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Design, synthesis, and bioactivity evaluation of novel 1-(4-(benzylsulfonyl)-2-nitrophenyl) derivatives as potential anti-inflammatory agents against LPS-induced acute lung injury

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ABSTRACT

Acute lung injury (ALI) is a devastating disease with a high mortality rate of 30%–40%. There is an unmet clinical need owing to limited treatment strategies and little clinical benefit. The pathology of ALI indicates that reducing the inflammatory response could be a highly desirable strategy to treat ALI. In this study, we designed and synthesized 36 novel 1-(4-(benzylsulfonyl)-2-nitrophenyl) derivatives and evaluated their anti-inflammatory activities by measuring the release of cytokines in lipopolysaccharide (LPS)-challenged J774A.1 cells. Compounds **19**, **20**, and **39** potently reduced the release of IL-6 and TNF-α in J774A.1 cells. Additionally, **39** improved LPS-induced ALI *in vivo* and inhibited cytokine production in lung tissues. Furthermore, **39** reduced inflammatory infiltration and downregulated p-p65 levels in lung tissues. Thus, compound **39** could serve as a new lead structure for the development of anti-inflammatory drugs to treat ALI.

Introduction

Acute respiratory distress syndrome (ARDS) and its milder form, acute lung injury (ALI), are common clinically critical diseases with a mortality rate of 30 %–40 %.[1] Dysfunction of the alveolar-capillary membrane, reduced alveolar fluid clearance, and enhanced inflammation are the terminal pathophysiological characteristics of ALI/ARDS, which result in pulmonary edema and hypoxemia.[2–3] Despite the extensive studies that have been conducted on ALI/ARDS, protective ventilation, including low tidal volume ventilation and high positive end-expiratory pressure levels, remains the best practice for clinical treatment.[4] However, ventilation has not reduced the mortality rate of ALI/ARDS, and long-term ventilation can exacerbate lung damage or even cause ventilator-induced lung injury.[5–6] Although supportive therapies, such as inhaled nitric oxide, can improve arterial oxygenation, none of these treatments can reduce inflammation in the lungs.[7] Steroidal anti-inflammatory drugs may decrease lung inflammation; although their efficacy in ALI/ARDS is controversial.[8] Pharmacological agents such as aspirin,[9–11] ibuprofen,[12] and celecoxib have shown no obvious benefit in clinical trials with respect to treating ALI. Therefore, developing an alternative strategy to treat ALI is highly desirable.

The pathogenesis of ALI/ARDS can be divided into 3 phases, namely, exudative, proliferative, and fibrotic.[6,13,14] The initial exudative phase is characterized by alveolar barrier damage and pulmonary edema. The process of lung repair begins in the proliferative phase after 1–2 weeks and overlaps with the exudative phase. It may progress to a nonreversible fibrotic phase in some patients. Enhanced inflammation is a key pathophysiological characteristic of ALI/ARDS in the exudative phase. An imbalance between the pro-inflammatory and anti-inflammatory

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mediators leads to disruption of the alveolar epithelia and results in pulmonary edema and hypoxemia.[6–7] Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are the two key cytokines that contribute most to the progression of ALI/ARDS.[15] IL-6 and TNF-α production has been reported to be elevated in the bronchoalveolar lavage fluid (BALF) in humans and in experimental ALI.[16] These findings indicate that inhibiting the excess production of pro-inflammatory cytokines, including IL-6 and TNF-α, can be a promising approach to treating ALI.

Sulfur-containing compounds have several biological effects, including anti-inflammatory, antibacterial, and anticancer effects (Fig. 1).[17] Austrasulfone is a sulfone-containing natural product known for its potent neuroprotective effect against neurotoxicity in 6-OHDA-induced neuroblastoma.[18] Compound **2** is the metabolite of the active organosulfur product of garlic, which protects against the development of cigarette smoke extract–induced lung injury by reducing inflammatory cytokine levels and augmenting antioxidant activity via the ERK/p38 MAPK and NF-κB pathways.[19] Belinostat (**3**), a novel enzyme histone deacetylase (HDAC) inhibitor with a sulfonamide-hydroxamide structure, is an approved therapeutic agent to treat relapsed/refractory peripheral *T*-cell lymphoma.[20] Anticancer agent **4** bearing a sulfonamide structure exhibited potent antitumor activity by degrading RBM 39, an essential mRNA-splicing factor.[21] AG-348 (**5**) is a novel small-molecule allosteric activator of pyruvate kinase that significantly upregulates both wild-type and mutant forms of erythrocyte pyruvate kinase (PKR), thereby increasing adenosine triphosphate (ATP) production and reducing 2,3-diphosphoglycerate levels.[22].

Compound **6** is a synthetic sulfone containing compound derived from Parasitic Worms *Acanthocheilonema viteae* product ES-62.[23] It showed anti-inflammatory effects *in vitro* and prevented the development of arthritis in mice by downregulating the myeloid differentiation primary response protein 88 (MyD88). In addition, this sulfone derivative was found to promote metabolic homeostasis in a mouse model of obesity and to significantly reduce disease development in fibrosis models.[24–25] Based on these findings, compound **6** was selected as a lead compound to develop active analogs for the treatment of inflammatory diseases. As shown in Fig. 2, inspired by compound **3**, we introduced a phenyl group to replace the ethyl chain in structure **6** to design a novel structure. As we introduced an aromatic ring in the target compounds, we further modified the R_2 group with substituent piperazinyl groups to introduce a hydrophilic moiety in the target compounds. Based on these modifications, we designed and synthesized 36 sulfone derivatives.

The synthetic route of designed compounds reported in this study was illustrated in Scheme 1. Briefly, commercially available 4-chloro-3-nitrobenzene sulfonyl chloride was reacted with sodium bicarbonate and sodium sulfite in water to provide the corresponding intermediate **7**. Next, different substituent benzyl bromide in dimethylformamide (DMF) were reacted with **7** to yield intermediates **8a-8e**. Then, **8a-8e** were reacted with different substituent piperazines in a base along with potassium iodide as a catalyst to obtain compounds **9**–**44**.

Reagents and conditions. (a) $NaHCO_3$, Na_2SO_3, H_2O, 80 °C, 2 h; (b) different substituents benzyl bromide, DMF, 80 °C 2 h; (c) KI, TEA, MeCN, 85 °C 6 h.

We first investigated the anti-inflammatory activities of the synthesized compounds by detecting the production of IL-6 and TNF-α after an LPS challenge of macrophages through ELISA assay.[26] Briefly, J774A.1 cells were pretreated with 20 μM of the synthesized compounds for 30 min. Compound **6** and dimethylsulfoxide (DMSO) served as the positive and negative controls, respectively. J774A.1 cells were then challenged with 0.5 μg/mL LPS for 24 h. IL-6 and TNF-α levels in the medium were measured using the corresponding ELISA kits according to the manufacturers' instructions.

The primary anti-inflammatory activities of the designed compounds are listed in Table 1. We first explore the R_1 moiety of the designed compounds. After introducing fluorine at the 3′ position of the phenyl ring, the IL-6 inhibition ratio increased from 21.24 % to 58.89 % (**11** vs **18**). The 4′ fluorine–substituted compound **25** showed lower anti–IL-6 release activity compared with **18**. Introducing a methyl group at 4′ decreased the IL-6 inhibition ratio of the ether (**32** vs **18**). However, introducing the electron-donating methoxy group at 3′ showed appreciable IL-6 inhibition and little effect on inhibiting TNF-α release (**39** vs **18**).

Next, we explored the R_2 moiety of the designed compounds. The different piperazinyl groups that were introduced increased the IL-6 inhibition ratio in J774A.1 cells and had little effect on TNF-α inhibition. The SAR of the piperazine substituent showed the following trend with respect to IL-6 inhibition: 4-nitrophenyl ≈ 3-nitrobenzyl > hydroxyethyl ≈ acetyl ≈ ethyl > methoxyethyl.

To further investigate the anti-inflammatory activities of active compounds, the most potent compounds **19**, **20**, **31**, and **39** were selected to explore the dose-dependent inhibitory effects against LPS-induced IL-6 and TNF-α release. J774A.1 cells were pretreated with each of the selected compounds at the indicated concentrations for 30 min before challenging cells with LPS. As shown in Fig. 3, except **31**, the other three active compounds exhibited a dose-dependent inhibition of the release of LPS-induced IL-6 and TNF-α. The IC_{50} values of **19, 20,** and **39** were determined as 14.73 μM, 6.01 μM, and 5.66 μM for IL-6; and 28.88 μM, 23.41 μM, and 19.62 μM for TNF-α, respectively. Moreover, compound **39**[27] showed the strongest inhibition against the expression of both IL-6 and TNF-α, prompting us to further evaluate its activity in a mouse model of ALI.

Encouraged by the potent *in vitro* inhibitory activity, compound **39** was selected to further study its anti-inflammatory effect *in vivo* in a mouse model of LPS-induced ALI.[28] C57BL/6*J* mice were orally pre-administered compound **39** and dexamethasone (DXM) at 20 mg/kg for 30 min. Then, an intratracheal injection of 5 mg/kg LPS was used to induce ALI in mice. Mice in the sham group received a similar volume of saline. After 6 h, mice with ALI were sacrificed, and the lung tissues, blood, and BALF were collected. Wright-Giemsa staining[29] was used to

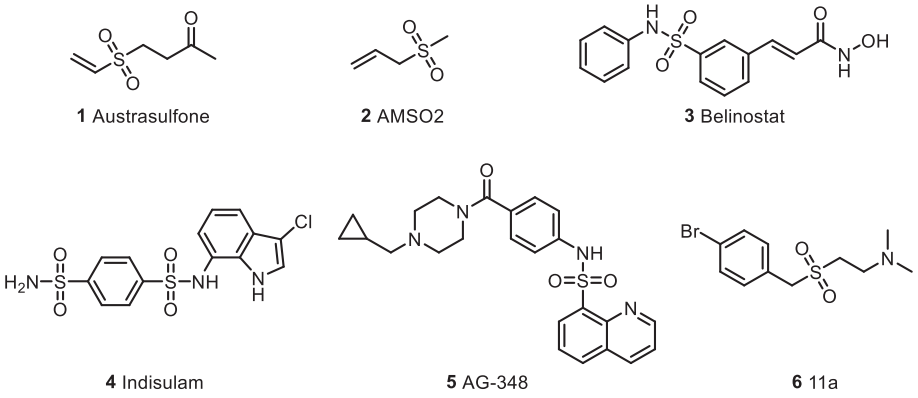


Fig. 1. Typical sulfur-containing compounds.

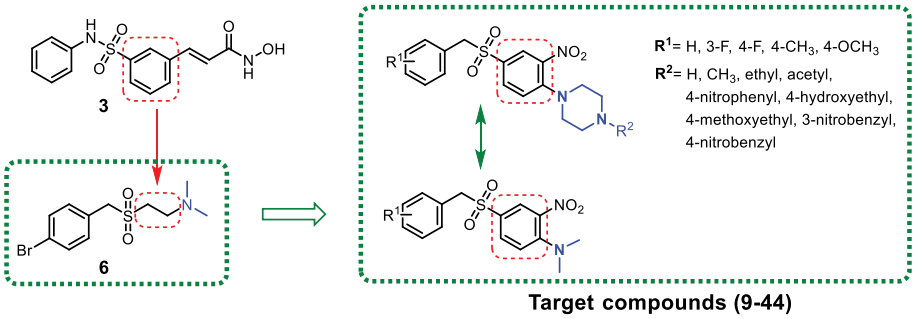
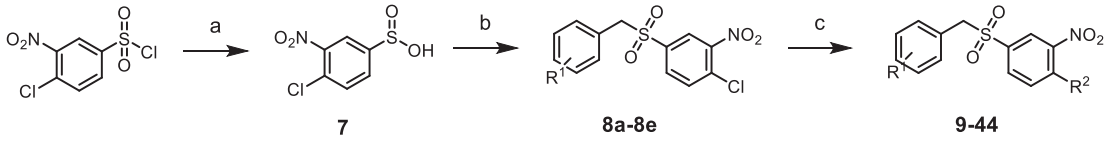


Fig. 2. Design strategy and modification for 1-(4-(benzylsulfonyl)-2-nitrophenyl) derivatives.



Scheme 1. Synthetic route of the designed compounds.

Table 1
Anti-inflammatory screening of the designed compounds using ELISA.

Index	R^1	R^2	IL-6 inhibition (%)	TNF-α inhibition (%)
9	H		NA*	NA
10	H	Methyl	53.04 ± 6.43	16.86 ± 6.22
11	H	Ethyl	21.24 ± 9.99	NA
12	H	Acetyl	40.41 ± 22.15	19.12 ± 18.41
13	H	4-nitrophenyl	51.52 ± 6.78	NA
14	H	2-hydroxyethyl	56.59 ± 6.72	29.04 ± 8.99
15	H	2-methoxyethyl	NA	NA
16	H	3-nitrobenzyl	57.27 ± 2.48	NA
17	3-F		NA	NA
18	3-F	Ethyl	58.89 ± 7.71	24.43 ± 3.20
19	3-F	Acetyl	62.38 ± 1.13	36.28 ± 6.29
20	3-F	4-nitrophenyl	68.87 ± 2.25	12.18 ± 1.67
21	3-F	2-hydroxyethyl	4.55 ± 4.14	NA
22	3-F	2-methoxyethyl	0.29 ± 7.28	NA
23	3-F	3-nitrobenzyl	59.44 ± 2.38	22.51 ± 1.17
24	4-F		NA	NA
25	4-F	Ethyl	31.39 ± 3.07	NA
26	4-F	Acetyl	33.81 ± 17.24	10.99 ± 15.03
27	4-F	4-nitrophenyl	62.47 ± 4.59	18.6 ± 12.53
28	4-F	2-hydroxyethyl	54.2 ± 3.02	29.96 ± 3.13
29	4-F	2-methoxyethyl	12.89 ± 11.47	NA
30	4-F	3-nitrobenzyl	59.52 ± 1.42	27.46 ± 1.48
31	4-F	4-nitrobenzyl	71.54 ± 4.62	26.45 ± 9.78
32	4-Me	Ethyl	52.95 ± 2.66	NA
33	4-Me	Acetyl	16.83 ± 5.92	NA
34	4-Me	4-nitrophenyl	NA	NA
35	4-Me	2-hydroxyethyl	NA	NA
36	4-Me	2-methoxyethyl	2.98 ± 2.92	NA
37	3-OMe		8.62 ± 6.10	NA
38	3-OMe	H	18.18 ± 10.91	NA
39	3-OMe	Ethyl	63.29 ± 6.75	33.13 ± 4.44
40	3-OMe	Acetyl	5.7 ± 13.95	NA
41	3-OMe	4-nitrophenyl	66.21 ± 5.39	NA
42	3-OMe	2-hydroxyethyl	15.23 ± 8.91	NA
43	3-OMe	2-methoxyethyl	NA	NA
44	3-OMe	3-nitrobenzyl	41.95 ± 9.53	NA
6			79.61 ± 3.55	NA

* NA = none activity.

determine the neutrophil count in the BALF. As shown in Fig. 4A, neutrophil counts increased in the LPS-challenged group, whereas pre-treatment with active compound **39** or DXM significantly decreased the neutrophil counts in the BALF of mice with ALI. These results indicated that **39** could alleviate ALI *in vivo*.

Pro-inflammatory cytokines are critical factors in the progression of

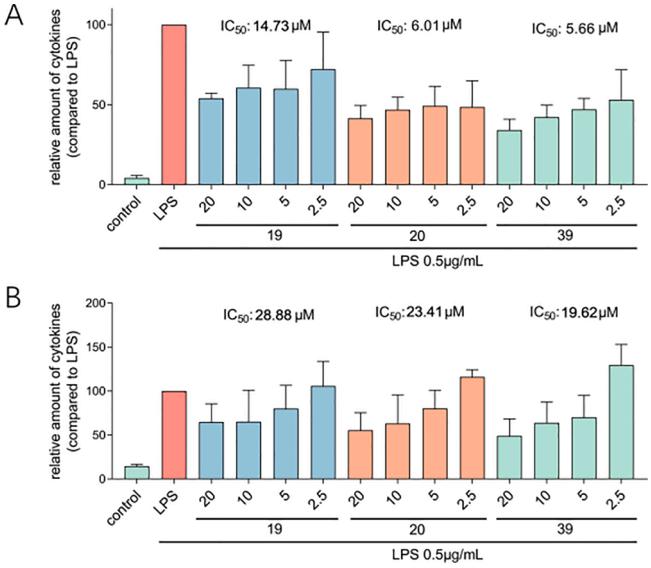


Fig. 3. (A) The active compounds inhibited LPS-induced IL-6 release upon J774A.1 cells in a dose-dependent manner. (B) The active compounds inhibited LPS-induced TNF-α release upon J774A.1 cells in a dose-dependent manner. Each bar represents the mean ± SEM in 3 independent experiments.

ALI, the production levels of TNF-α and IL-6 in the BALF and serum from LPS-induced mice with ALI were examined as biomarkers to determine the *in vivo* protective effect of **39** against ALI (Fig. 4B-4E). The release of TNF-α and IL-6 in the BALF and serum increased significantly after LPS stimulation; in contrast, pretreatment with **39** distinctly suppressed the levels of both cytokines, demonstrating its anti-inflammatory effect in mice with ALI. These findings revealed that **39** could alleviate ALI via its anti-inflammatory effect, thereby highlighting its potential in treating ALI and other inflammatory conditions.

In addition, the histopathological features of lung tissues of mice with ALI were studied after LPS induction.[26] As shown in Fig. 5, the lung tissues of mice from the LPS group exhibited a remarkable increase in alveolar septa thickness, pulmonary congestion, inflammatory infiltration, and tissue destruction compared with the tissues obtained from the control group. Pretreatment with **39** led to minimal histopathological changes. These results indicated that **39** effectively attenuated LPS-induced ALI in mice.

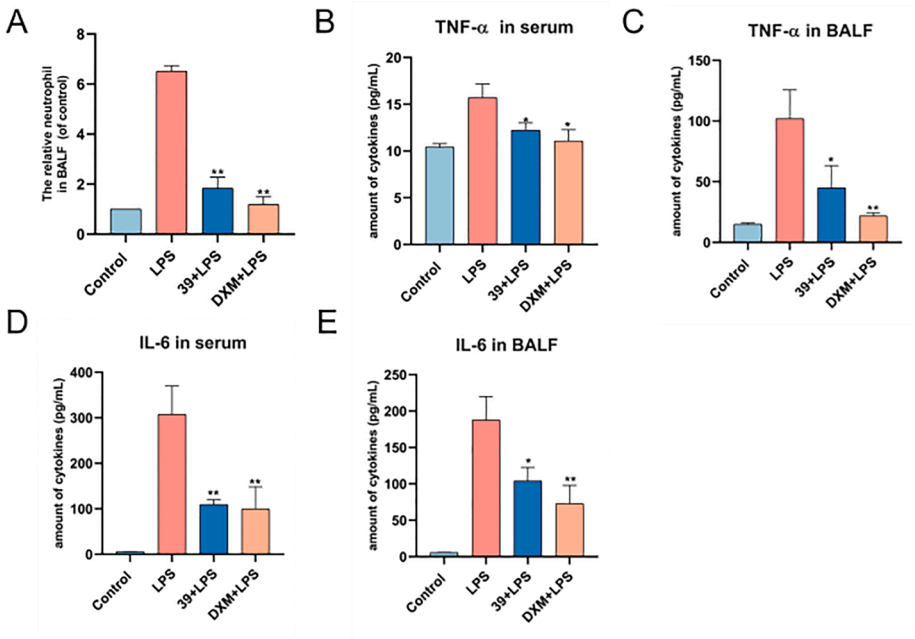


Fig. 4. (A) Relative number of neutrophils in the BALF. (B-C) TNF-α levels in the serum and BALF of mice with ALI. (D-E) IL-6 levels in the serum and BALF of mice with ALI. Each bar represents the mean ± SEM, n = 8, *, $p < 0.05$, **, $p < 0.01$ vs the LPS-only group.

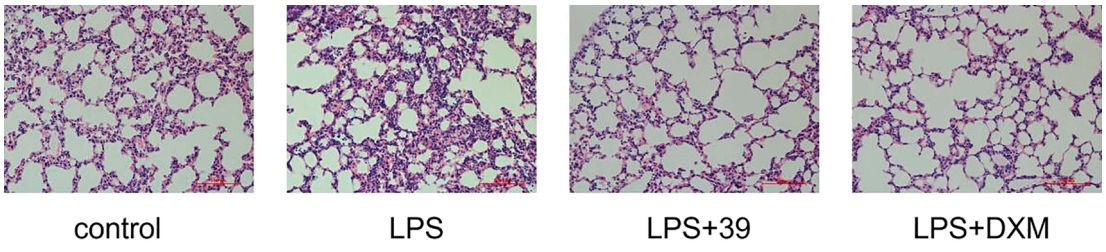


Fig. 5. Representative lung tissue showing the results of H&E staining.

An immunohistochemical assay[26] was used to explore the extent of neutrophil infiltration after the LPS challenge. Lung tissue sections were stained with F4/80 antibody, a biomarker of macrophages, to confirm the effect of compound **39** in alleviating ALI. As shown in Fig. 6A, LPS stimulation induced neutrophil infiltration in lung tissues compared with the sham group. The active compound and dexamethasone significantly reduced the percentage of macrophages in mice with ALI mice, indicating that compound **39** can inhibit LPS-induced neutrophil infiltration *in vivo*.

To further investigate the mechanism of **39** in attenuating ALI *in vivo*, lung tissues were analyzed using an immunohistochemical assay to stain p-p65. The NF-κB signaling pathway is involved in regulating innate immunity, and p65 is a key transcription factor regulating the NF-κB pathway. As shown in Fig. 6B, p-p65 expression significantly increased

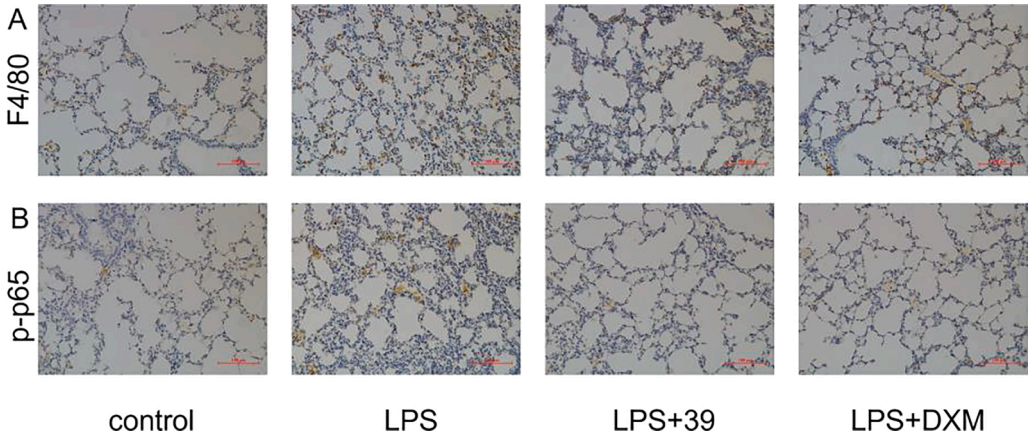


Fig. 6. (A) Representative lung tissues showing staining of the macrophage marker F4/80 (brown). Sections were counterstained with hematoxylin. (B) Representative lung tissues showing p-p65 staining (brown). Sections were counterstained with hematoxylin.

in the lung tissues of the LPS-stimulated group compared with p-p65 levels in the tissues obtained from the sham group. Pre-administration with active compound **39** or dexamethasone significantly reduced p-p65 levels in lung tissues. These results indicated that compound **39** could inhibit the NF-κB pathway and attenuate ALI in mice.

In summary, we designed and synthesized thirty-six 1-(4-(benzylsulfonyl)-2-nitrophenyl) derivatives and their anti-inflammatory activities were evaluated in LPS-challenged J774A.1 cells. Most compounds effectively inhibited TNF-α and IL-6 production in macrophages. In particular, compound **39** showed a dose-dependent inhibition of the release of TNF-α and IL-6. Furthermore, pretreatment with **39** attenuated LPS-induced ALI in mice by reducing macrophage infiltration into lung tissues, and cytokine production in the BALF and serum. Moreover, the active compound downregulated p65 expression in lung tissues. Collectively, these results suggest the potential of compound **39** as an anti-inflammatory agent to treat ALI. This work provides new lead structures for further development as anti-inflammatory drugs.

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.

Data availability

Data will be made available on request.

Acknowledgments

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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.2022.129097.

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27 Analytic data of potent compound **39**: Sodium bicarbonate (656.16 mg, 7.81 mmol), sodium sulfite (984.46 mg, 7.81 mmol), and 4-chloro-3-nitrobenzene sulfonyl chloride (1.00g, 3.91 mmol) were dissolved in water (20 mL) at 80 °C. Stirred for 2 h; the reaction was monitored using TLC. After the completion of the reaction, the solution was concentrated in a rotary evaporator. The crude product was recrystallized in ethanol to obtain the 4-chloro-3-nitrobenzenesulfinic acid (Yield: 90.02%). Then it (100.00 mg,0.45 mmol) was dissolved in DMF at 80 °C. 3-methoxybenzyl bromide (115.77 mg, 0.58 mmol) was added dropwise and stirred for 2 h. After the completion of the reaction, water was added and extracted with ethyl acetate (EA). The crude product was purified using flash column chromatography (DCM: MeOH=20:1) to get the 1-chloro-4-((3-methoxybenzyl)sulfonyl)-2-nitrobenzene (Yield: 44.89%). Then 1-chloro-4-((3-methoxybenzyl)sulfonyl)-2-nitrobenzene (30 mg,0.088 mmol) was dissolved in MeCN at 85 °C. Potassium iodide (19.17 mg, 0.12 mmol) and triethylamine (15.53 μL, 0.12 mmol) were added in portions and stirred for 6 h. After the completion of the reaction, water was added and extracted with EA. The crude product was purified using flash column chromatography (DCM: MeOH=20:1) to get Yellow powder in 54.58% yield. Purity: 98.56%; mp 112.0-113.3 °C. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.97 (d, J = 2.2 Hz, 1H), 7.56 (dd, J = 8.9, 2.3 Hz, 1H), 7.21 (dd, J = 8.8, 7.6 Hz, 1H), 7.04 (d, J = 8.9 Hz, 1H), 6.93 – 6.87 (m, 1H), 6.74 – 6.68 (m, 2H), 4.30 (s, 2H), 3.77 (s, 3H), 3.29 – 3.20 (m, 4H), 2.61 (t, J = 4.9 Hz, 4H), 2.51 (q, J = 7.2 Hz, 2H), 1.14 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-d) δ 159.76, 148.73, 139.20, 133.12, 129.77, 129.26, 128.28, 127.68,

123.17, 119.46, 116.31, 114.82, 63.16, 55.35, 52.22, 52.14, 50.55, 11.92. ESI-MS m/z: 420.2$[M+H]^+$.

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