Synthesis of Fluorine-Containing Phosphodiesterase 10A (PDE10A) Inhibitors and the In Vivo Evaluation of F-18 Labeled PDE10A PET Tracers in Rodent and Nonhuman Primate

Junfeng Li,† Xiang Zhang,‡ Hongjun Jin, † Jinda Fan,† Hubert Flores,‡ Joel S. Perlmutter,†,‡ and Zhude Tu†,*

†Department of Radiology and ‡Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110, United States

Supporting Information

ABSTRACT: A series of fluorine-containing PDE10A inhibitors were designed and synthesized to improve the metabolic stability of [11C]MP-10. Twenty of the 22 new analogues had high potency and selectivity for PDE10A: 18a–j, 19d–j, 20a–b, and 21b had IC50 values <5 nM for PDE10A. Seven F-18 labeled compounds [18F]18a–e, [18F]18g, and [18F]20a were radiosynthesized by [18F]-introduction onto the quinoline rather than the pyrazole moiety of the MP-10 pharmacophore and performed in vivo evaluation. Biodistribution studies in rats showed ~2-fold higher activity in the PDE10A-enriched striatum than nontarget brain regions; this ratio increased from 5 to 30 min postinjection, particularly for [18F]18a–d and [18F]20a. MicroPET studies of [18F]18d and [18F]20a in nonhuman primates provided clear visualization of striatum with suitable equilibrium kinetics and favorable metabolic stability. These results suggest this strategy may identify a [18F]-labeled PET tracer for quantifying the levels of PDE10A in patients with CNS disorders including Huntington’s disease and schizophrenia.

INTRODUCTION

Phosphodiesterase 10A (PDE10A) is a dual-specificity phosphodiesterase enzyme that is able to hydrolyze both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) to AMP and GMP, respectively. PDE10A mRNA is highly expressed in the brain; expression in peripheral tissues, with the exception of the testis, is low. Both PDE10A mRNA and protein are specifically enriched in the medium spiny neurons (MSNs) of the striatum. Abnormal striatal levels of PDE10A play a major role in schizophrenia, Huntington’s disease, addiction, and other neuropsychiatric disorders.

Positron emission tomography (PET) is a preclinical molecular imaging technique which permits quantitative noninvasive measurement of drug disposition and localization in animal models of human disease and an important tool for clinical studies. PDE10A inhibitors have shown promise as therapeutics in preclinical models of human disease; several have progressed to clinical trials. The highly potent (IC50 = 0.37 nM) and highly selective (>100-fold) PDE10A inhibitor 2-[4-(1-methyl-4-pyridin-4-yl)-1H-pyrazol-3-yl]-phenoxymethyl]-quinoline (MP-10) has been extensively evaluated as a therapeutic inhibitor of PDE10A and has completed several clinical trials in human subjects. [11C]MP-10 (Figure 1) was independently investigated by several groups as a PET tracer for quantifying PDE10A in vivo. Although variable success and significant species differences have been reported in preclinical studies, [11C]MP-10 remains a potentially viable PET tracer for clinical studies. Numerous other [11C]- and [18F]-labeled PDE10A inhibitors have been evaluated in preclinical rodent models, and although several candidate tracers have proceeded to studies in nonhuman primates and human subjects, the search for a PET tracer with ideal pharmacological properties is ongoing.

Although [11C]MP-10 provides a clear anatomic image of the brain with high accumulation in the striatum of nonhuman primates (NHP), tissue time-activity curves in macaques revealed an increasing trend of radioactivity accumulation in both the target region (striatum) and the reference region (cerebellum) which may limit its utility. Our metabolite analysis of [11C]MP-10 suggested that a brain penetrant radiometabolite was formed by O-dealkylation of the pyrazole moiety of [11C]MP-10. An additional consideration in the search for an improved PET tracer is that the longer half-life of [18F] places fewer time constraints on production and permits longer scan sessions that usually result in improved target:nontarget ratios and higher quality images. Although a potent and selective quinoline analogue of MP-10 was formed by introducing a [18F]-fluoroethyl group on the N-atom of the pyrazole moiety ([18F]JNJ41510417, Figure 1), slow equilibrium kinetics were reported in rodents and NHPs,

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possibly due to brain-penetrant radioactive metabolites that accumulate nonspecifically in the brain. To overcome this potential liability, we recently 11C-labeled the quinoline moiety instead of the pyrazole moiety of several new PDE10A inhibitors ([11C]1−4, Figure 1). We hypothesized that this strategy might lead to the identification of a new analogue with (i) increased in vivo metabolic stability, and/or (ii) a metabolic pathway which generates radioactive metabolites that do not cross the blood-brain barrier (BBB). Our earlier structure−activity relationship (SAR) study explored the introduction of a methoxy group (−OCH3) in the 3-, 4-, or 6-position of the 2-methylquinoline moiety of the MP-10 pharmacophore and the regioisomers of the new analogues. The most potent and selective compounds were the 3- and 4-methoxy substituted quinolines with an O atom bridge linkage; little difference was seen in the in vitro biological activity of regioisomers. Subsequent evaluation of the 11C-labeled lead tracers showed prolonged favorable target:background ratios in rats for the 3- and 4-methoxy substituted quinolines with high selectivity in the target region versus nontarget region and promising kinetic behavior in both the target region (striatum) and the reference regions of the NHP brain.

We have continued our exploration of 18F-labeled PET radiotracers for PDE10A by introducing fluoro-, fluoromethyl, fluoropropyl, or triazole fluorooethyl groups into the quinoline moiety of MP-10. We also explored the introduction of a short fluoroPEGylated (FPEG) unit. Incorporation of a short FPEG unit has been shown to (i) enable facile 18F-labeling of the target compound; (ii) improve the in vivo pharmacokinetics of molecules interacting with biologically interesting proteins or peptides; (iii) decrease the lipophilicity of molecules, which enhances their ability to cross the BBB. Radiolabeling of [11C]MP-10 also yielded the regioisomer ([11C]isoMP-10 Figure 1), a slightly less potent PDE10A inhibitor reported in the discovery of MP-10; [11C]isoMP-10 also showed selective binding in the rat striatum, though initial brain uptake was significantly lower than that of [11C]MP-10. We further expanded our synthetic efforts to include modification of the pyrazole moiety to generate regioisomers of the new fluorocontaining MP-10 quinoline analogues: this permitted expanded SAR studies. Here we report (i) the synthesis and screening of new analogues which allow 18F-labeling in the quinoline moiety rather than the pyrazole moiety; (ii) 18F-labeling of the potent and selective candidates and the in vivo evaluation of their suitability as PDE10A PET imaging agents using rats and macaques.

RESULTS AND DISCUSSION

Chemistry. The syntheses of target new PDE10A analogues was accomplished by a Mitsunobu reaction of benzyl alcohols on the quinoline moiety coupled with substituted phenols on pyrazole moiety 17a−b, or an O-alkylation of substituted methyl bromide on quinoline moiety with substituted phenols 17a−b. Following the above strategy, quinoline fragments of molecules including fluorene-containing intermediates 6a−j and 15a were first synthesized as shown in Scheme 1. Different approaches were used for the synthesis of compounds 6a−j and 15a: (i) aniline 5 was converted to the aryl fluoride 6a via diazonium fluoroborates under the Balz-Schiemann conditions; (ii) the alcohol 7 was converted directly into 6b by treatment with diethylnamnosulfur trifluoride (DAST); (iii) treatment of compound 8 with 1,1,1-trifluoro-N-phenyl-N-((trifluoromethyl)sulfonyl)methane-sulfonamide afforded the triflate; stille reaction of the triflate with allyltributyltin in the presence of tetrakis triphenylphosphine palladium and lithium bromide generated compound 9; hydroboration−oxidation of 9 to afford the alcohol, followed by fluorination generated compound 6c; (iv) alkylation of commercially available 10 (ethyl 4-hydroxy quinoline-2-carboxylate) with 2-fluoroethanol bromide afforded ethyl 4-(fluoroethoxy)quinoline-2-carboxylate (6d) in 60% yield; (v) alkylation of the phenol group of 10 with either a −Cl atom or −OTs group afforded the corresponding alcohols which were converted using DAST to the corresponding fluoroo-PEGylated intermediates 6e−g in modest yields (60−71%); (vi) alkylation of (2-methylquinolin-4-yl)methanol 7 with 2-fluoroethanol bromide

Figure 1. [11C]MP-10 and previously reported 11C- and 18F-labeled quinoline analogs.
under basic conditions afforded 4-((2-fluoroethoxy)methyl)-2-methylquinoline 6h; (vii) the aniline group of 5 was protected using di-tert-butyl dicarbonate to form the Boc-protected compound 11, subsequent N-alkylation using 2-fluoroethyl bromide afforded 6i; (viii) 10 was brominated using POBr3 to afford ethyl 4-bromoquinoline-2-carboxylate 12, which was converted to the azide compound and subjected to click reaction conditions,44 followed by DAST fluorination to afford compound 6j; (ix) using Friedlander synthesis,45 2-amino-benzaldehyde 13 reacted with 1-methoxypropan-2-one to afford the 3-methoxy-2-methylquinoline,46 subsequent demethylation utilizing BBr3 followed by O-alkylation afforded compound 15a.

The synthesis of intermediate compounds 16a–n was accomplished using strategies shown in Scheme 2: (i) bromination of 6a or 15d using NBS afforded compound 16a and 16n, respectively (Scheme 2, Method 1); (ii) the methylquinoline derivatives 6b–c, 6h–i, and 15a–c were oxidized using selenium dioxide (SeO2), followed by NaBH4 reduction, while the ethyl quinoline-2-carboxylates 6d–g, 6j were reduced using NaBH4 to afford the corresponding alcohols 16b–m (Scheme 2, Methods 2–3). The detailed methods for synthesis of intermediates are provided in the Supporting Information.

The synthesis of final compounds 18a–j, 19d–j, 20a–d, and 21b was accomplished as shown in Scheme 3. Substituted

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Scheme 1**

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\[ \begin{align*}
&5 \xrightarrow{a} 6a \\
&7 \xrightarrow{b} 6h \\
&8 \xrightarrow{c,d} 6b \\
&9 \xrightarrow{e,b} 6c \\
&10 \xrightarrow{f,g,b} 6d-g (n = 1-4) \\
&11 \xrightarrow{h} 6i \\
&12 \xrightarrow{i,l,b} 6j \\
&13 \xrightarrow{m,n} 14 \xrightarrow{f} 15a
\end{align*} \]
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**Reagents and conditions: (a) HBF4, NaNO2, −10 °C to rt; (b) DAST, CH3Cl, 0 °C to rt; (c) phenyl triflimide, DIMEA, DMF, rt; (d) LiBr, Pd[(C6H5)3P]4, THF, rt, then allyltributyltin 80 °C; (e) (i) 1.0 M borane THF complex, THF, 0 °C, (ii) NaOH, H2O2, sodium ascorbate, DMF, rt; (f) Cs2CO3, 1-bromo-2-fluoroethane, DMF, rt; (g) 2-(2-chloroethoxy)ethanol or 2-(2-(2-chloroethoxy)ethoxy)ethanol, K2CO3, DMF, 95–100 °C; or tetraethylene glycol p-toluenesulfonate, Cs2CO3, DMF, 60 °C; (h) 1-bromo-2-fluoroethane, KOH, MeCN, 80 °C; (i) (Boc)2O, TEA, DMAP, THF, rt; (j) phosphorus(V) oxybromide, K2CO3, MeCN, 92 °C; (k) NaN3, DMF, 90 °C; (l) 3-buten-1-ol, CuSO4+5H2O, sodium ascorbate, DMF, rt; (m) methoxyacetone, ethanolic KOH, ethanol, reflux; (n) BBr3, DMC, rt.

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phenols 17a–b were prepared as previously published (Scheme 3).35 16b–m were coupled with the phenol 17a or 17b with a Mitsunobu reaction39 to afford 18b–j, 20a–c, 19d–j, and 21b. Compounds 18a and 20d were made by O-alkylation of the substituted phenol 17a with substituted methyl bromide 16a or 16n. In vitro inhibition assays identified candidate potent and selective analogues for radiolabeling: 18a–e, 18g, and 20a. Schemes 4 and 5 outline the synthesis of the precursors for radiolabeling; the detailed methods for their synthesis are provided in the Supporting Information. Scheme 4 outlines the synthesis of precursors 23a, 23d, and 24a. The method described above for synthesis of 16d from 6d was used for the synthesis of 23a. A methoxymethyl ether (MOM) group was used to protect phenols 10 and 24b under basic conditions; the protected intermediates were reacted with the substituted phenol 17a under Mitsunobu reaction conditions to afford 27a–b. The MOM ether functional group was easily deprotected by TFA to afford precursors 23d and 24a.

The precursors 23b–c, 23e, and 23g were obtained by following Scheme 5. Commercially available tert-butylidydimethylsilyl (TBS) chloride was used as a silylation agent to protect alcohols 28a–d. The use of 1.7 equiv imidazole as base with 1.7 equiv of TBSCI and DMF as solvent proved effective and resulted in TBS-ethers 29a–d in high yield. Compounds 29a–d were converted to the respective alcohols 30a–d, which were coupled with the phenol 17a or 17b in the presence of Ph3P and di-tert-butyl azodicarboxylate (DBAD) via a Mitsunobu O-alkylation39 to afford compounds 31a–d. The TBS-ethers 31a–d were easily deprotected with a catalytic amount of tetrabutyl ammonium fluoride (TBAF); the intermediate alcohol was then chlorinated or sulfonated as indicated in Scheme 5 to afford precursors 23b–c, 23e, and 23g.

Radiochemistry. An overview of the radiolabeling of [18F]18a–e, [18F]18g, and [18F]20a is shown in Scheme 6. These tracers were labeled by the following strategies: (i) a one-step direct nucleophilic halogen exchange was used for labeling [18F]18a and [18F]18b; (ii) a one-step direct nucleophilic aliphatic substitution with a tosylate (Ts) or mesylate (Ms) leaving group was used for labeling [18F]18c, [18F]18e, and [18F]18g; (iii) a two-step strategy was used for labeling [18F]18d and [18F]20a using the 2-[18F]fluoroethyl tosylate 34 as a secondary labeling agent. The structures of the new fluorine-containing PDE10A inhibitors [18F]18a–e, [18F]18g, and [18F]20a are shown in Figure 2.

Scheme 6 shows the conditions used for the successful radiolabeling of [18F]18a–e, [18F]18g, and [18F]20a. Labeling of [18F]18b was initially planned by a direct nucleophilic aliphatic substitution using a tosylate (Ts) or mesylate (Ms) leaving group. However, because heating the precursor with [18F] at 85 °C for 15 min yielded only a trace of [18F]18b, an alternative strategy of halogen exchange reaction was pursued; this was successfully performed using the chloride precursor 23b. As shown in Scheme 6, a one-step displacement reaction using mesylate and tosylation leaving groups was employed for radiolabeling [18F]18b, [18F]18c, [18F]18e, and [18F]18g. A

Reagents and conditions: (a) NBS, CCl4, reflux; (b) SeO2, dioxane, reflux, then NaBH4, dioxane, EtOH, rt; (c) NaBH4, THF, MeOH, rt.

Scheme 2

Method 1

\[
\begin{align*}
6a, \ R^1 &= 4-F \\
15d, \ R^1 &= 6-OCH_2CH_2F
\end{align*}
\]

Method 2 and 3

\[
\begin{align*}
8b, \ R^2 &= CH_3, \ R^1 &= 4-CH_2F \\
8c, \ R^2 &= CH_3, \ R^1 &= 4-CH_2CH_2CH_2F \\
8d, \ R^2 &= COOEt, \ R^1 &= 4-CH_2F \\
8e, \ R^2 &= COOEt, \ R^1 &= 4-(OCH_2CH_2)F \\
8f, \ R^2 &= COOEt, \ R^1 &= 4-(OCH_2CH_2)F \\
8g, \ R^2 &= CH_2, \ R^1 &= 4-CH_2F \\
8h, \ R^2 &= CH_2, \ R^1 &= 4-(OCH_2CH_2)F \\
8i, \ R^2 &= CH_2, \ R^1 &= 4-N(Boc)CH_2F \\
8j, \ R^2 &= COOEt, \ R^1 &= 4-CH_3 \\
15a, \ R^2 &= CH_3, \ R^1 &= 3-CH_2CH_2F \\
15b, \ R^2 &= CH_3, \ R^1 &= 6-F \\
15c, \ R^2 &= CH_3, \ R^1 &= 6-CH_2F \\
16b, \ R^1 &= 4-CH_2F \\
16c, \ R^1 &= 4-CH_2CH_2CH_2F \\
16d, \ R^1 &= 4-OCH_2CH_2F \\
16e, \ R^1 &= 4-(OCH_2CH_2)F \\
16f, \ R^1 &= 4-(OCH_2CH_2)F \\
16g, \ R^1 &= 4-(OCH_2CH_2)F \\
16h, \ R^1 &= 4-CH_2CH_2CH_2F \\
16i, \ R^1 &= 4-N(Boc)CH_2F \\
16j, \ R^1 &= 4-CH_3 \\
16k, \ R^1 &= 3-OCH_2CH_2F \\
16l, \ R^1 &= 6-F \\
16m, \ R^1 &= 6-CH_2F
\end{align*}
\]
two-step strategy was used for the radiosynthesis of $[^{18}\text{F}]18d$ and $[^{18}\text{F}]20a$ using the tosylate $[^{18}\text{F}]34$ as a secondary labeling agent. First, $[^{18}\text{F}]KF/4,7,13,16,21,24$-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 2.2.2.) was substituted into the ditosylate $33$. Second, the resulting 2-fluoroethyl tosylate, $[^{18}\text{F}]34$, was directly used in a nucleophilic substitution by reaction with the precursors to afford $[^{18}\text{F}]18d$ and $[^{18}\text{F}]20a$. Details are provided in the Experimental Section. All tracers were obtained with high specific activity (SA), and with a radiochemical yield sufficient for in vivo studies. The radiochemical purity of each tracer was $>99\%$ and the chemical purity was $>95\%$. Details of the radiosynthesis for each $^{18}\text{F}$-labeled compound are provided in the Experimental Section.

**Reagents and conditions:** (a) 17a, NaH, DMF, rt; (b) 17a or 17b, DBAD, PPh$_3$, dioxane, 60 °C; (c) 17a or 17b, Ph$_3$P, DBAD, dioxane, 60 °C; then TFA, rt.
Scheme 4. Synthesis of Precursors (1)\textsuperscript{a}

\[ \text{Reagents and conditions: (a) NaBH}_4, \text{THF: MeOH (20:1), 0 °C to rt; (b) 17a, DBAD, PPh}_3, \text{dioxane, 60 °C; (c) MOM-Br, DIPEA, CH}_2\text{Cl}_2, 0 °C; (d) SeO}_2, \text{dioxane, reflux, then NaBH}_4, \text{dioxane, EtOH, rt; (e) NaBH}_4, \text{THF/MeOH; (f) TFA, DCM.} \]

Scheme 5. Synthesis of Precursors (2)\textsuperscript{a}

\[ \text{Reagents and conditions: (a) imidazole, TBSCl, DMF, 80 °C; (b) SeO}_2, \text{dioxane, reflux, then NaBH}_4, \text{dioxane, EtOH, rt; (c) NaBH}_4, \text{THF, MeOH, rt; (d) 17a, Ph}_3\text{P, DBAD, dioxane, 60 °C; (e) TBAF, THF, rt; (f) SOCl}_2, \text{chloroform, 0 °C; (g) MsCl, TEA, CH}_2\text{Cl}_2; (h) Ts-Cl, DMAP, DIEA, CH}_2\text{Cl}_2. \]
In Vitro Assays. We previously reported that the introduction of a 3- or 4-methoxy group on the quinoline fragment of MP-10 and isoMP-10 yielded potent and PDE10A selective analogues. Here we report our continued exploration of fluorine-containing quinoline analogues as PDE10A inhibitors. In vitro potency and selectivity of the new substituted quinoline analogues as PDE10A inhibitors are shown in Table 1; the top section of the table contains analogs of MP-10 while the lower section contains select corresponding regioisomers which are analogues of isoMP-10. Comparison of the in vitro assay results for MP-10 analogues and the regioisomers showed no significant...
difference for paired rosiglizos, although the slightly lower IC_{50} values for the analogues based on MP-10 suggest they may be modestly more potent. New analogues with a fluorine-containing group into the 4-position of the quinoline moiety of the MP-10 pharmacophore (18a–j) demonstrated high potency with IC_{50} values of 0.29 and 0.76 nM, respectively; however, replacement of the hydrogen at 6-position using larger fluorine-containing groups resulted in analogues with low potency (20a, –CH_2F, IC_{50} > 76 nM; 20d, –OCH_2CH_2F, IC_{50} = 33.5 ± 0.08 nM).

Selectivity of a PET ligand for PDE10A over the other subtypes is also an important consideration. Inhibition of PDE3A/B has been associated with arrhythmia and increased mortality, while PDE4 is widely expressed in the central nervous system. The potent new PDE10A inhibitors (20 compounds, PDE10A IC_{50} < 5 nM) were also evaluated for inhibition of PDE3A/B and PDE4A/B. The results for final compounds 18a–j, 19d–j, 20a–d, and 21b (Table 1) revealed that almost all compounds had very low potency for inhibiting PDE3A/B and PDE4A/B with IC_{50} values >750 nM, except 18c which had slight potency for inhibiting PDE4A/B (IC_{50} 460 nM for PDE4A and 380 nM for PDE4B). All of the new compounds, including 18c, had >210-fold selectivity ratios for PDE10A vs PDE3A/B and PDE4A/B. This suggests that 18a–j, 19d–j, 20a,b, and 21b are both potent and selective inhibitors of PDE10A.

Based on these screening results and our previous experience with the ^11C-labeled rosiglizos, seven analogues of MP-10 were identified as candidate ligands for ^19F-labeling: –F (18a), −(CH_2)_4F (18b), 18c), −OCH_2CH_2F (18d), −CH_2OCH_2CH_2F (18e), and –FPEG (18g, 20a). The precursors were synthesized (details are provided in the Supporting Information) for the subsequent radiolabeling and the in vivo evaluation as PET tracers for quantification of PDE10A in rodents and nonhuman primates.

**Biodistribution.** The biodistribution of [18F]18a–e, [18F] 18g, and [18F]20a was evaluated in normal adult male Sprague–Dawley rats at 5, 30, and 60 min postinjection (p.i.) Table 2 shows the uptake and washout in peripheral organs and whole brain for each of the seven tracers. The initial brain uptake values were 0.410 ± 0.063, 0.128 ± 0.012, 0.114 ± 0.015, 0.118 ± 0.012, 0.213 ± 0.039, 0.353 ± 0.052, and 0.992 ± 0.011%ID/g for [18F]18a, [18F]18b, [18F]18c, [18F]18d, [18F]18e, [18F]18g, and [18F]20a, respectively. At 5, 30, and 60 min p.i., brain uptake of each of the tracers was comparable to or higher than [11C]MP-10 (0.162 ± 0.025, 0.066 ± 0.008, and 0.056 ± 0.006%ID/g 5, 30, and 60 min p.i.), 39 modest but progressive accumulation of radioactivity in bone was observed for [18F]18a, [18F]18b, and [18F]18c: bone activity increased from 0.522 ± 0.116, 0.421 ± 0.063, and 0.222 ± 0.014%ID/g at 5 min to 6.300 ± 0.314, 5.620 ± 1.132, and 2.037 ± 0.303%ID/g at 60 min p.i., respectively. This progressive increase in bone activity was most likely the result of metabolic defluorination. These results suggest that functional groups including –F (18a), –CH_2F (18b), and –CH_2CH_2CH_2F (18c) on the quinoline fragment of MP-10 undergo in vivo metabolic defluorination in rodents. In contrast, [18F]18d, [18F]18e, [18F] 18g, and [18F]20a appeared more stable to in vivo defluorination.

The cerebellum and cortex are accepted reference (non-target) regions in kinetic modeling of PDE10A PET tracers. Therefore, in addition to high striatal uptake and retention, a candidate tracer for clinical imaging should show low uptake and rapid washout in the cerebellum and cortex; this will facilitate high target:non-target ratios and yield high quality images. The new radiotracers displayed generally heterogenous regional brain distribution with the highest uptake observed in the striatum; by 30 min p.i., the ratio of activity in the striatum versus nontarget brain regions was >2-fold for [18F]18a, [18F]18b, [18F]18c, [18F]18d, and [18F]20a (Figure 3). Between 5 and 30 min p.i., the striatum: cerebellum and striatum: cortex ratios increased for the three tracers, which suggests that they were rapidly cleared from nontarget regions and displayed specific binding within the PDE10A-enriched striatum.

Taken together, the rodent biodistribution and regional brain uptake and washout data identified 4-fluoroethoxy [18F]18d and 3-fluoroethoxy [18F]20a as the more promising candidates for further evaluation in nonhuman primates. Both tracers appeared stable to metabolic defluorination and showed favorable ratios in the striatum versus nontarget brain regions.

**MicroPET Imaging Studies of Cynomolgus Macaques.** The two fluorothoxy lead PET radioligands for imaging PDE10A, [18F]18d and [18F]20a, underwent PET imaging studies (n = 3 for each tracer) in male cynomolgus monkeys (Figure 4) using a microPET Focus 220 scanner. The representative summed images from 0 to 180 min were coregistered with MRI images to accurately identify the anatomical regions of interest (ROI’s) are shown in Figure 4. MicroPET studies of both [18F]18d and [18F]20a gave clear visualization of the PDE10A-enriched striatum. Both the caudate and putamen striatal regions had high uptake of [18F]18d and [18F]20a; the ratios of uptake in the striatum vs...
nontarget brain regions were >3-fold by 40 min p.i. In particular, for \([^{18F}]20a\), target:nontarget ratios reached >5-fold by 70 min p.i. More importantly, although the two tracers showed differences in striatal clearance, tissue time−activity curves of both \([^{18F}]18d\) and \([^{18F}]20a\) demonstrated improved washout kinetics from both target and nontarget regions compared with \([^{11C}]MP-10\). The improved washout kinetics suggest that the strategy of 18F-labeling the quinoline rather than the pyrazole moiety reduced the formation of radioactive metabolites that cross the BBB.

Metabolite Studies in Cynomolgus Macaques. Initial NHP brain imaging of \([^{18F}]18d\) and \([^{18F}]20a\) showed improved kinetics over previously published PET tracers for PDE10A. To further investigate the in vivo metabolic stability of these tracers, HPLC analysis was performed to identify the percentage of parent compound versus radiometabolites in solvent extracts of NHP plasma obtained during microPET imaging studies of \([^{18F}]18d\) and \([^{18F}]20a\). NHP arterial blood was obtained from cynomolgus macaques 5, 15, 30, 60, and 90 min p.i. for \([^{18F}]18d\) and \([^{18F}]20a\). Plasma was separated from packed red cells; ~65−75% of the radioactivity was in the plasma. Following solvent extraction and deproteination, ~90% of the plasma activity was in the supernatant and only 10% of the activity was in the protein pellet. The solvent extract was injected onto an HPLC to quantify the percentage of the radioactivity for the parent compound versus radiolabeled metabolites based on the different retention times. As shown in Table 3, HPLC analysis of NHP plasma revealed good...
metabolic stability for both $[^{18}F]18d$ and $[^{18}F]20a$. Particularly, at 30 min p.i., solvent extracts of arterial blood contained ~90% parent compound for $[^{18}F]18d$. The major radioactive metabolite of $[^{18}F]18d$ was more polar (retention time ~3.5 min) than the parent compound (retention time ~10.5 min), negligible amounts of a minor radioactive metabolite (retention time ~7.5 min) were also observed. For $[^{18}F]20a$, solvent extracts of arterial blood contained >90% parent compound 15 min p.i., 73% parent remained 30 min p.i. The major radioactive metabolite was more polar (retention time ~3.5 min) than the parent compound (retention time ~10.5 min). The minor radioactive metabolite was <14% of the recovered activity 90 min p.i. (retention time ~7.5 min). The results of the metabolite analysis confirmed that our new strategy of $[^{18}F]$-labeling the quinoline rather than the pyrazole moiety resulted in significant metabolic stability in NHP blood.

## CONCLUSION

In the present study, we optimized the structure of MP-10 to identify new compounds that can be $[^{18}F]$-labeled in the quinoline moiety instead of the pyrazole moiety. As a result, the 20 highly potent compounds $18a$–$j$, $19d$–$j$, $20a$–$b$, and $21b$ (IC$_{50} < 5$ nM) were identified which also displayed high selectivity for PDE10A against PDE3A/B and PDE4A/B. Among these potent compounds, $18a$–e, $18g$, and $20a$ were evaluated as potential PET radioligands for quantifying the level of PDE10A. $[^{18}F]18a$, $[^{18}F]18b$, $[^{18}F]18c$, $[^{18}F]18d$, $[^{18}F]18e$, and $[^{18}F]20a$ are, to our knowledge, the first MP-10 analogues successfully $[^{18}F]$-labeled in the quinoline instead of the pyrazole moiety. Rat biodistribution indicated $[^{18}F]18a$, $[^{18}F]18b$, $[^{18}F]18c$, $[^{18}F]18d$, $[^{18}F]18e$, and $[^{18}F]20a$ can cross the blood-brain-barrier and accumulate in the PDE10A-enriched striatum. $[^{18}F]18d$ and $[^{18}F]20a$ proceeded to further evaluation in NHPs. The preliminary microPET imaging studies in NHPs demonstrated that $[^{18}F]18d$ and $[^{18}F]20a$ provided very clear visualization of striatum. Although different patterns of striatal clearance were observed, both tracers showed rapid clearance from nontarget brain regions. Compared with $[^{18}C]MP-10$, $[^{18}F]18d$ and $[^{18}F]20a$ displayed more suitable washout kinetics in NHP brain. In addition, both tracers displayed favorable in vivo metabolic stability based on HPLC analysis of NHP plasma. These promising preliminary results suggest that our strategy has a high probability of identifying a clinical PET tracer for quantifying PDE10A in the human brain. Future NHP microPET imaging studies with $[^{18}F]18d$ and $[^{18}F]20a$ will explore the specificity of the tracer uptake.

In summary, the strategy of introducing fluorine-containing group in a suitable position of the 2-methylquinoline moiety of MP-10 identified new potent and selective PDE10A inhibitors;

### Table 2. Biodistribution of $[^{18}F]18a$, $[^{18}F]18b$, $[^{18}F]18c$, $[^{18}F]18d$, $[^{18}F]18e$, and $[^{18}F]20a$ in Male Sprague Dawley Rats

<table>
<thead>
<tr>
<th>organ</th>
<th>$[^{18}F]18a$</th>
<th>$[^{18}F]18b$</th>
<th>$[^{18}F]18c$</th>
<th>$[^{18}F]18d$</th>
<th>$[^{18}F]18e$</th>
<th>$[^{18}F]20a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>0.353 ± 0.063</td>
<td>0.376 ± 0.033</td>
<td>0.150 ± 0.021</td>
<td>0.471 ± 0.040</td>
<td>0.416 ± 0.062</td>
<td>0.201 ± 0.026</td>
</tr>
<tr>
<td>liver</td>
<td>6.87 ± 1.27</td>
<td>3.00 ± 0.91</td>
<td>1.60 ± 0.87</td>
<td>10.16 ± 1.86</td>
<td>4.01 ± 1.34</td>
<td>3.10 ± 0.61</td>
</tr>
<tr>
<td>kidney</td>
<td>1.887 ± 0.356</td>
<td>1.034 ± 0.131</td>
<td>0.533 ± 0.119</td>
<td>1.799 ± 0.207</td>
<td>1.351 ± 0.216</td>
<td>0.573 ± 0.134</td>
</tr>
<tr>
<td>muscle</td>
<td>0.339 ± 0.053</td>
<td>0.236 ± 0.019</td>
<td>0.092 ± 0.010</td>
<td>0.227 ± 0.042</td>
<td>0.174 ± 0.042</td>
<td>0.107 ± 0.032</td>
</tr>
<tr>
<td>fat</td>
<td>0.404 ± 0.102</td>
<td>0.576 ± 0.154</td>
<td>0.477 ± 0.099</td>
<td>0.343 ± 0.153</td>
<td>0.352 ± 0.081</td>
<td>0.222 ± 0.029</td>
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<tr>
<td>bone</td>
<td>0.522 ± 0.116</td>
<td>4.035 ± 0.153</td>
<td>6.300 ± 0.314</td>
<td>0.421 ± 0.063</td>
<td>4.055 ± 1.305</td>
<td>0.560 ± 0.113</td>
</tr>
<tr>
<td>brain</td>
<td>0.410 ± 0.063</td>
<td>0.191 ± 0.036</td>
<td>0.125 ± 0.009</td>
<td>0.128 ± 0.012</td>
<td>0.094 ± 0.023</td>
<td>0.069 ± 0.008</td>
</tr>
</tbody>
</table>

$[^{18}F]18a$, $[^{18}F]18b$, $[^{18}F]18c$, $[^{18}F]18d$, $[^{18}F]18e$, and $[^{18}F]20a$ were evaluated as potential PET radioligands for quantifying the level of PDE10A. $[^{18}F]18a$, $[^{18}F]18b$, $[^{18}F]18c$, $[^{18}F]18d$, $[^{18}F]18e$, $[^{18}F]18g$, and $[^{18}F]20a$ are, to our knowledge, the first MP-10 analogues successfully $[^{18}F]$-labeled in the quinoline instead of the pyrazole moiety. Rat biodistribution indicated $[^{18}F]18a$, $[^{18}F]18b$, $[^{18}F]18c$, $[^{18}F]18d$, $[^{18}F]18e$, and $[^{18}F]20a$ can cross the blood-brain-barrier and accumulate in the PDE10A-enriched striatum. $[^{18}F]18d$ and $[^{18}F]20a$ proceeded to further evaluation in NHPs. The preliminary microPET imaging studies in NHPs demonstrated that $[^{18}F]18d$ and $[^{18}F]20a$ provided very clear visualization of striatum. Although different patterns of striatal clearance were observed, both tracers showed rapid clearance from nontarget brain regions. Compared with $[^{18}C]MP-10$, $[^{18}F]18d$ and $[^{18}F]20a$ displayed more suitable washout kinetics in NHP brain. In addition, both tracers displayed favorable in vivo metabolic stability based on HPLC analysis of NHP plasma. These promising preliminary results suggest that our strategy has a high probability of identifying a clinical PET tracer for quantifying PDE10A in the human brain. Future NHP microPET imaging studies with $[^{18}F]18d$ and $[^{18}F]20a$ will explore the specificity of the tracer uptake.

In summary, the strategy of introducing fluorine-containing group in a suitable position of the 2-methylquinoline moiety of MP-10 identified new potent and selective PDE10A inhibitors;
this approach will help guide the design of potent and selective ligands with suitable lipophilicity as candidates for treating schizophrenia and other CNS diseases using PDE10A enzyme inhibition strategies. Further validation of these new analogues is ongoing to determine if either is a suitable PET tracer to quantify the level of PDE10A in the human brain.

**EXPERIMENTAL SECTION**

**General.** All reagents and chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. All anhydrous reactions were carried out in oven-dried glassware under an inert nitrogen atmosphere unless otherwise stated. When reactions involved extraction with DCM (CH₂Cl₂), chloroform (CHCl₃), or ethyl acetate (EtOAc), the organic extracts were dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. Free bases were converted into the corresponding oxalate salt by addition of oxalic acid for all final compounds. Melting points of salts were determined on the MEL-TEMP 3.0 apparatus and left uncorrected. ¹H NMR spectra were recorded at 400 MHz on a Varian Mercury-VX spectrometer with CDCCl₃ as solvents and tetramethylsilane (TMS) as the internal standard. Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc. Synthesis of starting materials and intermediates are shown in Supporting Information.


**Figure 3.** Ratios of striatum:target brain regions in rats. [¹⁸F]18a, [¹⁸F]18b, [¹⁸F]18c, [¹⁸F]18d, and [¹⁸F]18e displayed higher target:target ratios than [¹⁸F]18f and [¹⁸F]18g at 30 and 60 min p.i. for both striatum/cerebellum (top panel) and striatum/cortex (bottom panel).

**Table 1.** Chemical and physical properties of compounds ¹⁸F-18a–18e.

**Table 2.** Effect of PDE10A inhibition on the binding of [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice.

**Table 3.** Binding of [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Table 4.** Binding of [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 4.** Autoradiograms of mouse striatum, cortex, and cerebellum showing the distribution of [¹⁸F]-labeled compounds ¹⁸F-18a–18e.

**Figure 5.** Scatter plots of the striatum:target ratios for [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 6.** -Dose-response relationships between [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 7.** Time courses of [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 8.** -Dose-response relationships between [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 9.** Time courses of [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 10.** -Dose-response relationships between [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.

**Figure 11.** Time courses of [¹⁸F]-labeled compounds ¹⁸F-18a–18e in the striatum of mice following PDE10A inhibition.
Representative microPET studies of \([^{18}F]20a\) and \([^{18}F]20a\) in the brain of a male cynomolgus macaque: (a) \([^{18}F]20a\): PET image (top left), coregistered image (top middle), MR image (top right); (b) \([^{18}F]20a\): PET image (top left), coregistered image (top middle), MR image (top right); (c) brain TACs of \([^{18}F]18d\); (d) brain TACs of \([^{18}F]20a\). Both \([^{18}F]18d\) and \([^{18}F]20a\) gave clear visualization of the PDE10A-enriched striatum.

### Table 3. HPLC Analysis of NHP Plasma for \([^{18}F]18d\) and \([^{18}F]20a\)

<table>
<thead>
<tr>
<th>tracer</th>
<th>time p.i. (min)</th>
<th>% peak 1</th>
<th>% peak 2</th>
<th>% parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>([^{18}F]18d)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>15</td>
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<td>93</td>
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<tr>
<td></td>
<td>30</td>
<td>7</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>19</td>
<td>5</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>32</td>
<td>9</td>
<td>57</td>
</tr>
<tr>
<td>([^{18}F]20a)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>99</td>
</tr>
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</tr>
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<tr>
<td></td>
<td>90</td>
<td>39</td>
<td>14</td>
<td>45</td>
</tr>
</tbody>
</table>

*Peak 1 (radiometabolite) retention time \(\sim 3.5\) min. Peak 2 (radiometabolite) retention time \(\sim 7.5\) min.

Hz, 2H), 3.96 (s, 3H), 3.96–4.71 (m, 6H). mp: 166.0–166.2 °C. Anal. (C\(_{33}\)H\(_{35}\)FN\(_{4}\)O\(_{5}\)) C, H, N: Calcd C: 58.17, H: 4.88, N: 7.75. Found C: 58.35, H: 4.98, N: 7.94.

4-(2-(2-(2-Fluoroethoxy)ethoxy)ethoxy)ethoxy)-2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline (18h). Compound 16g was used as the starting material following the procedure described above for the preparation of 18b to synthesize 18h as a white solid (48 mg, 59%). 1H NMR (CDCl\(_3\)): \(\delta\) 8.46 (d, \(J = 5.9\) Hz, 2H), 8.21 (d, \(J = 8.3\) Hz, 1H), 7.98 (d, \(J = 8.4\) Hz, 1H), 7.69 (t, \(J = 7.6\) Hz, 1H), 7.56 (s, 1H), 7.48 (t, \(J = 7.6\) Hz, 1H), 7.38 (d, \(J = 8.7\) Hz, 2H), 7.15 (d, \(J = 5.9\) Hz, 2H), 7.05–6.95 (m, 3H), 5.32 (s, 2H), 4.61–4.55 (m, 1H), 4.49–4.42 (m, 1H), 4.35 (t, \(J = 4.6\) Hz, 2H), 4.00 (t, \(J = 4.6\) Hz, 2H), 3.95 (s, 3H), 3.82–3.62 (m, 2H). mp: 128.4–129.0 °C. Anal. (C\(_{33}\)H\(_{35}\)FN\(_{4}\)O\(_{5}\)) C, H, N: Calcd C: 57.96, H: 5.13, N: 7.31. Found C: 58.06, H: 5.31, N: 7.50.

N-(2-Fluoroethyl)-2-[(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl) quinolin-4-amine (18i). Compound 16i was used as the starting material following the procedure described above for the preparation of 18b, then used TFA for deprotection to afford 18i as a light yellow solid (42 mg, 63%). 1H NMR (CDCl\(_3\)): \(\delta\) 8.47 (d, \(J = 4.0\) Hz, 2H), 7.99 (d, \(J = 4.4\) Hz, 1H), 7.86–7.83 (m, 1H), 7.64–7.67 (m, 1H), 7.57 (s, 1H), 7.42–7.37 (m, 3H), 7.17–7.15 (m, 2H), 7.03–7.01 (m, 2H), 6.63 (s, 1H), 5.33 (s, 2H), 4.82–4.79 (m, 1H), 4.70–4.67 (m, 1H), 3.96 (s, 3H), 3.76–3.69 (m, 2H). mp: 210.6–210.7 °C. Anal. (C\(_{33}\)H\(_{35}\)FN\(_{4}\)O\(_{5}\)) C, H, N: Calcd C: 58.77, H: 4.45, N: 11.05. Found C: 58.98, H: 4.34, N: 11.23.

4-(2-(2-Fluoroethoxy)-1H-1,2,3-triazol-1-yl)-2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline (18j). Compound 16j was used as the starting material following the procedure described above for the preparation of 18b to synthesize 18j as a light yellow solid (59 mg, 64%). 1H NMR (CDCl\(_3\)): \(\delta\) 8.52–8.44 (m, 2H), 8.23–8.17 (m, 1H), 8.02–7.96 (m, 1H), 7.96–7.93 (m, 1H), 7.88–7.80 (m, 2H), 7.65–7.63 (m, 1H), 7.57 (s, 1H), 7.44–7.37 (m, 2H), 7.19–7.13 (m, 2H), 7.07–6.99 (m, 2H), 5.45 (s, 2H), 4.89 (t, \(J = 5.9\) Hz, 1H), 4.77 (t, \(J = 5.9\) Hz, 1H), 3.97 (s, 3H), 3.35 (t, \(J = 5.9\) Hz, 1H), 3.28 (t, \(J = 5.9\) Hz, 1H). mp: 214.2–214.6 °C. Anal. (C\(_{33}\)H\(_{35}\)FN\(_{4}\)O\(_{5}\)) C, H, N: Calcd C: 57.81, H: 4.12, N: 14.30. Found C: 57.97, H: 4.01, N: 14.42.
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4-(2-Fluoroethoxy)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-5-yl)phenoxy)methyl)quinoline (19d). Compound 16d with 17b (in place of 17a) were used as the starting materials following the procedure described above for the preparation of 18b to synthesize 19d as a white solid (86 mg, 70%).1 H NMR (CDCl3): δ 8.38 (d, J = 2.4 Hz, 2H), 8.29—8.27 (m, 1H), 8.03—8.01 (m, 1H), 7.82 (s, 1H), 7.75 (m, 1H), 7.57—7.53 (m, 1H), 7.26—7.22 (m, 2H), 7.16—7.14 (m, 2H), 7.04—7.02 (m, 2H), 5.48 (s, 2H), 4.89 (t, J = 5.8 Hz, 1H), 4.77 (t, J = 5.8 Hz, 1H), 3.74 (s, 3H), 3.35 (t, J = 5.8 Hz, 1H), 3.28 (t, J = 5.8 Hz, 1H). mp: 173.2—172.7 °C. Anal. (C27H23FN4O2·2H2C2O4) C, H, N: Calcd C: 59.45, H: 4.99, N: 11.00. Found C: 58.85, H: 4.45, N: 11.05.

4-(2-Fluoroethoxy)methyl-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-5-yl)phenyl) methyl)quinoline (19e). Compound 16e with 17b (in place of 17a) were used as the starting materials following the procedure described above for the preparation of 18b to synthesize 19e as a white solid (62 mg, 69%).1 H NMR (CDCl3): δ 8.39—8.38 (m, 2H), 8.14—8.12 (m, 1H), 8.02—8.00 (m, 1H), 7.82 (s, 2H), 7.77—7.74 (m, 1H), 7.62—7.58 (m, 2H), 7.32—7.23 (m, 2H), 7.18—7.16 (m, 2H), 7.05—7.04 (m, 2H), 5.43 (s, 2H), 5.12 (s, 2H), 4.73—4.71 (m, 2H), 4.61—4.59 (m, 1H), 3.94—3.92 (m, 1H), 3.87—3.85 (m, 1H), 3.75 (s, 3H). mp: 121.3—121.8 °C. Anal. (C29H24FN7O2·2H2C2O4) C, H, N: Calcd C: 59.26, H: 4.51, N: 8.64. Found C: 59.43, H: 4.31, N: 8.51.

4-(2-Fluoroethoxy)ethoxy)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-5-yl)phenyl)methyl)quinoline (19f). Compound 16f with 17b (in place of 17a) were used as the starting materials following the procedure described above for the preparation of 18b to synthesize 19f as a white solid (58 mg, 62%).1 H NMR (CDCl3): δ 8.39—8.38 (m, 2H), 8.24 (d, J = 4.0 Hz, 1H), 8.01 (d, J = 4.3 Hz, 1H), 7.81 (s, 1H), 7.73 (m, 1H), 7.52 (m, 1H), 7.26—7.04 (m, 7H), 5.38 (s, 2H), 4.69 (m, 1H), 4.53 (m, 1H), 4.41 (m, 2H), 4.06 (m, 2H), 3.94—3.93 (m, 1H), 3.85—3.83 (m, 1H), 3.75 (m, 3H). mp: 109.6—109.7 °C. Anal. (C28H25FN4O4·2H2C2O4) C, H, N: Calcd C: 58.77, H: 4.45, N: 11.05. Found C: 58.53, H: 4.71, N: 11.22.

4-(2-Fluoroethyl)-1H-1,2,3-triazol-1-yl)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-5-yl)phenoxymethyl)quinoline (19j). Compound 16j with 17b (in place of 17a) were used as the starting materials following the procedure described above for the preparation of 18b to synthesize 19j as a light yellow solid (62 mg, 67%).1 H NMR (CDCl3): δ 8.38—8.36 (m, 2H), 7.99—7.97 (m, 2H), 7.87—7.84 (m, 2H), 7.80 (s, 1H), 7.68—7.64 (m, 1H), 7.26—7.23 (m, 2H), 7.16—7.14 (m, 2H), 7.04—7.02 (m, 2H), 5.46 (s, 2H), 4.89 (t, J = 5.8 Hz, 1H), 4.77 (t, J = 5.8 Hz, 1H), 3.74 (s, 3H), 3.35 (s, J = 5.8 Hz, 1H), 2.98 (s, 1H). mp: 96.8. Found C: 58.85, H: 4.45, N: 11.05.

General Radiochemistry. We used both one- and two-step approaches for radiolabeling; methods for each are described below. 18F-Fluoride is produced by a18O(p, n)18F reaction through proton irradiation of enriched18O water (95%) using Washington University's RDS-111 cyclotron (Siemens/CTI Molecular Imaging, Knoxville, TN, 2015, 58, 8584—8600.
USA). [\(^{18}\text{F}\)]Fluoride is first passed through an ion-exchange resin then eluted with 0.02 M potassium carbonate (K\(_2\)CO\(_3\)) solution.

Following the one- or two-step incorporation of [\(^{18}\text{F}\)]fluoride, the reaction mixture was purified using a semipreparative HPLC system which contains a 5 mL injection loop, a semipreparative column, UV detector at 254 nm, and an in-line radioactivity detector which is used to identify the desired product for collection in a vial containing 50 mL sterile water. The diluted eluent was trapped on a C-18 Plus Sep-Pak cartridge, which was then rinsed with 10 mL sterile water; the product was eluted with 0.6 mL ethanol followed by 5.4 mL saline. After the diluted radiotracer was sterile-filtered through a 0.22 mm syringe filter into a sterile glass vial, an aliquot was removed for QC testing. The total synthesis time was ∼2 h for one-step labeling and ∼3 h for two-step labeling. Quality control was performed on an analytical HPLC system with UV detection at 254 nm. The radioactive dose was authenticated by coinjection with the nonradioactive reference compound.

**Synthesis of [\(^{18}\text{F}\)]18a: A One-Step General Procedure for Radiosynthesis of [\(^{18}\text{F}\)]18a, [\(^{18}\text{F}\)]18b, [\(^{18}\text{F}\)]18c, [\(^{18}\text{F}\)]18e, and [\(^{18}\text{F}\)]18g.** 4-(Fluoroethyl)-2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline ([\(^{18}\text{F}\)]18a). A sample of ∼74 GBq [\(^{18}\text{F}\)]fluoride/K\(_2\)CO\(_3\) was added to a reaction vessel containing Kryptofix [222] (6.5–7.0 mg), and dried by azotropic distillation at 110 °C using MeCN (3 × 1 mL) under a gentle flow of nitrogen. 1.0–3.0 mg of the bromide precursor was then dissolved in MeCN (200 µL) and was then transferred to the reaction vessel which was capped, vortexed, and heated in an oil bath at 170–175 °C for 20 min. After the heat source was removed, the reaction mixture was diluted with 3.0 mL of HPLC mobile phase and purified using an Agilent SB-C18 column with MeCN/0.1 M ammonium formate buffer pH 4.5 (48/52, v/v) as the mobile phase at a flow rate of 4.0 mL/min; the retention time for the desired product was 19–21 min. QC was performed on a Grace Alltima C-18 column (250 mm × 4.6 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (62/38, v/v) at 1.8 mL/min. The retention time of [\(^{18}\text{F}\)]18a was 5.1 min. The radiochemical purity was >99%, the chemical purity was >95%, and the yield was 10.1 µmol (decay-corrected to EOS).

4-Fluoroethyl methyl (2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline ([\(^{18}\text{F}\)]18b). The chloride precursor 23b was used for the one-step radio labeling of [\(^{18}\text{F}\)]18b as described above for [\(^{18}\text{F}\)]18a. The reaction temperature was 105–110 °C for 20 min. The reaction mixture was purified using an Agilent SB-C18 column with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (40/60, v/v) at 4.0 mL/min; the retention time for the desired product was 24–26 min. QC was performed using a Phenomenex Prodigy C-18 column (250 mm × 4.6 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (62/38, v/v) at 1.5 mL/min. The retention time of [\(^{18}\text{F}\)]18b was 4.9 min. The radiochemical purity was >99%, the chemical purity was >95%, the yield was 1.1–1.5% (decay-corrected), and the SA was >74 GBq/µmol (decay-corrected to EOS).

4-(3-(Fluoroethyl)propyl)-2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline ([\(^{18}\text{F}\)]18c). The mesylate precursor 23c was used for the one-step radio labeling of [\(^{18}\text{F}\)]18c as described above for [\(^{18}\text{F}\)]18a. The reaction temperature was 75–80 °C for 20 min. The reaction mixture was purified using an Agilent SB-C18 column with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (50/50, v/v) at 4.0 mL/min; the retention time for the desired product was 17–18 min. QC was performed using an Agilent SB-C18 column (250 mm × 4.6 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (65/35, v/v) at 1.5 mL/min. The retention time of [\(^{18}\text{F}\)]18c was 4.9 min. The radiochemical purity was >99%, the yield was 10.1–12.2% (decay-corrected) and the SA was >74 GBq/µmol (decay-corrected to EOS).

4-Fluoroethyl methyl (2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline ([\(^{18}\text{F}\)]18d). A sample of ∼74 GBq [\(^{18}\text{F}\)]fluoride/Kryptofix [222]/K\(_2\)CO\(_3\) which was capped, mixed, and heated in an oil bath at 170–175 °C for 20 min. After the heat source was removed, the reaction mixture was diluted with 3.0 mL of HPLC mobile phase and passed through an alumina Neutral Sep-Pak Plus cartridge. The reaction mixture was then purified using an Agilent SB-C18 column (250 mm × 10 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 6.5 (50/50, v/v) at 4.0 mL/min; the retention time of [\(^{18}\text{F}\)]fluoroethyl tosylate product was 9.5–10 min. The retention time of the precursor was 23–24 min. The product was diluted with 50 mL sterile water then trapped on a C-18 Sep-Pak Plus cartridge. The trapped product was eluted with diethyl ether (2.5 mL).

The eluted solution formed two phases, the top organic phase was removed and reserved, and the bottom aqueous phase was extracted with 1 mL of ether. The ether extracts were collected and washed through two Sep-Pak Plus dry cartridges in series into a reaction vessel; the ether was evaporated under a stream of nitrogen. 1.2 mg of phenol precursor 24a was then dissolved in 200 µL DMSO and transferred to a vial containing 2.0 mg Cs\(_2\)CO\(_3\) and vortexed for 1 min; this saturated Cs\(_2\)CO\(_3\) solution was added to the reaction vessel which was capped, mixed, and heated in an oil bath at 85–90 °C for 15 min. The reaction mixture was diluted with 3.0 mL HPLC mobile phase and purified using an Agilent SB C-18 column with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (40/60, v/v) at 4.0 mL/min; the retention time was 21.4 min. QC was performed using an Agilent Zorbax, SB-C18 column (250 mm × 4.6 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (62/38, v/v) at 1.8 mL/min. The retention time of [\(^{18}\text{F}\)]20a was 4.8 min. The radiochemical purity was >99%, the chemical purity was >95%, the yield was 65–72% (decay-corrected), and the SA was >74 GBq/µmol (decay-corrected to EOS).

4-(2-Fluoroethyl)oxy)-2-(4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline ([\(^{18}\text{F}\)]18d). The phenol precursor 23d was used for the two-step labeling of [\(^{18}\text{F}\)]18d as described above for [\(^{18}\text{F}\)]20a. The reaction temperature was 95–100 °C for 15 min. The reaction mixture was purified using an Agilent SB-C18 column with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (45/55, v/v) at 4.0 mL/min; the retention time was 16.2 min. QC was performed using an Agilent Zorbax, SB-C18 column (250 mm × 4.6 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (60/40, v/v) at 4.0 mL/min; the retention time was 21.8 min. QC was performed using a Grace Altima-C18 column (250 mm × 4.6 mm) with a mobile phase of MeCN/0.1 M ammonium formate buffer pH 4.5 (62/38, v/v) at 1.8 mL/min. The retention time of [\(^{18}\text{F}\)]18e was 4.4 min. The radiochemical purity was >99%, the chemical purity was >95%, the yield was 15–18% (decay-corrected), and the SA was >74 GBq/µmol (decay-corrected to EOS).
pH 4.5 (50/50, v/v) at 1.4 mL/min. The retention time of [18F] was 7.5 min. The radiochemical purity was >99%, the chemical purity was 95%, the yield was 30–35% (decay-corrected) and the SA was >74 GBq/µmol (decay-corrected to EOS).

In Vitro Assay. The PDE10A in vitro screening assay followed our previously published procedures. PDE activity was measured using the Phosphodiesterase [3H]cAMP Scintillation Proximity Assay (SPA) (Cat. #TRKQ72090, PerkinElmer, Waltham, MA) with minor modifications. Recombinant human PDE10A containing catalytic domain of human PDE10A (Swiss-Prot accession number Q9Y233) was purchased from Enzo Life Sciences Inc., Farmingdale, NY. Recombinant human PDE3A and 3B, PDE4A, and PDE4B were purchased from EMD Chemicals, Inc., San Diego, CA. Optimal concentrations of enzymes were tested in the linear range of the enzyme activation curves with substrate. Inhibitor compounds were tested along with compound whose IC_{50} values are known (e.g., MF-10) as an internal control for each PDE assay. All inhibitors were dissolved in DMSO, and serial dilutions were performed to obtain desired concentrations. Diluted compounds (10 µL/well) were added to a 96-well plate, after addition of 50 µL diluted PDE enzyme in buffer the plate was incubated on ice for 5 min. 50 µL of radiolabeled substrate ([3H]cAMP) at a fixed (1/3 of the K_m) concentration of 1.48 kBq/mL was then added to each well and plates were incubated on ice for an additional 20 min to give ~30% substrate turnover before termination of the reaction with 50 µL yttrium silicate SPA beads (8 mg/mL). Plates were allowed to settle for 1.5 h at rt, then counted on a Tri-Carb Microbeta Counter (PerkinElmer, Waltham, MA). CPM values for each well were plotted against inhibitor concentration in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) and inhibitor IC_{50} values were calculated by nonlinear regression with Prism’s one-site receptor competitive binding model.

All compounds were independently assayed at least twice and fitting R^2 values were ≥90%; results are reported as fitted IC_{50} ± SD.

Biodistribution and Regional Brain Uptake in Rats. All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals under protocols approved by the Washington University Animal Studies Committee. For the biodistribution studies, 1.48–2.22 MBq of the radiotracer was injected in the tail vein of mature male Sprague–Dawley rats (250–400 g) under anesthesia (2.5% isoflurane in oxygen). At 5, 30, and 60 min post-injection (p.i.), rats (n = 4 per study group) were anesthetized and euthanized. The whole brain was quickly removed and the hippocampus, striatum, cortex, thalamus, brain stem, and cerebellum were separated by gross dissection. The remainder of the brain was collected to determine total brain uptake. Peripheral tissues including blood, lung, heart, liver, kidney, muscle, fat, and bone were also collected. Samples were weighed and counted in an automated gamma counter (Beckman Gamma 8000 well counter) with a standard of diluted injectate, and the %ID/g was calculated.

MicroPET Brain Imaging Studies in Male Cynomolgus Monkeys. Three independent PET studies were done on adult male cynomolgus macaques (~4–6 kg) for each tracer with a microPET Focus 220 scanner (Concorde/CTI/Siemens Microsystems, Knoxville, TN). The animal was fasted for 12 h before PET study and initially anesthetized with ketamine (10 mg/kg) and glycopyrrolate (0.13 mg/kg) intramuscularly. Upon arrival at the scanner, the animal was intubated and percutaneous catheters were placed for venous tracer injection and arterial blood sampling. The head was positioned supine in the adjustable head holder with the brain in the center of the field of view. Anesthesia was maintained at 0.75–2.0% isoflurane in oxygen and core temperature maintained at ~37 °C. A 10 min transmission scan was performed to confirm positioning; this was followed by a 45 min transmission scan for attenuation correction. Subsequently, a 3 h dynamic emission scan was acquired after venous injection of 185–259 MBq of [18F] or [18F] 20a.

MicroPET Image Processing and Analysis. Acquired list mode data were histogrammed into a 3D set of sinograms and binned to the following time frames: 3 × 1 min, 4 × 2 min, 3 × 3 min, and 20 × 5 min. Sinogram data were corrected for attenuation and scatter. Maximum a posteriori (MAP) reconstructions were done with 18 iterations and a β value of 0.004. A 1.5 mm Gaussian filter was applied to smooth each MAP reconstructed image. These images were then coregistered with MRI images to accurately identify the anatomical regions of interest with Amira software (Visage Imaging, Inc., Carlsbad, CA). The 3D regions of interest were manually drawn through all planes of coregistered MRI images for the caudate, putamen, and cerebellum. The regions of interest were then overlaid on all reconstructed PET images to obtain time-activity curves (TACs). Activity measures were standardized to body weight and dose of radioactivity injected to yield standardized uptake value (SUV). HPLC Metabolite Analysis of NHP Plasma. Arterial blood samples (~1.5 mL) were collected 2, 15, 30, 60, and 90 min p.i. from a percutaneous catheter in a heparinized syringe and 1 mL aliquots were counted in a well counter then centrifuged to separate red blood cells from plasma. Plasma was deproteinized with 2 parts ice-cold methanol and plasma proteins were separated by centrifugation. The solvent extract (200 µL) was injected on an Agilent SB C-18 analytic HPLC column (250 mm × 4.6 mm, 5 µm), with a mobile phase of MeCN/0.1 M ammonium formate buffer, pH 4.5 ([18F]18d, 50/50, v/v; [18F] 20a, 52/48, v/v), flow rate 0.9 mL/min, mass peaks were detected by UV at 254 nm. HPLC fractions were collected at 1 min intervals for 16 min and counted on a well counter and decay-corrected. The percent parent and percent radiolabeled metabolites for [18F]18d and [18F]20a was calculated by dividing the recovered activity in the respective peaks by the sum of the total recovered off the column and multiplying by 100. The parent compound peaks were authenticated by coinjection with the respective nonradioactive standard.

ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01205.

General chemistry methods. Synthesis of intermediate compounds: 6a–j, 9, 11, 12, 14, 15a–d, and 16a–n. Synthesis of radiolabeling precursors 23a–e, 23g and 24a and intermediate compounds (PDF)

Molecular formula strings of final products and IC_{50} values for PDEs (CSV)

AUTHOR INFORMATION

Corresponding Author

E-mail: luz@mir.wustl.edu. Tel: 1-314-362-8487. Fax: 1-314-362-8555.

Notes

The authors declare no competing financial interest.

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cGMP, cyclic guanosine monophosphate; DAST, diethylaminoethyl chloride; N,N-diisopropylleucine; DMC, dimethyl carbonate; EOS, end of synthesis; EOH, ethanol; EtOAe, ethyl acetate; FPEG, fluoroPEGylated; MeCN, acetonitrile; MSNs, medium spiny neurons; MOM-br, bromo(methoxy)methane; MP-10, 2-((1-(4-picolin-4-yl)-1H-pyrrol-3-yl)[1H-pyranyl]- methyl)quinoline; NHP, nonhuman primate; phenyl triflimide, N-phenyl-bis(trifluoromethane sulfonamide); PDE3A, phosphodiesterase 3A; PDE3B, phosphodiesterase 3B; PDE4A, phosphodiesterase 4A; PDE4B, phosphodiesterase 4B; PDE10A, phosphodiesterase 10A; p.i, postinjection; QC, quality control; RCY, radiocadiochemical yield; ROI, region of interest; SA, specific activity; TBSCI, tert-butyldimethylsilyl chloride; TEA, triethylamine

REFERENCES


