Discovery of Potent Cyclic GMP Phosphodiesterase Inhibitors. 2-Pyridyl- and 2-Imidazolylquinazolines Possessing Cyclic GMP Phosphodiesterase and Thromboxane Synthesis Inhibitory Activities

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Moderate cyclic GMP phosphodiesterase (cGMP-PDE, PDE V) inhibitor 2-phenyl-4-anilinoquinazoline (**1**) was identified utilizing MultiCASE assisted drug design (MCADD) technology. Modification of compound **1** was conducted at the 2-, 4-, and 6-positions of the quinazoline ring for enhancement of cGMP-PDE inhibitory activity. The 6-substituted 2-(imidazol-1-yl)-quinazolines are 1000 times more potent in *in vitro* PDE V enzyme assay than the well-known inhibitor zaprinast. The 6-substituted derivatives of 2-(3-pyridyl)quinazoline **84** and 2-(imidazol-1-yl)quinazoline **86** exhibited more than 1000-fold selectivity for PDE V over the other four PDE isozymes. In addition, cGMP-PDE inhibitors **64**, **65**, and **73** were found to have an additional property of thromboxane synthesis inhibitory activity.

Introduction

Cyclic nucleotide phosphodiesterase (PDE) enzymes can be grouped into five isozymes according to their specificity toward hydrolysis of cyclic AMP (cAMP) or cyclic GMP (cGMP): their sensitivity to regulation by calcium or calmodulin and their selective inhibition by various compounds.[1] Current research interest in PDE has focused largely on cAMP-PDE III/IV isozymes.[2] With the discovery that both atrial natriuretic peptides (ANP) and endothelium-derived relaxing factor (EDRF), identified as NO, lower blood pressure via stimulation of vascular smooth muscle guanylyl cyclase resulting in increase in intracellular levels of cGMP, interest in cGMP-PDE V, the principal isozyme that breaks down cGMP, has increased.[3–5] Zaprinast, a moderately selective inhibitor of cGMP-PDE V, has demonstrated beneficial antiplatelet and vasodilatory activity in animal studies presumably as a result of inhibiting the hydrolysis of cGMP via PDE V.[6]

The purpose of this investigation was 2-fold: first, to design and synthesize cGMP-PDE V inhibitors with greater potency and PDE isozyme selectivity than zaprinast to provide a pharmacologic tool that would permit a more careful biological exploration of the role of this isozyme in the control of blood pressure, it is well known that thromboxane, the product of thromboxane A_2 (TxA_2) synthase, is an extremely potent vasoconstrictor and inducer of platelet aggregation,[7] and thus, second, if possible, to incorporate into the cGMP-PDE V inhibitor(s) TxA_2 synthesis inhibitory activity to determine whether this additional property would result in improved antiplatelet and/or vasodilatory activity than PDE V inhibition alone. The pharmacological properties of these molecules will be reported separately.

We utilized the previously described[8] MultiCASE assisted drug design (MCADD) technology as an aid to identify compound **1**. MCADD is an expert learning program which automatically identifies and defines structure–activity relationships (SAR) thereby identifying potentially causal relationships between molecular structural fragments and biological activity. The program is capable of analyzing structurally diverse data sets. The activating structural fragments or biophores can cut across disparate structural classes. Biophores can subsequently be utilized as starting points for compound design and subsequent synthesis. The program has been applied toward defining SAR analyses in a variety of therapeutic areas.[9] In this project, databases consisting of cGMP-PDE inhibitors as well as of TxA_2 synthase inhibitors were constructed and analyzed separately in order to derive structural fragments responsible for each respective activity. Our analysis indicated common structural features present in both of the well-known cGMP-PDE inhibitors zaprinast[10] and MY-5445,[11] which may be conducive to cGMP-PDE inhibitory activity. Both zaprinast and MY-5445 share the common MCADD molecular fragment "N=C–NH". The fragment was found to be independent of chemical class. Considering the environment within which the fragment occurs implies that the structural feature would be "N=C(NH)C=CH". Therefore, it was assumed that a target molecule incorporating this feature in a proper structural skeleton should also possess cGMP-PDE inhibitory property. This led to the identification of **1** (Figure 1) as a new cGMP-PDE inhibitor. It should be noted that compound **1** (IC_{50} = 5.6 μM) exhibits similar activity to zaprinast (IC_{50} = 2.7 μM) and MY-5445 (IC_{50} = 5.0 μM). This paper discusses the further structural modifications of the identified target molecule undertaken for the enhancement of cGMP-PDE V inhibitory activity. Furthermore

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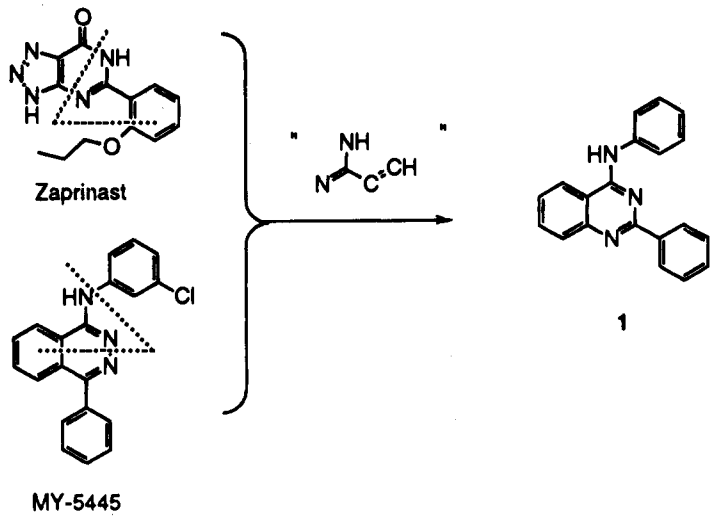
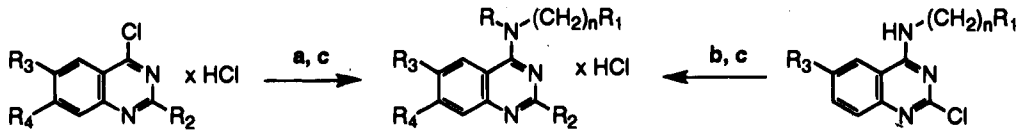


Figure 1. Identification of common structural features shared by zaprinast and MY-5445 led to design of a new cGMP-PDE inhibitor, 1.

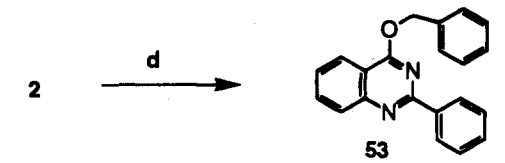
Scheme 1[a]



1, 25-52, 54-91, 93-95

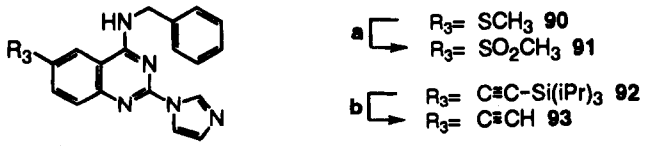
2, R_2= Ph, R_3=R_4= H
3, R_2= 2-thienyl, R_3=R_4= H
4, R_2= 2-furyl, R_3=R_4= H
5, R_2= CH=CHPh, R_3=R_4= H
6, R_2= cyclohexyl, R_3=R_4= H
7, R_2= 4-Py, R_3=R_4= H
8, R_2= 2-Py, R_3=R_4= H
9, R_2= 3-Py, R_3=R_4= H
10, R_2= 3-Py, R_3= Me, R_4= H
11, R_2= 3-Py, R_3= F, R_4= H
12, R_2= 3-Py, R_3= H, R_4= F
13, R_2= 3-Py, R_3= Cl, R_4= H
14, R_2= 3-Py, R_3= NO_2, R_4= H

15, n= 1, R_1= Ph, R_3= H
16, n= 1, R_1= 2-thienyl, R_3= H
17, n= 1, R_1= 2-furyl, R_3= H
18, n= 1, R_1= cyclohexyl, R_3= H
19, n= 2, R_1= Ph, R_3= H
20, n= 1, R_1= Ph, R_3= Cl
21, n= 1, R_1= Ph, R_3= Br
22, n= 1, R_1= Ph, R_3= OMe
23, n= 1, R_1= Ph, R_3= SMe
24, n= 1, R_1= Ph, R_3= C≡CSi(i-Pr)$_3$



[a] (a) $R_1(CH_2)_nNHR$; (b) imidazole or morpholine or piperazine, heat; (c) HCl; (d) $PhCH_2OH/NaH$.

Scheme 2[a]



[a] (a) H_2O_2/AcOH; (b) $N(nBu)_4F$.

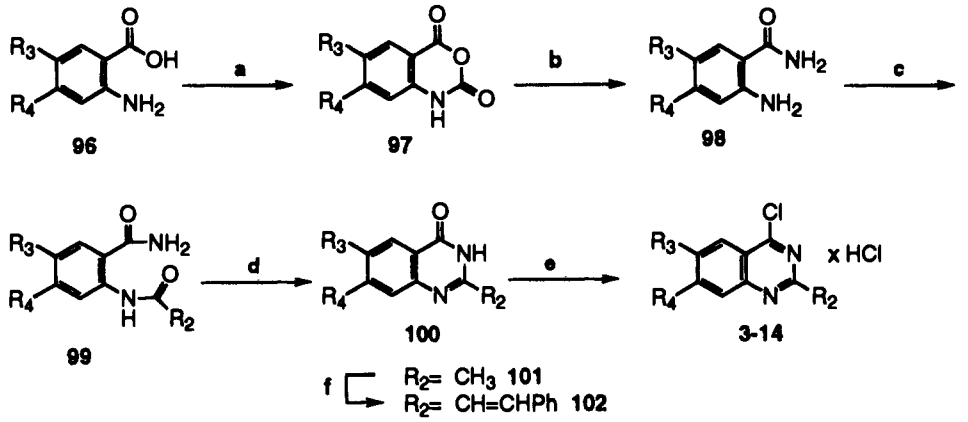
the incorporation of TxA_2 synthesis inhibitory activity to selected compounds is also discussed.

Chemistry

The syntheses of the title compounds described in Tables 1–3 were accomplished using Schemes 1 and 2. The 4-substituted 2-phenylquinazolines **1** and **25–52** (Scheme 1) were prepared in good yield by a nucleophilic displacement reaction of the commercially available 4-chloro-2-phenylquinazoline (**2**) with various primary or secondary amines.[12] In a similar manner, 4-chloroquinazolines **3–14** reacted readily with various amines to give the desired 2,4-disubstituted quinazolines **54–65**, **68–72**, **79–85**, and **94** in reasonable yields. In order to introduce a tertiary amino group at the 2-position, the appropriate 2-chloroquinazolines **15–24** were reacted with the desired amine under much stronger reaction conditions (**66–67**, **73–78**, **86–90**, **95**). For instance, **15** was heated with an excess amount of imidazole to give 2-substituted quinazoline **73** in good yield. The oxygen analog[13] 2-phenyl-4-(benzyloxy)-quinazoline (**53**) was prepared by the reaction of **2** with the sodium salt of benzyl alcohol.

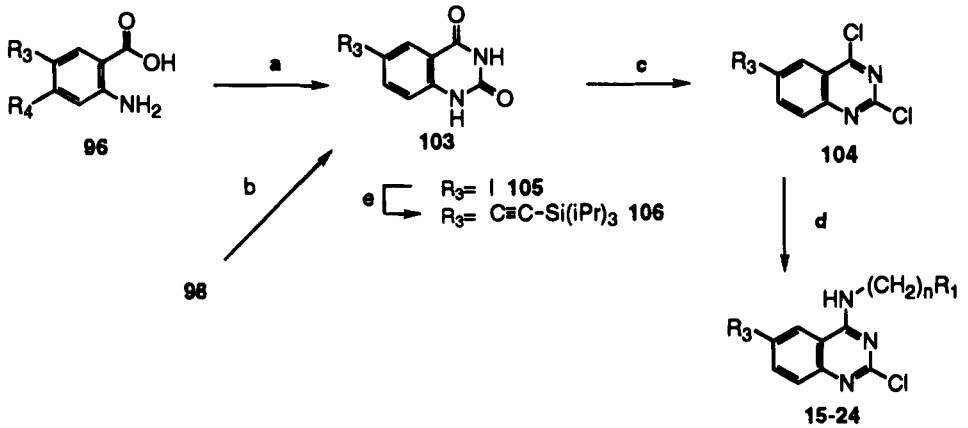
Scheme 2 outlines the syntheses of 6-sulfonyl- and 6-ethynylquinazolines, respectively. The 6-sulfonyl compound **91** was prepared by the oxidation of 6-methylthio precursor **90** with hydrogen peroxide in acetic acid. The 6-ethynyl compound **93** was obtained by desilation of

Scheme 3[a]



[a] (a) Phosgene; (b) NH_3; (c) RCOCl/reflux; (d) reflux; (e) $POCl_3$ or $SOCl_2$; (f) PhCHO.

Scheme 4[a]



[a] (a) KCNO/NaOH; (b) phosgene; (c) $POCl_3$/reflux; (d) $R_1(CH_2)_nNH_2$; (e) $(iPr)_3$Siacetylene/Ph_3P, $PdCl_2$, CuI/Et_2NH.

6-triisopropylethynyl compound **92** with tetra-*n*-butylammonium fluoride.

A standard synthetic method was adopted to prepare quinazolin-4-one **100**, a precursor to 4-chloroquinazolines (Scheme 3).[14,15] The substituted anthranilamide **98** was prepared by treating the corresponding anthranilic acid **96** with an excess of phosgene to yield isatoic anhydride **97** followed by treatment with ammonia. The reaction of anthranilamide **98** with an acid chloride gave the *N*-acylated product **99** which was cyclized with sodium methoxide in refluxing toluene to give the 2-substituted quinazolin-4-one **100**. Compound **100** underwent reaction with phosphorous oxychloride to form 4-chloroquinazoline derivatives **3**–**14**. Compound 2-styrylquinazolin-4-one (**102**)[15] was prepared from 2-methylquinazolin-4-one (**101**) by treatment with benzaldehyde according to literature procedure.

Scheme 4 depicts the preparation of 2-chloroquinazolines **15**–**24**. The 6-substituted quinazolin-2,4-dione **103** was prepared readily from the anthranilamide **98** by treatment with phosgene. Alternatively, literature procedure[16] was used to prepare 6-substituted quinazolin-2,4-dione **103** through an addition and cyclization reaction of the appropriate anthranilic acid **96** and potassium cyanate. The substituted 2,4-dichloroquinazoline **104** was prepared in excellent yield by refluxing quinazoline-2,4-dione **103** in phosphorous oxychloride.[17] Selective replacement of the 4-chloro group from 2,4-dichloroquinazoline (**104**) with a nucleophile (such as a primary amine) gave 4-substituted 2-chloroquinazolines **15**–**24** in excellent yield.

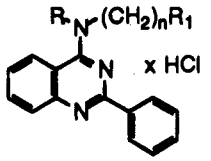
Considerable effort was made to introduce substitutions on the 6-position of the quinazoline, which was observed to be a potency tuning site. The synthesis of 6-[(triisopropylsilyl)ethynyl]quinazoline-2,4-dione (**106**) (Scheme 4) was accomplished by using a classic Heck coupling reaction between (triisopropylsilyl)acetylene and the requisite 6-iodoquinazoline-2,4-dione (**105**) in the presence of a catalytic amount of palladium chloride. The coupling product **106** was then converted to 6-[(triisopropylsilyl)ethynyl]-4-(benzylamino)-2-imidazolylquinazoline (**92**), a precursor to compound **93** (Scheme 2). The bulkier (triisopropylsilyl)acetylene group was used for its stability in multistep reactions.

Discussion

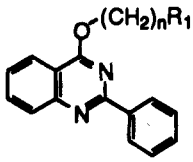
The biological methods used in the study are described in the Experimental Section. The different molecular forms of PDE were isolated using standard methods (PDE I from bovine aorta, PDE III and V from human platelets, PDE II from rat brain, and PDE IV from rat kidney).[6] All test compounds were dissolved in dimethyl sulfoxide (final concentration of 2.5%), and the enzyme activity was assessed by measuring the hydrolysis of [^{3}H]cAMP or [^{3}H]cGMP.[23] Zaprinast was used as a reference compound in the PDE inhibitory assays.

In order to develop the SAR for the cGMP-PDE inhibitory activity, modification of **1** was conducted in

Table 1. 2-Phenylquinazolines and cGMP-PDE Inhibitory Activity



1, 25-52



53

compd	n	R	R_1	mp, °C	yield, %[a]	method	mol formula[b]	IC_{50}, μM[c]
1	0	H	Ph	285[d] dec	95	A	$C_{20}H_{15}N_3 \cdot HCl \cdot 0.1H_2O$	5.6
25	0	Me	Ph	249–252	68	A	$C_{21}H_{17}N_3 \cdot HCl$	20
26	0	H	c-hexyl	153–155	22	A	$C_{20}H_{21}N_3$	3.9
27	0	H	c-pentyl	167–168	37	A	$C_{19}H_{19}N_3$	2.4
28	0	H	2-ClPh	149–150	50	A	$C_{20}H_{14}ClN_3 \cdot 0.2H_2O$	5.5
29	0	H	3-ClPh	230–243	57	A	$C_{20}H_{14}ClN_3 \cdot HCl \cdot H_2O$	1.0
30	0	H	4-ClPh	250–260	72	A	$C_{20}H_{14}ClN_3 \cdot 0.6H_2O$	2.15
31	0	H	3-NO_2Ph	208–210	50	A	$C_{20}H_{14}N_4O_2$	0.42
32	0	H	3-(OMe)Ph	244–246	50	A	$C_{21}H_{17}N_3O \cdot HCl$	0.76
33	0	H	3-(CO_2Me)Ph	149–151	34	A	$C_{22}H_{17}N_3O_2$	0.19
34	0	H	4-(CO_2Me)Ph	180–181	67	A	$C_{22}H_{17}N_3O_2$	0.86
35	0	H	3-Py[e]	224–225	7	A	$C_{19}H_{14}N_4 \cdot HCl \cdot 0.2H_2O$	13.0
36	0	H	1-pyrrolyl	170–172	46	A	$C_{18}H_{14}N_4$	6.0
37	0	H	3-(5-Me-isoxazolyl)	203–206	69	A	$C_{18}H_{14}N_4O \cdot 0.3H_2O$	3.6
38	1	H	Ph	128–129[f]	59	A	$C_{21}H_{17}N_3 \cdot 0.1H_2O$	0.32
39	1	Me	Ph	130–132	68	A	$C_{22}H_{19}N_3$	14
40	1	H	2-ClPh	137–138	71	A	$C_{21}H_{16}ClN_3 \cdot 0.3H_2O$	0.80
41	1	H	3-ClPh	130–132	50	A	$C_{21}H_{16}ClN_3 \cdot 0.2H_2O$	0.80
42	1	H	4-ClPh	145–148	68	A	$C_{21}H_{16}ClN_3$	0.74
43	1	H	3-NO_2Ph	135–137	60	A	$C_{21}H_{16}N_4O_2$	1.3
44	1	H	2-thienyl	119–120	20	A	$C_{19}H_{15}N_3S$	1.2
45	1	H	2-furyl	125–128	57	A	$C_{19}H_{15}N_3O$	4.7
46	1	H	2-Py	230 dec	99	A	$C_{20}H_{16}N_4 \cdot 2HCl \cdot 0.8H_2O$	11
47	1	H	3-Py	214–216	39	A	$C_{20}H_{16}N_4C \cdot 0.2H_2O$	8.6
48	1	H	2-THF	99–100	71	A	$C_{19}H_{19}N_3O$	8.8
49	2	H	Ph	119–120	33	A	$C_{22}H_{19}N_3 \cdot 0.2H_2O$	1.9
50	3	H	CO_2Et	91–94	35	A	$C_{20}H_{21}N_3O_2$	4.3
51	5	H	OH	116–118	55	A	$C_{19}H_{21}N_3O \cdot 0.3H_2O$	15
52	2	H	CH_3	105–107	85	A	$C_{17}H_{17}N_3$	6.2
53	1		Ph	121–122	50		$C_{21}H_{16}N_2O$	18.5
			zaprinast					2.7
			MY-5445					5.0

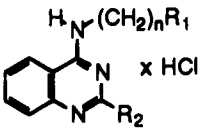
[a] Yields are not optimized. [b] Analyses for C, H, and N are within ±0.4% of the expected value for the formula. [c] IC_{50} values were determined from the logarithmic concentration–inhibition curve (five concentrations) against PDE V isolated from human platelets, and reference compound zaprinast was run in each assay. Ranges for data are within ±15%. [d] Freebase lit.[12c] mp 249 °C. [e] Py = pyridyl. [f] Lit.[12c] mp 118 °C.

a stepwise fashion. The initial focus of the investigation was to determine the effects of various groups at the C-4-position of 2-phenylquinazolines on the cGMP-PDE inhibitory activity (Table 1). The effects of the substituents at the 2-position on the cGMP-PDE inhibitory activities of the 2,4-disubstituted quinazolines were studied next (Table 2), and then substituent modifications at the 6- and 7-positions of the quinazoline nucleus were conducted to modulate the cGMP-PDE inhibitory activity (Table 3).

Modification of the 4-Position of 1. Replacement of the N-H group with a N-Me group (**25**, **39**) or an oxygen atom (**53**) reduced the cGMP-PDE inhibitory activity, indicating that this N-H amino group may play a role as a hydrogen bond donor. The cGMP-PDE inhibitory potency was increased with the addition of a spacer group between the phenyl and the amino N-H group, such as the benzylamino compound **38**. Inhibitory activity of the chain homolog phenethylamino **49** was in between that of the anilino (**1**) and benzylamino (**38**) compounds. Comparison of the activities of **29**–**34** indicated that the meta and para substitution on the 4-anilino group of **1** potentiated the inhibitory activity in the range of the 4-benzylamino compound **38**. The 4-anilino group could be replaced with a cyclohexylamino or cyclopentylamino group (**26**, **27**) without reduction of the inhibitory activity. These results indicate that at the 4-position of 2-phenylquinazoline, the potency is affected by the lipophilicity along with some steric requirement of the group. The replacement of the aryl group at the 4-position with a basic pyridyl group (**35**, **46**, **47**) was not well tolerated.

Modification of Position 2. Compounds **1** and **38** (anilino and benzylamino derivatives) were selected for the initial study of the effect of substituents at the 2-position (Table 2). Replacement of the phenyl group at the 2-position of **1** with heterocyclic groups, such as thienyl (**54**), furyl (**55**), and pyridyl (**58**), slightly potentiated the inhibitory activity. The same replacement at the 2-position of **38** with thienyl (**59**), furyl (**60**), and 2-pyridyl (**63**) resulted in the loss of some inhibitory activity; however, with imidazolyl (**73**), 3-pyridyl (**64**), and 4-pyridyl (**65**), the inhibitory activity was retained. The 4-benzylamino series imparted greater activity than the 4-anilino series for some of the 2-substituted quinazolines (**59** vs **54**, **61** vs **56**, **65** vs **58**). Overall, the heterocyclic group at the 2-position was compatible for cGMP-PDE inhibitory activity. The importance of this

Table 2. 2,4-Disubstituted Quinazolines and cGMP-PDE Inhibitory Activity



15, 54-80

compd	n	R_1	R_2	mp, °C	yield, %[a]	method	mol formula[b]	IC_{50}, μM[c]
54	0	Ph	2-thienyl	137–139	41	B	$C_{18}H_{13}N_3S$	2.4
55	0	Ph	2-furyl	183–4 dec	14	B	$C_{18}H_{13}N_3O{\cdot}0.15C_4H_{10}O$	2.3
56	0	Ph	CH=CHPh	216–217	93	B	$C_{22}H_{17}N_3$	9.0
57	0	Ph	c-hexyl	134–135	70	B	$C_{20}H_{21}N_3$	6.8
58	0	Ph	4-Py[d]	270–274	72	B	$C_{19}H_{14}N_4{\cdot}0.6H_2O$	1.2
59	1	Ph	2-thienyl	158–163	50	B	$C_{19}H_{15}N_3S{\cdot}0.1H_2O$	0.72
60	1	Ph	2-furyl	152–154	76	B	$C_{19}H_{15}N_3O{\cdot}0.2H_2O$	2.5
61	1	Ph	CH=CHPh	202–203	86		$C_{23}H_{19}N_3{\cdot}0.1H_2O$	1.3
62	1	Ph	c-hexyl	138–140	75	B	$C_{21}H_{23}N_3$	11.0
63	1	Ph	2-Py	140–155 dec	74	B	$C_{20}H_{16}N_4{\cdot}2HCl{\cdot}H_2O$	2.3
64	1	Ph	3-Py	137–138	16	B	$C_{20}H_{16}N_4$	0.45
65	1	Ph	4-Py	195–197	88	B	$C_{20}H_{16}N_4{\cdot}0.1H_2O$	0.31
66	1	Ph	4-morpholino	120–123	42	C	$C_{19}H_{20}N_4O$	4.3
67	1	Ph	4-Me-1-piperazinyl	130–132	43	C	$C_{20}H_{23}N_5{\cdot}0.1H_2O$	22.0
15	1	Ph	Cl	178–180	77	C	$C_{15}H_{12}ClN_3$	4.6
68	1	2-thienyl	3-Py	255 dec	93	B	$C_{18}H_{14}N_4S{\cdot}2HCl$	0.36
69	1	2-furyl	3-Py	210–219 dec	64	B	$C_{18}H_{14}N_4O{\cdot}2HCl{\cdot}H_2O$	1.7
70	1	3-Py	3-Py	257 dec	89	B	$C_{19}H_{15}N_5{\cdot}3HCl{\cdot}H_2O$	2.8
71	1	2-THF	3-Py	201–210	63	B	$C_{18}H_{18}N_4O{\cdot}2HCl{\cdot}H_2O$	5.0
72	1	c-Pr	3-Py	230–239 dec	77	B	$C_{17}H_{16}N_4{\cdot}2HCl{\cdot}1.1H_2O$	4.4
73	1	Ph	1-Im[e]	212–214	77	C	$C_{18}H_{15}N_5{\cdot}0.15H_2O$	0.26
74	1	2-thienyl	1-Im	234–235	29	C	$C_{16}H_{13}N_5S{\cdot}0.5H_2O$	0.21
75	1	2-furyl	1-Im	230	90	C	$C_{16}H_{13}N_5O{\cdot}2HCl{\cdot}0.8H_2O$	0.63
76	1	2-THF	1-Im	98–150	24	C	$C_{16}H_{19}N_5O{\cdot}2HCl{\cdot}1.3H_2O$	3.0
77	1	c-hexyl	1-Im	140–150	55	C	$C_{18}H_{21}N_5{\cdot}2HCl{\cdot}2.5H_2O$	0.23
78	2	Ph	1-Im	70–100 dec	65	C	$C_{19}H_{17}N_5{\cdot}2HCl{\cdot}0.6H_2O$	0.86
79	2	Ph	3-Py	220–250	65	B	$C_{21}H_{18}N_4{\cdot}2HCl{\cdot}H_2O$	0.54
80	2	2-(3-Me-pyrrolyl)	3-Py	140–142	49	B	$C_{20}H_{19}N_5{\cdot}0.3H_2O$	1.1

[a] Yields are not optimized. [b] Analyses for C, H, and N are within ±0.4% of the expected value for the formula. [c] IC_{50} values were determined from the logarithmic concentration–inhibition curve (five concentrations) against PDE V isolated from human platelets, and reference compound zaprinast was run in each assay. Ranges for data are within ±15%. [d] Py = pyridyl. [e] Im = imidazolyl.

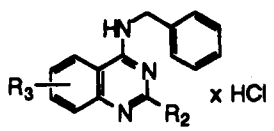
result will become apparent in the thromboxane section of the Discussion. The additional study of the series indicated that 2-heterocycle-substituted pyridyl or imidazolyl compounds were well tolerated with a variety of groups substituted at the 4-position (**68–80**). For the same 4-position substitutions (except for 4-benzylamino), the cGMP-PDE inhibitory activity of the 2-position imidazolyl or pyridyl compounds imparted greater activity than the 2-position phenyl compounds, and moreover, 2-position imidazolyl compounds were slightly more potent than the 2-position pyridyl compounds.

Modification of Positions 6 and 7. The 2-substituted imidazole (**73**) and 2-substituted pyridyl (**64**) compounds were selected for study of the effect of substituents at the 6- and 7-positions. The 6- and 7-positions of the quinazoline ring were found to modulate the cGMP-PDE inhibitory potency (Table 3). The results revealed that the 6-position of this series (**82**) was more sensitive than the 7-position (**83**) against cGMP-PDE. Extensive studies on the 6-position substitution were done in order to study the effect on potency. In general, as expected from the discussion in the previous section, the 2-imidazole compounds were more potent than the corresponding 2-pyridyl compounds. Although the 6-nitro compound **88** (IC_{50} = 0.0026 μM) is 6-fold more potent than the 6-methoxy derivative **89** (IC_{50} = 0.016 μM), the activity of the 6-methylsulfonyl **91** (IC_{50} = 0.11 μM) was much weaker than that of the 6-methylthio derivative **90** (IC_{50} = 0.014μM). These results indicate that electronic effect (σ) may not be directly related to the potency against cGMP-PDE. In the 2-(3-pyridyl)quinazoline series, there appeared to be some trend where increasing either the σ and/or π value of the substituents (H, **64** < F, **82** < Cl, **84** < NO_2, **85**) increased the potency toward cGMP-PDE; however, in the 2-imidazole series, this effect was not observed.

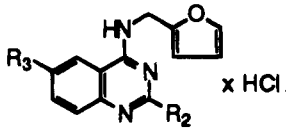
Since the direct electronic effect of 6-position substituents was not observed, we explored other possible molecular interactions attributing to the potency. The ethynyl group (**93**) with its weak electronic effect was taken under consideration because of its unique column shape π–π triple bond. It is noteworthy that ethynyl substitution at the 6-position (**93**) yielded the most potent cGMP-PDE inhibitor in this series with an IC_{50} of 0.5 nM. Our hypothesis according to this study is that the triple bond may provide additional binding through a stack effect. Further studies are required to understand this phenomenon.

At the time of completion of our work, Takase and his co-workers published their finding of the inhibitory effect of 4-[[(methylenedioxy)benzyl]amino]quinazolines against cGMP-PDE V.[4a] A similar observation was found based on their studies between the inhibitory activity toward cGMP-PDE and substituents at the 6-position of the 4-[[(methylenedioxy)benzyl]amino]-quinazoline ring.

Table 3. 2,4,6-Trisubstituted Quinazolines and cGMP-PDE Inhibitory Activity



81-91, 93



94-95

compd	R_3	R_2	mp, °C	yield, %[a]	method	mol formula[b]	IC_{50}, μM[c]
81	6-CH_3	3-Py[d]	265–269 dec	92	B	$C_{21}H_{18}N_4 \cdot 2HCl \cdot 0.6H_2O$	0.105
82	6-F	3-Py	200–202 dec	29	B	$C_{20}H_{15}FN_4 \cdot HCl \cdot 0.4H_2O$	0.165
83	7-F	3-Py	250	67	B	$C_{20}H_{15}FN_4 \cdot 2HCl$	0.48
84	6-Cl	3-Py	255	45	B	$C_{20}H_{15}ClN_4 \cdot 2HCl$	0.026
85	6-NO_2	3-Py	289–292 dec	77	B	$C_{20}H_{15}N_5O_2 \cdot 2HCl$	0.010
86	6-Cl	1-Im[e]	186 dec	73	C	$C_{18}H_{14}ClN_5 \cdot 2HCl \cdot 0.3H_2O$	0.0028
87	6-Br	1-Im	199–202 dec	36	C	$C_{18}H_{14}BrN_5 \cdot 2HCl \cdot 1.1H_2O$	0.0023
88	6-NO_2	1-Im	190 dec	26	C	$C_{18}H_{14}N_6O_2 \cdot 2HCl \cdot 1.5H_2O$	0.0026
89	6-OCH_3	1-Im	192–197	76	C	$C_{19}H_{17}N_5O \cdot 2HCl$	0.016
90	6-SCH_3	1-Im	192–195	73	C	$C_{19}H_{17}N_5S \cdot 2HCl \cdot 0.8H_2O$	0.014
91	6-SO_2CH_3	1-Im	125–130	52		$C_{19}H_{17}N_5O_2S \cdot 2HCl \cdot 2.5H_2O$	0.11
93[f]	6-C≡CH	1-Im	125–127	63		$C_{20}H_{15}N_5 \cdot 0.1H_2O$	0.00053
94	Cl	3-Py	218–222	68	B	$C_{18}H_{13}ClN_4O \cdot 2HCl \cdot H_2O$	0.12
95	OCH_3	1-Im	143–151	61	C	$C_{17}H_{15}N_5O_2 \cdot 2HCl$	0.072

[a] Yields are not optimized. [b] Analyses for C, H, and N are within ±0.4% of the expected value for the formula unless otherwise noted. [c] IC_{50} values were determined from the logarithmic concentration–inhibition curve (five concentrations) against PDE V isolated from human platelets, and reference compound zaprinast was run in each assay. Ranges for data are within ±15%. [d] Py = pyridyl. [e] Im = imidazolyl. [f] Tested as a hydrochloride salt.

Table 4. PDE Selectivity Data

	IC_{50}, μM[a]				
compd	I	II	III	IV	V
38	22.5	24.0	>100	8.2	0.32
59	13.0	21.0	>100	7.6	0.72
64	21.0	19.0	>100	9.0	0.45
65	7.0	17.0	>100	7.5	0.31
73	14.0	25.0	50	3.1	0.26
84	>50	>50	>50	>50	0.026
85	>3.0	>3.0	>3.0	>3.0	0.010
86	>3.0	>3.0	>3.0	>3.0	0.0028
89	2.8	>3.0	20	3.3	0.016
zaprinast	68	100	660	160	2.7
milrinone			1.5		110

[a] IC_{50} values were determined from the logarithmic concentration–inhibition curve. PDEs were isolated from several sources (PDE I from bovine aorta, PDE III and V from human platelets, PDE II from rat brain, and PDE IV from rat kidney).

Evaluation of some of the cGMP-PDE V inhibitory active compounds against other PDE isozymes was conducted (Table 4). The 2-aryl-4-quinazoline compounds **38**, **59**, **64**, **65**, and **73** were moderately selective (10–25-fold) for PDE V over PDE I, II, III, and IV isozymes. In this study, the reference cGMP-PDE V inhibitor zaprinast exhibited selectivity of 25-fold over other PDE isozymes. The 6-substituted quinazoline compounds **84–86** and **89** were more selective (175–1900-fold) for PDE V over the other four PDE isozymes. Specifically, compounds **84** and **86**, the 6-position-substituted derivatives of 2-(pyridyl)quinazoline **64** and 2-(imidazolyl)quinazoline **73**, respectively, are more than 1000-fold selective for PDE V over other PDE isozymes. The results indicate that the potency toward PDE V is modulated by the substituent at the 6-position. Although the 6-ethynyl compound **93** was a potent cGMP-PDE inhibitor, this molecule contains a reactive terminal aryl ethynyl group which has the propensity of undergoing Michael addition in a biological system. With the identification of other potent selective 6-substituted compounds, this compound, **93**, was not tested further.

In an effort to expand the therapeutic usefulness of TxA_2 synthase inhibitors, others have explored the incorporation of this property into molecules with other known activities. The TxA_2 synthase inhibition property has been combined with angiotensin-converting enzyme inhibition, 5-lipoxygenase inhibition, cAMP-PDE inhibition, and TxA_2 receptor antagonist; however, to the best of our knowledge, it has not been combined with cGMP-PDE inhibition.[7,18] The minimal structural parameters necessary for a selective TxA_2 synthase inhibitor have been described earlier and consist of a sterically unhindered 1*H*-imidazol-1-yl or 3-pyridyl nitrogen separated from a carboxylic acid terminus by 8–10 Å.[18a] The MCADD analysis indicated similar results as reported in the literature; however, it also indicated that appropriately placed imidazolyl and pyridyl moieties in the molecule might be sufficient to impart TxA_2 synthase inhibition property. Also there is an example of a (3-pyridylmethyl)benzoquinone as a TxA_2 synthase inhibitor in the literature which lacks the carboxylic acid moiety or the carboxylic acid isostere.[18c] If possible, we desired to incorporate the TxA_2 synthesis inhibitory property into the cGMP-PDE inhibitory molecule without the loss of the cGMP-PDE inhibitory activity. It was observed that substitutions of a variety of heterocycles at the 2-position of the quinazoline were tolerated for the cGMP-PDE activity (Table 2). Thus, it was reasoned that incorporation of a 1*H*-imidazol-1-yl or 3-pyridyl group at the 2-position of the quinazoline ring should yield molecules exhibiting TxA_2 synthesis inhibition with cGMP-PDE inhibition, which led to compounds **64** and **73**. These compounds were evaluated for inhibitory activity against human TxA_2 synthesis and cyclooxygenase (CO) in washed platelets *in vitro* (Table 5) and were found to be selectively inhibiting *in vitro* human thromboxane synthesis. These compounds also retained the cGMP-PDE inhibitory activity. With this finding and in keeping with our main objective, the 2-(1*H*-imidazol-1-yl)- and 2-(3-pyridyl)quinazoline series were further

Table 5. Inhibitory Activities of Compounds against Human TxA_2 Synthesis and Cyclooxygenase (CO) in Washed Platelets *in Vitro*

compd	IC_{50}, μM	
	TxA_2	CO
64	5.8	50
65	0.83	14
73	0.22	51
89	2.50	NT
OKY-046	3.3	NT

[a] IC_{50} values were determined from the logarithmic concentration–inhibition curve using the radiometric assay with [^{14}C]arachidonic acid.

Table 6. Inhibitory Activities of Compounds on the Thromboxane B_2 Production in A23187-Induced Rat Whole Blood *in Vitro* and *ex Vivo*[a]

compd	*in vitro* IC_{50}, μM	*ex vivo* ED_{50}, mg/kg
64	>10	>30
65	1.8	11
73	1.1	5.7
89	0.83	2.6

[a] IC_{50} and ED_{50} values were determined from the logarithmic concentration–inhibition curve and dose–inhibition curve, respectively, using enzyme immunoassay.

modified for increased cGMP-PDE inhibitory activity as discussed in the previous section. A compound, **89**, in the middle range of cGMP-PDE inhibitory potency was selected as a representative of the 6-substituted quinazoline series for evaluation in the thromboxane synthesis inhibition assay, and this compound was found to exhibit good inhibitory activity against the human TxA_2 synthesis in washed platelets. In the TxA_2 rat whole blood assay (Table 6), although the 2-(1*H*-imidazol-1-yl)quinazolines **73** and **89** exhibited good activity, the 2-(3-pyridyl)quinazoline **64** did not exhibit a desirable level of TxA_2 synthesis inhibition. The search for other pyridyl derivatives as TxA_2 synthesis inhibitors led to screening of 2-(4-pyridyl)quinazoline **65**. Although this 2-(4-pyridyl) compound offered good TxA_2 synthesis inhibitory activity, it was not pursued further because the imidazolylquinazoline series were equally potent and synthetically more accessible.

Conclusion

Modification of 2-phenyl-4-anilinoquinazoline (**1**), identified utilizing MCADD technology, was carried out to develop potent cGMP-PDE inhibitors. The cGMP-PDE V inhibitory activity of 6-substituted 2-imidazolylquinazolines is ca. 1000 times more potent than that of zaprinast. The potency of cGMP-PDE V was modulated by the substituent at the 6-position; the 6-substituted derivatives of 2-(pyridyl)quinazoline **64** and 2-(imidazolyl)quinazoline **73** are 1000-fold selective for PDE V over other PDE isozymes. Also, cGMP-PDE inhibitors (**64**, **65**, **73**) with an additional property of thromboxane synthesis inhibitory activity were identified.

Experimental Section

Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. The chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Infrared spectra were recorded on a Shimadzu IR-460 infrared spectrophotometer. The TLC analyses were run on E. Merck precoated silica gel 60F-254 plates of 0.25 mm thickness. Compounds of interest were detected either by ultraviolet lamp (Spectroline; 254 nm) or by staining with iodine. All reactions were carried out under a N_2 atmosphere. The *in vitro* phosphodiesterase assays were conducted in the laboratory of Ronald E. Weisharr, Coromed, Inc. (Troy, NY). The microanalyses were conducted at Oneida Research Services, Inc. (Whitesboro, NY).

Method A. 4-(Benzylamino)-2-phenylquinazoline (38). A solution of 4-chloro-2-phenylquinazoline[19] (**2**) (0.72 g, 3.0 mmol) and benzylamine (0.64 g, 6.0 mmol) in 20 mL of THF was heated to reflux for 6 h. After cooling to 23 °C and stirring for 18 h, the reaction mixture was diluted with ether and filtered. The filtered material was rinsed with ether, and the combined filtrate was concentrated under reduced pressure. The concentrate was purified over a silica gel column with 30% EtOAc–hexane as eluent. The product **38** was obtained as a white powder (0.55 g, 59% yield): mp 128–129 °C. ^{1}H NMR ($CDCl_3$): δ 5.01 (d, 2H), 5.94 (t, 1H), 7.30–7.52 (m, 9H), 7.62–7.78 (m, 2H), 7.95 (d, 1H), 8.56 (m, 2H). IR (KBr): 3300 (m), 1615 (w), 1562 (s), 1528 (s), 1374 (s), 768 (m), 738 (m) cm^{-1}. Anal. ($C_{21}H_{17}N_3 \cdot 0.1H_2O$) C, H, N.

Method B. 4-(Benzylamino)-2-(3′-pyridyl)quinazoline (64). 2-[*N*-(3-Pyridylcarbonyl)amino]anthranilamide. To a solution of anthranilamide (8.2 g, 60 mmol) and triethylamine (18.0 g, 180 mmol) in 100 mL of THF/CH_2Cl_2 (1:1) was added nicotinoyl chloride hydrochloride (10.8 g, 60 mmol). The mixture was allowed to stir at 23 °C for 6 h. The solution was then concentrated under reduced pressure. The concentrate was taken up in EtOAc and water and the mixutre filtered to give a crude product. The solid material was triturated with ether and filtered. The desired product was obtained as a yellow powder (11.5 g, 80% yield): mp 220–222 °C. ^{1}H NMR (DMSO-d_6): δ 7.22 (t, 1H), 7.64 (m, 2H), 7.93 (d, 2H), 8.27 (m, 1H), 8.48 (s, 1H), 8.69 (d, 1H), 8.83 (d, 1H), 9.12 (s, 1H).

2-(3′-Pyridyl)quinazolin-4-one. To a solution of 2-[*N*-(3-pyridylcarbonyl)amino]anthranilamide (11.5 g, 48 mmol) in 100 mL of toluene was added NaOMe (95%, 5.7 g, 100 mmol). The resulting solution was heated to reflux for 18 h. The reaction mixture was concentrated, and the residue was washed with aqueous NH_4Cl solution and CH_2Cl_2. The product was obtained as a gray powder (6.7 g, 63% yield): mp 275–276 °C (lit.[20] mp 276 °C). ^{1}H NMR (DMSO-d_6): δ 7.50–7.61 (m, 2H), 7.75–7.90 (m, 2H), 8.16 (d, 1H), 8.49 (m, 1H), 8.77 (d, 1H), 9.31 (s, 1H). IR (KBr): 3185 (w), 3045 (m), 2915 (w), 1677 (s), 1603 (m), 1558 (w), 1474 (m), 769 (m) cm^{-1}.

4-Chloro-2-(3′-pyridyl)quinazoline (9). A solution of 2-(3′-pyridyl)quinazolin-4-one (6.7 g, 30 mmol) and *N*,*N*-dimethylaniline (5.7 mL, 45 mmol) in 200 mL of benzene was heated to reflux, under a N_2 atmosphere, for 30 min with the removal of 15 mL of distillate. After cooling to 23 °C, $POCl_3$ (4.5 g, 29 mmol) was added and the resulting solution heated to reflux for 6 h. After cooling, the reaction was quenched with ice–water and the solution washed with dilute NaOH solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The concentrate was triturated with ether, and the solid was collected by filtration to give **9** (3.0 g, 41% yield): mp 178–179 °C. ^{1}H NMR ($CDCl_3$): δ 7.53 (t, 1H), 7.73 (t, 1H), 8.00 (t, 1H), 8.11 (d, 1H), 8.30 (d, 1H), 8.78 (bs, 1H), 8.93 (d, 1H), 9.82 (bs, 1H).

4-(Benzylamino)-2-(3′-pyridyl)quinazoline (64). A solution of **9** (0.48 g, 2.0 mmol) and benzylamine (0.42 g, 4.0 mmol) in 15 mL of THF was allowed to stir at 23 °C for 17 h. The solution was then diluted with ether and filtered. The filtrate was concentrated under reduced pressure, and the concentrate was purified over a silica gel column with 60% EtOAc–hexane as eluent. The isolated material was triturated with ether and the product collected as a solid (100 mg, 16% yield): mp 137–138 °C. ^{1}H NMR ($CDCl_3$): δ 5.01 (d, 2H), 6.20 (t, 1H), 7.26–7.49 (m, 6H), 7.71–7.79 (t, 3H), 7.95 (d, 1H), 8.68 (bs, 1H), 8.82 (m, 1H), 9.75 (bs, 1H). IR (KBr): 3305 (m), 1584 (s), 1520 (s), 1437 (m), 1410 (m), 1365 (s), 1325 (w), 765 (m), 694 (m) cm^{-1}. Anal. ($C_{20}H_{16}N_4$) C, H, N.

Method C. 4-(Benzylamino)-2-(imidazol-1-yl)-6-methoxyquinazoline (89). 2,4-Dichloro-6-methoxyquinazoline. To a suspension of 6-methoxyquinazoline-2,4-dione[21] (8.6 g, 44.7 mmol) in $POCl_3$ (35.5 mL, 380 mmol) was added *N*,*N*-

dimethylaniline (2.91 mL, 23 mmol). The reaction mixture was heated at 100 °C for 48 h. After cooling, the reaction mixture was slowly poured into 500 g of ice–water. The precipitate was collected, taken up in $CHCl_3$, dried over anhydrous $MgSO_4$, and concentrated. The concentrate was purified over a 200 g silica gel column, eluting with $CHCl_3$. The product was obtained as a pale yellow powder (6.6 g, 65% yield). ^1H NMR ($CDCl_3$): δ 4.01 (s, 3H), 7.42 (d, 1H), 7.62 (m, 1H), 7.93 (d, 1H).

4-(Benzylamino)-2-chloro-6-methoxyquinazoline (22). To a solution of 2,4-dichloro-6-methoxyquinazoline (1.99 g, 8.69 mmol) in 45 mL of CH_2Cl_2 was added benzylamine (1.14 mL, 10.4 mmol). After 1 h, triethylamine (1.25 mL, 9 mmol) was added to the reaction mixture and it was stirred at 23 °C for 20 h. The reaction mixture was diluted with $CHCl_3$, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The concentrate was triturated with ether/$CHCl_3$/hexane, and the product was obtained as a white powder in nearly quantative yield (2.65 g). ^1H NMR ($CDCl_3$): δ 3.89 (s, 3H), 4.88 (d, 2H), 5.94 (m, 1H), 6.89 (m, 1H), 7.30–7.47 (m, 6H), 7.74 (d, 1H).

4-(Benzylamino)-2-(imidazol-1-yl)-6-methoxyquinazoline Dihydrochloride (89). A mixture of **22** (2.07 g, 6.91 mmol), imidazole (1.8 g, 27 mmol), and phenol (17 g) was heated to 150 °C for 20 min. After cooling down to 23 °C, the reaction mixture was diluted with $CHCl_3$, washed with 1 N KOH solution twice and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The concentrate was triturated with $CHCl_3$/MeOH/ether solution and the solid collected by filtration. The product 4-(benzylamino)-2-(imidazol-1-yl)-6-methoxyquinazoline was obtained as a white powder (1.81 g, 79% yield): mp 221–224 °C. ^1H NMR (DMSO-d_6): δ 3.91 (s, 3H), 4.86 (m, 2H), 7.06 (s, 1H), 7.20–7.51 (m, 6H), 7.64 (d, 1H), 7.80 (m, 1H), 7.91 (s, 1H), 8.52 (s, 1H), 9.18 (m, 1H). IR (KBr): 1598 (s), 1552 (m), 1474 (s), 1403 (s), 1380 (m), 1354 (w), 1305 (m), 1284 (m), 1059 (m), 1033 (m), 910 (w), 833 (w) cm^{-1}.

To a suspension of the above free base (1.17 g, 3.54 mmol) in 2 mL of MeOH was added 6 mL of 10% HCl–MeOH solution. After stirring for 10 min, the mixture was concentrated and triturated with MeOH and ether. The product **89** was obtained as a pale yellow solid (1.38 g, 96% yield): mp 192–197 °C. ^1H NMR (DMSO-d_6): δ 3.94 (s, 3H), 4.97 (m, 2H), 7.20–7.42 (m, 3H), 7.46–7.60 (m, 3H), 7.72 (d, 1H), 7.87 (m, 1H), 7.94 (m, 1H), 8.40 (m, 1H), 9.75 (m, 1H), 9.98 (m, 1H). IR (KBr): 1630 (s), 1602 (s), 1563 (s), 1453 (m), 1420 (m), 1396 (s), 1343 (m), 1285 (m), 1257 (s), 1113 (m), 1020 (m), 778 (m), 703 (m) cm^{-1}. Anal. ($C_{19}H_{17}N_5O\cdot 2HCl$) C, H, N.

4-(Benzylamino)-2-(imidazol-1-yl)quinazoline (73). 4-(Benzylamino)-2-chloroquinazoline (15). To a solution of 2,4-dichloroquinazoline[17] (1.0 g, 5 mmol) in 50 mL of THF was added benzylamine (0.5 g, 5 mmol) in 10 mL of THF. The mixture became cloudy. After stirring for 30 min, 4 mL of 1 N NaOH solution was slowly added, and then the mixture was stirred for an additional 30 min. Anhydrous Na_2SO_4 was added, and the mixture was filtered. The filtrate was concentrated under reduced pressure, the residue was triturated with ether, and the product was collected to obtain a white solid (1.04 g, 77% yield): mp 178–180 °C. ^1H NMR ($CDCl_3$): δ 4.86 (d, 2H), 6.05 (s, 1H), 7.32–7.51 (m, 6H), 7.62–7.85 (m, 3H). Anal. ($C_{15}H_{12}ClN_3$) C, H, N.

4-(Benzylamino)-2-(imidazol-1-yl)-quinazoline (73). A mixture of **15** (0.81 g, 3.0 mmol), imidazole (0.81 g, 10 mmol), and phenol (3.0 g) was heated to reflux for 4.5 h. The mixture was then taken up in $CHCl_3$, washed twice with NaOH solution, dried over anhydrous K_2CO_3, and concentrated. The concentrate was triturated with ether and the product collected as a solid (0.7 g, 77% yield): mp 212–214 °C. ^1H NMR ($CDCl_3$): δ 4.92 (d, 2H), 6.32 (bs, 1H), 7.13 (s, 1H), 7.32–7.50 (m, 6H), 7.68–7.85 (m, 3H), 8.00 (s, 1H), 8.67 (s, 1H). Anal. ($C_{18}H_{15}N_5\cdot 0.15H_2O$) C, H, N.

4-(Benzyloxy)-2-phenylquinazoline (53). To a suspension of NaH (80% in oil, 75 mg, 2.4 mmol) in 10 mL of anhydrous THF was added a solution of benzyl alcohol (0.24 g, 2.2 mmol) in 8 mL of anhydrous THF via a syringe. After bubbling ceased, **2** (0.48 g, 2.0 mmol) was added all at once. The resulting mixture was stirred at 23 °C for 18 h. After evaporation of the solvent under reduced pressure, the residue was added to a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to afford a crude product which was purified by column chromatography on silica gel (20 g, 200–400 mesh), eluting with 5% EtOAc–hexane. The fraction with $R_f = 0.32$ was collected and evaporated to give the desired product as a white solid (0.31 g, 50% yield): mp 121–122 °C. ^1H NMR ($CDCl_3$): δ 5.79 (s, 2H), 7.35–7.63 (m, 9H), 7.83 (t, 1H), 8.00 (d, 1 H), 8.22 (d, 1H), 8.58–8.63 (m, 2H). IR (KBr): 3060 (w), 2950 (w), 1615 (m), 1574 (s), 1438 (s), 1351 (s), 771 (s) cm^{-1}. Anal. ($C_{21}H_{16}N_2O$) C, H, N.

4-(Benzylamino)-2-styrylquinazoline (61). 2-Styrylquinazolin-4-one (102). The compound was prepared according to literature procedure.[15] A mixture of 2-methylquinazoline (8.0 g, 50 mmol) and benzaldehyde (5.3 g, 50 mmol) in 100 mL AcOH was heated to reflux for 16 h. Upon cooling to 23 °C, the mixture was filtered and the solid rinsed with EtOH and CH_2Cl_2 to yield the desired product (7.5 g, 60% yield). ^1H NMR (DMSO-d_6): δ 7.02 (d, 1H), 7.40–8.24 (m, 10H), 12.35 (bs, 1H).

4-Chloro-2-styrylquinazoline (5). A mixture of **102** (7.0 g, 28.2 mmol) and 25 mL of $POCl_3$ was heated to reflux for 24 h. The material was treated with 2 mL of *N,N*-dimethylaniline, and the reaction mixture continued to reflux for an additional 1 h. The mixture was then concentrated and the residue taken up in $CHCl_3$, washed with water, dried over anhydrous $MgSO_4$, and concentrated. The concentrate was purified on a silica gel column with $CHCl_3$ as eluent. The purified material was triturated with ether/hexane and the product collected (5.1 g, 67% yield). ^1H NMR ($CDCl_3$): δ 7.48–8.62 (m, 11H).

4-(Benzylamino)-2-styrylquinazoline (61). A mixture of **5** (1.3 g, 5 mmol) and benzylamine (1.07 g, 10 mmol) in 25 mL of EtOH was heated to reflux for 26 h. A white precipitate formed after 1 h. The mixture was concentrated and taken up in 20 mL of $CHCl_3$ and 20 mL of aqueous NH_4Cl solution. The aqueous layer was extracted with $CHCl_3$, and the combined $CHCl_3$ extracts were dried over anhydrous Na_2SO_4 and concentrated. The concentrate was triturated with pentane, and the solid was recrystallized from EtOH (1.45 g, 86% yield): mp 202–203 °C. ^1H NMR ($CDCl_3$): δ 4.99 (d, 2H), 5.99 (t, 1H), 7.21–7.50 (m, 10H), 7.61–7.74 (m, 4H), 7.85 (m, 1H), 7.99–8.07 (d, 1H). IR (KBr): 3215 (w), 3055 (w), 2915 (w), 1640 (w), 1613 (m), 1571 (s), 1530 (s), 1389 (s), 959 (m), 747 (m), 699 (m) cm^{-1}. Anal. ($C_{23}H_{19}N_4\cdot 0.1H_2O$) C, H, N.

4-(Benzylamino)-2-(imidazol-1-yl)-6-(methylthio)-quinazoline Dihydrochloride (90). 6-(Methylthio)quinazoline-2,4-dione. To a solution of 2-amino-2-(methylthio)-benzoic acid[22] (4.9 g, 26.7 mmol) in 100 mL of water was added AcOH (1.9 mL, 32 mmol), and the mixture was heated to produce a suspension. The mixture was cooled in an ice bath, and KOCN (2.6 g, 32 mmol) dissolved in 30 mL of water was added dropwise. After stirring for 18 h, 20 g of NaOH was added. All the material dissolved initially, and then slowly precipitate formed. An additional 20 g of NaOH was added while the mixture cooled in an ice bath. The resulting mixture was filtered. The collected solid was stirred in water, and concentrated HCl was added. The resulting mixture was filtered to give the product as a solid (3.7 g, 66% yield). ^1H NMR (DMSO-d_6): δ 2.49 (s, 3H), 7.13 (d, 1H), 7.53–7.72 (m, 2H), 11.14 (s, 1H), 11.31 (s, 1H).

2,4-Dichloro-6-(methylthio)quinazoline. A mixture of 6-(methylthio)quinazoline-2,4-dione (3.6 g, 17 mmol), 30 mL of $POCl_3$, and 1 mL of *N,N*-dimethylaniline was heated to reflux for 5 h. After cooling, the mixture was poured over ice, and the resulting precipitate was filtered. The collected solid was taken up in CH_2Cl_2 and dried over anhydrous $MgSO_4$. The material was then filtered through a short plug of silica gel to remove base line material and concentrated. The residue was triturated with ether/hexane and filtered to afford a solid product (3.85 g, 92% yield): mp 158–160 °C. ^1H NMR ($CDCl_3$): δ 2.64 (s, 3H), 7.78–7.91 (m, 3H). ^1H NMR (DMSO-d_6): δ 2.69 (s, 3H), 7.82 (s, 1H), 7.90–8.10 (m, 2H). Anal.

($C_9H_6Cl_2N_2S$) Calcd: C, 44.10; H, 2.47; N, 11.43. Found: C, 44.03; H, 2.35; N, 11.34.

4-(Benzylamino)-2-chloro-6-(methylthio)quinazoline (23). To a mixture of 2,4-dichloro-6-(methylthio)quinazoline (2.45 g, 10.0 mmol), triethylamine (2.0 g, 20 mmol), and 50 mL of CH_2Cl_2 was added benzylamine (1.18 g, 11 mmol). After stirring for 3.5 h, the mixture was washed once with $NaHCO_3$ solution. The mixture was then dried over anhydrous $MgSO_4$, concentrated, and triturated with ether/hexane to produce a white solid (2.39 g, 83% yield). ^1H NMR ($CDCl_3$) δ 2.57 (s, 3H), 4.88 (d, 2H), 7.42 (m, 6H), 7.70 (m, 2H).

4-(Benzylamino)-2-(imidazol-1-yl)-6-(methylthio)quinazoline Dihydrochloride (90). A mixture of **23** (2.4 g, 8.3 mmol), imidazole 2.9 g, 43 mmol), and EtOH (5 mL) was heated. The EtOH was distilled off, and after 30 min, the reaction mixture was allowed to cool to 23 °C. The solidified mixture was triturated with water, filtered, and dried. The product 4-(benzylamino)-2-(imidazol-1-yl)-6-(methylthio)quinazoline was obtained as a solid (2.1 g, 73% yield): mp 198–201 °C. ^1H NMR (DMSO-d_6): δ 2.61 (s, 3H), 4.87 (d, 2H), 7.07 (s, 1H), 7.23–7.70 (m, 7H), 7.91 (s, 1H), 8.13 (s, 1H), 8.52 (s, 1H), 9.35 (t, 1H). IR (KBr): 3130 (w), 1596 (s), 1474 (s), 1401 (s), 1348 (m), 1322 (m), 1230 (w), 1057 (m), 877 (w), 750 (w), 697 (w) cm^{-1}.

To a solution of the above free base (0.55 g, 1.6 mmol) in 15 mL of methanol was added 1 mL of 10% HCl–MeOH. The mixture was concentrated and triturated with ether, and the product **90** was collected in a nearly quantitative yield (0.67 g): mp 192–195 °C. ^1H NMR (DMSO-d_6): δ 2.64 (s, 3H), 4.96 (d, 2H), 7.31–7.86 (m, 9H), 8.26 (s, 1H), 8.40 (s, 1H), 9.75 (t, 1H), 9.96 (s, 1H). IR (KBr): 3210 (w), 3040 (m), 2600 (m), 1630 (s), 1556 (s), 1495 (m), 1433 (m), 1510 (s), 1339 (m), 1203 (w), 1112 (w), 1091 (w), 1013 (w), 823 (w), 743 (m), 704 (m), 615 (w) cm^{-1}. Anal. ($C_{19}H_{17}N_5 \cdot 2HCl \cdot 0.8H_2O$) C, H, N.

4-(Benzylamino)-2-(imidazol-1-yl)-6-(methylsulfonyl)quinazoline Dihydrochloride (91). To 4-(benzylamino)-2-(imidazol-1-yl)-6-(methylthio)quinazoline (0.7 g, 2 mmol) in 7 mL of AcOH was added 30% H_2O_2 solution (3.0 mL, 26 mmol), and the mixture was stirred for 18 h. The mixture was poured into a solution of 50% NaOH (10.5 g, 130 mmol) and ice and extracted three times with 70 mL portions of $CHCl_3$. The combined organic extract was dried over anhydrous K_2CO_3, filtered, and concentrated. The concentrate was triturated with ether and the desired product 4-(benzylamino)-2-(imidazol-1-yl)-6-(methylsulfonyl)quinazoline collected as a solid (0.45 g, 60% yield): mp 277–280 °C. ^1H NMR (DMSO-d_6): δ 3.29 (s, 3H), 4.88 (d, 2H), 7.11 (s, 1H), 7.21–7.47 (m, 5H), 7.90 (d, 1H), 7.97 (s, 1H), 8.25 (d, 1H), 8.61 (s, 1H), 9.00 (s, 1H), 9.85 (t, 1H). IR (KBr): 3375 (w), 3010 (w), 1617 (s), 1594 (s), 1559 (s), 1472 (s), 1448 (s), 1354 (m), 1305 (s), 1265 (w), 1232 (w), 1151 (s), 1129 (m), 958 (w), 839 (w), 764 (m), 566 (w) cm^{-1}.

To a solution of the above free base (0.30 g, 0.79 mmol) in 15 mL of MeOH was added 1 mL of 10% HCl–MeOH. The mixture was concentrated and triturated with ether and the product **91** collected as a solid (0.31 g, 87%): mp 125–130 °C. ^1H NMR (DMSO-d_6): δ 3.34 (s, 3H), 4.97 (d, 2H), 7.31–7.50 (m, 5H), 7.85 (s, 1H), 7.93 (d, 1H), 8.32 (d, 1H), 8.44 (s, 1H), 9.14 (s, 1H), 9.98 (s, 1H), 10.12 (t, 1H). IR (KBr): 3230 (s), 3040 (s), 2705 (s), 2370 (m), 1616 (s), 1572 (s), 1524 (s), 1497 (m), 1399 (s), 1326 (s), 1258 (m), 1204 (w), 1147 (s), 1008 (m), 834 (w), 783 (s), 730 (w), 620 (w), 535 (m) cm^{-1}. Anal. ($C_{19}H_{17}N_5O_2S \cdot 2HCl \cdot 2.5H_2O$) C, H, N.

6-Ethynyl-4-(benzylamino)-2-(imidazol-1-yl)quinazoline (93). 6-[(Triisopropylsilyl)ethynyl]quinazoline-2,4-dione (106). In a 1 L three-neck flask were placed triphenylphosphine (1.31 g, 5 mmol), $PdCl_2$ (442 mg, 2.5 mmol), and 20 mL of diethylamine. After stirring for 1 h, 200 mL of diethylamine, 6-iodoquinazoline-2,4-dione[2e] (**105**) (28.8 g, 100 mmol), and CuI (380 mg, 2 mmol) were added to the mixture. After an additional 50 min, (triisopropylsilyl)acetylene (27 mL, 21.8 g, 120 mmol) was added and stirring continued for 18 h. The mixture was concentrated, triturated with water, acidified with 1 N HCl, and filtered. The solid was washed with hexane and allowed to dry. The solid was then purified over a silica gel column eluting with THF and triturated with hexane and the solid product collected (26.5 g, 77% yield). ^1H NMR (DMSO-d_6): δ 1.10 (s, 21H), 7.16 (d, 1H), 7.68 (m, 1H), 7.88 (m, 1H), 11.29 (s, 1H), 11.38 (s, 1H).

2,4-Dichloro-6-[(triisopropylsilyl)ethynyl]quinazoline. To a mixture of **106** (20 g, 58 mmol) in 200 mL of $POCl_3$ was added 2 mL of dimethylaniline. The solution was heated to reflux for 2 h. After cooling to 23 °C, the reaction was quenched by slowly pouring over ice. The precipitate was collected by filtration, washed with water, taken up in CH_2Cl_2, dried over anhydrous $MgSO_4$, and concentrated to obtain the product as a solid (19 g, 86% yield). ^1H NMR ($CDCl_3$): δ 1.16 (s, 21H), 7.88–8.04 (m, 2H), 8.31 (s, 1H). ^1H NMR (DMSO-d_6): δ 1.14 (s, 2H), 8.02–8.16 (m, 2H), 8.28 (m, 1H).

4-(Benzylamino)-2-chloro-6-[(triisopropylsilyl)ethynyl]quinazoline. To a solution of 2,4-dichloro-6-[(triisopropylsilyl)ethynyl]quinazoline (1.9 g, 5 mmol) in 50 mL of CH_2Cl_2 were added benzylamine (0.54 g, 5 mmol) and 1 mL of triethylamine. After stirring for 18 h, the mixture was washed with 50 mL of dilute K_2CO_3 and concentrated to yield the product as a solid (1.76 g, 78% yield): mp 186–189 °C. ^1H NMR ($CDCl_3$): δ 1.12 (s, 21H), 4.87 (d, 2H), 6.12 (t, 1H), 7.32–7.50 (m, 5H), 7.67–7.85 (m, 3H). ^1H NMR (DMSO-d_6): δ 1.12 (s, 21H), 4.74 (d, 2H), 7.25–7.42 (m, 5H), 7.56 (d, 1H), 7.82 (m, 1H), 8.51 (d, 1H), 9.46 (m, 1H).

4-(Benzylamino)-2-(imidazol-1-yl)-6-[(triisopropylsilyl)ethynyl]quinazoline (92). A mixture of 4-(benzylamino)-2-chloro-6-[(triisopropylsilyl)ethynyl]quinazoline (1.6 g, 3.5 mmol), imidazole (2.4 g, 68 mmol), and 5 mL of EtOH was heated with removal of EtOH by distillation. After 2 h, the mixture was cooled to 23 °C, triturated with water, and filtered. The solid was taken up in $CHCl_3$, washed with 1 N NaOH, dried over anhydrous K_2CO_3, and concentrated to obtain the product as a yellow solid (1.5 g, 89% yield): mp 186–188 °C. ^1H NMR (DMSO-d_6): δ 1.13 (s, 21H), 4.85 (s, 2H), 7.09 (s, 1H), 7.21–7.40 (m, 3H), 7.46 (m, 2H), 7.67 (d, 1H), 7.80 (d, 1H), 7.93 (s, 1H), 8.55 (d, 2H), 9.50 (s, 1H).

4-(Benzylamino)-6-ethynyl-2-(imidazol-1-yl)quinazoline (93). To a solution of **92** (1.5 g, 3.1 mmol) in 20 mL of THF was added 3.1 mL of 1.0 M $(nBu)_4NF$ in THF. After stirring for 1.5 h, the solution was concentrated, taken up in $CHCl_3$ and water, and filtered to remove suspended material which proved to be the product (0.77 g) with minor impurity. The filtrate was separated and the organic layer dried over anhydrous $MgSO_4$ and concentrated. The concentrate was triturated with ether and collected to give additional product with minor impurity (0.95 g). The combined products were recrystallized from EtOH to obtain the product **93** as a solid (0.64 g, 63% yield): mp 125–127 °C. ^1H NMR (DMSO-d_6): δ 4.35 (s, 1H), 4.86 (d, 2H), 7.01 (s, 1H), 7.35 (m, 3H), 7.50 (d, 1H), 7.68 (d, 1H), 7.93 (s, 1H), 8.57 (s, 2H), 9.43 (t, 1H). IR (KBr): 3285 (m), 1593 (s), 1555 (s), 1473 (s), 1447 (s), 1406 (s), 1349 (w), 1055 (w), 1027 (w), 900 (w), 836 (w) cm^{-1}. Anal. ($C_{20}H_{15}N_5 \cdot 0.1H_2O$) C, H, N.

To a suspension of **93** (0.6 g, 1.8 mmol) in 5 mL of MeOH was added 0.5 mL of 10% HCl–MeOH. The solution became clear, and then a precipitate formed. The solution was concentrated, and the residue was triturated with ether and collected to obtain the desired product 4-(benzylamino)-6-ethynyl-2-(imidazol-1-yl)quinazoline dihydrochloride as a yellow powder in nearly quantitative yield (0.71 g): mp 203–205 °C. ^1H NMR (DMSO-d_6): δ 4.43 (s, 1H), 4.97 (d, 2H), 7.33 (m, 3H), 7.50 (d, 2H), 7.55 (d, 1H), 7.85 (m, 2H), 8.40 (s, 1H), 8.74 (s, 1H), 9.88 (t, 1H), 9.97 (s, 1H). IR (KBr): 3400 (m), 3235 (m), 1598 (s), 1522 (w), 1445 (w), 1396 (s), 1350 (w), 899 (w), 842 (w) cm^{-1}. Anal. ($C_{20}H_{15}N_5 \cdot 2HCl$) Calcd: C, 60.31; H, 4.30; N, 17.58. Found: C, 60.39; H, 4.91; N, 17.51.

Isolating Phosphodiesterases (PDEs). The different molecular forms of PDE were isolated using standard methods previously described by Weishaar, et al.[6] PDEs were isolated from several sources (PDE I from bovine aorta, PDE III and V from human platelets, PDE II from rat brain, and PDE IV from rat kidney). Typically, connective tissue and adventitia were removed, and 2–4 g of tissue (or 1–2 units of platelets) was suspended in 10 vol of buffer A (20 mM Tris-HCl, pH 7.5, containing 2 mM $Mg(OAc)_2$, 1 mM dithiothreitol, and 5 mM Na_2EDTA) using a Brinkman polytron. The proteinase inhibitors leupeptin, pepstatin A, and phenylmethanesulfonyl fluo-

ride (PMSF) were also included in this buffer (final concentration of 100 nM each). The homogenate was centrifuged at 100000*g* for 60 min. The supernatant was then removed and filtered through four layers of cheesecloth. In preliminary experiments, the supernatant was applied to DEAE-cellulose and eluted with NaOAc. In subsequent experiments, the supernatant was applied to a DEAE-Trisacryl M column. The column was washed with several bed volumes of buffer B (20 mM Tris-HCl, pH 7.5, containing 2 mM $Mg(OAc)_2$, 1 mM dithiothreitol, and proteinase inhibitors) and eluted by two successive linear NaCl gradients (0.05–0.15 M, 300 mL total; 0.15–0.40 M, 200 mL total). Five milliliter fractions were collected and assayed for cAMP and cGMP PDE activity in the presence and absence of 0.3 mg of calmodulin and 10 mM $CaCl_2$. (This amount of calmodulin is sufficient to maximally stimulate calmodulin-dependent PDE activity.) Appropriate fractions were pooled and dialyzed overnight against 4 L of buffer C (200 mM Tris-HCl, pH 7.5). After complete separation, the PDEs were concentrated to 10% of the original volume. The protein was then diluted to 50% with ethylene glycol monoethyl ether and stored for up to 6 weeks at −20 °C.

Measuring Phosphodiesterase Activity. Phosphodiesterase activity was measured, as described by Thompson et al.,[23] in a reaction medium containing 40 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, and 1 mM dithiothreitol. The concentration of substrate ([^{3}H]cAMP or [^{3}H]cGMP) was 0.2 mM for all enzyme inhibitor studies. The concentration of substrate was 1.0 mM for PDE II. Test compounds were dissolved in DMSO at a final concentration of 2.5%. This concentration of DMSO inhibited enzyme activity by approximately 10%. The IC_{50} values (concentrations that produced 50% inhibition of substrate hydrolysis) for the compounds examined were determined from concentration–response curves with five concentrations (half-log increments) performed in duplicates on two occasions. Inhibitory responses to reference compounds were conducted in each assay.

Inhibitory Activities on Human Thromboxane A_2 Synthesis and Cyclooxygenase in Washed Platelets. Blood (50 mL) was withdrawn from the antecubital vein of healthy adult human volunteers. The blood was withdrawn into a syringe containing 5 mL of 3.8% trisodium citrate, pH 7.4. The blood was centrifuged at 300*g* for 10 min to obtain platelet rich plasma. The platelet rich plasma was centrifuged at 800*g* for 15 min. The platelet pellet was resuspended in saline containing 10 mM EDTA and sedimented by centrifugation. The final resuspension was performed in Krebs–Henseleit buffer without calcium, pH 7.4.

Test compounds were added into the washed platelets (185 μL, 1 × 10^6 platelets/mL) for 2 min at 37 °C.[24] After the incubation, [^{14}C]arachidonic acid (10 μL, 100 μM) was added to the washed platelets containing the compound and incubated for 2 min at 37 °C. The reaction was terminated by addition of ether–MeOH–1 M citric acid (30:4:1, v/v/v). The reaction mixture was centrifuged at 1000*g* for 2 min to separate the organic layer. The products were analyzed by TLC. The organic solution was placed at the bottom of the plate (Whatman silica gel 60A). The developing solvent was a mixture of organic layer of EtOAc–2,2,4-trimethylpentane–AcOH–water (110:50:20:100, v/v/v/v). After the development, the distribution of radioactivity on the plate was monitored by autoradiography. Radioactivity was measured using a densitomer. The percentage of inhibition was calculated from a percentage of counts recovered in the thromboxane B_2 and cyclooxygenase sections compared to the total counts recovered.

Inhibitory Activity on A23187-Induced Thromboxane Synthesis in Rat Whole Blood *in Vitro*. Blood was taken from the abdominal aorta of male Sprague–Dawley (SD) rats under ether anesthesia. Test compounds were added into 0.8 mL of whole blood and incubated at 37 °C for 5 min. After incubation, A23187 (30 μM) was added to the whole blood–compound mixture and the mixture incubated at 37 °C for 15 min to stimulate thromboxane synthesis. The reaction mixture was centrifuged at 18000*g* for 30 s to separate plasma. The plasma sample was treated with a Sep-pak (C18) column followed by thromboxane B_2 measurement using enzyme immunoassay kits (Cayman Chemical).

***Ex Vivo*.** Test compounds (suspended in 0.5% methyl cellulose) were orally administered to male SD rats fasted overnight. Thirty minutes after administration, heparinized blood was taken from the abdominal aorta under ether anesthesia. After incubation for 2 min at 37 °C, A23187 (30 μM) was added into the mixture of whole blood and the mixture was incubated at 37 °C for 15 min to stimulate the thromboxane synthesis. The reaction mixture was centrifuged at 18000*g* for 30 s to separate plasma. The plasma sample was treated with a Sep-pak (C18) column followed by thromboxane B_2 measurement using enzyme immunoassay kits (Cayman Chemical).

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