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Discovery of orally active pyrazoloquinolines as potent PDE10 inhibitors for the management of schizophrenia

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ABSTRACT

A series of pyrazoloquinoline analogs have been synthesized and shown to bind to PDE10 with high affinity. From the SAR study and our lead optimization efforts, compounds **16** and **27** were found to possess potent oral antipsychotic activity in the MK-801 induced hyperactive rat model.

Schizophrenia is a mental illness affecting ~1% of the world's population.[1] Although second generation antipsychotic drugs show effectiveness in treating positive psychotic symptoms (e.g., paranoid, visual delusions and hallucinations) of schizophrenia, they are ineffective against negative symptoms (e.g., absence of speech and lack of interest and drive) and cognitive disorder. However, even with current drug therapy, ~15% patients still have residual positive symptoms, and only a small portion of patients under treatment are able to lead independent lives.[2] In addition, current antipsychotic agents have shown adverse effects, including QT prolongation, weight gain, diabetes, and extrapyramidal syndrome (involuntary muscle movements).[3–5] There is an unmet medical need for improved treatment of schizophrenia.

The phosphodiesterases (PDEs) are a family of degradative enzymes that hydrolyze the second messengers, cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP), to terminate signal transduction. PDE10, a dual cAMP/cGMP phosphodiesterase, is expressed at high levels in the striatal medium spiny neurons, but is expressed at very low levels elsewhere in the brain and other tissues.[6] PDE10 inhibition mimics D2 dopamine receptor antagonism in the indirect striatopallidal output pathway through increasing cAMP and cGMP levels. This antagonistic effect could increase the activity of the striatonigral output and normalize the reduced striatal output that characterizes schizophrenia.[7]

Papaverine, a potent PDE10A inhibitor, was utilized as a pharmacological probe in mice, and exhibited efficacies in rat models of schizophrenia.[7,8] Several PDE10A inhibitors have been reported to exhibit efficacies in a range of antipsychotic models including the models established for negative symptoms and cognitive deficits.[7–9] Thus, selective PDE10 inhibitors provide a novel therapeutic approach for the treatment of schizophrenia.[9–11] Recent advances in X-ray co-crystal structures of PDE10 have triggered structure-based drug design for several pharmacophore models.[10,11]

From high-throughput screening of our chemical library, a series of analogs with a pyrazoloquinoline scaffold were identified to display PDE10A inhibitory activity. These compounds possessed C-4 N-substituted moieties. To further change and optimize the chemical properties of that series (4-aminopyridine series), chemistry was developed to prepare C-4 carbon-linked heterocyclic analogs. In this letter, we will describe syntheses and structure–activity relationships of C-4 carbon-linked pyrazoloquinoline analogs. The SAR study resulted in identification of highly potent and selective PDE10A ligands, demonstrating oral in vivo efficacy in the MK-801 induced hyperactivity rat model for anti-psychosis.

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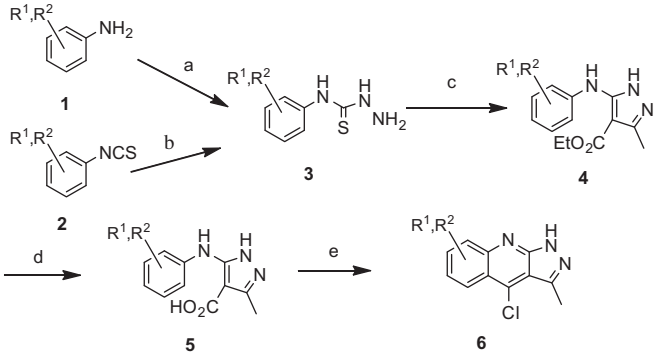
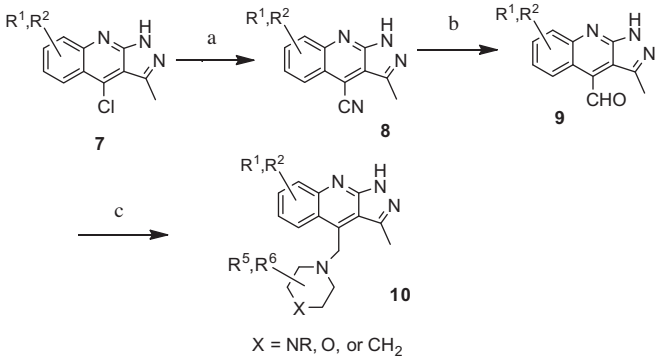


Scheme 1. Reagents and conditions: (a) NH_4OH, CS_2, EtOH, rt, 1 h; then $ClCH_2CO_2Na$, rt, 1 h; then NH_2NH_2, rt; (b) NH_2NH_2, DCM, rt; (c) ethyl 2-chloroacetoacetate, EtOH, rt; (d) KOH in EtOH–H_2O, reflux; (e) $POCl_3$, 100 °C.



Scheme 2. Reagents and conditions: (a) KCN, 110 °C, DMSO; (b) DIBAL, toluene; (c) amine, $NaBH(OAc)_3$.

The synthetic route to the pyrazoloquinoline core is summarized in Scheme 1. The commercially available aniline (**1**) was transformed to carbothio-hydrazine **3** in one pot. Alternatively **3** could be conveniently obtained from commercially available phenyl isothiocyanate (**2**). Then **3** was coupled with ethyl 2-chloroacetoacetate and cyclized to form pyrazole **4**.[9,10] Ester **4** was further hydrolyzed to acid **5**, and cyclization could occur under neat phosphorus(V) oxychloride condition at 100 °C to obtain **6**.

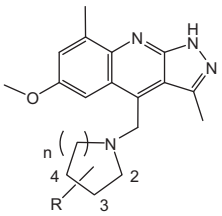
The C-4 heterocyclic-methyl analogs (**10**) were synthesized as described in Scheme 2. The C-4 chloro atom of **7** was replaced with a cyano group using potassium cyanide to afford **8**, followed by DIBAL reduction to give aldehyde **9**. Reductive amination of **9** with various heterocyclic amines afforded **10**. This is the first report of replacing the C-4 chloro atom with reactive one carbon unit for further transformation.

Target compounds were tested for their affinity at the cloned human recombinant PDE10A1 and/or other PDE isozymes (BPS Bioscience Inc.) by measuring their ability to compete with [^{3}H]cAMP (Amersham) as the radioligand.

The unsubstituted-piperidinyl analog (**11**) showed PDE10 binding affinity with a K_i of 804 nM. Introduction of a hydroxy group at the 4 position of the piperidine ring led to a ~14-fold increase of PDE10 binding affinity (**12**, K_i 57 nM, Table 1). The analogs with 4-fluoro or 3- or 4-trifluoromethyl piperidine (**13**–**15**) did not show desired potency (>100 nM). Three pyrrolidinylmethyl analogs were prepared to explore the relationship of reduced C-4 ring size and PDE10 affinity. The unsubstituted-pyrrolidinyl analog **16** displayed improved PDE10 affinity (K_i 24 nM) compared to that of piperidinyl **11**. Hydroxyl substituted analog **17** further improved the PDE10 binding affinity to 3 nM. The SAR trend is consistent with the SAR observed for piperidinyl analog **12**. However 3-hydroxymethyl substitution did not improve binding affinity in this series.

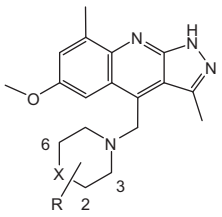
To explore whether the oxygen atom inside the C-4 ring could be tolerated, the morpholinemethyl analog (**19**–**23**, Table 2) was prepared. The unsubstituted-morpholine analog **19** exhibited highly potent PDE10 binding affinity with a K_i of 1 nM. Because **19** displayed high PDE10A binding affinity, it was soaked with PDE10A crystals for an X-ray co-crystallography study (deposition number: PDB code 3UI7). Compound **19** bound with PDE10A in the catalytic pocket (Figs. 1 and 2). The conserved glutamine Gln-726 formed strong bidentate hydrogen bonds with N-*H* and *N*-9 of the pyrazoloquinoline ring. The tricyclic ring of **19** was clamped by two phenylanalines, Phe-696 and Phe-729. The morpholine moiety extended into the deep pocket formed by Y524, H525, F696, and I692, and the oxygen atom formed an additional hydrogen bond with histidine (His-525). These binding interactions probably contributed to its high binding affinity. The methanethio group of methionine Met-713 was close to C-7. Therefore C-7 substitution might clash with Met-713, and reduce PDE10A binding affinity. The C-8 methyl group of **19** was proximate to Gly-725, and this might contribute to its good selectivity over other PDE isozymes. Other PDE isozymes possessed larger amino acids (e.g., Leu, Ile, etc.) instead of glycine at position 725, and the large

Table 1
SAR of the C-4 piperidinyl and pyrrolidinyl analogs



Compds	R	*n*	PDE10 K_i (nM)
Piperidines			
11	H	2	804
12	4-OH	2	57
13	4-F	2	202
14	3-CF_3	2	353
15	4-CF_3	2	871
Pyrrolidines			
16	H	1	24
17	3-OH (R)	1	3
18	3-CH_2OH	1	32

Table 2
SAR of the C-4 morpholinyl and piperazinyl analogs



Compds	X	R	PDE10 K_i (nM)
19	O	H	1
20	O	2-Me	3
21	O	2-CH_2OH	0.6
22	O	2,2-di-Me	171
23	O	*cis* 2,6-di-Me	26
24	NH	H	49
25	NH	2 =O	9
26	NH	2-Me (R)	30

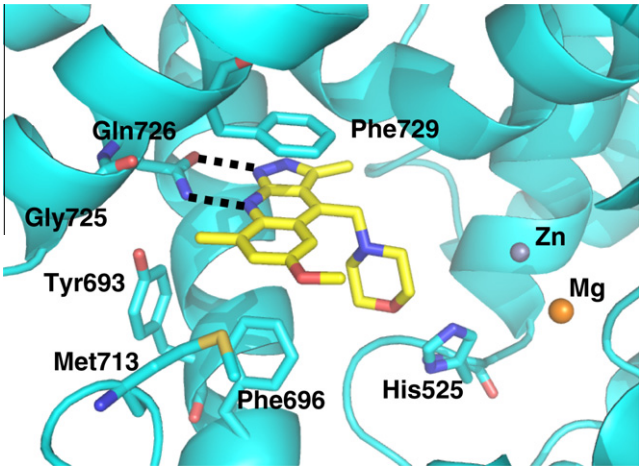


Figure 1. X-ray structure of PDE10 and **19** (yellow).

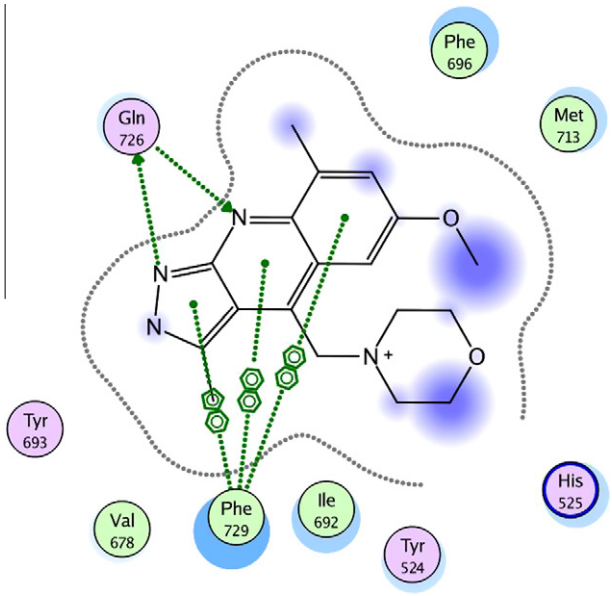


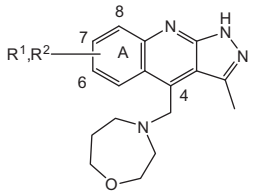
Figure 2. Binding mode and molecular interactions for **19** and PDE10 based on X-ray crystallography.

amino acids might clash with the C-8 methyl group of **19** and reduce binding affinity. The co-crystal structure explained some of the SAR and further assisted our future SAR development.

Additional SAR was conducted for the substituted morpholine analogs (**20**–**23**). Slight variation of 2-substituents (methyl or hydroxylmethyl for **20** and **21**, respectively) on the morpholine ring was tolerated. However binding affinity significantly dropped for the di-methyl analogs (**22** and **23**). The C-4 piperazinemethyl analogs (**24**–**26**) were also prepared for SAR investigation. Among them, the piperazin-2-one analog (**25**) showed the best binding affinity (9 nM). In general, polar substituents or replacements (OH, NH, O, or =O) on the C-4 cycloalkyl ring improved PDE10 binding affinity. This could be explained based on the X-ray co-crystal structure. In the X-ray structure, the C-4 cycloalkyl ring resided in the large hydrophilic pocket containing water molecules. Therefore hydrophilic substitution on the C-4 ring provided better binding interaction compared to that of non-polar substitution.

The homomorpholinemethyl analog (**27**),[12] a homolog of **19**, retained potent binding affinity (K_i 5 nM). To investigate the A-ring SAR, A-ring modified homomorpholine analogs (**28**–**36**, Table 3)

Table 3
SAR of the A-ring modification of **27**



Compds	R^1, R^2	PDE10 K_i (nM)
28	6-OMe	153
29	6-CF_3	104
27	6-OMe, 8-Me	5
30	6-OH, 8-Me	2
31	6-O$(CH_2)_2$OMe, 8-Me	1
32	6-O*i*Pr, 8-Me	8
33	6-OCF_3, 8-Me	19
34	6,8-di-OMe	14
35	6-Cl, 8-Me	41
36	6-Br, 8-OCF_3	37

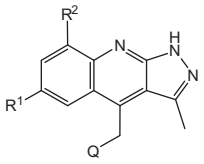
were prepared using different substituted anilines (**1**) as precursors. The C-5 substitution showed potential reduction of the stability of the compound (data not shown), and was not desired. The C-7 substitution usually resulted in reduction of the binding affinity due to clashing with Met713 residue of PDE10. Therefore only C-6 and C-8 substitutions were investigated. Both analogs **28** and **29** without 8-substitution showed poor binding affinity, K_i >100 nM. Compared to **27**, removal of the 8-methyl group (**28**) led to a ~20-fold reduction of the PDE10 binding affinity. This indicated that hydrophobic interaction between 8-methyl group and PDE10 assisted binding. Therefore 8-aliphatic substitution was preferred. Replacing 6-OMe group with other alkyl ethers such as O-*i*Pr or OCF_3 resulted in slightly reduced affinity (K_i values of 8 and 19 nM for **32** and **33**, respectively). Replacing the 8-methyl group of **27** with a methoxyl group lead to slightly reduced affinity (**34**, K_i 14 nM). PDE10 binding affinity diminished when the 6-position was substituted with halogen (Cl or Br) such as analogs **35** and **36**.

To investigate the relationship of the steric bulkiness of the C-4 ring of the molecule and PDE10 binding affinity, several bridged analogs including bridged pyrrolidine (**37** and **40**), piperidine (**38**), and morpholine (**39** and **41**) were prepared (Table 4). Overall these compounds showed significantly reduced PDE10 binding affinities, especially for **37** and **39**, ~46–50-fold reduction, compared to those of the non-bridged analogs (**16** and **19**, respectively). The morpholine analogs (**39** and **41**) again showed better potency, compared to the pyrrolidine or piperidine analogs (**37**, **38**, **40**). Therefore the strategy of increasing steric bulkiness did not lead to compounds with potent binding affinity.

Some compounds with potent PDE10 binding affinities were chosen for selectivity evaluation against other PDE isozymes (Table 5). In general these compounds were highly selective (>1000-fold) over PDE1A3, PDE2A, PDE3A, PDE4B2, PDE5A1, PDE7A1, PDE8A1, and PDE9A1. They were also selective over PDE6 and PDE11A3 with ~320- to 6000-fold selectivity.

Some compounds with high PDE10 binding affinity were evaluated for schizophrenia rat models based on inhibition of MK-801 induced hyperactivity. MK-801 is a NMDA-receptor antagonist, which produces schizophrenia-like symptoms in rats.[13] Selected compounds were screened at 30 mg/kg oral dosing, and the ones that showed reduction of hyperactivity in rats were further evaluated at 10 and 3 mg/kg (Table 6). Compounds **12**, **16**–**21**, and **27** reversed hyperactivity in the MK-801 treated rats, while **24**–**26** did not show efficacy in the same model. All of the active compounds except **18** reduced the hyperactivity higher than 100%, compared

Table 4
SAR of the C-4 bridged bi-cyclic analogs



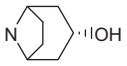
Compds	R^1, R^2	Q	PDE10 K_i (nM)
37	6-OMe, 8-Me		1200
38	6-OMe, 8-Me		265
39	6-OMe, 8-Me		46
40	6-Cl, 8-Me		229
41	6-Cl, 8-Me		60

Table 5
Binding affinity of some selective compounds versus other PDE isozymes

Compds	PDE5A1 K_i (μM)	PDE6 K_i (μM)	PDE11A3 K_i (μM)	Other PDEs[a] K_i (μM)
17	6.4	4.7	1.9	>10
19	10.6	2	14.6	>10
20	8.9	8.3	6.6	>10
21	5	0.3	1.2	ND
27	9.7	2.1	1.6	>10
30	20.8	2.4	4.9	>10
31	24.4	5.9	2.3	>10
32	>10	>10	>10	>10

[a] Other PDE isozymes: PDE1A3, PDE2A, PDE3A, PDE4B2, PDE7A1, PDE8A1, PDE9A1.

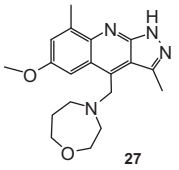
Table 6
In vivo screening data of selected analogs in anti-hyperactivity rat models

Compds	3 mpk	10 mpk	30 mpk
12	Inactive	Inactive	136%
16	96%[a]	107%	122%
17	Inactive	Inactive	102%
18	Inactive	Inactive	38%
19	Inactive	131%	109%
20	Inactive	88%	130%
21	Inactive	Inactive	104%
24	NT[b]	NT	Inactive
25	NT	NT	Inactive
26	NT	NT	Inactive
27	66%[c]	120%	103%
35	Inactive	Inactive	87%

The percentages indicate the reduced percentage of the induced hyperactivity.
[a] MED: 1 mg/kg.
[b] NT: Not tested.
[c] MED: 3 mg/kg.

to that of vehicle and were further evaluated. In the further testing, only compounds **16**, **19**, **20**, and **29** showed anti-hyperactive efficacy (reducing 107%, 131%, 88%, and 120%, respectively). Finally minimum effective doses of the most potent analogs (**16** and **27**) were determined as 1 and 3 mg/kg, respectively. Although the hydroxyl substitution (**17**, **19**) enhanced PDE10 binding affinity compared to that of the unsubstituted analogs (**16**, **21**), it however reduced in vivo efficacy in the MK-801 treated rat model. Hence, addition of the hydroxyl group to the C-4 heterocyclic was not desired.

Compound **16** did not show good AUC in the rat pharmacokinetic (PK) study (0.3 μM h). Compound **27** was further profiled for PK and in vitro safety studies. Compound **27** displayed reasonable rat AUC (3.8 μM h) and brain/plasma ratio (1.6) in the PK study. Compound **27** showed potent PDE10 inhibitory activity with K_i = 5 nM and >520-fold selectivity over other PDE isozymes. It showed minimal inhibition (5% at 10 μM) in hERG assay (Ionwork), and did not show significant induction effect in the hPXR reporter gene assay (0.17-fold relative to rifampicin). Compound **27** displayed no inhibition of CYP P450 enzymes (2D6, 2C9, and 3A4) up to 20 μM. Compound **27** exhibited reasonable pharmacokinetic profile with about 10-fold therapeutic window against hypolocomotion side effect (MED 30 mg/kg).



27

PDE10: Ki: 5 nM
other PDEs: >520 fold
rat PK: AUC: 3763 nM.h (0-6 h)
Brain: 42 ng/g (6 h)
Brain/Plasma ratio: 1.56 (6 h)
Rat Hyperactivity: MED: 3 mg/kg
Rat Hypolocomotion: MED: 30 mg/kg
Cyp Inh. (2D6, 3A4, 2C9): clean, >20 μM
hERG (iw): 5.3% inhibition (10 uM)
hPXR: 0.17 fold to rifampicin @ 1 μM
Protein binding (rat): 97%
Hepatocyte clearance:
8.3 uL/m/M cells (Human)
<1 uL/m/M cels (Rat)
6 uL/m/M cells (Monkey)

In conclusion, a novel series of potent PDE10 inhibitors based on the pyrazoloquinoline scaffold were discovered. Several of these compounds showed K_i levels in the single digit nM range, and one compound (**21**) showed K_i at sub nM level. These compounds showed good selectivity over other PDE isozymes. Among the potent PDE10 inhibitors identified, compounds **16** and **27** demonstrated potent oral anti-hyperactivity efficacy in the MK-801 rat model, with MED values of 1 and 3 mg/kg, respectively. Compound **27** was selected for further in vivo safety studies in animals.

Acknowledgments

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12. *Compound **27***: ^{1}H NMR ($CDCl_3$): δ 1.86 (tt, J = 6.1, 5.8 Hz, 2H), 2.74 (m, 2H), 2.82 (t, J = 5.8 Hz, 2H), 2.86 (s, 3H), 2.87 (s, 3H), 3.61 (m, 2H), 3.79 (t, J = 6.1 Hz, 2H), 3.93 (s, 3H), 4.39 (s, 2H), 7.32 (d, J = 2.8 Hz, 1H), 7.66 (d, J = 2.8 Hz, 1H), 11.41 (br s, 1H, NH). ESI-MS: 341 $[M+H]^+$, $C_{19}H_{24}N_4O_2$.
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