acetate (compound **6g**)*
Brown oil, 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.12 (dtd, *J* = 14.8, 8.3, 4.3 Hz, 2H), 7.03 (d, *J* = 2.4 Hz, 1H), 4.70–4.62 (m, 2H), 3.77 (d, *J* = 0.6 Hz, 2H), 2.42 (t, *J* = 2.5 Hz, 1H).

4.2.5. Synthesis of compounds 7a–g, 8a–f and 8h–j. Compound **4** (0.04 mmol) was dissolved in THF (1 mL), and then **6a–g** (0.04 mmol, 1.0 eq), VCNa (0.016

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mmol, 0.4 eq) and $CuSO_4$ aqueous solution (0.3 mL, 0.008 mmol, 0.2 eq) were successively added to the solution. The mixture was then stirred for 0.5 h at room temperature. After completion of the reaction, EtOAc was added to the mixture. The organic layer was washed with brine three times, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by CC, thus yielding **7a–g**. This procedure was also applied to the preparation of compounds **8a–f** and **8h–j**.

*(4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f][11]annulen-8-yl 2-(4-((4-acetamidophenoxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **7a**)*

White solid, 45% yield; HR-MS(ESI) *m/z*: 629.2942 $[M+Na]^+$ (calcd. for $C_{33}H_{42}N_4NaO_7$, 629.2946); ^1H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.16 (s, 1H), 7.48 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.08 (s, 1H), 5.42 (dd, *J* = 5.8, 3.7 Hz, 2H), 5.35 (s, 1H), 5.12 (d, *J* = 3.7 Hz, 2H), 4.94 (s, 1H), 4.66 (d, *J* = 11.2 Hz, 1H), 4.32 (d, *J* = 6.9 Hz, 1H), 2.90 (d, *J* = 12.3 Hz, 1H), 2.30 (d, *J* = 3.7 Hz, 2H), 2.00 (s, 3H), 1.94–1.78 (m, 2H), 1.65 (dd, *J* = 26.6, 12.6 Hz, 1H), 1.56 (s, 3H), 1.53–1.37 (m, 2H), 1.23 (s, 1H), 1.14 (s, 3H), 1.07 (s, 3H), 1.00 (d, *J* = 6.7 Hz, 2H), 0.87 (d, *J* = 6.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.21, 167.56, 154.18, 145.11, 143.33, 133.34, 126.24, 120.89, 115.13, 99.99, 78.40, 61.58, 55.37, 50.82, 48.50, 38.10, 35.81, 31.16, 29.45, 29.00, 25.35, 24.27, 21.75, 16.60, 14.90, 12.84.

*(4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f][11]annulen-8-yl 2-(4-((2,3-dioxoindolin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **7b**)*

Orange solid, 42% yield; HR-MS(ESI) *m/z*: 625.2653 $[M+Na]^+$ (calcd. for $C_{33}H_{38}N_4NaO_7$, 625.2633); ^1H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.95 (s, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.19 (dd, *J* = 7.9, 5.3 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 6.03 (d, *J* = 10.2 Hz, 1H), 5.39 (d, *J* = 6.9 Hz, 2H), 5.30 (s, 1H), 5.00 (s, 2H), 4.91 (s, 1H), 4.67 (s, 1H), 4.64 (d, *J* = 4.1 Hz, 1H), 4.31–4.25 (m, 1H), 3.95–3.89 (m, 1H), 2.24 (dd, *J* = 10.3, 3.3 Hz, 2H), 2.01 (d, *J* = 12.4 Hz, 1H), 1.87 (s, 2H), 1.56 (s, 3H), 1.48 (dd, *J* = 17.2, 7.2 Hz, 1H), 1.41 (dd, *J* = 11.8, 8.4 Hz, 1H), 1.16 (s, 3H), 1.06 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 2H), 0.84 (d, *J* = 6.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.78, 183.49, 167.35, 158.28, 150.62, 145.08, 141.95, 138.55, 133.77, 125.40, 124.96, 123.88, 118.04, 115.24, 111.63, 88.99, 78.34, 69.55, 60.21, 53.33, 50.85, 48.48, 38.08, 35.77, 35.45, 28.99, 28.68, 25.31, 21.69, 16.59, 14.88, 12.84.

*(1-(2-(((4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f][11]annulen-8-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl2-acetoxybenzoate (compound **7c**).*

White solid, 40% yield; HR-MS(ESI) *m/z*: 658.2742 $[M+Na]^+$ (calcd. for $C_{34}H_{41}N_3NaO_9$, 658.2735); ^1H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H), 8.00–7.87 (m, 1H), 7.76–7.64 (m, 1H), 7.41 (dd, *J* = 10.9, 4.4 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 6.07 (d, *J* = 10.4 Hz, 1H), 5.52–5.40 (m, 2H), 5.40–5.34 (m, 3H), 4.93 (s, 1H), 4.66 (d, *J* = 8.7 Hz, 1H), 4.31 (dd, *J* = 15.5, 7.4 Hz, 1H), 4.00 (s, 1H), 2.98–2.82 (m, 1H), 2.28 (ddd, *J* = 23.3, 10.7, 5.0 Hz, 2H), 2.16 (s, 3H), 2.11–1.95 (m, 2H), 1.94–1.79 (m, 2H), 1.72–1.61 (m, 1H), 1.55 (d, *J* = 9.0 Hz, 3H), 1.53–1.46 (m, 1H), 1.46–1.36 (m, 1H), 1.14 (s, 3H), 1.07 (s, 3H), 0.99 (d, *J* = 6.7 Hz, 2H), 0.86 (d, *J* = 6.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.78, 169.60, 167.46, 164.35, 150.39, 145.09, 141.89, 134.96, 133.78, 131.77, 126.92, 126.80, 124.57, 123.33, 115.29, 89.04, 78.40, 58.44, 53.35, 50.84, 48.50, 38.09, 35.81, 28.99, 28.69, 25.36, 21.70, 21.02, 16.59, 14.89, 12.84.

*(4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f][11]annulen-8-yl 2-(4-(((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **7d**)*

White solid, 46% yield; HR-MS(ESI) *m/z*: 716.2938 $[M+Na]^+$ (calcd. for $C_{40}H_{43}N_3NaO_8$, 716.2942); ^1H NMR (400 MHz, DMSO-d_6) δ 8.16 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.07 (s, 1H), 7.99 (dd, *J* = 5.2, 2.7 Hz, 2H), 7.89–7.81 (m, 1H), 7.77 (d, *J* = 8.3 Hz, 1H), 7.57–7.49 (m, 4H), 5.48 (d, *J* = 3.4 Hz, 1H), 5.36 (d, *J* = 5.6 Hz, 2H), 5.28 (d, *J* = 6.3 Hz, 2H), 4.71–4.56 (m, 2H), 4.42 (s, 1H), 4.35–4.20 (m, 1H), 2.99–2.84 (m, 1H), 2.34–2.18 (m, 1H), 2.15–2.04 (m, 1H), 2.00 (dd, *J* = 15.6, 8.8 Hz, 1H), 1.93–1.77 (m, 1H), 1.56 (s, 3H), 1.49 (d, *J* = 12.4 Hz, 1H), 1.45–1.35 (m, 1H), 1.23 (s, 2H), 1.14 (s, 3H), 1.06 (s, 3H), 0.98 (d, *J* = 6.7 Hz, 1H), 0.84 (d, *J* = 6.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.55, 167.46, 156.09, 155.25, 149.64, 145.10, 142.91, 139.32, 134.65, 133.79, 131.22, 130.82, 128.92, 128.91, 126.69, 125.64, 125.48, 123.96, 118.95, 115.24, 89.05, 78.41, 64.71, 53.34, 50.72, 48.49, 38.06, 35.82, 31.16, 28.99, 28.70, 25.36, 21.71, 16.60, 14.91, 12.84.

*(4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f][11]annulen-8-yl2-(4-((4-cinnamoylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **7e**)*

White solid, 43% yield; HR-MS(ESI) *m/z*: 702.3144 $[M+Na]^+$ (calcd. for $C_{40}H_{45}N_3NaO_7$, 702.3150); ^1H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.64 (dd, *J* = 8.3, 4.9 Hz, 2H), 7.61–7.51 (m, 4H), 7.46–7.38 (m, 4H), 7.09 (t, *J* = 7.4 Hz, 1H), 6.05 (d, *J* = 10.2 Hz, 1H), 5.44–5.39 (m, 1H), 5.37 (d, *J* = 5.2 Hz, 3H), 5.33–5.27 (m, 1H), 4.91 (s, 1H), 4.65 (dd, *J* = 10.6, 6.6 Hz, 1H), 4.27 (d, *J* = 7.0 Hz, 1H), 4.00–3.90 (m, 1H), 2.91 (t, *J* = 11.4 Hz, 1H), 2.33–2.17 (m, 2H), 2.04–1.95 (m, 1H), 1.90 (s, 1H), 1.78 (s, 1H), 1.69–1.60 (m, 1H), 1.57 (d, *J* = 8.1 Hz, 3H), 1.53–1.45 (m, 1H), 1.40

(dd, *J* = 12.6, 7.5 Hz, 1H), 1.23 (s, 1H), 1.16 (s, 4H), 1.05 (s, 4H), 0.97 (s, *J* = 6.7 Hz, 3H), 0.84 (s, *J* = 6.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.10, 167.15, 162.84, 159.50, 157.80, 154.01, 150.38, 142.33, 134.71, 130.55, 127.49, 126.97, 124.52, 123.88, 118.29, 115.65, 114.10, 87.31, 62.24, 55.63, 53.05, 51.11, 49.39, 37.29, 36.24, 35.28, 29.30, 29.00, 25.90, 20.54, 16.75, 14.50, 12.64.

*(1-(2-(((4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa [f][11]annulen-8-yl) oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl cinnamate (compound **7f**)*

White solid, 35% yield; HR-MS(ESI) *m/z*: 642.3145 [M+Na]$^+$ (calcd. for $C_{35}H_{45}N_3NaO_7$, 642.3150); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.79–7.63 (m, 3H), 7.42 (d, *J* = 4.7 Hz, 3H), 6.68 (dd, *J* = 16.0, 1.2 Hz, 1H), 6.07 (d, *J* = 10.3 Hz, 1H), 5.47–5.40 (m, 1H), 5.37 (d, *J* = 6.1 Hz, 1H), 5.33–5.27 (m, 2H), 4.94 (s, 1H), 4.68 (s, 1H), 4.35 (d, *J* = 6.7 Hz, 1H), 4.00 (dd, *J* = 6.7, 3.3 Hz, 1H), 2.92 (dd, *J* = 13.0, 8.8 Hz, 1H), 2.34–2.19 (m, 2H), 2.01 (d, *J* = 12.2 Hz, 1H), 1.94 (dd, *J* = 17.0, 7.2 Hz, 1H), 1.84 (d, *J* = 20.0 Hz, 1H), 1.65 (d, *J* = 13.2 Hz, 1H), 1.56 (s, 3H), 1.53–1.45 (m, 1H), 1.45–1.37 (m, 1H), 1.23 (s, 1H), 1.18 (d, *J* = 7.8 Hz, 1H), 1.14 (s, 3H), 1.07 (s, 3H), 1.00 (d, *J* = 6.7 Hz, 2H), 0.87 (d, *J* = 6.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.71, 167.54, 166.41, 151.38, 145.58, 145.11, 142.39, 134.37, 133.78, 131.08, 129.39, 128.91, 126.75, 118.08, 115.29, 89.02, 78.41, 69.46, 57.66, 53.36, 50.81, 48.50, 38.11, 35.80, 34.93, 28.99, 28.69, 25.34, 21.73, 16.59, 14.91, 12.84.

*(4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f][11] annulen-8-yl 2-(4-((2-(3H-indol-3-yl)acetoxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **7g**)*

White solid, 38% yield; HR-MS(ESI) *m/z*: 653.2944 [M+Na]$^+$ (calcd. for $C_{35}H_{42}N_4NaO_7$, 653.2946); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.11 (s, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 2.2 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.4 Hz, 1H), 5.76 (s, 1H), 5.46–5.40 (m, 1H), 5.36 (d, *J* = 15.4 Hz, 1H), 5.18 (d, *J* = 3.1 Hz, 2H), 4.94 (s, 1H), 4.72–4.60 (m, 1H), 3.77 (s, 2H), 2.91 (d, *J* = 11.8 Hz, 1H), 2.31 (dd, *J* = 10.1, 3.0 Hz, 1H), 2.24 (s, 1H), 1.84 (d, *J* = 15.2 Hz, 1H), 1.56 (s, 3H), 1.50 (d, *J* = 11.9 Hz, 1H), 1.42 (ddd, *J* = 12.1, 7.5, 3.4 Hz, 1H), 1.23 (s, 1H), 1.14 (s, 3H), 1.07 (s, 3H), 1.00 (d, *J* = 6.7 Hz, 2H), 0.87 (d, *J* = 6.7 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.74, 171.81, 167.54, 145.12, 142.34, 136.53, 133.77, 127.50, 126.70, 124.58, 121.52, 118.97, 115.29, 111.87, 107.18, 88.99, 78.42, 60.22, 57.73, 53.35, 50.77, 48.52, 38.12, 35.81, 31.05, 29.00, 28.70, 25.36, 21.23, 16.61, 14.92, 14.55, 12.85.

*(2'S,4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f] [11] annulene-9,2'-oxiran]-8-yl2-(4-((4-acetamidophenoxy) methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8a**).*

White solid, 49% yield; HR-MS(ESI) *m/z*: 645.2890 [M+Na]$^+$ (calcd. for $C_{33}H_{42}N_4NaO_8$, 645.2895); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.22 (s, 1H), 7.49 (d, *J* = 9.0 Hz, 2H), 7.03 (d, *J* = 7.3 Hz, 1H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.60 (d, *J* = 11.5 Hz, 1H), 6.34 (d, *J* = 4.7 Hz, 1H), 5.10 (s, 2H), 4.61–4.52 (m, 2H), 4.46 (d, *J* = 13.8 Hz, 1H), 4.34 (s, 1H), 4.29 (d, *J* = 4.3 Hz, 1H), 2.91 (dd, *J* = 13.1, 9.2 Hz, 1H),2.40 (dd, *J* = 10.1, 3.5 Hz, 1H), 2.32 (d, *J* = 6.3 Hz, 1H), 2.00 (s, 3H), 1.76 (s, 3H), 1.51 (d, *J* = 9.8 Hz, 2H), 1.46 (d, *J* = 6.7 Hz, 1H), 1.23 (s, 1H), 1.17 (dd, *J* = 8.8, 5.4 Hz, 2H), 1.10 (s, 3H), 1.08 (s, 3H), 0.88 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.77, 168.21, 154.22, 143.30, 142.47, 133.33, 132.03, 126.89, 120.90, 115.26, 88.58, 78.45, 71.03, 67.41, 59.14, 54.64, 54.01, 51.64, 48.20, 38.24, 35.32, 33.55, 29.48, 28.98, 25.87, 24.28, 20.33, 16.73, 14.56, 12.72.

*(2'S,4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa [f][11] annulene-9,2'-oxiran]-8-yl2-(4-((2,3-dioxoindolin-1-yl) methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8b**)*

Orange solid, 40% yield; HR-MS(ESI) *m/z*: 641.2570 [M+Na]$^+$ (calcd. for $C_{33}H_{38}N_4NaO_8$, 641.2582); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.65 (dd, *J* = 11.1, 4.4 Hz, 1H), 7.58 (d, *J* = 7.3 Hz, 1H), 7.23–7.06 (m, 2H), 7.00 (d, *J* = 7.2 Hz, 1H), 6.58 (d, *J* = 11.5 Hz, 1H), 6.28 (d, *J* = 4.6 Hz, 1H), 5.86 (s, 1H), 4.98 (q, *J* = 15.9 Hz, 2H), 4.58–4.46 (m, 2H), 4.39 (d, *J* = 13.8 Hz, 1H), 4.23 (s, 2H), 2.99 (dd, *J* = 13.2, 8.9 Hz, 1H), 2.38 (d, *J* = 6.6 Hz, 1H), 2.29 (d, *J* = 6.4 Hz, 1H), 1.74 (s, 3H), 1.59–1.35 (m, 6H), 1.23 (s, 1H), 1.10 (s, 3H), 1.07 (s, 3H), 0.86 (d, *J* = 6.1 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.66, 183.60, 158.26, 150.74, 143.23, 141.10, 138.55, 132.05, 126.17, 124.94, 123.84, 118.05, 111.73, 88.56, 78.45, 67.35, 59.30, 54.53, 54.09, 51.68, 48.27, 38.27, 35.32, 33.58, 29.49, 29.06, 25.89, 20.40, 16.64, 14.55, 12.69.

*(1-(2-(((2'S,4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2'-oxiran]-8-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl 2-acetoxybenzoate (compound **8c**)*

White solid, 44% yield; HR-MS(ESI) *m/z*: 674.2688 [M+Na]$^+$ (calcd. for $C_{34}H_{41}N_3NaO_{10}$, 674.2684); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.94 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.69 (d, *J* = 1.5 Hz, 1H), 7.42 (s, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 7.3 Hz, 1H), 6.60 (d, *J* = 11.6 Hz, 1H), 6.33 (d, *J* = 4.8 Hz, 1H), 5.86 (s, 1H), 5.34 (t, *J* = 14.2 Hz, 3H), 4.58 (s, 2H), 4.49 (s, 1H), 4.34 (s, 1H), 4.29 (d, *J* = 5.1 Hz, 1H), 2.98 (dd, *J* = 13.2, 9.1 Hz, 1H), 2.40 (dd, *J* = 10.6, 4.2 Hz, 1H), 2.32 (d, *J* = 6.4 Hz, 1H), 2.12 (s, 3H), 1.76 (s, 3H), 1.56–1.38 (m, 4H), 1.24 (s, 2H), 1.15 (d, *J* = 7.2 Hz, 2H), 1.10 (s, 3H), 1.07 (s, 3H), 0.88 (d, *J* = 6.5 Hz, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.78, 169.56, 164.45, 150.32, 143.22, 141.09, 134.90, 132.07, 131.84, 127.47,

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126.80, 124.54, 123.49, 88.64, 78.43, 67.33, 61.31, 59.11, 54.60, 54.08, 51.63, 48.19, 38.28, 35.28, 33.55, 29.41, 29.05, 25.85, 20.97, 20.35, 16.66, 14.61, 12.71.

*(2′S,4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11]annulene-9,2′-oxiran]-8-yl 2-(4-(((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8d**)*

White solid, 46% yield; HR-MS(ESI) *m/z*: 732.2890 $[M+Na]^+$ (calcd. for $C_{40}H_{43}N_3NaO_9$, 732.2892); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.16 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.09 (s, 1H), 8.01 (dd, *J* = 7.6, 2.0 Hz, 2H), 7.85 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.53 (dd, *J* = 11.0, 4.0 Hz, 5H), 7.01–6.91 (m, 1H), 6.58 (t, *J* = 14.4 Hz, 1H), 6.33 (s, 1H), 5.85 (s, 1H), 5.31 (s, 2H), 4.53 (d, *J* = 7.4 Hz, 1H), 4.49 (d, *J* = 13.8 Hz, 1H), 4.38 (d, *J* = 13.8 Hz, 1H), 4.28 (s, 2H), 3.01 (s, 1H), 2.39 (dd, *J* = 10.7, 4.3 Hz, 1H), 2.28 (d, *J* = 6.5 Hz, 1H), 1.74 (s, 3H), 1.53–1.48 (m, 1H), 1.48–1.40 (m, 3H), 1.35-1.30 (m, 3H), 1.23 (s, 1H), 1.11 (s, 4H), 1.06 (s, 4H), 0.88 (d, *J* = 6.5 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.74, 174.60, 156.02, 155.23, 143.17, 141.97, 139.10, 134.59, 132.06, 131.20, 130.87, 128.89, 127.17, 125.60, 125.49, 123.94, 118.91, 88.63, 78.43, 71.03, 67.66, 59.14, 54.65, 53.98, 51.63, 48.29, 38.31, 35.41, 33.60, 29.43, 29.05, 25.88, 20.37, 16.76, 14.73, 12.71.

*(2′S,4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7, 7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa [f][11]annulene-9,2′-oxiran]-8-yl 2-(4-((4-cinnamoylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8e**)*

White solid, 43% yield; HR-MS(ESI) *m/z*: 718.3090 $[M+Na]^+$ (calcd. for $C_{40}H_{45}N_3NaO_8$, 718.3095); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.69–7.64 (m, 2H), 7.61–7.55 (m, 3H), 7.45–7.38 (m, 4H), 7.10 (s, 1H), 6.97 (d, *J* = 7.3 Hz, 1H), 6.56 (d, *J* = 11.7 Hz, 1H), 6.29 (s, 1H), 5.85 (s, 1H), 5.35 (d, *J* = 3.3 Hz, 2H), 4.53 (d, *J* = 14.2 Hz, 1H), 4.41 (s, 1H), 4.26 (d, *J* = 4.8 Hz, 1H), 4.22 (s, 1H), 2.97 (dd, *J* = 13.0, 9.1 Hz, 1H), 2.40 (dd, *J* = 10.5, 3.9 Hz, 1H), 2.28 (d, *J* = 6.4 Hz, 1H), 1.76 (s, 3H), 1.53–1.45 (m, 2H), 1.44–1.35 (m, 3H), 1.23 (s, 2H), 1.08 (s, 3H), 1.05 (s, 3H), 0.99–0.91 (m, 3H), 0.89 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 200.69, 191.60, 157.30, 143.21, 142.53, 135.06, 133.85, 132.06, 130.82, 130.52, 129.68, 129.27, 128.98, 128.40, 127.20, 126.90, 121.50, 114.27, 88.70, 78.47, 70.96, 67.38, 59.11, 54.61, 54.11, 51.64, 48.27, 38.30, 35.40, 33.54, 29.47, 29.03, 25.89, 20.33, 16.75, 14.56, 12.71.

*(1-(2-(((2′S,4aR,6S,7S,7aR,8R,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4, 4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa [f][11]annulene-9,2′-oxiran]-8-yl)oxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl cinnamate (compound **8f**)*

White solid, 35% yield; HR-MS(ESI) *m/z*: 642.2775 $[M+Na]^+$ (calcd. for $C_{35}H_{45}N_3NaO_8$, 642.2786); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (d, *J* = 5.5 Hz, 1H), 7.71 (dd, *J* = 14.2, 10.1 Hz, 4H), 7.57 (d, *J* = 31.3 Hz, 1H), 7.51–7.34 (m, 4H), 6.68 (d, *J* = 16.0 Hz, 1H), 5.50 (d, *J* = 13.0 Hz, 1H), 5.44 (d, *J* = 5.3 Hz, 2H), 5.37–5.27 (m, 3H), 4.73 (d, *J* = 8.3 Hz, 1H), 4.44 (s, 1H), 2.92 (s, 1H), 2.08 (s, 1H), 1.99 (s, 2H), 1.74 (s, 3H), 1.49 (ddd, *J* = 38.3, 18.4, 10.6 Hz, 4H), 1.17 (s, 3H), 1.12 (s, 3H), 0.86 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.14, 167.10, 166.42, 150.33, 145.53, 142.33, 134.66, 134.40, 131.06, 129.39, 128.90, 126.90, 118.13, 87.35, 83.28, 62.85, 61.19, 57.73, 55.40, 53.06, 51.06, 49.51, 37.26, 35.29, 34.25, 29.33, 29.00, 25.89, 20.59, 16.75, 14.50, 12.62.

*(1aR,2′S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2′-oxiran]-8-yl 2-(4-(((2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8h**)*

White solid, 35% yield; HR-MS(ESI) *m/z*: 796.7700 $[M+Na]^+$ (calcd. for $C_{40}H_{43}N_3NaO_{13}$, 796.7709);^{1}H NMR (600 MHz, DMSO-d_6) δ 12.73 (s, 1H), 8.15 (s, 1H), 7.46 (s, 1H), 6.85 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.41 (d, *J* = 1.9 Hz, 1H), 6.20 (t, *J* = 8.8 Hz, 1H), 5.37 (dt, *J* = 25.5, 12.4 Hz, 3H), 5.18–5.09 (m, 2H), 4.42 (s, 1H), 2.89 (s, 1H), 2.45 (s, 1H), 2.07 (d, *J* = 14.9 Hz, 1H), 2.03–1.93 (m, 2H), 1.73 (s, 3H), 1.57–1.31 (m, 5H), 1.17 (s, 3H), 1.09 (s, 3H), 0.96 (d, *J* = 6.7 Hz, 1H), 0.90–0.74 (m, 5H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 178.37, 167.80, 164.62, 161.76, 156.82, 149.12, 145.59, 143.08, 136.39, 126.72, 121.53, 121.23, 116.02, 115.82, 104.62, 99.06, 94.04, 88.51, 78.50, 64.98, 59.15, 54.55, 51.50, 50.75, 48.04, 38.39, 35.27, 33.60, 29.46, 28.99, 25.85, 20.31, 16.60, 14.64, 12.73.

*(1aR,2′S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2′-oxiran]-8-yl 2-(4-(((5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8i**)*

White solid, 21% yield; HR-MS(ESI) *m/z*: $[M+Na]^+$ (calcd. for $C_{40}H_{43}N_3NaO_{12}$, 780.2739); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 10.88 (s, 1H), 10.24 (s, 1H), 8.14 (s, 1H), 7.94 (t, *J* = 7.2 Hz, 2H), 7.58 (s, 1H), 6.93–6.85 (m, 2H), 6.45 (d, *J* = 2.0 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 5.49 (s, 1H), 5.38 (dd, *J* = 27.8, 17.4 Hz, 3H), 5.18 (s, 2H), 4.71 (d, *J* = 8.2 Hz, 1H), 4.41 (s, 1H), 2.89 (s, 1H), 2.73 (s, 0H), 2.46 (s, 1H), 2.08 (d, *J* = 7.1 Hz, 1H), 1.91 (s, 1H), 1.73 (s, 3H), 1.53 (dd, *J* = 11.5, 8.3 Hz, 2H), 1.48–1.33 (m, 3H), 1.25 (d, *J* = 10.7 Hz, 1H), 1.17 (s, 3H), 1.10 (s, 3H), 0.82 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.43, 167.03, 164.64, 161.75, 160.56, 156.87, 156.63, 142.88, 136.29, 130.79, 126.86, 120.98, 115.90, 104.64, 99.10, 94.21, 87.33, 83.27, 78.59, 64.93, 53.05, 50.96, 37.25, 35.29, 30.07, 29.01, 25.89, 21.51, 16.72, 14.55, 12.59.

(1aR,2′S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,1

*0,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2′-oxiran]-8-yl 2-(4-((((S)-5-hydroxy-6,8-dimethyl-4-oxo-2-phenylchroman-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (compound **8j**)*

White solid, 20% yield; HR-MS(ESI) *m/z*: $[M+Na]^+$ (calcd. for $C_{42}H_{49}N_3NaO_{11}$, 794.3259); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 9.60 (s, 1H), 8.26 (d, *J* = 2.6 Hz, 1H), 7.55 (d, *J* = 13.1 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 5.57 (s, 1H), 5.53–5.41 (m, 3H), 5.33 (s, 1H), 4.95 (s, 2H), 4.75 (d, *J* = 8.1 Hz, 1H), 4.44 (s, 1H), 2.90 (s, 1H), 2.82 (dd, *J* = 17.1, 2.8 Hz, 1H), 2.07 (s, 1H), 2.00 (d, *J* = 8.5 Hz, 6H), 1.73 (s, 2H), 1.58–1.47 (m, 2H), 1.46–1.32 (m, 2H), 1.17 (s, 3H), 1.10 (s, 3H), 0.96 (d, *J* = 6.6 Hz, 1H), 0.94–0.88 (m, 1H), 0.83 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 199.05, 167.09, 163.65, 158.70, 158.22, 158.15, 143.02, 129.44, 128.55, 126.72, 115.69, 110.78, 109.73, 105.20, 87.35, 78.64, 65.89, 60.22, 53.01, 51.07, 42.70, 37.23, 35.28, 29.46, 29.00, 25.90, 21.23, 16.74, 14.49, 12.61, 9.23, 8.68.

4.2.6 Synthesis of compounds 9 and 10. Compounds **9** and **10** were synthesized according to our previous method [36].

4.2.7 Synthesis of compounds 11 and 12. Compounds **11** and **12** were synthesized according to our previous method [18].

*(1aR,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-8-(prop-2-yn-1-yloxy)-4a,5,6,7,7a,8,9,10,11,11a-decahydro-1H-cyclopenta[a]cyclopropa[f][11]annulen-4(1aH)-one (compound **11**)*

45% yield; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.46 (s, 1H), 4.81 (d, *J* = 14.2 Hz, 1H), 4.64 (s, 1H), 4.53 (d, *J* = 8.4 Hz, 1H), 4.42 (s, 1H), 4.33 (qd, *J* = 15.8, 2.4 Hz, 2H), 4.24 (t, *J* = 9.1 Hz, 1H), 4.07 (s, 1H), 4.03 (t, *J* = 3.3 Hz, 1H), 2.80 (dd, *J* = 12.9, 9.0 Hz, 1H), 2.54 (dd, *J* = 13.3, 6.8 Hz, 1H), 2.13 (dd, *J* = 9.8, 3.3 Hz, 1H), 2.01–1.82 (m, 4H), 1.55 (s, 3H), 1.53–1.37 (m, 4H), 1.17 (ddd, *J* = 12.2, 8.2, 4.3 Hz, 1H), 1.14 (s, 4H), 1.07 (s, 3H), 1.05 (d, *J* = 6.7 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 2H).

*(1aR,2′S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-8-(prop-2-yn-1-yloxy)-1,4a,5,6,7,7a,8,10,11,11a-decahydrospiro[cyclopenta[a]cyclopropa[f][11]annulene-9,2′-oxiran]-4(1aH)-one (compound **12**)*

32% yield; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53 (d, *J* = 11.7 Hz, 1H), 6.23 (s, 1H), 4.97 (s, 1H), 4.76 (d, *J* = 17.8 Hz, 1H), 4.54 (d, *J* = 17.8 Hz, 1H), 4.38 (d, *J* = 9.2 Hz, 1H), 3.02 (dd, *J* = 12.9, 8.3 Hz, 1H), 2.38 (dd, *J* = 13.5, 5.2 Hz, 1H), 2.08–1.94 (m, 3H), 1.74 (s, 3H), 1.71–1.48 (m, 4H), 1.25 (s, 1H), 1.18 (d, *J* = 8.3 Hz, 3H), 1.13 (s, 1H), 1.10 (s, 3H), 1.01 (s, 3H), 0.97–0.90 (m, 1H), 0.89–0.80 (m, 2H).

4.2.8 Synthesis of compounds 6d1 and 6d2. Compound **6d1** was synthesized from **5d** and methyl bromoacetate according to the synthetic method for compounds **6a–g**.

The crude product of **6d1** (0.8 mmol, 1.0 eq) was dissolved in THF (30 mL), and $LiOH·H_2O$ aqueous solution (10 mL, 1.6 mmol, 2.0 eq) was then added to the solution. The mixture was stirred for 3–4 h at room temperature. After completion of the reaction, THF was removed by reduced pressure. The residue was diluted with water, and the pH was slowly adjusted to 2–3 with 1 M HCl. The resulting solid was filtered and washed with water, then dried in an oven, thus yielding **6d2** as a white solid (75% for two steps).

*2-((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)acetic acid (compound **6d2**)*

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.10 (dd, *J* = 7.7, 1.8 Hz, 2H), 7.85–7.79 (m, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.63–7.56 (m, 3H), 7.53 (t, *J* = 7.5 Hz, 1H), 4.38 (s, 2H).

4.2.9 Synthesis of compounds 6d3 and 6d4. Compound **6d3** was synthesized from **5d** and 1,3-dibromopropane according to the synthetic method for compounds **6a–g**. Compound **6d4** was synthesized from **6d3** according to the synthetic method for compound **2**.

*3-(3-bromopropoxy)-2-phenyl-4H-chromen-4-one (compound **6d3**).*

Light yellow solid, 44% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.09–8.01 (m, 2H), 7.69 (tt, *J* = 9.4, 2.0 Hz, 1H), 7.59–7.49 (m, 4H), 7.46–7.37 (m, 1H), 4.18 (t, *J* = 5.8 Hz, 2H), 3.51 (t, *J* = 6.6 Hz, 2H), 2.33–2.15 (m, 2H).

4.2.10 Synthesis of compounds 6d5 and 6d6. Compound **6d5** was synthesized from **5d** and N-Boc-glycine according to the synthetic method for compound **1**.

Compound **6d5** (0.34 mmol) was dissolved in DCM (2 mL), and the reaction was placed in an ice bath. TFA (2 mL) was added to the solution after 15 min. The reaction was stirred for 30 min. After completion of the reaction, THF and DCM were removed by reduced pressure. The crude product (**6d6**'s TFA salt) was used in the following reactions without further purification (95% yield).

*4-oxo-2-phenyl-4H-chromen-3-yl 2-((tert-butoxycarbonyl)amino)acetate (compound **6d5**).*

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.88 (dd, *J* = 6.6, 2.9 Hz, 2H), 7.78–7.69 (m, 1H), 7.62–7.51 (m, 4H), 7.45 (t, *J* = 7.6 Hz, 1H), 5.10 (s, 1H), 4.29 (d, *J* = 5.5 Hz, 2H), 1.45 (s, 9H).

4.2.11 Synthesis of compounds 7d1 and 8d1. Compound **7d1** (or **8d1**) was synthesized from **9** (or **10**) and 3-hydroxyflavone according to the synthetic method for compound **1**.

(1aR,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-

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*cyclopenta[a]cyclopropa[f] [11] annulen-8-yl (4-oxo-2-phenyl-4H-chromen-3-yl) succinate (compound **7d1**)*

White solid, 47% yield; HR-MS(ESI) *m/z*: 677.2709 $[M+Na]^+$ (calcd. for $C_{39}H_{42}NaO_9$, 677.2721); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.91 (ddd, *J* = 8.1, 6.2, 1.7 Hz, 3H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.65–7.53 (m, 5H), 6.03 (d, *J* = 10.4 Hz, 1H), 5.50 (s, 1H), 4.87 (s, 1H), 4.62 (s, 1H), 4.04 (d, *J* = 7.4 Hz, 1H), 4.01–3.95 (m, 1H), 2.95 (dd, *J* = 13.2, 9.4 Hz, 1H), 2.68 (t, *J* = 7.4 Hz, 2H), 2.38 (td, *J* = 7.3, 4.2 Hz, 2H), 2.31 (dd, *J* = 10.5, 3.3 Hz, 1H), 2.20 (dd, *J* = 14.3, 5.6 Hz, 1H), 2.06–1.91 (m, 2H), 1.91–1.81 (m, 3H), 1.72 (d, *J* = 13.3 Hz, 1H), 1.57 (s, 3H), 1.53–1.37 (m, 3H), 1.24–1.18 (m, 1H), 1.15 (s, 3H), 1.11 (s, 3H), 0.97 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.73, 172.31, 171.60, 170.72, 156.21, 155.58, 151.44, 145.85, 135.28, 133.99, 133.38, 132.11, 129.77, 129.46, 128.61, 126.27, 125.57, 123.22, 119.21, 114.32, 89.19, 78.81, 67.18, 53.81, 48.80, 37.94, 36.00, 35.06, 32.88, 32.78, 29.02, 28.75, 25.48, 21.82, 16.68, 15.04, 12.88.

*(1aR,2'S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2'-oxiran]-8-yl (4-oxo-2-phenyl-4H-chromen-3-yl) succinate (compound **8d1**)*

White solid, 48% yield; HR-MS(ESI) *m/z*: 693.2661 $[M+Na]^+$ (calcd. for $C_{39}H_{42}NaO_{10}$, 693.2670); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.99–7.88 (m, 3H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.67–7.54 (m, 5H), 6.20 (d, *J* = 9.2 Hz, 1H), 5.36 (s, 1H), 4.21 (d, *J* = 5.3 Hz, 1H), 3.87 (s, 1H), 3.02–2.85 (m, 3H), 2.83–2.65 (m, 2H), 2.44 (d, *J* = 3.2 Hz, 1H), 2.08 (d, *J* = 11.5 Hz, 2H), 1.80 (s, 2H), 1.74 (s, 3H), 1.59–1.43 (m, 2H), 1.35 (d, *J* = 6.9 Hz, 1H), 1.16 (s, 3H), 1.15 (s, 3H), 1.12–1.05 (m, 1H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.87–0.72 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.79, 172.00, 171.53, 170.56, 156.16, 155.57, 149.99, 135.29, 135.00, 133.41, 132.06, 129.68, 129.43, 128.75, 126.27, 125.55, 123.20, 119.22, 88.74, 78.76, 67.01, 59.31, 54.53, 51.76, 48.30, 38.27, 35.34, 33.55, 29.51, 29.00, 26.81, 25.85, 20.46, 16.73, 14.73, 12.75.

4.2.12 Synthesis of compounds 7d2 and 8d2. Compound **7d2** (or **8d2**) was synthesized from lathyrol (or epoxylathyrol) and **6d2** according to the synthetic method for compound **1**.

*(1aR,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a]cyclopropa[f] [11] annulen-8-yl 2-((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)acetate (compound **7d2**)*

White solid, 59% yield; HR-MS(ESI) *m/z*: 635.2611 $[M+Na]^+$ (calcd. for $C_{37}H_{40}NaO_8$, 635.2615); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18–8.12 (m, 2H), 8.09 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.88–7.81 (m, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 7.61–7.48 (m, 5H), 6.01 (d, *J* = 10.2 Hz, 1H), 5.39 (s, 1H), 5.01–4.84 (m, 2H), 4.79 (s, 1H), 4.54 (s, 1H), 4.12 (d, *J* = 6.8 Hz, 1H), 3.72 (d, *J* = 3.3 Hz, 1H), 2.96–2.81 (m, 1H), 2.14 (dd, *J* = 10.4, 3.2 Hz, 2H), 1.92–1.72 (m, 3H), 1.71–1.60 (m, 1H), 1.55 (s, 3H), 1.43 (dt, *J* = 20.1, 10.4 Hz, 3H), 1.13 (s, 4H), 1.08 (s, 3H), 0.90 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.70, 174.18, 168.64, 155.07, 154.65, 151.65, 145.20, 139.54, 134.64, 133.90, 131.34, 130.96, 129.15, 128.91, 125.60, 125.36, 123.79, 118.84, 114.77, 89.06, 78.39, 68.37, 68.01, 53.58, 48.67, 37.92, 35.90, 34.66, 28.98, 28.67, 25.43, 21.71, 16.66, 14.84, 12.87.

*(1aR,2'S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2'-oxiran]-8-yl 2-((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)acetate (compound **8d2**)*

White solid, 24% yield; HR-MS(ESI) *m/z*: 629.2726 $[M+H]^+$ (calcd. for $C_{37}H_{41}O_9$, 629.2745); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (dt, *J* = 7.5, 3.2 Hz, 2H), 8.10 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.85 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 1H), 7.60–7.48 (m, 5H), 6.19 (d, *J* = 9.3 Hz, 1H), 5.42 (s, 1H), 4.97 (dt, *J* = 31.0, 9.7 Hz, 2H), 4.11 (d, *J* = 6.8 Hz, 1H), 3.62 (dd, *J* = 6.3, 2.9 Hz, 1H), 2.91 (dd, *J* = 13.0, 9.0 Hz, 1H), 2.46 (d, *J* = 3.0 Hz, 1H), 2.08 (d, *J* = 7.9 Hz, 2H), 1.95–1.84 (m, 2H), 1.73 (s, 4H), 1.66 (d, *J* = 14.3 Hz, 1H), 1.59–1.37 (m, 4H), 1.33–1.21 (m, 2H), 1.17 (s, 3H), 1.11 (s, 3H), 0.97–0.91 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.76, 174.24, 168.85, 155.13, 154.72, 150.00, 139.53, 134.99, 134.64, 131.33, 130.97, 129.20, 128.95, 125.61, 125.36, 123.82, 118.89, 88.62, 78.56, 68.44, 67.40, 59.13, 54.62, 51.66, 48.20, 38.24, 35.32, 33.56, 29.48, 29.00, 25.89, 20.35, 16.71, 14.59, 12.74.

4.2.13 Synthesis of compounds 7d3 and 8d3. Compound **7d3** (or **8d3**) was synthesized from **11** (or **12**) and **6d4** according to the synthetic method for compounds **7a–g**.

*3-(3-(4-((((1aR,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a] cyclopropa[f][11]annulen-8-yl)oxy) methyl)-1H-1,2,3-triazol-1-yl)propoxy)-2-phenyl-4H-chromen-4-one (compound **7d3**)*

White solid, 48% yield; HR-MS(ESI) *m/z*: 716.3314 $[M+Na]^+$ (calcd. for $C_{41}H_{47}N_3NaO_7$, 716.3306); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (d, *J* = 7.9 Hz, 1H), 8.06 (dd, *J* = 7.4, 2.1 Hz, 2H), 8.02 (s, 1H), 7.88–7.82 (m, 1H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.65–7.58 (m, 3H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.41 (d, *J* = 10.3 Hz, 1H), 4.80–4.75 (m, 1H), 4.71 (d, *J* = 15.2 Hz, 2H), 4.65 (d, *J* = 11.6 Hz, 2H), 4.44 (t, *J* = 7.1 Hz, 3H), 4.34 (s, 1H), 4.15 (t, *J* = 3.2 Hz, 1H), 4.03 (dd, *J* = 6.6, 3.5 Hz, 3H), 2.91–2.72 (m, 1H), 2.56 (dd, *J* = 13.0, 6.6 Hz, 1H), 2.17 (dd, *J* = 11.9, 5.2 Hz, 4H), 1.97–1.77 (m, 4H), 1.56 (s, 3H), 1.48 (d, *J* = 12.9 Hz, 2H), 1.44–1.36 (m, 2H), 1.23 (s, 2H), 1.17 (dd, *J* = 9.3, 4.9 Hz, 2H), 1.12 (s, 3H), 1.03 (s, 3H), 0.92 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.12, 174.44, 156.07, 155.30,

150.21, 145.43, 140.08, 134.65, 133.90, 131.47, 130.91, 129.17, 128.99, 125.61, 125.48, 124.04, 124.00, 118.97, 110.22, 88.38, 88.23, 69.44, 65.64, 65.50, 56.31, 50.10, 46.87, 38.01, 35.94, 35.41, 30.86, 29.00, 28.47, 25.37, 22.12, 16.66, 15.05, 12.94.

*(1aR,2'S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-8-((1-(3-((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)-1,4a,5,6,7,7a,8,10,11,11a-decahydrospiro[cyclopenta[a]cyclopropa[f][11]annulene-9,2'-oxiran]-4(1aH)-one (compound **8d3**)*

White solid, 45% yield; HR-MS(ESI) *m/z*: 732.3281 $[M+Na]^+$ (calcd. for $C_{41}H_{47}N_3NaO_8$, 732.3255); ^1H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 8.07 (dd, *J* = 9.3, 3.7 Hz, 2H), 8.04–7.99 (m, 3H), 7.93 (s, 1H), 7.85–7.76 (m, 2H), 7.71 (dd, *J* = 7.9, 5.2 Hz, 2H), 7.63–7.52 (m, 6H), 7.50–7.43 (m, 2H), 6.90 (d, *J* = 11.2 Hz, 1H), 4.89 (d, *J* = 3.0 Hz, 1H), 4.68–4.59 (m, 3H), 4.54 (d, *J* = 11.1 Hz, 1H), 4.40 (t, *J* = 7.0 Hz, 1H), 4.30 (d, *J* = 11.3 Hz, 1H), 4.07–3.91 (m, 5H), 2.68 (dd, *J* = 11.3, 5.5 Hz, 1H), 2.14 (tt, *J* = 12.1, 6.2 Hz, 4H), 1.92 (s, 1H), 1.79 (d, *J* = 7.3 Hz, 3H), 1.77–1.70 (m, 2H), 1.59–1.45 (m, 2H), 1.32–1.12 (m, 5H), 1.07 (s, 3H), 0.92 (t, *J* = 6.9 Hz, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.14, 174.36, 155.83, 155.22, 144.14, 140.05, 134.74, 134.54, 131.38, 130.84, 129.09, 128.91, 125.50, 125.41, 124.46, 123.95, 118.85, 91.62, 76.39, 73.15, 69.44, 69.33, 65.65, 60.52, 59.58, 51.15, 46.92, 46.85, 38.07, 34.67, 32.17, 30.83, 28.93, 28.69, 26.23, 20.06, 15.88, 13.75, 13.51.

4.2.14 Synthesis of compounds 7d4 and 8d4. Compound **7d4** (or **8d4**) was synthesized from **9** (or **10**) and **6d6** according to the synthetic method for compound **1**.

*(1aR,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-9-methylene-4-oxo-1a,4,4a,5,6,7,7a,8,9,10,11,11a-dodecahydro-1H-cyclopenta[a] cyclopropa[f][11]annulen-8-yl 4-oxo-4-((2-oxo-2-((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)ethyl)amino)butanoate (compound **7d4**)*

White solid, 20% yield; HR-MS(ESI) *m/z*: 734.2922 $[M+Na]^+$ (calcd. for $C_{41}H_{45}NNaO_{10}$, 734.2936);^1H NMR (400 MHz, DMSO-d_6) δ 8.14–8.06 (m, 1H), 7.97–7.87 (m, 3H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.60 (dt, *J* = 22.6, 7.5 Hz, 4H), 6.06 (d, *J* = 10.3 Hz, 1H), 5.38 (s, 1H), 4.83 (s, 1H), 4.58 (s, 1H), 4.23 (d, *J* = 6.4 Hz, 1H), 4.12 (s, 2H), 4.00 (s, 1H), 3.03–2.83 (m, 3H), 2.63 (qd, *J* = 17.2, 9.4 Hz, 2H), 2.29 (dd, *J* = 10.2, 3.1 Hz, 1H), 2.18 (d, *J* = 10.7 Hz, 1H), 1.95 (dd, *J* = 18.1, 11.5 Hz, 2H), 1.73 (s, 2H), 1.56 (s, 3H), 1.44 (ddd, *J* = 16.3, 11.8, 9.4 Hz, 3H), 1.13 (s, 3H), 1.12 (s, 3H), 0.96 (t, *J* = 8.8 Hz, 3H), 0.83 (dd, *J* = 9.5, 7.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.63, 171.89, 171.69, 171.52, 170.49, 167.83, 156.13, 155.56, 145.72, 135.30, 133.39, 132.10, 129.67, 129.42, 128.73, 126.28, 125.53, 123.19, 119.22, 114.34, 89.19, 78.67, 67.68, 53.64, 48.78, 40.74, 37.93, 35.98, 34.88, 29.01, 28.76, 25.45, 21.81, 16.68, 15.03, 12.86.

*(1aR,2'S,4aR,6S,7S,7aR,8R,11aS,E)-4a,7-dihydroxy-1,1,3,6-tetramethyl-4-oxo-1,1a,4,4a,5,6,7,7a,8,10,11,11a-dodecahydrospiro[cyclopenta[a]cyclopropa[f][11] annulene-9,2'-oxiran]-8-yl 4-oxo-4-((2-oxo-2-((4-oxo-2-phenyl-4H-chromen-3-yl)oxy)ethyl)amino) butanoate (compound **8d4**)*

White solid, 36% yield; HR-MS(ESI) *m/z*: 727.2995 $[M+H]^+$ (calcd. for $C_{41}H_{46}NO_{11}$, 727.2993);^1H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 7.1 Hz, 1H), 8.00–7.87 (m, 3H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.61 (dt, *J* = 14.8, 6.1 Hz, 5H), 6.20 (d, *J* = 9.2 Hz, 1H), 5.37 (s, 1H), 4.22 (d, *J* = 6.5 Hz, 1H), 4.14 (s, 2H), 3.94–3.76 (m, 1H), 2.94 (dd, *J* = 10.9, 6.6 Hz, 3H), 2.74 (dd, *J* = 15.5, 7.1 Hz, 2H), 2.44 (d, *J* = 3.0 Hz, 1H), 2.07 (d, *J* = 12.4 Hz, 2H), 1.79 (s, 2H), 1.73 (s, 4H), 1.59–1.42 (m, 2H), 1.41–1.31 (m, 1H), 1.16 (s, 3H), 1.15 (s, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.89–0.72 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.80, 172.00, 171.86, 171.53, 170.57, 167.62, 156.17, 155.57, 150.00, 135.30, 134.99, 133.40, 132.07, 129.68, 129.43, 128.76, 126.28, 125.54, 123.20, 119.23, 88.73, 78.76, 67.00, 59.31, 54.53, 51.75, 48.30, 40.76, 38.27, 35.33, 33.55, 29.50, 29.00, 26.81, 25.85, 20.45, 16.73, 14.73, 12.75.

4.2.15 Synthesis of compounds 6h–j. Compounds **5a–g** (0.34 mmol, 1.0 eq) were dissolved in DMF (2 mL), and K_2CO_3 (0.17 mmol, 0.5 eq) was then added at room temperature. Propargyl bromide (0.17 mmol, 0.5 eq) was added to the mixture after 0.5 h. The reaction was stirred for 8–9 h at room temperature. After completion of the reaction, EtOAc was added to the mixture. The organic layer was washed with brine three to five times, then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by CC, thus yielding **6h–j**. The attachment positions of propynyl groups were determined with HMBC. Assignments of ^{13}C and ^1H chemical shifts of quercetin, kaempferol and farrerol were made according to Gyeltshen et al. [52], Buyinza et al. [53] and Devkota et al. [54], respectively.

*2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(prop-2-yn-1-yloxy)-4H-chromen-4-one (compound **6h**)*

Light yellow solid, 47% yield; ^1H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 10.90 (s, 1H), 9.81 (s, 1H), 9.35 (s, 1H), 7.55 (d, *J* = 1.9 Hz, 1H), 7.53–7.48 (m, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.43 (d, *J* = 1.8 Hz, 1H), 6.21 (d, *J* = 1.8 Hz, 1H), 4.87 (d, *J* = 2.0 Hz, 2H), 3.51 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.14, 164.70, 161.68, 156.98, 156.78, 149.26, 145.59, 135.37, 121.55, 121.26, 116.23, 116.01, 104.36, 99.17, 94.12, 79.72, 79.17, 59.31.

*5,7-dihydroxy-2-(4-hydroxyphenyl)-3-(prop-2-yn-1-yloxy)-4H-chromen-4-one (compound **6i**)*

White solid, 54% yield; ^1H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 1H), 10.90 (s, 1H), 10.29 (s, 1H), 8.00 (d, *J* = 8.9 Hz, 2H), 6.93 (d, *J* = 8.9 Hz, 2H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 4.89 (d, *J* = 2.4 Hz, 2H), 3.51 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.16,

164.73, 161.66, 160.72, 156.92, 156.83, 135.35, 130.96, 121.02, 115.94, 104.39, 99.20, 94.27, 79.74, 79.17, 59.38.

*(S)-5-hydroxy-6,8-dimethyl-2-phenyl-7-(prop-2-yn-1-yloxy)chroman-4-one (compound **6j**)*
Light yellow solid, 45% yield; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.16 (s, 1H), 9.60 (s, 1H), 7.33 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 5.48 (dd, *J* = 12.8, 2.8 Hz, 1H), 4.61 (d, *J* = 2.3 Hz, 2H), 3.61 (t, *J* = 2.4 Hz, 1H), 3.31 (dd, *J* = 17.1, 12.8 Hz, 1H), 2.81 (dd, *J* = 17.1, 3.0 Hz, 1H), 2.03 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 199.10, 163.06, 158.56, 158.14, 158.10, 129.37, 128.54, 115.68, 110.84, 109.74, 105.27, 79.65, 78.98, 78.63, 60.61, 42.63, 9.39, 8.83.

4.3. Biology

4.3.1 Cell cultures. The RAW264.7 cell line was purchased from the American Type Culture Collection. The cells were grown in 25 cm^2 cell culture flasks with Dulbecco's modified Eagle's medium containing 15% fetal bovine serum and 1% penicillin-streptomycin solution in an incubator (37°C, 5% CO_2).

4.3.2 NO assay on LPS-induced cells. The level of NO release by LPS-induced RAW264.7 cells was measured with Griess reagent (Beyotime, China). RAW264.7 cells were seeded in 96-well plates with 35,000 cells per well overnight. Subsequently, **8d1** was added in a concentration gradient for pretreatment, and 0.5 μg/mL LPS was added for 24 h. At room temperature, an equal volume of cell supernatant was mixed with Griess reagents I and II. After incubation for 10 minutes, a microplate reader was used to detect the absorption of the mixture at 540 nm for subsequent calculations.

4.3.3 Cell viability assays. The viability of RAW264.7 cells was assessed with cell counting kit-8 (CCK-8) assays (APExBIO, USA). First, RAW264.7 cells were seeded into 96-well plates with 25,000 cells per well and cultured to approximately 50–60% confluence. After treatment with **8d1** for 3 h, the preprotected cells were stimulated with LPS at 0.5 μg/mL for 21 h. Then 10 μL of CCK-8 reagent was added to each well, and the cells were continually incubated for 30–45 min. Finally, the optical density values were measured at 450 nm with a microplate reader.

4.3.4 Western blotting. The cells were lysed in RIPA buffer with 0.1% PMSF on ice for 30 min. The samples were boiled at 95°C for 5 min, then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane via a wet-transfer system. The membranes were blocked with 5% BSA and then incubated with specific primary antibodies overnight at 4°C. The next day, the membranes were incubated with HRP-conjugated secondary antibody for 2 h at 4°C. Detection was performed with a BeyoECL Star chemiluminescence kit (Beyotime, China) according to the manufacturer's instructions.

4.3.5 Preparation of nuclear and cytoplasmic extracts. RAW264.7 cells were cultured in six-well plates and pretreated for 3 h with **8d1** (10 μM) or DMSO, then induced with 0.5 μg/mL LPS for 24 h. The nuclear and cytoplasmic extraction reagents MeiLun Nuclear and Cytoplasmic Protein Extraction Kit (MeiLunbio, China) were used according to the manufacturer's protocol. Subsequently, 5× loading buffer was added to the samples, which were then boiled at 95°C for 5–10 min. GAPDH was used as the cytoplasmic internal control, and Histone H3 was used for the nuclear internal control. The protein levels of NF-κB in cytoplasmic or nuclear fractions were determined by western blotting.

4.3.6 Immunofluorescence analysis of LC3 puncta and nuclear translocation of NF-κB. RAW264.7 cells were seeded on TC-treated cell slides (Solarbio, China) at the desired cell density and treated as described as above. After being washed with PBS three times, the cells were fixed in 4% paraformaldehyde for 20 min. The cells were treated with freshly prepared 0.2% Triton X-100 in PBS for 10 min. Blocking was performed with 5% BSA, which was incubated with samples at room temperature for 1 h. Subsequently, rabbit anti-NF-κB and anti-LC3B (Abcam, USA) were reacted with cells at 4°C overnight. Subsequently, Cy3-conjugated goat anti-rabbit antibody was incubated with the cell slides for 1 h at room temperature. DAPI was then used to stain nuclei in the dark for 5 min. Images were recorded under a fluorescence microscope.

4.3.7 Statistical analysis. Statistical analysis was performed as described in our previous work [36].

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CONFLICTS OF 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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