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N-Acylhydrazones as inhibitors of PDE10A

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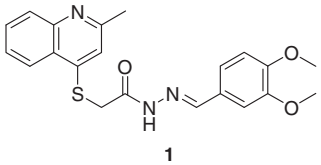
ABSTRACT

Cyclic nucleotide phosphodiesterases (PDEs) are represented by a large superfamily of enzymes. A series of hydrazone-based inhibitors was synthesized and shown to be novel, potent, and selective against PDE10A. Optimized compounds of this class were efficacious in animal models of schizophrenia and may be useful for the treatment of this disease.

Cyclic nucleotide phosphodiesterases (PDEs) are a superfamily of enzymes that hydrolyze the second messengers cyclic adenosine and cyclic guanosine 3',5'-monophosphate (cAMP and cGMP). Inhibition of PDEs has been an active area of drug discovery.[1] Selective inhibitors of the individual PDE families have widely been sought for a broad indication of therapeutic uses. Potential therapeutic uses include the treatment of allergies,[2a] COPD,[2b] persistent pulmonary hypertension of the newborn,[2c] carcinoma,[2d] angina,[2e] cardiovascular regulation,[2f] depression,[2g] and erectile dysfunction.[2h] PDE10A was identified independently by three groups in 1999 and shows a dual activity of hydrolysis of both cAMP and cGMP.[3–5] PDE10A is an attractive target because it may offer a novel therapy for the treatment of neurological and psychiatric disorders including Parkinson's disease, Huntington's disease, schizophrenia, and disorders that would benefit from increasing levels of cAMP and cGMP within neurons.[6] Increasing neuronal cAMP and cGMP may benefit patients with neurological and psychiatric disorders through striatal activation and behavioral suppression.

To date, a number of drug-like small molecules have been reported to inhibit PDE10A, although few have entered clinical trials. There have also been published reports of X-ray and computational data obtained for PDE10 complexed with ligands.[7–9] In an effort to identify inhibitors of PDE10A, a high-throughput screening campaign was conducted and provided screening hits which contained an *N*-acylhydrazone linker typified by potent screening hit **1** (Fig. 1). Hydrazone-based molecules have been popular as potential therapeutics for tuberculosis,[10a,10b] and as small molecule inhibitors of numerous targets such as human glucagon receptor,[10c] and pituitary adenylate cyclase-activating polypeptide receptor.[10d] Here we report the structure–activity relationship (SAR) study of a novel class of highly selective hydrazone-based small molecules that inhibit PDE10A in the low nanomolar range



1

IC_{50} 0.049 μM

Figure 1. Schematic representation of **1**.

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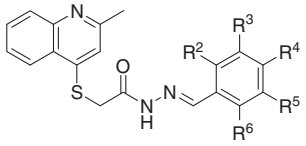
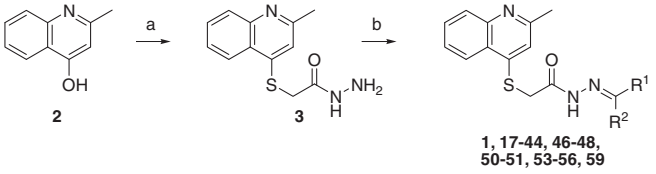
Table 1
Dimethoxyphenyl region SAR of substituted *N*-acylhydrazones



Compd	R^2	R^3	R^4	R^5	R^6	PDE10A IC_{50}[a] (μM)
1	H	OMe	OMe	H	H	0.049
17	H	OMe	H	H	H	1.2
18	H	H	OMe	H	H	1.4
19	H	OMe	H	OMe	H	0.15
20	H	OMe	Cl	H	H	0.087
21	H	OMe	F	H	H	0.19
22	H	OMe	Br	H	H	0.093
23	H	OMe	Me	H	H	0.10
24	H	OMe	CN	H	H	0.16
25	H	OMe	CF_3	H	H	0.25
26	H	$OCHF_2$	$OCHF_2$	H	H	0.31
27	H	OEt	OMe	H	H	0.26
28	H	CF_3	OMe	H	H	0.42
29	H	F	OMe	H	H	0.62
30	H	Cl	OMe	H	H	0.22
31	H	OCF_3	OMe	H	H	1.7
32	H	O-^{i}Pr	OMe	H	H	0.66
33	H	OMe	OMe	H	Cl	0.20
34	H	OMe	OMe	H	Br	0.077
35	H	OMe	OMe	H	F	0.19
36	Cl	OMe	OMe	H	H	4.6
37	Br	OMe	OMe	H	H	4.8
38	H	OMe	OMe	OMe	H	0.010
39	H	OMe	OMe	CN	H	0.011
40	H	OMe	OMe	Br	H	0.03
41	H	OMe	OMe	Cl	H	0.045
42	H	OMe	OMe	F	H	0.051
43	H	OMe	CN	OMe	H	0.014
44	H	OMe	Br	OMe	H	0.0048

[a] Values were calculated from the average of at least two experiments.

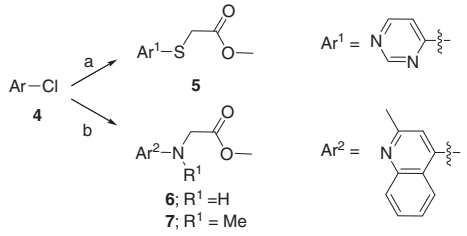


Scheme 1. Reagents and conditions: (a) (i) Lawesson's reagent, THF, 80 °C, 30%; (ii) methyl bromoacetate, K_2CO_3, DMF, 88%; (iii) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 80%; (b) R^1-C(O)R^2, EtOH, HOAc (cat.), reflux.

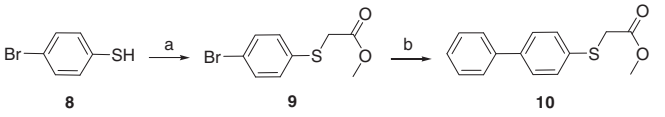
and show oral animal efficacy in behavioral models for schizophrenia.

The synthetic route used to synthesize the analogs in Table 1 is depicted in Scheme 1. Commercially available 2-methylquinolin-4-ol (**2**) was treated with Lawesson's reagent to provide the corresponding thiol which was then converted to intermediate **3** in good yield. Intermediate **3** was readily condensed with a variety of aldehydes or ketones in the presence of catalytic acetic acid to provide the corresponding acylhydrazones in typically >70% yield. In all cases only the thermodynamically stable geometrical *E*-isomer was detected and isolated. Compounds **17–44**, **46–48**, **50**, **51**, **53–56**, **59** and the oxygen linked compound, **57**, were synthesized in a similar fashion as outlined above.

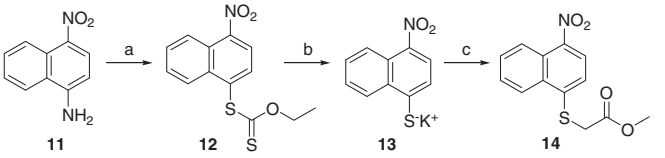
In order to access a broader range of synthetic precursors it was of interest to explore additional chemistry routes. As shown in Scheme 2, aryl chlorides could readily be reacted with either a thiolate anion or amine functionality to provide intermediates **5**, **6**, and **7**. Further transformations using previously described conditions provided final compounds **45**, **58** and **60**, respectively.



Scheme 2. Reagents and conditions: (a) methyl thioglycolate, *N*,*N*-diisopropylethylamine, DMF; (b) glycine methylester HCl or sarcosine methylester HCl, Et_3N, EtOH, reflux.



Scheme 3. Reagents and conditions: (a) methyl bromoacetate, K_2CO_3, DMF; (b) $PhB(OH)_2$, $Pd(PPh_3)_4$ (5 mol %), Na_2CO_3, THF/H_2O.



Scheme 4. Reagents and conditions: (a) $NaNO_2$, HCl (aq), 5 °C; potassium ethyl xanthate, 40 °C; (b) KOH, EtOH, reflux; (c) methyl bromoacetate, K_2CO_3, DMF.

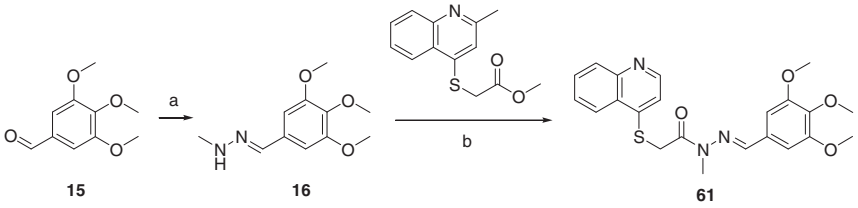
Biphenyl analog **49** was synthesized from 4-bromothiophenol **8** by alkylation with methyl bromoacetate to provide **9** followed by Suzuki coupling to give thioacetate **10** as shown in Scheme 3. Further synthetic transformations via the route depicted in Scheme 1 provided compound **49**.

Nitronaphthalene analog **52** was synthesized from naphthylamine **11** as shown in Scheme 4. Compound **11** was converted to xanthate **12** followed by hydrolysis to potassium thiolate **13**. Alkylation with methyl bromoacetate provided precursor **14** which was used to synthesize analog **52** in two steps.

N-Substituted analog **61** was synthesized as depicted in Scheme 5. Thus, treatment of aldehyde **15** with methyl hydrazine provided intermediate **16**. Condensation of **16** with the requisite methyl ester then gave the desired *N*-methyl analog **61**.

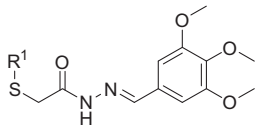
As part of a lead optimization strategy, acyl hydrazines were screened in an in vitro enzymatic assay for their ability to inhibit PDE10A.[11] In vitro potency optimization focused on modifying the three regions of the inhibitor: the heteroaromatic portion (quinoline), the dimethoxy-phenyl region, and the central linker.

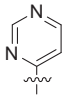
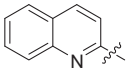
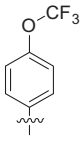
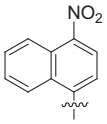
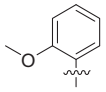
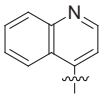
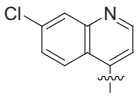
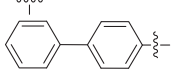
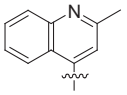
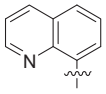
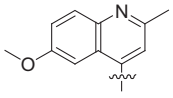
Initial SAR studies focused on modifications to the substituted phenyl ring while the 3-methyl quinoline and linker regions were held constant (Table 1). Based on previous reports of PDE10A inhibitors, it was known that methoxy substituted phenyl groups could be important recognition elements for enzyme binding.[7,9] In order to gain insight into the pharmacophore of screening hit **1**, it was modified by removing one of the methoxy groups (**17, 18**) and by changing the substitution pattern (**19**). These modifications resulted in a 3 to 28-fold loss in potency against PDE10A. Due to concerns about the metabolic liability of the 4-methoxy group from in vitro liver microsome studies (data not shown), it was of interest to search for less labile functionalities. Compounds **20–25** illustrate that replacement of the 4-methoxy group with chloro, fluoro, bromo, methyl, cyano or trifluoromethyl group led to less



Scheme 5. Reagents and conditions: (a) methyl hydrazine, EtOH, reflux; (b) EtOH, HOAc (cat.), reflux.

Table 2
Heteroaromatic region SAR of substituted *N*-acylhydrazones



Compd	R^1	PDE10A IC_{50}[a] (μM)	Compd	R^1	PDE10A IC_{50}[a] (μM)
45		1.07	**51**		0.14
46		0.86	**52**		0.10
47		0.66	**53**		0.024
48		0.55	**54**		0.028
49		0.26	**55**		0.010
50		0.19	**56**		0.008

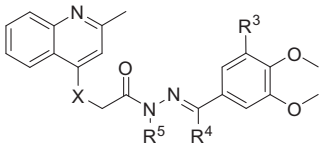
[a] Values were calculated from the average of at least two experiments.

active analogs. Replacement of the 3-methoxy group also provided less potent inhibitors as demonstrated by compounds **27**–**32** compared to parent compound **1**. Bis-difluoromethoxy compound **26** gave an IC_{50} of only 0.31 μM. Incorporation of a halogen at either the 1- or 6-positions on the phenyl ring (**33–37**) gave analogs with IC_{50} values in the range of 0.077–4.8 μM. In contrast, 3,4,5-trisubstituted phenyl analogs where R^5 was methoxy, cyano, bromo, chloro or fluoro (compounds **38–42**) gave equivalent or better potency against PDE10A as compared to compound **1**. Finally, replacement of the 4-methoxy group of **38** was examined via the synthesis of 4-cyano derivative **43** and 4-bromo compound **44**. While both gave excellent in vitro activity, compound **44** was both the most potent example with an IC_{50} of 4.8 nM and provided improved metabolic stability (data not shown).

A second area of SAR effort focused on modification of the 3-methyl quinoline portion of the molecule. It was determined early in the course of SAR studies that an aromatic group, and preferentially a functionalized bicyclic ring system, provided potent inhibitors against PDE10A. Table 2 shows representative examples of the substituents explored. In the majority of cases, only modest PDE10A inhibition was observed with mono-aromatic ring systems such as **45–48**. In contrast, fused bicyclic aromatic ring systems provided examples with substantially better activity. For instance, 4-substituted quinoline based compounds (**53–56**) provided the most potent inhibitors. Examples of 2- and 8-substituted quinolines such as compounds **50** and **51** failed to provide significant improvements in potency and were not pursued. Installation of other aromatic systems such as biphenyl and naphthyl compounds **49** and **52** also failed to provide necessary improvements in potency.

The final area of SAR exploration studied modifications to the hydrazone linker region (Table 3). A comparison of compounds **1** and **59** illustrates that even a relatively small alkyl group substituted in place of hydrogen at the benzylidene carbon resulted in approximately a threefold loss of potency. It was also of interest to determine if the hydrazone nitrogen could tolerate substitutions. A comparison of compounds **55** and **61** illustrates that substitution of the NH hydrogen with a methyl group also resulted in almost a threefold loss of potency. Lastly, it was of great interest to know the effect of replacement of the heteroatom linker. It was

Table 3
Linker modifications



Compd	X	R^3	R^4	R^5	PDE10A IC_{50}[a] (μM)
1	S	H	H	H	0.049
57	O	H	H	H	1.6
58	NH	H	H	H	8.0
59	S	H	Me	H	0.18
55	S	OMe	H	H	0.010
60	NMe	OMe	H	H	0.36
61	S	OMe	H	Me	0.026

[a] Values were calculated from the average of at least two experiments.

Table 4
Rat pharmacokinetic profile of compound **56**

PK properties[a]	Value
$T_{1/2}$ (h), iv	11.3
CL (L/h/kg), iv	3.3
Vss (L/kg), iv	17.4
T_{max} (h), po	0.25
C_{max} (ng/ml), po	539
F (%)	21

[a] iv dose: 4 mg/kg, po dose: 9 mg/kg.

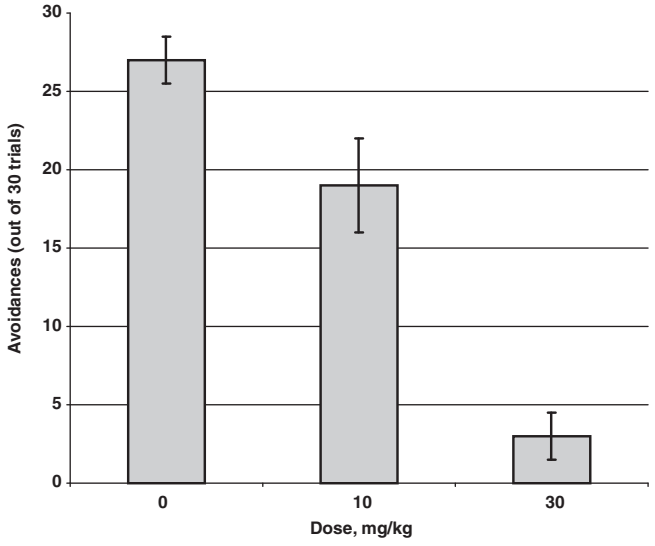


Figure 2. Attenuation of conditioned avoidance response by **56**. Mice were trained to avoid foot-shock by moving to the other side of a shuttle-box in response to conditional stimulus (CS). Training proceeded until mice demonstrated at least 25 avoidances out of 30 trials. Compound **56** was administered intraperitoneally in 30 mM H_3PO_4, 0.2% tween 80, 5% DMSO 20 min before testing. Control group received vehicle injection. Eight mice per group were used. Paired-sample *t*-test was used to compare performance of the same animals on the day of the experiment and on the previous day when they were injected with the vehicle. p <0.05 for 10 mg/kg and p <0.001 for 30 mg/kg.

clearly demonstrated by comparison of **1**to **57** and **58** that replacement of sulfur with either oxygen or an unsubstituted amino moiety led to a significant loss of inhibition. Attempts to incorporate a substituted amino moiety into the linker also led to reduced potency as illustrated by compound **60**. Based on these observations

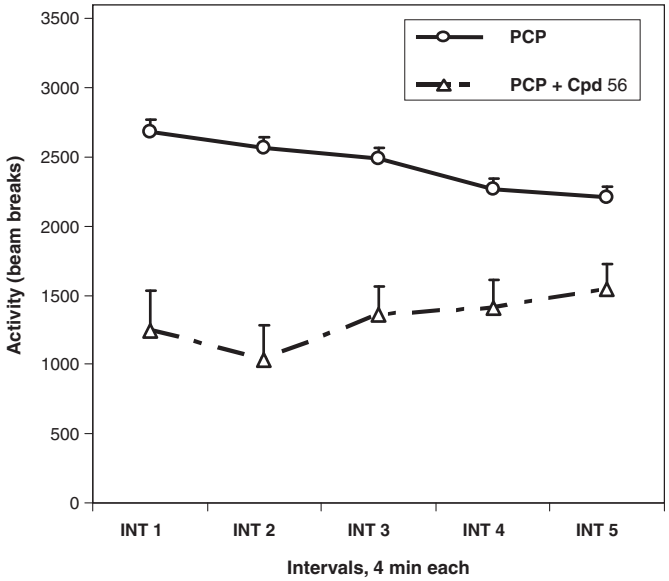


Figure 3. Compound **56** reduced PCP-induced hyperactivity. Activity was monitored by number of beam breaks in an open field novel environment. Compound (20 mg/kg) or vehicle (30 mM H_3PO_4, 0.2% tween 80, 5% DMSO) were administered IP 20 min before testing, and PCP was injected by the same route 10 min before testing. Normal activity in this experiment ranges between 500 and 1000. Eight mice per group were used, *t*-test for each interval showed p <0.005.

only a limited number of non-sulfur based analogs were prepared and tested.

As part of a lead optimization strategy, acylhydrazones were screened in in vitro enzymatic assays for their ability to inhibit PDE10A[12] while maintaining selectivity against a broader panel of PDEs. It was found that compound **56** possessed excellent selectivity for PDE10A against PDE1A-PDE11A (excluding PDE6A which was not tested). Selectivity ratios varied from 110-fold to >1250-fold. In no case were any inhibitors found to be more selective against another PDE as compared to PDE10A.

Selected compounds were also evaluated in pharmacokinetic (PK) studies in rats (Table 4). In vitro observations indicated that the compounds had adequate stability in simulated gastic fluid to be absorbed orally. Compound **56** demonstrated high clearance, and bioavailability was modest (21%). However, the PK profile was favorable enough to warrant further evaluation in behavioral models.

Compound **56** was assessed in two different behavioral models shown to have predictive validity for schizophrenia. Figure 2 shows that in the conditioned avoidance response (CAR) model[13] the compound demonstrated a dose-dependent and statistically significant reduction upon intraperitoneal (IP) injection at doses of 10 and 30 mg/kg.

The attenuation of hyperlocomotion induced by phencyclidine (PCP) has also been shown to have predictive validity for anti-psychotic drugs.[14] Figure 3 shows that in this assay, **56** significantly reduced PCP-induced hyperactivity at a dose of 20 mg/kg (IP).

In conclusion, the first reported synthesis and evaluation of hydrazone-based inhibitors of PDE10A have been described. SAR studies led to the discovery of compound **56**, an excellent inhibitor that showed a high degree of enzyme selectivity against other PDEs. The compound was absorbed orally and demonstrated in vivo efficacy in two animal behavioral models of schizophrenia. This compound also showed a positive effect using an in vivo cognition model (data not shown). The findings from this study will be useful for further optimization and will be the subject of additional reports in due course.

Acknowledgements

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11. PDE10 Assay. Compounds were tested for PDE10 potency by measuring the inhibition of PDE10 hydrolysis of [3H]cGMP to [3H]GMP. Generally, eight dilutions of compound were assayed in 50 mM Tris–HCl pH 7.5, 8.3 mM $MgCl_2$, 0.5 mg/mL BSA, 1.7 mM EGTA, 16 nM [3H]cGMP and 1% DMSO. To start the reaction, mouse PDE10 (BPS BioSciences, CA) was added to a final concentration of 25 ng/ml. The reaction was incubated at 30 °C for 20 min. PDE10 hydrolysis was terminated by the addition of yttrium silicate beads (GE Healthcare, RPNQ0150) and counted on a Wallac Microbeta scintillation counter 1–2 h following the addition of the beads. Data was analyzed using XLfit (Microsoft) from which IC_{50} values were obtained.
12. The inhibition of other PDE enzymes by the PDE10A inhibitors was evaluated under the same conditions described above for PDE10A. Fractional inhibition was evaluated at four concentrations (0.1, 1, 10, and 100 μM). In cases where inhibition at the highest concentration was less than 50%, the lower limit value in the logistic model was fixed to 0% activity.
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