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# 4-Sulfonyloxy/alkoxy benzoxazolone derivatives with high anti-inflammatory activities: Synthesis, biological evaluation and action mechanism via p38/ERK-NF-κB/iNOS pathway

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**Data Availability Statement**

The data that support the findings of this study are openly available in [repository name e.g "figshare"] at http://doi.org/[doi], reference number [reference number].

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**Abstract:** In an effort to discover new agents with high anti-inflammatory activity, 22 new 4-sulfonyloxy/alkoxy benzoxazolone derivatives were synthesized, characterized and evaluated for their anti-inflammatory activities against lipopolysaccharide (LPS)-induced nitric oxide (NO) production and TNF-α expression in RAW 264.7 cells *in vitro*. Most of these compounds displayed greater inhibitory ability against NO production than the lead compound 4-*o*-methyl-benzenesulfonyl benzoxazolone, and the most active compound **2h** exhibited the strongest inhibitory activity against NO, IL-1β, and IL-6 production with $IC_{50}$ values 17.67, 20.07 and 8.61 μM, respectively. The effects of **2h** were comparable or stronger than those of the positive control celecoxib. Compound **2h** also displayed higher activity *in vivo* than celecoxib in a mouse model of xylene-induced ear edema, based on their inhibitory rates of 42.69 and 30.87%, respectively. Further molecular analysis revealed that compound **2h** significantly reduced the iNOS levels in cell supernatant and suppressed the protein expression of iNOS, p-p38, p-ERK, and nuclear NF-κB. The results indicated that the anti-inflammatory effect of **2h** might be realized through the regulation of ERK- and p38-mediated mitogen-activated protein kinase (MAPK)-NF-κB/iNOS signaling, thereby reducing the excessive

release of NO, IL-1β, and IL-6. Our findings demonstrated that compound **2h**, a new benzoxazolone derivative, could inhibit activation of the MAPK-NF-κB/iNOS pathway, supporting its potential as a novel anti-inflammatory agent.

**Keywords:** 4-sulfonyloxy/alkoxy benzoxazolone derivatives, anti-inflammatory, MAPK-NF-κB/iNOS pathway

## 1. Introduction

Inflammation, a defensive process activated by stimuli such as pro-inflammatory cytokines (TNF-α, IL-1β) and lipopolysaccharides (LPS) (He, 2015; Kim, 2016), is closely related to multiple diseases, including neurodegenerative disorder (Chen, 2017), cancer (Pennel, 2019), cardiovascular disease (Xavier, 2018), rheumatoid arthritis (Antwi, 2017), hypertension (Nandeesha, 2015), and psoriasis (Kitahata, 2018). Traditional non-steroidal anti-inflammatory drugs have been intensively used as anti-inflammatory and analgesic agents based on their inhibitory effects against COX. However, unfavorable side effects, such as nephrotoxicity, gastrointestinal lesions, and cardiac toxicity (Pirlamarla, 2016; Liu, 2016), limited their application. Therefore, novel anti-inflammatory drugs with new target or strong effects with low toxicity are urgently required.

Inflammatory mediators that can aggravate the inflammatory response, such as TNF-α, IL-1β, IL-6, PGE2, and NO, play important role during the inflammatory process (Ying, 2013; Sittisart, 2016). Nitric oxide (NO), an important vasodilatation factor, exhibits diverse biological functions. Excessive NO production caused by iNOS activation can increase vascular permeability when inflammation occurs (Paesano, 2005; Ma, 2015). Meanwhile, high NO levels promote the activation of NF-κB pathways (Gul, 2018), increasing the leakage of inflammatory cytokines such IL-1β, TNF-α, and IL-6 that activate mitogen-activated protein kinase (MAPK) and JAK/STAT pathways (Abarikwu, 2014). Therefore, increased levels of NO, IL-1β, IL-6, and TNF-α are the most typical characteristics of the inflammatory process (Taniguchi, 2014; Ballak, 2015), and suppressing their factors has become a strategy for the development of anti-inflammatory drugs.

Benzoxazole derivatives have occupied a prominent role in medicinal chemistry based on their diverse and significant biological properties, including anti-inflammatory, anti-arrhythmic, anti-cancer, anti-microbial, and anti-HIV activities (Abdelazeem, 2015; Tang, 2013; Önkol, 2004; Murty, 2011). A series of 4-phenylsulfonyl benzoxazolone derivatives was synthesized by our group, and the lead compound 4-o-methyl-benzenesulfonyl benzoxazolone (MBB), with definitive anti-inflammatory activity *in vitro* and *in vivo*, was obtained (Tang, 2018). However, the activity of MBB was inferior to that of positive control drugs such as celecoxib. To strengthen the structure-activity relationship, hydrocarbyl, heterocyclic, and amino groups were introduced to obtain novel 4-sulfonyloxy substituted benzoxazolone derivatives (**2a-2j**) in this study. Meanwhile, 4-alkoxy substituted benzoxazolone derivatives were synthesized using the nucleophilicity of hydroxyl groups to obtain compounds **3a-3d** and **4a-4h** to expand the variety of available compounds.

## 2. Experimental part

### *2.1 Chemistry*

All sulfonyl chlorides were obtained from J&K Scientific. 2-amino-1,3-benzenediol was commercially procured from Beijing HvsF United Chemical Materials Ltd. Melting points were determined on a digital microscopic melting apparatus and were uncorrected. $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400-MHz instrument in $CDCl_3$ or $CD_3OD$, and chemical shifts were reported in ppm. ESI-MS Data were recorded on a Bruker Esquire3000$^{+}$ spectrometer.

### *2.2 General procedure for the synthesis of target compounds*

#### *2.2.1 Synthesis of 4-hydroxy-benzoxazolone (compound 1)*

2-Aminoresorcinol (1.25 g, 10 mmol) was reacted with triphosgene (BTC, 2.80 g, 9.4 mmol) under a nitrogen atmosphere at reflux for 6 h to gain the key intermediate compound **1**. The reaction was monitored via TLC, and the resulting liquid was purified using chromatography on silica gel with 6 % methanol in dichloromethane (yield, 73.8 %). Mp 240 ℃, ESI-MS m/z calculated for $C_7H_5NO_3$ $[M-H]^-$: 150.2. $^{1}H$-NMR ($CD_3OD$, 600 MHz) δ: 6.64 (d, J = 8.0 Hz, 1H, Ar-H); 6.73 (d, J =8.0 Hz, 1H, Ar-H); 6.87 (t, J = 8.2 Hz, 1H, Ar-6-H); 10.12 (s, 1H, OH); 11.54 (d, 1H, NH).

#### *2.2.2. General procedure for the synthesis of compounds 2a-2j*

Triethylamine (80 mg, 0.79 mmol) was added to the solution of **1** (100 mg, 0.66 mmol) in anhydrous acetone at a temperature of -5-0℃. After 30 min, sulfonyl chloride (0.79 mmol) was added slowly through a constant pressure drop funnel and refluxed at 70℃ until **1** was completely consumed as confirmed via TLC. The target compound was purified by chromatography using a mixture of ethyl acetate and petroleum ether. Detailed analytical data are presented in the Supporting Information.

#### *2.2.3. General procedure for the synthesis of compounds 3a-3d*

Different halogenated hydrocarbons (0.79 mmol) in acetone (2.5 mL) were added dropwise to a stirred mixture of **1** (100 mg, 0.66 mmol) in acetone with 2-4% NaOH and then refluxed at 70℃ for 5-8 h. The water layer was separated, and the solvent of the organic layer was removed via vacuum filtration. The residue was purified by column chromatography using a mixture of ethyl acetate and hexane. Detailed analytical data are presented in the Supporting Information.

#### *2.2.4. General procedure for the synthesis of compounds 4a-4h*

The target compounds **4a-4h** were obtained by reacting **1** (100 mg, 0.66 mmol) with excessive amounts of halogenated hydrocarbons (1.5 mmol) under an acetone solution containing $K_2CO_3$ at 60°C. After vacuum filtration to remove $K_2CO_3$, the organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated under reduced pressure. The residue was purified via column chromatography with 20-40% hexane in ethyl acetate. Detailed analytical data are presented in the Supporting Information.

***2.3. Biological evaluation in vitro***

LPS was obtained from Sigma-Aldrich (St Louis, MO, USA). MTT, TNF-α, IL-1β, and IL-6 ELISA kits were purchased from BosterBio (Wuhan, Hubei, China). An NO assay kit was obtained from Beyotime Biotechnology (Shanghai, China).

***2.3.1 Cell culture***

RAW 264.7 cells were obtained from BosterBio (Wuhan, Hubei, China). These cells were grown in DMEM containing 10% FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin under a 5% $CO_2$ humidified atmosphere at 37°C.

***2.3.2 NO assay***

RAW 264.7 cells were seeded into a 24-well culture plate at a density of 70-80% per well and incubated for 24 h. Then, the cells were treated with the target compounds and LPS (1 μg/mL) for 24 h. The supernatant was collected and incubated with standard nitrite solution, substrate solution A, and substrate solution B at 37°C. Finally, absorbance was detected at 540 nm according to the Griess operational specification.

***2.3.3 Assay of TNF-α, IL-1β, IL-6, and iNOS expression***

TNF-α, IL-1β, IL-6, and iNOS levels were measured using ELISA kits according to the manufacturer's instructions. RAW 264.7 cells were cultivated in six-well plates at a density of $1 \times 10^6$ cells per well and subsequently incubated with different concentrations of compounds and LPS (1 μg/mL) for 24 h. The cell culture supernatant was centrifuged and collected to measure cytokine production.

***2.3.4 Western blotting***

Primary antibodies against iNOS, NF-κB, p38, p-p38, ERK, p-ERK, β-actin, and conjugated anti-rabbit secondary antibodies were purchased from Bioworld Technology Inc. RAW 264.7 cells were treated with compound **2h** (12.5, 25, and 50 μM/L) and LPS (1 μg/mL) for 24 h. After removing the supernatant, the cells were collected, washed twice with PBS, lysed, and assessed for protein levels using a BCA protein assay kit. The protein was subjected to

SDS-PAGE and transferred to nitrocellulose filter membranes (BosterBio). Membranes were blocked with 5% skimmed milk powder in TBST (pH 7.4) and incubated with the antibodies overnight at 4°C. After washing with TBST, membranes were exposed to HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature, followed by detection using enhanced chemiluminescence (BosterBio).

***2.3.5 Real-time quantitative PCR***

RAW 264.7 cells were incubated with compound **2h** (12.5, 25, and 50 μM/L) and LPS (1 μg/mL) for 24 h, and the total RNA was isolated using TRIzol reagent (TaKaRa). Complementary DNA was synthesized using a reverse transcription kit (TaKaRa) according to the manufacturer's instructions. PCR was performed using SYBR Premix Ex Taq™ (TaKaRa).

***2.3.6 Assay of toxicity in vitro***

Cytotoxicity was tested using the MTT method. Specifically, the optical density of purple formazan dissolved in DMSO was determined after 24 h of incubation of cells with different concentrations of test compounds.

***2.4. Biological evaluation in vivo***

Fifty ICR mice (25 males and 25 females) purchased from Experimental Animal Center, Shanxi Medical University (Shanxi, China) were randomly divided into five groups. After adaptation for 3 days, the test compounds dissolved in 0.5% CMC-Na were orally administered at a dose of 25 mg/kg, and animals in the control group received 0.5% CMC-Na alone. One hour later, xylene (20 mL) was applied to the right ear to induce an inflammatory response. Then, all animals were euthanized 0.5 h later via cervical dislocation, and 8-mm round sections of treated (right) and untreated (left) ears were removed from each mouse. The weights of the right and left ears were recorded, and the edema inhibitory rate was calculated. All animal experiments were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

***2.5. Molecular docking studies of 2h***

The binding mode of **2h** with iNOS protein was performed using the molecular docking module in Sybyl-2.0 (Tripos Associates, St. Louis, MO, USA). To assess the structural similarity with chlorzoxazone, the iNOS protein (PDB code: 1M8D), which was the crystal structure of chlorzoxazone-bound iNOS, was chosen to perform molecular docking studies. The crystal structure of iNOS was downloaded from the PDB database. 3D structures of compound **2h** were generated and optimized using the Sybyl-2.0 program. The protein iNOS conformations and energy minimizations were prepared using the Tripos force field by the Powell method. Meanwhile, the protein was prepared

for docking simulation by extracting the ligand chlorzoxazone (chains A), deleting all the water molecules, adding hydrogen atoms, and assigning the AMBER charge and AMBER7 FF99 force field.

## 3. Results and discussion

### *3.1 Chemistry*

The synthetic strategy to prepare 22 target compounds is illustrated in **Scheme 1**. First, the condensation reaction was utilized to synthesize the **1** by treating 2-aminoresorcinol with Bis (trichloromethyl) carbonate under a nitrogen atmosphere in tetrahydrofuran, and the yield was 73.8 %. The first series of target compound, namely 4-sulfonyloxy-benzoxazolone derivatives **(2a-2j)** were obtained via the sulfonylation of compound **1** with sulfonyl chlorides in anhydrous acetone and triethylamine under refluxing conditions, and the yield was 45-85%. By replacing the hydrogen atom at the 4-position of compound **1** with a different alkyl radical, 4-alkoxy-benzoxazolone derivatives (**3a–3d**) were obtained via the Williamson reaction of intermediate **1** with halohydrocabon in a refluxing acetone solution containing 2-5% NaOH. To avoid the side reactions stemming from the reactivity of the hydrogen atom at the N-position, the halohydrocarbon should be added dropwise to **1** with a strict equivalence ratio of 0.8-1. Finally, **1** was subjected to a substitution reaction of the hydrogen atom at the 4-position and N-position via treatment with excess halohydrocarbon under acetone solution containing $K_2CO_3$ at 60 ℃ to provide the third series of target compounds (**4a-4h**) with yields of 30-60%. All target compounds (**Table 1**) were purified by column chromatography on silica gel and characterized using $^1$H NMR, $^{13}$C NMR, and ESI-MS.

### *3.2 Inhibitory effects of the target compounds against LPS-induced NO and TNF-α release*

The synthesized compounds were evaluated for their inhibitory effects against LPS-induced TNF-α and NO release in mouse RAW 267.4 macrophages. The results illustrated that most of these compounds inhibited NO release at a concentration of 25 μM, but the inhibition rate for TNF-α release was generally less than 10% (**Table 2)**. Notably, **2h** and **4h** were the most effective compounds, dramatically reducing NO release by 86.06 and 58.33% respectively, which were higher than the rate for the lead compound MBB and comparable to the rate for positive control celecoxib. Therefore, these active compounds **2h** and **4h** were selected for the further anti-inflammatory analyses. The structure-activity relationship suggested that an amino group (**2d** and **2h**) can strengthen the inhibition of NO release, and aromatic functional groups (**2d**, **2h** and **2j**) could also increase the inhibition of NO release for the rigid structural characteristics, which may permit easier docking with the target protein. Meanwhile, we also found that the introduction of aromatic rings increased the inhibitory activity (**3c**, **3d**, **4h**, and **4j**) when position 4 of benzoxazolone

was replaced with an alkoxy group, and lengthening of the carbon chain might appropriately increase the inhibitory activity (**3d** and **4h**).

***3.3 Anti-inflammatory effects of compounds 2h and 4h on xylene-induced ear edema in mice in vivo***

Based on the previous *in vitro* test results, compounds **2h** and **4h** were investigated for anti-inflammatory activity against xylene-induced mouse ear edema *in vivo*. As displayed in **Table 3**, compound **2h** reduced edema by 42.69%, surpassing the effects of celecoxib (30.87%).

***3.4 Inhibition effects of compounds 2h and 4h on the release of NO, TNF-α, IL-1β, and IL-6 by LPS-treated RAW 267.4 cells in vitro***

Inflammatory signaling induced by LPS can result in the excess production of NO and a variety of cytokines, such as TNF-α, IL-1β, and IL-6, which are the immediate signs of the inflammatory response. To investigate whether compounds **2h** and **4h** could inhibit cytokines and NO release, NO, TNF-α, IL-1β, and IL-6 levels in LPS-stimulated RAW264.7 cell supernatant were determined after treatment with different concentrations of compounds by ELISA. The results (**Fig. 1**) indicated that compounds **2h** and **4h** had no cytotoxic effects at concentrations less than 100 μM, and the concentration range of 0-100 μM was selected for further activity study. The NO assay (**Table 4, Fig. 2**) illustrated that **2h** and **4h** significantly inhibited NO release in a concentration-dependent manner with similarly efficacy as celecoxib. However, the compounds exerted no effects on TNF-α release, with $IC_{50}$ values exceeding 50 μM. Excitingly, compound **2h** had comparable effects on IL-1β release ($IC_{50}$ = 20.07 μM) and stronger effects on IL-6 release ($IC_{50}$ = 8.61 μM) than celecoxib (17.94 and 36.04 μM, respectively).

***3.5 Effect of compound 2h on iNOS expression in LPS-treated RAW 267.4 cells in vitro***

Aberrant NO synthesis catalyzed by iNOS, which is one of the most pivotal signals during LPS-induced inflammation in macrophages, can accelerate the severity of inflammation. Thus, inhibiting the expression of iNOS has been recognized as a potential strategy for treating inflammatory diseases. In an effort to study the effect of the highly active compound **2h** on iNOS expression, ELISA, RT-PCR, and Western blotting were performed. As expected, LPS obviously increased iNOS levels in the cell supernatant, and compound **2h** inhibited iNOS expression in a concentration-dependent manner ($IC_{50}$ = 40.96 μM, **Fig. 3A**). Meanwhile, RT-PCR and Western blotting revealed that iNOS expression was strongly diminished in cells at both the mRNA and protein levels after treatment with 12.5 μM **2h** (**Fig. 3B-D**). These results revealed that the anti-inflammatory effect of **2h** was closely associated with its inhibitory effects on iNOS expression.

### *3.6 Effect of compound 2h on p38/ERK-NF-κB signaling*

The aforementioned results demonstrated that compound **2h** could significantly downregulate iNOS expression and thereby decrease the release of NO and cytokines, but the regulatory mechanism was unclear. To clarify the mechanism, Western blotting was performed to detect the inflammatory signal molecule. The translocation of NF-κB from the cytoplasm to the nucleus is the most significant factor that induces the overexpression of inflammatory mediators and enzymes. As shown in **Fig. 4A**, the nuclear translocation of NF-κB was significantly increased following LPS treatment, and this translocation was reduced by treatment with compound **2h**. ERK and p38 are well-known MAPKs implicated in the regulation of NF-κB expression of via phosphorylation. Therefore, the effects of **2h** on ERK and p38 phosphorylation were also assessed. ERK and p38 were phosphorylated after the treatment with LPS, and treatment with compound **2h** obviously decreased the LPS-induced phosphorylation of ERK and p38 in a concentration-dependent manner (**Fig. 4B and 4C**). These results suggested that the compound could regulate ERK- and p38-mediated MAPK pathways to inhibit the translocation of NF-κB from the cytoplasm to the nucleus, thereby decreasing iNOS expression and inhibiting the release of NO and other inflammatory mediators.

### *3.7 Molecular modeling*

Based on the findings that most 4-sulfonyloxy/alkoxy benzoxazolone derivatives displayed specific NO inhibitory activities, especially compound **2h**, we speculated that these compounds might be novel iNOS inhibitors that can suppress NO release and thereby decrease the activation of inflammatory signaling pathways. Thus, compound **2h** was subjected to molecular docking studies to identify the mode of its interaction with the intended receptor. It must be emphasized that the marketed drug chlorzoxazone, which has a similar skeletal structure as the target compounds, can bind with iNOS to form a chlorzoxazone-iNOS crystal structure (PDB entry 1M8D). In our previous research, 25 μM chlorzoxazone was demonstrated to reduce NO, TNF-α, IL-6, and IL-1β levels by 45.1, 25.59, 35.4, and 31.25%, respectively (Tang, 2018). Therefore, PDB entry 1M8D was selected for this modeling study. In the chlorzoxazone-iNOS crystal structure, an oxygen atom could form a hydrogen bond with MET368, and an amino group could form an additional hydrogen bond with TRP366 (**Fig. 5A).** Compound **2h** bound via the nitrogen atom of the oxazole ring with PRO344, the carbonyl oxygen of the oxazole ring with VAL346, and sulfonyloxy radicals with MET368 (**Fig. 5B and 5C)**. Comparing the molecular models of chlorzoxazone and compound **2h**, we found that the mode of interaction with iNOS was significantly changed by the introduction of a sulfonyloxy group and the change of the molecular size. We speculated that the increased number of hydrogen bonds form might play important role

associated with the activity.

## 4. Conclusions

In this study, a series of 4-sulfonyloxy and 4-alkoxy substituted benzoxazolone derivatives was synthesized, and their inhibitory activity against NO and TNF-α release by LPS-stimulated RAW 267.4 cells were tested. The results revealed that most of these compounds exhibited stronger inhibitory ability against NO release than MBB. We first found that compound **2h** displayed the strongest anti-inflammatory activity against NO release, and it also significantly reduced edema formation *in vivo* with greater activity than celecoxib. More interestingly, **2h** exerted comparable effects on NO and IL-1β production and stronger effects on IL-6 production than celecoxib, but the compound has no effects on COX activity. Furthermore, the results of ELISA, PCR, and Western bloting illustrated that compound **2h** significantly reduced iNOS expression, and this inhibitory effect was regulated by ERK- and p38-mediated MAPK signaling, which inhibited the nuclear translocation of NF-κB. Considering the strong inhibitor activity on iNOS expression and its structural similarity with chlorzoxazone, compound **2h** was subjected to a molecular docking study to elaborate its interaction with iNOS. The results indicated that **2h** formed three hydrogen bonds with the amino acid residues of iNOS, which might lead to direct or indirect inhibitory effects. Based on these promising findings, 4-substituted benzoxazolone derivatives might be a novel class of anti-inflammatory agents that could block MAPK-NF-κB/iNOS pathway activation and inhibit the release of inflammatory mediators.

## Conflicts of interest

Authors declare no conflict of interest.

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**Figure, Table and Scheme captions**

**Scheme 1** The synthetic route of the target compounds. Reagents and conditions. (i) tetrahydrofuran, $N_2$, 60℃, 4h; (ii) acetone, triethylamine, 60℃, 3-6h; (iii) acetone, NaOH (2-5%), 60℃, 5h;(iv) acetone, $K_2CO_3$, 60℃, 8h;

**Table 1** Structures of derivatives 2a-2e, 3a-3d and 4a-4h

**Table 2** The inhibition rate of NO and TNF-α in LPS-induced macrophages *RAW264.7* cells

**Table 3** Anti-inflammatory effects of compounds 2h and 4h on xylene-induced ear edema in mice

**Table 4** Effect of compounds 2h and 4h on the expression of NO, TNF-α, IL-1β and IL-6 by LPS-induced RAW 267.4 cells *in vitro*

**Fig 1** Effects of compound 2h and 4h on RAW264. 7 cell viability. RAW264. 7 cells were incubated with compound 2h and 4h for 24h, and the cell viability was evaluated by MTT method. The data was expressed as means ± S.D. from three independent experiments. (compared with the control, *$p < 0.05$)

**Fig 2** Effects of compound 2h and 4h on the expression of NO, TNF-α, IL-6 and IL-1β. RAW264.7 cells were incubated with the test compound 2h (A), 4h (B), the positive drug MBB (C) and Celecoxib (D) for 24h, and the supernatant were used to determine the inhibition rate of NO, TNF-α, IL-6 and IL-1β.

**Fig 3** Effect of compound 2h on the expression of iNOS. RAW264.7 cells were incubated with compound 2h at 12.5,

25 and 50 μM for 24h. The supernatant were used to determine the enzyme activity of iNOS (A), and the cells were collected to evaluate the mRNA level (B) and the protein level (C, D) of iNOS. The data was expressed as means ± S.D. from three independent experiments (compared with the control, *p < 0.05, **p < 0.01).

**Fig 4** Effects of compound 2h on the protein expression of p-38, ERK and NF-κB. RAW264.7 cells were incubated with compound 2h at 12.5, 25 and 50 μM for 24h, and the cells were collected to evaluate the protein level of NF-κB (A), p-38(B) and ERK(C). The data was expressed as means ± S.D. from three independent experiments (compared with the LPS group, *p < 0.05, **p < 0.01).

**Fig 5** Docking of the compound 2h with iNOS. The binding mode of target compound with iNOS protein was performed using molecular docking module in Sybyl-2.0. PDB entry 1M8D, was selected for this modeling study. (A) Chlorzoxazone. (B,C) Compound 2h.

**Table 1** Structures of derivatives 2a-2j, 3a-3d and 4a-4h

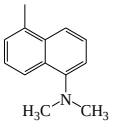
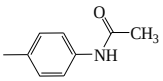
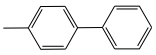
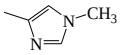
| Compd | $R_1$ | Compd | $R_1$ | Compd | $R_2$ | Compd | $R_2$ |
|---|---|---|---|---|---|---|---|
| **2a** | $-CH_2CH_2CH_3$ | **2f** |  | **3a** | $-CH_2\ CH_2CH_2CH_3$ | **4c** | $-CH_2CH_2CH_3$ |
| **2b** | $-CH_2\ (CH_3)_2$ | **2g** |  | **3b** | $-CH_2CH_2CH_2Cl$ | **4d** | $-CH_2\ CH_2CH_2CH_3$ |
| **2c** | $-\ CH_2C_6H_5$ | **2h** |  | **3c** | $-CH_2\ C_6H_5$ | **4e** | $-CH_2\ (CH_3)CH_2CH_3$ |
| **2d** |  | **2i** |  | **3d** | $-CH_2CH_2\ C_6H_5$ | **4f** | $-CH_2CH_2CH_2Cl$ |
| **2e** |  | **2j** |  | **4a** | $-CH_3$ | **4g** | $-CH_2\ C_6H_5$ |

**Table 2** The inhibition rate of NO and TNF-α in LPS-induced macrophages *RAW264.7* cells

| Compound No. | % Inhibition [a,b] | |
|---|---|---|
| | NO | TNF-α |
| **Celecoxib** | 89.48±9.07 | 66.48±1.35 |
| **MBB** | 14.4±9.07 | - |
| **2a** | 35.46±3.68 | - |
| **2b** | 18.14±4.17 | - |
| **2c** | - | 31.09±9.37 |
| **2d** | 34.80±3.19 | 9.40±7.36 |
| **2e** | 12.58±5.94 | - |
| **2f** | 9.48±2.52 | - |
| **2g** | - | - |
| **2h** | 86.06±7.47 | 18.46±3.79 |
| **2i** | 24.67±6.21 | - |
| **2j** | 36.02±3.69 | - |
| **3a** | 10.02±1.61 | - |
| **3b** | 40.65±8.4 | - |
| **3c** | 31.31±2.51 | - |
| **3d** | 49.82±12.7 | - |
| **4a** | 19.45±8.27 | - |
| **4b** | 15.52±5.98 | - |
| **4c** | 18.69±7.38 | - |
| **4d** | - | 14.25±4.82 |
| **4e** | 14.06±6.51 | 19.82±2.97 |
| **4f** | 15.97±7.41 | - |
| **4g** | 35.7±13.58 | - |
| **4h** | 58.33±3.43 | 14.38±4.78 |

[a] % Inhibition of 25 μM concentrations.

[b] All dates represent mean ± S.D. from three independent experiments.

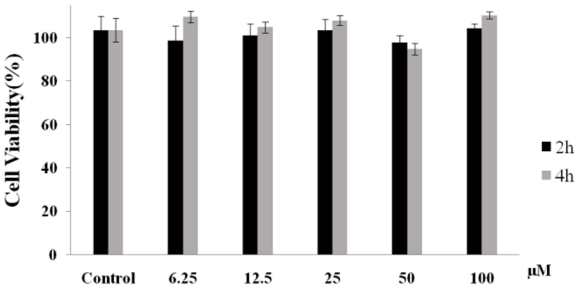
**Table 3** Anti-inflammatory effects of compounds 2h and 4h on xylene-induced ear edema in mice

| Compounds | Dose (mg/kg) | Weight of left ear (mg) | Weight of right ear (mg) | Weight difference (mg) | Edema inhibitory rate (%) |
|---|---|---|---|---|---|
| Control | - | 8.89±1.51 | 21.75±2.38 | 12.86±1.89 | - |
| Celecoxib | 25 | 10.62±1.64 | 19.51±1.08 | 8.89±1.72** | 30.87±9.350 |
| MBB | 25 | 9.32±1.82 | 21.16±2.13 | 11.84±2.10* | 7.93±3.04 |
| 2h | 25 | 9.38±0.52 | 16.75±2.43 | 7.37±2.13** | 42.69±9.55 |
| 4h | 25 | 9.79±1.94 | 21.72±2.71 | 11.93±2.77* | 7.23±3.65 |

Compared with the control group, *$p < 0.05$, **$p < 0.01$.

**Table 4** Effect of compounds 2h and 4h on the expression of NO, TNF-α, IL-1β and IL-6 by LPS-induced RAW 267.4 cells *in vitro*

| Compound No. | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | NO | TNF-α | IL-6 | IL-1β |
| Celecoxib | 17.89±3.19 | 41.18±4.28 | 36.04±5.28 | 17.94±2.91 |
| MBB | 44.36±8.27 | 109.42±15.31 | 48.04±11.31 | 54.14±4.29 |
| 2h | 17.67±2.15 | 89.39±9.13 | 8.61±1.04 | 20.07±2.11 |
| 4h | 31.74±0.16 | 79.96±7.43 | 57.63±9.20 | 45.14±4.94 |

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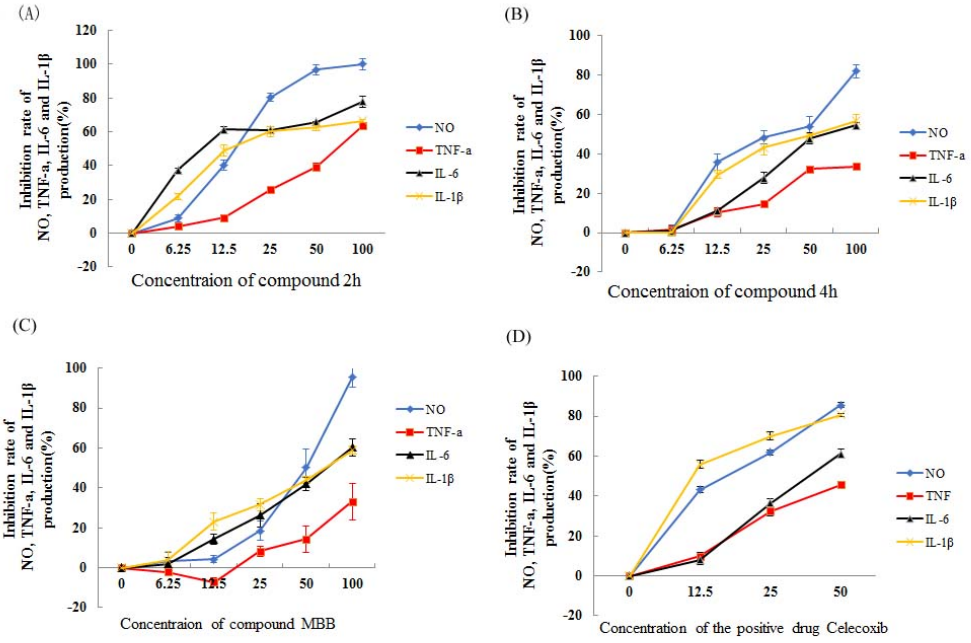


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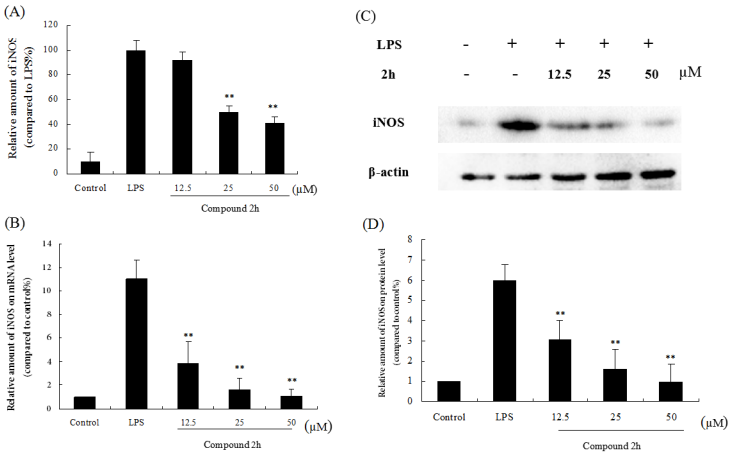


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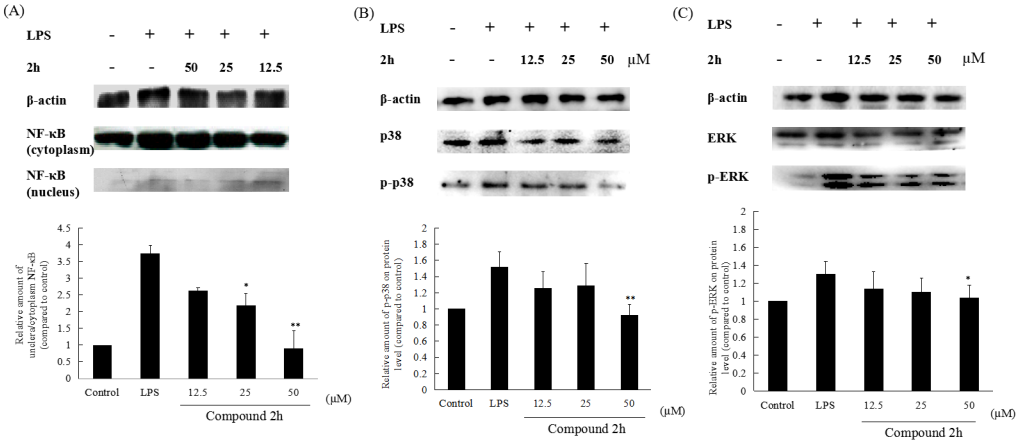


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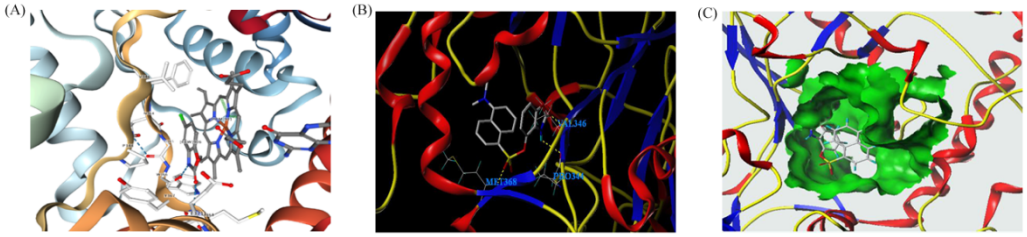


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