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Synthesis and in Vitro Evaluation of New Analogues as Inhibitors for Phosphodiesterase 10A

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Abstract

A series of analogues were synthesized by optimizing the structure of papaverine. The *in vitro* PDE10A binding affinity (IC_{50}) values for these new analogues were measured; for compounds that have IC_{50} value less than 60 nM for PDE10A, the binding affinities (IC_{50} value) for PDE3A and PDE3B were tested. Of these analogues, compounds **6a**, **6b**, **6n**, **8b**, **8c** and **11** displayed relatively higher PDE10A potency with IC_{50} value in the range of 28–60 nM. The most potent compound 1-(4-(2-(2-fluoroethoxy)ethoxy)-3-methoxybenzyl)-6,7-dimethoxyisoquinoline (**8c**) has the IC_{50} value of 28 ± 1.2 nM for PDE10A, 2200 ± 437 nM for PDE3A and 2520 ± 210 nM for PDE3B. Compared to papavarine, compound **8c** displayed similar PDE10A potency but improved selectivity to PDE10A versus PDE3A and PDE3B. To identify high potent PDE10A inhibitor, further optimization of the structures of these analogues is necessary.

Keywords

PDE10A; CNS; Schizophrenia

1. Introduction

Abnormal striatal output is caused by the dysfunction of striatopallidal transmission, which is the major contributor to the pathophysiology of central nervous system (CNS) disorders including schizophrenia, Parkinson's disease, Huntington's disease, Torrette's syndrome, and drug abuse [1–4]. The cyclic nucleotide phosphodiesterase enzymes have 11 gene families that contain 21 phosphodiesterase genes. The cyclic nucleotide phosphodiesterase 10A (PDE10A) is one of the 11 gene families and is highly expressed in the primary input neurons of the basal ganglia circuit, which are striatal medium-sized spiny neurons (MSNs) [5]. The inhibition of PDE10A alternates the basal ganglia circuit output and increases striatopallidal MSN activation, which represents a new therapeutic approach for the treatment of some of CNS diseases [5–9].

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PDEs regulate levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by hydrolysis of the cyclic nucleotide into their respective nucleotide monophosphates. PDE10A is a unique dual specificity phosphodiesterase that can convert both cAMP to adenosine monophosphate (AMP) and cGMP to guanosine monophosphate (GMP) [10–12].

Across species, compared to other PDE families, the PDE10A protein has a high degree of homology and is uniquely localized in mammals. PDE10 enzyme is expressed only in the testis and the brain [10–12]. Within the brain, the expression of the PDE10A enzyme is the highest in striatum (caudate and putamen), nucleus accumbens, and the olfactory tubercle of MSNs [10, 11, 13, 14]. Based on the fact that PDE10A expression parallels D₂ localization in the brain, inhibition of PDE10A may have similar functional effects as D₂ inhibition. The inhibition of PDE10A may mediate the clinical antipsychotic effects. However, there are distinct differences between PDE10A inhibition and classical antagonists of D₂ receptors. The D₂ receptor has signaling components besides cAMP [15] and the inhibition of PDE10A interferes with cAMP levels that may negatively modulate rather than directly antagonize dopamine signaling. The inhibition of PDE10A may avoid the limitations that are observed with strong D₂ antagonism to treat schizophrenia and other CNS diseases. Furthermore, PDE10A is also expressed in D₁ expressing striatal neurons [14]. The activation of the D₁ receptor leads to the stimulation of adenylate cyclases, which results in the increase of cAMP levels. Therefore, the inhibition of PDE10A is likely to have effects that simulate D₁ receptor agonism. Together, PDE10A inhibition will increase cAMP in cells and can be expected to increase cGMP levels. cGMP activates a number of target proteins in the cells like cAMP and it also interacts with the cAMP signaling pathways. Consequentially, inhibition of PDE10A to elevate cAMP and cGMP could be a novel therapeutic strategy for the treatment of associated neurological and psychiatric disorders and may avoid unwanted side effects such as extrapyramidal symptoms associated with conventional therapeutics.

Over the past 10 years, tremendous efforts have been made to develop PDE10A inhibitors for the treatment of schizophrenia and associated mental disorders [16–20]. The compounds in Figure 1 have attracted more attention [18, 19, 21]. Although papaverine has only moderate potency (IC₅₀ = 36 nM) and poor selectivity (9 fold) over other PDE isoforms, papaverine was the first compound used to explore the role of PDE10A in the CNS. It was reported that papaverine potentiated the cataleptic effect of the D₂ receptor antagonist haloperidol in rats, but did not cause catalepsy on its own. Papaverine as well as more potent and more selective PDE 10A inhibitors PQ-10, MP-10 and TP-10, were all able to reduce hyperactivity in rats induced by phenylcyclohexylpiperidine (PCP) and amphetamine [8]. Furthermore, PDE10A inhibition with papaverine reverses subchronic PCP-induced deficits in attention set-shifting in rats [22]. Papaverine has also shown efficacy in rat novel object recognition [23]. Together, these data suggest that PDE10A inhibition might alleviate cognitive deficits associated with schizophrenia and psychosis.

In this paper, we will report the synthesis and biological binding affinity measurement of the new analogues as PDE10A inhibitors based on the modification of the molecular structure of papaverine. Our investigation was inspired by (1) papaverine has PDE10A inhibition function with moderate potency for PDE10A and low selectivity; (2) the rapid *in vivo* metabolism of papaverine limits it serving as a PET ligand for imaging in PDE10A *in vivo* [24]; and (3) the demand of a suitable PET probe for imaging the PDE10A enzyme *in vivo*.

2. Results and discussion

2.1. Chemistry

Papaverine, 1-(3,4-dimethoxybenzyl-6,7-dimethoxy)isoquinoline, which possesses four methoxy groups in its molecular structure, is now known to be a potent inhibitor of phosphodiesterase 10A (PDE10A) and has been reported to increase cognitive performance in the rat model of schizophrenia. Radioactive [³H]papaverine, [¹⁴C]papaverine and [¹¹C]papaverine have successfully been made to conduct pharmacologic studies. To develop the F-18 labeled PDE10A PET tracer, we synthesized a series of analogues by replacing the methoxy group(s) with fluoroethoxyl group or other groups.

The synthesis of the target analogues **6a–n**, **8a–c** and **11** was shown in Scheme 1–3. Substituted benzaldehydes **1a** and **1b** were condensed with nitromethane to generate 2-phenyl-1-nitroethene derivatives **2a** and **2b** [25], which reacted with sodium methoxide to give (1-methoxy-2-nitroethyl)benzene derivatives **3a** and **3b** [26]. It was found that the conversion of compounds **2a** and **2b** to **3a** and **3b** was complete in just 10 min when one equivalent of compound **2** was added into two equivalents of sodium methoxide solution in methanol. Reduction of the nitro group of **3a** and **3b** with LiAlH₄ in THF gave amines **4a** and **4b** [26]. Due to the higher hydrophilicity of **4a** and **4b**, the extraction of **4a** and **4b** with organic solvent from their aqueous solution was very difficult. To resolve this problem, the excess lithium aluminum hydride was decomposed by carefully adding a very small amount of water followed by filtering the reaction mixture through celite to remove the precipitate, which was washed with THF. The THF was directly evaporated under vacuum without washing with water. When this procedure was followed, the yields of **4a** and **4b** were improved from 40% to 90%. Amines **4a** and **4b** were coupled with the corresponding substituted benzyl carboxylic acid using 1,1'-carbonyldiimidazole as a coupling agent to give amide **5a–m** and **5o**. Commercially available 2-amino-1-(3,4-dimethylphenyl)ethanol reacted with 2-substituted acetyl chloride to afford intermediate compounds **5n** and **5p**. Cyclization reaction of **5a–p** with POCl₃ in acetonitrile afforded compounds **6a–p**. Compounds **6a–n** were converted into oxalic salts for in vitro affinity measurements. Based on our experiments, the yield of the cyclization reaction was very good when the reaction time was limited to 30 min, otherwise the yield was much lower. Hydrogenolysis to remove the benzyl group in **6o** and **6p** afforded phenol intermediates **7a** and **7b** which were reacted with 2-bromo-1-fluoroethane or 1-chloro-2-(2-fluoroethoxy)ethane via *O*-alkylation to afford compounds **8a–c**. Compounds **8a–c** were converted into oxalic salts for determining their binding affinities. To test whether replacing the isoquinoline ring with a quinazoline ring increases the binding affinity of PDE10A, compound **7** was synthesized according to Scheme 3. Starting with 4-chloro-6,7-dimethoxyquinazoline, **9** was treated with (4-methoxybenzyl) magnesium chloride, **10** in the presence of Manganese (II) chloride in THF to afford compound **11** which was converted into its oxalate.

2.2. In vitro assessment of affinities

The binding affinities of PDE10A for the newly synthesized analogues were first determined by measuring IC₅₀ values. It was reported that papaverine was not only a relatively potent inhibitor of PDE10A, but also exhibited moderate cross-reactivity versus PDE3A and PDE3B (*K_i* as 279, and 417 nM, respectively) [19]. The use of PDE3A/B inhibitor could lead to arrhythmia and increased mortality [27, 28]. Therefore, compounds that had IC₅₀ value less than 60 nM for PDE10A, were further determined by their binding affinities for PDE3A/B. The PDE activities were measured according to the procedure described in the experimental section. The binding affinities of the analogues were shown in Table 1.

To develop fluorine analogues, we took the following approach. First, we replaced the 6-methoxy in papaverine with a 6-fluoroethoxy group and replaced the 3-methoxy with a fluorine or methyl group. It was found that compounds **6a** and **6b** have a modest affinity for PDE10A (IC_{50} value for **6a** was 49 nM and for **6b** was 58 nM). However, after replacing the 4-methoxy with a 4-methylthio group (**6c**), the IC_{50} value for PDE10A dropped to 112 nM. Second, when the 6-methoxy was retained and the 7-methoxy was replaced with a 7-fluoroethoxy, compounds **6d–m** and **8a** were generated. Compared to **6a–c** and **8b**, the PDE10A binding affinities for **6d** vs. **6a**, **6e** vs. **6b**, **8a** vs. **8b** were reduced by 5 fold; for **6f** vs. **6c**, it was reduced by approximately 2 fold. The binding affinities were 49 ± 1.1 , 58.5 ± 1.1 , 112 ± 1.2 and 55 ± 1.2 nM for **6a**, **6b**, **6c** and **8b**, 198 ± 1.1 , 244 ± 1.1 , 237 ± 1.1 and 246 ± 1.1 nM for **6d**, **6e**, **6f** and **8a** respectively. Similar or even greater reduction in the PDE10A binding affinities was found for analogues **6g–m** and **8a**. Third, we retained both the 6 and 7-methoxy groups and replaced the 3-methoxy or 4-methoxy on the substituted benzyl group and found that both the **6n** and **8b** displayed modest affinities for PDE10A (IC_{50} value as 55 ± 1.0 nM for **6n** and 55 ± 1.2 nM for **8b**). Extending the 4-fluoroethoxy group to a 4-(2-(2-fluoroethoxy))ethoxy group increased PDE10A affinity by 2-fold, from 55 ± 1.2 nM for **8b** to 28 ± 1.2 nM for **8c**). Fourth, we replaced 6,7-dimethoxyisoquinoline with 6,7-dimethoxy quinazoline, while removing the 3-methoxy group, which generated **11** that had a modest PDE10A affinity of $IC_{50} = 48.8 \pm 1.07$ nM.

3. Conclusions

In the current study, the papaverine structure was modified by incorporating fluorine containing substituted group(s) and replacing the isoquinoline ring with a quinazoline ring. The *in vitro* PDE10A binding affinity results of these compounds indicated that the compound **8c**, 1-(4-(2-(2-fluoroethoxy)ethoxy)-3-methoxybenzyl)-6,7-dimethoxyisoquinoline has a comparable binding affinity for PDE10A to that of papaverine. Compared to papaverine, compound **8c** has a much lower binding affinity for PDE3A (2200 nM for **8c** vs 279 nM for papaverine) and a lower binding affinity for PDE3B (2517 nM for **8c** vs 417 nM for papaverine) than that of papaverine [19]. Compound **8c** is a fluorine containing compound that can take advantage of the isotopic properties of F-18 (half-life is 110 min, widely commercial availability) compared to that of C-11 (half-life of 20 min; C-11 tracers have to be made in-house at a cyclotron facility). Fluorine-18 labeled probes are more suitable for conducting the imaging studies of PDE10A in living animals. The radioactive [^{18}F]**8c** will be radiosynthesized for *in vivo* evaluation to test its potential as a PET tracer for imaging the PDE10A *in vivo*.

4. Experimental

4.1 Chemistry

1H NMR spectra were recorded on a Varian 300 MHz NMR spectrometer. Chemical shifts are reported in δ values with respect to tetramethylsilane (TMS) as an internal reference standard. The following abbreviations are used for multiplicity of NMR signals: br s = broad singlet, s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplet, t = triplet, m = multiplet, q = quartet. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, and were within $\pm 0.4\%$ of the calculated values. Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant No. P41RR0954). All reactions were carried out under an inert atmosphere of nitrogen.

Procedure A: synthesis of compounds 1a and 1b

4-(2-Fluoroethoxy)-3-methoxybenzaldehyde (1a): Potassium carbonate (30.0 g, 0.22 mol) was added to a solution of 4-hydroxy-3-methoxybenzaldehyde (15.2 g, 0.1 mol) and 2-bromo-1-fluoroethane (25.4 g, 0.2 mol) in acetone (100 mL). The reaction mixture was refluxed overnight until the reaction was complete as determined by thin layer chromatography (TLC). After the solvent was removed, 200 mL of water was added to the flask, and the mixture was extracted with ethyl acetate (50 mL \times 3). The combined organic layers were dried over anhydrous sodium sulfate. Filtered and concentrated, the crude product was purified by silica gel column chromatography with EtOAc/MeOH (95/5, v/v) as mobile phase to give **1a** (15.3 g, 77%) as a pale yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 3.94 (s, 3 H), 4.37 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.83 (dt, $J = 47.4, 4.2$ Hz, 2 H), 7.01 (d, $J = 8.1$ Hz, 1 H), 7.44–7.47 (m, 2 H), 9.87 (s, 1 H).

3-(2-Fluoroethoxy)-4-methoxybenzaldehyde (1b): Starting with 3-hydroxy-4-methoxybenzaldehyde, Procedure A was followed to afford compound **1b** (5.9 g, 80%) as a pale yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 3.97 (s, 3 H), 4.34 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.83 (dt, $J = 47.4, 4.2$ Hz, 2 H), 7.01 (d, $J = 8.4$ Hz, 1 H), 7.43 (d, $J = 2.1$ Hz, 1 H), 7.51 (dd, $J = 8.4, 2.1$ Hz, 1 H), 9.86 (s, 1 H).

Procedure B: synthesis of compounds 2a and 2b

(E)-1-(2-Fluoroethoxy)-2-methoxy-4-(2-nitrovinyl)benzene (2a): A stirred mixture of **1a** (12.5 g, 63.1 mmol), nitromethane (12.0 mL, 221.7 mmol) and anhydrous ammonium acetate (12.0 g, 155.8 mmol) in glacial acetic acid (28 mL) was refluxed for 30 min. After cooling to room temperature, the reaction mixture was poured into water (200 mL). The solid was collected by filtration to give **2a** (13.0 g, 85%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 3.93 (s, 3 H), 4.34 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.82 (dt, $J = 47.4, 4.2$ Hz, 2 H), 6.94 (d, $J = 8.1$ Hz, 1 H), 7.03 (d, $J = 2.1$ Hz, 1 H), 7.15 (dd, $J = 8.1, 2.1$ Hz, 1 H), 7.53 (d, $J = 13.8$ Hz, 1 H), 7.96 (d, $J = 13.8$ Hz, 1 H).

(E)-2-(2-fluoroethoxy)-1-methoxy-4-(2-nitrovinyl)benzene (2b): Starting with compound **1b**, procedure B was followed to afford compound **2b** (7.8 g, 78%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 3.97 (s, 3 H), 4.34 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.83 (dt, $J = 47.4, 4.2$ Hz, 2 H), 7.01–7.13 (m, 2 H), 7.51 (d, $J = 8.4$ Hz, 1 H), 7.56 (d, $J = 13.5$ Hz, 1 H), 7.90 (d, $J = 13.5$ Hz, 1 H).

Procedure C: synthesis of compounds 3a and 3b

1-(2-Fluoroethoxy)-2-methoxy-4-(1-methoxy-2-nitroethyl)benzene (3a): A solution of **2a** (3.3 g, 13.7 mmol) in dry THF (50 mL) was added in one portion to a stirred solution of sodium methoxide (25% in MeOH, 9 mL) at room temperature. The resulting mixture was stirred for 5 min and poured into water. The crude product was collected by filtration to give **3a** (2.6 g, 70%) as a pale yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 3.27 (s, 3H), 3.89 (s, 3H), 4.29 (dt, $J = 27.3, 3.9$ Hz, 2 H), 4.38 (dd, $J = 12.6, 3.6$ Hz, 1 H), 4.61 (dd, $J = 12.3, 9.9$ Hz, 1 H), 4.70–4.92 (m, 3 H), 6.88–6.95 (m, 3 H).

2-(2-Fluoroethoxy)-1-methoxy-4-(1-methoxy-2-nitroethyl)benzene (3b): Starting with **2b**, procedure C was followed to afford compound **3b** (7.3 g, 78%) as a pale yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 3.26 (s, 3 H), 3.88 (s, 3 H), 4.24–4.40 (m, 3 H), 4.60 (dd, $J = 10.8, 9.9$ Hz, 1 H), 4.71–4.91 (m, 3 H), 6.90–6.97 (m, 3 H).

Procedure D: synthesis of compounds 4a and 4b

2-(4-(2-Fluoroethoxy)-3-methoxyphenyl)-2-methoxyethanamine (4a): A solution of **3a** (2.6 g, 9.5 mmol) in THF (45 mL) was added to lithium aluminum hydride (1.1 g, 29.1 mmol) in dry THF (15 mL) dropwise. The reaction mixture was refluxed for 6–8 h. The excess lithium aluminum hydride was decomposed by carefully adding a small amount of water. The suspension was filtered through (Celite) and washed with THF. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography with CH₂Cl₂/MeOH/Et₃N (95/5/1, v/v/v) as mobile phase to give **4a** (1.53 g, 66%) as a pale yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 2.81(dd, *J* = 13.6, 4.8 Hz, 1 H), 2.92 (dd, *J* = 13.2, 7.5 Hz, 1 H), 3.27 (s, 3 H), 4.08 (dd, *J* = 7.5, 4.2 Hz, 1 H), 3.88 (s, 3H), 4.28 (dt, *J* = 27.3, 4.5 Hz, 2 H), 4.78 (dt, *J* = 47.4, 4.5 Hz, 2 H), 6.83–6.91 (m, 3 H).

2-(3-(2-Fluoroethoxy)-4-methoxyphenyl)-2-methoxyethanamine (4b): Starting with **3b**, procedure D was followed to afford compound **4b** (1.90 g, 82%). ¹H NMR (300 MHz, CDCl₃) δ 2.81(dd, *J* = 13.2, 4.2 Hz, 1 H), 2.90 (dd, *J* = 13.2, 7.5 Hz, 1 H), 3.25 (s, 3 H), 3.88 (s, 3 H), 4.06 (dd, *J* = 7.5, 4.2 Hz, 1 H), 4.28 (dt, *J* = 27.9, 3.9 Hz, 2 H), 4.78 (dt, *J* = 47.7, 3.9 Hz, 2 H), 6.87 (s, 3 H).

Procedure E: synthesis of compounds 5a–o

2-(3-Fluoro-4-methoxyphenyl)-N-(2-(3-(2-fluoroethoxy)-4-methoxyphenyl)-2-methoxyethyl)acetamide (5a): In a typical conversion, 1,1'-Carbonyldiimidazole (CDI, 0.32 g, 2 mmol) was added to a solution of 2-(4-fluoro-3-methoxyphenyl)acetic acid (0.37 g, 2 mmol) in 20 mL of dichloromethane, and the mixture was stirred at room temperature for 2 h, at which point, 2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethanamine **4a** (0.48 g, 2.0 mmol) was added and the mixture was stirred overnight. The reaction mixture was washed with saturated sodium carbonate solution, dried over anhydrous sodium sulfate. After concentrating, the residue **5a** was used in the next step without further purification. ¹H NMR (300 MHz, CDCl₃) δ 3.17–3.26 (m, 4 H), 3.47 (s, 2 H), 3.59 (m, 1 H), 3.87 (s, 3 H), 3.89 (s, 3 H), 4.13 (dd, *J* = 8.4, 4.5 Hz, 1 H), 4.26 (dt, *J* = 27.9, 4.2 Hz, 2 H), 4.77 (dt, *J* = 47.4, 4.2 Hz, 2 H), 5.74 (br, 1 H), 6.78–6.99 (m, 6 H).

N-(2-(3-(2-Fluoroethoxy)-4-methoxyphenyl)-2-methoxyethyl)-2-(4-methoxy-3-methylphenyl)acetamide (5b): Procedure E was followed to prepare compound **5b**. ¹H NMR (300 MHz, CDCl₃) δ 2.20 (s, 3 H), 3.1–3.22 (m, 4 H), 3.47 (s, 2 H), 3.58 (m, 1 H), 3.83 (s, 3 H), 3.86 (s, 3 H), 4.12 (dd, *J* = 8.4, 4.5 Hz, 1 H), 4.24 (dt, *J* = 27.3, 4.5 Hz, 2 H), 4.77 (dd, *J* = 47.4, 4.5 Hz, 2 H), 5.77 (br, 1 H), 6.70–7.01 (m, 6 H).

N-(2-(3-(2-Fluoroethoxy)-4-methoxyphenyl)-2-methoxyethyl)-2-(4-(methylthio)phenyl)acetamide (5c): Procedure E was followed to prepare compound **5c**. ¹H NMR (300 MHz, CDCl₃) δ 2.49 (s, 3 H), 3.15–3.23 (m, 4 H), 3.51 (s, 2 H), 3.59 (m, 1 H), 3.87 (s, 3 H), 4.11 (m, 1 H), 4.24 (dt, *J* = 27.6, 4.5 Hz, 2 H), 4.77 (dt, *J* = 47.1, 4.5 Hz, 2 H), 5.74 (br, 1 H), 6.73–6.85 (m, 3 H), 7.13–7.25 (m, 4 H).

2-(3-Fluoro-4-methoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)acetamide (5d): Procedure E was followed to prepare compound **5d**. ¹H NMR (300 MHz, CDCl₃) δ 3.20–3.28 (m, 4 H), 3.49 (s, 2 H), 3.62 (m, 1 H), 3.85 (s, 3 H), 3.89 (s, 3 H), 4.13 (m, 1 H), 4.27 (dt, *J* = 27.3, 4.2 Hz, 2 H), 4.79 (dt, *J* = 47.4, 4.2 Hz, 2 H), 5.77 (br, 1 H), 6.72–6.94 (m, 6 H).

N-(2-(4-(2-Fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)-2-(4-methoxy-3-methylphenyl)acetamide (5e): Procedure E was followed to prepare compound **5e**. ¹H NMR (300 MHz, CDCl₃) δ 2.21 (s, 3 H), 3.15–3.23 (m, 4 H), 3.48 (s, 2 H), 3.59 (m, 1 H),

3.83 (s, 3 H), 3.84 (s, 3 H), 4.13 (m, 1 H), 4.26 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.78 (dt, $J = 47.4, 4.2$ Hz, 2 H), 5.78 (br, 1 H), 6.71–6.86 (m, 4 H), 6.98–7.01 (m, 2 H).

N-(2-(4-(2-Fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)-2-(4-(methylthio)phenyl)acetamide (5f): Procedure E was followed to afford compound **5f**. ^1H NMR (300 MHz, CDCl_3) δ 2.52 (s, 3 H), 3.21–3.27 (m, 4 H), 3.55 (s, 2 H), 3.63 (m, 1 H), 3.87 (s, 3 H), 4.13 (m, 1 H), 4.30 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.71 (dt, $J = 47.4, 4.2$ Hz, 2 H), 5.77 (br, 1 H), 6.73–6.86 (m, 3 H), 6.89–7.26 (m, 4 H).

2-(2-Fluoro-4-methoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)acetamide (5g): Procedure E was followed to prepare compound **5g**. ^1H NMR (300 MHz, CDCl_3) δ 3.14–3.26 (m, 4 H), 3.51 (s, 2 H), 3.62 (m, 1 H), 3.80 (s, 3 H), 3.85 (s, 3 H), 4.15 (m, 1 H), 4.26 (dt, $J = 27.6, 4.2$ Hz, 2 H), 4.78 (dt, $J = 47.1, 4.2$ Hz, 2 H), 5.85 (br, 1 H), 6.62–6.87 (m, 5 H), 7.15 (m, 1 H).

2-(5-Bromo-2-methoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)acetamide (5h): Procedure E was followed to prepare compound **5h**. ^1H NMR (300 MHz, CDCl_3) δ 3.04–3.13 (m, 4 H), 3.43 (s, 2 H), 3.55 (m, 1 H), 3.74 (s, 3 H), 3.76 (s, 3 H), 4.03 (m, 1 H), 4.24 (dt, $J = 27.9, 4.2$ Hz, 2 H), 4.70 (dt, $J = 47.4, 4.2$ Hz, 2 H), 6.06 (br, 1 H), 6.63–6.79 (m, 4 H), 7.19 (s, 1 H), 7.30 (m, 1 H).

2-(3-Bromo-4-methoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)acetamide (5i): Procedure E was followed to prepare compound **5i**. ^1H NMR (300 MHz, CDCl_3) δ 3.16–3.27 (m, 4 H), 3.47 (s, 2 H), 3.61 (m, 1 H), 3.85 (s, 3 H), 3.89 (s, 3 H), 4.15 (m, 1 H), 4.27 (dd, $J = 27.3, 4.2$ Hz, 2 H), 4.78 (dd, $J = 47.1, 4.2$ Hz, 2 H), 5.75 (br, 1 H), 6.70–6.88 (m, 4 H), 7.15 (m, 1 H), 7.43 (s, 1 H).

N-(2-(4-(2-Fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)-2-(3-fluorophenyl)acetamide (5j): Procedure E was followed to prepare compound **5j**. ^1H NMR (300 MHz, CDCl_3) δ 3.14–3.26 (m, 4 H), 3.56 (s, 2 H), 3.64 (m, 1 H), 3.85 (s, 3 H), 4.15 (m, 1 H), 4.27 (dt, $J = 27.6, 4.5$ Hz, 2 H), 4.70 (dt, $J = 47.1, 4.5$ Hz, 2 H), 5.80 (br, 1 H), 6.73–6.80 (m, 3 H), 6.96–7.04 (m, 3 H), 7.27–7.35 (m, 1 H).

N-(2-(4-(2-Fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)-2-(3-(2-fluoroethoxy)-4-methoxyphenyl)acetamide (5k): Procedure E was followed to prepare compound **5k**. ^1H NMR (300 MHz, CDCl_3) δ 3.17–3.25 (m, 4 H), 3.49 (s, 2 H), 3.58 (m, 1 H), 3.85 (s, 3 H), 3.88 (s, 3 H), 4.13 (m, 1 H), 4.19–4.33 (m, 4 H), 4.86–4.88 (m, 4 H), 5.76 (br, 1 H), 6.70–6.87 (m, 6 H).

2-(3,5-Dimethoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)acetamide (5l): Procedure E was followed to prepare compound **5l**. ^1H NMR (300 MHz, CDCl_3) δ 3.18–3.24 (m, 4 H), 3.50 (s, 2 H), 3.60 (m, 1 H), 3.78 (s, 6 H), 3.85 (s, 3 H), 4.12 (m, 1 H), 4.26 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.78 (dt, $J = 47.4, 4.5$ Hz, 2 H), 5.84 (br, 1 H), 6.38 (m, 3 H), 6.70–6.86 (m, 3 H).

2-(4-Fluoro-3-methoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-methoxyethyl)acetamide (5m): Procedure E was followed to prepare compound **5m**. ^1H NMR (300 MHz, CDCl_3) δ 3.18–3.25 (m, 4 H), 3.51 (s, 2 H), 3.61 (m, 1 H), 3.85 (s, 3 H), 3.88 (s, 3 H), 4.12 (m, 1 H), 4.27 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.78 (dt, $J = 47.4, 4.5$ Hz, 2 H), 5.79 (br, 1 H), 6.69–6.87 (m, 4 H), 7.00–7.11 (m, 2 H).

N-(2-(3,4-dimethoxyphenyl)-2-hydroxyethyl)-2-(3-(2-fluoroethoxy)-4-methoxyphenyl)acetamide (5n): Procedure E was followed to afford compound **5n**.

The ^1H NMR (300 MHz, CDCl_3) is δ 3.33 (m, 1 H), 3.51 (s, 2 H), 3.59 (m, 1 H), 3.89 (m, 9 H), 4.24 (dt, $J = 28.8, 4.5$ Hz, 2 H), 4.74 (dd, $J = 8.1, 3.6$ Hz, 1 H), 4.78 (dt, $J = 47.4, 4.5$ Hz, 2 H), 5.81 (br, 1 H), 6.78–6.86 (m, 6 H).

2-(4-(Benzyloxy)-3-methoxyphenyl)-N-(2-(4-(2-fluoroethoxy)-3-methoxyphenyl)-2-ethoxyethyl)acetamide (5o): Procedure E was followed to prepare compound **5o**. The ^1H NMR (300 MHz, CDCl_3) is δ 3.14–3.20 (m, 3 H), 3.49 (s, 2 H), 3.60 (m, 1 H), 3.84 (s, 3 H), 3.87 (s, 3 H), 4.15 (m, 1 H), 4.22 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.82 (dt, $J = 47.4, 4.5$ Hz, 2 H), 5.16 (s, 2 H), 5.79 (br, 1 H), 6.67–6.87 (m, 4 H), 7.20–7.50 (m, 7 H).

Procedure F: synthesis of compound 5p

2-(4-(Benzyloxy)-3-methoxyphenyl)-N-(2-(3,4-dimethoxyphenyl)-2-hydroxyethyl)acetamide (5p): Saturated sodium carbonate solution (5 mL) was added to a mixture of 2-amino-1-(3,4-dimethoxyphenyl)ethanol hydrochloride (0.41 g, 1.75 mmol) in ether (10 mL) and the mixture was stirred for 5 min. To the mixture, 2-(4-(benzyloxy)-3-methoxyphenyl)acetyl chloride (0.57 g, 1.96 mmol) in ether was added dropwise. The mixture was stirred for 2 h. The solid was collected by filtration to give **5p** (0.70 g, 88%). ^1H NMR (300 MHz, CDCl_3) δ 3.13 (br, 1 H), 3.28–3.38 (m, 1 H), 3.52 (s, 2 H), 3.53–3.62 (m, 1 H), 3.86 (s, 9 H), 4.75 (m, 1 H), 5.15 (s, 2 H), 5.80 (br, 1 H), 6.66–6.87 (m, 6 H), 7.25–7.46 (m, 5 H).

Procedure G: synthesis of compounds 6a–p

1-(3-Fluoro-4-methoxybenzyl)-6-(2-fluoroethoxy)-7-methoxyisoquinoline oxalate (6a): A mixture of **5a** and POCl_3 (0.5 mL) in acetonitrile (20 mL) was stirred under reflux for 30 min. After being cooled to room temperature and quenched with 25 mL of saturated sodium bicarbonate, the mixture was extracted with methylene chloride (10 mL \times 3) and dried over MgSO_4 . After evaporation of the solvent, the residue was purified by column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (20/1, v/v) as mobile phase to give the isoquinoline **6a** (0.41 g, 66%) as a white solid. To a solution of the **6a** in ethyl acetate/methanol (5 mL) was added 1 equivalent of oxalic acid in MeOH (5 mL), and the resulting mixture was stirred for 1 h at room temperature to give a crude oxalate salt. The white solid was filtered, washed thoroughly with ethyl acetate and methanol, separately. The free base was converted into oxalate salt for in vitro affinity measurements and elemental analysis. Mp (oxalate salt): 179.7–180.9 $^\circ\text{C}$; ^1H NMR (300 MHz, free base, CDCl_3) δ 3.83 (s, 3 H), 3.89 (s, 3 H), 4.39 (dt, $J = 27.0, 4.2$ Hz, 2 H), 4.51 (s, 2 H), 4.87 (dt, $J = 47.7, 4.2$ Hz, 2 H), 6.81–7.00 (m, 3 H), 7.08 (s, 1 H), 7.28 (s, 1 H), 7.42 (d, $J = 5.7$ Hz, 1 H), 8.35 (d, $J = 5.7$ Hz, 1 H). ^{13}C NMR (75 MHz, free base, CDCl_3) δ 40.4, 55.1 (d, $J = 33$ Hz), 66.9 (d, $J = 20.5$ Hz), 76.2, 80.4 (d, $J = 168.6$ Hz), 103.5, 105.9, 112.6, 115.3 (d, $J = 18.2$ Hz), 117.8, 122.2, 123.0 (d, $J = 3.4$ Hz), 131.7 (d, $J = 5.6$ Hz), 132.2, 140.1, 145.1 (d, $J = 11.4$ Hz), 149.2, 150.5, 151.4 (d, $J = 244.8$ Hz), 156.3. Anal. Calcd for $\text{C}_{20}\text{H}_{19}\text{F}_2\text{NO}_3 \cdot \text{H}_2\text{C}_2\text{O}_4$: C, 58.80; H, 4.71; N, 3.12. Found: C, 58.75; H, 4.64; N, 3.09.

6-(2-Fluoroethoxy)-7-methoxy-1-(4-methoxy-3-methylbenzyl)isoquinoline oxalate (6b): Starting with **5b**, procedure G was followed to prepare compound **6b** as a white solid. Mp (oxalate salt): 172.9–175.0 $^\circ\text{C}$; ^1H NMR (300 MHz, free base, CDCl_3) δ 2.14 (s, 3 H), 3.76 (s, 3 H), 3.89 (s, 3 H), 4.38 (dt, $J = 27.0, 4.2$ Hz, 2 H), 4.50 (s, 2 H), 4.86 (dt, $J = 47.4, 4.2$ Hz, 2 H), 6.71 (d, $J = 8.7$ Hz, 1 H), 7.04–7.07 (m, 3 H), 7.38 (s, 1 H), 7.39 (d, $J = 5.7$ Hz, 1 H), 8.36 (d, $J = 6.0$ Hz, 1 H). ^{13}C NMR (75 MHz, CDCl_3) δ 31.1, 41.0, 55.8 (d, $J = 47.8$ Hz), 68.2 (d, $J = 20.5$ Hz), 77.4, 80.4 (d, $J = 168.6$ Hz), 105.3, 107.0, 110.4, 119.4, 126.9, 127.0, 130.3, 130.6, 131.1, 134.0, 135.3, 139.1, 150.8, 154.0, 157.8. Anal. Calcd for $(\text{C}_{21}\text{H}_{22}\text{FNO}_3 \cdot \text{H}_2\text{C}_2\text{O}_4)$: C, 62.02; H, 5.43; N, 3.14. Found: C, 61.75; H, 5.52; N, 3.12.

6-(2-Fluoroethoxy)-7-methoxy-1-(4-(methylthio)benzyl)isoquinoline oxalate (6c):

Starting with **5c**, procedure G was followed to prepare compound **6c** (0.23 g, 72%) as a white solid. Mp (oxalate salt): 138.5–140.0 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 2.42 (s, 3 H), 3.88 (s, 3 H), 4.38 (dt, *J* = 27.0, 4.2 Hz, 2 H), 4.55 (s, 2 H), 4.86 (dt, *J* = 47.4, 4.2 Hz, 2 H), 7.06 (s, 1 H), 7.14–7.21 (m, 4 H), 7.38 (s, 1H), 7.41 (d, *J* = 6.0 Hz, 1 H), 8.36 (d, *J* = 6.0 Hz, 1 H). Anal. Calcd for C₂₀H₂₀FNO₂S•H₂C₂O₄•0.5H₂O: C, 57.88; H, 5.08; N, 3.07. Found: C, 57.89; H, 5.03; N, 2.93.

1-(4-Fluoro-3-methoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6d):

Starting with **5d**, procedure G was followed to prepare **6d** (0.21 g, 66%) as a white solid. Mp (oxalate salt): 207.7–208.7 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 3.83 (s, 3 H), 4.00 (s, 3 H), 4.27 (dt, *J* = 27.3, 3.9 Hz, 2 H), 4.50 (s, 2 H), 4.81 (dt, *J* = 47.1, 3.9 Hz, 2 H), 6.82–7.00 (m, 3 H), 7.07 (s, 1 H), 7.31 (s, 1 H), 7.44 (d, *J* = 5.7 Hz, 1 H), 8.37 (d, *J* = 5.7 Hz, 1 H). Anal. Calcd for C₂₀H₁₉F₂NO₃•H₂C₂O₄: C, 58.80; H, 4.71; N, 3.12. Found: C, 58.78; H, 4.73; N, 3.23.

7-(2-Fluoroethoxy)-6-methoxy-1-(4-methoxy-3-methylbenzyl)isoquinoline oxalate (6e):

Starting with **5e**, procedure G was followed to prepare compound **6e** (0.13 g, 61%) as a white solid. Mp (oxalate salt): 179.4–180.2 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 2.14 (s, 3 H), 3.76 (s, 3 H), 3.99 (s, 3 H), 4.26 (dt, *J* = 27.3, 4.5 Hz, 2 H), 4.48 (s, 2 H), 4.80 (dt, *J* = 47.4, 4.5 Hz, 2 H), 6.70 (d, *J* = 7.8 Hz, 1 H), 7.00–7.10 (m, 3 H), 7.39 (s, 1 H), 7.41 (d, *J* = 5.7 Hz, 1 H), 8.37 (d, *J* = 5.7 Hz, 1 H). Anal. Calcd for C₂₁H₂₂FNO₃•H₂C₂O₄: C, 62.02; H, 5.43; N, 3.14. Found: C, 62.09; H, 5.51; N, 3.26.

7-(2-Fluoroethoxy)-6-methoxy-1-(4-(methylthio)benzyl)isoquinoline oxalate (6f):

Starting with **5f**, procedure G was followed to prepare compound **6f** (0.15 g, 70%) as a white solid. Mp (oxalate salt): 199.5–200.5 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 2.43 (s, 3 H), 3.99 (s, 3 H), 4.26 (dt, *J* = 27.0, 4.2 Hz, 2 H), 4.53 (s, 2 H), 4.80 (dt, *J* = 47.4, 4.2 Hz, 2 H), 7.07 (s, 1 H), 7.16 (m, 4 H), 7.32 (s, 1 H), 7.43 (d, *J* = 5.7 Hz, 1 H), 8.37 (d, *J* = 5.7 Hz, 1 H). Anal. Calcd for C₂₀H₂₀FNO₂S•H₂C₂O₄: C, 59.05; H, 4.96; N, 3.13. Found: C, 59.06; H, 4.93; N, 3.10.

1-(2-Fluoro-4-methoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6g):

Starting with **5g**, procedure G was followed to prepare compound **6g** (0.13 g, 67%) as a pale solid. Mp (oxalate salt): 203.5–204.2 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 3.74 (s, 3 H), 3.99 (s, 3 H), 4.35 (dt, *J* = 27.3, 4.2 Hz, 2 H), 4.49 (s, 2 H), 4.85 (dt, *J* = 47.4, 4.2 Hz, 2 H), 6.55 (dd, *J* = 8.7, 1.8 Hz, 1 H), 6.64 (dd, *J* = 12.0, 2.7 Hz, 1 H), 7.00–7.13 (m, 2 H), 7.40–7.43 (m, 2 H), 8.36 (d, *J* = 5.7 Hz, 1 H). Anal. Calcd for C₂₀H₁₉F₂NO₃•H₂C₂O₄: C, 58.80; H, 4.71; N, 3.12. Found: C, 58.90; H, 4.75; N, 3.11.

1-(5-Bromo-2-methoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6h):

Starting with **5h**, procedure G was followed to prepare compound **6h** (0.14 g, 49%) as a white solid. Mp (oxalate salt): 195.7–197.3 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 3.90 (s, 3 H), 4.00 (s, 3 H), 4.27 (dt, *J* = 27.1, 4.5 Hz, 2 H), 4.52 (s, 2 H), 4.81 (dt, *J* = 47.4, 4.5 Hz, 2 H), 6.78 (d, *J* = 9.0 Hz, 1 H), 7.07 (s, 1 H), 7.16–7.43 (m, 4 H), 8.37 (d, *J* = 5.7 Hz, 1 H). Anal. Calcd for C₂₀H₁₉BrFNO₃•H₂C₂O₄: C, 51.78; H, 4.15; N, 2.74. Found: C, 51.90; H, 4.21; N, 2.80.

1-(3-Bromo-4-methoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6i):

Starting with **5i**, procedure G was followed to prepare compound **6i** (0.16 g, 47%) as a white solid. Mp (oxalate salt): 208.4–209.2 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 3.83 (s, 3 H), 4.00 (s, 3 H), 4.29 (dt, *J* = 27.3, 4.5 Hz, 2 H), 4.49 (s, 2 H), 4.83 (dt, *J* = 47.4, 4.5 Hz, 2 H), 6.78 (d, *J* = 8.1 Hz, 1 H), 7.07 (s, 1H), 7.13 (dd, *J* = 8.1, 1.8 Hz, 1 H), 7.32 (s, 1 H), 7.43

(d, $J = 5.7$ Hz, 1 H), 7.49 (d, $J = 1.8$ Hz, 1 H), 8.37 (d, $J = 5.7$ Hz, 1 H). Anal. Calcd for $C_{20}H_{19}BrFNO_3 \cdot H_2C_2O_4$: C, 51.78; H, 4.15; N, 2.74. Found: C, 51.92; H, 4.21; N, 2.80.

1-(3-Fluorobenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6j): Starting with **5j**, procedure G was followed to prepare compound **6j** (0.12 g, 35%) as a white solid. Mp (oxalate salt): 182.4–183.4 °C; 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.92 (s, 3 H), 4.18 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.49 (s, 2 H), 4.73 (dt, $J = 47.4, 4.2$ Hz, 2 H), 6.76–6.87 (m, 2 H), 6.95–7.00 (m, 2 H), 7.10–7.21 (m, 2 H), 7.37 (d, $J = 5.7$ Hz, 1 H), 8.36 (d, $J = 5.7$ Hz, 1 H). Anal. Calcd for $C_{19}H_{17}F_2NO_2 \cdot H_2C_2O_4$: C, 60.14; H, 4.57; N, 3.34. Found: C, 59.98; H, 4.65; N, 3.42.

7-(2-Fluoroethoxy)-1-(3-(2-fluoroethoxy)-4-methoxybenzyl)-6-methoxyisoquinoline oxalate (6k): Starting with **5k**, procedure G was followed to prepare compound **6k** (0.14 g, 58%) as a white solid. Mp (oxalate salt): 171.2–172.0 °C; 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.74 (s, 3 H), 3.99 (s, 3 H), 4.16–4.35 (m, 4 H), 4.49 (s, 2 H), 4.70–4.80 (m, 4 H), 6.55–7.13 (m, 4 H), 7.40–7.43 (m, 2 H), 8.36 (d, $J = 5.7$ Hz, 1 H). Anal. Calcd for $C_{22}H_{23}F_2NO_4 \cdot H_2C_2O_4$: C, 58.42; H, 5.11; N, 2.84. Found: C, 58.58; H, 5.22; N, 2.94.

1-(3,5-Dimethoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6l): Starting with **5l**, procedure G was followed to prepare compound **6l** (75 mg, 22%) as a white solid. Mp (oxalate salt): 176.2–176.7 °C; 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.70 (s, 6 H), 3.97 (s, 3 H), 4.26 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.50 (s, 2 H), 4.78 (dt, $J = 47.4, 4.5$ Hz, 2 H), 6.28 (t, $J = 2.4$ Hz, 1 H), 6.41 (m, 2 H), 7.04 (s, 1 H), 7.36 (s, 1 H), 7.41 (d, $J = 5.7$ Hz, 1 H), 8.36 (d, $J = 5.7$ Hz, 1 H). Anal. Calcd for $C_{21}H_{22}FNO_4 \cdot H_2C_2O_4$: C, 59.87; H, 5.24; N, 3.04. Found: C, 59.91; H, 5.22; N, 3.07.

1-(4-Fluoro-3-methoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline oxalate (6m): Starting with **5m**, procedure G was followed to prepare compound **6m** (65 mg, 36%) as a white solid. Mp (oxalate salt): 154.8–155.6 °C; 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.76 (s, 3 H), 3.99 (s, 3 H), 4.26 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.48 (s, 2 H), 4.80 (dt, $J = 47.4, 4.5$ Hz, 2 H), 6.70 (d, $J = 7.8$ Hz, 1 H), 7.00–7.10 (m, 3 H), 7.39 (s, 1 H), 7.41 (d, $J = 5.7$ Hz, 1 H), 8.37 (d, $J = 5.7$ Hz, 1 H). Anal. Calcd for $C_{20}H_{19}F_2NO_3 \cdot H_2C_2O_4$: C, 58.80; H, 4.71; N, 3.12. Found: C, 58.82; H, 4.81; N, 3.21.

1-(3-(2-Fluoroethoxy)-4-methoxybenzyl)-6,7-dimethoxyisoquinoline (6n): Starting with **5n**, procedure G was followed to prepare compound **6n** (125 mg, 50%) as a pale solid. Mp (oxalate salt): 149.7–150.6 °C; 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.81 (s, 3 H), 3.90 (s, 3 H), 4.01 (s, 3 H), 4.16 (dt, $J = 27.9, 4.5$ Hz, 2 H), 4.52 (s, 2 H), 4.70 (dt, $J = 47.1, 4.5$ Hz, 2 H), 6.78–6.89 (m, 3 H), 7.05 (s, 1 H), 7.32 (s, 1 H), 7.43 (d, $J = 5.7, 1$ Hz), 8.36 (d, $J = 5.7$ Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.2, 56.3, 56.6, 56.9, 69.3 (d, $J = 21.6$ Hz), 80.9 (d, $J = 169.6$ Hz), 105.0, 106.0, 112.6, 115.5, 121.6, 122.1, 122.9, 128.5, 131.5, 136.9, 152.7, 154.7, 157.0, 160.7, 163.3. Anal. Calcd for $C_{21}H_{22}FNO_4 \cdot H_2C_2O_4$: C, 59.87; H, 5.24; N, 3.04. Found: C, 59.78; H, 5.26; N, 3.04.

1-(4-(Benzyloxy)-3-methoxybenzyl)-7-(2-fluoroethoxy)-6-methoxyisoquinoline (6o): Starting with **5o**, procedure G was followed to prepare compound **6o** (0.16 g, 40%) as a white solid. 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.77 (s, 3 H), 3.99 (s, 3 H), 4.25 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.50 (s, 2 H), 4.85 (dt, $J = 47.4, 4.5$ Hz, 2 H), 5.09 (s, 2 H), 6.70–6.80 (d, $J = 7.8$ Hz, 4 H), 7.06 (s, 1 H), 7.29–7.44 (m, 6 H), 8.37 (d, $J = 5.7$ Hz, 1 H).

1-(4-(Benzyloxy)-3-methoxybenzyl)-7-(2-methoxy)-6-methoxyisoquinoline (6p): Starting with **5p**, procedure G was followed to prepare compound **6p** (0.23 g, 29%) as a white solid. 1H NMR (300 MHz, free base, $CDCl_3$) δ 3.77 (s, 3 H), 3.86 (s, 3 H), 4.00 (s, 3 H),

4.44 (d, $J = 5.7$ Hz, 2 H), 4.52 (s, 2 H), 5.09 (s, 2 H), 6.75 (s, 1 H), 6.76 (s, 1 H), 6.83 (s, 1 H), 7.05 (s, 1 H), 7.30–7.44 (m, 8 H), 8.70 (d, $J = 7.5$ Hz, 1 H).

Procedure H: synthesis of compounds **7a** and **7b**

4-((7-(2-Fluoroethoxy)-6-methoxyisoquinolin-1-yl)methyl)-2-methoxyphenol (7a): A mixture of **6o** (0.45 g, 1.0 mmol), hydrochloric acid (4.0 M, 3 mL), and ethanol (5 mL) was refluxed for 2h. After diluting the reaction mixture with ethyl acetate (20 mL), saturated NaHCO₃ solution was added and then extracted with ethyl acetate (30 mL × 3). The organic layer was dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified on a silica gel column with CH₂Cl₂/MeOH (20/1, v/v) as mobile phase to give **7a** (180 mg, 50%) as a white solid. ¹H NMR (300 MHz, CDCl₃) is δ 3.75 (s, 3 H), 3.99 (s, 3 H), 4.26 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.50 (s, 1 H), 4.81 (dt, $J = 47.4, 4.5$ Hz, 2 H), 5.58 (br, 1 H), 6.7–6.82 (m, 3 H), 7.06 (s, 1 H), 7.39 (s, 1 H), 7.43 (d, $J = 5.7$ Hz, 1 H), 8.37 (d, $J = 5.7$ Hz, 1 H).

4-((6,7-Dimethoxyisoquinolin-1-yl)methyl)-2-methoxyphenol (7b): Starting with **6p**, following the above procedure H to afford **7b** (0.12 g, 49%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 3.75 (s, 3 H), 3.90 (s, 3 H), 4.00 (s, 3 H), 4.52 (s, 2 H), 5.65 (br, 1 H), 6.76 (s, 1 H), 6.82 (s, 2 H), 7.05 (s, 1 H), 7.35 (s, 1 H), 7.42 (d, $J = 5.7$ Hz, 1 H), 8.36 (d, $J = 5.7$ Hz, 1 H).

Synthesis of compounds **8a** and **8b**

7-(2-Fluoroethoxy)-1-(4-(2-fluoroethoxy)-3-methoxybenzyl)-6-methoxyisoquinoline oxalate (8a): Starting with **7a**, procedure A was followed to prepare **8a** (0.15 g, 42%) as a pale solid. The oxalate salt of **8a** was prepared from **8a** according to the procedure described for the oxalate salt of **6a**. Mp (oxalate salt): 167.2–168.0 °C; ¹H NMR (300 MHz, free base, CDCl₃) is δ 3.76 (s, 3 H), 3.99 (s, 3 H), 4.20 (dt, $J = 27.3, 4.5$ Hz, 2 H), 4.26 (dt, $J = 27.3, 4.2$ Hz, 2 H), 4.52 (s, 2H), 4.73 (dt, $J = 47.4, 4.2$ Hz, 2 H), 4.80 (dt, $J = 47.7, 4.5$ Hz, 2 H), 6.75–6.83 (m, 3 H), 7.07 (s, 1H), 7.38 (s, 1 H), 7.43 (d, $J = 5.4$ Hz, 1 H), 8.38 (d, $J = 5.4$ Hz, 1 H). Anal. Calcd for C₂₂H₂₃F₂NO₄•H₂C₂O₄: C, 58.42; H, 5.11; N, 2.84. Found: C, 58.45; H, 5.14; N, 2.75.

1-(4-(2-Fluoroethoxy)-3-methoxybenzyl)-6,7-dimethoxyisoquinoline oxalate (8b): Starting with **7b**, procedure A was followed to prepare **8b** (0.17 g, 55%) as a white solid. The oxalate salt of **8b** was prepared from **8b** according to the procedure described for the oxalate salt of **6a**. Mp (oxalate salt): 157.3–158.2 °C. ¹H NMR (300 MHz, CDCl₃) δ 3.76 (s, 3 H), 3.90 (s, 3 H), 4.01 (s, 3 H), 4.21 (dt, $J = 27.6, 3.9$ Hz, 2 H), 4.54 (s, 2 H), 4.74 (dt, $J = 47.7, 3.9$ Hz, 2 H), 6.80–6.86 (m, 3 H), 7.05 (s, 1 H), 7.33 (s, 1 H), 7.43 (d, $J = 5.4$ Hz, 1 H), 8.37 (d, $J = 5.4$ Hz, 1 H). ¹³C NMR (75 MHz, CDCl₃) δ 37.4, 56.4, 56.6, 56.9, 69.0 (d, $J = 21.6$ Hz), 82.2 (d, $J = 169.6$ Hz), 105.0, 106.0, 113.2, 115.3, 120.9, 122.6, 122.9, 130.0, 131.6, 136.9, 147.6, 150.8, 154.6, 157.0, 163.3. Anal. Calcd for C₂₁H₂₂FNO₄•H₂C₂O₄: C, 59.87; H, 5.24; N, 3.04. Found: C, 59.70; H, 5.21; N, 2.96.

Procedure I: synthesis of compound **8c**

1-(4-(2-(2-Fluoroethoxy)ethoxy)-3-methoxybenzyl)-6,7-dimethoxyisoquinoline oxalate (8c): A mixture of **7b** (0.2 g, 0.62 mmol), 1-chloro-2-(2-fluoroethoxy)ethane (0.2 g, 1.59 mmol), and K₂CO₃ (0.25 g, 1.81 mmol) in DMF (10 mL) was stirred at room temperature overnight. The mixture was poured into water (50 mL) and extracted with ethyl acetate (10 mL × 3). The organic layer was dried over MgSO₄. After evaporation of the solvent, the residue was purified by silica gel column chromatography with CH₂Cl₂/MeOH (20/1, v/v) as mobile phase to give **8c** (0.14 g, 54%) as a white solid. The oxalate salt of **8c** was prepared from **8c** according to the procedure described for the oxalate salt of **6a**. Mp

(oxalate salt): 152.2–153.0 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 3.74 (s, 3 H), 3.83–3.90 (m, 7 H), 4.00 (s, 3 H), 4.14 (t, *J* = 5.4 Hz, 2 H), 4.53 (s, 2 H), 4.55 (dt, *J* = 47.7, 4.2 Hz, 2 H), 6.80–6.84 (m, 3 H), 7.05 (s, 1 H), 7.33 (s, 1 H), 7.43 (d, *J* = 5.7 Hz, 1 H), 8.37 (d, *J* = 5.7 Hz, 1 H). ¹³C NMR (75 MHz, CDCl₃) δ 37.5, 56.4, 56.6, 56.9, 69.1, 70.0, 70.5 (d, *J* = 21.8 Hz), 83.3 (d, *J* = 168.4 Hz), 105.1, 106.0, 113.0, 114.7, 120.9, 121.6, 123.0, 139.4, 131.6, 136.9, 148.0, 152.6, 154.7, 157.0, 163.3. Anal. Calcd for C₂₃H₂₆FNO₅•H₂C₂O₄: C, 59.40; H, 5.58; N, 2.77. Found: C, 59.26; H, 5.60; N, 2.78.

Procedure J: synthesis of compound 11

6,7-Dimethoxy-4-(4-methoxybenzyl)quinazoline oxalate (11): To a solution of 4-chloro-6,7-dimethoxyquinazoline, **9** (0.25 g, 1.1 mmol) and manganese (II) chloride (12.6 mg, 0.1 mmol) in THF (20 mL) was added 4-methoxybenzyl magnesium chloride, **10** (3.3 mmol). After the reaction mixture was stirred at room temperature for 6 h, water (30 mL) was added, and the mixture was extracted with ethyl acetate (20 mL × 3). The organic layer was dried over anhydrous sodium sulfate. After concentrating, the residue was purified by silica gel column with CH₂Cl₂/MeOH (20/1, v/v) as mobile phase to give **11** (0.21 g, 58%) as a white solid. The oxalate salt of **11** was prepared from **11** according to the procedure described for the oxalate salt of **6a**. Mp (oxalate salt): 127.6–128.4 °C; ¹H NMR (300 MHz, free base, CDCl₃) δ 3.76 (s, 3 H), 3.93 (s, 3 H), 4.03 (s, 3 H), 4.48 (s, 2 H), 6.81–6.85 (m, 2 H), 7.19–7.30 (m, 4 H), 9.09 (s, 1 H). ¹³C NMR (75 MHz, CDCl₃) δ 40.9, 55.3, 56.1, 56.4, 102.6, 107.3, 114.2, 119.5, 129.8, 130.1, 148.5, 150.2, 153.7, 155.6, 158.5, 166.3. Anal. Calcd for C₁₈H₁₈N₂O₃: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.53; H, 5.81; N, 8.99.

4.2. *In vitro* Assessment of affinities

To determine the potency of the new synthesized analogues, first, compounds were screened *in vitro* for their affinities toward PDE10A to determine IC₅₀ values. Compounds having a high affinity for PDE10A (IC₅₀ values < 60 nM), were further assessed for their selectivity for PDE10A versus other PDE isoforms, PDE3A and PDE3B. All compounds were independently assayed at least two times.

4.2.1. PDE10A enzyme assay protocol—The screening method followed published procedures [10–12, 19, 29]. PDE activity was measured using the Phosphodiesterase [³H]cAMP Scintillation Proximity Assay (SPA) (Cat. #TRKQ7090, Perkin Elmer, Waltham, MA) with minor modifications to the manufacturer's protocol. Briefly, the effect of PDE inhibitors is determined by assaying a fixed amount of enzyme in the presence of varying compound concentrations and a low [³H]cAMP substrate concentration; the substrate concentration used in the assay is 1/3 of the *K_m* concentration, allowing for comparisons of IC₅₀ values across a panel of different PDE enzymes. Reactions are initiated with enzyme, incubated to give ~30% substrate turnover, and terminated with yttrium silicate SPA beads. Plates are sealed, allowed to settle, and counted on a Trilux Micro-Beta Counter (PerkinElmer, Waltham, MA). Radioactivity units can be converted to percent activity of an uninhibited control (100%), plotted against inhibitor concentration and inhibitor IC₅₀ values were calculated.

4.2.2. Measure the *in vitro* affinities for PDE 3A and PDE 3B to determine selectivity of PDE10A vs PDE3A and PDE3B for analogues with high PDE10A affinity—Compounds with high affinity for PDE10A (IC₅₀ < 60 nM) were further assessed for their PDE10A selectivity over other PDE3A and PDE3B. The screening method is the same as the protocol for PDE10A except the assays will use other PDE isoforms and their concentrations are adjusted based on the *K_i* value for the different PDE isoforms.

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Abbreviations

AMP	Adenosine monophosphate
Anal	Analysis
Calcd	Calculated
cAMP	cyclic Adenosine monophosphate
CDI	1,1'-Carbonyldiimidazole
CIMS	Chemical ionization mass spectrometry
CNS	Central nervous system
cGMP	cyclic Guanosine monophosphate
DCC	N,N'-Dicyclohexylcarbodiimide
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
GMP	Guanosine monophosphate
MSNs	Medium-sized spiny neurons
ND	Not determined
PA	Papaverine
PCP	Phenylcyclohexylpiperidine
PDE10A	Phosphodiesterase 10A
PDE3A	Phosphodiesterase 3A
PDE3B	Phosphodiesterase 3B
PET	Positron emission tomography
PLD	Phospholipase D
THF	Tetrahydrofuran
TLC	Thin layer chromatography

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Research highlights

- Successfully synthesized and evaluated a series of analogues as PDE10A inhibitors.
- Identified six compounds which have PDE10 binding affinities ranging between 28–60 nM.
- Compound **8c** has high affinity for PDE10A and high selectivity for PDE10A vs. PDE3A/B.
- Compound **8c** can be radiosynthesized with fluorine-18 serving as PDE10A PET probe.

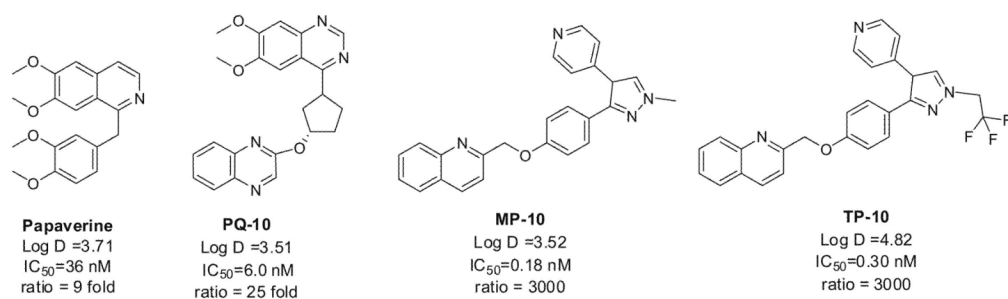
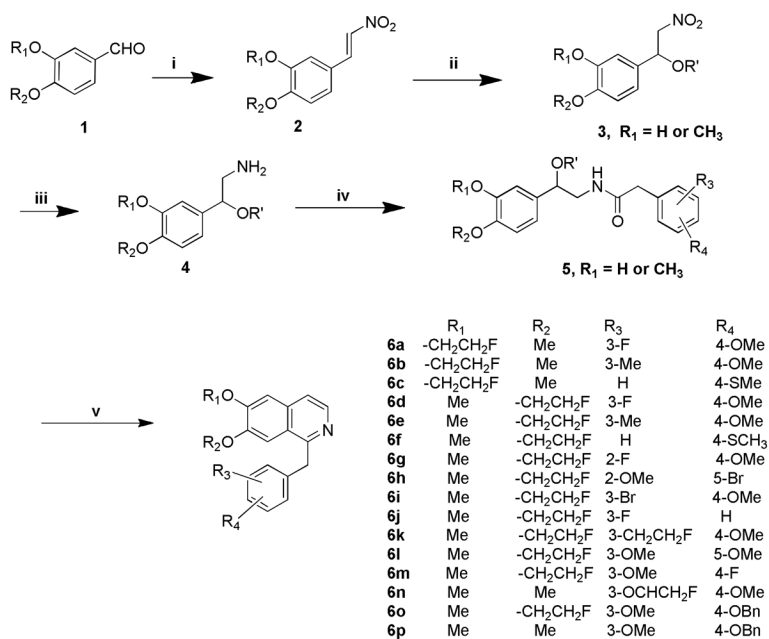
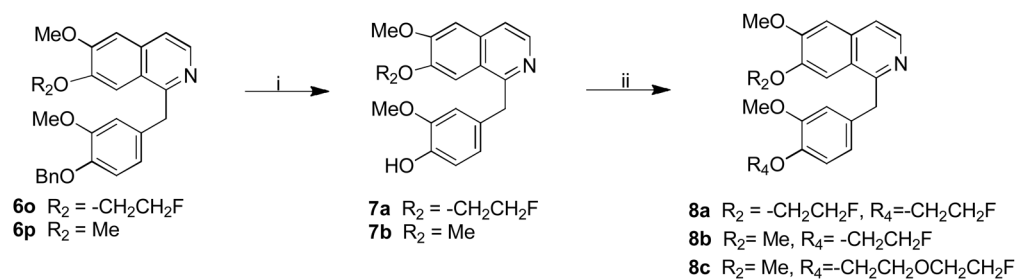


Figure 1.
Structures and *in Vitro* Assays of the Lead Compounds

**Scheme 1.**

Reagents and conditions:

i) MeNO₂, NH₄OAc, HOAc; ii) MeONa/MeOH, THF; iii) LiAlH₄, THF;iv) CDI, CH₂Cl₂, 2-(substituted phenyl)acetic acid or 2-(substituted phenyl)acetic chloride;v) POCl₃, acetonitrile

**Scheme 2.**

Reagents and conditions:

i) 1:1 HCl(aq), EtOH; ii) 2-bromo-1-fluoroethane or 1-chloro-2-(2-fluoroethoxy)ethane, K_2CO_3 , DMF

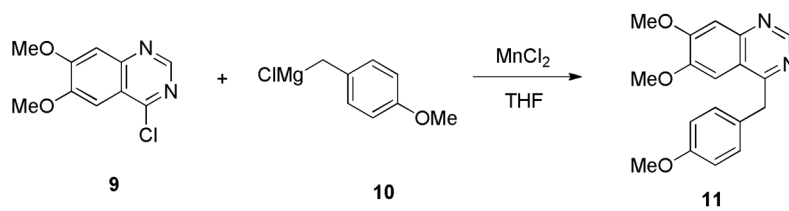
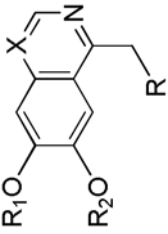
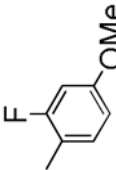
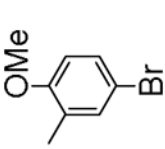
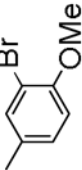
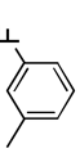
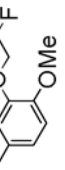
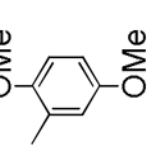
**Scheme 3.**

Table 1

Structure and Affinities of New Analogues^a

	Chemical Structure		IC ₅₀ (nM)			Log p ^b
	X	R	PDE10A	PDE3A	PDE3B	
						
Papaverine	C	Me	21±1.2	917 ± 100	1030 ± 88	3.71
6a	C	-CH ₂ CH ₂ F	49±1.1	1290 ± 97	1250 ± 220	4.13
6b	C	-CH ₂ CH ₂ F	58.5±1.1	1190 ± 130	1670 ± 240	4.57
6c	C	-CH ₂ CH ₂ F	112±1.2	ND ^c	ND ^c	4.69
6d	C	Me	198±1.1	ND ^c	ND ^c	4.13
6e	C	Me	244±1.1	ND ^c	ND ^c	4.57
6f	C	Me	237±1.1	ND ^c	ND ^c	4.68

	Chemical Structure				IC ₅₀ (nM)			Log P ^b
	X	R ₁	R ₂	R	PDE10A	PDE3A	PDE3B	
6g	C	Me	-CH ₂ CH ₂ F		500±1.2	ND ^c	ND ^c	4.23
6h	C	Me	-CH ₂ CH ₂ F		872±1.3	ND ^c	ND ^c	5.15
6i	C	Me	-CH ₂ CH ₂ F		248±1.1	ND ^c	ND ^c	4.69
6j	C	Me	-CH ₂ CH ₂ F		469±1.3	ND ^c	ND ^c	4.26
6k	C	Me	-CH ₂ CH ₂ F		200±1.1	ND ^c	ND ^c	4.17
6l	C	Me	-CH ₂ CH ₂ F		262±1.1	ND ^c	ND ^c	3.92

	Chemical Structure				IC ₅₀ (nM)			Log P ^b
	X	R ₁	R ₂	R	PDE10A	PDE3A	PDE3B	
6m	C	Me	-CH ₂ CH ₂ F		303±1.1	ND ^c	ND ^c	4.13
6n	C	Me	Me		55±1.0	1940 ± 490	2510 ± 140	3.93
8a	C	Me	-CH ₂ CH ₂ F		246±1.1	ND ^c	ND ^c	4.17
8b	C	Me	Me		55±1.2	1740 ± 390	2170 ± 260	3.93
8c	C	Me	Me		28±1.2	2200 ± 440	2520 ± 210	3.54
11	C	Me	Me		48.8±1.07	3980 ± 510	3100 ± 270	2.53

^aIC₅₀ is defined as the concentration of the inhibitor required to reduce the [³H]cAMP hydrolysis activity of recombinant human PED10A by 50% with scintillation proximity assay.

^bCalculated value at pH = 7.4 by ACD/I-Lab ver. 7.0 (Advanced Chemistry Development, Inc., Canada).

^cNot determined.