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Research Article

Synthesis and radiolabelling of [^{123}I]-4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide, a potential dopamine D_3 antagonist for SPECT studies

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Summary

Schizophrenia is a devastating mental disorder characterized by relapsing psychotic episodes accompanied with emotional, professional and social decline. The classical dopamine hypothesis of schizophrenia postulates that hyperactivity of dopaminergic neurotransmission is responsible for the positive symptoms of the disorder. More exactly hyperactivity of the dopamine D_3 receptor system is thought to be involved in the pathology of schizophrenia. Therefore a new ^{123}I-labelled compound was developed which may allow *in vivo* visualization of the D_3 receptor by SPECT. [^{123}I]-4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide was synthesized and labelled by electrophilic aromatic substitution of the tributylstannyl derrivative. The radiochemical yield was 82–85% and the specific activity was >2.96 Ci/μmol. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: Dopamine; D_3 receptor; SPECT; radiotracer

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Introduction

The classical dopamine hypothesis of schizophrenia is based on the correlation between clinical doses of antipsychotic drugs, with prototype haloperidol, and their potency to block the dopamine D_2 receptor.[1,2] The dopamine receptors have been divided into 5 subtypes based on molecular biology data, the D_1-like receptors D_1 and D_5 and the D_2-like receptors D_2, D_3 and D_4. The latter class has recently received substantial attention, particularly since this receptor class is thought to be the target for atypical antipsychotic drugs, with prototype clozapine. Atypical neuroleptics are known to bind to a much lesser degree to the dopamine D_2 receptors in the striatum than typical neuroleptics although their antipsychotic efficacy is equal to that of typical neuroleptics.[3] Experimental studies have already shown a predominant action of atypical neuroleptics in the limbic regions. D_3, D_4 and D_5 subtypes of dopamine receptors seem to be predominantly located in the limbic system and cortical areas. Atypical neuroleptics have a more selective action on these receptor subtypes than do typical neuroleptics.[4] In the past several radiotracers for the D_2-like receptors like [^{123}I]-epidepride, [^{123}I]-IBZM, [^{11}C]-raclopride, [^{11}C]-nemonapride and [^{11}C]-methylspiperone have been developed, however these showed no selectivity between the different receptors of this class.[5,6] Very few radiotracers have been developed which show selectivity for the dopamine D_3 receptor like 7-OH-PIPAT.[7]

The aim of this study was to synthesize a tracer for SPECT studies of the dopamine D_3 receptor. In order to develop a tracer for these receptors a number of demands have to be met. First of all the tracer needs to have high affinity for the dopamine D_3 receptor due to the low density of these receptors, furthermore the selectivity over the other dopamine receptor subtypes and other receptor types in general needs to be good and finally the lipophilicity should not be too high in order to avoid non-specific binding. Selection of [^{123}I]-4-iodo-*N*-(4-(4-(2-methoxyphenyl)-1-piperazinyl) butyl)-benzamide as a potential tracer for the D_3 receptor was based upon data collected by Murray *et al.*[8] These data indicate that the corresponding bromated derivative has high affinity for the D_3 receptor ($pK_i = 9.3$) and shows a 100 fold selectivity over the D_2 ($pK_i = 7.4$) and D_4 ($pK_i = 7.3$) receptors. Apart from dopamine receptors there is some interaction with the 5-HT_{1A} receptor ($pK_i = 7.9$) and the α_1 receptor ($pK_i = 7.9$).[8] From these data we can assume that the iodinated derivative will also show selectivity for the D_3 receptor moreover since

antagonists with larger groups in the same position also show D_3 selectivity (e.g. aminobenzyl: pK_i $D_3 = 9.7$, pK_i $D_2 = 7.7$).[8]

Experimental

General

All reagents were purchased from commercial sources and were used without further purification. Reactions were monitored by thin layer chromatography (TLC, Polygram Sil G/UV_{254}, Machery-Nagel, Germany). Chromatographic purification of unlabelled compounds was performed on silica gel using the solvent systems indicated in the text. For mixed solvent systems, ratios are given with respect to volumes. Proton NMR spectra were recorded on a Varian 300 MHz FT-NMR spectrometer (Department of Medicinal Chemistry, Ghent University). Chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in either chloroform-d_3, acetone-d_6 or methylsulfoxide-d_6. High resolution mass spectroscopy performed using a Micromass Q-Tof-2 mass spectrometer (Rega Institute for Medicinal Research, Leuven University). [^{123}I] Sodium iodide (in 0.05 M NaOH) was purchased from DuPont Pharma (Brussels, Belgium). HPLC analysis of the radioligand was performed using a Waters 515 HPLC pump, a Waters 2487 UV detector (254 nm), and a Ludlum model 2200 sealer ratemeter. The column used was a reverse-phase base-deactivated column (Nucleosil C18 BDS 4.6 × 250 mm, 5 μm particle size; Alltech) and the mobile phases and flow rates are indicated in the text below.

*Synthesis of 2-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butyl)-isoindole-1,3-dione (**2**)*

1-(2-methoxyphenyl)-piperazine (2.3 g, 12 mmol) was dissolved in dimethylformamide (10 ml). Anhydrous sodium carbonate (1.27 g, 12 mmol) was pulverized and added, the mixture was heated to 80°C during 15 min. *N*-4-bromobutylphtalimide (3.38 g, 12 mmol) was added in small portions. The temperature was maintained at 80°C during 3 h and the reaction was monitored by TLC (dichloromethane/methanol/ammonia, 97/3/5). The formation of a new product with $R_f = 0.64$ was observed. The reaction mixture was then purified by extracting with ethyl acetate (100 ml) and the organic layer was washed with water

(2 × 100 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. 3.58 g of a yellow solid was obtained. This solid was used without further purification.

*Synthesis of 4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butylamine (**3**)*

2-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butyl)-isoindole-1,3-dione (**2**) (3.54 g, 9 mmol) was dissolved in absolute ethanol (200 ml). After addition of hydrazine hydrate (1 ml, 20 mmol) the mixture was refluxed for 3 h. Subsequently the mixture was cooled to room temperature. The reaction mixture was diluted with dichloromethane (100 ml) and washed with water. The organic layer was isolated, dried with sodium sulphate and evaporated under reduced pressure. The residue was analysed with TLC (dichloromethane/methanol/ammonia: 87/13/5) and three products were observed, the starting product ($R_f = 0.9$) and two newly formed products (R_f: 0.5 and 0.2). The mixture was purified by column chromatography (silica, 200 × 30 mm) with dichloromethane/methanol/ammonia (95/2/5) as eluent. The eluted fractions were collected and analysed with mass spectrometry and ^{1}H-NMR. The product with $R_f = 0.2$ was determined to be the desired product. The collected fractions were evaporated to yield 1.8 g (yield = 80%) of a white solid.

^{1}H-NMR (d_6-DMSO): 6.8–7.0 (m, 4 H, Ar*H*), 3.9 (s, 3 H, OCH_3), 3.2 (m, 4 H, piperazine*H*), 2.8 (t, 2 H, -CH_2-NH_2-), 2.6 (m, 4 H, piperazine*H*), 2.4 (t, 2 H, N-CH_2-CH_2), 1.8–2.0 (2q, 4 H, -CH_2-CH_2-). ESI-MS *m*/*z*: 264 ($[M + H]^+$).

*Synthesis of 4-bromo-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide (**4**)*

4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butylamine (**3**) (1.77 g, 6.73 mmol) was dissolved in dichloromethane (18 ml). Triethylamine (1.08 g, 10.68 mmol) was added and the temperature of the mixture was cooled to 0°C. 4-Bromobenzoylchloride (1.66 g, 7.57 mmol) was dissolved in dichloromethane (1.4 ml) and added dropwise at such a rate as to maintain the temperature below 10°C. Then the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (100 ml) and washed with a saturated sodium bicarbonate solution. The organic layer was then washed with water, dried with anhydrous sodium sulphate and the solvent was removed under reduced pressure. TLC analysis (dichloromethane/methanol/

ammonia: 87/13/5) of the mixture showed a newly formed product ($R_f = 0.8$). Purification was conducted on a silica column (silica, 200×30 mm) starting with pure dichloromethane and gradually (steps of 0.5%, 300 ml of each eluent) changing to dichloromethane/methanol (98/2). The eluted fractions were evaporated, a white solid was obtained (2.7 g yield = 90%). ^{1}H-NMR (d_6-DMSO): 7.8–7.9(dd, 4 H, Ar*H*), 6.7–7.0 (m, 4 H, Ar*H*), 3.8 (s, 3 H, OCH_3), 3.7 (t, 2 H, -CH_2-NH-CO), 3.3 (m, 4 H, piperazine*H*), 2.9 (m, 4 H, piperazine*H*), 2.8 (t, 2 H, N-CH_2-CH_2), 1.6–1.8 (2q, 4 H, -CH_2-CH_2-). ESI-MS *m*/*z*: 446-448 ($[M + H]^+$).

*Synthesis of N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-4-tributylstannyl-benzamide (***5***)*

4-bromo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide (**4**) (2.7 g, 6.04 mmol) was dissolved in dry toluene (100 ml). Hexabutylditin (10.44 g) and a catalytic amount ($\pm$ 3 mg) of tetrakistriphenylphosfinepalladium were added and the mixture was refluxed under nitrogen for 15 h. The reaction mixture was analysed by TLC (dichloromethane/methanol/ammonia: 87/13/5) and showed a newly formed product ($R_f = 0.65$). The mixture was purified on a silica column (silica, 200×30 mm) using dichloromethane/methanol (99/1) as eluent. The eluted fractions were collected, a yellowish, oily substance was obtained (2.8 g yield = 71%).

^{1}H-NMR (d_1-$CHCl_3$): 7.5-7.7 (dd, 4 H, Ar*H*), 7 (m, 4 H, A*rH*), 3.9 (s, 3 H, OCH_3), 3.1 (t, 2 H, -CH_2-NH-CO), 2.7 (s, 4 H, piperazine*H*), 2.9 (m, 4 H, piperazine*H*), 2.8 (t, 2 H, *N*-CH_2-CH_2), 1.3–1.5 (2q, 4 H, -CH_2-CH_2-), 0.8–0.9 (t, 27 H, tributylstannyl). EXACT MASS *m*/*z*: 658.3392 ($[M + H]^+$) (calculated: 658.3394).

*Synthesis of 4-iodo-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide (***1***)*

4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butylamin (**3**) (1.00 g, 3.80 mmol) was dissolved in dichloromethane (10 ml). Triethylamine (0.61 g, 6.03 mmol) was added and the temperature of the mixture was cooled to 0°C. 4-Iodobenzoylchloride (1.14 g, 4.28 mmol) was dissolved in dichloromethane (0.8 ml) and added dropwise at such a rate as to maintain the temperature below 10°C. Then the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (100 ml) and washed with a saturated sodium

bicarbonate solution. The organic layer was then washed with water, dried with anhydrous sodium sulphate and the solvent was removed under reduced pressure. TLC analysis (dichloromethane/methanol/ammonia: 87/13/5) of the mixture showed a newly formed product ($R_f = 0.8$). Purification was conducted on a silica column (silica, 200 × 30 mm) starting with pure dichloromethane and gradually (steps of 0.5%, 300 ml of each eluent) changing to dichloromethane/methanol (98/2). The eluted fractions were evaporated, a white solid was obtained (1.5 g yield = 88%). ^{1}H-NMR (d_6-DMSO): 7.9–8.0 (dd, 4 H, Ar*H*), 6.7–7.1 (m, 4 H, Ar*H*), 3.9 (s, 3 H, OCH_3), 3.7 (t, 2 H,-CH_2-NH-CO), 3.3 (m, 4 H, piperazine*H*), 2.9 (m, 4 H, piperazine*H*), 2.8 (t, 2 H, N-CH_2-CH_2), 1.6–1.8 (2q, 4 H, -CH_2-CH_2-). EXACT MASS *m*/*z*: 494.1309 ([M + H]$^+$) (calculated: 494.1306).

*Synthesis of [^{123}I]-4-iodo-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butyl)-benzamide (^{123}**I-1**)*

The iodination was conducted by electrophilic aromatic substitution of the tributylstannyl derivative (**5**). *N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-**4**-tributylstannyl-benzamide (**5**) (650 µg, 1 µmol) was dissolved in ethanol (50 µl). n.c.a. [^{123}I]NaI in sodium hydroxide solution (15 µL 0.01 M), chloramine T (282 µg, 1 µmol) and glacial acetic acid (5 µl) were added. The mixture was stirred and left to react for 10 min at room temperature. Afterwards an aqueous solution of sodium metabisulphite (15 µl 285 µg, 1.5 µmol) was added to quench the reaction.[9] The mixture was purified by HPLC with ethanol/acetate buffer (0.05 M, pH 5) (35/65) as mobile phase at a flow rate of 1 ml/min. The radiolabelled product was collected ($R_t = 16$ min) and analysed with the same HPLC system (see Figure 1).

Determination of the lipophilicity

Lipophilicity (log *P*) was determined by adding 100 µCi of the labelled compound to a mixture of 100 ml water and 100 ml octanol. After this mixture was shaken thoroughly, 50 ml of the octanol was taken and another 50 ml water is added. The mixture was shaken again and 1 ml of both the water and the octanol were counted for radioactivity. Log *P* was calculated by dividing the counts in the octanol by the counts in the water.

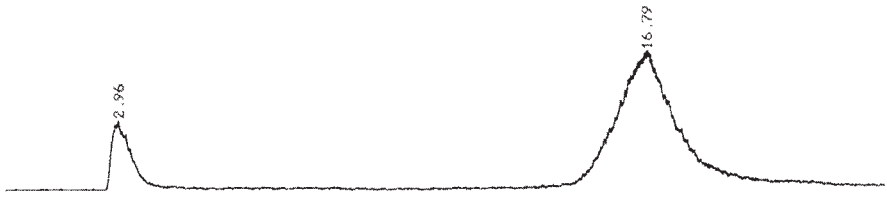
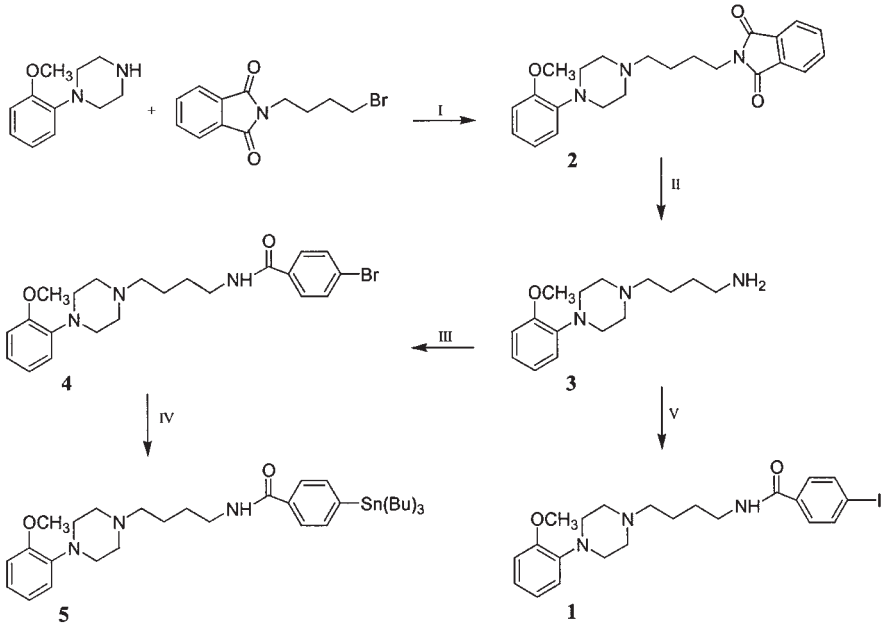


Figure 1. Radiochromatogram of a [^{123}I]-4-iodo-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide (^{123}I-1) synthesis

Results and discussion

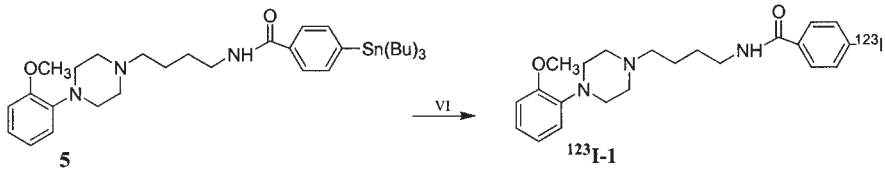
Synthesis of the precursor molecule and the cold product is shown in Scheme 1. These molecules were synthesized by coupling 1-(2-methoxyphenyl)-piperazine and *N*-4-bromobutylphtalimide followed by deprotection of the formed phtalimide moiety using hydrazine hydrate. The primary amine was then coupled to the appropriate



Scheme 1. Synthesis of *N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-4-tributylstannyl-benzamide and 4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)-butyl)-benzamide (I) sodium carbonate and dimethylformamide, (II) hydrazine hydrate and ethanol, (III) 4-bromobenzoylchloride, triethylamine and dichloromethane, (IV) hexabutyiditin, tetrakistriphenylphosfinepalladium and toluene, (V) 4-iodobenzoylchloride, triethylamine and dichloromethane

halogen benzoylchloride. The iodinated product was synthesized in good overall yield of about 53%. Further reaction of the bromine compound with hexabutylditin in the presence of tetrakistriphenylphosfinepalladium gave the tributylstannyl precursor. The overall yield for the precursor synthesis was about 38%.

The radiolabelling of the precursor molecule was conducted by electrophilic iododestannylation of the tributylstannyl precursor as shown in Scheme 2 and gave a radiochemical yield of 82–85%. The radiochemical purity of the collected fraction was > 95%. Since no UV signal was obtained from the synthesized product specific activity was obtained by determination of the detection limit of the UV detector, this showed the specific activity to be > 2.96 Ci/μmol. Stability was tested by leaving the product in solution at room temperature and reïnjecting it into the HPLC system. Up to 48 h after synthesis the radiochemical purity remained > 95%. The total amount of radioactivity obtained was up to 10 mCi, sufficient to perform SPECT studies in human volunteers. The log *P* value was measured to be 2.2.



5 ^{123}I-1

Scheme 2. [^{123}I]-4-iodo-N-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide. (VI) [^{123}I]-NaI, chloramine T, acetic acid, sodiummetabisulphite and ethanol

Conclusion

We reported the synthesis and radiolabelling of [^{123}I] 4-iodo-*N*-(4-(4-(2-methoxyphenyl)-piperazin-1-yl)butyl)-benzamide as a potential radiotracer for *in vivo* visualization of the dopamine D_3 receptor antagonist. The compound was labelled in a good yield (82–85%) and high specific activity (> 2.96 Ci/μmol) and will be evaluated by means of *in vitro* and *in vivo* experiments.

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