MedChemComm

Accepted Manuscript

View Article Online
DOI: 10.1039/C8MD00156A

Journal Name

ARTICLE

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Synthesis and evaluation of 6-heteroarylamino-2,4,5-trimethylpyridin-3-ols as inhibitors of TNF-α-induced cell adhesion and inflammatory bowel disease

Sang Won Park,[†a] Suhrid Banskota,[†b] Pallavi Gurung,[b] You Jin Jin,[a] Han-eol Kang,[a] Chhabi Lal Chaudhary,[b] Sang Yeul Lee,[a] Byeong-Seon Jeong,*[b] Jung-Ae Kim,*[b] and Tae-gyu Nam*[a]

Inflammatory bowel disease (IBD) is an inflammatory disease in gastrointestinal tract with complex pathogenesis. Here, we synthesized 6-heteroarylamino analogues to inhibit TNF-α-induced adhesion of monocyte to colon epithelial cells which is implicated in initial inflammation process of IBD. The best analogue, **16a**, showed IC_{50} = 0.29 μM, which is about five orders of magnitude better than that of 5-aminosalicylic acid (5-ASA), a positive control. Oral administration of **6f** and **16a** dramatically ameliorated 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colon inflammation in rat. The ameliorating effects were accompanied by high level of recovery in colon and body weights and in myeloperoxidase (MPO) level. Consistently, the compounds suppressed the expression of intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein 1 (MCP-1). Moreover, they significantly suppressed the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 while increasing the level of IL-10, an anti-inflammatory cytokine.

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory pathology occurring in gastrointestinal tract with major subtypes being Crohn's disease and ulcerative colitis.[1] Although it is considered a low-mortality disease, it can severely devastate quality of life of patients. It is spreading all around the world with over 1 million people in USA and 2.5 million in Europe being estimated to have IBD.[2] Recently, unprecedented growing rates are observed even in traditionally low-incidence regions including developing countries. Several drugs are currently used in clinic to treat symptoms of IBD.[2-4]

Although the causes of IBD have not been clearly understood, it is believed that complex association of genetic, environmental, and immune factors underlies the disease.[1,5] In gut, mucosal layer performs a first-line defense conducting a number of innate immune functions. Under harmful conditions such as disturbance of microbiotic and cytokine balance, intestinal epithelial cells participate in the initiation and dissemination of inflammation by secreting pro-inflammatory cytokines and chemokines.[5] Among them, tumor necrosis factor-α (TNF-α) is secreted from monocytes and colon epithelial cells. TNF-α, in turn, induces colon epithelial cells to express other inflammatory cytokines and adhesion molecules in higher level. It recruits more inflammatory cells, including immune cells, into the damaged intestinal epithelium. This process is one of the critical steps in the initiation of inflammation and tissue injury in IBD.[6,7]

Beside TNF-α, other pro-inflammatory cytokines also contribute to the aggravation of colitis. Interleukin-6 (IL-6) plays a crucial role in the uncontrolled intestinal inflammatory process by enhancing STAT-3 nuclear translocation where it induces anti-apoptotic genes such as Bcl-xL. The development of T cells' resistance to apoptosis further aggravates intestinal inflammation.[8] IL-1β level is increased in colonic tissues of IBD patients,[9] and its levels are positively linked with the disease severity of IBD.[10] In contrast to the enhanced pro-inflammatory cytokines, decrease in IL-10, an anti-inflammatory cytokine mainly produced by innate immune cells such as monocytes and regulatory T cells, also aggravates IBD[11] by losing its ability to inhibit bacterial product-mediated induction of pro-inflammatory cytokines, such as TNF-α and IL-1β.[12] Considering these network and interaction between pro- and anti-inflammatory cytokines, a compound that corrects or restores the altered balance between the pro- and anti-inflammatory cytokines in IBD would be the most desirable therapeutic drug.

Although several molecular targets have been proposed for IBD,[13-16] TNF-α currently seems to be the most successful target mainly because of the high efficacy of anti-TNF-α monoclonal antibodies, such as infliximab and adalimumab. As virtually no small molecule has been approved for IBD since the anti-TNF-α antibodies, there are huge medical unmet needs for chemical version of anti-IBD agents. A few small molecule anti-IBD agents have been reported in literatures or in clinical trials.[14,17-20]

[a.] Department of Pharmacy and Institute of Pharmaceutical Science and Technology, Hanyang University, Ansan, Gyeonggi-do 15588, Republic of Korea.
[b.] College of Pharmacy and Institute for Drug Research, Yeungnam University, Gyeongsan 38541, Republic of Korea.
† Equally contributed as first author.
∗ Corresponding author. Tel.: +82-53-810-2814; fax: +82-53-810-4654; e-mail: jeongb@ynu.ac.kr (B.-S. J); Tel.: +82-53-810-2816; fax: +82-53-810-4654; e-mail: jakim@yu.ac.kr (J.-A. K.); Tel.: +82-31-400-5807; fax: +82-31-400-5958; e-mail: tnam@hanyang.ac.kr (T.-g. N.)
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8MD00156A

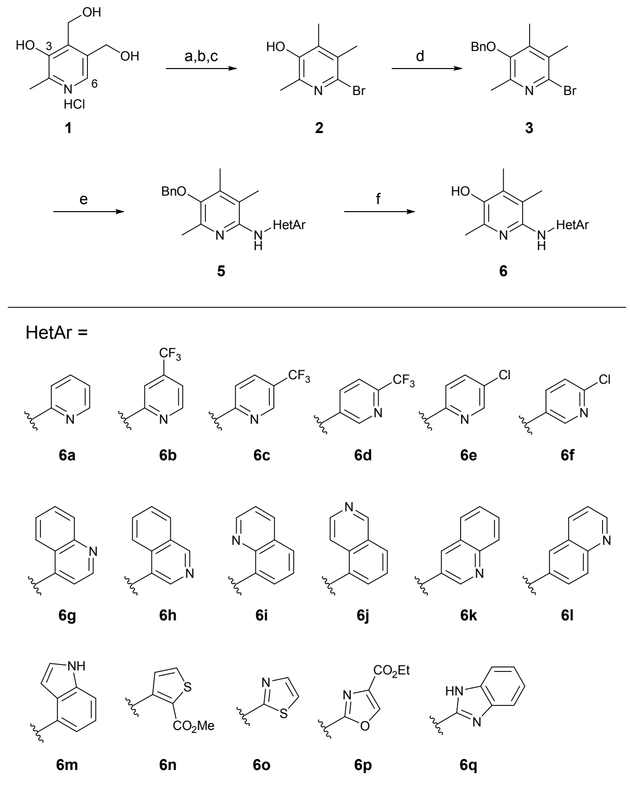
We have previously reported the inhibitory activity of 6-alkyl- and 6-phenyl-amino-2,4,5-trimethylpyridin-3-ols against colitis.[21] Some of them inhibited TNF-α-induced monocyte adhesion to colon epithelial cell better than 5-aminosalicylic acid (5-ASA *a.k.a.* mesalazine), a positive control and the active metabolite of sulfasalazine (SSZ) which is a medication used for the treatment of IBD. Oral administration of the best compound showed about 30-times better efficacy than sulfasalazine, a prodrug of 5-ASA, in a rat model of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis,[25] a widely used animal model of IBD.

In this study, we further expanded the scope of side chain (HetAr group in Scheme 1) by installing heteroarylamino groups on *C*(6)-position to render more polarity. In fact, cLogP values of heteroaryl compounds shown here are smaller than those of the corresponding phenyl analogues by about one unit. Compounds shown here inhibited TNF-α-induced adhesion of monocytes to HT-29 human colonic epithelial cells.[22-24] In order to demonstrate *in vivo* efficacy, selected compounds were tested in the rat model of TNBS-induced colitis. Mode of action of the anti-colitis activities were further characterized by the relevant molecules *in vivo*.

Results and Discussion

Synthesis

6-Heteroarylamino-2,4,5-trimethylpyridin-3-ols (**6a–6q**) were synthesized using a synthetic route we have developed (Scheme 1)[26] but with novel heteroarylamines **4**. In short, $SOCl_2$ with catalytic amount of DMF converted two primary hydroxy groups of pyridoxine·HCl (**1**) to chlorides which were then removed reductively using Zn and acetic acid under refluxing condition to give 2,4,5-trimethylpyridin-3-ol. *C*(6)-position was brominated by electrophilic aromatic bromination with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) to afford compound **2** and phenolic OH group was then protected with benzyl group to give compound **3**. Bromide in the compound **3** was replaced with various heteroarylamines **4** under Buchwald-Hartwig amination reaction conditions to afford compounds **5**. Lastly, benzyl protective group was removed either by catalytic hydrogenolysis or by BCl_3 depending on the substituents to give 6-heteroarylamino-2,4,5-trimethylpyridin-3-ols (**6a–6q**).

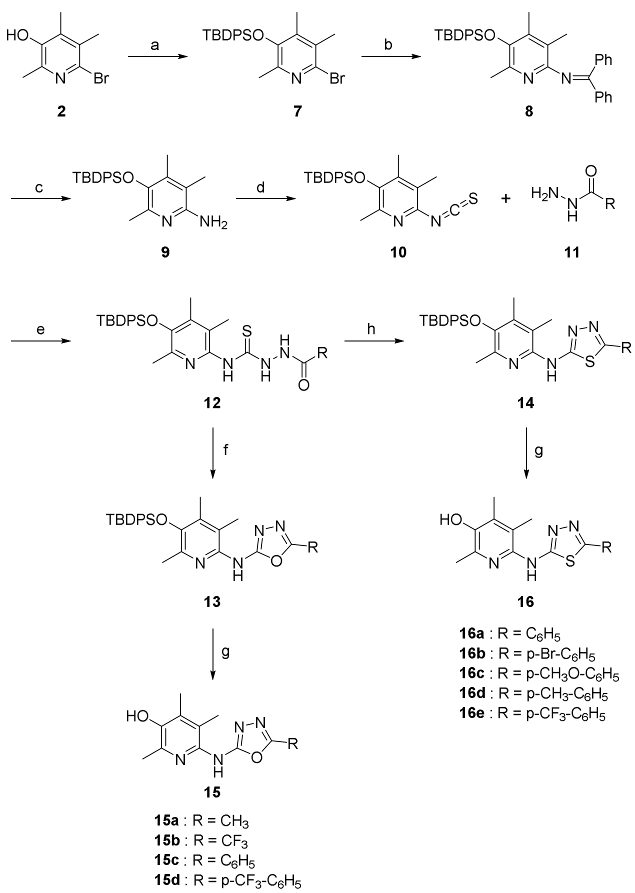


Scheme 1. Synthetic scheme and structure of 6-heteroarylamino-2,4,5-trimethylpyridin-3-ols (6). Reagents and conditions: (a) $SOCl_2$, DMF, reflux, 30 min, 93%; (b) Zn, AcOH, reflux, 3 h, 92%; (c) DBDMH, THF, r.t., 3 h, 80%; (d) $PhCH_2Cl$, K_2CO_3, DMF, r.t., 12 h, 97%; (e) H_2N-HetAr (4), $Pd_2(dba)_3$, BINAP, NaO^tBu, toluene, reflux or H_2NHetAr (4), $Pd(OAc)_2$, Xantphos, Cs_2CO_3, toluene, reflux; (f) H_2, Pd/C, MeOH, r.t. or BCl_3, CH_2Cl_2, r.t.

In cases of 6-(1,3,4-oxadiazol-2-yl)amino analogues 15 and 6-(1,3,4-thiadiazol-2-yl)amino analogues 16, the oxadiazole and thiadiazole groups did not survive debenzylation conditions used in the final step of Scheme 1. Therefore, we introduced *tert*-butyldiphenylsilyl (TBDPS) group instead of benzyl group for the protection of phenolic OH of compound 2 (Scheme 2). TBDPS-protected compound 7 was coupled with benzophenone imine, a synthetic equivalent for ammonia, under Buchwald-Hartwig amination reaction condition to give 8. Free primary amino group was liberated from the imine of 8 by acidic methanolysis to afford compound 9 which was then treated with thiophosgene and Hünig's base to give isothiocyanate 10. Treatment of 10 with various *N*-acylhydrazides 11 leads to the corresponding *N*-acyl thiosemicarbazides 12 which serve as key intermediate for the preparation of both 2-amino-1,3,4-oxadiazoles (13) and 2-amino-1,3,4-thiadiazoles (14).[27] A desulfurative cyclization method using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was successfully employed for preparation of 6-(1,3,4-oxadiazol-2-yl)amino analogues 13. On the other hand, an efficient reaction system (i.e., *p*-toluenesulfonyl chloride (*p*-TsCl) with triethylamine in a polar solvent, *N*-methyl-2-pyrrolidine (NMP)) for dehydrative cyclization of 12 gave 6-(1,3,4-thiadiazol-2-yl)amino compounds 14.

View Article Online
DOI: 10.1039/C8MD00156A

Finally, deprotection of TBDPS of the cyclized compounds 13 and 14 with tetra-*n*-butylammonium fluoride afforded oxadiazole/thiadiazole-containing pyridinol compounds **15** and **16**, respectively.



Scheme 2. Synthesis of 6-(1,3,4-oxadiazol-2-yl)amino analogues (**15**) and 6-(1,3,4-thiadiazol-2-yl)amino analogues (**16**). Reagents and conditions: (a) TBDPSCl, imidazole, DMF, r.t., 24 h, 87%; (b) Ph_2C=NH, $Pd_2(dba)_3$, BINAP, NaO^{t}Bu, toluene, reflux, 5 h, 89%; (c) HCl-MeOH, THF-MeOH, r.t. 24 h, 93%; (d) $CSCl_2$, ^{i}Pr_2NEt, r.t., 1 h, 98%; (e) CH_2Cl_2, r.t.; (f) EDCI, DMSO, 60 °C; (g) *n*-Bu_4NF, THF, 0 °C; (h) *p*-TsCl, Et_3N, NMP, r.t.

Inhibition of TNF-α-induced cell adhesion

TNF-α-induced attachment and infiltration of immune cells to colon epithelium is one of the hallmark events leading to IBD.[6,7] To quantitatively assess inhibitory activity of the compounds against this adhesion process, we set up a cell-based screening system in which monocytes (U937 cells) and colon epithelial cells (HT-29 cells) were co-cultured in the presence of TNF-α.[26] Treatment of TNF-α indeed induced the adhesion of monocytic cells to colonic epithelial cells, indicated by increased fluorescence that was pre-loaded in U937 cells (Supporting Information). 5-ASA showed very weak inhibition (3.5%) at 1 μM concentration and only moderate inhibition (50.5%) even at 20 mM against the adhesion (Table 1). Meanwhile, 6-heteroarylamino-2,4,5-trimethylpyridin-3-ols (**6**, **15** and **16**) exerted up to 78% inhibition at 1 μM, equivalent to three to five orders of magnitude stronger activities than that of 5-ASA. Of note, 1 μM concentration of the compounds **6f**, **6k**, and **16a** showed superior activity to 20 mM of 5-ASA.

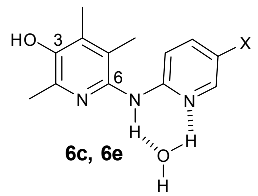
Table 1. Inhibitory activity against TNF-α-induced monocyte adhesion to colon epithelial cells.

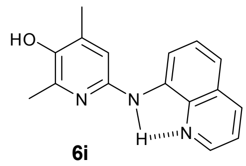
Comp.	Inhibition (%)†	Comp.	Inhibition (%)†
5-ASA (1 μM)	3.5 ± 2.4 *	6m	31.9 ± 6.4 *
5-ASA (20 mM)	50.5 ± 1.8 *	6n	23.7 ± 2.4 *
6a	8.5 ± 1.2 *	6o	23.7 ± 6.1 *
6b	22.8 ± 10.9	6p	38.5 ± 1.3 *
6c	34.8 ± 16.3	6q	59.9 ± 4.7 *
6d	50.6 ± 10.6 *	15a	26.2 ± 4.6 *
6e	59.0 ± 8.4 *	15b	35.8 ± 5.0 *
6f	75.4 ± 3.4 *	15c	1.4 ± 3.0
6g	60.2 ± 5.8 *	15d	55.7 ± 4.9 *
6h	55.4 ± 4.5 *	16a	78.7 ± 2.5 *
6i	10.4 ± 5.9	16b	13.7 ± 2.3 *
6j	52.8 ± 6.9 *	16c	13.2 ± 1.9 *
6k	63.4 ± 1.6 *	16d	16.6 ± 3.1 *
6l	56.6 ± 1.1 *	16e	52.3 ± 1.1 *

† Data are represented as inhibition % at 1 μM concentration of compound and as mean ± SEM. All experiments independently carried out at least three times. * $P < 0.05$ compared to vehicle-treated control group.

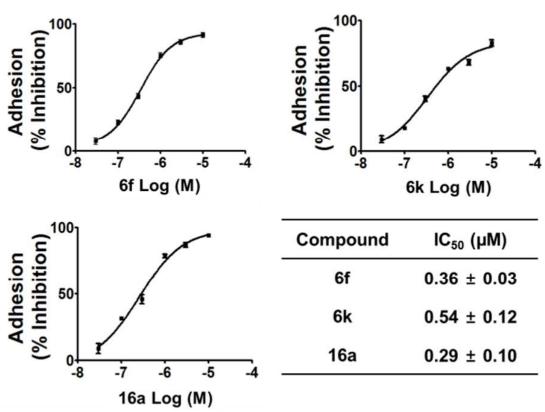
2-Pyridyl analogue **6a** showed little activity (8.5%) compared to its phenyl analogue (73.6%) published by us[21] in the inhibition of TNF-α-induced adhesion. However, installation of -CF_3 group on *m*-position of 2-pyridyl group (**6b**) increased the activity by 2.7-fold from **6a**, while the same group on *para*-position increased the activity by 4.1-fold (**6c**). Interestingly, with the identical *p*-CF_3 substituent, 3-pyridyl analogue (**6d**) showed 6.0-fold higher activity than **6a**. Replacement of *p*-CF_3 group of **6c** with weaker electron-withdrawing *p*-Cl increased the activity to 59.0% (**6e**). Upon change of 2-pyridyl substitution of **6e** to 3-pyridyl position with the same *p*-Cl (**6f**), the activity reached the highest point (75.4%). Among pyridine and quinoline analogues (**6a – 6l**), analogues that can assist a cyclic hydrogen bond network with or without H_2O between *C*(6)-amino group and heteroaryl group (*e.g.*, **6c** and **6e**) generally showed lower activity than their counterparts that have unfavorable structure for the cyclic network (*e.g.*, **6d** and **6f**). Quinoline analogues, **6g**–**6l**, showed the same trend; **6i** being the only one that possibly forms the cyclic hydrogen bond showed marginal activity (10.4%) while the rest of quinolones analogues showed several fold higher activity (52.8–63.4%).

View Article Online
DOI: 10.1039/C8MD00156A





Indole (**6m**), thiophene (**6n**), thiazole (**6o**) and oxazole (**6p**) analogues showed modest range of activity while benzimidazole analogue (**6q**) showed high activity (59.9%). Next, we decided to explore 1,3,4-oxadiazole (**15**) and 1,3,4-thiadiazole (**16**) series which have three heteroatoms in the cycles. Among oxadiazole series (**15**), *p*-trifluoromethylphenyl analogue (**15d**) showed the highest activity (55.7%) while the rest showed low to modest activity. Of thiadiazoles (**16**), the simplest phenyl substitution (**16a**) showed tremendously high activity (78.7%). There seems to be no clear structure-activity relationship (SAR) among this series in terms of electronic effect of the substitution. Although electron-withdrawing substitution (**16e**) is more active than electron-donating substitution (**16c**), neither compounds is more active than the phenyl substitution (**16a**). We then measured half maximal inhibitory concentration (IC_{50}, μM) of three representative compounds: **6f**, **6k**, and **16a**, whose activities are aligned in a narrow range in a high activity group in Table 1. As shown in Figure 1, their IC_{50} values are turned out 0.36, 0.54 and 0.29 μM, respectively. The order of their IC_{50}'s is exactly consistent with that of a single concentration cell-based assay, demonstrating high degree of correlation between the assays and validity of our methodology. The best IC_{50} is up to about 7×10^4-fold better than that of 5-ASA ($IC_{50} \approx 20$ mM).



Compound	IC_{50} (μM)
6f	0.36 ± 0.03
6k	0.54 ± 0.12
16a	0.29 ± 0.10

Figure 1. IC_{50} values of the selected compounds, **6f**, **6k** and **16a**. Each data represents mean ± S.E.M for three independent experiments performed in triplicate.

In vivo activity against TNBS-induced colitis model and mode of action

Next, we conducted a TNBS-induced rat colitis model to assess anti-colitis activity *in vivo*. We chose two compounds that showed the best activity in Table 1 for this assay, **6f** and **16a**, which represent distinctive heterocyclic scaffolds. As a macroscopic marker to represent a disease phenotype, we measured recovery level on colon and body weights upon drug treatment. Upon rectal administration of TNBS, rats showed colitis signs, such as bloody diarrhea, and wasting conditions with sluggish and weak movement.[27] Compared to rats in the sham-operated group, TNBS-treated rats showed a significant reduction in body weight followed by stagnated increase. In contrast, rats receiving oral administration of **6f** or **16a** (1 mg/kg) showed significant recovery of the TNBS-induced decrease in body weight (Fig. 2A). Besides, 16a showed 79% recovery in colon weight at 1 mg/kg dose, which is superior macroscopic activity to SSZ (300 mg/kg), 6f and our previous compound[21] which showed about 72% recovery in colon weight at 1 mg/kg dose, demonstrating significant efficacy of new scaffold (Fig. 2C). Colon tissues in the TNBS-treated lesion site showed significant inflammation, as revealed by edema and adhesion in gross morphology examination (Fig. 2B), and increased wet weight per colon length (Fig. 2C). In addition, myeloperoxidase (MPO) level in colon tissues is directly related to neutrophil infiltration into tissues, serving as a biochemical marker of inflammation.[28,29] TNBS treatment induced a tremendous increase in MPO activity (Fig. 2D), and the increased MPO activity by TNBS (456.67 ± 41.78 ng/mL) was significantly suppressed by treatment with 6f (127.02 ± 19.24 ng/mL) and 16a (107.37 ± 14.09 ng/mL). The inhibitory effect of these compounds at 1 mg/kg was better than that of 300 mg/kg SSZ (MPO level of 209.65 ± 4.13) (Fig. 2D).

Although fundamental molecular targets of the compounds responsible for the phenotype are to be investigated, we further examined in this study the inhibitory effects of the compounds on the expression levels of cytokines responsible for inflammatory response. It will provide more consistency to our mechanism based on the assay against TNF-α-induced cell adhesion. In ELISA (Fig. 2E) and western blotting (Fig. 2F−2I) analyses, TNBS treatment dramatically increased expression of monocyte chemoattractant-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and pro-inflammatory cytokines, TNF-α, IL-1β and IL-6, whereas anti-inflammatory cytokine, IL-10, was significantly decreased in the inflamed colon tissues (Fig. 2F, 2I). However, oral administration of 6f or 16a significantly restored the TNBS-altered protein expressions. All those changes consistently and strongly support the anti-IBD activity of our compounds. Indeed, the inhibitory effect of 1 mg/kg 6f or 16a was much stronger than that of 300 mg/kg SSZ. We believe that data in this report also confirmed the validity of our phenotypic screening where inhibitory activity of compounds against pathological role of TNF-α can be monitored.

View Article Online
DOI: 10.1039/C8MD00156A

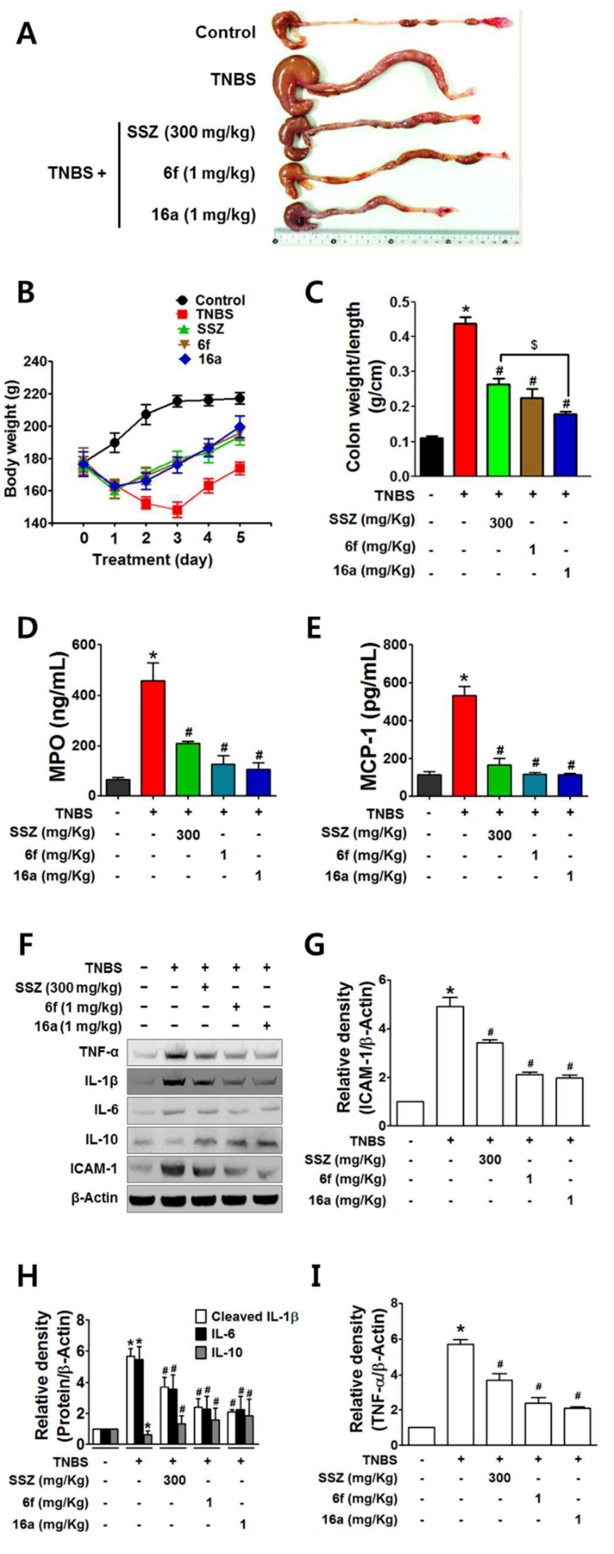


Figure 2. 6-Heteroarylamino-2,4,5-trimethylpyridin-3-ol (**6f** and **16a**) ameliorates the clinical features of TNBS-induced colitis in rats. Colitis was induced by rectal administration of TNBS. The control group received 50% ethanol as a vehicle. Data represent the mean ± SEM for five rats per group. **P* < 0.05 compared to the vehicle-treated control group. #*P* < 0.05 compared to the TNBS-treated group. (**A**) Macroscopic appearance of the large intestine. (**B**) Body weight was recorded daily from day 0 to day 5. (**C**) Colon weight per unit length of colon (distal 5–6 cm segment). (**D**) MPO activity of colon tissue measured with an MPO assay kit. (**E**) Expression of MCP-1 of colon tissue. (**F–I**) Inhibitory effects of compounds **6f** and **16a** on TNBS-altered protein expressions of TNF-α, IL-1β, IL-6, IL-10, and ICAM-1 in rat colon tissues. The bar diagrams, **G**, **H** and **I**, represent the quantitative change in the expressions of the proteins. **P* < 0.05 vs. the vehicle-treated controls. #*P* < 0.05 vs. TNBS-treated group.

Conclusions

We synthesized 6-heteroarylamino-2,4,5,-trimethylpyridin-3-ols and tested them as anti-colitis agents *in vitro* and *in vivo*. Most analogues showed superior inhibition to 5-ASA against TNF-α-induced adhesion of monocytes to colon epithelial cells at >10^3-fold lower concentration. In TNBS-induced *in vivo* rat colitis model, oral administration of 1 mg/kg **16a** showed the most active recovery profiles in body and colon weight among aminopyridinol series including 6-alkylamino and 6-phenylamino analogues we previously reported. The activity was also demonstrated by significant decrease of MPO activity. In addition, **16a** significantly decreased the expression of inflammatory molecules and pro-inflammatory cytokines while increasing anti-inflammatory cytokine. The effects of 1 mg/kg dose of 6f and 16a were much stronger than 300 mg/kg SSZ in those profiles, demonstrating their remarkable potency. Although the detailed mechanism of action should be studied further, it is strongly suggested that 6-heteroaylamino-2,4,5-trimethylpyridin-3-ol can be an excellent anti-IBD scaffold.

Conflicts of interest

There authors declare no competing interest.

Acknowledgements

This work was supported by grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI15C0542).

References

1 B. Khor, A. Gardet, R. J. Xavier, *Nature* 2011, **474**, 307.
2 G. G. Kaplan, *Nat Rev Gastroenterol. Hepatol*. 2015, **12**,720.
3 M. F. Neurath MF, *Nat Rev Gastroenterol. Hepatol.* 2017, **14**, 269.
4 I. Bravata, M. Allocca, G. Fiorino, S. Danese,T. zik, C. Maaser, A. Lugering, M. Kagnoff, L. Mayer, S. Targan, W. Domschke, *Inflamm. Bowel Dis.* 2006, **12**, 1068.
5 C. M. Olesen, M. Coskun, L. Peyrin-Biroulet, O. H, Nielsen, *Pharmacol. Ther.* 2016, **159**, 110.
6 G. Kojouharoff, W. Hans, F. Obemeier, D. N. Mannel, T. Andus, J. Scholmerich, V. Gross, W. Falk, *Clin. Exp. Immunol.* 1997, **107**, 353.

View Article Online
DOI: 10.1039/C8MD00156A

7 R. Atreya, M. F. Neurath, *Clin. Rev. Allergy Immunol.* 2005, **28**, 187.
8 M. Ligumsky, P. L. Simon, F. Karmeli, D. Rachmilewitz, *Gut* 1990, **31**, 686.
9 M. Coccia, O. J. Harrison, C. Schiering, M. J. Asquith, B. Becher, F. Powrie, K. J. Maloy, *J. Exp. Med.* 2012, **209**,1595.
10 E. O. Glocker, D. Kotlarz, C. Klein, N. Shah, B. Grimbacher, *Ann. N.Y. Acad. Sci.* 2011, **1246**, 102.
11 K. N. Couper, D. G. Blount, E. M. Riley, *J. Immunol.* 2008, **180**, 5771.
12 L. Peyrin-Biroulet, W. Sandborn, B. E. Sands, *et. al.*, *Am. J. Gastroenterol.* 2015, **110**, 1324.
13 B. E. Sands, *Dig Dis.* 2014, **32**:supple 1:74.
14 M. Allocca, M. Jovani, G. Fiorino, S. Schreiber, S. Danese, *Curr. Drug Targets* 2013,**14**, 1508.
15 D. J. Gibson, E. J. Ryan, G. A. Doherty, *Inflamm. Bowel Dis.* 2013, **19**, 2716.
16 K. Hasumi, S. Sato, T. Saito, J. Y. Kato, K. Shirota, J. Sato, H. Suzuki, S. Ohta, *Bioorg. Med. Chem.* 2014, **22**, 4162.
17 N. Hayakawa, M. Noguchi, S. Takeshita, *et. al.*, *Bioorg. Med. Chem.* 2014, **22**, 3021.
18 W. J. Sandborn, S. Ghosh, J. Panes, I. Vranic, W. Wang, W. Neizychowski, *et. al.*, *Clin. Gastroenterol. Hepatol.* 2014, **12**, 1485.
19 G. Monteleone, M. F. Neurath, S. Ardizzone, *et. al.*, *N. Engl. J. Med.* 2015, **372**, 1104.
20 S. Banskota, H. E. Kang, D. G. Kim, S. W. Park, H. Jang, U. Karmacharya, B. S. Jeong, J. A. Kim, T. G. Nam, *Bioorg. Med. Chem. Lett.* 2016, **26**, 4587.
21 D. Thapa, J. S. Lee, S. Y. Park, Y. H. Bae, S. K. Bae, J. B. Kwon, K. J. Kim, M. K. Kwak, Y. J. Park, H. G. Choi, J. A. Kim, *J. Pharmacol. Exp. Ther.* 2008, **327**, 353.
22 N. Kobayashi, A. Ikesue, S. Majumdar, T. J. Siahaan, *J. Pharmacol. Exp. Ther.* 2006, **317**, 309.
23 S. Park, S. C. Regmi, S. Y. Park, E. K. Lee, J. H. Chang, S. K. Ku, D. H. Kim, J. A. Kim, *Eur. J. Pharmacol.* 2014, **735**, 184.
24 A. R. Jurjus, N. N. Khoury, J. M. Reimund, *J. Pharmacol. Toxicol. Methods* 2004, **50**, 81.
25 D. G. Kim, Y. Kang, H. Lee, E. K. Lee, T. G. Nam, J. A. Kim, B. S. Jeong, *Eur. J. Med. Chem.* 2014, **78**, 126.
26 S. J. Yang, S. H. Lee, H. J. Kwak, Y. D. Gong, *J. Org. Chem.* 2013, **78**, 438.
27 J. E. Krawisz, P. Sharon, W. F. Stenson, *Gastroenterology* 1984, **87,** 1344.
28 E. Sekizuka, M. B. Grisham, M. A. Li, E. A. Deitch, D. N. Granger, *Gastroenterology* 1988, **95**, 1528.