CHEMISTRY & BIODIVERSITY

Accepted Article

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To be cited as: *Chem. Biodiversity* **2024**, e202400031

Link to VoR: https://doi.org/10.1002/cbdv.202400031

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10.1002/cbdv.202400031

Discovery of 6-Acylamino/Sulfonamido Benzoxazolone with IL-6 Inhibitory Activity as Promising Therapeutic Agents for Ulcerative Colitis

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Abstract: Ulcerative colitis has been widely concerned for its persistent upward trend, and the sustained overproduction of pro-inflammatory cytokines such as IL-6 remains a crucial factor in the development of UC. Therefore, the identification of new effective drugs to block inflammatory responses is an urgent and viable therapeutic strategy for UC. In our research, twenty-three 6-acylamino/sulfonamido benzoxazolone derivatives were synthesized, characterized, and evaluated for anti-inflammatory activity against NO and IL-6 production in LPS-induced RAW264.7 cells. The results demonstrated that most of the target compounds were capable of reducing the overexpression of NO and IL-6 to a certain degree. For the most active compounds **3i, 3j** and **3l**, the inhibitory activities were superior or equivalent to those of the positive drug celecoxib with a dose-dependent relationship. Furthermore, animal experiments revealed that active derivatives **3i, 3j** and **3l** exhibited definitive therapeutical effect on DSS induced ulcerative colitis in mice by mitigating weight loss and DAI score while decreasing levels of pro-inflammatory cytokines such as IL-6 and IFN-γ, simultaneously increasing production of anti-inflammatory cytokines IL-10. In addition, compounds **3i**, **3j** and **3l** could also inhibit the oxidative stress to alleviate ulcerative colitis by decreasing MDA and MPO levels. These finding demonstrated that compounds **3i, 3j** and **3l** hold significant potential as novel therapeutic agents for ulcerative colitis.

Key words: Benzoxazolone, anti-inflammatory, ulcerative colitis

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Introduction

Ulcerative colitis (UC) is a subtype of inflammatory bowel diseases (IBD), characterized by persistent and diffuse inflammation in the mucosa and submucosa of the colon and rectum[1]. Clinical manifestations of UC include increased frequency of abdominal pain, diarrhea, blood stool, and progressive loss of peristaltic function[2], significantly impacting the quality of life. The etiology of UC is multifactorial, involving genetic changes, environmental stimulus, intestinal microflora dysbiosis, defects in the intestinal mucosal barrier, and immune system dysregulation[3,4]. Although aminosalicylates, glucocorticoid and immunomodulators are commonly used for clinical management across mild to severe UC cases[5], long-term medication can also lead to repeated attack or associated adverse effects[6,7]. Importantly, there has been a global surge in the incidence and prevalence of ulcerative colitis (UC)[8,9] which now poses as a risk factor for developing intestinal cancer. Therefore, it is imperative to develop alternative strategies to tackle UC.

As severe and chronic inflammation diseases, the sustained overproduction of inflammatory cytokines like IFN-γ, TNF-α, IL-1β, IL-6[10-12], plays a pivotal role as cell-signaling molecules driving the pathogenesis of UC. Increased production of IL-6 has been observed in serum and tissue biopsies from human or experimental UC studies and positively correlates with the severity of UC[13,14]. Binding of IL-6 to its soluble receptor (sIL-6R) forms the IL-6/sIL-6R complex which stimulates gp130 expressing and induces gp130 dimerization. Activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K) pathways[15] also occurs through IL-6 signal transduction via gp130. The IL-6/gp130 signaling axis is implicated in the pathogenesis of various inflammatory diseases including UC, thus effectively blocking the production IL-6 represents a feasible strategy for the treatment of UC.

We have previously reported that benzoxazole compounds substituted with sulfamine functional group at 2 or 4 positions exhibited determinate high IL-6 inhibitory activity[16]. As our ongoing studies on the potential use of benzoxazole as anti-inflammatory agents, twenty-three 6-acylamino/sulfonamido substituted benzoxazole derivatives were synthesized and evaluate anti-inflammatory against IL-6. Additionally, the therapeutic potential of highly active compounds *in vivo* for UC was also explored. Our findings will provide a valuable foundation for the application of IL-6 inhibitors in colitis treatment and further structural optimization.

Results and discussion

Synthesis of benzoxazole derivatives

As described in Scheme 1, 6-aminobenzoxazolone was used as a raw material to react with acyl chloride or sulfonyl chloride under alkaline conditions of K_2CO_3 to produce the target compound. The procedure was simple with an average percent yield of 50-60%. The structures of all twenty-three target compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, and ESI-MS.

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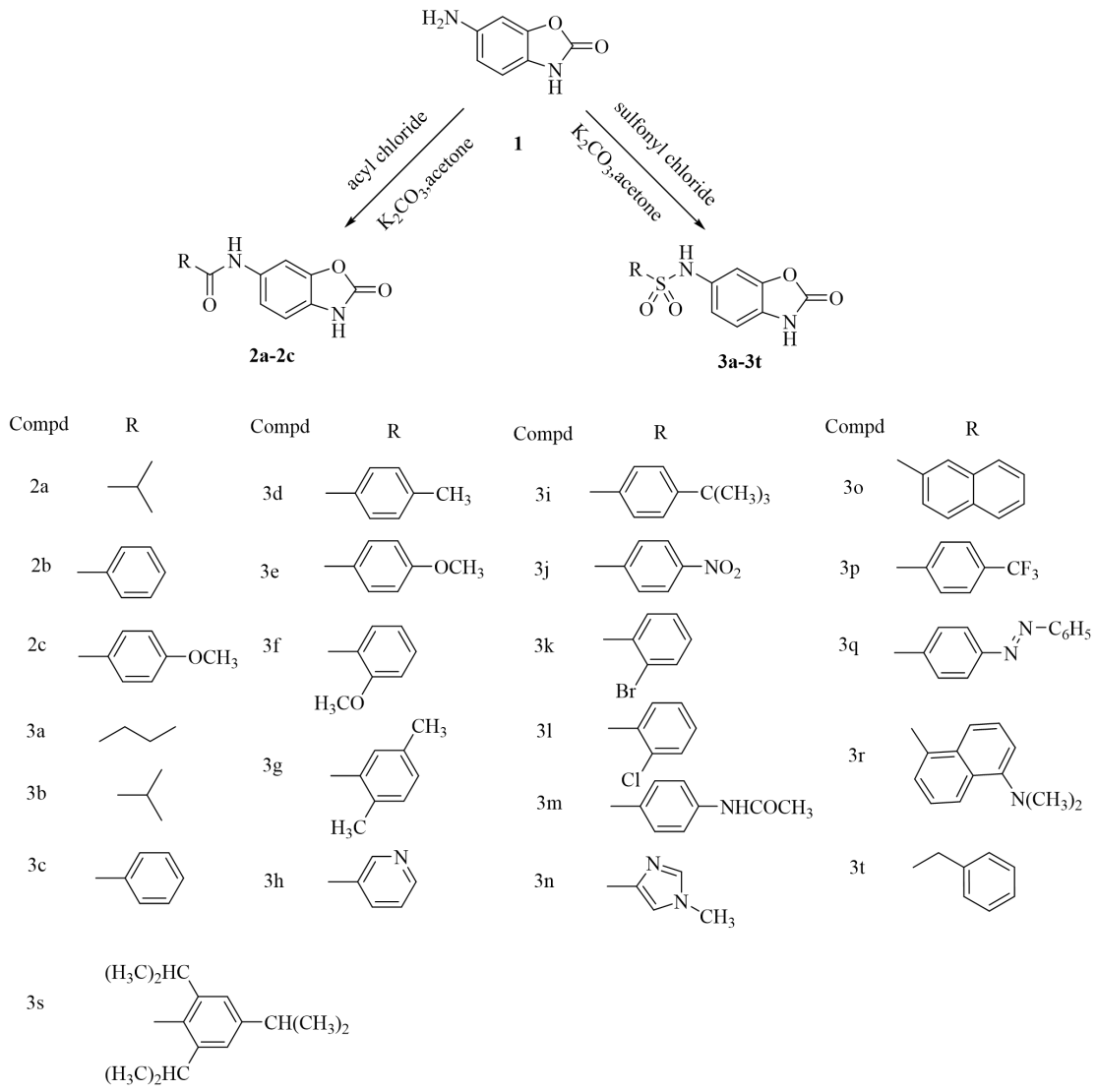

Scheme 1 The synthetic route of the target compounds

Initial anti-inflammatory screening against LPS-induced NO and IL-6 release

All the synthesized compounds were screened for their anti-inflammatory activity *in vitro* on LPS-induced mouse RAW 267.4 macrophages cells. Initially, the cell viability of the compounds was assessed at 10 μM using the MTT method to ensure that subsequent activities were not due to toxicity but rather their own intrinsic properties. As shown in Figure 1A, most of the compounds exhibited a cell viability of over 90%, expect compounds 2a, 3e, 3h, 3m and 3s. Inflammation triggers an increase in pro-inflammatory cytokines such as NO and IL-6 by activated macrophages, leading to inflammatory injury. Therefore, LPS, a powerful activators of macrophages, was used to treat with RAW 267.4 macrophages cells at 1 μg/mL to stimulate the inflammatory response, and then primary screening tests were conducted at a concentration of 10 μM to evaluate the inhibitory activity of target compounds on NO and IL-6 production, except compounds 3h, 3m and 3s. Meanwhile, to validate the reliability and reproducibility of our established model, clinical medicine celecoxib was selected as a positive control drug. The results (Figure 1B, 1C) revealed that the majority of the compounds presented a moderate inhibitory effect against NO and IL-6,

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which was either superior or equivalent to that of the positive drug. Structure-activity relationship analysis showed that when benzoxazolone was modified by introducing sulfonamido at 6 position to give compounds 3b, 3c and 3e, there appeared to be no enhancement in IL-6 inhibitory activity compared to compounds 2a, 2b and 2c with acylamino substitution, however, these modifications resulted in a more effective inhibition of NO. This finding was consistent with our previous discovery on 2-substituted benzoxazolone derivatives[16]. In addition, we also found that electronic effects had no significant impact on anti-inflammatory activity as seen in compounds such as 3i and 3j. Surprisingly, compounds 3p and 3r, which had the same substituent groups but different substituted position compared with our previous reported compounds[16], displayed markedly distinct activities which suggested that these compounds might have different targets warranting further investigation.

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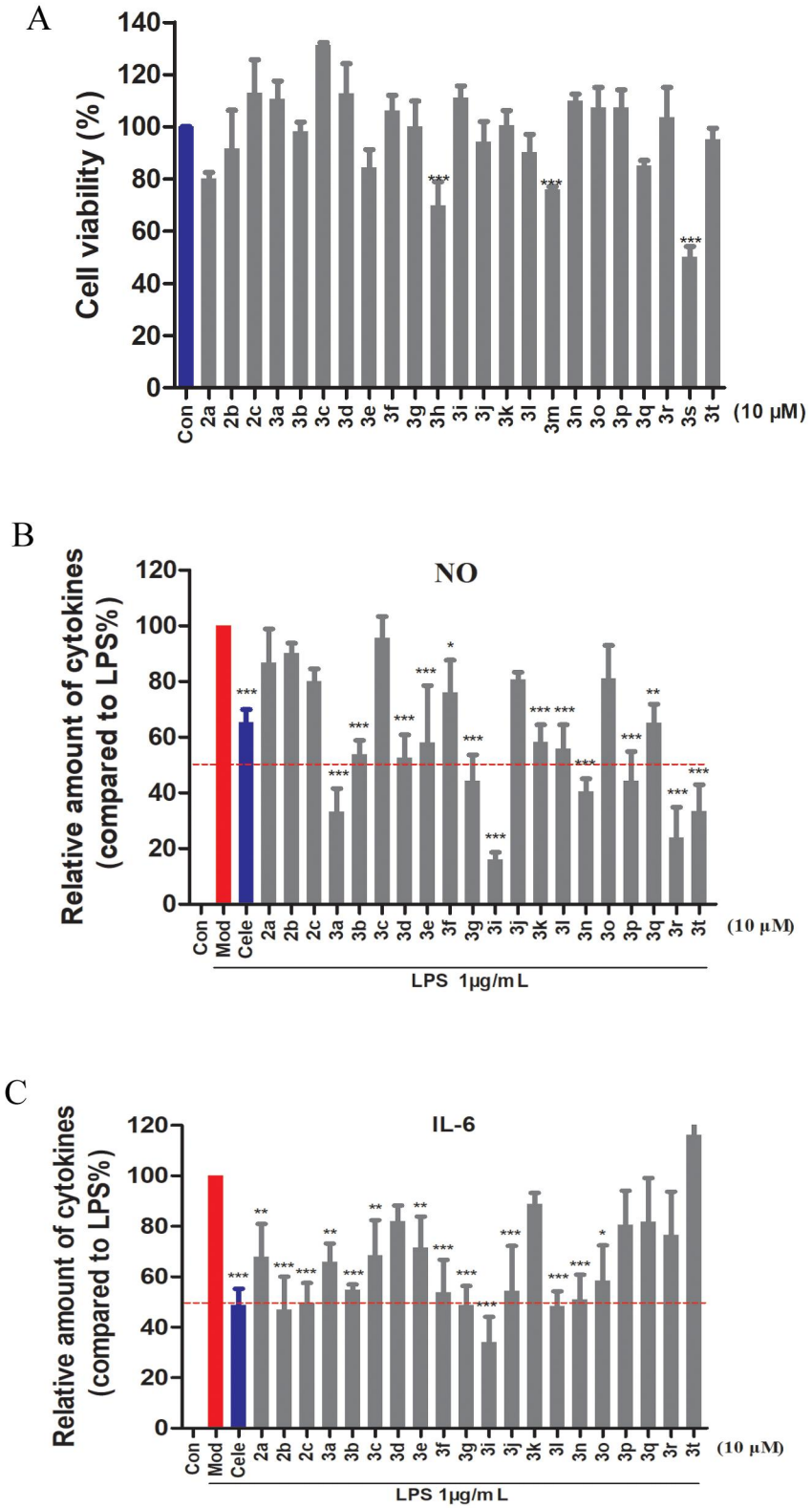

Figure 1 Inhibition effects of the synthesized compounds on NO and IL-6 at 10 μM in LPS-induced RAW 264.7 cells. (A) Cell viability evaluation by MTT method. (B) NO levels measured by Griess method. (C) IL-6 levels

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measured by ELISA. Data are represented as the mean ± SD of three independent experiments. (*p < 0.05 compared to model; **p < 0.01 compared to model; ***p < 0.001 compared to model)

Active compounds 3i, 3j and 3l inhibited LPS-induced NO and IL-6 release in a dose-dependent manner

Among all the derivatives, compounds 3i, 3j and 3l exhibited significant potential activity with NO and IL-6 inhibition rates exceeding 50%. To investigate the dose-dependent inhibitory effect on NO and IL-6, the active compounds 3i, 3j and 3l were selected to treat with RAW 267.4 macrophages cells at various concentrations ranging from 1.25 to 20 μM. As shown in Fig 2A and 2B, compounds 3i, 3j and 3l exhibited a dose-dependent inhibition effect on LPS-induced overproduction of both NO and IL-6. The IC_{50} values for compounds 3i, 3j and 3l were found to be 12.88, 6.03, and 7.06 μM for IL-6, and 8.18 μM of compound 3j for NO, respectively.

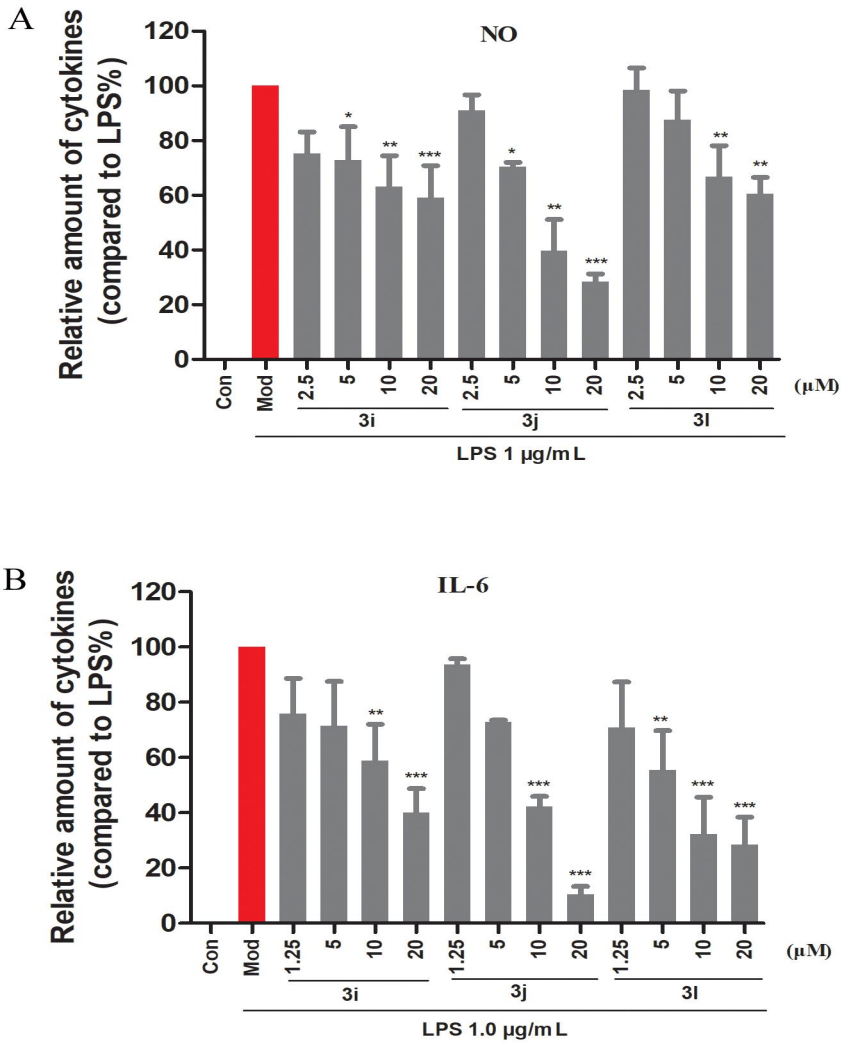

Figure 2 Inhibition effects of compounds 3i, 3j, and 3l on NO (A) and IL-6 (B) in LPS-induced RAW 264.7 cells. Data are represented as the mean ± SD of three independent experiments (*p < 0.05 compared to model; **p < 0.01 compared to model; ***p < 0.001 compared to model).

Active compounds 3i, 3j and 3l ameliorated the symptoms of dextran sulfate sodium (DSS)-induced ulcerative colitis in mice

Literature reports have demonstrated the significant roles of overproduction of NO and IL-6 in ulcerative colitis[12]. Our previous *in vitro* experiments have shown that compound 3i, 3j and 3l could effectively inhibit the secretion of NO and IL-6 to perform anti-inflammatory activity. Subsequently, the anti-inflammatory activities *in vivo* of active compounds 3i, 3j and 3l were tested on DSS-induced ulcerative colitis in mice. As shown in Fig 3A, the bodyweight of DSS-induced mice was gradually reduced compared to control group during the 7 days of experiment along with severe pathological symptoms such as diarrhea and hematochezia. Meanwhile, DAI results also showed a obvious increase in DAI value for DSS-induced colitis group (Fig 3B). While treatment with compounds 3i, 3j and 3l at a dose of 25 mg/kg could alleviate the weight loss and mitigate diarrhea severity while markedly reducing the DAI score from day 5 which were all superior to the positive drug salazosulfapyridine (SASP, 200 mg/kg).

In addition, DSS decreased the colonic length to 5.28 ± 0.35 cm, while compounds 3i, 3j and 3l restored the colon lengths to 5.92 ± 0.29, 6.4 ± 0.68, and 6.84 ± 0.83 cm, respectively (Fig 3C and 3D). Further pathological examination revealed severe damage in the colon tissue of the DSS-treated group including crypt disappearance, submucosal edema, inflammatory infiltration, mucosal epithelial cell and tissue necrosis. Compared with the model group, compounds 3i, 3j and 3l significantly ameliorated these pathologic changes induced by DSS (Fig 3E). Furthermore, treatment with 3i and 3j decreased inflammatory cell infiltration and mucosal injury while increasing goblet cells.

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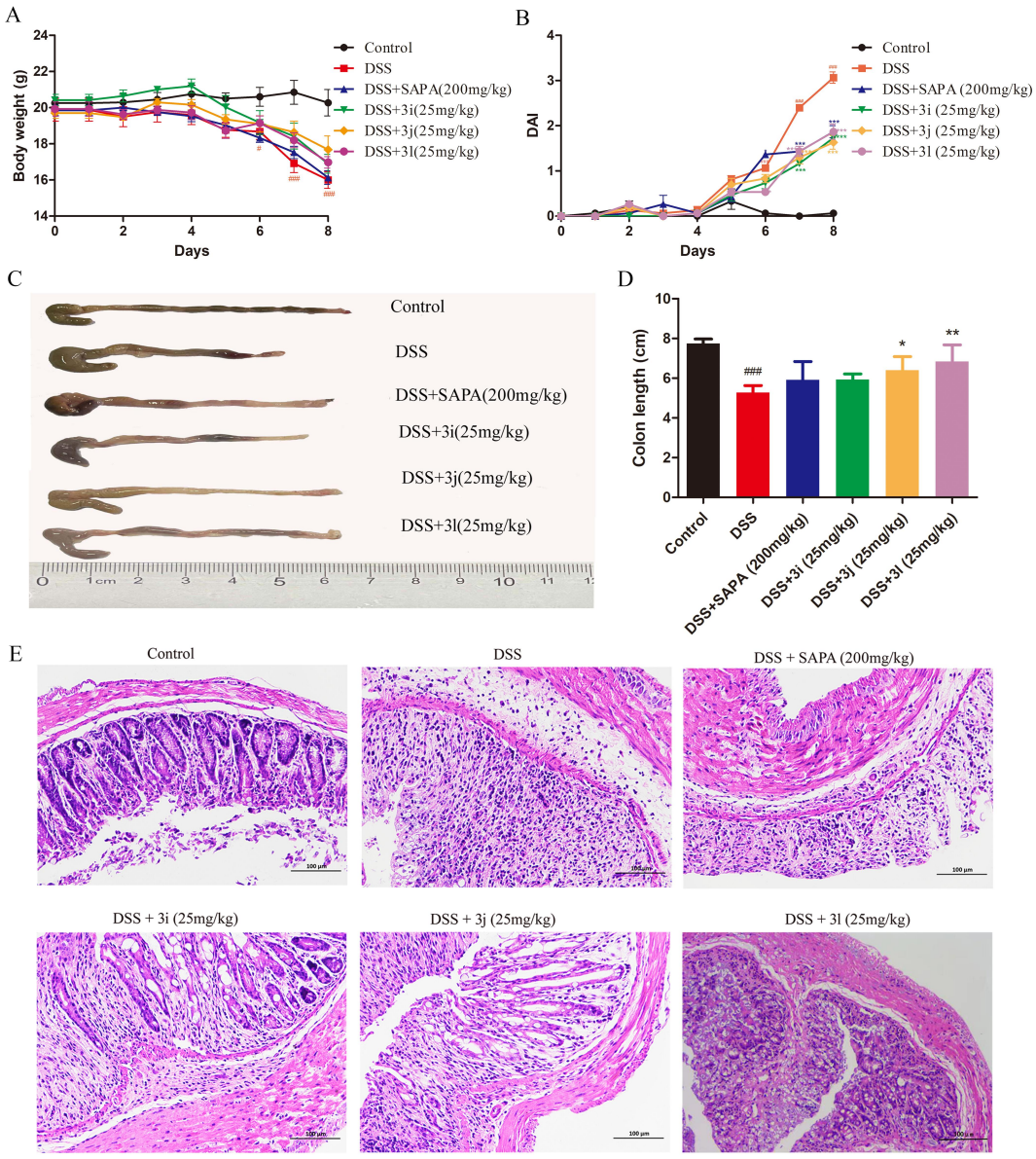

Figure 3 Effect of compound 3i, 3j and 3l on DSS-induced ulcerative colitis in mice. (A) The daily weight of mice was recorded. (B) The daily DAI of mice was calculated. (C-D) The colon length of mice was measured. Data are represented as the mean ± SD. (n=6) (E) Hematoxylin and eosin (H & E) were used for pathological analysis of colon tissues. Sulfasalazine (SASP, 200 mg/kg) was used as a positive control drug. $^{\#}p < 0.05$ compared with control group; $^{\#\#}p < 0.01$ compared with control group; $^{\#\#\#}p < 0.001$ compared with control group; $^{*}p < 0.05$ compared with DSS group; $^{**}p < 0.01$ compared with DSS group; $^{***}p < 0.001$ compared with DSS group.

Active compounds 3i, 3j and 3l decreased the MDA, MPO activity to mitigated oxidative stress damage induced by DSS

Lipid peroxidation (MDA) emerged as a pivotal marker of oxidative stress, exhibiting a significant increase in UC colon tissue. Myeloperoxidase (MPO), a peroxidase responsible for producing excessive amounts of oxidants and inducing oxidative tissue damage, was found to be upregulated. As shown in Fig 4, the model group displayed increased MDA and MPO activity in colonic tissue compared to the control group, while active compounds 3i, 3j and 3l exhibited a

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remarkable reduction in both MDA and MPO levels that were equivalent to the positive drug. The results implied that our derivatives could able to inhibit the oxidative stress effectively to alleviate UC.

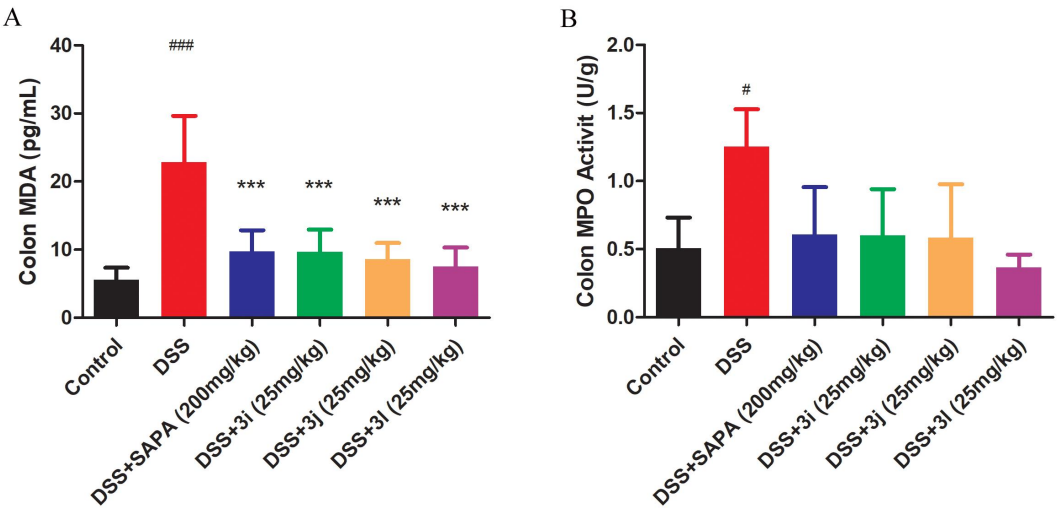

Figure 4 Effect of compound 3i, 3j and 3l on expression of MDA (A) and MPO activity(B) in colon tissues of DSS-induced ulcerative colitis mice. Data are represented as the mean ± SD. (n=6). Sulfasalazine (SASP, 200 mg/kg) was used as a positive control drug. $^{\#}p < 0.05$ compared with control group; $^{\#\#}p < 0.01$ compared with control group; $^{\#\#\#}p < 0.001$ compared with control group; $^{*}p < 0.05$ compared with DSS group; $^{**}p < 0.01$ compared with DSS group; $^{***}p < 0.001$ compared with DSS group.

Active compounds 3i, 3j and 3l modulated the expression of IL-6, IL-10 and IFN-γ in serum to inhibit inflammatory response

Levels of inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, are closely associated with the initiation and persistence of ulcerative colitis. It was found that active compounds 3i, 3j and 3l could reduce the levels of inflammatory cytokines IL-6 and IFN-γ (Fig 5A, 5B). Notably, expressions of IL-6 was markedly decrease in compounds 3i and 3j (25 mg/kg) treated groups which was superior to the positive drug SASP (200 mg/kg). Meanwhile, we observed a remarkable increase in the expression of anti-inflammatory factor IL-10 following treatment with our derivatives. In contrast, SASP only slightly enhanced IL-10 levels without significant difference compared to the model group (Fig 5C). These results demonstrated that our derivatives possessed inhibitory effects on excessive pro-inflammatory cytokine production while promoting anti-inflammatory factors to modulate the inflammatory response.

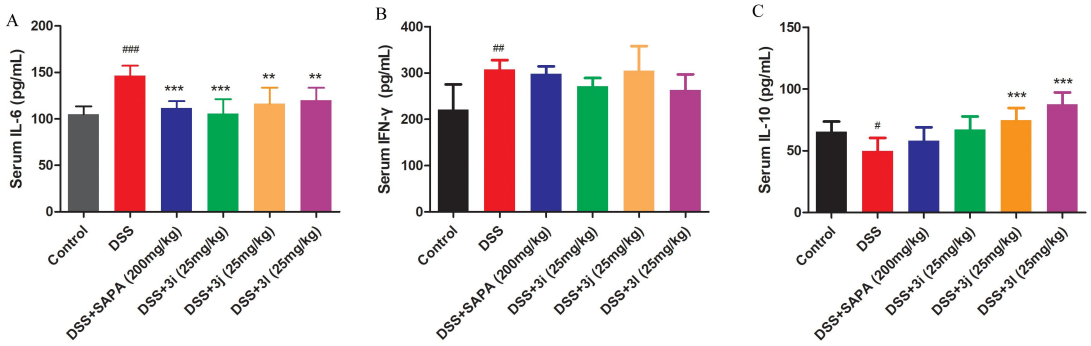

Figure 5 Effect of compound 3i, 3j and 3l on expression of IL-6 (A), IFN-γ(B) and IL-10 (C) in serum of DSS-induced ulcerative colitis mice. Data are represented as the mean ± SD. (n=6). Sulfasalazine (SASP, 200 mg/kg) was used as a positive control drug. $^{\#}p < 0.05$ compared with control group; $^{\#\#}p < 0.01$ compared with control group; $^{\#\#\#}p < 0.001$ compared with control group; $^{*}p < 0.05$ compared with DSS group; $^{**}p < 0.01$ compared with DSS group; $^{***}p < 0.001$ compared with DSS group.

Conclusions

In conclusion, twenty-three 6-acylamino/sulfonamido substituted benzoxazole derivatives were designed and synthesized, which not only increased the chemical diversity of benzoxazole, but also led to the discovery of three promising anti-inflammatory derivatives 3i, 3j and 3l that exhibited excellent IL-6 inhibitory activity with IC_{50} values 12.88, 6.03, and 7.06 μM, respectively. Further pharmacological studies demonstrated that these compounds could ameliorate DAI score, decrease the inflammatory cell infiltration and mucosal injury, reduce the MDA, MPO, IL-6, IFN-γ levels while increasing the expression of IL-10 on DSS-induced ulcerative colitis in mice which were better than the positive drug salazosulfapyridine (SASP, 200 mg/kg). In general, compounds 3i, 3j and 3l with high IL-6 inhibited activity exhibited a protective effect on ulcerative colitis which could serve as new structure for developing anti-inflammatory drugs for UC treatment.

Materials and Methods

Chemistry

General methods and material

Melting points were determined on a X-4 microscopic thermometer without any corrections applied. Electronspray ionization mass spectra in positive mode (ESI-MS) were recorded on a Water ZQ2000 mass spectrometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker 600 MHz instrument (Bruker Company, Germany) in DMSO solvent. All the reactions were monitored by thin layer chromatography (TLC) on silica gel GF254 plates, and the target compounds were purified through by column chromatography using silica gel (200-300 mesh, Qingdao, China). All chemical reagents and solvents used in this study were purchased from commercial sources and utilized without purification. The ELISA kits used for the *in vitro* activity assays were purchased from Boster Biological Technology.

General procedure for the preparation of compound 2a-2c and 3a-3t

46 mg (0.33 mmol) K_2CO_3 was added to a stirred solution of 6-aminobenzoxazol-2(3H)-one (50 mg, 0.33mmol) in acetone, and stirred at room temperature for 30 min. Subsequently, a solution of different chloride/sulfonyl chloride (0.4 mmol) in dry acetone was added to undergo refluxing at 60℃ for 4-6 h . The reaction progress was monitored by TLC, and the target compounds were purified using chromatography.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)isobutyramide **2a** Yellow solid; yield(%): 19.9%; mp(℃): 145.6~147.3℃; ESI-MS(m/z): 221.33 ($[M+H]^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.80 (s, 1H), 7.63 (s, 1H), 7.18 (d, J = 7.8 Hz, 1H), 6.94 (d, J = 6.4 Hz, 1H), 2.55 (m, 1H), 1.09 (d, J = 6.8 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 175.34, 156.76, 144.27, 133.88, 130.12, 114.70, 109.97, 101.70, 35.34, 22.55, 19.99.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzamide **2b** Yellow solid; yield(%): 70.9%; mp(℃): 158.8~159.7℃; ESI-MS(m/z): 253.25 ($[M-H]^-$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.58 (s, 1H), 10.30 (s, 1H), 7.95 (d, J = 7.2 Hz, 2H), 7.83 (s, 1H), 7.63- 7.57 (m, 1H), 7.54 (t, J = 7.5 Hz, 2H), 7.49 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 165.88, 155.11, 143.58, 135.31, 134.38, 132.03, 128.86, 128.06, 126.72, 116.49, 109.87, 103.27.

4-methoxy-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzamide **2c** Yellow solid; yield(%): 82.3%; mp(℃): 69.2~70.9℃; ESI-MS(m/z): 283.22 ($[M-H]^-$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.55 (s, 1H), 10.13 (s, 1H), 7.95 (d, J = 8.8 Hz, 2H), 7.82 (s, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 8.8 Hz, 3H), 3.84 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 165.23, 162.38, 155.12, 143.58, 134.58, 129.98, 127.31, 126.51, 116.42, 114.08, 109.81, 103.24, 55.90.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)propane-1-sulfonamide **3a** White solid; yield(%): 46.9%; mp(℃): 123~125℃; ESI-MS(m/z):257.13 ($[M+H]^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.18 (d, J = 8.6 Hz, 1H), 6.59 (s, 1H), 6.45 (d, J = 10.8 Hz, 1H), 3.71 (t, J = 7.8 Hz, 2H), 1.80-1.72 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 150.20, 147.57, 142.98, 117.34, 113.51, 110.03, 96.38, 54.91, 16.83, 12.68.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)propane-2-sulfonamide **3b** Brown solid; yield(%): 44.5%;mp(℃): 119.4~120.8 ℃; ESI-MS(m/z): 257.30 ($[M+H]^+$); ^{1}H NMR (600 MHz, DMSO-d_6):δ 7.17 (d, J = 8.7 Hz, 1H), 6.58 (s, 1H), 6.45 (d, J = 8.7 Hz, 1H), 3.92 (m, 1H), 1.36 (d, J = 6.8 Hz, 6H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 150.21, 147.59, 142.94, 117.78, 113.62, 110.07, 96.34, 55.99, 15.96.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3c** White solid; yield(%): 72.4%; mp(℃): 205.3~207.2℃; ESI-MS(m/z): 289.11 ($[M-H]^-$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.58 (s, 1H), 10.18 (s, 1H), 7.72 (d, J = 7.3 Hz, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.54 (t, J =7.7 Hz, 2H), 7.01 (s, 1H), 6.95 (d, J = 8.3 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H); ^{13}C NMR (151MHz, DMSO-d_6): δ 154.81, 143.77, 139.65, 133.35, 132.38, 129.69, 127.85, 127.17, 117.74, 110.34, 104.26.

4-methyl-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3d** White solid; yield(%): 98%; mp(℃): 259.1~261.2℃; ESI-MS(m/z): 303.23 ($[M-H]^-$); ^{1}H NMR(600 MHz, DMSO-d_6):δ 11.57 (s, 1H), 10.10 (s, 1H), 7.60 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 7.00 (s, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 2.33 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 154.81, 143.76, 143.67, 136.83, 132.56, 130.11, 127.70, 127.22, 117.53, 110.34, 104.05, 31.76.

4-methoxy-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3e** White solid; yield(%):43.11%; mp(℃): 243~244.1 ℃; ESI-MS(m/z): 321.58 ($[M+H]^+$);^{1}H NMR (600 MHz, DMSO-d_6): δ 11.56 (s, 1H), 10.02 (s, 1H), 7.64 (d, J = 8.9 Hz, 2H), 7.05 (d, J = 8.9 Hz, 2H), 7.00

(s, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 162.87, 154.82, 143.76, 132.71, 131.28, 129.39, 127.64, 117.51, 114.81, 110.33, 104.02, 56.07.

2-methoxy-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3f** Yellow solid; yield(%):52.5%; mp(℃): 220.1~220.9℃; ESI-MS(m/z): 321.21 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.01 (d, J = 7.9 Hz, 1H), 7.80-7.70 (m, 1H), 7.29-7.15 (m, 3H), 6.55 (s, 1H), 6.46 (d, J = 8.6 Hz, 1H), 3.63 (s, 3H)；^{13}C NMR (151 MHz, DMSO-d_6): δ 157.62, 149.52, 147.44, 142.75, 137.82, 131.64, 124.25, 121.04, 117.56, 114.17, 113.98, 110.08, 96.16, 56.76.

2,5-dimethy-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3g** Brown solid; yield(%): 28.3%; mp(℃): 155.3~156.9℃; ESI-MS(m/z):319.25 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.00 (d, J = 8.2 Hz, 1H), 7.36-7.29 (m, 3H), 6.57 (s, 1H), 6.50 (d, J = 8.7 Hz, 1H), 2.45 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 149.50, 147.59, 146.70, 142.93, 138.60, 134.09, 132.29, 131.56, 127.76, 117.23, 113.88, 110.28, 96.53, 21.39, 20.12.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)pyridine-3-sulfonamide **3h** White solid; yield(%):46.38%; mp(℃): 223.5~225.5℃; ESI-MS(m/z): 292.26 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.63 (s, 1H), 10.37 (s, 1H), 8.83 (s, 1H), 8.79 (d, J = 6.8 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.60 (t, J = 8.0 Hz, 1H), 7.03 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 154.79, 153.97, 147.57, 143.84, 135.93, 135.25, 131.64, 128.36, 124.80, 118.28, 110.45, 104.86.

4-(tert-butyl)-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3i** Yellow solid; yield(%): 60.7%; mp(℃): 157.3~158.3℃; ESI-MS(m/z):369.07 ([M+Na]); ^{1}H NMR(600 MHz, DMSO-d_6): δ 7.95 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.6 Hz, 1H),6.53-6.49 (m, 2H),1.28 (s, 9H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 159.45, 149.64, 148.01, 143.06, 133.72, 127.85, 127.40, 116.21, 113.95, 110.23, 96.33, 35.68, 31.03.

4-nitro-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3j** Yellow solid; yield(%): 25.97%; mp(℃): 259.1~261.1℃; ESI-MS(m/z): 334.19 ([M-H]$^-$);^{1}H NMR (600 MHz, DMSO-d_6): δ 11.63 (s, 1H), 10.48 (s, 1H), 8.36 (d, J = 8.8 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.04 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 154.78, 150.30, 145.02, 143.82, 131.42, 130.11, 128.82, 125.07, 118.46, 110.48, 104.97.

2-bromo-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3k** White solid; yield(%):52.86%; mp(℃): 201~203℃; ESI-MS(m/z): 368.80 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.34 (d, J = 9.7 Hz, 1H), 7.96 (d, J = 9.1 Hz, 1H), 7.78-7.70 (m, 2H), 7.29(d, J = 8.7 Hz, 1H), 6.59 (s, 1H), 6.49 (d, J = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 149.21, 147.78, 142.86, 137.22, 136.37, 135.76, 134.05, 129.15, 120.33, 117.36, 114.36, 110.26, 96.39.

2-chloro-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3l** Yellow solid; yield(%): 60.1%; mp(℃): 192.7~194.1℃; ESI-MS(m/z): 325.18 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.32 (d, J = 8.0 Hz, 1H), 7.86-7.69 (m, 3H), 7.29 (d, J = 8.7 Hz, 1H), 6.59 (s, 1H), 6.49 (d, J = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 149.20, 147.81, 142.87, 137.36, 134.07, 133.65, 132.83, 131.95, 128.73, 117.18, 114.16, 110.27, 96.41.

4-acetamido-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3m** White solid; yield(%): 19.9%; mp(℃):315.1~316.4℃; ESI-MS(m/z): 346.24([M-H]$^-$);^{1}H NMR (600 MHz, DMSO-d_6): δ 11.57 (s, 1H), 10.30 (s, 1H), 10.04 (s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.8 Hz, 2H), 6.99 (s, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 2.06 (s, 3H); ^{13}C NMR

(151 MHz, DMSO-d_6): δ 169.47, 154.82, 143.76, 143.57, 133.17, 132.59, 128.43, 127.72, 119.00, 117.67, 110.30, 104.19, 24.56.

1-methyl-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)-1H-imidazole-4-sulfonamide **3n** Brown solid; yield(%): 70.4%;mp(℃): 215.2~216.8℃; ESI-MS(m/z): 295.27 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.30 (s, 1H), 7.85 (s, 1H), 7.29 (d, J = 8.7 Hz, 1H), 6.53 (s, 1H), 6.47 (d, J = 8.7 Hz, 1H), 3.73 (s, 3H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 149.38, 147.72, 142.85, 141.46, 135.02, 129.04, 116.90, 114.22, 110.09, 96.18, 34.35.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)naphthalene-2-sulfonamide **3o** Yellow solid; yield(%):60.82%; mp(℃): 135.2~136.7℃; ESI-MS(m/z): 340.97 ([M+H]$^+$);^{1}H NMR (600 MHz, DMSO-d_6): δ 8.59 (d, J = 7.8 Hz, 1H), 8.48 (d, J = 8.6 Hz, 1H), 8.46 (d, J = 8.3 Hz, 1H), 8.19 (d, J = 6.9 Hz, 1H), 7.82 (t, J = 7.5 Hz, 1H), 7.71(m, 2H), 7.48 (d, J = 8.6 Hz, 1H), 6.55 (d, J = 8.0 Hz, 2H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 149.32, 147.93, 142.91, 137.51, 134.26, 133.36, 131.23, 130.21, 129.91, 128.04, 127.83, 125.13, 123.06, 116.54, 113.95, 110.31, 96.46.

4-trifluoromethyl-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3p** White solid; yield(%): 60.33%; mp(℃): 267.3~268.8℃; ESI-MS(m/z): 357.08 ([M-H]$^-$); ^{1}H NMR(600 MHz, DMSO-d_6): δ 11.65 (s, 1H), 10.43 (s, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 8.4 Hz, 2H), 7.05 (s, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 6.6 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 170.79, 154.79, 143.82, 143.50, 131.67, 128.28, 128.20, 126.99, 118.16, 110.45, 104.73.

4-phenyldiazenyl-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3q** Brown solid; yield(%):54.76%; mp(℃): 277.1~278.7.℃; ESI-MS(m/z): 393.10 ([M-H]$^-$); ^{1}H NMR(600 MHz, DMSO-d_6): δ 11.63 (s, 1H), 10.35 (s, 1H), 7.99 (d, J = 8.6 Hz, 2H), 7.93-7.90 (m, 4H), 7.64-7.62 (m, 3H), 7.06 (s, 1H), 6.97 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 6.4 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 170.79, 154.81, 154.34, 152.31, 143.80, 141.38, 132.93, 132.00, 130.08, 128.74, 128.15, 123.56, 123.36, 118.16, 110.41, 104.69.

5-(dimethylamino)-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)naphthalene-1-sulfonamide **3r** White solid; yield(%): 87.7%; mp(℃): 206.3~208.1℃; ESI-MS(m/z): 384.01 ([M+H]$^+$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.64 (d, J = 8.5 Hz, 1H), 8.56 (d, J = 7.4 Hz, 1H), 8.06 (d, J = 8.7 Hz, 1H), 7.78 (t, J = 8.0 Hz, 1H), 7.57 (t, J = 8.2 Hz, 1H), 7.45 (d, J = 9.2 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 6.57-6.50 (m, 2H), 2.82 (s, 6H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 170.80, 152.52, 149.30, 147.90, 142.87, 133.26, 131.58, 130.04, 129.45, 129.39, 124.11, 117.05, 116.66, 116.24, 113.93, 110.31, 96.46, 45.46.

2,4,6-triisopropyl-*N*-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)benzenesulfonamide **3s** Yellow solid; yield(%):57.3%; mp(℃): 162.6~163.9℃; ESI-MS(m/z): 439.14 ([M+Na]); ^{1}H NMR(600 MHz, DMSO-d_6): δ 7.37 (s, 2H), 7.32 (d, J=8.6 Hz, 1H), 6.62 (s, 1H), 6.54 (d, J=8.9 Hz, 1H), 4.01 (m, 2H), 2.97 (m, 1H), 1.23 (d, J = 6.94 Hz, 6H), 1.12 (d, J = 7.06 Hz, 12H); ^{13}C NMR (151 MHz, DMSO-d_6): δ 155.75, 152.04, 149.99, 147.60, 142.92, 130.29, 124.81, 116.84, 113.45, 110.43, 96.51, 33.95, 29.38, 24.46, 23.65.

N-(2-oxo-3-hydrobenzo[d]oxazol-6-yl)-1-phenylmethanesulfonamide **3t** Yellow solid; yield(%): 44.4%; mp(℃): 69.1~71.2℃; ESI-MS(m/z): 303.22([M-H]$^-$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.12 (s, 1H), 7.43-7.38 (m, 5H), 6.78 (d, J = 8.2 Hz, 1H), 6.57 (s, 1H), 6.42 (d, J = 8.2 Hz, 1H), 4.38 (s, 2H)； ^{13}C NMR (151 MHz, DMSO-d_6): δ 198.44, 155.15, 144.76, 143.34, 131.65, 129.05, 128.51, 128.35, 121.30, 110.39, 97.44, 62.95.

Biology assays *in vitro*

Cell culture

The RAW 264.7 cells, purchased from Boster Biological Technology, were cultured at 37°C under 5% CO_2 humidified atmosphere in DMEM medium supplemented with 10% FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin.

Cytotoxicity assay

The cells were seeded onto 96-well culture plates and allowed to adhere to the target compounds at a concentration of 10 μM for 24 h. Then 20 μL of MTT solution (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 °C. After the medium was removed, 150 μL DMSO was added to dissolve the resulting purple formazan, and the absorbance at 570 nm was recorded using microplate reader (Thermo Co. Ltd. Model 680) to calculate cell survival rate.

Nitric oxide assay

The RAW264.7 cells were cultured in 96-well plates and incubated with LPS (1 μg/ml) and target compounds (10 μM) for 24h to gain cell supernatant. Subsequently, the nitric oxide levels were measured immediately using the Griess method according to the instructions of the test kits. Following an incubation period of of 10 min with Griess reagent, nitrite levels were quantified at 540 nm using a microplate reader (Thermo Co. Ltd. Model 680).

Detection of IL-6 secretion

The production of pro-inflammatory cytokines IL-6 was evaluated by enzyme-linked immunosorbent assay (ELISA). RAW264.7 cells were cultured in 48-well plates and subsequently treated with LPS in the presence or absence of the test compounds (10 μM) at 37 °C. The cell supernatant was obtained after incubation for 24 h to detect the IL-6 level following the manufacturer's instructions. The absorbance at 450 nm was measured by a microplate reader (Bio-Rad Laboratories, CA, USA) to determine the relative amount compared to LPS treated group.

Biology assays *in vivo*

DSS-induced ulcerative colitis in mice

Male C57BL/6 mice (SPF, weight of 20±2 g) were purchased from Shanxi Medical University. The animals were adaptively fed in the laboratory environment for 7 days before formal experiments. Forty-eight mice were randomly divided into 6 groups (n = 8 per group): control group, DSS group, DSS+3i (25 mg/kg), DSS+3j (25 mg/kg), DSS+3l (25mg/kg), and DSS+positive drug SASP (salicylazosulfapyridine, 200 mg/kg, Shanghai Xinyi Tianping Pharmaceutical Co., LTD) group. Mice in the control group were free to drink water for 7 days, and DSS groups received 3% DSS (w/v) solution freely for consecutive 7 days. Meanwhile, all the mice in drug treated groups orally administrated with test compounds dissolved in 0.5% CMC-Na. Body weight and fecal bleeding were recorded daily to calculate disease activity index (DAI). On the eight day, the mice were euthanized under anesthesia and the colon tissues and blood samples were collected for analysis.

Histopathologic examination of colon tissues

The collected colon tissues fixed in a 4% paraformaldehyde solution were embedded in paraffin and cut into 5 μm sections. Subsequently, hematoxylin and eosin (H&E) staining was performed

to observe the pathological changes under a microscope, which was performed by Wuhan Sevicebio Technology Biotechnology Co., LTD.

Detection of MPO and MDA activity

The colon tissues were washed with PBS and homogenized in a saline solution to gain intestinal tissue homogenate. Then the activities of MPO and MDA were measured according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute) . Finally, the absorbance was determined using a microplate reader at 460 nm and 532 nm, respectively.

Measurement of cytokines

The collected blood was centrifuged at 3000 rpm for 15 minutes at 4°C to obtain the serum. Subsequently, the expressions of IL-6, IL-10 and IFN-γ in serum were detected using enzyme-linked immunosorbent assay kits (Jiangsu Meimian Industrial Co., LTD) following the manufacturer's instructions.

Statistical analysis

All the data were presented as the mean ± standard error of three independent experiments, and the statistical analysis of data was processed using GraphPad Prism 5.0. Statistical tests of One-way ANOVA were used and a p value < 0.05 was considered to be statistically significant.

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.

Author Contribution Statement

Rui Ge and Jiaqi Song: activity assays *in vitro* and *in vivo.* Zhen Cao: design, synthesis, and structural identification. Shurong Ban: article modification. Li Tang: article writing, corresponding author. Qingshan Li: projects design, corresponding author.

Acknowledgement

This work was financially supported by the National Natural Science Foundation of China (NO. 81602976), Shanxi Province Science Foundation (NO. 202203021211235), and Shanxi Key Laboratory of Innovative Drug for the Treatment of Serious Diseases Based on Chronic Inflammation, China (NO. 2021sxcxyw07).

Ethical Approval

All of the experimental procedures involving animals were conducted in accordance with the Institutional Animal Care guidelines of Shanxi Medical University. The animal production license number is SCXK (Jin) 2019-0004.

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Figure, Table and Scheme captions

Scheme 1 The synthetic route of the target compounds

Figure 1 Inhibition effects of the synthesized compounds on NO and IL-6 at 10 μM in LPS-induced RAW 264.7 cells. (A) Cell viability evaluation by MTT method. (B) NO levels measured by Griess method. (C) IL-6 levels measured by ELISA. Data are represented as the mean ± SD of three independent experiments. (*$p < 0.05$ compared to model; **$p < 0.01$ compared to model; ***$p < 0.001$ compared to model)

Figure 2 Inhibition effects of compounds 3i, 3j, and 3l on NO (A) and IL-6 (B) in LPS-induced RAW 264.7 cells. Data are represented as the mean ± SD of three independent experiments (*$p < 0.05$ compared to model; **$p < 0.01$ compared to model; ***$p < 0.001$ compared to model).

Figure 3 Effect of compound 3i, 3j and 3l on DSS-induced ulcerative colitis in mice. (A) The daily weight of mice was recorded. (B) The daily DAI of mice was calculated. (C-D) The colon length of mice was measured. Data are represented as the mean ± SD. (n=6) (E) Hematoxylin and eosin (H & E) were used for pathological analysis of colon tissues. Sulfasalazine (SASP, 200 mg/kg) was used as a positive control drug. $^{\#}p < 0.05$ compared with control group; $^{\#\#}p < 0.01$ compared with control group; $^{\#\#\#}p < 0.001$ compared with control group; $^{*}p < 0.05$ compared with DSS group; $^{**}p < 0.01$ compared with DSS group; $^{***}p < 0.001$ compared with DSS group.

Figure 4 Effect of compound 3i, 3j and 3l on expression of MDA (A) and MPO activity(B) in colon tissues of DSS-induced ulcerative colitis mice. Data are represented as the mean ± SD. (n=6). Sulfasalazine (SASP, 200 mg/kg) was used as a positive control drug. $^{\#}p < 0.05$ compared with control group; $^{\#\#}p < 0.01$ compared with control group; $^{\#\#\#}p < 0.001$ compared with control group; $^{*}p < 0.05$ compared with DSS group; $^{**}p < 0.01$ compared with DSS group; $^{***}p < 0.001$ compared with DSS group.

Figure 5 Effect of compound 3i, 3j and 3l on expression of IL-6 (A), IFN-γ(B) and IL-10 (C) in serum of DSS-induced ulcerative colitis mice. Data are represented as the mean ± SD. (n=6). Sulfasalazine (SASP, 200 mg/kg) was used as a positive control drug. $^{\#}p < 0.05$ compared with control group; $^{\#\#}p < 0.01$ compared with control group; $^{\#\#\#}p < 0.001$ compared with control group; $^{*}p < 0.05$ compared with DSS group; $^{**}p < 0.01$ compared with DSS group; $^{***}p < 0.001$ compared with DSS group.

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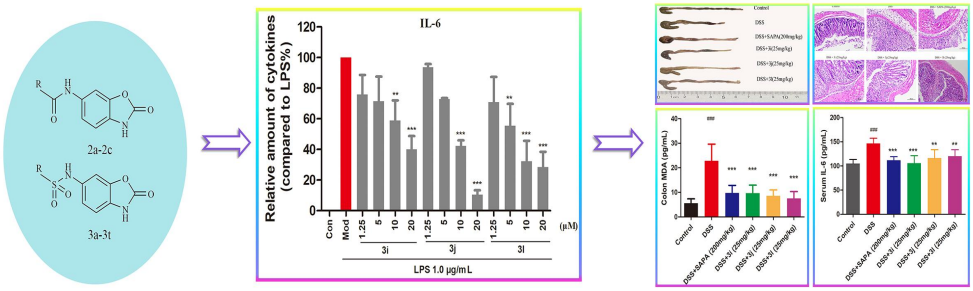
10.1002/cbdv.202400031

Graphical abstract



Tweetable summary

Twenty-three 6-acylamino/sulfonamido benzoxazolone derivatives were prepared and identified. Compounds **3i, 3j** and **3l** showed the highest inflammatory activity and determinate therapeutical effect on ulcerative colitis which provide a new therapeutic agents for ulcerative colitis.