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Research Article

Synthesis and *in vivo* evaluation in mice of ([123]I)-(4-fluorophenyl)(1-(3-iodophenethyl)piperidin-4-yl) methanone as a potential SPECT-tracer for the serotonin 5-HT_{2A} receptor

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Abstract: This work reports the synthesis, radiolabelling and *in vivo* evaluation in NMRI mice of [^{123}I]-(4-fluorophenyl)[1-(3-iodophenethyl)piperidin-4-yl]methanone ([^{123}I]-3-I-CO) as a potential SPECT tracer for the 5-HT_{2A} receptor. The tributylstannylprecursor was synthesized with a 15% overall yield. Radiolabelling was performed using an electrophilic iododestannylation with yields of 85%. Radiochemical purity was always >95%. Log *P* was determined to be 3.10 ± 0.10. The tracer showed good uptake in mouse brain (6.3 ± 1.3% ID/g tissue at 10 min p.i., 2 ± 0.36% ID/g tissue at 1 h p.i.). These results warrant further research in larger animals to determine suitability of [^{123}I]-3-I-CO as a 5-HT_{2A} tracer. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: 5-HT_{2A} receptor; SPECT; radiotracer; serotonin; antagonist

Introduction

The serotonin 5-HT_{2A} receptor is an important target in nuclear medicine and psychiatry. Indeed, alteration of central 5-HT_{2A} receptors has been reported in several psychiatric conditions, such as schizophrenia, stress, aggression, depression and anorexia, both in humans[1–5] and in animals.[6–8] Currently, several tracers are used for *in vivo* imaging of the 5-HT_{2A} receptor. Most of them are PET-tracers: [^{18}F]-altanserin;[5,9,10] [^{18}F]-setoperone;[4,11] [^{11}C]-MDL100907[12,13] (Figure 1). Disadvantages associated with these PET-tracers are slow kinetics[14] and possible interference of lipophilic radiometabolites.[15,16]

Currently only one SPECT tracer for the 5-HT_{2A} receptor, [^{123}I]-R91150 (Figure 1) is used in clinical practice, with varying results.[1,2,17,18] *In vitro* testing with [^{123}I]-R91150 is problematic due to aspecific binding issues.

Due to the limitations of the existing tracers, development of novel tracers targeting the 5-HT_{2A} receptor is warranted. Preferably, these tracers should have no radiolabelled metabolites, high specific binding to the 5-HT_{2A} receptor and low aspecific binding.

This work reports the synthesis, radiosynthesis and preliminary *in vivo* evaluation in mice of [^{123}I]-(4-fluorophenyl)[1-(3-iodophenethyl)piperidin-4-yl] methanone ([^{123}I]-3-I-CO, **7**) as a potential SPECT tracer for the 5-HT_{2A} receptor. 3-I-CO is a 5-HT_{2A} antagonist with good affinity for the 5-HT_{2A} receptor (K_i = 0.51 nM) and a selectivity of at least a factor 20 over other 5-HT receptor subtypes.[19]

Results and discussion

Synthesis scheme of the tributylstannylprecursor for [^{123}I]-3-I-CO is shown in Figure 2.

3-Bromophenylacetic acid (**1**) was reduced to the corresponding alcohol (**2**) with borohydride-tetrahydrofuran, after which the hydroxyl-function was replaced by bromine using phosphorus tribromide to yield 3-bromophenethyl bromide (**3**). The ethylbromide (**3**) was coupled with 4-(4-fluorobenzoyl)piperidine (**4**) by a nucleophilic substitution reaction at 120°C, yielding [1-(3-bromophenethyl)piperidin-4-yl](4-fluorophenyl)-methanone (**5**). Finally, the bromine-atom of (**5**) was replaced by a tributylstannyl-group by reaction with hexabutylditin to obtain (**6**). Overall synthesis yield of

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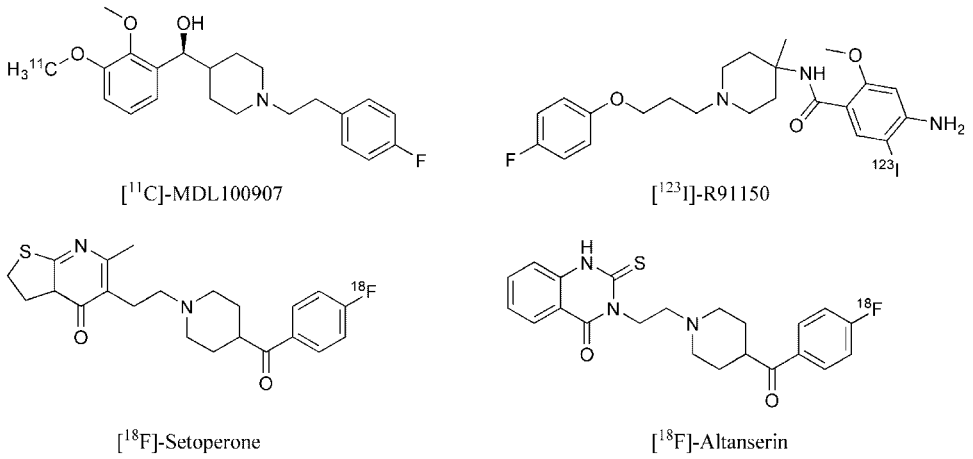

Figure 1 5-HT_{2A} tracers currently used in patients.

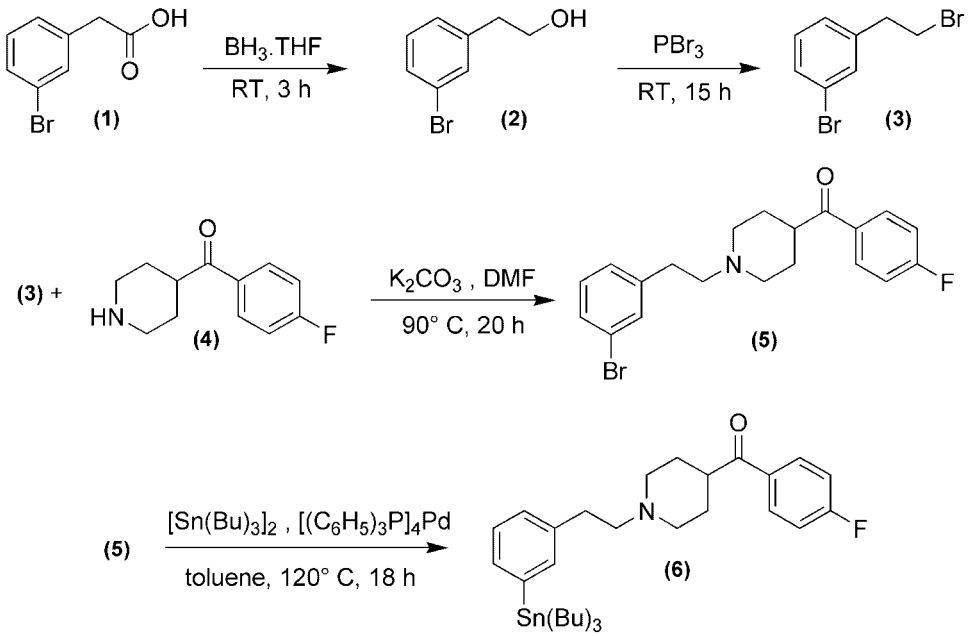

Figure 2 Synthesis of the tributylstannylprecursor.

(**6**) was only about 15%, mainly due to the low yield of the final step.

The cold iodinated molecule (used as a reference during HPLC analysis) was obtained by reacting (**6**) with iodine in chloroform. Yield was 30%.

The radiolabelling was performed using an electrophilic iododestannylation on the tributylstannylprecursor (**6**) (Figure 3) with chloramine-T. A radiochemical yield of 85% was obtained.

Radiochemical purity was always >95%. Identification of the collected product was performed by comparing the retention times on HPLC between the tracer and the cold reference sample. Retention times were identical.

Partition coefficient was determined using the 'shake-flask' method. A log *P* value of 3.10 ± 0.10 was obtained.

The results of the biodistribution study in male NMRI mice are shown in Table 1.

Figure 4 shows the uptake of [^{123}I]-3-I-CO in mouse brain and the blood radioactivity concentration. A maximum concentration in brain of $6.3 \pm 1.3\%$ ID/g tissue was obtained 10 min post injection, at 1 h post injection the radioactivity concentration was $2 \pm 0.36\%$

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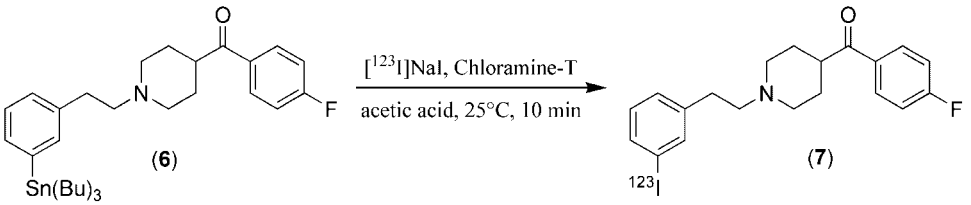

Figure 3 Radiosynthesis of [^{123}I]-3-I-CO.

Table 1 Biodistribution of [^{123}I]-3-I-CO in NMRI mice

Tissue	30 s	60 s	2 min	5 min	10 min	30 min	1 h	2 h
Blood	5.60 ± 1.03	3.48. ± 0.25	2.58 ± 0.19	2.01 ± 0.34	2.37 ± 0.20	1.80 ± 0.29	1.51 ± 0.04	0.80 ± 0.50
Brain	4.37 ± 0.48	4.31 ± 0.32	4.79 ± 0.13	5.94 ± 1.56	6.26 ± 1.36	3.37 ± 0.61	1.94 ± 0.37	0.98 ± 0.03
Heart	15.9 ± 1.82	10.9 ± 0.77	7.04 ± 0.91	4.83 ± 1.41	3.96 ± 0.73	2.11 ± 0.38	1.56 ± 0.07	1.22 ± 0.12
Lungs	29.6 ± 4.77	28.6 ± 1.95	21.1 ± 2.11	18.0 ± 2.72	13.2 ± 4.21	5.40 ± 0.46	3.68 ± 0.19	3.31 ± 0.83
Stomach	1.45 ± 0.33	2.01 ± 0.54	3.46 ± 0.45	5.49 ± 3.21	7.90 ± 3.63	5.90 ± 0.50	11.4 ± 3.96	18.1 ± 3.11
Spleen	3.24 ± 2.24	3.99 ± 0.49	5.52 ± 1.07	9.85 ± 0.16	10.1 ± 0.97	5.42 ± 1.15	5.64 ± 1.61	4.70 ± 2.79
Liver	5.40 ± 3.07	8.96 ± 1.55	12.8 ± 1.39	19.9 ± 5.79	21.7 ± 3.29	13.5 ± 1.36	9.64 ± 0.10	6.93 ± 0.63
Kidney	15.7 ± 4.26	17.7 ± 5.23	19.2 ± 1.01	16.5 ± 4.33	11.2 ± 2.84	8.15 ± 0.30	8.96 ± 3.59	5.24 ± 0.48
Small Int.	1.92 ± 0.58	2.94 ± 0.14	3.29 ± 0.39	4.43 ± 0.67	6.34 ± 1.73	5.67 ± 0.97	11.5 ± 6.99	19.0 ± 4.49
Large Int.	0.82 ± 0.25	1.32 ± 0.28	1.45 ± 0.40	2.20 ± 0.82	2.26 ± 0.85	1.77 ± 0.36	1.84 ± 0.53	4.21 ± 3.16
Blatter	1.86 ± 0.72	2.06 ± 0.62	2.89 ± 0.75	4.48 ± 1.91	5.53 ± 2.03	10.7 ± 8.95	16.4 ± 7.31	16.2 ± 7.11
Fat tissue	1.20 ± 0.85	1.30 ± 0.20	1.66 ± 1.22	1.17 ± 0.26	1.29 ± 0.36	2.54 ± 0.48	3.54 ± 0.24	4.95 ± 1.83

Radioactivity concentrations are expressed as percentage of the injected dose per gram tissue (% ID/g tissue). Results are expressed as mean ± s.d. ($n = 3$).

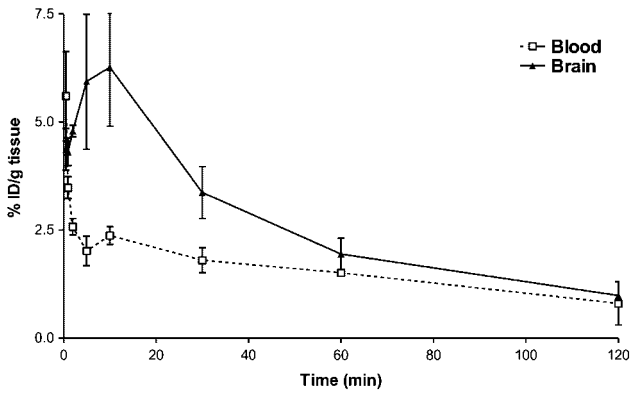

Figure 4 Biodistribution of [^{123}I]-3-I-CO in mouse brain. Results are expressed as mean ± s.d. ($n = 3$).

ID/g tissue. Blood activity always remained lower than brain activity. High uptake was also seen in lungs, liver and kidney (Table 1).

Experimental

General

All chemicals and reagents were purchased from Sigma-Aldrich (St-Louis, MO, USA) or Acros Organics (Geel, Belgium) and were used without further purification unless specified otherwise. HPLC and flash chromatography solvents were purchased from Chemlab NV (Belgium).

Organic reactions were monitored by normal phase thin layer chromatography (TLC) with UV detection at 254 nm where possible (TLC, Polygram Sil G/UV$_{254}$, 200 μm, Machery-Nagel, Germany).

Purification of non-radioactive products was achieved with flash column chromatography on silica gel (240–400 mesh, 60 Å, Sigma-Aldrich, Belgium) using a glass column (36 mm × 460 mm, Büchi) and a

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Waters LC-8A preparative HPLC pump at a flow of 60 ml/min. Solvent systems are indicated in the text. For mixed solvent systems, ratios are given with respect to volumes.

^{1}H-NMR spectra were recorded on a Varian 300 MHz FT-NMR spectrometer (Laboratory for Medicinal Chemistry, Ghent University, Belgium). Chemical shifts were recorded in ppm (δ) relative to an internal tetramethylsilane (TMS) standard in either acetone-d_6, dimethylsulfoxide-d_6 or chloroform-d_3.

Mass spectrometry was performed on a Waters Micromass ZMD mass-spectrometer with electrospray-ionization (ESI) probe. Samples were dissolved in methanol at a concentration of 0.050 mg/ml.

No carrier added (n.c.a.) [^{123}I]-NaI (formulated in 0.05 M NaOH) was purchased from GE Healthcare Biosciences (Diegem, Belgium).

Tracer purification and quality control were performed with a Waters Breeze system, consisting of a Waters 1525 HPLC pump and a Waters 2487 UV detector (detection at 254 nm). A scaler ratemeter (model 2200, Ludlum Measurements Inc, Sweetwater, Texas, USA) connected to a NaI-scintillation probe (model 44-2, 1 in × 1 in NaI crystal, Ludlum Measurements Inc, Sweetwater, TX, USA) was used for radioactivity detection. Purification and analysis of the radioligand were performed on a reverse-phase semi-preparative HPLC column (Alltech Apollo C_{18} 5 μm, 7 × 250 mm with 7 × 33 mm precolumn). A 200 μl stainless steel HPLC loop was used. Mobile phase consisted of 50/50 acetonitrile/phosphate buffer (0.02 M, pH 7). The flow rate was 4 ml/min.

Male Naval Medical Research Institute (NMRI) mice were obtained from Charles River Laboratories. All animal experiments were conducted following the principles of laboratory animal care and the Belgian law on the protection of animals. Our research protocol was approved by the Ghent University Hospital ethical committee (ECP 05/14).

Synthesis of precursor and cold reference compound

2-(3-bromophenyl)ethanol (2). 3-Bromophenylacetic acid (5 g, 23 mmol) (**1**) was added dropwise to a cooled solution of borohydride–tetrahydrofuran complex (BH3.THF, 35 mmol) under nitrogen atmosphere. The mixture was slowly warmed to room temperature and stirred for 2 h. The reaction was quenched by adding a 50/50 mixture of water and acetic acid (50 ml). THF was removed under reduced pressure. Saturated $NaHCO_3$ solution (100 ml) was added, and the mixture was extracted with dichloromethane (3 × 100 ml). The organic phases were combined, washed once with brine (100 ml), dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. A light yellow oil was obtained (3.7 g, 80%) which was used without further purification.

^{1}H-NMR (d_3-CH_3Cl, δ): 7.40–7.25 (m, 2H, 2-ArH and 4-ArH), 7.15–7.05 (m, 2H, 5-ArH and 6-ArH), 4.78 (t, 1H, -OH), 3.86 (m, 2H, R-CH_2-OH), 2.74 (t, 2H, Ar-CH_2-R). ESI-MS: 183 (MH^+ minus H_2O).

1-bromo-3-(2-bromoethyl)benzene (3). 2-(3-bromophenyl)ethanol (4 g, 20 mmol) (**2**) was cooled to 0°C under nitrogen atmosphere, and phosphorus tribromide (6.8 g, 2.4 ml, 25 mmol) was added slowly. The reaction was stirred at room temperature for 16 h. The reaction mixture was poured onto crushed ice (100 g). Saturated $NaHCO_3$ solution (100 ml) was added and the mixture was stirred for 30 min. The mixture was extracted with chloroform (3 × 100 ml) and the combined extracts were washed with saturated $NaHCO_3$ solution (100 ml). The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was dissolved in dichloromethane (200 ml) and filtered through silica gel. After removal of the solvent under reduced pressure, a slightly orange oil was obtained (4.7 g, 90%).

^{1}H-NMR: 7.30–7.20 (m, 2H, 2-Ar-H and 4-Ar-H), 7.10–7.0 (m, 2H, 5-Ar-H, 6-Ar-H), 3.63 (t, 2H, R-CH_2-Br), 3.05 (t, 2H, Ar-CH_2-R).

(1-(3-bromophenethyl)piperidin-4-yl)(4-fluorophenyl) methanone (5). To a solution of 4-(4-fluorobenzoyl) piperidine (2.5 g, 12 mmol) (**4**) in dry dimethylformamide (DMF, 40 ml) under nitrogen atmosphere 1-bromo-3-(2-bromoethyl)benzene (4 g, 15 mmol) (**3**) was added, followed by K_2CO_3 (4.2 g, 30 mmol). The mixture was stirred and heated to 90°C in an oil bath for 18 h. After cooling to room temperature, the reaction mixture was filtered and the filter cake was washed with DMF (3 × 25 ml). The combined filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography with 90:10:5 dichloromethane/methanol/triethylamine. An orange solid was obtained (60%).

^{1}H-NMR (d_6-DMSO, δ): 8.10 (m, 2H, 2-ArF and 6-ArF), 7.37 (m, 2H, 3-ArF and 5-ArF), 7.30–7.25 (m, 2H, 2-ArBr and 4-ArBr), 7.10–7.0 (m, 2H, 5-ArBr and 6-ArBr), 3.0 (q, 1H, F-Ar-CO-CH-R_2), 2.70–2.65 (m, 4H, Br-Ar-CH_2-CH_2-R), 2.24 (m, 4H, N-$(CH_2)_2$), 1.65 (m, 4H, F-Ar-CO-CH-$(CH_2)_2$-R). ESI-MS: 390/392 (MH^+).

(1-(3-tributylstannylphenethyl)piperidin-4-yl)(4-fluorophenyl)methanone (6). [1-(3-bromophenethyl)piperi-

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din-4-yl](4-fluorophenyl)methanone (100 mg, 0.25 mmol) (**5**) was dissolved in anhydrous toluene (10 ml) under nitrogen atmosphere. A catalytic amount of tetrakistriphenylphosphinepalladium (10 mg) was added, followed by hexabutylditin (0.4 ml, 0.75 mmol). The mixture was heated in the dark on an oil bath at 120°C for 18 h. After cooling to room temperature, dry toluene (20 ml) was added and the mixture was filtered over celite. The filter cake was washed with dry toluene (2×15 ml), and the combined filtrates were stripped of solvent under reduced pressure. The residue was purified by preparative thin layer chromatography, using dichloromethane:methanol:triethylamine (90:10:5) as eluent. The band corresponding to the product was scraped off and extracted with methanol. After evaporation of the solvent under reduced pressure, pure **6** was obtained (45 mg, 30%).

^{1}H-NMR (d_6-DMSO, δ): 8.10 (m, 2H, 2-ArF and 6-ArF), 7.37 (m, 2H, 3-ArF and 5-ArF), 7.2–7.1 (m, 4H, Sn(Bu)$_3$-Aryl-H), 3.0 (q, 1H, F-Ar-CO-CH-R$_2$), 2.70–2.65 (m, 4H, Br-Ar-CH$_2$-CH$_2$-R), 2.24 (m, 4H, N-(CH$_2$)$_2$), 1.65 (m, 4H, F-Ar-CO-CH-(CH$_2$)$_2$-R), 1.2–0.8 (m, 27H, tributylstannyl). ESI-MS: 602 (MH^+)

(1-(3-iodophenethyl)piperidin-4-yl)(4-fluorophenyl) methanone. [1-(3-tributylstannylphenethyl)piperidin-4-yl](4-fluorophenyl)methanone (50 mg, 0.08 mmol) was dissolved in anhydrous chloroform (10 ml) under nitrogen atmosphere. Iodine (0.2 ml, 1 M solution in chloroform) was slowly added. The mixture was stirred for 30 min at room temperature. The reaction was quenched by addition of sodium metabisulphite (1.5 ml, 5% solution in water) and potassium fluoride (1.5 ml, 1 M solution in methanol). The resulting mixture was stirred for 30 min and both phases were separated. The aqueous phase was extracted with chloroform (3×10 ml). The combined organic phases were washed with brine (15 ml), dried over anhydrous sodium sulphate and filtered. Chloroform was removed under reduced pressure, and the residue was purified by preparative TLC with dichloromethane:methanol:-triethylamine (90:10:5) as eluent. The band corresponding to the product was scraped off and extracted with methanol. After solvent removal under reduced pressure, an orange solid was obtained (11 mg, 0.025 mmol, 30% yield).

^{1}H-NMR (d_6-DMSO, δ): 8.10 (m, 2H, 2-ArF and 6-ArF), 7.50–7.60 (m, 2H, 2-ArI and 4-ArI), 7.37 (m, 2H, 3-ArF and 5-ArF), 7.0–7.11 (m, 2H, 5-ArI and 6-ArI), 3.0 (q, 1H, F-Ar-CO-CH-R$_2$), 2.70–2.65 (m, 4H, Br-Ar-CH$_2$-CH$_2$-R), 2.24 (m, 4H, N-(CH$_2$)$_2$), 1.65 (m, 4H, F-Ar-CO-CH-(CH$_2$)$_2$-R). ESI-MS: 438 (MH^+)

Radiosynthesis and purification of (^{123}I)-3-I-CO

[^{123}I]-3-I-CO was prepared using an electrophilic iododestannylation on the tributylstannylprecursor as shown in Figure 3. The precursor (**6**) (100 μg, 0.16 μmol) was dissolved in ethanol (50 μl). n.c.a. [^{123}I]-NaI (in 0.05 M sodium hydroxide, 5–30 μl, 37–500 MBq), chloramine-T (CT, 15 μl of a 18.8 mg/ml solution in H_2O) and glacial acetic acid (GAA, 20 μl) were added. The mixture was reacted at room temperature for 10 min. The reaction was quenched by addition of sodium metabisulphite (15 μl of a 19 mg/ml solution in H_2O) and HPLC mobile phase (100 μl, 50/50 acetonitrile/phosphate buffer pH 7) was added. The mixture was applied to a reverse-phase HPLC column (Alltech Apollo C18 7×250 mm, 5 μm) for purification and eluted with a mixture of 50% acetonitrile and 50% phosphate buffer (0.02M, pH7) at a flow rate of 4 ml/min. The fraction corresponding to [^{123}I]-3-I-CO ($T_R = 70$ min) was collected, diluted with water and passed over a C_{18}-cartridge (Alltech Maxi-Clean SPE Prevail C_{18}, previously activated with water and methanol). The cartridge was rinsed with water (5 ml) and the tracer was eluted with ethanol (1 ml). The ethanol was evaporated under a flow of nitrogen and the tracer was redissolved in 5% ethanol in water. The solution was sterilized by sterile filtration (Schleicher & Schuell, FP 013/AS 0.22 μm filter) for use in biodistribution studies. The radiochemical purity of the product was determined with the same reverse-phase HPLC system used for purification.

Measurement of partition coefficient (log P)

The partition coefficient of [^{123}I]-3-I-CO was measured according to the 'shake flask' method.[20] About 30 μl (0.1 mCi) of [^{123}I]-3-I-CO was added to a tube containing n-octanol (5 ml) and phosphate buffered saline (PBS, pH = 7.4, 5 ml). The tube was shaken in a vortex mixer for 5 min followed by centrifugation (5 min). The aqueous layer was discarded to remove free iodine, and fresh PBS (5 ml) was added to the tube. The tube was shaken and centrifuged; 0.5 ml aliquots of both phases were taken and counted for radioactivity with an automated gamma counter (Cobra Autogamma, five 1 in × 1 in NaI(Tl) crystals, Packard Canberra). The aqueous phase in the tube was discarded, and 4.5 ml of fresh PBS was added. The tube was shaken and centrifuged, aliquots were taken and counted, and the aqueous layer was again discarded. This process was repeated once more after adding 4 ml of fresh PBS. Radioactivity counts were decay corrected. The partition coefficient was calculated as P = (counts/g in

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1-octanol)/(counts/g in PBS). All the experimental measurements were performed in triplicate.

Biodistribution in NMRI mice

A biodistribution study of [^{123}I]-3-I-CO was performed in NMRI mice. Adult male NMRI mice (weight 20–25 g) were injected in the tail vein with 20–50 μCi of [^{123}I]-3-I-CO. At various time points post injection (20, 40 s, 1, 2, 5, 10, 30, 60, 120 min, $n = 3$ per time point) the mice were sacrificed. Blood was collected and the animals were rapidly dissected. Blood samples and organs of interest were weighed and counted for radioactivity in an automated gamma counter. The results were decay corrected and tissue radioactivity concentrations were expressed as a percentage of the injected dose per gram of tissue (% ID/g, mean ± standard deviation).

Conclusion

This work reports the synthesis, radiolabelling and *in vivo* evaluation in NMRI mice of [^{123}I]-(4-fluorophenyl)[1-(3-iodophenethyl)piperidin-4-yl]methanone, a potential SPECT tracer for visualization of the 5-HT$_{2A}$ receptor. The tributylstannylprecursor was synthesized with an overall yield of 15%. The precursor was radiolabelled with excellent yield (85%). Radiochemical purity was always >95%. Log *P* was measured by the 'shake-flask' method and was 3.10 ± 0.10. The tracer showed good uptake in mouse brain (6.3 ± 1.3% ID/g tissue at 10 min p.i., 2 ± 0.36% ID/g tissue at 1 h p.i.). These results warrant further research to assess the potential of [^{123}I]-3-I-CO as a 5-HT$_{2A}$ tracer. More specifically, regional biodistribution and displacement studies in larger animals (rats, rabbits) are needed to demonstrate specific binding in 5-HT$_{2A}$ receptor-rich brain regions.

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