Cyclic GMP Phosphodiesterase Inhibitors. 2. Requirement of 6-Substitution of Quinazoline Derivatives for Potent and Selective Inhibitory Activity

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We synthesized various 4-[[3,4-(methylenedioxy)benzyl]amino]quinazolines substituted at the 5- to 8-positions and evaluated their inhibitory activities toward cyclic GMP phosphodiesterase (cGMP-PDE) from porcine aorta. Monosubstitution at the 6-position was essential for the inhibitory activity, and the preferred substituents were compact and hydrophobic: methoxy (**3b**, IC_{50} = 0.23 μM), methyl (**3c**, 0.10 μM), chloro (**3d**, 0.019 μM), thiomethyl (**3f**, 0.031 μM), and cyano (**3p**, 0.090 μM) groups. Compounds **3b–d,f,p** lacked inhibitory activity toward other PDE isozymes (all IC_{50} values > 100 μM), and their relaxing activities in porcine coronary arteries were well correlated with the inhibitory activities toward cGMP-PDE (r = 0.88, $p < 0.05$). One of these compounds, **3b**, elevated the intracellular cGMP level in isolated porcine coronary arteries without causing any change in the cAMP level. We consider that this series of compounds dilates coronary arteries via potent and specific inhibition of cGMP-PDE.

Introduction

Phosphodiesterases (PDEs) which hydrolyze cyclic nucleotides, cAMP and cGMP, have been classified into five isozyme families,[1] but research has been hampered by the lack of potent and selective inhibitors of several isozyme families,[2] including cGMP phosphodiesterase (cGMP-PDE, type V).[3] The well-known cGMP-PDE inhibitors zaprinast and MY-5445 (Chart 1) have only moderate potencies and selectivities.

We have reported a novel potent inhibitor of cGMP-PDE, 4-[[3,4-(methylenedioxy)benzyl]amino]-6,7,8-trimethoxyquinazoline (**3a**; Chart 2), and have shown by structure–activity relationship (SAR) studies that the 4-[3,4-(methylenedioxy)benzyl]amino group (part A) is essential for potent inhibitory activity.[4] However, since we had fixed the 6-, 7-, and 8-substituents of the quinazoline moiety as trimethoxy groups (part B), we could not examine the role of the substituents at these positions. In addition, **3a** was insufficiently selective for cGMP-PDE because it also had a moderate inhibitory activity toward Ca^{2+}–calmodulin-dependent PDE (CaM-PDE, type I).

In the present study, we synthesized various quinazoline derivatives substituted at the 5- to 8-positions, fixing the 4-substituent as a [3,4-(methylenedioxy)benzyl]amino group. Corresponding 3-[3,4-(methylenedioxy)benzyl]-quinazolin-4(3*H*)-ones were also synthesized. These compounds were evaluated for cGMP-PDE inhibitory activity, and potent inhibitors were further evaluated for inhibitory activities toward the other PDE isozymes. We also examined the relaxing effects of the potent and selective inhibitors as well as the effect of a representative compound on cyclic nucleotide levels in isolated porcine coronary arteries.

Chemistry

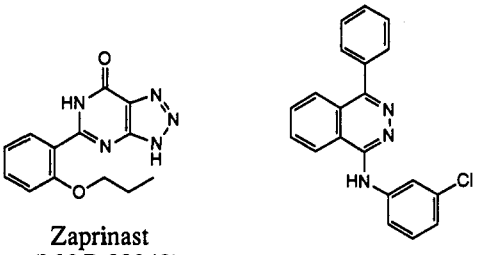
General synthetic procedures were as follows. The quinazolin-4(3*H*)-ones (**1b–n**), obtained from anthranilic acid derivatives by heating with formamide, were chlorinated with $POCl_3$. The resultant 4-chloroquinazolines (**2b–n**) were reacted with piperonylamine in the presence of Na_2CO_3 or triethylamine to give **3b–n**. Compound **1o** and its chlorinated compound **2o** were synthesized according to the literature[5] followed by the same reaction as described above to give **3o**. The syntheses of **4f,i,o** and **5f** were similar to those of **4a** and **5a**, respectively, in the previous paper[4] (Scheme 1).

Compound **3f** was oxidized with *m*-chloroperbenzoic acid (MCPBA) at 0 °C to afford **6**, which was further oxidized likewise at room temperature to afford **7** (Scheme 2). Both reactions proceeded in fairly good yields.

Compounds **8** and **9** were obtained from **3g** by standard synthetic reactions. Condensation of **9** and amines with diethyl phosphorocyanidate (DEPC) afforded **10**, **11**, and **12** (Scheme 3).

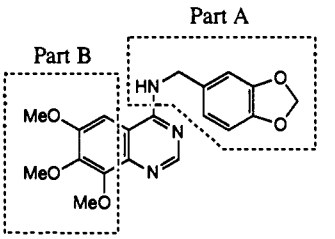
6-Cyanoquinazoline derivatives are synthetically new, and 4-chloro-6-cyanoquinazoline (**2p**) was expected to be useful as an intermediate for introducing various substituents at the 4-position in further studies. We therefore

Chart 1



Zaprinast (M&B 22948) MY-5445

Chart 2



3a

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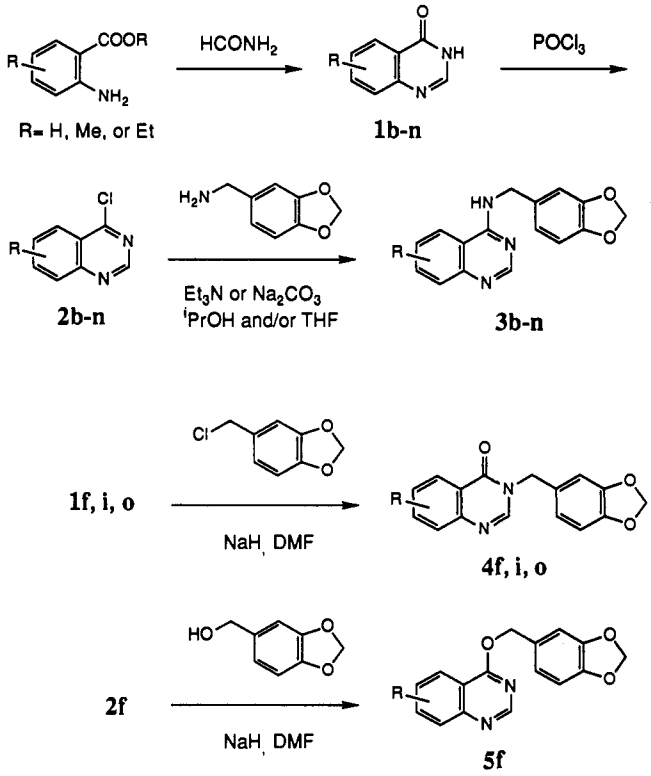
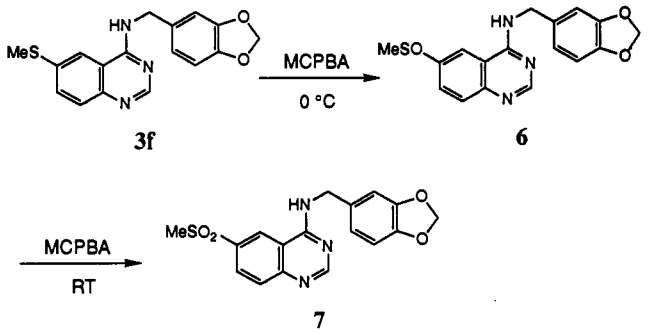
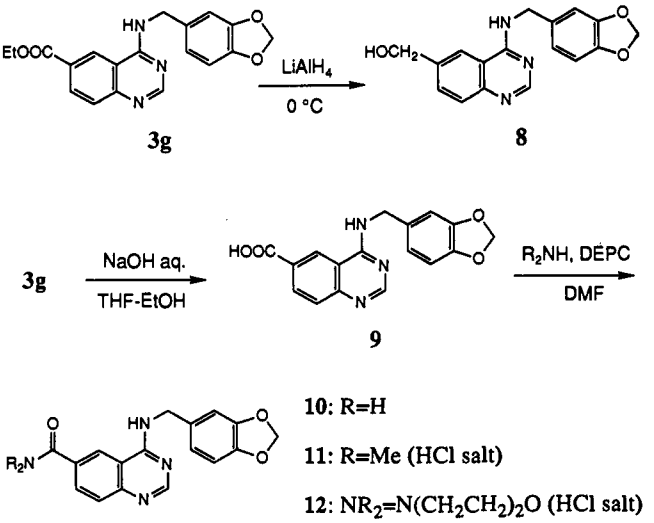
Scheme 1



Scheme 2



Scheme 3



searched for a facile and efficient synthetic route to **2p**. The chloro atom of ethyl 5-chloro-2-nitrobenzoate was easily substituted by a nucleophile such as thiomethoxide or imidazole (unpublished results) but could not be substituted with cyanide under any conditions that we tried (various cyanides such as CuCN and various solvents;

Scheme 4

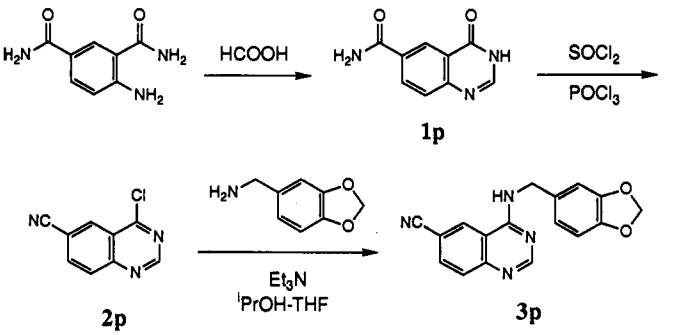
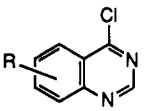


Table 1. New 4-Chloroquinazolines Synthesized



2e-g, j, k, m, p

compd	R	formula[a]	mp, °C (recrystn solv)[b]	yield, %[c]
2e	6-OEt	$C_{10}H_9ClN_2O$	120–121 (A)	93
2f	6-SMe	$C_8H_7ClN_2S$	134–135 (B)	94
2g	6-COOEt	$C_{11}H_9ClN_2O_2$	107–108 (B)	31
2p	6-CN	$C_9H_4ClN_3$	219–220 (C)	35
2j	8-OMe	$C_9H_7ClN_2O$	134–135 (B)	71
2k	5,6-$(MeO)_2$	$C_{10}H_9ClN_2O_2$[d]	111–112 (B)	57
2m	6,8-$(MeO)_2$	$C_{10}H_9ClN_2O_2$	180–181 (B)	92

[a] Analyses for C, H, and N were within ±0.4% of the expected values for the formula. [b] Key: A = hexane, B = AcOEt–hexane, and C = AcOEt. [c] Yields were the values after column chromatography and were not optimized. [d] C: calcd, 53.47; found, 52.55. N: calcd, 12.47; found, 11.95.

data not shown). Then, dehydration of the amide group in appropriate synthetic intermediates was examined. 4-Aminoisophthalamide was condensed with formic acid to afford 6-carbamoylquinazolin-4(3*H*)-one (**1p**). We found it difficult to obtain the dehydrated compound or the chlorinated compound, using $SOCl_2$ or $POCl_3$ alone, respectively, so we tried simultaneous dehydration and chlorination of **1p** by refluxing with a mixture of $SOCl_2$ and $POCl_3$. The product, **2p**, was easily isolated and purified. It reacted with piperonylamine in the same manner as the other 4-chloroquinazolines, to afford **3p** (Scheme 4).

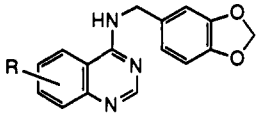
Chemical data of the new 4-chloroquinazolines are listed in Table 1.

Pharmacological Results and Discussion

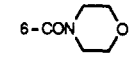
Cyclic GMP-PDE and the other four PDE isozymes were isolated from porcine aorta and used in the inhibition assays,[2] the conditions of which are briefly described in the Experimental Section. Compound **3a** was used as an active control in each screening assay for the inhibition of cGMP-PDE.

The cGMP-PDE inhibitory activities of the 4-[[3,4-(methylenedioxy)benzyl]amino]quinazoline derivatives are listed in Table 2. Only compounds substituted at the 6-position of the quinazoline ring exhibited potent inhibitory activity toward cGMP-PDE. The preferred substituents were methoxy (**3b**, IC_{50} = 0.23 μM), methyl (**3c**, IC_{50} = 0.10 μM), chloro (**3d**, IC_{50} = 0.019 μM), thiomethyl (**3f**, IC_{50} = 0.031 μM), and cyano (**3p**, IC_{50} = 0.090 μM) groups. It is clear that electronic effects are not involved because the above groups include both electron-donating and electron-withdrawing groups. We consider that a hydrophobic substituent with certain steric limitations is

Table 2. Structures, Properties, and cGMP-PDE Inhibitory Activities of 4-[[3,4-(Methylenedioxy)benzyl]amino]quinazolines



3a-p, 6-12

compd	R	formula[a]	mp, °C (recrystn solv)[b]	yield, %[c]	IC_{50}, μM[d]
3a	6,7,8-$(OMe)_3$				0.31 ± 0.05 (n = 11)
3b	6-OMe	$C_{17}H_{15}N_3O_3$	207–208 (A)	86	0.23 ± 0.03 (n = 10)
3c	6-Me	$C_{17}H_{15}N_3O_2$	203–204 (A)	68	0.10 ± 0.02 (n = 11)
3d	6-Cl	$C_{16}H_{12}N_3O_2$	199–200 (A)	76	0.019 ± 0.004 (n = 4)
3e	6-OEt	$C_{18}H_{17}N_3O_3$	190–191 (A)	44	0.38
3f	6-SMe	$C_{17}H_{15}N_3O_2S$	174–175 (A)	83	0.031 ± 0.008 (n = 4)
6	6-S(O)Me	$C_{17}H_{15}N_3O_3S \cdot H_2O$	154–155 (A)	80	0.38
7	6-SO_2Me	$C_{17}H_{15}N_3O_4S \cdot 0.2H_2O$	192–193 (A)	81	0.81
3g	6-COOEt	$C_{19}H_{17}N_3O_4$	156–157 (A)	96	0.62
8	6-CH_2OH	$C_{17}H_{15}N_3O_3 \cdot 0.2H_2O$	176–177 (C)	34	0.93
9	6-COOH	$C_{17}H_{13}N_3O_4$	247–248 dec (D)	98	7.5
10	6-$CONH_2$	$C_{17}H_{14}N_4O_3$	289–291 dec (E)	73	0.77
11[e]	6-$CONMe_2$	$C_{19}H_{18}N_4O_3 \cdot HCl$	228–229 (F)	79	2.3
12[e]		$C_{21}H_{20}N_4O_4 \cdot HCl \cdot 0.5H_2O$	208–209 (F)	87	2.7
3p	6-CN	$C_{17}H_{12}N_4O_2$	243–244 (B)	89	0.090 ± 0.009 (n = 3)
3h	H	$C_{16}H_{13}N_3O_2$	197–198 (A)	69	4.2
3i	7-Cl	$C_{16}H_{12}ClN_3O_2$	209–210 (A)	62	1.1
3j	8-OMe	$C_{17}H_{15}N_3O_3$	204–205 (A)	76	5.9
3k	5,6-$(OMe)_2$	$C_{18}H_{18}N_3O_4$	122–123 (B)	74	0.73
3l	6,7-$(OMe)_2$	$C_{18}H_{17}N_3O_4$	221–222 (B)	77	0.84
3m	6,8-$(OMe)_2$	$C_{18}H_{18}N_3O_4$	217–218 (A)	88	0.81
3n	6,7-OCH_2O-	$C_{17}H_{13}N_3O_4 \cdot 0.2H_2O$	229.231 (B)	55	1.6
3o	6-SMe, 7-OMe	$C_{18}H_{17}N_3O_3S$[f]	200–205 dec (B)	39	0.35

[a] Analyses for C, H, and N were within ±0.4% of the expected values for the formula unless otherwise noted. [b] Key: A = $CHCl_3$–hexane, B = AcOEt–hexane, C = THF–hexane, D = THF–EtOH–H_2O, E = DMF–H_2O, and F = EtOH–Et_2O. [c] Yields were not optimized. [d] IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least three points). In cases where repeated determinations were made, values are given as mean ± SEM (number of experiments). [e] HCL salt. [f] N: Calcd, 11.82; found, 11.05.

required on the basis that: (1) the inhibitory activity tended to be attenuated with increase of substituent size (**3b**, **3e**; **6**, **7**) and (2) hydrophilic groups such as hydroxyl (**8**), carboxyl (**9**), and carbamoyl (**10**) groups that possess an acidic proton are disfavored. However, the substituents present in the more potent compounds, chloro, thiomethyl, and cyano groups, may also act as hydrogen-bonding acceptors, and this feature may also contribute to their potency.

The unsubstituted compound (**3h**) or the compounds with 7- or 8-substitution instead of 6-substitution (**3i,j**) showed only weak inhibitory activities. Furthermore, the presence of a 5-, 7-, or 8-substituent resulted in loss of the inhibitory activities, even in the presence of an effective substituent at the 6-position (**3k–o**); the inhibitory potencies of these compounds were even weaker than that of **3a**, the 6,7,8-trisubstituted compound, though the reason for this is not clear.

If the methylenedioxy group of the inhibitors interacts with the catalytic site of cGMP-PDE, the structural similarity of the quinazoline to the guanine part of cGMP[4] may imply that the 6-substituent on the quinazoline corresponds to the 2-amino group of the guanine. According to the results of our SAR studies, we postulate that the 2-amino group of cGMP interacts with cGMP-PDE in such a hydrophobic and steric manner, unlike the 2-amino group of the guanine moiety which is regarded as a hydrogen-bonding donor in the DNA double helix. Further SAR studies are needed to elucidate the above hypothesis of the superimposition.

3-[3,4-(Methylenedioxy)benzyl]quinazolin-4(3*H*)-ones were also synthesized and evaluated for cGMP-PDE inhibitory activity (Table 3). The comparatively potent inhibitory activity of **4a** was lost in the 6- or 7-monosubstituted or 6,7-disubstituted derivatives (**4f,i,o**). We concluded that the SARs of this series of compounds do not correspond to those of the 4-substituted quinazoline derivatives described above. The 4-[3,4-(methylenedioxy)-benzyl]oxy group was ineffective even when a suitable substituent was present at the 6-position (**5f**).

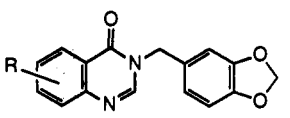
The potent cGMP-PDE inhibitors, **3b–d,f,p**, had little effect on the other PDE isozymes (all IC_{50} values > 100 μM, Table 4). In particular, they completely lacked inhibitory activity toward CaM-PDE, whereas the trimethoxy derivative **3a** had a moderate inhibitory activity (IC_{50} = 5.5 μM).

Papaverine (a nonselective PDE inhibitor) and buquineran[6] (a selective inhibitor of cGMP-inhibited PDE, type III) bear a structural resemblance to the prototype compound **3a** (Chart 3), but these compounds possess 6,7-dimethoxy groups in the heteroaromatic nucleus. These results suggest that the SAR of the 6-monosubstituted quinazolines are specific and only applicable to the inhibitory activity toward cGMP-PDE.

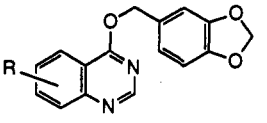
Compounds **3b–d,f,p** were then examined for relaxing effects in isolated porcine coronary arteries precontracted with $PGF_{2\alpha}$ (10^{-5} M).[7] The EC_{50} values of these compounds (relaxation-EC_{50}) are included in Table 4. They were well correlated with the inhibitory activities toward cGMP-PDE ($r = 0.88$, $p < 0.05$) as shown in Figure 1 by a linear regression analysis of -log(relaxation-EC_{50}) vs -log(type V-IC_{50}). This correlation suggests that the physiological action of these compounds to dilate coronary arteries is a consequence of their inhibition of cGMP-PDE.

Finally, we evaluated the effects of **3b** on the cyclic nucleotide levels in isolated porcine coronary arteries (Figure 2). Compound **3b** significantly elevated cGMP

Table 3. Structures, Properties, and cGMP-PDE Inhibitory Activities of 3-[3,4-(Methylenedioxy)benzyl]quinazolin-4(3*H*)-ones and 4-[[3,4-(Methylenedioxy)benzyl]oxy]quinazolines



4a, f, i, o



5a, f

compd	R	formula[a]	mp, °C (recrystn solv)[b]	yield, %[c]	IC_{50}, μM[d]
4a	6,7,8-(OMe)$_3$				0.53
4f	6-SMe	$C_{17}H_{14}N_2O_3S$	162–163 (B)	64	2.3
4i	7-Cl	$C_{16}H_{11}ClN_2O_3$	147–148 (B)	67	8.7
4o	6-SMe, 7-OMe	$C_{18}H_{18}N_2O_4S$[e]	210–215 dec (B)	7	3.6
5a	6,7,8-(OMe)$_3$				1.0
5f	6-SMe	$C_{17}H_{14}N_2O_3S$	104–105 (A)	69	1.4

[a–d] See the corresponding footnotes a–d in Table 2. [e] C: calcd, 60.66; found, 61.77.

Table 4. Inhibitory Activities of **3a–d,f,p** on Five PDE Isozymes and Their Relaxing Effects in Isolated Porcine Coronary Arteries Precontracted with $PGF_{2\alpha}$

	IC_{50}, μM[a]					
compd	V	I	II	III	IV	EC_{50}, μM[b]
3a	0.36 ± 0.09	5.5 ± 2.5	8.7 ± 1.2	>100	>100	2.13 ± 0.35 (n = 28)
3b	0.23 ± 0.03	>100	>100	>100	>100	1.19 ± 0.54 (n = 16)
3c	0.10 ± 0.02	>100	>100	>100	>100	1.56 ± 0.55 (n = 14)
3d	0.019 ± 0.004	>100	>100	>100	>100	0.19 ± 0.06 (n = 11)
3f	0.031 ± 0.008	>100	>100	>100	>100	0.12 ± 0.03 (n = 8)
3p	0.090 ± 0.009	>100	>100	>100	>100	0.30 ± 0.10 (n = 8)

[a] (I) Ca^{2+}–calmodulin-dependent PDE; (II) cGMP-stimulated PEE; (III) cGMP-inhibited PDE (IC_{50} of milrinone = 0.74 ± 0.10 μM); (IV) cAMP-specific PDE (IC_{50} of rolipram = 0.72 ± 0.17 μM); (V) cGMP-PDE. All IC_{50} values were determined from the logarithmic concentration–inhibition plot (at least three points) and are given as mean ± SEM from at least three experiments. [b] EC_{50} values were determined from the logarithmic cumulative concentration–relaxation plot and are given as mean ± SEM (number of experiments).

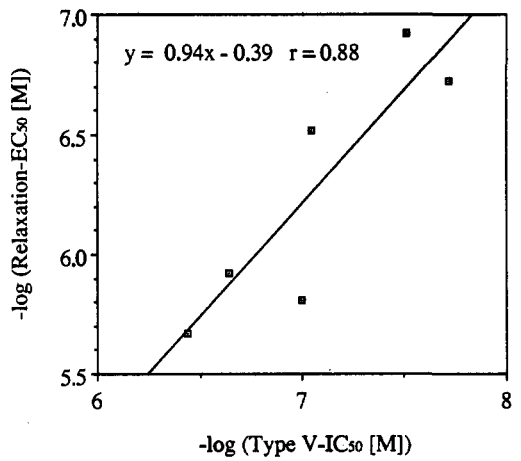
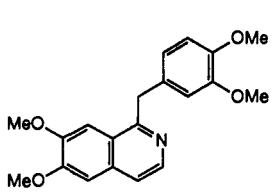
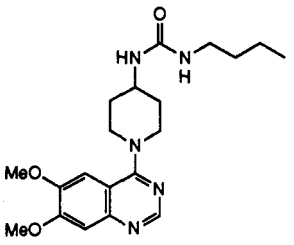


Figure 1. Correlation of relaxing effects in isolated coronary arteries with cGMP-PDE inhibitory activities (least-squares regression analysis).

Chart 3



Papaverine



Buquineran

level without causing any significant change in cAMP level, as was reported for **3a** in the previous paper.[4]

In conclusion, we have found that 6-monosubstituted quinazoline derivatives related to **3a** exhibited greatly potentiated inhibitory activity toward cGMP-PDE and the steric size and hydrophobic character of the substituent seemed to be important. Unlike **3a**, these compounds lacked inhibitory activity toward CaM–PDE, but they retained potent relaxing activity on porcine coronary arteries, which was well correlated with the inhibitory activity toward cGMP-PDE. One of the potent and selective inhibitors, **3b**, markedly elevated the intracellular cGMP level. These results suggest that this series of compounds dilates coronary arteries via potent and specific inhibition of cGMP-PDE. The cardiohemodynamic effects of these selective inhibitors *in vivo* are under investigation.

Experimental Section

Melting points (mp) were determined on an electrothermal capillary melting point apparatus and are uncorrected. 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 Analytical Chemistry Section of Eisai Tsukuba Research Laboratories.

4-Chloro-6-(methylthio)quinazoline (2f). General Procedure. A suspension of 6-(methylthio)quinazolin-4(3*H*)-one (**1f**; 4.00 g, 20.8 mmol) in $POCl_3$ (40 mL) was heated under reflux for 3 h and concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered through a small amount of silica gel, and evaporated to give 4.12 g (94%) of **2f**. An analytical sample was recrystallized from EtOAc–hexane in the similar experiment: mp 134–135 °C; ^1H NMR ($CDCl_3$) δ 2.61 (3H, s), 7.80 (1H, dd, J = 8.8, 2.0 Hz), 7.97 (1H, d, J = 8.8 Hz), 8.03 (1H, d, J = 2.0 Hz), 8.99 (1H, s); MS m/e (FAB) 211 (MH^+). Anal. ($C_9H_7ClN_2S$) C, H, N.

4-[[3,4-(Methylenedioxy)benzyl]amino]-6-(methylthio)-quinazoline (3f). General Procedure. A mixture of **2f** (4.12 g, 19.6 mmol), piperonylamine (3.70 g, 24.5 mmol), and Na_2CO_3 (3.50 g, 33.0 mmol) in 2-propanol (100 mL) was refluxed overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (eluted with

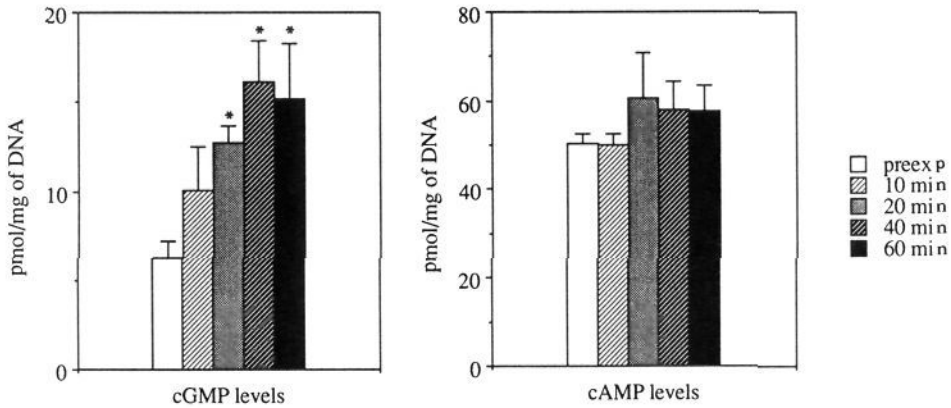


Figure 2. Effects of **3b** on the intracellular cGMP and cAMP levels in isolated porcine coronary arteries. The methods are described in the Experimental Section. The concentration of the drug was 30 μM in each case. Each value is the mean ±SEM (n = 5 or 6), expressed in pmol/mg of DNA. *: $p < 0.05$ vs preexposure (one-way analysis of variance followed by Duncan's multiple comparison test).

EtOAc–hexane) and recrystallized from $CHCl_3$–hexane to give **3f** as pale yellow crystals (5.32 g, 83%): mp 174–175 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (3H, s), 4.79 (2H, d, J = 5.2 Hz), 5.93 (2H, s), 6.77 (1H, d, J = 8.0 Hz), 6.89 (1H, d, J = 8.0 Hz), 6.94 (1H, s), 7.62 (1H, dd, J = 8.8, 2.0 Hz), 7.75 (1H, d, J = 8.8 H), 7.97 (1H, d, J = 2.0 Hz), 8.10 (1H, br s), 8.56 (1H, s); MS m/e (FAB) 326 (MH^+). Anal. ($C_{17}H_{15}N_3O_2S$) C, H, N.

3-[3,4-(Methylenedioxy)benzyl]-6-(methylthio)quinazolin-4(3*H*)-one (4f). General Procedure. To a mixture of 0.50 g (2.6 mmol) of **1f** and 0.55 g (3.2 mmol) of piperonyl chloride in 20 mL of DMF was added 0.15 g (3.8 mmol) of 60 wt % sodium hydride, and the mixture was stirred at 70 °C for 3 h. After cooling, H_2O was added and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluted with EtOAc–hexane) and recrystallized from EtOAc–hexane to give 0.54 g (64%) of **4f** as colorless needles: mp 162–163 °C; ^{1}H NMR ($CDCl_3$) δ 2.58 (3H, s), 5.11 (2H, s), 5.95 (2H, s), 6.78 (1H, d, J = 7.4 Hz), 6.85 (1H, d, J = 7.4 Hz), 6.86 (1H, s), 7.61 (1H, dd, J = 8.8, 2.0 Hz), 7.65 (1H, d, J = 8.8 Hz), 8.06 (1H, d, J = 2.0 Hz), 8.10 (1H, s); MS m/e (FAB) 327 (MH^+). Anal. ($C_{17}H_{14}N_2O_3S$) C, H, N.

4-[[3,4-(Methylenedioxy)benzyl]oxy]-6-(methylthio)quinazoline (5f). To a suspension of 0.21 g (5.3 mmol) of 60 wt % sodium hydride in 10 mL of DMF was added 0.80 g (5.3 mmol) of piperonyl alcohol, and the mixture was stirred at 50 °C for 10 min. Then, 1.00 g (4.8 mmol) of **2f** was added, and the reaction mixture was stirred at 70 °C for 3 h. After cooling, H_2O was added and the aqueous mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluted with EtOAc–hexane) and recrystallized from $CHCl_3$–hexane to give **5f** as pale yellow crystals (1.07 g, 69%): mp 104–105 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (3H, s), 5.56 (2H, s), 6.00 (2H, s), 6.85 (1H, d, J = 8.0 Hz), 7.01 (1H, dd, J = 8.0, 1.6 Hz), 7.03 (1H, d, J = 1.6 Hz), 7.72 (1H, dd, J = 8.8, 1.6 Hz), 7.88 (1H, d, J = 8.8 Hz), 7.89 (1H, d, J = 1.6 Hz), 8.78 (1H, s); MS m/e (FAB) 327 (MH^+). Anal. ($C_{17}H_{14}N_2O_3S$) C, H, N.

4-[[3,4-(Methylenedioxy)benzyl]amino]-6-(methylsulfinyl)quinazoline (6). To a solution of **3f** (1.80 g, 5.53 mmol) in $CHCl_3$ (100 mL) was added dropwise a solution of MCPBA (1.20 g, 6.95 mmol) in $CHCl_3$ (30 mL) with stirring and ice cooling. After stirring for 2 h at 0 °C, the reaction mixture was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and filtered. Purification by flash chromatography (eluted with EtOAc–acetone) and recrystallization from $CHCl_3$–hexane afforded **6** as *pale yellow crystals (1.51 g, 80%): mp 154–155 °C; ^{1}H NMR* ($CDCl_3$) δ 2.75 (3H, s), 4.80 (2H, d, J = 5.2 Hz), 5.96 (2H, s), 6.80 (1H, d, J = 8.0 Hz), 6.89 (1H, d, J = 8.0 Hz), 6.91 (1H, s), 7.06 (1H, br s), 7.64 (1H, d, J = 8.8 Hz), 7.98 (1H, d, J = 8.8 Hz), 8.43 (1H, s), 8.74 (1H, s); MS m/e (FAB) 342 (MH^+). Anal. ($C_{17}H_{15}N_3O_3S \cdot H_2O$) C, H, N.

4-[[3,4-(Methylenedioxy)benzyl]amino]-6-(methylsulfonyl)quinazoline (7). To a solution of **6** (1.00 g, 2.93 mmol) in $CHCl_3$ (50 mL) was added dropwise a solution of MCPBA (0.65 g, 3.8 mmol) in $CHCl_3$ (20 mL) at room temperature. After stirring for 3 h, the reaction mixture was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and filtered. Purification by flash chromatography (eluted with EtOAc) and recrystallization from $CHCl_3$–hexane afforded **7** as yellow crystals (0.85 g, 81%): mp 192–193 °C; ^{1}H NMR ($CDCl_3$) δ 3.13 (3H, s), 4.80 (2H, d, J = 5.2 Hz), 5.95 (2H, s), 6.79 (1H, d, J = 8.0 Hz), 6.91 (1H, d, J = 8.0 Hz), 6.95 (1H, s), 8.05 (1H, d, J = 8.8 Hz), 8.17 (1H, d, J = 8.8 Hz), 8.72 (1H, s), 8.81 (1H, br s), 8.98 (1H, s); MS m/e (FAB) 358 (MH^+). Anal. ($C_{17}H_{15}N_3O_4S \cdot 0.2H_2O$) C, H, N.

6-(Hydroxymethyl)-4-[[3,4-(methylenedioxy)benzyl]amino]quinazoline (8). To a suspension of 0.10 g (2.6 mmol) of $LiAlH_4$ in THF (20 mL) was added dropwise a solution of **3g** (0.35 g, 1.0 mmol) in THF (20 mL) at room temperature. After the mixture was stirred at room temperature for 6 h, H_2O (0.1 mL), 10% aqueous NaOH (0.1 mL), and H_2O (0.3 mL) were added dropwise to the reaction mixture, successively. The mixture was refluxed for 15 min and then cooled, dried over $MgSO_4$, filtered through a small amount of silica gel, and concentrated under reduced pressure. The residual solid was recrystallized from THF–hexane to give 0.16 g (51%) of **8** as pale yellow crystals: mp 176–177 °C; ^{1}H NMR (DMSO-d_6) δ 4.62 (2H, d, J = 5.6 Hz), 4.65 (2H, d, J = 5.6 Hz), 5.36 (1H, t, J = 5.6 Hz), 5.94 (2H, s), 6.82 (1H, s), 6.82 (1H, s), 6.92 (1H, s), 7.63 (1H, d, J = 8.4 Hz), 7.70 (1H, d, J = 8.4 Hz), 8.20 (1H, s), 8.41 (1H, s), 8.74 (1H, t, J = 5.6 Hz); MS m/e (FAB) 310 (MH^+). Anal. ($C_{17}H_{15}N_3O_3 \cdot 0.2H_2O$) C, H, N.

6-Carboxy-4-[[3,4-(methylenedioxy)benzyl]amino]quinazoline (9). To a solution of 2.50 g (7.12 mmol) of **3g** in THF (40 mL) and EtOH (40 mL) was added 1 N aqueous NaOH (40 mL). After stirring at room temperature over night, the reaction mixture was neutralized with 1 N aqueous HCl (40 mL). The precipitated crystals were collected by filtration, washed with H_2O, and dried to give 1.23 g (53%) of pure **9** as white crystals: mp 247–248 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.86 (2H, d, J = 5.6 Hz), 5.99 (2H, s), 6.89 (1H, d, J = 8.0 Hz), 6.92 (1H, d, J = 8.0 Hz), 7.02 (1H, s), 7.92 (1H, d, J = 8.8 Hz), 8.46 (1H, d, J = 8.8 Hz), 8.96 (1H, s), 9.20 (1H, s), 10.88 (1H, br s); MS m/e (FAB) 324 (MH^+). Anal. ($C_{17}H_{13}N_3O_4$) C, H, N.

6-Carbamoyl-4-[[3,4-(methylenedioxy)benzyl]amino]quinazoline (10). To a stirred solution of 0.40 g (1.2 mmol) of **9** in DMF (10 mL) was added 0.30 mL (2.0 mmol) of DEPC at 0 °C, and NH_3 gas was bubbled into the mixture at 0 °C for 30 min. After the ice bath was removed and the mixture was stirred at room temperature for 3 h, H_2O was added to the reaction mixture, and the precipitated crystals were collected by filtration, *washed with H_2O, and dried to give 0.29 g (73%) of pure **10*** as white crystals; mp 289–291 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.68 (2H, d, J = 6.0 Hz), 5.97 (2H, s), 6.85 (1H, d, J = 8.0 Hz), 6.88 (1H, d, J = 8.0 Hz), 6.97 (1H, s), 7.55 (1H, br s), 7.70 (1H, d, J = 8.4 Hz), 7.97 (1H, br s), 8.18 (1H, dd, J = 8.4, 1.6 Hz), 8.50 (1H, s), 8.84 (1H, d, J = 1.6 Hz), 8.92 (1H, br t, J = 6.0 Hz); MS m/e (FAB) 323 (MH^+). Anal. ($C_{17}H_{14}N_4O_3$) C, H, N.

6-[(Dimethylamino)carbonyl]-4-[[3,4-(methylenedioxy)benzyl]amino]quinazoline Hydrochloride (11). To a mix-

ture of 9 (0.36 g, 1.1 mmol), dimethylamine hydrochloride (0.15 g, 1.8 mmol), and Et_3N (0.50 mL, 3.6 mmol) in DMF (5 mL) was added DEPC (0.26 mL, 1.7 mmol), and the whole was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography (eluted with CH_2Cl_2–MeOH) and crystallized from EtOH (containing HCl gas)–ether to give 0.34 g (79%) of 11 as white crystals: mp 228–229 °C; ^{1}H NMR (DMSO-d_6) δ 2.99 (3H, s), 3.05 (3H, s), 4.86 (2H, d, J = 5.6 Hz), 5.99 (2H, s), 6.87 (1H, d, J = 8.0 Hz), 6.92 (1H, dd, J = 8.0, 1.6 Hz), 7.03 (1H, d, J = 1.6 Hz), 7.92 (1H, d, J = 8.4 Hz), 8.06 (1H, dd, J = 8.4, 1.6 Hz), 8.75 (1H, d, J = 1.6 Hz), 8.96 (1H, s), 10.91 (1H, t, J = 5.6 Hz); MS m/e (FAB) 351 (MH^+). Anal. ($C_{19}H_{18}N_4O_3$·HCl) C, H, N.

4-[[3,4-(Methylenedioxy)benzyl]amino]-6-(morpholinocarbonyl)quinazoline hydrochloride (12) was similarly obtained from 9 in 87% yield: mp 208–209 °C; ^{1}H NMR (DMSO-d_6) δ 3.60 (4H, br s), 3.69 (4H, br s), 4.86 (2H, d, J = 5.6 H), 5.99 (2H, s), 6.87 (1H, d, J = 8.4 Hz), 6.92 (1H, dd, J = 8.4, 1.6 Hz), 7.03 (1H, d, J = 1.6 Hz), 7.93 (1H, d, J = 8.8 Hz), 8.05 (1H, dd, J = 8.8, 1.6 Hz), 8.75 (1H, d, J = 1.6 Hz), 8.96 (1H, s), 10.94 (1H, t, J = 5.6 Hz); MS m/e (FAB) 393 (MH^+). Anal. ($C_{21}H_{20}N_4O_4$·HCl·0.5H_2O) C, H, N

6-Carbamoylquinazolin-4(3*H*)-one (1p). A suspension of 4-aminoisophthalamide (5.00 g, 27.9 mmol) in formic acid (100 mL) was heated under reflux for 3 h. After removal of the solvent, the crystalline residue was washed with EtOH and ether, respectively, and dried to give 5.22 g (99%) of 1p as white crystals: mp >260 °C; ^{1}H NMR (DMSO-d_6) δ 7.50 (1H, br s), 7.71 (1H, d, J = 8.4 Hz), 8.16 (1H, s), 8.25 (1H, br s), 8.26 (1H, dd, J = 8.4, 2.0 Hz), 8.67 (1H, d, J = 2.0 Hz), 12.31 (1H, br s); MS m/e (FAB) 190 (MH^+). Anal. ($C_9H_7N_3O_2$·0.25H_2O) C, H, N.

4-Chloro-6-cyanoquinazoline (2p). A suspension of 1p (1.00 g, 5.3 mmol) in $POCl_3$ (30 mL) and $SOCl_2$ (30 mL) was heated under reflux for 36 h. The reaction mixture obtained as a yellow clear solution was concentrated under reduced pressure. The residue was suspended in EtOAc and washed with saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered through a small amount of silica gel, and evaporated. Recrystallization from EtOAc afforded 0.35 g (35%) of 2p as pale yellow crystals: mp 219–220 °C; ^{1}H NMR ($CDCl_3$) δ 8.12 (1H, dd, J = 8.8, 1.8 Hz), 8.21 (1H, dd, J = 8.8, 0.6 Hz), 8.69 (1H, dd, J = 1.8, 0.6 Hz), 9.19 (1H, s); MS m/e (FAB) 190 (MH^+). Anal. ($C_9H_4ClN_3$) C, H, N.

Enzyme Source and Screening Assay. Five PDE isozymes were separated from the supernatant homogenates of porcine aorta by DEAE-Toyopearl 650S chromatography in the presence of 0.1 mM Ca^{2+} followed by rechromatography in the absence of Ca^{2+} and affinity chromatography on immobilized rolipram[8] or cGMP: Ca^{2+}–calmodulin-dependent PDE (CaM-PDE, type I), cGMP-stimulated PDE (type II), cGMP-inhibited PDE (type III), cAMP-specific PDE (type IV), and cGMP-PDE (type V).[2]

PDE activity was determined by a modification of a previously described two-step radioisotropic procedure.[9] [^{3}H]cGMP or [^{3}H]cAMP at a concentration of 1 μM was used as a substrate. The substrate used in the inhibition assay for each isozyme is as follows: type I, cGMP; type II, cAMP; type III, cAMP; type IV, cAMP; type V, cGMP. The tested compounds were dissolved in DMSO and then diluted with assay buffer, at concentrations ranging from 10^{-8} to 10^{-4} M. The final concentration of DMSO was less than 0.4% (v/v) so that it had no interferences in the screening assay system.[4]

Relaxing Effect on Isolated Coronary Arteries Precontracted with $PGF_{2\alpha}$. Porcine coronary arteries were removed, cleaned of adjacent tissues, and cut into rings with special care not to damage 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 (final concentration, 7×10^{-9} M)-induced relaxation of strips precontacted 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 tested compound were constructed. Relaxation was calculated as a percentage of the contractile response to $PGF_{2\alpha}$.

Measurement of the Intracellular cGMP and cAMP Levels in Smooth Muscle Cells of Isolated Coronary Arteries. Porcine coronary arteries were removed, cleaned of adjacent tissues, cut transversally into rings, denuded of endothelial cells, and incubated in Krebs–Henseleit solution (37 °C, pH 7.4, bubbled with 95% O_2–5% CO_2). The coronary arterial strips were preincubated for 40 min, during which period the incubation medium was changed three times. After this, $PGF_{2\alpha}$ (final concentration, 10^{-5} M) was added and the strips were incubated for 60 min. Then, a tested compound (final concentration, 30 μM) was added for a period of 0, 10, 20, 40, or 60 min. The pieces of artery were quickly frozen in liquid N_2 and stored at −80 °C before being homogenized in 1 mL of 10% TCA. Each homogenate was centrifuged, and the supernatant was extracted with water-saturated ethyl ether. The organic layer was discarded, and the aqueous solution was subjected to radioimmunoassay for cyclic nucleotides.[10] Cyclic nucleotide levels were expressed with respect to DNA, which was extracted from the pellets and assayed fluorometrically by previously described methods.[11]

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Supplementary Material Available: Additional analytical and experimental data for the compounds (19 pages). Ordering information is given on any current masthead page.

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