Fluorinated Benzamide Neuroleptics. 1. Radiosynthesis of (S)-N-[(1-Ethyl-2-Pyrrolidinyl)Methyl]-5-(2[F-18]Fluoroethyl)-2-Methoxybenzamide: A Potential Fluorine-18 Labeled PET Radiotracer for Dopamine D2 Receptors.§

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Summary

The synthesis of a tosylate precursor for the radiosynthesis of a potential PET radiotracer, (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(2-[^{18}F]fluoroethyl)-2-methoxybenzamide is reported. Reaction of ^{18}F-fluoride with the tosylate provides the radiolabeled product in 10-25% yield. Specific activity of the radiotracer varied between 600-800 Ci/mmol after purification of the product mixture by reverse phase HPLC .

Keywords: Radiofluorination, Neuroleptic, PET tracer, Dopamine D2 receptor.

Introduction

Dopamine D2 receptors have been imaged *in vivo* by positron emission tomography (PET) in both primates and humans using a variety of positron-emitting radioligands (1,2). Substituted benzamides have been reported to show specific, high affinity and reversible binding for the dopamine D2 receptors (3). Raclopride, which is a substituted benzamide has been labeled with carbon-11 and used for PET scans in primates and humans (4,5). Due to the short half-life of carbon-11 (20.4 min) development of a fluorine-18 (110 min) labeled benzamide derivative would provide a radiotracer which will be more tolerant to delays in synthesis time and also permit longer PET data acquisition times. Efforts to generate a fluorine-18 labeled derivative of raclopride have been pursued in several laboratories (6). Fluoroalkylation at the pyrrolidine nitrogen of raclopride lowers its affinity towards the D2 receptor and therefore renders it incapable of being developed as a PET tracer. Also reported is the development of fluorinated derivatives of eticlopride which has a higher binding affinity than raclopride for the D2 receptor. Although the fluoroethyl derivative of eticlopride has high affinity for the D2 receptor *in vitro* , it showed poor *in vivo* selectivity in the rat brain (7).

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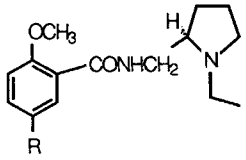
§ Presented in part at the 36th Annual Meeting of the Society of Nuclear Medicine, St. Louis, June 13-16, 1989.

0362–4803/90/050609–08$05.00

Received November 1, 1989
Revised December 8, 1989

We are involved in developing fluoroalkyl analogs of the related, clinically used antipsychotic sulpiride **1**, as possible selective D2 receptor antagonists. The aminosulfonyl moiety of sulpiride is replaced with a fluoroalkyl group in these analogs in order to avoid proximity of the fluorine atom to the pyrrolidine nitrogen. Other appropriate substituents in the C-3 position of the benzamide nucleus have also been incorporated. These fluorinated derivatives remarkably show high affinity (IC_{50} of 10^{-7} to 10^{-8} M) for the D2 receptor binding sites labeled with ^{3}H-spiperone (Mukherjee et. al., manuscript in preparation). Fluorine-18 analogs of these compounds are therefore likely to be good candidates for development as radiotracers for PET.

In this report the radiosynthesis of F-18 labeled **2**, (S)-N-[(1-ethyl-2-pyrrolidinyl)-methyl]-5-(2[^{18}F]fluoroethyl)-2-methoxybenzamide as a PET radiotracer for dopamine D2 receptors is described.

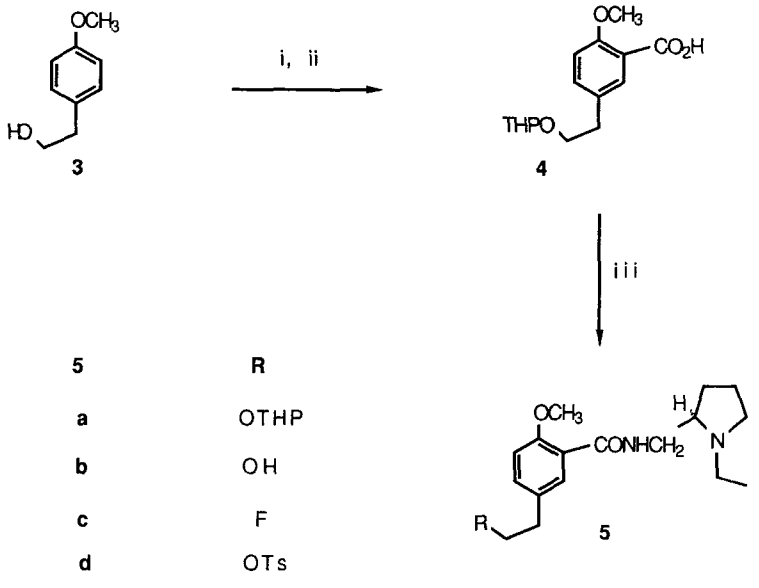


1 R= SO_2NH_2 Sulpiride

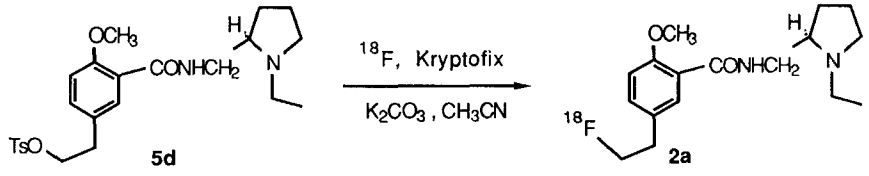
2 R= CH_2CH_2F

Materials and Methods

4-Methoxyphenethyl alcohol and 2-(aminomethyl)-N-ethylpyrrolidine were purchased from Aldrich Chem. Co. All other reagents were of analytical grade and used as such. (S)-2-(aminomethyl)-N-ethylpyrrolidine was resolved as a ditartarate salt ($\alpha = -38^{o}$) from the racemic mixture by the reported procedure (8). [^{18}F]fluoride was made via the $^{18}O(p,n)^{18}F$ reaction using $[^{18}O]H_2O$ in a Cyclotron Corporation CS-15 cyclotron (9). HPLC was carried out on a Gilson Gradient System consisting of two Gilson pumps and two detectors, a UV detector fixed at 280 nm and a radiation flow detector with a NaI(Tl) crystal. Solvent A of the gradient was 0.01N ammonium acetate, pH 4.5 with acetic acid and solvent B was methanol. C_{18} semi-prep (250x10 mm) column from Alltech Assoc Inc. was used for reverse phase HPLC and Macherey-Nagel silica plates supplied by Alltech Assoc. Inc. were used for preparative TLC. Proton NMR spectra were obtained on a Varian EM-360 60 MHz spectrometer and electron impact mass spectra were obtained on a VG Instruments Inc. Model 7250 mass spectrometer. A Perkin-Elmer Lamda UV/VIS spectrophotometer was used to measure UV absorption.



Scheme-1 i) DHP, PPTS, CH_2Cl_2 ; ii) n-BuLi, -78°C, CO_2; iii) DCC, DMAP, $CHCl_3$



Scheme-2 Direct fluoride-18 labeling of tosylate.

In order to assess lipophilicity of the compounds, octanol-water partition coefficients were measured by the reported method (10). Octanol (2 mL) and 0.066M phosphate-buffered saline, pH 7.4 (2 mL) were presaturated with one another by vortexing for 10 minutes. The respective compound was added to this mixture and thoroughly vortexed for 30-60 minutes after which the samples were centrifuged and the two layers separated and analysed. The apparent lipophilicity, log P for **2** (250 μg) was obtained spectrophotometrically. Fluorine-18 activity was counted for obtaining log P of **2a** (50-100 μCi). Log P of **2** was measured to be 0.81±0.02 and that of **2a** was 0.80±0.01. Values are the mean of at least three measurements.

2-Methoxy-5-(2-tetrahydropyranyloxyethyl)benzoic acid (**4**):

4-Methoxyphenethyl alcohol (10 g; 66 mmol) was mixed with dihydropyran (5.6 g; 66 mmol) in dichloromethane (100 mL). To this was added catalytic amounts of pyridinium p-toluenesulfonate (50 mg) and the mixture stirred for 4 hours at ambient temperature. The mixture was then washed with 10% sodium carbonate followed by water. The organic layer was then dried with magnesium sulfate and the solvent removed *in vacuo*. The tetrahydropyranyl ether was obtained in 96% yield and was used without further purification.

The tetrahydropyranyl ether (2 g; 8.5 mmol) was taken in anhydrous tetrahydrofuran (20 mL) and the mixture cooled to -78°C. Into this cooled mixture, n-butyllithium (8.5 mmol) was added over a nitrogen atmosphere. The reaction was allowed to proceed at -78°C for 30 minutes and then warmed upto ambient temperature and stirred for 30 minutes. The mixture was then poured over dry ice in ether. Solvent was then removed *in vacuo*. The residue was dissolved in water and extracted with ether. The aqueous layer was then acidified and extracted with ethyl acetate. The ethyl acetate was removed *in vacuo* to provide the acid **4** (0.9 g; 38%). NMR ∂ ppm: 8.25 (s, 1H, COOH), 7.85 (d, 1H, H-6), 6.7-7.4 (dd, 2H, H-3, H-4), 4.4 (s, 1H, O-CH-O), 3.85 (s, 3H, OCH_3), 3.3-3.7 (m, 4H), 2.5-2.85 (t, 2H, benzylic CH_2), 1.1-1.7 (br, 6H). MS (m/z, %): 280 (M^+, 1%), 263 ($[M\text{-}OH]^+$, 1%, 178 ($[M\text{-}C_5H_8O\text{-}H_2O]^+$, 92%), 85 ($C_5H_9O^+$, 100%).

(S)-N-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-(2-hydroxyethyl)-2-methoxybenzamide (**5b**):

The acid **4** (1.0 g; 3.6 mmol), (S)-2(aminomethyl)-N-ethylpyrrolidine (0.46 g; 3.6 mmol) and catalytic amounts of dimethylaminopyridine (0.04 g) were dissolved in chloroform (15 mL). The mixture was cooled to 0-5°C and into this stirring mixture dicyclohexylcarbodiimide (0.74 g; 3.6 mmol) was added. The reaction was allowed to stir at 0-5°C for 4 hours and then allowed to stir overnight at ambient temperature. The precipitated dicyclohexylurea was filtered and the filtrate washed with saturated sodium bicarbonate followed by water. The chloroform layer was then dried with magnesium sulfate and the solvent removed *in vacuo*. The residue was purified by chromatography to give the benzamide **5a** (0.87 g; 64%).

The tetrahydropyranyl ether (0.2 g; 0.5 mmol) was dissolved in ethanol-water (95:5) mixture (2 mL). To this was added oxalic acid so as to bring the pH to approximately 3.0 and the mixture stirred at 50°C for 2 hours. The deprotected alcohol **5b** was obtained in quantitative yield. NMR ∂ ppm: 8.4 (hump, 1H, CONH), 8.0-8.1 (d, 1H, H-6), 6.7-7.4 (dd, 2H, H-3, H-4), 4.9 (hump, 1H, OH), 3.9 (s, 3H, OCH_3), 3.7-4.0 (t, 2H, CH_2OH), 2.65-2.95 (t, 2H, benzylic CH_2),

1.4-3.4 (m, 11H), 0.95-1.3 (t, 3H, CH_3). MS (m/z, %): 306 (M^+, 5%), 277 ($[M-C_2H_5]^+$, 17%), 179 ($M-C_7H_{15}N_2$, 88%), 111 ($C_7H_{13}N^+$, 90%), 98 ($C_6H_{12}N_2^+$, 100%).

(S)-N-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-(2-fluoroethyl)-2-methoxybenzamide(**2**)

Diethylaminosulfur trifluoride (13 μL; 0.1 mmol) was mixed with dichloromethane (0.8 mL) and the mixture cooled to -40°C. Into this stirring mixture was added dropwise a solution of the substituted phenethyl alcohol **5b** (31 mg; 0.1 mmol) in dichloromethane (0.5 mL) over a period of 10 minutes. The reaction was allowed to proceed at -40°C for one hour and then warmed to ambient temperature and stirred for 18 hours. The organic layer was extracted with water. The pH of the aqueous layer was brought up to 10 and then extracted with ethyl acetate to provide the crude product. Chromatographic separation of the mixture provided pure **2**, in 70-80% yield. NMR ∂ ppm: 8.5 (hump, 1H, CONH), 8.1 (d, 1H, H-6), 6.85-7.45 (dd, 2H, H-3, H-4), 4.85-5.15 (t, 1H of CH_2F), 4.05-4.35 (t, 1H of CH_2F), 3.95 (s, 3H, OCH_3), 2.65-2.95 (t, 2H, benzylic CH_2), 1.5-3.4 (m, 11H), 0.95-1.35 (t, 3H, CH_3). MS (m/z, %): 308 (M+, 1%), 279 ($[M-C_2H_5]^+$, 1%), 181 ($[M-C_7H_{15}N_2]^+$, 9%), 98 ($C_6H_{12}N^+$, 100%), 111 ($C_7H_{13}N^+$, 7%).

(S)-N-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-(2-toluenesulfonyloxyethyl)-2-methoxybenzamide (**5c**):

The alcohol **5b** (0.2 g; 0.65 mmol) and pyridine (0.1 mL) were taken in dichloromethane (2 mL) and the mixture cooled to 0-5°C. Into this p-toluenesulfonyl chloride (0.12 g; 0.65 mmol) was added. The reaction was stirred for 2 hours at 0-5°C followed by 3 hours at ambient temperature. The reaction mixture was then washed with water and the organic layer dried in vacuo. The residue was taken up in ether and washed with 10% sodium hydroxide and water and dried over magnesium sulfate. The ether solution was filtered and the filtrate evaporated in vacuo to dryness. The tosylate **5c** was obtained in 70% yield and was separated from the alcohol by preparative tlc. NMR ∂ ppm: 8.35 (hump, 1H, CONH), 7.85 (d, 1H, H-6), 7.1-7.75 (dd, 4H, tosyl H-2,3,5,6), 6.6-7.25 (dd, 2H, H-3, H-4), 3.95-4.25 (t, 2H, CH_2-OSO_2), 3.85 (s, 3H, OCH_3), 2.7-3.05 (t, 2H, benzylic CH_2), 2.4 (s, 3H, tosyl CH_3), 1.5-3.4 (m, 11H), 0.9-1.25 (t, 3H, CH_3).

(S)-N-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-(2-[^{18}F]fluoroethyl)-2-methoxybenzamide (**2a**)

Aqueous ^{18}F-fluoride (10-25 mCi) was treated with potassium carbonate (4.8 mg) and kryptofix (26 mg) in a micro reaction vial. The mixture was dried by heating at 85-90°C under a stream of nitrogen. Last traces of water were removed azeotropically with acetonitrile. Into this

dried kryptofix-$K^{18}F$ complex, a solution (5.0 mg in 500 µL of acetonitrile) of the tosylate **5d** was added. This mixture was refluxed at 85-90°C for 30 minutes. The acetonitrile was blown dry and the residue taken up in ethyl acetate (3 mL) and washed with water (1 mL). The ethyl acetate layer was then passed through a mini-column of neutral alumina. The recovered ethyl acetate was evaporated to dryness and the residue taken up in 1:1 methanol-water and injected into the HPLC. Separation was carried out on an Alltech C_{18} column, using gradient elution with 95% 0.01 N ammonium acetate, pH 4.5 (solvent A) and 5% methanol (solvent B) at a flow rate of 3 mL/min, changing linearly to 5% solvent A and 95% solvent B in 20 minutes, and eluted for 10 additional minutes with this mixture (5% solvent A: 95% solvent B). The retention time of tosylate **5d** was 21.0 minutes while that of **2a** was 17.6 minutes. No other major radioactive peaks were observed in the chromatogram. The yields varied between 10-25% in several experiments for **2a**. Specific activity of **2a** was determined spectrophotometrically by measuring its absorbance at 298 nm and using the molar extinction coefficient determined for **2** (log Σ_{298} = 4.08, in methanol). The specific activity of **2a** after three passes through the HPLC varied between 600-800 Ci/mM. The observed low specific activity was due to traces of a mass peak that coeluted with **2a** and were inseparable under the gradient conditions used.

Results and Discussion

Carboxylation of the tetrahydropyranyl ether of **3** with n-butyllithium and CO_2 at -78°C gave low to moderate yields of the substituted benzoic acid **4**. Longer reaction times did not increase the yield significantly. The tetrahydropyranyl ether was found not to be stable to conditions employed for the formation of the acid chloride using thionyl chloride. We therefore employed dicyclohexylcarbodiimide to condense the acid with (S)-2-aminomethyl-N-ethylpyrrolidine. Yields of this condensation were in the range of 50-60%. One of the problems accounting for the low yield was the formation of O-acyl urea. Increasing amounts of the catalyst, 4-dimethylaminopyridine did not increase the yields of the product. The tetrahydropyranyl ether of **5a** was cleaved with oxalic acid in ethanol-water mixture cleanly to provide the corresponding phenethyl alcohol **5b**. The alcohol was converted to the fluoro derivative **5c** by treatment with diethylaminosulfur trifluoride (11). The tosylate precursor **5d** for F-18 labeling purposes was prepared by treating the alcohol **5b** with p-toluenesulfonyl chloride and pyridine in isolated yields of 70-75%.

^{18}F-fluoride was solubilized with kryptofix and potassium carbonate in acetonitrile taken in

micro reaction vials. Reaction of this ^{18}F-fluoride was carried out in small volumes of acetonitrile (typically 100-500 μL) with 3-5 mg of the tosylate **5d** for 30 minutes at 85-90°C. The yields were in the range of 10-25%. Purification of **2a** by reverse phase HPLC showed no other major radioactive peak, although **2a** had to be passed at least three times through the HPLC in order to remove traces of UV absorbing peaks (**5d** and an unidentified non-radioactive major side product in the reaction eluting at 19.5 minutes). Chemical purity of **2a** was reduced due to traces of coeluting mass peaks. Specific activity of **2a** (600-800 Ci/mmole) was computed using the extinction coefficient of **2** and assuming the coeluting peak to have a similar extinction coefficient. Purification of **2a** will have to be carried out under better HPLC eluting conditions in order to increase the specific activity.

Sulpiride has been shown to penetrate into the brain very poorly due to its low lipid solubility (12). The octanol-water partition coefficient (log P) reported for sulpiride is -0.50 (13). A log P value of 2.0±0.5 has been suggested to provide optimum brain uptake for central nervous system agents (14). The value obtained for **2** and **2a** in our experiments is 0.81±0. 02 and 0.80±0.01 respectively. Replacement of the sulfonamide group in sulpiride **1** with the fluoroethyl group in **2** makes the compound more lipophilic. The greater apparent lipophilicity of **2a** compared to that of sulpiride is likely to result in a greater brain uptake of **2a** than sulpiride. An increased striatal uptake of **2a** (compared to that of sulpiride) is therefore expected. However, confirmation of this hypothesis will have to await biodistribution studies of **2a** in rats.

In conclusion, we have reported the development of a new F-18 labeled benzamide neuroleptic in moderate yields of 10-25% with specific activities in the range of 600-800 Ci/mmole. Biodistribution of the radiotracer will be carried out in rats in order to confirm specific loclization in the striatum and the possibility of carrying out preliminary PET studies. Improvements of the isolated yields are being investigated with respect to the various reaction conditions, such as reaction time, reflux temperature of the reaction and nature of the leaving group. Enhancement of specific activities by improving elution parameters of HPLC in order to avoid coelution of unidentified mass peaks is also in progress.

Acknowledgements

We are particularly indebted to Drs. S. J. Gatley and O. T. DeJesus for their valuable assistance and discussions. We also wish to acknowledge support provided by the U. S. Department of Energy under contract No. DE-FGO2-86ER60438 and Brain Research Foundation of the University of Chicago.

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