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

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ABSTRACT

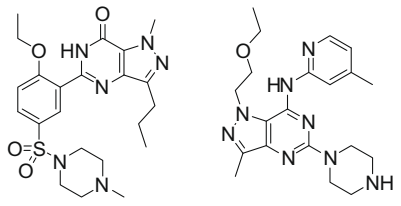
1*H*-Pyrazolo[4,3-*d*]pyrimidines were previously disclosed as a potent second generation class of phosphodiesterase 5 (PDE5) inhibitors. This work explores the advancement of more selective and potent PDE5 inhibitors resulting from the substitution of 2-(2,2,2-trifluoroethoxy)ethyl at the 1 position in the so-called alkoxy pocket.

Sildenafil (Fig. 1, **1**), sold as Viagra® and Revatio®, is successfully used in the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension (PAH). Sildenafil acts by competitive inhibition of phosphodiesterase 5 (PDE5).[1] PDE5 regulates guanosine cyclic 3′,5′-monophosphate (cGMP) levels by converting cGMP to GMP. cGMP interacts with protein kinase G which leads to the reduction of intracellular Ca^{+} levels and vasodilation.[2] By inhibiting PDE5, cGMP levels rise resulting in vasodilation of the vascular endothelium allowing for the treatment of ED and PAH.

Sildenafil is a potent PDE5 inhibitor (IC_{50} = 3.5 nM) and selective against other PDE isoforms. The most active isoform is PDE6 (IC_{50} = 30 nM) which is expressed in the retina and may be related to visual effects which are sometimes reported. Sildenafil is quickly absorbed and has a 4–6 h duration of effect.[3] Efforts to improve upon the selectivity of PDE5 inhibitors and extend their plasma half-life have been previously reported.[4] Compound **2** is an example of a new class of PDE5 inhibitors with good potency (1.84 nM) and selectivity (PDE6 IC_{50} = 946 nM) that served as a representative starting point in this novel template.[5] Our goal was to enhance the potency of these pyrazolo[4,3-*d*]pyrimidines while enhancing the selectivity to prevent possible side effects while extending the half-life to allow for chronic dosing of a PDE5 inhibitor.

Initially we decided to investigate the ethoxyethyl moiety at the N-1 position of the pyrazolopyrimidine due to its potential susceptibility to metabolic degradation due to its alkoxy substituent and its potential to influence selectivity as indicated by previous SAR.[5] The synthesis of these analogs is illustrated in Figures 2 and 3. The attachment of the alkoxy piece is done early in the synthesis. A Mitsunobu reaction of the alkoxyethanol (**3**) piece is performed with 4-nitro-1*H*-pyrazole-5-carboxamide (**4**) to afford the N-alkylated pyrazole (**5**). The nitro group in compound **5** is reduced to the amine **6** via hydrogenation. Cyclization of compound **6** to the dione **7** was accomplished by reaction with carbonyldiimidazole. Finally the common intermediate for all analogs was prepared by chlorination of **7** using phosphorus oxychloride in the presence of a catalytic amount of dimethylformamide (DMF) to produce the required pyrazolopyrimidine dichloride **8**.

The final analogs (**10–29**) can easily be prepared from dichloride **8** in a facile manner which allowed for the rapid development of SAR for this series (Fig. 3). 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



1 **2**

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

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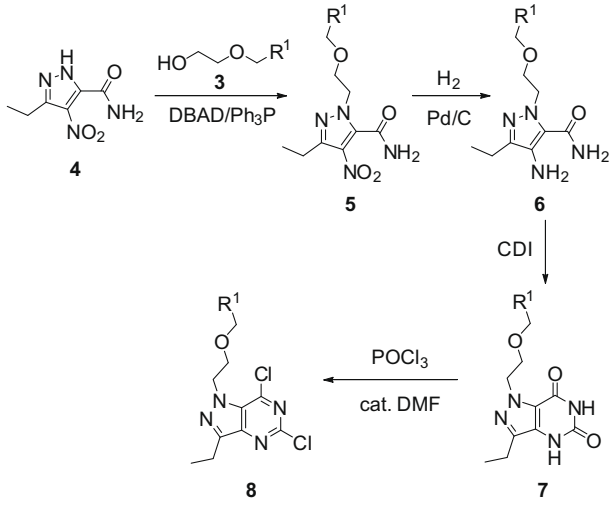


Figure 2. Preparation of intermediate dichloride **8**.

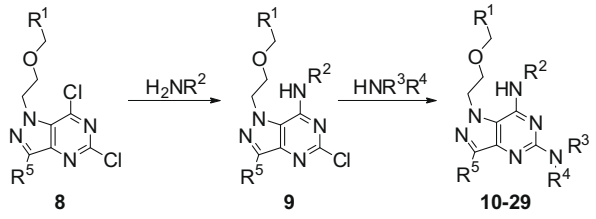
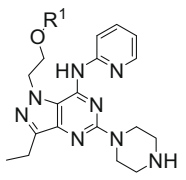


Figure 3. Preparation of final analogs.

the second and more nucleophilic amine (HNR^3R^4) in excess to the above reaction mixture to produce the final product (**10–29**) 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.

As a comparator to the previous work we prepared compound **10** with an ethoxyethyl substituent (Table 1). Compound **10** exhibited good PDE5 potency[6] (400 pM) and reasonable metabolic stability with 78% of the compound intact after a 30 min incubation with human liver microsomes (HLM). We sought to see the effect of extending the alkoxy substituent. When replacing the ethoxy with an *n*-propoxy substituent (**11**) we observed similar potency (300 pM) with improved PDE6 and PDE11 selectivity indicating the alkoxy pocket is shallower in PDE6 and PDE11. Not surprisingly compound **11** had poorer metabolic stability (58%) due to its higher lipophilicity. We prepared a bulkier *i*-propoxy analog (**12**) which lost a little potency (860 pM) compared to **10** but did not improve the selectivity as much as **11**. We prepared the trifluoroethoxy analog (**13**) in an effort to block metabolism at the ethoxy chain. This was successful as our HLM stability assay improved to 91% even though the log *P* increased. Another interesting observation with **13** was that the trifluoroethyoxy group greatly enhanced the selectivity against PDE6 and PDE11.

Table 1
SAR at the N-1 position. (R^5 = Et)



Compd	R^1	PDE5 inhibition IC_{50}[a] (nM)	PDE6/PDE5 ratio[b]	PDE11/PDE5 ratio[b]	HLM stability[c]
10	Et	0.40	24×	44×	78%
11	*n*-Pr	0.30	180×	160×	58%
12	*i*-Pr	0.86	50×	52×	nd
13	CH_2CF_3	0.65	150×	450×	91%

[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.

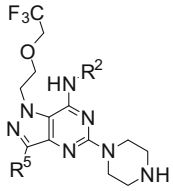
We pursued the trifluoroethoxyethyl analogs further due to their excellent selectivity and improved metabolic profile. We started by exploring the SAR of the C3 and C7 positions (Table 2). The high level of selectivity observed with the trifluoroethyoxyethyl analog **13** was observed in these analogs as well. We explored the pyridine substituent's effect on selectivity by adding a small methyl substituent in an effort to keep log *P*'s manageable. The 6-methylpyridyl analogs (**14** and **15**) exhibited greater PDE6 selectivity while maintaining PDE5 potency compared to **13**. When the methyl group is moved to the 4 position of the pyridine (**16** and **17**) some PDE5 activity is lost while PDE11 selectivity is reduced indicating subtle changes in the binding pockets of the PDE isoforms. The methyl group at the C3 position (**14** and **16**) exhibits slightly better potency and selectivity compared to the ethyl group (**15** and **17**).

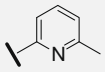
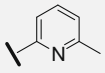
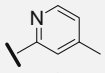
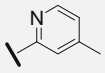
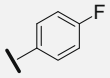
A *p*-fluoroaniline analog (**18**) exhibited greatly improved PDE5 potency (89 pM) while increasing PDE6 and PDE11 selectivity and being metabolically stable. The downside of this type of analog is the increase in log *P*, the potential for hERG interactions and concerns over aniline derived reactive metabolites. Pyrimidine **19** modulates the basicity of the pyridine nitrogen and an enhancement in PDE5 potency (150 pM) while improving PDE11 selectivity while having excellent metabolic stability.

We examined the SAR at the C-5 position while maintaining the pyrimidine at the C-7 position and the trifluoroethoxyethyl group at the N-1 position (Table 3). Expanding the piperazine ring to the homopiperazine shows a sharp drop off in potency (**20** and **21**) indicating the importance of ring size and placement of the basic nitrogen. Methylation of the homopiperazine nitrogen (**22**) does little to affect the PDE5 potency of these molecules. Introduction of the dimethylated aminopiperidine affords a nice improvement in potency indicating that this pocket has room to place larger substituents to maintain potency and selectivity. The introduction of a methyl group at the 3-position of the piperazine (**24** and **25**) led to an improvement of PDE5 potency by presumably taking advantage of enhanced lipophilic contact to the binding pocket. When R^5 is ethyl (**25**), PDE11 selectivity is greatly improved compared to piperazine **19** while PDE6 selectivity is maintained. The enantiomer of **24**, **26** is sixfold less potent than **24** and shows some decrease in PDE6 and PDE11 selectivity. An example of an acyclic analog (**27**) shows weaker PDE5 potency compared to the cyclic analogs. Examination of a couple non-basic analogs led to modest potencies indicating importance of potential H-bonding interactions with the basic amine. The morpholine (**28**) and piperazinone (**29**) analogs exhibited good potency and selectivity.

With a basic center and an aromatic ring in the molecule we were concerned about potential interactions with the human ether-a-go-go-related gene (hERG) which have the potential to lead to QT prolongation related toxicities which warranted further study.[7] Compounds **14**, **19**, **24** and **25** offered a good cross section of compounds with a combination of good potency, selectivity and metabolic stability. We initially used a competition assay using radiolabeled dofetilide to determine the compound's likelihood to interact with the hERG channel (Table 4). At a compound concentration of 10 μM each molecule inhibited at a value less than

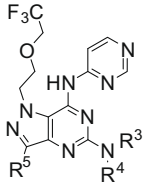
Table 2
SAR at the C-3 and C-7 position

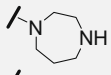
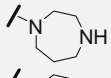
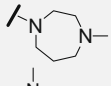
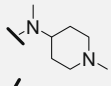
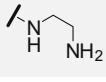


Compd	R^2	R^5	PDE5 inhibition IC_{50}[a] (nM)	PDE6/PDE5 ratio[b]	PDE11/PDE5 ratio[b]	HLM[c] (%)
14		Me	0.78	420×	440×	83
15		Et	1.01	310×	440×	63
16		Me	1.84	510×	280×	75
17		Et	3.28	220×	170×	80
18		Et	0.09	470×	2200×	100
19		Et	0.15	120×	2800×	96

[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.

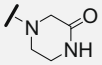
Table 3
SAR of the C-5 position



Compd	NR^3R^4	R^5	PDE5 inhibition IC_{50}[a] (nM)	PDE6/PDE5 ratio[b]	PDE11/PDE5 ratio[b]	HLM[c]
20		Me	9.79	35×	204×	nd
21		Et	3.15	69×	635×	nd
22		Et	6.48	309×	287×	nd
23		Et	0.35	285×	5650×	31%
24		Me	0.14	312×	14,700×	76 min[d]
25		Et	0.07	125×	27,000×	42 min[d]
26		Me	0.94	187×	2100×	68%
27		Et	9.52	>210×	>210×	nd

(*continued on next page*)

Table 3 (*continued*)

Compd	NR^3R^4	R^5	PDE5 inhibition IC_{50}[a] (nM)	PDE6/PDE5 ratio[b]	PDE11/PDE5 ratio[b]	HLM[c]
28		Et	3.92	107×	5100×	nd
29		Et	2.96	>675×	>675×	34%

[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.
[d] HLM half-life.

Table 4
Safety and in vivo pharmacology

Compd	Dofetilide[a] (%)	hERG[b]	IV Dog PK[c]			SHR[d]
			$t_{1/2}$	Cl	V_{dss}	
14	40.0	560 nM	nd	nd	nd	nd
19[e]	10.7	5.1 μM	6.7	20.2	8.0	+
24[e]	24.7	900 nM	4.9	22	9.4	++
25[f]	18.8	1.4 μM	5.1	18.5	7.8	++

[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, ++ decrease of >15 mmHg.

50%. As expected, the removal of the basic center leads to a loss of dofetilide binding (% inh @ 10 μM: **28**, 1%; **29**, 3%). Generally the pyridyl analogs tended to have higher dofetilide binding, presumably due to the pyridine's higher log *P* and the pyridine being more basic than the pyrimidine.

To confirm the relevance of the dofetilide competition binding assay we took select compounds into a hERG patch clamp assay to determine the compound's interaction with the hERG channel (Table 4). We found that the rank order of activity observed with the dofetilide assay was the same for the hERG assay. Once again the pyridine **14** had the greatest hERG activity (560 nM). Note that it appears that the hERG activity is underestimated by the dofetilide assay for these molecules. Pyrimidine **19** showed 10.7% inhibition at 10 μM in the dofetilide assay where it had an IC_{50} = 5.1 μM in hERG. Therefore the hERG safety margin was less than originally predicted by the dofetilide assay. The ratio of PDE5 IC_{50} to hERG IC_{50} for compound **19** is over 34,000×.

We examined the pharmacokinetic (PK) properties of these molecules using dogs (Table 4).[8] Compounds in this series generally exhibit excellent solubility, presumably due to the basic nitrogen. Pyrimidines **19**, **24** and **25** have similar profiles with moderate clearance (~20 mL/min/kg) and volume (8–9 L/kg) leading to terminal half-lifes of 5–7 h, consistent with once a day dosing in human.

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 **19**, **24** and **25** all exhibited a lowering of blood pressure after oral and IV administration of compound. For example after IV dosing of 1.5 mg/kg of compound **25**, we observed a maximum decrease in mean arterial blood pressure (MAP) of 27 mmHg at 2 h post dose. The MAP remained at a reduction of 23 mmHg 6 h post dose. The measured free fraction of compound **25** at 6 h post dose indicates blood levels 72-fold over the PDE5 IC_{50} levels. When compound **25** is dosed at 5 mg/kg orally, the MAP decreased by 27 mmHg at its maximum effect. The reduction in MAP was sustained over 20 h post dose. At 20 h the free plasma exposure of compound **25** is 25-fold over the measured IC_{50} levels indicating its promise for once a day dosing.

We have presented a number of potent and selective PDE5 inhibitors that exhibit promising PK profiles consistent with projected once a day dosing in humans. More importantly these compounds exhibit good oral efficacy as measured by the reduction of MAP, which is sustained as predicted by the PK profile allowing for further investigation of the chronic dosing of PDE5 inhibitors.

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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.