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# Discovery of potent, selective, and metabolically stable 4-(pyridin-3-yl)cinnolines as novel phosphodiesterase 10A (PDE10A) inhibitors

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ABSTRACT

We report the discovery of 6,7-dimethoxy-4-(pyridin-3-yl)cinnolines as novel inhibitors of phosphodiesterase 10A (PDE10A). Systematic examination and analyses of structure–activity-relationships resulted in single digit nM potency against PDE10A. X-ray co-crystal structure revealed the mode of binding in the enzyme's catalytic domain and the source of selectivity against other PDEs. High in vivo clearance in rats was addressed with the help of metabolite identification (ID) studies. These findings combined resulted in compound **39**, a promising potent inhibitor of PDE10A with good in vivo metabolic stability in rats and efficacy in a rodent behavioral model.



Phosphodiesterase 10A belongs to a family of phosphodiesterase (PDE) enzymes whose role is to hydrolyze the 3′–5′ phosphodiester bonds of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to produce adenosine monophosphate (AMP) and guanosine monophosphate (GMP), respectively. Consequently, phosphodiesterases effectively regulate cell signalling by modulating the levels of critical secondary messengers cAMP/cGMP.[1] The eleven PDE isozyme families identified to date vary in their affinities for cAMP and cGMP and differ in their relative tissue distributions.[2] In particular, the predominance of PDE10A in the medium spiny neurons of the striatum captured our interest.[3] Inhibition of PDE10A enzyme activity in the striatum could present a new approach to treat neurological disorders whose pathology has been linked to striatal brain regions. Several groups have reported findings to suggest inhibition of PDE10A could have therapeutic benefit in treatment of schizophrenia and potentially other neurodegenerative disorders.[4] PDE10A knock out ($PDE10A^{-/-}$) mice demonstrated reduced hyperactivity response in the locomotor activity model where rodents were challenged with PCP to trigger schizophrenic like behaviors.[5] Of the PDE10A inhibitors reported to date,[6] TP-10 (Fig. 1) was comprehensively described to be effective in several rodent behavioral models of schizophrenia such as the locomotor activity model, the Conditioned Avoidance Response model (CAR), and the Prepulse Inhibition model (PPI).[7] These findings further supported the hypothesis that the symptoms of schizophrenia could be ameliorated by a PDE10A inhibitor. The ultimate therapeutic utility of the novel biological target PDE10A will be demonstrated from clinical data. Notably, PF-02545920 (MP-10) is currently being evaluated in clinical trials for the treatment of schizophrenia.[8]

Our search for a novel and potent inhibitor of PDE10A enzyme activity began with compound **1** that exhibited PDE10A potency of 590 nM (Table 1). Removal of the chlorine atom resulted in significant loss of activity (**2**), indicating the importance of substitution at the 2-pyridyl position for PDE10A activity. Replacement of the chlorine atom with a methyl group (**3**) improved potency. Whereas bulky trifluoromethyl group (**4**) had a modest impact on activity, a small nitrile substituent (**5**) increased potency. Unsubstituted amine (**6**) was also tolerated. Alkylation of the amine with an isopropyl group produced a single digit nM potency inhibitor (**7**). Nitrogen atom appeared to be the optimal linker as replacement with carbon (**8**) or oxygen (**9**) decreased potency.

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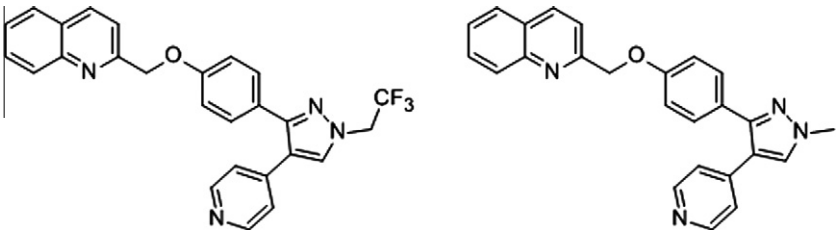
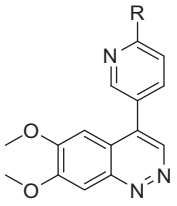


**Figure 1.** TP-10 and MP-10.

**Table 1**
SAR of 2-pyridine analogs

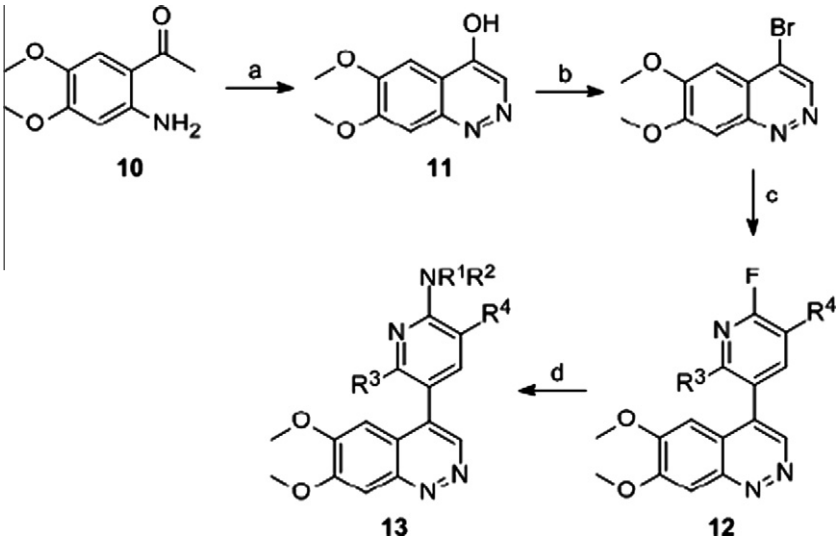


| Compound# | R | PDE10 $IC_{50}$ (nM) |
|---|---|---|
| **1** | Cl | 590 |
| **2** | H | 16300 |
| **3** | $CH_3$ | 181 |
| **4** | $CF_3$ | 320 |
| **5** | CN | 92.8 |
| **6** | $NH_2$ | 124 |
| **7** | $NH^iPr$ | 7.6 |
| **8** | $CH_2{}^iPr$ | 40.6 |
| **9** | $O^iPr$ | 24.4 |

The improvement in potency with small modifications of substituents on the pyridine ring was surprisingly dramatic. These early findings convinced us of need for a focused SAR effort and the need for an efficient synthesis to the 6,7-dimethoxy-4-(pyridin-3-yl)cinnoline core that would enable rapid analoging.[9] The synthetic route began with commercially available 1-(2-amino-4,5-dimethoxyphenyl)ethanone (**10**). Scheme 1 Cyclization with sodium nitrite afforded 6,7-dimethoxycinnolin-4-ol (**11**) in 71% yield. Bromination with phosphorous oxybromide followed by Suzuki coupling with the appropriately substituted (6-fluoropyridine-yl)boronic acid produced the 4-(6-fluoropyridin-3-yl)-6,7-dimethoxycinnoline scaffold (**12**). The synthesis of final compounds (**13**) was accomplished by heating the cinnoline pyridyl fluoride scaffold in DMSO with the appropriate amine.

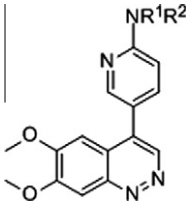
Keeping the amine linker fixed, we proceeded to examine the effects of various substituents (Table 2). Compared to compound **7**, the hydrogen bond donor on the amine did not appear to be essential to the activity (**14**). Increasing the chain length (**15**) and inserting an oxygen atom (**16**) into the alkyl chain did not result in significant impact on potency. Cyclopropyl group (**17**) decreased its PDE10A activity while cyclobutyl (**18**) and cyclopentyl (**19**) groups produced comparable or slight improvement in potency. Extending the cyclopropyl substituent by one carbon (**20**) resulted in a tenfold improvement in potency versus compound **17**. Electron-withdrawing groups like allyl (**21**) and trifluoromethyl (**22**) did not further increase activity. Cyclic amine substituents were also examined. Variation of ring size (**23**, **24**), location of oxygen atom (**25**), and presence of a hydrogen bond donating group (**26**) all afforded less potent compounds.

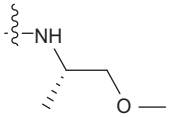
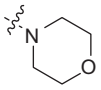
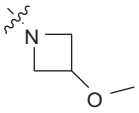
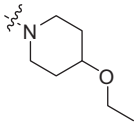
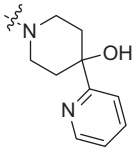
With single digit nM potency achieved, we began to profile select compounds in the in vivo rat pharmacokinetic (PK) studies. Compounds **16** and **24**, for example, were initially selected due to low in vitro rat liver microsomal clearance of 51 and 76 μL/min/mg, respectively. However, higher than expected in vivo clearance (1 mg/kg dose, IV), was observed (5.53 L/h/kg for **16**, 6.35 L/h/kg for **24**). Metabolite ID studies in rat hepatocytes showed extensive metabolism of the aniline substituents. We reasoned that a




**Scheme 1.** Reagents and conditions. (a) $NaNO_2$, HCl, water, 75 °C, 71% yield; (b) $POBr_3$, MeCN, 70 °C, 77% yield; (c) $Cs_2CO_3$, $Pd(PPh_3)_4$, DME, water; 40–95% yield; (d) amine, $K_2CO_3$, DMSO, 90 °C.

**Table 2**
Investigation of aniline substitution



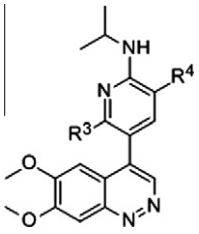

| Compound# | $NR^1R^2$ | PDE10 $IC_{50}$ (nM) |
|---|---|---|
| **7** | –N($^i$Pr)H | 7.6 |
| **14** | –N($^i$Pr)Me | 5.8 |
| **15** | –N($^i$Bu)H | 7.5 |
| **16** |  | 10.1 |
| **17** | –N($^c$Pr)H | 36.9 |
| **18** | –N($^c$Bu)H | 10.9 |
| **19** | –N($^c$Pentyl)H | 4.4 |
| **20** | –N($CH_2{}^c$Pr)H | 3.0 |
| **21** | –N(Allyl)H | 16.4 |
| **22** | –N($CH_2CF_3$)H | 12.2 |
| **23** |  | 21.4 |
| **24** |  | 18.6 |
| **25** |  | 13 |
| **26** |  | 13.2 |

polar substituent on the aniline should reduce the metabolic liability in that region. Gratifyingly, compound **26** emerged with good in vitro and in vivo data. The cyclic amine alcohol exhibited rat liver microsomal clearance of 28 μL/min/mg and rat in vivo clearance of 0.146 L/h/kg.

Since cyclic amines generally afforded less potent compounds than acyclic amines (Table 2), but provided viable PK profiles, we next turned to examine substitution on other positions of the pyridine ring in hopes of regaining potency in the single digit nM range (Table 3). Introduction of a methyl group *ortho* to the pyridine nitrogen (**27**) resulted in significant loss of potency. Adding the same functional group *ortho* to the aniline amine (**28**), however, led to an improvement in potency. Replacement with a chlorine atom was not as favorable (**29**). Bulkier substituents such as ethyl (**30**), cyclopropyl (**31**), and difluoroethyl (**32**) demonstrated similar potency improvements. Electron-withdrawing groups like fluorine (**33**), nitrile (**34**), and vinyl (**35**) were less tolerated. Unlike primary amide (**36**) which afforded decreased PDE10A activity, tertiary (**37**) and primary alcohols (**38**) all generated similar improvements in potency as compared to methyl analog **28**.

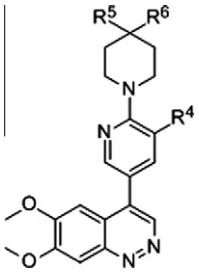
Finally, we combined the findings above to determine if they would result in a potent and metabolically stable PDE10A inhibitor suitable for testing in behavioral models (Table 4). To improve potency, we were gratified to find that addition of a small functional

**Table 3**
Examination of disubstitution on pyridine ring




| Compound# | $R^3$ | $R^4$ | PDE10 $IC_{50}$ (nM) |
|---|---|---|---|
| **7** | H | H | 7.6 |
| **27** | Me | H | 125 |
| **28** | H | Me | 2.0 |
| **29** | H | Cl | 4.0 |
| **30** | H | $CH_2CH_3$ | 1.1 |
| **31** | H | $^c$Pr | 1.5 |
| **32** | H | $CHF_2$ | 2.4 |
| **33** | H | F | 9.7 |
| **34** | H | CN | 17.3 |
| **35** | H | Vinyl | 7.0 |
| **36** | H | $C(O)NH_2$ | 16.3 |
| **37** | H | $C(CH_3)_2OH$ | 2.6 |
| **38** | H | $CH_2OH$ | 1.6 |

**Table 4**
SAR of piperidine ring




| Compound# | $R^4$ | $R^5$ | $R^6$ | PDE10 $IC_{50}$ (nM) | CL (L/hr/kg) |
|---|---|---|---|---|---|
| **26** | H | OH | 2-Pyridyl | 13.2 | 0.15 |
| **39** | Me | OH | 2-Pyridyl | 2.6 | 0.18 |
| **40** | Cl | OH | 2-Pyridyl | 5.8 | 0.38 |
| **41** | Me | OH | $^c$Pr | 1.8 | 0.86 |
| **42** | Me | OH | Me | 3.2 | 2.15 |
| **43** | Me | $C(CH_3)_2OH$ | H | 4.9 | 1.29 |
| **44** | $CHF_2$ | $C(CH_3)_2OH$ | H | 1.2 | 0.80 |
| **45** | Cl | $C(CH_3)_2OH$ | H | 6.2 | 1.84 |
| **46** | $^c$Pr | $C(CH_3)_2OH$ | H | 1.3 | 0.94 |

group at the $R^4$ position such as methyl (**39**) and chloro (**40**) increased PDE10A potency to single digit nM compared to compound **26**. Compounds **39** and **40** also maintained the low rat in vivo clearance of compound **26**. Replacement of the bulky 2-pyridyl groups on the piperidine with smaller cyclopropyl (**41**) and methyl (**42**) groups, and extending the alcohol with a gem-dimethyl methylene group (**43**–**46**) all maintained the single digit nM potency. In vivo rat clearance of compounds **41**, **43**–**44**, and **46** remained acceptable.

For proof of concept, compound **39** was tested in the Conditioned Avoidance Response (CAR) behavioral model[10] to assess the efficacy of our compounds in a rodent model of schizophrenia. Sprague-Dawley rats were tested 1 h post dosing at 3, 5.6, and 10 mg/kg given by oral gavage (Fig. 2). Compound **39** was able to suppress the avoidance response in rats with a MED of 5.6 mg/kg.

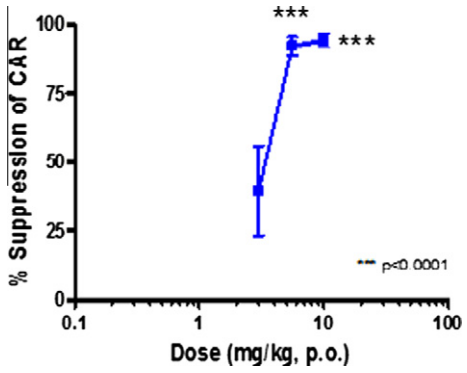



**Figure 2.** Conditioned Avoidance Response study with compound **39**.

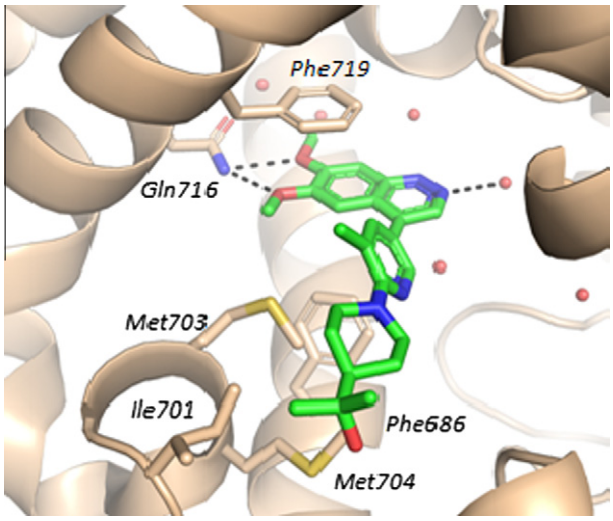



**Figure 3.** X-ray co-crystal structure of compound **43** in the catalytic domain of PDE10A enzyme.

Counterscreen assays showed compounds **39–46** exhibited 350×–5000× fold selectivity against PDE3 and several were greater than 2000× fold selective against other PDE isomers (1–9, 11) as well. A co-crystal structure of representative compound **43** in the human PDE10A catalytic domain elucidated the key bonding interactions (Fig. 3).[11] The molecule appeared to be anchored by a bifurcating hydrogen bonding interaction between both methoxy groups on the cinnoline core and the conserved Gln716. The substituents on the aniline extends to a shelf-like area in the enzyme consisting of four amino acids: Phe686, Ile701, Met703, and Met704. Comparison against other phosphodiesterase isozymes revealed that amino acid residues in this region of the catalytic binding domain differed amongst the PDEs. Thus, this interaction of the piperidine alcohol with the PDE10A enzyme in that region was likely the source of its high selectivity.

In conclusion, we have described the identification of a novel, potent, efficacious and selective inhibitor of PDE10A. Systematic investigation of structure–activity-relationships resulted in over 500× fold improvement in potency, increasing the activity of our initial lead from $IC_{50}$ of 590 nM (**1**) to 1 nM (**44**, **46**). The issue of high rat in vivo clearance was addressed with installation of alcohol substituted cyclic amines (**26**, **39–41**, **43–44**, **46**). Our selective inhibitor of PDE10A (**39**) was demonstrated to be efficacious in a rodent behavioral model of schizophrenia.

## References and notes


1. (a) Soderling, S. H.; Bayuga, S. J.; Beavo, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7071; (b) Bender, A. T.; Beavo, J. A. *Pharmacol. Rev.* **2006**, *58*, 488; (c) Lerner, A.; Epstein, P. M. *Biochem. J.* **2006**, *393*, 21; (d) Conti, M.; Beavo, J. *Annu. Rev. Biochem.* **2007**, *76*, 481.
2. Lakics, V.; Karran, E. H.; Boess, F. G. *Neuropharmacology* **2010**, *59*, 367.
3. Xie, Z.; Adamowicz, W. O.; Eldred, W. D.; Jakowski, A. B.; Kleiman, R. J.; Morton, D. G.; Stephenson, D. T.; Strick, C. A.; Williams, R. D.; Menniti, F. S. *Neuroscience* **2006**, *139*, 597.
4. (a) Menniti, F. S.; Faraci, W. S.; Schmidt, C. J. *Nat. Rev. Drug Disc.* **2006**, *5*, 660; (b) Halene, T. B.; Siegel, S. J. *Drug Discovery Today* **2007**, *12*, 870; (c) Menniti, F. S.; Chappie, T. A.; Humphrey, J. M.; Schmidt, C. J. *Curr. Opin. Invest. Drugs* **2007**, *8*, 54; (d) Hebb, A. L.; Robertson, H. A. *Curr. Opin. Pharmacol.* **2007**, *7*, 86; (e) Nishi, A.; Kuroiwa, M.; Miller, D. B.; O'Callaghan, J. P.; Bateup, H. S.; Shuto, T.; Sotogaku, N.; Fukuda, T.; Heintz, N.; Greengard, P.; Snyder, G. L. *J. Neurosci.* **2008**, *28*, 10460; (f) Kehler, J.; Nielsen, J. *Curr. Pharm. Design* **2011**, *17*, 137; Kleiman, R. J.; Kimmel, L. H.; Bove, S. E.; Lanz, T. A.; Harms, J. F.; Romegialli, A.; Miller, K. S.; Willis, A.; Des Etages, S.; Kuhn, M.; Schmidt, C. J. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 64.
5. Siuciak, J. A.; McCarthy, S. A.; Chapin, D. S.; Fujiwara, R. A.; James, L. C.; Williams, R. D.; Stock, J. L.; McNeish, J. D.; Strick, C. A.; Menniti, F. S.; Schmidt, C. J. *Neuropharmacology* **2006**, *51*, 374.
6. (a) Cantin, L.-D.; Magnuson, S.; Gunn, D.; Barucci, N.; Breuhaus, M.; Bullock, W. H.; Burke, J.; Claus, T. H.; Daly, M.; DeCarr, L.; Gre-Willse, A.; Hoover-Litty, H.; Kumarasinghe, E. S.; Li, Y.; Liang, S. X.; Livingston, J. N.; Lowinger, T.; MacDougall, M.; Ogutu, H. O.; Olague, A.; Otto-Morgan, R.; Schoenleber, R. W.; Tersteegen, A.; Wickens, P.; Zhang, Z.; Zhu, J.; Zhu, L.; Sweet, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2869; (b) Chappie, T. A.; Humphrey, J. M.; Allen, M. P.; Estep, K. G.; Fox, C. B.; Lebel, L. A.; Liras, S.; Marr, E. S.; Menniti, F. S.; Pandit, J.; Schmidt, C. J.; Tu, M.; Williams, R. D.; Yang, F. V. *J. Med. Chem.* **2007**, *50*, 182; (c) Kehler, J.; Ritzén, A.; Greve, D. R. *Exp. Opin. Ther. Patents* **2007**, *17*, 147; (e) Höfgen, N.; Stange, H.; Schindler, R.; Lankau, H.-J.; Grunwald, C.; Langen, B.; Egerland, U.; Tremmel, P.; Pangalos, M. N.; Marquis, K. L.; Hage, T.; Harrison, B. L.; Malamas, M. S.; Brandon, N. J.; Kronbach, T. *J. Med. Chem.* **2010**, *53*, 4399; (f) Kehler, J.; Ritzen, A.; Langgård, M.; Petersen, S. L.; Farah, M. M.; Bundgaard, C.; Chirstoffersen, C. T.; Nielsen, J.; Kilburn, J. P. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3738; (g) Helal, C. J.; Kang, Z.; Hou, X.; Pandit, J.; Chappie, T. A.; Humphrey, J. M.; Marr, E. S.; Fennell, K. F.; Chenard, L. K.; Fox, C.; Schmidt, C. J.; Williams, R. D.; Chapin, D. S.; Siuciak, J.; Lebel, L.; Menniti, F.; Cianfrongna, J.; Fronseca, K. R.; Nelson, F. R.; O'Connor, R.; MacDougall, M.; McDowell, L.; Liras, S. *J. Med. Chem.* **2011**, *54*, 4536.
7. Schmidt, C. J.; Chapin, D. S.; Cianfrogna, J.; Corman, M. L.; Hajos, M.; Harms, J. F.; Hoffman, W. E.; Lebel, L. A.; McCarthy, S. A.; Nelson, F. R.; Proulx-LaFrance, C.; Majchrzak, M. J.; Ramirez, A. D.; Schmidt, K.; Seymour, P. A.; Siuciak, J. A.; Tingley, F. D., III; Williams, R. D.; Verhoest, P. R.; Menniti, F. S. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 681.
8. (a) Verhoest, P. T.; Chapin, D. S.; Corman, M.; Fonseca, K.; Harms, J. F.; Hou, X.; Marr, E. S.; Menniti, F. S.; Nelson, F.; O'Connor, R.; Pandit, J.; Proulx-LaFrance, C.; Schmidt, A. W.; Schmidt, C. J.; Suiciak, J. A.; Liras, S. *J. Med. Chem.* **2009**, *52*, 5188; (b) Clinicaltrials.gov identifier NCT01175135.
9. (a) For more details on synthetic preparations and PDE10A assay conditions, CAR model, see: Hu, E.; Kunz, R.; Nixey, T.; Hitchock, S. Preparation of quinoline derivatives and their aza analogs as phosphodiesterase 10 inhibitors. WO2009025839.; (b) Hu, E.; Kunz, R.; Chen, N.; Nixey, T.; Hitchcock, S. Preparation of quinoline derivatives and their aza analogs as phosphodiesterase 10 inhibitors. WO 2009025823.; (c) Arrington, M. P.; Liu, R.; Hopper, A. T.; Conticello, R. D.; Nguyen, T. M.; Gauss, C. M.; Burli, R.; Hitchcock, S. A.; Hu, E.; Kunz, R. Cinnoline derivatives as phosphodiesterase 10 inhibitors, their preparation, pharmaceutical compositions, and use in therapy. WO 2007098169.
10. Wadenberg, M.-L. G.; Hicks, P. B. *Neuosci. Biobehav. Rev.* **1999**, *23*, 851.
11. (a) Wang, H.; Liu, Y.; Hou, J.; Zheng, M.; Robinson, H.; Ke, H. *Proc. Natl. Acad. Sci.* **2007**, *104*, 5782; (b) Coordinates of co-crystal structure of compound 43 in PDE10 have been deposited in the Protein Data Bank. PDB ID code is 4DDL.