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The discovery of potent, selective, and orally bioavailable PDE9 inhibitors as potential hypoglycemic agents

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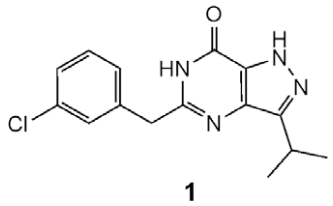
ABSTRACT

Starting from a non-selective pyrazolo-pyrimidone lead, the sequential use of parallel medicinal chemistry and directed synthesis led to the discovery of potent, highly selective, and orally bioavailable PDE9 inhibitors. The availability of these tools allowed for a thorough evaluation of the therapeutic potential of PDE9 inhibition.

Phosphodiesterase 9 (PDE9) is one of three cGMP specific enzymes out of the nineteen known PDE isoforms.[1] PDEs regulate intracellular cAMP and/or cGMP levels and have received a great deal of attention as drug targets for treating a wide range of conditions.[2] Although they are a very drug-able class of targets, they do carry certain challenges. Their ubiquitous expression in many tissue types can lead to toleration and side effect issues. Likewise, the presence of several PDE isoforms in the same cell may thwart efforts to modulate cyclic nucleotide levels due to redundant degratory pathways. PDE9 is a particularly complex isoform due to the more than twenty splice variants that have been identified.[3]

Our interest in PDE9 inhibitors as potential antidiabetic agents originated from knock out (KO) studies in mice. When placed on a high fat diet, these mice developed a phenotype that included reduced insulin resistance, reduced weight gain, and lower fat mass. A program was initiated to determine whether these effects could be mimicked with a small molecule inhibitor. At the origin of this project, there were no reported inhibitors of PDE9.[4] Cross-screening of representative examples from previous PDE inhibitor programs identified a potent but non-selective inhibitor **1**. In addition to PDE9, the compound had significant activity at PDE 1a, 1b, 1c and 5.

Although not initially prepared by parallel synthesis, it was recognized that compound **1** could be accessed by a parallel synthesis protocol we had previously developed (Scheme 1). This method allows the condensation of 2-aminoheterocycliccarboxamides[5] with carboxylic acids in a two-step activation/acylation and cyclization sequence.[5b]



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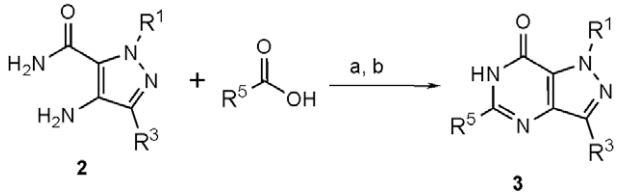

2 **3**

Scheme 1. Reagents and condition: (a) CDI, pyridine, DMA; (b) KO*t*Bu, IPA, 80 °C.

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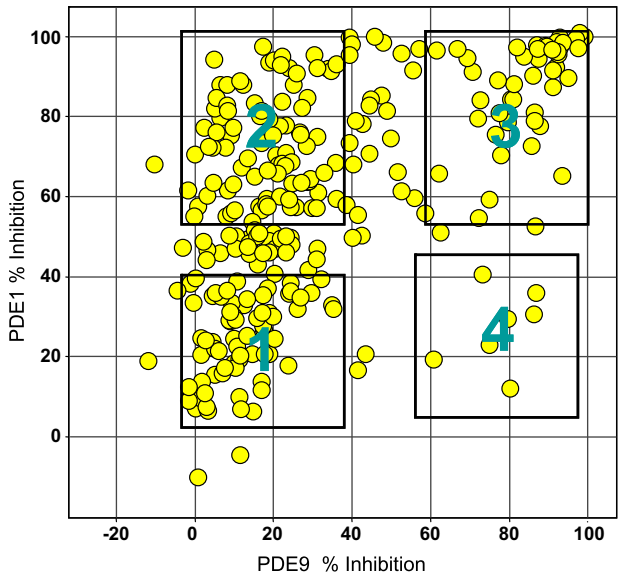


Figure 1. Profile of diverse 1st array in PDE 1 and 9 assays (1 μM concentration).

The first array combined the same isopropyl-substituted template as in compound **1** but explored a wide diversity of carboxylic acids, resulting in 272 5-substituted pyrazolopyrimidinone derivatives. These analogs were profiled in a single point inhibition assay against both PDE1 and PDE9 (results displayed in Fig. 1) and through endpoint assay determination for selected examples. A number of SAR conclusions were drawn from this array based on a number of common features of the C5 substituent in the compounds from the four marked segments:

(1) Compounds showing weak activity against both PDE1 and 9 were mostly derived from arylcarboxylic acids.

(2) Compounds showing strong activity against PDE1 but weakly active against PDE9 featured alpha-branched benzyl groups.

(3) Potent inhibitors of both PDE1 and 9 included a range of 3- and 4-substituted benzyl groups.

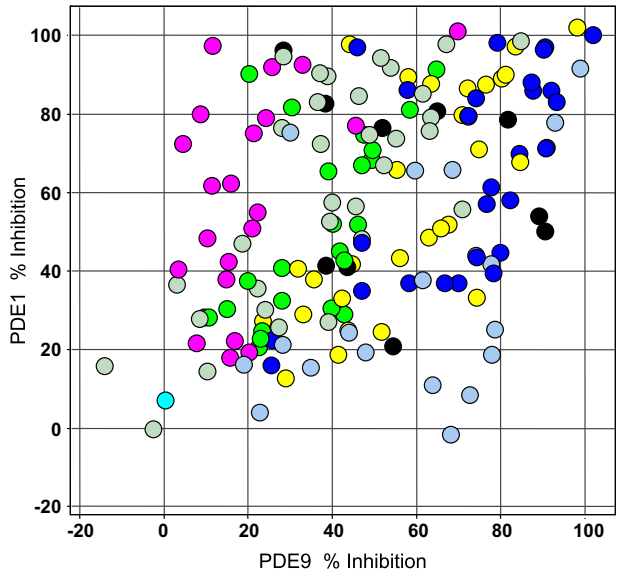


Figure 2. Profile of directed 2nd array in PDE 1 and 9 assays (1 μM concentration). Colored by R^1,R^3: H, cPent; H, iBu; H, iPr; H, nBu; H, tBu; H, pyridin-2-yl; H, pyridin-3-yl; Me, nPr.

(4) The target segment was poorly populated but contained several 2-substituted benzyl substituents.

The second array combined eight templates with a selection of monomers informed with data from the first library, plus additional 2-substituted phenylacetic acids from our monomer collection, resulting in 164 novel analogues. Figure 2 shows that the substituents on the pyrazole template also play a part in determining selectivity. Interestingly, introduction of a 1-methylsubstituent or increasing the steric bulk at the 3-position (isopropyl to *t*-butyl) reduced selectivity. Selectivity was enhanced by replacement of the isopropyl substituent by a 3-pyridyl group, albeit at the expense of inferior physicochemical properties. Constraint of the isopropyl in a cyclopentyl ring provided little advantage in either potency or selectivity. Two of the most interesting hits (**4** and **5** in Table 1) from the libraries were profiled against the PDE1 isoforms, which confirmed the importance of *ortho* substitution on selectivity. Compound **5** became the focus of further SAR efforts. Areas for improvement included increasing potency and selectivity as well as polarity and solubility.

Despite lacking a protein crystal structure early in the program, it was inferred that the pyrimidone portion of the core was forming key hydrogen bonds to the conserved glutamine in the catalytic site, similar to PDE5, and was therefore considered essential.[6] Small aliphatic groups were preferred at C3, although this SAR will not be presented within the scope of this publication. One obvious area for modification was the benzyloxy group, which was a metabolic and solubility liability. Replacement of the benzyl group with a variety of amines and other polar substituents was well tolerated suggesting that this region was exposed to solvent. This hypothesis was supported by homology modeling and later confirmed by X-ray crystallography.[7]

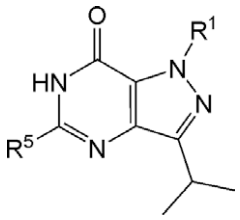
Compound **6** emerged as an analog of interest due to its improved selectivity and solubility. As such, it was deemed adequately potent and selective to begin in vivo testing. After substantial investigation, it was found that no acute glucose lowering could be detected. However, sub-chronic dosing in *ob*/*ob* mice showed robust glucose lowering and reduced weight gain (Table 2). Poor pharmacokinetics in mice required the use of very high doses admixed with powdered chow to achieve sustained plasma drug exposure. The fact that insulin was also reduced suggested improved glucose disposal. This profile was not unlike the KO phenotype and gave us the confidence to continue with the program.

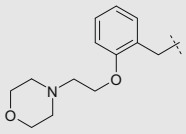
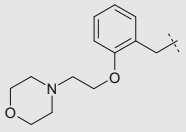
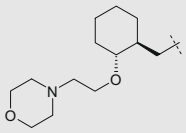
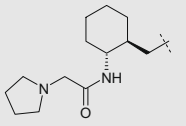
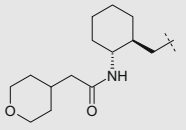
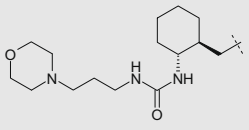
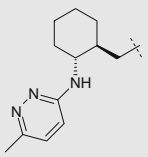
Given that the core structure in **6** was the same as found in sildenafil, we attempted to leverage the SAR learned in that program. Methylation at N1 for example, was found to increase PDE5 potency and selectivity over PDE1.[8] Unfortunately, this modification, analog **7**, resulted in loss of both potency and selectivity for PDE9 confirming the results from the libraries. It was noted that **7** showed substantial improvement in permeability. The inability to mask the pyrazole NH on the core would have implications later in the program (vide infra).

The improved selectivity of the saturated analog **8** over PDE1a and PDE1b prompted further exploration in the cyclohexyl series. An added benefit of this modification was that it attenuated the metabolic lability of the doubly benzylic methylene group found in **6**.

To expand the SAR, the amino cyclohexyl intermediate **20** was targeted. Its synthesis required an efficient preparation of the acid **19** which initially proved elusive. A scalable route was eventually developed as shown in Scheme 2. The key steps were using dissolving metal reduction of oxime **16** to achieve selectivity for the desired *trans* isomer, and the use of the trifluoroacetamide protecting group which provided the highly crystalline intermediate **18** which facilitated purification. The absolute configuration of the active enantiomer of the final products was unambiguously

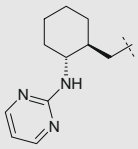
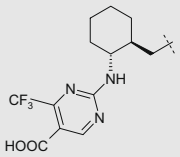
Table 1
Activity and selectivity of PDE9 inhibitors



Compound	R^5	R^1	PDE9 IC50[a] (μM)	PDE1a IC50 (μM)	PDE1b IC50 (μM)	PDE1c IC50 (μM)	*c*Log*P*	MDCK Papp (10^{-6} cm/s)
1	3-Chloro-benzyl	H	0.01	0.054	0.038	0.004	3.14	17
4	2-OCF_3-benzyl	H	0.056	2.5	2.65	0.52	3.46	10.8
5	2-Benzyloxy-benzyl	H	0.082	1.43	2.63	1.34	4.12	nd
6		H	0.041	3.9	4.95	1.13	2.47	15
7		Me	0.53	0.79	7.81	0.97	2.1	34
8		H	0.087	>10	>10	2.05	2.40	6.3
9		H	0.036	>10	>10	>10	2.28	0.5
10		H	0.019	>10	>10	3.1	1.36	0.75
11		H	0.046	>10	>10	4.2	2.30	0.7
12		H	0.023	>10	>10	7.6	3.06	1.7

(*continued on next page*)

Table 1 (*continued*)

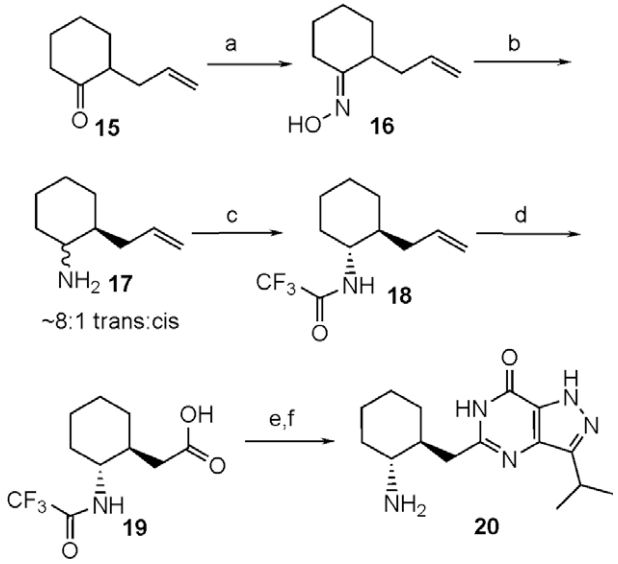
Compound	R^5	R^1	PDE9 IC50[a] (μM)	PDE1a IC50 (μM)	PDE1b IC50 (μM)	PDE1c IC50 (μM)	*c*Log*P*	MDCK Papp (10^{-6} cm/s)
13[b]		H	0.035	7.7	9.8	4.4	2.69	13
14[b]		H	0.007	>10	>10	>10	3.04	nd

[a] Values represent the mean of at least three experiments. Generally, SEMs were ±50% of the mean value. See the Supplementary data for details.
[b] Single enantiomers.

Table 2
Subchronic (4 day) dosing of compound **6** in ob/ob mice

Dose[a] (mg/kg)	Glu[b] (%)	Ins[b] (%)	BW[b] (%)	[Free drug][c] (nM)
100	−32	−35	−4.3	140
250	−40	−35	−6.4	243
500	−55	−59	−11	361

Change relative to control.
[a] Compound dosed in feed.
[b] Control values on day four: glucose: 416 ± 33 mg/dl; insulin: 23 ± 3.5 ng/mL; body weight: 44 ± 0.8 g.
[c] Free drug concentration at terminal bleed. See Supplementary data for details.



Scheme 2. Reagents and conditions: (a) NH_2OH HCl, pyridine, EtOH, rt; (b) Li, NH_3, THF, *t*BuOH, −78 °C; (c) CF_3CO_2Me, CH_2Cl_2; (d) $KMnO_4$, $NaIO_4$, acetone, water; (e) **2**, T3P, Et_3N, EtOAc, rt; (f) KO*t*Bu, 1-propanol, 80 °C.

determined by utilizing enantiomerically enriched **15** of known configuration.[9]

The availability of **20** enabled the rapid production of potent and selective analogs utilizing a variety of linking groups (e.g., amines, amides, sulfonamides, ureas, etc.). A representative selection of these derivatives is shown in Table 1. It soon became clear that despite excellent potency, selectivity and solubility profiles, analogs containing an amide or similar linker, were plagued with low oral bioavailability. This attribute (presumably due to poor permeability reflected in the low MDCK Papp values) was most likely due to two factors: high polar surface area and number of hydrogen bond donors. This data helped to refine our design strategy. Although substitution off the nitrogen was not restricted from a potency point of view, substituents leading to compounds with drug-like properties were much more limited. The pyrimidine analogs **13** and **14** provided the optimal balance of pharmacological and physicochemical properties, which resulted in excellent oral bioavailability (Table 3). Unfortunately, neither of these analogs possessed any in vivo glucose lowering activity despite achieving equivalent or higher tissue and plasma free drug levels compared to **6**. After extensive studies, it was postulated that the activity seen with **6** was due to an, as of yet unidentified, off-target mechanism.

Table 3
In vivo pharmacokinetics

	Compound **13**	Compound **14**
Rat Cl (ml/min/kg)	46	19.8
Rat Vdss (L/kg)	0.85	0.12
Rat F (%)	100	100
Dog Cl (ml/min/kg)	3.9	nd
Dog Vdss (L/kg)	0.9	nd

In summary, starting from a non-selective lead, a series of potent, selective, soluble and orally bioavailable PDE9 inhibitors have been identified. Keys to this success was the discovery of a solvent-accessible region of the catalytic site which allowed for fine tuning the physicochemical properties of the lead, as well as minimizing polar surface area and hydrogen bond donors to improve permeability. Although these compounds failed to validate PDE9 as a diabetes target, they remain useful tools for examining other potential indications for this cGMP-modulating enzyme.

Supplementary data

Experimental details for the preparation of test compounds as well as biological protocols and full PDE panel selectivity data for compounds **6**, **13** and **14**, are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.024.

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