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An improved preparation of [^{18}F]FPBM: A potential serotonin transporter (SERT) imaging agent

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ABSTRACT

Introduction: In vivo positron emission tomography (PET) imaging of the serotonin transporter (SERT) is a valuable tool in drug development and in monitoring brain diseases with altered serotonergic function. We have developed a two-step labeling reaction for the preparation of the high serotonin affinity ligand [^{18}F]FPBM ([^{18}F]2-(2′-((dimethylamino)methyl)-4′-(3-fluoropropoxy)phenylthio)benzenamine, **1**).

Method: To improve and automate the radiolabeling of [^{18}F]FPBM, **1**, an intermediate, [^{18}F]3-fluoropropyltosylate, [^{18}F]**4**, was prepared first, and then it was reacted with the phenol precursor (4-(2-aminophenylthio)-3-((dimethylamino)methyl)phenol, **3**) to afford [^{18}F]FPBM, **1**. To optimize the labeling, this O-alkylation reaction was evaluated under different temperatures, using different bases and varying amounts of precursor **3**. The desired product was obtained after a solid phase extraction (SPE) purification.

Results: This two-step radiolabeling reaction successfully produced the desired [^{18}F]FPBM, **1**, with an excellent radiochemical purity (>95%, n = 8). Radiochemical yields were between 31% and 39% (decay corrected, total time of labeling: 70 min, n = 8). The SPE purification cannot completely remove pseudo-carriers in the final dose of [^{18}F]FPBM, **1**. The concentrations of major pseudo-carriers were measured by UV-HPLC (476–676, 68–95 and 50–71 μg for precursor **3**, *O*-hydroxypropyl and *O*-allyloxy derivatives, **5** and **6**, respectively). To investigate the potential inhibition of SERT binding of these pseudo-carriers, we performed in vitro competition experiments evaluated by autoradiography. Known amounts of 'standard' FPBM, **1**, of the pseudo-carriers, **5** and **6**, were added to the HPLC-purified [^{18}F]**1** dose. The inhibition of 'standard' FPBM, **1**, binding to the SERT binding sites, using monkey brain sections, were measured (EC_{50} = 13, 46, 7.1 and 8.3 nM, respectively for **1**, precursor **3**, O-hydroxypropyl and O-allyloxy derivative of **3**).

Conclusion: An improved radiolabeling method by a SPE purification for preparation of [^{18}F]FPBM, **1**, was developed. The results suggest that it is feasible to use this labeling method to prepare [^{18}F]FPBM, **1**, without affecting in vivo SERT binding.

1. Introduction

The serotonin transporter (SERT), along with transporters for dopamine (DAT) and noradrenaline (NET), is one of the three-monoamine reuptake transporters in the brain. Due to its central role in maintaining the balance of the serotonergic system, SERT has been associated with many different functions in healthy people and in CNS disorders [1–3]. Altered SERT function has been implicated in a variety of psychiatric diseases, such as major depressive disorder, obsessive–compulsive disorder, alcohol dependence, and others [4–7]. Thus, SERT is a major target for drug therapy against depression, as well as other psychiatric disorders. In vivo positron emission tomography (PET) imaging of SERT binding sites in the brain may be useful as a method for probing pathophysiological and therapeutic mechanisms in psychiatric disorders, such as major depression [8–10], obsessive-

Abbreviations: DASB, (N,N-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine; (+)-McN5652, trans-1,2,3,5,6,10-<beta>-hexahydro-6-[4-(methylthio)phenyl-[pyrrolo-[2,1-<alpha>]isoquinoline; PET, positron emission tomography; SPECT, single photon emission computed tomography; nor-<beta>-CIT, 2<beta>-carbomethoxy-3<beta>-(4-iodophenyl); SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor; St, striatum; Th, thalamus; 5-HT, serotonin; ADAM, 2-((2-((dimethylamino)methyl)- phenyl)thio)-5-iodophenylamine; FPBM, 2-(2′-((dimethylamino)methyl)-4′-(3-fluoropropoxy)phenylthio)benzenamine; Cd, Caudate nucleus; CER, Cerebellum; DM, Dorsomedial hypothalamic nucleus; DR, Dorsal raphe nucleus; GP, Globus pallidus; ic, Internal capsule; HIP, Hippocampus; MD, Mediodorsal thalamic nucleus; Me, Medial amygdaloid nucleus; SN, Substantia nigra; VL, Ventrolateral thalamic nucleus.

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compulsive disorder [11], eating disorders [12], and addiction [13]. The imaging study may also be employed for examining neurodegenerative diseases (such as Alzheimer's disease and Parkinson's disease) that involve the serotonergic system [14–18].

A number of SERT ligands for in vivo imaging have been developed [19–34]. Ligands with a core structure of diarylsulfide showed the most promising results as in vivo imaging agents (Fig. 1). Currently, [^{11}C]DASB is the most frequently used PET SERT imaging agent because of its good selectivity, high reproducibility, and simple quantification [6]. [^{11}C]DASB is a ^{11}C-labeled radiotracer that is limited by the short physical half-life ($T_{1/2}$ = 20 min), moderate cortical test–retest reliability, and the lack of competition with endogenous serotonin. An [^{18}F]SERT imaging agent may be valuable because of a longer physical half-life ($T_{1/2}$ = 110 min) and better opportunity for a widespread application. Significant efforts have been made to develop such a ^{18}F-labeled radiotracer for SERT imaging [29,34–39].

Currently, there are two promising ^{18}F-labeled ligands, [^{18}F]ADAM (N,N-dimethyl-2-(2-amino-4-[^{18}F]fluorophenylthio) benzylamine) [33] and (2-(2′-((dimethylamino)methyl)-4′-(3-[^{18}F]-fluoropropoxy) phenylthio)benzenamine), [^{18}F]FPBM, **1**, [14,40]for imaging SERT (Fig. 1). These ligands have been successfully tested in rodents and monkeys. Results from the first human study of [^{18}F]ADAM [33] showed that it is safe and effective. The regional specific uptake in the human brain correlated well with the known distribution of SERT. The optimal imaging time (about 120 min) was a bit long but acceptable for routine clinical use. One major drawback of using [^{18}F]ADAM as a SERT imaging agent, is its relatively low and variable radiochemical yield (1.5–14.8%, EOS). In addition, the preparation requires a high-performance liquid chromatography (HPLC) purification step, which is time consuming.

Recently, [^{18}F]FPBM, **1**, has been shown to possess a high selective binding (K_i = 0.38 nM), high brain uptake (0.99% dose/g at 2 min postinjection), and an excellent in vivo target-to-nontarget ratio (7.7 at 120 min post injection) [14,21,40]. Previously, the labeling of this diarylsulfide was done by a nucleophilic fluorination with K[^{18}F]F/$K_{2.2.2}$ via precursor **2**, followed by the reduction of the nitro group to an amine group (Scheme 1). The desired product, [^{18}F]FPBM, **1**, was further purified by a high-performance liquid chromatography (HPLC) and solid phase extraction (SPE). This radiolabeling method also suffered, similarly as [^{18}F]ADAM, from low and variable radiochemistry yield [21]. Reported herein is an improved radiolabeling method for the preparation of [^{18}F]FPBM, **1**, with different precursor and through the use of a SPE purification.

2. Materials and methods

2.1. General

All reagents and solvents were purchased commercially (Aldrich, Acros and Alfa Co) and were used without further purification, unless otherwise indicated. Reactions of non-radioactive chemical reactions were monitored by a thin-layer chromatography (TLC) analysis with pre-coated plates of silica gel 60 F_{254}. Electrospray ionization mass spectra were recorded with LC MSD TOF, Agilent Technologies. [^{18}F] fluoride aqueous solution was purchased from IBA (IBA Molecular North America, Inc.). Solid-phase extraction cartridges (SEP Pak® Light QMA, Oasis® HLB 3 cc) were obtained from Waters (Milford, MA, USA).

2.2. Chemistry

4-(2-aminophenylthio)-3-((dimethylamino)methyl)phenol (precursor **3** or compound **3**) and 2-(2′-((dimethylamino)methyl)-4′-(3-fluoropropoxy) phenylthio)benzenamine (FPBM, **1**), as well as the pseudo-carriers **5** (O-allyloxy derivative of **3**) and **6** (O-hydroxypropyl derivative of **3**) were synthesized according to methods previously reported [21,40,41] (Scheme 1).

2-(4-(allyloxy)-2((dimethylamino)methyl)phenylthio)benzenamine, **5**: ^{1}H NMR (400 MHz, δ ppm) 2.33 (s, 6H), 3.59 (s, 2H), 4.45–4.52 (m, 2H), 5.37 (d, J = 1.6 Hz, 1H), 5.42 (d, J = 2.6 Hz, 1H), 5.60–6.08 (m, 1H), 6.68–6.72 (m, 3H), 6.93–6.96 (m, 2H), 7.13–7.37 (m, 1H), 7.39 (d, J = 0.8 Hz, 1H). MS m/z: 315.1526 ($M + H^+$).

3-(4-(2-aminophenylthio)-3-((dimethylamino)methyl)phenoxy)-propan-1-ol, **6**: ^{1}H NMR (400 MHz, δ ppm) 1.34–1.41 (m, 3H), 2.00–2.06 (m, 2H), 2.33 (s, 6H), 3.01–3.08 (m, 2H), 3.59 (s, 2H), 3.85 (t, J = 5.8 Hz, 2H), 4.10 (t, J = 5.6 Hz, 2H), 6.67–6.71 (m, 3H), 6.90–6.95 (m, 2H), 7.10–7.17 (m, 1H), 7.37–7.41 (m, 1H). MS m/z: 333.1678 ($M + H^+$).

2.3. Radiolabeling of [^{18}F]**1** (Scheme 1)

2.3.1. Preparation of [^{18}F]KF/ Kryptofix [2.2.2]

The [^{18}F]fluoride (10–20 mCi) produced by cyclotron, using an ^{18}O(p, n)^{18}F reaction, was trapped on a Sep Pak® Light QMA cartridge. The activity was eluted with 0.65 mL solution (0.60 mL acetonitrile and 0.05 mL water solution containing 7.2 mg Kryptofix [2.2.2] ($K_{2.2.2}$) and 1.3 mg potassium carbonate) into a 10 mL test tube. The resulting solution was blown to dryness at 90 °C under a stream of argon. The residual of K[^{18}F]F/Kryptofix [2.2.2] solution was further azeotropically dried (twice with 2 mL anhydrous acetonitrile under a stream of argon).

2.3.2. Preparation of [^{18}F]3-fluopropyltosylate, [^{18}F]**4**.

2.0 mg 1,3-propanediol-di-p-tosylate were dissolved in 1 mL acetonitrile. This solution was added to the dried [^{18}F]KF/$K_{2.2.2}$ prepared above. The mixture was heated at 90 °C for 5 min. After adding 5 mL water, the resulting mixture was loaded on an Oasis® HLB 3 cc cartridge (preconditioned with 10 mL ethanol and 10 mL water). The solution was pushed through and the cartridge was washed with 5 mL water. The desired [^{18}F]3-fluoropropyltosylate ([^{18}F]**4**) was eluted with 1.5 mL acetonitrile.

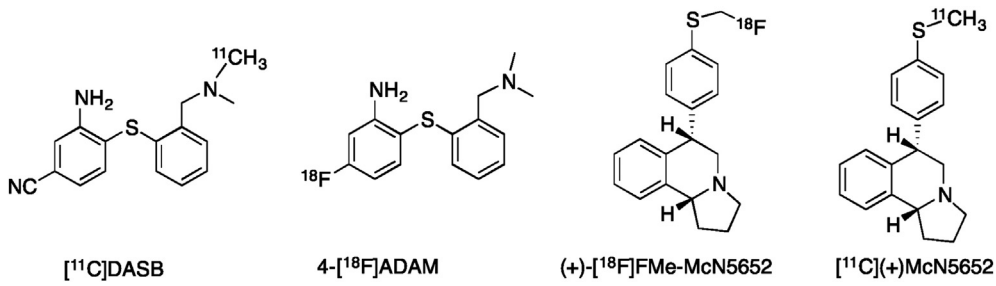

Fig. 1. Chemical structures of several known imaging agents for serotonin transporter (SERT).