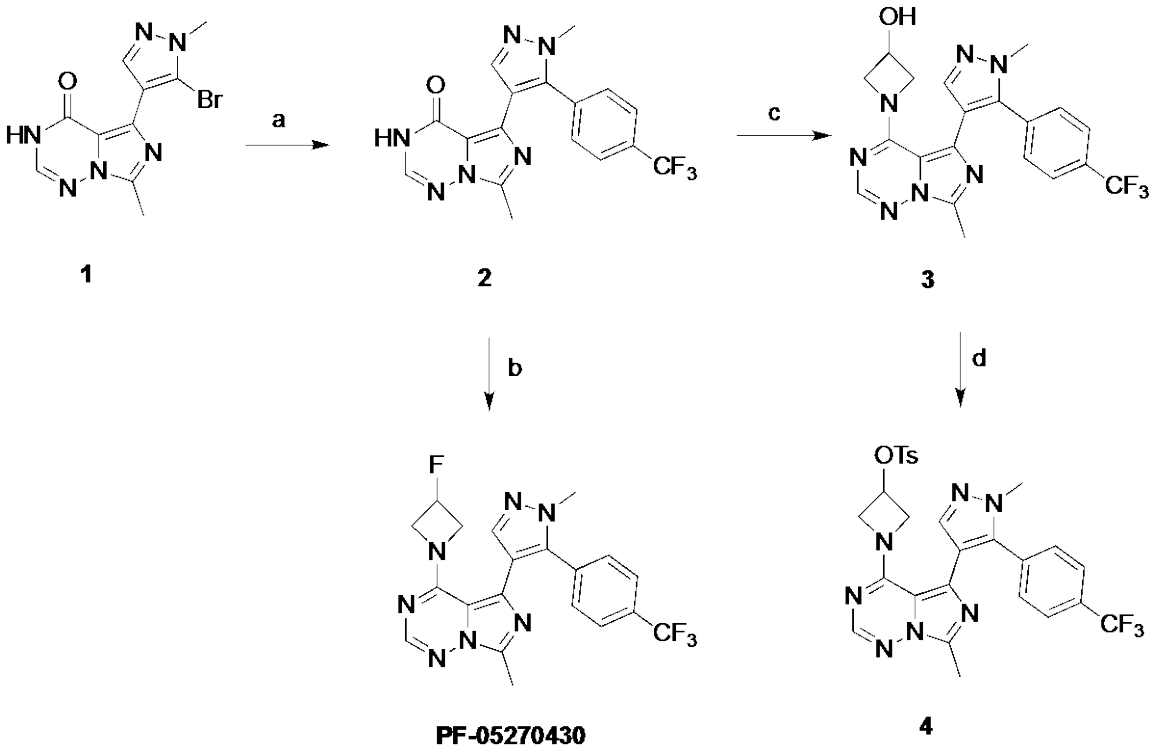
Supplemental Table 1. Potency, selectivity and in vitro/in vivo PK profile of PF-05270430

Target	IC_{50} (nM)	Property	Value
PDE2A	0.53	CNS PET MPO[a]	4.81
PDE10A	3,900	RRCK[b]	21.5 x 10^{-6} cm/sec
PDE1B, 3A, 4D	>30,000	MDR BA/AB[c]	1.32
PDE5A	26,000	Fu_b (rat)[d]	0.077
PDE6A	>10,000	Fu_p (rat)[e]	0.24
PDE7B	12,000	B/P_total (rat)[f]	1.56
PDE8B, 9A, 11	>10,000	B/P_free (rat)[g]	0.50

[a]Multiparameter optimization index for CNS PET ligands; [b]Index for ligand passive permeability in RRCK cells; [c]Measure of ligand efflux potential through multi-drug resistance cells; [d]Fraction unbound in rat brain; [e]Fraction unbound in rat plasma; [f]Ratio of total drug levels in rat brain and plasma, determined from pharmacokinetics in Wistar-Han rats 0-2 h following a single 1 mg/kg intravenous dosing; [g]Ratio of free drug levels in rat brain and plasma, calculated based on B/P_total and fractions unbound in rat brain and plasma.



Reagents and conditions: a. 2 mol% $Pd(PPh_3)_4$, K_3PO_4, EtOH/H_2O, reflux; b. 1,2,4-triazole, $POCl_3$, Et_3N, CH_3CN, then 4-fluoroazetidine•HCl, Et_3N, CH_2Cl_2; c. 1,2,4-triazole, $POCl_3$, Et_3N, CH_3CN, then 4-hydroxyazetidine•HCl, Et_3N, CH_2Cl_2; d. TsCl, Et_3N, CH_2Cl_2.

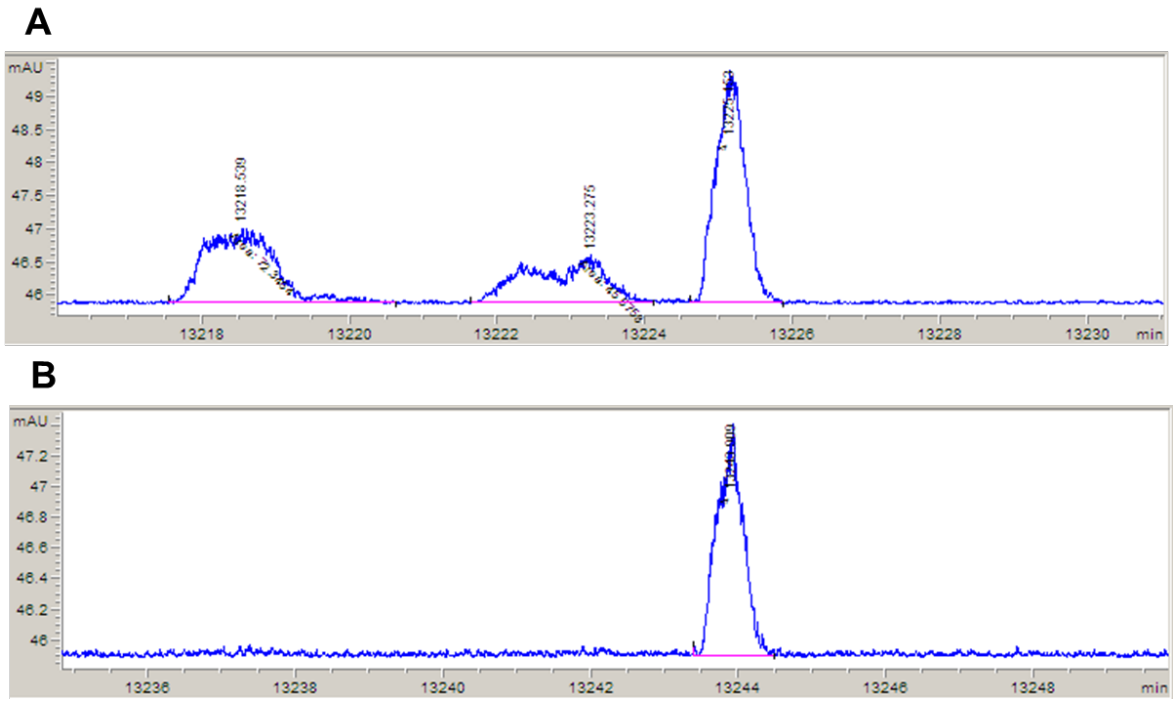
Supplemental Figure 1. Synthesis of PF-05270430 and the tosylate precursor for ^{18}F-PF-05270430.

Metabolite study in rats

Two male Sprague–Dawley (SD) rats (Charles River) with body weights of 306 and 343.5 grams were used to study the plasma and brain metabolites of ^{18}F-PF-05270430. Rats were briefly anesthetized (2-3% isoflurane in O_2) for tail vein injection of radiotracer (69.1 and 67.1 MBq), then allowed to recover for conscious uptake of the radiotracer. Rats were anesthetized again by isoflurane (5% in O_2) at 30 min post injection, blood was collected by cardiac puncture followed by decapitation. The brain was removed and the entire striatum dissected for analysis.

Blood samples were processed by centrifuging for 5 min at 5,000 rpm in a VWR Clinical 50 centrifuge. Plasma was separated from the blood pellet, then diluted with 1-2 mL of de-ionized water and filtered through a Pall Life Science GHP 0.45 µm syringe filter (13 mm). Rat striatum samples were processed by adding 0.5 mL of acetonitrile and homogenizing with a Polytron homogenizer. The homogenate was centrifuged at 14,000 rpm for 10 min. An aliquot of the supernatant and the entire pellet were removed, weighed and counted for radioactivity in a Wallac 1470 Wizard gamma counter (PerkinElmer, Waltham, MA) for 1 min per sample using a 25-1800 keV energy window. The supernatant was diluted with water and filtered as described above for the blood samples. Both the plasma and brain samples were spiked with 20-100 nmol of reference standard PF-05270430 and analyzed by HPLC via the method of Hilton et al. with minor modifications (*1*). Briefly, the samples were loaded onto a 5 mL HPLC injector loop and injected onto a capture column (Chiral Technologies BioTrap 500 MS, 4 × 20 mm, cat # 39H17). The capture column was eluted with 1% aqueous acetonitrile for 4 min at a flow rate of 2 mL/min and then back-flushed onto a Thermo Scientific Hypersil Gold aQ column (4.6 × 150 mm, 5 µm, cat. # 504955) eluting with a mixture of 40/60 acetonitrile:0.1% formic acid (v/v) at a flow rate of 1.5 mL/min. At the end of the collection the column was eluted with a mixture of 90/10

acetonitrile:0.1% formic acid (v/v) to ensure there were no other non-polar radioactive species remaining on the Hypersil column. The effluents were monitored through a flow detector (Bioscan PET Metabolite coincidence detector). The parent fraction was determined by the decay-corrected area associated with the radioactive peak of ^{18}F-PF-05270430 on the HPLC chromatogram (Supplemental Figure S2). Recovery of activity from the brain samples was between 83 and 95%.



Supplemental FigureS2. Representative HPLC chromatograms from metabolite analysis of ^{18}F-PF-05270430 in rats at 30 min post tracer injection: A) in plasma; B) in striatum.

Metabolite analysis in rhesus monkey plasma

The unmetabolized fraction of the tracer in arterial blood samples from the rhesus monkey studies were analyzed using the column switching HPLC method (*1*). Whole blood samples in EDTA tubes were centrifuged at 2,300 g at 4 °C for 5 min to separate the plasma. Plasma samples were then treated with urea (8 M) and loaded onto a capture column (19 × 4.6 mm) packed with

Phenomenex SPE C18 Strata-X sorbent and eluted with 1% acetonitrile in water at a flow rate of 2 mL/min for 4 min, after which the activity trapped on the capture column was back-flushed onto an analytical HPLC column (Phenomenex Luna(2) C18 column, 5 μm, 4.6 x 250 mm) eluting with 45% acetonitrile in 0.1 M ammonium formate at a flow rate of 1.55 mL/min. The HPLC eluent was collected in fractions with an automated fraction collector (Model CF-1, Spectrum Chromatography, Houston, TX) and counted in an automatic gamma well counter (Wizard2, PerkinElmer, Waltham, MA). The unmetabolized parent fraction with retention time of ~7 min after column switching was determined as the ratio of the sum of radioactivity in fractions containing the parent compound to the total amount of radioactivity collected, and fitted with bounded sum of exponentials or inverted gamma approach.

Supplemental Table 2. % Blockade of ^{18}F-PF-05270430 binding with PF-05180999 in rhesus monkeys

Region	%V_T (MA1)		%BP_{ND} (MA1)		%BP_{ND} (SRTM)	
	Dose (0.2 mg/kg)	**Dose (2.0 mg/kg)**	**Dose (0.2 mg/kg)**	**Dose (2.0 mg/kg)**	**Dose (0.2 mg/kg)**	**Dose (2.0 mg/kg)**
	TM762	**BM090**	**TM762**	**BM090**	**TM762**	**BM090**
Cerebellum	-22%	-28%	-	-	-	-
Occipital cortex	-28%	-42%	-177%	-186%	**-**	-194%
Temporal cortex	-31%	-24%	-43%	15%	-55%	17%
Cingulate cortex	-44%	-20%	-62%	20%	-68%	24%
Frontal cortex	-40%	-20%	-47%	18%	-56%	20%
Caudate	-22%	33%	-1%	76%	-2%	76%
Putamen	-24%	34%	-3%	72%	-4%	72%
Nucleus accumbens	-9%	29%	16%	70%	15%	69%
High-binding regions*	-18±8%	32±3%	4±11%	73±3%	3±10%	72±3%

* High binding regions: putamen, caudate and nucleus accumbens.
**** %blockade of BP_{ND} (SRTM) in the occipital cortex calculated from subject BM090 only, because the estimates in subject TM762 were unstable (relative standard error > 100%).

PF-05180999 plasma pharmacokinetic analysis

Venous blood samples were collected in EDTA treated tubes. Plasma was isolated after centrifugation and stored at −80 °C prior to analysis.

Plasma standard curves were prepared via serial dilution at concentrations of 0.61−2500 ng/mL. Aliquots of plasma sample (30 µL) were precipitated with 180 µL of acetonitrile containing an internal standard. Samples were vortexed for 2 min and then centrifuged at 3,500 rpm for 7 min. The supernatant (150 µL) was transferred to a 96-well plate where 10 µL was injected onto a Phenomenex Kinetic C18 column (Torrence, CA). Liquid Chromatography Tandem Mass Spectrometry (LC−MS/MS) analysis was carried out using a high performance liquid chromatography system consisting of Shimadzu LC20AD pumps (Shimadzu Scientific Instruments, Columbia, MD) with a CTC PAL autosampler (Leap Technologies, Carrboro, NC) interfaced to an API 4000 LC−MS/MS quadrupole tandem mass spectrometer (AB Sciex Inc., Ontario, Canada). The mobile phase consisted of solvent A (water with 0.1% formic acid in 10 mM ammonium formate) and solvent B (acetonitrile with 0.1% formic acid). The gradient was as follows: solvent B was held at 5% for 0.3 min, linearly ramped from 5% to 80% in 1.1 min, held at 80% for 0.3 min, and then stepped to 5% over 0.01 min. The mass spectrometer was operated using positive electrospray ionization. All raw data were processed using Analyst Software ver. 1.4.2 (AB Sciex Inc., Ontario, Canada).

Supplemental Table 3. Blockade of ^{18}F-PF-05270430 binding in the striatum and plasma exposure with PF-05180999 in cynomolgus monkeys

Animal ID*	Condition	PF-05180999 dose (mg/kg)	Striatum BP_{ND} (SRTM)**	PF-05180999 plasma concentration (ng/mL)	%Blockade in striatum
CM1	Baseline	0.0	1.406	-	-
CM2	Baseline	0.0	1.890	-	-
CM2	Blocking	0.2	1.826	40.2	3%
CM1	Blocking	0.2	1.112	52.0	21%
CM1	Blocking	0.4	0.849	87.9	40%
CM2	Blocking	0.4	0.762	111.7	60%
CM1	Blocking	0.5	0.637	80.0	55%
CM2	Blocking	0.5	0.883	108.7	53%
CM1	Blocking	0.6	0.580	128.7	59%
CM2	Blocking	0.6	0.534	148.5	72%
CM1	Blocking	2.0	0.454	208.5	68%
CM2	Blocking	2.0	0.569	240.0	70%

* CM1, cynomolgus monkey 1; CM2, cynomolgus monkey 2
** The striatum BP_{ND} calculated by averaging the values in putamen and caudate for each scan.

REFERENCES

1. Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF. Column-switching HPLC for the analysis of plasma in PET imaging studies. *Nucl Med Biol.* 2000;27:627-630.