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Brief Article

Design, Synthesis, and Biological Evaluation of 3-[4-(2-Hydroxyethyl)piperazin-1-yl]-7-(6-methoxypyridin-3-yl)-1-(2-propoxyethyl)pyrido[3,4-*b*]pyrazin-2(1*H*)-one, a Potent, Orally Active, Brain Penetrant Inhibitor of Phosphodiesterase 5 (PDE5)

Robert O. Hughes,*,† D. Joseph Rogier,† E. Jon Jacobsen,† John K. Walker,† Alan MacInnes,† Brian R. Bond,† Lena L. Zhang,† Ying Yu,† Yi Zheng,† Jeanne M. Rumsey,† Jennie L. Walgren,† Sandra W. Curtiss,† Yvette M. Fobian,† Steven E. Heasley,† Jerry W. Cubbage,† Joseph B. Moon,† David L. Brown,† Brad A. Acker,† Todd M. Maddux,† Mike B. Tollefson,† Brent V. Mischke,† Dafydd R. Owen,‡ John N. Freskos,† John M. Molyneaux,† Alan G. Benson,† and Rhadika M. Blevis-Bal†

† *Pfizer Global Research and Development, Chesterfield Parkway West, St. Louis, Missouri 63017, and* ‡ *Pfizer Global Research and Development, Ramsgate Road, Sandwich CT139NJ, U.K.*

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We recently described a novel series of aminopyridopyrazinones as PDE5 inhibitors. Efforts toward optimization of this series culminated in the identification of 3-[4-(2-hydroxyethyl)piperazin-1-yl]-7-(6-methoxypyridin-3-yl)-1-(2-propoxyethyl)pyrido[3,4-*b*]pyrazin-2(1*H*)-one, which possessed an excellent potency and selectivity profile and demonstrated robust in vivo blood pressure lowering in a spontaneously hypertensive rat (SHR) model. Furthermore, this compound is brain penetrant and will be a useful agent for evaluating the therapeutic potential of central inhibition of PDE5. This compound has recently entered clinical trials.

Introduction

Gathered over the past decade, significant clinical and commercial experience has demonstrated the safety, toleration, and efficacy of PDE5 inhibitors, such as sildenafil, for the treatment of male erectile dysfunctionon (MED[a]).[1] Furthermore, experimental studies and clinical trials indicate that PDE5 inhibitors are effective in the treatment of various other diseases such as Raynaud's disease, gastrointestinal disorders, and stroke and exert cardioprotective effects.[2]

With this potential in mind and initially targeting the discovery of compounds suitable for use as antihypertensive agents, we initiated a program directed toward the discovery of long-acting, selective inhibitors of PDE5. Previously, we reported on the identification of the aminopyridopyrazinone class of PDE5 inhibitors.[3] Among the desirable features of this class of PDE5 inhibitors was the excellent selectivity over other PDE isoforms.[4] Initial optimization of the profile of this class of compounds led to neutral inhibitor 3-[(*trans*-4-hydroxycyclohexyl)amino]-7-(6-methoxypyridin-3-yl)-1-(2-propoxyethyl)pyrido[3,4-*b*]pyrazin-2(1*H*)-one,[5] **1** (Figure 1), which possessed an excellent preclinical profile including robust, sustained reduction in blood pressure (BP) in the SHR model of hypertension. Upon further study, however, this compound caused unacceptable toxicity[6] which precluded its further development. To overcome this shortcoming, one strategy that we pursued was to develop compounds from different physicochemical classes[7] in the anticipation that toxicological profile of these compounds would diverge from that of **1**. To implement this strategy, we focused on modifications to the southwest (SW) region of the molecule. As determined by crystallographic analysis, the SW substituent points toward the solvent; therefore, modifications to this region of the molecule would likely have minimal effect on the PDE potency and selectivity. Herein, we described our efforts to identify a basic compound from the aminopyridopyrazinone class. These efforts culminated in the discovery of clinical candidate **13**.

We considered the previously described basic compounds **2** and **3**[5] as good starting points worthy of further optimization. As summarized in Table 1 both compounds demonstrate excellent potency against PDE5, derived from human platelets, and good selectivity over PDE6 and PDE11. Both compounds also possessed high ($>100\ \mu$M) aqueous solubility. Encouragingly, in the case of the **3**, the affinity for the hERG channel (4.5 μM) was only modest. Unfortunately, both compounds suffered from high clearance in the rat. Accordingly, our goal was to improve the pharmacokinetic profile of this subset of compounds while maintaining the favorable aspects: potency, selectivity, solubility, and hERG margin. As illustrated in Figure 2, we speculated that reducing the flexibility and rotational degrees of freedom in the SW substituent by constraining it into a ring system (as piperazine or a homopiperazine) would result in an improvement in metabolic stability. That is, we hypothesized that positive impact of the removal of three rotatable bonds from **2** or **3** on the pharmacokinetic profile of these compounds would outweigh any deleterious effects from the slight increase in lipophilicity of the new molecules.

Results and Discussion

The synthetic route into this class of molecules is summarized in Scheme 1. Treatment of **4** with *n*-BuLi followed by

*To whom correspondence should be addressed. Phone: (636) 247-9303. Fax: (636) 247-5620. E-mail: Robert.O.Hughes@pfizer.com.

[a] Abbreviations: BP, blood pressure; SHR, spontaneously hypertensive rat; PDE, phosphodiesterase; MED, male erectile dysfunction; CNS, central nervous system; PK, pharmacokinetics; SD, Sragueâ€“Dawley.

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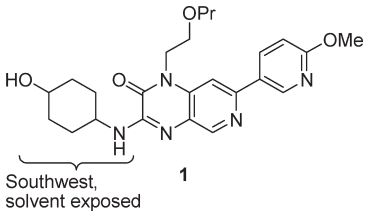
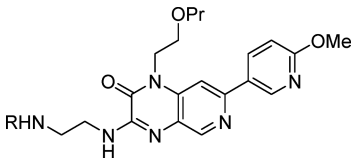


Figure 1. Structure of **1**.

Table 1. Selected Data for **2** and **3**[g]



Compound	R	PDE5[a]	PDE6-fold[b]	PDE11-fold[c]	Sol[d]	hERG[e]	Cl[f]
2		0.25	4840	>8000	>100	ND	170
3		1.12	242	>1785	>100	4500	582

[a] PDE5 IC_{50} (nM). [b] PDE6 selectivity defined as (PDE6 IC_{50})/(PDE5 IC_{50}). [c] PDE11 selectivity defined as (PDE11 IC_{50})/(PDE5 IC_{50}). [d] Aqueous solubility measured at pH 7.4 (μM). [e] hERG IC_{50} (nM) determined in a patch-clamp experiment. [f] Clearance ((mL/min)/kg) measured in the Sragué−Dawley (SD) rat following a 1 mpk iv dose. [g] PDE IC_{50} values are the averages of at least three determinations. hERG IC_{50} is from one determination.

quenching with *N*-fluorobenzenesulfonimide (NFSI) gave the desired fluoropyridine, **5**, in 60% yield. Addition of propyloxyethylamine to **5** was brought about in refluxing EtOH to give the diamino ether **6** in 82% yield. The action of HCl to remove the Boc group of **6** followed by treatment with methyl 2-chloro-2-oxoacetate gave, upon heating, the desired dione **7**. Reaction of dione **7** with oxalyl chloride in the presence of a catalytic amount of DMF gave chloroimidate **8** (66% for three steps) which was reacted with cyclic amines to give **9** (50−90%). A Suzuki reaction between the chloroaminopyridopyrazinone (**9**) and 6-methoxypyridin-3-ylboronic acid completed the synthesis in good overall yield.

As shown in Table 2, constraint of the SW substituent was well tolerated in terms of PDE5 potency. Indeed, comparison of **2** and **10** (0.25 nM → 3.28 nM) and **3** and **13** (1.12 nM → 0.20 nM) reveals little impact of cyclization on potency. In all cases selectivity over PDE11 was high. However, we observed that in some cases selectivity over PDE6 eroded. Specifically, homopiperazines **15** and **16** were less than 100-fold selective over PDE6 as was bicyclo derivative **11**. *N*-Ethyl derivative **12** and *N*-hydroxylethyl derivative **13** possessed excellent selectivity over PDE6. However, **10**, lacking a piperazine substituent, was less selective against PDE6. Interestingly, homologation of **13** to **14** resulted in similar potency against PDE5 but a 2.5-fold loss in PDE6 selectivity. Piperazine analogues **10**, **12**, and **13** were evaluated in the hERG patch-clamp experiment. Compounds **12** (1450-fold) and **13** (8950-fold) showed excellent selectivity for PDE5 over hERG activity.

Table 3 summarizes pharmacokinetic parameters for **2**, **3**, **10**, and **13**. All four compounds were found to be stable in rat and human microsomal systems. Additionally, all four compounds displayed similar protein binding in rat and humans with free fractions ranging from 18% to 30%. We were gratified to find a dramatic improvement in the in vivo clearance measured in the rat. Supporting our hypothesis that reduced conformational flexibility would lead to improved PK, **10** and **13** were significantly improved over the respective acyclic counterparts. Interestingly, the improvement in the in vivo PK profile was not predicted by the in vitro microsomal assays.

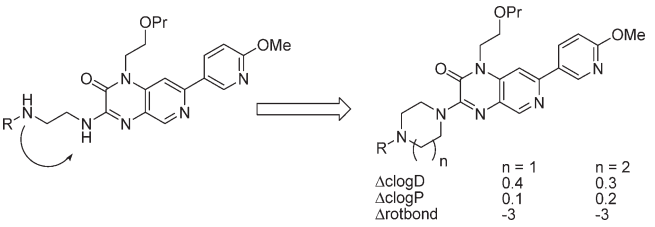
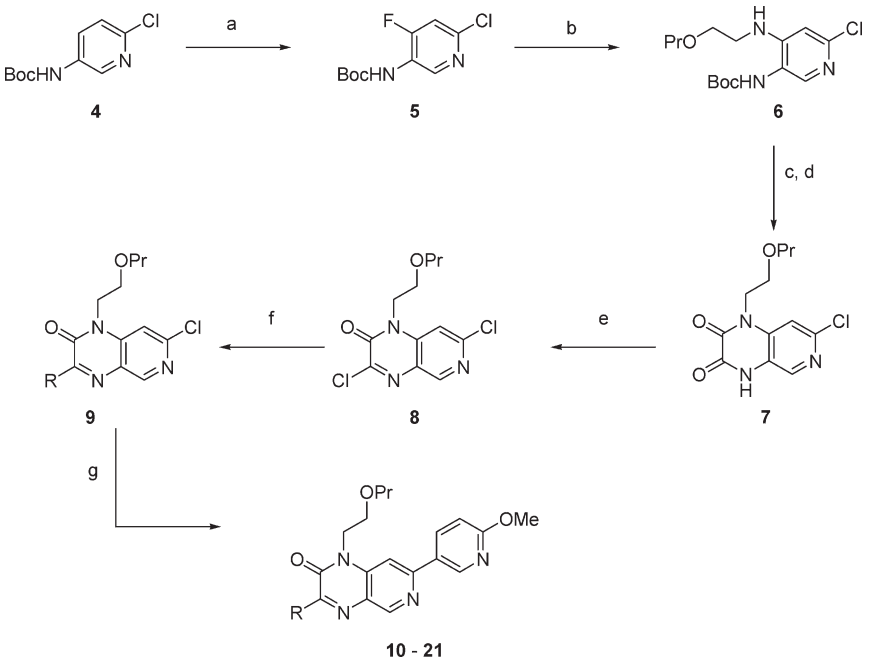


Figure 2. Proposed cyclization of **2** and **3** to improve pharmacokinetic parameters. ΔclogP, ΔclogD, and Δrotbonds are from $Property_{cyclized} - Property_{acyclic}$.

During our optimization of the neutral alcohol subclass of aminopyridopyrazinones, which led to **1**, we noted that methyl branching of the SW side chain resulted in improvements in the PK profile.[6] Accordingly, we sought to examine the effect of this modification on **13**. Table 4 summarizes the key results of this effort. Overall, methylation of the hydroxyl ethyl side chain of **13** was not beneficial. In the case of **17**, **19**, and **21** selectivity over PDE6 was reduced (49-fold, 64-fold, and 68-fold, respectively). Compound **20**, which displayed a similar PDE5, PDE6, and PDE11 profile to **13**, was significantly more potent at blocking the hERG channel (54 nM, 160-fold PDE5 IC_{50}). Similar data were collected for **18**: good PDE5, PDE6, and PDE11 data but a significantly reduced hERG margin (1000-fold) relative to **13**. Likely the increase in lipophilicity associated with the introduction of addition methyl groups is driving increased affinity to the hERG channel. As a result of these combined properties, **17**−**21** were not further studied.

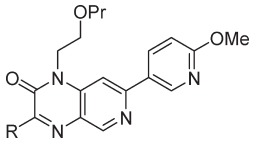
In parallel with our efforts to expand the SAR around **13**, we elected to more extensively profile this analogue. Compound **13** showed excellent selectivity versus all other PDE isoforms and against a wide panel of binding assays.[8] Additional pharmacokinetic studies were also encouraging. In the dog, **13** exhibited low clearance (5.8 (mL/min)/kg) and high bioavailability (92%). In a bile duct canulated rat study less than 2% of parent was recovered over 6 h of monitoring. Furthermore, in rats and dogs **13** showed negligible renal clearance (< 2%). Taken together, these data suggest that the primary route of clearance for **13** is through hepatic metabolism. The metabolite profile in rat, dog, and human microsomes and hepatocytes was similar, and the major metabolite was loss of the *n*-propoxy group. Finally, consistent with this compound's excellent in vitro permeability, **13** demonstrated 4-fold higher brain concentrations than total plasma concentration 2 h after a single subcutaneous dose of 10 mpk in rat, suggesting the potential for significant CNS penetration of this compound. As **13** demonstrated an attractive potency, selectivity, and PK profile, we undertook a more detailed evaluation of the PK/pharmacodynamic (PD) relationships for this compound. For these studies we utilized reduction in BP in the conscious SHR as the pharmacodynamic readout. A single oral dose (2 mpk) of **13** reduced systolic BP by

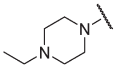
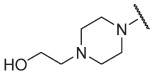
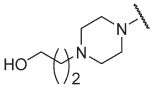
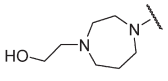
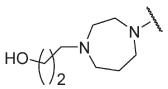
Scheme 1. General Synthetic Route for the Synthesis of **10**−**21**[a]



[a] (a) TMEDA, *n*-BuLi, Et_2O, −60 → −10 °C, 2 h; then NFSI, THF −60 → 0 °C, 1 h, 60%; (b) 2-propoxyethanamine, EtOH, reflux, 22 h, 82%; (c) 4 N HCl, 1,4-dioxane, room temp, 1 h; (d) MeOC(O)C(O)Cl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C → room temp, 4 h; then PhMe, 105 °C; (e) $(COCl)_2$, DMF(cat), CH_2Cl_2, room temp, 30 min, 66% for three steps; (f) amines, Et_3N, THF, room temp, 4 h, 50−90%; (g) 6-methoxypyridin-3-ylboronic acid, $(Ph_3P)_4P$ (10 mol %), Na_2CO_3, 1,4-dioxane−EtOH, 100 °C, 2 h, 50−85%.

Table 2. PDE5 Potency and PDE6, PDE11, and hERG Selectivity Data for Compounds **10**−**16**



Compound	R	PDE5[a]	PDE6-fold[b]	PDE11-fold[c]	hERG[d]
10		3.28	51	2460	670
11		2.38	93	456	ND
12		0.22	198	4920	320
13		0.20	158	2460	1790
14		0.18	62	4260	ND
15		0.72	70	>2780	ND
16		1.08	94	>1860	ND

[a] PDE5 IC_{50} (nM). [b] PDE6 selectivity defined as (PDE6 IC_{50})/(PDE5 IC_{50}). [c] PDE11 selectivity defined as (PDE11 IC_{50})/(PDE5 IC_{50}). [d] hERG IC_{50} (nM) determined in a patch-clamp experiment.

Table 3. Selected Metabolic and Pharmacokinetic Parameters for **2**, **3**, **10**, and **13**

	microsomal stability		protein binding		rat PK	
compd	rat (%)[a]	human (%)[a]	rat (%)	human (%)	Cl[b]	*F* (%)[c]
2	98	97	83.4	69.4	170	ND
3	100	95	77.4	78.8	582	ND
10	86	86	84.2	81.2	42	ND
13	89	85	82.9	67.8	37	69

[a] Percent remaining after 30 min of incubation. [b] Clearance ((mL/min)/kg) measured in the SD rat following a 1 mpk iv dose. [c] Bioavailability measured the SD rat following a 1 mpk po dose.

25 mmHg, which was sustained for approximately 24 h (data not shown). This study demonstrated that maximal BP lowering efficacy is achieved when free plasma exposures of **13** are above ~10-fold the PDE5 enzyme IC_{50}. To further refine this exposure−response relationship, changes in BP were monitored following steady state iv infusion of escalating doses of **13**. Data from this study enabled a detailed analysis of the exposure−response relationship on an individual animal basis. Figure 3 shows steady state exposure plotted against the corresponding BP lowering efficacy for each individual infusion period. These data enhanced our confidence that maximal BP lowering efficacy would be achieved for **13** with a free plasma concentration of 2.3 nM (11-fold PDE5 IC_{50}). Importantly, **13** was well tolerated in toxicological studies. On the basis of the excellent, overall profile, **13** was selected for clinical development. In healthy human volunteers **13** plasma concentrations increased proportionally with dose. Absorption of **13** was rapid with a median T_{max} estimate of 1−2 h across all doses studied. Following attainment of C_{max}, plasma **13** concentrations declined over time with a mean apparent elimination half-life of approximately 17−25 h across dose levels.

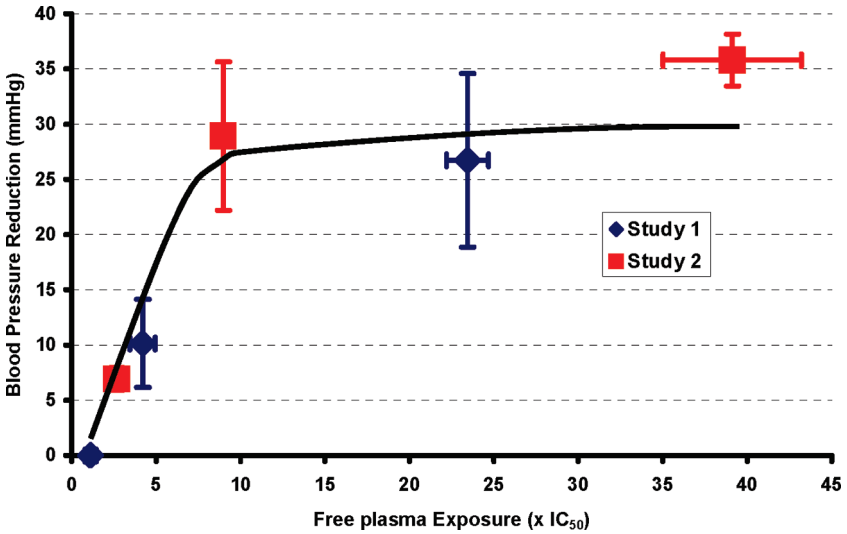
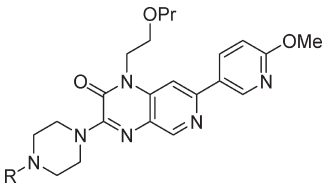
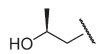
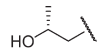
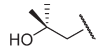
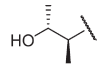


Figure 3. PK/PD relationship for **13** in the conscious SHR.

Table 4. PDE5 Potency and PDE6, PDE11, and hERG Selectivity Data for Compounds **13**–**21**



Compound	R	PDE5[a]	PDE6-fold[b]	PDE11-fold[c]	hERG[d]
13		0.20	158	2460	1790
17		0.31	49	1405	ND
18		0.23	134	2384	230
19		0.21	64	1370	ND
20[e]		0.33	134	1740	54
21[e]		0.30	68	2090	ND

[a] PDE5 IC_{50} (nM). [b] PDE6 selectivity defined as (PDE6 IC_{50})/(PDE5 IC_{50}). [c] PDE11 selectivity defined as (PDE11 IC_{50})/(PDE5 IC_{50}). [d] hERG IC_{50} (nM) determined in a patch-clamp experiment. [e] Racemic. Relative chemistry known.

Conclusion

We have described the optimization of a basic series of aminopyridopyrazinones as potent, selective, and safe inhibitors of PDE5. This effort culminated with the identification of the brain penetrant **13**, 3-[4-(2-hydroxyethyl)piperazin-1-yl]-7-(6-methoxypyridin-3-yl)-1-(2-propoxyethyl)pyrido[3,4-*b*]-pyrazin-2(1*H*)-one, which will enable the clinical evaluation of the role of PDE5 in the CNS. Additional data on this compound will be reported in due course.[9]

Supporting Information Available: Procedures for the preparation of **13**; analytical data for **10**–**21**; details of the PDE5, PDE6, and PDE11 assays; additional selectivity data for **13**; procedures for the in vivo experiment summarized in Figure 3; method for determining brain concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) Accordingly, we routinely only monitored the selectivity of compounds against the closely related PDE6 and PDE11.
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(6) In rats, **1** caused vacuolization of the pancreatic acinar cells.
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(8) **13** was screened against other members of the PDE family (1A, 1B, 1C, 2, 3A, 3B, 4A, 4B, 4C, 4D, 7, 7B, 8A, 8B, and 9) and showed no significant inhibition (IC_{50} > 2000 nM in all cases). Additional selectivity data were obtained at CEREP. See Supporting Information for additional details.

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