Discovery of New Orally Active Phosphodiesterase (PDE4) Inhibitors

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A series of 4-anilinopyrazolopyridine derivatives were synthesized and biologically evaluated as inhibitors of phosphodiesterase (PDE4). Chemical modification of 3, a structurally new chemical lead that was found in our in-house library, was focused on 1- and 3-substituents. Full details of the discovery of a new orally active chemical lead 5 are presented. Structure–activity relationship data, pharmacological evaluation, and the subtype selectivity study are also presented.

Key words phosphodiesterase (PDE4) inhibitor; subtype selective; orally active; 4-anilinopyrazolopyridine

Phosphodiesterase 4 (PDE4) is an enzyme that is responsible for the inactivation of cyclic adenosine monophosphate (cAMP).[1,2] The anti-inflammatory effects of PDE4 inhibitors have been well established in a variety of animal models.[3] However, clinical use of these inhibitors has not been achieved yet because of dose-limiting side effects such as nausea and vomiting that restrict therapeutic application.[4]

In previous reports, we have described the design and synthesis of bicyclo[3·3·0]octane derivatives[5] and piperidine derivatives,[6] which are interesting new classes of PDE4 inhibitors with therapeutic potential. Structure activity relationship (SAR) studies of PDE4 inhibitors based on the structural features of Ariflo **1**[7] have revealed that its further modification yields a series of PDE4 inhibitors with an improved therapeutic index relative to the classical inhibitor, rolipram **2** (Fig. 1). To create efficient new PDE4 inhibitors with a higher therapeutic potential, discovery and evaluation of a new chemical lead with a completely different structure was considered to provide another approach to chemical modification. Here we report on the discovery of two new orally active pyrazolopyridines **5** and **9** (Fig. 2). SAR data are also presented.

Chemistry

The synthesis of the test compounds listed in Tables 1—3 is outlined in Chart 1.[8—11] Compounds **27a**, **d**—**e**, **g**—**i**, **p**, **x**, and **z** were commercially available. The other 1-methylpyrazoles, **27c** and **27f**, were prepared by condensation of methylhydrazine to the corresponding Michael acceptors **33** and **34**, respectively. 3-Methylpyrazoles, **27j**—**o**, **q**—**w**, and **y** were prepared from **35** and the corresponding hydrazine derivatives. Michael addition of **27a**—**z** to diethyl ethoxymethylenemalonate gave **28a**—**z**, respectively, after which ring closure was accomplished with phosphorus oxychloride under reflux to afford chlorides **29a**—**z**, respectively. Alkaline hydrolysis of **29a**—**z** gave carboxylic acids **30a**—**z**, respectively. Heating of **30a**—**z** with thionyl chloride, followed by treatment with aqueous ammonia, resulted in production of the amides **31a**—**z**, respectively. Replacement of the chloro moiety of **31a**—**z** with appropriate anilines led to the test compounds **5** and **8**—**26**. *N*-Methylation of the 3-methoxyaniline moiety afforded **7**. Compounds **3**, **4**, and **6** were prepared from **31a** according to the procedure described for preparation of **5** from **31a**.

Results and Discussion

A series of pyrazolopyridine derivatives were synthesized and evaluated for inhibition of phosphodiesterase type 4 prepared from U937 cells,[12] which were derived from human monocytes. The results of the assays are expressed as IC_{50} values, *i.e.*, the test compound concentration that achieved 50% inhibition relative to the vehicle. Test compounds were also evaluated for inhibition of LPS-induced production of tumor necrosis factor-α (TNF-α) in rats.[13] The results are expressed as ID_{50} values, *i.e.*, the dose that showed 50% inhibition relative to the vehicle.

During the course of screening of PDE4 inhibitors, compound **3** showed moderate potency in both the *in vitro* and *in vivo* assays. Preliminary SAR data are summarized in Table 1. As illustrated by the SAR study of **3**—**7**, the *meta*-substituted aniline analog **5** exhibited the highest activity in both the *in vitro* and *in vivo* evaluations. The *ortho*-isomer **4** also showed moderate *in vitro* activity, but the *para*-isomer **6** did not show any *in vitro* activity at 0.1 μM. *N*-Methylation of the aniline moiety of **3** afforded **7**, which showed no LPDE4 inhibitory activity at 0.3 μM.

We next focused on further optimization of the 1- and 3-methyl moieties of **5**. First, optimization was started with

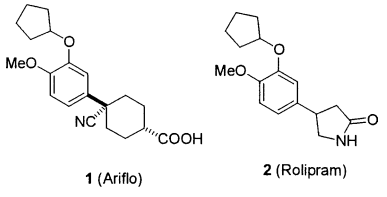

Fig. 1. Structures of Ariflo **1** and Rolipram **2**

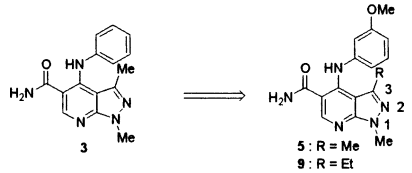

Fig. 2. Discovery of a New Chemical Lead **3** and Identification of Orally Active PDE4 Inhibitors **5** and **9**

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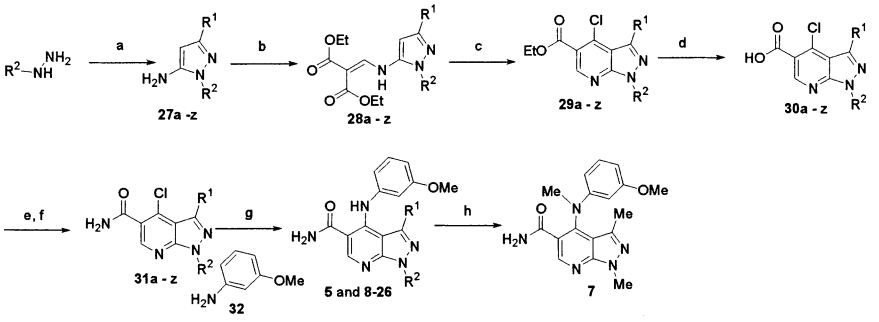


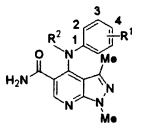
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v
R^1 =	Me	H	Et	*t*Bu	*c*Pr	*c*Pen	Ph	2-Thienyl	4-ClPh	Me	Me	Me	Me	Me	Me	Me	Me	Me	Me	Me	Me	Me
R^2 =	Me	Me	Me	Me	Me	Me	Me	Me	Me	Et	*n*Pr	*n*Bu	*n*Pen	*c*Pen	*c*Hex	Ph	2-ClPh	2-MePh	2-OMePh	3-ClPh	3-MePh	3-OMePh

	w	x	y	z
R^1 =	Me	Me	Me	*c*Pr
R^2 =	4-ClPh	4-MePh	4-OMePh	Ph

Reagents: (a) $EtCOCH_2CN$ **33** or $cPenCOCH_2CN$ **34** or $CH_3(NH_2)C{=}CHCN$ **35**, Et_3N, EtOH, reflux, (then AcONa, AcOH, 90 °C); (b) diethyl ethoxymethylenemalonate, 120 °C; (c) $POCl_3$, 120 °C; (d) KOHaq, dioxane; (e) $SOCl_2$, 80 °C; (f) NH_4OH, THF; (g) 3-methoxyaniline, dioxane, reflux; (h) Ag_2O, MeI, pyridine, toluene, CH_3CN

Chart 1

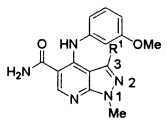
Table 1. Activity Profile of Pyrazolopyridine Derivatives



Compd.	R^1	R^2	LPDE4[a] IC_{50} (μM)	Inhibition of TNF-α[b] ID_{50} (mg/kg, *p.o.*)
3	H	H	0.04	(48%)[c]
4	2-OMe	H	0.08	(68%)[c]
5	3-OMe	H	0.005	2.4
6	4-OMe	H	>0.1	NT[d]
7	3-OMe	Me	>0.3	NT[d]

a) Inhibition of PDE4 prepared from U937 cells (a cell line derived from human monocytes). IC_{50} represent a mean of $n=2$. *b*) ID_{50} for inhibition of LPS-induced TNF-α production in rats ($n=7$) 0.5 h after oral dosing of a test compound. *c*) Inhibition % at 10 mg/kg, *p.o.* *d*) Not tested.

Table 2. Activity Profile of Pyrazolopyridine Derivatives



Compd.	R^1	LPDE4[a] IC_{50} (μM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, *p.o.*)
8	H	0.03	NE[b]
9	Et	0.003	3.1
10	*t*Bu	>0.3	NT[c]
11	*c*Pr	0.002	NE[b]
12	*c*Pen	0.005	2.9
13		0.2	NT[c]
14		0.04	NE[b]
15		0.2	NT[c]

a) See corresponding footnotes from Table 1. *b*) Not effective at 10 mg/kg, *p.o.* *c*) Not tested.

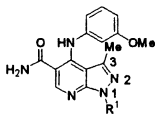
chemical modification of the 3-methyl moiety of **5**, as shown in Table 2.

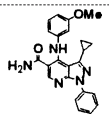
Replacement of the 3-methyl moiety of **5** with hydrogen, ethyl, *t*-butyl, cyclopropyl, or cyclopentyl moieties gave **8**—**12**, respectively, which were nearly 6-fold less potent, nearly equipotent, far less potent, 2-fold more potent, and nearly equipotent as inhibitors of LPDE4, respectively. Among compounds **8**—**12**, it was found that compounds **9** and **12** showed *in vivo* efficacy at ID_{50} values of 3.1 mg/kg, (*p.o.*) and 2.9 mg/kg, (*p.o.*), respectively, while **11** showed weaker *in vivo* activity than **12** despite its higher *in vitro* potency. Replacement of the 3-methyl moiety of **5** with aromatic substituents such as phenyl, 2-thienyl, and *para*-chlorophenyl moieties resulted in **13**—**15**, respectively, showing 40-fold, 8-fold, and 40-fold lower LPDE4 inhibitory activity. Compound **14**, which exhibited the strongest *in vitro* activity among compounds **13**—**15**, did not show any *in vivo* activity at an oral dose of 10 mg/kg, unlike its strong *in vitro* activity.

Next, chemical modification of the 1-methyl moiety of **5** was carried out. Replacement of the 1-methyl moiety of **5** with an alkyl moiety, such as an ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, cyclopentyl, or cyclohexyl moiety, gave **16**—**21**, which were nearly 6-fold less potent, equipotent, 2-fold less potent, 1.6-fold less potent, 2-fold less potent, and 20-fold less potent inhibitors of LPDE4, respectively. Compounds **16**—**20** demonstrated nearly 50% inhibition at an oral dose of 10 mg/kg in the LPS-induced TNF-α production assay in rats, while the 1-cyclohexyl analog **21**, which had the weak-

est *in vitro* activity among **16**—**21**, did not show *in vivo* efficacy at 10 mg/kg. Replacement of the 1-methyl moiety of **5** with an aromatic moiety, such as a phenyl, 1-(*ortho*-substituted)phenyl, 1-(*meta*-substituted)phenyl, or 1-(*para*-substisuted)phenyl moiety, resulted in **22**, **23a**—**c**, **24a**—**c**, and **25a**—**c**, respectively. Among these analogs, the 1-phenyl analog **22** demonstrated equipotent *in vitro* activity to that of **5**, but failed to show *in vivo* efficacy at an oral dose of 10 mg/kg. Introduction of a substituent, such as a chloro, methyl, or methoxy group, into the newly introduced phenyl moiety of **22** was found to be deleterious for both *in vitro* and *in vivo* activity, as illustrated by **23a**—**c**, **24a**—**c**, and **25a**—**c**. Two of these compounds, **24a** and **25b**, were selected for the *in vivo* evaluation. As a result, compound **24a** showed no efficacy at an oral dose of 10 mg/kg, while **25b** showed 49% inhibition of TNF-α production. In a final optimization process, the best substituents for position-1 and poisition-3 were selected and incorporated into one molecule. Simultaneous replacement of the 3-methyl moiety of **5** with a cyclopropyl moiety and the 1-methyl moiety with a 1-phenyl moiety led to **26**, which showed 2-fold weaker LPDE4 inhibitory activity and was much less potent *in vivo* (33% inhibition at an oral dose of 10 mg/kg). As shown in Tables 2 and 3, the above chemical modification of **5** gave compounds **9**, **12**, **17**, and **19**, which exhibited nearly equipotent *in vitro* activity relative to that of **5**. Among them, compounds **9** and **12** were more potent and nearly equipotent *in vivo*, while **17** and **19** showed much weaker *in vivo* activity relative to **5**. In particular, the 1-phenyl analog **22** showed no *in vivo* efficacy at an oral dose of 10 mg/kg despite its equipotent *in vitro* activity relative to that of **5**.

Table 3. Activity Profile of Pyrazolopyridine Derivatives



Compd.	R^1	LPDE4[a] IC_{50} (μM)	Inhibition of TNF-α[a] ID_{50} (mg/kg, *p.o.*)
16	Et	0.03	(54%)[b]
17	*n*Pr	0.004	(56%)[b]
18	*n*Bu	0.01	(47%)[b]
19	*n*Pen	0.008	(58%)[c]
20	*c*Pen	0.01	(48%)[b]
21	*c*Hex	0.1	NE[e]
22		0.004	NE[e]
23a (X=Cl)		0.04	NT[d]
23b (X=Me)		0.08	NT[d]
23c (X=OMe)		0.07	NT[d]
24a (X=Cl)		0.01	NE[e]
24b (X=Me)		0.02	NT[d]
24c (X=OMe)		0.02	NT[d]
25a (X=Cl)		0.05	NT[d]
25b (X=Me)		0.04	(49%)[b]
25c (X=OMe)		0.03	NT[d]
26		0.01	NE[e]

a) See corresponding footnotes from Table 1. *b*) Inhibition % at 10 mg/kg, *p.o.* *c*) Inhibition % at 3 mg/kg, *p.o.* *d*) Not tested. *e*) Not effective at 10 mg/kg, *p.o.*

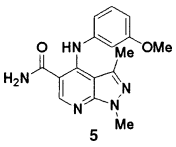
Further biological evaluation of compounds **3**, **5**, and **9**, which were selected based on their oral activity against LPS-induced TNF-α production, was carried out as shown in Table 4. These compounds were evaluated for inhibition of slow reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction in guinea pigs.[14,15] The results are expressed as ID_{50} values, *i.e.*, the dose that gave 50% inhibition relative to the vehicle. These compounds were also evaluated for inhibition of TNF-α production in human whole blood (HWB)[16] to estimate their clinical potential. The results are expressed as IC_{50} values, *i.e.*, the test compound concentration that gave 50% inhibition relative to the vehicle. It was found that the inhibition of bronchoconstriction in actively sensitized guinea pigs was not always consistent with the inhibition of LPS-induced TNF-α production in rats, probably because of differences in pharmacokinetics due to cross-species comparison. Compounds **5** and **9** demonstrated 50% inhibition of SRS-A-induced bronchoconstriction, with ID_{50} values of 6.8 mg/kg, (*p.o.*) and 7.9 mg/kg, (*p.o.*), respectively, while their ID_{50} values for TNF-α production were 2.4 mg/kg and 3.1 mg/kg, respectively. Compound **3** showed 67% inhibition of SRS-A-mediated bronchoconstriction at an oral dose of 30 mg/kg, and it showed 48% inhibition of TNF-α production in rats at an oral dose of 10 mg/kg. Regarding inhibition of gastric emptying in rats,[17] the ID_{50} values of **3** and **5** were higher than that of Ariflo **1**, while their IC_{50} values for LPS-induced TNF-α production assay in HWB were lower than that of **1**. Compounds **3**, **5**, and **9** were further evaluated to access their side effect profile in a ferret emesis model. It was shown that **3** and **5** did not cause emesis at oral

Table 4. Activity Profile of Pyrazolopyridine Derivatives

Compd.	Inhibition of bronchoconstriction[a] ID_{50} (mg/kg, *p.o.*)	Inhibition of TNF-α production[b] ID_{50} (mg/kg, *p.o.*)	Inhibition of gastric emptying[c] ID_{50} (mg/kg, *p.o.*)	Inhibition of TNF-α production in HWB[d] IC_{50} (μM)	Ferret emesis[e] (vomiting/tested) 3 (mg/kg, *p.o.*)	10 (mg/kg, *p.o.*)	30 (mg/kg, *p.o.*)
1 (Ariflo)	4.5	1.7	5.7	18	NT[h]	NT[h]	NT[h]
3	(67%)[g]	(48%)[f]	(5%)[f]	13	NT[h]	NT[h]	0/2
5	6.8	2.4	(23%)[f]	3.0	0/2	0/2	1/2
9	7.9	3.1	(30%)[f]	1.2	NT[h]	NT[h]	NT[h]

a) Inhibition of SRS-A-mediated bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs (n=3—6); OVA challenge 0.15 mg/kg 1 h after oral dosing of a test compound. *b*) See corresponding footnotes from Table 1. *c*) Inhibition of gastric emptying in rats (n=5). *d*) Inhibition of LPS-induced TNF-α production in human whole blood. IC_{50} represent a mean of n=3. *e*) Vomiting test in fasted ferrets. *f*) Inhibition % at 10 mg/kg, *p.o.* *g*) Inhibition % at 30 mg/kg, *p.o.* *h*) Not tested.

Table 5. The Profile against PDEs of **5**



5

Compd.	% Inhibition at 10 μM
PDE1[a]	27
PDE2[b]	24
PDE3[b]	54
PDE5[b]	14
PDE6[c]	10

Source: *a*) bovine heart, *b*) human platelets, *c*) bovineretinal rod outersegments.

doses of up to 30 mg/kg and 10 mg/kg, respectively.

Compounds **3**, **5**, and **9** demonstrated unexpectedly poor bioavailability (BA) despite their relatively good oral activity (the BA of **3**, **5**, and **9** was 8.2%, 1.8%, and 3.1%, respectively). One of the reasons for this result was estimated to be ascribed to their potent inhibitory activity of LPS-induced TNF-α production in rat whole blood. For example, compound **5** exhibited IC_{50} value of 0.18 μM (data is not given in the table) in rat whole blood.

Subtype selectivity of **5** was also investigated, as displayed in Table 6. The inhibitory activity of **5** against PDE1, 2, 3, 5, and 6 at 10 μM was 27%, 24%, 54%, 14% and 10%, respectively.[18—21] Accordingly, compound **5** seems to be a subtype-selective PDE4 inhibitor.

Conclusion

Design and synthesis of new orally active subtype-selective PDE4 inhibitors were achieved by structural optimization of chemical lead **3**, which was found among our in-house library. From the compounds tested, compound **5** demonstrated best results with regard to oral activity and side effects, but it showed unexpectedly poor oral bioavailability. Further chemical modification of **5** to improve its bioavailability may be promising to identify an orally bioavailable PDE4 inhibitor with a higher therapeutic potential.

Experimental

General Procedure Analytical samples were homogeneous as confirmed by thin-layer chromatography (TLC), and yielded spectroscopic data consistent with the assigned structures. All 1H-NMR spectra were obtained with a Varian Gemini-200 or MERCURY-300 spectrometer. The chemical shift values are reported in ppm (δ) and coupling constants (*J*) in Hertz (Hz). Fast atom bombardment (FAB) and electron ionisation (EI) mass spectra were obtained with a JEOL JMS-DX303HF or JMS-700 spectrometer. IR spectra were measured using a Perkin-Elmer FTIR 1760X or JASCO FTIR-430 spectrometer. Column chromatography was carried out using silica gel [Merck silica gel 60 (0.063—0.200 mm), Wako Gel C200, Fuji Silysia FL60D, or Fuji Silysia BW-235]. TLC was also performed on silica gel (Merck TLC plate, silica gel 60 F254).

3-Ethyl-1-methyl-1*H*-pyrazol-5-amine (27c) The following reaction was carried out under argon atmosphere. To a stirred solution of methylhydrazine (3.88 ml, 73.0 mmol) in EtOH (16 ml) was added **33** (8.10 g, 73.0 mmol) under refluxing for 2 h. The reaction mixture was concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (EtOAc) to give **27c** (6.84 g, 54.7 mmol, 75%) as a pale orange powder. ^{1}H-NMR (200 MHz, $CDCl_3$) 5.38 (s, 1H), 3.61 (s, 3H), 3.45 (br s, 2H), 2.52 (q, *J*=7.4 Hz, 2H), 1.20 (t, *J*=7.4 Hz, 3H).

The following compounds **27f** and **27j**—**o** were prepared according to the same procedures as described for the preparation of **27c**.

1-(2-Chlorophenyl)-3-methyl-1*H*-pyrazol-5-amine (27q) The following reaction was carried out under argon atmosphere. To a stirred mixture of 2-chlorophenylhydrazine hydrochloride (5.00 g, 27.9 mmol) and **35** (2.29 g, 27.9 mmol) in EtOH (160 ml) was added Et_3N (3.97 ml, 27.9 mmol) under refluxing for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. To a stirred solution of the residue in AcOH (16 ml) was added AcONa (229 mg, 2.79 mmol). The reaction mixture was stirred at 90 °C for 6.5 h, poured into ice water, neutralized with 5 N NaOH and extracted with a mixture of EtOAc/Et_2O. The organic layer was washed with water and brine, died over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1/1) to give **27q** (4.07 g, 19.6 mmol, 70%) as an ivory powder. ^{1}H-NMR (200 MHz, $CDCl_3$) 7.56—7.36 (m, 4H), 5.48 (s, 1H), 3.58 (br, 2H), 2.24 (s, 3H).

The following compounds **27r**—**w** and **27y** were prepared from the corresponding hydrazine derivatives according to the same procedures as described for the preparation of **27q** from **35** and 2-chlorophenylhydrazine.

Diethyl 2-{[(1,3-Dimethyl-1*H*-pyrazol-5-yl)amino]methylene}malonate (28a) A mixture of 5-amino-1,3-dimethylpyrazole **27a** (20.0 g, 180 mmol) and diethyl ethoxymethylenemalonate (38.9 g, 180 mmol) was stirred at 120 °C for 1 h. The reaction mixture was poured into *n*-hexane and the precipitates were collected by filtration to give **28a** (45.3 g, 161 mmol, 89%) as an ivory powder. ^{1}H-NMR (300 MHz, $CDCl_3$) 11.00 (bd, *J*=12.3 Hz, 1H), 8.14 (d, *J*=12.3 Hz, 1H), 5.86 (s, 1H), 4.32 (q, *J*=7.2 Hz, 2H), 7.24 (q, *J*=7.1 Hz, 2H), 3.74 (s, 3H), 2.23 (s, 3H), 1.38 (t, *J*=7.2 Hz, 3H), 1.32 (t, *J*=7.2 Hz, 3H).

The following compounds **28b**—**z** were prepared from the corresponding pyrazole derivatives **27b**—**z**, respectively according to the same procedures as described for the preparation of **28a** from **27a**.

Ethyl 4-Chloro-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (29a) The following reaction was carried out under argon atmosphere. A mixture of **28a** (28.1 g, 100 mmol) and $POCl_3$ (140 ml, 1.50 mol) was heated at 120 °C under stirring for 18 h. The reaction mixture was poured into ice water and the precipitates were collected by filtration to give **29a** (19.6 g, 77.5 mmol, 77%) as an ivory powder. ^{1}H-NMR (300 MHz, $CDCl_3$) 8.95 (s, 1H), 4.45 (q, *J*=7.2 Hz, 2H), 4.07 (s, 3H), 2.76 (s, 3H), 1.44 (t, *J*=7.2 Hz, 3H).

The following compounds **29b**—**z** were prepared from the corresponding enamine derivatives **28b**—**z**, respectively according to the same procedures as described for the preparation of **29a** from **28a**.

4-Chloro-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid (30a) To a stirred mixture of **29a** (19.6 g, 77.5 mmol) and 85% KOH (50.0 g, 760 mmol) in DME (152 ml) was added H_2O (120 ml). After being stirred at room temperature for 18 h, the reaction mixture was neutralized with 4 N HCl. The precipitates were collected by filtration and washed with H_2O to give **30a** (17.3 g, 76.9 mmol, 99%) as a white powder. ^{1}H-NMR (300 MHz, DMSO-d_6) 8.90 (s, 1H), 4.00 (s, 3H), 3.33 (br s, 1H), 2.67 (s, 3H).

The following compounds **30b**—**z** were prepared from the corresponding ester derivatives **29b**—**z**, respectively according to the same procedures as described for the preparation of **30a** from **29a**.

4-Chloro-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (31a) The following reaction was carried out under argon atmosphere. A mixture of **30a** (2.00 g, 8.89 mmol) and $SOCl_2$ (3.24 ml, 44.5 mmol) was heated at 80 °C under stirring for 1 h, the reaction mixture was concentrated *in vacuo*. The resulting residue was used for the next step without further purification.

To a stirred mixture of 28% NH_4OH (12 ml) and THF (30 ml) was added a solution of the acyl chloride in THF (10 ml) at 0 °C. After being stirred at 0 °C for 10 min, the reaction mixture was concentrated *in vacuo*. The residue was poured into H_2O and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was triturated with Et_2O to give **31a** (1.98 g, 8.84 mmol, 99%) as a white powder. ^{1}H-NMR (300 MHz, DMSO-d_6) 8.52 (s, 1H), 8.02 (br s, 1H), 7.78 (br s, 1H), 3.98 (s, 3H), 2.64 (s, 3H).

The following compounds **31b**—**z** were prepared from the corresponding carboxylic acid derivatives **30b**—**z**, respectively according to the same procedures as described for the preparation of **31a** from **30a**.

4-[(3-Methoxyphenyl)amino]-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (5) To a mixture of **31a** (899 mg, 4.01 mmol) in dioxane (28 ml) was added aniline **32** (1.48 g, 12.0 mmol). After being stirred at 120 °C for 2 h, the reaction mixture was poured into Et_2O and the precipitates were collected by filtration. The resulting powder was triturated with H_2O to give **5** (686 mg, 2.21 mmol, 55%) as white crystals. mp 259—

260 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.97 (s, 1H), 8.74 (s, 1H), 8.21 (br s, 1H), 7.56 (br s, 1H), 7.26—7.17 (m, 1H), 6.75—6.60 (m, 3H), 3.89 (s, 3H), 3.71 (s, 3H), 1.69 (s, 3H); IR (KBr) 3363, 3196, 1676, 1619, 1585, 1521, 1500, 1385; HR-MS (EI) Calcd for $C_{16}H_{17}N_5O_2$ 311.1382, Found 311.1369.

The following compounds **3**—**4**, **6** and **8**—**26** were prepared from the corresponding 4-chloropyrazolopyridine derivatives **31a**—**z**, respectively according to the same procedures as described for the preparation of **5** from **31a**.

4-Anilino-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (3) Yield 63%; Off-white crystals; mp 276—277 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 11.0 (s, 1H), 8.74 (s, 1H), 8.21 (br, 1H), 7.55 (br, 1H), 7.36—7.28 (m, 2H), 7.17—7.07 (m, 3H), 3.88 (s, 3H), 1.59 (s, 3H); IR (KBr) 3406, 3185, 2925, 1677, 1619, 1586, 1521, 1500, 1422, 1384, 1146; HR-MS (FAB) Calcd for $C_{15}H_{16}N_5O$ 282.1355, Found 282.1381.

4-[(2-Methoxyphenyl)amino]-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (4) Yield 46%; White crystals; mp 260—262 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.70 (s, 1H), 8.71 (s, 1H), 8.18 (br s, 1H), 7.49 (br s, 1H), 7.16—7.08 (m, 2H), 6.95 (d, J=7.5 Hz, 1H), 6.88—6.79 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 1.59 (s, 3H); IR (KBr) 3435, 3350, 3205, 1655, 1615, 1588, 1566, 1521, 1486, 1441, 1403, 1382, 1354, 1258, 1114, 1022; HR-MS (EI) Calcd for $C_{16}H_{17}N_5O_2$ 311.1382, Found 311.1365.

4-[(4-Methoxyphenyl)amino]-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (6) Yield 91%; White crystals; mp 269—278 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 11.11 (s, 1H), 8.71 (s, 1H), 8.16 (br s, 1H), 7.49 (br s, 1H), 7.08 (d, J=6.9 Hz, 2H), 6.92 (d, J=6.9 Hz, 2H), 3.85 (s, 3H), 3.75 (s, 3H), 1.54 (s, 3H); IR (KBr) 3401, 3189, 1679, 1618, 1585, 1519, 1493, 1440, 1385, 1254; HR-MS (EI) Calcd for $C_{16}H_{17}N_5O_2$ 311.1382, Found 311.1423.

4-[(3-Methoxyphenyl)amino]-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (8) Yield 67%; White crystals; mp 235—237 °C; ^{1}H-NMR (300 Hz, DMSO-d_6) 11.49 (s, 1H), 8.72 (s, 1H), 8.14 (br s, 1H), 7.46 (br s, 1H), 7.38 (t, J=7.5 Hz, 1H), 7.00—6.85 (m, 3H), 6.67 (s, 1H), 3.89 (s, 3H), 3.75 (s, 3H); IR (KBr) 3384, 3026, 2925, 1653, 1601, 1522, 1490, 1407, 1343, 1283, 1269, 1196, 1160, 1049, 977; HR-MS (EI) Calcd for $C_{15}H_{15}N_5O_2$ 297.1226, Found 297.1229.

3-Ethyl-4-[(3-methoxyphenyl)amino]-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (9) Yield 56%; Ivory crystals; mp 209—211 °C; ^{1}H-NMR (300 Hz, $CDCl_3$) 10.87 (s, 1H), 8.74 (s, 1H), 8.21 (br s, 1H), 7.56 (br s, 1H), 7.17 (t, J=8.1 Hz, 1H), 6.70—6.60 (m, 2H), 6.58 (m, 1H), 3.90 (s, 3H), 3.68 (s, 3H), 1.98 (q, J=7.2 Hz, 2H), 0.93 (t, J=7.2 Hz, 3H); IR (KBr) 3354, 3180, 2939, 1670, 1586, 1522, 1491, 1380, 1266, 1209, 1155, 1039, 975; HR-MS (EI) Calcd for $C_{17}H_{19}N_5O_2$ 325.1539, Found 325.1549.

3-*tert*-Butyl-4-[(3-methoxyphenyl)amino]-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (10) Yield 100%; Ivory crystals; mp 224—228 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 8.62 (s, 1H), 8.14 (s, 1H), 7.73 (br s, 1H), 7.39 (br s, 1H), 7.03 (t, J=8.4 Hz, 1H), 6.43—6.35 (m, 1H), 6.27—6.20 (m, 2H), 3.99 (s, 3H), 3.64 (s, 3H), 1.33 (s, 9H); IR (KBr) 3435, 3333, 3298, 3196, 1667, 1577, 1557, 1502, 1299, 1287, 1154; HR-MS (EI) Calcd for $C_{19}H_{23}N_5O_2$ 353.1852, Found 353.1839.

3-Cyclopropyl-4-[(3-methoxyphenyl)amino]-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (11) Yield 97%; Ivory crystals; mp 234—237 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 11.00 (s, 1H), 8.74 (s, 1H), 8.22 (br s, 1H), 7.57 (br s, 1H), 7.19 (t, J=7.8 Hz, 1H), 6.69—6.60 (m, 3H), 3.87 (s, 3H), 3.68 (s, 3H), 1.26—1.14 (m, 1H), 0.70—0.60 (m, 2H), 0.44—0.34 (m, 2H); IR (KBr) 3399, 3191, 1664, 1617, 1595, 1565, 1522, 1490, 1397, 1264, 1156; HR-MS (EI) Calcd for $C_{18}H_{19}N_5O_2$ 337.1539, Found 337.1526.

3-Cyclopentyl-4-[(3-methoxyphenyl)amino]-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (12) Yield 75%; White crystals; mp 183—185 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.82 (s, 1H), 8.74 (s, 1H), 8.21 (br s, 1H), 7.56 (br s, 1H), 7.17 (d, J=8.1 Hz, 1H), 6.70—6.61 (m, 2H), 6.56 (d, J=8.1 Hz, 1H), 3.91 (s, 3H), 3.69 (s, 3H), 2.25—2.10 (m, 1H), 1.65—1.43 (m, 6H), 1.35—1.15 (m, 2H); IR (KBr) 3350, 3175, 1668, 1586, 1561, 1519, 1490, 1455, 1398, 1318, 1266, 1156; HR-MS (EI) Calcd for $C_{20}H_{23}N_5O_2$ 365.1852, Found 365.1845.

4-[(3-Methoxyphenyl)amino]-1-methyl-3-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (13) Yield 99%; White crystals; mp 209—213 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) 10.47 (s, 1H), 8.64 (s, 1H), 7.35—7.31 (m, 2H), 7.11—7.06 (m, 3H), 6.75 (t, J=8.1 Hz, 1H), 6.37—6.32 (m, 1H), 6.26—6.19 (m, 2H), 5.90—5.75 (br s, 2H), 4.14 (s, 3H), 3.59 (s, 3H); IR (KBr) 3351, 3194, 1677, 1587, 1519, 1494, 1470, 1400, 1357, 1309, 1268, 1241, 1156, 1036, 974; HR-MS (EI) Calcd for $C_{21}H_{19}N_5O_2$ 373.1539, Found 373.1552.

4-[(3-Methoxyphenyl)amino]-1-methyl-3-(2-thienyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (14) Yield 79%; Yellow crystals; mp 183—189 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 11.12 (1H), 8.84 (s, 1H), 8.29 (br s, 1H), 7.66 (br s, 1H), 7.25 (dd, J=5.1, 0.9 Hz, 1H), 6.92 (dd, J=3.6, 0.9 Hz, 1H), 6.81 (t, J=7.5 Hz, 1H), 6.66 (dd, J=5.1, 3.6 Hz, 1H), 6.37—6.24 (m, 3H), 4.04 (s, 3H), 3.55 (s, 3H); IR (KBr) 3344, 3200, 1659, 1587, 1520, 1494, 1401, 1270, 1155; HR-MS (EI) Calcd for $C_{19}H_{17}N_5O_2S$ 379.1103, Found 379.1111.

3-(4-Chlorophenyl)-4-[(3-methoxyphenyl)amino]-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15) Yield 74%; Yellow crystals; mp 186—189 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 11.22 (s, 1H), 8.85 (s, 1H), 8.30 (br s, 1H), 7.65 (br s, 1H), 7.26 (d, J=8.4 Hz, 2H), 7.10 (d, J=8.4 Hz, 2H), 6.80—6.70 (m, 1H), 6.28—6.20 (m, 3H), 4.04 (s, 3H), 3.54 (s, 3H); IR (KBr) 3349, 3150, 1671, 1586, 1560, 1508, 1494, 1399, 1355, 1316, 1266, 1154; HR-MS (EI) Calcd for $C_{21}H_{18}ClN_5O_2$ 407.1149, Found 407.1146.

1-Ethyl-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (16) Yield 8% in 3 steps; Gray crystals; mp 196—198 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.93 (s, 1H), 8.71 (s, 1H), 8.22—8.15 (br s, 1H), 7.60—7.50 (br s, 1H), 7.20 (dd, J=8.7, 7.8 Hz, 1H), 6.70—6.67 (m, 2H), 6.63 (d, J=8.7 Hz, 1H), 4.31 (q, J=7.2 Hz, 2H), 3.70 (s, 3H), 1.69 (s, 3H), 1.35 (t, J=7.2 Hz, 1H); IR (KBr) 3424, 3195, 2926, 1655, 1585, 1515, 1491, 1440, 1408, 1383, 1356, 1299, 1247, 1156, 1039, 948; HR-MS (EI) Calcd for $C_{17}H_{19}N_5O_2$ 325.1539, Found 325.1547.

4-[(3-Methoxyphenyl)amino]-3-methyl-1-propyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (17) Yield 28% in 2 steps; Ivory crystals; mp 169—171 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.94 (s, 1H), 8.71 (s, 1H), 7.18 (br s, 1H), 7.55 (br s, 1H), 7.23—7.17 (m, 1H), 6.71—6.62 (m, 3H), 4.23 (t, J=6.6 Hz, 2H), 3.69 (s, 3H), 1.80 (tq, J=6.6, 7.2 Hz, 2H), 1.69 (s, 3H), 0.81 (t, J=7.2 Hz, 3H); IR (KBr) 3343, 3183, 2961, 1661, 1586, 1561, 1512, 1437, 1407, 1382, 1354, 1285, 1237, 1156, 1040, 972; HR-MS (EI) Calcd for $C_{18}H_{21}N_5O_2$ 339.1695, Found 339.1701.

1-Butyl-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (18) Yield 23% in 2 steps; Pale yellow crystals; mp 157—159 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.94 (s, 1H), 8.71 (s, 1H), 8.19 (br s, 1H), 7.55 (br s, 1H), 7.23—7.17 (m, 1H), 6.73—6.60 (m, 3H), 4.28 (t, J=7.0 Hz, 2H), 3.69 (s, 3H), 1.77 (quint., J=7.0 Hz, 2H), 1.68 (s, 3H), 1.20 (tq, J=7.0, 7.5 Hz, 2H), 0.87 (t, J=7.5 Hz, 3H); IR (KBr) 3620, 3350, 3185, 2958, 1933, 1871, 2767, 2549, 1737, 1669, 1586, 1513, 1489, 1464, 1438, 1407, 1382, 1355, 1287, 1254, 1232, 1194, 1151, 1118, 1079, 1038; HR-MS (EI) Calcd for $C_{19}H_{23}N_5O_2$ 353.1852, Found 353.1861.

4-[(3-Methoxyphenyl)amino]-3-methyl-1-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (19) Yield 75%; Pale gray crystals; mp 148—150 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.92 (s, 1H), 8.70 (s, 1H), 8.18 (br, 1H), 7.54 (br, 1H), 7.23—7.17 (m, 1H), 6.71—6.67 (m, 2H), 6.65—6.61 (m, 1H), 4.26 (t, J=7.2 Hz, 2H), 3.69 (s, 3H), 1.84—1.73 (m, 2H), 1.69 (s, 3H), 1.36—1.13 (m, 4H), 0.82 (t, J=7.1 Hz, 3H); IR (KBr) 3385, 3195, 2939, 1669, 1615, 1590, 1561, 1514, 1489, 1440, 1409, 1383, 1355, 1280, 1151, 1038; HR-MS (EI) Calcd for $C_{20}H_{25}N_5O_2$ 367.2008, Found 367.2014.

1-Cyclopentyl-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (20) Yield 73%; Ivory crystals; mp 153—157 °C; ^{1}H-NMR (300 Hz, DMSO-d_6) 10.91 (s, 1H), 8.70 (s, 1H), 8.18 (br s, 1H), 7.54 (br s, 1H), 7.19 (t, J=8.4 Hz, 1H), 6.75—6.60 (m, 3H), 5.23 (quintet, J=7.4 Hz, 1H), 3.70 (s, 3H), 2.10—1.75 (m, 6H), 1.70 (s, 3H), 1.75—1.60 (m, 2H); IR (KBr) 3341, 3184, 2957, 2870, 1657, 1586, 1559, 1509, 1441, 1382, 1354, 1283, 1257, 1196, 1154, 1045, 916; HR-MS (EI) Calcd for $C_{20}H_{23}N_5O_2$ 365.1852, Found 365.1862.

1-Cyclohexyl-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (21) Yield quant; White amorphous solid; ^{1}H-NMR (300 MHz, $CDCl_3$) 10.54 (s, 1H), 8.51 (s, 1H), 7.22—7.15 (m, 1H), 6.73—6.66 (m, 3H), 5.90—5.70 (br s, 2H), 4.78—4.68 (m, 1H), 3.76 (s, 3H), 2.05—1.85 (m, 6H), 1.80 (s, 3H), 1.75—1.20 (m, 4H); IR (KBr) 3346, 3193, 2933, 2856, 1656, 1587, 1509, 1454, 1383, 1357, 1308, 1261, 1235, 1155, 1046, 971; HR-MS (EI) Calcd for $C_{21}H_{25}N_5O_2$ 379.2008, Found 379.2004.

4-[(3-Methoxyphenyl)amino]-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (22) Yield 62%; White crystals; mp 189—193 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) 10.56 (s, 1H), 8.60 (s, 1H), 8.12—8.09 (m, 2H), 7.53—7.48 (m, 2H), 7.26—7.18 (m, 2H), 6.78—6.69 (m, 3H), 5.90—5.70 (br s, 2H), 3.77 (s, 3H), 1.77 (s, 3H); IR (KBr) 3396, 3195, 1659, 1593, 1561, 1508, 1490, 1465, 1439, 1404, 1384, 1361, 1308, 1229, 1197, 1151, 1127, 1037, 945; HR-MS (EI) Calcd for $C_{21}H_{19}N_5O_2$ 373.1539, Found 373.1535.

1-(2-Chlorophenyl)-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (23a) Yield 78%; Beige crystals; mp 230—231 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.93 (br, 1H), 8.67 (s, 1H), 8.23 (br, 1H), 7.70 (dd, J=7.7, 1.8 Hz, 1H), 7.66—7.49 (m, 4H), 7.25 (dd, J=7.7, 7.7 Hz, 1H), 6.78—6.67 (m, 3H), 3.72 (s, 3H), 1.78 (s, 3H); IR (KBr) 3331, 3196, 1651, 1592, 1489, 1384, 1359, 1300, 1264, 1225, 1195, 1151, 1105, 1036, 956; HR-MS (EI) Calcd for $C_{21}H_{18}ClN_5O_2$ 407.1149, Found 407.1172.

4-[(3-Methoxyphenyl)amino]-3-methyl-1-(2-methylphenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (23b) Yield 71%; Ivory crystals; mp 133—136 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.95 (s, 1H), 8.67 (s, 1H), 8.21 (br s, 1H), 7.59 (br s, 1H), 7.45—7.30 (m, 4H), 7.24 (t, J=8.1 Hz, 1H), 6.80—6.60 (m, 3H), 3.72 (s, 3H), 2.05 (s, 3H), 1.78 (s, 3H); IR (KBr) 3188, 1654, 1587, 1560, 1509, 1302, 1296, 1151; HR-MS (EI) Calcd for $C_{22}H_{21}N_5O_2$ 387.1695, Found 387.1698.

1-(2-Methoxyphenyl)-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (23c) Yield 79% in 3 steps; White crystals; mp 205—206 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.87 (s, 1H), 8.63 (s, 1H), 8.25—8.15 (br s, 1H), 7.62—7.53 (br s, 1H), 7.52—7.46 (m, 1H), 7.38—7.34 (m, 1H), 7.27—7.21 (m, 2H), 7.10—7.05 (m, 1H), 6.74—6.66 (m, 3H), 3.73 (s, 3H), 3.70 (s, 3H), 1.77 (s, 3H); IR (KBr) 3431, 3176, 1668, 1584, 1561, 1516, 1467, 1438, 1406, 1383, 1357, 1298, 1278, 1238, 1152, 1043, 1021, 951; HR-MS (EI) Calcd for $C_{22}H_{21}N_5O_3$ 403.1644, Found 403.1663.

1-(3-Chlorophenyl)-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (24a) Yield 99%; White crystals; mp 93—95 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.95 (br, 1H), 8.84 (s, 1H), 8.39 (t, J=2.0 Hz, 1H), 8.28 (br, 1H), 8.22—8.18 (m, 1H), 7.70 (br, 1H), 7.55 (dd, J=8.1, 8.1 Hz, 1H), 7.38—7.33 (m, 1H), 7.22 (t, J=8.0 Hz, 1H), 6.79—6.68 (m, 3H), 3.72 (s, 3H), 1.79 (s, 3H); IR (KBr) 3413, 3195, 1710, 1685, 1589, 1561, 1508, 1484, 1435, 1407, 1385, 1291, 1152, 1038; HR-MS (EI) Calcd for $C_{21}H_{18}C_{11}N_5O_2$ 407.1149, Found 407.1134.

4-[(3-Methoxyphenyl)amino]-3-methyl-1-(3-methylphenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (24b) Yield 92%; Brown crystals; mp 133—140 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.92 (s, 1H), 8.81 (s, 1H), 8.26 (br s, 1H), 8.00—7.95 (m, 2H), 7.67 (br s, 1H), 7.40 (t, J=7.8 Hz, 1H), 7.21 (t, J=7.8 Hz, 1H), 7.12 (d, J=7.8 Hz, 1H), 6.80—6.65 (m, 3H), 3.71 (s, 3H), 2.39 (s, 3H), 1.80 (s, 3H); IR (KBr) 3429, 2923, 1731, 1652, 1588, 1559, 1506, 1489, 1402, 1383,1358, 1298, 1223, 1197, 1150, 1044; HR-MS (EI) Calcd for $C_{22}H_{21}N_5O_2$ 387.1695, Found 387.1697.

1-(3-Methoxyphenyl)-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (24c) Yield 28%; Brown crystals; mp 88—89 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.92 (s, 1H), 8.82 (s, 1H), 8.35—8.20 (br s, 1H), 7.82—7.79 (m, 2H), 7.73—7.60 (br s, 1H), 7.42 (t, J=8.1 Hz, 1H), 7.22 (t, J=8.1 Hz, 1H), 6.89—6.85 (m, 1H), 6.75—6.68 (m, 3H), 3.82 (s, 3H), 3.71 (s, 3H), 1.80 (s, 3H); IR (KBr) 3424, 2925, 1656, 1594, 1561, 1507, 1491, 1404, 1385, 1359, 1285, 1225, 1151, 1043; HR-MS (EI) Calcd for $C_{22}H_{21}N_5O_3$ 403.1644, Found 403.1638.

1-(4-Chlorophenyl)-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (25a) Yield 42%; Beige crystals; mp 180—181 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.94 (br, 1H), 8.81 (s, 1H), 8.29 (br, 1H), 8.26 (d, J=9.0 Hz, 2H), 7.69 (br, 1H), 7.58 (d, J=9.0 Hz, 2H), 7.22 (dd, J=8.1, 8.1 Hz, 1H), 6.77—6.67 (m, 3H), 3.71 (s, 3H), 1.79 (s, 3H); IR (KBr) 3478, 3178, 1665, 1595, 1561, 1507, 1491, 1464, 1404, 1386, 1303, 1259, 1221, 1153, 1091, 1037, 952; HR-MS (EI) Calcd for $C_{21}H_{18}ClN_5O_2$ 407.1149, Found 407.1156.

4-[(3-Methoxyphenyl)amino]-3-methyl-1-(4-methylphenyl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (25b) Yield 81%; White crystals; mp 131—133 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.91 (s, 1H), 8.79 (s, 1H), 8.26 (br s, 1H), 8.03 (d, J=8.1 Hz, 2H), 7.66 (br s, 1H), 7.31 (d, J=8.1 Hz, 2H), 7.21 (t, J=8.4 Hz, 1H), 6.75—6.67 (m, 3H), 3.71 (s, 3H), 2.35 (s, 3H), 1.79 (s, 3H); IR (KBr) 3364, 2925, 1655, 1590, 1559, 1514, 1491, 1440, 1404, 1383, 1358, 1305, 1150, 1044, 943; HR-MS (EI) Calcd for $C_{22}H_{21}N_5O_2$ 387.1695, Found 387.1714.

1-(4-Methoxyphenyl)-4-[(3-methoxyphenyl)amino]-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (25c) Yield 73% in 3 steps; Gray crystals; mp 205—207 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) 10.93 (s, 1H), 8.78 (s, 1H), 8.30—8.20 (br s, 1H), 8.00 (d, J=9.0 Hz, 2H), 7.67—7.58 (br s, 1H), 7.22 (t, J=8.1 Hz, 1H), 7.08 (d, J=9.0 Hz, 2H), 6.75—6.67 (m, 3H), 3.80 (s, 3H), 3.71 (s, 3H), 1.79 (s, 3H); IR (KBr) 3464, 3351, 2837, 1660, 1594, 1561, 1516, 1491, 1408, 1385, 1297, 1242, 1171, 1153, 1038, 952; HR-MS (EI) Calcd for $C_{22}H_{21}N_5O_3$ 403.1644, Found 403.1638.

3-Cyclopropyl-4-[(3-methoxyphenyl)amino]-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (26) Yield 82%; White crystals; mp 159—163 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) 10.50 (s, 1H), 8.61 (s, 1H), 8.12 (t, J=7.5 Hz, 2H), 7.49 (t, J=8.1 Hz, 2H), 7.31—7.17 (m, 2H), 6.78—6.62 (m, 3H), 6.00—5.60 (br s, 2H), 1.37—1.25 (m, 1H), 0.90—0.81 (m, 2H), 0.53—0.48 (m, 2H); IR (KBr) 3376, 1655, 1593, 1559, 1506, 1397, 1306, 1151, 1051, 954; HR-MS (EI) Calcd for $C_{23}H_{21}N_5O_2$ 399.1695, Found 399.1717.

4-[(3-Methoxyphenyl)(methyl)amino]-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (7) The following reaction was carried out under argon atmosphere. To a stirred mixture of **5** (100 mg, 0.322 mmol), toluene (10 ml) and MeCN (5 ml) were added Ag_2O (112 mg, 0.485 mmol) and MeI (568 mg, 4.00 mmol). After being stirred at 0 °C for 15 h, the precipitates were removed by filtration through a pad of celite, and washed with toluene. The filtrate was concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 50/1) to give **7** (98.4 mg, 0.303 mmol, 94%) as a yellow amorphous solid. ^{1}H-NMR (300 MHz, DMSO-d_6) 8.64 (s, 1H), 7.62 (br s, 1H), 7.43 (br s, 1H), 7.03 (t, J=8.1 Hz, 1H), 6.37—6.33 (m, 1H), 6.17—6.10 (m, 2H), 3.96 (s, 3H), 3.64 (s, 3H), 3.27 (s, 3H), 2.02 (s, 3H); IR (KBr) 3463, 3385, 3142, 1678, 1580, 1491, 1378, 1271, 1214, 1169, 1118, 1052, 959; HR-MS (EI) Calcd for $C_{17}H_{19}N_5O_2$ 325.1539, Found 325.1534.

Assay of Human PDE4 Activity The method of Reeves *et al.*[22] was modified to isolate phosphodiesterase type 4 isozyme (PDE4). The enzyme was prepared from U937 cells derived from human monocytes, and was stored at −20 °C after preparation. Measurement of PDE4 activity was performed using this stored enzyme after it was diluted with distilled water containing bovine serum albumin. The substrate solution was prepared by adding ^{3}H-cAMP (300000 dpm (5000 Bq)/assay) and 100 μmol/l cAMP solution to 100 mmol/l Tris–HCl (pH 8.0) containing 5 mmol/l ethylene glycol-bis (β-aminoethyl ether) and *N*,*N*,*N*′,*N*′-tetraacetic acid. The substrate solution was mixed with the enzyme solution containing a test compound dissolved in dimethylsulfoxide (DMSO), and incubation was done for 30 min at 30 °C. Assays were performed in duplicate at three to four different concentrations of each test compound, and the IC_{50} values were determined.

Inhibition of LPS-Induced Plasma TNF-α Production in Rats Male Crj:CD(SD)IGS rats aged 6 weeks (n=7) were fasted overnight, and the test compounds (0.01—0.1 mg/10 ml/kg) were administered orally at 1 h before intravenous injection of 1 μg/kg of LPS (*Escherichia coli* Serotype 055 B5). The plasma TNF-α level was measured with a commercially available ELISA kit (R&D Systems) at 90 min after LPS challenge. The percent inhibition (the dosage required to inhibit plasma TNF-α production by 50%) was determined by the following formula:

$$\% \text{ inhibition} = 100 - (C\text{-}S)/(L\text{-}S) \times 100$$

C: Plasma TNF-α concentration in LPS-treated animals pretreated with a test compound. L: Plasma TNF-α concentration in LPS-treated animals pretreated with saline. S: Plasma TNF-α concentration in saline-treated animals pretreated with saline.

SRS-A Mediated Bronchoconstriction in Guinea Pigs Male Hartley guinea pigs aged 7 weeks (n=5) were actively sensitized by intraperitoneal administration of 1 mg of ovalbumin (OVA) containing 5×10^9 killed *Bordetella pertussis* organisms on day 0. On day 14, the bronchoconstrictor response was measured using a modified version of the method of Konzett and Rüssler. Bronchoconstriction was induced by an intravenous injection of OVA (0.15—0.5 mg/kg). Sensitized animals were treated with both a cyclooxygenase inhibitor (indomethacin at 5 mg/kg i.v., 3 min before OVA) and an antihistamine (pyrilamine at 1 mg/kg i.v., 1 min before OVA) to ensure that endogenous SRS-A was solely responsible for bronchoconstriction. Test compounds were administered orally at 1 h before antigen challenge. Bronchoconstrictor response was measured for 15 min and the result was represented as the area under the curve (*AUC* 0—15 min).

Gastric Emptying in Rats Male Sprague-Dawley rats were fasted overnight and were orally administered test compounds or 0.5 w/v% methylcellulose (10 ml/kg). In addition, 0.05 mg/ml of phenol red solution was orally administered in a volume of 1.5 ml at twenty minutes after dosing with the test compounds. Forty minutes after administration of the test compounds, both the cardia and pylorus of the stomach were ligated under anesthesia with sodium pentobarbital (75 mg/kg, i.p.), and then the stomach was isolated without leakage of phenol red. The stomach was cut open and the phenol red solution was drained into a beaker containing 100 ml of 0.1 N NaOH. Part of the solution was filtrated (pore size: 0.45 μm) and the absorbance at 546 nm was measured to determine the amount of dye remaining in the stomach. Then the gastric emptying rate was calculated by the following formula:

$$\text{gastric emptying rate} = 100 \times (0.75 - \text{concentration of dye in the stomach})/0.75$$

A value of 0.75 μg/ml was equal to a concentration of 0.05 mg/ml, which was achieved by adding 1.5 ml of phenol red to 100 ml of 0.1 N NaOH. The 50% inhibition rate for gastric emptying by the test compounds was calculated by defining gastric emptying after vehicle administration as 100%.

Inhibitory Activity on LPS-Induced TNF-α Production in Human Whole Blood Under the supervision of a physician, blood was collected into a heparinized tube (final concentration: 10 U/ml heparin sodium) from a forearm vein in three healthy male donors. A solution of the test compound (10 μl) dissolved in DMSO was added to 180 μl of whole blood, and the mixture was pre-incubated for 30 min at 37 °C. Then 10 μl of 2 μg/ml of LPS was added and incubated for 6 h at 37 °C, after which the plasma TNF-α concentration was measured with a human TNF-α ELISA kit (DIACLONE). Assays were performed in duplicate at three to four different concentrations of each test compound, and the IC_{50} values were determined.

Ferret Emetic Study Male ferrets (weighting about 1.2 kg) were fasted overnight and test compounds were administered orally. Their behavior was observed throughout a 1 h period after gavage. Results were expressed as the number of animals that vomited relative to the animals tested.

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