Optimization of Substituted *N*-3-Benzylimidazoquinazolinone Sulfonamides as Potent and Selective PDE5 Inhibitors

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A previous report from these laboratories identified the *N*-3-benzylimidazoquinazolinone nucleus as a more selective PDE5 inhibitor template compared to the pyrazolopyrimidine of sildenafil. This paper describes in detail the structure−activity relationships of a set of sulfonamide analogues, several of which are both more potent and more selective PDE5 inhibitors in vitro than sildenafil. The synthesis, in vitro enzyme activity and selectivity, and in vitro functional and preclinical pharmacokinetic assessment of molecules in this series are described.

Introduction

Sildenafil (**1**, Viagra) (Chart 1) is the first and only orally active phosphodiesterase type 5 (PDE5) inhibitor available for the treatment of erectile dysfunction (ED).[1] PDE5 is the major cGMP hydrolyzing enzyme in human *corpus cavernosum*, and inhibition of this intracellular enzyme elevates levels of the cyclic nucleotide. Upon sexual stimulation, release of nitric oxide from nonadrenergic, noncholinergic neurons activates guanylyl cyclase, and PDE5 inhibition enhances cGMP accumulation in the tissue. Increased cGMP levels cause a decrease in intracellular calcium concentration, and this leads to relaxation of vascular smooth muscle and *corpus cavernosum* tissue, resulting in increased arterial blood flow to the penis and ultimately erection.

The age-associated incidence of ED and the potentially large patient population, estimated to be as many as 30 million men in the United States alone,[2] have combined with the success of sildenafil to provide strong stimuli for discovery and development of additional PDE5 inhibitors. Chart 1 illustrates selected chemotypes that have been reported as potent (PDE5 IC_{50} < 5 nM) PDE5 inhibitors.[3] One important issue facing a new agent is selectivity versus other isozymes in the phosphodiesterase superfamily.[4] In the literature, **3**−**6** have selectivity data reported for PDE1−3.[3a−d] We have previously compared **1** and **2** against PDE1−6 and shown that **2** is significantly more specific in vitro than sildenafil.[5] PDE1 and 6 are cGMP-hydrolyzing phosphodiesterases found in the vasculature and retina, respectively,[6] and inhibition of these isozymes may be associated with some of the adverse side effects of sildenafil therapy, e.g., headache and facial flushing (possibly PDE1) and visual disturbances (PDE6). An improved, second-generation PDE5 inhibitor would be one with greater potency and specificity for PDE5, resulting in an agent with potentially fewer PDE-associated side effects and greater efficacy as a treatment for ED.

We recently reported the discovery of **2**, which was shown to be as efficacious as **1** in a functional assay of PDE5 activity (potentiation of relaxation of rabbit *corpus cavernosum* tissue). This compound was derived from the benzylimidazoquinazolinone sulfonamide **7** (Chart 2), which itself was developed following targeted screening.[5] The improved PDE5 selectivity of **2** and **7** was associated with the *N*-3-benzyl substituent. This paper describes structure−activity relationships (SAR) of the *N*-3-benzyl moiety, as well as SAR at the sulfonamide locus, leading to a group of compounds that are equal to **2** in terms of in vitro potency and selectivity. Selected compounds in this series demonstrate functional activity in the rabbit *corpus cavernosum* tissue strip model and have been evaluated in preclinical pharmacokinetic animal models.

Chemistry

The carboxylic acids **10a**−**c** needed for coupling with benzimidazoles **12a**−**g** (Scheme 2) were prepared as shown in Scheme 1. The specific amines illustrated here were selected following library-based evaluation of over 80 different sulfonamides **8** (X = H).[7] Benzimidazoles **12a**−**g** were prepared as described previously.[5] Coupling of these two fragments was done either via the acid chloride (method A) or via a carbodiimide-mediated approach (method B, Scheme 2). Cyclization of the intermediate benzamide was accomplished using potassium *tert*-butoxide/*tert*-butyl alcohol (Scheme 2).[5] The overall yield for these transformations was good to excellent in most cases.

To assess the effect of the linker (sulfonamide vs amide) on in vitro PDE5 potency, a sulfonamide analogue (**16**) of **2** and amide derivatives (**18a**,**b**) of sulfonamides **13g**,**j**, respectively, were prepared (Scheme 3). The sulfonyl chloride intermediate **15**, needed for **16**, was prepared by chlorosulfonation of **14**. This crude material was reacted with ammonia to furnish **16**. Carboxylic acid **17**[5] was coupled with 4-ethylpiperazine and (*R*)-3-(dimethylamino)pyrrolidine to furnish **18a**,**b**, respectively (Scheme 3).

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Chart 1

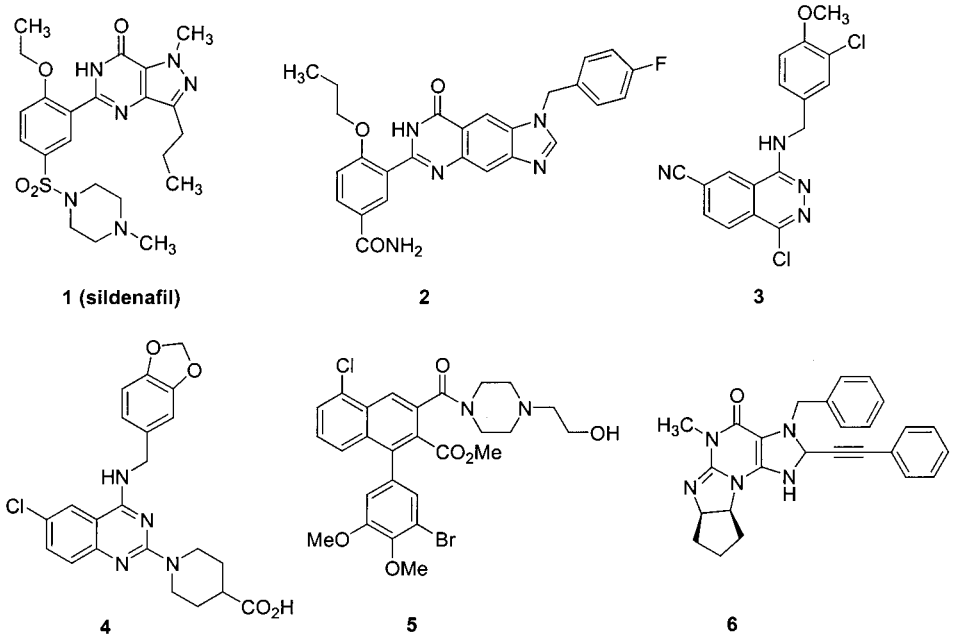
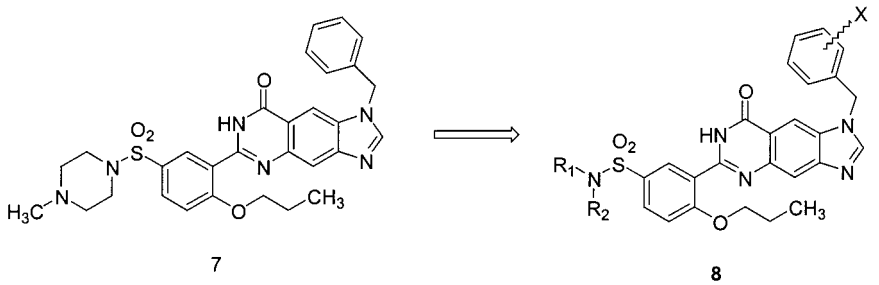
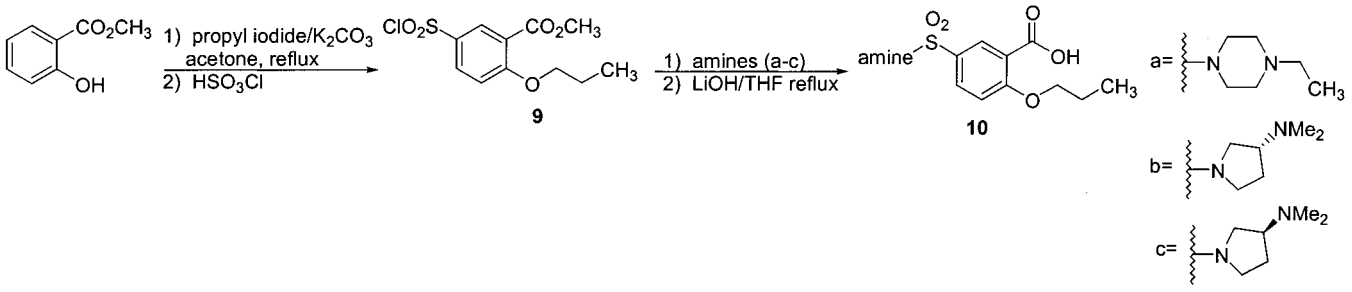


Chart 2



Scheme 1

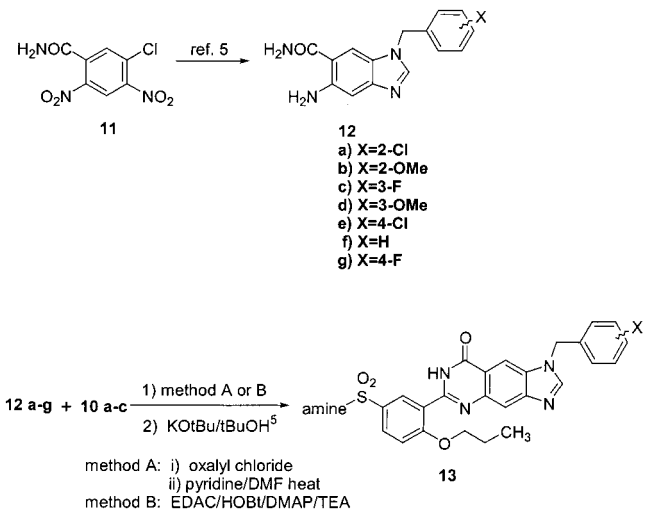


Results

In Vitro PDE5 Activity. Compounds **13a**−**k**, **16**, and **18a**,**b** were screened against PDE1−6 as previously described.[5] The data in Table 1 indicate that there is a measurable preference for an *N*-ethylpiperazine moiety (e.g. **13b**) over the *N*-methyl derivative (**7**). The pyrrolidine sulfonamides **13h**,**i** illustrate a stereospecific effect of the amine substituent on PDE5 potency, with the *R* enantiomer preferred approximately 20-fold over the *S* isomer. Comparison of the 4-fluorobenzyl derivative **13a** with **7** suggests that in this series of sulfonamides, a substituted benzyl moiety has a potency-enhancing effect. In the 2-methoxy analogue **13d**, the substituent exerts a more significant effect, resulting in a compound equipotent with **2**. A similar tendency was observed in the (*R*)-pyrrolidine sulfonamides, leading to **13j**, which is one of the most potent PDE5 inhibitors reported to date.

Selectivity for PDE5 over other PDEs is uniformly enhanced by the *N*-3-benzyl moiety, resulting in compounds with little, if any, affinity for PDE1−4. In the case of PDE6, the effect of the benzyl substituent and sulfonamide was more complex. *N*-methyl sulfonamide **13a**, with a 4-fluorobenzyl substituent, was 60-fold selective for PDE5 compared to the unsubstituted analogue **7**, which was 20-fold selective. The 50-fold selectivity shown by *N*-ethyl sulfonamide **13b** was due primarily to improved PDE5 potency, rather than decreased affinity for PDE6 when compared to **13a**. Comparison of the PDE6 IC_{50} values for *N*-ethylpiperazine (**13b**) and pyrrolidine (**13h**) sulfonamides clearly indicated that the latter had the undesired effect of improving PDE6 potency. Interestingly, in this series,

Scheme 2



Compound	Amine	X
a	4-Me-piperazine	4-F
b	4-Et-piperazine	H
c	4-Et-piperazine	2-Cl
d	4-Et-piperazine	2-OMe
e	4-Et-piperazine	3-F
f	4-Et-piperazine	3-OMe
g	4-Et-piperazine	4-F
h	(R)-3-NMe_2-pyrrolidine	H
i	(S)-3-NMe_2-pyrrolidine	H
j	(R)-3-NMe_2-pyrrolidine	4-F
k	(R)-3-NMe_2-pyrrolidine	4-Cl

Table 1. PDE5 IC_{50} and Selectivity Ratios for Other PDEs[a]

	IC_{50} (nM)	IC_{50} ratio				
compd	PDE5	PDE1/5	PDE2/5	PDE3/5	PDE4/5	PDE6/5
1	1.6 ± 0.5	140	>10^4	3500	2600	8
2	0.48 ± 0.1	>10^5	>10^5	>10^5	4200	60
7	5.3 ± 1.1	3400	>10^4	8800	600	20
13a	3.3 ± 0.5	>10^4	>10^4	>10^4	>10^4	60
13b	1.1 ± 0.6	>10^4	>10^4	>10^4	>10^4	50
13c	1.6 ± 0.2	>10^4	>10^4	>10^4	>10^4	40
13d	0.62 ± 0.09	>10^4	>10^4	>10^4	>10^4	90
13e	1.4 ± 0.3	>10^4	>10^4	>10^4	>10^4	80
13f	1.1 ± 0.3	>10^4	7000	>10^4	3500	50
13g	1.7 ± 0.9	>10^4	>10^4	>10^4	>10^4	80
13h	1.1 ± 0.05	>10^4	>10^4	>10^4	4000	30
13i	27 ± 9	NT[c]	NT	NT	NT	3
13j	0.20 ± 0.06	>10^4	>10^4	>10^4	9600	70
13k	0.52 ± 0.1	>10^4	>10^4	>10^4	2500	50
16	3.2 ± 0.5	>10^4	>10^4	>10^4	700	20
18a	35[b]	NT	NT	NT	NT	NT
18b	40[b]	NT	NT	NT	NT	NT

[a] Enzyme sources: PDE1, bovine heart; PDE2, rat kidney; PDE3, human platelet; PDE4, rat kidney; PDE5, human platelet; PDE6, bovine retina. All entries are averages based on at least 3 determinations, except where noted. [b] Average of 2 determinations. [c] NT = not tested.

a 4-fluoro substituent (**13j**: PDE6 IC_{50} = 14 nM) seemed to improve PDE6 affinity (**13h**: PDE6 IC_{50} = 33 nM), unlike the effect observed in the piperazine sulfonamides (e.g. **13a**).

The data in Table 1 indicate that amide and sulfonamide linker SARs appear to be different with the same set of amines. Using ammonia, an amide (**2**) is preferred over a sulfonamide (**16**). However, with piperazine and (dimethylamino)pyrrolidine, the sulfonamide moiety is clearly preferred: compare for example **13g**/**18a** and **13j**/**18b**. We hypothesize that the geometry of the sulfonamide linkage allows the more complex amines to interact favorably with the enzyme. In contrast, the planar primary carboxamide is preferred by the enzyme, compared to the corresponding sulfonamide.

In Vitro Functional Evaluation. We had previously noted that addition of an *N*-3-benzyl moiety had variable effects on the efficacy of PDE5 inhibitors in the rabbit *corpus cavernosum* strip assay, where amide **2** was as active as sildenafil, while sulfonamide **7** was only weakly efficacious.[5] In this test, the compounds are delivered in solution to a strip of rabbit *corpus cavernosum* tissue suspended in an aqueous tissue bath. To show activity in this model, molecules must efficiently diffuse into cells where the enzyme is located. The data in Table 2 indicate that only a few of these sulfonamides are active in this functional test. There is no obvious association between in vitro PDE5 potency and efficacy, as **13b** with an IC_{50} of 1.1 nM was inactive, while **13f** with the same IC_{50} had moderate activity. Similarly, **2** and **13j** are equipotent in vitro, but **2** was more efficacious in this functional assay. Of the three molecules that showed measurable efficacy at 30 nM (**13d**,**f**,**k**), only one, **13d**, showed a dose response when tested at 300 nM (data not shown). From these results, we conclude that activity in the tissue strip assay is not a simple consequence of in vitro inhibition of PDE5. These results cannot be explained by significant differences in lipophilicity because the cLogP of sulfonamides **13a**−**k** as well as that of **7** and amide **2** are all within 0.6 units of each other.[9] Because of the activity of carboxamide **2** in the rabbit *corpus cavernosum* strip assay, a pharmacokinetic evaluation was carried out. The drug was administered as a solution to fasted dogs at a dose of 8 mg/kg. The compound showed poor oral bioavailability ($F \leq 10\%$) and as a consequence was not considered for further study in animal models of ED.

Conclusions

In this paper, we have demonstrated a general strategy for the preparation of highly PDE5-selective sulfonamide-based *N*-3-benzylimidazoquinazolinones, some of which are more potent than sildenafil against the isolated enzyme. Substitution of the benzyl group improves in vitro potency relative to the parent compounds. Two different amines were identified which gave potent, selective PDE5 inhibitors, and stereoselective effects on PDE5 potency were demonstrated using chiral amines. All of these compounds are more selective than sildenafil in vitro against other phosphodiesterase isozymes. In particular, substantially improved specificity against other cGMP-hydrolyzing phosphodiesterases (PDE1,6) has been achieved. The SAR results in this work indicate that the *N*-3-benzyl moiety is the primary feature associated with this increase in specificity, especially relative to PDE6. In the sulfonamide series, this can be amplified or attenuated depending on the amine used. There appears to be a divergence between the SAR data obtained for sulfonamides and carboxamides using the same amine, and the details of this phenomenon remain to be elucidated. Our data suggests that the amine moiety interacts within the active site of PDE5 and does not project into bulk solvent. In vitro functional efficacy in this series can be obtained, but

Scheme 3

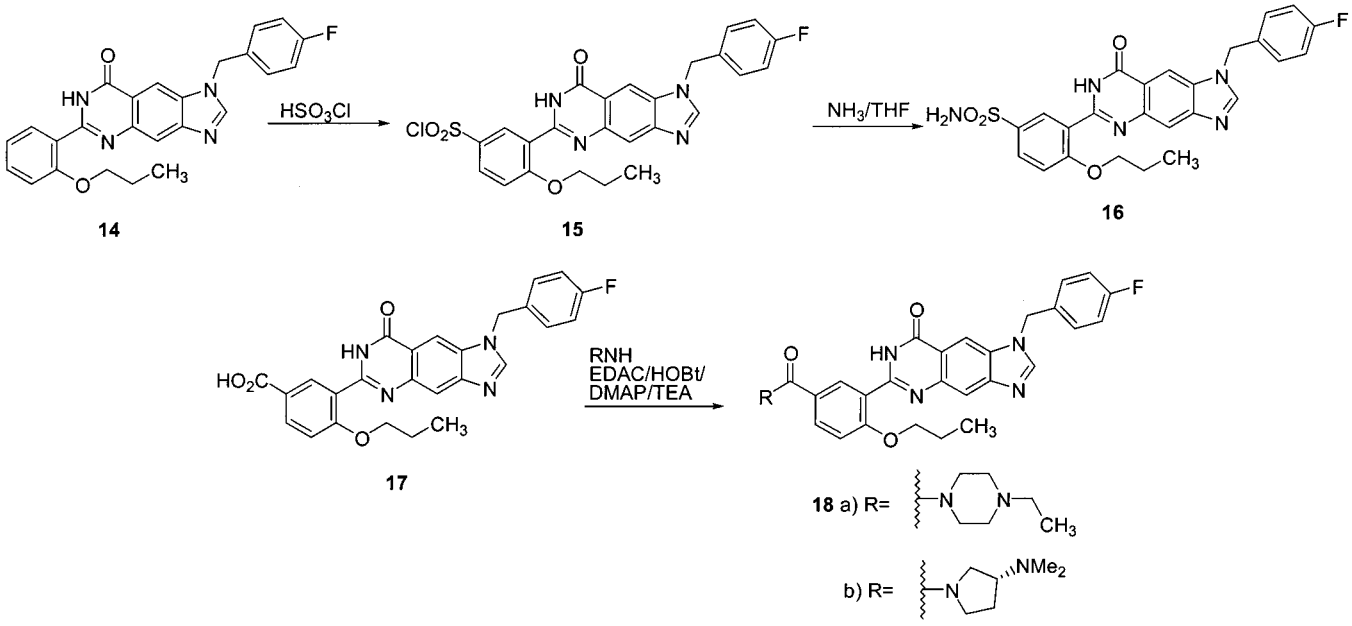


Table 2. Rabbit *Corpus Cavernosum* Functional Assay[a]

compd	% control relaxation integral (30 nM)	compd	% control relaxation integral (30 nM)
1	150 ± 20	**13e**	100 ± 10
2	140 ± 10	**13f**	130 ± 10
7	120 ± 10	**13g**	100 ± 10
13a	90 ± 10	**13h**	110 ± 10
13b	90 ± 6	**13j**	120 ± 5
13c	90 ± 10	**13k**	130 ± 30
13d	140 ± 20	**16**	100 ± 5

[a] Control (untreated) response = 100%. Values are based on the response of at least 4 independent tissue strips.

activity in the *corpus cavernosum* model requires efficient cell penetration of the inhibitor. This result highlights the challenges associated with identifying an efficacious PDE5 inhibitor that may be useful in vivo.

Experimental Section

General details have been previously described.[5]

Methyl 4-chlorosulfonyl-2-propoxybenzoic Acid (9). To a solution of methyl salicylate (25 g, 0.16 mol) in 300 mL of acetone were added 34 g of potassium carbonate (0.25 mol) and 84 g (0.49 mol) of 1-iodopropane. The mixture was heated to reflux for 24 h, then cooled and filtered. The filtrate was concentrated and the residue taken up in 500 mL of ether. The ether solution was washed twice with water and twice with brine, then dried and concentrated to give a faintly yellow oil. This material was a single spot by TLC (1:1 methylene chloride/hexane, R_f 0.4) and was used without further purification.

The crude propoxy ether was added dropwise at 0 °C to a mixture of 35 mL of chlorosulfonic acid and 9.6 mL of thionyl chloride over 30 min. The dark red reaction was stirred overnight and was allowed to warm slowly to room temperature. The reaction was cautiously poured over 500 g of ice and stirred to deposit a yellow solid which was recrystallized from cyclohexane to furnish 13 g (0.045 mol, 28% yield) of fine white needles: mp 58−59 °C; ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 163.9, 135.6, 132.7, 131.8, 121.5, 113.6, 71.6, 52.9, 22.6, 10.7.

General Procedure for Synthesis of Carboxylic Acids 10a−c. The appropriate amine (1.1 equiv) was dissolved in methylene chloride (5−20 mL) along with 1.3 equiv of triethylamine and cooled to 0 °C. Sulfonyl chloride **9** was dissolved in methylene chloride (10−15 mL) and added dropwise over 30 min to this solution. The reaction was stirred at 0 °C for 1−1.5 h, then washed twice with water and twice with brine. The organic solution was dried and concentrated to furnish the product sulfonamide esters. These materials were used without further purification and were found to be single spots by TLC analysis (1:1 acetone/methylene chloride).

The appropriate methyl ester was dissolved in 40−50 mL of THF with 10−15 mL of water and 1 equiv of LiOH hydrate. The reaction was heated to reflux overnight and solvents were removed by rotary evaporation to furnish a quantitative yield of the lithium salt of the carboxylic acid as a white solid.

5-[(4-Ethyl-4-piperazinyl)sulfonyl]-2-propoxybenzoic acid lithium salt (10a): LRMS [MH$^+$] 357; ^{1}H NMR (400 MHz, D_2O) δ 7.73 (d, 1H, J = 2.4 Hz), 7.65 (1H, dd, J = 2.5, 9.1 Hz), 7.57 (1H, d, J = 2.5 Hz), 7.14 (1H, d, J = 9.1 Hz), 4.05 (t, 2H, J = 6.5 Hz), 3.03 (br s, 4H), 2.52 (br. s, 4H), 2.40 (apparent q, 2H, J = 7.2, 14.4 Hz), 1.79−1.87 (m, 2H), 1.03 (t, 3H, J = 7.2 Hz).

(*R*)-5-[(3-(Dimethylamino)pyrrolidinyl)sulfonyl]-2-propoxybenzoic acid lithium salt (10b): HPLC retention time 2.14 min; LRMS [MH$^+$] 357.

(*S*)-5-[(3-(Dimethylamino)pyrrolidinyl)sulfonyl]-2-propoxybenzoic acid lithium salt (10c): HPLC retention time 2.14 min; LRMS [MH$^+$] 357.

The preparation of *N*-3-benzylbenzimidazoles **12a−g** was carried out as reported previously.[5] The yield reported for each compound is the net yield for the three-step transformation from the 2,4-dinitro-5-chlorobenzamide precursor.

5-Amino-1-[[(2-chlorophenyl)methyl]amino]-1*H*-benzimidazole-6-carboxamide (12a): yield 100%; LRMS [MH$^+$] 301; NMR (400 MHz, CD_3OD) δ 9.54 (s, 1H), 8.36 (s, 1H), 7.68 (s, 1H), 7.35−7.42 (m, 4H), 5.75 (s, 2H).

5-Amino-1-[[(2-methoxyphenyl)methyl]amino]-1*H*-benzimidazole-6-carboxamide (12b): yield 87%; LRMS [MH$^+$] 297; NMR (400 MHz, CD_3OD) δ 9.67 (s, 1H), 8.68 (s, 1H), 8.00 (s, 1H), 7.67 (dd, 1H, J = 1.5, 7.4 Hz), 7.38−7.42 (m, 1H), 7.03−7.12 (m, 2H), 5.77 (s, 2H), 3.86 (s, 3H).

5-Amino-1-[[(3-fluorophenyl)methyl]amino]-1*H*-benzimidazole-6-carboxamide (12c): yield 100%; LRMS [MH$^+$] 285; NMR (400 MHz, CD_3OD) δ 9.74 (s, 1H), 8.57 (s, 1H), 8.00 (s, 1H), 7.42−7.50 (m, 1H), 7.29−7.39 (m, 2H), 7.10−7.20 (m, 1H), 5.86 (s, 2H).

5-Amino-1-[[(3-methoxyphenyl)methyl]amino]-1*H*-benzimidazole-6-carboxamide (12d): yield 94%; LRMS [MH$^+$] 297; NMR (400 MHz, CD_3OD) δ 9.51 (s, 1H), 8.40 (s, 1H), 7.67 (s, 1H), 7.36 (t, 1H, J = 7.8 Hz), 6.96−7.07 (m, 3H), 5.70 (s, 2H), 3.81 (s, 3H).

5-Amino-1-[[(4-Chlorophenyl)methyl]amino]-1*H*-benzimidazole-6-carboxamide (12e): yield 72%; LRMS (M + H) 301; NMR (400 MHz, CD_3OD) δ 9.55 (s, 1H), 8.44 (s, 1H), 7.76 (s, 1H), 7.55−7.59 (m, 2H), 7.16−7.21 (m, 2H), 7.03−7.12 (m, 2H), 5.75 (s, 2H).

Physical data for **12f**,**g** have been previously reported.[5]

Coupling of benzimidazoles **12a**−**g** with carboxylic acids **10a**−**c** using methods A and B (Scheme 2) have been previously described along with the cyclization of the resulting benzamides to afford the target imidazoquinazolinones.[5] The specific coupling method used and net yield for these two steps are indicated with each compound. Physical data for target compounds **13a**−**k** are as follows.

1-[[3-[1-[(4-Fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-methylpiperazine (13a): method B; yield 80%; mp 227−228 °C; NMR (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 8.97 (d, 1H, J = 2.4 Hz), 8.29 (apparent d, 2H, J = 7.5 Hz), 8.17 (s, 1H), 7.86 (dd, 1H, J = 2.4, 8.7 Hz), 7.04−7.26 (partially obscured by solvent, m, 7H), 5.44 (s, 2H), 4.26 (t, 2H, J = 6.5, 13 H), 3.13, (br s, 4H), 2.51 (br s, 4H), 2.27 (s, 3H), 2.02−2.07 (m, 2H), 1.18 (t, 3H, J = 7.5 Hz). Anal. Calcd for $C_{30}H_{31}N_6O_4SF{\cdot}1.3H_2O$: C, H, N.

1-[[3-[7,8-Dihydro-8-oxo-1-(phenylmethyl)-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-ethylpiperazine (13b): method A; yield 64%; mp 135−137 °C; NMR (400 MHz, $CDCl_3$) δ 10.8 (s, 1H), 8.93 (s, 1H), 8.31 (s, 1H), 8.25 (s, 1H), 8.18 (s, 1H), 7.84 (dd, 1H, J = 2.5, 8.7 Hz) 7.30−7.40 (m, 2H), 7.18−7.28 (m, 2H) 7.16 (d, 1H, J = 8.9 Hz), 5.46 (s, 2H), 4.24 (t, 2H, J = 6.5 Hz), 3.13 (br s, 4H), 2.56 (br s, 4H), 2.40 (apparent q, 2H, J = 7.2, 14.4 Hz), 1.98−2.07 (m, 2H), 1.16 (t, 3H, J = 7.4 Hz), 1.02 (t, 3H, J = 7.2 Hz). Anal. Calcd for $C_{31}H_{34}N_6O_4S{\cdot}H_2O$: C, H, N.

1-[[3-[1-[(2-Chlorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-ethylpiperazine (13c): method A; yield 49%; mp 225−226 °C; NMR (400 MHz, $CDCl_3$) δ 10.8 (s, 1H), 8.97 (d,-1H, J = 2.1 Hz), 8.28 (d, 1H, J = 3 Hz), 8.2 (s, 1H), 7.86 (dd, 1H, J = 2.1, 8.6 Hz), 7.1−7.4 (m, 6H), 5.4 (s, 2H), 4.3 (t, 2H, J = 6.4 Hz), 3.1 (br s, 4H), 2.5 (br s, 4H), 2.4 (dd, 2H, J = 7.1, 14.3 Hz), 2.02−2.07 (m, 2H), 1.17 (t, 3H, J = 6.4 Hz), 1.02 (t, 3H, J = 7.1 Hz). Anal. Calcd for $C_{31}H_{33}N_6O_4SCl{\cdot}1.2H_2O$: C, H, N, Cl.

1-[[3-[7,8-Dihydro-1-[(2-methoxyphenyl)methyl]-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-ethylpiperazine (13d): method A; yield 70%; mp 180−182 °C; NMR (400 MHz, $CDCl_3$) δ: 10.7 (s, 1H), 8.97 (dd, 1H, J = 2.2 Hz), 8.43 (s, 1H), 8.23 (apparent d, 2H, J = 7.5 Hz), 7.86 (dd, 1H, J = 2.3, 8.7 Hz), 7.15−7.34 (m, partially obscured by solvent, 3H), 6.93 (apparent t, 2H, J = 7.8 Hz), 5.44 (s, 2H), 4.26 (t, 2H, J = 6.5 Hz), 3.89 (s, 3H), 3.13 (br s, 4H), 2.55 (br s, 4H), 2.40 (apparent q, 2H, J = 7.1, 14.3 Hz), 2.01−2.09 (m, 2H), 1.18 (t, 3H, J = 7.5 Hz), 1.02 (t, 3H, J = 7.2 Hz). Anal. Calcd for $C_{32}H_{36}N_6O_5S{\cdot}H_2O$: C, H, N, S.

1-[[3-[1-[(3-Fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-ethylpiperazine (13e): method B; yield 93%; mp 115−117 °C; NMR (400 MHz, $CDCl_3$) δ 10.7 (s, 1H), 8.97 (d, 1H, J = 2.3 Hz), 8.61 (d, 1H, J = 2.4 Hz), 8.28 (apparent d, 2H, J = 2.1 Hz), 8.20 (s, 1H), 7.86 (dd, 1H, J = 2.3, 8.7 Hz), 7.33 (apparent q, 1H, J = 5.9, 7.9 Hz), 7.17 (d, 1H, J = 8.8 Hz), 7.00−7.06 (m, 2H), 6.90 (d, 1H, J = 9.1 Hz), 5.47 (s, 2H), 4.26 (t, 2H, J = 6.5 Hz), 3.13 (br s, 4H), 2.55 (br. S, 4H), 2.41 (apparent q, 2H, J = 7.1, 14.3 Hz), 2.04 (apparent q, 2H, J = 6.9, 14.1 Hz), 1.17 (t, 3H, J = 7.5 Hz), 1.02 (t, 3H, J = 7.2 Hz). Anal. Calcd for $C_{31}H_{33}N_6O_4SF{\cdot}2H_2O$: C, H, N, F.

1-[[3-[7,8-Dihydro-1-[(3-methoxyphenyl)methyl]-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-ethylpiperazine (13f): method A; yield 55%; mp 117−118 °C; NMR (400 MHz, $CDCl_3$) δ 10.7 (s, 1H), 8.97 (d, 1H, J = 2.4 Hz) 8.33 (s, 1H), 8.27 (s, 1H), 8.18 (s, 1H), 7.86 (dd, 1H, J = 2.4, 8.7 Hz), 7.24−7.28 (partially obscured by solvent, m, 1H), 7.16 (d, 1H, J = 8.8 Hz), 6.86 (dd, 1H, J = 2.4, 8.0 Hz), 6.81 (d, 1H, J = 7.9 Hz), 6.74 (d, 1H, J = 2.4 Hz), 5.43 (s, 2H), 4.25 (t, 2H, J = 6.5 Hz), 3.76 (s, 3H), 3.13 (br s, 4H), 2.55 (br s, 4H), 2.40 (apparent q, 2H, J = 7.2, 14.4 Hz), 2.01−2.07 (m, 2H), 1.17 (t, 3H, J = 7.5 Hz), 1.02 (t, 3H, J = 7.2 Hz). Anal. Calcd for $C_{32}H_{36}N_6O_5S{\cdot}1.2H_2O$: C, H, N.

1-[[3-[1-[(4-Fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-4-ethylpiperazine (13g): method B; yield 52%; mp 155−158 °C; NMR (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 8.97 (d, 1H, J = 2.4 Hz) 8.30 (s, 1H), 8.27 (s, 1H), 8.18 (s, 1H), 7.86 (dd, 1H, J = 2.4, 8.7 Hz), 7.15−7.26 (m, partially obscured by solvent, 3H), 7.05 (apparent t, 1H, J = 6.7 Hz), 5.44 (s, 2H), 4.26 (t, 2H, J = 6.5 Hz), 3.13 (br s, 4H), 2.55 (br s, 4H), 2.41 (apparent q, 2H, J = 7.1, 14.2 Hz), 2.00−2.09 (m, 2H), 1.18 (t, 3H, J = 7.4 Hz), 1.02 (t, 3H, J = 7.2 Hz). Anal. Calcd for $C_{31}H_{33}N_6O_4SF{\cdot}0.5H_2O$: C, H, N, F.

(*R*)-1-[[3-[7,8-Dihydro-8-oxo-1-(phenylmethyl)-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-3-(dimethylamino)pyrrolidine (13h): method B; yield 71%; mp >300 °C (HCl salt); NMR (400 MHz, $CDCl_3$) δ 10.79 (s, 1H), 8.97 (d, 1H, J = 2.4 Hz), 8.35 (s, 1H), 8.22−8.26 (m, 2H), 7.95 (dd, 1H, J = 2.2, 8.8 Hz), 7.34−7.39 (m, 2H), 7.21−7.24 (m, partially obscured by solvent, 4H), 5.48 (s, 2H), 4.28 (t, 2H, J = 6.5 Hz), 3.43−3.65 (m, 4H), 3.32−3.39 (m, 1H), 2.74 (br s, 6H), 2.30−2.37 (m, 1H), 2.01−2.06 (m, 2H), 1.17 (t, 3H, J = 7.4 Hz). Anal. Calcd for $C_{31}H_{34}N_6O_4S{\cdot}H_2O$: C, H, N.

(*S*)-1-[[3-[7,8-Dihydro-8-oxo-1-(phenylmethyl)-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonyl]-3-(dimethylamino)pyrrolidine (13i): method B; yield 67%; mp 190 °C dec (HCl salt); NMR (400 MHz, $CDCl_3$) δ 10.78 (s, 1H), 9.00 (d, 1H, J = 2.4 Hz), 8.33 (s, 1H), 8.26 (s, 1H), 8.18, (s, 1H), 7.94 (dd, 1H, J = 2.3, 8.7 Hz), 7.34−7.39 (m, 2H), 7.18−7.24 (m, partially obscured by solvent, 4H), 5.47 (s, 2H), 4.27 (t, 2H, J = 6.4 Hz), 3.62−3.66 (m, 1H), 3.48−3.52 (m, 1H), 3.31−3.38 (m, 1H), 3,11−3.22 (m, 1H), 2.80−3.00 (m, 1H), 2.35 (br s, 6H), 2.01−2.06 (m, 2H), 1.17 (t, 3H, J = 7.4 Hz). Anal. Calcd for $C_{31}H_{34}N_6O_4S{\cdot}2.2H_2O$: C, H, N.

(*R*)-1-[[3-[1-[(4-Fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]-sulfonyl]-3-(dimethylamino)pyrrolidine (13j): method B; yield 69%; mp 223−225 °C; NMR (400 MHz, $CDCl_3$) δ 10.80 (s, 1H), 9.02 (d, 1H, J = 2.3 Hz), 8.30 (s, 1H), 8.28 (s, 1H), 8.18 (s, 1H), 7.93 (dd, 1H, J = 2.3, 8.7 Hz), 7.22−7.25 (m, 1H), 7.17 (d, 1H, J = 8.8 Hz), 7.04−7.08 (apparent t, 1H, J = 8.6 Hz), 5.44 (s, 2 H), 4.27 (t, 2H, J = 6.5 Hz), 3.63 (apparent q, 1H, J = 7.2, 9.1 Hz), 3.44−3.49 (m, 1H), 3.35 (apparent t, 1H, J = 7.2 Hz), 3.01 (apparent t, 1H, J = 9.1 Hz), 2.63−2.70 (m, 1H), 2.19 (s, 6H), 2.02.-2.09 (m, 3H), 1.65−1.72 (m, 3H), 1.18 (t, 3H, J = 7.2 Hz). Anal. Calcd for $C_{31}H_{33}N_6O_4SF{\cdot}H_2O$: C, H, N.

(*R*)-1-[[3-[1-[(4-Chlorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]-sulfonyl]-3-(dimethylamino)pyrrolidine (13k): method B; yield 75%; mp 220−222 °C; NMR (400 MHz, $CDCl_3$) δ 10.80 (s, 1H), 9.02 (d, 1H, J = 2.5 Hz), 8.28 (s, 1H), 8.27 (s, 1H), 8.18 (s, 1H), 7.93 (dd, 1H, J = 2.4, 8.7 Hz), 7.32−7.35 (m, 2H) 7.17 (apparent d, 1H, J = 8.6 Hz), 5.44 (s, 2 H), 4.27 (t, 2H, J = 6.5 Hz), 3.63 (apparent q, 1H, J = 7.0, 9.3 Hz), 3.45 (apparent q, 1H, J = 2.8, 9.4 Hz), 3.32−3.37 (m, 1H), 3.01 (apparent t, 1H, J = 9.1 Hz), 2.63−2.70 (m, 1H), 2.19 (s, 6H), 2.02.-2.07 (m, 3H), 1.65−1.71 (m, 3H), 1.18 (t, 3H, J = 7.4 Hz). Anal. Calcd for $C_{31}H_{33}N_6O_4SCl{\cdot}H_2O$: C, H, N, Cl.

1-[3-[7,8-Dihydro-8-oxo-1-(phenylmethyl)-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]sulfonamide (16). Benzimidazole **12g** was coupled with 2-propoxybenzoic acid and cyclized to quinazolinone **14** as described earlier.[5] Compound **14** (0.10 g, 0.23 mmol) was added portionwise over 15 min to an ice-cold solution of 5 mL of chlorosulfonic acid. The reaction was stirred at 0 °C for 5 h, then cautiously poured over ice to precipiatate a tan solid which was collected by filtration, washed with water and dried. Without further purification, this material was dissolved in 8 mL of a 0.5 M solution of ammonia in dioxane. The reaction was stirred at room temperature for 4 days, then concentrated to a tan solid which was purified by flash chromatography eluting with 9:1

methylene chloride:methanol to furnish 0.012 g of the desired compound as a white solid (0.025 mmol, 12% net yield): NMR (400 MHz, DMSO) δ 9.3−9.4 (br s, exch. with D2O), 8.81 (s, 1H), 8.32 (s, 1H), 8.13 (s, 1H), 8.00 (s, 1H), 7.92 (d, 1H, $J = 10$ Hz), 7.30−7.46 (m, 5H), 7.21 (apparent t, 2H, $J = 8.4$ Hz), 5.68 (s, 2H), 4.10 (t, 2H, $J = 6.5$ Hz), 1.73 (m, 2H), 0.94 (t, 3H, $J = 7.1$ Hz); HRMS calcd for 507.1376, obsd (MH^+) 508.1454.

1-Ethyl-4-[[3-[1-[(4-fluorophenyl)methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]-carbonyl]piperazine (18a) and (*R*)-1-[[3-[1-[(4-Fluorophenyl)-methyl]-7,8-dihydro-8-oxo-1*H*-imidazo[4,5-*g*]quinazolin-6-yl]-4-propoxyphenyl]carbonyl]-3-(dimethylamino)pyrrolidine (18b). Prepared as a part of a library of analogues of **2** by the following procedure: Carboxylic acid **17** was dissolved in pyridine at room temperature. To this were added 1.2 equiv of the appropriate amine, HOBt hydrate and EDAC-HCl, along with 0.15 equiv of DMAP. The reaction was stirred at room temperature for 4 h. The reaction was poured into ice water to precipipate the desired products which were collected by filtration, washed with water and 1.0 N NaOH then dried to furnish the products which were then analyzed by HPLC and LC/MS for purity and appropriate mass. Using the standard HPLC and LC/MS conditions described earlier,[5] **18a** had a retention time of 3.04 min, was at least 95% pure, and showed an M + H peak of 569. Similarly, **18b** had a retention time of 2.99 min, was at least 90% pure, and showed an M + H peak of 569. These materials were tested without further purification as PDE5 inhibitors using the method described below.

PDE Activity Assay. Enzymatic activity is assayed using a commercially available phosphodiesterase scintillation proximity (SPA) assay kit with either [^{3}H]cGMP or [^{3}H]cAMP as the substrate depending on the PDE of interest (Amersham product #TRKQ 7090 for cAMP kit; #TRKQ 7100 for cGMP kit). The manufacturer's protocol was followed explicitly except that the reactions are carried out at room temperature and 3 mM nonradioactive cyclic nucleotide was included in the suspension of SPA beads to stop the synthesis of additional radioactive products. The radioactive product of the reaction, [^{3}H]nucleotide monophosphate, preferentially bound to the SPA beads, excited the scintillant embedded in the beads, and was quantified on a Packard TopCount liquid scintillation counter. Nonspecific binding to the beads (the assay blank) was quantified by adding a 10000-fold excess of nonradioactive substrate to the reaction mixture prior to the addition of enzyme to prevent the synthesis of radioactive product. The assay blank was subtracted from the activity of the enzyme measured using the SPA kit as described above. The activity in samples that received test compound was calculated as a percent of the control activity measured in samples that only received the vehicle.

Enzyme Preparations. For PDE3,5 activity, the enzyme source was sonicated human platelet homogenates prepared by the method of Seiler et al.[10] PDE5 accounted for approximately 90% of the [^{3}H]cGMP hydrolytic activity in the homogenates and PDE3 accounted for over 80% of the [^{3}H]-cAMP hydrolytic activity. PDE1 from bovine heart was purchased from Sigma and assayed with 1 μM calmodulin and 4 mM calcium present in the reaction buffer and [^{3}H]cGMP as the substrate. PDE2,4 were purified from rat kidney cytosol by Mono-Q anion-exchange chromatography as described.[11] The substrate for both of these isoforms was [^{3}H]cAMP. Nonradioactive cGMP (1 μM) was also included in the reaction buffer for the PDE2 assay because the cAMP hydrolytic activity of this isoform was stimulated by cGMP. Rod outer segment membranes were purified from bovine retinas as described.[12] PDE6 was stripped from the rod outer segment membranes by hypotonic washes and trypsin-activated prior to the assay as described.[13] Trypsin treatment activated PDE6 by proteolytically degrading the inhibitory γ-subunit.

Data Analysis. Each data set was fit to a curve for inhibition at a single site using the nonlinear regression analysis in the Activity Base/XLFit software package, and IC_{50} values were calculated by this analysis. Selectivity ratios were calculated as the ratio of the IC_{50} value for inhibition of one PDE divided by the IC_{50} value for inhibition of PDE5.

Rabbit *Corpus Cavernosum* Strip Assay. Physiological salt solutions: A bicarbonate-buffered salt solution (PSS) was used, containing (in mM): 118.4 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.9 $CaCl_2$, 25.0 $NaHCO_3$, and 10.1 D-glucose. The solution additionally contained 3 μM indomethacin, 1 μM atropine, and 5 μM guanethidine. Concentrated stock solutions (10 mM) of test compounds were prepared and serially diluted to the appropriate concentrations in DMSO. Appropriate solvent/time controls were run in parallel.

Tissue preparation: Adult male New Zealand white rabbits weighing 3.4−3.7 kg were sacrificed by intravenous Nembutal injection. The entire penis (up to pelvic bone) was quickly removed and placed in ice-cold aerated PSS. The skin and connective tissue were carefully removed. The *corpus spongiosum* was cut from the groove under the corpus cavernosum. The *tunica albuginea* was dissected from the *corpus cavernosum*. The *corpus cavernosum* was carefully cut along the midline and connective tissue was cleaned from the "septal" region. The two corpora were longitudinally cut in half; each of these strips was then cut in half along the transverse axis, resulting in eight strips approximately 2 mm × 5 mm. Each strip was individually mounted for isometric force recording using silk suture in 10-mL tissue baths. One end was secured between the two platinum plates of a field stimulating electrode, which in turn was connected to a micrometer for control of tissue length. The field stimulating electrode was attached to a Stimu-splitter and Grass stimulator. The other end of the suture was connected to a Grass FT.03 force displacement transducer. The strips were bathed in PSS maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. Data were simultaneously recorded on a Grass recorder and a Biopac System which additionally allowed measurement of the integral at each frequency. A preload tension of 1 g was applied to each strip. Following the equilibration period, the strips were contracted with 3 μM PE until a steady state level of force was attained. For the first frequency−response curve, 3 μM DMSO (solvent control) was added to each strip for 10 min. A frequency−response curve of the following parameters was then performed: 24 V in the form of square wave pulses of 0.2-ms duration delivered as 10-s train and a 4-min interval between stimuli. A frequency of 32 Hz was chosen to measure the effects of test compounds based on the signal-to-noise response. After a 4-min recovery at 32 Hz, the strips were washed extensively and allowed at least 50 min to recover during which time tension adjustments and several washes were done. The frequency−response curve was repeated in the presence of test compound. Following another recovery period, a third frequency−response curve was performed in the presence of a higher concentration of test compound.

Data analysis: All force determinations were made assuming that the lowest level of force following a washout period defined 100% relaxation and the phenylephrine contraction prior to DMSO or test compound addition was 0% relaxation. The direct relaxation induced by test compound, measured after the 10-min incubation period, was calculated relative to these values. The peak relaxation responses at 32 Hz were also measured relative to these values. Values were then normalized to the maximum peak relaxation attained during the control frequency−response curve. The integral for the area under the curve at 32 Hz was determined for a set time period (3.66 s from immediately prior to stimulation). The integral for the relaxation response was then calculated as the difference between the integral for the time period immediately prior to the start of stimulation and that for the frequency. All responses were normalized to the maximum attained during the first frequency−response curve.

Each data set was fit to a sigmoidal dose−response curve by nonlinear regression using the GraphPad Prism software package.

Supporting Information Available: Elemental analysis data for compounds **13a**−**h**,**j**,**k**. This material is available free of charge via the Internet at http://pubs.acs.org.

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