4-(3-Chloro-4-methoxybenzyl)aminophthalazines: Synthesis and Inhibitory Activity toward Phosphodiesterase 5

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We synthesized various 4-(3-chloro-4-methoxybenzyl)aminophthalazines substituted at the 1- and 6-positions and evaluated their inhibitory activity toward phosphodiesterase 5 (PDE5) and their vasorelaxant activity in isolated porcine coronary arteries precontracted with prostaglandin F2α (10^{-5} M). The preferred substituents at the 1-position of the phthalazine were 4-hydroxypiperidino, 4-hydroxymethylpiperidino, 4-(2-hydroxyethyl)piperidino, and 4-oxopiperidino. Among these compounds, [4-(3-chloro-4-methoxybenzyl)amino-1-(4-hydroxy)piperidino]-6-phthalazinecarbonitrile monohydrochloride (**13**) exhibited potent PDE5 inhibitory activity (IC_{50} = 0.56 nM) with >1700-fold high selectivity over other PDE isozymes (PDE1−4). Compound **13** exhibited the most potent vasorelaxant action (EC_{50} = 13 nM) in this series of compounds. Compound **13** reduced mean pulmonary arterial pressure by 29.9 ± 3.1% when administered intravenously at 30 μg/kg to the chronically hypoxic rats and had an apparent oral bioavailability of about 19.5% in rats and was selected for further biological evaluation.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) have been classified into at least seven distinct isozyme families,[1] of which one, cGMP-PDE (PDE5), specifically hydrolyzes cGMP. Zaprinast[2] and MY-5445[3] are classical PDE inhibitors possessing a moderate selectivity for PDE5. Recently, selective and potent PDE5 inhibitors such as UK-92480,[4] 1,3-dimethyl-6-(2-propoxy-5-methanesulfonylamidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (DMPPO),[5] 1-arylnaphthalene lignans,[6] and quinazoline derivatives[7−9] (Figure 1) have been reported to show vasorelaxing, antihypertensive, and penile-erection-promoting activities.

In the previous paper we reported that some 4-benzylamino-1-chloro-6-substituted-phthalazines (**2**−**4**) potently inhibited PDE5 and proposed a pharmacophore for PDE5 inhibition.[10] In the present study, to find more potent PDE5 inhibitors we synthesized a variety of 4-(3-chloro-4-methoxybenzyl)aminophthalazines substituted at the 1- and 6-positions, via the chlorides (**2**−**6**). We evaluated their inhibitory activities toward PDE5. Some of these compounds were also examined for relaxant activity in isolated porcine coronary arteries precontracted with prostaglandin F2α (PGF2α), in comparison with E4021.

Chemistry

The general synthetic procedure was as follows (Scheme 1). 1,4,6-Trisubstituted phthalazines were synthesized by the reaction of 4-benzylamino-1-chloro-6-substituted-phthalazine (**2**−**5**) and amine in the presence of diisopropylethylamine.

The syntheses of **2**−**5** were reported in the previous paper.[10] In the case of 6,7-dichlorophthalazine (**6**),

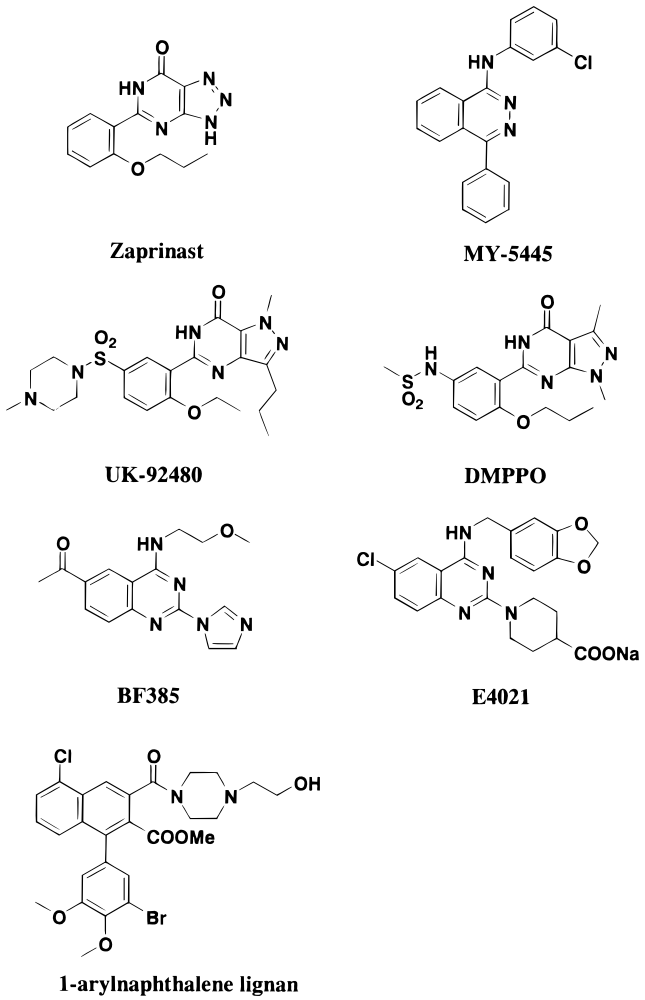

Figure 1.

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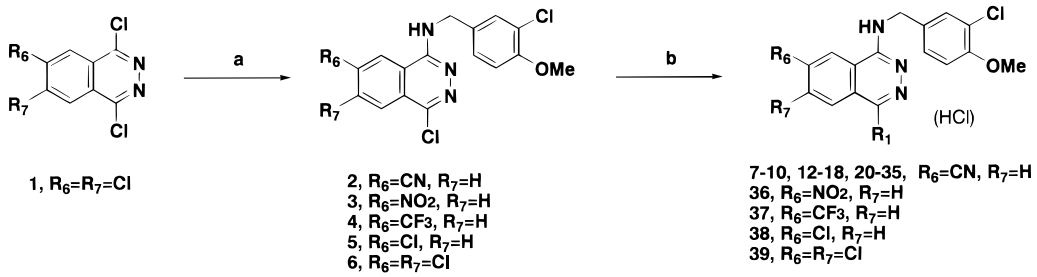
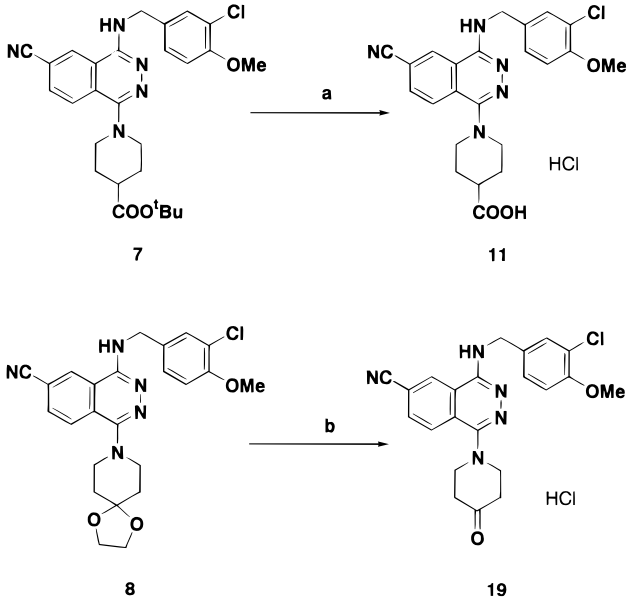
Scheme 1[a]



[a] Reagents: (a) 3-chloro-4-methoxybenzylamine hydrochloride, Et_3N, 2-propanol; (b) amine, iPr_2EtN, NMP (then HCl).

Scheme 2[a]



[a] Reagents: (a) HCOOH then HCl; (b) TFA then HCl.

a known 1,4,6,7-tetrachlorophthalazine (**1**)[11] and 3-chloro-4-methoxybenzylamine hydrochloride were refluxed in 2-propanol in the presence of triethylamine.

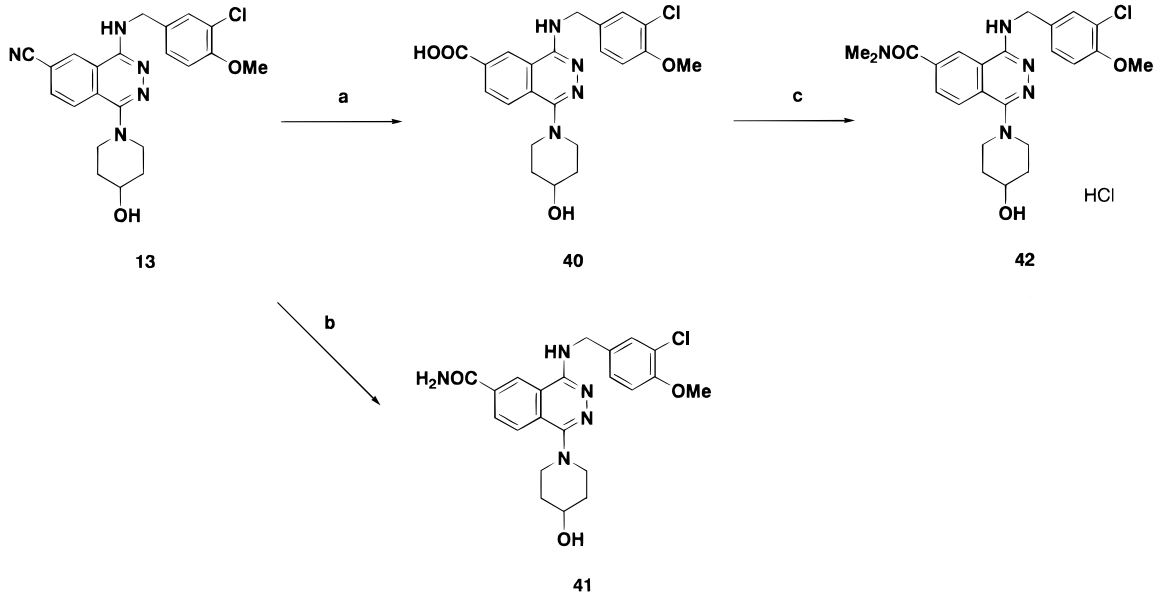
Compounds **11** and **19** were synthesized via the protected *tert*-butyl ester (**7**) and ethyleneketal (**8**), respectively (Scheme 2). Compounds **40** and **41** were synthesized from a cyano compound (**13**). Compound **42** was synthesized by the coupling of **40** and dimethylamine (Scheme 3).

Pharmacological Results and Discussion

The biological methods used are described in the Experimental Section. E4021 or sildenafil citrate were used as the active control in each PDE5-inhibitory activity assay.

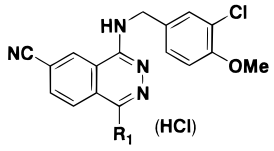
The pharmacological results obtained for the 4-[(3-chloro-4-methoxybenzyl)amino]-6-phthalazinecarbonitrile derivatives are listed in Table 1. 1-Piperidine derivatives (**9**−**20**) and thiomorpholino derivatives (**21**−**23**) generally exhibited more potent PDE5-inhibitory activity than **2**. The carboxylic acid (**11**) and sulfonamide (**20**), which have an acidic proton, were weaker inhibitors than the unsubstituted piperidine (**9**). The effects of neutral and relatively small substituents at the 4-position of piperidine and 1-position of thiomorpholine, i.e., unsubstituted (**9**), hydroxyl (**13**), keto (**19**), sulfide (**21**), S-oxide (**22**), and S-dioxide (**23**), were almost equivalent as regards PDE5-inhibitory activity. Some of the hydroxy compounds (**13**−**15**) exhibited more potent vasorelaxant effects than **2**, and the 4-hydroxyl compound (**13**) exhibited the most potent vasorelaxant effect (EC_{50} = 13 nM) among them. The 4-ketone (**19**) was equipotent to the 4-hydroxyl compound (**13**) in vasorelaxant effect.

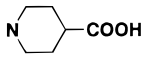
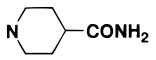
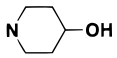
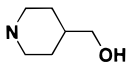
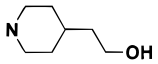
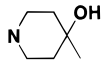
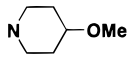
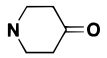
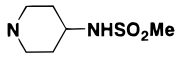
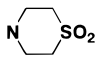
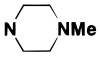
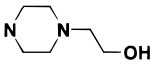
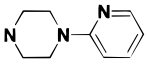
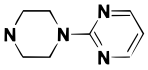
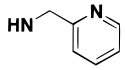
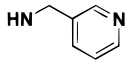
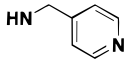
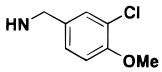
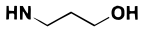
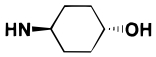
Scheme 3[a]



[a] Reagents: (a) aq 1 N NaOH, MeOH, reflux; (b) aq 1 N NaOH, EtOH, THF, rt; (c) Me_2NH, DCC, HOBt, Et_3N, CH_3CN, H_2O then HCl.

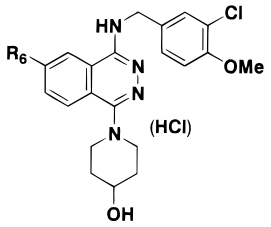
Table 1. Pharmacological Results of 1-Substituted-4-[(3-chloro-4-methoxybenzyl)amino]-6-phthalazinecarbonitriles



Compound	R_1	PDE5 IC_{50}, nM[a]	Vasorelaxant Effect EC_{50}, nM[b]
2	Cl	3.0	150
9		0.55	39
10		1.9	130
11		3.1	170
12		0.50	nt[c]
13		0.56	13
14		0.93	19
15		0.70	30
16		0.70	130
17		1.2	nt
18		1.1	100
19		0.53	13
20		14	nt
21		0.32	nt
22		0.70	50
23		0.79	nt
24		1.23	870
25		0 95	320
26		1.7	100
27		11	nt
28		19	nt
29		1.5	nt
30		2.7	nt
31		6.8	nt
32		6.7	nt
33		150	nt
34		2.6	nt
35		12	nt
sildenafil citrate		2.2	38 (n=3)
E4021		3.7	980

[a] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least four points). [b] EC_{50} values were determined from the logarithmic concentration−inhibition curve. The value is given as the mean of two experiments. [c] nt means not tested.

Table 2. Pharmacological Results of 6-Substituted-4-[(3-chloro-4-methoxybenzyl)amino]-1-(4-hydroxypiperidino)phthalazines



compd	R_6	PDE5 IC_{50}, nM[a]	vasorelaxant effect EC_{50}, nM[b]
13	CN	0.56	13
36	NO_2	0.30	30
37	CF_3	0.73	120
38	Cl	4.4	780
39	6,7-Cl_2	0.86	nt[c]
40	COOH	600	nt
41	$CONH_2$	2.0	nt
42	$CONMe_2$	58	nt

[a–c] See footnotes in Table 1.

Among piperazine derivatives (**24**−**28**), **24**−**26** exhibited potent inhibitory activity. The substituent effects were similar to those of piperidines (**24** vs **9**, **26** vs **15**), though the piperazine derivatives were slightly weaker PDE5 inhibitors than the piperidine derivatives. 4-(2-Pyridyl)piperazine (**27**) and 4-(2-pyrimidyl)piperazine (**28**) lacked potent inhibitory activity, so a bulky substituent at the 4-position of piperazine seems to decrease PDE5-inhibitory ability. Compounds (**30**−**35**) substituted with a primary amino group were less potent than **2**.

Next, we examined the effect of substituents at the 6-position of phthalazine with the 1-substituent fixed as 4-hydroxypiperidine. The results are listed in Table 2. The nitro (**36**) and trifluoromethyl (**37**) compounds were almost equipotent as PDE5 inhibitors to the nitrile (**13**). The chloro compound (**38**) was less potent. These results were parallel to our previous findings.[10] The 6,7-dichloro compound (**39**) was unexpectedly potent. Carboxylic acid (**40**), carbamoyl (**41**), and dimethylamide (**42**) derivatives of **13** showed loss of PDE5-inhibitory activity. These results were parallel to our findings on quinazoline derivatives.[8b]

We selected **13** as the optimum compound for PDE5-inhibitory activity and vasorelaxant effect. It is compared with other PDE inhibitors in Table 3. Compound **13** exhibited highly selective PDE5-inhibitory activity

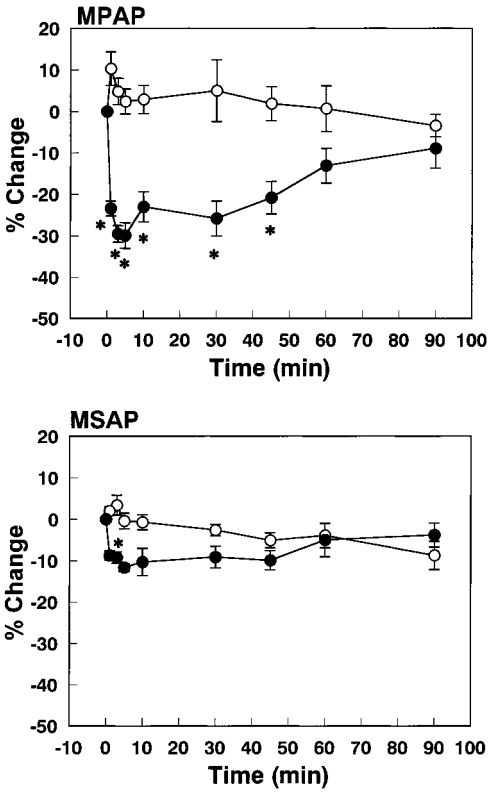


Figure 2. Time courses of effects of compound **13** on mean pulmonary (MPAP) and systemic (MSAP) arterial pressures in chronically hypoxic pulmonary hypertensive rats. Compound **13** at a dose of 30 μg/kg (closed circles, $n = 6$) or vehicle (open circles, $n = 6$) was intravenously injected to conscious rats. Values are mean ± SEM. *Significant difference from corresponding vehicle values, $P < 0.05$ (by repeated-measures ANOVA).

over 1700 times versus PDE1−4. This result confirms that the pharmacophore we proposed in the previous paper[10] is appropriate for selective PDE5-inhibitory activity. Compound **13** exhibited slight inhibitory activity toward PDE2 ($IC_{50} = 0.984$ μM) and PDE4 ($IC_{50} = 1.17$ μM) but was ineffective toward PDE1 ($IC_{50} = 26.2$ μM) and PDE2 ($IC_{50} = 17.5$ μM).

Next, we examined the effects of **13** on mean pulmonary (MPAP) and systemic (MSAP) arterial pressures in chronically hypoxic pulmonary hypertensive rats. The time courses of the hemodynamic effects of an intravenous injection of **13** at 30 μg/kg are shown in Figure 2. In chronically hypoxic rats before an intravenous injec-

Table 3. Inhibitory Activities of **13** on Five PDE Isozymes[a]

compd	IC_{50}, μM[b] (95% confidence interval) PDE5	PDE1	PDE2	PDE3	PDE4
13	0.000560 (0.000442−0.000710)	26.2 (20.0−34.4)	0.984 (0.841−1.15)	17.5 (11.4−26.9)	1.17 (1.01−1.36)
zaprinast	1.11 (0.733−1.68)	31.2 (26.5−36.7)	90.2 (68.1−119)	>100	>100
milrinone	>100	>100	>100	0.843 (0.581−1.23)	13.0 (10.4−16.3)
dipyridamole	0.574 (0.244−1.35)	74.5 (51.1−108)	3.24 (2.54−4.14)	44.0 (31.8−60.7)	5.27 (3.58−7.78)
rolipram	>100	>100	>100	>100	3.63 (2.56−5.13)

[a] The five PDE isozymes were isolated from porcine tissues as follows: PDE1, aorta; PDE2, adrenal; PDE3, platelets; PDE4, liver; PDE5, platelets. [b] IC_{50} values were determined from the logarithmic concentration−inhibition curves (at least four points) and are mean values of three experiments.

Table 4. Pharmacokinetic Parameters of **13** after 0.3 mg/kg Administration in Rats

route	*n*	t_{max}, h	$t_{1/2}$, h	C_{max}, ng/mL	AUC, ng h/mL	BA, %
iv	4	-	2.20	-	166.0	-
po	4	0.25	-	25.54	32.4	19.5

tion of **13** at a dose of 30 μg/kg, the mean pulmonary and systemic arterial pressures were 27.7 ± 1.5 and 104 ± 3 mmHg, respectively. Compound **13** caused significant decreases in MPAP at doses of more than 3 μg/kg and MSAP only at a dose of 30 μg/kg.

Maximum hypotensive responses of MPAP and MSAP to **13** were 29.9 ± 3.1% and 9.2 ± 1.4% and were noted 5 and 3 min after the intravenous injection, respectively. The lowering effect of **13** on MPAP lasted over 45 min, whereas its effect on MSAP was transient. No changes in heart rate were observed.

We evaluated the pharmacokinetic parameters of **13**. As shown in Table 4, **13** had an apparent oral bioavailability of about 19.5%. The plasma protein binding for 30 ng/mL of **13** was 95.7 ± 1.3%.

In conclusion, we have synthesized various 4-(3-chloro-4-methoxybenzyl)aminophthalazines substituted at the 1- and 6-positions via the 1-chlorides. In this series of compounds, **13** exhibited potent PDE5-inhibitory activity (IC_{50} = 0.56 nM) and high selectivity for PDE5 over other PDE isozymes (PDE1−4), amounting to >1700-fold. Compound **13** also exhibited the most potent vasorelaxant action (EC_{50} = 13 nM) in this series of compounds. Compound **13** reduced mean pulmonary arterial pressure by 29.9 ± 3.1% when administered intravenously at 30 μg/kg to the chronically hypoxic rats and had an apparent oral bioavailability of about 19.5% in rats and was selected for further biological evaluation.[12,13]

Experimental Section

Melting points (mp) were determined on an electrothermal capillary melting point apparatus and on a hot-stage apparatus, without correction. All ^{1}H NMR spectra were measured on a Varian (400 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) and elemental analyses were performed at the Analytical Chemistry Section of Eisai Tsukuba Research Laboratories.

***N*-(3-Chloro-4-methoxybenzyl)-*N*-(4,6,7-trichloro-1-phthalazinyl)amine (6).** A mixture of **1** (2.2 g, 8.2 mmol), 3-chloro-4-methoxybenzylamine hydrochloride (1.8 g, 8.7 mmol), Et_3N (2.6 mL, 18.7 mmol), and 2-propanol (100 mL) was heated at reflux for 6 h. The mixture was concentrated under reduced pressure, and the residue was dissolved in EtOAc and THF. The solution was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by recrystallization (EtOH−THF) to give **6** (2.16 g, 66%) as a pale yellow powder: ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s), 4.77 (2H, d, *J* = 4.8 Hz), 5.29 (1H, t, *J* = 4.8 Hz), 6.91 (1H, d, *J* = 8.0 Hz), 7.32 (1H, dd, *J* = 8.0, 2.4 Hz), 7.45 (1H, d, *J* = 2.4 Hz), 7.89 (1H, s), 8.28 (1H, s); MS *m/e* (FAB) 404 (MH^+).

4-[(3-Chloro-4-methoxybenzyl)amino]-1-(1,2,3,6-tetrahydro-1-pyridinyl)-6-phthalazinecarbonitrile Hydrochloride (10). General Procedure. A mixture of **2** (1.0 g, 2.8 mmol), 1,2,3,6-tetrahydro-1-pyridine (1.2 g, 14.5 mmol), $^i Pr_2EtN$ (2.5 mL, 14.4 mmol), and NMP (10 mL) was heated at 170 °C for 4.5 h and then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the free base (620 mg, 55%). The free base (620 mg, 1.5 mmol) was recrystallized from a mixture of 1 N HCl (1.8 mL), H_2O (6.4 mL), and EtOH (8 mL) to give **10** (420 mg, 62%) as a yellow powder: mp 240−243 °C (dec); ^{1}H NMR ($CDCl_3$) δ 2.38−2.46 (2H, m), 3.35−3.38 (2H, m), 3.80−3.84 (2H, m), 3.85 (3H, s), 4.74 (2H, d, *J* = 5.6 Hz), 5.85 (1H, d, *J* = 10 Hz), 5.93 (1H, d, *J* = 10 Hz), 7.16 (1H, d, *J* = 8.4 Hz), 7.48 (1H, dd, *J* = 8.4, 2.0 Hz), 7.63 (1H, d, *J* = 2.0 Hz), 8.23 (1H, d, *J* = 8.4 Hz), 8.47 (1H, d, *J* = 8.4 Hz), 9.49 (1H, s), 10.46 (1H, br s); MS *m/e* (FAB) 406 (MH^+). Anal. ($C_{22}H_{20}ClN_5O$·HCl) C, H, N.

1-[4-[(3-Chloro-4-methoxybenzyl)amino]-6-cyano-1-phthalazinyl]-4-piperidinecarboxylic Acid Hydrochloride (11). A mixture of **7** (1.20 g, 2.4 mmol) and formic acid (20 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated at reduced pressure. The residue was purified by silica gel column chromatography to give the free base (1.05 g, 98%). The free base (1.0 g, 2.2 mmol) was recrystallized from a mixture of 1 N HCl (2.3 mL), H_2O (2.7 mL), and EtOH (5 mL) to give **11** (890 mg, 82%) as a yellow powder: mp >270 °C; ^{1}H NMR (DMSO-d_6) δ 1.88−1.93 (2H, m), 1.96−2.03 (2H, m), 2.50−2.59 (1H, m), 2.92−3.01 (2H, m), 3.50−3.58 (2H, m), 3.85 (3H, s), 4.74 (2H, d, *J* = 5.2 Hz), 7.16 (1H, d, *J* = 8.4 Hz), 7.48 (1H, dd, *J* = 8.4, 2.4 Hz), 7.63 (1H, d, *J* = 2.4 Hz), 8.26 (1H, d, *J* = 8.4 Hz), 8.46 (1H, dd, *J* = 8.4, 1.2 Hz), 9.49 (1H, d, *J* = 1.2 Hz); MS *m/e* (FAB) 452 (MH^+). Anal. ($C_{23}H_{22}ClN_5O_3$·HCl·0.5H_2O) C, H, N.

4-[(3-Chloro-4-methoxybenzyl)amino]-1-(4-oxopiperidino)-6-phthalazinecarbonitrile Hydrochloride (19). A mixture of **8** (10.0 g, 21.5 mmol) and TFA (40 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated at reduced pressure. The residue was taken up in water and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography and recrystallized from EtOH−H_2O to give the free base (7.29 g, 80%). The free base (7.20 g, 17.1 mmol) was recrystallized from a mixture of 1 N HCl (20.5 mL) and EtOH (50 mL) to give **19** (4.45 g, 57%) as a yellow powder: mp 206 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.62−2.68 (4H, m), 3.55−3.61 (4H, m), 3.85 (3H, s), 4.77 (2H, d, *J* = 5.6 Hz), 7.15 (1H, d, *J* = 8.6 Hz), 7.49 (1H, dd, *J* = 8.6, 2.0 Hz), 7.64 (1H, d, *J* = 2.0 Hz), 8.40 (1H, d, *J* = 8.4 Hz), 8.50 (1H, dd, *J* = 8.4, 1.2 Hz), 9.55 (1H, d, *J* = 1.2 Hz); MS *m/e* (FAB) 422 (MH^+). Anal. ($C_{22}H_{20}ClN_5O_2$·HCl·0.25H_2O) C, H, N.

4-[(3-Chloro-4-methoxybenzyl)amino]-1-(4-hydroxypiperidino)-6-phthalazinecarboxylic Acid (40). A mixture of **13** (1.0 g, 2.4 mmol), aqueous 1 N−NaOH (40 mL), and MeOH (100 mL) was heated at reflux for 6 h. To this mixture was added H_2O (100 mL) and aqueous 1 N HCl (40 mL) to give a precipitate. The resultant precipitate was collected by filtration and washed with H_2O to give **40** (860 mg, 82%) as a yellow solid: mp 205−207 °C; ^{1}H NMR (DMSO-d_6) δ 1.62−1.73 (2H, m), 1.90−1.96 (2H, m), 2.90−2.97 (2H, m), 3.35−3.45 (2H, m), 3.66−3.74 (1H, m), 3.82 (3H, s), 4.64 (2H, s), 4.77 (1H, bs), 7.10 (1H, d, *J* = 8.4 Hz), 7.36 (1H, dd, *J* = 8.4, 2.0 Hz), 7.48 (1H, d, *J* = 2.0 Hz), 8.08 (1H, d, *J* = 8.6 Hz), 8.38 (1H, dd, *J* = 8.6, 1.4 Hz), 8.54 (1H, bs), 9.03 (1H, s); MS *m/e* (FAB) 443 (MH^+). Anal. ($C_{22}H_{23}ClN_4O_4$·0.7H_2O) C, H, N.

4-[(3-Chloro-4-methoxybenzyl)amino]-1-(4-hydroxypiperidino)-6-phthalazinecarboxamide (41). To a solution of **13** (500 mg, 1.2 mmol) in EtOH (6 mL) and THF (6 mL) was added aqueous 1 N NaOH (2.4 mL). The mixture was stirred for 15 h at room temperature. It was then diluted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography and recrystallized from CH_3CN to give **41** (230 mg, 44%) as a yellow powder: mp 167−169 °C; ^{1}H NMR (DMSO-d_6) δ 1.62−1.72 (2H, m), 1.88−1.96 (2H, m), 2.85−2.95 (2H, m), 3.30−3.43 (2H, m), 3.68 (1H, m), 3.82 (3H, s), 4.63 (2H, d, *J* = 5.7 Hz), 4.73 (1H, d, *J* = 4.0 Hz), 7.09 (1H, d, *J* = 8.4 Hz), 7.36 (1H, dd, *J* = 8.4, 2.0 Hz), 7.46 (1H, d, *J* = 2.0 Hz), 7.72 (1H, br s), 7.74 (1H, t, *J* = 5.7 Hz),

7.98 (1H, d, $J = 8.6$ Hz), 8.11 (1H, br s), 8.25 (1H, dd, $J = 8.4$, 1.3 Hz), 8.78 (1H, d, $J = 1.3$ Hz); MS *m/e* (FAB) 442 (MH^+). Anal. ($C_{22}H_{24}ClN_5O_3 \cdot 0.8H_2O$) C, H, N.

***N*$_6$,*N*$_6$-Dimethyl-4-[(3-chloro-4-methoxybenzyl)amino]-1-(4-hydroxypiperidino)-6-phthalazinecarboxamide Hydrochloride (42).** To a suspension of **40** (300 mg, 0.68 mmol), dimethylamine hydrochloride (110 mg, 1.35 mmol), DCC (210 mg, 1.0 mmol), and 1-hydroxybenzotriazole (140 mg) in triethylamine (0.24 mL) and acetonitrile (10 mL) was added H_2O (1 mL), affording a clear solution. The mixture was heated at 60 °C for 8 h, during which time a precipitate was formed. After cooling, the precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the free base (260 mg, 82%) as a yellow solid. The free base (260 mg, 0.55 mmol) was recrystallized from 1 N HCl, EtOH, EtOAc, and IPE to give **42** (260 mg, 93%) as a yellow powder: mp 179−184 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.65−1.75 (2H, m), 1.92−2.00 (2H, m), 2.94 (3H, s), 3.06 (3H, s), 3.00−3.10 (2H, m), 3.45−3.53 (2H, m), 3.73−3.80 (1H, m), 3.85(3H, s), 4.71 (2H, d, $J = 5.6$ Hz), 7.16 (1H, d, $J = 8.6$ Hz), 7.44 (1H, d, $J = 8.6$ Hz), 7.59 (1H, bs), 8.12 (1H, dd, $J = 8.6$, 1.0 Hz), 8.17 (1H, d, $J = 8.6$ Hz), 8.86 (1H, bs), 10.10 (1H, bs), 13.85 (1H,bs); MS *m/e* (FAB) 470 (MH^+). Anal. ($C_{24}H_{28}ClN_5O_3 \cdot HCl \cdot H_2O$) C, H, N.

Enzyme Source and Phosphodiesterase Activity Assay. PDE5 was separated from the supernatant of a homogenate of porcine platelets or porcine lung by DEAE-Toyopearl 650S chromatography.[9]

PDE activity was determined by a modification of a previously described two-step radioisotopic procedure.[14] [^{3}H]cGMP at a concentration of 1 μM was used as a substrate. The tested compounds were dissolved in DMSO and diluted with the assay buffer to give concentrations ranging from 10^{-10} to 10^{-6} M. The final concentration of DMSO was 1% (v/v).

Vasorelaxant Effect in Isolated Porcine Coronary Arteries Precontracted with PGF2α. Porcine coronary arteries were removed, freed from adjacent tissues, and cut into rings with great care to avoid damage to the endothelium. The rings were longitudinally opened and mounted in organ baths containing 10 mL of Krebs−Henseleit solution (37 °C, pH 7.4, bubbled with 95% O_2−5% CO_2). The coronary arterial strips were allowed to equilibrate under a resting tension of 1 g. The presence of intact endothelial cells was confirmed by bradykinin-induced (final concentration, 10^{-8} M) relaxation in preparations precontracted with KCl (final concentration, 50 mM). The strips were contracted with PGF2α (final concentration, 10^{-5} M), and after the attainment of a plateau contraction, cumulative concentration−relaxation curves for a test compound were constructed. Relaxation was calculated as a percentage of the contractile response to PGF2α.

Acute Hemodynamic Effects of Compound 13 in Conscious Rats with Pulmonary Hypertension. Male adult Sprague−Dawley rats were exposed to reduced barometric pressure (380 mmHg) in a hypobaric chamber for 3 weeks to induce hypoxic pulmonary hypertension as described previously.[13] After a 3 week exposure to hypoxia, the rats were anesthetized with intramuscular ketamine (90 mg/kg) and pentobarbital sodium (15 mg/kg) for catheterization of the pulmonary and right carotid arteries and right jugular vein. The catheters were filled with heparinized saline and tunneled subcutaneously to the back of the neck. The rats were allowed to recover for 48 h in room air. After recovery, the conscious rats (210−262 g, $n = 30$) were used for hemodynamic measurements. Mean pulmonary and systemic arterial pressures were measured with pressure transducers (TP-2001; Nihon Kohden, Tokyo, Japan).

The hemodynamic measurements were performed under normoxic conditions. Either 1, 3, 10, or 30 μg/kg of **13** or its vehicle (5% glucose solution containing 5 mM phosphoric acid) was intravenously injected into conscious chronic hypoxic rats. Pulmonary and systemic arterial pressures were monitored for 90 min. At the end of the study, the rat was killed by an overdose of pentobarbital sodium.

All data are expressed as mean ± SEM. The value for each hemodynamic parameter after the injection of **13** was compared with the value after the injection of vehicle by using repeated-measures ANOVA followed by Dunn-type multiple comparison. *P* values of <0.05 were considered significant.

Pharmacokinetic Studies. The pharmacokinetics of **13** was studied in male Sprague−Dawley rats. In the iv and po studies, **13** was dissolved in 10% lactose−phosphoric acid (pH 2). Rats were sacrificed by exsanguination under ether anaesthetized at 5 (iv only), 15, and 30 min and 1, 1.5 (po only), 2, 4, 6, 8, and 24 h after dosing, and blood samples (1 mL) were collected. Plasma was obtained by centrifugation of blood at 3000*g* for 15 min and stored at −20 °C until analysis. The concentration of **13** was determined by HPLC following liquid−liquid extraction from plasma.

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