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Identification, synthesis and SAR of amino substituted pyrido[3,2*b*]pyrazinones as potent and selective PDE5 inhibitors

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ABSTRACT

A new class of potent and selective PDE5 inhibitors is disclosed. Guided by X-ray crystallographic data, optimization of an HTS lead led to the discovery of a series of 2-aryl, (*N*8)-alkyl substituted-6-aminosubstituted pyrido[3,2*b*]pyrazinones which show potent inhibition of the PDE5 enzyme. Synthetic details and some structure–activity relationships are also presented.

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Cyclic guanidine monophosphate (cGMP) is a second messenger for nitric oxide and atrial natriuretic peptide. Increasing levels of cGMP activates protein kinase G which through phosphorylation of other downstream proteins promotes vasorelaxation. Phosphodiesterase type 5 (PDE5) is widely expressed in smooth muscle, lung, kidney and platelets and plays a major role in regulating intracellular levels of cGMP through degradation of cGMP to the inactive 5′-GMP.[1] For this reason, PDE5 inhibitors were originally studied as potential anti-hypertensive or antianginal agents. The discovery of the potent PDE5 inhibitor sildenafil (Viagra®), Figure 1, led to breakthrough therapies for male erectile dysfunction and has spurred development of additional PDE5 agents such as vardenafil (Levitra®) and tadalafil (Cialis ®).[2,3] More recently, sildenafil was approved for the treatment of pulmonary hypertension (Revatio®) further illustrating the potential of PDE5 inhibitors as therapeutic agents.[4]

As the potential therapeutic uses of PDE5 inhibitors grow there is an increasing need to develop new classes of inhibitors more suitable for chronic usage. One liability of the current marketed
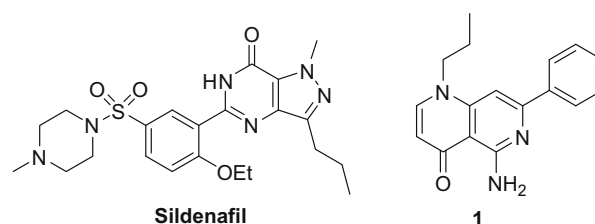

Figure 1.

class of inhibitors is a less than ideal selectivity profile against other members of the PDE family.[5–7]

As part of our on-going effort[8] to identify potent, selective, and long-acting PDE5 inhibitors, a high-throughput screen of the Pfizer compound file was initiated. The screen yielded the phenyl substituted quinolin-4-one derivative **1** (Fig. 1) as a potent PDE-5 inhibitor (IC_{50} = 51 nM) but with poor selectivity for PDE6 (IC_{50} = 6 nM).

To facilitate further optimization of this lead, we obtained an X-ray crystal structure of **1** bound in the active site of PDE5[9] (see Fig. 2). A comparison between **1** and sildenafil in the active site revealed the *n*-propyl group of **1** fits into the lipophilic pocket
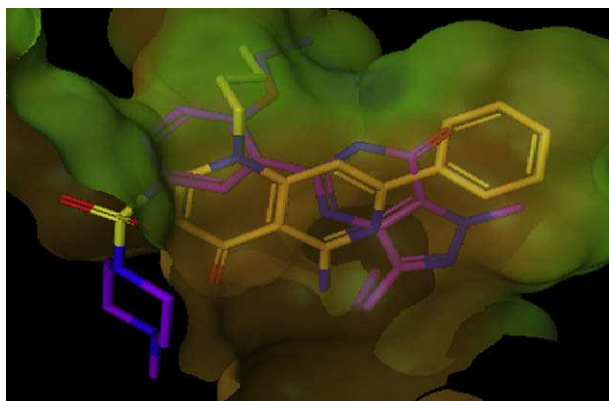
Figure 2. Overlay of co-crystal structure for compound **1** (orange) and sildenafil (purple) in the active site.

occupied by the ethoxy group of sildenafil. Additionally, the phenyl ring of **1** pointed into a deep lipophilic pocket not occupied by sildenafil. Finally, inspection of the crystal structure revealed that the solvent exposed space, occupied by the sulfonylpiperazine group in sildenafil, was unoccupied by **1** suggesting a potential handle for physical property and PK modulation via the incorporation of polar functionality (Fig. 3).

Our enthusiasm for **1** was tempered, however, by concerns over the drug ability of the quinolin-4-one scaffold. In addition, **1** was plagued by an inefficient and lengthy synthesis which presented limited handles for systematic modification. The crystal structure of **1** suggested that replacement of the quinolin-4-one system, to address these concerns, with similarly substituted 6, 6 fused ring systems should be tolerated. We first explored this idea by examining a small set of related 2-quinolones represented by **2** (Fig. 2). We were pleased to find compound **2** maintained activity on PDE5 (IC$_{50}$ = 25 nM) and, more importantly, demonstrated some selectivity against PDE6 (sixfold). While encouraged by this result we also viewed the 2-quinolone scaffold to be a non-optimal template suffering from high lipophilicity and the presence of a potential Michael acceptor. Building off compound **2**, we next turned our attention to the dihydropyrazinone core exemplified by compound **3**. Gratifyingly, **3**, which has a lower log D and lacks the Michael acceptor, also maintained similar potency and selectivity to **2** (PDE5 IC$_{50}$ = 2 nM, PDE6 IC$_{50}$ = 20 nM); however the aqueous solubility of this series was still poor. Similar to what we observed in the analysis of **1** the crystal structure of **3** (not shown) bound to PDE5 suggested that the lower left region of the molecule pointed to solvent and would allow for the incorporation of an ionizable center to potentially improve solubility. Of the 6, 6 ring systems which we envisioned capable of projecting substituents toward
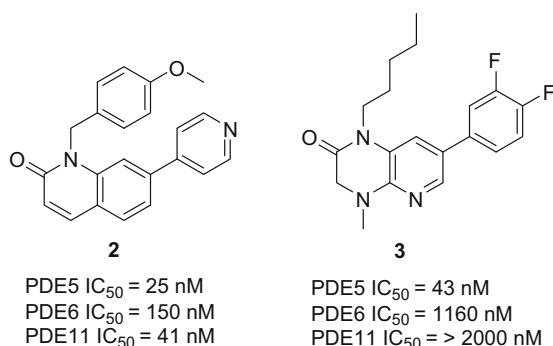
solvent, the amino-pyrido[3,2b]pyrazinone (Fig. 4) was particularly attractive as a starting point for further optimization.

In addition to allowing for the incorporation of a third substituent, the pyrazinones could be obtained through a flexible synthesis that would allow for rapid generation of SAR for each of the three regions. Furthermore, compound **4** would strike a good balance between decreasing lipophilicity and maintaining the same binding mode observed with the previous leads as depicted in Figure 5.

Toward these ends, we initiated efforts to prepare analogs in the pyridazinone template. Synthesis of the pyridopyrazinones was carried out according to Scheme 1. Reaction of a primary amine

Figure 4. Representative pyridopyrazinone scaffold to serve as the template for our analog program.
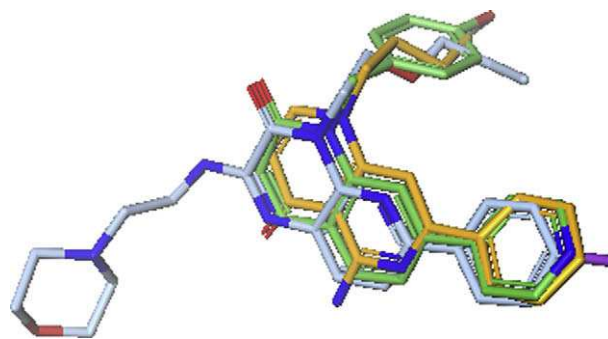
Figure 5. Overlay of co-crystal structure of compound **1** (green) with the minimized conformation of **3** (orange) and prototype pyridazinone inhibitor **22** (blue).

2
PDE5 IC$_{50}$ = 25 nM
PDE6 IC$_{50}$ = 150 nM
PDE11 IC$_{50}$ = 41 nM

3
PDE5 IC$_{50}$ = 43 nM
PDE6 IC$_{50}$ = 1160 nM
PDE11 IC$_{50}$ = > 2000 nM

Figure 3. Structure and PDE data for a set of rationally designed inhibitors.
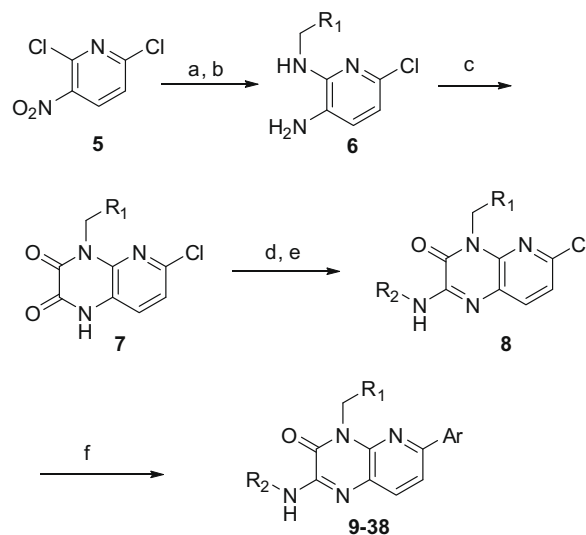
Scheme 1. General synthetic scheme to the pyridopyrazinone scaffold. Reagents and conditions: (a) R^{1}CH$_2$NH$_2$, Na$_2$CO$_3$, iPrOH, 0–20 °C; (b) 55 PSI H$_2$, Raney Ni; (c) oxalic acid, 4 N HCl, reflux 16 h; (d) oxalyl chloride, DMF, CH$_2$Cl$_2$; (e) R^{2}NH$_2$, Et$_3$N, CH$_2$Cl$_2$; (f) aryl boronic acid, Pd(Ph$_3$)$_4$, Na$_2$CO$_3$ glyme:H$_2$O (5:1), reflux 24 h.

Table 1
Structure–activity relationships for the R^1 and R^2 positions
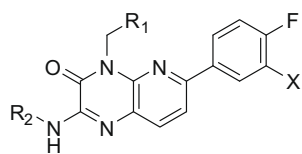
#	R1	R2	X	PDE isoforms[a]		
				5	6	11
9	phenyl-OMe	morpholine-propyl	H	1.3	10.1	345
10	phenyl-OMe	morpholine-propyl	F	2.0	11.6	482
11	3-pyridyl	tetrahydropyran-methyl	H	2.5	375	474
12	pyridyl	tetrahydropyran-methyl	H	20.3	920	970
13	3,5-difluorophenyl	tetrahydropyran-methyl	H	2.7	910	390
14	cyclohexyl	H	H	28.5	114	145
15	cyclohexyl	tetrahydropyran-methyl	H	3.0	459	324
16	cyclohexyl	morpholine-propyl	H	4.6	240	405
17	cyclohexyl	morpholine-propyl	F	1.6	195	213
18	cyclohexyl	pyridyl-methyl	H	13.4	871	558
19	cyclohexyl	dimethylamide	H	3.5	>2000	90.9
20	isopropoxy-methyl	morpholine-propyl	F	52.5	979	1,660
21	ethoxy-methyl	tetrahydropyran-methyl	H	149	ND[b]	ND[b]
22	propoxy-methyl	tetrahydropyran-methyl	H	22.4	>2000	>2000
23	propoxy-methyl	morpholine-propyl	H	33.3	1850	>2000
24	isopropyl	tetrahydropyran-methyl	H	224	ND[b]	ND[b]

[a] Assay protocols can be found in Ref. 10 IC_{50} values are reported in nM.
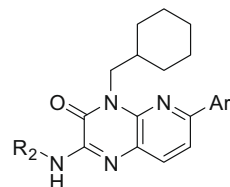[b] ND; Not Determined.

with 2,6-dichloro-3-nitropyridine (**5**) occurred selectively at the 2-position and gave amino-substituted pyridine **6**. Reduction of the nitro group followed by reaction with oxalic acid/HCl gave rise to the intermediate dione **7**. In turn, **7** was exposed to oxalyl chloride in the presence of DMF to give a chloroimidate intermediate which upon exposure to either a primary or secondary amine under mildly basic conditions yielded amino-substituted pyridopyrazinone compound **8**. With both the R^1 and R^2 variables introduced the synthesis was completed by standard Suzuki coupling of **8** with various aryl boronic acids to give analogs **9–38**.

Table 1 presents structure–activity relationships (SAR) of a representative set of analogs aimed at probing the breadth and scope of both the R^1 and R^2 substituents. Initially, we focused on analogs with the aryl substituent fixed as either the 4-fluorophenyl or 3,4-difluorophenyl group. In general, the SAR highlighted that PDE5 activity was more sensitive to the nature of the R^1 substituent than to the R^2 substituent. We identified several analogs with PDE5 potency in the low nanomolar range with varying degrees of selectivity against PDE 6 and 11. For example, when R^1 was *p*-methoxyphenyl (e.g., **9–10**) we obtained potent analogs in the single digit nanomolar range with good selectivity (~100×) against PDE11 but only modest selectivity against PDE6. The 3,5-difluorophenyl analog, **13** was found to be potent against PDE5 but also shows good selectivity against both PDE6 and PDE11. Pyridyl groups were also explored with the 3-pyridyl analog, **11** being preferred as it

Table 2
Structure–activity relationships for the R^1 and Ar positions when R^1 is cyclohexylmethyl

#	R2	Ar	PDE isoforms[a]		
			5	6	11
25	morpholine-propyl	dimethylisoxazole	1.0	41.6	286
26	tetrahydropyran-methyl	pyridyl	0.9	33.5	36.5
27	dimethylamide	methoxypyridyl	0.3	72.0	7.7
28	tetrahydropyran-methyl	methoxypyridyl	0.3	32.0	8.1
29	morpholine-propyl	methoxypyridyl	0.4	25.2	5.9
30	tetrahydropyran-methyl	methoxypyrimidyl	0.3	36.3	8.2
31	tetrahydropyran-methyl	hydroxypyridyl	2.9	112	79.2

[a] Assay protocols can be found in Ref. 10 IC_{50} values are reported in nM.

Table 3
Structure–activity relationships for the R^2 and Ar positions when R^1 is *n*-propylethoxy
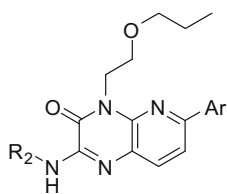
#	R_2	Ar	PDE isoforms[a]		
			5	6	11
32			36.5	1980	>2000
33			33.9	1170	>2000
34			14.5	1190	1210
35			2.3	350	1230
36			2.4	317	1540
37			26.7	>2000	>2000
38			534	ND[b]	ND[b]

[a] Assay protocols can be found in Ref. 10. IC_{50} values are reported in nM.
[b] ND; not determined.

was found to be more active and show greater sparing of PDE6 and PDE11 compared to 2-pyridyl analog **12**.

We also explored the saturated cyclohexyl ring which generally gave potent analogs (e.g., **15–17**) with good (>100×) selectivity for PDE6 and PDE11. We also observed some impact on activity and/or selectivity due to the nature of R^2 substituent (e.g., **18–19**). When R^1 was a simple alkyl group or alkyl ether a drop in PDE5 potency was observed which was also accompanied by an equal or greater loss in PDE6 and PDE11 activity. The chain length proved critical for activity. The ethoxyisopropyl analog, **20**, and ethoxyethyl analog, **21**, both exhibit only modest inhibition of PDE5. However, when the chain was lengthened by one atom to ethoxypropyl, a nearly sixfold improvement in activity, relative to **21**, was observed with only minimal or no increase in either PDE6 or PDE11 activity (e.g., **22** and **23**).

We next probed the SAR around the aryl substituent. We elected to focus on the scaffolds where R^1 was fixed as either the cyclohexyl or ethoxypropyl group. Table 2 summarizes the SAR observed in the template where R^1 was cyclohexyl. Within this series PDE5 potency was found to be relatively unaffected by the aryl group but selectivity was found to be negatively impacted. The

4-methoxypyridyl group was found to be optimal in terms of PDE5 potency (e.g., **27–29**) yielding a number of analogs in the sub-nanomolar range. Unfortunately, selectivity falls off especially for PDE11 which was found to be significantly diminished in this series. Additionally, these compounds suffered from poor aqueous solubility. For example, the aqueous solubility of **29** was found to be <3 μM, at pH 7.4.

The results for when R^1 was ethoxypropyl are summarized in Table 3. In general, these analogs tended to be less potent (∼5–10-fold) than their corresponding cyclohexyl variants found in Table 2 (see for e.g., **29** vs **35** for a comparison). We also noted a similar trend with respect to the 2-methoxypyridine substituent which was again found to be optimal with regard to PDE5 potency as highlighted by examples **34–36**. More importantly, however, these compounds demonstrated good selectivity against PDE6 (>100×) and excellent selectivity against PDE11 (>600×). Poor solubility was still as issue with this series as **36** was measured to have a solubility of 4.6 μM, at pH 7.4. Finally, as a representative example for the series, we also assessed the rat[11,12] pharmacokinetic (PK) profile of **36**. This compound exhibited high clearance (67.6 ml/min/kg) resulting in a modest half-life (∼3 h) indicating further refinement of the template would be required. Efforts to further optimize this scaffold to improve the PK properties, as well as the physical–chemical properties, will be discussed in the following Letter of this journal.

In conclusion, we report the discovery of a novel series of 2-amino substituted pyrido[3,2b]pyrazinone analogs that show potent and selective inhibition of PDE5. Starting from a novel lead, **1**, identified in a high-throughput screen and relying heavily on X-ray crystal structures we developed the pyridopyrazinone template **4** through a series of lead-hopping exercises. We further optimized the template to identify a handful of potent and selective analogs.

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9. The coordinates have been deposited in the PDB under accession code 3HDZ.
10. Brown, D. L.; Owen, D. R.; Phillips, C.; Palmer, M. J.; Bell, A. S.; Freskos, J. N.; Fobian, Y. M.; Walker. J. K.; Hughes, R.; Jacobsen, E. J.; Tollefson, M. B.; Brown, D. G.; Mischke, B. V.; Molyneaux, J. M.PCTWO 07/020521.
11. The Pfizer Institutional Animal Care and Use Committee reviewed and approved the animal use in these studies. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.
12. PK experiments were conducted in Sprague Dawley rats under the following conditions; IV: 2 mpk (*n* = 3) vehicle: 70% PEG400/20% 0.05 M citrate buffer/10% ethanol, pH 5; PO: 2 mpk (*n* = 3) vehicle: 0.5% methylcellulose/0.1% Tween 80 in 50 nM citric acid, pH 5.