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Synthesis, radiolabeling and preliminary in vivo evaluation of [^{18}F]FE-PE2I, a new probe for the dopamine transporter

Magnus Schou [a,*], Carsten Steiger [a], Andrea Varrone [a,b], Denis Guilloteau [c], Christer Halldin [a,b]

[a] Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry Section, Stockholm, Sweden
[b] Stockholm Brain Institute, Stockholm, Sweden
[c] Inserm U619, Université François Rabelais de Tours, France

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ABSTRACT

A new dopamine transporter (DAT) ligand, (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbofluoroethoxy-3β-(4′-methyl-phenyl) nortropane (FE-PE2I, **6**), derived from PE2I (**1**), was prepared and found to be a potent inhibitor of rodent DAT in vitro. Compound **6** was radiolabelled with fluorine-18 ($t_{1/2}$ = 109.8 min) for PET studies in monkeys. In vivo PET measurements showed a regional distribution in brain that corresponds to the known distribution of DAT. This binding was specific, reversible and the kinetics of [^{18}F]**6** binding in brain were faster than for its lead compound, [^{11}C]**1**. The possible presence of a hydroxymethyl-radiometabolite formed by oxidation in the 3β-benzylic position of [^{18}F]**6** warrants further detailed evaluation of the metabolism of [^{18}F]**6**. [^{18}F]**6** is a potential radioligand for imaging DATs in the human brain with PET.

The dopaminergic (DA) reuptake system has since long been of central interest in relation to the pathophysiology of several neuropsychiatric and neurodegenerative disorders as well as being a target for drugs of abuse. Since the first positron emission tomography (PET) imaging study of the DA transporter (DAT) was conducted with [^{11}C]nomifensine in a hemi-parkinsonian patient in 1987,[1] there have been several studies demonstrating the utility of DAT imaging for diagnostic applications. In addition, by using PET and [^{11}C]cocaine, knowledge has been gained in the bio-distribution and pharmacokinetics of this drug inside the living human brain that would not have been obtained otherwise.[2]

Given the high concentration of DAT in brain (B_{max} ~ 200 pmol/g in human putamen),[3] and that the binding potential (BP) of a PET radioligand is proportional to the concentration of target sites (BP = B_{max}/K_d), the DAT is a relatively straight-forward target for radioligand development. Consequently, there are several successful examples of radioligands that enable DAT imaging in vivo, most of which have been derived from cocaine. PE2I (**1**, Fig. 1), FECNT (**2**), FECT (**3**) and β-CIT (**4**) are examples of such radioligands.[4–7] Although radioligands exist that have been used in clinical settings already, none considered optimal for the accurate quantification of DATs is available today. Problems associated with current radioligands are slow kinetics, lack of selectivity and/or problems with blood brain barrier (BBB) permeable radiometabolites.[4,8,9]

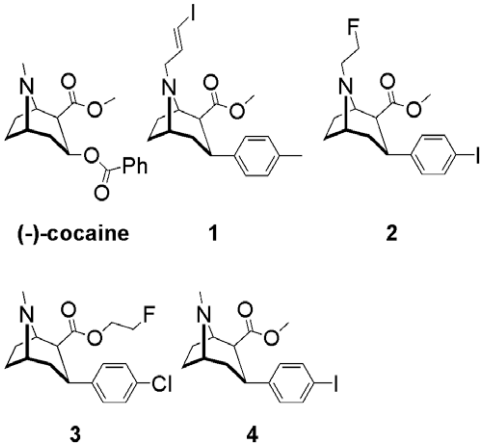

Figure 1. Structures of cocaine and some DAT radioligands developed for PET.[4–7]

In view of our aim to develop an improved DAT radioligand that could overcome some of the abovementioned problems, we decided to use **1** as a lead compound. Compound **1** has excellent pharmacological properties, being a potent (K_i = 17 nM) and selective DAT inhibitor.[10] In addition, [^{11}C]**1** has been used for PET imaging of DATs in both pre-clinical and clinical settings, thereby showing the potential for analogs of [^{11}C]**1** to be useful as PET imaging agents. However, two clear disadvantages also exist for

* Corresponding author. Present address: AstraZeneca R&D Södertälje, Medicinal Chemistry, S-151 85 Södertälje, Sweden.
E-mail address: magnus.schou@astrazeneca.com (M. Schou).

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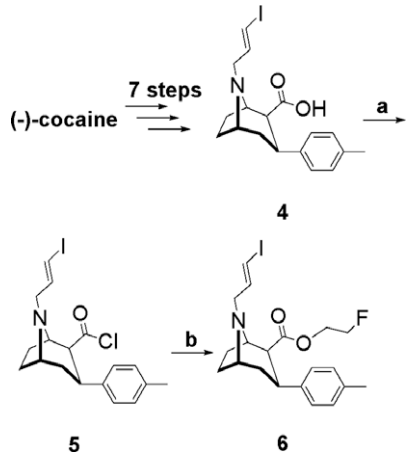
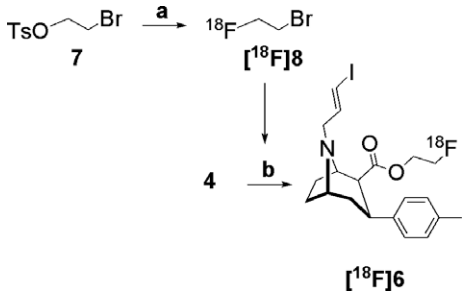
[^{11}C]**1**. These originate in its metabolism and its slow binding kinetics in brain. In a detailed report on the metabolism of [^{11}C]**1**, Shetty et al. showed the presence of two radiometabolites of [^{11}C]**1** inside the rodent brain.[9] These radiometabolites are produced by sequential oxidation of the benzylic position of the 3β-phenyl ring, first to the alcohol and then to the corresponding acid. Radiometabolites in brain pose a threat to the accurate quantification of a PET measurement, since the PET camera merely measures radioactivity in brain and cannot distinguish between chemical entities. It is therefore important to perform radiometabolite measurements and, if possible, introduce the radiolabel in a position of the molecule that prevents formation of BBB permeable radiometabolites.

A potential disadvantage of using **1** as a lead compound for radioligand development is thus that analogs of **1** may show similar benzylic oxidation radiometabolites as those of [^{11}C]**1**. However, Shetty et al. also found only a small level of the 2β-carboxylic acid in the rat (all in urine), suggesting that the ester moiety of **1** is relatively metabolically stable. In the light of these results, we decided to use the 2β-carboxy position for introducing a fluoroethyl group, since this would serve for several purposes. The first being to avoid extensive formation of the BBB permeable product, 2-[^{18}F]fluoroethanol, which was shown in rodent brain during PET studies with [^{18}F]**2** (in that case produced by oxidative N-dealkylation).[8] The second purpose for introducing an ^{18}F-label into an analog of **1**, is that it facilitates imaging at later time-points (due to the longer half-life of ^{18}F ($t_{1/2}$ = 109.8 min, compared to ^{11}C ($t_{1/2}$ = 20.4)), which is of special interest since [^{11}C]**1** displays slow kinetics in the human brain in vivo.[11] In addition, it is also well-known that substitution in the 2β ester position on this scaffold is well tolerated at the DAT (i.e., this moiety can be modified with retention of affinity). Another general advantage with fluorine-18 is its lower positron energy as compared with carbon-11 (650 keV vs 960 keV, respectively), which results in improved spatial resolution of the obtained PET images. This is important when imaging the substantia nigra (SN), which is a small region of the brain responsible for dopamine production in brain and therefore of central interest in research on the DA system.

The synthesis of the target compound **6** is depicted in Scheme 1 and started from naturally occurring cocaine. The first seven steps from cocaine to **4** are well described in the literature.[10,12] Shortly, hydrolysis of the benzoate moiety in cocaine and subsequent elimination of the resultant alcohol in a mixture of methanol and hydrochloric acid produced anhydroecgonine methyl ester. Reacting this unsaturated ester with *p*-tolylmagnesium bromide furnished the Michael addition product, which was demethylated and alkylated with the *E*-isomer of tributyltin propenyl chloride. Iodination and hydrolysis of the methyl ester yielded **4** in 18% overall yield from cocaine.[12]



Scheme 1. Reagents and conditions: (a) $POCl_3$, DCM, rt, 4 h; (b) 2-fluoroethanol, Et_3N, DCM, 0 °C→rt.



Scheme 2. Reagents and conditions: (a) [^{18}F]F^-, K2.2.2, K_2CO_3, *o*-DCB, 135 °C, 10 min; (b) TBAH, DMF, 85 °C, 20 min.

Compound **6** was assayed for affinity at the DAT using competition assays with [^{125}I]PE2I on rodent tissue. As expected, **6** had retained its affinity at rodent DAT (K_i = 12 ± 1.7 nM (n = 3) vs K_i = 16 nM for **1**). To further evaluate the potential **6** as a candidate PET radioligand for imaging DAT in the human brain, we radiolabelled **6** with fluorine-18. The precursor for radiolabelling, [^{18}F]fluoroethyl bromide ([^{18}F]**8**) was prepared from [^{18}F]fluoride ion by adapting a previously described method (Scheme 2).[13] Briefly, bromoethyl tosylate (**7**) in *o*-dichlorobenzene was added to a dried [K2.2.2][^{18}F]fluoride–carbonate complex. After a 10 min reaction, [^{18}F]**8** was distilled off into an ice-cold reaction vessel containing DMF. [^{18}F]**8** was obtained in 38% radiochemical yield (uncorrected for decay) from [^{18}F]fluoride within 45 min after end of bombardment (EOB).

[^{18}F]**6** was obtained by heating an alkaline mixture of **4** and [^{18}F]**8** for 20 min. After completed heating, [^{18}F]**6** was purified by semi-preparative HPLC. After isolation of the product fraction and evaporation of solvents, [^{18}F]**6** was formulated in a mixture of physiologically buffered saline, ethanol and propylene glycol before final sterilization by filtration (0.22 μm Millex® GV filter; Millipore, Sweden). The overall radiochemical yield of [^{18}F]**6** from [^{18}F]fluoride was 7% (uncorrected for decay) and the total synthesis time was 90 min. The radiochemical purity of [^{18}F]**6** was >95% and the specific radioactivity was 113–385 GBq/μmol. The structure of [^{18}F]**6** was also confirmed by comparison of the fragmentation spectra of reference **6** and the carrier associated with[^{18}F]**6** (major fragments, m/z (relative abundance): 394.15 (12%), 366.15 (18%), 248.05 (15%), 166.97 (20%)).

Two PET measurements were conducted in cynomolgus monkeys with [^{18}F]**6** for 180 min each. Monkey 1 underwent one baseline study (59 MBq). Monkey 2 underwent a displacement study with intravenous administration of GBR 12909 (2.5 mg/kg) at 30 min after injection of [^{18}F]**6** (67 MBq). The results are shown in Figure 2 (below).

After injection of [^{18}F]**6**, radioactivity readily entered brain in a high amount, with approximately 540% SUV present in the caudate at 13 min after injection. The rank order for regional radioactivity uptake in brain was caudate > putamen > thalamus ~ midbrain ~ frontal cortex ~ cerebellum. The ratios of radioactivity to the cerebellum peaked at 105 min and were 7.5 and 7.9 for the caudate and putamen, respectively. In the displacement experiment, the binding ratio to cerebellum decreased with 81% (to 1.4) in the caudate and 83% (to 1.3) in the putamen at the end of the PET measurement. Thus, the distribution of radioactivity in brain was reversible, specific and in accordance with the known distribution of DATs in primate brain. Rather surprisingly, [^{18}F]**6** also showed much faster kinetics in brain than [^{11}C]**1**,[7,11] which

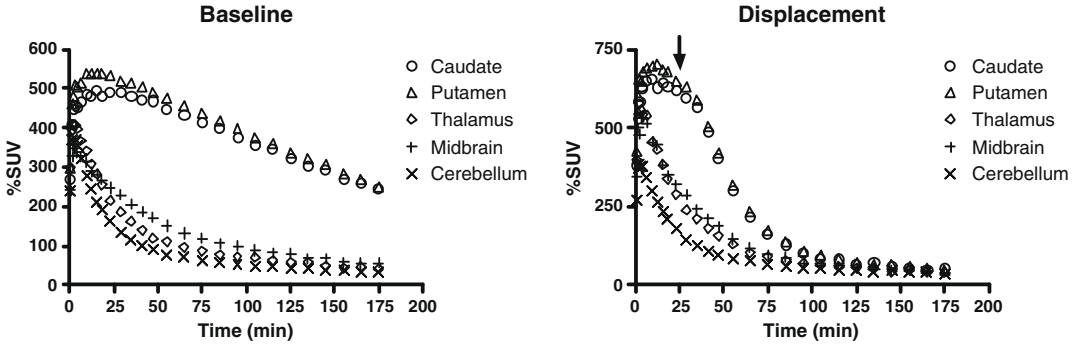


Figure 2. Time–activity curves following injection with [^{18}F]**6**. SUV represents standard uptake values (%injected dose per gram tissue multiplied with body weight). The arrow denotes the administration of the displacement compound, GBR12909.

is of benefit to the patient by reducing the time spent in the PET camera.

The time-course for the metabolism of [^{18}F]**6** was studied by radio-HPLC on acetonitrile extracts from venous monkey plasma.[14] The recovery from the analytical procedure was greater than 91%. Two major radiometabolite fractions were observed, probably corresponding to oxidation products of the 3β-benzylic position (i.e., to the hydroxyl and carboxylic acid derivatives of [^{18}F]**6**) as previously shown for the closely structurally related [^{11}C]**1**.[9] Although it appears that the 3β-benzylic oxidation product is formed to a lower extent as compared after injection of [^{18}F]**6** to after injection of [^{11}C]**1**, the presence of M1 in plasma warrants further detailed evaluation of the metabolites of [^{18}F]**6** by radio-LC and LC–MS/MS. This, as well as a more detailed description of the time-course for metabolite formation in monkeys, will be reported elsewhere.

In summary, a new fluoroethyl analogue of PE2I, compound **6**, was prepared and found to be a potent inhibitor of rodent DAT in vitro. As a candidate radioligand, **6** was radiolabelled with ^{18}F at high specific radioactivity and in good radiochemical yield. The binding of [^{18}F]**6** to DAT was specific, reversible and showed faster kinetics in brain than [^{11}C]**1**. The possible presence of a hydroxymethyl-radiometabolite formed by oxidation in the 3β-benzylic position of [^{18}F]**6** warrants a further detailed evaluation by LC–MS/MS. This will be reported elsewhere.

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