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Research Article

Radiosynthesis and *in vivo* study of [^{18}F]1-(2-fluoroethyl)-4-[(4-cyanophenoxy)methyl]piperidine: a promising new sigma-1 receptor ligand

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Summary

The novel sigma-1 receptor PET radiotracer [^{18}F]1-(2-fluoroethyl)-4-[(4-cyanophenoxy)methyl]piperidine ([^{18}F]WLS1.002, [^{18}F]-2) was synthesized ($n = 6$) by heating the corresponding *N*-ethylmesylate precursor in an anhydrous acetonitrile solution containing [^{18}F]fluoride, Kryptofix K_{222} and potassium carbonate for 15 min. Purification was accomplished by reverse-phase HPLC methods, providing [^{18}F]-2 in 59 $\pm$ 8% radiochemical yield (EOB), with specific activity of 2.89 $\pm$ 0.80 Ci/μmol (EOS) and radiochemical purity of 98.3 $\pm$ 2.1%. Rat biodistribution studies revealed relatively high uptake in many organs known to contain sigma-1 receptors, including the lungs, kidney, heart, spleen, and brain. Good clearance from normal tissues was observed over time. Blocking studies (60 min) demonstrated high (> 80%) specific binding of [^{18}F]-2 in the brain, with reduction also noted in other organs known to express these sites. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: sigma receptor; radiotracer; fluorine-18; PET

Introduction

Over the past 20 years, much research has been carried out with the aim of determining the biological role and therapeutic value of sigma receptor ligands.[1–9] Sigma receptors are thought to be involved in several diseases of the central nervous system (CNS), such as schizophrenia, depression, dementia, ischemia, and even in peripheral nervous system diseases.[1] Sigma receptors are classified into two main subtypes; these are termed sigma-1 and sigma-2. Sigma-1 receptors are the best characterized of the two subtypes and are

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thought to regulate glutamate NMDA receptor function as well as the release of neurotransmitters, such as dopamine. The sigma-1 receptor is thought to be involved in certain neuropsychiatric disorders, and in learning and memory processes.[10,11] In addition, sigma-1 receptors are expressed in relatively high densities in many human cancer cell types, including malignancies of the breast, lung and prostate.[1,12,13] Our laboratory is developing imaging agents suitable for *in vivo* tomographic assessment of sigma-1 receptors in animal models and in humans. Such agents would allow for the determination and direct comparison of sigma receptor subtype densities in normal humans and in subjects with various neurological disorders or cancer.[14]

Several receptor tracers incorporating radioisotopes of carbon, fluorine, iodine, and hydrogen have been reported. These include [^{18}F]-α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol,[15] [^{123}I]-iodobenzamides,[16] [^{125}I]-iodobenzovesamical,[17] [^{125}I]-iodophenyl-3-(adamantyl)guanidine,[18] [^{18}F]haloperidol,[19] *N*-[^{11}C]-benzyl-*N*-normetazocine,[20] [^{18}F]memantine,[21] [^{11}C]nemonapride,[22] [^{11}C]NE100,[23] [^{18}F]FE-SA4503,[24] and [^{11}C]SA6298.[25] In our previous work, we reported a series of selective sigma-1 receptor compounds that could be labeled with common PET and SPECT radioisotopes, including the title compound.[26] From this series, [^{18}F]1-(3-fluoropropyl)-4-(4-cyanophenoxymethyl)piperidine ([^{18}F]FPS) [K_D= 0.5 nM, log P = 2.8] has been evaluated in rodents, baboons and more recently in humans. PET scanning in[27,28] health participants recently revealed that [^{18}F]FPS does not reach transient equilibrium by 4 h post-administration, supporting the development of a lower affinity tracer for brain imaging studies (Waterhouse *et al*., unpublished data). As an extension of this effort, we report here the synthesis and first *in vivo* evaluation of [^{18}F]1-(2-fluoroethyl)-4-[(4-cyanophenoxy)methyl]piperidine ([^{18}F]WLS1.002, [^{18}F]-2) (K_D= 5 nM, log P = 2.4), a structurally similar but lower affinity tracer in comparison to [^{18}F]FPS.

Materials and methods

General

Proton NMR spectra were recorded on a Brucker 400 MHz FT-NMR spectrometer (Department of Chemistry, Columbia University). Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in either chloroform-d_3, and coupling constants (J) are reported in Hertz (Hz). Chromatographic purification of unlabeled compounds was performed using silica gel (Aldrich, 200–400 mesh, ASTM) and solvent systems indicated in the following text. 4-[4-Cyanophenoxy)methyl]piperidine[3] was prepared using a reported method, and the spectroscopic and melting point data matched that previously published.[26] All other reagents were purchased from commercial sources and were used without further purification.

With respect to radiochemistry, high specific activity [^{18}F]fluoride was provided by Columbia University PET Center. It was produced from enriched [^{18}O] water by the ^{18}O(p,n)^{18}F nuclear reaction. High-performance liquid chromatography (HPLC) purification and quality control analyses were performed via a system comprised of a Waters 515 HPLC pump, a Waters PDA UV detector, and a Bicron Flow-Scint radiation detector. The HPLC columns were packed with[26,27] reverse-phase base-deactivated stationary phase (Phenomenex, ODS, analytical: 4.6×250 mm, 5 μm particle size; semipreparative: 10×250 mm, 10 μm particle size), and the mobile phases and flow rates used are indicated in the text below.

Synthesis

1-(2-hydroxyethyl)-4-[(4-cyanophenoxy)methyl]piperidine.[4] To a solution of 4-[(4-cyanophenoxy)methyl]piperidine[3] (300 mg, 1.39 mmol) in dichloromethane (20 ml) was added 3-bromoethanol (187 mg, 106 μl, 1.50 mmol) and potassium carbonate (770 mg, 5.56 mmol). The resulting mixture was stirred at room temperature for 24 h and deionized (DI) water (15 ml) was added. The compound was extracted with dichloromethane (3×10 ml), and the organic layers were combined, dried over sodium sulfate, and evaporated under reduced pressure. The product was purified by column chromatography (100% ethanol, $R_f = 0.25$) to afford a clear, colorless oil product that solidified to a white solid (256 mg, 1.00 mmol, 72%); ^{1}H-NMR: δ (ppm) 7.60 (d, J= 8.4 Hz, 2H), 6.96 (d, J= 8.4 Hz, 2H), 3.87 (d, J= 5.9 Hz, 2H), 3.66 (t, J= 5.3 Hz, 2H), 3.02 (d, J= 11.6 Hz, 2H), 2.60 (t, J= 5.3 Hz, 2H), 2.17 (d, J= 11.6 Hz, 2H), 1.88 (m, 3H), 1.47 (m, 2H).

1-(2-methylsulfonylethyl)-4-[(4-cyanophenoxy)methyl]piperidine.[5] 1-(2-Hy-droxyethyl)-4-[(4-cyanophenoxy)methyl]piperidine[4] (150 mg, 0.59 mmol) was dissolved in a mixture of triethylamine (2 ml) and dichloromethane (15 ml) and to this was added methansulfonyl chloride (79 mg, 0.69 mmol). The resulting solution was stirred for 1 h at room temperature. The volatile components were removed under reduced pressure, and the product was purified by column chromatography (silica gel; 100% ethyl acetate, $R_f = 0.28$) to provide product 5 as a clear, white solid (126 mg, 64%), m.p. = 86–88°C; ^{1}H-NMR: 7.60 (dd, J = 2.00, 7.04 Hz, 2H), 6.95 (dd, J = 2.00, 7.04 Hz, 2H), 3.87 (d, J= 5.88 Hz, 2H), 3.65 (m, 2H), 3.02 (m, 2H), 2.80 (m, 2H), 2.19 (m, 2H), 1.87 (m, 3H), 1.50 (m, 2H).

1-(2-fluoroethyl)-4-[(4-cyanophenoxy)methyl]piperidine.[2] To dichloromethane (6 ml) was added 4-[4-cyanophenoxy)methyl]piperidine (280 mg, 1.3 mmol), potassium carbonate (716 mg, 5.18 mmol) and 2-bromo-1-fluoropropane (104 μl, 1.09 mmol). The resulting solution was stirred at room temperature for 24 h. The reaction mixture was then diluted with DI water (100 ml) and the

product extracted into dichloromethane (2×20 ml). The organic extracts were combined, dried over magnesium sulfate and the solvent was removed *in vacuo* to provide the crude product as a yellow oil. The product was purified by column chromatography (silica gel, mobile phase:ethyl acetate/ethanol (9/2 (v/v)) to provide a white solid (311 mg, 91%), m.p. = 46–47°C; ^{1}H-NMR: δ (ppm) 7.60 (d, J= 8.9 Hz, 2H), 6.95 (d, J= 8.9 Hz, 2H), 4.61 (m, 2H), 3.87 (d, J= 5.9 Hz, 2H), 3.06 (d, J= 9.2 Hz, 2H), 2.76 (m, 2H), 2.16 (t, J= 9.2 Hz, 2H), 1.86 (m, 3H), 1.50 (m, 2H). LCMS: (M/Z, %), 263.1 ($M + H^+$, 100%).

Radiolabeling

[^{18}F]1-(2-fluoroethyl)-4-[(4-cyanophenoxy)methyl]piperidine, ([^{18}F]-2). The [^{18}F]fluoride was trapped on a quaternary ammonium (QMA) cartridge, and was subsequently eluted into a 5 ml V-vial by passing an aqueous of 5 mg K_2CO_3 and 2 mg Kryptofix$_{222}$ (1.1 ml total volume) through the cartridge. Azeotropic drying in a reaction vial under argon with acetonitrile (4×1 ml) furnished the dry complex that was used for the fluorination reaction. The mesylate precursor (1.8–2.1 mg) in anhydrous acetronitrile (1 ml) was added to the reaction vial. The mixture was heated at 90°C for 15 min, DI water was added and the mixture was heated for another 10 min at this temperature to convert the non-reacted precursor to the corresponding alcohol for easy purification. Isolation of [^{18}F]-2 was achieved by diluting the crude reaction mixture with DI water (100 ml) and trapping the product on a C-18 Sep-Pak. The crude product was eluted into the vial with acetonitrile (1 ml). The acetonitrile solution containing crude product was diluted with 2 ml 0.1 M ammonium acetate buffer, and this solution was directly injected onto the HPLC system (mobile phase: 0.1 M ammonium acetate solution/acetonitrile 70/30 (v/v); flow rate: 10 ml/min, R_t = 27–29 min) to provide purified [^{18}F]-2. The HPLC product fraction containing [^{18}F]-2 was mixed with DI water (100 ml) and was passed across the C-18 Sep-Pak. After washing with 10 ml of DI water, the final product was recovered into a sample vial by slowly flushing the C-18 Sep-Pak with 1 ml of absolute ethanol.

Chemical and radiochemical purity, and specific activity was assessed using analytical HPLC methods (mobile phase: 0.1 M ammonium acetate solution/acetonitrile 78/22 (v/v), Phenomenex® ODS column, flow rate: 2 ml/min) to test a sample ($\sim$1 mCi) of the final product. For the internal standard identity test, a standard of 2 (10 µl of 10 µg/ml in 10% ethanol/water) was mixed with an aliquot of product prior to analysis. To prepare solutions suitable for *in vivo* administration, the ethanol solution containing [^{18}F]-2 was diluted with sterile saline and then passed through a 22 µm filter into a 10 ml sterile evacuated vial.

Biological

All *in vivo* experimental procedures were approved by the NYSPI Institutional Animal Care and Use Committee and were carried out in compliance with State and Federal laws governing animal experimentation.

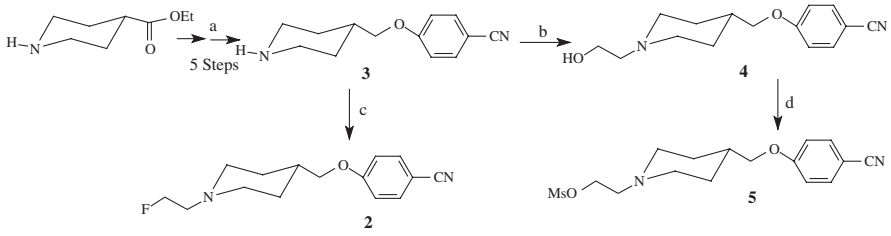
Rat distribution and blocking studies. For the biodistribution and blocking studies, [^{18}F]-2 (15 μCi, 100 μl saline including $<5\%$ (v/v) ethanol) was administered to awake 10–14 week old male Sprague–Dawley rats (225–280 g) via tail vein injection. At pre-selected times, the rats were killed by decapitation while anesthetized with CO_2 gas. For the blocking study, rats were administered either saline (100 μl), or the sigma-1 subtype selective antagonist BD 1008 (1 mg/kg) 5 min prior to radiotracer administration. For all studies, selected organs and tissues were removed post mortem, weighed, and assayed for radioactivity. The percent of injected dose (% ID) and the percent of injected dose of [^{18}F]-2 per gram of tissue (% ID/g) were determined for each sample by comparison with a 5% aliquot of the injected dose as a standard. For the blocking study, the significance between groups was evaluated using a two-tailed *t*-test. The criterion for significance was $p \leqslant 0.05$.

In vitro binding assays. [^{18}F]FPS or [^{18}F]2 (1 nM, approximately 1 μCi/tube) was incubated for 1.5 h in a total volume of 1.0 ml phosphate buffer (pH = 8.0, 22°C) in the presence of rat forebrain membranes (3 mg/tube). The effect of different concentrations of the haloperidol (as a standard), FPS or 2 (10^{-12}–10^{-4} M) on tracer binding was examined. All assays were performed in triplicate. The inhibition constant of haloperidol was calculated to confirm appropriate assay conditions.

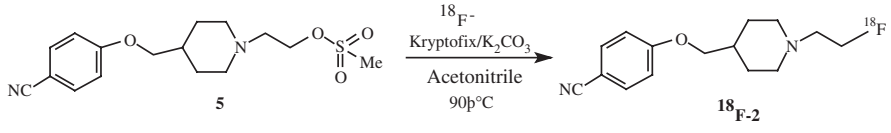
Experiments will be terminated by the addition of 3 ml ice cold buffer, and unbound tracer removed by washing twice more (approx 4 ml/wash) with cold buffer using a 48 well cell harvester (Brandel). Membranes were placed into separate 7 ml tubes and assayed for radioactivity with a gamma counter (Cobra II, Packard Instruments). Analysis of binding data (K_i for haloperidol, and K_D for SFE and FPS) was performed using non-linear regression (Prism 3.0.cx, Graphpad).

Results and discussion

The title compound was synthesized using straightforward established methods (Scheme 1). 4-((4-cyanophenoxy)methyl)piperidine, 3, was prepared in five steps from ethyl isonepecotate as previously described.[27,28] Alkylation of 3 with 2-bromo-1-fluoroethane in the presence of potassium carbonate provided standard compound 2 in good yield. Likewise, compound 4 was prepared by alkylating 3 with 2-bromo-1-ethanol. The alkyl mesylate precursor 5 was synthesized by treating 4 with methanesulfonyl chloride in



Scheme 1. Synthesis of standard 2 and precursor 5. Reagents and conditions: (a) see References;[27,28] (b) 1-Bromo-2-ethanol, K_2CO_3, CH_2Cl_2; (c) 1-Bromo-2-ethane, K_2CO_3, CH_2Cl_2; (d) MsCl, Et_3N, CH_2Cl_2



Scheme 2. Radiosynthesis of target compound [^{18}F]-2

the presence of triethyl amine. This precursor was purified by normal-phase flash column chromatography in good yield. The material was stable at room temperature for at least 1 month; the longest period of examination.

After drying the [^{18}F]-fluoride as described in the experimental section, [^{18}F]-2 was synthesized by heating 5 at 90°C for 15 min in an anhydrous solution of [^{18}F]fluoride and Kryptofix–potassium carbonate mixture in acetonitrile (Scheme 2). Purification of [^{18}F]-2 was accomplished using a semipreparative reverse-phase HPLC. The average isolated product yield after HPLC purification was $59 \pm 8\%$ EOB ($n = 6$). The average time for radioligand synthesis and HPLC purification was about 100 min.

The specific activity of the product was 2.89 ± 0.80 Ci/μmol EOS and the radiochemical purity was $98.3 \pm 2.1\%$. No significant chemical impurities were detected by HPLC (UV, 250 nm). The identity of the [^{18}F]-2 was supported by co-elution of the radiolabeled product and unlabeled standard, 2.

In vitro binding assays provided K_D values of 0.5 and 5.0 nM for FPS and 2, respectively. These values are lower from previously published result inhibition constant values, and this is likely due to the use of a phosphate buffer instead of *Tris* buffer. We found in past studies that *Tris* buffer (0.1 M) interfered with the binding of sigma tracers to the sigma-1 receptor, and that IC_{50}s are lower (representing higher affinities) when a phosphate buffer is used. In any case these data reveal that 2 exhibits lower affinity compared to FPS in rat forebrain tissues. As previously published, the sigma-2 receptor affinities for FPS and SFE are 144 and 361 nM, respectively.[26]

[^{18}F]-2 was administered via tail vein injection to awake male rats at a concentration of 15 μCi in 100 μl sterile saline. For blocking studies, either saline or the sigma-1 subtype selective antagonist BD 1008 was administered. The results of the biodistribution in selected organs at 5, 15, 30, 60, 150 and 330 min post-injection (PI) are presented in Table 1. In this table the results of BD 1008 blocking experiment at 60 min post-administration of [^{18}F]-2 are presented.

From Table 1, high initial uptake of radioactivity was observed in most organs, peaking by 5 min followed by clearance over time. In the brain, radioactivity concentrations observed over time included 1.41 ± 0.05% ID/g at 5 min, 1.08 ± 0.23% ID/g at 60 min and 0.33 ± 0.03% ID/g at 330 min PI. This is in stark contrast to the higher affinity sigma-1 tracer [^{18}F]FPS (K_D= 0.5 nM), where no significant decrease of the tracer from the brain occurs over the same time period in adult male rats.[25,28] In organs known to contain sigma-1 receptor, including the lungs, kidneys, heart and spleen, uptake was between 1.01 and 7.71% ID/g at 5 min, with the radioactivity quickly clearing thereafter from most organs. The relative amount of radioactivity in bone was low and did not significantly increase over time, indicating that this tracer is relatively stable to *in vivo* defluorination. Relative uptake in fat was also low. Finally, we note that concentration of radioactivity in the blood remained low, just 0.14 ± 0.03% ID/g at 5 min and 0.07 ± 0.02% ID/g at 60 min. Administration of the sigma-1 selective compound BD 1008 via tail vein 5 min before tracer injection reduced brain activity by 80% at 60 min. Likewise, the amount of radioactivity in peripheral organs including

Table 1. Biodistribution of [^{18}F]-2 in awake male rats (n = 4–5)

Time	Blood	Kidney	Muscle	Heart	Liver	Spleen
5	0.14 ± 0.03	2.99 ± 0.10	0.63 ± 0.12	1.75 ± 0.15	0.68 ± 0.17	1.01 ± 0.21
15	0.11 ± 0.02	2.05 ± 0.50	0.36 ± 0.26	0.85 ± 0.15	0.80 ± 0.28	2.11 ± 0.70
30	0.07 ± 0.01	1.45 ± 0.19	0.35 ± 0.02	0.66 ± 0.06	1.16 ± 0.13	1.78 ± 0.11
60	0.07 ± 0.02	1.20 ± 0.29	0.23 ± 0.08	0.45 ± 0.05	1.31 ± 0.22	1.63 ± 0.29
150	0.06 ± 0.01	0.73 ± 0.25	0.19 ± 0.05	0.26 ± 0.09	1.95 ± 0.73	1.08 ± 0.33
330	0.04 ± 0.01	0.35 ± 0.02	0.09 ± 0.02	0.11 ± 0.01	1.13 ± 0.37	0.36 ± 0.02
60 (Block)	0.17 ± 0.04	0.60 ± 0.38	0.19 ± 0.03	0.21 ± 0.10	2.68 ± 0.24	0.30 ± 0.06
Time	Brain	Lung	Testes	Intestine	Fat	Bone
5	1.41 ± 0.06	7.71 ± 0.93	0.27 ± 0.06	1.40 ± 0.32	0.30 ± 0.12	0.32 ± 0.12
15	1.15 ± 0.35	4.63 ± 1.01	0.28 ± 0.09	1.23 ± 0.57	0.29 ± 0.13	0.38 ± 0.17
30	1.17 ± 0.09	3.21 ± 0.39	0.32 ± 0.06	1.47 ± 0.10	0.30 ± 0.08	0.50 ± 0.09
60	1.08 ± 0.23	1.78 ± 0.64	0.33 ± 0.11	1.24 ± 0.32	0.23 ± 0.36	0.39 ± 0.16
150	0.96 ± 0.30	0.84 ± 0.19	0.45 ± 0.07	1.04 ± 0.51	0.31 ± 0.11	0.39 ± 0.09
330	0.33 ± 0.03	0.31 ± 0.05	0.34 ± 0.03	1.22 ± 0.24	0.18 ± 0.04	0.21 ± 0.07
60 (Block)	0.21 ± 0.01	0.38 ± 0.09	0.67 ± 0.07	0.35 ± 0.04	0.45 ± 0.06	0.19 ± 0.01

Data are mean of % ID/g ± SD.

the heart, lungs, kidney, spleen, intestines was also significantly reduced. These data support that saturable binding of the tracer occurs *in vivo*.

Currently one fluorine-18-labeled sigma receptor tracer has been evaluated in rodents and non-human primates; namely [^{18}F]FE-SA4503 that possesses a high affinity for both sigma-1 (K_i= 6.48 nM) and sigma-2 subtypes (K_i= 2.11 nM).[24] The regional brain uptake and clearance rates of [^{18}F]FE-SA4503 in the rat CNS appears similar to that of [^{18}F]SFE. However, the binding of [^{18}F]FE-SA4503 is likely to both sigma receptor subtypes and, in addition, the saturable binding of [^{18}F]FE-SA4503 appears is lower than for SFE (50% compared to 80%).

Conclusion

We have successfully synthesized [^{18}F]1-(2-fluoroethyl)-4-[(4-cyanophenoxy)-methyl]piperidine ([^{18}F]-2) as a potential sigma-1 receptor ligand for *in vivo* use. The radiosynthesis of this novel compound proved to be a highly reproducible and feasible method. The radioligand has been prepared in high specific activity and good radiochemical purity in sufficient quantities to permit *in vitro* and *in vivo* evaluations. The rat biodistribution study showed this compound had significant uptake in the lungs, kidney, heart, spleen, and brain. These data demonstrate that [^{18}F]-2 exhibits promising characteristics for *in vivo* use and may provide a superior brain imaging radiotracer due to its lower affinity (K_i= 5 nM) and faster clearance rates from the brain compared to [^{18}F]FPS. The blocking study (60 min) showed a greater than 80% reduction in [^{18}F]-2 brain uptake by sigma-1 selective ligand BD 1008, and the activity in other sigma-1 receptor expressing organs was also reduced, indicating saturable binding in peripheral tissues as well. Further studies of this new promising sigma-1 tracer are warranted.

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