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1-(2-Ethoxyethyl)-1*H*-pyrazolo[4,3-*d*]pyrimidines as potent phosphodiesterase 5 (PDE5) inhibitors

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ABSTRACT

1*H*-Pyrazolo[4,3-*d*]pyrimidines are a class of potent and selective second generation phosphodiesterase 5 (PDE5) inhibitors. This work explores the potency, selectivity and efficacy of 1-(2-ethoxyethyl)-1*H*-pyrazolo[4,5-*d*]pyrimidines as PDE5 inhibitors resulting in the advancement of a clinical candidate.

Sildenafil (Fig. 1, **1**), sold as Viagra® and Revatio®, acts by competitive inhibition of phosphodiesterase 5 (PDE5)[1] and is successfully used in the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension (PAH). Guanosine cyclic 3',5'-monophosphate (cGMP) levels are regulated by PDE5 through the conversion to GMP leading to the reduction of intracellular Ca^{+} levels and vasodilation.[2] By inhibiting PDE5, cGMP levels rise resulting in vasodilation of the vascular endothelium leading to benefit in conditions such as ED and PAH.

The effort to discover a novel PDE5 inhibitor with the potential for once a day dosing for chronic conditions was previously reported.[3] In the previous Letter[4] we report our efforts that led to promising and novel PDE5 inhibitors which exhibited an extended half-life and sustained efficacy consistent with projected once a day dosing in humans. Compound **2** is a potent PDE5 inhibitor (IC_{50} = 0.07 nM) with excellent selectivity (PDE6 IC_{50} = 8.9 nM) that exhibited efficacy in spontaneously hypertensive rats (SHR) at a dose of 5 mg/kg orally. We had some concerns about the trifluoroalkoxy substituent. First, the log *P* of the trifluoroethoxy is about 0.25 units higher than the ethoxy group potentially leading toward increase interaction with the hERG channel. Second, there was concern that the metabolic degradation product, trifluoroethanol, could cause adverse effects upon prolonged use.[5] As a result of these concerns we decided to proactively explore the feasibility of the ethoxyethyl substituent at the N-1 position.

The synthesis of these analogs was previous reported and illustrated in Figure 2.[4] The final analogs (**10–42**) can easily be prepared from the dichloride **8** in an efficient manner. Typically the first amine added to dichloride **8** was a deactivated amine, such as an aminopyridine. An excess of amine could be added and heated under conventional or microwave conditions to afford selective addition to the C-7 position leading to monoamine product **9**. It was possible to add a second and more nucleophilic amine (HNR^3R^4) in excess to the above reaction mixture to produce the final product (**10–42**) with selective addition at C-5 position and no adducts observed from the first amine. Extraction and reverse phase HPLC afforded pure products for testing.

We examined the SAR of the C-7 position with the ethoxyethyl substituent in the alkoxy pocket at N-1 (Table 1). We focused on aminopyridine substituents based upon our previous data in similar series. Aminopyridine **10** exhibited good PDE5 potency[6] (500 pM) with modest selectivity against PDE6 (78×) and PDE11 (62×). We wanted to determine whether we could push the

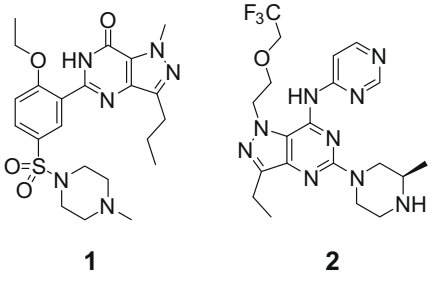

Figure 1. Structure of first generation (sildenafil, **1**) and second generation (pyrazolopyrimidine, **2**) PDE5 inhibitor.

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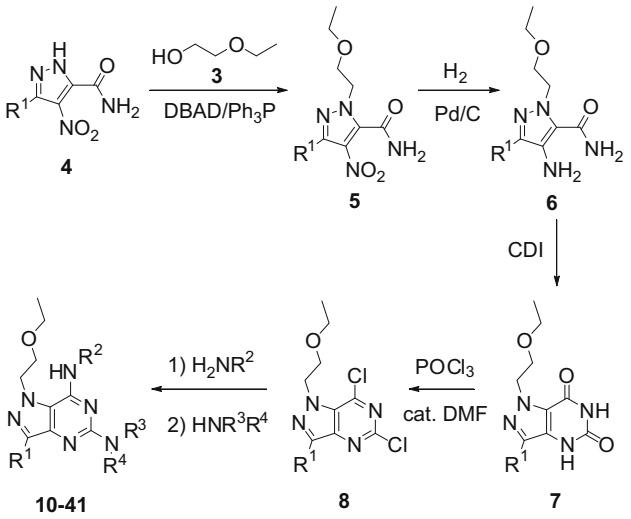


Figure 2. Preparation of PDE5 inhibitors.

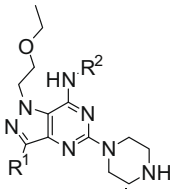
selectivity even higher due to concerns surrounding potential visual side effects due to PDE6 inhibition. Extension of the C-3 methyl to an ethyl (**11**) affords similar PDE5 potency (400 pM) with a loss of PDE6 (24×) and PDE11 selectivity (44×).

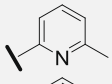
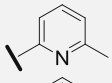
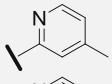
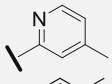
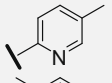
When the pyridine is replaced by a phenyl (**12**) we observe a boost in PDE5 inhibition (70 pM) and improvement in selectivity as we saw in the trifluoroethoxyethyl series previously reported. We did not pursue this analog due to the potential of producing a toxic metabolite from the aniline moiety.

Next we examined the addition of a single methyl group to the pyridine ring and observed the effect on both potency and selectivity. Methyl substitution at the 4- and 6-positions of the pyridine ring were preferred with 6-methyl substitution (**13** and **14**) delivering good potency and reasonable selectivities. When we switch to the 4-methylpyridine the C-3 substituent has a greater influence on selectivity. When C-3 is a methyl group (**15**) potency diminishes slightly from the 6-methylpyridine analog (**13**). More interestingly the selectivity against PDE6 and PDE11 is greatly reduced for compound **15**. When the C-3 methyl is extended to an ethyl group (**16**) not only does potency improve (380 pM) but so does PDE6 selectivity (100×). We presume this is due to a more optimal hydrophobic contact of compound **16**'s ethyl group in conjunction with the properly placed 4-methylpyridine. When the methyl on the pyridine is placed at either the 3- or 5-position (**17** and **18**) both potency and selectivity diminishes indicating the importance of the methyl substitution of the pyridine toward binding in each of the PDE isoforms. Another observation of this series (Table 1) was that the metabolic stability appears to be sufficient to support once a day dosing.

With the 4-methylpyridine offering good potency and reasonable selectivity we sought to explore the C-5 substituent while

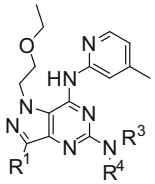
Table 1
SAR at the C-3 and C-7 position

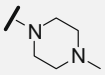
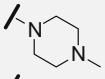
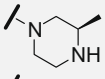
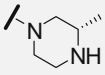
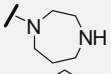
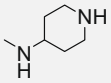
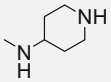
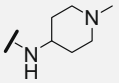
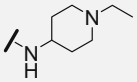
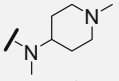
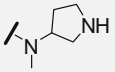
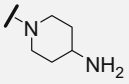
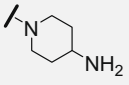
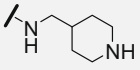
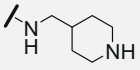
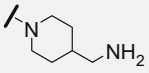
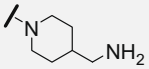


Compd	R^2	R^1	PDE5 Inh IC_{50}[a] (nM)	PDE6/PDE5 Ratio[b]	PDE11/PDE5 Ratio[b]	HLM[c]
10		Me	0.50	78×	62×	nd
11		Et	0.40	24×	44×	78%
12		Et	0.07	140×	160×	81%
13		Me	0.65	150×	61×	nd
14		Et	0.66	84×	52×	nd
15		Me	1.4	45×	13×	nd
16		Et	0.38	100×	24×	67%
17		Et	3.3	2.7×	1.1×	89%
18		Et	5.5	46×	18×	nd

[a] PDE5, PDE6 and PDE11 assay protocols can be found in Ref. 6.
[b] Ratio of IC_{50}'s.
[c] Human liver microsome stability, % compound remaining after 30 min.

Table 2
SAR of the C-5 position

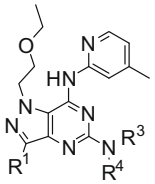


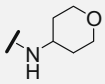
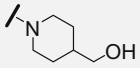
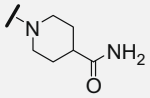
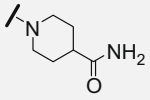
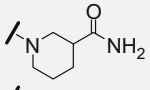
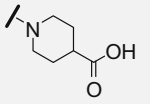
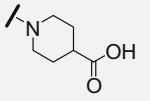
Compd	NR^3R^4	R^1	PDE5 Inh IC_{50}[a] (nM)	PDE6/PDE5 Ratio[b]	PDE11/PDE5 Ratio[b]	HLM[c]
19		Me	2.6	140×	130×	54%
20		Et	0.89	91×	99×	35%
21		Et	2.5	46×	31×	61%
22		Et	1.2	62×	8.3×	40%
23		Et	0.76	130×	28×	67%
24		Me	2.3	>320×	110×	84%
25		Et	0.20	310×	110×	60%
26		Et	0.72	58×	58×	nd
27		Et	2.0	190×	110×	41%
28		Et	0.087	110×	540×	36%
29		Et	0.42	50×	97×	26%
30		Me	2.3	350×	160×	90%
31		Et	0.78	170×	82×	nd
32		Me	0.35	260×	44×	nd
33		Et	0.011	110×	36×	nd
34		Me	0.070	220×	30×	nd
35		Et	0.007	170×	47×	nd

[a] PDE5, PDE6 and PDE11 assay protocols can be found in Ref. 6.
[b] Ratio of IC_{50}'s.
[c] Human liver microsome stability, % compound remaining after 30 min, nd = not determined.

maintaining the 4-methylpyridine (Table 2). Methylation of the piperazine (**19** and **20**) results in a modest loss of PDE5 potency compared to the desmethylpiperazine (**15** and **16**), however, there was a positive effect in PDE6 and PDE11 selectivity. Not surprisingly the metabolic stability of these compounds is reduced. The 3-methylpiperazine analogs (**21** and **22**) did not improve upon

Table 3
Non-basic SAR at the C-5 position



Compd	NR^3R^4	R^1	PDE5 Inh IC_{50}[a] (nM)	PDE6/PDE5 Ratio[b]	PDE11/PDE5 Ratio[b]	HLM[c]
36		Me	1.2	190×	130×	15%
37		Et	0.07	100×	120×	28%
38		Me	0.19	260×	320×	26%
39		Et	0.08	210×	210×	1%
40		Et	0.08	47×	56×	<1%
41		Me	0.05	89×	410×	nd
42		Et	0.04	100×	530×	87%

[a] PDE5, PDE6 and PDE11 assay protocols can be found in Ref. 6.
[b] Ratio of IC_{50}'s.
[c] Human liver microsome stability, % compound remaining after 30 min.

Table 4
Safety and in vivo pharmacology

Compd	Dofetilide[a] (%)	hERG[b]	IV Dog PK[c]			SHR[d]
			$t_{1/2}$	Cl	V_{dss}	
20	42	nd	5.2	30	11.4	nd
24	64	2.8 μM	nd	nd	nd	+
31	11	nd	11.7	48	50.4	+
42	61	1.1 μM	5.9	12.6	3.8	++

[a] Percent inhibition of [^{3}H]-dofetilide binding to the hERG protein stably expressed on HEK-293 cells following a 10 lM dose of test compound.
[b] hERG patch clamp electrophysiology assay, IC_{50}.
[c] Compound dosed at 0.2[f]–0.5[e] mpk in 10 kg beagles. Halflife ($t_{1/2}$) in h, clearance (Cl) in mL/min/kg, volume of distribution (V_{dss}) in L/kg.
[d] Compound dosed orally in spontaneously hypertensive rats (SHR) while monitoring MAP, + = decrease of 10–15 mmHg sustained <6 h, ++ decrease of >15 mmHg sustained >6 h.

the piperazine's potency or selectivity. Expansion to the homopiperazine ring (**23**) did not dramatically change the potency, selectivity or metabolic stability.

We examined a number of other basic amines to replace the piperazine (Table 2). Aminopiperidine (**24** and **25**) exhibit improved PDE6 (>300×) and PDE11 (>100×) selectivity. Interestingly there is approximately a 10× shift between the C-3 methyl analog (2.3 nM) and the C-3 ethyl analog (0.20 nM). This is not too different than the 4-methylpiperazine observation and hints at the subtle hydrophobic interactions in the binding pocket.

Methylation of the piperidine nitrogen (**26**) results in a loss of PDE5 potency and PDE6/11 selectivity while ethylation of the same nitrogen (**27**) continues the erosion of PDE5 potency but offers a recovery of PDE6/11 selectivity. Methylation of both nitrogens in aminopiperidine (**28**) affords an improvement in potency (87 pM) while maintaining excellent selectivity. Methylaminopyrrolidine (**29**) had good PDE5 potency but a loss of selectivity. Unfortunately the alkylated amines exhibit poor metabolic stability, presumably as a result of N-dealkylation. When the piperidine nitrogen was directly connected to the C-5 position (**30** and **31**) the potency was similar to its isomer (**24** and **25**) where the C-3 ethyl analogs exhibited greater PDE5 potency.

To further probe the tolerance of this pocket we added an additional atom. This resulted in excellent potencies and good PDE6 selectivity. As before the PDE5 potency is greater for the C-3 ethyl analogs versus the methyl analogs (**33** vs **32** and **35** vs **34**), however, the PDE6 selectivity is better for the methyl analogs. Interesting the PDE11 selectivity is marginal for all of these analogs, once again indicating the subtleties of the different binding pockets.

Since PDE5 inhibitors have had some correlation with the potential to interact with the human ether-a-go-go-related gene (hERG) we wanted to see if non-basic moieties would be tolerated.[7] Replacing aminopiperidine (**24**) with aminotetrahydropyran

(**36**) showed a similar potency and selectivity profile indicating that a basic nitrogen is not required for binding affinity (Table 3). Unfortunately compound **36** metabolizes quickly (15% remaining after 30 min). Piperidine methylalcohol **37** delivers excellent PDE5 potency (70 pM) and good PDE6/11 selectivity (100×/120×) but metabolizes quickly. 4-Amidopiperidines (**38** and **39**) exhibit similarly good potencies and improved PDE6/11 selectivity but metabolize extremely quick as well. The 3-amidopiperidine (**40**) has excellent potency but now indicates a change in selectivity indicating the potential differences in the depth and width of the PDE6 and PDE11 binding pockets.

We prepared carboxylic acid derivatives to further explore the true nature of the binding pocket but to also alter the binding profile to the hERG channel. This modification was also expected to significantly decrease the volume of distribution and alter the PK profile of these compounds. Compounds **41** and **42** both exhibit excellent potencies (50 pM and 40 pM) while maintaining adequate PDE6 selectivity and improved PDE11 selectivity (>400×). On a positive note, when compound **42** was incubated with human liver microsomes, 87% of compound remained after 30 min.

Table 4 indicates additional profiling we performed on several representative compounds. We initially used a competition assay using radiolabeled dofetilide to determine the compound's likelihood to interact with the hERG channel. At a compound concentration of 10 μM no molecule inhibited at a value greater than 70%. We followed up compounds with a value greater than 50% with a hERG patch clamp assay. We found hERG values to be slight more potent than what is predicted by the dofetilide assay. Based on a ratio of hERG/PDE5 IC_{50} values of compound **24** (>3100×) and compound **42** (>27,000×), we had few cardiac safety concerns going forward with these compounds.

We examined the pharmacokinetic (PK) properties of these molecules using dogs (Table 4).[8] Methylpiperazine **20** has moderately high clearance (30 mL/min/kg) and volume (11.4 L/kg). Aminopiperidine **31** had an even higher clearance (48 mL/min/kg) and volume (50.4 mL). As previously stated we prepared the acids with the hopes that it would alter the PK profile away from the high volumes seen with the amines. This indeed was the case. Acid **42** showed an improved clearance (12.6 mL/min/kg) and reduced volume (3.8 L/kg) resulting in a half-life of 5.9 h.

These compounds were taken into an in vivo model of efficacy (Table 4). We used spontaneously hypertensive rats (SHR) which were monitored for compound levels and blood pressure. Encouragingly pyrimidines **24**, **31** and **42** all exhibited a lowering of blood pressure after oral administration of compound. This is consistent with low clearance and also the high solubility observed with compounds from this series. Amines **24** and **31** show a maximum decrease in mean arterial blood pressure (MAP) of 10–15 mmHg at 1–2 h post dose. The MAP returned to basal levels in under 6 h as indicated by the higher clearances observed in dog PK. Acid **42** shows a maximum reduction in MAP of 30 mmHg which is sustained for at least 24 h. The measured free fraction of compound **42** at 24 h post dose indicates blood levels 18-fold over the PDE5 IC_{50} levels.

We have presented an alternate to the trifluoroethoxyethyl compounds that were previously reported. We successfully indentified a number of potent and selective PDE5 inhibitors that that exhibit promising PK profiles in the presence of the potentially metabolizable ethoxyethyl substituent. In an efficacy model using SHR we observed sustained lowering of blood pressure which show promise as a long-acting therapeutic when the acid is incorporated. Compound **42** was taken forward into phase 1 clinical trials for further evaluation.

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8. 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.