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Discovery of dronedarone and its analogues as NLRP3 inflammasome inhibitors with potent anti-inflammation activity

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ABSTRACT

Inhibiting NLRP3 inflammasome activation is a prospective therapeutic strategy for uncontrolled inflammatory diseases. It is the first time that dronedarone, a multiply ion channel blocker, was identified as a NLRP3-inflammasome inhibitor with an IC_{50} value of 6.84 μM against IL-1β release. A series of novel 5-amide benzofuran derivatives were designed and synthesized as NLRP3-inflammasome inhibitors. Compound **8c** showed slightly increased activity (IC_{50} = 3.85 μM) against IL-1β release. Notably, treatment with **8c** could significantly inhibit NLRP3-mediated IL-1β release and ameliorate peritoneal inflammation in a mouse model of sepsis. Collectively, **8c** is a promising lead compound for further chemical development as a NLRP3 inhibitor with anti-inflammation effects.

Targeting NLRP3 inflammasome is a new and hot research field with strong potential to treat inflammatory diseases.[1,2] Over-activation of NLRP3 inflammasome is closely related to various diseases, such as peritonitis, hyperinflammation following virus infection, inflammatory bowel disease, Alzheimer's disease (AD), stroke, gout and nonalcoholic steatohepatitis (NASH).[3–8] Notably, NLRP3$^{-/-}$ mice exhibited improved pathology in a mice model for these diseases.[3] NLRP3 inflammasome is a multiprotein complex that has been extensively studied.[9] It is an oligomeric protein formed by activation of the sensor protein NLRP3 and then recruitment of ASC and procaspase-1.[10] NLRP3 activation relies on intracellular secondary signals triggered pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs),[11,12] unlike other inflammasome sensors (AIM2, NLRP1, or NLRC4) as the direct recognition of external stimuli.[10] To date, there are various intracellular secondary signals to activate NLRP3, such as intracellular ion fluxes, lysosomal resident cathepsin B, reactive oxygen species (ROS), and ubiquitin/deubiquitination post-translation modification of NLRP3.[13] Among these, intracellular ion fluxes are common and critical triggers signal of NLRP3 inflammasome activation.[14]

Ion fluxes, including K^+ efflux,[10,13] Ca^{2+} mobilization,[12] and Cl^- efflux,[15] have been early proposed to be related to the release of proinflammatory cytokine IL-1β mediated by NLRP3 inflammasome activation. Indeed, recent studies found that many ion channel blockers could inhibit NLRP3 inflammasome activation (Figure 1). For example, glyburide, a selective inhibitor of ATP-sensitive K^+ channel, inhibits IL-1β release in LPS-activated human monocytes with an IC_{50} of 12 μM.[16,17] Another K^+ channel blocker β-hydroxybutyrate (BHB) inhibits IL-1β release in LPS-primed mouse bone marrow-derived macrophages (BMDMs) with an IC_{50} of 2 ~ 10 mM.[18] As a Ca^{2+} channel blocker, nimodipine inhibits Aβ-stimulated IL-1β release from microglia cells with ~ 48% inhibition at 36 nM.[19] The Cl^- channel blocker ethacrynic acid inhibits ATP-induced release of IL-1β in human monocytes with the IC_{50} of 3 μM.[20] These findings suggest that blocking ion channel blockers such as K^+, Ca^{2+}, or Cl^- is a potential strategy against NLRP3 inflammasome activation.

Inspiringly, multiply blocking of ion fluxes may have beneficial effects on inhibition of NLRP3 inflammasome activation. Dronedarone, a marketed drug, is a multiply ion channel blocker, which can inhibit K^+, Ca^{2+} and Na^+ fluxes.[21,22] As expected, in the model of LPS and nigericin-stimulated NLRP3 inflammasome activation in J774A.1 cells,[23] dronedarone showed good inhibitory potency toward NLRP3 inflammasome mediated IL-1β release with IC_{50} of 6.84 μM. The potential of dronedarone to inhibit NLRP3 inflammasome drove us to identify NLRP3 inhibitors with increased potency.

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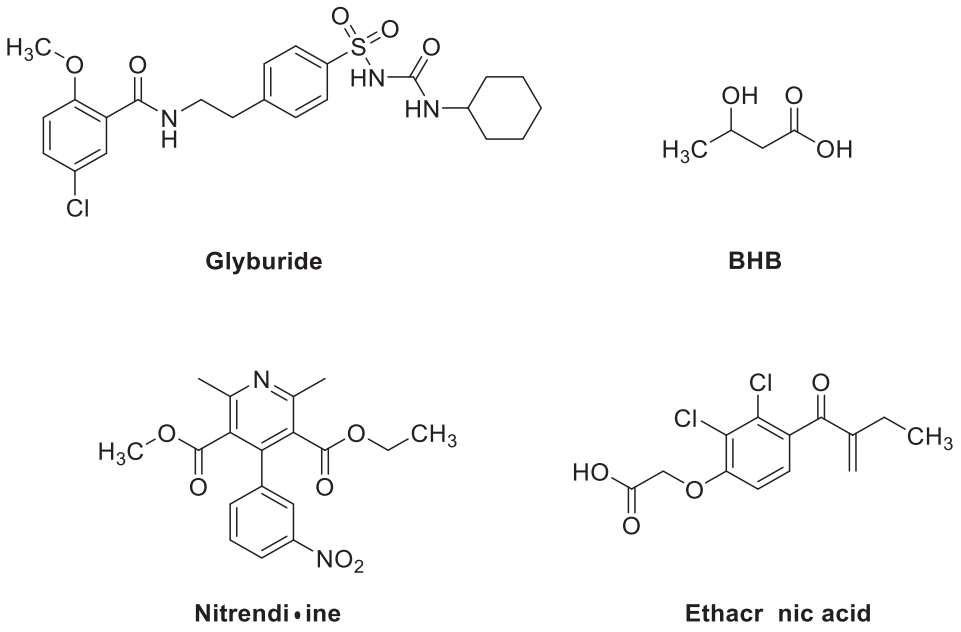


Fig. 1. Reported inhibitors of IL-1β as ion channel blockers.

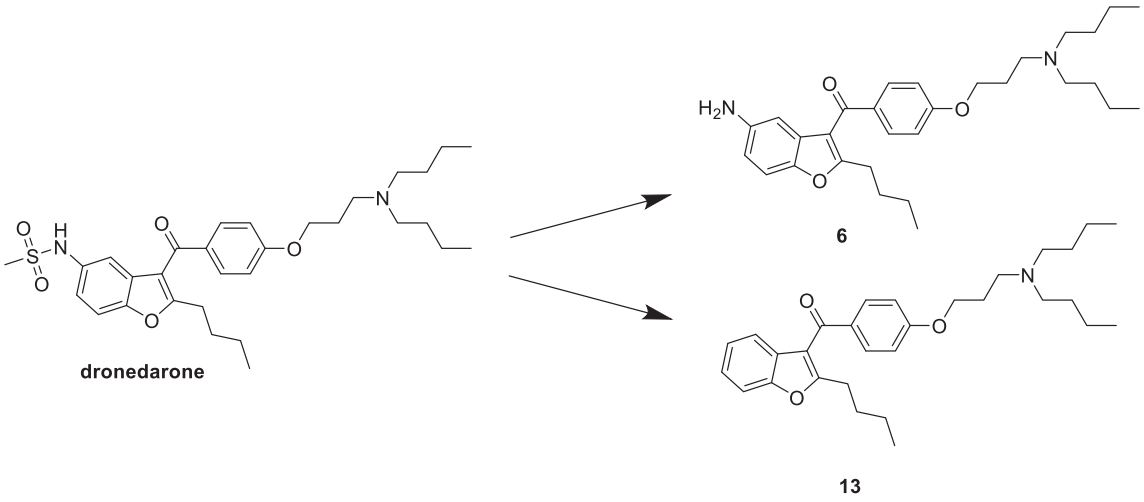
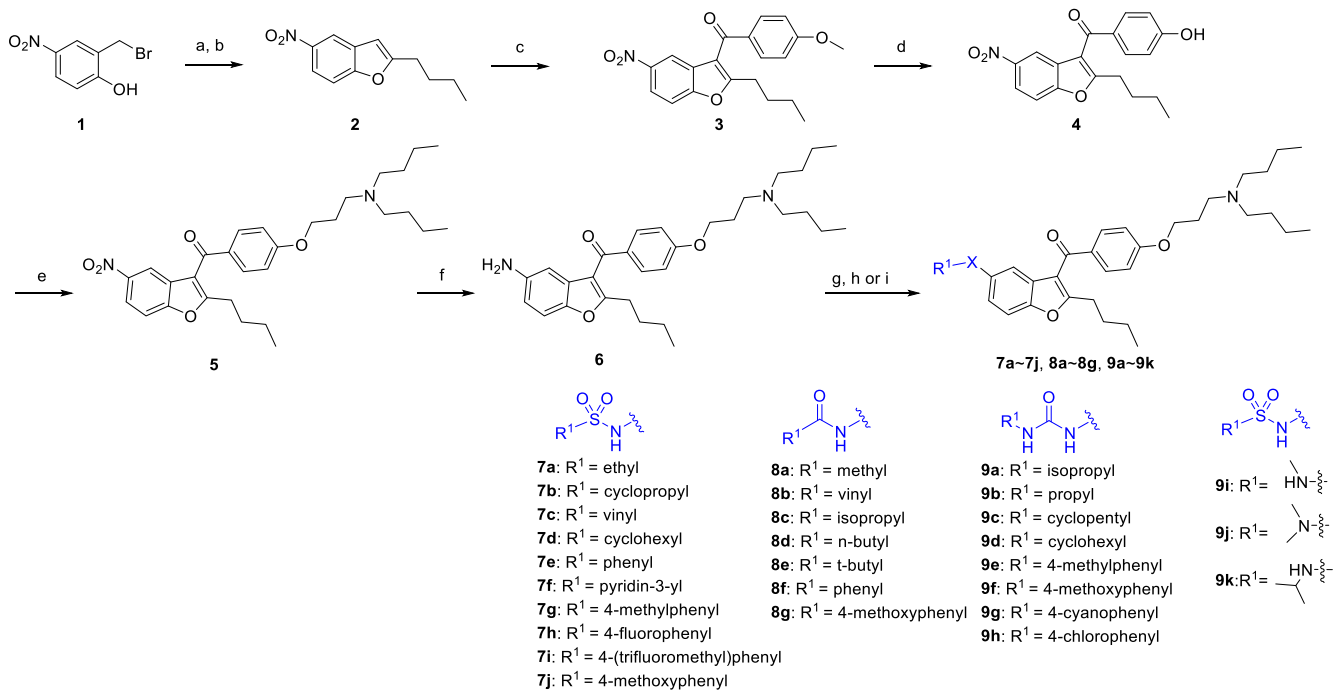


Fig. 2. Structures of dronedarone, **6** and **13**.

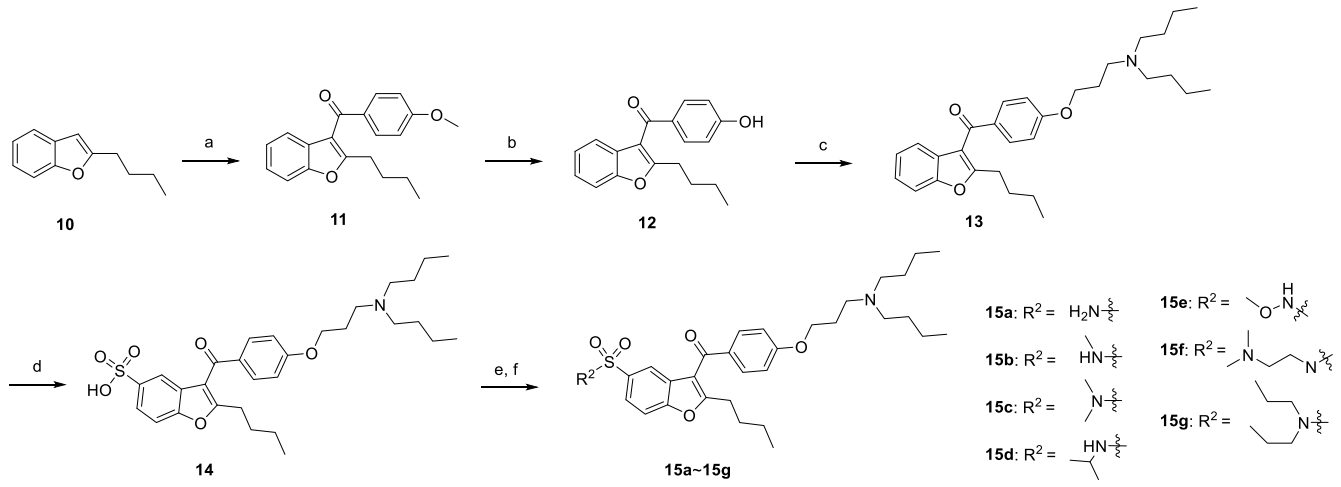
In the beginning, we found that the intermediate **6** and **13** (Figure 2), removal of methanesulfonyl or methanesulfonamide, led to the loss of potency on inhibiting IL-1β release mediated by NLRP3 inflammasome ($IC_{50} > 100$ μM). As the sulfonamide moiety seems to significantly affect NLRP3 inflammasome inhibiting activity, we focused our interest on modifying the C-5 aminosulfonyl group of dronedarone.

The preparation of various derivatives of dronedarone is illustrated in Scheme 1 and Scheme 2 according to similar reported procedures.[24,25] Commercially available 2-(bromomethyl)-4-nitrophenol underwent Wittig reaction[25] to provide benzofuran derivative **2**. Friedel-Craft acylation reaction[26] of **2** with 4-methoxybenzoyl chloride in the presence of $AlCl_3$ to afford the intermediate **3** which, upon demethylation using $AlCl_3$ in chlorobenzene, provided intermediate **4**. O-alkylation of **4** with *N*-butyl-N-(3-chloropropyl)butan-1-amine in the presence of potassium carbonate offered **5**, which was further reduced with iron power to provide the amine **6**.[25] Compound **7a** ~ **7j**, **8a** ~ **8g**, **9a** ~ **9k** were obtained from intermediate **6** with various substrate (sulfonyl chlorides, acyl chlorides, isocyanic acids or aminosulfonyl chlorides) by N-acylation[24] or nucleophilic addition[27] in the yield of 12%~85%. As shown in Scheme 2, similar to the previous synthetic route from **2** to **5**, intermediate **13** was obtained from **10** in three steps. Next, sulfonylation reaction[27] of **13** and chlorosulfonic acid afforded intermediate **14**. Treatment of **14** with oxalyl chloride,[28] and then condensation with the corresponding amines[29] provided compound **15a** ~ **15g** in the yield of 25%~62%.

To evaluate the ability of the compounds to inhibit NLRP3 inflammasome activation, all the synthesized compounds were tested for their ability to inhibit the release of IL-1β mediated via the NLRP3 inflammasome activation using LPS and nigericin-stimulated J774A.1 cells. As shown in Table 1, the prototype dronedarone showed good inhibitory activity with an IC_{50} value of 6.84 μM and was used as a benchmark to compare the potency of other analogs. As mentioned earlier, the sulfonyl at the C5-position amino group significantly affects the activities against NLRP3 inflammasome, so we first investigated the effect of different sizes of R substituents on sulfonamide. In general, small R group on the C-5 aminosulfonyl moiety resulted in favorable inhibitory activity, which increased in an order of cyclopropyl < vinyl < Et < Me with IC_{50} values of 45.5, 25.3, 19.0, and 6.84 μM, respectively. The six-member



Scheme 1. Chemical synthesis of **7a** ~ **7j**, **8a** ~ **8g**, **9a** ~ **9k**. Reagents and conditions: (a) Ph_3P, DCM, reflux, 1 h; (b) $CH_3(CH_2)_3COCl$, Et_3N, PhMe, reflux, 1 h; (c) 4-methoxybenzoyl chloride, $AlCl_3$, DCM, rt, 24 h; (d) $AlCl_3$, PhCl, 80 °C, 2 h; (e) *N*-butyl-N-(3-chloropropyl)butan-1-amine, K_2CO_3, KI, DMF, 65 °C, 5 h; (f) Fe, NH_4Cl, H_2O, EtOH, reflux, 1 h; (g) various sulfonyl chlorides, PhMe, Et_3N, rt, 2 h; (h) various acyl chlorides, DCM, Et_3N, rt, 2 h; (i) various isocyanic acids, DCM, Et_3N, 0 °C, 2 h.

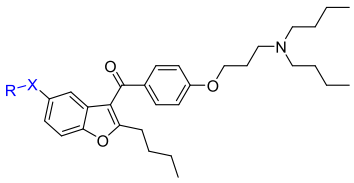


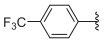
Scheme 2. Chemical synthesis of **15a** ~ **15g**. Reagents and conditions: (a) 4-methoxybenzoyl chloride, $AlCl_3$, DCM, rt, 24 h; (b) $AlCl_3$, PhCl, 80 °C, 2 h; (c) *N*-butyl-N-(3-chloropropyl)butan-1-amine, K_2CO_3, KI, DMF, 65 °C, 5 h; (d) $ClSO_3H$, rt, 12 h; (e) Oxalyl chloride, DMF, DCM, 0 °C, 2 h; (f) various amine, Et_3N, rt, 12 h.

ring substituents (cyclohexyl, 3-pyridyl, and phenyl) resulted in a 1.5 to 5.0-fold decrease of activities, as compared to that of dronedarone. The *para*-substituted phenyl derivatives **7g** ~ **7j** (Table 1), had no improvement in potency. The reversal of the C-5 aminosulfonyl group of dronedarone led to compound **15b** which is 3.2 times less potent as an inhibitor of NLRP3 inflammasome. The attempt to change different types of amino groups (**15a** ~ **15g**) failed to improve the inhibitory activities. Bioisosteric replacement is usually a tool of the utmost importance widely used in analogs design. Therefore, the replacement of methylsulfonamide function by an acetamido group showed similar inhibitory activity. Furthermore, various substitutions of R group in this series exhibited comparable activity to that of the parent compound, and 8c showed more potent activity with an IC_{50} value of 3.85 μM. The other sulfonamide bioisosteres, urea and aminosulfonamide function bioisosteres, had relatively negative effects on inhibition of IL-1β releasing via NLRP3 inflammasome. For example, the subseries of urea **9a** ~ **9h** and aminosulfonamide **9i** ~ **9k** exhibited low inhibitory with IC_{50} values of 13.3 ~ 74.8 μM and 7.65 ~ 13.4 μM, respectively. The potent activity of compound **8c** indicated that the compound has the potential to treat NLRP3 inflammasome-associated inflammatory diseases.

As the compound **8c** showed the most potent inhibition of IL-1β release, **8c** was further selected to investigate its preliminary mechanism. Compound **8c** exhibited a concentration-dependent inhibition of IL-1β release in J774A.1 cells (Figure 3A). The result of immunoblot analysis (Figure 3B) showed that **8c** dose-dependently decreased the amount of caspase-1 p20 and IL-1β in supernatants from the **8c**-treated cells. Compound **8c** did not affect the expression levels of pro-IL-1β, pro-

Table 1
Inhibitory potency against the IL-1β production of designed compounds.



Compound	R =	X =	IC_{50} (μM)	Compound	R =	X =	IC_{50} (μM)
Dronedarone	Me		6.84	**8a**	Me		6.77
7a	Et		19.0	**8b**			26.7
7b			45.5	**8c**			3.85
7c			25.3	**8d**			5.82
7d			25.0	**8e**			9.03
7e			10.7	**8f**			13.3
7f			33.4	**8g**			>100
7g			25.0	**9a**			13.3
7h			12.7	**9b**			19.2
7i			81.6	**9c**			25.0
7j			27.9	**9d**			74.8
15a	H		26.7	**9e**			71.2
15b	Me		22.2	**9f**			24.5
15d			42.5	**9g**			18.5
15e			83.8	**9h**			16.1
15f			>100	**9i**			11.4
15c*			61.1	**9j**			13.4
15g*			>100	**9k**			7.65

"*" represents structure R and X is combined in one structure.

caspase-1, NLRP3, or ASC in cell lysates. These findings suggest compound **8c** indeed suppresses NLRP3 Inflammasome-mediated caspase-1 activation and IL-1β secretion via acting on the inflammasome activation step.

Furthermore, we investigated whether **8c** could ameliorate the sepsis in animal models (Figure 4). Mice were injected intraperitoneally with LPS and showed significantly increased secretion of proinflammatory cytokines IL-1β, IL-6 and TNF-α in both the serum and peritoneal lavage fluid compared to the control group.[23,30] Treatment with **8c** (100 mg/kg/d) significantly reduced the level of NLRP3-inflammasome mediated IL-1β in both serum (42%) and peritoneal lavage fluid (22%). We noticed that compound **8c** had no or only slightly inhibitory effects on IL-6 level in vivo (2% in peritoneal fluid and 0% in serum), as well as TNF-α in peritoneal fluid. TNF-α in serum was an exception which showed an 18% reduction as compared to LPS-induced model group. These results suggest that the inhibitor **8c** may markedly suppress NLRP3 inflammasome-related acute inflammation in sepsis.

In conclusion, dronedarone, a multiply ion channel blocker, was identified as a novel NLRP3-inflammasome inhibitor. It exhibited a good inhibitory effect on NLRP3-inflammasome mediated IL-1β release with an IC_{50} value of 6.84 μM. A series of 5-amide benzofuran derivatives were designed, synthesized, and evaluated for IL-1β inhibitory activity. Of the compounds tested, **8c** showed slightly increased biological profile exhibiting an IL-1β inhibitory activity (IC_{50}) of 3.85 μM. Further

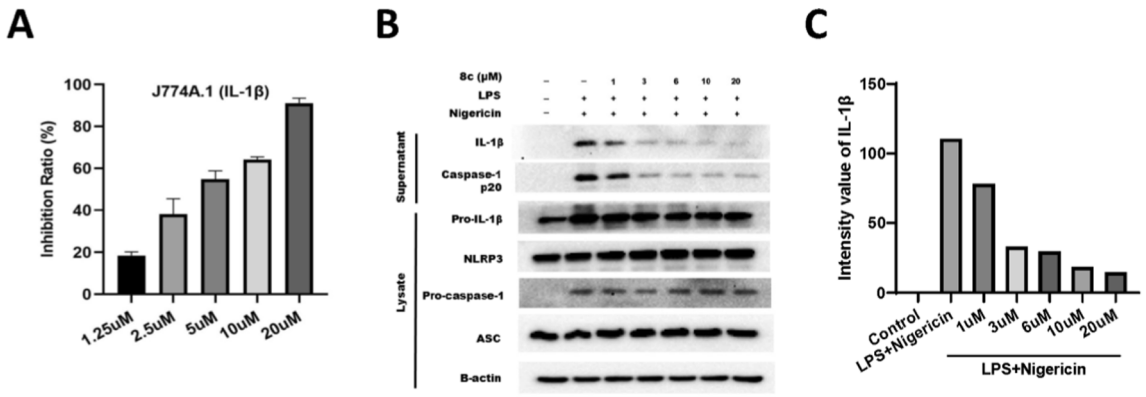


Fig. 3. Compound **8c** reduces NLRP3 inflammasome-dependent IL-1β secretion. (A) LPS-primed J774A.1 cells were treated with indicated dose of **8c** for 1 h and then stimulated with nigericin for another 1 h. The level of IL-1β release in cell supernatant detected by ELISA. (B-C) Western blot analyzed the level of NLRP3-related proteins in cell supernatant and lysate of J774A.1 cells.

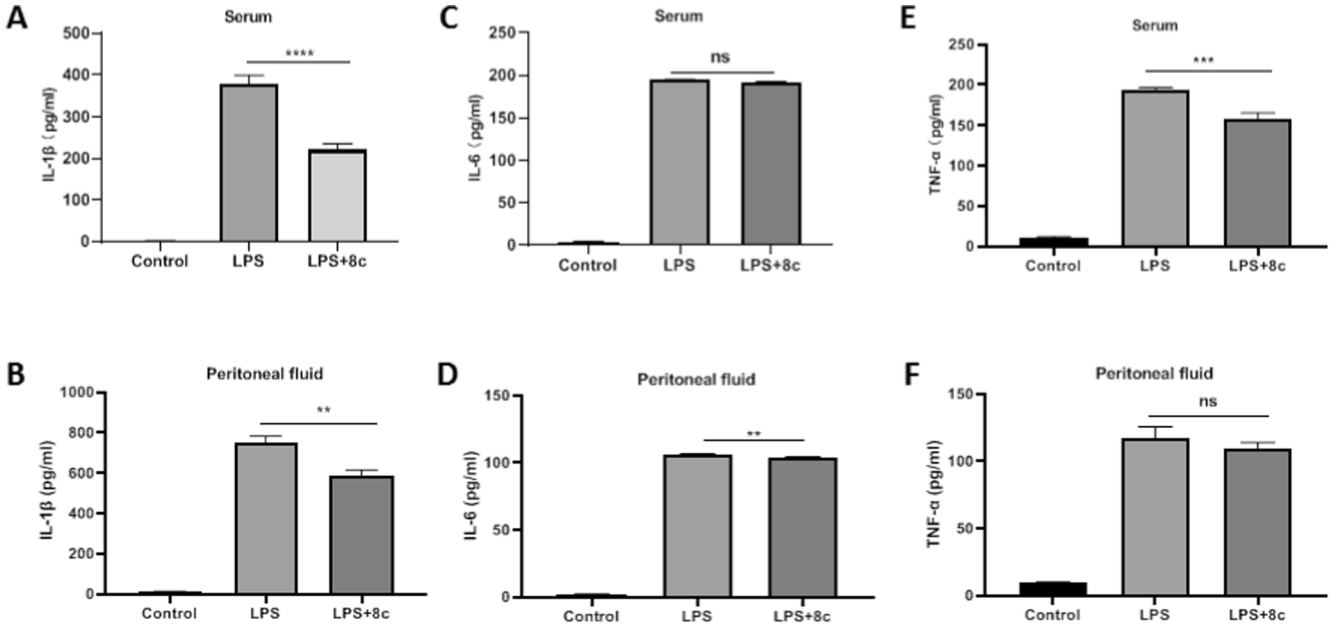


Fig. 4. Compound **8c** ameliorates LPS-induced sepsis in vivo. (A and B) IL-1β level from C57BL/6 mice that were treated with 100 mg/kg compound **8c** before LPS injection in the serum (A) or peritoneal lavage fluid (B) from mice intraperitoneally injected with LPS in the presence or absence of compound **8c**. (C and D) ELISA of IL-6 in the serum (C) or peritoneal lavage fluid (D) from mice intraperitoneally injected with LPS in the presence or absence of compound **8c**. (E and F) ELISA of TNF-α in the serum (E) or peritoneal lavage fluid (F) from mice intraperitoneally injected with LPS in the presence or absence of compound **8c**. Results are representative of n = 5 and are means ± SEM. Compared with LPS treated group, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Western blotting study found compound **8c** suppresses NLRP3 inflammasome via acting on the inflammasome activation step. Importantly, in a sepsis model in mice, the treatment with **8c** indeed significantly inhibited NLRP3-mediated IL-1β release and ameliorate peritoneal inflammation. Collectively, the results strongly support further chemical development of **8c** as a promising lead for NLRP3 inhibitors and exploration of their therapeutic potential for inflammatory diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128160.

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