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Pyrazoloquinolines as PDE10A inhibitors: Discovery of a tool compound

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ABSTRACT

A series of pyrazoloquinolines, possessing (hetero)arylhydroxymethyl substituents at the quinoline C-4 position were evaluated as PDE10A inhibitors. Among these, methylpyrimidyl analogue **15** was identified as having good rodent and monkey exposure, and a MED of 10 mg/kg in an in vivo model.

Schizophrenia is a complex mental disorder that is estimated to affect 0.5% of the world's population.[1] The predominant course of treatment is administration of antipsychotic agents, with these drugs generally categorized as being either 'typical' or 'atypical' antipsychotics. Both classes however largely rely on antagonism of the dopamine D_2 receptor to achieve the desired response.[2] Although effective, these medications frequently produce undesirable side effects, including but not limited to weight gain, sedation, and extrapyramidal syndrome (EPS). Phosphodiesterase 10A (PDE10A) is a dual cAMP/cGMP hydrolyzing enzyme that has recently emerged as a promising target for the treatment of schizophrenia. Since it is believed that inhibition of PDE10A might produce antipsychotic efficacy without interacting directly on dopamine receptors, a drug relying on PDE10A inhibition may exhibit a more desirable side effect profile than currently available treatments.[3–5]

In order to advance our PDE10A program, a tool compound was needed for complete assessment of both efficacy and side effect liabilities. Such a compound needs to be a potent enzyme inhibitor with adequate oral exposures in rodent and monkey. We recently described the discovery of **1** (Fig. 1) as a potent inhibitor of PDE10A.[6–8] Compound **1** inhibited MK-801 induced hyperactivity[9] with a MED of 3 mg/kg in the rat following oral dosing. Unfortunately, **1** had no oral exposure in monkey and hence its EPS profile could not be measured, as we intended to perform the assessment in the monkey.[10] To improve exposure in monkey with this class of compounds, we continued SAR development using compound **1** as a starting point. Since it was known that oxidation of the homomorpholine ring in **1** was a major metabolic pathway, we sought to replace this group with aromatic and heteroaromatic substituents under the hypothesis that this might improve oral exposure. Monkey hepatocyte clearance rate was used as an estimate of exposure in plasma, as we were mindful that a loose relationship existed between the two parameters.[11] This proved to be a useful tool to prioritize testing in vivo, and we set a goal of clearance being <15 μL/min/million cells.

The compounds described in this report were prepared using the general synthesis depicted in Scheme 1. For the present study, we were interested only in analogues with C-8 methyl and C-6 methoxy/chloro substituents on the quinoline core. Thiosemicarbazide intermediates **4** and **5** were thus prepared upon treatment of the corresponding aniline with carbon disulfide and hydrazine. Condensation with ethyl 2-chloroacetoaceate gave, following extrusion of sulfur, pyrazole esters **6** and **7**. Saponification and cyclization with $POCl_3$ then yielded pyrazoloquinolines **10** and **11**. Substitution of the chloride for a bromide was accomplished upon hydrolysis (HCl, MeOH) followed by bromination ($POBr_3$, DMF). This three step sequence gave a higher overall yield than the direct conversion of **8** to **12** using $POBr_3$. In order to obtain the desired targets, the bromoquinolines were allowed to react with *n*-BuLi, followed by a range of heteroaromatic ketones and aldehydes. The yields for this step were generally modest.

The SAR of representative analogues in the *C*-6-methoxy quinoline series is presented in Table 1. All compounds prepared were racemates unless otherwise noted. The PDE inhibitory activity

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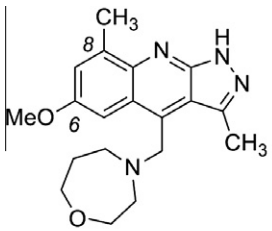


1

PDE10A K_i = 5 nM

other PDEs > 500 fold selective

rat PK AUC = 3763 nM.h (0-6 h)

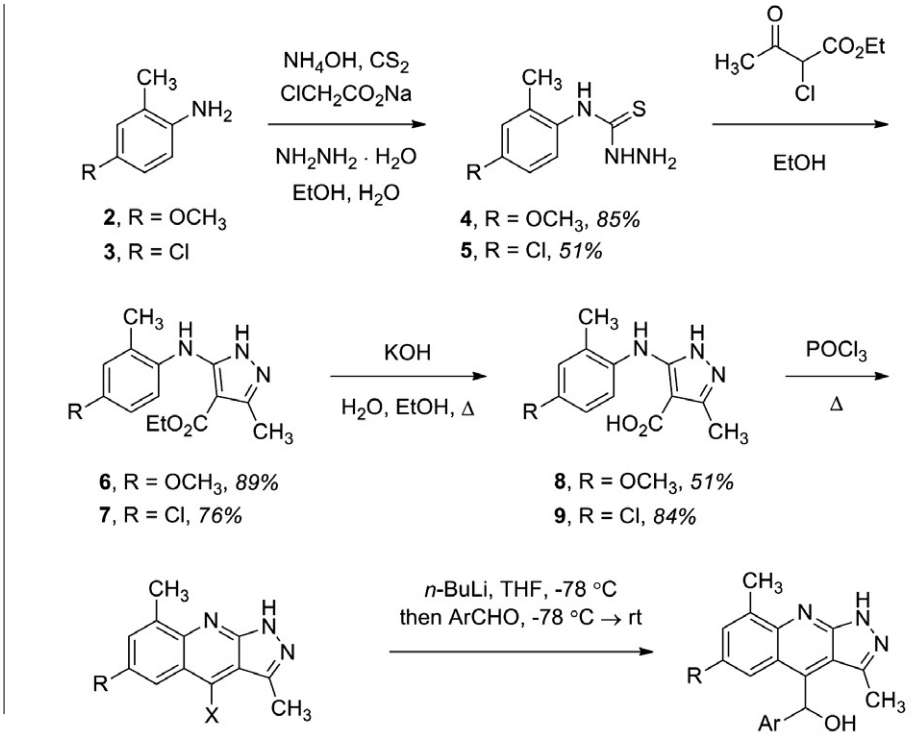
brain concentration = 42 ng/g (6 h)

brain/plasma ratio = 1.56 (6 h)

MK-801 induced rat hyperactivity MED = 3 mpk

hepatocyte clearance = 8 (h), <1 (r), 6 (m)

Figure 1. Select data for compound **1**.



2, R = OCH_3

3, R = Cl

4, R = OCH_3, *85%*

5, R = Cl, *51%*

6, R = OCH_3, *89%*

7, R = Cl, *76%*

8, R = OCH_3, *51%*

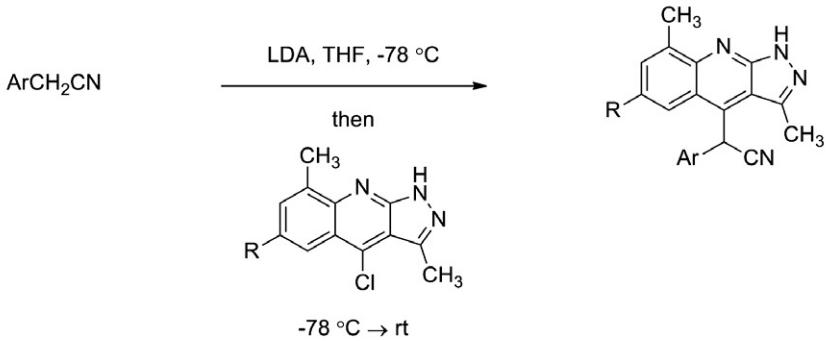
9, R = Cl, *84%*

10, X = Cl, R = OCH_3, *quant*

11, X, R = Cl, *95%*

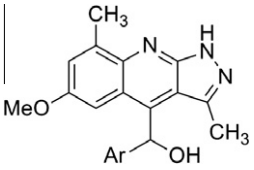
12, R = OCH_3, *65% over 2 steps*

13, R = Cl, *32% over 2 steps*



Scheme 1. Synthesis of pyrazoloquinolines.

Table 1
PDE10A Inhibitory activity of pyrazoloquinolines possessing various C-4 benzyl groups



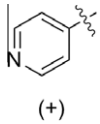
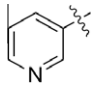
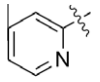
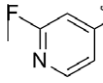
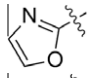
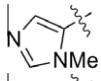
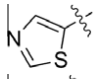
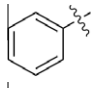
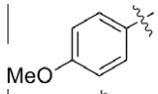
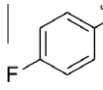
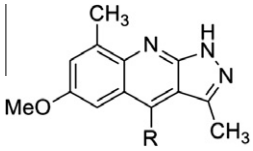
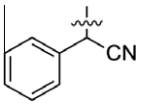
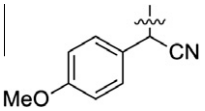
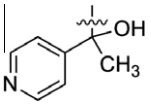
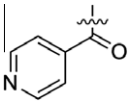
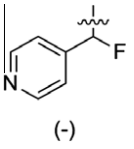
Entry	Ar	PDE10A K_i (nM)	CYP3A4 Inhib. (IC_{50}, μM)	Monkey hepatocyte clearance (μL/min/million cells)
1	 (+)	0.06	3.0 (co); 2.9 (pre)	66
2		10	2.7 (co); <0.2 (pre)	67
3		54	10.6 (co); 4.6 (pre)	NA
4		0.07	4.2 (co); 0.6 (pre)	66
5		0.6	7.8 (co); 4.4 (pre)	63
6		21	4.9 (co); 1.2 (pre)	22
7		2	0.7 (co); <0.2 (pre)	NA
8		50	NA	NA
9		2	1.2 (co); <0.2 (pre)	60
10		83	NA	63

Table 2
PDE10A Inhibitory activity of pyrazoloquinolines possessing various linkers at the C-4 position



Entry	R	PDE10A K_i (nM)	CYP3A4 Inhib. (IC_{50}, μM)	Monkey hepatocyte clearance (μL/min/million cells)
1		12	~20 (co); 0.8 (pre)	64
2		2	12.9 (co); 0.3 (pre)	NA
3		4	14.2 (co); 7.1 (pre)	66
4		1	NA	66
5	 (-)	0.3	12.0 (co); 0.3 (pre)	62

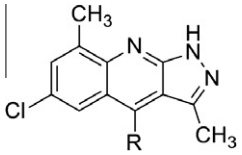
of target compounds was determined using a scintillation proximity assay with [^{3}H]cAMP as substrate measuring the hydrolysis of cAMP to AMP with recombinant human PDEs 1–11. Inhibitors having PDE10A K_i <50 nM were selected for further evaluation. During the course of this investigation, we became aware that many of these analogues exhibited significant CYP3A4 inhibition. Therefore, compounds were routinely screened for both reversible (preincubation of the analogue with the CYP3A4 enzyme) and time-dependent (coincubation of the analogue with CYP3A4) inhibition.[12] As an initial criteria, we were interested in analogues that exhibited both time-dependent and reversible CYP3A4 IC_{50} >10 μM.

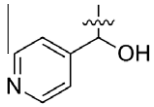
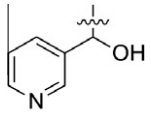
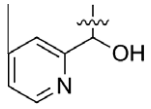
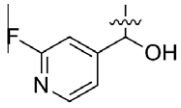
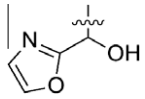
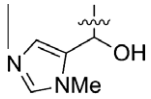
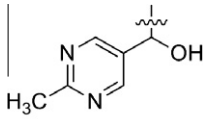
Pyrazoloquinolines possessing hydroxymethylpyridyl substituents were uniformly active. When the nitrogen atom was moved from the 4- to 3- or 2- position, a loss in PDE10A inhibitory activity was observed (Table 1, entries 1–3). This may be explained by the ability of the 4-pyridyl isomer to better interact, either directly or through a water bridge, with a nearby zinc atom in the PDE10 catalytic site.[6] The optically active 4-pyridyl analogue was obtained by resolving the racemic mixture and is one of the most potent compounds identified. Unfortunately, both the 4- and 3-pyridyl analogues exhibited high monkey hepatocyte clearance with significant CYP3A4 inhibition. We hypothesized that the CYP3A4 inhibition may be due to an interaction of the CYP enzyme with the nonbonding electrons of the pyridine nitrogen.[13] Thus, we prepared the *ortho*-fluoro substituted pyridine (Table 1, entry 4). Unfortunately, no improvement in CYP inhibition was observed, although PDE10A potency was maintained. In order to further explore this, a number of analogues in which the pyridine was replaced with an azole were prepared. Selected examples are displayed in entries 5–7. In all cases this modification failed to give any significant improvement in CYP inhibition. It was generally observed that analogues in which the C-4 heterocycle was replaced with a phenyl ring were less active than their heteroaromatic counterparts, and exemplary data is presented in entries 7–9. A notable exception was the 4-methoxy phenyl species (Table 1, entry 9), although this compound was a potent CYP3A4 inhibitor. Thus it appeared that in this subseries lowering the basicity of, or replacing the heteroaryl ring does not reduce CYP3A4 inhibition.

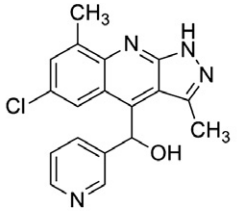
We next investigated the effect of replacing the benzylic hydroxyl group of the analogues described above with other functional groups. Although this would not necessarily be expected to reduce the level of CYP inhibition, it was hypothesized that such structural modifications would improve hepatocyte clearance values.[14] Thus, the alcohol underwent oxidation to the corresponding ketone upon treatment with IBX. The hydroxyl group was also converted to a 2° alkyl fluoride upon reaction with DAST. In order to prepare analogues with a benzylic cyano group, 4-chloroquinolines were allowed to react with anions derived from benzyl nitriles (Scheme 1). The resulting SAR studies are listed in Table 2.

Two compounds possessing benzyl nitrile subunits (Table 2, entries 1 and 2) were active in the PDE10A inhibition assay, although

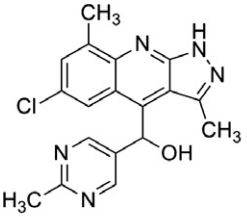
Table 3
PDE10A Inhibitory activity of pyrazoloquinolines possessing C-6-chloro substitution



Entry	R	PDE10A K_i (nM)	CYP3A4 Inhib. (IC_{50}, μM)	Monkey hepatocyte clearance (μL/min/million cells)
1		0.2	0.5 (co); 0.3 (pre)	25
2	 **14**	11	2.1 (co); 1.1 (pre)	46
3		39	12.2 (co); 1.8 (pre)	NA
4		1	>20 (co); >20 (pre)	37
5		0.4	1.0 (co); 7.4 (pre)	NA
6		21	0.8 (co); 0.3 (pre)	NA
7	 **15**	3	>20 (co); 7.9 (pre)	8



14



15

PDE10A K_i = 11 nM
other PDEs > 500 fold selective
rat PK AUC = 11040 nM.h (0-6 h)
brain concentration = 115 ng/g (6 h)
brain/plasma ratio = 1.5 (6 h)
MK-801 induced rat hyperactivity MED = 10 mpk
hepatocyte clearance = 5 (h), 26 (r), 46 (m)

PDE10A K_i = 5 nM
other PDEs > 500 fold selective
rat PK AUC = 4286 nM.h (0-6 h)
brain concentration = < 14 ng/g (6 h)
brain/plasma ratio = < 0.18 (6 h)
MK-801 induced rat hyperactivity MED = 10 mpk
hepatocyte clearance = 2 (h), 11 (r), 8 (m), 7 (d)
monkey PK AUC = 11496 nM.h (0-6 h)
$t_{1/2}$ = 7.1 h

Figure 2. Selected data from analogues **14** and **15**.

they were strong CYP3A4 inhibitors. In the case of the unsubstituted phenyl ring analogue (entry 1), no improvement in clearance was observed. We also explored 4-pyridyl derivatives, since these were the most potent compounds in the hydroxy series. Although K_i values were in the single digit nanomolar range, the 3° alcohol (Table 2, entry 3), ketone (Table 2, entry 4), and alkyl fluoride (Table 2, entry 5) exhibited high clearance values in monkey hepatocytes. Disappointingly, modification of the benzylic substituent had no effect on clearance rate.

Finally, we examined the SAR in the *C*-6-chloro pyrazoloquinoline series. Analogues in this series generally exhibited lower monkey hepatocyte clearance rates relative to their C-6 methoxy counterparts, and selected results are presented in Table 3. Unsubstituted pyridines remained active PDE10A inhibitors (Table 3, entries 1–3), although CYP3A4 inhibition was a recurring problem. The addition of a fluorine atom adjacent to the nitrogen atom was an effective strategy for decreasing CYP inhibition (Table 3, entry 4). This may be due to decreasing the basicity of the pyridine nitrogen,[13] and suggested that analogues with less basic heterocyles might be less potent CYP3A4 inhibitors in this subseries, if not in the C-8 methoxy collection described in Table 1. Unfortunately, an oxazole (Table 3, entry 5) and imidazole (Table 3, entry 6) both exhibit significant CYP inhibition. Finally, a methyl-substituted pyrimidine (Table 3, entry 7, **15**) had a PDE10A K_i = 3 nM with acceptable clearance rate in monkey hepatocytes and reasonable CYP3A4 profile.

Based on this data, methylpyrimidine **15** was selected for further evaluation (Fig. 2). This compound inhibited MK-801 induced hyperactivity in rat with an MED of 10 mg/kg when given orally.[9,15] Analogue **15** does not exhibit CYP450 induction in rat liver slice studies and is approximately within our desired criteria of CYP450 inhibition. Additionally, this compound was inactive against a panel of 23 kinases (IC_{50} >30 μM). On 3 mg/kg oral dosing, **15** exhibited excellent exposure (AUC = 11,496 nM h) with a half life of 7.1 h in monkey. It also displayed good oral exposure in rodent with an AUC of 4286 nM h over 6 h at 10 mg/kg dose. An ex vivo study (after sacrificing the rodent at the 6 h time point) revealed that the amount of **15** present in the brain was below the limit of detection. However, the concentration of **15** in the plasma at the 6 h time point (220 nM) was also minimal, making a determination of brain to plasma ratio impossible. As a closely related analogue, pyridine **14** was also profiled. Similar to **15**, compound **14** inhibited MK-801 induced hyperactivity with a MED of 10 mg/kg. This analogue exhibited excellent exposure in the rat (AUC = 11,040 nM h) over 6 h. Notably, the plasma concentration at the 6 h time point of the study was 810 nM, making a determination of the brain to plasma ratio feasible. At the 6 h time point, the amount of **14** in the brain was 115 ng/g and the brain to plasma ratio was 1.5. In fact, several analogues in this series had brain to plasma ratios >0.5, helping support our hypothesis that the reduction in MK-801 hyperactivity is a result of PDE10A inhibition. The overall profiles of **14** and especially **15** render these analogues valuable tool compounds to investigate PDE10A inhibition as a treatment for schizophrenia.

In conclusion, we have described a series of pyrazoloquinolines, possessing (hetero)arylhydroxymethyl substituents at the quinoline C-4 position as PDE10A inhibitors. 4-Pyridyl analogues were found to be extremely potent, but exhibited high clearance rates in monkey hepatocytes. Replacing the benzylic hydroxyl group with other functionalities failed to lower these values. Replacing the quinoline C-6 methoxy group with a chloro substituent generally led to reduction of the clearance rate in monkey hepatocytes. Within this series, the methylpyrimidyl compound **15** emerged as a tool compound with a good overall profile. Complete evaluation of this compound in a variety of rodent and monkey models, including efficacy and side effect profiles, would assist in developing PDE10A inhibitors for the treatment of schizophrenia.

Acknowledgments

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