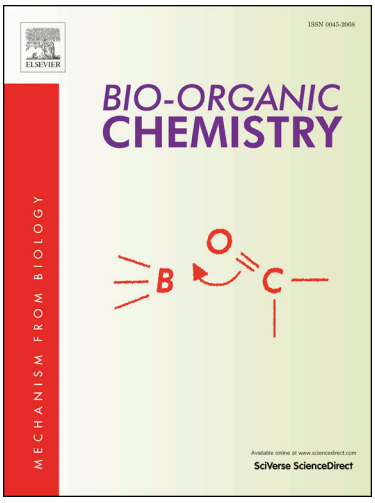
Journal Pre-proofs



Design, synthesis and biological evaluation of novel arylpropionic esters for the treatment of acute kidney injury

Jiawei Zuo, Shi-Meng Wang, Xia Jiang, Mengxin Cao, Ziwen Zhang, Tianlu Shi, Hua-Li Qin, Wenjian Tang

PII: S0045-2068(20)31753-3
DOI: https://doi.org/10.1016/j.bioorg.2020.104455
Reference: YBIOO 104455

To appear in: *Bioorganic Chemistry*

Received Date: 26 August 2020
Revised Date: 29 October 2020
Accepted Date: 31 October 2020

Please cite this article as: J. Zuo, S-M. Wang, X. Jiang, M. Cao, Z. Zhang, T. Shi, H-L. Qin, W. Tang, Design, synthesis and biological evaluation of novel arylpropionic esters for the treatment of acute kidney injury, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.104455

Design, synthesis and biological evaluation of novel arylpropionic esters for the treatment of acute kidney injury

Jiawei Zuo [a,c], Shi-Meng Wang [b,d], Xia Jiang [a], Mengxin Cao [a], Ziwen Zhang [a], Tianlu Shi [c], Hua-Li Qin [d,*], Wenjian Tang [a,*]

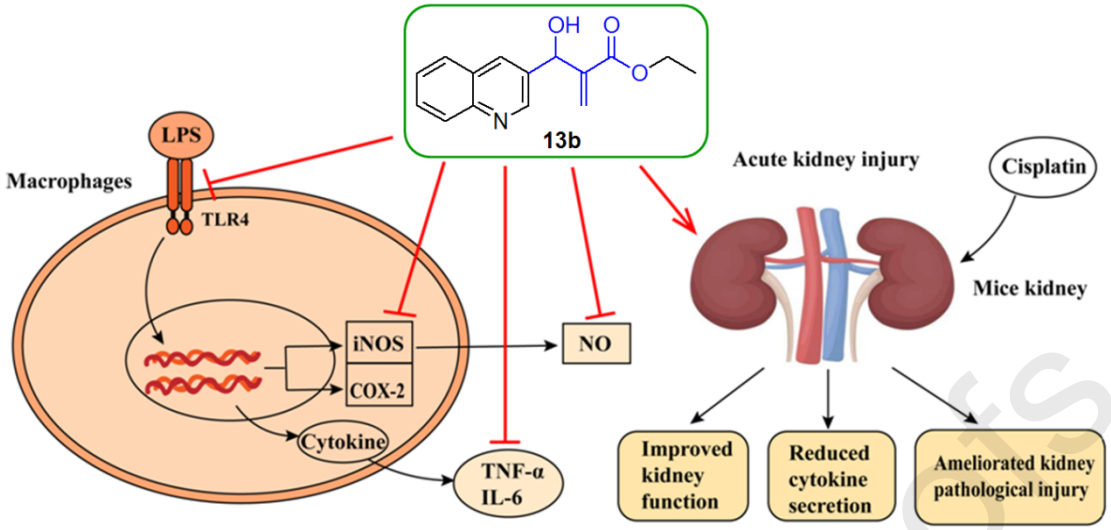
[a] *School of Pharmacy, Anhui Province Key Laboratory of Major Autoimmune Diseases, Anhui Medical University, Hefei 230032, China*

[b] *School of Life Science, Wuchang University of Technology, Wuhan 430223, PR China*

[c] *First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230001, China*

[d] *School of Chemistry, Chemical Engineering and Life Science, Wuhan University of Technology, Wuhan 430070, China*

- Novel arylpropionic esters exhibited significant anti-inflammatory activity.
- This arylpropionic ester scaffold bearing multi-functional groups.
- **13b** could suppress iNOS, COX-2 expression and NO, TNF-α and IL-6 production.
- **13b** significantly improved cisplatin-induced acute kidney injury.



Novel arylpropionic ester scaffold exhibited significant anti-inflammation. *In vitro*, **13b** decreased the levels of pro-inflammatory factors TNF-α and IL-6, inhibited the expression of related proteins TLR4, iNOS and COX-2. Moreover, **13b** effectively maintained normal metabolic function and improved the pathological damage of kidney tissue in AKI mice.

Abstract： Acute kidney injury (AKI) is associated with a strong inflammatory response, and inhibiting the response effectively prevents or ameliorates AKI. A series of novel arylpropionic esters were designed, synthesized and evaluated their biological activity in LPS-stimulated RAW264.7 cells. Novel arylpropionic esters bearing multi-functional groups showed significant anti-inflammatory activity, in which, compound **13b** exhibited the most potent activity through dose-dependent inhibiting the production of nitric oxide (NO, IC_{50} = 3.52 μM), TNF-α and IL-6 (84.1% and 33.6%, respectively), as well as suppressing the expression of iNOS, COX-2 and TLR4 proteins. In C57BL/6 mice with cisplatin-induced AKI, compound **13b** improved kidney function, inhibited inflammatory development, and reduced pathological damage of kidney tissues. In brief, this arylpropionic ester scaffold may be developed as anti-inflammatory agents.

Keywords: Arylpropionic acid; quinoline; acute kidney injury; anti-inflammatory; multi-functional group

*Corresponding author.
E-mail: qinhuali@whut.edu.cn (H.L. Qin); ahmupharm@126.com (W.J. Tang).

1. Introduction

Acute kidney injury (AKI) is a global public health concern associated with high morbidity, mortality, and healthcare costs [1]. Platinum drugs are widely used for the treatment of ovarian, breast, stomach, lung, prostate cancers, and so on, but they usually cause particular renal toxicity due to its special metabolism in the kidney [2,3]. Clinical manifestations have proved that AKI is associated with intrarenal and systemic inflammation, which is essential for eliminating microbial pathogens and repairing tissue after diverse forms of injury [4-6]. The pathological features of AKI include tubular and vascular cell damage, oxidative stress and intense inflammatory reaction [7]. Intense researches also showed that many anti-inflammatory drugs for AKI were proved effective in animal models but failed to translate to humans over the past decades, however, with the increase of AKI patients, there was an urgent need for new drugs and treatment [8].

The discovery of novel anti-inflammatory agents has attracted significant attention of medicinal chemists. As shown in Fig. 1, acrivastine and ibuprofen are marketed drugs with anti-inflammatory activity. Acrivastine, an α,β-unsaturated propenoic acid derivative, is a non-sedating antihistamine used for treatment of hayfever, urticaria, and rhinitis, while ibuprofen, an arylpropionic acid derivative, is a commonly used NSAID with anti-inflammatory, analgesic, and antipyretic effects. In medical discovery, natural products showed benign therapeutic potency and fewer adverse effects. Cinnamic acid is a secondary metabolite in the phenylpropanoid pathway and the source of hydroxycinnamic acid derivatives like coumaric, caffeic, ferulic, and sinapic acids in either ester or glycosidic forms. Cinnamic acid displayed a wide range of pharmacological activities, such as anti-inflammatory, anticancer, antimicrobial activities, including salvianolic acid B with four phenylpropanoid units and chlorogenic acid with one phenylpropanoid unit (Fig. 1) [9-12]. Therefore, arylpropionic ester scaffold was often used to design and find new anti-inflammatory agents [13-15].

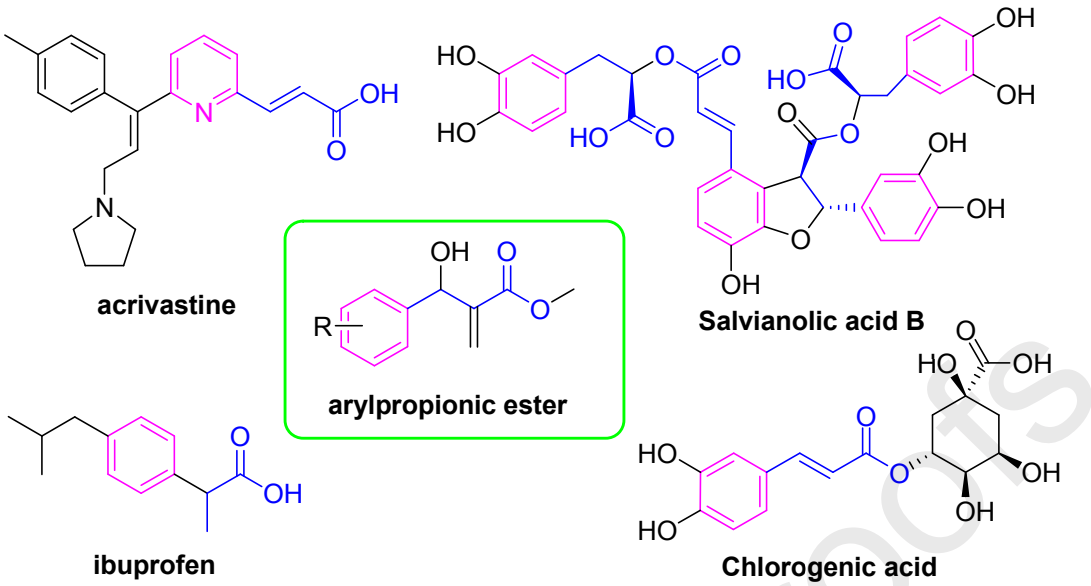


Fig. 1. The analogues of arylpropionic esters in this study.

Multi-functional compounds are designed broadly as hybrid or conjugated drugs from two or more pharmacophores/drugs having multiple pharmacological actions in treatment of multi-factorial diseases [16]. Novel arylpropionic ester with 3-hydroxy and 2-methylene, one of Baylis-Hillman adducts, is a valuable scaffold with promising biological molecules, including potent antibacterial and antifungal activities [17-20]. As shown in Fig. 1, 3-hydroxy-2-methylene-3-phenylpropionic ester is an arylpropionic ester derivative with polyfunctional groups, however, its anti-inflammatory activity was not reported. In this work, we would explore the anti-inflammatory activity of novel arylpropionic esters.

Macrophages, significant innate immune sentinel, play a role of promoting renal fibrosis and repairing damage in kidney injury [21]. In the response to AKI, pro-inflammatory macrophages (M1) can secrete inflammatory cytokines and induce kidney damage and fibrosis, while in some micro-circumstances, anti-inflammatory macrophages (M2) release regenerative trophic factors and reduce inflammation, promote wound healing and kidney recovery [22-24]. Nitric oxide (NO) is synthesized from l-arginine by the inducible nitric oxide synthase (iNOS or NOS2) in the pathogenesis and control of tumors, chronic degenerative diseases and autoimmune processes [25,26]. Pharmacological and genetic methods also indicated that cytokines

including TNF-α and IL-6 play a central role in the activation of inflammation responses and the pathogenesis of injury in cisplatin-induced nephrotoxicity [27,28]. Thus, the anti-inflammatory activity of novel arylpropionic esters could be screened through inhibiting the NO production, the release of pro-inflammatory factors TNF-α and IL-6 in lipopolysaccharide (LPS)-activated macrophages. Further structural optimization will be carried out to find lead compounds.

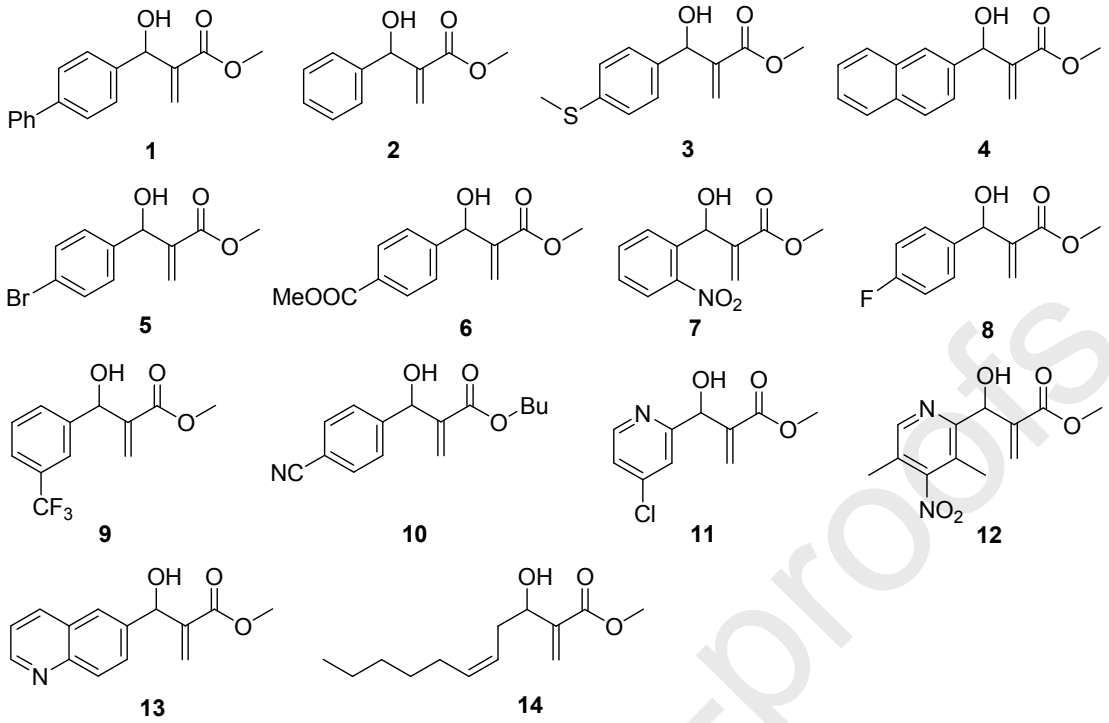
In the inflammatory response of AKI, it is very important to control the release of related proteins and pro-inflammatory factors, which effectively reduce the damage of renal tissue and maintain normal renal function. The nephritis of rodents has provided evidence that iNOS also functions as a negative feedback regulator of the autoimmune Th1 cell response and thereby protects the host against immunopathological sequelae [29]. As a common inducible enzyme, the expression of COX-2 in the kidney is relatively high and plays an important role in water/salt metabolism, regulating renin release [30-33], kidney development [19,34,35], and mediating kidney damage [36-38]. In addition, toll-like receptors (TLRs) are a class of proteins, and the continuous signaling of TLR can lead to autoimmune diseases and chronic inflammatory diseases, so the tight regulation of TLR signaling pathways is very important [39]. Under the pathological conditions of AKI, the expression of TLR4 is significantly increased, and inflammation can promote the development of kidney tissue damage and renal failure [2,40]. Therefore, it is significant to clarify the protective effect on the active lead in AKI model mice.

2. Results and discussion

2.1. Chemistry

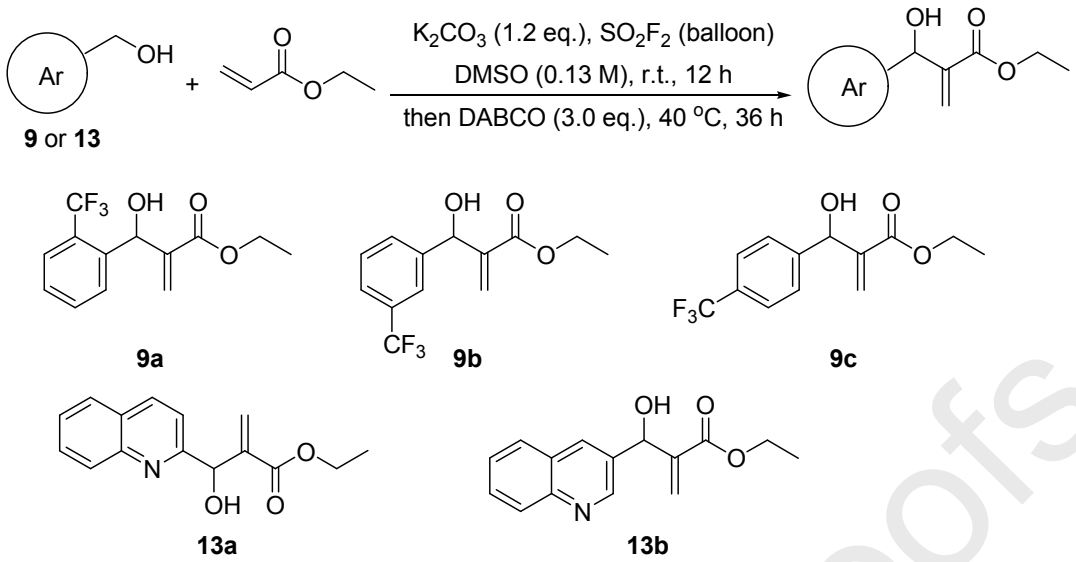
In our recent work, a series of arylpropionic esters (**1–14**) were prepared through a cascade dehydrogenative Baylis–Hillman reaction of the C(sp^3)–H of primary alcohols with the C(sp^2)–H of electron-deficient olefins mediated by SO_2F_2 (Scheme 1) [41-43]. As shown in Table 1, initial screening of novel arylpropionic esters revealed that compounds **9** and **13** alleviated the increase of LPS-induced NO release, which implied

their potent anti-inflammatory activity.



Scheme 1. Chemical structures of novel arylpropionic esters **1–14**.

To further study the structure-activity relationship (SAR), the constitutional isomers of compounds **9** and **13** were synthesized through a cascade dehydrogenative Baylis-Hillman reaction of the C(sp^3)–H of primary alcohols with the C(sp^2)–H of electron-deficient olefins for forming allylic alcohols mediated by SO_2F_2. As shown in Scheme 2, compounds **9a–9c**, **13a** and **13b** were synthesized through this mild method without the requirement of transition metals.



Scheme 2. Synthesis of compounds **9a–9c**, **13a** and **13b**.

2.2. Cytotoxicity assessment

In cases where novel arylpropionic esters are of no toxicity or low toxicity, it is necessary to evaluate their biological activity. The RAW264.7 cells were treated with the same concentration of synthesized compounds (10 μM) for 24 h and the viability of the cells was measured by MTT assay. As shown in Fig. 2, compared with the negative control, most of arylpropionic esters did not notably affect cell viability, except for compounds **4**, **9b** and **9c** having slight effect. Therefore, the concentration of compounds was suitable for further evaluation of biological activity.

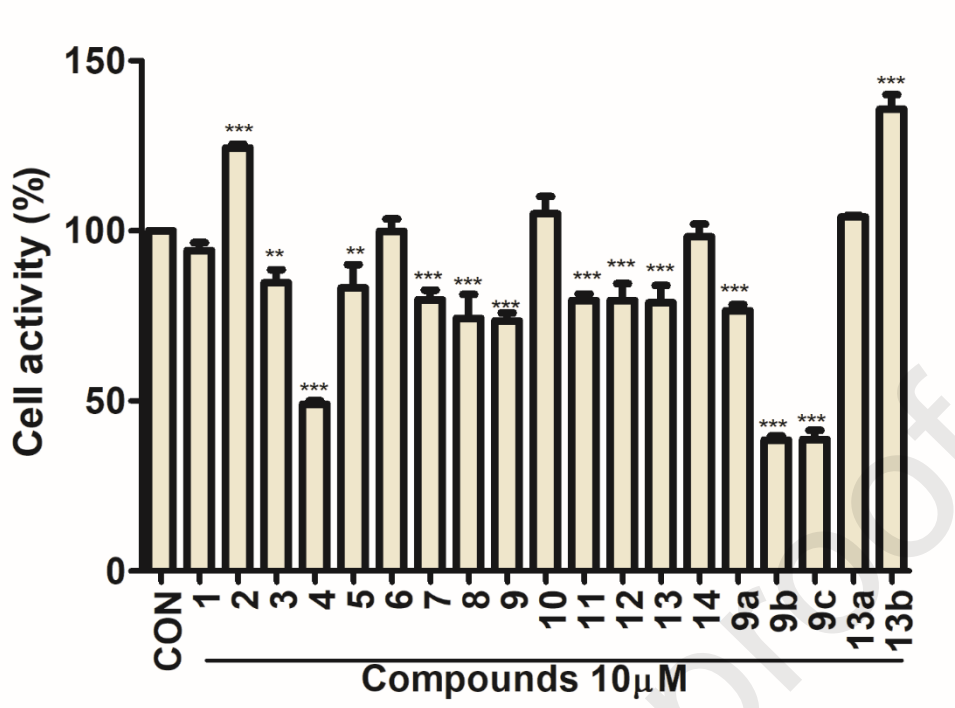


Fig. 2. Cell viability was detected by MTT assay after RAW264.7 cells were pretreated with synthesized compounds at the same concentration (10 μM) for 24 h. Results were expressed as percentage of cell survival *vs* untreated cell (control) and shown as mean ± SD (n = 3). Statistical significance was calculated using one-way ANOVA and Bonferroni post hoc tests. $^{***}p < 0.001$ compared with the control group; $^{**}p < 0.01$ compared with the control group.

2.3. Inhibition of NO production in LPS-stimulated RAW264.7 cells

In the inflammatory response, activated macrophages induce the expression of nitric oxide synthase (iNOS) and produce NO, and excessive release of NO is regarded as an important factor in inflammatory responses [44,45]. To evaluate the anti-inflammatory effect of arylpropionic esters, Griess reagent was used to detect the level of the LPS-induced NO release in RAW264.7 cells. After pretreatment with compounds at the concentration of 5 μM, the production of LPS-induced NO in the supernatant was significantly reduced (Fig. 3). Novel arylpropionic esters showed a dose-dependent inhibition of NO production, and IC_{50} values of synthesized compounds further were

calculated.

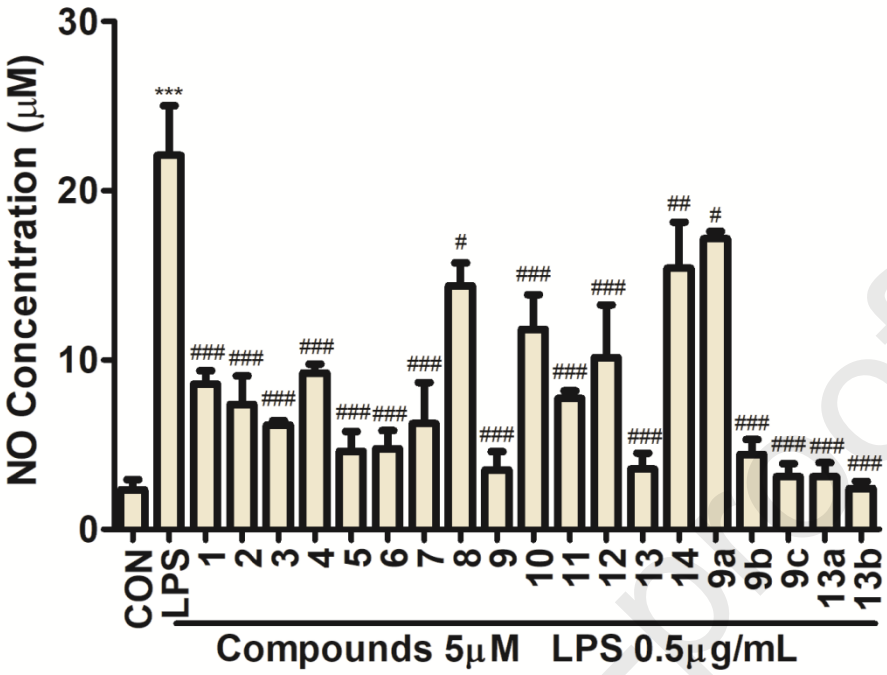


Fig. 3. The inhibitory effects of synthesized compounds on NO production in LPS-stimulated RAW 264.7 cells. RAW264.7 cells were pretreated with compounds at the same concentration (5 μM) for 1 h, then incubated with LPS (0.5 μg/mL) for 24 h. NO production in supernatant was detected by Griess reagent assay. $^{***}p < 0.001$ compared with the control group; $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ compared with the LPS group.

As shown in Table 1, novel arylpropionic esters showed significant anti-inflammatory activity, amongst them, compounds **9c**, **13a** and **13b** exhibited the most potent NO inhibitory activity (IC_{50} = 4.12, 3.78 and 3.52 μM, respectively). Arylpropionic ester scaffold with 3-hydroxy and 2-methylene may be the key factor of anti-inflammatory activity (Fig. 1). Aryl with different structures showed micromole anti-inflammatory activity, when aryl was substituted with alkyl, the activity decreased (IC_{50} = 12.43 μM for compound **14**). Ester group of arylpropionic ester has hardly effect on the activity, for example, IC_{50} = 3.88 and 4.65 μM for compounds **9** (methyl ester) and **9b** (ethyl ester), respectively. Substituent position of aryl affected the activity, for example, the activity of *o*-CF_3 of phenyl (IC_{50} = 15.77 μM for **9a**) was much less than those of *m*-CF_3 and *p*-CF_3 (IC_{50} = 4.65 and 4.12 μM for **9b** and **9c**, respectively).

Substituent position of quinoline ring hardly affected the activity (IC_{50} = 3.79, 3.78 and 3.52 μM for 6- (**13**), 2- (**13a**) and 3-position (**13b**), respectively), thus, compounds **13**, **13a** and **13b** exhibited almost same inhibitory effect on NO production (Fig. 4A). The effects of compounds **13a** and **13b** on the levels of pro-inflammatory factors TNF-α and IL-6 induced by LPS would be further evaluated.

Table 1

The IC_{50} values or inhibition rates (%) of the inhibition of NO, TNF-α and IL-6 production.

Compound	IC_{50} (μM) [a]	Inhibition (%) [b,c]	
	NO	TNF-α	IL-6
1	6.62 ± 1.61	28.5	14.4
2	5.71 ± 0.70	67.7	36.9
3	5.84 ± 1.64	67.1	3.6
4	6.15 ± 1.45	*na*	21.0
5	6.01 ± 2.18	21.5	32.2
6	5.03 ± 1.32	10.7	42.5
7	4.43 ± 0.65	52.8	85.3
8	14.07 ± 1.21	28.8	15.7
9	3.88 ± 1.16	23.3	31.4
10	11.21 ± 1.90	49.8	44.3
11	5.87 ± 0.97	54.9	23.6
12	7.54 ± 1.12	52.4	35.5
13	3.79 ± 0.85	53.8	44.5
14	12.43 ± 2.20	45.5	51.0
9a	15.77 ± 1.36	71.4	22.5
9b	4.65 ± 1.14	52.5	*na*
9c	4.12 ± 1.33	44.2	28.5

13a	3.78 ± 0.92	68.6	54.9
13b	3.52 ± 0.88	84.1	33.6

[a] The detection of NO production with Griess reagent;

[b] The inhibition of cytokines were performed using ELISA kits and the inhibition rates were calculated at 10 μM concentration of the compound;

[c] The IC_{50} values were means of three determinations; the inhibition rate values were means of two determinations; *na*, no activity.

2.4. Determination of pro-inflammatory cytokines

Activated macrophages produce excessive amounts of multiple pro-inflammatory cytokines, including TNF-α, IL-6 and IL-12, leading to serious systemic diseases with a high mortality [46]. Therefore, to further evaluate the effects of novel arylpropionic esters on the LPS-induced TNF-α and IL-6 production, RAW264.7 cells were cultured with LPS in the presence of compounds for 24 h, and the levels of cytokines in the cell supernatant were determined using ELISA kits. All the test compounds exhibited the ability to inhibit the production of TNF-α and/or IL-6, and the percentage in assay was calculated. As shown in Table 1, most of compounds significantly inhibited the levels of TNF-α and IL-6, amongst them, compounds **13a** and **13b** showed a dose-dependent inhibition on TNF-α and IL-6 production (Figs. 4B and 4C). Compound **13b** had better inhibitory effect on TNF-α, while compound **13a** had better inhibitory effect on IL-6. These results showed that novel arylpropionic esters attenuate an excessive immune reaction in RAW264.7 macrophages by blocking the increase of pro-inflammatory cytokines. Thus, compound **13b** was selected to further explore the anti-inflammatory mechanisms.

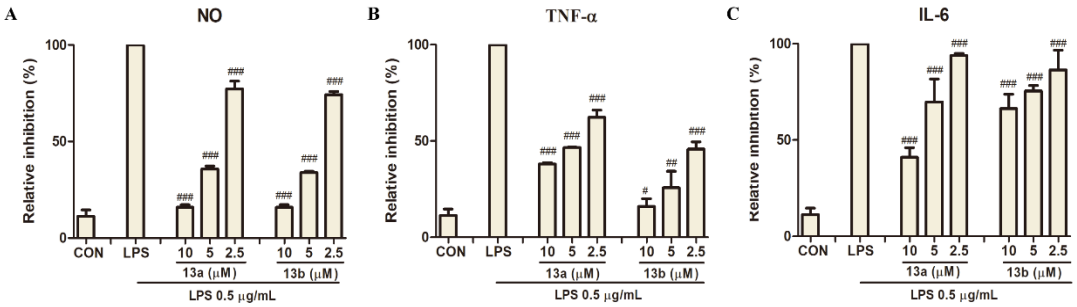


Fig. 4. Compounds **13a** and **13b** inhibited LPS-induced NO, IL-6 and TNF-α production in RAW264.7 cells. Compounds (10, 5, 2.5 μM) were pre-treated RAW264.7 cells for 1 h, and then incubation with LPS (0.5 μg/mL) for 24 h. NO production and cytokine levels (TNF-α and IL-6) in the supernatant were measured by Griess and ELISA kits, respectively. $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ compared with the LPS group.

*2.5. Anti-inflammatory mechanism of compound **13b***

The inflammatory process involves a variety of molecular mechanisms, in which two of the most important are NO and cyclooxygenase 2 (COX-2). Decreased bioavailability of NO and increased oxidative stress partially mediate renal vasoconstriction and medullary hypoxia, which may be associated with AKI [47]. Increased NO release may activate soluble guanidine cyclase, leading to cGMP signaling and up-regulating COX-2 expression [48]. The molecular synergy between iNOS and COX-2 may represent the main mechanism of inflammatory response. Western blot assay was used to verify the effect of compound **13b** on the expression of iNOS-COX-2 in LPS-activated RAW264.7. After stimulation with 0.5 μg/mL LPS for 24 h, the expression of iNOS and COX-2 in the LPS group increased significantly compared with the control group, while compound **13b** inhibited the expression of iNOS and COX-2 in a concentration-dependent manner (Fig. 5). Drugs that block the interaction of iNOS–COX-2 may have anti-inflammatory effects, and they work synergistically with COX-2 inhibitors to reduce dosage and side effects. Toll-like receptors (TLRs) are widely expressed on kidney epithelial cells and leukocytes, regulating innate and adaptive immune responses. TLR4 is an important mediator of

cisplatin-induced production of cytokines (such as TNF-α), inflammation, and subsequent kidney damage [49]. As shown in Fig. 5, compound **13b** effectively inhibited TLR4 protein expression in LPS-stimulated RAW264.7 cells. Thus, TLR4-targeted signaling pathway may be a feasible therapeutic strategy to prevent cisplatin-induced AKI in patients.

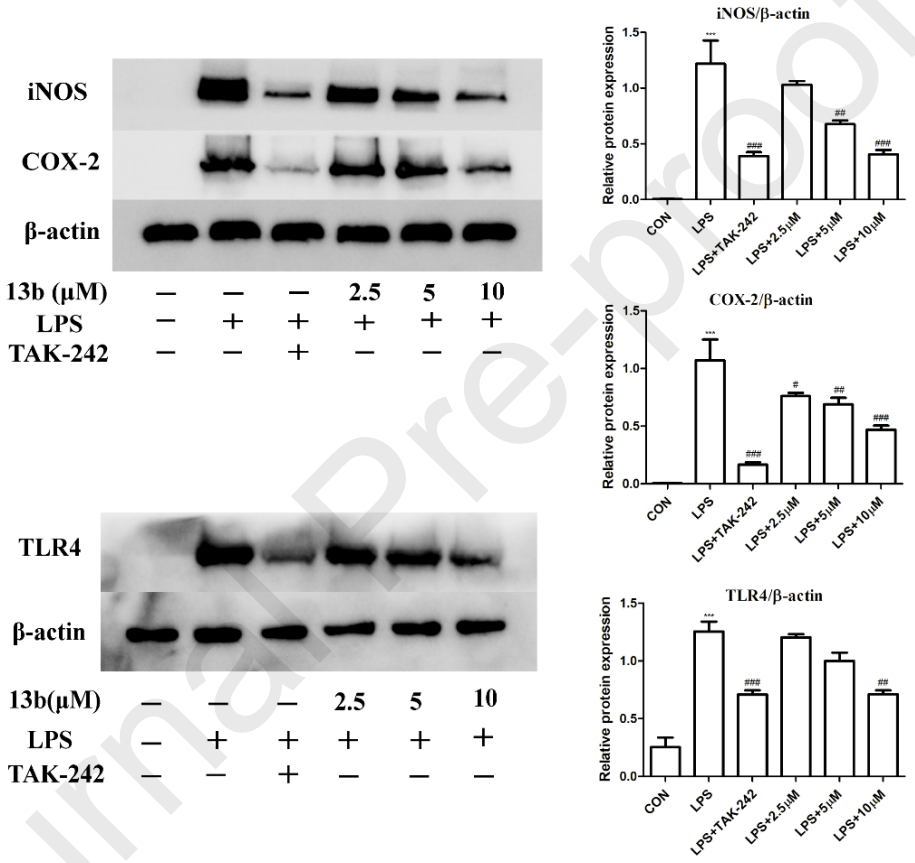


Fig. 5. Effects of compound **13b** on LPS-induced iNOS, COX-2 and TLR4 protein expression in RAW264.7 cells. After compound **13b** (2.5, 5, 10 μM) pretreatment for 1 h, LPS (0.5 μg/mL) was incubated for 24 h, and the protein expression in RAW264.7 cells was detected by Western blotting. TAK-242 was the inhibitor of TLR4. $^{***}p < 0.001$ compared with the control group; $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ compared with LPS group.

2.6. In vivo study of the mouse model of AKI

*2.6.1. The acute toxicity in vivo of compound **13b***

We randomly divided C57BL/6 mice into two groups, each with 6 mice, and evaluated the acute toxicity of compound **13b** *in vivo*. One group was given a single dose of 300 mg/kg, and the other group was given a single dose of 100 mg/kg by intragastric administration to further observe the acute poisoning of mice. After administration of compound **13b**, the mice showed reduced spontaneous activity, gathered together, little response to external stimuli, and no obvious changes in body weight. No mice died and all were in fine condition.

2.6.2. Kidney function biomarkers and cytokines in serum

Clinical data have shown that approximately one third of patients develop AKI after cisplatin treatment, accompanied by decreased glomerular filtration rate, increased blood urea nitrogen (BUN) and serum creatinine (SCR), and electrolyte imbalance [50,51]. Urea and creatinine are important indicators of kidney function examination, reflecting the degree of damage to glomerular filtration function. As the diagnostic criteria of nephrotoxicity, urea and creatinine reflects the late manifestations of the disease, but early intervention is critical for the effective treatment of AKI [52]. The *in vivo* activity of compound **13b** was evaluated in C57BL/6 mice of cisplatin-induced AKI. The results showed that compared with the control group, the levels of SCR and BUN in the cisplatin-treated group were significantly increased, and renal dysfunction occurred (Figs. 6A and 6B). However, compared with the model group, the values of SCR and BUN in compound **13b-**treated mice were decreased (Figs. 6A and 6B). Compound **13b** could ameliorate kidney damage and maintain glomerular filtration function. Besides, the increasing release of pro-inflammatory cytokines in serum of the kidney injury mice was significantly inhibited by compound **13b**. As shown in Figs. 6C and 6D, the levels of pro-inflammatory mediators TNF-α and IL-6 in the serum of compound **13b**-treated mice were significantly lower than those in model group. In all in, compound **13b** has obvious effect on maintaining renal function and inhibiting

inflammation of cisplatin-induced AKI in model mice.

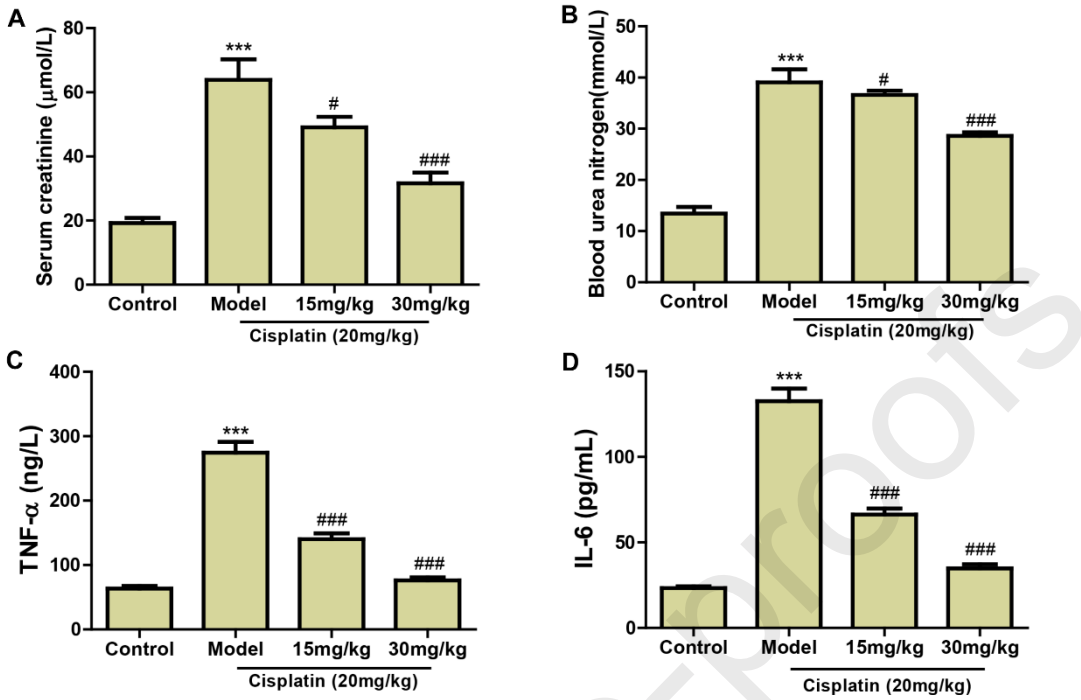


Fig. 6. The protective effect of compound **13b** on cisplatin-induced AKI in C57BL/6 mice. After pre-treatment with compound **13b** (15 and 30 mg/kg), the mice were injected intraperitoneally with cisplatin (20 mg/kg) and sacrificed 72 h later. Detection of kidney function markers (SCR and BUN) and pro-inflammatory factors (TNF-α and IL-6) levels in serum. $^{***}p < 0.001$ compared with control group; $^{###}p < 0.001$, $^{#}p < 0.05$ compared with model group.

2.6.3. Kidney histopathological analysis

As an important characteristic of AKI, tubular damage may eventually lead to kidney tubular interstitial fibrosis [53,54]. Cisplatin caused kidney damage and increased kidney specific gravity, while compound **13b** significantly decreased the kidney specific gravity after injury (Fig. 7). In cisplatin-induced AKI mice, kidney tissues were removed and fixed in 10% formaldehyde. After dehydration in gradient concentration of alcohol, the tissues were embedded in paraffin and sliced. Sections were stained by H&E and PAS. Finally, image them under an optical microscope. As shown in Fig. 8,

in the blank control group, normal morphology of the glomeruli and proximal tubules were clearly shown in the kidneys of mice. While in cisplatin-induced AKI mice, the kidneys were severely damaged, resulting in renal tubular epithelial injury, inflammatory cell infiltration, renal tubular cell swelling, and tubular dilatation. Therefore, the treatment of compound **13b** significantly improved the necrosis of kidney tissue and inflammatory infiltrating cells caused by cisplatin in model mice.

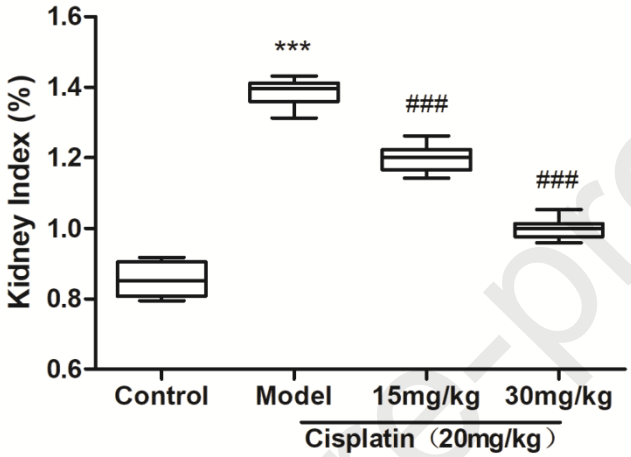


Fig. 7. The effect of compound **13b** on kidney index. Compound **13b** (15 and 30 mg/kg) was pre-treated for mice, and cisplatin (20 mg/kg) solution was injected intraperitoneally. After 72 h, the mice and kidney tissues of every group were weighed. $^{***}p < 0.001$ compared with control group; $^{###}p < 0.001$ compared with model group.

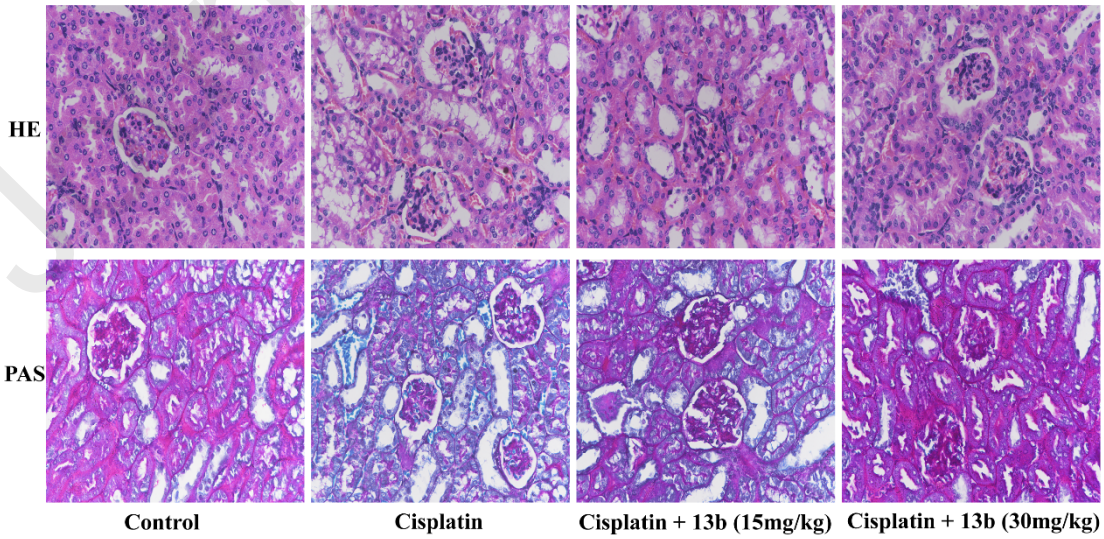


Fig. 8. The effect of compound **13b** on the pathological changes of kidney tissue caused

by cisplatin (20 mg/kg). The kidney was stained with H&E and PAS for histological examination.

3. Conclusions

A series of novel arylpropionic esters were designed, synthesized and evaluated their biological activities. These arylpropionic esters bearing multi-functional groups showed benign anti-inflammatory activity through inhibiting NO production and the levels of pro-inflammatory factors TNF-α and IL-6. SAR showed that aryl of arylpropionic ester scaffold was necessary on the anti-inflammatory activity, alkyl decreased the activity, ester had hardly effect on the activity; substituent position of benzene ring affected the activity, while that of quinoline ring hardly affected the activity. In LPS-treated macrophages, compound **13b** showed significant anti-inflammatory activity by dose-dependent inhibiting the production of NO (IC_{50} = 3.52 μM), decreasing the levels of TNF-α and IL-6 (84.1% and 33.6%, respectably), as well as inhibiting the expression of iNOS, COX-2 and TLR4 protein. In vivo study in C57BL/6 mice of cisplatin-induced AKI showed that compound **13b** decreased the levels of BUN, SCR, TNF-α and IL-6 in serum to inhibit the inflammatory response, improved the pathological damage of the kidney tissue to maintain the metabolic function of the kidney. In short, the preliminary anti-inflammatory mechanism showed the protective effect of compound **13b** on AKI in C57BL/6 mice. Novel arylpropionic esters have great potential to be developed as a promising AKI therapeutic agent through reducing and healing the inflammatory damage.

4. Experimental section

4.1. Chemistry

All reagents were purchased from commercial sources and were used without further purification. Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker

AV-400 instrument in $CDCl_3$. Chemical shifts are reported in parts per million (δ) downfield from the signal of tetramethylsilane (TMS) as internal standards. Coupling constants are reported in Hz. The multiplicity is defined by *s* (singlet), *d* (doublet), *t* (triplet), or *m* (multiplet). High resolution mass spectra (HRMS) were obtained on an Agilent 1260-6221 TOF mass spectrometry. Column and thin-layer chromatography (CC and TLC, resp.) were performed on silica gel (200-300 mesh) and silica gel GF_{254} (Qingdao Marine Chemical Factory) respectively.

*4.2. General procedure for the preparation of compounds **9a–9c**, **13a** and **13b**.*

A mixture of arylmethanol (**9** or **13**, 1.0 mmol), K_2CO_3 (1.2 mmol, 1.2 eq.) and DMSO (8 mL, 0.13 M) under an atmosphere of SO_2F_2 (balloon) was stirred at room temperature for 12 h before DABCO (1,4-diazabicylo [2.2.2] octane) (3.0 mmol, 3 eq.) and ethyl acrylate (3.0 mmol, 3 eq.) were added. The resulting mixture was stirred at 60ºC for an additional 36 h. The mixture was concentrated under the reduced pressure. The residue was purified by flash column chromatography (petroleum/EtOAc = 1/1, v/v) to obtain title compound.

*4.2.1. Ethyl 3-hydroxy-2-methylene-3-(2-trifluoromethylphenyl)propionate (**9a**)*. Light yellow oil, yield 87%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 8.8 Hz, 2H), 7.58 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 6.39 (s, 1H), 6.03 (s, 1H), 5.57 – 5.51 (s, 1H), 4.20 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 141.8, 139.3, 132.3, 128.9, 128.6, 127.3, 126.9, 125.9, 124.3, 67.87, 61.2, 14.0; TOF-HRMS: *m/z* $[M + Na]^+$ calcd for $C_{15}H_{15}NO_3$: 297.0709; found: 297.0708.

*4.2.2. Ethyl 3-hydroxy-2-methylene-3-(3-trifluoromethylphenyl)propionate (**9b**)*. Light yellow oil, yield 83%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.61 – 7.51 (m, 2H), 7.47 (t, J = 7.7 Hz, 1H), 6.38 (s, 1H), 5.82 (s, 1H), 5.60 (s, 1H), 4.19 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 142.4, 141.5, 130.7, 129.9, 128.9, 126.7, 124.6, 124.0, 123.4, 73.0, 61.2, 14.0; TOF-HRMS: *m/z* $[M + Na]^+$ calcd for $C_{15}H_{15}NO_3$: 297.0709; found: 297.0708.

*4.2.3. Ethyl 3-hydroxy-2-methylene-3-(4-trifluoromethylphenyl)propionate (**9c**).* Light yellow oil, yield 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 6.37 (s, 1H), 5.81 (s, 1H), 5.59 (s, 1H), 4.19 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 145.4, 141.6, 123.0, 126.9 (2C), 126.6, 125.4 (2C), 125.2, 73.0, 61.2, 14.0; TOF-HRMS: *m/z* $[M + Na]^+$ calcd for $C_{15}H_{15}NO_3$: 297.0709; found: 297.0707.

*4.2.4. Ethyl 3-hydroxy-2-methylene-3-(quinolin-2-yl)propionate (**13a**).* Light yellow oil, yield 79%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (dd, J = 16.7, 8.5 Hz, 2H), 7.82 (d, J = 8.1 Hz, 1H), 7.72 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.58 – 7.51 (m, 1H), 7.46 (d, J = 8.5 Hz, 1H), 6.40 (s, 1H), 5.99 (s, 1H), 5.79 (s, 1H), 4.24 – 4.12 (m, 2H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 159.6, 146.4, 142.1, 137.4, 130.1, 128.9, 127.8, 127.8, 127.8, 126.8, 119.1, 72.1, 61.1, 14.3; TOF-HRMS: *m/z* $[M + H]^+$ calcd for $C_{15}H_{15}NO_3$: 258.1125; found: 258.1122.

*4.2.5. Ethyl 3-hydroxy-2-methylene-3-(quinolin-3-yl)propionate (**13b**).* Light yellow powder, yield 81%; m.p. 96-97ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, J = 2.1 Hz, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.10 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 8.2, 1.0 Hz, 1H), 7.73 – 7.67 (m, 1H), 7.58 – 7.51 (m, 1H), 6.43 (s, 1H), 5.95 (s, 1H), 5.79 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 149.8, 147.3, 141.7, 134.5, 133.9, 129.6, 128.8, 128.0, 127.7, 126.9, 126.4; TOF-HRMS: *m/z* $[M + H]^+$ calcd for $C_{15}H_{15}NO_3$: 258.1125; found: 258.1125.

4.3. Biological evaluation

4.3.1. Cell culture

The RAW264.7 cell line was obtained from BeNa Culture Collection Company. The Mouse peritoneal macrophages were cultured in DMEM (Hyclone, USA) containing 10% FBS ((Biological Industries, Israel), 100 U/mL penicillin and 100 mg/mL

streptomycin (Beyotime). Unless otherwise indicated, the RAW264.7 cells were grown in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. After the macrophages have reached the logarithmic growth phase, they are usually cultured with 1 % serum for 24 h and then subjected to subsequent experiments.

4.3.2. Cell viability assay

Each compound was tested for their *in vitro* cytotoxicity on the RAW264.7 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric dye reduction method. In 96-well plates, the cells (1×10^4 cells/100 μL) were seeded and maintained at 37°C in 5% CO_2 for 24 h, and then the well was treated with concentration of 10 μM for 24 h. Then, 20 μL of MTT solution (prepared in PBS solution, 5 mg/mL) was added to every well and incubated for 4 h. After this process, all the liquid was discarded in the well, added 150 μL of DMSO to each well, incubated for 20 min in a constant temperature shaker, then detected at 492 nm with a microplate reader (MQX200, Bio-Tek, USA).

4.3.3. Nitric oxide (NO) and cytokine assay

The supernatant of compounds-treated the RAW264.7 cell cultures were collected and used to measure nitric oxide (NO) and cytokines (TNF-α and IL-6) by Griess reagent (Beyotime, China) and enzyme-linked immunosorbent assay (ELISA) kits (Huamei, Wuhan) according to the manufacturer's protocol. RAW264.7 cells were seeded into 48-well plate with 7×10^4 cells per well and incubated for 24 h, and then cells were retreated with compounds (2.5, 5 or 10 μM) for 1 h, incubated with LPS (0.5 μg/mL) for 24 h [55].

4.3.4. Western blot assay

Western blot is commonly used to detect protein expression in cells or tissues. RAW264.7 cells were plated in 6-well plates with 2×10^6 cells per well and incubated

for 24 h. Next, the cells were treated with or without compound **13b** (2.5, 5, 10 μM) for 1 h before exposure to LPS (0.5 μg/mL) for 24 h. In normal, cells were washed with phosphate-buffered saline (PBS) and were lysed in 200 μL RIPA cell lysis buffer that contains PMSF and phosphatase inhibitors (Beyotime, china) on ice for 30 min. The lysates were boiled for 10 min at 95°C and electrophoresed in 10% SDS-PAGE polyacrylamide gels and then transferred to PVDF membrane (GE Healthcare, UK). The proteins were incubated with antibodies against β-actin, iNOS, COX-2 and TLR4 and further incubated for 1 h with HRP-conjugated secondary antibodies. Finally, Protein bands exposed under enhanced chemiluminescence detection reagents (Millipore, MA, USA) conditions. Those bands were scanned and intensity quantified using Image J (1.45s, USA).

4.3.5. Animal experiments

Male C57BL/6 mice (weighing 18-25 g) were purchased and fed with standard rodent chow and water adapted for 7 days at the Animal Center of Anhui Medical University (Hefei, China). All experiments about animal were according to the Guide for Care and Use of Laboratory Animals of National Institutes of Health. The experimental animals were divided into four groups: normal control group, model group, cisplatin + **13b** (15 mg/kg) group and cisplatin + **13b** (30 mg/kg) group which contain 10 mice. Mice in the **13b** group were given compound of **13b** (15 mg/kg and 30 mg/kg) with intragastric administration for 3 consecutive days, and the other groups were given the same amount of normal saline. The third day, all mice except the normal group received a single intraperitoneal injection of cisplatin solution (20 mg/kg) (aladdin, China) after 1h of intragastric administration. The normal group, mice were injected intraperitoneally with the same dose of saline. Under the same conditions, mice continued administration either the **13b** compound or equal volume of saline for 3 days, followed by obtaining serum and kidney tissue. Maximal kidney injury, as assessed by kidney function biomarkers and histologic measurements, was observed at 72 h following intraperitoneal injections of cisplatin [56].

On the third day after cisplatin administration, all mice were anesthetized with ether, immediately blood and kidney were collected for further analysis. After standing on ice for more than half an hour, the blood was centrifuged at 1500 rpm, 4°C and 5 min to obtain serum. The levels of serum blood urea nitrogen (BUN) and serum creatinine (SCR) were measured by commercially supplied kits (Nanjing Jiancheng Bioengineering Institute, China) which according to the manufacturer's instructions. The secretion of inflammatory cytokines (TNF-α, IL-6) in blood of all mice was detected with ELISA kits (Huamei, Wuhan). Most of the kidney tissues of the mice were stored at -80°C, and a small part was fixed with 10% formalin buffer for 24 h at 25°C and then embedded in paraffin. To evaluate the biological activity of compound **13b** *in vivo*, the degree of kidney injury in each group of mice was determined by hematoxylin-eosin (H&E) and periodic acid-schiff (PAS) staining.

4.4. Statistical analysis

All date in the figures are expressed as means ± standard error of the mean (SEM). The results in the text were analyzed by one-way analysis of variance (ANOVA). A value of *P* less than 0.05 indicated that the difference is statistically significant. All experimental results are repeated at least two or three times.

Acknowledgments

Financial support was provided by Natural Science Fundation of Anhui provincial Department of Education (KJ2019ZD21), and Anhui Provincial Natural Science Foundation (2008085MH261, 2008085MH272).

Notes

The authors declare no competing financial interest.

Abbreviations used

AKI, acute kidney injury; LPS, lipopolysaccharide; NO, nitric oxide; TNF-a, tumor

necrosis factor-α; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; TLRs, toll-like receptors; TLR4, toll-like receptor 4; SuFEx, sulfur (VI) fluoride exchange; NSAID, nonsteroidal anti-inflammatory drugs; NOS2, nitric oxide synthase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ELISA, enzyme linked immunosorbent assay; BUN, blood urea nitrogen; SCR, serum creatinine; H&E, hematoxylin-eosin; PAS, periodic acid-schiff; DMEM, dulbecco's modification of eagle's medium dulbecco.

Appendix A. Supplementary material

The copies of representative ^{1}H and ^{13}C NMR spectra can be found at http://

References

[1] A. Zuk, J.V. Bonventre, Acute Kidney Injury, Annu. Rev. Med. 67 (2016) 293-307.

[2] N. Pabla, Z. Dong, Cisplatin nephrotoxicity: mechanisms and renoprotective strategies, Kidney Int. 73 (2008) 994-1007.

[3] A. Yonezawa, K. Inui, Organic cation transporter OCT/SLC22A and H (+)/organic cation antiporter MATE/SLC47A are key molecules for nephrotoxicity of platinum agents, Biochem. Pharmacol. 81 (2011) 563-568.

[4] S.M. Bagshaw, Short- and long-term survival after acute kidney injury, Nephrol. Dial. Transplant. 23 (2008) 2126-2128.

[5] K. Matsushita, T. Saritas, M.B. Eiwaz, N. McClellan, I. Coe, W. Zhu, M.Z. Ferdaus, L.Y. Sakai, J.A. McCormick, M.P. Hutchens, The acute kidney injury to chronic kidney disease transition in a mouse model of acute cardiorenal syndrome emphasizes the role of inflammation, Kidney Int. 97 (2020) 95-105.

[6] H. Rabb, M. Griffin, D. McKay, S. Swaminathan, P. Pickkers, M. Rosner, J. Kellum, C. Ronco, Inflammation in AKI: Current Understanding, Key Questions, and Knowledge Gaps, J. Am. Soc. Nephrol. 27 (2016) 371-379.

[7] F. Tögel, C. Westenfelder, Kidney protection and regeneration following acute injury: progress through stem cell therapy, Am. J. Kidney Dis. 60 (2012) 1012-1022.

[8] A. Zarjou, A. Agarwal, Sepsis and Acute Kidney Injury, J. Am. Soc. Nephrol. 22

(2011) 999-1006.

[9] S. Hwang, Y. Kim, Y. Park, H. Lee, K. Kim, Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells, Inflamm Res. 63 (2014) 81-90.

[10] L. Zhang, Y. Fan, H. Su, L. Wu, Y. Huang, L. Zhao, B. Han, G. Shu, M. Xiang, J. Yang, Chlorogenic acid methyl ester exerts strong anti-inflammatory effects via inhibiting the COX-2/NLRP3/NF-κB pathway, Food Funct. 9 (2018) 6155-6164.

[11] S. Wang, L. Hu, X. Gao, H. Guo, G. Fan, Anti-inflammatory activity of salvianolic acid B in microglia contributes to its neuroprotective effect, Neurochem Res. 35 (2010) 1029-1037.

[12] J. Zhao, X.-C. Yang, M. Fujino, N. Ichimaru, W. Que, X.-K. Li, S. Takahara, Salvianolic acid B ameliorates liver injury in a murine aGvHD model by decreasing inflammatory responses via upregulation of HO-1, Transpl. Immunol. 55 (2019) 101203.

[13] M. Krasavin, A. Lukin, N. Zhurilo, A. Kovalenko, I. Zahanich, S. Zozulya, Novel agonists of free fatty acid receptor 1 (GPR40) based on 3-(1,3,4-thiadiazol-2-yl) propanoic acid scaffold, J. Enzyme. Inhib. Med. Chem. 31 (2016) 1404-1410.

[14] G. Palermo, A. Favia, M. Convertino, M. De Vivo, The Molecular Basis for Dual Fatty Acid Amide Hydrolase (FAAH)/Cyclooxygenase (COX) Inhibition, Chem. Med. Chem. 11 (2016) 1252-1258.

[15] A. Gouda, E. Beshr, F. Almalki, H. Halawah, B. Taj, A. Alnafaei, R. Alharazi, W. Kazi, M. AlMatrafi, Arylpropionic acid-derived NSAIDs: New insights on derivatization, anticancer activity and potential mechanism of action, Bioorg. Chem. 92 (2019) 103224.

[16] Y. Bansal, O. Silakari, Multifunctional compounds: Smart molecules for multifactorial diseases, Eur. J. Med. Chem. 76 (2014) 31-42.

[17] S. Bhowmik, S. Batra, Applications of Morita-Baylis-Hillman Reaction to the Synthesis of Natural Products and Drug Molecules, Curr. Org. Chem. 18 (2014) 3078-3119.

[18] S. Singh, S. Bhat, Antimicrobial potential of 3-hydroxy-2-methylene-3-

phenylpropionic acid derivatives, Acta. Pharm. 61 (2011) 447-455.

[19] W. da Silva, D. Rodrigues, R. de Oliveira, R. Mendes, T. Olegário, J. Rocha, T. Keesen, C. Lima-Junior, M. Vasconcellos, Synthesis and activity of novel homodimers of Morita-Baylis-Hillman adducts against *Leishmania donovani*: A twin drug approach, Bioorg. Med. Chem. Lett. 26 (2016) 4523-4526.

[20] S. Sousa, J. Rocha, T. Keesen, E. Silva, P. de Assis, J. de Oliveira, S. Capim, F. Xavier, B. Marinho, F. Silva, C. Lima-Junior, M. Vasconcellos, Synthesis of 16 New Hybrids from Tetrahydropyrans Derivatives and Morita-Baylis-Hillman Adducts: In Vitro Screening against *Leishmania donovani*, Molecules 22(2) (2017) 207.

[21] X.M. Meng, D.J. Nikolic-Paterson, H.Y. Lan, Inflammatory processes in renal fibrosis, Nat. Rev. Nephrol. 10 (2014) 493-503.

[22] Q. Cao, Y. Wang, D. Harris, Pathogenic and protective role of macrophages in kidney disease, Am. J. Physiol. Renal Physiol. 305 (2013) F3-11.

[23] P. Nelson, A. Rees, M. Griffin, J. Hughes, C. Kurts, J. Duffield, The renal mononuclear phagocytic system, J. Am. Soc. Nephrol. 23 (2012) 194-203.

[24] S. Huen, L. Huynh, A. Marlier, Y. Lee, G. Moeckel, L. Cantley, GM-CSF Promotes Macrophage Alternative Activation after Renal Ischemia/Reperfusion Injury, J. Am. Soc. Nephrol. 26 (2015) 1334-1345.

[25] S. Anavi, O. Tirosh, iNOS as a metabolic enzyme under stress conditions, Free Radic. Biol. Med. 146 (2020) 16-35.

[26] C. Bogdan, Nitric oxide and the immune response, Nat. Immunol. 2 (2001) 907-916.

[27] S. Faubel, E. Lewis, L. Reznikov, D. Ljubanovic, T. Hoke, H. Somerset, D. Oh, L. Lu, C. Klein, C. Dinarello, C. Edelstein, Cisplatin-induced acute renal failure is associated with an increase in the cytokines interleukin (IL)-1beta, IL-18, IL-6, and neutrophil infiltration in the kidney, J. Pharmacol. Exp. Ther. 322 (2007) 8-15.

[28] G. Ramesh, W. Reeves, TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity, J. Clin. Invest. 110 (2002) 835-842.

[29] C. Bogdan, The multiplex function of nitric oxide in (auto)immunity, J. Exp. Med. 187 (1998) 1361-1365.

[30] A. Rios, H. Vargas-Robles, A. Gámez-Méndez, B. Escalante, Cyclooxygenase-2 and kidney failure, Prostaglandins Other Lipid Mediat. 98 (2012) 86-90.

[31] J. Hernández, H. Astudillo, B. Escalante, Angiotensin II stimulates cyclooxygenase-2 mRNA expression in renal tissue from rats with kidney failure, Am. J. Physiol. Renal Physiol. 282 (2002) F592-598.

[32] R. Harris, M. Zhang, H. Cheng, Cyclooxygenase-2 and the renal renin-angiotensin system, Acta. Physiol. Scand. 181 (2004) 543-547.

[33] J. Schnermann, J.P. Briggs, Tubular control of renin synthesis and secretion, Pflugers Arch. 465 (2013) 39-51.

[34] H. Liu, W. Ye, G. Guan, Z. Dong, Z. Jia, T. Yang, Developmental regulation of calcineurin isoforms in the rodent kidney: association with COX-2, Am J Physiol Renal Physiol. 293 (2007) F1898-1904.

[35] P. Slattery, S. Frolich, Y. Schreiber, R.M. Nusing, COX-2 gene dosage-dependent defects in kidney development, Am. J. Physiol. Renal Physiol. 310 (2016) F1113-1122.

[36] Z. Jia, N. Wang, T. Aoyagi, H. Wang, H. Liu, T. Yang, Amelioration of cisplatin nephrotoxicity by genetic or pharmacologic blockade of prostaglandin synthesis, Kidney Int. 79 (2011) 77-88.

[37] S. Agrawal, A. Guess, M. Chanley, W. Smoyer, Albumin-induced podocyte injury and protection are associated with regulation of COX-2, Kidney Int. 86 (2014) 1150-1160.

[38] P. Ranganathan, C. Jayakumar, R. Mohamed, Z. Dong, G. Ramesh, Netrin-1 regulates the inflammatory response of neutrophils and macrophages, and suppresses ischemic acute kidney injury by inhibiting COX-2-mediated PGE2 production, Kidney Int. 83 (2013) 1087-1098.

[39] K. Hamidzadeh, S.M. Christensen, E. Dalby, P. Chandrasekaran, D.M. Mosser, Macrophages and the Recovery from Acute and Chronic Inflammation, Annu. Rev. Physiol. 79 (2017) 567-592.

[40] A. Rousta, S. Mirahmadi, A. Shahmohammadi, D. Nourabadi, M. Khajevand-Khazaei, T. Baluchnejadmojarad, M. Roghani, Protective effect of sesamin in lipopolysaccharide-induced mouse model of acute kidney injury via attenuation of

oxidative stress, inflammation, and apoptosis, Immunopharmacol. Immunotoxico. 40 (2018) 423-429.

[41] Y. Jiang, N.S. Alharbi, B. Sun, H.-L. Qin, SO_2F_2 mediated cascade dehydrogenative Morita–Baylis–Hillman reaction of the C(sp3)–H of primary alcohols with the C(sp2)–H of electron-deficient olefins for the assembly of allylic alcohols, RSC Advances 9 (2019) 29784-29787.

[42] G. Zha, W. Fang, Y. Li, J. Leng, X. Chen, H. Qin, SOF-Mediated Oxidative Dehydrogenation and Dehydration of Alcohols to Alkynes, J. Am. Chem. Soc. 140 (2018) 17666-17673.

[43] G.F. Zha, W.Y. Fang, J. Leng, H.L. Qin, A Simple, Mild and General Oxidation of Alcohols to Aldehydes or Ketones by SO_2F_2/K_2CO_3 Using DMSO as Solvent and Oxidant, Adv. Synth. Catal. 361 (2019) 2262-2267.

[44] A. Weigert, A. von Knethen, D. Fuhrmann, N. Dehne, B. Brüne, Redox-signals and macrophage biology, Mol. Aspects. Med. 63 (2018) 70-87.

[45] B. Li, Y. Yang, L. Chen, S. Chen, J. Zhang, W. Tang, 18alpha-Glycyrrhetinic acid monoglucuronide as an anti-inflammatory agent through suppression of the NF-kappaB and MAPK signaling pathway, MedChemComm. 8 (2017) 1498-1504.

[46] M. Rossol, H. Heine, U. Meusch, D. Quandt, C. Klein, M.J. Sweet, S. Hauschildt, LPS-induced Cytokine Production in Human Monocytes and Macrophages, Crit. Rev. Immunol. 31 (2011) 379-446.

[47] H. Lin, T. Lee, S. Lin, Y. Yeh, T. Lu, C. Hsu, DDAH-2 alleviates contrast medium iopromide-induced acute kidney injury through nitric oxide synthase, Clin. Sci. 133 (2019) 2361-2378.

[48] S. Kim, D. Huri, S. Snyder, Inducible nitric oxide synthase binds, S-nitrosylates, and activates cyclooxygenase-2, Science 310 (2005) 1966-1970.

[49] B. Zhang, G. Ramesh, S. Uematsu, S. Akira, W.B. Reeves, TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity, J. Am. Soc. Nephrol. 19 (2008) 923-932.

[50] L. Patzer, Nephrotoxicity as a cause of acute kidney injury in children, Pediatr. Nephrol. 23 (2008) 2159-2173.

[51] G.S. Oh, H.J. Kim, A. Shen, S.B. Lee, D. Khadka, A. Pandit. S. HS, Cisplatin-induced Kidney Dysfunction and Perspectives on Improving Treatment Strategies, Electrolyte Blood Press. 12 (2014) 55-65.

[52] D. Moledina, C. Parikh, Phenotyping of Acute Kidney Injury: Beyond Serum Creatinine, Semin. Nephrol. 38 (2018) 3-11.

[53] L.S. Chawla, P.L. Kimmel, Acute kidney injury and chronic kidney disease: an integrated clinical syndrome, Kidney int. 82 (2012) 516-524.

[54] D. Martin-Sanchez, O. Ruiz-Andres, J. Poveda, S. Carrasco, P. Cannata-Ortiz, M.D. Sanchez-Nino, M. Ruiz Ortega, J. Egido, A. Linkermann, A. Ortiz, A.B. Sanz, Ferroptosis, but Not Necroptosis, Is Important in Nephrotoxic Folic Acid-Induced AKI, J. Am. Soc. Nephrol. 28 (2017) 218-229.

[55] B. Li, S. Cai, Y.A. Yang, S.C. Chen, R. Chen, J.B. Shi, X.H. Liu, W.J. Tang, Novel unsaturated glycyrrhetic acids derivatives: Design, synthesis and anti-inflammatory activity, Eur. J. Med. Chem. 139 (2017) 337-348.

[56] K.P. Kang, D.H. Kim, Y.J. Jung, A.S. Lee, S. Lee, S.Y. Lee, K.Y. Jang, M.J. Sung, S.K. Park, W. Kim, Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation, Nephrol. Dial. Transplant. 24 (2009) 3012-3020.

Declaration of interests

✓ ☐ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

The authors declare that they have no conflict of interets.

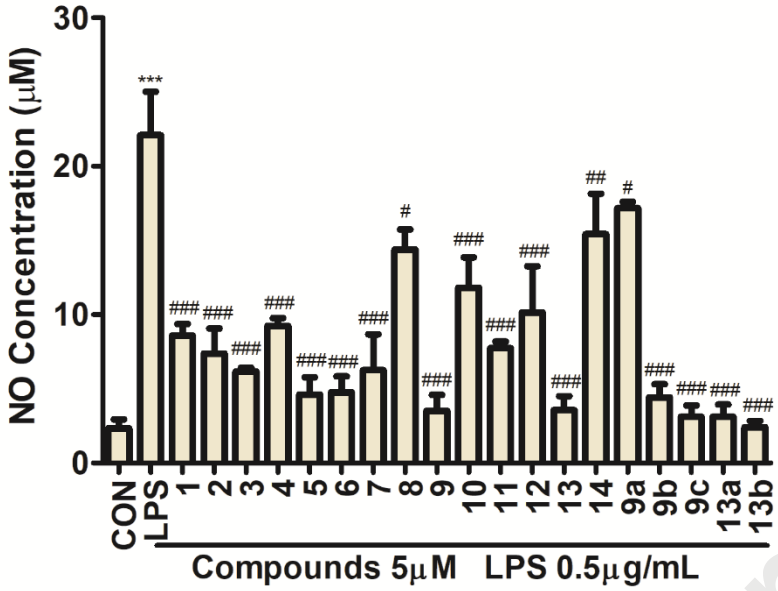


Fig. 3. The inhibitory effects of synthesized compounds on NO production in LPS-stimulated RAW 264.7 cells. RAW264.7 cells were pretreated with compounds at the same concentration (5 μM) for 1 h, then incubated with LPS (0.5 μg/mL) for 24 h. NO production in supernatant was detected by Griess reagent assay. $^{***}p < 0.001$ compared with the control group; $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$ compared with the LPS group.