4-Benzylamino-1-chloro-6-substituted Phthalazines: Synthesis and Inhibitory Activity toward Phosphodiesterase 5

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We synthesized various 4-benzylamino-1-chloro-6-substituted phthalazines (**15**) and 4-benzylamino-1-chloro-7-substituted phthalazines (**16**) and evaluated their inhibitory activity toward phosphodiesterase 5 (PDE5) purified from porcine platelets. The PDE5-inhibitory activities of **15** were greater than those of the isomers (**16**). The preferred substituent at the 4-position of phthalazine was a (3-chloro-4-methoxybenzyl)amino group, and those at the 6-position were cyano, nitro, and trifluoromethyl groups. Compounds **15a** (IC_{50} = 4.8 nM), **15f** (3.5 nM), and **15i** (5.3 nM) were more potent inhibitors than E4021 (8.6 nM). Compounds **15a** and **15f** also showed vasorelaxant activity in isolated porcine coronary arteries precontracted with prostaglandin $F_{2\alpha}$ (10^{-5} M). The EC_{50} values for vasorelaxant action of **15a**, **15f**, and E4021 were 150, 160, and 980 nM, respectively. These results show that novel PDE5 inhibitors possessing a potent vasorelaxant effect may exist among phthalazine derivatives.

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)-pyrazolo[3,4-*d*]pyrimidin-4-(5*H*)-one (DMPPO),[5] and quinazoline derivatives[6−8] (Figure 1) have been reported to show vasorelaxing,[9] antihypertensive, and penile erection-promoting activities.

In the present study, as a continuation of our work on quinazolines[7,8] we synthesized 4-benzylamino-1-chloro-6-substituted phthalazines (Figure 2) and evaluated their inhibitory activities toward PDE5. Some of these compounds were also examined for relaxant activity in isolated porcine coronary arteries precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), in comparison with E4021.

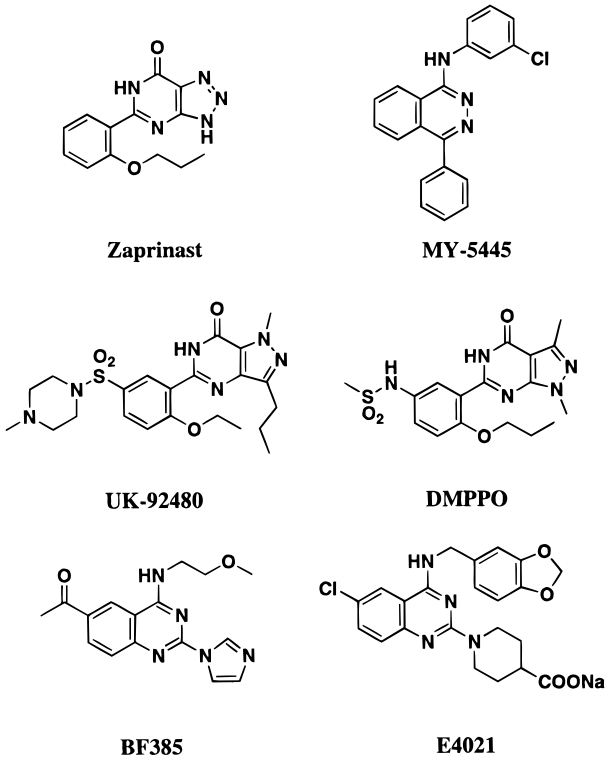

Figure 1.

Chemistry

New 4-benzylamino-1-chloro-6-substituted phthalazines were synthesized by the reaction of 1,4-dichloro-6-substituted phthalazines and benzylamines.

The 1,4-dichloro-6-substituted phthalazines were obtained according to Scheme 1. Compounds **5a** and **5b** were synthesized from corresponding phthalic anhydrides (**3a**) or phthalimide (**3b**) through phthalazine ring formation by hydrazine monohydrate and chlorination by phosphorus oxychloride. Compound **5c** was synthesized from 2-chloro-5-trifluoromethylbenzoic acid (**1**) through the corresponding phthalimide (**3c**) by the method of Lawton and McRitchie.[10] Compound **5d** was prepared through the corresponding phthalimides synthesized by means of the Sandmeyer reaction of 4-aminophthalimide (**2**). The synthesis of **5d** was also accomplished by an alternative route (Scheme 2). The reaction of trimellitic anhydride chloride (**6**) and ammonia water gave a mixture of 2,4-dicarbamoylbenzoic acid and 2,5-dicarbamoylbenzoic acid, and this mixture was heated in NMP to afford 4-carbamoylphthalimide (**7**). After formation of the phthalazine ring, dehydration and chlorination were simultaneously accomplished by refluxing in a mixture of phosphorus oxychloride and thionyl chloride. This route does not require a large quantity of KCN, so it is more practical than the route via the Sandmeyer reaction described above.

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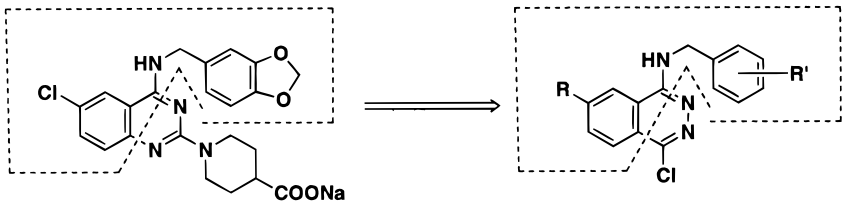
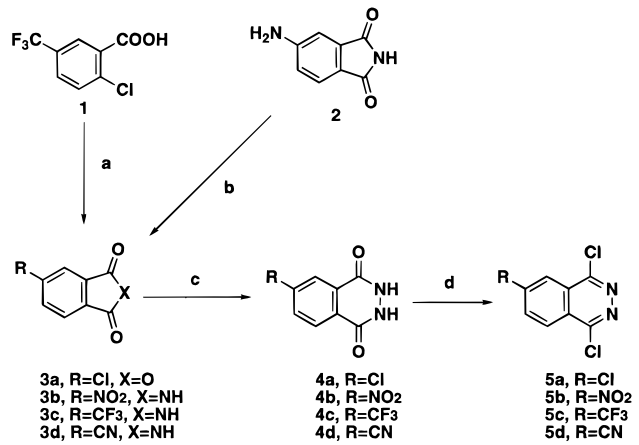


E4021

Figure 2. Proposed pharmacophore for PDE5-inhibitory activity.

Scheme 1[a]



[a] Reagents: (a) CuCN, DMF then $FeCl_3$, HCl; (b) $NaNO_2$, HCl then KCN, CuCl; (c) $NH_2NH_2 \cdot H_2O$, EtOH; (d) $POCl_3$, iPr_2EtN.

Benzylamines were synthesized according to Scheme 3. Chlorination of 4-methoxybenzaldehyde (**10a**) with sulfuryl chloride in the presence of a catalytic amount of pyridine regioselectively gave the chlorinated compound (**12a**). Compound **12a** was transformed into the formamide (**13a**) by heating in a mixture of formamide and formic acid, and acidic hydrolysis gave the benzylamine (**14a**). Reaction of 2-chloroanisole (**11**) and acetyl chloride regioselectively gave **12c**. Compound **12c** was transformed into **14c** in the same manner described above.

The results of the reaction of **5** and **14** are summarized in Table 1. Reactions of 1,4-dichloro-6-substituted phthalazines and amines have not previously been reported. The reactions in NMP with DBU did not require heating, while in the other reactions heating was necessary. Under these conditions, the reactions were completed within a relatively short time. The structures of the isomers (**15a**−**i**, **16a**−**i**) were determined by means of NOE experiments.

The formation of the isomer **15** exceeded that of **16**.

Pharmacological Results and Discussion

PDE5 was purified from porcine platelets for use in the inhibition assays, the conditions of which are briefly described in the Experimental Section. E4021 was used as the active control in each screening assay. The PDE5-inhibitory activities of the title compounds (**15**) and some isomers (**16**) are listed in Table 2.

The compounds (**15**) exhibited moderate or potent PDE5-inhibitory activities. Their inhibitory activities were more potent than those of the corresponding isomers **16** (**15a** vs **16a**, **15g** vs **16g**, **15h** vs **16h**). These results suggest that the spatial relation between the 6-substituent and the benzylamino moiety is important for the inhibitory activity, and support the proposed pharmacophore in Figure 2.

The substituents of the benzylamino moiety were selected on the basis of our results with quinazolines. That is, 4-(3-chloro-4-methoxybenzyl)amino- and 4-[(3,4-methylenedioxy)benzyl]aminoquinazolines were more potent than the other compounds. We selected 3-chloro-4-methoxybenzylamino, its derivatives, and 3,4-methylenedioxybenzylamino groups.

The 4-[(3,4-methylenedioxy)benzyl]amino derivatives (**15b**, **15g**) showed moderate PDE5-inhibitory activities. On the other hand, 4-(3-chloro-4-methoxybenzyl)amino derivatives (**15a**, **15f**, **15h**, **15i**) were more potent than **15b** and **15g**, and some of them were as potent as E4021. When **R** is CN (**15a**−**15e**), **15a** is far more potent than the other derivatives. This may be attributed to the importance conformational restrictions (**15a** vs **15c**, **15a** vs **15e**) and the effect of favorable hydrophobic interactions in appropriately oriented and bulky substituents (**15a** vs **15b**, **15a** vs **15d**).

With regard to the effects of **R**, CN (**15a**), NO_2 (**15f**), and CF_3 (**15i**) are more potent than Cl (**15h**), but the reasons for this are unclear.

Vasorelaxant effects of **15a** and **15f** were then examined in isolated porcine coronary arteries precontracted with PGF2α (10^{-5} M). The EC_{50} values of these compounds are included in Table 2. In this assay, the EC_{50} values of **15a** and **15f** are less than that of E4021.

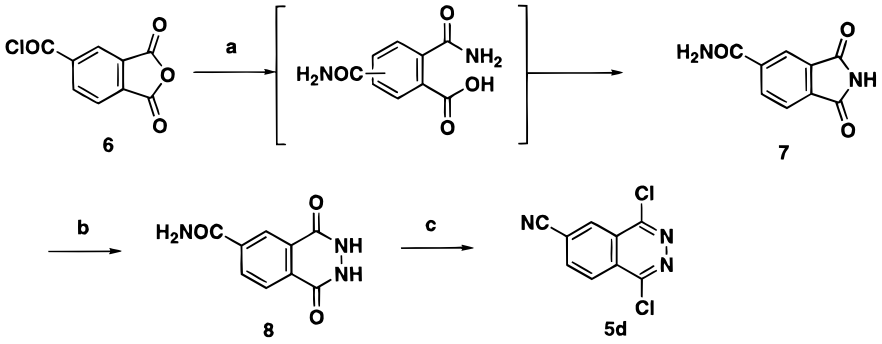
In conclusion, we have synthesized various 4-benzylamino-1-chloro-6-substituted phthalazines (**15**) and 1-benzylamino-4-chloro-7-substituted phthalazines (**16**). The inhibitory activities of **15** toward PDE5 were greater than those of **16**. These results support the pharmacophore proposed for PDE5-inhibitory activities. In particular, **15a**, **15f**, and **15i** showed potent inhibitory activities and their IC_{50} values were less than that of E4021. Compounds **15a** and **15f** exhibited a relaxant effect on isolated porcine coronary arteries precontracted with $PGF_{2\alpha}$ (10^{-5} M). We continue to search for more potent and orally active phthalazine derivatives.

Experimental Section

Melting points (mp) were determined on an electrothermal capillary melting point apparatus and on a hot-stage apparatus, without correction. All ^1H 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.

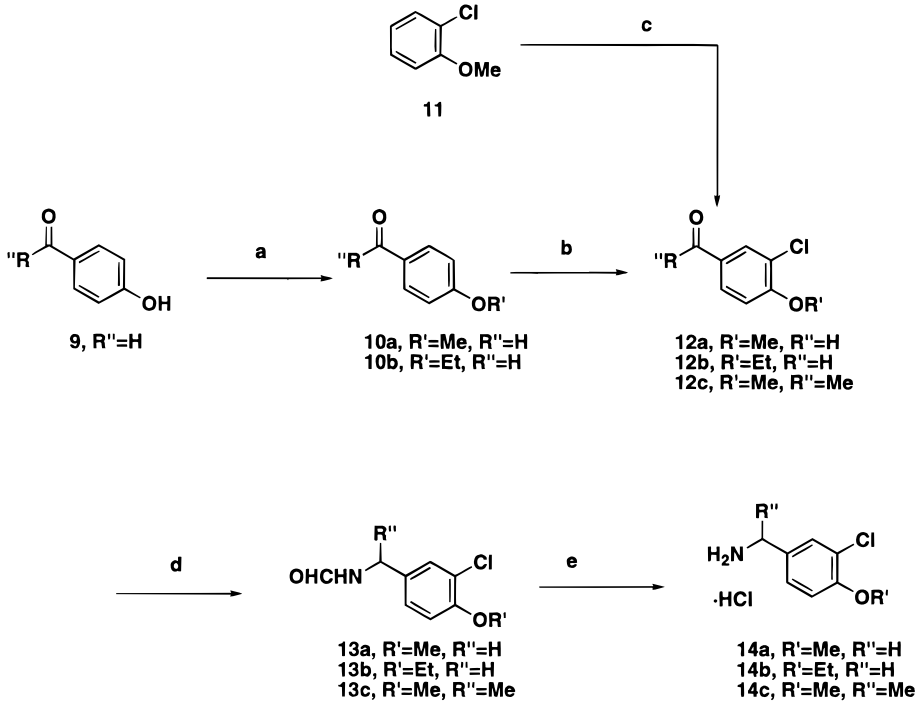
4-Trifluoromethylphthalimide (3c). A mixture of **1** (15.0 g, 66.9 mmol), CuCN (9.00 g, 100 mmol), and DMF (24 mL) was stirred, heated at reflux for 1 h, and then cooled. To the

Scheme 2[a]



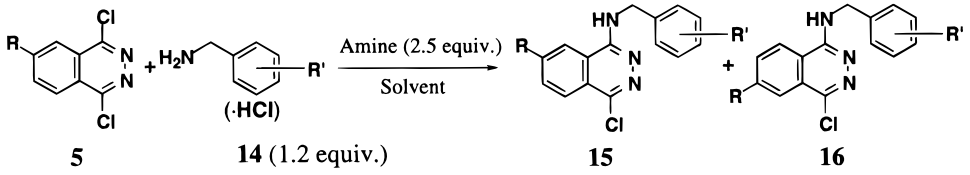
[a] Reagents: (a) 29% NH_4OH, acetone then heat in NMP; (b) $NH_2NH_2 \cdot H_2O$, NMP; (c) $POCl_3$, $SOCl_2$.

Scheme 3[a]



[a] Reagents: (a) $(EtO)_2SO_2$, K_2CO_3, CH_3CN; (b) SO_2Cl_2, pyridine; (c) AcCl, $AlCl_3$, CH_2Cl_2; (d) HCOOH, $HCONH_2$; (e) HCl, EtOH.

Table 1. Results of Reaction of **5** and **14**

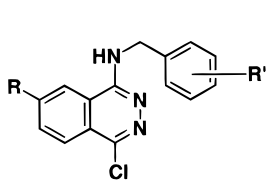


run	phthalazines **5**	benzylamine **14** (**R′**)	conditions	isolated yield[b] (%) **15**	**16**
1	**5d**	**14a**	Et_3N, THF, reflux, 6 h	**15a**, 56	**16a**, 25
2	**5d**	3,4-methylenedioxy	DBU, NMP, rt, 2 h	**15b**, 44	**16b**, 15
3	**5d**	4-MeO	DBU, NMP, rt, 8 h[a]	**15c**, 56	**16c**, 20
4	**5d**	**14b**	Et_3N, THE, reflux, 7 h	**15d**, 46	**16d**, 24
5	**5d**	**14c**	DBU, NMP, rt, 4 h[a]	**15e**, 45	**16e**, 18
6	**5b**	**14a**	Et_3N, THF, reflux, 6 h	**15f**, 55	**16f**, 20
7	**5b**	3,4-methylenedioxy	Et_3N, EtOH, reflux, 6 h	**15g**, 35	**16g**, 23
8	**5a**	**14a**	DBU, NMP, rt, 4.5 h[a]	**15h**, 38	**16h**, 31
9	**5c**	**14a**	DBU, NMP, rt, 2 h	**15i**, 44	**16i**, 28

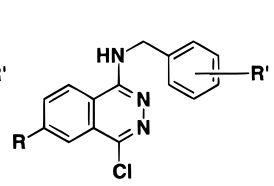
[a] Reaction finished within 2 h. [b] 1,4-Dibenzylaminophthalazines were slightly observed in the reaction mixtures.

suspension was added a mixture of $FeCl_3$ (22.5 g, 139 mmol) and concentrated HCl (7.5 mL) in H_2O (37.5 mL) over 5 min. The mixture was stirred and heated at 65 °C for 1 h, then chilled, diluted with ice water (150 mL), and filtered. The dark brown solid was triturated with 5% aqueous $NaHCO_3$, collected by filtration, and dried to give **3c** (13.6 g, 94%) as a brown

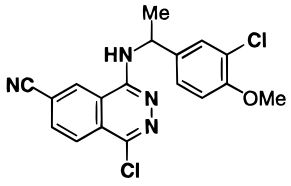
Table 2. Structures, Properties, PDE5-Inhibitory Activities, and Relaxing Effects of Phthalazines



15



16



15e

compd	R	R′	formula[a]	mp, °C (recrystn solv)[b]	IC_{50}, nM[c]	EC_{50}, nM[d]
15a	CN	3-Cl-4-MeO	$C_{17}H_{12}Cl_2N_2O$	213.0−214.5 (A)	4.8	150
15b	CN	3,4-methylenedioxy	$C_{17}H_{11}ClN_4O_2$	163.0−164.0 (A)	100	nt
15c	CN	4-MeO	$C_{17}H_{13}ClN_4O$	201.0 dec (B)	140	nt
15d	CN	3-Cl-4-EtO	$C_{18}H_{14}Cl_2N_4O$	194.0−195.0 (A)	49	nt
15e	CN	α-Me-3-Cl-4-MeO	$C_{18}H_{14}Cl_2N_4O$	204.0−205.0 (B)	98	nt
15f	NO_2	3-Cl-4-MeO	$C_{16}H_{12}ClN_4O_3$	217.0−217.5 (C)	3.5	160
15g	NO_2	3,4-methylenedioxy	$C_{16}H_{11}ClN_4O_4$	186.5−188.0 dec (D)	63	nt
15h	Cl	3-Cl-4-MeO	$C_{16}H_{12}Cl_3N_3O$	197.0−198.5 (D)	54	nt
15i	CF_3	3-Cl-4-MeO	$C_{17}H_{12}Cl_2F_3N_3O$	126.0−127.5 (B)	5.3	nt
16a	CN	3-Cl-4-MeO	$C_{17}H_{12}Cl_2N_4O{\cdot}0.1H_2O$	122.0−123.5 (A)	140	nt
16g	NO_2	3,4-methylenedioxy	$C_{16}H_{11}ClN_4O_4$	240.0−242.0 dec (E)	9200	nt
16h	Cl	3-Cl-4-MeO	$C_{16}H_{12}Cl_3N_3O$	168.0−169.5 (D)	810	nt
E4021					8.6 ($n = 2$)	980

[a] Analyses for C, H, and N were within ±0.4% of the expected values for the formula. [b] Key: A = EtOAc, B = EtOH, C = EtOAc−hexane, D = EtOH−EtOAc, E = EtOH−THF. [c] IC_{50} values were determined from the logarithmic concentration-inhibition curve (at least four points). In cases where repeated determinations were made, the value is given as the mean (number of experiments). [d] EC_{50} values were determined from the logarithmic concentration−relaxation curve. The methods are described in the Experimental Section.

solid: ^{1}H NMR (DMSO-d_6) δ 8.04 (1H, d, J = 5.6 Hz), 8.14 (1H, s), 8.21 (1H, d, J = 5.6 Hz), 11.70 (1H, s).

4-Cyanophthalimide (3d). Concentrated HCl (57 mL) was added to a suspension of **2** (40.0 g, 247 mmol) in H_2O (300 mL) with stirring and ice cooling. To this suspension was added dropwise a solution of $NaNO_2$ (20.6 g, 299 mmol) in H_2O (69 mL) while the temperature was held below 5 °C. The mixture was cooled to −20 °C. Toluene (300 mL) was added, and the mixture was neutralized with $NaHCO_3$ to prepare a diazonium salt solution.

A solution of KCN (105.7 g, 1.63 mol) in H_2O (206 mL) was added dropwise to a suspension of CuCl (63.4 g, 640 mmol) in H_2O (250 mL) with vigorous stirring and ice cooling. The mixture was stirred for another 1 h at the same temperature, and then EtOAc (500 mL) was added, followed by the above diazonium salt solution in portions. The mixture was stirred for 1 h with ice cooling and filtered through a Celite pad. The filtrate was washed with H_2O, saturated aqueous $NaHCO_3$, diluted aqueous HCl, and brine and dried over anhydrous $MgSO_4$. The organic solution was concentrated under reduced pressure to give **3d** (41.0 g, 97%) as a reddish brown solid: mp 237−238 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (1H, dd, J = 7.5, 1.0 Hz), 8.29 (1H, dd, J = 7.5, 1.5 Hz), 8.36 (1H, dd, J = 1.5, 1.0 Hz), 11.73 (1H, s); MS *m/e* (FAB) 173 (MH^+).

2,3-Dihydro-1,4-phthalazine-6-carbonitrile (4d). General Procedure. Hydrazine monohydrate (25 mL) was added to a suspension of **3d** (80.0 g, 465 mmol) in EtOH (1000 mL), and the mixture was stirred for 5 h at room temperature. The mixture was then concentrated to half volume under reduced pressure, diluted with H_2O (1000 mL), and acidified with diluted HCl to afford a precipitate, which was collected by filtration to give **4d** (71.0 g, 82%) as a brown solid: ^{1}H NMR (DMSO-d_6) δ 8.19 (1H, br s), 8.27 (1H, dd, J = 8.0, 1.6 Hz), 8.48 (1H, br s), 11.39 (2H, br s); MS *m/e* (FAB) 166 (MH^+).

1,4,6-Trichlorophthalazine (5a). General Procedure. To a suspension of **4a** (24.9 g, 127 mmol) in $POCl_3$ (150 mL) was added ^{i}Pr$_2$EtN (25 mL), and the mixture was heated at reflux for 3 h and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and poured into ice−water. The solution was filtered through a Celite pad to remove a small amount of insoluble material, and the organic layer of the filtrate was separated. The organic layer was washed with saturated aqueous $NaHCO_3$, diluted HCl, and brine and dried over anhydrous $MgSO_4$. The solution was filtered through a silica gel pad, and the filtrate was concentrated under reduced pressure to give **5a** (24.4 g, 82%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.01 (1H, dd, J = 8.8, 2.0 Hz), 8.29 (1H, d, J = 8.8 Hz), 8.31 (1H, d, J = 2.0 Hz).

4-Carbamoylphthalimide (7). A solution of **6** (21.1 g, 100 mmol) in acetone (25 mL) was added dropwise to a 29% ammonia solution (200 mL) with stirring and ice cooling, and the mixture was stirred for 1 h at the same temperature. The excess NH_3 was evaporated off under reduced pressure, and the mixture was acidified with concentrated HCl, with cooling. The precipitate was collected by filtration, washed with H_2O, and dried to give a mixture of 2,4-dicarbamoylbenzoic acid and 2,5-dicarbamoylbenzoic acid (18.5 g, 89%).

A suspension of the above mixture (16.0 g, 77 mmol) in NMP (80 mL) was heated at 150 °C for 3 h. The mixture was cooled, and H_2O (200 mL) was added to afford a precipitate. The precipitate was collected by filtration, washed with H_2O, and dried to give **7** (13.3 g, 91%) as a pale brown solid: ^{1}H NMR (DMSO-d_6) δ 7.70 (1H, br s), 7.90 (1H, dd, J = 7.2, 1.2 Hz), 8.28−8.31 (2H, m), 8.32 (1H, br s), 11.48 (1H, br s).

6-Carbamoyl-2,3-dihydro-1,4-phthalazinedione (8). Hydrazine monohydrate (0.8 mL) was added to a suspension of **7** (2.0 g, 11 mmol) in NMP (12 mL), and the mixture was stirred for 0.5 h at room temperature. Then 3 N aqueous HCl (5.5 mL) and H_2O (50 mL) were added. The precipitated solid was collected by filtration and dried to give **8** (2.0 g, 94%) as a pale brown solid: ^{1}H NMR (DMSO-d_6) δ 7.68 (1H, br s), 8.12 (1H, br d, J = 8.4 Hz), 8.32 (1H, dd, J = 8.4, 1.6 Hz), 8.39 (1H, br s), 8.59 (1H, br s), 11.69 (2H, br s).

1,4-Dichlorophthalazine-6-carbonitrile (5d). A suspension of **8** (1.0 g, 4.9 mmol) in $POCl_3$ (20 mL) and $SOCl_2$ (20 mL) was heated at reflux overnight and then concentrated under reduced pressure, and the residue was dissolved in CH_2Cl_2. The solution was washed with H_2O, dried over anhydrous $MgSO_4$, and filtered through a silica gel pad. The filtrate was concentrated under reduced pressure to give **5d** (0.76 g, 70%) as a pale brown solid: ^{1}H NMR ($CDCl_3$) δ 8.24 (1H, dd, J = 8.4, 1.6 Hz), 8.47 (1H, dd, J = 8.4, 0.8 Hz), 8.68 (1H, dd, J = 1.6, 0.8 Hz).

4-Ethoxybenzaldehyde (10b). To a mixture of **9** (25.3 g, 207 mmol) and diethyl sulfate (40 mL, 306 mmol) in CH_3CN (400 mL) was added K_2CO_3 (45 g, 326 mmol), and the mixture was heated at reflux for 0.5 h and then poured into water. The whole was extracted with EtOAc. The organic layer was

washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give **10b** (30 g, 96%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.2 Hz), 4.12 (2H, q, J = 7.2 Hz), 6.97−7.02 (2H, m), 7.81−7.86 (2H, m), 9.88 (1H, s).

3-Chloro-4-methoxybenzaldehyde (12a). General Procedure. A mixture of **10a** (420 g, 3.09 mol) and pyridine (6 mL) was treated dropwise with SO_2Cl_2 (510 g, 3.78 mol) with stirring, while the temperature was held below 30 °C. The mixture was stirred at room temperature for 0.5 h and at 70 °C for 4 h. Excess SO_2Cl_2 was evaporated off, and IPE (500 mL) and hexane (5 L) were added to the residue to afford a precipitate. The precipitate was collected by filtration, washed with hexane, and dried to give **12a** (379 g, 72%) as a pinkish solid: ^{1}H NMR ($CDCl_3$) δ 3.99 (3H, s), 7.05 (1H, d, J = 8.6 Hz), 7.78 (1H, dd, J = 8.6, 2.0 Hz), 7.91 (1H, d, J = 2.0 Hz), 9.86 (1H, s).

3-Chloro-4-methoxyacetophenone (12c). To a suspension of **11** (10.0 g, 70.1 mmol) and $AlCl_3$ (28.0 g, 210 mmol) in CH_2Cl_2 (150 mL) was added AcCl (16.5 g, 210 mmol) at 0 °C. The mixture was stirred for 2 h at 0 °C, then poured into ice water, and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, then dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give **12c** (12.9 g, quant.) as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.55 (2H, s), 3.97 (3H, s), 6.96 (1H, d, J = 8.6 Hz), 7.86 (1H, dd, J = 8.6, 2.2 Hz), 7.99 (1H, d, J = 2.2 Hz).

***N*-(3-Chloro-4-methoxybenzyl)formamide (13a). General Procedure.** A mixture of **12a** (551 g, 3.23 mol), formamide (1.5 L), and formic acid (1 L) was heated at 130 °C for 6 h, then poured into ice water, and extracted with EtOAc. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, H_2O, and brine. After having been dried over anhydrous $MgSO_4$, the solution was filtered through a silica gel pad, and the filtrate was concentrated under reduced pressure to about 1 L. Hexane (500 mL) was added to the residue to afford a precipitate. The precipitate was collected by filtration and washed with a mixture of EtOAc and hexane (1:1) to give **13a** (410 g, 64%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.89 (3H, s), 4.41 (2H, d, J = 5.6 Hz), 5.85 (1H, br s), 6.88 (1H, d, J = 8.4 Hz), 7.16 (1H, dd, J = 8.4, 2.4 Hz), 7.30 (1H, d, J = 2.4 Hz), 8.26 (1H, s).

3-Chloro-4-methoxybenzylamine Hydrochloride (14a). General Procedure. A mixture of **13a** (410 g, 2.06 mol), EtOH (2.6 L), and concentrated HCl (260 mL) was heated at reflux for 3 h, during which time a precipitate was formed. The mixture was cooled, and Et_2O (4 L) was added to it. The resultant precipitate was collected by filtration and washed with Et_2O to give **14a** (406 g, 95%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 3.85 (3H, s), 3.94 (2H, s), 7.18 (1H, d, J = 8.4 Hz), 7.44 (1H, dd, J = 8.4, 2.0 Hz), 7.61 (1H, d, J = 2.0 Hz), 8.39 (3H, br s).

1-Chloro-4-[[3,4-(methylenedioxy)benzyl]amino]-6-phthalazinecarbonitrile (15b) and 1-Chloro-7-[[3,4-(methylenedioxy)benzyl]amino]-7-phthalazinecarbonitrile (16b). General Procedure. To a solution of **5d** (1.0 g, 4.5 mmol) and piperonylamine (810 mg, 5.4 mmol) in NMP (10 mL) was added DBU (1.7 g, 11.2 mmol). The mixture was stirred at room temperature for 2 h, then poured into H_2O, and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluted with toluene−THF) to give **15b**, pale yellow crystals, 670 mg (44%), as the less polar product and **16b**, pale yellow crystals, 230 mg (15%), as the more polar product.

15b: mp 163.0−164.0 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.78 (2H, d, J = 5.2 Hz), 5.37 (1H, t, J = 5.2 Hz), 5.96 (2H, s), 6.81 (1H, d, J = 8.0 Hz), 6.92 (1H, dd, J = 8.0, 1.8 Hz), 6.94 (1H, d, J = 1.8 Hz), 8.05 (1H, dd, J = 8.4, 1.4 Hz), 8.11−8.12 (1H, m), 8.30 (1H, dd, J = 8.4, 0.6 Hz); MS *m/e* (FAB) 339 (MH^+). Anal. ($C_{17}H_{11}ClN_4O_2$) C, H, N.

16b: mp 165.0−167.0 °C (EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.78 (2H, d, J = 5.2 Hz), 5.34 (1H, t, J = 5.2 Hz), 5.96 (2H, s), 6.80 (1H, d, J = 7.6 Hz), 6.91 (1H, dd, J = 7.6, 1.2 Hz), 6.94 (1H, d, J = 1.2 Hz), 7.86 (1H, dd, J = 8.4, 0.4 Hz), 8.01 (1H, dd, J = 8.4, 1.6 Hz), 8.54 (1H, dd, J = 1.6, 0.4 Hz).

Enzyme Source and Phosphodiesterase Activity Assay. PDE5 was separated from the supernatant of a homogenate of porcine platelets by DEAE-Toyopearl 650S chromatography.[8]

PDE5 activity was determined by a modification of a previously described two-step radioisotopic procedure.[11] [^{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 5% (v/v).

Vasorelaxant Effect in Isolated Porcine Coronary Arteries Precontracted with $PGF_{2\alpha}$. 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 aorta strips were allowed to equilibrate under a resting tension of 1 g. The presence of intact endothelial cells was confirmed by bradykinin (final concentration, 7×10^{-9} M)-induced relaxation in preparations precontracted with KCl (final concentration, 50 mM). The strips were contracted with $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$.

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