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Synthesis and biological evaluation of parthenolide derivatives with reduced toxicity as potential inhibitors of the NLRP3 inflammasome

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Abstract:

Parthenolide (PTL) can target NLRP3 inflammasome to treat inflammation and its related disease, but its cytotoxicity limits further development as an anti-inflammatory drug. A series of PTL analogs and their Michael-type adducts were designed and synthesized, and most of them showed high activities against the NLRP3 inflammasome pathway. The most potent compound **8b** inhibited the release of IL-1β with IC_{50} values of 0.3 μM in J774A.1 cell and 1.0 μM in primary glial cells, respectively. Moreover, **8b** showed low toxicity against J774A.1 cell (IC_{50} = 24.1 μM) and HEK-293T (IC_{50} = 69.8 μM) with a ~8 folds increase of therapeutic index compared to its parent PTL. The preliminary mechanism study revealed that **8b** mediated anti-inflammation is associated with the NLRP3 inflammasome signal pathway. Based on these investigations, we propose that **8b** might be a potential drug candidate for ultimate development of the anti-inflammation drug.

Keywords: Parthenolide; NLRP3 inflammasome inhibitor; NO donor; low cytotoxicity

Inflammation is a part of the immune system's defense response triggered by infection or tissue damage[1]. However, dysregulated and chronic inflammation can cause inflammatory tissue reactions, and eventually lead to a diverse range of non-communicable diseases[2]. Increasing evidence indicates that over-activation of NLRP3 inflammasome is responsible for various diseases, such as inflammatory diseases — peritonitis, hyperinflammation following virus infection and inflammatory bowel disease, and inflammation-related diseases — Alzheimer's disease (AD), stroke, gout and nonalcoholic steatohepatitis (NASH)[3]. In particular, knockout of NLRP3 can ameliorate the pathology in a mice model for these diseases. The findings indicate that NLRP3 is an attractive drug target for curing NLRP3 inflammasome-driven diseases.

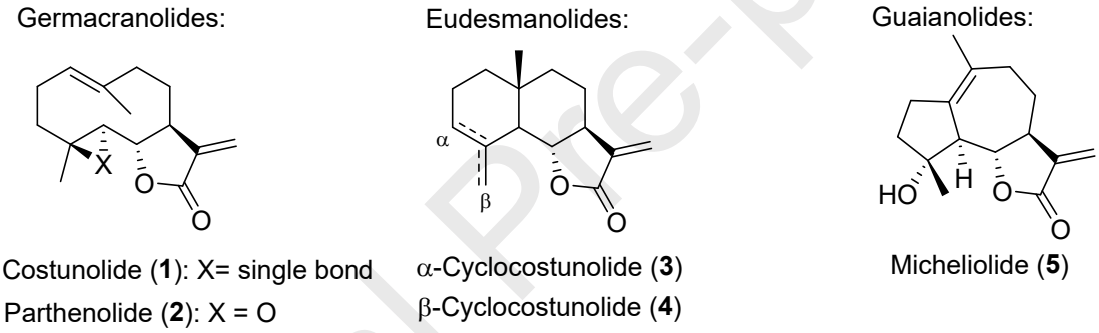


Figure 1. Selected naturally occurring SLs.

Parthenolide (PTL, **2**), a germacranolide-type sesquiterpene lactone (SL) of a 10,5-ring structure, was found to have potent anti-inflammatory effects for decades[4-6]. However, only recently has it attracted extraordinary research interest due to it targeting NLRP3 inflammasome[7]. In addition, PTL powerfully alleviates neuroinflammation[8] and ameliorates brain injury in vivo[9]. PTL of great potency inspires our curiosity about other types of SL, including eudesmanolides and guaianolides (Figure 1). For another, SLs suffer from the high cytotoxicity drawback[10-12] that limits their further development for treating inflammatory disease. Structurally, all of SLs contain α-methylene-γ-lactone moiety with high reactivity and side-chain with lipophilicity[12]. Indirect evidence links

their cytotoxicity to the a-methylene-g-lactone moiety[13, 14]. Apparently, the α,β-unsaturated carbonyl system, a Michael acceptor, can increase SL's toxicity toward cells via covalently binding to biological nucleophiles[12, 15].

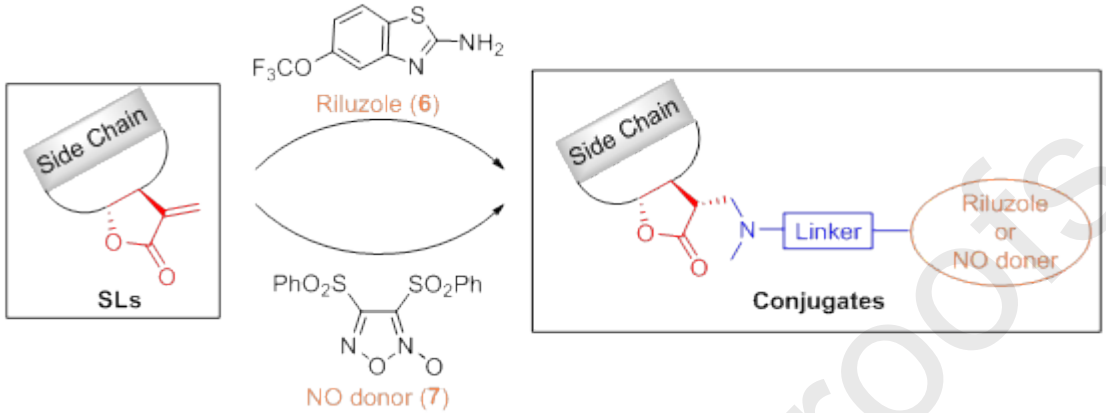
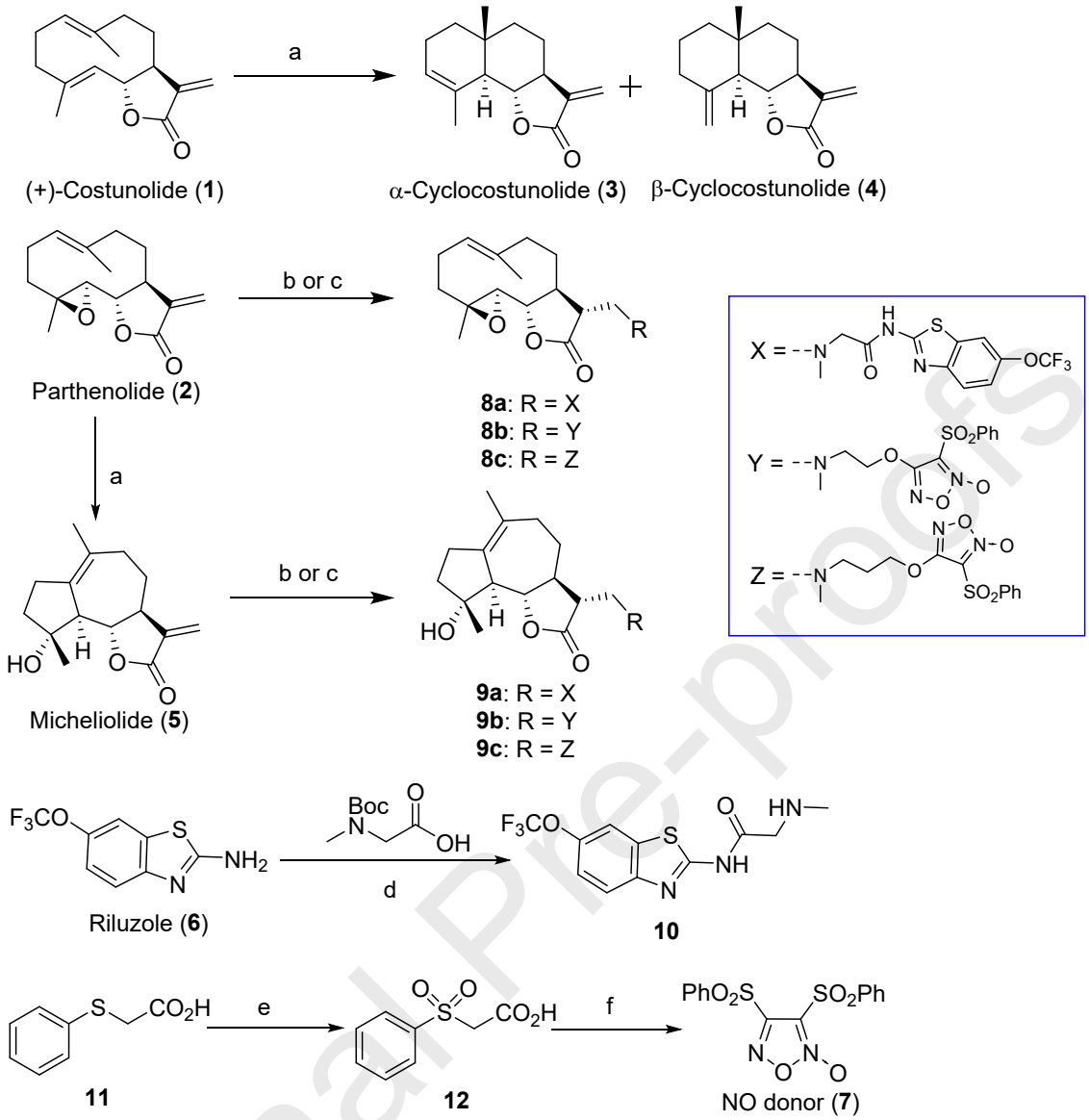


Figure 2. Design of SL derivatives based on reducing cytotoxicity

The key impact of α,β-unsaturated carbonyl moiety drew our attention towards its modification to reduce cytotoxicity and to improve anti-inflammatory activity. It was also reported that most Michael adducts of SLs (amino[16-18] or thiol[19]) exhibited less cytotoxicity compared to its parent SL. Therefore, as shown in Figure 2, the adjunction of cytoprotective fragment, such as riluzole[20] or NO donor[21,22], may be a feasible means to improve the safety of drugs.

Herein, we synthesized SLs of various carbocyclic skeletons and used dialkylamine-containing cytoprotective agents for conjugation with SL to resolve the aforementioned potency and cytotoxicity problems.



Scheme 1. Synthesis of diverse types of SLs and their derivatives.

[a]Reagents and conditions: (a) p-TsOH, CH_2Cl_2, rt, 8 h; (b) **11**, TEA, CH_2Cl_2, rt, 10 h; (c) 2-(methylamino)ethanol or 3-(methylamino)-1-Propanol, TEA, CH_2Cl_2, rt, 10 h; then adding THF, NaH, and **5**. (d) HATU, DIEA, DMF, rt, 8h; then HCl in MeOH; (e) 30% H_2O_2, AcOH, rt, 4 h; (f) fuming HNO_3, 90 °C, 4 h.

Firstly, the preparation of sesquiterpene lactones of various carbocyclic skeletons is illustrated in Scheme 1. Starting with the natural product (+)-costunolide (**1**), treatment of **1** with p-toluenesulfonic acid (p-TsOH) in $CHCl_2$ underwent transannular cyclization

to generate eudesmanolides **3** and **4** in yields of 36% and 51%, respectively[23]. Under the same condition, the guaianolide micheliolide (**5**) was yielded from parthenolide (2)[24]. The intermediates **10** or **7** was readily obtained from compound **6** or **11** according to a reported procedure[25, 26]. A Michael addition reaction of PTL or MCL with **10** provided the riluzole–PTL or MCL conjugates **8a** and **9a**. Alternatively, the conjugates **8b**–**c** and **9b**–**c** was prepared by the nucleophilic substitution reaction of **7** and Michael adducts of PTL or MCL with 2-(methylamino)ethanol.

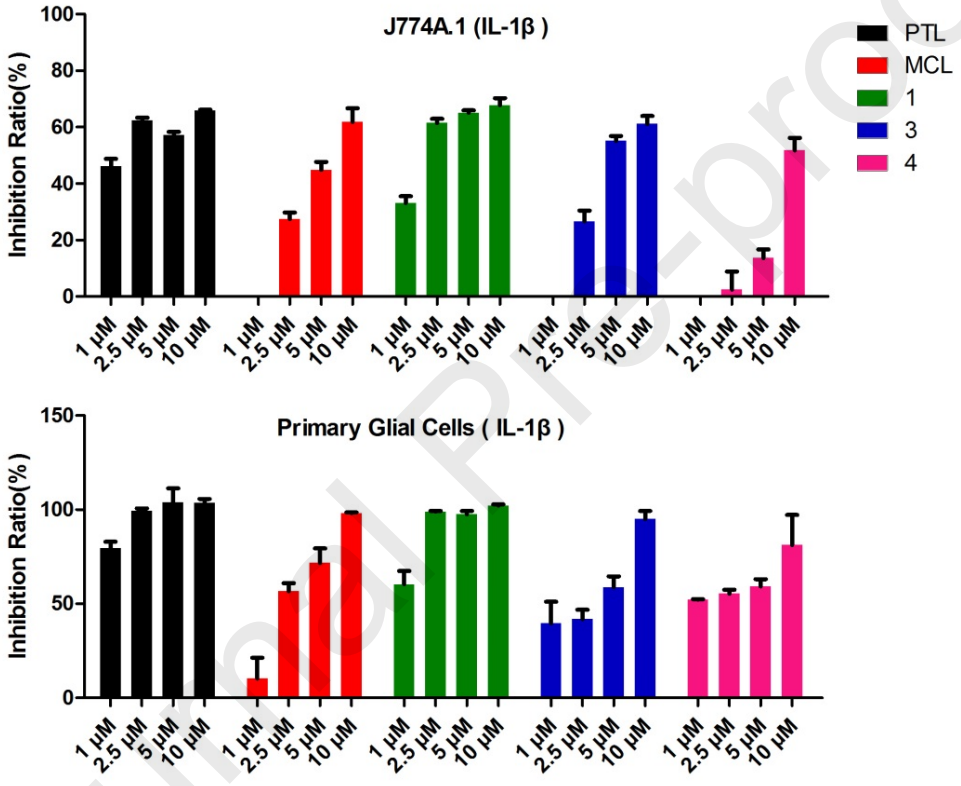


Figure 3. SLs inhibits the IL-1β release in J774A.1 and primary glial cells upon stimulation with LPS/Nigericin.

Next, we established a cell model using the murine macrophages cell line J774A.1[27] and primary glial cells[28] in which the release of IL-1β is mediated via the NLRP3 inflammasome activation upon priming with lipopolysaccharide (LPS). Firstly, the natural sesquiterpene lactones **1** – **5** were tested for their abilities to inhibit the release of IL-1β. As shown in Figure 3, all types of sesquiterpene lactones showed high

concentration-dependent inhibition of IL-1β release in both J774A.1 and primary glial cells. It is obvious that germacranolides of a 5,10-ring skeleton, such as compound **1** and **2**, had higher inhibitory potency than eudesmanolides of a 5,6,6-ring skeleton (**3** and **4**) and micheliolide (**5**) of a 5,7,5-ring skeleton.

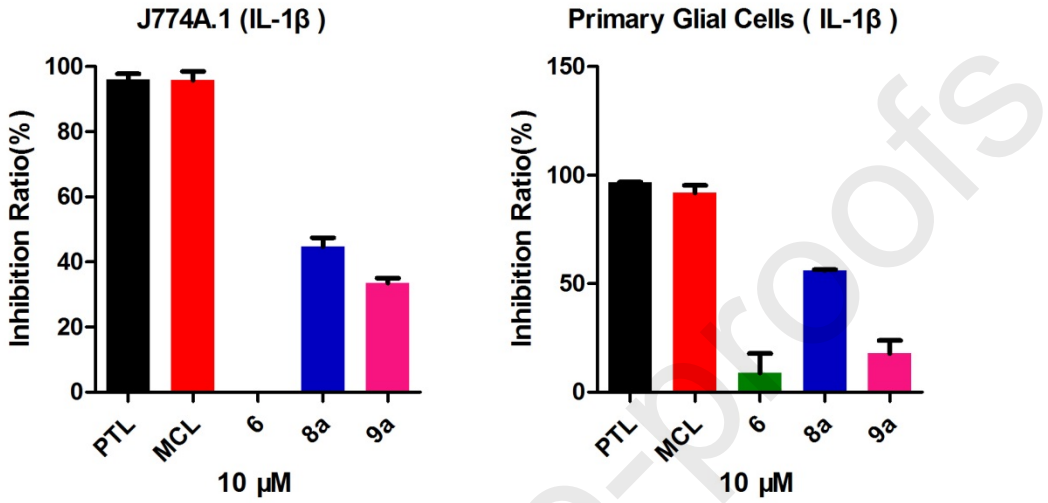


Figure 4. Compound **8a** and **9a** inhibit the IL-1β release in J774A.1 cells and primary glial cells upon stimulation with LPS/Nigericin upon stimulation with LPS/Nigericin.

Table 1. Cytotoxicity and Inhibitory Potency against the IL-1*β* Production in J774A.1 and Primary Glial Cells of Designed Derivatives

Cmpd	Inhibition of IL-1*β* (IC_{50}, μM)		Cytotoxicity (TC_{50}, μM)		TI [a]
	J774A.1	Glial cells	J774A.1	HEK-293T	
PTL	1.4	1.8	14.3	29.9	10.2
7	0.93	1.9	11.4	22.4	12.2
MCL	2.3	5.9	21.9	51.7	9.5
8b	0.30	1.0	24.1	69.8	80.3
8c	0.40	2.2	66.6	166.1	166.5
9b	0.50	1.7	59.5	56.5	119.0
9c	0.49	2.3	68.5	105.5	139.8

[a] Therapeutic index (TI) calculated for TC_{50} (J774A.1) *versus* IC_{50} (J774A.1)

After the evaluation of inhibitory activity on IL-1β release in J774A.1 and primary glial cells for the sesquiterpene lactones, the two highly potent and readily available compounds, PTL and MCL, were selected for further research. The preliminary screening results showed that riluzole (**6**) had no inhibition of IL-1β release in J774A.1 cell and slight activity in primary glial cells at 10 μM (Figure 4). Unsurprisingly, the conjugate of PTL or MCL with **6**, **8a** or **9a**, led to a significant drop in the inhibition of IL-1β release. Despite this, no complete loss of activity by modification of α, β-unsaturated double bond indicates the design is workable if the linked molecule possesses good activity against NLRP3. The notion is supported by the results of **8b**–**c** and **9b**–**c**, as a conjugation of PTL or MCL with NO donor **7**. As shown in Table 1, PTL, MCL and **7** exhibited good potency on inhibiting IL-1β release in J774A.1 with IC_{50} values of 1.4 μM, 2.3 μM and 0.93 μM, respectively, but with relatively high cytotoxicity (PTL: 14.3 μM; MCL: 21.9 μM; **7**: 11.4 μM). As expected, all the four synthesized conjugates **8b**–**c** and **9b**–**c** significantly reduced cytotoxicity against J774A.1 and HEK-293T, and resulted in a remarkable increase of inhibitory potency. Notably, their conjugates **8b** showed the highest inhibitory potency in J774A.1 (IC_{50} = 0.30 μM) and primary glial cells. The cytotoxicity data clearly demonstrated a 1.5-folds and 2.3-folds decrease of cytotoxicity against J774A.1 and HEK-293T, respectively, as compared to that of PTL. The therapeutic index of **8b** is up to 80.3, which is important for a wide therapeutic window. Although the chain extension to aminopropanol as in **8c** led to lower toxicity, a slightly decreased potency was observed. A similar case was found in the conjugates **9b** and **9c**. Further tests in primary glial cells confirmed **8b** as the most potent inhibitor against NLRP3 inflammasomes (IC_{50} = 1.0 μM).

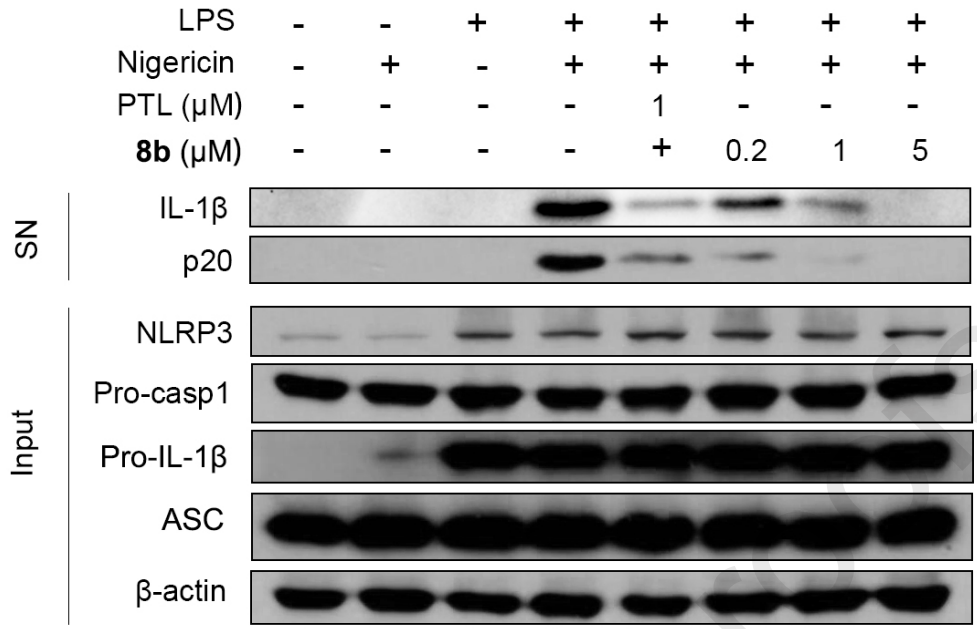


Figure 5. Compound **8b** reduces NLRP3-inflammasome-derived IL-1β secretion. Medium SN and cell extracts were analyzed by immunoblotting, as indicated

Taking account that compound **8b** showed the most potent inhibition of IL-1β release, it was further selected for investigation of its preliminary mechanism. Western blots showed that treatment with **8b** dose-dependently decreased the amount of caspase-1 p20 and IL-1β in supernatants (Fig. 5). Compound **8b** did not affect the expression levels of pro-IL-1β, pro-caspase-1, NLRP3, or ASC in cell lysates (Fig. 5). These findings suggest compound **8b** suppresses NLRP3 Inflammasome-mediated caspase-1 activation and IL-1β secretion.

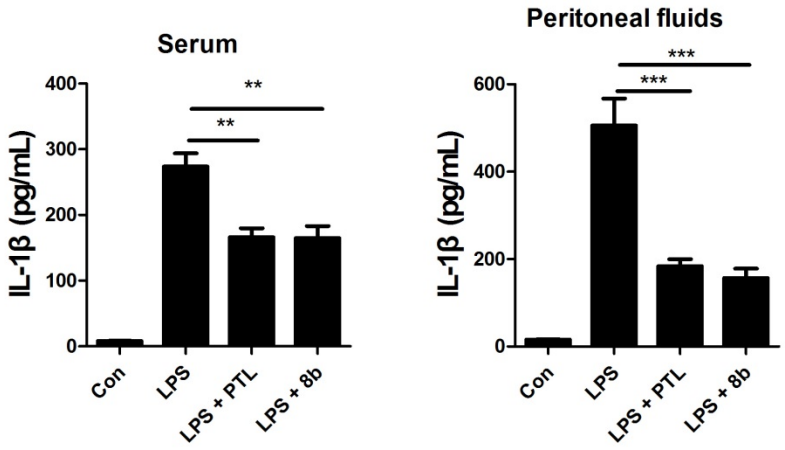


Figure 6. Compound **8b** ameliorates peritoneal inflammation in vivo.

Finally, we investigated whether **8b** could inhibit the NLRP3-driven inflammation in animal models of LPS-induced inflammatory responses in vivo[29]. mice were injected intraperitoneally with LPS and showed significantly increased IL-1β in lavage fluid. As shown in Figure 6, parthenolide was tested as a positive control. The level of IL-1β in both serum and peritoneal fluid was obviously reduced by the treatment of **8b** (30mg/kg/d). Notably, compounds **8b** exhibited activity comparable to that of PTL in the LPS-challenged mice. This could be due to different molecular weight as well as pharmacokinetic properties.

In conclusion, the rapid formation of eudesmanolides and guaianolides can be achieved via germacranolides. SLs of the various skeleton, especially PTL and MCL, showed great inhibitory potency on NLRP3-mediated IL-1β release. Design of the linkage between PTL or MCL with NO donor **7** leads to four conjugates with high potency and low cytotoxicity. It is worth noting that compound **8b** was the most potent against the release of IL-1β with a high TI value of 80. These in-vitro results encouraged us to further evaluate its in-vivo efficacy on NLRP3-driven inflammation. Indeed, the treatment with **8b** improved the high IL-1β levels caused by peritoneal inflammation in mice. Collectively, the results of **8b** together with that of **9b** prove the effectiveness of our

design for lowering Michael receptor-induced toxicity. These data also warrant further development of **8b** and analogs as potential agents for the treatment of peritoneal inflammation as well as other NLRP3 inflammasome-driven 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.

Acknowledgments

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Appendix A. Supplementary data

The following are the Supplementary data to this article. Supporting data to this article can be found online.

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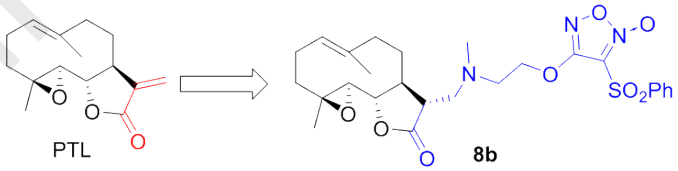
Graphical Abstract



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