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# Hybrids of aurantiamide acetate and isopropylated genipin as potential anti-inflammatory agents: the design, synthesis and biological evaluation

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## ABSTRACT

A novel series of hybrids designed on the basis of aurantiamide acetate and isopropylated

genipin were synthesized and biologically evaluated as anti-inflammatory agents. Among them, compound **7o** exhibited the best inhibitory activity against TNF-$\alpha$ secretion ($IC_{50}$ = 16.90 $\mu$M), and was selected for further *in vitro* and *in vivo* functional study. The results demonstrated that **7o** was capable of suppressing the expression of LPS-induced iNOS and COX-2, as well as reducing the production of NO at the concentration of 5 $\mu$M, which may be resulted from its regulation of NF-$\kappa$B signaling and MAPK signaling. Moreover, compound **7o** exhibited favourable *in vivo* anti-inflammatory activity with an inhibition rate of 53.32% against xylene-induced ear swelling in mice at the dose of 5 mg/kg.

**Keywords:** Anti-inflammatory, Aurantiamide acetate, Synthesis, NF-$\kappa$B, MAPK

## 1. Introduction

As a fundamental defense mechanism of host protection against injury or infection, the inflammatory process also results in tissue damage and causes a myriad of diseases such as arthritis, atherosclerosis and diabetes[1-2]. Mounting researches showed that Nuclear factor kappa B (NF-$\kappa$B) and Mitogen-activated protein kinase (MAPK) signaling pathways play vital roles in inflammatory diseases[3-7]. NF-κB paticipates in regulating the expression of downstream targets including COX-2, iNOS, as well as inducing the generation of pivotal inflammatory factors such as TNF-$\alpha$, IL-6[8-10]. MAPK is a serine/threonine protein kinase which is mediating the production of TNF-$\alpha$, NO and other inflammatory factors, thereby being crucial for the initiation and progression of inflammation as well[11].

Aurantiamide acetate **(1, Fig. 1)** originating from medicinal plants exhibits a broad spectrum of biological activities, including antioxidant, anti-inflammatory and neurotrophic activities. Among these, the anti-inflammatory effect stems from its capability to modulate the NF-$\kappa$B and MAPK pathways, leading to dose-dependent suppression of NO and TNF-$\alpha$ production, as well as iNOS and COX-2 expression[12]. Throughout the past decades, structural modification of **1** has predominantly focused on the terminal phenyl moiety and the phenylalanine residue **(2, Fig. 1)**[13].

Upon modifying the phenyl of the phenylalanine residue and unmasking its carboxylic functionality, Sircar[14] and co-workers discovered a series of N-benzoylphenylalanine derivatives. Compound **3 (Fig. 1)**, as a representative, has entered the clinical research owing to its excellent anti-inflammatory activity and drug-like properties. As illustrated by the chemical structure of **3**, the $\alpha$-amino acid residue is crucial for activity, and unmasking its carboxylic functionality does not dramatically weaken the activity.

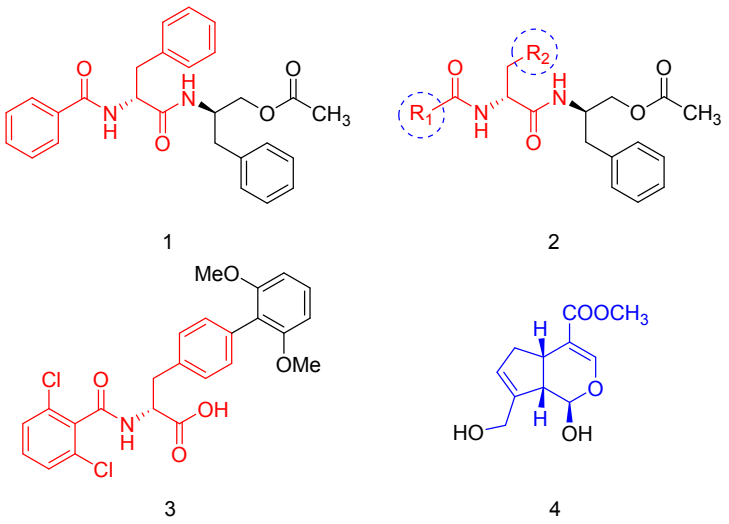



**Figure 1** The structures of compounds **1-4**

Genipin **(4, Fig. 1)** is the main biologically active component of Geniposide with diverse pharmacological activities, including anti-thrombotic, anti-tumor, anti-inflammatory and hepatoprotective activities. Recent studies have shown that Genipin could significantly downregulate LPS-induced expression of inflammation-related enzymes and suppress the production of pro-inflammatory factors by regulating NF-$\kappa$B and MAPK signaling pathways[15-17]. The hydroxyl at the C-3 position and the hemiacetal at the C-10 position in Genipin are the main reasons for its poor stability, low lipid solubility and limited pharmacological activity in physiological solution. Therefore, we further optimized the structure of Genipin according to the report of Li Qingshan et al.[18].

As stated above, both **1** and **4** may serve as an anti-inflammatory agent by the capability to interfere with NF-$\kappa$B and MAPK signaling pathways. Hence, a series of aurantiamide

acetate-isopropylated genipin hybrids have recently designed via combing their key pharmacophores. Firstly, according to the chemical structure of **3**, the phenylalanine carboxylic functionality of **1** was unmasked for providing a site for derivatization **(Fig. 3)**. Then, the primary alcoholic hydroxyl group was replaced by an amino group for generating amide after condensation with the phenylalanine derivative, which was more stable than the ester functionality. Additionally, we also investigated whether incorporation of a trans-carbon-carbon double bond between the terminal phenyl and the amide group had an impact on activity[19-23].

Herein, we communicate the synthesis and biological evaluation of these aurantiamide acetate-genipin hybrids as potential anti-inflammatory agents. Their anti-inflammatory activity was evaluated by lipopolysaccharide (LPS)-induced cytokine production in RAW264.7 cells. Furthermore, the most potent compound **7o** was used to illustrate the preliminary mechanism of anti-inflammatory action.

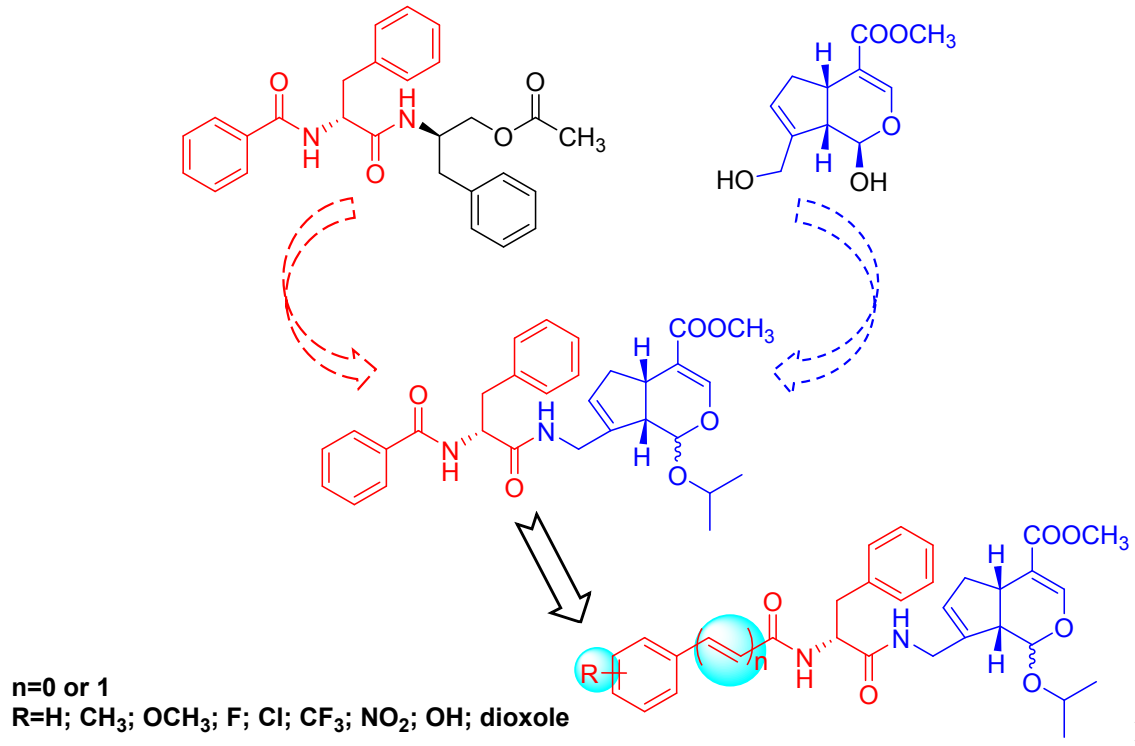


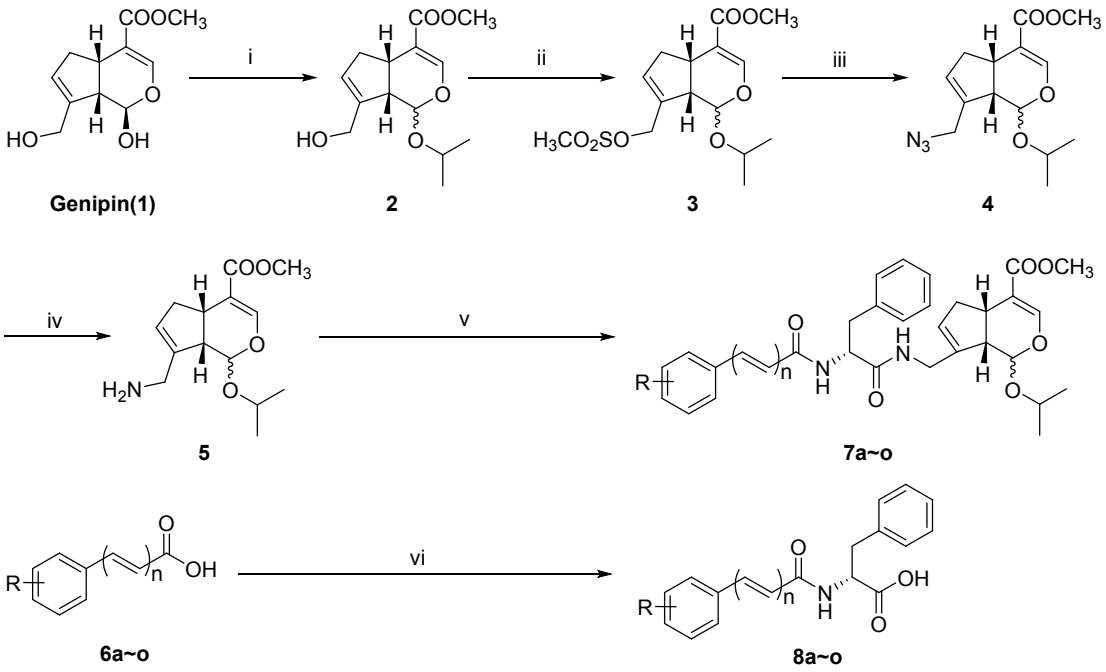

**Figure 2**

Design rationale of target compounds

## 2. Results and discussion

### 2.1. Chemistry

The synthetic route of target compounds **7a-7o** was outlined in **Scheme 1**. Genipin, as the starting material was subjected to O-alkylation to afford **2**. Its hydroxyl functionality was converted to azide after methylsulfonylation and the further treatment with $NaN_3$. Afterwards, reduction of the azide group furnished the key intermediate **5**. The key fragment **8a-o** was prepared via transformation of **6a-o** to corresponding acyl chlorides and subsequent condensation with *L*-phenylalanine in the presence of NaOH. Finally, condensation of **5** with **8a-o** provided **7a-7o** as the target compounds.




Compounds

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | n=0 | **a** | **b** | **c** | **d** | **e** | **f** | **g** | **h** | **i** | **j** | **k** | **l** |
| | | H | *p*-$CH_3$ | *m*-$CH_3$ | *p*-$OCH_3$ | *m*-$OCH_3$ | *p*-Cl | *m*-Cl | *p*-F | *m*-F | *p*-$NO_2$ | *p*-$CF_3$ | dioxole |
| | n=1 | **m** | **n** | | **o** | | | | | | | | |
| | | *m*-F | *m*-$OCH_3$ | | *p*-OH,*m*-$OCH_3$ | | | | | | | | |

Reagents and conditions: (i) *p*-TsOH·$H_2O$, *iso*-PrOH, reflux; (ii) $CH_3SO_2Cl$, $Et_3N$, DCM, rt; (iii) $NaN_3$, DMF, rt; (iv) $SnCl_2$, MeOH, rt; (v) **8a~o**, HOBT, EDCl, $Et_3N$, DCM, rt; (vi) $SOCl_2$, reflux.

**Scheme 1** The synthetic route of target compounds

2.2. The inhibitory effect on TNF-*α* secretion

Since TNF-*α* is an important pro-inflammatory cytokine, we first investigated the inhibitory activity of the target compounds against LPS-induced TNF-*α* secretion in RAW264.7 cells with Genipin and Aurantiamide acetate as reference compounds. **Tables 1** demonstrated that positive control groups and Aurantiamide acetate analogues were the ability to reduce TNF-*α* production. Among them, **7d**， **7h**， **7i**， **7o** performed better than Aurantiamide acetate in inhibiting LPS-induced TNF-*α* expression. Particularly, compound **7o** exerted the most potent inhibitory activity against TNF-*α* secretion throughout this series ($IC_{50}$= 16.90±1.23 *μ*M). Thus, it was selected for further *in vitro* and *in vivo* biological evaluation.

**Table 1** Inhibitory effect of Target compounds on LPS induced TNF-*α* secretion in RAW264.7

| Compound | $IC_{50}$（*μ*M） | Compound | $IC_{50}$（*μ*M） |
|---|---|---|---|
| **7a** | 39.11±2.17 | **7j** | 28.16±1.59 |
| **7b** | 51.59±3.66 | **7k** | 34.97±1.54 |
| **7c** | 57.68±3.84 | **7l** | 25.12±1.40** |
| **7d** | 18.44±1.26** | **7m** | 27.83±1.44 |
| **7e** | 24.13±1.58** | **7n** | 86.04±1.94 |

| | | | |
|---|---|---|---|
| **7f** | 31.45±1.86 | **7o** | 16.90±1.23** |
| **7g** | 25.73±1.37** | **4** | 98.83±5.97 |
| **7h** | 18.14±1.13** | **1** | 23.65±1.37 |
| **7i** | 19.58±1.29** | | |

**Compared with Aurantiamide** **p＜0.01，Values are expressed as the mean ± SD (n=5).**

2.2 Compound **7o** inhibits inflammatory mediators in RAW264.7 cells

Considering the essential role played by NO in the inflammation, as well as its close correlation with the expression of COX-2 and iNOS, we then investigated the effect of **7o** on LPS-induced NO production in RAW264.7 cells. As shown in **Fig. 3**, compound **7o** was capable to inhibit the NO generation in a concentration-dependent manner. At the concentration as low as 5 $\mu$M, it remarkably inhibited the NO production. As shown in **Fig. 3**, the impact of **7o** on iNOS and COX-2 expressions in RAW264.7 was then detected by western blot assay. Compound **7o** dramatically downregulated the expressions of both iNOS and COX-2 at the concentration of 5 $\mu$M. Besides, the downregulation was dose-dependent. These results illustrated that compound **7o** could reduce LPS-induced inflammatory response in RAW264.7 cells.

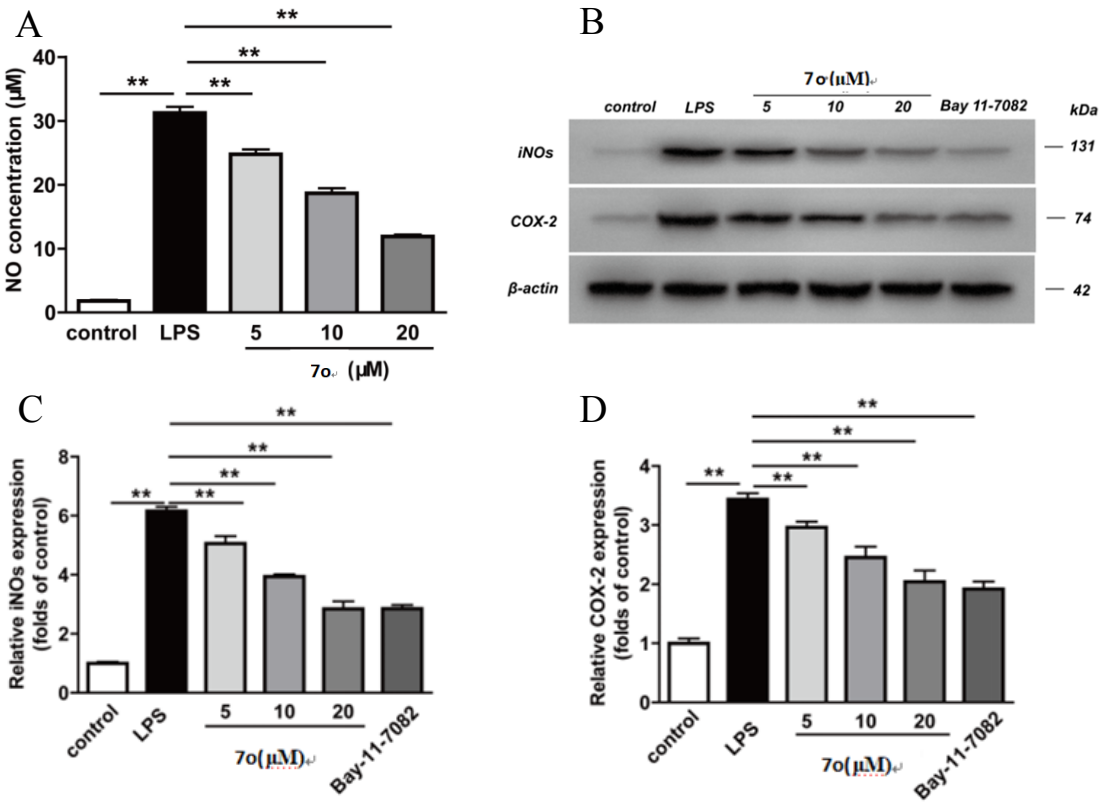



**Figure 3** Compound **7o** reduced NO production in RAW264.7 cells

**Fig. 3** Effects of **7o** on LPS-induced iNOS and COX-2 expression in RAW264.7 cells. The cells were pre-treated with different concentrations of **7o** for 2h and then were stimulated with LPS (200 ng/mL) or without LPS for 8 h. Bay 11-7082 is the NF-*κ*B inhibitor. Representative bands were shown in (A), Quantitative analysis of iNOS expressions in (B), Quantitative analysis of COX-2 expressions in (C). The results were presented as mean ± SD (n = 3).*p < 0.05, **p < 0.01.

## 2.3 Compound **7o** suppresses NF-*κ*B activation in RAW264.7 cells.

As an important pathway regulating the production of proinflammatory cytokines, NF-*κ*B signaling can be activated by LPS-stimulation, which leads to the phosphorylation and degradation of I*κ*B to NF-*κ*B p65. Then p65 translocates to the nucleus and interacts with target promoters to cause the release of inflammatory factors such as TNF-*α*. As shown in **Fig. 4**, the levels of I*κ*B, phos-I*κ*B, phos-p65 were detected by western blot assay to evaluate the suppressive effect of **7o** on NF-*κ*B signaling after LPS-stimulation. Taken together, **7o** was capable of modulating NF-*κ*B

signaling.

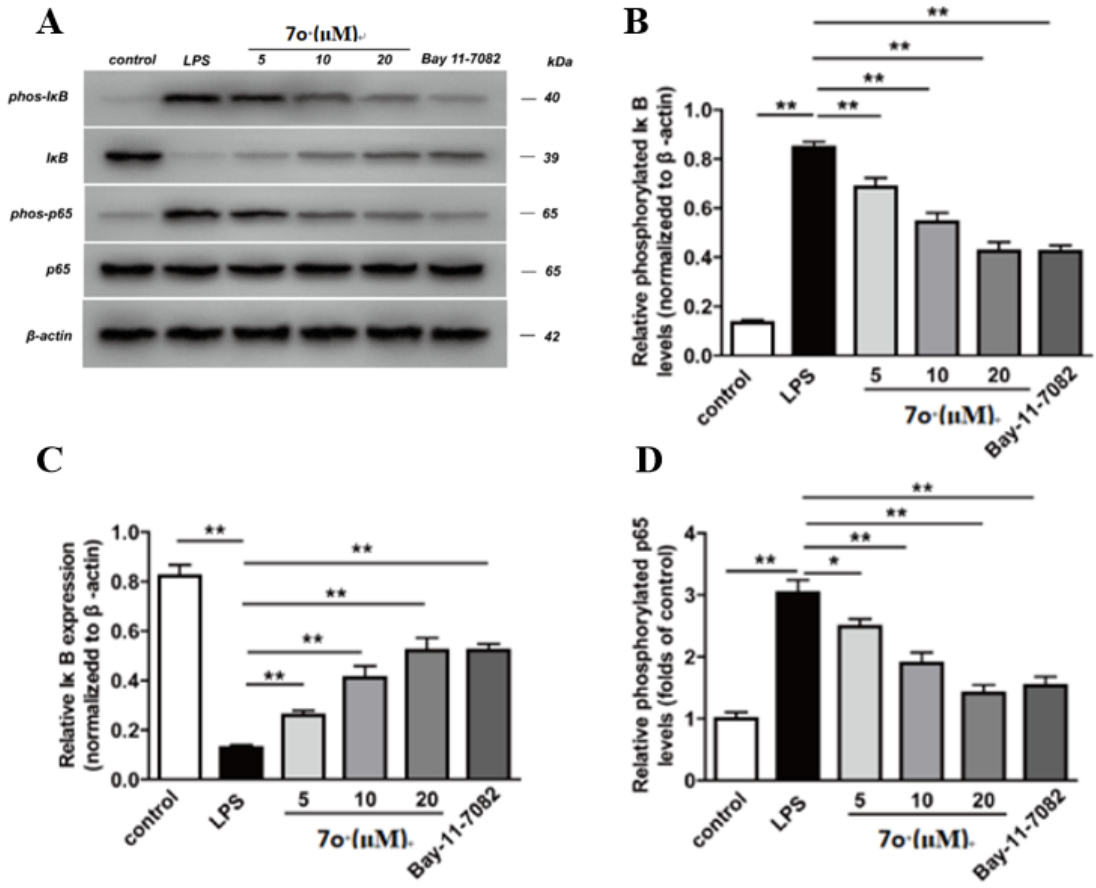



**Fig. 4** Compound **7o** inhibited LPS-induced NF-$\kappa$B activation in RAW264.7 cells. RAW264.7 cells were co-incubated with **7o** (5, 10, 20 $\mu$M) and LPS (200 ng/mL) for 2 h. Representative bands were shown in (A); The levels of I$\kappa$B expression (B) and the phosphorylation levels of I$\kappa$B (C) were normalized to $\beta$-actin; Phosphorylation levels of p65 (D) were normalized to control. The results were presented as mean ± SD (n = 3). *p < 0.05, **p < 0.01.

## 2.4 Compound **7o** inhibits MAPK signaling activation in RAW264.7 cells.

MAPKs are a family of signal transduction proteins, including p38, ERK, and JNK, and play crucial roles in the expression of iNOS and COX-2. Besides, they can induce the production of NO and TNF-$\alpha$. To determine the effect of compound **7o** on the LPS-induced activation of MAPKs, the expressions of phos-ERK, phos-JNK, and phos-p38 were examined. As was expected, compound **7o** inhibited p38 activation in the MAPK signaling pathway in a concentration-dependent manner, but had no obvious effect on phosphorylation of JNK or ERK

(**Fig. 5**). Therefore, the anti-inflammatory activity of compound **7o** was speculated to have a relationship with its negative effects on p38 activation.

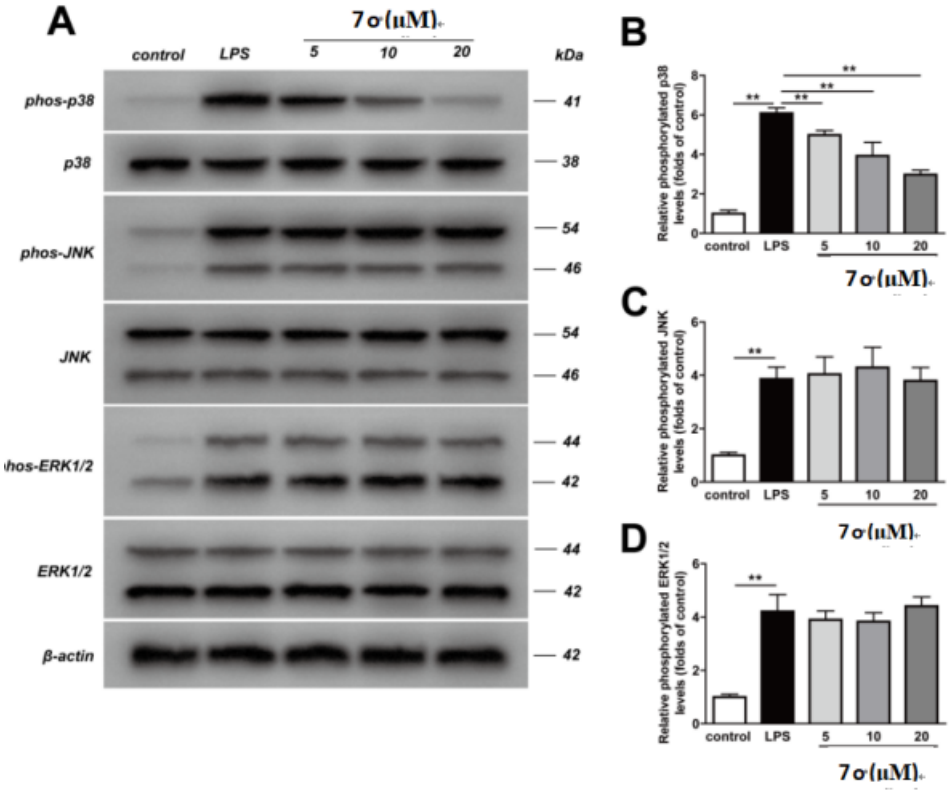



**Fig. 5** Compound **7o** inhibited LPS-induced MAPK-signalling activation in RAW264.7 cells. Representative bands were shown in (A). Phosphorylation levels of p38 (B), JNK (C) and ERK1/2 (D) were normalized to control. The results were presented as mean ± SD (n = 3). *p < 0.05, **p < 0.01.

2.5 Anti-inflammatory activity in vivo

**Table 2** The effect of different compounds on xylene-induced ear swelling in mice.

| **Group** | Dose(mg/kg) | Swelling degree (mg) | Inhibition (%) |
|---|---|---|---|
| **Blank** | **-** | 0.12±0.66 | |

| | | | |
|---|---|---|---|
| **Model** | **-** | 14.74±1.28 | |
| **7o** | **5** | 6.88±1.33** | 53.32 |
| **Aurantiamide acetate** | **5** | 7.84±2.29** | 46.81 |
| **Dexamethasone** | **0.5** | 4.92±0.59** | 66.65 |

* p<0.05, ** p<0.01 vs Model.

swelling degree (mg) = MR - ML.

Inhibition (%) = ((MR-ML) model-(MR-ML) treated)/(MR-ML) model * 100%.

Values are expressed as the mean ± SD (n=6).

The *in vivo* anti-inflammatory activity of compound **7o** was further determined by the allergic contact dermatitis model. The results were expressed as percent inhibition of mouse ear swelling over the untreated control group and were shown in **Table 2.** Based on the data, compound **7o** showed better anti-inflammatory activity than aurantiamide acetate at the dose of 5 mg/kg. In other words, compound **7o** could inhibit mouse ear swelling and show good anti-inflammatory activity.

## 3. Conclusions

In conclusion, a novel series of hybrids designed on the basis of aurantiamide acetate and isopropylated genipin were synthesized and biologically evaluated as anti-inflammatory agents. Among these compounds, compound **7o** exhibited the best inhibitory activity against TNF-$\alpha$ secretion ($IC_{50}$=16.90 $\mu$M). Then the mechanisms of action of compound **7o** were investigated in further studies. The results demonstrated that **7o** was capable of suppressing the LPS-induced expression of LPS-induced iNOS and COX-2, as well as the production of NO, which may be

resulted from its regulation of NF-$\kappa$B and MAPK signaling pathways. Moreover, compound **7o** also showed great anti-inflammatory activity *in vivo*, and might serve as promising lead candidates for anti-inflammatory agent development.

## 4. Experimental section

### 4.1. Chemistry

Melting points were determined on SGWX-4 melting point apparatus (Precision Science Inc., Shanghai, China), which were uncorrected. NMR spectra were measured at 400 or 600 MHz for $^{1}$H NMR, and 100 or 150 MHz for $^{13}$C NMR, respectively, on Bruker DRX-400(Bruker Inc, CH) in DMSO-$d_6$, using TMS as internal standard. ESI-MS data was collected on an LCQ Advantage MAX spectrometer (Finnigan Inc. USA). Column chromatography was performed with silica gel (200 mesh, Qingdao Marine Chemical Inc., Qingdao, China). TLC was carried out on glass pre-coated silica gel GF 254 plates (Qingdao Marine Chemical Inc.). Unless otherwise noted, all chemicals were purchased from commercially available sources.

### 4.2. General procedure for preparation of compound (**5**)

#### 4.2.1 methyl (4*aS*,7*aS*)-7-(hydroxymethyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(2)**

In a three-neck flask, Genipin (2.0 g, 8.84 mmol), *p*-Toluenesulfonic acid monohydrate (2.0 g, 10.51 mmol) and Isopropyl alcohol (20 ml) were added. The reaction mixture was heated to 80 ℃ for 2 h with stirring (TLC, PE/EA=4:1). After the reaction was completed, water (100 ml) was added. The reaction solution was extracted with dichloromethane (3 × 50 mL) and combined organic layers, dried with sodium sulfate and evaporated in vacuo to afford a yellow oil. The oil

was purified by chromatography (PE/EA=4:1) to give the title compound as white solid. Yield 84.4%, m.p. 89.5 ℃-92.7 ℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.46 (s, 1H, =C**H**), 5.76-5.68 (m, 1H, =C**H**), 4.71 (d, *J* = 7.8 Hz, 1H, C**H**), 4.10 (d, *J* = 15.0 Hz, 1H, C**H$_2$**OH), 3.99-3.92 (m, 2H), 3.61 (s, 3H, COOC**H$_3$**), 2.98 (d, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.70-2.64 (m, 1H, C**H**), 2.43 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.05-1.95 (m, 1H, C**H**), 1.16 (d, *J* = 6.0 Hz, 3H, CHC**H$_3$**), 1.09 (d, *J* = 6.0 Hz, 3H, CHC**H$_3$**); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ: 170.08, 155.23, 147.84, 128.62, 113.38, 102.78, 74.54, 62.66, 54.04, 48.83, 41.21, 38.64, 26.43, 24.73; ESI-MS for **C$_{14}$H$_{20}$O$_5$** m/z: 268.87 $[M+H]^+$.

4.2.2 methyl (4*aS*,7*aS*)-1-isopropoxy-7-(((methylsulfonyl)oxy)methyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(3)**

Compound **2** (2.0 g, 7.45 mmol), $Et_3N$ (1.6 mL, 11.51 mmol) was dissolved in DCM (30 mL), Methanesulfonyl chloride (0.9 g, 7.86 mmol) was added dropwise under ice-cooling. After the addition was completed, the reaction mixture was continued to stir for 1 h. After the reaction was completed, DCM (50 ml) was added, then washed with water (20 mL×3), dried with sodium sulfate and evaporated in vacuo to afford a yellow oil (2.5g). Yield 96.9%. No purification for the next reaction.

4.2.3 methyl (4*aS*,7*aS*)-7-(azidomethyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(4)**

Compound **3** (2.0 g, 7.45 mmol), $NaN_3$ (2.4 g, 37.25 mmol) was dissolved in DMF (30 mL), The reaction mixture was stirred at room temperature for 3 h. After the reaction was completed, the reaction solution was poured into 100 mL of ice water, then extracted with ether (50 mL×3) and combined organic layers, dried with sodium sulfate and evaporated in vacuo to afford

Compound **4**. No purification for the next reaction.

4.2.4 methyl (4*aS*,7*aS*)-7-(aminomethyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(5)**

In the flask, compound **4** was dissolved in Methanol (20 mL), Stannous chloride dihydrate (2.05 g, 10.83 mmol) was added. The mixture was stirred at room temperature for 3 h. After the reaction was completed, the reaction solution was poured into 100 mL of ice water and diluted with 5% NaOH (liq.) was slowly added dropwise to pH = 8, then extracted with Ethyl acetate (50 mL×3) and combined organic layers, dried with sodium sulfate and evaporated in vacuo. The residues were purified by chromatography (DCM/Methanol=20:1) to give the title compound as brown oil (1.43 g). Yield 74.1%, $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ: 7.44 (s, 1H, =C**H**), 5.68-5.62 (m, 1H, =C**H**), 4.69 (d, *J* = 7.8 Hz, 1H, C**H**), 3.99-3.93 (m, 1H, C**H**$CH_3$), 3.61 (s, 3H, COOC**H$_3$**), 3.34 (d, *J* = 16.8 Hz, 1H, C**H$_2$**$NH_2$), 3.18 (d, *J* = 16.8 Hz, 1H, C**H$_2$**$NH_2$), 2.97 (q, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.69-2.58 (m, 1H, C**H**), 2.45 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.15 (s, 2H, $CH_2$N**H$_2$**), 1.98-1.93 (m, 1H, C**H**), 1.16 (d, *J* = 6.0 Hz, 3H, CHC**H$_3$**), 1.09 (d, *J* = 6.0 Hz, 3H, CHC**H$_3$**); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ: 170.06, 155.16, 149.18, 127.90, 113.38, 102.96, 74.53, 53.97, 49.18, 44.59, 41.28, 38.65, 26.38, 24.65; ESI-MS for **$C_{14}H_{21}NO_4$** m/z: 267.94 $[M+H]^+$.

4.3. General procedure for preparation of compound (**8a-8o**)

A solution of substituted benzoic acid or substituted benzoenoic acid (16.38 mmol), $SOCl_2$ (15 mL) were refluxed for 5 h, then evaporated in vacuo to afford corresponding acyl chloride, *L*-phenylalanine (2.25 g, 13.65 mmol) was dissolved in 2 M NaOH (20 mL), and corresponding acyl chloride was slowly added dropwise under ice-cooling. After the dropwise addition, the reaction mixture was continued to stir for 2 h(DCM/Methanol=5:1). After the reaction was

completed, the reaction solution was diluted with 2M HCl (liq.) was slowly added dropwise to pH = 5, then filtrated and dried to give the title compound **8a-8o**.

4.4. General procedure for preparation of compound (**7a-7o**)

To a solution of **8a-8o** (2 mmol), EDC·HCl (0.422 g, 2.2 mmol), HOBt (0.297 g, 2.2 mmol) and **5** (0.564 g, 2 mmol.) in DMF (20 mL), was added $Et_3N$ (0.83 mL). The reaction mixture was stirred at room temperature for 2 h and monitored by TLC (DCM/Methanol=10:1). After the reaction was completed, the reaction mixture was added 10% citric acid (30 mL) and filtered. The residue was purified via silica using methanol/DCM = 1:20 to provide the target compound **7a-7o**.

4.4.1 methyl (4*aS*,7*aS*)-7-((2-benzamido-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7a)**. white soild, yield, 45.2%, m.p. 143.5℃-145.6 ℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.54 (s, 1H, ArCON**H**), 8.22 (s, 1H, CON**H**), 7.77 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.49-7.44 (m, 2H, =C**H**, Ar**H**), 7.40 (t, *J* = 7.8 Hz, 2H, Ar**H**), 7.32 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.22 (t, *J* = 7.8 Hz, 2H, Ar**H**), 7.13 (t, *J* = 7.8 Hz, 1H, Ar**H**), 5.59-5.47 (m, 1H, =C**H**), 4.76-4.66(m, 2H, OC**H**O, NHC**H**CO), 4.04-3.91(m, 2H, CC**H**$_2$NH), 3.75-3.64 (m, 1H, C**H**(CH$_3$)$_2$), 3.60 (s, 3H, COOC**H$_3$**), 3.10-3.04 (m, 1H, ArC**H**$_2$CH), 3.02-2.94 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.67-2.57 (m, 1H, C**H**), 2.42 (t, *J* =7.8 Hz, 1H, C**H$_2$**), 2.03-1.93 (m, 1H, C**H**), 1.20-1.08 (m, 6H, CH(C**H$_3$**)$_2$);$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ: 174.13, 170.06, 169.34, 155.30, 144.05, 141.54, 137.14, 134.33, 132.25, 131.22, 131.13, 130.51, 129.30, 129.28, 111.29, 102.78, 74.78, 58.14, 54.09, 49.41, 41.74, 41.21, 40.45, 38.59, 26.47, 24.80; ESI-MS for **$C_{30}H_{34}N_2O_6$** m/z: 518.99 $[M+H]^+$.

4.4.2 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((2-(4-methylbenzamido)-3-phenylpropanamido)methyl)-1,4a,5,7a-tetra

hydrocyclopenta[*c*]pyran-4-carboxylate **(7b)**. white soild, yield, 40.8%, m.p. 153.2-155.4 ℃; $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 8.44 (s, 1H, ArCON**H**), 8.19 (s, 1H, CON**H**), 7.68 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.45(s, 1H, =C**H**), 7.31 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.24-7.17 (m, 4H, Ar**H**), 7.12 (t, *J* = 7.2 Hz, 1H, Ar**H**), 5.58-5.50 (m, 1H, =C**H**), 4.75-4.65 (m, 2H, OC**H**O, NHC**H**CO), 4.05-3.90 (m, 2H, CC**H**$_2$NH), 3.75-3.63 (m, 1H, C**H**(CH$_3$)$_2$), 3.60 (s, 3H, COOC**H$_3$**), 3.09-3.03 (m, 1H, ArC**H**$_2$CH), 3.02-2.94 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.68-2.56 (m, 1H, C**H**), 2.42 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.30 (s, 3H, ArC**H$_3$**), 2.04-1.92 (m, 1H, C**H**), 1.20-1.08 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 174.21, 170.06, 169.18, 155.29, 144.20, 144.06, 141.56, 134.34, 132.26, 131.74, 131.11, 130.53, 129.30, 129.25, 113.29, 102.79, 74.78, 58.08, 54.08, 49.41, 41.73, 41.21, 40.46, 38.60, 26.47, 24.80, 24.03; ESI-MS for **C$_{31}$H$_{36}$N$_2$O$_6$** m/z: 533.12 [M+H]$^+$.

4.4.3 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((2-(3-methylbenzamido)-3-phenylpropanamido)methyl)-1,4a,5,7a-tetra hydrocyclopenta[*c*]pyran-4-carboxylate **(7c)**. white soild, yield, 49.1%, m.p. 132.3-134.7 ℃; $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 8.47 (s, 1H, ArCON**H**), 8.19 (s, 1H, CON**H**), 7.62-7.51 (m, 2H, Ar**H**), 7.45 (s, 1H, =C**H**), 7.34-7.25 (m, 4H, Ar**H,**), 7.22 (t, *J* = 7.2 Hz, 2H, Ar**H**), 7.12 (t, *J* = 7.2 Hz, 1H, Ar**H**), 5.58-5.48 (m, 1H, =C**H**), 4.76-4.65 (m, 2H, OC**H**O, NHC**H**CO), 4.05-3.90 (m, 2H, CC**H**$_2$NH), 3.75-3.63 (m, 1H, C**H**(CH$_3$)$_2$), 3.60 (s, 3H, COOC**H$_3$**), 3.10-3.03 (m, 1H, ArC**H**$_2$CH), 3.02-2.92 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.68-2.56 (m, 1H, C**H**), 2.42 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.30 (s, 3H, ArC**H$_3$**), 2.03-1.93 (m, 1H, C**H**), 1.22-1.07 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 174.14, 170.05, 169.44, 155.29, 144.05, 141.52, 140.45, 137.14, 134.86, 132.25, 131.12, 131.10, 131.05, 129.32, 129.27, 127.64, 113.28, 102.80, 74.79, 58.06, 54.07, 49.39, 41.74, 41.21, 40.45, 38.60, 26.47, 24.79, 23.99; ESI-MS for **C$_{31}$H$_{36}$N$_2$O$_6$** m/z: 533.01 [M+H]$^+$.

4.4.4 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((2-(4-methoxybenzamido)-3-phenylpropanamido)methyl)-1,4a,5,7a-tet

rahydrocyclopenta[*c*]pyran-4-carboxylate **(7d)**. white soild, yield, 50.6%, m.p. 143.3-145.6 ℃; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 8.39 (s, 1H, ArCON**H**), 8.19 (s, 1H, CON**H**), 7.76 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.45 (s, 1H, =C**H**), 7.31 (d, *J* = 7.8 Hz, 2H, Ar**H**), 7.21 (t, *J* = 7.8, 2H, Ar**H**), 7.11 (t, *J* = 7.8 Hz, 1H, Ar**H**), 6.93 (d, *J* = 7.8 Hz, 2H, Ar**H**), 5.56-5.50 (m, 1H, =C**H**), 4.75-4.62 (m, 2H, OC**H**O, NHC**H**CO), 4.04-3.90 (m, 2H, CC**H**$_2$NH), 3.75 (s, 3H, ArOC**H$_3$**), 3.74-3.63 (m, 1H, C**H**(CH$_3$)$_2$), 3.60 (s, 3H, COOC**H$_3$**), 3.08-3.02 (m, 1H, ArC**H**$_2$CH), 3.01-2.92 (m, 2H, ArC**H**$_2$CH, ,C**H**$_2$), 2.67-2.56 (m, 1H, C**H**), 2.41 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 1.99-1.95 (m, 1H, C**H**), 1.20-1.07 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 174.30, 170.06, 168.80, 164.48, 155.29, 144.08, 141.61, 132.36, 132.25, 131.11, 129.34, 129.27, 129.24, 116.42, 113.28, 102.79, 74.78, 58.41, 58.09, 54.08, 49.40, 41.72, 41.20, 40.45, 38.59, 26.47, 24.80; ESI-MS for **C$_{31}$H$_{36}$N$_2$O$_7$** m/z: 549.12 [M+H]$^+$.

4.4.5 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((2-(3-methoxybenzamido)-3-phenylpropanamido)methyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7e)**. white soild, yield, 51.8%, m.p. 107.8-110.5 ℃; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 8.53 (s, 1H, ArCON**H**), 8.21 (s, 1H, CON**H**), 7.45 (s, 1H, =C**H**), 7.36-7.29 (m, 5H, Ar**H**), 7.22 (t, *J* = 7.2 Hz, 2H, Ar**H**), 7.12 (t, *J* = 7.2 Hz, 1H, Ar**H**), 7.03 (d, *J* = 7.2 Hz, 1H, Ar**H**), 5.53-5.47 (m, 1H, =C**H**), 4.76-4.63 (m, 2H, OC**H**O, NHC**H**CO), 4.05-3.90 (m, 2H, CC**H**$_2$NH), 3.75 (s, 3H, ArOC**H$_3$**), 3.73-3.62 (m, 1H, C**H**(CH$_3$)$_2$), 3.60 (s, 3H, COOC**H$_3$**), 3.10-3.04 (m, 1H, ArC**H**$_2$CH), 3.02-2.93 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.68-2.56 (m, 1H, C**H**), 2.42 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.03-1.98 (m, 1H, C**H**), 1.22-1.07 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 174.10, 170.05, 169.10, 162.09, 155.30, 144.04, 141.52, 138.60, 132.35, 132.26, 131.12, 129.32, 129.28, 122.76, 120.18, 115.68, 113.29, 102.78, 74.78, 58.31, 58.14, 54.07, 49.40, 41.74, 41.20, 40.45, 38.59, 26.46, 24.79; ESI-MS for **C$_{31}$H$_{36}$N$_2$O$_7$** m/z: 549.14 [M+H]$^+$.

4.4.6 methyl (4*aS*,7*aS*)-7-((2-(4-chlorobenzamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetra hydrocyclopenta[*c*]pyran-4-carboxylate **(7f)**. white soild, yield, 48.9%, m.p. 167.5-169.6 ℃; $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 8.65 (s, 1H, ArCON**H**), 8.22 (s, 1H, CON**H**), 7.82-7.72 (m, 2H, Ar**H**), 7.49-7.39 (m, 3H, Ar**H**), 7.31-7.25 (m, 2H, Ar**H**), 7.22-7.16 (m, 2H, Ar**H**), 7.13-7.06 (m, 1H, Ar**H**), 5.55-5.45 (m, 1H, =C**H**), 4.72-4.62 (m, 2H, OC**H**O, NHC**H**CO), 4.98-3.88 (m, 2H, CC**H**$_2$NH), 3.73-3.65 (m, 1H, C**H**(CH$_3$)$_2$), 3.58 (s, 3H, COOC**H$_3$**), 3.07-3.01 (m, 1H, ArC**H**$_2$CH), 2.98-2.91 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.63-2.55 (m, 1H, C**H**), 2.42-2.36(m, 1H, C**H$_2$**), 1.99-1.91 (m, 1H, C**H**), 1.15-1.09 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 173.99, 170.05, 168.72, 155.30, 144.02, 141.46, 139.16, 135.85, 132.45, 132.22, 131.32, 131.13, 129.33, 129.30, 113.28, 102.77, 74.77, 58.19, 54.09, 49.40, 41.74, 41.20, 40.44, 38.59, 26.47, 24.79; ESI-MS for **C$_{30}$H$_{33}$ClN$_2$O$_6$** m/z: 553.06 [M+H]$^+$.

4.4.7 methyl (4*aS*,7*aS*)-7-((2-(3-chlorobenzamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetra hydrocyclopenta[*c*]pyran-4-carboxylate **(7g)**. white soild, yield, 47.7%, m.p. 136.2-137.6 ℃; $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ 8.72 (s, 1H, ArCON**H**), 8.26 (s, 1H, CON**H**), 7.83 (s, 1H, Ar**H**), 7.72 (d, *J* = 7.2 Hz, 1H, Ar**H**), 7.54 (d, *J* = 7.2 Hz, 1H, Ar**H**), 7.48-7.40 (m, 2H, Ar**H**), 7.34-7.27 (m, 2H, Ar**H**), 7.25-7.18 (m, 2H, Ar**H**), 7.15-7.09 (m, 1H, Ar**H**), 5.56-5.50 (m, 1H, =C**H**), 4.77-4.65 (m, 2H, OC**H**O, NHC**H**CO), 4.05-3.89 (m, 2H, CC**H**$_2$NH), 3.77-3.64 (m, 1H, C**H**(CH$_3$)$_2$), 3.59 (s, 3H, COOC**H$_3$**), 3.12-3.05 (m, 1H, ArC**H**$_2$CH), 3.02-2.91 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.66-2.55 (m, 1H, C**H**), 2.44-2.38 (m, 1H, C**H$_2$**), 2.04-1.93 (m, 1H, C**H**), 1.26-1.06 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 173.90, 170.05, 167.94, 155.29, 144.01, 141.43, 139.11, 136.10, 134.16, 133.25, 132.21, 131.14, 130.35, 129.33, 129.32, 129.28, 113.28, 102.77, 74.78, 58.20, 54.07, 49.41, 41.76, 41.21, 40.41, 38.59, 26.46, 24.79; ESI-MS for **C$_{30}$H$_{33}$ClN$_2$O$_6$** m/z: 553.07 [M+H]$^+$.

4.4.8 methyl (4*aS*,7*aS*)-7-((2-(4-fluorobenzamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetra hydrocyclopenta[*c*]pyran-4-carboxylate **(7h)**. white soild, yield, 47.2%, m.p. 112.8-114.7 ℃; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 8.64 (s, 1H, ArCON**H**), 8.29 (s, 1H, CON**H**), 7.83 (t, *J* = 7.2 Hz, 2H, Ar**H**), 7.44 (s, 1H, =C**H**), 7.30 (d, *J* = 7.2 Hz, 2H, Ar**H**), 7.26-7.15 (m, 4H, Ar**H**), 7.10 (t, *J* = 7.2 Hz, 1H, Ar**H**), 5.56 -5.46 (m, 1H, =C**H**), 4.75-4.61 (m, 2H, OC**H**O, NHC**H**CO), 4.04-3.88 (m, 2H, CC**H**$_2$NH), 3.73-3.61 (m, 1H, C**H**(CH$_3$)$_2$), 3.58 (s, 3H, COOC**H$_3$**), 3.07-3.01 (m, 1H, ArC**H**$_2$CH), 2.99-2.90 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.63-2.54 (m, 1H, C**H**), 2.39 (t, *J* = 7.2 Hz, 1H, C**H$_2$**), 2.01-1.92 (m, 1H, C**H**), 1.20-1.06 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 174.12, 170.06, 168.25, 166.95 ($J_{C\text{-}F}$ = 247.2Hz), 155.35, 144.06, 141.53, 133.53, 133.18 ($J_{C\text{-}F}$ = 8.8Hz), 132.24, 131.14, 129.29, 129.19 ($J_{C\text{-}F}$ = 3.3Hz), 118.16 ($J_{C\text{-}F}$ = 21.8Hz), 113.22, 102.79, 74.79, 58.22, 54.11, 49.33, 41.74, 41.15, 40.42, 38.62, 26.48, 24.79; ESI-MS for **C$_{30}$H$_{33}$FN$_2$O$_6$** m/z: 537.01 [M+H]$^+$.

4.4.9 methyl (4*aS*,7*aS*)-7-((2-(3-fluorobenzamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetra hydrocyclopenta[*c*]pyran-4-carboxylate **(7i)**. white soild, yield, 46.9%, m.p. 121.1-123.7 ℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.73 (s, 1H, ArCON**H**), 8.32 (s, 1H, CON**H**), 7.67 (d, *J* = 7.8 Hz, 1H, Ar**H**), 7.64-7.59 (m, 1H, Ar**H**), 7.54-7.46 (m, 2H, Ar**H**, =C**H**), 7.39-7.32 (m, 3H, Ar**H**), 7.26 (d, *J* = 8.3 Hz, 2H, Ar**H**), 7.20-7.13 (m, 1H, Ar**H**), 5.64-5.50 (m, 1H, =C**H**), 4.83-4.64 (m, 2H, OC**H**O, NHC**H**CO), 4.11-3.93 (m, 2H, CC**H**$_2$NH), 3.82-3.70 (m, 1H, C**H**(CH$_3$)$_2$), 3.64 (s, 3H, COOC**H$_3$**), 3.19-3.10 (m, 1H, ArC**H**$_2$CH), 3.06-2.96 (m, 2H,ArC**H**$_2$CH, C**H**$_2$), 2.72-2.60(m, 1H, C**H**), 2.46 (t, *J* = 7.7 Hz, 1H, C**H$_2$**), 2.07-1.97 (m, 1H, C**H**), 1.24-1.04 (m, 6H, CH(C**H$_3$)$_2$**).$^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 171.33, 167.44, 165.36, 163.51, 161.08, 152.69, 140.11($J_{C\text{-}F}$ = 259.3Hz), 136.79, 130.74($J_{C\text{-}F}$ = 8.0Hz), 129.61, 128.53, 126.69, 124.10($J_{C\text{-}F}$ = 2.9Hz), 118.72, 118.51, 114.67($J_{C\text{-}F}$ = 22.8Hz), 110.65, 100.16, 72.16, 55.61, 51.47, 46.77, 37.81, 35.98, 23.85, 22.17; ESI-MS for **C$_{30}$H$_{33}$FN$_2$O$_6$** m/z: 537.13 [M+H]$^+$.

4.4.10 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((2-(4-nitrobenzamido)-3-phenylpropanamido)methyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7j)**. white soild, yield, 52.8%, m.p. 151.1-154.4 ℃; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 8.95 (s, 1H, ArCON**H**), 8.31 (s, 1H, CON**H**), 8.25 (d, *J* = 8.4 Hz, 2H, Ar**H**), 7.99 (d, *J* = 8.4 Hz, 2H, Ar**H**), 7.45 (s, 1H, =C**H**), 7.31 (d, *J* = 7.2 Hz, 2H, Ar**H**), 7.22 (t, *J* = 7.2 Hz, 2H, Ar**H**), 7.12 (t, *J* = 7.2 Hz, 1H, Ar**H**), 5.58-5.53 (m, 1H, =C**H**), 4.76-4.69 (m, 2H, OC**H**O, NHC**H**CO), 4.06-3.91 (m, 2H, CC**H**$_2$NH), 3.77-3.64 (m, 1H, C**H**(CH$_3$)$_2$), 3.60 (s, 3H, COOC**H$_3$**), 3.13-3.07 (m, 1H, ArC**H**$_2$CH), 3.02-2.93 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.66-2.58 (m, 1H, C**H**), 2.42 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.04-1.92 (m, 1H, C**H**), 1.14 (m, 6H, CH(C**H$_3$)$_2$**); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ: 173.74, 170.05, 167.71, 155.30, 152.11, 143.98, 142.74, 141.32, 132.23, 132.01, 131.16, 129.35, 129.31, 126.47, 113.28, 102.78, 74.78, 58.32, 54.07, 49.41, 41.78, 41.20, 40.48, 38.61, 26.47, 24.79; ESI-MS for **C$_{30}$H$_{33}$N$_3$O$_8$** m/z: 564.13 [M+H]$^+$.

4.4.11 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((3-phenyl-2-(4-(trifluoromethyl)benzamido)propanamido)methyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate**(7k)**. white soild, yield, 47.8 %, m.p. 148.3-151.1 ℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.89 (s, 1H, ArCON**H**), 8.34 ( s, 1H, CON**H**), 8.00 (d, *J* = 8.1 Hz, 2H, Ar**H**), 7.83 (d, *J* = 8.1 Hz, 2H, Ar**H**), 7.49 (s, 1H, =C**H**), 7.35 (d, *J* =7.6 Hz, 2H, Ar**H**), 7.29-7.23 (m, 2H, Ar**H**), 7.20-7.12 (m, 1H, Ar **H**), 5.63-5.57 (m, 1H, =C**H**), 4.83-4.71 (m, 2H, OC**H**O, NHC**H**CO), 4.13-3.94 (m, 2H, C C**H**$_2$NH), 3.84-3.70 (m, 1H, C**H**(CH$_3$)$_2$), 3.64 (s, 3H, COOC**H$_3$**), 3.18-3.10 (m, 1H, ArC**H**$_2$ CH), 3.07-2.96 (m, 2H, ArC**H**$_2$CH, C**H**$_2$), 2.73-2.59 (m, 1H, C**H**), 2.46 (t, *J* = 7.9 Hz, 1 H, C**H$_2$**), 2.10-1.96 (m, 1H, C**H**), 1.26-1.12 (m, 6H, CH(C**H$_3$)$_2$**). $^{13}$C NMR (101 MHz, D MSO-$d_6$) δ 171.23, 167.44, 165.54, 152.69, 141.38, 138.77, 131.61($J_{C-F}$ = 32.3Hz),129.62, 1 28.79, 128.53, 126.71, 125.66($J_{C-F}$ = 3.2Hz), 121.69($J_{C-F}$ = 274.2 Hz), 110.65, 100.15, 72.1 6, 55.62, 51.46, 46.77, 38.57, 37.83, 35.98, 23.85, 22.17; ESI-MS for **C$_{31}$H$_{33}$F$_3$N$_2$O$_6$** m/z: 585.26 [M+H]$^-$.

4.4.12 methyl (4*aS*,7*aS*)-7-((2-(benzo[*d*][1,3]dioxole-5-carboxamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7l)**. white soild, yield, 55.2%, m.p. 167.8-169.2 ℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.45 (s, 1H, ArCON**H**), 8.25 (s, 1H, CON**H**), 7.54-7.46 (m, 1H, Ar**H**), 7.42 (d, *J* = 8.1 Hz, 1H, =C**H**), 7.38-7.30 (m, 3H, Ar**H**), 7.29-7.21 (m, 2H, Ar**H**), 7.21-7.11 (m, 1H, Ar**H**), 7.00-6.90 (m, 1H, Ar**H**), 6.09 (s, 2H,OC**H$_2$**O), 5.59-5.55 (m, 1H, =C**H**), 4.82-4.72 (m, 1H, NHC**H**CO), 4.72-4.62 (m, 1H, OC**H**O), 4.13-3.91 (m, 2H, CC**H$_2$**NH), 3.85-3.74 (m, 1H, C**H**(CH$_3$)$_2$), 3.64 (s, 3H, COOC**H$_3$**), 3.19-2.92 (m, 3H, ArC**H$_2$**CH, C**H$_2$**), 2.77-2.58 (m, 1H, C**H**), 2.47-2.41 (m, 1H, C**H$_2$**), 2.09-1.94 (m, 1H, C**H**), 1.29-1.12 (m, 6H, CH(C**H$_3$**)$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 171.60, 167.45, 165.80, 152.69, 150.16, 147.62, 141.43, 138.95, 129.62, 128.51, 126.65, 122.97, 110.65, 108.20, 107.94, 102.09, 100.16, 72.17, 55.57, 51.48, 46.76, 38.57, 37.81, 35.97, 23.85, 22.17; ESI-MS for **C$_{31}$H$_{34}$N$_2$O$_8$** m/z: 563.14 [M+H]$^+$.

4.4.13 methyl (4*aS*,7*aS*)-7-((2-((*E*)-3-(4-fluorophenyl)acrylamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7m)**. white soild, yield, 40.9%, m.p. 178.6-180.4 ℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.39 (s, 1H, ArCON**H**), 8.29 (s, 1H, CON**H**), 7.64-7.56 (m, 2H, Ar**H**), 7.49 (s, 1H, =C**H**), 7.36 (d, *J* = 15.8 Hz, 1H, ArC**H**=CH), 7.30-7.22 (m, 6H, Ar**H**), 7.21-7.16 (m, 1H, Ar**H**), 6.66 (d, *J* = 15.9 Hz, 1H, ArCH=C**H**), 5.49-5.44(m, 1H, =C**H**), 4.77-4.63 (m, 2H, NHC**H**CO, OC**H**O), 4.11 -3.83 (m, 2H, CC**H$_2$**NH), 3.81-3.69 (m, 1H, C**H**(CH$_3$)$_2$), 3.63 (s, 3H, COOC**H$_3$**), 3.09-2.93 (m, 2H, ArC**H$_2$**CH), 2.89-2.79(m, 1H, C**H$_2$**), 2.71-2.56 (m, 1H, C**H**), 2.42 (t, *J* = 7.8 Hz, 1H, C**H$_2$**), 2.04-1.92 (m, 1H, C**H**), 1.28-1.15 (m, 6H, CH(C**H$_3$**)$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 171.26, 166.26($J_{C\text{-}F}$ = 236.67Hz), 161.90, 152.70, 141.30, 138.35, 138.16, 131.96($J_{C\text{-}F}$ = 3.1Hz), 130.16($J_{C\text{-}F}$ = 8.3Hz), 129.62, 128.55, 126.73, 122.34, 116.37($J_{C\text{-}F}$ = 23.3Hz), 110.64, 100.03, 72.14, 56.49, 54.73, 51.48, 46.69, 38.55, 36.00,

23.86, 22.15; ESI-MS for $\mathbf{C_{32}H_{35}FN_2O_6}$ m/z: 563.17 $[M+H]^+$.

4.4.14 methyl (4*aS*,7*aS*)-1-isopropoxy-7-((2-((*E*)-3-(4-methoxyphenyl)acrylamido)-3-phenylpropanamido)methyl)-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7n)**. white soild, yield, 38.3%, m.p. 183.3-185.0℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.33-8.24 (m, 2H,ArCON**H**,CON**H**), 7.52-7.46 (m, 3H, Ar**H**, =C**H**), 7.37-7.22 (m, 5H, Ar**H**, ArC**H**=CH), 7.21-7.15 (m, 1H, Ar**H**), 7.01-6.94 (m, 2H, Ar**H**), 6.56 (d, *J* = 15.8 Hz, 1H, ArCH=C**H**), 5.46-5.41 (m, 1H, =C**H**), 4.81-4.63 (m, 2H, NHC**H**CO, OC**H**O), 4.19-3.89 (m, 2H, CC$\mathbf{H}_2$NH), 3.78 (s, 3H, OC$\mathbf{H}_3$), 3.77-3.69 (m, 1H, C**H**$(CH_3)_2$), 3.63 (s, 3H, COOC$\mathbf{H_3}$), 3.10-2.92 (m, 2H, ArC$\mathbf{H}_2$CH), 2.92-2.79 (m, 1H, C$\mathbf{H}_2$), 2.70-2.56 (m, 1H, C**H**), 2.42 (t, *J* = 7.8 Hz, 1H, C$\mathbf{H_2}$), 2.10-1.89 (m, 1H, C**H**), 1.26-1.14 (m, 6H, CH(C$\mathbf{H_3}$)$\mathbf{_2}$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 171.37, 167.44, 165.49, 160.78, 152.69, 141.33, 139.07, 138.43, 129.62, 129.54, 128.54, 127.86, 126.67, 119.95, 114.84, 110.64, 100.18, 72.14, 55.71, 54.71, 51.48, 46.69, 38.56, 35.99, 23.86, 22.16; ESI-MS for $\mathbf{C_{33}H_{38}N_2O_7}$ m/z: 575.26 $[M+H]^+$.

4.4.15 methyl (4*aS*,7*aS*)-7-((2-((*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylamido)-3-phenylpropanamido)methyl)-1-isopropoxy-1,4a,5,7a-tetrahydrocyclopenta[*c*]pyran-4-carboxylate **(7o)**. white soild, yield, 35.6%, m.p. 88.6-92.1℃; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.45 (s, 1H, O**H**), 8.33-8.12 (m, 2H, ArCON**H**, CON**H**), 7.49 (s, 1H, =C**H**), 7.32-7.22 (m, 5H, Ar**H**, C**H**=CH), 7.21-7.14 (m, 1H, Ar**H**), 7.10 (s, 1H, Ar**H**), 6.97 (d, *J* = 8.2 Hz, 1H, Ar**H**), 6.80-6.75 (m, 1H, Ar**H**), 6.54 (d, *J* = 15.7 Hz, 1H, ArCH=C**H**), 5.54-5.42 (m, 1H, =C**H**), 4.80-4.59 (m, 2H, NHC**H**CO, OC**H**O), 4.08-3.90 (m, 2H, CC$\mathbf{H}_2$NH), 3.80 (s, 3H, OC$\mathbf{H}_3$), 3.78-3.70 (m, 1H, C**H**$(CH_3)_2$), 3.63 (s, 3H, COOC$\mathbf{H_3}$), 3.11-2.93 (m, 2H, ArC$\mathbf{H}_2$CH), 2.90-2.78 (m, 1H, C$\mathbf{H}_2$), 2.70-2.57 (m, 1H, C**H**), 2.42 (t, *J* = 7.9 Hz, 1H, C$\mathbf{H_2}$), 2.10-1.91 (m, 1H, C**H**), 1.31-1.09 (m, 6H,CH(C$\mathbf{H_3}$)$\mathbf{_2}$). $^{13}$C NMR (101 MHz,

DMSO-$d_6$) δ 171.40, 167.44, 165.65, 152.69, 148.76, 148.24, 141.33, 139.74, 138.43, 129.61, 128.54, 126.67, 122.05, 119.18, 116.08, 111.11, 110.64, 100.18, 72.15, 55.92, 54.66, 51.47, 46.70, 39.05, 38.53, 35.99, 23.86, 22.16; ESI-MS for $\mathbf{C_{33}H_{38}N_2O_8}$ m/z: 590.94 $[M+H]^+$.

### 4.5 Cell Culture

RAW264.7 cells (ATCC, Rockville, MD, USA) were maintained in RPMI 1640 medium supplemented with 100 U/ml of penicillin, 100 $\mu$g/ml of streptomycin, and 10% FBS Cells were grown at 37 ℃ and 5% $CO_2$ in humidified air.

### 4.6 Assay for TNF-$\alpha$ inhibition

TNF-$\alpha$ production was measured by Enzyme-Linked Immuno-Sorbent Assay (ELISA). In brief, RAW264.7 macrophage cells ($1\times10^5$ cells/well) were plated in 96-well plates, until the 90% cells fusion, then cells were starved by being cultured in serum free medium for another 2 h to eliminate FBS influence. The cells were then treated with compounds for 2 h before exposure to 200 ng/ml LPS for 8 h. The culture medium was used to assay the cytokine production with mouse ELISA kit (TNF-$\alpha$; BOSTER Biological Technology co.ltd, China) according to manufacturer's instructions.

### 4.7 Assay for NO inhibition

NO production was quantified by nitrite accumulation in the culture medium using the Griess reaction. Briefly, RAW264.7 cells were pre-treated with vehicle or **7o** (0-20uM dose range) for 2 h, and then stimulated with or without LPS (200 ng/ml) for 8 h. At the indicated time points, the cells were harvested and washed, and cell lysates were prepared. The lysates were mixed with Griess reagent for 30 min measured at 540 nm. The cells treated with diluent were used as negative controls for the background levels of nitrite production, while sodium nitrite at different concentrations was used as the positive controls for the establishment of a standard curve.

### 4.8 Western blotting

The cell in 96-well plates were treated as described above and then stimulated with LPS (200ng/mL) for 24h. The cells were harvested and lysed in an extraction lysis buffer (Beyotime Biotechnology, Shanghai, China) containing protease inhibitors. The protein concentration was determined using a BCA protein assay kit (Thermo Scientific, 23227). The whole cell lysates were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Each membrane was incubated with Tris-buffered saline (pH 7.6, containing 0.05% Tween-20 and 5% non-fat milk). The nitrocellulose membrane was incubated with the primary antibody against p-JNK1/2, JNK1/2, p-p38, p38, p-ERK1/2, ERK1/2, I$\kappa$Ba, NF-$\kappa$B p65, COX-2, iNOS or $\beta$-Actin (Abcam, Cambridge, UK). Immunoreactive bands were detected by incubating with horseradish peroxidase-conjugated secondary antibodies, and visualised using enhanced chemiluminescence reagents (Bio-Rad, Hercules, CA).

### 4.9 Animals

The title compound was evaluated for its anti-inflammatory activity using xylene-induced ear swelling in mice. In brief, Dexamethasone was administered orally at a dose level of 0.5 mg/kg and other compounds were administered orally at a dose level of 5 mg/kg(suspended in 0.5% Na CMC given p. o.) every day for a total of six days. DNFB (10 $\mu$L of 1% (w/v)) was given for sensitization and challenged with DNFB on the right ear 6d later. Round ear piece of 9mm diameter was cut from both right and left ear 24 h after challenge, and weighted separately. Degree of ear swelling was expressed as the weight difference of right and left ear piece and was determined using the following formula:

Ear swelling = right ear weight - left ear weight

Inhibition% = (average swelling degree of the model control group - average swelling degree of the administration group) / average swelling degree of the model control group × 100%.

### 4.10 Statistical analysis

Results are expressed as mean standard deviation (SD) and were analysis statistically with analysis of variance (ANOVE), and the Tukey method were assessed differences between groups. A value of $p < 0.05$ is considered to be statistically significant.

**Conflicts of interest**

The authors declare no conflicts of interest.

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