Bioorg. Med. Chem. Lett. 43 (2021) 128059

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and activity of *N*-(5-hydroxy-3,4,6-trimethylpyridin-2-yl) acetamide analogues as anticolitis agents via dual inhibition of TNF-α- and IL-6-induced cell adhesions

Ujjwala Karmacharya [a], Sushil Chandra Regmi [a], Bhuwan Prasad Awasthi [a], Prakash Chaudhary [a], Ye Eun Kim [b], Iyn-Hyang Lee [a], Tae-gyu Nam [b,*], Jung-Ae Kim [a,*], Byeong-Seon Jeong [a,*]

[a] College of Pharmacy and Institute for Drug Research, Yeungnam University, Gyeongsan 38541, Republic of Korea
[b] Department of Pharmacy and Institute of Pharmaceutical Science and Technology, Hanyang University, Ansan, Gyeonggi-do 15588, Republic of Korea

ARTICLE INFO

Keywords:
Inflammatory bowel disease
TNF-α
IL-6
Amidopyridinol
TNBS-induced colitis

ABSTRACT

Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are the critical pro-inflammatory cytokines involved in the pathogenesis of inflammatory bowel disease (IBD). Inhibition of these cytokines and related signaling pathways has been a target for the development of IBD therapeutics. In the current study, 6-acetamido-2,4,5-trimethylpyridin-3-ol (**1**) and various analogues with the amido scaffold were synthesized and examined for their inhibitory activities in *in vitro* and *in vivo* IBD models. The parent compound **1** (1 μM) showed an inhibitory activity against TNF-α- and IL-6-induced adhesion of monocytes to colon epithelial cells, which was similar to tofacitinib (1 μM), a JAK inhibitor, but much better than mesalazine (1,000 μM). All the analogues showed a positive relationship (R^2 = 0.8943 in a linear regression model) between the inhibitory activities against TNF-α-induced and those against IL-6-induced adhesion. Compound **2**–**19** turned out to be the best analogue and showed much better inhibitory activity against TNF-α- and IL-6-induced adhesion of the cells than tofacitinib. In addition, oral administration of compound **1** and **2**–**19** resulted in a significant suppression of clinical signs of TNBS-induced rat colitis, including weight loss, colon tissue edema, and myeloperoxidase activity, a marker for inflammatory cell infiltration in colon tissues. More importantly, compound **2**–**19** (1 mg/kg) was more efficacious in ameliorating colitis than compound **1** and sulfasalazine (300 mg/kg), the commonly prescribed oral IBD drug. Taken together, the results suggest that compound **2**–**19** can be a novel platform for dual-acting IBD drug discovery targeting both TNF-α and IL-6 signaling.

Inflammatory bowel disease (IBD) is the chronic, relapsing, and abrogating inflammation of the gastrointestinal tract and is mainly classified into Crohn's disease (CD) and ulcerative colitis (UC) based on pathologic features [1,2]. The etiology of IBD is complex, however, the altered immune function of both innate and adaptive immunity is regarded as a major player in the pathogenesis of IBD [3]. Although different subtypes of adaptive immune T cells are involved in CD (a Th1/Th17-associated) versus UC (a Th2-like disease) [4], abnormally high level of expression of proinflammatory cytokines are common in CD and UC, in particular, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [5–7].

TNF-α exerts a variety of detrimental effects on the intestinal mucosa, such as disruption of barrier function, promotion of intestinal epithelial cell death, and induction of other pro-inflammatory cytokines [8,9]. Upon TNF-α binding, TNF receptor type 1 (TNFR1) activates signaling molecules, TNF-R1-associated death domain protein (TRADD), receptor-interacting protein 1 (RIP1), and TNFR-associated factor 2 (TRAF2), which sequentially lead to activation of nuclear factor-κB (NF-κB), a redox-sensitive transcription factor [10,11]. In the signaling pathway, NADPH oxidase (NOX) is also activated. NOX-derived reactive oxygen species (ROS) in phagocytic and non-phagocytic cells have been shown to play an important role in TNF-α-mediated immune responses [12–15]. In TNF-α-treated cells, TNF-α–ROS–NF-κB pathway is also involved in de novo synthesis of IL-6 and STAT3 [16].

IL-6 binds and activates a heterodimeric signaling complex consisting of the IL-6 α-receptor (IL-6Rα) and the signal-transducing β-subunit

* Corresponding authors.
E-mail addresses: tnam@hanyang.ac.kr (T.-g. Nam), jakim@yu.ac.kr (J.-A. Kim), jeongb@ynu.ac.kr (B.-S. Jeong).

https://doi.org/10.1016/j.bmcl.2021.128059
Received 4 February 2021; Received in revised form 29 March 2021; Accepted 19 April 2021
Available online 23 April 2021

glycoprotein 130 (gp130) [17]. The expression of gp130 is found in every tissue and cell types, whereas IL-6R is predominantly expressed in certain types of cells, such as several leukocyte subpopulations, megakaryocytes, and hepatocytes. Heterodimeric association of gp130 with IL-6Rα triggers phosphorylation of the Janus kinases, JAK1, JAK2, and tyrosine kinase 2, which are constitutively associated with gp130, leading to the activation (phosphorylation) of STAT3 [18]. Active STAT3 subsequently translocates from the cytoplasm to the nucleus, serving a critical role as a transcription factor [19]. TNF-α induction is reported as one of the consequent gene expressions by IL-6–STAT3 pathway [20]. Also, IL-6 signaling activates NF-κB, which is critical for induction of inflammatory molecules in colon epithelium [21]. TNF-α-induced upregulations of TNF-α and IL-6 suggest a crosstalk between TNF-α and IL-6 [22].

Although anti-TNF-α biologics, such as infliximab and adalimumab, and anti-IL-6 receptor antibody, such as tocilizumab, have been successfully developed [23–26], anti-TNFα antibody drugs become the most effective therapeutics in the clinic among the biologics and orally available drugs including tofacitinib, a JAK inhibitor [27–29]. In search of small molecule version alternatives, we have reported several classes of 2,4,5-trimethylpyridin-3-ol analogues (**A**~**F** in Fig. 1), derived from pyridoxine·HCl (also known as vitamin B_6·HCl) with anti-colitis activity [30–32]. They mainly tether modifications on C(6)-position with various functional group linkers such as amino-, ureido-, thioureido-, sulfonamido-, carbamato-, and alkoxy-groups with trimethylpyridinol moiety being retained. In a consecutive study, we have reported the structural change of pyridinol ring moiety and its effect on anti-colitis activity where 6-acetamido-2,4,5-trimethylpyridin-3-ol (**1** in Fig. 2) was used as a reference structure for modification [33]. Compound **1** was observed to be far superior to mesalazine/sulfasalazine and in a similar level to tofacitinib in the activities against both *in vitro* TNF-α-induced adhesion and in vivo DSS-induced colitis model. Mesalazine is an orally available IBD drug for patients with mild to moderate level of severity. Because one of the action mechanism of mesalazine is suppression of nuclear factor-κB [34], which is involved in the transcription of a variety of inflammatory genes, it was used as a positive control to compare anti-inflammatory action of compounds. Our efforts on the discovery of effective anti-colitis agents continued. Various analogues with the amido scaffold (**1**) were designed and synthesized in pursuit of the diversity of side chains tethering on the C(6)-position. In the present study, we categorized the amido group into four classes (G^1~G^4), as shown in the general structure **2** (Fig. 2) and examined if there is a relationship between chemical structure and activity. We also investigated a correlation by a statistical analysis between the inhibitory activity of this series of compounds against TNF-α-induced adhesion and against IL-6-induced adhesion.

Described in Scheme 1 is the general synthetic method for amido analogues starting from pyridoxine which has been well-established by us [35]. Briefly, the two benzylic hydroxyl groups of pyridoxine were cut off and converted into two methyl groups as in compounds **3**, and then benzyl protection followed by ring bromination afforded the substrate **4** for the introduction of nitrogen-containing functionality at C(6)-position. The key compound **8** for the preparation of various amido derivatives was obtained by palladium-catalyzed amination reaction with benzophenone imine as the ammonia equivalent and successive methanolysis. We used an alternative synthetic method for compound **8** which has also been developed by us and includes a nucleophilic aromatic substitution reaction of pyridine-*N*-oxide compound **6** with phthalimide. Twenty-six acid chlorides which can be classified into roughly four categories (G^1 ~ G^4) as shown in Fig. 2 were reacted with the key intermediate compound **8** to give amido products **9**. Subsequent debenzylation toward the final compounds **2** was conducted using either hydrogenolysis or BCl_3 in the presence of pentamethylbenzene as a non-Lewis-basic cation scavenger. The chemical structure of each compound is shown in Table 1.

As shown in Table 1, high level of positive correlation was observed between inhibitory activities against TNF-α-induced cell adhesion and those against IL-6-induced one throughout the table, demonstrating the crucial role of both cytokines in cell adhesion event. The adhesion of monocytic cells to colonic epithelial cells can be initial pathological characteristics of IBD. The parent compound, **1,** showed 56% inhibition against TNF-α-induced adhesion at 1 μM concentration (Table 1). It also inhibited IL-6-induced adhesion with the activity slightly lower than that against TNF-α-induced adhesion. Mesalazine, the active metabolite of sulfasalazine (SSZ), showed very weak inhibition against both TNF-α- and IL-6-induced adhesions even at 1000 μM concentration. Interestingly, tofacitinib showed quite an inhibitory activity against both TNF-α- and IL-6-induced adhesion (49.1% and 58.6%, respectively), indicating JAK/STAT inhibition can result in cross activity against TNF-α-induced and IL-6-induced cell adhesions. Of the compound **1** analogues, six

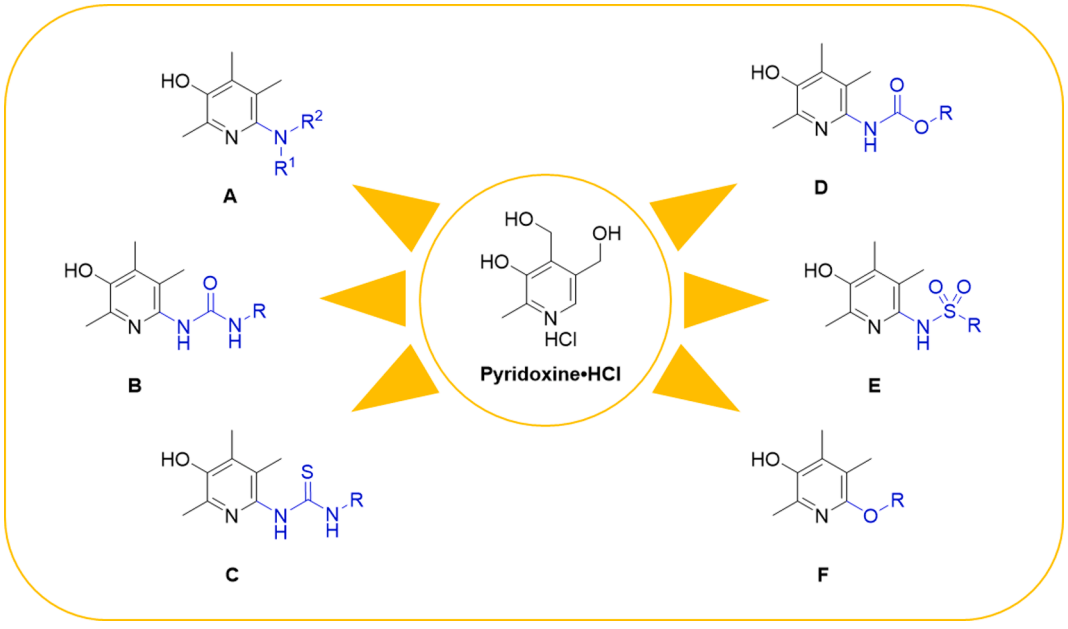


Fig. 1. General structures of 2,4,5-trimethylpyridin-3-ol analogues (**A** ~ **E**) which were synthesized from pyridoxine·HCl and used for the discovery of anti-colitis agents by us.

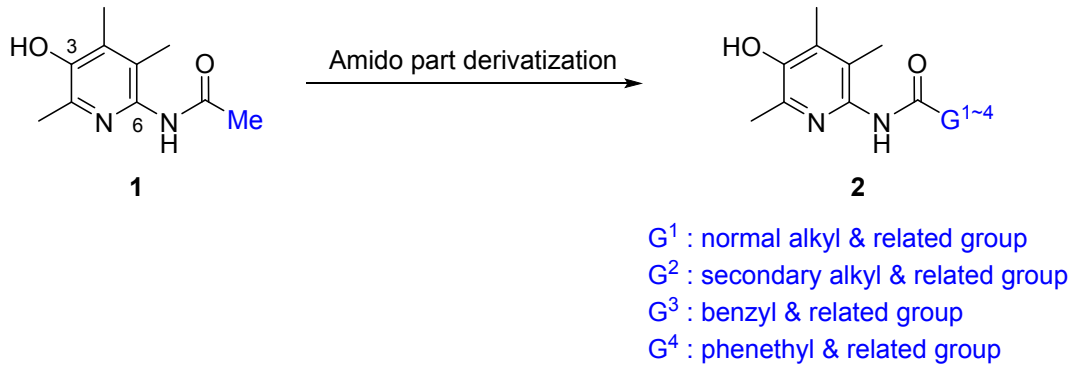
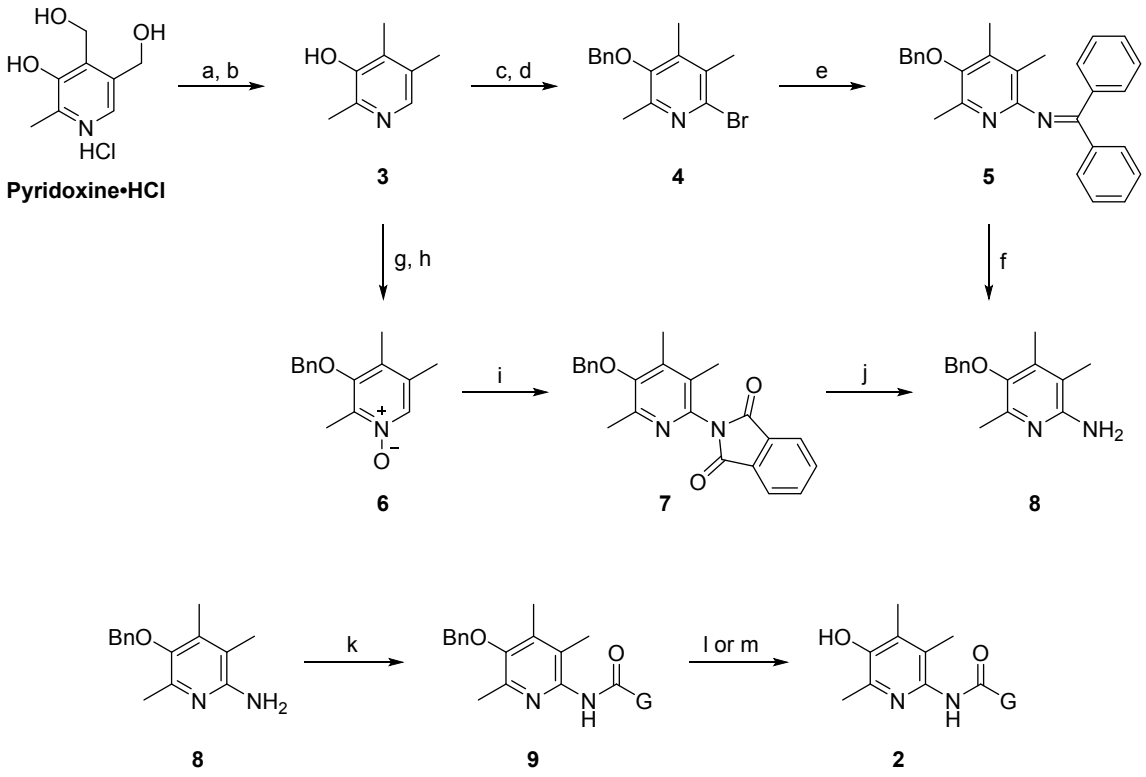


Fig. 2. Analogues of 6-acetamido-2,4,5-trimethylpyridin-3-ol (**1**) with diverse side chains for anti-colitis study.



Scheme 1. Synthetic method for derivatization of amino moiety of 6-amido-2,4,5-trimethylpyridinol. Reagents and conditions: (a) $SOCl_2$, DMF, reflux, 30 min, 93%; (b) Zn, AcOH, reflux, 3 h, 92%; (c) DBDMH, THF, r.t., 3 h, 80%; (d) $PhCH_2Cl$, K_2CO_3, DMF, r.t., 12 h, 97%; (e) HN = CPh_2, $Pd_2(dba)_3$, BINAP, $NaOBu^t$, PhMe, reflux, 12 h, 83%; (f) HCl in MeOH, THF, r.t., 12 h, 83%; (g) $PhCH_2Cl$, K_2CO_3, DMF, r.t., 12 h, 71%; (h) *m*-CPBA, CH_2Cl_2, r.t., 1 h, 94%; (i) *p*-TsCl, *i*-Pr_2NEt, phthalimide, CH_2Cl_2, r.t., 12 h, 70%; (j) H_2NNH_2, THF-EtOH, r.t., 1 h, 90%; (k) G-COCl, Et_3N, CH_2Cl_2; (l) H_2, Pd/C, MeOH, r.t.; (m) BCl_3, $CHMe_5$, CH_2Cl_2, 0 °C~r.t.

compounds (**2–2**, **2–10**, **2–12**, **2–14**, **2–15**, and **2–19**) showed higher inhibition against TNF-α-induced adhesion than **1**. Also, these compounds showed better activity than **1** in IL-6-induced adhesion.

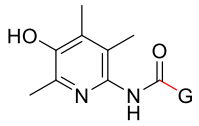
All the compounds in Table 1 showed a positive correlation between the inhibitory activities against TNF-α-induced and those against IL-6-induced adhesion (Table 1 and Fig. 3). For the statistical analysis of the correlation, a linear regression model was performed. The simple linear regression equation with y (% inhibition against IL-6 induced adhesion) as the response variable and × (% inhibition against TNF-α-induced adhesion) as the explanatory variable was

$y = 0.82\,x + 0.54$ ($R^2 = 0.8943$)

For every one-unit increase in x, the y increased by 0.82 on average. In other words, increasing x by 10% is associated with an increase in the y of 8.2% ($P < 0.0001$). That is, these series compounds inhibit monocyte-epithelial cell adhesion induced by TNF-α or IL-6, and the degree of inhibition against each cytokine-induced adhesion is a proportional relationship (Fig. 3).

Of the analogues in G^1 groups, there seems to be no simple structure–activity relationship (SAR) regarding the length of alkyl chain. C_3 (normal-, chloro-, and unsaturated-C_3 chain) and C_{15} side chains (**2–1**, **2–8**, **2–9**, and **2–4**, respectively) showed low activity while C_1, C_7, and C_{11} side chains showed high activity (**1**, **2–2**, and **2–11**, respectively). Branched alkyl side chains in G^1 group generally showed considerable activity > 50% (**2–5** and **2–6**). Analogues in G^2 group generally showed high activity except for *t*-butyl side chain (**2–11**). More specifically, G^2 group analogues with a methine carbon directly attached to amide carbonyl showed high activity (**2–10**, **2–12**, **2–13**, and **2–14**) while a quaternary carbon-bearing analogue (**2–11**) showed low activity. Of the G [3] group analogues, only a simple benzyl side chain (**2–15**) showed considerable activity while all the halogenated analogues showed low activity. Halogenated benzyl side chains (**2–16** and **2–17**) are especially detrimental to the activity. G^3 group has a methylene spacer between

Table 1
Inhibitory activity of compounds at 1 μM concentration against TNF-α- and IL-6-induced adhesion of human monocytic cells (U937) to human colonic epithelial cells (HT-29).



2

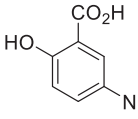
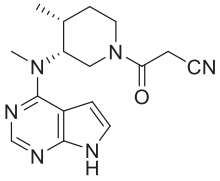
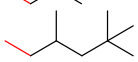
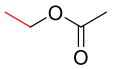
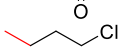
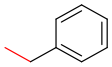
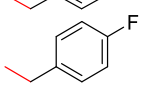
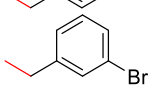
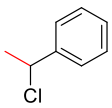
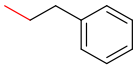
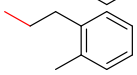
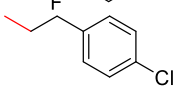
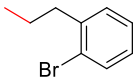
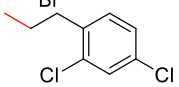
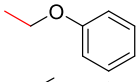
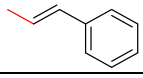
Compound	G		% inhibition in adhesion assay (1 μM)[a]	
	Category	Structure	TNF-α-induced	IL-6-induced
Mesalazine			9.9 ± 14.7 (1,000 μM)	1.7 ± 7.8 (1,000 μM)
Tofacitinib			49.1 ± 0.1*	58.6 ± 2.3*
1		$-CH_3$	56.0 ± 6.3*	47.2 ± 4.1*
2–1	G^1	$-CH_2CH_2CH_3$	10.0 ± 6.1	17.5 ± 9.4*
2–2	G^1	$-CH_2(CH_2)_5CH_3$	61.5 ± 7.2*	54.8 ± 6.6*
2–3	G^1	$-CH_2(CH_2)_9CH_3$	55.8 ± 7.7*	44.3 ± 8.0*
2–4	G^1	$-CH_2(CH_2)_{13}CH_3$	31.7 ± 10.5	17.4 ± 7.1
2–5	G^1		52.3 ± 4.2*	48.0 ± 4.4*
2–6	G^1		54.5 ± 10.4*	46.3 ± 4.7*
2–7	G^1		6.8 ± 9.1	9.7 ± 5.7*
2–8	G^1		24.7 ± 18.0	17.8 ± 4.2
2–9	G^1		31.9 ± 0.8*	21.3 ± 6.9
2–10	G^2		64.2 ± 1.4*	53.9 ± 4.4*
2–11	G^2		37.5 ± 12.2	21.1 ± 6.0*
2–12	G^2		60.6 ± 12.4*	52.7 ± 9.1*
2–13	G^2		42.8 ± 7.8*	34.8 ± 5.1*
2–14	G^2		64.5 ± 12.5*	56.7 ± 6.2*
2–15	G^2		56.6 ± 8.0*	50.2 ± 4.3*
2–16	G^2		7.2 ± 4.8*	18.1 ± 5.2*
2–17	G^3		6.2 ± 10.7	9.2 ± 8.4*
2–18	G^3		36.6 ± 0.9*	22.5 ± 3.6

Table 1 (*continued*)

Compound	G		% inhibition in adhesion assay (1 μM)[a]	
	Category	Structure	TNF-α-induced	IL-6-induced
2–19	G^4		72.5 ± 13.2*	69.6 ± 9.8*
2–20	G^4		20.2 ± 1.4*	12.9 ± 4.5
2–21	G^4		13.9 ± 6.6*	18.0 ± 7.0*
2–22	G^4		30.6 ± 4.9*	20.8 ± 4.6*
2–23	G^4		35.3 ± 1.7*	22.0 ± 5.5*
2–24	G^4		41.5 ± 3.6*	35.9 ± 5.1*
2–25	G^4		10.8 ± 15.7	11.4 ± 9.2*
2–26	G^4		25.2 ± 14.7	14.2 ± 5.7

*P < 0.05 versus vehicle-treated group.
[a] Data are shown as 'mean ± SEM' of at least three independent experiments performed in triplicate.

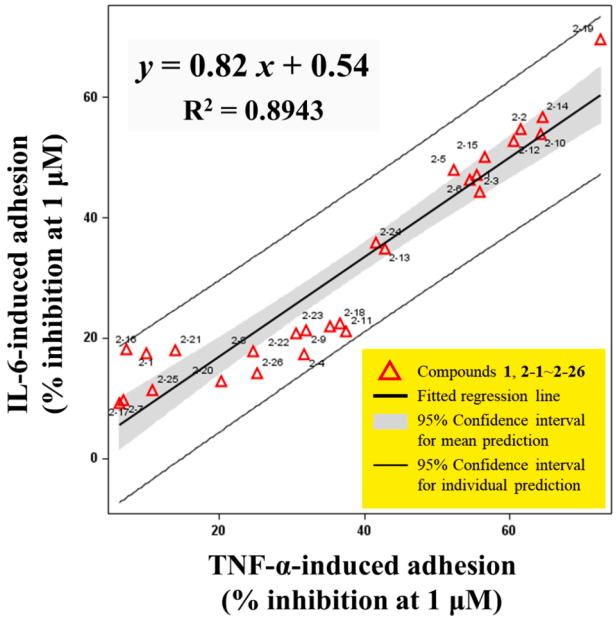


Fig. 3. The regression of % inhibition against IL-6 induced adhesion (y) on % inhibition against TNF-α-induced adhesion (x). Analyses were conducted using SAS procedures (SAS version 9.4, SAS Institute, Cary, NC, USA) and significance was defined at $P < 0.05$.

amide carbonyl carbon and a phenyl ring, and the addition of one more methylene spacer to that of G^3 group rendered G^4 group. Halogen substituents undermining the activity in G^3 group displayed the same effects on the analogues with phenethyl side chains in G^4 group. Only a simple phenethyl side chain (**2–19**) showed the best activity both against TNF-

α- and IL-6-induced cell adhesion (72.5% and 69.6%, respectively) while the other analogues within the group showed low to moderate activity. We chose compounds **1** and **2**–**19** as the parent structure and the best analogue, respectively, for the in vivo experiment using a TNBS-induced rat colitis model.

Administration of TNBS to rat colon through rectum induced bloody diarrhea, decreased activity, and a significant reduction followed by stagnated increase in body weight (Fig. 4A-1). In contrast, oral administration of compound **1** showed a significant recovery of the TNBS-induced decrease in body weight in a dose-dependent manner (Fig. 4A-1). Also, compound **1** suppressed congestion, adhesion (Fig. 4A-2), and increase in tissue weight/ length (Fig. 4A-3) in the colon treated with TNBS. The effects of compound **1** (10 mg/kg) was better than SSZ (300 mg/kg). Oral administration of compound **2**–**19** (1 mg/kg) showed a better ameliorating effect on TNBS-induced changes in body weight (Fig. 4B-1), colon morphology (Fig. 4B-2), and colon weight/ length (Fig. 4B-3). Measurement of tissue myeloperoxidase (MPO) level, which is directly related to neutrophil content and used for quantitation of inflammation [36], also showed the superior activity of compound **2**–**19** to compound **1** or SSZ (Fig. 4C).

In this study, we synthesized 6-acetamido-2,4,5-trimethylpyridin-3-ol analogues with the structural variation on amido-substituent and examined their inhibitory activity in colitis models *in vitro* and *in vivo*. High level of positive relationship was found in between the inhibitory activity against TNF-α-induced and that against IL-6-induced adhesion of monocytes to colon epithelial cells. Many compounds in this study showed superior inhibition to mesalazine at three orders of magnitude lower concentration. In TNBS-induced in vivo rat colitis model, oral administration of compound **2**–**19** with phenethyl chain on amido part at 1 mg/kg showed excellent recovery profiles in body and colon weight

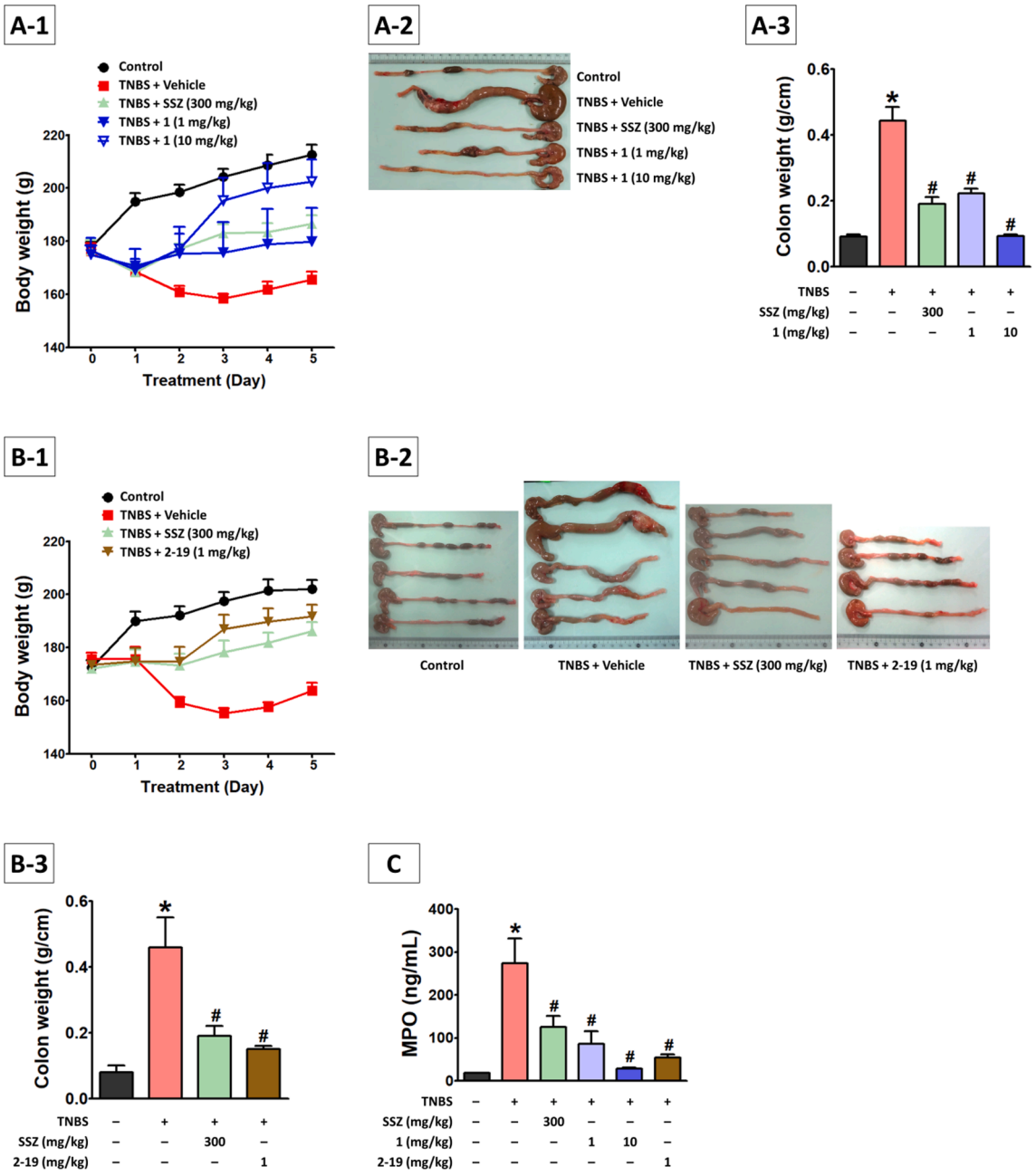


Fig. 4. Inhibitory effect of compounds **1** and **2**–**19** on TNBS-induced rat colitis. TNBS was administered via rectum, and compounds **1** and **2**–**19** were orally administered daily for 5 days. MPO level in colon tissues was determined by using MPO assay kit. $^{*}P < 0.05$ vs control (Mock) group. $^{\#}P < 0.05$ vs vehicle-treated TNBS colitis group. SSZ: sulfasalazine.

and MPO level, which was better than the recovery activity of 300 mg/kg SSZ. Although the detailed mechanism of action should be studied further, it is strongly suggested that compound **2–19** can be an excellent anti-IBD scaffold targeting both TNF-α and IL-6 signaling.

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.

Acknowledgement

This work was supported by the 2019 Yeungnam University Research Grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128059.

References

[1] Laass MW, Roggenbuck D, Conrad K. Diagnosis and classification of Crohn's disease. *Autoimmen Rev*. 2014;13:467–471.
[2] Neurath MF. Current and emerging therapeutic targets for IBD. *Nat Rev Gastroenterol Hepatol*. 2017;14:269–278.
[3] Oliva-Hemker M, Fiocchi C. Etiopathogenesis of inflammatory bowel disease: the importance of the pediatric perspective. *Inflamm Bowel Dis*. 2002;8:112–128.
[4] Fuss IJ, Heller F, Boirivant M, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest*. 2004;113:1490–1497.
[5] Strober W, Lúdvíksson BR, Fuss IJ. The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn disease. *Ann Intern Med*. 1998;128:848–856.
[6] Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood*. 2008;112:1557–1569.
[7] Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol*. 2014;5:491.
[8] Neurath MF. Cytokines in inflammatory bowel disease. *Nat Rev Immunol*. 2014;14:329–342.
[9] Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. *Crit Rev Eukaryot Gene Expr*. 2010;20:87–103.
[10] Festjens N, Vanden Berghe T, Vandenabeele P. Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. *Biochim Biophys Acta*. 2006;1757:1371–1387.
[11] Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science*. 2002;296:1634–1635.
[12] Kadayat TM, Banskota S, Gurung P, et al. Discovery and structure-activity relationship studies of 2-benzylidene-2,3-dihydro-1H-inden-1-one and benzofuran-3(2H)-one derivatives as a novel class of potential therapeutics for inflammatory bowel disease. *Eur J Med Chem*. 2017;137:575–597.
[13] Banskota S, Regmi SC, Kim JA. NOX1 to NOX2 switch deactivates AMPK and induces invasive phenotype in colon cancer cells through overexpression of MMP-7. *Mol Cancer*. 2015;14:123.
[14] Blaser H, Dostert C, Mak TW, Brenner D. TNF and ROS Crosstalk in Inflammation. *Trends Cell Biol*. 2016;26:249–261.
[15] Lambeth JD, Krause KH, Clark RA. NOX enzymes as novel targets for drug development. *Semin Immunopathol*. 2008;30:339–363.
[16] Brasier AR. The nuclear factor-kappaB-interleukin-6 signalling pathway mediating vascular inflammation. *Cardiovasc Res*. 2010;86:211–218.
[17] Garbers C, Hermanns HM, Schaper F, et al. Plasticity and cross-talk of interleukin 6-type cytokines. *Cytokine Growth Factor Rev*. 2012;23:85–97.
[18] Boulanger MJ, Chow DC, Brevnova EE, Garcia KC. Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex. *Science*. 2003;300:2101–2104.
[19] Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci (Lond)*. 2012;122:143–159.
[20] de Jong PR, Schadenberg AW, van den Broek T, et al. STAT3 regulates monocyte TNF-alpha production in systemic inflammation caused by cardiac surgery with cardiopulmonary bypass. *PLoS ONE*. 2012;7, e35070.
[21] Wang L, Walia B, Evans J, Gewirtz AT, Merlin D, Sitaraman SV. IL-6 induces NF-kappa B activation in the intestinal epithelia. *J Immunol*. 2003;171:3194–3201.
[22] Tanabe K, Matsushima-Nishiwaki R, Yamaguchi S, Iida H, Dohi S, Kozawa O. Mechanisms of tumor necrosis factor-alpha-induced interleukin-6 synthesis in glioma cells. *J Neuroinflammation*. 2010;7:16.
[23] Valin A, Del Rey MJ, Municio C, et al. IL6/sIL6R regulates TNFα-inflammatory response in synovial fibroblasts through modulation of transcriptional and post-transcriptional mechanisms. *BMC Mol Cell Biol*. 2020;21:74.
[24] Song YN, Zheng P. Efficacy and safety of tumor necrosis factor-α blockers for ulcerative colitis: A systematic review and meta-analysis of published randomized controlled trials. *J Food Drug Anal*. 2015;23:1–10.
[25] Fausel R, Afzali A. Biologics in the management of ulcerative colitis - comparative safety and efficacy of TNF-α antagonists. *Ther Clin Risk Manag*. 2015;11:63–73.
[26] Lis K, Kuzawińska O, Bałkowiec-Iskra E. Tumor necrosis factor inhibitors - state of knowledge. *Arch Med Sci*. 2014;10:1175–1185.
[27] Kang S, Tanaka T, Kishimoto T. Therapeutic uses of anti-interleukin-6 receptor antibody. *Int Immunol*. 2015;27:21–29.
[28] Andreakos E, Taylor PC, Feldmann M. Monoclonal antibodies in immune and inflammatory diseases. *Curr Opin Biotechnol*. 2002;13:615–620.
[29] Nishimoto N, Kishimoto T. Inhibition of IL-6 for the treatment of inflammatory diseases. *Curr Opin Pharmacol*. 2004;4:386–391.
[30] Banskota S, Kang HE, Kim DG, et al. In vitro and in vivo inhibitory activity of 6-amino-2,4,5-trimethylpyridin-3-ols against inflammatory bowel disease. *Bioorg Med Chem Lett*. 2016;26:4587–4591.
[31] Park SW, Banskota S, Gurung P, et al. Synthesis and evaluation of 6-heteroarylamino-2,4,5-trimethylpyridin-3-ols as inhibitors of TNF-α-induced cell adhesion and inflammatory bowel disease. *MedChemComm*. 2018;9:1305–1310.
[32] Chaudhary CL, Gurung P, Jang S, et al. Synthesis, activity and mechanism of alkoxy-, carbamato-, sulfonamido-, thioureido-, and ureido-derivatives of 2,4,5-trimethylpyridin-3-ol against inflammatory bowel disease. *J Enzyme Inhib Med Chem*. 2020;35:1–20.
[33] Chaudhary CL, Chaudhary P, Dahal S, et al. Inhibition of colitis by ring-modified analogues of 6-acetamido-2,4,5-trimethylpyridin-3-ol. *Bioorg Chem*. 2020;103,104130.
[34] Iacucci M, de Silva S, Ghosh S. Mesalazine in inflammatory bowel disease: a trendy topic once again? *Can J Gastroenterol*. 2010;24:127–133.
[35] Lee H, Banskota S, Kim DG, et al. Synthesis and antiangiogenic activity of 6-amido-2,4,5-trimethylpyridin-3-ols. *Bioorg Med Chem Lett*. 2014;24:3131–3136.
[36] Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology*. 1984;87:1344–1350.