Synthetic Studies on Condensed-Azole Derivatives. I. Synthesis and Anti-asthmatic Activities of ω-Substituted Alkylthioimidazo[1,2-*b*]pyridazines

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A series of novel ω-substituted alkylthioimidazo[1,2-*b*]pyridazines was designed and synthesized in an effort to find a novel anti-asthmatic agent. The anti-asthmatic activity of these compounds was evaluated on the basis of their ability to inhibit thromboxane A_2 synthetase and platelet activating factor (PAF)-induced bronchoconstriction in guinea pigs. None of these compounds significantly inhibited thromboxane A_2 synthetase, though, sulfonamide derivatives potently inhibited PAF-induced bronchoconstriction. Among them, 3-(imidazo[1,2-*b*]pyridazin-6-yl)thiopropanesulfonamide (5) showed the most potent inhibitory effect. The anti-asthmatic effects of compound 5 in experimental models were superior to those of theophylline.

Key words anti-asthmatic effect; PAF-induced bronchoconstriction; imidazo[1,2-*b*]pyridazin; theophylline; structure–activity relationship

A number of 5- and 6-membered condensed-azole ring systems having a nitrogen atom in the bridge head, for example imidazo[1,2-*a*]pyridine (I), imidazo[1,5-*a*]pyridine (II) and imidazo[1,2-*b*]pyridazine (III), have the characteristic property that a cation generated by protonation or quaternization is delocalized over the entire ring (Fig. 1).[1)]

Some drugs having a condensed-azole ringsystem, such as zolimidine[2)] (antiulcer drug) and zolpidem[3)] (hypnotic) bearing imidazo[1,2-*b*]pyridine and ibudilast[4)] (anti-asthmatic drug) bearing pyrazolo[1,5-*a*]pyridine (Fig. 2) are already on the market.

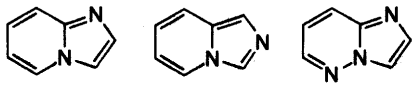
In the course of our study on 5- and 6-membered condensed-azole ring systems, which have this interesting electronic property, we found that 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-methoxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate hydrochloride (cefozopran, Fig. 3)[5)] showed more potent antibacterial activity against both gram-positive and gram-negative bacteria than many third generation injectable cephalosporins. This compound is now under clinical trials.

We next focused our attention on anti-asthmatic activity. In recent years, it has been established that arachidonic acid metabolites, such as prostaglandins, leukotrienes (LTs) and thromboxanes (TXs), play important roles *in vivo*.[6)] TXA_2 has potent vasoconstricting and bronchoconstricting activities.[7)] TXA_2 synthetase inhibitors may have therapeutic utility in asthma, and several inhibitors of TXA_2 synthetase have already been evaluated in clinical studies. Ozagrel[8)] (Fig. 4) was the first compound from this new class of anti-asthmatic agents to be marketed. Studies on the structure–activity relationships of these TXA_2 synthetase inhibitors, indicated that the stronger inhibitors among these compounds are those that contain a carboxyalkyl chain of 5—7 carbon atoms attached to a nitrogen atom in the heterocyclic ring.[9)] To our knowledge, only one compound possessing a condensed-azole, CGS-13080[10)] (Fig. 4) consisting of imidazo[1,5-*a*]pyridine has been reported in the field of TXA_2 synthetase inhibitors. We hypothesized that changing the heterocyclic ring from imidazo[1,5-*a*]pyridine to another condensed-azole ring, especially imidazo[1,2-*b*]pyridazine which is found in cefozopran, would yield a novel TXA_2 synthetase inhibitor.

We describe here the synthesis and anti-asthmatic activity of a series of ω-substituted alkylthioimidazo[1,2-*b*]pyridazines.

Chemistry

The synthesis of the ester (**1**), carboxylic acid (**2**) and amide (**3**) derivatives of butylthioimidazo[1,2-*b*]pyr-



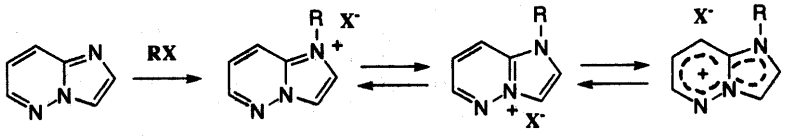


Fig. 1

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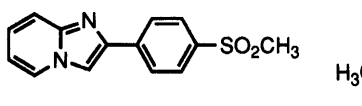
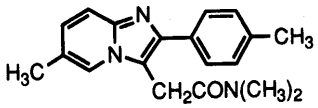
idazines listed in Table 1 was carried out *via* the route shown in Chart 1. After esterification of 5-mercaptovaleric acid (**16**),[11] the obtained ethyl 5-mercaptopentanoate (**17**) was reacted with 6-chloroimidazo[1,2-*b*]pyridazine (**18**)[12] in the presence of sodium ethoxide to afford the ester derivative (**1**). The carboxylic acid (**2**), prepared by hydrolysis of **1**, was converted to the amide (**3**) by treatment with aqueous ammonia *via* acyl chloride. The sulfonamide derivative (**4**) containing a side-chain of 2 carbon atoms was prepared from sodium 2-mercaptoethanesulfonate (**19**) by the route shown in Chart 2. Compound **19** was reacted with **18** in the presence of sodium methoxide to afford the sodium sulfonate (**20**) which was treated with phosphoryl chloride and then ammonia to give the sulfonamide (**4**). The sulfonamide derivatives (**5**—**15**) having a side-chain of 3—7 methylene groups listed in Table 1 were synthesized *via* the routes shown Chart 3. The chloro- or bromoalkylsulfonyl chlorides (**21**—**25**)[13] were treated with ammonia gas under ice-cooling to afford the sulfonamides (**26**, **31**, **34**—**36**).[13] After treatment with potassium hydrogen sulfide, these sulfonamides were reacted with **18** in the presence of sodium methoxide to give **5**, **10** and **13**—**15**. Compounds **21** and **22** were reacted with various amines under ice-cooling to yield the chloroalkylsulfonamides (**27**—**29**, **32**, **33**). The sulfonamides were treated with potassium hydrogen sulfide and then **18** to afford the N-alkylsubstituted sulfonamide derivatives (**6**—**8**, **11**, **12**). The N-arylsubstituted sulfonamide (**30**) was obtained by refluxing of the sulfonyl chloride (**21**) in benzene containing aniline. The N-arylsubstituted sulfonamide derivative (**9**) was prepared from **30** in a manner similar to that used to prepare **6**.

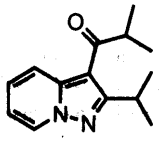


Zolimidine



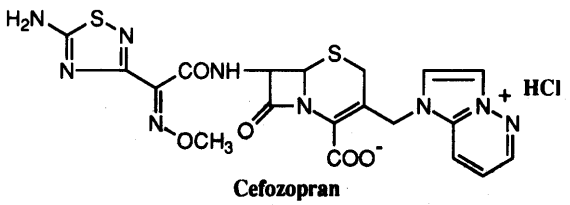
Zolpidem

Fig. 2



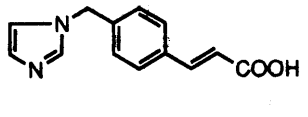
Ibudilast

Fig. 3

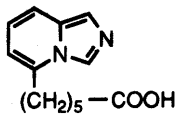


Cefozopran

Fig. 4



Ozagrel



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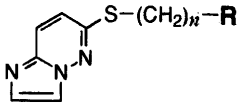
Pharmacological Results and Discussion

The ω-substituted alkylthioimidazo[1,2-*b*]pyridazines obtained in this study were evaluated *in vitro* for their ability to inhibit TXA_2 synthetase and *in vivo* for their ability to inhibit platelet activating factor (PAF)-induced bronchoconstriction in guinea pigs. Since TXA_2 is extremely volatile and readily converted *in vitro*, the

Table 1. Physical Data for ω-Substituted Alkylthioimidazo[1,2-*b*]pyridazines



Compd. No.	*n*	R	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Yield (%)
1	4	$-COOC_2H_5$	122—124	$C_{13}H_{17}N_3O_2S \cdot HCl$	49.44	5.74	13.30	49.36	5.87	13.32	51
2	4	$-COOH$	174—176	$C_{11}H_{13}N_3O_2S \cdot HCl$	45.91	4.90	14.60	45.83	4.96	14.80	95
3	4	$-CONH_2$	200—203	$C_{11}H_{14}N_4O_2S \cdot HCl$	46.07	5.27	19.54	46.03	5.54	19.46	50
4	2	$-SO_2NH_2$	145—147	$C_8H_{10}N_4O_2S_2$	37.20	3.90	21.69	37.00	3.89	21.41	45
5	3	$-SO_2NH_2$	147—148	$C_9H_{12}N_4O_2S_2$	39.69	4.44	20.57	39.62	4.42	20.50	32
6	3	$-SO_2NHCH_3$	114—116	$C_{10}H_{14}N_4O_2S_2 \cdot H_2O$	39.46	5.30	18.41	39.41	5.28	18.29	20
7	3	$-SO_2NH-$	120—121	$C_{12}H_{16}N_4O_2S_2$	46.13	5.16	17.93	46.08	5.16	17.86	19
8	3	$-SO_2N(CH_3)_2$	110—111	$C_{11}H_{16}N_4O_2S_2$	43.98	5.37	18.65	43.90	5.25	18.60	10
9	3	$-SO_2NH-$	159—160	$C_{15}H_{16}N_4O_2S_2$	51.70	4.63	16.08	51.76	4.58	16.01	8
10	4	$-SO_2NH_2$	219—221	$C_{10}H_{14}N_4O_2S_2 \cdot HCl$	37.21	4.68	17.36	37.24	4.40	17.36	51
11	4	$-SO_2NHCH_3$	164—167	$C_{11}H_{16}N_4O_2S_2 \cdot HCl$	39.22	5.09	16.63	39.42	5.04	16.34	60
12	4	$-SO_2NH-$	145—148	$C_{13}H_{18}N_4O_2S_2 \cdot HCl$	43.03	5.28	15.44	43.03	5.28	15.28	40
13	5	$-SO_2NH_2$	180—182	$C_{11}H_{16}N_4O_2S_2 \cdot HCl$	39.22	5.09	16.63	39.20	5.09	16.38	22
14	6	$-SO_2NH_2$	98—100	$C_{12}H_{18}N_4O_2S_2 \cdot 2H_2O$	45.27	6.96	17.60	45.28	6.93	17.91	51
15	7	$-SO_2NH_2$	141—143	$C_{13}H_{20}N_4O_2S_2$	47.54	6.14	17.06	47.74	6.29	17.09	48

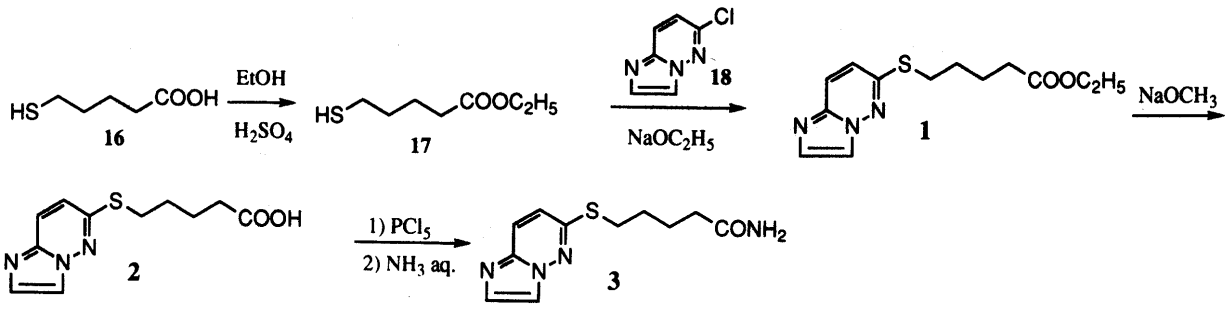


Chart 1

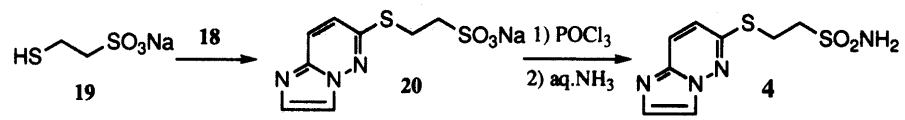


Chart 2

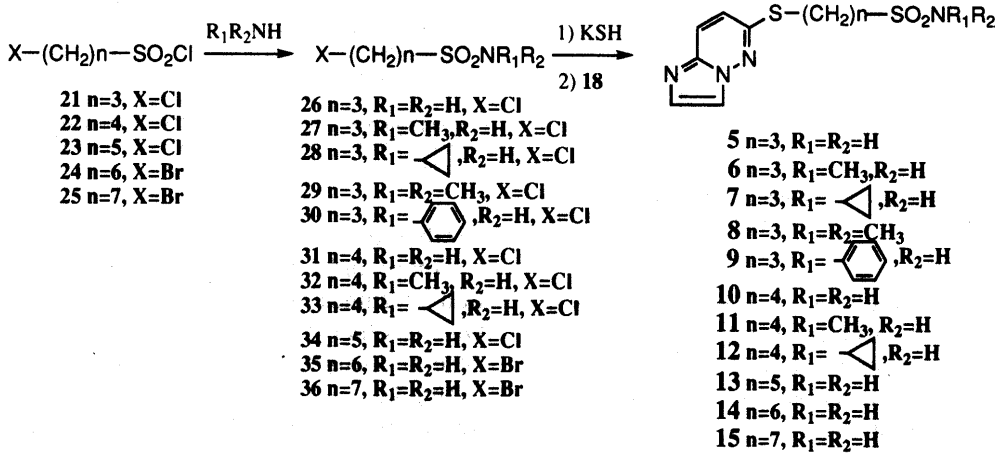


Chart 3

inhibition of TXA_2 synthetase activity was assayed by measuring the inhibition of the generation of 12(*S*)-hydroxy-5,8,10-heptadecatrienoic acid (HHT) from arachidonic acid.[14]

TAX_2 Synthetase Inhibition Table 2 shows the inhibition of HHT generation by ω-substituted butylthioimidazo[1,2-*b*]pyridazines with various terminal functional groups. These compounds inhibited HHT generation at a concentration of 10^{-4} M. As shown by Ford *et al.*,[10] varying the terminus of ω-substituted imidazo[1,5-*a*]-pyridines produced an increase in the TXA_2 synthetase inhibition in the order $COOEt < CONH_2 < COOH$, with the carboxyl group being the most preferable. In contrast, the carboxylic acid (**2**) obtained in this study was the weakest inhibitor, and increased activity was observed with the ester (**1**) and amide (**3**) in that order. Further, the sulfonamide (**10**) showed activity comparable to that of the amide (**3**). Introduction of a methyl (**11**) or a cyclopropyl group (**12**) into the sulfonamide moiety of **10** increased the activity. However, the inhibitory activity was 100-fold weaker than that of the known TXA_2 synthetase inhibitor Ozagrel.

Inhibition of PAF-Induced Bronchoconstriction The imidazo[1,2-*b*]pyridazines were evaluated for anti-asthematic activity using PAF-induced bronchoconstriction in guinea pigs (Table 2). Imidazo[1,2-*b*]pyridazines showed inhibitory activity at a dose of 3 mg/kg (i.v.), and the sulfonamide (**10**) was the most potent. No correlation was observed between inhibition of HHT generation *in vitro* and PAF-induced bronchoconstriction *in vivo*. It is known that not only TXA_2 but also several other chemical mediators are involved in PAF-induced bronchoconstriction in guinea pigs.[15] Ozagrel, a strong inhibitor of TXA_2 synthetase, does not inhibit PAF-induced bronchocon-

Table 2. Variation in Inhibition of HHT Generation and Anti-asthmatic Effect with Terminal Functionality of Imidazo[1,2-*b*]pyridazines

Compd. No.	% inhibition		
	In vitro HHT generation		*In vivo* PAF-induced bronchoconstriction
	10^{-4} M	10^{-5} M	3 mg/kg (i.v.)[a]
1	31	0	24
2	11	3	24
3	46	10	59**
10	46	−3	71**
11	49	11	64**
12	62	12	34*
Ozagrel	100	100	7

a) Compounds were given intravenously 2 min before PAF treatment. Significance of differences (Dunnett's test): * $p<0.05$, ** $p<0.01$ (*vs.* control).

Table 3. Variation in Anti-asthmatic Effect with Side-Chain Length of Sulfamoylalkylthioimidazo[1,2-*b*]pyridazines

Compound No.	% inhibition of PAF-induced bronchoconstriction[a]
4	42*
5	71**
10	54**
13	51**
14	49**
15	46*

a) Compounds were given orally at a dose of 30 mg/kg 1 h before PAF treatment. Significance of differences (Dunnett's test): * $p<0.05$, ** $p<0.01$ (*vs.* control).

Table 4. Variation in Anti-asthmatic Effect with Substituted Sulfonamides of Imidazo[1,2-*b*]pyridazine

Compound No.	% inhibition of PAF-induced bronchoconstriction[a]
5	71**
6	41*
7	47*
8	52*
9	5

a) Compounds were given orally at a dose of 30 mg/kg 1 h before PAF treatment. Significance of differences (Dunnett's test): * $p<0.05$, ** $p<0.01$ (*vs.* control).

striction at a dose of 3 mg/kg (i.v., 7%), and compound **10** was more potent than Ozagrel against PAF-induced bronchoconstriction. It seems unlikely that the potent bronchodilatory activity of compound **10** is due to its inhibition of TXA_2 synthetase.

We next examined the optimum side-chain length of the imidazo[1,2-*b*]pyridazin-6-yl-thioalkylsulfonamides by oral administration using PAF-induced bronchoconstriction. As shown in Table 3, these compounds are also orally active. The length of the side chain influenced the potency, and compound **5** with a side chain 3 carbons in length showed the most potent inhibitory effect.

The effects of substituents on the sulfonamide moiety of **5** were examiend by oral administration using PAF-induced bronchoconstriction, and the results are summarized in Table 4. Introduction of a methyl or cyclopropyl group (**6** and **7**) tended to decrease activity. The dimethyl derivative (**8**) retained activity, but was weaker than **5**. The introduction of an aryl group into the sulfonamide (**9**) reduced the activity.

These results suggest that the non-substituted sulfonamide is the most suitable for anti-asthmatic activity.

Anti-asthmatic Activities of Compound 5 The bronchodilating activity of compound **5** on the contraction of guinea pig trachea strips induced by several spasmogens was examined. The results were compared with those obtained with theophylline, which has been used in the treatment of asthma for many years, and are summarized in Table 5. Compound **5** at concentrations of 10^{-5} to 10^{-3} M inhibited the spasmogen-induced contractile response of guinea pig trachea strips in a concentration-dependent manner, and the IC_{50} values were 0.07 (histamine), 0.08 (U-46619) and 0.12 (carbachol) mM. The bronchodilating activity of compound **5** was comparable to that of theophylline.

Table 5. Effects of Compound **5** and Theophylline on the Contraction of Guinea Pig Trachea Strips Induced by Various Spasmogens

Agonist	IC_{50} (mM)	
	Compound **5**	Theophylline
Histamine	0.07	0.06
U-46619	0.08	0.07
Carbachol	0.12	0.44

Table 6. Effects of Compound **5** and Theophylline on Experimental Allergic Asthma Induced by Antigen Inhalation in Guinea Pigs Passively Sensitized with Rabbit Anti-egg Albumin Serum

Compound	No. of animals	Symptom[a]				Mean score	Mortality died/total
		0	I	II	III		
Control	7	—	—	—	**7**	3.0±0.0	7/7
Mepyramine (3 mg/kg)	7	—	**1**	—	**6**	2.7±0.3	4/7
5 + mepyramine (3 mg/kg)	7	**1**	**5**	**1**	—	1.0±0.2**	0/7
Theophylline + mepyramine (3 mg/kg)	7	—	**3**	—	**4**	2.1±0.4	3/7

Compounds were given orally at a dose of 30 mg/kg 1 h before antigen challenge. *a*) 0, no symptoms; I, dyspnea; II, cyanosis; III, collapse or death. ** $p<0.01$ *vs.* control.

Compound **5** inhibited PAF-induced bronchoconstriction in guinea pigs at a dose of 30 mg/kg (*p.o.*, 71%). On the other hand, the inhibition by theophylline at a dose of 30 mg/kg (*p.o.*, 43%) was weaker. Compound **5** (30 mg/kg, *p.o.*) reduced the asthmatic symptoms induced by antigen inhalation in conscious guinea pigs passively sensitized with anti-egg albumin serum (Table 6), whereas theophylline (30 mg/kg, *p.o.*) had no effect on these symptoms. These results suggest that compound **5** is superior to theophylline.

Finally, the mechanism of the anti-asthmatic effect of ω-sulfamoylalkylthioimidazo[1,2-*b*]pyridazines was examined. Compound **5** did not inhibit cyclooxygenase of RBL-1 cells or 5-lipooxygenase activity of rat platelets at 10^{-5} M. Furthermore, compound **5** at 50 μg/ml did not show antagonistic activity at TXA_2, PAF or LTD_4 receptors. These results suggest that the mechanism of the anti-asthmatic activity of compound **5** differs from that of known anti-asthmatic agents.

In conclusion, we obtained ω-sulfamoylalkylthioimidazo[1,2-*b*]pyridazines which have novel structures, representing a new class of bronchodilators, and which may be of significant value in the treatment of asthma and other respiratory diseases.

Experimental

The melting points were determined on a Yanagimoto hot plate micro melting point apparatus and are uncorrected. IR spectra were taken with a Hitachi 215 spectrophotometer. ^{1}H-NMR spectra were recorded with a Varian Gemini-200 (200 MHz) spectrometer using tetramethylsilane as the internal standard. Chromatography was carried out with Merck Silica gel 60 (70—230 mesh).

Ethyl 5-Mercaptopentanoate (17) A solution of 5-mercaptovaleric acid[11] (**16**, 4.3 g) in EtOH (20 ml) containing CH_2Cl_2 (10 ml) and H_2SO_4 (0.4 ml) was refluxed for 5 h. After the solvent was evaporated *in vacuo*, H_2O (50 ml) was added to the residue followed by extraction with CH_2Cl_2 (50 ml). The organic layer was separated, washed with H_2O, dried over

Table 7. IR and ^{1}H-NMR Data for ω-Substituted Alkylthioimidazo[1,2-*b*]pyridazines

Compd. No.	IR (KBr) cm^{-1}	^{1}H-NMR (DMSO-d_6)
1	2668, 1730, 1500, 1469, 1365, 1281, 1179	1.17 (3H, t, J=7 Hz), 1.68—1.77 (4H, m), 2.36 and 3.25 (each 2H, t, J=7 Hz), 4.05 (2H, q, J=7 Hz), 7.65 and 8.23 (each 1H, d, J=9.5 Hz), 8.18 and 8.52 (each 1H, d, J=2 Hz)
2	2920, 1723, 1470, 1147	1.64—1.78 (4H, m), 2.30 and 3.26 (each 2H, t, J=7 Hz), 7.65 and 8.22 (each 1H, d, J=10 Hz), 8.15 and 8.50 (each 1H, d, J=2 Hz)
3	3085, 1674, 1467, 1413	1.65—1.76 (4H, m), 2.12 and 3.24 (each 2H, t, J=7 Hz), 6.75 and 7.30 (each 1H, br s), 7.56 and 8.19 (each 1H, d, J=9.5 Hz), 8.09 and 8.48 (each 1H, d, J=2 Hz)
4	3340, 1535, 1452, 1337, 1279, 1143, 1136	3.63 (4H, br s), 6.69 and 7.79 (each 1H, d, J=10 Hz), 6.82 (2H, br s), 7.64 and 7.99 (each 1H, s)
5	3345, 1605, 1535, 1470, 1335, 1155, 1110	2.15 (2H, m), 3.15 and 3.33 (each 2H, t, J=8 Hz), 6.85 (2H, br s), 7.14 and 7.97 (each 1H, d, J=9.5 Hz), 7.68 and 8.19 (each 1H, s)
6	3040, 1535, 1445, 1325, 1150, 1125	2.31—2.39 (2H, m), 2.83 (3H, d, J=5 Hz), 3.23 and 3.37 (each 2H, t, J=7 Hz), 4.47 (1H, m), 6.84 and 7.77 (each 1H, d, J=10 Hz), 7.67 and 7.86 (each 1H, d, J=7 Hz)
7	3030, 2795, 1535, 1450, 1325, 1294, 1140	0.67—0.78 (4H, m), 2.26—2.40 (2H, m), 2.63—2.67 (1H, m), 3.25—3.40 (4H, m), 4.82 (1H, br s), 6.85 and 7.76 (each 1H, d, J=9 Hz), 7.67 and 7.86 (each 1H, d, J=1 Hz)
8	3040, 1445, 1360, 1320, 1135	2.33—2.40 (2H, m), 2.45 (6H, s), 3.05—3.12 (2H, m), 3.43—3.50 (2H, m), 6.84 and 7.77 (each 1H, d, J=9.5 Hz), 7.67 and 7.86 (each 1H, d, J=1 Hz)
9	1600, 1525, 1495, 1335, 1120	2.20—2.32 (2H, m), 3.13—3.31 (4H, m), 6.81 and 7.73 (each 1H, d, J=10 Hz), 7.20—7.26 (5H, m), 7.62 and 7.83 (each 1H, d, J=1 Hz)
10	3275, 1532, 1453, 1323, 1135	1.83—1.87 (4H, m), 3.04 and 3.23 (each 2H, m), 6.79 (2H, br s), 7.12 and 7.95 (each 1H, d, J=9.5 Hz), 7.67 and 8.18 (each 1H, s)
11	3015, 1513, 1462, 1310, 1150	1.82—1.86 (4H, m), 2.57 (3H, br s), 3.07 and 3.29 (each 3H, t, J=7 Hz), 6.93 (1H, br s), 7.64 and 8.24 (each 1H, d, J=9.5 Hz), 8.16 and 8.53 (each 1H, d, J=2 Hz)
12	3135, 1568, 1470, 1317, 1143, 1112	0.52—0.60 (4H, m), 1.84—1.88 (1H, m), 2.40—2.43 (1H, m), 3.12 and 3.29 (each 2H, t, J=6.5 Hz), 7.44 (1H, br s), 7.64 and 8.24 (each 1H, d, J=9.5 Hz), 8.16 and 8.52 (each 1H, d, J=1 Hz)
13	3450, 1470, 1320, 1310, 1145, 1120	1.55—1.58 (2H, m), 1.73—1.80 (4H, m), 2.99 and 3.25 (each H, t, J=7 Hz), 6.77 (2H, br s), 7.61 and 8.22 (each 1H, d, J=9.5 Hz), 8.15 and 8.51 (each 1H, d, J=1.5 Hz)
14	3310, 1529, 1446, 1329, 1268, 1140	1.43—1.47 and 1.67—1.76 (each 4H, m), 2.92—3.00 and 3.16—3.25 (each 2H, m), 6.74 (2H, br s), 7.10 and 7.94 (each 1H, d, J=9.5 Hz), 7.67 and 8.18 (each 1H, s)
15	3275, 1531, 1449, 1318, 1274, 1123	1.31—1.45 (6H, m), 1.63—1.74 (4H, m), 2.95 and 3.19 (each 2H, t, J=7.5 Hz), 6.72 (2H, br s), 7.10 and 7.94 (each 1H, d, J=9.5 Hz), 7.66 and 8.16 (each 1H, s)

$MgSO_4$ and evaporated *in vacuo* to give 4.94 g of **17** (94%, colorless oil). IR (neat): 2940, 1738, 1449, 1375, 1185 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.5 Hz), 1.66—1.75 (4H, m), 2.32 (2H, t, J=7 Hz), 2.55 (2H, m), 4.14 (2H, q, J=7.5 Hz).

Ethyl 4-(Imidazo[1,2-*b*]pyridazin-6-yl)thiobutanecarboxylate Hydrochloride (1) To a NaOEt solution prepared from Na (0.7 g) and EtOH (100 ml), **17** (4.9 g) and 6-chloroimidazo[1,2-*b*]pyridazine[12] (**18**, 4.69 g) were added, and the mixture was refluxed for 7 h with stirring. After the solvent was evaporated *in vacuo*, H_2O (50 ml) was added to the residue followed by extraction with CH_2Cl_2 (50 ml). The organic layer was separated, washed with H_2O, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (95:5). The eluate was concentrated *in vacuo* to 5 ml, and saturated methanolic HCl (1 ml) was added to the solution. The resulting crystalline precipitate was collected by filtration to give 4.88 g of **1** (51%).

4-(Imidazo[1,2-*b*]pyridazin-6-yl)thiobutanecarboxylic Acid Hydrochloride (2) A mixture of **1** (2.5 g) and a 28% methanolic NaOMe solution (1.34 ml) in MeOH (25 ml) containing H_2O (0.4 ml) was refluxed for 3 h with stirring. After the solvent was evaporated *in vacuo*, the residue was dissolved in H_2O (30 ml). The solution was acidified to pH 4 with concentrated (conc.) HCl, and the resulting precipitate was collected by filtration. The precipitate was dissolved in a small amount of MeOH and saturated methanolic HCl (1 ml) was added to the solution. The crystalline precipitate was collected by filtration to give 2.16 g of **2** (95%).

4-(Imidazo[1,2-*b*]pyridazin-6-yl)thiobutaneamide Hydrochloride (3) A stirred suspension of **2** (2 g) in C_6H_6 (40 ml) was treated with PCl_5 (1.66 g) in portions under ice-cooling. The reaction mixture was stirred for 30 min under ice-cooling and further stirred at room temperature for 1 h. The resulting precipitate was collected by filtration and washed with diisopropyl ether. The precipitate was added in portions to ice-cooled 25% aq. NH_3 (30 ml). This mixture was stirred for 30 min under ice-cooling, then for 2 h at room temperature. The precipitate formed was collected by filtration and dissolved in a small amount of MeOH, then saturated methanolic HCl (1 ml) was added to the solution. The crystalline precipitate was collected by filtration to give 1.14 g of **3** (50%).

Sodium 2-(Imidazo[1,2-*b*]pyridazin-6-yl)thioethanesulfonate (20) A 28% methanolic NaOMe solution (3.72 ml) was added to a suspension of sodium 2-mercaptoethanesulfonate (**19**, 3.04 g) in MeOH (25 ml), followed by stirring at room temperature for 15 min. Then **18** (2.80 g) in MeOH (15 ml) was added, and the whole was refluxed for 5 h with stirring. The resulting precipitate was collected by filtration and washed with diisopropyl ether to give 4.18 g of **20** (81%). mp 263—266 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.79—2.85 and 3.38—3.42 (each 2H, m), 7.55 and 8.18 (each 1H, d, J=10 Hz), 8.08 and 8.15 (each 1H, d, J=2 Hz). *Anal.* Calcd for $C_8H_8N_3O_3S_2Na$: C, 34.16; H, 2.87; N, 14.94. Found: C, 34.20: H, 2.95; N, 14.83.

2-(Imidazo[1,2-*b*]pyridazin-6-yl)thioethanesulfonamide (4) A mixture of **20** (2.8 g) and $POCl_3$ (15 ml) was heated at 120 °C for 3 h with stirring. After the solvent was evaporated *in vacuo*, CH_2Cl_2 (70 ml) was added to the residue followed by filtration to remove insoluble material. The filtrate was evaporated *in vacuo* to give 2.22 g of 2-(imidazo[1,2-*b*]-pyridazin-6-yl)thioethanesulfonyl chloride (colorless oil, 80%), ^{1}H-NMR (DMSO-d_6) δ: 2.79—2.85 and 3.38—3.42 (each 2H, m), 7.75 and 8.28 (each 1H, d, J=10 Hz), 8.26 and 8.63 (each 1H, d, J=2 Hz). A solution of the sulfonyl chloride in THF (50 ml) was bubbled with NH_3 gas for 40 min under ice-cooling with stirring. After the precipitate was filtered off, the filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (95:5) and the product was recrystallized from Et_2O to give 1.34 g of **4** (45%) as colorless crystals.

3-(Imidazo[1,2-*b*]pyridazin-6-yl)thiopropanesulfonamide (5) A solution of 3-chloropropanesulfonyl chloride[13] (**21**, 25 g) in CH_2Cl_2 (200 ml) was bubbled with NH_3 gas for 1 h under ice-cooling with stirring. After the precipitate was filtered off, the filtrate was washed with H_2O, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was recrystallized from *n*-C_6H_{12} to give 21 g of 3-chloropropanesulfonamide[13] (**26**, 91%). mp 64—65 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.95—2.15 (2H, m), 2.58 and 3.07 (each 2H, t, J=7.5 Hz), 6.82 (2H, br s). A mixture of **26** in MeOH (150 ml) and a 2 N KSH–EtOH solution (150 ml) were stirred at 70 °C for 1 h. A 28% methanolic NaOMe solution (11.3 ml) and **18** (8.0 g) were added to the reaction mixture, followed by refluxing for 3 h with stirring.

After the solvent was evaporated *in vacuo*, H_2O was added to the residue. The mixture was neutralized to pH 7 with conc. HCl and then extracted with EtOAc–THF (1 : 1, 100 ml × 3). The extract was washed with H_2O, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (95 : 5) and recrystallized from MeOH to give 11.2 g of **5** (32%).

Compounds **10**, **13**, **14** and **15** were prepared by the same procedures as employed to prepare **5**, using 4-chlorobutanesulfonamide[13] (**31**), 5-chloropentanesulfonamide[13] (**34**) 6-bromohexanesulfonamide (**35**) and 7-bromoheptanesulfonamide (**36**), respectively, instead of **26**. The chemical data for these compounds (**10** and **13**—**15**) are summarized in Tables 1 and 7.

***N*-Methyl 4-(Imidazo[1,2-*b*]pyridazin-6-yl)thiopropanesulfonamide (6)** A 40% methylamine–MeOH solution (0.93 ml) in EtOAc (15 ml) was added dropwise to an ice-cooled mixture of **21** (2.66 g) in EtOAc (15 ml) with stirring. After the mixture was stirred for 0.5 h under ice-cooling, 0.1 N HCl (30 ml) was added to it. The organic layer was separated, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in MeOH (20 ml), and a 2 N KSH–EtOH solution (15 ml) was added. The reaction mixture was stirred at 70 °C for 1 h. A 28% methanolic NaOMe solution (3 ml) and **18** (2.3 g) in MeOH (10 ml) were added to the mixture followed by refluxing for 1.5 h. After the solvent was evaporated *in vacuo*, 0.1 N HCl (30 ml) was added to the residue followed by extraction with EtOAc (30 ml). The organic layer was separated, washed with H_2O, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (95 : 5) and recrystallized from MeOH–Et_2O to give 858 mg of **6** (20%).

Compounds **7** and **8** were prepared by the procedures employed to prepare **6**, using cyclopropylamine and dimethylamine, respectively, instead of methylamine. Starting from 4-chlorobutanesulfonylchloride (**22**) instead of **21**, compounds **11** and **12** were similarly prepared using methylamine and cyclopropylamine, respectively. The chemical data for these compounds are summarized in Tables 1 and 7.

***N*-Phenyl 4-(Imidazo[1,2-*b*]pyridazin-6-yl)thiopropanesulfonamide (9)** A mixture of **21** (2.66 g) and aniline (2.8 g) in C_6H_6 (30 ml) was refluxed for 1 h with stirring. The subsequent procedures were the same as those employed in the synthesis of **6**. Compound **9** (423 mg, 8%) was obtained after recrystallization from MeOH–Et_2O.

Generation of 12(*S*)-Hydroxy-5,8,10-heptadecatrienoic Acid (HHT)[14] Blood was collected in 3.2% sodium citrate (1 ml for 9 ml of blood) from anesthetized rats (Jcl: wistar, male, 12—15 wks) *via* the abdominal aorta. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from the blood by centrifugation at 2000 rpm for 15 s and at 3000 rpm for 5 s at room temperature. PRP was adjusted to 10^6 platelets/μl, and 0.225 ml of PRP was added to 25 μl of arachidonic acid (5 mg/ml) and 2.5 μl of test compound solution. This mixture was allowed to react for 15 min at 37 °C, and then 1.1 ml of EtOH was added. After shaking, the mixture was centrifuged at 2000 rpm for 10 min, 1 ml of supernatant was diluted with 1 ml of water, and HHT was measured by HPLC: column, YMC PAK A-302 (4.6 × 150 mm); mobile phase, MeCN : MeOH : H_2O : AcOH (400 : 200 : 200 : 1); flow rate, 1 ml/min; detection, 240 nm.

The value of % inhibition of HHT generation was determined by means of the following formula.

$$\%\ \text{inhibition of HHT generation} = (\text{peak area of control} - \text{peak area with test compound})/\text{peak area of control}.$$

PAF-Induced Bronchoconstriction in Guinea Pigs The bronchoconstriction induced by PAF (1 μg/kg, i.v.) was measured according to the method of Konzett–Rössler[16] using groups of 5 or 6 hartley guinea pigs (male, body weight about 450 g). The guinea pigs were anesthetized with urethane (1.5 g/kg, i.p.) and fixed in a supine position. After tracheotomy, a tracheal cannula was inserted and connected to a respirator (Harvard apparatus, rodent respirator). The side arm of the tracheal tube was connected to a bronchospasm transducer (Ugobasile 7020). The conditions for respiration were set as a constant volume of 3—7 ml, a rate of 70/min and a constant inflation pressure of 10 cm H_2O. The change in overflow air volume was recorded on a Recti-Hori-8c (San-ei Sokki) through a transducer. After treatment with gallamine triethiodide (1 mg/kg, i.v.) PAF dissolved in saline was injected into the jugular vein through a cannula at a dose of 1 μg/kg, and the induced bronchoconstriction was recorded for 15 min. Compounds were given intravenously 2 min before PAF treatment or orally 1 h before PAF treatment.

Spasmogen-Induced Contraction of Tracheal Strips Male Hartley guinea pigs (about 400 g) were killed by a sharp blow to the neck and exsanguinated. Tracheae were removed and tracheal strips were prepared using the method of Takagi *et al.*[17] A strip was placed in a 10-ml organ bath containing aerated Tyrode's solution at 37 °C. An initial tension of 1 g was loaded and then contractions induced by spasmogens were isotonically recorded on a Rectigraph-8s (Sanei Instruments Co., Ltd.) *via* an FD transducer (SB-IT, Nihon Kohden). Compounds were cumulatively added to the bath after the maximum contractile response to histamine (10^{-5} M), U-46619 (10^{-8} M) or carbachol (10^{-6} M) had been achieved.

Experimental Allergic Asthma Induced by Inhalation of Antigen in Conscious Passively Sensitized Guinea Pigs Experimental allergic asthma was provoked by inhalation of antigen in male guinea pigs passively sensitized by intravenous injection of 0.5 ml of rabbit anti-egg albumin (EA) serum; animals were challenged by inhalation of antigen 1 d after sensitization. The asthmatic symptoms were evaluated according to the method of Yamamura *et al.*[18] The animals were placed individually in a transparent cylindrical box and challenged by exposure to an antigen aerosol (0.5% EA solution) using an ultrasonic nebulizer (Nihon-Kohden, TUR-3000). The severity of symptoms produced by inhalation of the antigen aerosol for 3 min was graded as "0" (no symptoms), "I" (dyspnea), "II" (cyanosis) and "III" (collapse or death). These were converted into numerical scores of 0 to 3 for statistical evaluation. Compounds dissolved in saline were given orally 1 h before the inhalation of antigen.

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