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Research Article

Synthesis, radiosynthesis and preliminary *in vivo* evaluation of [^{123}I]-(4-fluorophenyl) {1-[2-(2-iodophenyl)ethyl]piperidin-4-yl}methanone, a potential 5-HT$_{2A}$-antagonist for SPECT brain imaging

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Summary

Many people suffer from psychiatric illnesses like depression and anorexia. Relevant to these diseases is amongst others a malfunctioning of brain 5-HT$_{2A}$-receptors. To allow *in vivo* quantification of these receptors with Single Photon Emission Computerized Tomography (SPECT), a radiolabelled ligand with high 5-HT$_{2A}$ affinity is needed.

This work reports the radiosynthesis of [^{123}I]-(4-fluorophenyl) {1-[2-(2-iodophenyl)ethyl]piperidin-4-yl}methanone, the synthesis of its precursor, (4-fluorophenyl) {1-[2-(2-bromophenyl)ethyl]piperidin-4-yl}methanone, and the preliminary *in vivo* evaluation of the tracer. The precursor was synthesized with a total yield of 40%. Radiolabelling was performed using a halogen exchange reaction and the yield was 70%. Radiochemical purity was $>95\%$, and specific activity was at least 2.4 Ci/μmol. Log *P* was measured to be 2.52. The tracer showed uptake in mice brain (3.5% I.D./g tissue at 3 min post injection) and therefore will be evaluated further by regional brain biodistribution and displacement studies in rabbits.

Key Words: serotonin; 5-HT$_{2A}$-receptor; SPECT; radiotracer

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Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter in the central nervous system. Cerebral 5-HT-receptors have been implicated in the pathogenesis of major mood and psychotic disorders, such as depression and schizophrenia.[1] Relevant to these diseases are problems associated with the 5-HT_{2A} G-protein-coupled receptor, found amongst others in high densities in cortical areas, striatum and hippocampus in all species.[2] To evaluate 5-HT_{2A}-receptor malfunctioning, *in vivo* quantification by positron emission tomography (PET) or SPECT is needed. These techniques provide highly sensitive methods for measuring *in vivo* neurochemical and pharmacological effects at specific target–receptor proteins. Several radioligands have been used to assess the 5-HT_{2A}-receptor[4] including [^{11}C]-*N*-methylspiperone, [^{11}C]-MDL1009075, [^{18}F]-setoperone, [^{123}I]-R91150. The PET-tracers are expensive, requiring a cyclotron on-site. [^{123}I]-R91150 shows high aspecific binding, thereby limiting its *in vitro* use. Development of better SPECT radiotracers with high specific brain uptake, selectivity for 5-HT_{2A}-receptors, and low aspecific binding would therefore be very useful. A log *P* value between 2 and 3 is desirable for minimizing aspecific binding issues.

This work reports the synthesis and preliminary *in vivo* evaluation in mice of [^{123}I]-(4-fluorophenyl){1-[2-(2-iodophenyl)ethyl]piperidin-4-yl}methanone ([^{123}I]-**5b**), a potential SPECT-tracer for *in vivo* imaging of the 5-HT_{2A}-receptor. The compound shows nanomolar affinity for the 5-HT_{2A}-receptor[6] ($K_i = 1.95$ nM), 20-fold selectivity over the 5-HT_{2C}-receptor and antagonistic properties.

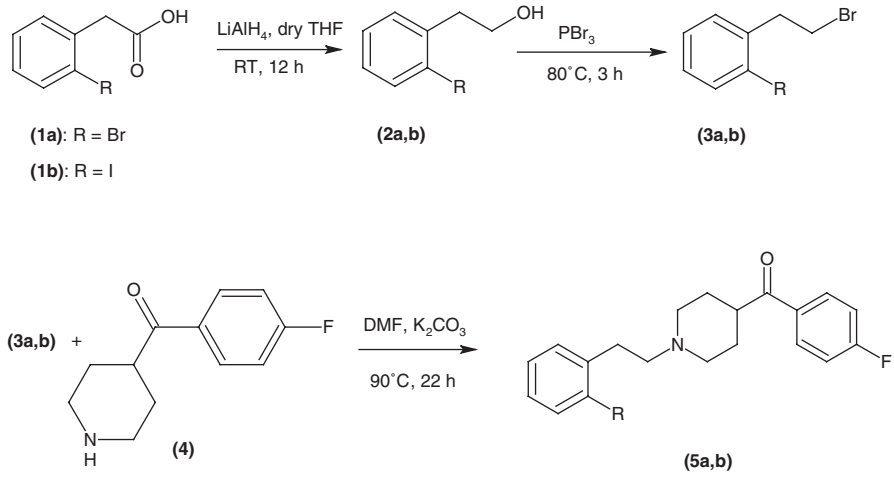
Results and discussion

Synthesis of the precursor molecule and the cold product as reference compound is shown in Scheme 1.

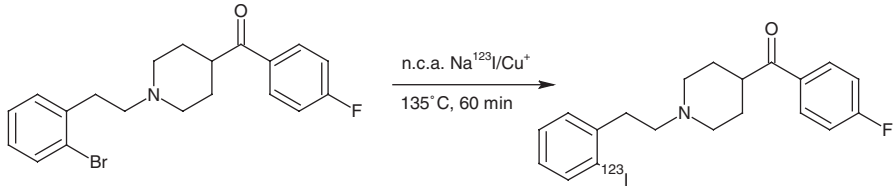
The appropriate halogenated phenylacetic acid derivatives (**1a**, **1b**) were reduced to the alcohols (**2a**, **2b**) with lithium aluminum hydride, after which the hydroxyl moiety was replaced by bromine using phosphorus tribromide, thus creating a much better leaving group. These ethylbromides (**3a**, **3b**) were then coupled with 4-(4-fluorobenzoyl)piperidine (**4**) by nucleophilic substitution.[6]

The iodinated and brominated products were both obtained in overall yields of 40%. The radiolabelling was conducted by a Cu^+ catalyzed nucleophilic exchange on the bromoprecursor (Scheme 2). A radiochemical yield of 70% was obtained.

The radiochemical purity of the tracer was $>95\%$. Specific activity was calculated to be at least 2.4Ci/μmol.



Scheme 1. Synthesis of precursor and reference compound



Scheme 2. Radiosynthesis of [^{123}I]-5b

Identification of the collected tracer was performed by comparing retention times on HPLC between the radioactive labelled product and the unlabelled iodinated molecule **5b**.

Tracer stability at room temperature was tested by reinjecting the tracer into the HPLC system; the radiochemical purity was $>95\%$ at 24 h after synthesis.

The log *P* value was measured by the shake-flask method to be 2.52, a suitable value for blood–brain–barrier penetration.[11]

Biodistribution studies demonstrated brain uptake of the compound in mice (3.5% I.D./g tissue at 3 min p.i.) (Figure 1). Other organs are not shown.

Experimental

General

All reagents were purchased from commercial sources (Sigma Aldrich Fluka, Acros Organics, Belgium) and were used without further purification, unless stated otherwise. 4-(4-Fluorobenzoyl)piperidine was commercially available

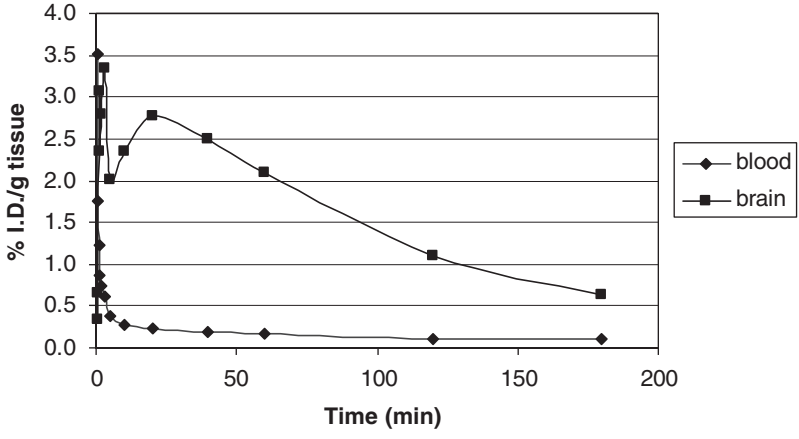


Figure 1. Biodistribution of [^{123}I]-5b in NMRI mice

as the *p*-toluenesulfonate. It was extracted as the base and purified by recrystallization from acetonitrile.

Reactions were monitored by thin layer chromatography under UV (254 nm) where possible (TLC, Polygram Sil G/UV$_{254}$, Machery-Nagel, Germany).

Purification of unlabelled compounds was achieved by column chromatography with silica gel (Sigma Aldrich, 200-400 mesh), using the solvent systems 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 (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 dimethylsulfoxide-d$_6$.

Mass spectrometry was performed on a Waters Micromass ZMD mass-spectrometer with electrospray-ionization probe. Samples were dissolved in methanol.

n.c.a. (no carrier added) [^{123}I] NaI (in 0.05 M NaOH) was purchased from Bristol-Myers-Squibb (Brussels, Belgium). HPLC purification and analysis of the radioligand were performed using a Waters 515 HPLC pump, a Waters 2487 UV detector (254 nm), and a Ludlum 2200 scaler ratemeter. Purification and analysis of radioligand were performed on a reversed-phase analytical HPLC column (Alltech Alltima C18 4.6 × 250 mm, 5 μm). Mobile phase was 50/50 acetonitrile/phosphate buffer (0.02 M, pH 6.5). A flow rate of 4 ml/min was applied.

For biodistribution studies, male NMRI (Naval Medical Research Institute) mice were used. Our research protocol was approved by the ethical committee (ECP 03/22).

Synthesis of precursor and reference compound

2(2-Bromophenyl)ethanol **(2a)**. Lithiumaluminumhydride (2.85 g, 75 mmol) was slowly added to dry tetrahydrofuran (100 ml) under nitrogen. The suspension was stirred and cooled to 0°C. A solution of 2-bromophenylacetic acid (1a) (10.7 g, 50 mmol) in dry tetrahydrofuran (50 ml) was added dropwise. The addition funnel was rinsed with dry tetrahydrofuran, and the ice-bath was removed. The mixture was stirred at ambient temperature for 12 h. The reaction mixture was cooled to 0°C, and quenched by adding ethylacetate (50 ml) dropwise, followed by methanol (20 ml). 15% NaOH in water (30 ml) was added to the mixture, which was then filtered over celite. The precipitate was washed with tetrahydrofuran (3×50 ml). The filtrate was evaporated under reduced pressure. Dichloromethane (250 ml) was added to the residue and the organic layer was separated, washed with brine (100 ml), dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. A yellowish oil was obtained (6.81 g, 69%).

^{1}H-NMR (d_6-DMSO, δ): 7.56 (d, 1 H, Br–ArH), 7.26 (d, 1 H, Br–ArH), 7.14-7.06 (q, 2 H, Br–ArH), 3.88 (t, 2 H, R–CH_2–OH), 2.87 (t, 2 H, Ar–CH_2–R), 1.54 (s, 1 H, –OH).

ESI-MS *m*/*z*: 201-203 ($[M+H]^+$).

2(2-Iodophenyl)ethanol **(2b)**. **2b** was prepared in exactly the same manner as **2a**, starting from 2-iodophenylacetic acid. Yield was 60%.

1H-NMR (d_6-DMSO, δ): 7.75 (d, 1H, I–ArH), 6.90 (d, 1H, I–ArH), 6..94-7.37 (q, 2H, I–ArH), 3.86 (t, 2H, R–CH_2–OH), 2.87 (t, 2H, Ar–CH_2–R), 1.54 (s, 1H, –OH).

ESI-MS *m*/*z*: 249 ($[M+H]^+$).

1-Bromo-2(2-bromoethyl)benzene **(3a)**. 2-(2-Bromophenyl)ethanol (**2a**) (5 g, 25 mmol) was added to a 25 ml vial, and the vial was cooled to 0°C under nitrogen. Phosphorus tribromide (8.12 g, 2.8 ml, 30 mmol) was added dropwise. The reaction mixture was heated at 80°C for 2 h. The mixture was poured onto crushed ice, 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 washed once with saturated $NaHCO_3$-solution (100 ml) and once with brine (100 ml). The solution was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. A yellowish oil was obtained (6.31 g, 96%).

^{1}H-NMR (d_6-DMSO, δ): 7.79 (d, 1H, Br–ArH), 7.25 (d, 1H, Br–ArH), 7.18-7.09 (q, 2H, Br–ArH), 3.48 (t, 2H, R–CH_2–Br), 2.96 (t, 2H, Ar–CH_2–R).

1-Iodo-2(2-bromoethyl)benzene **(3b)**. **3b** was prepared in exactly the same manner as **3a**, starting from **2b**. Yield was 90%.

^{1}H-NMR (d_6-DMSO, δ): 8.10 (d, 1H, I–ArH), 7.65 (d, 1H, I–ArH), 7.03-6.92 (q, 2H, I–ArH), 3.52 (t, 2H, R–CH_2–Br), 3.05 (t, 2H, Ar–CH_2–R).

(4-Fluorophenyl){1-[2-(2-bromophenyl)ethyl]piperidin-4-yl}methanone **(5a)**. To a solution of 4-(4-fluorobenzoyl)piperidine (**4**) (2.07 g, 10 mmol) in dry DMF (50 ml) under a nitrogen atmosphere was added 1-bromo-2-(2-bromoethyl)benzene (**3a**) (3.93 g, 15 mmol), followed by K_2CO_3 (5.5 g, 40 mmol). The mixture was heated at 90°C for 22 h. After cooling to ambient temperature, the mixture was filtered, and the precipitate was washed with DMF (20 ml). The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography with 20:80:10 EtOAc/hexane/Et_3N to give **5a** as a yellow solid (2.32 g, 60%).

^{1}H-NMR (d_6-DMSO, δ): 7.90 (q, 2H, F–ArH), 7.60 (d, 1H, Br–ArH), 7.22 (d, 1H, Br–ArH), 7.13 (m, 2H, F–ArH).7.09-7.00 (q, 2H, Br–ArH), 3.30-3.20 (m, 4H), 3.10 (t, 1H), 2.80 (t, 2H, Ar–CH_2–CH_2), 2.70 (t, 2H, Ar–CH_2), 2.10-1.92 (m, 4H).

ESI-MS *m/z*: 389/391 $[M+H]^+$.

(4-Fluorophenyl){1-[2-(2-iodophenyl)ethyl]piperidin-4-yl}methanone **(5b)**. **5b** was prepared in exactly the same manner as **5a**, starting from **3b**. Yield was 52%.

1H-NMR (d_6-DMSO, δ): 7.90 (q, 2H, F–ArH), 7.76 (d, 1H, I–ArH), 7.28 (t, 1H, I–ArH), 7.03-6.92 (m, 2H, F–ArH), 6.93-6.77 (m, 2H, I–ArH), 3.30-3.20 (m, 4H), 3.10 (t, 1H), 2.90 (t, 2H, Ar–CH_2–CH_2), 2.60 (t, 2H, Ar–CH_2), 2.10-1.92 (m, 4H).

ESI-MS *m/z*: 438 $[M+H]^+$.

Radiosynthesis

The nucleophilic exchange labelling on the bromoprecursor was performed using a Cu^+-catalysed method[7–9]. Two stock solutions were prepared: Solution A containing 32.5 mg $CuSO_4$ /10 ml water; Solution B, containing 9 mg $SnSO_4$, 25 mg gentisinic acid and 50 mg citric acid /10 ml water. Both solutions were flushed with nitrogen to remove oxygen. The bromoprecursor **5a** (1 mg, 2.6 μmol) was dissolved in 100 μl ethanol under nitrogen; 500 μl of solution B and 65 μl of solution A were added to the vial. The vial was flushed with nitrogen, sonified for 15 min, and [^{123}I] NaI (0.1–5mCi) was added.

The vial was heated at 135°C for 1 h in an aluminum heating mantle, and cooled to ambient temperature. After cooling, the reaction mixture was injected onto an HPLC column for purification (Alltech Alltima RPC18, 4.6 × 250 mm), with 50/50 acetonitrile/phosphate buffer (0.02 M, pH6.5) as eluent at 4 ml/min. The desired radiolabelled product (R_t = 19.3 min) was collected, and diluted with water to bring the acetonitrile concentration below 10%. The mixture was passed through a Sep-Pak cartridge (Waters Sep-Pak Light tC18), and rinsed with 5 ml saline. The cartridge was previously activated with 1 ml methanol and rinsed with 1 ml saline. The tracer was eluted

with 1 ml ethanol. An aliquot was reinjected onto the same HPLC system for quality control and stability testing. For biodistribution studies, the tracer was formulated in an ethanol/saline solution (containing less then 10% ethanol), and filtered through a 0.2 μm filter (Schleicher&Schuell, FP 013/AS).

Since no UV-signal was obtained from the synthesized product, specific activity was calculated by determination of the detection limit of the UV-detector, using a calibration curve with cold **5b**.

Determination of partition coefficient

Determination of the partition coefficient was performed according to published literature.[10,11] Briefly, about 10 μl (0.1 mCi) of the radioligand was added to a separatory funnel containing *n*-octanol (100 ml) and phosphate buffer (0.02 M, pH 7.4, 100 ml). The mixture was shaken manually for 3 min and the layers were separated. The aqueous layer was discarded, to remove any hydrophilic impurities present. The *n*-octanol layer (100 ml) was transferred to a second separatory funnel containing phosphate buffer (100 ml). The mixture was shaken for 3 min, and the layers were separated. A 5 ml aliquot of both layers was counted for radioactivity. The aqueous layer was discarded. Once again, the *n*-octanol layer (95 ml) was transferred to a new separatory funnel containing phosphate buffer (95 ml), the funnel shaken for 3 min, the layers separated, and a 5 ml aliquot of both layers was taken and counted for radioactivity. This process was repeated once more. The radioactivity counts were decay-corrected, and the partition coefficient was calculated: P = counts in *n*-octanol/counts in buffer. Reported log P value represents the mean of three determinations.

Biodistribution study in NMRI mice

A biodistribution study of the radiotracer was performed in mice. Adult white male NMRI mice weighing 20–25 g were each injected with 1-2μCi of the radiotracer [^{123}I]-**5b** in the tail vein. The mice were sacrificed at selected time points after injection ($n = 3$ per time point). Blood and tissues of different organs (brain, heart, lung, liver, kidney, e.a.) were rapidly removed and weighed. Radioactivity of the samples was measured in an automated γ-counter (Cobra, Packard Canberra). Tissue radioactivity concentrations were expressed as percent of injected dose per gram of tissue (% I.D./g tissue).

Conclusion

This work reported the synthesis and radiolabelling of (4-fluorophenyl) {1-[2-(2-iodophenyl)ethyl]piperidin-4-yl} methanone, a potential radiotracer for *in vivo* visualization of the 5-HT_{2A}-receptor with SPECT. The precursor was synthesized in an overall yield of 40%. The tracer was labelled in good yield

(70%). The specific activity was at least 2.4 Ci/μmol. Log *P* was 2.52. The tracer showed good brain uptake in mice (3.5% I.D./g tissue at 3 min p.i.). Regional biodistribution and displacement studies in rabbit brain are further required to demonstrate specific binding in brain regions expressing 5-HT_{2A}-receptors.

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