Published in final edited form as:

Bioorg Med Chem Lett. 2015 February 15; 25(4): 919–924. doi:10.1016/j.bmcl.2014.12.054.

Synthesis and in vitro characterization of cinnoline and benzimidazole analogues as phosphodiesterase 10A inhibitors

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Abstract

Fifteen cinnoline analogues and six benzimidazole phosphodiesterase 10A (PDE10A) inhibitors were synthesized as potential PET radiopharmaceuticals and their in vitro activity as PDE10A inhibitors was determined. Nine out of twenty-one compounds were potent inhibitors of PDE10A with IC₅₀ values ranging from 1.5 to 18.6 nM. Notably, the IC₅₀ values of compounds **26a**, **26b**, and **33c** were 1.52 ± 0.18, 2.86 ± 0.10, and 3.73 ± 0.60 nM, respectively; these three compounds also showed high in vitro selectivity (> 1,000-fold) for PDE10A over PDE 3A/3B, PDE4A/4B. The high potency and selectivity of these three compounds suggests that they could be radiolabeled with PET radionuclides for further evaluation of their in vivo pharmacological behavior and ability to quantify PDE10A in the brain.

Keywords

Benzimidazole analogues; Cinnoline analogues; Phosphodiesterase 10A; PET

Phosphodiesterase 10A (PDE10A) is a dual specificity enzyme that hydrolyzes both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) and is an important modulator of intracellular levels of these cyclic nucleotides. PDE10A has been cloned and characterized from mouse and human tissue; it is highly expressed in striatum with modest expression in other brain regions and low expression outside the central nervous system (CNS).^{1–4} PDE 10A inhibitors have been investigated as anti-psychotics in schizophrenia and therapeutic agents for treating movement disorders associated with Huntington's disease and Parkinson disease and other neurological disorders characterized by the decrease in the activity of the neurons in the basal ganglia.^{5–7} Because positron emission tomography (PET) imaging is a powerful non-invasive tool for quantitatively measuring changes in CNS biomarkers under baseline conditions and after treatment, a

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Supplementary Data

Supplementary material associated with this article including the detailed chemical synthesis, yields, NMR data and the in vitro assay methods are provided in the supporting online information.

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potent and selective PDE10A tracer with suitable pharmacokinetic behavior would be valuable in evaluating these therapeutic interventions. ^{11}C -papaverine ($\text{IC}_{50} = 36 \text{ nM}$)⁸, [^{11}C]MP-10 ($\text{IC}_{50} = 0.18 \text{ nM}$) and structurally similar analogues have been investigated as PET radiopharmaceuticals in rodents and nonhuman primates, though pharmacokinetic properties and unfavorable metabolism limited their utility in preclinical models of human disease.^{8–11} The MP-10 analogue ^{18}F -JNJ42259152 was approved for clinical investigation in human subjects. Although preliminary data showed promising kinetics in normal volunteers, the development of kinetic models was challenging due to the presence of brain-penetrating metabolites.^{12, 13} Despite the lack of correlation between PDE10A expression and clinical severity in a recent exploratory study of ^{18}F -JNJ42259152 in subjects with HD and healthy controls,¹⁴ continued efforts to develop structurally diverse PDE10A inhibitors may identify PET ligands with improved in vivo pharmacological behavior. In this manuscript, we report the synthesis and in vitro characterization of analogues having either cinnoline (**1**) or benzimidazole (**2**) pharmacophores. (Figure 1) Several new lead compounds reported here and a previously reported cinnoline analogue have IC_{50} values $< 20 \text{ nM}$. The diverse structures represented in these compounds may result in the identification of tracers with kinetic and metabolic profiles suitable for measuring PDE10A in the brain with PET.

The syntheses of cinnoline PDE10A analogues **13a–d** and **14a–b** were accomplished according to Scheme 1. Commercially available 1-(4-(benzyloxy)-3-methoxyphenyl)-ethanone (**3**) was nitrated to afford compound **4**, then reduced to give amine **6**. Either **5**, the commercially available 1-(2-amino-4, 5-dimethoxyphenyl)ethanone or the amine **6** underwent diazotization/cyclization, followed by treatment with phosphorus oxychloride to afford intermediates **9** or **10**, respectively. The chloro intermediates were treated with 2-fluoro-3-substituted boronic acid, followed by 4-substituted-4-piperidinol to give the desired cinnoline PDE10A analogues **13a–d**. The benzyl group in compounds **13b** or **13d** was removed by using hydrogen under 10% Pd/C to afford hydroxyl containing compounds **14a** or **14b**.

Compounds **26a–i**, which possess substitution groups in the pyridine ring of cinnoline, were synthesized as shown in Scheme 2. Compound **26a** was previously reported by Hu et al.¹⁵ The synthesis of **26a–f** and **26h–i** used procedures similar to those described above for **13a–b** and **14a–b**. The synthesis of compound **26g** was accomplished by the conversion of 4-(pyridin-3-yl)piperidin-4-ol hydrochloride to compound **25** by alkylation and Miyaura borylation, followed by Suzuki reaction to afford the final product, **26g**.

The syntheses of benzimidazole PDE10A analogues **33a–f** were accomplished according to Scheme 3. Treatment of benzimidazole **27** with 4-methoxy benzoyl chloride under basic conditions to generate compound **28**, followed by *N*-methylation of benzimidazole afforded compound **29**. Deprotection of the anisole with boron tribromide afforded phenol intermediate **30**, which was treated with 3-bromo-2-chloropyridine to give key intermediate **31**. Compounds **33a–c** were obtained by Suzuki coupling of intermediate **31** with substituted boronic acids. Benzimidazole derivatives **33d–e** were synthesized by two steps: **31** was first converted into the stannyl containing compound **32**; this was followed by Stille coupling of compound **32** with substituted acyl chlorides to give compounds **33d–e**.

In vitro assays were performed following standard literature techniques developed by other investigators; the procedure used here and in our prior studies is detailed in the supplementary methods.^{7, 16} Briefly, a scintillation proximity assay (SPA) was used to measure the IC₅₀ values of all inhibitors. The tritiated cyclic nucleotide substrate concentration used in the enzyme assay was 1/3 of the K_m. The potency of each analogue as a PDE inhibitor was determined by measuring the activity of a fixed amount of recombinant human PDE enzyme in the presence of varying inhibitor concentrations. After background subtraction, counts for samples containing the inhibitors were plotted against inhibitor concentration to calculate the IC₅₀ values using non-linear regression analysis. All compounds were independently assayed at least three times.

The IC₅₀ values of the fifteen cinnoline analogues (**13a–d**, **14a–b**, and **26a–i**) and six benzimidazole analogues (**33a–f**) for PDE10A are shown in Table 1 and Table 2. Among the cinnoline analogues, for compounds **13a**, **13b**, and **14a**, the potency of substituted inhibitors for PDE10A showed in a decreasing order OCH₃ > OBn > OH; the IC₅₀ values of **13a**, **13b**, and **14a** were 18.4, 4,250, and 10,800 nM, respectively. Although compounds **13c**, **13d**, and **14b** have a pyridine-3-yl group instead of a methyl group at the R³ position, a similarly decreasing trend for PDE10A inhibition was observed for compounds **13c**, **13d**, and **14b** with IC₅₀ values of 14, 1,550, and 6,620 nM, respectively. The known compound **13c**¹⁷ was also synthesized and its IC₅₀ value was determined to ensure the tests results are comparable with published values. Compound **26a** has methyl group instead of the –H group at the R³ position compound **13c**; this led to the 9.2-fold increased potency of the IC₅₀ value from 14.0 nM for **13c** to 1.52 nM for **26a**. The pyridine-3-yl group at R⁴ position of **26a** was replaced by a 2-fluoro-pyridin-5-yl group to give **26b**; the chlorine-substituted analogue **26e** was also synthesized. The IC₅₀ values of **26b** and **26e** for PDE10A were 2.86 and 9.80 nM respectively, which are similar to the IC₅₀ value we determined for **26a** (1.52 nM). When the methoxyl group at R¹ in **26b** was replaced by a benzyloxyl group in **26f**, the potency decreased with IC₅₀ values increasing from 2.86 nM for **26b** to 350 nM for **26f**. This result was consistent with the results observed for **13a** and **13b**, **13c** and **13d**. When methoxyl groups at R¹ and R² were either replaced by a cyclized group (–OCH₂O–) in **26c** or removed in **26d**, compounds **26c** and **26d** lost inhibition activity for PDE10A completely. In comparing **26g** and **13c**, when the –H group in R³ was substituted with a –Cl group the potency of the inhibitor improved slightly from 14.0 nM for **13c** to 5.52 nM for **26g**. Compounds **26h** and **26i** were obtained by replacing the methoxyl group of R¹ in **26b** with a fluoropropoxyl and 2-(oxymethyl)-quinoliny group, respectively. The IC₅₀ values increased from 2.86 nM for **12b** to 518 and 79.3 nM for **26h** and **26i**, respectively. This result is consistent with the results observed for compounds **13a**, **13b**, **13c** and **13d**. Throughout these measurements, the methoxyl group at R¹ position of cinnoline structural analogues was critical in retaining high potency for inhibiting PDE10A. The replacement of a methoxyl group at R¹ with other groups such as –H, –OCH₂O–, –OBn, fluoropropoxy, 2-(oxymethyl)-quinoliny led to a significant reduction or a total loss in PDE10A inhibition activity.

The benzimidazole analogues are structurally distinct from cinnoline analogues; other benzimidazole inhibitors with high potency toward PDE10A have been previously

reported.¹⁸ Six benzimidazole analogues (**33a–f**) were synthesized and their IC₅₀ values for PDE10A were determined. In vitro data indicated that compounds **33a**, **33b**, and **33c** possessing a phenyl, 4-methoxyphenyl or heteroaryl substitution group for R¹ had higher inhibition potency for PDE10A; the IC₅₀ values were 18.6 ± 5.3, 8.0 ± 1.38, and 3.73 ± 0.60 nM for compounds **33a**, **33b**, and **33c**, respectively. Optimization of the substituted group in R¹ may improve potency for PDE10A. However, imposing a carbonyl group between the aryl or heteroaryl group and the skeleton of the benzimidazole resulted in significantly decreased activity toward PDE10A. Both **33d** and **33e** lost inhibition activity for PDE10A; the IC₅₀ value of **33f** increased to 1900 nM as shown in Table 2.

Compounds **26a**, **26b**, and **33c** are very potent inhibitors of PDE10A with IC₅₀ values < 5 nM, so further in vitro assays were conducted to determine their selectivity. It has been reported that inhibition of PDE3A/B may cause arrhythmia and increased mortality,^{19, 20} inhibition of PDE4A may increase heart and respiratory rates.²¹ Although such undesirable side-effects pose fewer safety concerns for clinical radiotracers in comparison with therapeutic agents, specificity is important. Therefore, the IC₅₀ values of **26a**, **26b** and **33c** for PDE3A, PDE3B, PDE4A, and PDE4B were measured to determine their selectivity as PDE10A inhibitors. The enzyme assays revealed that all three lead compounds had very weak inhibition activity toward PDE3A/3B, PDE4A/B with > 1000-fold selectivity for PDE10A vs. PDE3A/B and PDE4A/B, as shown in Table 3.

Overall, fifteen cinnoline and six benzimidazole analogues were synthesized and screened for their in vitro activity as PDE10A inhibitors. Nine of the twenty-one compounds showed high in vitro potency for PDE10A with IC₅₀ values ranging from 1.52 to 18.6 nM. The three most potent compounds **26a**, **26b**, and **33c** have IC₅₀ values of 1.52 ± 0.18, 2.86 ± 0.10, and 3.73 ± 0.60 nM, respectively; in addition, these three compounds have > 1000-fold selectivity for PDE10A over PDE3A/B and PDE4A/B and are structurally suitable compounds for labeling with PET radionuclides. Further studies will be needed to characterize the pharmacological behavior of the PET ligands in vivo and determine their potential as PET tracers for assessing PDE10A in living subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the National Institute of Mental Health (NIMH) and the National Institute of Neurological Disorders (NINDS) under MH092797, NS061025 and NS075527, and through funding from the Mallinckrodt Institute of Radiology (MIR) at the Washington University School of Medicine. The authors would also like to thank Lynne Jones for assistance in preparing the manuscript.

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Figure 1.
Structures of lead PDE10A inhibitors

Scheme 1. Synthesis of cinnoline analogues

Reagents and conditions:

(a) HNO₃, SnCl₄, CH₂Cl₂, -70°C; (b) Fe, NH₄HCO₃, toluene, water, reflux; (c) NaNO₂, acetic acid, 70% H₂SO₄, Et₃N; (d) POCl₃, PCl₅; (e) 2-fluoropyridine-5-boronic acid, Pd(PPh₃)₄, Cs₂CO₃, diglyme; (f) i. 4-(pyridin-3-yl)piperidin-4-ol or 4-methylpiperidin-4-ol, K₂CO₃, DMSO, reflux; or ii. K₂CO₃, DMSO, reflux, then H₂, 10% Pd/C, methanol, 75 psi.

Scheme 2. Synthesis of cinnoline derivatives with substitution in the pyridine ring

Reagents and conditions:

- (a) H_2 (75 psi), 10% Pd/C, MeOH; (b) 2-(bromomethyl)quinoline or 1-bromo-3-fluoropropane, NaH, THF. (c) 2-fluoro-3-methylpyridine-5-boronic acid, $\text{Pd}(\text{PPh}_3)_4$, Cs_2CO_3 , 1,2-dimethoxyethane; (d) 5-bromo-2,3-dichloro-pyridin, K_2CO_3 , DMSO, 110 °C; (e) bis(pinacolato)diboron, KOAc, (1,1'-bis(diphenylphosphino)ferrocene)Pd(II) dichloride, dioxane, 120 °C; (f) piperidinol, K_2CO_3 , DMSO, 100 °C; (g) **9**, $\text{Pd}(\text{PPh}_3)_4$, Cs_2CO_3 , 1,2-dimethoxyethane.

Scheme 3. Syntheses of new benzimidazole analogues

Reagents and conditions:

(a) 4-Methoxybenzoyl chloride, pyridine, Et₃N, NaOH, reflux; (b) CH₃I, NaH, DMF; (c) BBr₃, DCM; (d) 3-bromo-2-chloropyridine, K₂CO₃, Cs₂CO₃, DMSO; (e) boronic acid ester, K₂CO₃, Pd(PPh₃)₄; (f) hexamethyltin, K₂CO₃, Pd(PPh₃)₄, toluene; (g) substituted acyl chloride or aromatic or heteroaromatic boronic acid, palladium.

Table 1

The PDE10A inhibition activity (IC_{50} nM) of cinnoline analogues

Compound#	R ³	R ²	R ³	R ⁴	IC_{50} (nM) ^a
13a	-OCH ₃	-OCH ₃	H	CH ₃	18.4 ± 6.0
13b	-OBn	-OCH ₃	H	CH ₃	4,250 ± 520
13c	-OCH ₃	-OCH ₃	H	pyridin-3-yl	14.0 ± 2.0
13d	-OBn	-OCH ₃	H	pyridin-3-yl	1,550 ± 260
14a	-OH	-OCH ₃	H	CH ₃	10,800 ± 1,500
14b	-OH	-OCH ₃	H	pyridine-3-yl	6,620 ± 600
26a	-OCH ₃	-OCH ₃	-CH ₃	pyridine-3-yl	1.52 ± 0.18
26b	-OCH ₃	-OCH ₃	-CH ₃	2-fluoro-pyridin-5-yl	2.86 ± 0.10
26c	-O-CH ₂ -O-		-CH ₃	2-fluoro-pyridin-5-yl	NA ^b
26d	-H	-H	-CH ₃	2-fluoro-pyridin-5-yl	NA ^b
26e	-OCH ₃	-OCH ₃	-CH ₃	2-chloro-pyridin-5-yl	9.8 ± 1.3
26f	-OBn	-OCH ₃	-CH ₃	2-fluoro-pyridin-5-yl	350 ± 23
26g	-OCH ₃	-OCH ₃	-Cl	pyridin-3-yl	5.52 ± 0.33
26h	2-(oxymethyl)-quinolinyl	-OCH ₃	-CH ₃	2-fluoro-pyridin-5-yl	518 ± 86
26i	fluoropropoxy	-OCH ₃	-CH ₃	2-fluoro-pyridin-5-yl	79.3 ± 3.6

^a IC_{50} value (mean ± SD nM) were determined in at least three experiments;

b_{NA} = no inhibition activity

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Table 2The PDE10A inhibition activity (IC₅₀ nM) of benzimidazole analogues

Compound #	R ¹	IC ₅₀ (nM) ^a
33a	phenyl	18.6 ± 5.3
33b	4-methoxyphenyl	8.00 ± 1.38
33c	pyridine-3-yl	3.73 ± 0.60
33d	benzoyl	NA ^a
33e	4-methoxybenzoyl	NA ^a
33f	<i>N</i> -acetylpiperidin-4-ethanoyl	>1.9×10 ³

^aIC₅₀ value (mean ± SD nM) were determined in at least three experiments;^bNA = no inhibition activity.

Table 3

The inhibition activity (IC_{50} nM) of compounds **26a**, **26b**, and **33c** towards PDE 3A&B, PDE4A & B*^a

Compound #	PDE10A (nM)	PDE3A (nM)	PDE3B (nM)	PDE4A (nM)	PDE4B (nM)
26a	1.52 ± 0.18	3,070 ± 180	4,110 ± 360	7,830 ± 800	8,730 ± 730
26b	2.86 ± 0.10	3,700 ± 250	6,540 ± 520	25,400 ± 3,600	30,600 ± 400
33c	3.73 ± 0.60	167,000 ± 52,000	234,000 ± 66,000	403,000 ± 6,700	25,900 ± 2,900

^a IC_{50} value (mean ± SD nM) were determined in at least three experiments