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Discovery of novel 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivatives as potential anti-inflammatory agents

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Discovery of novel 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivatives as potential anti-inflammatory agents

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Abstract: A novel 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivative **5** with good anti-inflammatory activity was identified from our in-house library. Based on hit compound **5**, two series of 2-(piperidin-4-yl)-1H- benzo[d]imidazole derivative **6a-g** and **7a-h** were designed and synthesized as novel anti-inflammatory agents. Most of synthesized compounds exhibited good inhibitory activity on NO and TNF-α production in LPS-Stimulated RAW 264.7 Macrophages, in which the compound **6e** showed most potent inhibitory activity on NO (IC_{50} = 0.86 μM) and TNF-α (IC_{50} = 1.87 μM)

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production. Further evaluation revealed that compound **6e** displayed more potent in vivo anti-inflammatory activity than ibuprofen did on xylene-induced ear edema in mice. Additionally, western blot analysis revealed that compound **6e** could restore phosphorylation level of IκBα and protein expression of p65 NF-κB in LPS-stimulated RAW 264.7 macrophages.

Keywords: Inflammation; benzimidazole; 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivatives; anti-inflammatory activity.

Abbreviations: **NSAIDs** (non-steroidal anti-inflammatory drugs), **FLAP** (5-lipoxygenase-activating protein), **BK** (Bradykinin), **PPA** (polyphosphoricacid), **DIPEA** (diisoproplethyl amine), **DMF** (dimethylformamide), **PSA** (polar surface area), **LPS** (lipopolysaccharides), **TNF-α**(Tumour necrosis factor-alpha), **NO** (Nitric oxide), **ELISA** (enzyme-linked immunosorbant assay), **DMEM** (Dulbecco's modified Eagle medium), **DMSO** (dimethyl sulfoxide), **FBS** (fetal bovine serum), **OD** (optical density), **MTT** (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide).

Inflammation is a protective immune response against harmful stimuli including damaged cells, irritants, or pathogens (1). However, dysfunctional inflammation response will cause tissue damage, leading to an emergence of chronic inflammation, which is a risk factor for a host of chronic diseases such as autoimmune disorders, neurodegenerative disorders, and cancer (2, 3). Current treatment of inflammation involves extensive use of non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (4). However, long term administration of NSAIDs is associated with serious adverse effects, including cardiovascular toxicity, gastrointestinal ulcerations, platelet dysfunction, and renal and hepatic toxicity (5, 6). Meanwhile, high doses and prolonged usage of glucocorticoids cause serious side effects such as Cushing syndrome, osteoporosis, and reduced rate of bone growth in children (7). Thus, there is clearly an unmet medical need for more effective and safe anti-inflammatory agents.

Benzimidazole derivatives exhibit various biological activity, including antiparasitic(8), antiulcer(9), antihypertensive(10, 11), antitumor(12, 13), antibacterial(14), etc, and have therefore attracted

considerable pharmaceutical interest (15-18). Recently, benzimidazole scaffold has gained increasing importance as a pharmacophore for designing anti-inflammatory and analgesic agents (**Fig.1**) (19, 20). For example, benzoimidazole derivative **1** was found to be a highly selective CB2 agonist of Ki value 3.3 nM in attenuating pain and neuroinflammation(21). Compound **2** was identified as a potent 5-lipoxygenase-activating protein (FLAP) inhibitor (IC_{50} = 0.12 μM.), which is currently considered as a promising and clinically relevant strategy for pharmacological intervention with inflammatory diseases (22).

Also, 1-benzylbenzimidazole **3** was described as a Bradykinin (BK) B1 receptor antagonist with excellent affinity (EC_{50} = 2 nM) and **3** was used as anti-inflammatory analgesic (23). In addition, bilastine **4** has shown to possess anti-inflammatory activity (24). Based on above findings, benzimidazole is a privileged structure that can be used in drug design as anti-inflammatory agents.

With promising anticipation on benzimidazole derivatives as stated above, we started a preliminary study on investigating the anti-inflammatory effects of novel benzimidazole derivatives, and a novel 2-(piperidin-4-yl)-1H- benzo[d]imidazole derivative **5** with good anti-inflammatory activity was identified from our in-house library. Compound **5** exhibited similar anti-inflammatory activity with that of ibuprofen in xylene-induced ear swelling assay in mice. Further evaluation revealed that compound **5** showed potent activity in inhibitions of NO and TNF-a production in LPS-Stimulated RAW 264.7 macrophages with IC_{50} value of 10.35 μM and 7.70 μM, respectively. Physicochemical properties calculation revealed that compound **5** with a moderate molecular weight of 393.48 and log P value of 3.56 might display good drug-like properties. Therefore, compound **5** would serve as a promising hit compound for further optimization. Considering short side chain of ethyl group at 1-position of compound **5**, we firstly modified this site by introducing long side chains and aromatic rings (compound **6a-g**, **Fig. 1**). Since phenylacetic acid derivatives also exhibited excellent anti-inflammatory activity (25), a series of phenylacetic acid derivatives (compound **7a-h**, **Fig. 1**) were designed. In this paper, by modifying this hit compound **5**, two series of 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivative **6a-g** and **7a-h** were designed and synthesized, and their anti-inflammatory activity were evaluated.

Methods and Materials

General chemistry

All chemicals (reagent grade) used were purchased from Sigma-Aldrich (USA) and Sinopharm Chemical Reagent Co. Ltd (China). Melting points were measured on capillary tube and were uncorrected. IR spectra (in KBr pellets) were taken using Shimadzu FT-IR-8400S spectrophotometer. ^{1}H NMR spectra were measured on Bruker AV-300 or ACF 500 spectrometer at 25 °C and referenced to TMS. Chemical shifts were reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns were designed as s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses were performed on CHN-O-Rapid instrument (Elementar, Hanau, Germany). Analytical thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF 254).

Detailed synthetic procedures and characterization data for all new compounds **9-14, 5, 6a-g** and **7a-h** are provided in the Supporting Information (Appendix S1) of this article.

Cell culture and treatment

Mouse RAW 264.7 macrophages were obtained from the Shanghai Institute of Cell Biology (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL of penicillin and 100 μg/mL of streptomycin (Amresco) at 37°C in 5% CO_2/95% air. LPS purchased from Sigma (St. Louis, MO) was dissolved in FBS. Test compounds were dissolved in dimethyl sulfoxide (DMSO) for in vitro experiments.

Cell viability test

Cell viability was assessed using a modified 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay(26). Briefly, cells (3×10^5 cells/well) were seeded in a 96 well plate and treated with test compounds at 80 μM with LPS (100 ng/mL) for 24 h. Then, 100 μL of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well and further incubated for 4 h at 37 °C. Subsequently, 100 μL of DMSO was added to each well to dissolve any deposited formazan. The optical density (OD) of each well was measured at 540 nm with CORONA SH-1000 microplate reader (Ibaraki-Ken, Japan). All experiments were carried out in triplicate.

Measurement of nitrite (NO) levels

Measurement of nitrite (NO) levels was performed according to a described method (27). RAW 264.7 cells were seeded in 96-well plates at a density of 3.0×10^5/well. Cells were pretreated with DMSO and test compounds (0-80 μM dose range) for 2 h, and then incubated with LPS (100 ng/mL) for 24 h. The culture media were collected to measure nitrite concentrations using Griess reaction by adding 100 μL Griess reagent (1% [w/v] sulfanilamide in 5% phosphoric acid and 0.1% [w/v] N-1-naphthyl-ethylendiamide-dihydrochloride (NED) in H_2O)). The absorbance at 550 nm was measured in CORONA SH-1000 microplate reader (Ibaraki-Ken, Japan) and a series of known concentrations of sodium nitrite were used as the standards.

Measurement of TNF-α production using ELISA assay

Measurement of TNF-α production using ELISA assay was performed according to a described method (28). RAW 264.7 Cells were pretreated with test compounds (0-80 μM) or vehicle control for 2 h, and then treated with LPS (100 ng/ml) for 24 h. After treatment, the culture medium was collected separately and centrifuged at 1000 rpm for 10 min. After centrifugation, the supernatant was separated and stored at -80 °C until further use. Levels of TNF-α were determined by enzyme-linked immunosorbant assay (ELISA) using mouse TNF-α ELISA Kits (R&D, Minneapolis USA).

Experiment of xylene-induced ear edema in mice

Xylene-induced ear edema in mice was performed according to a previously described method (29). Overnight fasted Male Kunming (KM) mice (20 ± 2 g) (12 h) were randomly divided into groups (n=8/group). One group of mice, served as a control group, was given vehicle (0.5% CMC in water in a volume of 20 mL/kg) only. The rest of groups, which were served as experimental groups, were given two doses (4 and 12 mg/kg) of test compounds and ibuprofen suspended in vehicle (20 mL/kg) orally (p.o.) for 5 days. 30 min after the last administration, 0.1 mL of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. One hour after the application of xylene, mice were killed and both ears were removed. Circular sections were taken, using a cork borer with a diameter of 9 mm, and measured. The degree of ear swelling was calculated based on the weight of left ear without xylene. Ear edema (ΔW) was calculated as follows: $\Delta W = W_R - W_L$. The weights of right (W_R) and left (W_L) ears were measured (mg) respectively of the same

mouse. Edema inhibitory activity was calculated according to the following formula: Percentage inhibition (%) = (Wcontrol − Wtreated) / Wcontrol × 100.

Measurement of protein expressions (p-IκB, IκB, p65 NF-κB) using Western Blot

RAW 264.7 Cells were pretreated with test compounds (10 μM) or vehicle control for 2 h, and then treated with LPS (100 ng/ml) for 24 h. Protein extraction of cytosolic and nuclear fractions was performed by using a cytosolic/nuclei isolation kit (KeyGEN, Nanjing, China), according to a multiple centrifugation method. Protein concentration was determined using an enhanced BCA protein assay (Beyotime, China). Equal quantities of cytosolic/nuclear proteins from each treatment (80 μg of total protein/lane) were resolved on 10% SDS-PAGE and then transferred to polyvinylidene fluoride membranes (Millipore, MA, USA). Membranes were blocked in TBST (Tris-buffered saline (TBS) containing 0.1% Tween-20) containing 5% skimmed milk power for 1 h at 37 °C. The membranes were then treated individually with specific antibody diluted in TBST containing 5% skimmed milk power, which includes p-IκB, IκB, p65 NF-κB (#9936, 1:1000) and α-tubulin (#2144, 1:1000) antibodies (CST, MA, USA). After incubating with horseradish peroxidase-conjugated secondary antibody (1:5000, Bioworld, MN, USA), immunoreactive bands were visualized by TMB reagent (KPL, Maryland, USA).

Statistical analysis

Data were expressed as means ± S.D. of at least three independent experiments performed in triplicate. Statistical analysis was performed using a one-way analysis of variance (ANOVA). The difference between the mean values of two groups was assessed by the Student–Newman–Keuls test. Multiple group comparisons were performed using a Dunnett's test. SPSS version 12 was used for all statistical analyses. Column charts were generated using Prism 5.0 software (GraphPad Software, INC, La Jolla, CA). P-values of less than 0.05 were considered significant.

Results and discussion

Chemistry

The synthetic route adopted to obtain compound **5** and the target compounds **6a-g** and **7a-h** is diagrammed in **Scheme 1**. Cyclization of commercially available benzene-1,2-diamine (**8)** with

piperidine-4-carboxylic acid in polyphosphoricacid (PPA) afforded compound **9.** Compound **9** was protected with t-butyloxycarboryl (Boc) group at the piperidine nitrogen to give compound **10**, which was further alkylated with NaH and alkyl halides (R_1X) to give compounds **11a-h.** Subsequently, deprotection of Boc from **11a-h** yielded compounds **12a-h**. Compounds **14i-ii** were obtained from substitution of commercially available 4-hydroarmotic acid ester **13i-ii** with 1,2-dibromoethane. The final coupling of compounds **12a-h** and compounds **14i-ii** using diisoproplethyl amine (DIPEA) in dimethylformamide (DMF) gave **15a-h** and **16a-h,** which were hydrolyzed with LiOH to afford compound **5** and the target compounds **6a-g** and **7a-h.** The target compounds **6a-g** and **7a-h** are presented in Table 1, and their structures were fully characterized by spectroscopic methods and the elemental analysis.

Physicochemical properties.

Physicochemical properties of drugs strongly influenced the absorption, distribution, biological availability, and pharmacological activity of drugs(25). Lipophylicity and polar surface area (PSA) and Lipinski Rule of five parameters were important physicochemical parameters of target compounds that were calculated and shown a result that all these new compounds except compounds **6g** and **7h** met the criteria of Lipinski's "Rule of Five" and might have favorable physicochemical properties. The results were presented in **Table 1**.

Inhibition of NO Production in LPS-Stimulated Macrophages.

Nitric oxide (NO) is an important pro-inflammatory mediator in the pathogenesis of inflammation(30). Excessive production of NO was found to be detrimental to host tissues (31). Thus, pharmacological inhibition of NO is considered to be a promising therapeutic strategy for the treatment of inflammatory disorders(32). Therefore, the effect of target compounds **6a-g** and **7a-h** on NO inhibition at 50 μM was firstly determined by using the lipopolysaccharides (LPS)-stimulated Raw 264.7 machrophages (27)**.** Dexamethasone and ibuprofen were chosen as positive controls. These compounds by which the inhibition ratio of NO production exceeded 40% were chosen for the IC_{50} value test. The results were listed in **Table 2**.

In general, most compounds shown more potent or comparable activity as compared with ibuprofen. Hit compound **5** with R_1 as ethyl group showed potent inhibitory activity (IC_{50} = 10.35 μM), being 5-fold more potent than positive control ibuprofen (IC_{50} = 56.34 μM). In the series of benzoimidazole derived benzoic acid **6a-g**, when ethyl group was replaced with long-chain groups such as butyl (**6a**), methoxyethyl (**6b**), ethoxyethyl (**6c**) and phenoxyethyl (**6d**), the inhibitory activity decreased dramatically. Compound **6e**, containing a benzyl group, showed the most potent activity with IC_{50} values 0.86 μM, which was tenfold more potent than hit compound **5**, and was 50-fold more potent than ibuprofen. However, substituted benzyl groups **6h** and **6g** showed slightly decreased potency compared to benzyl group **6e**. In the case of benzoimidazole derived phenylacetic acid **7a-h**, all of the compounds exhibited lower inhibitory activity, of which compound **7b** (IC_{50} = 25.39 μM) showed twofold less potent activity than hit compound **5**.

Comparing with dexamethasone (IC_{50}= 10.57 μM), Hit compound **5** showed comparable inhibitory activity (IC_{50} = 10.35 μM). Compounds **6a** (IC_{50}= 20.59 μM), **6f** (IC_{50}= 24.47 μM), **6g** (IC_{50}= 26.74 μM), **7b** (IC_{50}= 25.39 μM) and **7e** (IC_{50}= 28.67 μM) were two-fold less potent than positive control dexamethasone. Compound **6e** showed the most potent inhibitory activity with IC_{50} values 0.86 μM, which was tenfold more potent than hit compound **5** and positive control dexamethasone, respectively. In this regard, Compounds **6a**, **6e**, **6f**, **6g**, **7b** and **7e** were selected for further studies of anti-inflammatory activity.

To exclude the possibility that the inhibition of inflammatory response were due to cytotoxicity caused by treatment of these compounds, the cell viability was determined by MTT assay (26). RAW 264.7 macrophages were treated with individual compounds at 80 μM with LPS for 24 h. As shown in **Table 2**, compared with the positive control dexamethasone and ibuprofen, all compounds did not show significant cytotoxicity (the viability of Raw 264.7 cells: >95%) at the concentrations of 80 μM.

Inhibition of TNF-α production in in LPS-Stimulated Macrophages.

Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine that plays an important role in many inflammatory processes. Over-production of TNF-α is associated with the pathogenesis of a range of inflammatory diseases(33). Thus, further evaluation on effectiveness of compounds **6a**, **6e**, **6f**,

6g, **7b** and **7e** in inhibition of TNF-α production in RAW 264.7 macrophages was performed. Dexamethasone and ibuprofen were served as positive controls. Macrophages were incubated with LPS for 24 h in the presence of the compounds at a range of concentration (0-80 μM). The levels of TNF-α in the culture medium supernatant were detected by enzyme-linked immunosorbant assay (ELISA) (28).

The result in **Table 2** indicated that hit compound **5** shown strong inhibition on TNF-α production with IC_{50} value of 7.70 μM. Its activity was more potent than ibuprofen (IC_{50} = 50.47 μM), but was less potent than dexamethasone (IC_{50} = 2.45 μM). Compounds **6a**, **6f, 6g**, **7b** and **7e** exhibited moderate inhibitory effect on TNF-α production. Compound **6e** showed the most potent inhibitory effects on TNF-α production with IC_{50} value of 1.87 μM, which was more potent than positive control dexamethasone. Compound **6e** showed most potent inhibitory activity on both NO (IC_{50} = 0.86 μM) and TNF-α (IC_{50} = 1.87 μM) production. Next, the *in vivo* anti-inflammatory activity of compound **6e** was performed.

***In vivo* anti-inflammatory activity on xylene-induced ear edema in mice**

In vivo anti-inflammatory activity of hit compound **5** and compound **6e** was evaluated by the xylene-induced ear edema in mice(29). Topical application of xylene induced cutaneous inflammation at the ears of mice, which caused a significant increase in earplug weight of the right ear when compared to that of vehicle-treated left ear. Treating the mice with ibuprofen in doses of 4 mg/kg and 12 mg/kg significantly gave rise to an inhibition of 26.77% and 39.34% in earplug weight (**Fig. 2**). Moreover, oral administration of compounds **5** and **6e** at 4 and 12 mg/kg significantly decreased earplug weight of xylene-treated mice with a dose-dependent manner. Hit compound **5** displayed similar inhibition rate (26.23% and 37.70%) with ibuprofen, while compound **6e** showed the highest anti-inflammatory inhibition rate with 33.30% and 50.60%, respectively. These findings indicated that compound **6e** contains reasonable anti-inflammatory activity and is more potent than ibuprofen.

Inhibition of NF-κB pathways

Finally, in order to determine whether the inhibition of NF-κB pathways contributed to the anti-inflammation, we evaluated the effects of hit compound **5** and the most active compound **6e** on phosphorylation levels of IκBα and protein expressions of p65 NF-κB in LPS-stimulated RAW264.7 macrophages by western blot analysis (**Fig. 3**). LPS stimulation significantly up-regulated the phosphorylation level of IκB (p-IκB) and protein expression of p65 NF-κB, which could be restored by compounds **5** (p-IκB: 36%, p65 NF-κB: 48%) and **6e** (p-IκB: 68%, p65 NF-κB: 81%) at 10 μM, as well as positive control dexamethasone (p-IκB: 56%, p65 NF-κB: 70%) at 10 μM. More importantly, **6e** was more potential in regulating expressions of p-IκB, IκB and p65 NF-κB than dexamethasone, nevertheless, the effect of hit compound **5** was weaker than that of dexamethasone. Thus, data suggest that compound **6e** could restore the phosphorylation of IκB (p-IκB) and protein expressions of p65 NF-κB in LPS-stimulated RAW264.7 macrophages.

Conclusion

In this study, by modifying the hit compound **5**, two series of 2-(piperidin-4-yl)-1H-benzo[d]imidazole derivative **6a-g** and **7a-h** were designed, synthesized and tested for anti-inflammatory activity. Firstly, physicochemical parameters calculation revealed that most of new compounds met the criteria of Lipinski's "Rule of Five" and might have favorable physicochemical properties. Then, all compounds were tested for the effect on NO inhibition and parts of them were tested their inhibition of TNF-a production by using the LPS-stimulated Raw 264.7 machrophages. Compound **6e** displayed the most potent inhibitory activity on NO (IC_{50} = 0.86 μM) and TNF-α (IC_{50} = 1.87 μM) production. Furthermore, *in vitro* anti-inflammatory evaluation on xylene-induced ear edema in mice revealed that compound **6e** was more potent than ibuprofen. Finally, western blot analysis revealed that compound **6e** could restore phosphorylation levels of IκBα and protein expressions of p65 NF-κB in LPS-stimulated RAW 264.7 macrophages. Taking all data into account, compound **6e** could be served as a promising lead for novel anti-inflammatory agents

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Figures and schemes captions

Figure 1 Structure of reported anti-inflammatory benzimidazole compounds and hit compound 5, and design of the target compounds.

Figure 2 The effects of compounds 5 and 6e on xylene-induced ear edema in mice. Compounds **5** and **6e**, and ibuprofen (4, 12 mg/kg, p.o.) were administered 30 min before topical application of xylene (0.1 ml) to the right ears of mice. Values are expressed as means ± S.D (n=8). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus vehicle group.

Figure 3 The effects of compouds 5 and 6e on phosphorylation levels of IκBα and protein expressions of p65 NF-κB in LPS-stimulated RAW264.7 macrophages. RAW264.7 macrophages were preteated with **5**, **6e** and dexamethasone at 10 μM for 2 h, and stimulated by 100 ng/mL LPS for another 24 h. Protein levels of p-IκB, IκB, p65 NF-κB and α-tubulin were detected by Western blot analysis. The protein level of p-IκB was normalized to that of IκB while the protein level of p65 NF-κB was normalized to that of α-tubulin. Values are expressed as means ± S.D (n=4). $^{###}P<0.001$ versus normal group; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus LPS vehicle group.

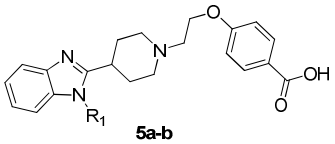
Scheme 1. Synthesis of the compound 5 and the target compounds 6a-g and 7a-h. Reagents and conditions: (a) piperidine-4-carboxylic acid, PPA, 150 °C, 5 h; (b) $(Boc)_2O$, NaOH, 0 °C, 16 h; (c) R_1X, NaH, DMF, , 80 °C, 3 h; (d) HCl (gas), ethyl acetate/dichloromethane (3:1), 0 °C, 5 h; (e) 1,2-dibromoethane, K_2CO_3, KI, CH_3CN, 70°C, 18 h; (f) DIPEA, DMF, 80 °C, 16 h; (g) LiOH, MeOH/

tetrahydrofuran/ H_2O(1: 2: 2), 70 °C, 16 h.

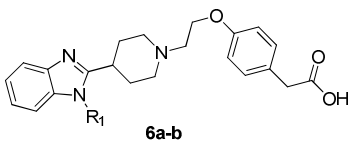
Table 1. Structures and calculated physicochemical parameters of target compounds 6a-g and 7a-h.

Table 2. Cell viability and inhibitory activity of target compounds 6a-g and 7a-h on NO and TNF-α production in LPS-Stimulated RAW 264.7 Cells.

Table 1. Structures and calculated physicochemical parameters of target compounds 6a-g and 7a-h.



5a-b



6a-b

Compounds	R_1	MW	LogP[a]	HBA	HBD	RB	TPSA Å^2	MR[cm^3/mol]
		≤ 500	≤ 5	≤ 10	≤ 5	≤ 10	≤ 140	
5	ethyl	393.479	3.56	5	1	7	65.37	111.41
6a	1-butyl	421.532	4.46	5	1	9	65.37	120.6
6b	2-methoxyethyl	423.505	3.07	6	1	9	74.6	117.53
6c	2-ethoxyethyl	437.531	3.41	6	1	10	74.6	122.33
6d	2-phenoxyethyl	485.574	4.89	6	1	10	74.6	137.25
6e	benzyl	455.548	4.96	5	1	8	65.37	131.1
6f	4-methoxybenzyl	485.574	4.83	6	1	9	74.6	138.35
6g	4-chlorobenzyl	489.993	5.51	5	1	8	65.37	135.71
7a	ethyl	407.505	3.56	5	1	8	65.37	115.72
7b	1-butyl	435.559	4.46	5	1	10	65.37	124.92
7c	2-methoxyethyl	437.531	3.07	6	1	10	74.6	121.84
7d	2-ethoxyethyl	451.558	3.41	6	1	11	74.6	126.64
7e	2-phenoxyethyl	499.601	4.83	6	1	11	74.6	141.57
7f	benzyl	469.575	4.96	5	1	9	65.37	135.42
7g	4-methoxybenzyl	499.601	4.77	6	1	10	74.6	142.67
7h	4-chlorobenzyl	504.02	5.46	5	1	9	65.37	140.02

MW: Molecular weight (g/mol); LogP: n-octanolewater partition coefficient are calculated by Chemdraw 11.0; HBD: number of hydrogen bond donors; HBA: number of hydrogen bond acceptors; RB: number of rotatable bonds; TPSA: topological polar surface area; MR:

Table 2. Cell viability and inhibitory activity of target compounds 6a-g and 7a-h on NO and TNF-α production in LPS-Stimulated RAW 264.7 Cells

compds	Inhibition (%) at 50 μM	Inhibition of NO IC_{50} (μM) [a]	Inhibition of TNF-α IC_{50} (μM) [a]	Cell viability (%) [b]
5	107.08±3.06	10.35±0.46	7.70±1.06	98.87±1.36
6a	112.56±4.22	20.59±1.26	18.03±1.52	96.85±0.87
6b	50.08±2.23	37.32±2.17	ND	95.46±0.29
6c	38.97±1.83	ND	ND	97.34±1.47
6d	47.95±2.83	34.32±1.23	ND	96.85±0.59
6e	92.91±3.87	0.86±0.12	1.87±0.21	98.45±1.83
6f	81.65±4.25	24.47±1.82	19.24±1.32	94.32±2.93
6g	56.15±1.63	26.76±1.92	24.38±2.46	96.87±0.33
7a	36.67±2.46	ND	ND	95.74±2.71
7b	64.33±3.76	25.39±1.42	13.50±1.52	96.86±2.63
7c	37.84±1.48	ND	ND	97.56±1.98
7d	36.67±1.23	ND	ND	97.29±2.12
7e	46.15±2.37	28.67±2.25	33.49±2.34	94.67±2.98
7f	36.37±1.41	ND	ND	99.12±0.43
7g	45.64±2.23	37.83±3.41	ND	95.76±1.57
7h	45.13±2.38	40.83±2.68	ND	97.26±2.61
dexamethasone	82.56±1.18	10.57±0.42	2.45±0.16	97.45±0.83
Ibuprofen	41.73±2.08	56.34±3.22	50.47±3.18	98.87±0.43

Values are the mean values of three experiments. [a] IC_{50} values were not determined (ND). [b] Cell viability after treatment with 80 μM of each compound was expressed as a percentage (%) of the value obtained from LPS alone group.