Novel Dual Inhibitors of 5-Lipoxygenase and Thromboxane A_2 Synthetase: Synthesis and Structure-Activity Relationships of 3-Pyridylmethyl-Substituted 2-Amino-6-hydroxybenzothiazole Derivatives

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As part of our search for novel antiinflammatory drug candidates, we have designed and synthesized a series of 3-pyridylmethyl-substituted 2-amino-6-hydroxybenzothiazoles. Introduction of a 3-pyridylmethyl group into the 2-amino group (type-A) or the benzene ring (type-B) of 2-amino-6-hydroxybenzothiazoles imparted dual inhibitory activity against the production by glycogen-induced peritoneal cells of rat (*in vitro*) of leukotriene B_4 (LTB_4) and thromboxane A_2 (TXA_2), while not significantly inhibiting that of prostaglandin E_2 (PGE_2). The observed inhibition of the former two arachidonic acid metabolites was indicated to be the result of a direct action on 5-lipoxygenase and TXA_2 synthetase by a cell-free *in vitro* assay. On the other hand, the inhibitory activities against PGE_2 production were for most compounds very weak, indicating that they did not inhibit cyclooxygenase. Structure-activity relationship studies concerning the position of the 3-pyridylmethyl group revealed that type-B compounds generally showed about 10-fold stronger inhibitory activity against TXA_2 synthetase than type-A compounds. The position of the 3-pyridylmethyl group played an important role in TXA_2 synthetase inhibition. When some of these compounds (**8**, **13a**, **26a** (E3040), **26b**, **27b**, and **28b**) were orally administered in the rat TNB/ethanol-induced chronic colitis model (100 mg/kg), the production of both LTB_4 and TXB_2 in the rat colon was reduced (*ex vivo*). In addition, one type-B compound, 6-hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole (**26a**), demonstrated a therapeutic effect at treatments of 100 mg/kg po once daily for 11 days and showed almost comparable activity to sulfasalazine at a dose of 500 mg/kg, the reference drug for inflammatory bowel diseases, in this *in vivo* model.

Introduction

Much effort has been directed at investigating the relationship between inflammatory diseases and arachidonic acid metabolites, such as the leukotrienes (LTs), prostaglandins (PGs), and thromboxanes (TXs).[1-4] During the past decade, it has become clear that these mediators play important roles in some inflammatory or allergic diseases, either acting alone or in combination.[4,5] Extensive research has been undertaken to develop dual inhibitors of 5-lipoxygenase (5-LO) and cyclooxygenase (CO) with the expectation of superior effectiveness compared to individual enzyme inhibitors.[6-9] However, at present there are no practicable dual inhibitors for the treatment of inflammatory diseases, and only nonsteroidal antiinflammatory drugs (NSAIDs) are used clinically. It also should be noted that new dual inhibitors affecting both 5-LO and thromboxane A_2 (TXA_2) synthetase are in development.[10-13]

Recently, it has been reported that the production of the arachidonic acid metabolites, leukotriene B_4 (LTB_4), TXB_2 (the stable metabolite of TXA_2), and prostaglandin E_2 (PGE_2), is markedly increased in the colonic mucosa tissue of patients with inflammatory bowel disease (IBD).[14,15] Much attention has been paid to LTB_4 and TXA_2, since the effectiveness of 5-LO inhibitors or TXA_2 synthetase inhibitors has been reported in experimentally induced colitis.[16,17] Judging from the ineffectiveness of NSAIDs such as indomethacin, the involvement of PGs in the pathogenesis of IBD is still not clear;[18-20] however, it should be taken into consideration that some PGs, such as PGE_2, are thought to have protective effects on the colonic mucosa.[21]

Previously, we reported 6-hydroxybenzothiazole derivatives (E6080) which exhibit inhibition of 5-LO.[22,23] In an effort to improve the therapeutic usefulness of 5-LO inhibitors, we have explored the design of compounds with additional inhibitory activity against TXA_2 synthetase by the introduction of a pyridine or imidazole moiety, both of which are known to selectively bind the iron residue of TXA_2 synthetase.[24,25]

In this paper, we describe the synthesis and pharmacological properties of a series of 3-pyridylmethyl-substituted 2-amino-6-hydroxybenzothiazoles as candidate novel dual inhibitors of both 5-LO and TXA_2 synthetases, not suppressing the production and release of PGE_2, and useful for the treatment of IBD.

Chemistry

There are two possible ways to introduce a pyridine ring into the skeleton of 2-amino-6-hydroxybenzothiazole. We designed and synthesized the following two types of compounds in which the positions of the 3-pyridylmethyl substituent are different. Type-A compounds: compounds with a 3-pyridylmethyl substituent at the 2-amino group of 2-amino-6-hydroxybenzothiazoles. Type-B compounds: compounds with a 3-pyridylmethyl substituent on the benzene ring of 2-amino-6-hydroxybenzothiazoles.

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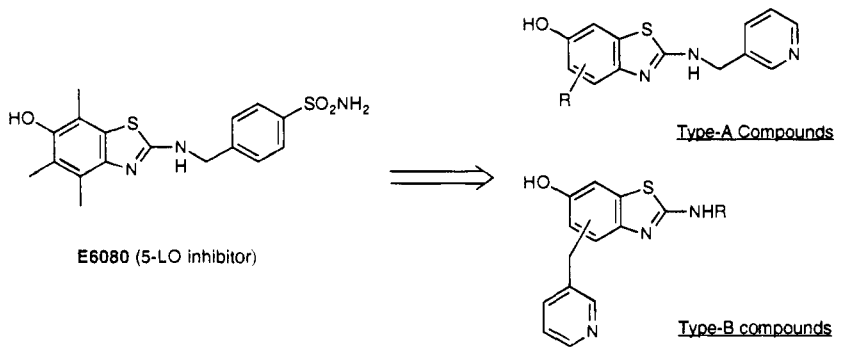
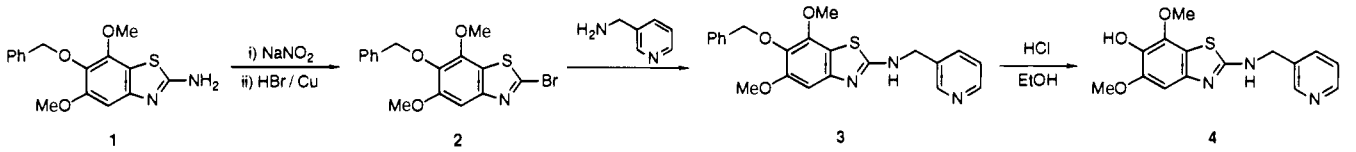
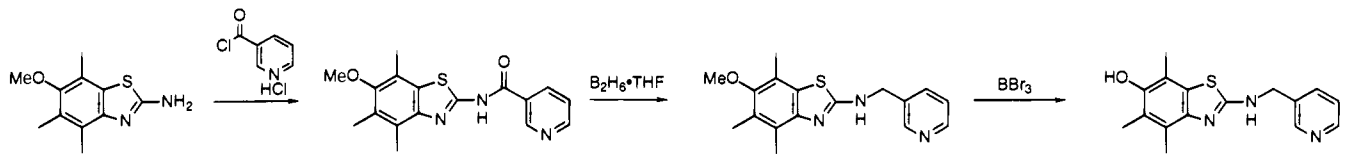
Chart 1



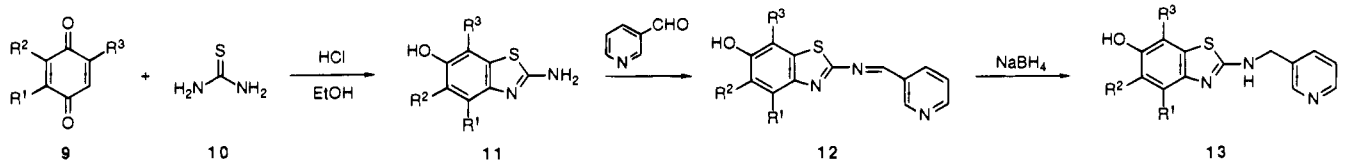
Scheme 1. Synthesis of Type-A Compound **4**



Scheme 2. Synthesis of Type-A Compound **8**



Scheme 3. Synthesis of Type-A Compounds **13**



Synthesis of Type-A Compounds (4–13). 2-Amino-6-hydroxybenzothiazoles were synthesized by following the methods described previously.[26] There are three synthetic pathways for the preparation of type-A compounds from 2-amino-6-hydroxybenzothiazoles. In the first method, shown in Scheme 1, the amino group of 2-amino-6-(benzyloxy)-5,7-dimethoxybenzothiazole (**1**) was converted into the corresponding bromide **2** by the Sandmeyer reaction and **2** was reacted with 3-(aminomethyl)pyridine to afford the benzothiazole **3**. Deprotection of the methoxy group with hydrochloric acid gave the 6-hydroxybenzothiazole **4** (Scheme 1).

Alternatively, the amino group of 2-amino-6-methoxy-4,5,7-trimethylbenzothiazole (**5**) was reacted with nicotinoyl chloride hydrochloride in pyridine to give the amide compound **6**. Compound **6** was reduced with borane, and its methoxy group was deprotected with BBr_3 to afford the 6-hydroxybenzothiazole **8** (Scheme 2).

2-Amino-6-hydroxybenzothiazoles **11** could be also prepared according to the method of Lau[27] from corresponding 1,4-benzoquinones **9**. The 1,4-benzoquinones **9** were cyclized in the presence of thiourea (**10**) and hydrochloric acid to give 2-amino-6-hydroxybenzothiazoles **11** in one step. The benzothiazoles **11** obtained were reacted with 3-pyridinecarboxaldehyde to give the Schiff bases **12**. The Schiff bases **12** thus obtained were reduced to the desired benzothiazoles **13** with sodium borohydride (Scheme 3).

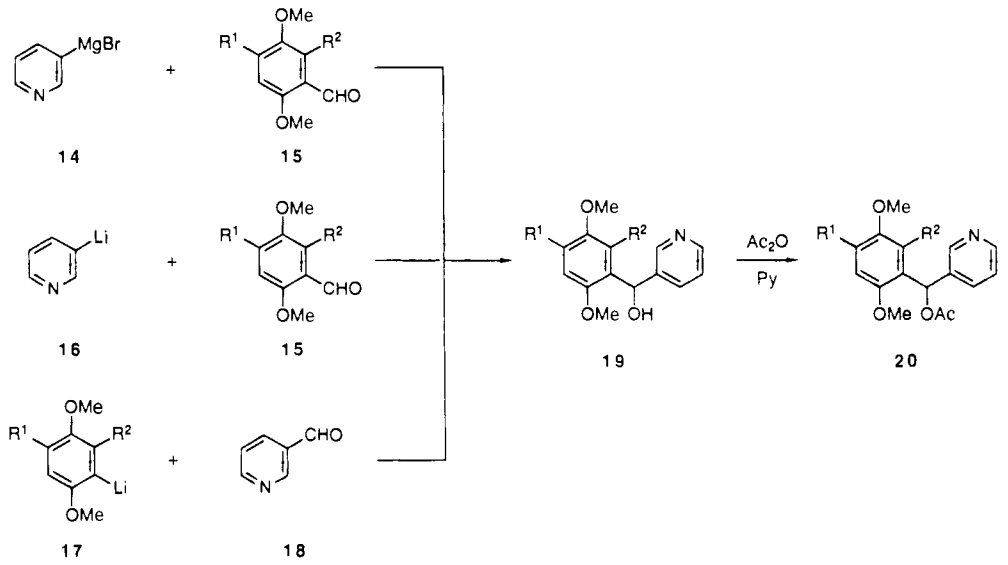
Synthesis of Type-B Compounds (26–28). The requisite intermediates, secondary alcohols **19** for the synthesis of type-B compounds, were prepared by the three methods shown in Scheme 4. The first method is a Grignard reaction of 2,5-dimethoxybenzaldehyde with 3-pyridylmagnesium bromide. 3-Pyridyllithium can also be used instead of the Grignard reagent to give the same secondary alcohol. Alternatively, the same product could be obtained by the coupling of 3-pyridinecarboxaldehyde with 1,4-benzohydroquinone dimethyl ether in the presence of *n*-butyllithium.

The acetates **20** obtained from **19** were oxidized by cerium(IV) ammonium nitrate (CAN) to afford the corresponding quinones **21**. Quinones **21** were reacted with thioureas **22**, according to the method of Lau,[27] to give benzothiazoles **23**. The acetoxy group (**23**) was removed with zinc/acetic acid to afford the desired compounds **26** (method A, Scheme 5).

In an alternative route, the acetates **20** were hydrogenated with palladium on carbon to give compounds **24**. Compounds **24** were oxidized by CAN and reacted with thioureas **22** to afford the corresponding benzothiazoles **26** (method B, Scheme 5).

In order to examine substituent effects on biological activities, we also prepared the 5- and 7-positional isomers (**27** and **28**, respectively) (Chart 2).

Scheme 4. Synthesis of Secondary Alcohols **19**



Scheme 5. Synthesis of Type-B Compounds

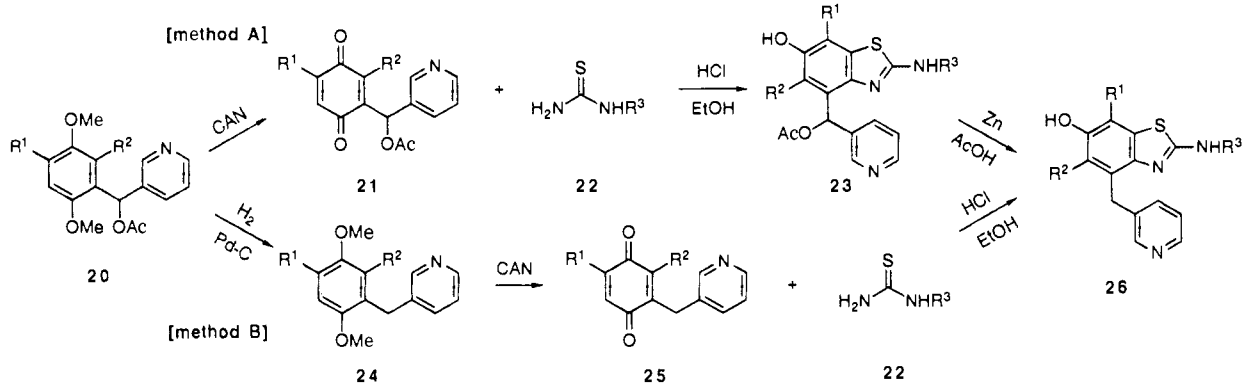
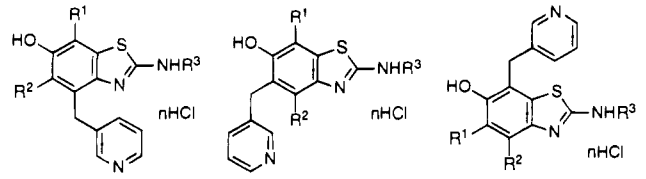


Chart 2



It should be noted that benzothiazoles **23** could also be prepared by the method in which thiouronium salts **29** are isolated.[23] The reaction of 1,4-benzoquinones **21** with an equimolar amount of thioureas **22** in the presence of hydrochloric acid afforded thiouronium salts **29**. The isolated salts **29** underwent cyclization in the presence of a catalytic amount of 1,4-benzoquinone, giving benzothiazole derivatives **23** in good yields (Scheme 6).

Pharmacological Results and Discussion

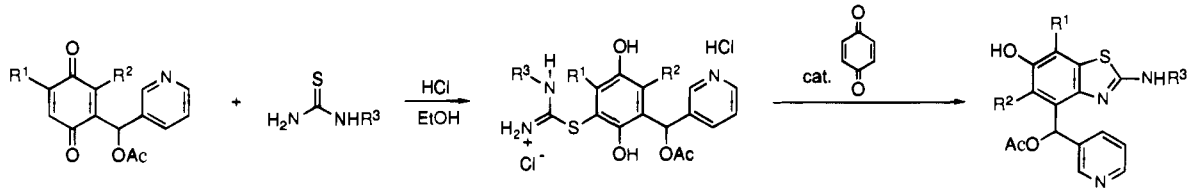
We evaluated the biological activities of the compounds by *in vitro* assay, monitoring the inhibition of $LTB_4/TXB_2/PGE_2$ production by glycogen-induced peritoneal cells of rat[28] and/or human whole blood.[29] Inhibitory activities against isolated enzyme were also measured. Some compounds selected after the *in vitro* assay were also subjected to *ex vivo* and *in vivo* assay using the rat TNB/ethanol-induced chronic colitis model (*vide infra*).[30]

(1) ***In Vitro* Studies.** All of the compounds prepared were initially evaluated for their inhibitory activities against the production of LTB_4 and TXB_2 using glycogen-induced peritoneal cells of rat, and their relative potencies have been expressed as the IC_{50} values (μM) (Table 1). As shown in Table 1, all of the compounds except **13b,c** were found to be potent inhibitors of the production of LTB_4, with IC_{50} values of the order 10^{-7} M. This is almost the same or stronger than that of the lead compound, E6080 (IC_{50} value of 0.08 μM from glycogen-induced peritoneal cells of rat).

Although the lipophilicity of some series of compounds is known to closely correlate with their inhibition of LTB_4 production,[10,31] we observed that in this case an alkyl substituent on the amino group of the benzothiazole (**26a–c**) had only a minor effect on inhibitory activities against the production of LTB_4. However introduction of an additional aromatic group into the benzene ring (**13b–c**) tended to lower potency. On the other hand, benzothiazoles with a 3-pyridylmethyl group in their benzene ring (type-B compounds) exhibited the most potent inhibition of LTB_4 production. We therefore consider that in this series the presence of a group which can conjugate to the benzene ring of the benzothiazole reduces the inhibitory activity against the production of LTB_4.

The IC_{50} values for the inhibition of production of TXB_2 vary from 10^{-9} to 10^{-6} M for type-B compounds, while those of type-A compounds are within the range

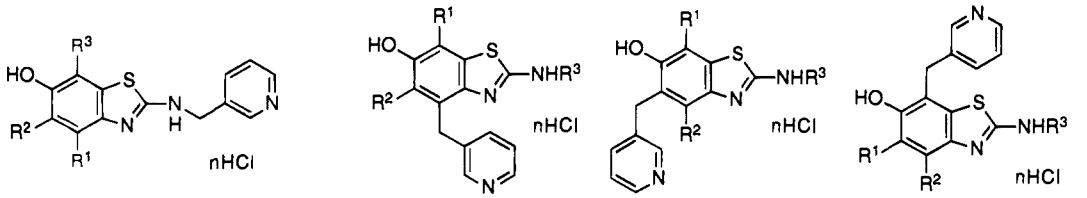
Scheme 6. Synthesis of Type-B Compounds via the Method in which Thiouronium Salts Were Isolated



21 22 29 23

Table 1. Inhibitory Activity against Eicosanoid Production by Glycogen-Induced Peritoneal Cells of Rat[a]

Type-A Compounds **Type-B Compounds**



4, 8, 13 26 27 28

compd	R^1	R^2	R^3	n	mp, °C (recrystn solvent)[b]	IC_{50}, μM[c] LTB_4	TXB_2	PGE_2	formula[d]
4	H	OMe	OMe	0	142–143 (A)	0.09	5.5	>100[e]	$C_{15}H_{15}N_3O_3S$[f]
8	Me	Me	Me	0	194–195 (B)	<0.1 (89%)	2.0	>100[e]	$C_{16}H_{17}N_3OS \cdot 0.3H_2O$
13a	Me	H	Me	0	156–157 (C)	<0.1 (55%)	4.4	>100[e]	$C_{15}H_{15}N_3OS \cdot 0.21H_2O$
13b	Ph	H	H	0	207–208 (C)	7.0	1.4	9.6	$C_{19}H_{15}N_3OS$[g]
13c	–CH=CH–CH=CH–		Me	0	194–195 (C)	1.6	4.0	5.5	$C_{18}H_{15}N_3OS$
26a	Me	Me	Me	0	236–238 (D)	0.10	0.09	>100[e]	$C_{16}H_{17}N_3OS$
26b	Me	Me	Et	2	194–195 (E)	<0.1 (64%)	0.10	>100[e]	$C_{17}H_{21}Cl_2N_3OS$
26c	Me	Me	*n*-Pr	2	198–199 (B)	<0.1 (78%)	0.09	>100[e]	$C_{18}H_{23}Cl_2N_3OS \cdot 0.6H_2O$
26d	Me	Me	H	2	171–172 (B)	0.19	0.002	NT	$C_{15}H_{17}Cl_2N_3OS \cdot 2H_2O$
26e	Me	OMe	H	2	208–209 (B)	0.17	0.11	>100[e]	$C_{15}H_{17}Cl_2N_3O_2S \cdot 1.85H_2O$[h]
26f	Me	OMe	Me	2	168–169 (B)	0.17	1.3	NT	$C_{16}H_{19}Cl_2N_3O_2S$
26g	Et	OMe	H	2	153–154 (B)	0.15	0.18	NT	$C_{16}H_{19}Cl_2N_3O_2S \cdot 2.5H_2O$
26h	Et	OMe	Me	0	191–193 (B)	<0.1 (51%)	0.33	>100[e]	$C_{17}H_{19}N_3O_2S \cdot 0.17H_2O$
27a	Me	Me	Me	2	170–171 (D)	0.30	<0.1 (65%)	>100[e]	$C_{16}H_{17}N_3OS$[g]
27b	Me	Me	Et	0	219–220 (D)	0.23	0.65	>100[e]	$C_{17}H_{19}N_3OS$
28a	Me	Me	H	2	235–237 (D)	0.25	5.3	>100[e]	$C_{15}H_{17}Cl_2N_3OS$
28b	Me	Me	Et	0	188–189 (E)	<0.1 (64%)	2.0	>100[e]	$C_{17}H_{19}N_3OS \cdot 0.32H_2O$

[a] Aliquots (5×10^5 cells) of rat peritoneal leukocytes were incubated (5 min, 37 °C) with test compounds prior to addition of A23187 (4 μM, 10 min, 37 °C). Aliquots of the supernatant were analyzed for LTB_4, TXB_2, and PGE_2. See Experimental Section for details. [b] Recrystallization solvent: A, AcOEt; B, EtOH–H_2O; C, AcOEt–*n*-hexane; D, EtOH; E, EtOH–*n*-hexane. [c] IC_{50} values were derived from inhibition experiments in which data points were measured in duplicate and concentration–effect curves by probits method. Values in parentheses indicate the % inhibition at 0.1 μM. [d] Elemental analyses for C, H, and N are within ±0.4% of the theoretical values. [e] The production of PGE_2 was potentiated at 1 μM. [f] N: calcd, 13.24; found, 12.70. [g] Formula was obtained by high-resolution mass spectroscopy. [h] H: calcd, 5.12; found, 5.77. NT: not tested.

of 10^{-6} M. When we compared the inhibitory activities of these two classes of compounds, type-B generally showed about 10-fold stronger inhibitory activity against the production of TXB_2 than type-A except for **28a,b**. The observed difference in inhibitory activities of type-A and type-B compounds may be attributed to a nonbonded interaction between the enzyme and the aromatic residue of the compounds.[32] In type-B compounds, the phenol ring of the benzothiazole adjacent to the 3-pyridylmethyl group may play an important role as a π donor in TXA_2 synthetase inhibition, while in type-A compounds and **28a,b**, this nonbonded interaction may be disturbed by some steric factors.

The above results clearly show that type-B compounds possess potent and well-balanced dual inhibition of LTB_4 and TXB_2 production by glycogen-induced peritoneal cells of rat. It was shown that selected type-B compounds (**26a,b**, **27b**, and **28b**) inhibit the production of these mediators at the enzyme level, as they also possess inhibitory activities against both 5-LO from RBL-1 cells and TXA_2 synthetase from human platelets, whereas they do not inhibit the cyclooxygenase from the seminal vesicle of sheep (**27b** was not tested) (Table 2). Activity differences between the 3-pyridylmethyl positional isomers were also evaluated using by **26b**, **27b**, and **28b**. While the inhibitory activity against LTB_4 production from human neutrophils is almost the same for all these compounds, **26b** (*meta* isomer) exhibited more potent inhibition than **27b** and **28b** (*ortho* isomer) against cell-free *in vitro* TXA_2 synthetase. These results suggest that steric or electric demands around the hydroxy group of the benzothiazole might be an important factor in TXA_2 synthetase inhibition.

Except for **13b,c**, these compounds enhanced the production of PGE_2 at 1 μM while inhibiting against the production of TXB_2 by glycogen-induced peritoneal cells of rat. This can be attributed to their inhibition of TXA_2 synthetase without affecting cyclooxygenase, resulting in enhanced production of PGE_2 by CO from accumulated PGH_2.

Table 2. Summary of *in Vitro* Activities (IC_{50}, μM)[a]

	human whole blood[b]			human neutrophils[c]	microsome of RBL-1 cells[d]	human platelets[e]	microsome of human platelets[f]	seminal vesicle of sheep[g]
compd	LTB_4	TXB_2	PGE_2	LTB_4	5-LO	TXB_2	TXA_2 synthetase	CO
26a (E3040)	0.83	1.1	>90	0.21	0.23	0.08	0.009	>300
26b	1.1	0.56	>91	<0.1 (57%)	0.91	0.06	0.010	>300
27b	NT	NT	NT	<0.1 (60%)	NT	2.3	0.39	NT
28b	0.50	34.8	>100	<0.1 (60%)	0.27	4.3	0.81	NT
A-64077[h]	0.52	>100	>100	0.81	0.93	>100	>100	90
E6080	24	42	>100	0.03	0.21	>100	NT	NT

[a] IC_{50} values were derived from inhibition experiments in which data points were measured in duplicate and concentration–effect curves by probits method. Values in parentheses indicate the % inhibition at 0.1 μM. [b] Human whole blood (0.4 mL) was incubated (5 min, 37 °C) with test compounds prior to addition of A23187 (40 μM, 20 min, 37 °C). Aliquots of plasma were analyzed for LTB_4, TXB_2, and PGE_2. [c] Human PMNs (1×10^5 cells) were incubated (5 min, 37 °C) with test compounds prior to addition of A23187 (4 μM, 10 min, 37 °C). Aliquots of the supernatant were analyzed for LTB_4. [d] Homogenates of RBL-1 cells were incubated (5 min, 37 °C) with test compounds prior to addition of arachidonic acid (0.2 mM, 10 min, 37 °C). Aliquots of the reaction mixtures were analyzed for 5-HETE. [e] Human platelets (6×10^7 cells) were incubated (5 min, 25 °C) with test compounds prior to addition of PGH_2 (1 μg/mL, 3 min, 25 °C). Aliquots of the supernatant were analyzed for TXB_2. [f] Microsome fraction (20 μg of protein) from human platelets was incubated (5 min, 25 °C) with test compounds prior to addition of PGH_2 (1 μg/mL, 1 min, 25 °C). Aliquots of the reaction mixtures were analyzed for TXB_2. [g] Microsome fraction (17 μg of protein) from sheep seminal vesicles was incubated (10 min, 25 °C) with test compounds prior to addition of arachidonic acid (20 μM, 20 min, 25 °C). Aliquots of the reaction mixtures were analyzed for PGE_2. [h] 5-LO inhibitor from Abbott. NT: not tested.

Table 3. Inhibitory effects on the Production and Release of LTB_4, TXB_2, and PGE_2 from TNB[a]/Ethanol-Induced Inflamed Colonic Mucosa in Rats (*ex Vivo*)

	dose,		suppression ratio, %[b]		
compd	mg/kg	n	LTB_4	TXB_2	PGE_2
4	100	4	19 ± 14	55 ± 6	10 ± 20
8	100	5	94 ± 2	72 ± 2	30 ± 7
13a	100	5	94 ± 1	36 ± 12	30 ± 14
26a (E3040)	100	4	73 ± 4	64 ± 2	0[c]
26b	100	4	94 ± 4	85 ± 5	0[d]
27b	100	4	83 ± 4	67 ± 2	8 ± 15
28b	100	4	89 ± 1	53 ± 8	41 ± 7
E6080	100	5	86 ± 5	0[e]	0[f]

[a] Trinitrobenzenesulfonic acid. [b] Colitis was induced by intracolonic administration of 0.25 mL of 50% ethanol containing 30 mg of TNB. Each compound (test groups) or vehicle (control group) was treated once, 7 days after intracolonic administration of TNB/50% ethanol. LTB_4, TXB_2, and PGE_2 were measured after *in vitro* incubation of the colon. Suppression ratio refers to the difference between the amount of eicosanoids produced in the test groups and the control group. Results are shown as means ± SEM. [c] Enhancement (51 ± 7%) was observed. [d] Enhancement (7 ± 17%) was observed. [e] Enhancement (22 ± 25%) was observed. [f] Enhancement (3 ± 17%) was observed.

(2) *Ex Vivo* Studies. As noted earlier, it has been suggested that production of inflammatory mediators such as LTs, TXs, and PGs in the colon is closely related to the symptomos of IBD.[15] We evaluated the effects of those compounds selected by the *in vitro* studies on the production of inflammatory mediators from the colon in the rat TNB/ethanol-induced chronic colitis model[30] to compare their *ex vivo* properties. Test compounds were orally administered once, 7 days after the induction of colitis, and 6 h later the rats were sacrificed. The colons excised from the rats were incubated with A23187 (calcium ionophore). The amount of eicosanoids released in the incubation mixtures was determined by radioimmunoassay. The suppression ratios (%) were calculated on the basis of the difference in the amounts of these eicosanoids between the control group that received vehicle and the test groups (Table 3).

Among type-A compounds, **4**, **8**, and **13a** inhibited the production of LTB_4 and TXB_2 in the colon. Altering the methyl or methoxy substituents at the benzene ring showed little effect on the inhibition of TXB_2 production. On the other hand, the methoxy derivative **4** showed a reduced inhibitory activity against the production of LTB_4. These results suggest that hydrophilic substituent groups at the benzene ring increase the inhibition activity of the production of LTB_4 in the *ex vivo* model. These compounds also weakly inhibited the production of PGE_2 in the colon.

Type-B compounds generally showed stronger inhibitory activities against the production of LTB_4 and TXB_2. It should be noted that **26a,b** and **27b** did not suppress the production of PGE_2, while **28b**, which bears a 3-pyridylmethyl group at its 7-position, inhibited the production of the latter in the colon.

In the *ex vivo* model, a lead compound, E6080 (5-LO inhibitor),[22,23] at a dose of 100 mg/kg suppressed the production of LTB_4 from the colon 6 h after oral administration but had no effect on the production of TXB_2 and PGE_2. These results are consistent with its *in vitro* IC_{50} values.

From these studies, the most promising compounds, **26a,b** and **27b**, which showed superior activities both *in vitro* and *ex vivo*, were considered to be potential therapeutic agents for the treatment of IBD, and they were further evaluated by *in vivo* screening.

(3) *In Vivo* Studies. The compounds (**8**, **13a**, **26a,b**, **27b**, and **28b**) to be examined were orally administered to TNB/ethanol-induced chronic colitis rats once daily for 11 consecutive days, starting 3 days after induction of TNB/ethanol-colitis. These rats were subjected to autopsy on the 14th day after TNB injection. The effects of the compounds were judged by measuring the following clinical parameters: myeloperoxidase (MPO) activity (U/g)[33] as a biochemical marker of neutrophil content in the tissue and the lesion area (mm^2) in the inflamed colon (Table 4). The results are shown in Table 4, along with results for predonisolone and sulfasalazine (SASP), which are clinically used for the treatment of IBD.

All the compounds inhibited both MPO activity and lesion area in the colon of the TNB/ethanol-induced chronic colitis rats. The results indicate that these compounds could accelerate healing of colonic damage in this model. It is noteworthy that **26a** at a dose of 100 mg/kg showed almost comparable activity to SASP at a dose of 500 mg/kg.

Table 4. Therapeutic Effects on Rat TNB/Ethanol-Induced Colitis Model[a] (*in Vivo*)

compd	dose, mg/kg/day	*n*	% inhibition of MPO, U/g[b]	% inhibition of lesion area, mm^2 [c]
8	100	7	31 ± 10	81 ± 14
13a	100	7	51 ± 11	71 ± 13
26a (E3040)	100	5	61 ± 17	39 ± 15
26b	100	6	41 ± 12	57 ± 14
27b	100	7	34 ± 12	48 ± 17
28b	100	6	43 ± 11	64 ± 14
predonisolone	10	5	60 ± 14	19 ± 12
sulfasalazine (SASP)	500	4	60 ± 18	16 ± 10

[a] Colitis was induced by intracolonic administration of 0.25 mL of 50% ethanol containing 30 mg of TNB. The treatment with each compound (test groups) or vehicle (control group) was started from 3 days after induction of colitis and continued daily for 11 days. The other group received 0.25 mL of 50% ethanol intracolonically, in place of TNB/ethanol solution (ethanol group). [b] Myeloperoxidase (MPO) activity in the colon. % inhibition = [1 − (MPO in test group − MPO in ethanol group)/(MPO in control group − MPO in ethanol group)] × 100. Results are shown as means ± SEM. [c] Colonic damage was measured macroscopically and expressed in mm^2/colon. % Inhibition = [1 − (lesion area in test group − lesion area in ethanol group)/(lesion area in control group − lesion area in ethanol group)] × 100. Results are shown as means ± SEM.

In conclusion, compound **26a** was chosen for further clinical evaluation because it stands out as having potent inhibitory activities against production of LTB_4 and TXB_2 (*in vitro* and *ex vivo*) and antiinflammatory activity as demonstrated in the rat TNB/ethanol-induced chronic colitis model (*in vivo* model).

Characterization of Compound 26a (E3040). Compound **26a** exhibited well-balanced inhibition toward production of both LTB_4 and TXB_2 but did not inhibit production of PGE_2 in *in vitro* screening. It markedly reduced the production of LTB_4 and TXB_2 in the colon of TNB/ethanol-induced chronic colitis rats. It was also effective in the TNB/ethanol-induced chronic colitis model in this *in vivo* model. The present results indicate that 6-hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole (**26a**) is a potent, orally active, dual inhibitor of 5-LO and TXA_2 synthetase and may be useful for the treatment of human IBD.

Experimental Section

Chemistry. Reagent and solvents were purchased from usual commercial sources. Silica gel (Kieselgel 60, Merck) was used for column chromatography and silica gel (Kieselgel 60 F_{254}, Merck) for analytical thin layer chromatography (TLC). Compounds were detected on TLC by UV light (254 nm). Melting points were measured on a Yanagimoto micromelting apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX-90Q (90 MHz) or Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer. All organic extracts were dried over anhydrous $MgSO_4$, and the solvent was removed with a rotary evaporator under reduced pressure.

6-(Benzyloxy)-5,7-dimethoxy-2-[(3-pyridylmethyl)amino]benzothiazole (3a). A mixture of 6-(benzyloxy)-2-bromo-5,7-dimethoxybenzothiazole (**2**) (380 mg, 1.0 mmol) and 3-(aminomethyl)pyridine (320 mg, 3.0 mmol) was heated and stirred at 120 °C for 4 h. After addition of water, the mixture was extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated to afford **3** (360 mg, 0.9 mmol, 90%) as a gray solid, which was used in the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 3H), 3.96 (s, 3H), 4.68 (s, 2H), 5.01 (s, 2H), 5.47 (br s, 1H), 6.94 (s, 1H), 7.26–7.40 (m, 4H), 7.49 (d, J = 7.0 Hz, 2H), 7.76 (d, J = 7.5 Hz, 1H), 8.56 (dd, J = 1.7, 4.8 Hz, 1H), 8.66 (d, J = 1.7 Hz, 1H).

6-Hydroxy-5,7-dimethoxy-2-[(3-pyridylmethyl)amino]benzothiazole (4). To a solution of **3** (360 mg, 0.88 mmol) in ethanol (10 mL) was added concentrated hydrochloric acid (5 mL), and the mixture was heated under reflux for 2 h. It was then neutralized with a saturated aqueous solution of sodium hydrogen carbonate and extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated. The resulting solid was recrystallized from ethyl acetate to afford **4** (180 mg, 0.57 mmol, 65%) as an off-white solid: mp 142–143 °C (AcOEt); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.77 (s, 3H), 3.81 (s, 3H), 4.56 (d, J = 5.5 Hz, 2H), 6.86 (s, 1H), 7.37 (dd, J = 4.8, 7.5 Hz, 1 H), 7.77 (d, J = 7.5 Hz, 1H), 8.31 (t, J = 5.5 Hz, 1H), 8.36 (s, 1H), 8.47 (dd, J = 1.7, 4.8 Hz, 1H), 8.59 (d, J = 1.7 Hz, 1H). Anal. ($C_{15}H_{15}N_3O_3S$) C, H; N: calcd, 13.24; found, 12.70.

6-Methoxy-4,5,7-trimethyl-2-(3-pyridinecarboxamido)benzothiazole (6). After a mixture of 2-amino-6-methoxy-4,5,7-trimethylbenzothiazole (**5**) (2.2 g, 9.9 mmol), nicotinoyl chloride hydrochloride (2.7 g, 15.2 mmol), and pyridine (3 mL) in tetrahydrofuran (50 mL) was heated at 60 °C for 2 h, the reaction was quenched by adding water. The precipitates were collected by filtration, washed with water, and dried to afford **6** (2.7 g, 8.2 mmol, 83%) as an off-white solid, which was used for the next step without further purification: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.35 (s, 3H), 2.50 (s, 3H), 2.60 (s, 3H), 3.76 (s, 3H), 7.41 (dd, J = 7, 5 Hz, 1H), 8.32–8.52 (m, 1H), 8.74 (d, J = 5 Hz, 1H), 9.32 (s, 1H).

6-Methoxy-4,5,7-trimethyl-2-[(3-pyridylmethyl)amino]benzothiazole (7). Compound **6** (300 mg, 0.92 mmol) was suspended in tetrahydrofuran (30 mL). Then a 1.0 M solution of borane/THF complex in tetrahydrofuran (10 mL, 10 mmol) was added, and the mixture was heated under reflux for 30 min. After addition of 20 mL of 1 N hydrochloric acid, the mixture was further heated and stirred at 60 °C for 15 min. The mixture was neutralized with a saturated aqueous solution of sodium hydrogen carbonate and extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated to give **7** (250 mg, 0.80 mmol, 87%) as an off-white solid, which was used for the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.27 (s, 3H), 2.33 (s, 3H), 2.48 (s, 3H), 3.68 (s, 3H), 4.65 (s, 2H), 5.40 (br s, 1H), 7.29 (dd, J = 4.8, 7.5 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 8.55 (dd, J = 1.7, 4.8 Hz, 1H), 8.67 (d, J = 1.7 Hz, 1H).

6-Hydroxy-4,5,7-trimethyl-2-[(3-pyridylmethyl)amino]benzothiazole (8). To a solution of **7** (250 mg, 0.80 mmol) in dichloromethane (10 mL) was added a 1.0 M solution of boron tribromide in dichloromethane (5 mL, 5.0 mmol). After being stirred at room temperature for 30 min, the mixture was neutralized with a saturated aqueous solution of sodium hydrogen carbonate and extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated. The resulting solid was recrystallized from ethanol–water to afford **8** (100 mg, 0.33 mmol, 41%) as a white solid: mp 194–195 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.12 (s, 3H), 2.18 (s, 3H), 2.35 (s, 3H), 4.55 (d, J = 5.7 Hz, 2H), 7.36 (dd, J = 4.8, 7.5 Hz, 1H), 7.81 (d, J = 7.5 Hz, 1H), 7.90 (s, 1H), 8.16 (t, J = 5.7 Hz, 1H), 8.46 (dd, J = 1.7, 4.8 Hz, 1H), 8.62 (d, J = 1.7 Hz, 1H). Anal. ($C_{16}H_{17}N_3OS$) C, H, N.

6-Hydroxy-4,7-dimethyl-2-[(3-pyridylmethyl)amino]benzothiazole (13a). A mixture of 2-amino-6-hydroxy-4,7-dimethylbenzothiazole hydrochloride (**11a**)[27] (10.0 g, 44.0 mmol), 3-pyridinecarboxaldehyde (9.4 g, 88.0 mmol), and ammonium acetate (33.4 g, 44.0 mmol) in toluene (500 mL) was vigorously stirred and heated under reflux for 5 h, while the water was removed with a Dean–Stark extractor. After cooling to room temperature, the precipitates were collected by filtration, washed with water, and dried to afford the crude imine **12a** (12.4 g, 44.0 mmol). To a suspension of **12a** (10.7 g, 37.8 mmol) in ethanol (200 mL) at 0 °C was added sodium borohydride (1.33 g, 35.0 mmol) in portions. The mixture was stirred at 0 °C for 3 h and then neutralized by adding 10% hydrochloric acid in portions. After extraction with ethyl acetate, the organic extract was washed with brine, dried, and evaporated.

The crude residue was purified by flash column chlomatography on silica gel (solvent: ethyl acetate:*n*-hexane = 1:3–4:1). The resulting solid was recrystallized from ethyl acetate–*n*-hexane to afford **13a** (5.2 g, 18.2 mmol, 41%) as a white solid: mp 156–157 °C (AcOEt–*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (s, 3H), 2.43 (s, 3H), 4.51 (d, J = 6.0 Hz, 2H), 6.55 (s, 1H), 7.34 (dd, J = 4.5, 7.5 Hz, 1H), 7.78 (br d, J = 7.5 Hz, 1H), 8.17 (t, J = 6.0 Hz, 1H), 8.43 (d, J = 4.5 Hz, 1H), 8.57 (s, 1H), 8.90 (s, 1H). Anal. ($C_{15}H_{15}N_3OS$) C, H, N.

6-Hydroxy-4-phenyl-2-[(3-pyridylmethyl)amino]benzothiazole (13b). Compound **11b** (320 mg, 1.3 mmol) was treated according to the same procedure described in the preparation of **13a** to afford **13b** (80 mg, 0.24 mmol, 18%) as a white solid: mp 207–208 °C (AcOEt–*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.49 (d, J = 7.0 Hz, 2H), 7.00 (s, 1H), 7.30–7.46 (m, 5H), 7.64 (s, 1H), 7.66 (s, 1H), 7.76 (br d, J = 7.0 Hz, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.54–8.60 (m, 2H); HRMS ($C_{19}H_{15}N_3OS$) calcd, 334.1014 (MH^+); found, 334.1009.

5-Hydroxy-4-methyl-2-[(3-pyridylmethyl)amino]naptho[1,2-*d*]thiazole (13c). Compound **11c** (2.0 g, 7.5 mmol) was treated according to the same procedure described in the preparation of **13a** to afford **13c** (800 mg, 2.5 mmol, 33%) as a white solid: mp 194–195 °C (AcOEt–*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.41 (s, 3H), 4.68 (d, J = 4.5 Hz, 1H), 7.38 (dd, J = 4.5, 7.5 Hz, 1H), 7.39–7.48 (m, 2H), 7.87 (br d, J = 7.5 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.31 (d, J = 8.0 Hz, 1H), 8.42 (dd, J = 1.5, 4.5 Hz, 1H), 8.47 (d, J = 1.5 Hz, 1H), 8.71 (s, 1H), 8.32 (br s, 1H). Anal. ($C_{18}H_{15}N_3OS$) C, H, N.

[4-[6-Hydroxy-5,7-dimethyl-2-(methylamino)benzothiazolyl]](3-pyridyl)methyl Acetate (23a) (Method A). To a stirred solution of 1-methyl-2-thiourea (**22a**) (480 mg, 5.3 mmol) in ethanol (20 mL) and concentrated hydrochloric acid (1.4 mL) was added dropwise [2-(3,5-dimethyl-1,4-benzoquinonyl)](3-pyridyl)methyl acetate (**21a**) (3.0 g, 10.5 mmol) dissolved in ethanol (total volume: 12 mL). After the mixture had been stirred at room temperature for 24 h, the precipitates were collected by filtration, dissolved in 10 mL of water, and neutralized with a saturated aqueous solution of sodium hydrogen carbonate. After extraction with ethyl acetate, the organic extract was washed with brine, dried, and evaporated to give **23a** (1.0 g, 2.8 mmol, 53%) as a white solid, which was used in the next step without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (s, 3H), 2.20 (s, 3H), 2.35 (s, 3H), 3.05 (s, 3H), 7.20 (dd, J = 4.5, 7.5 Hz, 1H), 7.54 (br d, J = 5.7 Hz, 1H), 8.05 (s, 1H), 8.45 (d, J = 4.5 Hz, 1H), 8.52 (d, J = 1.5 Hz, 1H).

6-Hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole (26a) (Method A). To a solution of **23a** (500 mg, 1.4 mmol) dissolved in acetic acid (5 mL) was added zinc powder (750 mg, 11.4 mmol), and the mixture was heated under reflux for 5 h. After addition of water, the mixture was extracted with ethyl acetate. The organic extract was washed with brine, dried, and evaporated. The resulting solid was recrystallized from ethanol to give **26a** (260 mg, 0.87 mmol, 62%) as a white solid: mp 236–238 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10 (s, 3H), 2.23 (s, 3H), 2.90 (d, J = 4.5 Hz, 3H), 4.27 (s, 2H), 7.22 (dd, J = 4.5, 7.5 Hz, 1H), 7.50 (br d, J = 7.5 Hz, 1H), 7.63 (br s, 1H), 7.89–7.94 (m, 1H), 8.30 (br d, J = 5.0 Hz, 1H), 8.46 (br s, 1H). Anal. ($C_{16}H_{17}N_3OS$) C, H, N.

6-Hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole Diydrochloride (26a·2HCl) (Method B). To a stirred solution of 1-methyl-2-thiourea (**22a**) (480 mg, 5.3 mmol) in ethanol (15 mL) and concentrated hydrochloric acid (1.2 mL) was added dropwise **25a** (2.1 g, 9.2 mmol) dissolved in ethanol (total volume: 10 mL). After the mixture had been stirred at room temperature for 24 h, the precipitate was collected by filtration, washed with ethanol, and dried. The resulting solid was recrystallized from ethanol to afford **26a** dihydrochloride (1.4 g, 3.8 mmol, 83%) as a white solid: mp 296–298 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.26 (s, 3H), 3.00 (d, J = 0.5 Hz, 3H), 4.53 (s, 2H), 7.95 (t, J = 7.0 Hz, 1H), 8.32 (d, J = 7.0 Hz, 1H), 8.77 (br s, 1H), 8.75 (br s, 1H). Anal. ($C_{16}H_{19}Cl_2N_3OS$) C, H, N.

2-(Ethylamino)-6-hydroxy-5,7-dimethyl-4-(3-pyridylmethyl)benzothiazole Dihydrochloride (26b). Compound **25b** (1.2 g, 5.3 mmol) and 1-ethyl-2-thiourea (**22b**) (280 mg, 2.7 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26b** (400 mg, 1.0 mmol, 39%) as a white solid: mp 194–195 °C (EtOH–*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (t, J = 7.0 Hz, 3H), 2.14 (s, 3H), 2.28 (s, 3H), 3.51 (dt, J = 7.0, 5.0 Hz, 2H), 4.60 (s, 2H), 7.98 (t, J = 7.0 Hz, 1H), 8.31 (d, J = 7.0 Hz, 1H), 8.75 (s, 1H), 8.78 (d, J = 7.0 Hz, 1H). Anal. ($C_{17}H_{21}Cl_2N_3OS$) C, H, N. In some cases, the resulting dihydrochloride was dissolved in water and the free thiazoles were obtained by neutralization with aqueous sodium hydrogen carbonate solution.

6-Hydroxy-5,7-dimethyl-2-(propylamino)-4-(3-pyridylmethyl)benzothiazole Dihydrochloride (26c). Compound **25c** (5.2 g, 22.9 mmol) and 1-propyl-2-thiourea (**22c**) (1.4 g, 11.5 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26c** (3.7 g, 9.2 mmol, 80%) as a light yellow solid: mp 198–199 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, J = 7.5 Hz, 3H), 1.58 (q, J = 7.0 Hz, 2H), 2.10 (s, 3H), 2.24 (s, 3H), 3.40 (br s, 2H), 4.56 (s, 2H), 7.94 (dd, J = 5.5, 8.0 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.72 (s, 1H), 8.74 (d, J = 5.5 Hz, 1H). Anal. ($C_{18}H_{23}Cl_2N_3OS$) C, H, N.

2-Amino-6-hydroxy-5,7-dimethyl-4-(3-pyridylmethyl)-benzothiazole Dihydrochloride (26d). Compound **25d** (7.0 g, 30.8 mmol) and thiourea (**22d**) (1.2 g, 15.4 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26d** (5.0 g, 14.0 mmol, 91%) as a white solid: mp 171–172 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 3H), 2.25 (s, 3H), 4.50 (s, 2H), 7.94 (dd, J = 7.0, 6.0 Hz, 1H), 8.20 (d, J = 7.0 Hz, 1H), 8.71 (s, 1H), 8.75 (d, J = 6.0 Hz, 1H). Anal. ($C_{15}H_{17}Cl_2N_3OS$) C, H, N.

2-Amino-6-hydroxy-5-methoxy-7-methyl-4-(3-pyridylmethyl)benzothiazole Dihydrochloride (26e). Compound **25e** (44.0 g, 183.0 mmol) and thiourea (**22d**) (6.9 g, 90.8 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26e** (20.0 g, 53.5 mmol, 59%) as a white solid: mp 208–209 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.21 (s, 3H), 3.65 (s, 3H), 4.40 (s, 2H), 7.17 (s, 1H), 7.30 (s, 1H), 7.43 (s, 1H), 7.94 (dd, J = 8.0, 5.6 Hz, 1H), 8.35 (d, J = 8.0 Hz, 1H), 8.75 (d, J = 5.6 Hz, 1H), 8.80 (br s, 1H). Anal. ($C_{15}H_{17}Cl_2N_3O_2S$) C, N; H: calcd, 5.12; found, 5.77.

6-Hydroxy-5-methoxy-7-methyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole Dihydrochloride (26f). Compound **25f** (4.1 g, 16.9 mmol) and 1-methyl-2-thiourea (**22a**) (760 mg, 8.4 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26f** (640 mg, 5.2 mmol, 62%) as a white solid: mp 168–169 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.94 (s, 3H), 3.66 (s, 3H), 4.39 (s, 2H), 7.94 (dd, J = 8.0, 5.6 Hz, 1H), 8.37 (br d, J = 8.0 Hz, 1H), 8.73 (d, J = 5.6 Hz, 1H), 8.81 (br s, 1H). Anal. ($C_{16}H_{19}Cl_2N_3O_2S$) C, H, N.

2-Amino-7-ethyl-6-hydroxy-5-methoxy-4-(3-pyridylmethyl)benzothiazole Dihydrochloride (26g). Compound **25g** (50.0 g, 194 mmol) and thiourea (**22d**) (7.4 g, 97.0 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26g** (18.3 g, 58.2 mmol, 60%) as a yellow solid: mp 153–154 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (t, J = 7.6 Hz, 3H), 2.62 (q, J = 7.6 Hz, 2H), 3.64 (s, 3H), 4.35 (s, 2H), 7.92 (dd, J = 8.0, 5.6 Hz, 1H), 8.31 (br d, J = 8.0 Hz, 1H), 8.73 (d, J = 5.6 Hz, 1H), 8.77 (br s, 1H). Anal. ($C_{16}H_{19}Cl_2N_3O_2S$) C, H, N.

7-Ethyl-6-hydroxy-5-methoxy-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole (26h). Compound **25h** (4.0 g, 15.6 mmol) and 1-methyl-2-thiourea (**22a**) (700 mg, 7.8 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **26h** (800 mg, 5.5 mmol, 70%) as a white solid: mp 191–193 °C (EtOH–H_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.2 Hz, 3H), 2.75 (q, J = 7.2 Hz, 2H), 3.05 (s, 3H), 3.73 (s, 3H), 4.29 (s, 2H), 5.05 (br, s, 1H), 5.70 (br s, 1H), 7.12 (dd, J = 8.0, 4.8 Hz, 1H), 7.65 (br, d, J = 8.0 Hz, 1H), 8.37 (dd, J = 4.8, 1.2 Hz, 1H), 8.64 (d, 1.6 Hz, 1H). Anal. ($C_{17}H_{19}N_3O_2S$) C, H, N.

6-Hydroxy-4,7-dimethyl-2-(methylamino)-5-(3-pyridylmethyl)benzothiazole (27a). 2,5-Dimethyl-6-(3-pyridylmethyl)-1,4-benzoquinone (2.0 g, 8.8 mmol) and 1-methyl-2-thiourea (**22a**) (400 mg, 4.4 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **27a** (680 mg, 2.3 mmol, 52%) as a white solid: mp 170–171 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.13 (s, 3H), 2.29 (s, 3H), 2.90 (d, J = 4.8 Hz, 3H), 4.25 (s, 2H), 7.95 (dd, J = 8.0, 4.8 Hz, 1H), 8.31 (d, J = 8.0 Hz, 1H), 8.68 (s, 1H), 8.75 (d, J = 4.8 Hz, 1H), 8.93 (m, 2H); HRMS ($C_{16}H_{17}N_3OS$) calcd, 300.1171 (MH^+); found, 300.1175.

2-(Ethylamino)-6-hydroxy-4,7-dimethyl-5-(3-pyridylmethyl)benzothiazole (27b). 2,5-Dimethyl-6-(3-pyridylmethyl)-1,4-benzoquinone (5.0 g, 22.0 mmol) and 1-ethyl-2-thiourea (**22b**) (1.15 g, 11.0 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **27b** (2.3 g, 7.3 mmol, 66%) as a white solid: mp 219–220 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.18 (t, J = 7.1 Hz, 3H), 2.24 (s, 3H), 2.34 (s, 3H), 3.34 (m, 2H), 4.06 (s, 2H), 7.23 (dd, J = 7.8, 4.8 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.63 (t, J = 5.1 Hz, 1H), 8.15 (br s, 1H), 8.33 (dd, J = 4.8, 1.5 Hz, 1H), 8.41 (d, J = 2.2 Hz, 1H). Anal. ($C_{17}H_{19}N_3OS$) C, H, N.

2-Amino-6-hydroxy-4,5-dimethyl-7-(3-pyridylmethyl)-benzothiazole Dihydrochloride (28a). 2,3-Dimethyl-5-(3-pyridylmethyl)-1,4-benzoquinone (12 g, 52.9 mmol) and thiourea (**22d**) (2.0 g, 26.4 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **28a** (4.8 g, 13.4 mmol, 51%) as an off-white solid: mp 235–237 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.20 (s, 3H), 2.39 (s, 3H), 4.30 (s, 2H), 7.95 (dd, J = 7.8, 4.8 Hz, 1H), 8.28 (d, J = 7.8 Hz, 1H), 8.75 (s, 1H), 8.78 (d, J = 4.8 Hz, 1H). Anal. ($C_{15}H_{17}Cl_2N_3OS$) C, H, N.

2-(Ethylamino)-6-hydroxy-4,5-dimethyl-7-(3-pyridylmethyl)benzothiazole (28b). 2,3-Dimethyl-5-(3-pyridylmethyl)-1,4-benzoquinone (5.1 g, 22.5 mmol) and 1-ethyl-2-thiourea (**22b**) (1.15 g, 11.0 mmol) were treated according to the same procedure described in the preparation of **26a** (method B) to afford **28b** (2.2 g, 7.0 mmol, 64%) as a white solid: mp 188–189 °C (EtOH–n-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.13 (t, J = 7.0 Hz, 3H), 2.14 (s, 3H), 2.35 (s, 3H), 3.30 (m, 2H), 3.98 (s, 2H), 7.23 (dd, J = 7.8, 4.8 Hz, 1H), 7.53 (ddd, J = 7.8, 4.8, 2.4 Hz, 1H), 8.11 (s, 1H), 8.33 (d, J = 4.8 Hz, 1H), 8.40 (d, J = 2.4 Hz, 1H). Anal. ($C_{17}H_{19}N_3OS$) C, H, N.

Inhibition of Eicosanoid Production (*in Vitro*). Glycogen-Induced Peritoneal Cells of Rats. Mixed peritoneal leukocytes containing polymorphonuclear leukocytes (PMNs) and mononuclear leukocytes were elicited from male F344 rats (Charles River) by an ip injection of 10 mL of 6% glycogen solution (type II, Sigma), according to Moroney et al.[28] The cells were suspended in Hanks balanced salt solution (HBSS) free of Ca^{2+} and Mg^{2+} at a concentration of 5×10^6 cells/mL. Aliquots (0.1 mL) of the cell suspensions were preincubated for 5 min at 37 °C with test compound or vehicle (0.1% DMSO/0.1% BSA) in 96-well plates (Coastar). The reaction was initiated by adding A23187 (4 μM, Calibiochem, CA). At the end of incubation (10 min at 37 °C), the reaction was terminated by adding BW755C (100 μM). The incubation mixtures were centrifuged (110g, 5 min), and aliquots of the supernatants were analyzed for LTB_4, TXB_2, and PGE_2 by radioimmunoassay (Amersham, NEM).

Human Whole Blood. Human fresh blood was obtained from healthy volunteers. The blood was anticoagulated with heparin (10 U/mL). Aliquots (0.4 mL) were preincubated with test compound or vehicle (0.1% DMSO/0.1% BSA) for 5 min at 37 °C. A23187 (40 μM) was added, and the blood samples were incubated for an additional 20 min. Plasma was separated by centrifugation and analyzed for LTB_4, TXB_2, and PGE_2 by radioimmunoassay.

Human Peripheral Blood Neutrophils. Human blood was anticoagulated with heparin (10 U/mL). Red cells were first removed by dextran sedimentation (6% dextran, 37 °C, 60 min). Neutrophils were sedimented by percol (Sigma) gradient density centrifugation and resuspended in HBSS free of Ca^{2+} and Mg^{2+} at a concentration of 1×10^6 cells/mL. Aliquots (0.1 mL) were preincubated with test compound or vehicle (0.1% DMSO/0.1% BSA) for 5 min at 37 °C. Eicosanoid synthesis was induced by adding A23187 (4 μM). After 10 min, the cells were pelleted by centrifugation (110g, 10 min). Cell-free supernatants were assayed for LTB_4 by radioimmunoassay.

Human Platelets. Platelet-rich plasma was obtained from citrated whole blood by centrifugation (110g, 10 min) and mixed with anticoagulant solution (ACD-A solution, Terumo Co., Ltd., Japan). The platelet suspension was centrifuged (1000g, 10 min) and resuspended in Tris-HCl–saline, pH 7.4, containing 10 μM indomethacin at a concentration of 3×10^8 cells/mL. Aliquots (0.2 mL) of the platelet suspension were preincubated with test compound or vehicle alone (0.1% DMSO/0.1% BSA) for 5 min at 25 °C before addition of PGH_2 (1 μg/mL). After 3 min at 25 °C, the reaction was terminated by adding 0.8 mL of a 55 mM citrate/100% ethanol solution. The cell suspensions were centrifuged (110g, 10 min), and the supernatants were assayed for TXB_2 by radioimmunoassay.

RBL-1 Cell 5-Lipoxygenase. RBL-1 cells (2×10^6 cells/mL), suspended in 50 mM phosphate buffer, pH 7.4, containing 0.25 M sucrose and 1 mM EDTA, were homogenized by sonication (Branson Sonifer 185, 5 microtip setting) at 0 °C. Aliquots (0.5 mL) of the homogenates were preincubated for 5 min at 37 °C with test compound or vehicle (0.1% DMSO/0.1% BSA) in the presence of 2 mM glutathione. The reaction was initiated by addition of arachidonic acid (0.2 mM). After the mixture was incubated for 10 min at 37 °C, the reaction was stopped by adding 0.05 μL of 2 N formic acid. Then, 0.2 mL of $CHCl_3$/MeOH (4:1) and 0.2 mL of saturated NaCl solution were added. The samples were centrifuged for 5 min at 110g, and the organic phases were analyzed for 5-HETE by HPLC (C_{18} ODS, Nucleosil, MeOH:H_2O:AcOH (75:25:0.01), 1.5 mL/min).

Human Platelet Thromboxane Synthetase. Platelets were separated from platelet-rich plasma by centrifugation (1000g, 10 min, 4 °C), as described above, and resuspended in phosphate-buffered saline (Dulbecco's PBS), pH 7.4. Platelets were homogenized by sonication (Branson Sonifer 185, 5 microtip setting) at 0 °C. The microsome fraction was separated by centrifugation (105000g, 60 min) and resuspended in 50 mM Tris-HCl–saline, pH 7.4, containing 10 μM indomethacin. Aliquots (20 μg of protein) of the enzyme preparation were preincubated for 5 min at 25 °C with test compound or vehicle (0.1% DMSO/0.1% BSA). The reaction was initiated by adding PGH_2 (1 μg/mL). After the mixture was incubated for 1 min at 25 °C, the reaction was terminated by adding 0.8 mL of a 55 mM citric acid/100% ethanol solution. The incubation mixture was assayed for TXB_2 by radioimmunoassay.

Sheep Seminal Vesicle Cyclooxygenase. Lyophilized sheep seminal vesicle microsomes served as the source of cyclooxygenase. The enzyme preparation was dissolved in 100 mM Tris-HCl, pH 7.4, containing 5 mM tryptophan and 5 mM glutathione. Aliquots (17 μg of protein) of the enzyme preparation were preincubated for 10 min at 25 °C with test compound or vehicle (0.1% DMSO/0.1% BSA). The reaction was initiated by adding arachidonic acid (20 μM). After the mixture was incubated for 20 min at 25 °C, the reaction was terminated by adding indomethacin (100 μM). The supernatant of the incubation mixture was analyzed for PGE_2 by radioimmunoassay.

Inhibition of Eicosanoid Production in TNB Colitis Rat Colon (*ex Vivo*). Male F344 rats (Charles River), aged 9 weeks, were used after fasting for 2 days. Colitis was induced under light ether anesthesia by a single intracolonic administration of 0.25 mL of 50% ethanol containing 30 mg of trinitrobenzenesulfonic acid (TNB, TCI), as previously described.[30] Either test compound (suspended in 0.5% MC solution) or vehicle was given once, 7 days after the induction of colitis, and 6 h later the rats were sacrificed. Colons excised from the rats were incubated in Tyrode solution containing A23187 (2 μg/mL) for 15 min at 37 °C. The amount of eicosanoids released in the incubation mixtures was determined by radioimmunoassay, according to Dreyling et al.[34] The suppression ratios (%) were calculated on the basis of the

difference in the amounts of these eicosanoids between the control group that received vehicle and the test groups.

Therapeutic Effects on TNB Colitis (*in Vivo*). TNB colitis was induced in rats as described above. Either test compound (suspended in 0.5% MC solution) or vehicle was administered orally once daily for 11 days beginning 3 days after the rectal instillation of TNB/ethanol. Colitis rats that received vehicle served as the control group. The other group received 0.25 mL of 50% ethanol intracolonically, in place of the TNB/ethanol solution (ethanol group). On day 14, the rats were sacrificed. Colonic damage was evaluated by determining myeloperoxidase activity in the tissue as described by Krawisz et al.[33] as a biochemical marker of neutrophil content in the tissue. The therapeutic ratio (% inhibition) was calculated according to the following equation: % inhibition = [1 − (MPO in test group − MPO in ethanol group)/(MPO in control group − MPO in ethanol group)] × 100. Colonic damage was measured macroscopically and expressed in mm^2/colon, using a stereomicroscope. The therapeutic ratio (% inhibition) was calculated according to the following equation: % inhibition = [1 − (lesion area in test group − lesion area in ethanol group)/(lesion area in control group − lesion area in ethanol group)] × 100.

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