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The discovery of potent, selective, and orally active pyrazoloquinolines as PDE10A inhibitors for the treatment of Schizophrenia

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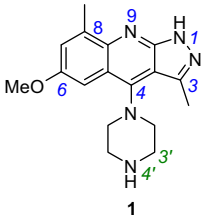
ABSTRACT

High-throughput screening identified a series of pyrazoloquinolines as PDE10A inhibitors. The SAR development led to the discovery of compound **27** as a potent, selective, and orally active PDE10A inhibitor. Compound **27** inhibits MK-801 induced hyperactivity at 3 mg/kg with an ED_{50} of 4 mg/kg and displays a ~6-fold separation between the ED_{50} for inhibition of MK-801 induced hyperactivity and hypolocomotion in rats.

Schizophrenia is a complex thought disorder affecting cognition, emotion, perception and behavior. It is characterized by 'positive' symptoms, including hallucinations, delusions, and disorganized speech and behavior; 'negative' symptoms, including lack of motivation, an inability to express emotion, and apathy, and cognitive dysfunction. First generation anti-psychotic drugs (APs) are dopamine 2 (D2) receptor antagonists. While effective in treating positive symptoms, these drugs are ineffective in treating negative symptoms and cognitive dysfunction and commonly cause extrapyramidal syndrome (EPS), a serious side effect characterized by a constellation of involuntary movements. Second generation APs target the D2 receptor, as well as other receptors and have a lower incidence of EPS. However, they are also ineffective in treating negative symptoms and cognitive dysfunction and are associated with other severe side effects, including weight gain, diabetes, and QT prolongation.[1–3]

Phosphodiesterases (PDEs) regulate intracellular cAMP and/or cGMP levels and have been identified as potential therapeutic targets for treating a wide variety of conditions.[4–12] PDE10A is a dual substrate PDE highly expressed in medium spiny neurons of the striatum.[13,14] By blocking the degradation of cAMP and cGMP, a PDE10A inhibitor should mimic the effect of a D2 receptor antagonist and a D1 receptor agonist, an ideal profile for an AP agent. PDE10A inhibitors are under investigation as antipsychotic agents and have been shown to be efficacious in a variety of preclinical models that may predict efficacy in the treatment of schizophrenia.[15]

High-throughput screening identified a series of pyrazoloquinolines as PDE10A inhibitors. Pyrazoloquinoline derivatives have been reported as PDE5 inhibitors previously.[16] Compound **1** is a potent hPDE10A inhibitor with a PDE10A inhibitory activity of 11 nM and selectivity greater than 70-fold over other PDE enzymes including PDE5A (Fig. 1). It has high ligand efficiency[17] (LE = 0.47) with a calculated lipophilicity (*c*Log*P*) of 3.8 and polar surface area (PSA) of 66. In vitro metabolite identification conducted by incubating compound **1** with rat microsome suggested that O-demethylation is the major metabolic pathway in the rat. In the mouse and



1

PDE10A K_i= 11 nM, LE= 0.47
PDE5A Ki > 100,000 nM
Other PDEs $K_i \geq 770$ nM
Rat PK AUC (PO,10 mg/kg, 0-6 h)[19]= 3.3 μM.hr
Brain conc: 123 ng/g, B/P ratio= 1.2 (6-h)
Rat PPB (% bound): 84%

MW= 311,cLogP= 3.8, PSA= 66

Figure 1. Structure, in vitro and PK data of compound **1**.

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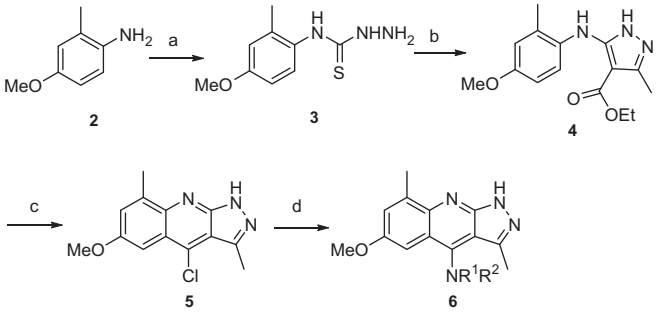
human microsomes, the major metabolic pathway is hydroxylation of the piperazine ring. Screening of compound **1** against panels of receptors and protein kinases indicated that it was active against serotonin receptors 5HT1b, 5-HT3, and 5-HT7 (54–85% inhibition at 10 μM) and casein kinase 1 delta (CK1δ IC_{50} = 320 nM). Compound **1** was evaluated for its anti-psychotic effect in the inhibition of MK-801-induced hyperactivity assay.[18] In this study compound **1** was not active at 30 mg/kg in the rat dosed orally. Therefore the SAR development was aimed at improving the off-target selectivity, and in vivo activity of the series.

Target compounds were prepared according to the procedure described in Scheme 1. Thiosemicarbazide intermediate **3** was thus prepared upon treatment of the corresponding aniline with carbon disulfide and hydrazine. Condensation with ethyl 2-chloroacetoacetate gave, following extrusion of sulfur, pyrazole ester **4**. Saponification and cyclization with $POCl_3$ then yielded the chloride **5**. In order to obtain the title compounds **6**, the chloride **5** was allowed to react with a range of amines with or without solvent at 125 °C or under microwave condition at 165 °C.[20]

The PDE inhibitory activity was determined using a scintillation proximity assay with [^{3}H]cAMP as substrate measuring the hydrolysis of cAMP to AMP with recombinant human PDEs 1–11. Selected compounds were evaluated for PK using the cassette-accelerated rapid rat screen.[19] Anti-psychotic efficacy was evaluated by measuring inhibition of MK-801-induced hyperactivity in the rat.[18] The potential for hypolocomotion was evaluated in a spontaneous locomotor activity test[21] to differentiate between efficacy, as defined by reversal of the MK-801 induced behavior, and hypolocomotoric effects of the compound.

The co-crystal structure of compound **1** bound within the catalytic subunit of PDE10A is shown in Figure 2. Several key interactions between compound **1** and the protein were observed. First, the N-1-H and N-9 of the inhibitor form two hydrogen bonds with the conserved Gln726. Second, the planar pyrazoloquinoline occupies the hydrophobic clamp defined by Phe729 and Phe696, achieving favorable π–π stacking through face-to-face interactions with Phe729 and edge-to-face with Phe696. The 8-methyl group lies underneath Phe729 and displays a hydrophobic interaction with the protein. The 6-methoxy group is exposed to the solvent. Although the piperazine points toward the metal ions, they are separated by a large space filled with a PO_4^{-2} and water molecules, which allows for possible SAR development in this area.

Our initial SAR efforts were directed to probe the importance of the specific interactions within this chemotype. Introduction of a methyl group on the N-1 of compound **1** resulted in a significant loss in potency (PDE10A IC_{50} = 858 nM), presumably due to disruption of the H-bond interaction between **1** and Gln726. Similarly, removal of the methyl at the C-8 position (**7** and **8**) decreased the



Scheme 1. Reagents and conditions: (a) (1) NH_4OH, CS_2, EtOH, rt; (2) $ClCH_2CO_2H$; (3) NH_2NH_2, rt, 77% (three steps); (b) $CH_3C(O)CHClC(O)OEt$, EtOH, rt, 64%; (c) (1) KOH, EtOH, reflux, 5 h; (2) $POCl_3$, $(CH_2)_2Cl_2$, 57% (two steps); (d) NR^1R^2, 125 °C or microwave 165 °C, 10–40%.

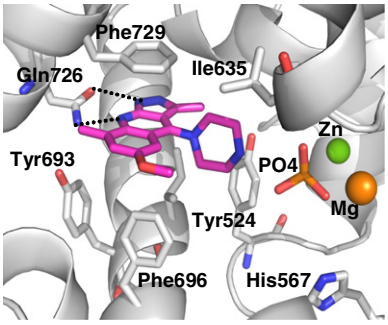


Figure 2. Co-crystal structure of 1 and PDE10A (PDB ID code 3UUO).

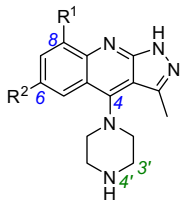
potency, presumably due to the absence of a hydrophobic interaction (Table 1). This clearly demonstrates that these interactions are important and need to be maintained. The in vitro potency does not appear to be improved by the addition of a methoxy or chlorine at the C-6 of the pyrazoloquinoline as in **7** and **10**. Replacement of the methoxy group at the C-6 position with a chlorine (**9** and **10**) to address the potential metabolic demethylation produced compounds with similar PDE10A activity. Surprisingly, this modification reduced plasma drug exposure of **9** in the rat. The area under curve (AUC) is 0.14 μM h in the rat PK screen.

Extensive SAR exploration was centered at the C-4 position (Table 2). Since the 6-OMe-8-Me substitution pattern led to the best in vitro and PK profile comparing compounds **7**–**10**, this substitution was maintained for the SAR development to identify the optimal C-4 substitution. Replacement of piperazine with a piperidine (**11**) or thiomorpholine (**14**) led to a loss of potency. Morpholine (**12**), homomorpholine (**13**), and homopiperazine (**15**) are well tolerated at the C-4 position. Compound **13** has an excellent in vitro and PK profile with selectivity greater than 1450-fold over other PDE enzymes and good plasma drug exposure (AUC = 11 μM h, B/P = 0.3) in the rat PK screen. It inhibited MK-801-induced hyperactivity in the rat at 30 mg/kg dosed orally.

Encouraged by these results, we revisited C-6 position of **13** to address the potential metabolic demethylation issue. While H, CH_3, F, Cl, Br, OH, and pyrrolidine are tolerated at the C-6 position, these modifications decreased PDE10A inhibitory activity (K_i = 24–140 nM) and plasma drug exposure for tested compounds.

The subsequent SAR development was aimed at improving the in vitro potency by incorporating a substituent on piperazine, piperidine, morpholine, or homopiperazine in **1**, **11**, **12** and **15**. Small alkyl groups are tolerated at C-3′ or C-4′ of **1**, **11**, **12** or **15**. Polar substituents on piperazine, morpholine, piperidine or homopiperazine improve PDE10A activity. The biological activities of a

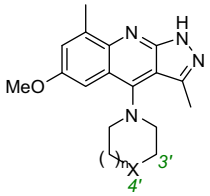
Table 1
PDE10A inhibitory activity of C-6 and/or C-8 modification analogs



Compd	R^1	R^2	PDE10A K_i[a] (nM)
7	H	OMe	585
8	H	H	550
9	Me	Cl	35
10	H	Cl	419

[a] Values are means of at least two experiments.

Table 2
PDE10A inhibitory activity of 4-piperazine modification analogs



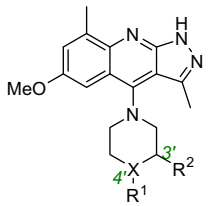
Compd	X	*n*	PDE10A K_i[a] (nM)
11	CH_2	1	363
12	O	1	23
13	O	2	3.6
14	S	1	196
15	NH	2	15

[a] Values are means of at least two experiments.

representative selection of analogs are shown in Table 3. Compound **16**, the (S)-3′-Me-4′-C(O)NH_2 analog of **1** has an improved PDE10A inhibitory activity (K_i = 3.3 nM) and plasma drug exposure (AUC = 9.5 μM h) in the rat PK screen as compared to **1**. However, it was not active in the in vivo study at 30 mg/kg dosed orally. This lack of in vivo activity may be due to a low brain level. The brain drug concentration at an hour after 30 mg/kg oral dosing was 0.027 μg/g. In comparison with **11** or **12**, the PDE10A activity was enhanced by the introduction of a variety of polar groups at the C-3′ or C-4′ position (compounds **17**–**23**). In the rat PK study, no drug concentration of these compounds was detected in the brain, presumably due to the presence of three or four hydrogen bond donors in the molecule.

Concurrent SAR development was focused on acyclic amines at the C-4 position of pyrazoloquinoline. A variety of secondary amines are tolerated at the C-4 position. The biological activities of a representative selection of secondary amine analogs are shown in Table 4. Increasing the linker length between the C-4-N and pyridine (**24** vs **26**) or tetrahydropyran (**27**, **28** and **29**), or moving the oxygen of the tetrahydropyran from the C-4 to the C-2 position (**27** and **30**) led to a loss of potency. However, increasing the linker length between C-4-N and the piperidine (**33** and **34**) improved potency. Compound **31** is significantly more potent than **30** and **32**, presumably due to favorable polar interactions between the NH

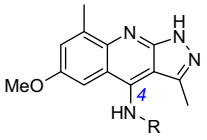
Table 3
PDE10A inhibitory activity of ring substitution analogs of 1, 11, and 12

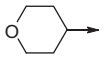
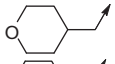
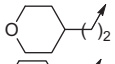
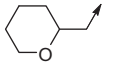
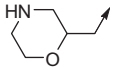
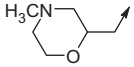
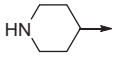
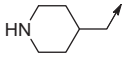


Compd	X	R^1	R^2	PDE10A K_i[a] (nM)	Br C_{6h} (ng/g)
16	N	C(O)NH_2	CH_3 (S)	3.3	<10
(±)–**17**	O		CH_2OH	9.6	<10
18	C	NH_2	H	1	<10
19	C	OH	H	14	<10
20	C	CH2NH_2	H	0.5	nd
21	C	C(O)NH_2	H	3	<10
22	C	NHC(O)NH_2	H	27	<10
(±)–**23**	C	H	CH_2OH	29	<10

[a] Values are means of at least two–three experiments (nd = not determined).

Table 4
PDE10A inhibitory activity of the C-4 secondary amine analogs



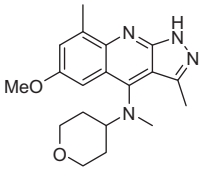
Compd	R	PDE10A K_i[a] (nM)	PDE6 K_i[a] (nM)
24	4-Py-CH_2	0.6	211
25	2-Py-CH_2	6	1811
26	4-Py-$(CH_2)_2$	70	554
27		12	278
28		52	697
29		409	na
30		130	843
31		5	13882
32		280	na
33		55	48% @ 3.6 μM
34		15	14782

[a] Values are means of at least two experiments.

of the morpholine on **31** and PDE10A. While these modifications produced potent and selective PDE10A inhibitors, these compounds either exhibited CYP3A4 (**24** and **25**) or had low plasma and/or no brain drug exposure in the rat PK screen (**31** and **34**).

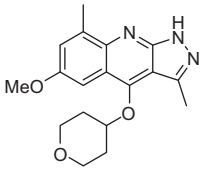
Many of the C-4 secondary amine analogs are potent PDE6 inhibitors. PDE6 activity has known effects on visual disturbances and is therefore an activity to be avoided.[22] A molecular docking study of **27** and PDE6 suggests a flipped binding mode of the tricyclic ring, which places the C-4-NH and C-6-OMe of the pyrazoloquinoline next to the conserved Gln773 side chain in PDE6.[23] Within this altered mode, the C-4-NH makes a hydrogen bond with Gln773, but the amine is confined to a relatively tight space. Thus any bulky substitution at the C-4-NH could decrease PDE6 potency. Consistent with this binding mode, introduction of a methyl group on the C-4-NH of **27** or replacing the NH of **27** with an oxygen decreased the PDE6 inhibitory activity (**35** and **36**, Fig. 3). Compound **36** is a potent and selective PDE10A inhibitor with high plasma drug exposure in the rat PK screen (AUC = 10.6 μM h). In the in vivo study, it inhibited MK-801-induced hyperactivity in the rat at 30 mg/kg dose orally, but was not active at lower dose (10 mg/kg).

Compound **27** has a desirable PK profile and good physicochemical properties. It is not a *P*-glycoprotein substrate in the rat. The in vitro hepatocyte clearance trend for rat, dog, and monkey for this compound appears to track with the in vivo exposure. It inhibited MK-801-induced hyperactivity in the rat with an ED_{50} of 4 mg/kg. A rat hypolocomotion study was conducted to evaluate the sedation effect of **27**. The ED_{50} of this compound is 23 mg/kg demonstrating a modest separation between efficacy and hypolocomotion. Compound **27** had undetectable plasma levels (limit of quantitation = 2 ng/ml) in the monkey, thus the evaluation of the EPS side effect was hindered as this study is conducted using Cebus monkeys (Fig. 4).



35

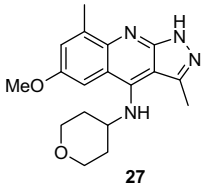
PDE10 K_i= 41 nM
PDE6 K_i= 7383 nM



36

PDE10 K_i= 3.1 nM
PDE6: 30% inhibition @ 1 μM

Figure 3. Compounds **35** and **36**.



27

MW= 326, cLogP= 3.5, PSA= 72

PDE10A K_i = 12 nM, LE= 0.45
PDE6 K_i= 278 nM
PDE5A K_i= 17324 nM
Other PDEs $K_i \geq$ 4793 nM
Rat PK AUC (PO, 10 mg/kg)= 11.8 μM.h
Brain concentration: 1.1 μg/g, B/P ratio= 0.7 (6-h)
Dog PK AUC (PO, 2 mg/kg)= 12.5 μM.h
Monkey PK AUC (PO, 3 mg/kg)= 0 (LOQ= 2 ng/ml)
Hepatocyte clearance (μL/m/M cell): 1.7, 2.9, 0.8, 54 (human, rat, dog, monkey)
Rat PPB (% bound): 93%
Caco2 Efflux ratio= 0.7
Inhibition of MK-801 induced hyperactivity in the rat: ED_{50}= 4 mg/kg
Rat hypolocomotion: ED_{50}= 23 mg/kg

Figure 4. In vitro, in vivo, and PK data of compounds **27**.

In summary, crystal structure-aided SAR development has identified a series of pyrazoloquinolines as potent and selective PDE10A inhibitors. In general, these compounds have desirable physicochemical properties (MW <400, *c*Log*P* <4, PSA <100). Compound **27** is a potent PDE10A inhibitor with good to excellent selectivity over other PDE enzymes except for PDE6. Compared with the lead compound, **27** has an improved selectivity over CK1δ IC_{50} = 4000 nM). It is efficacious in an animal model believed to mimic the positive symptoms of schizophrenia, and possesses a modest separation between the efficacy and hypolocomotion. The disclosure of further SAR development at the C-4 position of pyrazoloquinoline to improve the PDE6 selectivity, in vivo activity and monkey PK for the EPS study will be the subject of future publications.

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18. Male CD rats were habituated to the locmotor activity (LMA) chambers 24 h before testing. On test day, they were co-administered MK-801 (0.3 mg/kg, sc) and the PDE10 inhibitor (30 mg/kg, po) and placed in the testing apparatus. Inhibition of MK-801 induced activity was measured as a percent of activity in the PDE10 inhibitor + MK-801group relative to the vehicle + MK-801 group.
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