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Research Article

Synthesis and preliminary *in vivo* evaluation of 4-[^{18}F]fluoro-*N*-{2-[4-(6-trifluoromethylpyridin-2-yl) piperazin-1-yl]ethyl}benzamide, a potential PET radioligand for the 5-HT$_{1A}$ receptor

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Summary

4-Fluoro-*N*-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}benzamide is a full 5-HT$_{1A}$ agonist with high affinity ($pK_i = 9.3$), selectivity and a $c\log P$ of 3.045. The corresponding PET radioligand 4-[^{18}F]fluoro-*N*-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}benzamide was synthesized by nucleophilic aromatic substitution on the nitro precursor. The fluorinating agent K[^{18}F]F/Kryptofix 2.2.2 was both dried (9 min, 700 W) and incorporated in the precursor (5 min, 700 W) using a commercially available microwave oven. In a total synthesis time of 60 min, an overall radiochemical yield of 18% (SD = 5, $n = 7$, EOS) was obtained. Radiochemical purity was always higher than 99% and specific activity always higher than 81.4 GBq/μmol (2.2 Ci/μmol). Initial brain uptake in mice was 2.19% ID (5.47% ID/g, 2 min) but decreased rapidly (0.17% ID, 0.45% ID/g (60 min)). During the first 20 min *p.i.*, radioactivity concentration of the brain was significantly higher than that of blood demonstrating good brain entry of the tracer.

Key Words: Org 13063; 5-HT$_{1A}$ agonist; ^{18}F labelling; biodistribution study

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Introduction

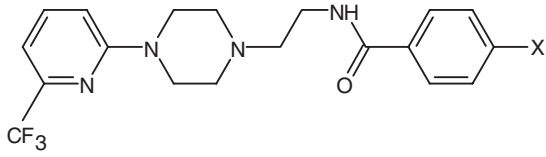
The neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) plays an important role in a variety of physiological, behavioral and cognitive functions e.g. gastrointestinal motility, cardiovascular function, pain perception, thermoregulation, sleep, mood and sexual behavior. Impairments of the serotonergic functions have been implicated in the etiology of a variety of mental disorders including psychotic disorders, mood and anxiety disorders (obsessive compulsive disorder, panic disorder, phobia, etc.) schizophrenia, sleep disorders and dementia.[1–3]

The early discovery of 8-OH-DPAT (8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)-tetraline) and buspirone as 5-HT_{1A} (partial) agonists, makes this 5-HT receptor subtype the most intensively studied. Clinically, it is meant to be involved in the pathogenesis of several neuropsychiatric disorders e.g. anxiety and depression, disorders in the regulation of aggression and substance abuse disorders.[3,4]

The nuclear imaging modalities PET (positron emission tomography) and SPECT (single photon emission computed tomography) offer the unique opportunity to study *in vivo* and non-invasively, the 5-HT_{1A} receptor, using a selective radioligand. At the moment, only 5-HT_{1A} receptor PET tracers are available with the lead component [carbonyl-^{11}C]WAY100635 (*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexane[^{11}C]carboxamide, $pK_i = 9.1$[5]) which is capable of delineating the 5-HT_{1A} receptor in human brain.[6] [Carbonyl-^{11}C]desmethyl-WAY100635 ([^{11}C]DWAY, *N*-{2-[4-(2-hydroxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexane[^{11}C]-carboxamide, $IC_{50} = 1.4$ nM[7]) seems to possess even better *in vivo* properties as demonstrated by its significantly higher brain radioactivity signal providing improved imaging statistics, advantages in biomathematic modeling and the preclusion of [^{11}C]-DWAY as a metabolite interfering with PET measurements.[8]

Because of the better nuclear properties of the ^{18}F isotope and the more reliable radiosynthetic procedures, ^{18}F labelled WAY100635 analogs were rapidly developed with [^{18}F]MPPF ([^{18}F]FBWAY, 4-[^{18}F]fluoro-*N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide, $pK_i = 8.5$[5]) as most important example.[9–11] The lower affinity of MPPF for the 5-HT_{1A} receptor in comparison to WAY100635 could make this ligand sensitive to endogenous serotonin concentration which could enable us to detect differences in serotonin release.[11]

A completely different structure, based on 8-OH-DPAT, is found in the antagonist [^{11}C]NAD299 ((R)-3-*N*,*N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-[^{11}C]carboxamide hydrogen (2R,3R)-tartrate monohydrate, $pK_i = 9.2$[12]) that was shown to be able to delineate the 5-HT_{1A} receptor in non-human primates.[12–14]



X=F 1.F X=I 1.I

X=^{18}F 1.^{18}F X=^{123}I 1.^{123}I

Figure 1. Chemical structure of 1.F (Org 13063), 1.^{18}F, 1.I and 1.^{123}I

Org 13063 or 4-fluoro-*N*-{2-[4-(6-trifluoromethyl-pyridin-2-yl)piperazin-1-yl]ethyl}benzamide (1.F, Figure 1) is a full 5-HT$_{1A}$ agonist with high affinity ($pK_i = 9.3$) and selectivity (pK_i: 5-HT$_{1B}$ = 5.4, 5-HT$_{2A}$ = 6.27, 5-HT$_{2C}$ = 6.07, 5-HT$_6$ = 5.53, 5-HT$_7$ = 6.72, SERT < 5.1, D_1 < 5.2, D_{2a} = 5.93, D_3 = 7.05, D_4 = 8.57, DAT < 5.1, α_1 = 6.5, α_{2A} = 5.62, α_{2B} = 5.5, α_{2C} = 5.34, NET < 5.0, M_1 < 5.2, M_2 < 5.2, M_3 < 5.18, M_4 < 5.44, ORL-1 < 5.28, μ = 5.64, κ = 5.31, δ < 5.39).[15] Lipophilicity was calculated to be 3.045.[†] Its iodine derivative (1.I, Figure 1) was found to have lower *in vitro* affinity ($pK_i = 8.2$) without loss of selectivity[15] and was considerably more lipophilic ($c\log P = 3.879$[†]). Biodistribution studies of its radioiodinated analogue (1.^{123}I, Figure 1) were performed in rats leading to a 0.22% ID in the brain at 5 min *p.i.* At none of the selected time points, radioactivity concentration of brain was significantly higher than that of blood. Furthermore, in rabbits, the radioactivity distribution did not reflect the regional distribution of the 5-HT$_{1A}$ receptor in the brain nor could it be blocked by 5-HT$_{1A}$ selective ligands.[16,17]

The imaging of G-protein coupled receptors (GPCR) with agonist radioligands has been very controversial. So far, no agonist or partial agonist has yielded significant specific labelling of the 5-HT$_{1A}$ receptor *in vivo* despite numerous efforts e.g. [^{11}C]Org 13052,[18] [^{11}C]LY274601[19] and 8{{3-[4-(2-[^{11}C]methoxyphenyl)piperazin-1-yl]-2-hydroxypropyl}oxy}thiochroman.[20] [^{11}C]Org 13052 ($pK_i = 10$) is a partial agonist with low intrinsic activity (IA = 0.2). Brain uptake of radioactivity in rat was rather low (0.8% ID/g between 10 and 30 min), regional differences were minor and a spatially uniform reduction in the amount of ^{11}C was observed after treatment of animals with 8-OH-DPAT.[18,21] [^{11}C]LY274601 ($pK_i = 9.2$) is a full agonist with high blood-brain-barrier penetration in mice (6.8% ID, 30 min). However hippocampus/cerebellum ratios were very poor ($\pm$ 1.2) and uptake in target areas was hardly affected after pretreatment with 8-OH-DPAT. The radioligand was metabolized quickly with a half life of

[†] Calculated with Biobyte $c\log P$ 4.0.

15 min.[19] 8{{3-[4-(2-[^{11}C]methoxyphenyl)piperazin-1-yl]-2-hydroxypropyl}oxy}thiochroman ($pK_i = 8.3$) is a partial agonist and was *in vivo* evaluated in rat and cat. Results showed low accumulation in the brain dominated by non-specific binding.[20]

It has been postulated that agonists only bind to receptors that are coupled to a G-protein (high affinity state) which represent only a variable fraction of the total receptor amount available for binding of antagonists. Agonist radioligands require therefore a higher affinity (smaller K_d) than antagonists to achieve equal binding potential (B_{max}/K_d) in receptor-containing areas of the brain. In addition, the binding of agonists is generally transient, since agonist binding converts the receptor into a G-protein dissociated conformational state that usually has low affinity for the agonist. Nevertheless, an effective agonist radioligand for brain 5-HT_{1A} receptors would be a useful tool for the selective investigation of the functional GPCR since it is likely that, in certain diseases, only the high affinity state GPCR is altered.[21,22]

We hereby report the synthesis and *in vivo* evaluation in mice of 4-[^{18}F]fluoro-*N*-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}benzamide (1.^{18}F, Figure 1).

Results and discussion

The precursor 1.NO_2 was synthesized (Figure 2) through a four-step synthesis starting from 2-chloro-6-trifluoromethylpyridine (2). Coupling of this pyridine derivative (2) with piperazine, followed by a Gabriel synthesis, gave the primary ethylamine (5). The latter was coupled with the appropriate benzoylchloride to give the nitro (1.NO_2) derivative of Org 13063. Overall

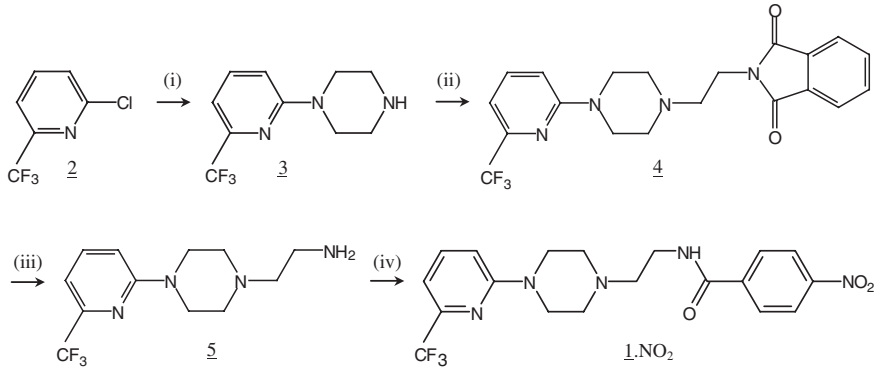


Figure 2. Synthesis of the precursor compound (i) piperazine, reflux, 22 h (ii) 2-Br-ethylphtaalimide, Na_2CO_3, 80°C, 3 h (iii) 1. hydrazine hydrate, reflux, 3 h; 2. HCl, reflux, 4 h (iv) 4-NO_2-benzoylchloride, TEA, RT, 3 h

chemical yield of this precursor synthesis was 8% and resulted in chemically pure, brown crystals.

4-[^{18}F]Fluoro-*N*-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}-benzamide (1.^{18}F) was synthesized by nucleophilic aromatic substitution on 1.NO_2 with the fluorinating agent K[^{18}F]F/Kryptofix 2.2.2 (K2.2.2) (Figure 3). Both drying and incorporation of [^{18}F]F$^-$ were initially performed by conventional heating. The use of aluminium heating blocks for the azeotropic distillation process (3×1 ml CH_3CN, 3×10 min, 110°C, He flow) and the nucleophilic labelling reaction (30 min, 150°C) rendered low radiochemical yields (8% (SD = 3, $n = 3$)) and long production times (105 min). Therefore, the possible benefit of microwave heating was examined. The evaporation process of the azeotropic distillations originally was difficult to control (bumping) resulting in a loss of radioactivity of up to 30%. Optimization of the reaction vial position in the microwave oven, power and time of heating and flow rate of the He gas was performed taking into account that the first two parameters (vial position and microwave power) were to be compatible with the subsequent labelling reaction. This resulted in the preparation of K[^{18}F]F/K2.2.2 in a vial placed against the door of the microwave oven working at 700 W within 9 min ($P_{He} = 0.2$ bar). Under these circumstances, the subsequent radiosynthesis only took 5 min giving a radiochemical yield of 42% (SD = 3, $n = 7$).

The ^{18}F labelling of a molecule containing a trifluoromethyl group could pose problems because of the potential isotopic fluoride exchange. The possible incorporation of ^{18}F in the CF_3 group of 1.NO_2 was examined by performing a radiosynthesis using *N*-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}benzamide as precursor in stead of 1.NO_2 under the exact same labelling conditions as described above. This radiosynthesis did not

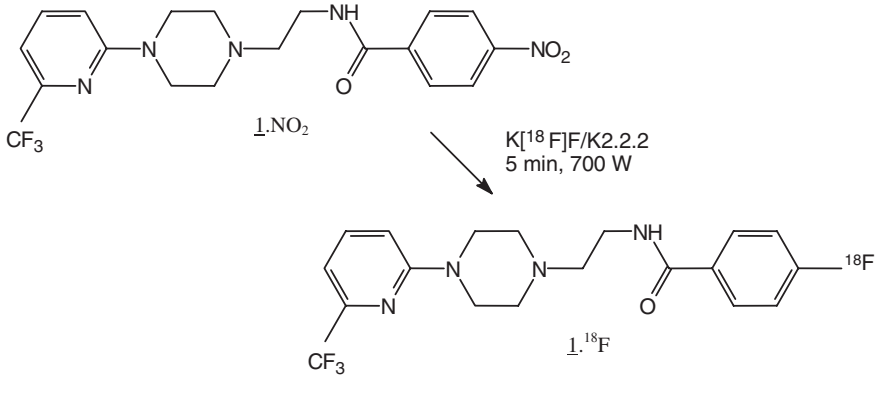


Figure 3. Radiosynthesis of 1.^{18}F

result in any labelled products hereby excluding the possibility of labelling the CF_3 moiety under our labelling conditions. The exchange of the NO_2 group of the precursor 1.NO_2 for a ^{19}F originating from the large excess of precursor CF_3 cannot be excluded but cannot be too extensive seeing the relatively good specific activity of the produced tracer (SA = 81.4 GBq/μmol).

The use of DMSO as reaction medium makes it impossible to directly inject the crude reaction mixture onto the HPLC system. Classically, this problem is solved by performing a solid phase extraction redissolving precursor and tracer compound in a HPLC compatible solvent. We solved this problem simply by diluting the reaction mixture with cold, acidic H_2O. This H_2O was acidified (pH = 2.2) to prevent precipitation of the precursor and cooled in an ice bath to prevent degradation of the tracer under acidic aqueous circumstances. This dilution-cooling step did not introduce an extra operation in the synthesis protocol and was less time consuming and less expensive than the traditional solid phase extraction.

The large excess of precursor compared to the fluorinating agent K[^{18}F]F/K2.2.2 is used to drive this type of reaction to completion. However, this seriously hampered the subsequent HPLC separation of precursor and tracer compound. Ideally, the tracer should elute before its precursor to avoid contamination of the tracer with small amounts of precursor. Several eluentia were tested (NaOAc/EtOH, KH_2PO_4/EtOH, NaOAc/CH_3CN, KH_2PO_4/THF) on a Symmetry Prep column but only the use of the tertiary eluent NaOAc(0.05 M, pH = 5)/MeOH/THF (55/28/17) at 5 ml/min combined speed, good resolution and elution of 1.^{18}F (t_R = 29 min) before 1.NO_2 (t_R = 35 min). This semi-preparative HPLC set up made radiochemical purity higher than 99%. Specific activity was always higher than 81.4 GBq/μmol (2.2 Ci/μmol). In a typical production, 4.07 GBq (110 mCi) of injectable tracer solution was produced starting from 22.2 GBq (600 mCi) within 60 min (overall radiochemical yield = 18% (SD = 5, n = 7, EOS)). Stability tests showed that the tracer remained more than 98% radiochemically pure during the first 24 h after production.

Biodistribution studies were performed in mice leading to a 2.19% ID (5.47% ID/g) in the brain at 2 min *p.i.* decreasing rapidly afterwards (0.17% ID, 0.45% ID/g (60 min)). During the first 20 min *p.i.*, the radioactivity concentration (% ID/g) of the brain was significantly higher than that of blood (Table 1). Brain to blood ratios turned out to be much better for 1.^{18}F than for 1.^{123}I, probably because of its lower lipophilicity ($c\log P$ (1.^{18}F) = 3.045 versus $c\log P$ (1.^{123}I) = 3.879). Further experiments are in progress to investigate the selective and specific binding of the tracer to the 5-HT_{1A} receptor in the brain.

Table 1. Average %ID/g tissue and standard deviations as a function of time (min)

		Time (min)								
Tissue		1	2	5	10	20	40	60	120	180
Blood		3.293	2.546	1.715	1.515	1.189	0.599	0.538	0.363	0.333
	SD	0.649	0.355	0.112	0.324	0.152	0.126	0.201	0.007	0.061
Brain		6.766	5.468	3.944	2.900	1.533	0.561	0.452	0.328	0.266
	SD	1.529	1.018	1.059	0.603	0.054	0.172	0.154	0.111	0.087

Experimental

Material and methods

Org 13063 was obtained from Organon NV (The Netherlands). Chemicals were purchased from Sigma-Aldrich-Fluka (Belgium), Fluorochem (United Kingdom) or Merck (Belgium) and were of the highest available purity. They were used without further purification. He gas (Alphagaz He®) was purchased from Air Liquid (Belgium) and was dried over molecular sieves (3 Å). H_2O was deionized with a Milli-Q water system (Millipore). [^{18}O]H_2O (95 + %) was purchased from Campro Scientific (The Netherlands).

Chemical reactions were monitored by TLC using coated silica gel plates (Polygram SIL G/UV_{254} — Machery-Nagel) and mobile phases as mentioned in the text. The different spots were visualized using either UV light and/or ninhydrine (0.2% in EtOH). Preparative TLC was performed using coated silica glass plates (SIL G-200 UV_{254} (2 mm) — Machery-Nagel) and mobile phases as mentioned. Column chromatography was performed with silica gel (50–200 μm, Süd Chemie) and mobile phases as mentioned in the text.

^{1}H NMR spectra were obtained on a 500 MHz spectrometer (Bruker, Accuspec 3000). Chemical shifts are reported in ppm units downfield from internal $(CH_3)_4Si$. Electrospray Ionisation Mass Spectrometry (ESI MS) was performed on a Micromass ZMD (Waters, Belgium). Exact mass measurements were performed on a quadrupole-time of flight mass spectrometer (Q-Tof-2, Micromass, UK) equipped with a standard electrospray ionization (ESI) interface (Rega Institute, Belgium).

For microwave heating, a commercially available oven (Samsung M935, 1000 W) was used. Borosilicate vials (Maxi-vial, 10 ml, Alltech, Belgium) equipped with screw caps and TFE/silicone liners were used in the microwave experiments.

Purification of the tracer was performed by semi-preparative HPLC using a Symmetry Prep C18 column (Waters, 300 × 7.8 mm, 7 μm) and NaOAc (0.05 M, pH = 5)/MeOH/THF: 55/28/17 at a flow rate of 5 ml/min. Analytical HPLC was performed using a Symmetry C18 column (Waters, 150 × 4.6 mm,

5 μm) and NaOAc (0.05 M, pH = 4.5)/MeOH/THF: 62/23/18. All HPLC runs were carried out making use of a Waters 510 pump, a UV/VIS detector at $\lambda = 254$ nm (PU 4110 UV/VIS — Philips) and a NaI(Tl) detector (Bicron Frisktech®, probe 1 × 1 in). Radiochromatograms were recorded on a two channel integrator (C-R5A Chromatopac, Shimadzu). C18 Sep-Pak® cartridges were purchased from Waters Corporation.

Radioactivity measurements were performed on a single channel NaI(Tl) detector (Canberra Packard, type 2007P, 3 × 3 in) or on a Cobra B5005 auto gamma (Canberra Packard).

Synthesis of 1-(6-trifluoromethylpyridin-2-yl)piperazine (3)

Piperazine (2.871 g, 33 mmol, 99%) was dissolved in EtOH (20 ml, pa) and brought to reflux. A solution of 2-chloro-6-trifluoromethylpyridine (2, 1.997 g, 11 mmol) in EtOH (10 ml, pa) was added slowly. The stirred reaction mixture was refluxed and monitored with TLC (CH_2Cl_2/CH_3OH/HOAc: 50/10/3). After 22 h, the reaction was stopped, the solvent was evaporated and H_2O (100 ml) was added. 1-(6-Trifluoromethylpyridin-2-yl)-piperazine (3) was extracted from the H_2O with EtOAc (2 × 100 ml). The organic layer was washed with H_2O (2 × 100 ml) and evaporated. To the resulting brown oil, H_2O (20 ml) was added and stirred for 3 h. 3 was extracted with CH_2Cl_2 (2 × 50 ml). The organic layer was dried with Na_2SO_4 (anhydrous) and evaporated to give a yellow oil. Chemical yield was 85%. ^{1}H NMR (360 MHz, $CDCl_3$): 1.80 (1H, s, NH), 2.96 (4H, t), 3.59 (4H, t), 6.75 (1H, d), 6.92 (1H, d), 7.56 (1H, t); ESI MS 232.1 $[MH]^+$.

Synthesis of 2-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}isoindole-1,3-dione (4)

A stirred mixture of 3 (5.077 g, 21.959 mmol) and Na_2CO_3 (2.327 g, 21.959 mmol) in DMF (30 ml, pa) was heated to 80°C. 2-Bromo-ethylphthalimide (5.873 g, 21.959 mmol, 95%) was added while the temperature was kept at 80°C. The reaction was monitored with TLC (CH_2Cl_2/CH_3OH/NH_3: 90/16/21) and stopped after 3 h by adding cold H_2O (300 ml). 2-{2-[4-(6-Trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}isoindole-1,3-dione (4) was extracted from the aqueous phase with EtOAc (2 × 100 ml). The organic layer was washed with H_2O (2 × 200 ml) and acidified with HCl by means of a Kipp device. The hydrochloride salt of 4 was filtered off and washed with EtOAc. The precipitation was dissolved in H_2O (100 ml) which subsequently was made basic (pH = 14). 4 was extracted with CH_2Cl_2 (2 × 100 ml). The organic layer was dried with Na_2SO_4 (anhydrous) and evaporated. The formed, white precipitation was redissolved in warm CH_3OH. Cooling this solution, formed 4 as white crystals. Chemical yield was 65%. ^{1}H NMR (360 MHz, $CDCl_3$):

2.62 (4H, t), 2.69 (2H, t), 3.53 (4H, t), 3.87 (2H, t), 6.74 (1H, d), 6.92 (1H, d), 7.55 (1H, t), 7.72 (2H, d), 7.85 (2H, d); ^{1}H NMR (300 MHz, d_6-DMSO): 2.51 (4H, t), 2.58 (2H,t), 3.45 (4H, t), 3.74 (2H, t), 7.00 (1H, d), 7.07 (1H, d), 7.70 (1H, t), 7.80-7.88 (4H, m); ESI MS 405.2 $[MH]^+$.

Synthesis of 2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethylamine (5)

To a stirred solution of 4 (8.661 g, 21.417 mmol) in EtOH (28 ml, pa) at reflux, was slowly added hydrazine hydrate (1.049 ml, 21.417 mmol, 99%). The reaction was monitored with TLC (CH_2Cl_2/CH_3OH/NH_3: 90/16/21). After 3 h, H_2O (13 ml) and HCl (6.5 ml, 37%) were added. After another 4 h of reflux, the formed solid was filtered off and washed with EtOH/H_2O/HCl (37%): 28/13/6.5. EtOH was evaporated and the pH of the remaining aqueous fraction was adjusted to 13 with NaOH (30 ml, 20%). 2-[4-(6-Trifluoromethylpyridin-2-yl)piperazin-1-yl]ethylamine (5) was extracted with CH_2Cl_2 (2×100 ml). The combined organic layers were dried with Na_2SO_4 (anhydrous) and evaporated. A yellow precipitation was formed. Further purification was performed by column chromatography (CH_2Cl_2/CH_3OH/NH_3: 90/16/21). Chemical yield was 26%. ^{1}H NMR (360 MHz, $CDCl_3$): 1.78 (2H, s, NH), 2.49 (2H, t), 2.55 (4H, t), 2.85 (2H, t), 3.61 (4H, t), 6.76 (1H, d), 6.93 (1H, d), 7.56 (1H, t); ESI MS: 275.2 $[MH]^+$, 258.3 $[MH\text{-}NH_2]^+$.

Synthesis of 4-NO_2-N-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl] ethyl} benzamide (1.NO_2)

Compound 5 (274.3 mg, 1 mmol) was dissolved in CH_2Cl_2 (2 ml). After addition of TEA (221 µl, 1.586 mmol), the reaction mixture was cooled to 0°C. 4-NO_2-benzoylchloride (213.2 mg, 1.126 mmol, 98%) in CH_2Cl_2 (2 ml) was added dropwise. The reaction was monitored with TLC (CH_2Cl_2/CH_3OH/NH_3: 90/16/21). This reaction mixture was stirred for 3 h at ambient temperature. The organic layer was washed with H_2O, dried with Na_2SO_4 (anhydrous) and evaporated. Purification was performed by column chromatography (CH_2Cl_2/CH_3OH/NH_3: 90/16/21). Chemical yield was 60%. ^{1}H NMR (360 MHz, $CDCl_3$): 2.65 (4H, t), 2.70 (2H, t), 3.60 (6H, m), 6.80 (1H, d), 6.95 (2H, d), 7.60 (1H, t), 7.95 (2H, d), 8.30 (2H, d); ESI-MS $[M+H]^+$: 424; Exact mass (ESI-MS) $[M+H]^+$: found 424.1596, calculated 424.1589.

Production of the fluorinating agent K[^{18}F]F/K2.2.2

No carrier added [^{18}F]F^- was produced with 18 MeV protons (5 µA, 120 min) via the ^{18}O(p,n)^{18}F reaction on enriched [^{18}O]H_2O. Radioactivity was trapped on an anion exchange resin (Dowex 1×8, 200–400 mesh) and eluted with a K_2CO_3 (5.4 mg, 39 µmol)/K2.2.2 (22 mg, 58 µmol) aqueous solution (350 µl, mQ H_2O). After 3 successive azeotropic distillations with CH_3CN (3×1 ml,

dry) in the microwave oven (3×3 min, 700 W) under continuous He flow ($P_{He} = 0.2$ bar, flow rate = 50 ml/min), the fluorinating agent K[^{18}F]F/K2.2.2 could be used for labelling.

Radiosynthesis and purification of 4-[^{18}F]fluoro-N-{2-[4-(6-trifluoromethylpyridin-2-yl)piperazin-1-yl]ethyl}benzamide (1.^{18}F)

Compound 1.NO_2 (10.0 mg, 24 μmol) in DMSO (0.9 ml, dry) was added to the reaction vial containing the fluorinating agent K[^{18}F]F/K2.2.2 and reacted in a microwave oven for 5 min at 700 W. The reaction mixture was subsequently added to ice cold H_2O (1.1 ml, 1.68 M HOAc, pH = 2.2) and loaded onto the loop (2 ml) of the semi-preparative HPLC. The eluted tracer fraction was diluted 5 times with H_2O and loaded onto a C18 Sep-Pak® cartridge (previously activated with EtOH (5 ml) and H_2O (10 ml)). The C18 Sep-Pak® cartridge was rinsed with H_2O (10 ml). Subsequently, the tracer was eluted with EtOH (1 ml) and diluted with H_2O (9 ml).

Quality control

Analysis of the tracer solution was performed by analytical HPLC (see Material and Methods Section). Stability testing was carried out by reinjection onto the same analytical HPLC set up.

Animal studies

All animal studies were carried out in compliance with the Animal Ethical Committee of the Ghent University and the Belgian laws. NMRI mice ($m = 20$–25 g, male) were used which had *ad libitum* access to food and water before and during the experiments.

Biodistribution studies in mice

Mice ($n = 3 \times 9$) were injected directly in the tail vein with 1.^{18}F ($\pm$ 37 kBq (1 μCi), $\pm$ 200 μl H_2O/EtOH, max. 10% EtOH) and sacrificed by decapitation at set time points *p.i.* (1, 2, 5, 10, 20, 40, 60, 120 and 180 min). All animals were dissected; blood and urine samples were collected. The dissected tissues were blotted to remove adhering blood and weighed in tarred counting tubes. The present radioactivity was measured on a Cobra B5005 auto gamma counter. All values were corrected for decay and background. The injected dose (ID) was calculated by determining the radioactivity per gram injection solution on the same counting device and weighing the injection syringe, before and after injection.

Results are expressed as percentage of the injected dose per gram tissue (% ID/g) or as percentage of the injected dose (% ID). Averages and standard deviations (SD) were calculated. The *t*-test (two sided, paired, $n = 3$, $\alpha = 0.05$)

was used for determining significant differences between results of different organs.

Conclusions

4-[^{18}F]Fluoro-*N*-{2-[4-(6-trifluoromethylpyridin-2-yl]piperazin-1-yl]ethyl}benzamide was synthesized by nucleophilic aromatic substitution on the nitro precursor in a microwave oven (5 min, 700 W) in sufficient overall radiochemical yield (18% (EOS)), radiochemical purity (> 99%) and specific activity (> 81.4 GBq/μmol (2.2 Ci/μmol)) to start *in vivo* evaluation.

Biodistribution studies in mice were performed leading to 2.19% ID (5.47% ID/g) in the brain at 2 min *p.i.* During the first 20 min, radioactivity concentration of brain was significantly higher than that of blood. Brain to blood ratios turned out to be much better for 1.^{18}F than for 1.^{123}I, probably because of its lower lipophilicity. Although the affinity of 1.^{18}F is 10 times higher than that of 1.^{123}I, only little retention of radioactivity could be demonstrated in the mouse brain. Further experiments are in progress to investigate the selectivity and the specificity of the binding of the tracer in the brain.

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