Improving success in the development of novel PET ligands for CNS indications

Elizabeth Beck *for the Pfizer Neuroscience PET unit*

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Medicinal Chemistry

Positron Emission Tomography (PET):

- A non-invasive imaging method that provides high resolution (2-3 mm) and quantitative information on specific target areas.

- PET requires a radioligand labeled with a positron emitting nuclide, typically **^{11}C** ($t_{1/2}$ = 20 min) or **^{18}F** ($t_{1/2}$ = 110 min).

Main applications of PET in Neuroscience:

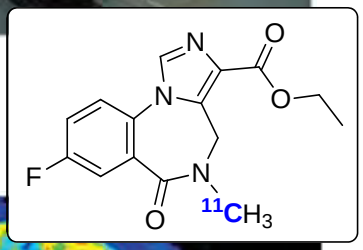
- **Bio-distribution** information after administration of only a few µg tracer
- **Measures receptor occupancy** (RO)
  - Support Proof of Mechanism
  - Define clinical go/no go criteria
  - Optimize clinical dose selection
- **Diagnostic tool** early detection, characterization and to monitor disease progression.

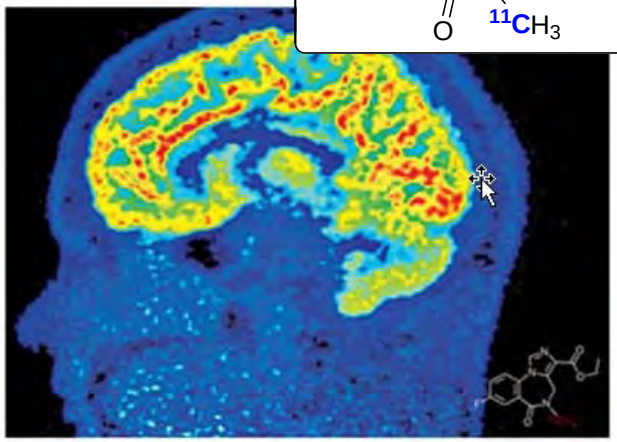
Oncology:

- [^{18}F]-FDG-PET: imaging fast growing tumors: diagnosis, staging and monitoring treatment of cancer

Typical Pet facility

[^{11}C]flumazenil





Radio labeled [^{11}C]flumazenil delineates benzodiazepine receptors in the brain

PET Ligand Design Criteria

- **Pharmacology:**

Target density / Mode of action

Affinity:: B_{max} (nM)/K_d > 10 (**B_{max}**: max Conc. of target binding site)

Selectivity: >30-100x selective

- **Safety:**

Lower safety hurdle: single dose IV micro dosing

- Structure alerts/Gentox may be tolerated
- Establish safety window at multiple of projected clinical dose (μg scale)

- **Pharmacokinetics:**

Brain permeability

No brain-penetrating radioactive metabolites

Low non-specific binding

In-silico tools and high through-put assays to predict:
- good brain permeability
- minimal non-specific binding

- **Synthesis:**

Synthesis limited to **[^{11}C]** or **[^{18}F]**;

Rapid synthesis - due to short $T_{1/2}$

Proximity to cyclotron – limited reagents

Improve do-ability of labeling
→ enable greater flexibility in the design of ligands

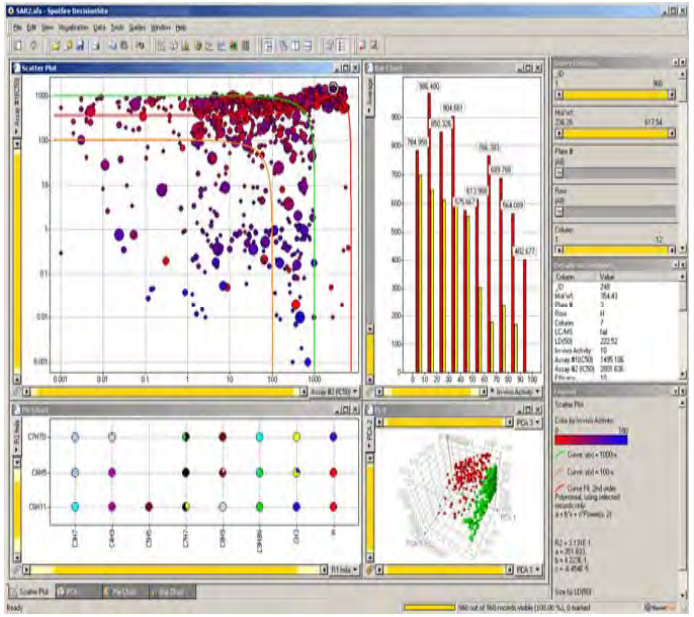
Our approach to address current knowledge gap around Pharmacokinetics

62 Validated PET ligands
("**Yes**" category)

15 ligands failed due to NSB
("**No**" category)

Physicochemical properties:
MWt, tPSA, cLogP, LogD, PKa, HBDOR, CNS MPO, CNS MPO2

In silico ADME models*:
cRRCK (permeability)
cMDR (P-gp liability)
cHLM (microsomal clearance)
cFu_b (brain free fractions)
cFu_p (plasma free fractions)



Spotfire Analysis

- Identify key differences in the properties of validated PET ligands (Yes) and negative controls (No) and define a chemical space that would enable a higher probability of success for novel CNS PET ligand development.

**Experimental data was used in analysis if available*

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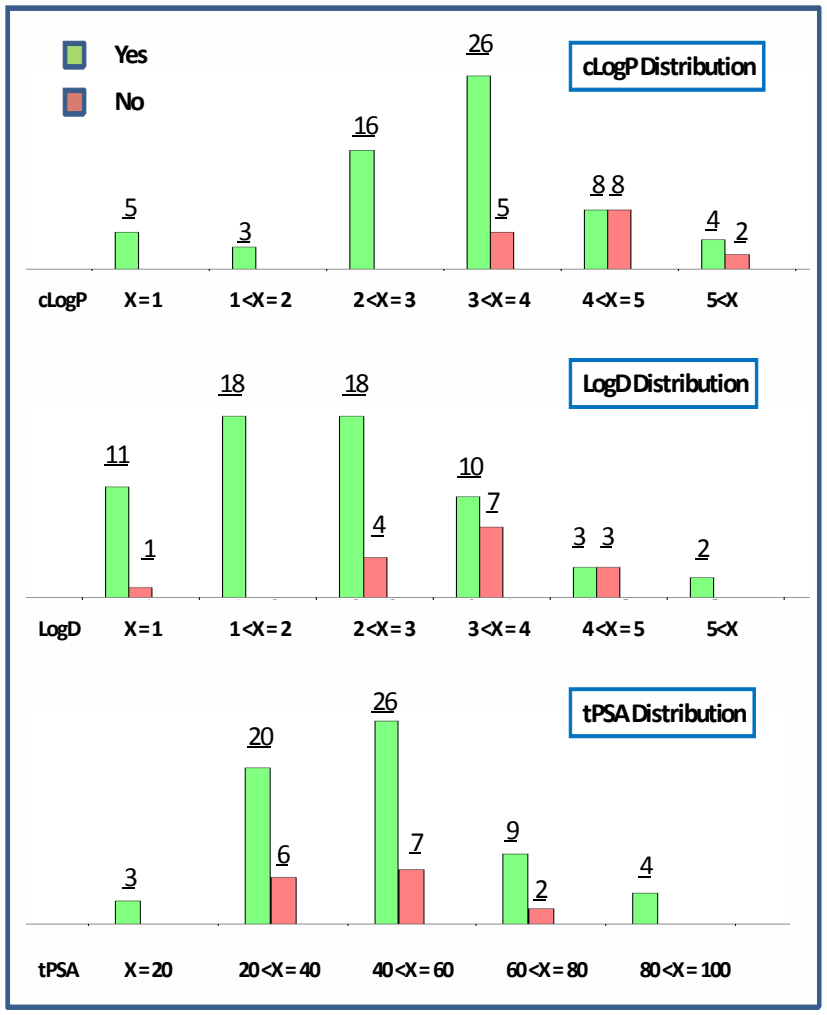
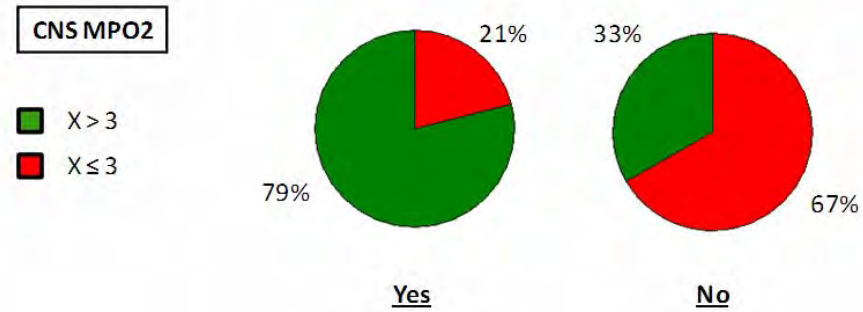
Physicochemical properties comparison between successful and failed PET ligands





MPO = Multi-parameter optimization

- Based on 6 properties: LogP, LogD, TPSA, MW, HBD and pKa
- Monotonic decreasing function is employed with inflection points that designate 'desirable' ranges.
- Scores from 0-1 are calculated for each property and summed to provide the final MPO score (range 0-6)

Physiochemical properties

- CNS MPO2 > 3
- LogD < 3

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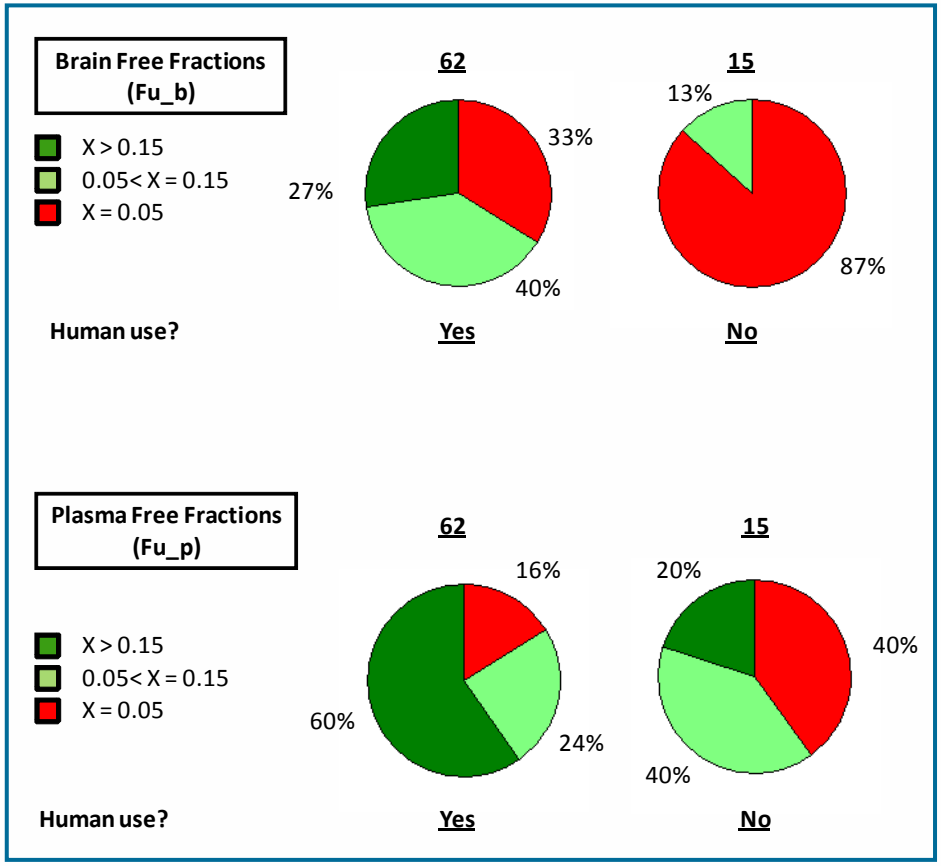
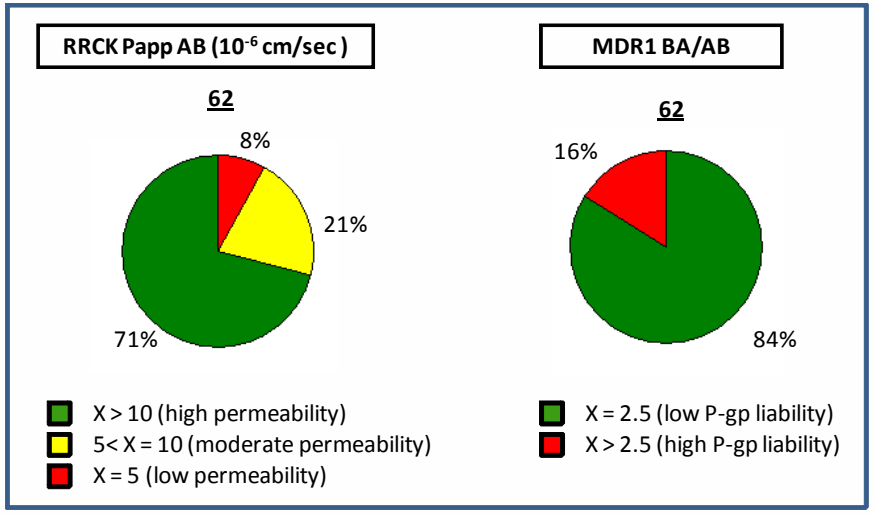
CNS MPO2: Wager et. al. *ACS Chem. Neurosci.* **2010**, *1*, 420-449

Brain and plasma free-fractions as a good predictor for non-specific binding

Non-specific binding: Free-fractions



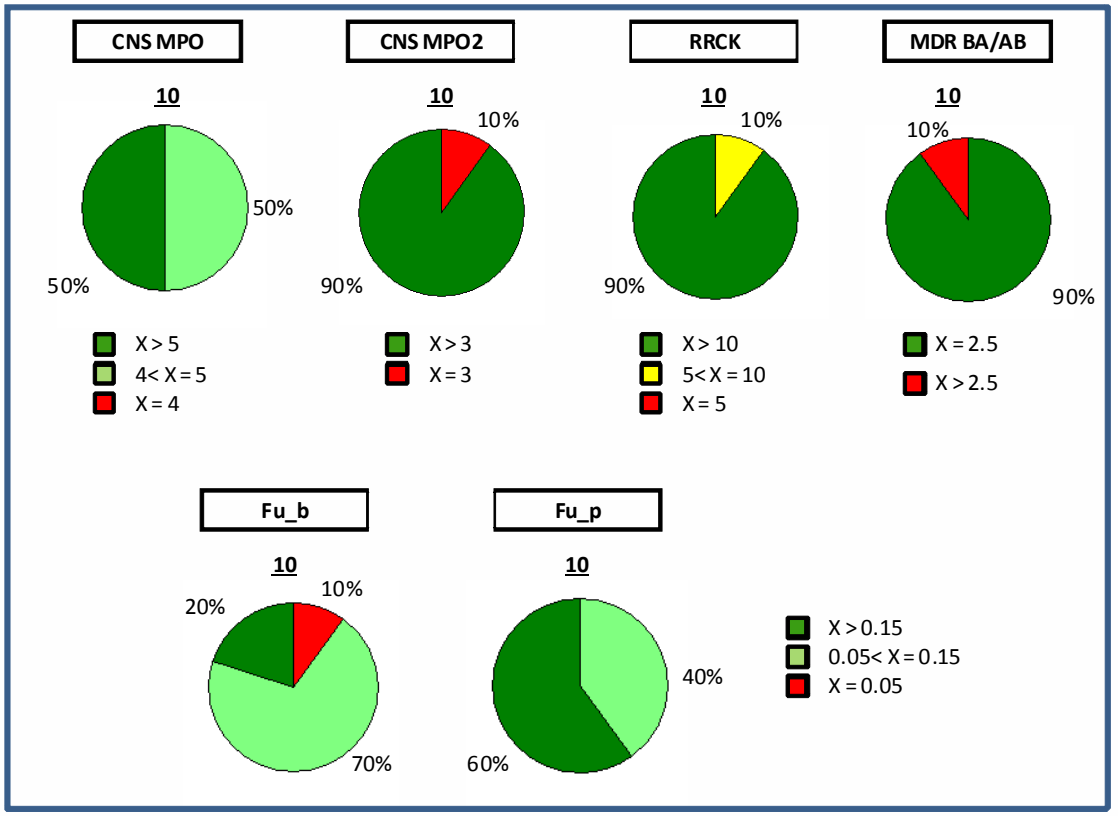
Brain Permeability: RRCK, MDR



Low non-specific binding	Brain permeability
▪ high risk of NSB if cFu_b < 5%, cFu_p < 5% ▪ cFu_b, cFu_p > 5% Preferably cFu_p > 15%	▪ RRCK AB > 5 x10^{-6} cm/sec (mod-high permeability) ▪ MDR BA/AB < 2.5 (low Pgp liability)

Top 10 PET ligands reinforce our defined design criteria

Ligands	Target Type	Target Receptor
S_Raclopride	GPCR	D2/D3
Fallypride	GPCR	D2
Flumazenil	GPCR	GABA-A
[^{11}C]DASB	Transporter	SERT
CFT	Transporter	DAT
MDL100907	GPCR	5HT2A, SERT
WAY100635	GPCR	5HT1A antagonist
[^{11}C]Carfentanil (CFN)	GPCR	mu-opioid
PHNO	GPCR	D2/D3 agonist
[^{11}C]ABP688	GPCR	mGluR5 NAM



Pharmacology

- B_{max}/K_d > 10
- 30-100x selectivity over other receptors

Physiochemical properties

- CNS MPO2 > 3
- LogD < 3

Low non-specific binding

- high risk of NSB if cFu_b < 5%, cFu_p < 5%
- cFu_b, cFu_p > 5% Preferably cFu_p > 15%

Brain permeability

- RRCK AB > 5 x10^{-6} cm/sec (mod-high permeability)
- MDR BA/AB < 2.5 (low Pgp liability)

Application in identification of novel PDE2 ligand

Physiochemical properties

- CNS MPO2 > 3
- LogD < 3

Pharmacology

- B_{max}/K_d > 10
- 30-100x selectivity over other receptors

Low NSB

- cFu_b and cFu_p > 5%; Preferably cFu_p > 15%

Brain permeability

- RRCK AB > 5
- MDR BA/AB < 2.5

- Data mining process for a PDE2A PET-ligand:

~1200 compounds

MDR1 BA/AB <2.5
RRCK P_{app}AB >5

~350 compounds

cFu_b and cFu_p > 5%

~200 compounds

CNS MPO2 >3

~150 compounds

IC50 < 10 nM

~20 compounds

Structure amenability to radiolabeling

8 leads

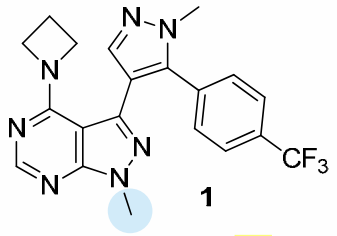
PDE2A: dual substrate phosphodiesterase

- Inhibitors increase cyclic nucleotide levels and positively amplify NMDA signaling in key brain areas associated with cognition and motivation
- High expression in cortex, hippocampus, striatum, medial habenula. ***B_{max} (striatium) = 235 nM***

Utility of PDE2A Pet ligand:

- Correlate target occupancy measurements with efficacy end-points for novel PDE-2A inhibitors
- Translational tool to enable clinical evaluation

In-vitro and in-vivo PK properties



1

PDE2 IC_{50} (nM)	2.3
Selectivity (over other PDEs)	>500x
CNS PET MPO	4.94
RRCK AB (10^{-6} cm/s)	21.5
MDR BA/AB	1.46
cFu_p (rat)	32%
cFu_b (rat)	9.5%
cFu_p (human)	23%

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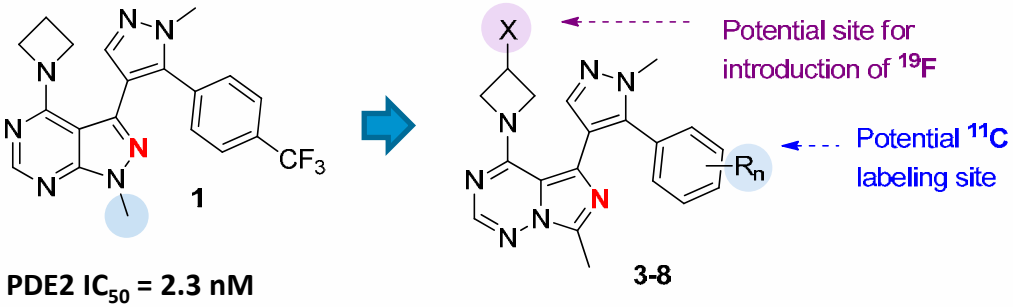
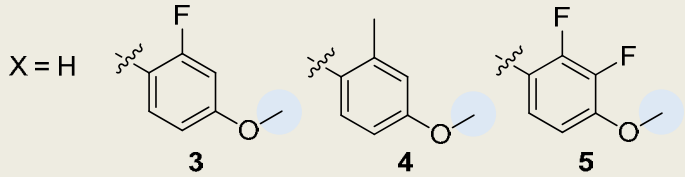
Design of six PET-specific PDE2A analogues

Core optimization to improve H-bond acceptor strength → PET specific SAR

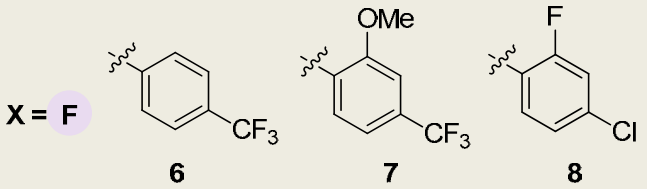


PDE2 IC_{50} = 2.3 nM

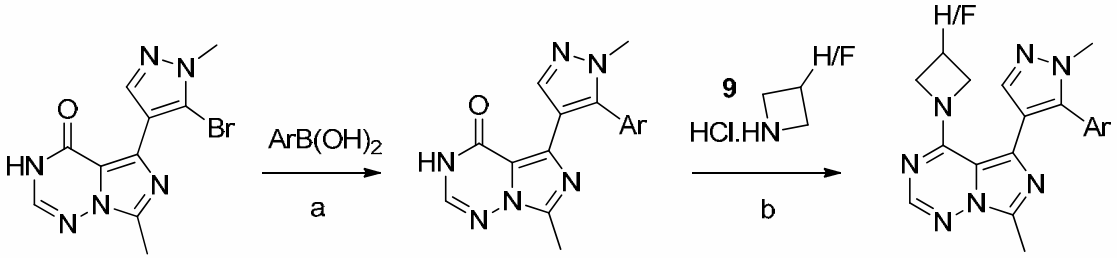
Potential to incorporate ^{11}C via O-methylation:



Potential to incorporate ^{19}F via nucleophilic displacement:



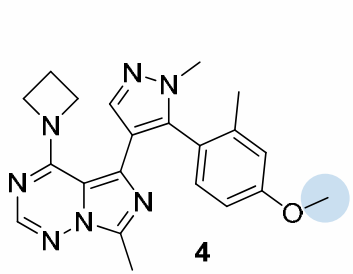
Synthesis of PET analogues **3**-**8**:



Reagents and conditions:
(a) 2 mol % $Pd(PPh_3)_4$, K_3PO_4, EtOH/water, reflux;
(b) 1,2,4-triazole, $POCl_3$, Et_3N, CH_3CN, then Et_3N, **9**, CH_2Cl_2

Experimental properties of PDE2A PET analogues and [^{18}F] synthesis

In-vitro and in-vivo PK properties



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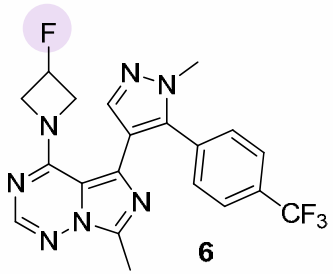
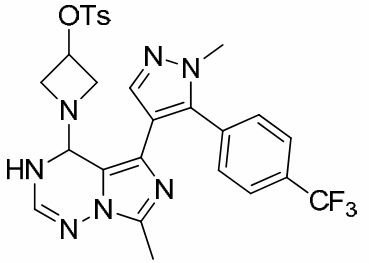


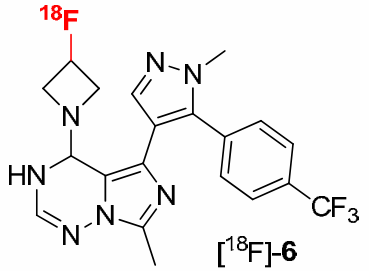
6

	4	6
PDE2 IC_{50} (nM)	0.58	0.53
RRCK AB (10^{-6} cm/s)	42	21
MDR BA/AB	1.23	1.71
rFu_p (rat)	29%	24%
rFu_b (rat)	17%	7.7%
hFu_p (human)	23%	17%
B/P_total (rat)	0.34	1.56
B/P_free (rat)	0.2	0.5

Synthesis of [^{18}F]-6



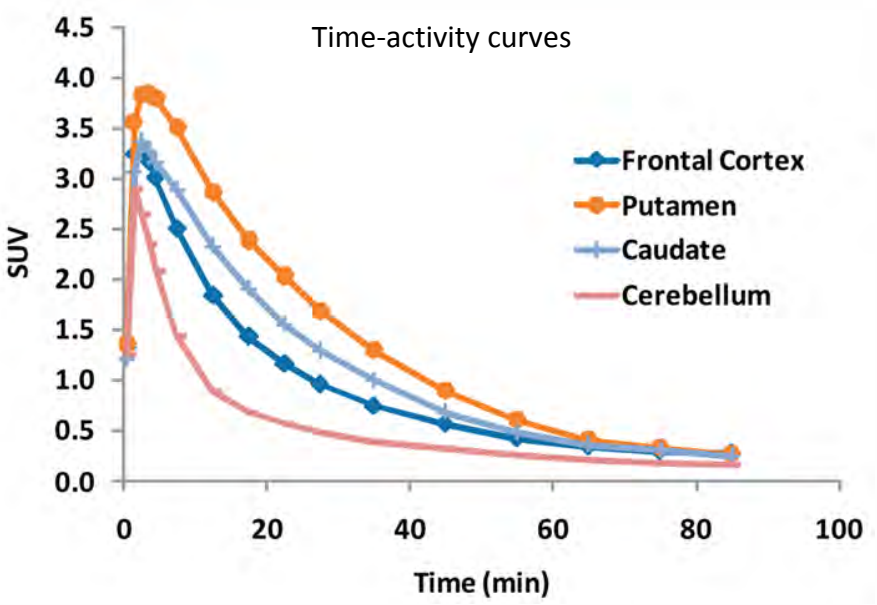
TBA[^{18}F],
t-amyl alcohol,
110 °C, 30 min,



[^{18}F]-**6**

Radiochemical purity: 99.5%
Specific activity: 540 ± 152 GBq/mol
(14592 ± 4095 Ci/mmol)

Baseline time-activity curves and images from [^{18}F]-6 in cyaomolgus monkeys



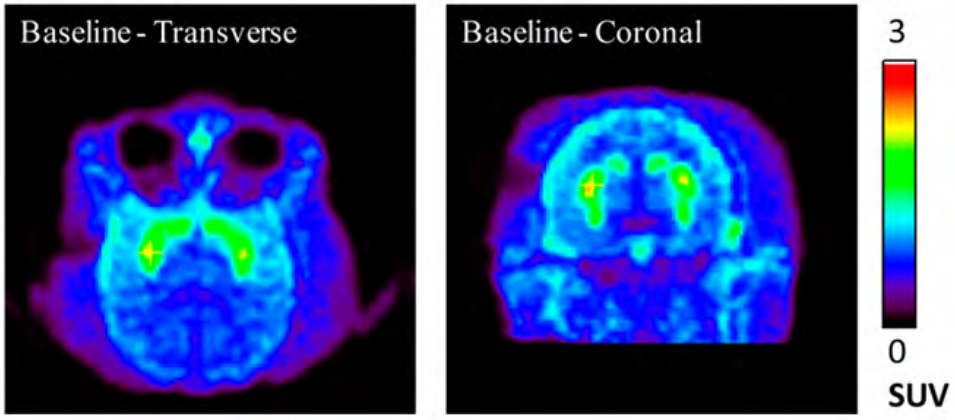
Baseline studies:

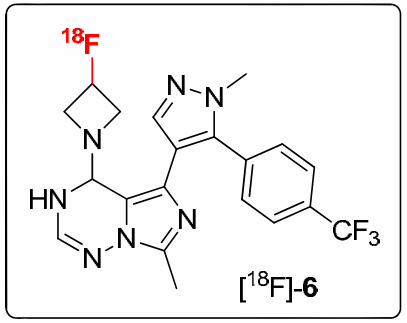
-Demonstrate rapid and high up-take in striatum (putamen and caudate) and low up-take in cerebellum – consistent with distribution pattern of PDE2A

- Cerebellum used as a reference region for NSB: [^{18}F]-**6** has a in-vivo binding potential of 1.51 ± 0.18 (n=2) in striatum

Blocking study:

- Signal in striatum was blocked by a PDE2A inhibitor in a dose-responsive manner → used to measure target occupancy in primates





Reconstructed baseline images (summed between 0-60 mins)

Expanding the synthetic tool box for ^{18}F-labeling

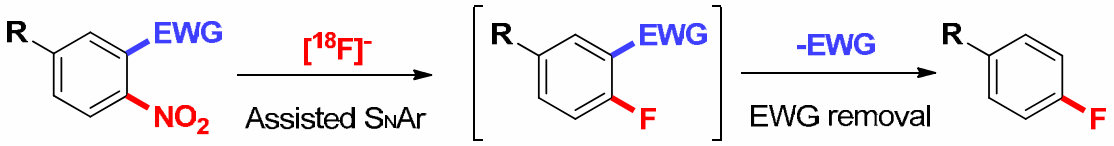
GOAL: Expand the scope of late stage nucleophilic fluorination reactions to unactivated arenes

Criteria:

- Nucleophilic fluorination using no-carrier-added $^{18}F^-$
- Late-stage, fast process compatible with highly functionalized molecules
- Complimentary scope to recent advances (heterocycles, basic amines)
- Practical one-pot protocol suitable for automation

^{18}F fluorination: Ritter *Science* **2011**, *334*, 639, Ross/Coene *J. Am. Chem. Soc.* **2007**, *129*, 8018
Reviews: Littich/Scott. Angew. *Chem. Int. Ed.* **2012**, *51*, 1106. Tredwell/Gouverneur *Angew. Chem. Int. Ed.* **2012**, *51*, 11426.

Our approach:



Nucleophilic fluorination: good precedent with ^{18}F on *electron deficient* aromatics

EWG removal: could we develop a robust and rapid method to remove an accessory EWG?

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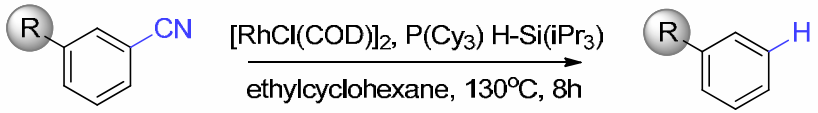
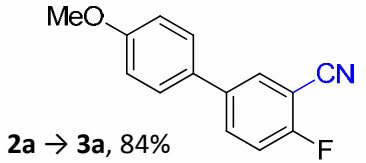
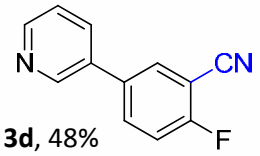
Development of a fast and general decyanation



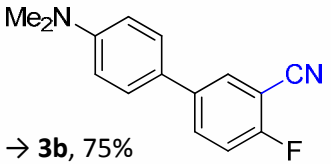
Chatani et. al. *J. Am. Chem. Soc.* **2009**, *131*, 3174



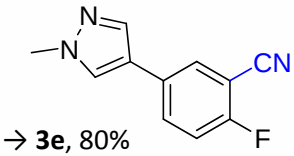
2a → **3a**, 84%



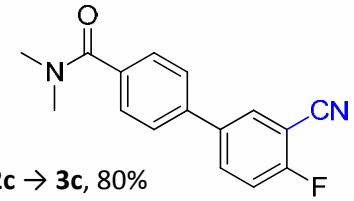
2d → **3d**, 48%



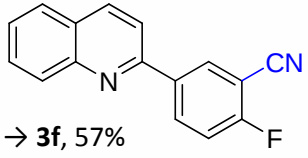
2b → **3b**, 75%



2e → **3e**, 80%



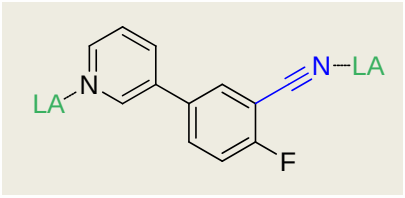
2c → **3c**, 80%

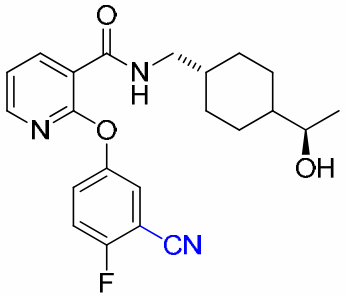


2f → **3f**, 57%

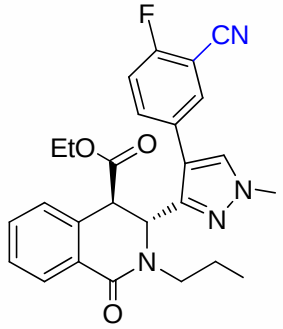
Lewis-acid additive:

- Reduces reaction inhibition
- Reaction acceleration

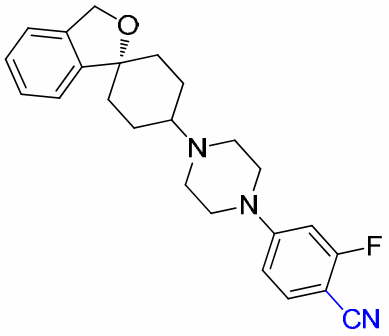




2g→ **3g**, 40%



2h→ **3h**, 69%



2i → **3i**, 60%

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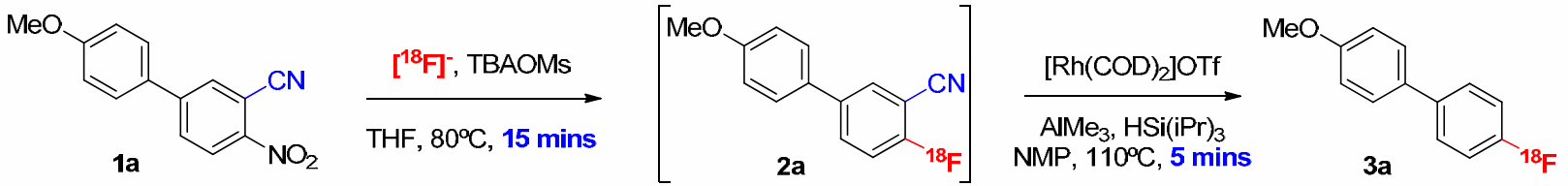
Application of [^{18}F] fluorination/decyanation sequence on model substrate

Radiosynthesis



	[^{18}F] Separation cartridge	Yield	Radiochem purity	Radio-HPLC Yield
Manual	PS-HCO3	57%	99%	99.8%
	QMA	22%	95%	95.0%
Automated Reactor	QMA	RCY = 5-12%, Chemical purity 90% High specific activity (>20,000 Ci/mmol)		

- **Provides a novel and practical [^{18}F]-labelling strategy that is complimentary to the recent advances in late-stage electrophilic fluorination and enables greater flexibility in the design of future PET ligands**

Summary

- PET is a powerful translational tool for preclinical and clinical evaluations of candidate compounds.
  - enables measurement of target occupancy via blocking studies with inhibitors.

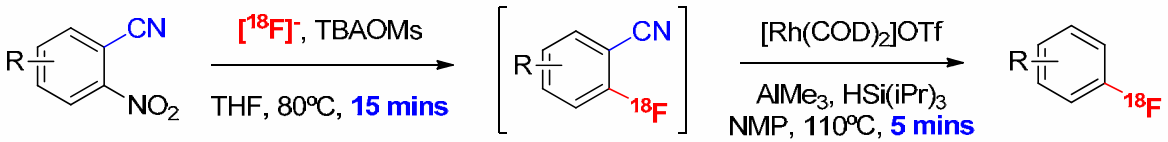
Our Approaches to improving success in PET ligand development:

- A set of preferred design and selection parameters to enable prospective design and prioritization:

Pharmacology	Physicochemical Properties:	Low non-specific binding	Brain Permeability
•Bmax/Kd > 10 •>30-100x Selectivity over other receptors.	•CNS MPO2 > 3 •LogD = 3	• high risk of NSB if cFu_b = 0.05 and cFu_p = 0.05; •cFu_b and cFu_p > 0.05; preferably cFu_p > 0.15.	• RRCK AB > 5 x 10^{-6} cm/sec (moderate to high permeability) • MDR BA/AB = 2.5 (low Pgp liability)

Zhang et al. *J. Med. Chem.*, **2013**, *56*, 4568–4579

- Expanding the synthetic tool box for ^{18}F-labeling through development of a new strategies for late stage fluorination of aromatics o within complex molecules.



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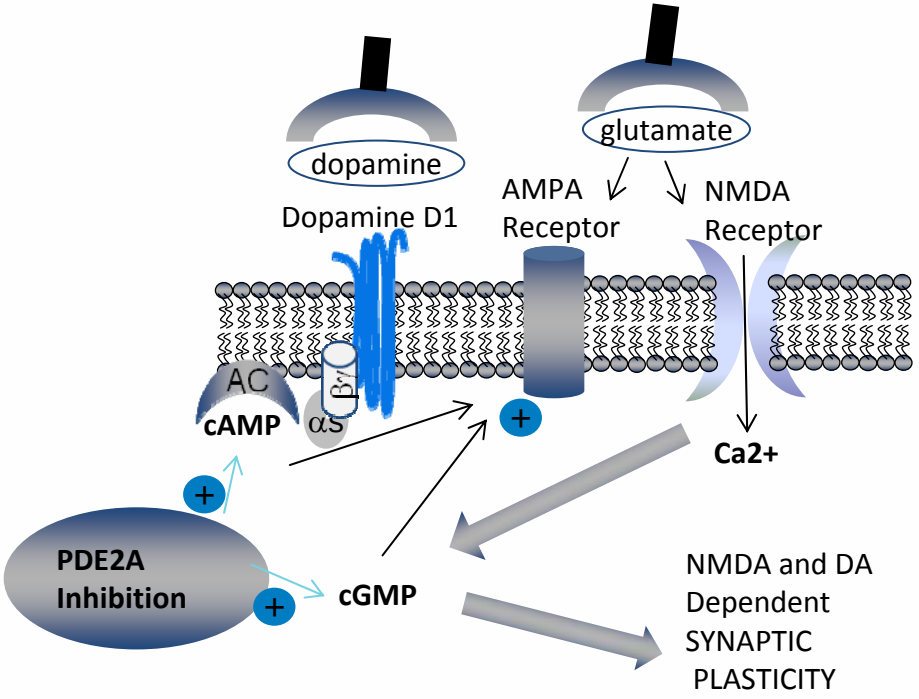
Pfizer WORLDWIDE RESEARCH & DEVELOPMENT
Medicinal Chemistry

BACK-UPS

Identification of a novel PDE2A PET ligand

PDE2A Inhibitors: Positive Amplifiers of NMDA and DA Signaling



PDE2A localization

Stephenson et al. JHC 2009; 57(10):933-49

- High expression in cortex, hippocampus, striatum, medial habenula
- Critical regions for cognition, motivation
- All behaviors impacted in schizophrenia

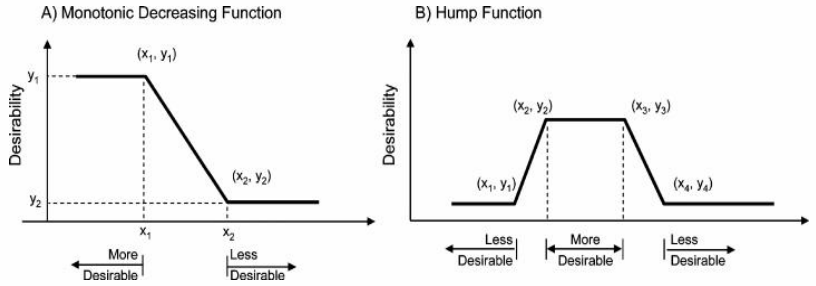
▪ Develop a selective PDE2A PET ligand to serve as a translational tool

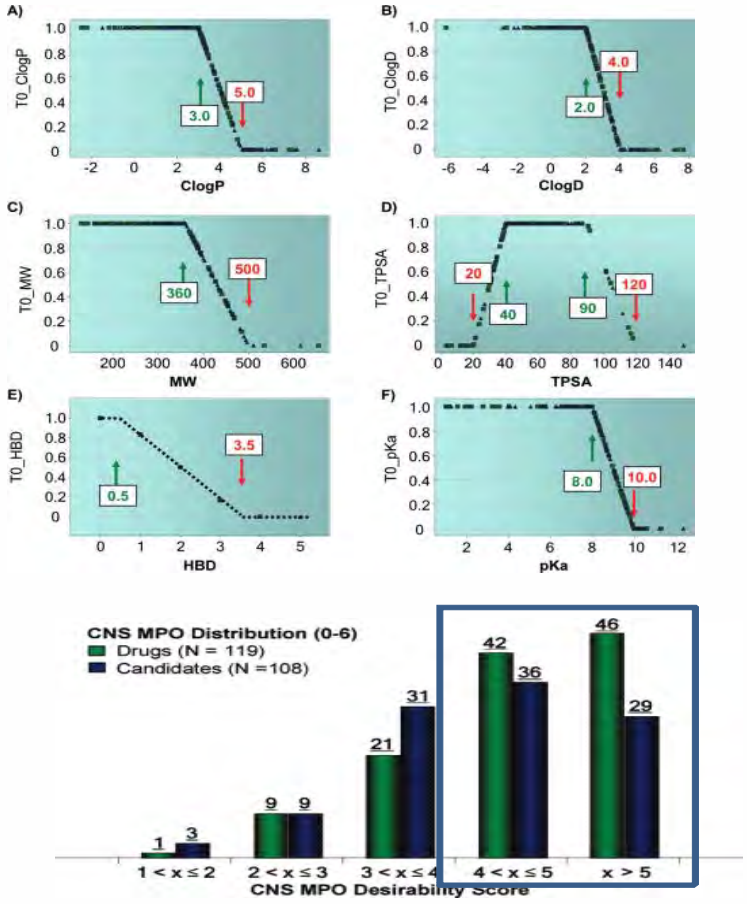
- Pre-clinical pharmacology studies (eg. correlating target occupancy measurements with efficacy end-points for novel PDE-2A inhibitors)
- Clinical evaluation (Translation of RO-PD correlations between species)

TOOLS: CNS MPO – Expand design space by aligning all 6 physiochemical properties and avoiding hard cutoffs

Properties	Transformation (T0)	Weight	CNS MPO [a]		CNS MPO2 [b]	
			More desirable range (T0= 1.0)	Less desirable range (T0= 0.0)	More desirable range (T0= 1.0)	Less desirable range (T0= 0.0)
cLogP	Monotonic decreasing	1.0	cLogP = 3	cLogP > 5	cLogP = 2.8	cLogP > 4.0
cLogD	Monotonic decreasing	1.0	cLogD = 2	cLogD > 4	cLogD = 1.7	cLogD > 2.9
MWt	Monotonic decreasing	1.0	MWt = 360	MWt> 500	MWt = 305.3	MWt > 345.9
tPSA	Hump Function	1.0	40< tPSA = 90	tPSA = 20; tPSA > 120	44.8< tPSA = 63.3	tPSA = 32.3; tPSA > 86.2
HBD	Monotonic decreasing	1.0	HBD = 0.5	HBD > 3.5	HBD = 1	HBD > 2
pKa	Monotonic decreasing	1.0	pKa = 8	pKa > 10	pKa = 7.2	pKa > 9.5

[a] inflection values are defined based on medicinal chemistry experiences and literature sources; [b] CNS MPO2 inflection values are defined by a statistical analysis of physicochemical property distribution of marketed CNS drugs.





▪ ***In Silico* ADME models** (cRRCK, cMDR BA/AB, cFu_b, cFu_p) used in the analysis were statistical models developed based on data points of structurally diverse compounds generated in Pfizer in-house ADME high throughput screening. Experimental data was used in the analysis if available (20/62 PET ligands, 3/15 negative controls).

CNS MPO: desirability inflection points

Physicochemical Properties, Transformed Function Utilized, Weighting, and Parameter Ranges for CNS MPO and CNS PET MPO

			CNS MPO[a]		CNS PET MPO[b]	
properties	transformation (T0)	weight	more desirable range (T0 = 1.0)	less desirable range (T0 = 0.0)	more desirable range (T0 = 1.0)	less desirable range (T0 = 0.0)
ClogP	monotonic decreasing	1.0	ClogP ≤ 3	ClogP > 5	ClogP ≤ 2.8	ClogP > 4.0
ClogD	monotonic decreasing	1.0	ClogD ≤ 2	ClogD > 4	ClogD ≤ 1.7	ClogD > 2.8
MW	monotonic decreasing	1.0	MW ≤ 360	MW > 500	MW ≤ 305.3	MW > 350.5
TPSA	hump function	1.0	40 < TPSA ≤ 90	TPSA ≤ 20; TPSA > 120	44.8 < TPSA ≤ 63.3	TPSA ≤ 32.3; TPSA > 86.2
HBD	monotonic decreasing	1.0	HBD ≤ 0.5	HBD > 3.5	HBD ≤ 1	HBD > 2
pK_a	monotonic decreasing	1.0	pK_a ≤ 8	pK_a > 10	pK_a ≤ 7.2	pK_a > 9.5

CNS PET MPO inflection values are defined by a statistical analysis of the physicochemical properties of 119 marketed CNS drugs.

- Median values and the 75th percentile values are used to define the more desirable and less desirable ranges, respectively, for ClogP, ClogD, MW, HBD, and pKa.
- The more desirable range of TPSA is defined by the median value (44.8) and the 75th percentile value (63.3), while the less desirable range is defined by 25th percentile (32.3) and 90th percentile (86.2) values

How does PET work?

PET-ligands give off thousands of detectable, locatable decays per second
- PET sensitivity gives 1-5mm resolution images.

Use of radioactive tracers is low risk with:
a)High specific activity labels
b)Sensitive scanners (**very low doses** needed)
c)Reasonably **short half-life**

PET isotopes need:
a) Pure '**beta**' decay
b) Low beta energy

Pet nuclide decays in the body by positron emission - emitted positron is not detected directly:

- travels a short distance (5-20mm)
- collides with an electron in the surrounding tissue.
- This annihilation event produces 2 gamma ray photons (g) of 511 ke V that travels 180^{o} to each other.
- Simultaneous detection of g-rays enables approximate location of PET probe in body to be located