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Synthesis and Preliminary in vivo Evaluation of 4-[^{123}I]iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide, a Potential SPECT Radioligand for the 5-HT$_{1A}$ Receptor

M. Vandecapelle[1*], F. De Vos[1], F. Dumont[1], K. Audenaert[2], D. Leysen[3], R.A. Dierckx[2] and G. Slegers[1]

[1] Department of Radiopharmacy, Ghent University, Ghent, Belgium
[2] Division of Nuclear Medicine, Ghent University Hospital, Ghent, Belgium
[3] Department of Medicinal Chemistry, Scientific Development Group, Organon NV, Oss, The Netherlands

SUMMARY

4-[^{123}I]Iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.^{123}I), a potential SPECT 5-HT$_{1A}$ radioligand, was synthesized by electrophilic iododestannylation of the tributyltin derivative with n.c.a. [^{123}I]NaI and chloramine-T under acidic conditions (20 min, ambient temperature). Radiochemical yield averaged around 60 % (s_d=8, n=22). After HPLC purification, chemical purity was higher than 95 % and radiochemical purity higher than 99 %. Specific activity was always higher than 222 GBq/μmol (6 Ci/μmol). The calculated log P is 4.28. Initial brain uptake in NMRI mice was 1.16 % ID (2 min) and decreased slowly afterwards (0.51 % ID (2 h)). 60 min *p.i.*, the majority of the radioactivity in plasma and brain was present as 1.^{123}I, respectively 75.15 % and 96.48 %.

Key words: Org 13063, 5-HT$_{1A}$ agonist, ^{123}I labelling, SPECT, brain uptake, metabolite analysis

INTRODUCTION

The neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) plays an important role in a variety of physiological, behavioural and cognitive functions e.g. gastrointestinal

* Author for correspondence: Harelbekestraat 72, B-9000 Ghent, Belgium;
Telephone: +32 (0)9 2648066; fax: +32 (0)9 2648071; e-mail: marleen.vandecapelle@rug.ac.be

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motility, cardiovascular function, pain perception, thermoregulation, sleep, mood and sexual behaviour. Impairments of serotonergic neurotransmitter functions have been implicated in the aetiology of a variety of mental disorders including psychotic disorders, mood and anxiety disorders (obsessive compulsive disorder, panic disorder, phobia,...), sleep disorders and dementia (1, 2, 3).

Over the past few years, special efforts have been put in the development of a 5-HT_{1A} radioligand. Clinically, this receptor subtype is meant to be involved in the pathogenesis of several neuropsychiatric disorders e.g. anxiety and depression, disorders in the regulation of aggression and substance abuse disorders (3, 4).

PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) offer the unique opportunity to study the 5-HT_{1A} receptor using a selective radioligand. At the moment, mainly PET tracers are available to study the 5-HT_{1A} receptor. [Carbonyl-^{11}C]-WAY100635 (N-{2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl}-N-2-pyridinylcyclohexanecarboxamide, pK_i=9.1 (5)) is, despite major metabolization, capable of delineating the 5-HT_{1A} receptor in human brain (6) (^{11}C: β^+, $t_{½}$=20.4 min). [^{18}F]-MPPF (4-fluoro-N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-2-pyridinylbenzamide=[^{18}F]-FBWAY, pK_i=8.5 (5)) suffers from the same extensive metabolization but seems nevertheless to be a good ^{18}F labelled alternative for [carbonyl-^{11}C]-WAY100635 (7, 8) (^{18}F: β^+, $t_{½}$=109.7 min). Several other ^{18}F labelled derivatives of WAY100635 are being developed e.g. [^{18}F]-FCWAY (9) and pyridine derivatives of [^{18}F]-MPPF (10). A completely different structure is found in [^{11}C]-NAD299 ((R)-3-N,N-dicyclobutylamino-8-fluoro-3,4-dihydro-2H-1-benzopyran-5-carboxamide hydrogen (2R,3R)-tartrate monohydrate, pK_i=9.2 (11)) which was shown to be able to delineate the 5-HT_{1A} receptor in non-human primates (11, 12).

Org 13063 or 4-fluoro-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl} benzamide (1.F, figure 1) is a full 5-HT_{1A} agonist with high affinity (pK_i=9.3) and selectivity (1000 fold (towards 5-HT_{2A}) and higher) for this receptor subtype (13). The iodine derivative (1.I, figure 1) was found to have lower affinity (pK_i=8.2) but showed no loss of selectivity (14) and has a calculated lipophilicity value (clog P) of 4.28 (15).

The labelling of agonists for G-protein coupled receptors has been very controversial: no agonist or partial agonist has yielded significant specific labelling of the 5-HT_{1A} receptor *in vivo* despite numerous efforts with very high affinity and selective ligands. Examples of failed agonists or partial agonists in the 5-HT_{1A} field (for *in vivo* imaging) include [^{11}C]8-OH-DPAT, [^{11}C]Org 13052, [^{11}C]OSU 191, [^{125}I]trans-8-OH-PIPAT. In contrast some antagonists have been used with great success even when their *in vitro* affinity is no better than the agonists. It has been postulated that such differences between agonists and antagonists arise from the dissociation of G-protein receptor complexes upon binding of an agonist ending with free receptor complex subunits with low affinity for the agonist. Thus allowing the fast dissociation of the ligand from the receptor (16, 17). Another possible explanation for the *in vivo* failure of agonistic radioligands is the fact that agonists have to compete with the endogenous neurotransmitter serotonin for the same binding sites while the antagonistic radioligands can bind also to the low affinity state receptor (18). However from a clinical and functional point of view, it would be highly valuable to develop a 5-HT_{1A} radioligand that is capable of only visualizing the high affinity state of this receptor.

We hereby report the synthesis and preliminary *in vivo* evaluation of 4-[^{123}I]iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.^{123}I) (^{123}I: γ, $t_{1/2}$=13.2 h).

RESULTS AND DISCUSSION

4-[^{123}I]Iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.^{123}I, figure 2) was synthesized by electrophilic iododestannylation of the tributyltin precursor (1.$SnBu_3$, figure 1). The synthesis of this tributyltin precursor and the reference iodine molecule (1.I) is shown in figure 1. After the acidic hydrolysis of the amide moiety of Org 13063 (1.F), the primary amine (2) was coupled with the appropriate halogen benzoylchloride to give the bromine (1.Br) or the iodine (1.I) derivative of Org 13063. Reaction of the bromine compound with Sn_2Bu_6 in the presence of $Pd(PPh_3)_4$ catalyst, resulted in the production of the tributyltin precursor (1.$SnBu_3$).

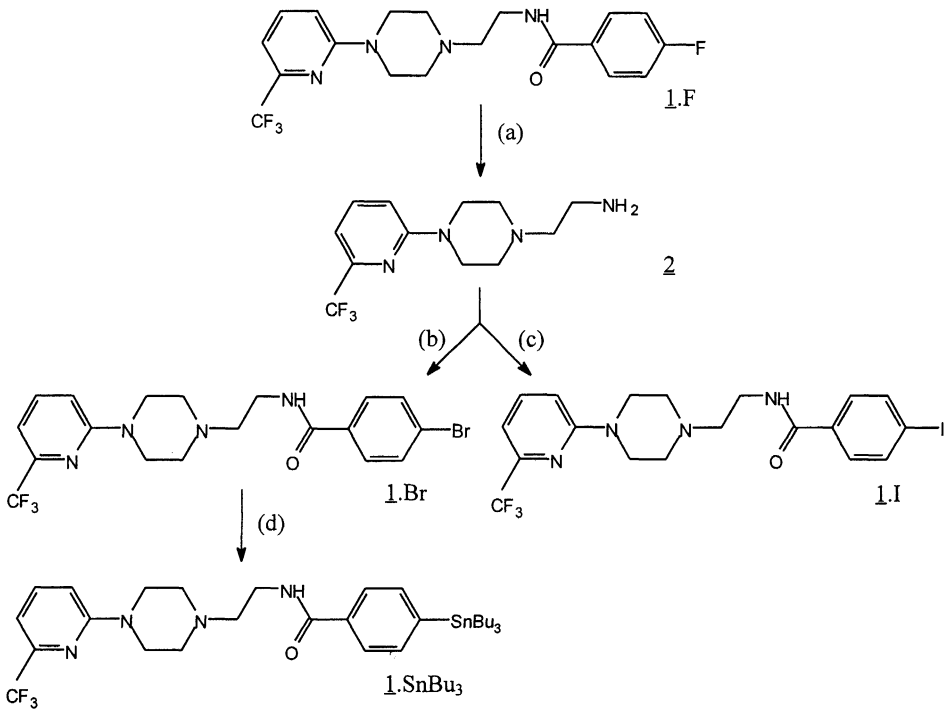

Figure 1: Synthesis of the tributyltin precursor (1.$SnBu_3$) and the reference iodine molecule (1.I)
Reaction conditions: (a) HCl (0.5 M), reflux, 96 h (b) 4-Br-benzoylchloride, TEA, RT, 3 h
(c) 4-I-benzoylchloride, TEA, RT, 3 h (d) Sn_2Bu_6, $Pd(PPh_3)_4$, reflux, 16 h, N_2

Originally, 2 was synthesized through a three step reaction starting from piperazine and 2-chloro-6-trifluoromethylpyridine (19). Due to the poor availability and the high cost of the latter product, we searched for an alternative route. The here proposed reaction (figure 1, step (a)) yielded 2 in only one step instead of three. Because of the difficulty of completely separating the final tracer (1.^{123}I) from Org 13063 (1.F), the purification of 2 should be carried out carefully to completely remove Org 13063 to prevent its presence in the final tracer solution.

Because the bromine derivative of Org 13063 was first synthesized, we tried the nucleophilic exchange reaction (20) directly on this compound. However, no 1.^{123}I could be detected. We have to remark that these nucleophilic exchange attempts were only limited and that we switched very quickly to the electrophilic exchange labelling reaction on the tributyltin compound.

4-[^{123}I]Iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.^{123}I) was synthesized by electrophilic iododestannylation of the tributyltin precursor (1.$SnBu_3$) with n.c.a. [^{123}I]NaI and chloramine-T (CAT) (figure 2).

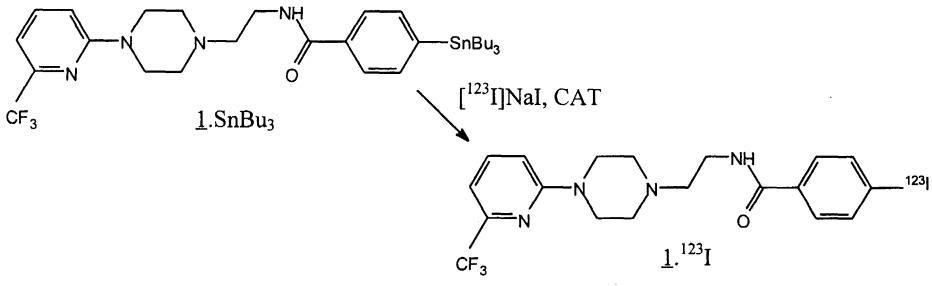

Figure 2: Radiosynthesis of 1.^{123}I

The different reaction parameters of this labelling method were separately and successively optimized (figure 3). For experiments performed more than once, averages and standard deviations (s_d) were calculated. The aim of this optimization was only to have enough tracer to start the *in vivo* evaluation.

Varying reaction time en temperature showed that the highest radiochemical yield (r.c.y.) was obtained after keeping the reaction mixture at ambient temperature for 30 min (figure 3 (a)). We preferred keeping the duration of this radiosynthesis as short as possible and continued our experiments with 20 min reaction time.

To guarantee a good HPLC purification, the total reaction volume should be kept as low as possible. The volume of the precursor solution was therefore reduced from 100 to 50 μL. We observed that the minimum amount of precursor needed for reaching reasonable yields, was 0.1 mg (0.15 μmol) (figure 3 (b)). A precursor amount of 3 mg (1.5 μmol) made the reaction mixture turbid and the radiochemical yield decreased.

In the previously discussed experiments, CAT was dissolved in H_2O. This solvent was replaced by acetone because of the observed longer storage life of the latter solution. This switch was shown to have no significant impact on the achieved radiochemical yields (data not shown). The influence of the total amount of CAT was only moderate in the studied concentration area (figure 3 (c)).

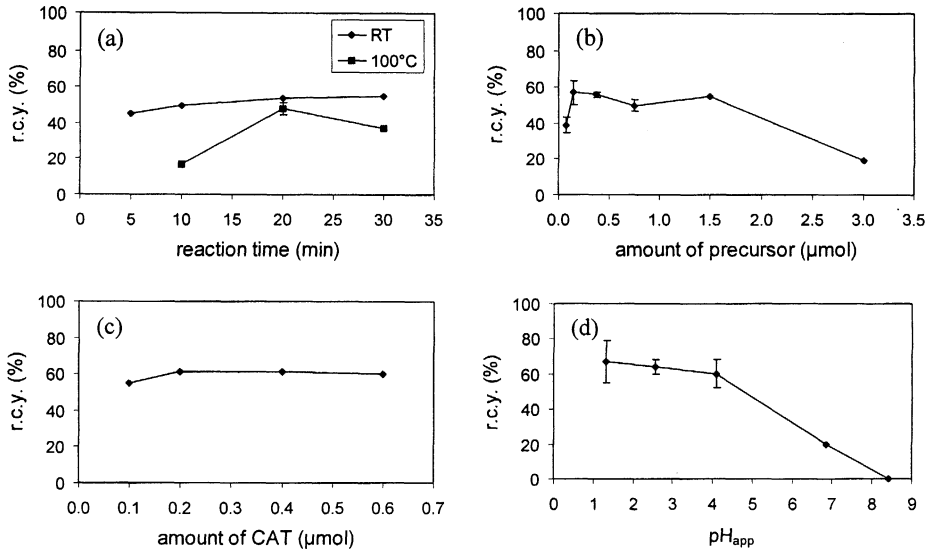

Figure 3: Influence of different reaction parameters on radiochemical yield (r.c.y.)
(a) reaction time and temperature (b) amount of precursor (c) amount of CAT (d) pH_{app}

Finally, the influence of the acidity of the reaction mixture on the labelling yield was investigated (figure 3 (d)). The previously used acetic acid was replaced by phosphoric acid. Addition of different phosphate solutions created different pH_{app} of the reaction mixture. As we expected, highest radiochemical yields were obtained under acidic reaction conditions because of the use of CAT as oxidant. We preferred labelling 1.$SnBu_3$ at pH_{app} 4.1 because of the better stability of both the tracer and its precursor at higher pH_{app} if not immediately injected on HPLC.

This optimization procedure resulted in the production of the desired radioligand with a radiochemical yield (r.c.y.) averaging around 60 % (s_d=8, n=22). Purification of the synthesized tracer was performed by HPLC. Originally, we used an Alltima C18 column (150 x 4.6 mm, 3 µm) combined with CH_3CN/KH_2PO_4 (0.1 M, pH=6.5): 55/45 as eluens (flow rate=1 mL/min). The incompatibility of CH_3CN with intravenous injections would have made a time consuming evaporation or Solid Phase Extraction of the tracer elution fraction necessary. Therefore, we switched to a more practical HPLC system consisting of a Spherisorb C18 column (150 x 4.6 mm, 3 µm) and $EtOH/KH_2PO_4$ (0.1 M, pH=6.5): 50/50 as eluens (flow rate=0.5 mL/min). The retention time (R_t) of the tracer on this HPLC system was ± 45 min (figure 4).

The precursor could not be detected with this HPLC system, the peak right in front of the tracer (R_t=± 35 min) is probably the chlorine derivative associated with the use of CAT as oxidant.

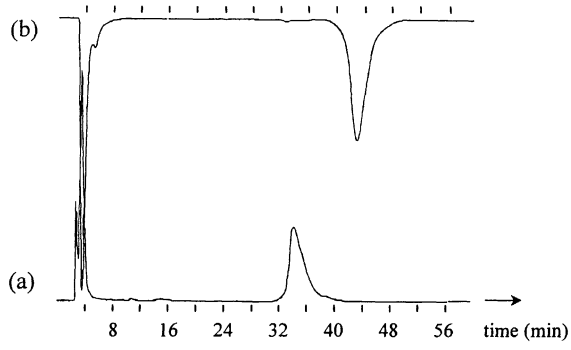

Figure 4: Radiochromatogram of a 1.^{123}I synthesis
(a) UV signal, (b) NaI(Tl) signal

This HPLC set up made chemical purity higher than 95 % and radiochemical purity higher than 99 %. Pharmaceutical preparation was limited to a simple dilution with sterile water. Specific activity (S.A.) was always higher than 222 GBq/μmol (6 Ci/μmol). Stability tests showed that the tracer remained more than 98 % radiochemically pure during the first 24 h after production. In a typical production, we started with 296 MBq (8.00 mCi) of n.c.a. [^{123}I]NaI and synthesized 129.5 MBq (3.50 mCi) of injectable tracer solution within 70 min.

Biodistribution studies in mice showed that the injected radioactivity enters the brain quickly and sufficiently (1.16 % ID s_d=0.18, 2 min). The slow decrease afterwards (0.51 % ID s_d=0.10, 2 h) suggests that the tracer is bound (non) specifically in the brain. However, at none of the selected time points, the % ID/g tissue was significantly higher than that of blood (table 1). This is probably more caused by a slow blood wash out than by a too limited brain uptake. This slow clearance is probably caused by the relatively high lipophilicity value of the iodinated molecule.

Highest radioactivity concentrations were observed in the liver (21.95 % ID/g s_d=3.97, 10 min). After the liver, small (16.89 % ID/g s_d=4.74, 2 h) and large intestine (14.75 % ID/g s_d=2.31, 3 h) reached successively their highest radioactivity concentrations. As these radioactivity values are coming from the intestine contents,

this profile is caused by biliary excretion in the gastrointestinal tract. The radioactivity release through renal and enteric excretion happens quickly and completely: no accumulation of radioactivity was observed (data not shown).

	0.33	0.66	1	1.5	2	3	5	10	20	40	60
Blood	13.70	8.36	6.56	4.43	4.18	3.64	2.71	2.88	2.16	1.75	1.72
	2.06	3.07	1.56	0.87	0.77	0.48	0.18	0.44	0.39	0.44	0.72
Brain	2.41	3.03	2.87	2.57	2.68	2.61	2.45	2.95	2.39	2.03	2.44
	0.61	0.39	0.50	0.11	0.51	0.42	0.27	0.10	0.41	0.47	1.06
	90	120	150	180	240	360	540	720	900	1440	2880
Blood	1.69	1.63	0.81	0.79	0.80	0.43	0.22	0.05	0.09	0.13	0.02
	0.31	0.62	0.44	0.18	0.16	0.21	0.12	0.03	0.02	0.12	0.02
Brain	1.87	1.23	0.75	0.77	0.73	0.21	0.06	0.02	0.01	0.04	0.01
	0.29	0.37	0.22	0.02	0.26	0.11	0.01	0.04	0.02	0.04	0.02

Table 1: Average % ID/g tissue and standard deviations as a function of time *p.i.* (min)

Radiochromatographic analysis of *in vivo* plasma and brain samples of mice sacrificed at different time points *p.i.*, showed 4 radioactive elution peaks, 3 of which could be identified as successively being [^{123}I]I$^-$, 4-[^{123}I]I-benzoate and the parent compound 1.^{123}I. 60 min *p.i.*, 75.15 % (s_d=0.66) of the plasma radioactivity was still present as 1.^{123}I, 14.13 % (s_d=2.08) as [^{123}I]I$^-$, 4.33 % (s_d=0.66) as 4-[^{123}I]I-benzoate and 2.96 % (s_d=0.30) as the non identified metabolite (figure 5 (a)). Radioactivity in brain samples 60 min *p.i.*, consisted for 96.48 % (s_d=0.08) of 1.^{123}I, 0.77 % (s_d=0.10) of [^{123}I]I$^-$, 0.68 % (s_d=0.01) of 4-[^{123}I]I-benzoate and 1.02 % (s_d=0.05) of the non identified metabolite (figure 5 (b)). The unidentified compound is probably a hydroxyl derivative of 1.^{123}I because of its somewhat shorter retention time than 1.^{123}I and its passage through the blood-brain barrier.

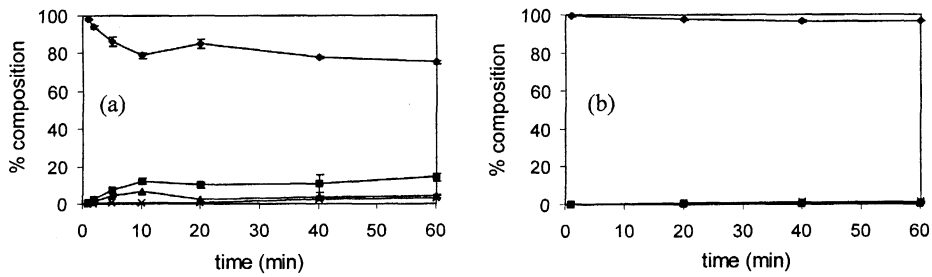

Figure 5: Chemical composition in terms of percentage of radioactivity present in (a) plasma and (b) brain samples (♦: 1.^{123}I, ■: [^{123}I]I$^-$, ▲: 4-[^{123}I]I-benzoate, ×: unidentified labelled metabolite)

The extraction procedure was validated by radiochromatographic analysis of spiked plasma and brain samples of non injected mice. Respectively 99.27 % (s_d=0.42) and 99.15 % (s_d=0.61) of the eluating radioactivity consisted of 1.^{123}I. The extraction yield of the validation experiment for plasma and brain samples was respectively 95.90 % (n=1) and 87.33 % (s_d=0.99, n=3).

EXPERIMENTAL

Materials and Methods

Org 13063 was obtained from Organon NV (Oss, The Netherlands). All other chemicals were purchased from Aldrich or UCB and were of the highest available purity. They were used without further purification except for toluene that was dried over molecular sieves (3 Å).

Chemical reactions were monitored by TLC using coated silica gel plates (Polygram SIL G/UV_{254} - Machery-Nagel) and CH_2Cl_2/CH_3OH/NH_3: 90/16/21 as mobile phase (19). The different spots were visualized using either UV light or ninhydrine. Column chromatography was performed with silica gel (50-200 μm, Süd Chemie) and mobile phases as mentioned. HPLC was carried out using a Waters 510 pump and HPLC columns and eluentia as mentioned. The effluent was monitored with an UV/VIS detector at λ=254 nm (PU 4110 UV/VIS - Philips) and a NaI(Tl) detector (Bicron Frisktech™, probe 1x1 inch). Effluent fractions were collected with a fraction collector (Pharmacia, RediFrac).

Radioactivity measurements were performed on a single channel NaI(Tl) detector (Canberra Packard, type 2007P, 3x3 inch).

^{1}H NMR spectra were obtained on a 500 MHz spectrometer (Brüker, Accuspec 3000). Chemical shifts are reported in ppm units downfield from internal $(CH_3)_4Si$. Electrospray Ionisation Mass Spectrometry (ESI MS) was performed on a HP 1100 instrument (Hewlett Packard). Exact mass measurements were performed on a quadrupole - time of flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface (KUL, Rega Institute, Belgium).

[^{123}I]NaI was purchased from Nycomed-Amersham (Cygne, The Netherlands) as n.c.a. [^{123}I]NaI (S.A.=9.25 TBq/μmol (25 Ci/μmol)) in NaOH (0.05 M).

All animal studies were carried out in compliance with the Animal Ethical Committee of the Ghent University and the Belgian laws. NMRI mice (m=20–25 g, either sex) were used.

Synthesis of 2-[4-(6-trifluoromethyl-2-pyridinyl]-1-piperazinyl]ethylamine (2)

Org 13063 (1.F, 500 mg, 1.261 mmol) was refluxed in a HCl solution (500 mL, 0.5 M) for 96 h. The solvent was partially evaporated and made alkaline by addition of a NaOH solution (35%). The formed 2 was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried with Na_2SO_4 (anhydric) and evaporated. Purification was performed by column chromatography (CH_2Cl_2/CH_3OH/NH_3: 90/16/21). Chemical yield was 85 %. ^{1}H NMR ($CDCl_3$): 2.49 (2H, t), 2.55 (4H, t), 2.57 (2H, s), 2.85 (2H, t), 3.61 (4H, t), 6.76 (1H, d), 6.93 (1H, d), 7.56 (1H, t); ESI MS (m/z): 275, 258; Exact mass (ESI-MS) $[M+H]^+$: found 275.1489, calculated 275.1483

Synthesis of 4-bromo- or 4-iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.Br or 1.I)

2 (274.3 mg, 1 mmol) was dissolved in CH_2Cl_2 (2 mL). After addition of TEA (158.9 mg, 1.586 mmol), the reaction mixture was cooled to 0° C. Synthesizing 1.Br or 1.I, respectively 4-bromo-benzoylchloride (247.1 mg, 1.126 mmol) or 4-iodo-benzoylchloride (300.0 mg, 1.126 mmol) in CH_2Cl_2 (2 mL) was added dropwise. This reaction mixture was stirred for 3 h at ambient temperature. The organic layer was washed with H_2O, dried with Na_2SO_4 (anhydric) and evaporated. Purification was performed by column chromatography (CH_2Cl_2/CH_3OH/NH_3: 90/16/21). Chemical yield was 72 % (1.Br) and 65 % (1.I). ^{1}H NMR ($CDCl_3$): 2.6 (6H, m), 3.55 (2H, q), 3.65 (4H, t), 6.78 (1H, d), 6.96 (1H, d), 7.55 (2H, t), 7.6 (1.Br)/7.5 (1.I) (1H, t), 7.65 (1.Br)/7.73 (1.I) (2H, d), 7.95 (1H, t); ESI MS (m/z): 457-458 (1.Br), 505 (1.I); Exact mass (ESI-MS) for 1.I $[M+H]^+$: found 505.0714, calculated 505.0713; Exact mass (ESI-MS) for 1.Br $[M+H]^+$: found 457.0850, calculated 457.0851

Synthesis of 4-tributyltin-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]-ethyl}benzamide (1.SnBu$_3$)

A solution of 1.Br (25.00 mg, 54.67 μmol), Sn_2Bu_6 (71.98 mg, 131.6 μmol) and catalytic amounts of $Pd(PPh_3)_4$ in dry toluene (4 mL), was stirred under reflux and N_2 atmosphere for 16 h. The solvent was evaporated and the residue was purified with column chromatography (flushing with hexane, elution of 1.SnBu$_3$ with EtOAc). Chemical yield was 20 %. ^{1}H NMR ($CDCl_3$): 2.6 (6H, m), 3.55 (2H, q), 3.65 (4H, t), 6.78 (1H, d), 6.96 (1H, d), 7.55 (2H, t), 7.6 (1H, t), 7.75 (2H, d), 7.95 (1H, t); ESI MS (m/z): 667.2-669.2; Exact mass (ESI-MS) $[M+H]^+$: found 669.2806, calculated 669.2801

Radiosynthesis, purification and analysis of 4-[^{123}I]iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.^{123}I)

(a) Radiochemical yield as a function of the reaction temperature and time

To a solution of 1.SnBu$_3$ (1 mg, 1.5 μmol) in EtOH (100 μL) was successively added a solution of CAT (111.0 μg, 0.394 μmol) in H_2O (10 μL), glacial HOAc (10 μL) and n.c.a. [^{123}I]NaI in NaOH (70 μL, 0.05 M). Reaction temperature was either ambient temperature or 100°C and reaction time was varied from 5 until 30 min. The reaction was quenched by the addition of $NaHSO_3$ (50 μL, saturated). Labelling yield was determined by HPLC analysis of 20 μL of the reaction mixture.

(b) Radiochemical yield as a function of the amount of precursor

Different amounts of precursor (0.075-3 μmol) were dissolved in EtOH (50 μL). CAT (111.0 μg, 0.394 μmol) in H_2O (10 μL), glacial HOAc (10 μL) and n.c.a. [^{123}I]NaI in NaOH (70 μL, 0.05 M) were added and the reaction mixture was allowed to react at ambient temperature for 20 min. The reaction was stopped by adding $NaHSO_3$ (50 μL, saturated). Labelling yield was determined by HPLC analysis of 20 μL of the reaction mixture.

(c) Radiochemical yield as a function of the amount of CAT

1.SnBu$_3$ (0.100 mg, 0.150 μmol) in EtOH (50 μL) was mixed with different solutions of CAT (0.1-0.6 μmol) in acetone (10 μL). Glacial HOAc (10 μL) and n.c.a. [^{123}I]NaI

in NaOH (70 μL, 0.05 M) were added. After 20 min at ambient temperature, the reaction was stopped by the addition of $NaHSO_3$ (50 μL, saturated). Labelling yield was determined by HPLC analysis of 20 μL of the reaction mixture.

(d) Radiochemical yield as a function of the pH_{app}

The pH of a phosphate solution (1 M starting from H_3PO_4 (85 %)) was adjusted by addition of different amounts of a NaOH solution (35 %). The phosphate solutions (pH=0.3-7.0, 10 μL) were added to a solution of 1.$SnBu_3$ (0.100 mg, 0.150 μmol) in EtOH (50 μL) and CAT (111.0 μg, 0.394 μmol) in acetone (10 μL). N.c.a. [^{123}I]NaI in NaOH (70 μL, 0.05 M) was added. The pH_{app} of the resulting solutions varied from 1.3 to 8.4. After 20 min at ambient temperature, the reaction was stopped by the addition of $NaHSO_3$ (50 μL, saturated). Labelling yield was determined by HPLC analysis of 20 μL of the reaction mixture.

(e) Optimized radiosynthesis, purification and analysis of 4-[^{123}I]iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl)-1-piperazinyl]ethyl}benzamide (1.^{123}I)

1.$SnBu_3$ (0.100 mg, 0.150 μmol) was dissolved in EtOH (50 μL). CAT (111.0 μg, 0.394 μmol) in acetone (10 μL), phosphate buffer (10 μL, pH=2) and n.c.a. [^{123}I]NaI in NaOH solution (70 μL, 0.05 M) were added successively. Allowing this mixture to react for 20 min at ambient temperature, the reaction was quenched by addition of $NaHSO_3$ (50 μL, saturated).

Purification was performed by HPLC (Spherisorb C18 (150 x 4.6 mm, 3 μm), EtOH/KH_2PO_4 (0.1 M, pH=6.5): 50/50, flow rate=0.5 mL/min). 1.^{123}I (R_t=± 45 min) was collected in fractions of 0.5 min (250 μL) and diluted sixfold with distilled water so that the final concentration of EtOH was below 10 %.

Determination of the specific activity, chemical and radiochemical purity was performed by HPLC under the same conditions as the purification of 1.^{123}I.

Stability tests were performed by reinjection of the radioligand fraction unto the same HPLC system.

Biodistribution study in mice

Mice (n=3x22) were injected with 1.^{123}I (74 or 148 kBq (2 or 4 μCi)/100 μl H_2O, max 10 % EtOH) in the lateral tail vein. At preset time points *p.i.* (20 s to 48 h), the animals were sacrificed by decapitation under halothane anestesia. All animals were dissected; blood and urine samples were collected. The different organs were washed and dried; bladder was emptied while stomach, small and large intestine were not. Everything was weighed and the present radioactivity measured. Body remainder values were corrected for geometry. All values were corrected for decay and background. The total amount of injected radioactivity was calculated by summation of the counts for the different tissues and organs.

Results are expressed as percentage of the injected dose per gram tissue (% ID/g) or as percentage of the injected dose (% ID). Averages and standard deviations (s_d) were calculated. The t-test (two sided, paired, n=3, α=0.05) was used for determining significant differences between results of different organs.

Metabolite analysis of mice plasma and brain samples

Mice (n=3x7) were injected with 1.^{123}I (± 3.7 MBq (100 μCi), 200 μL H_2O, max 10% EtOH) via de lateral tail vein. At set time points *p.i.* (1, 2, 5, 10, 20, 40, 60 min), the animals were sacrificed by decapitation under halothane anestetia. For all mice, blood samples were collected and except for the mice of 2 and 5 min, brain was removed.

The blood samples were centrifuged (5 min, 3000 g) and plasma was separated from the blood cells. Plasma aliquots (200 μL) were diluted with 800 μL of CH_3CN, mixed on a vortex (10 s) and centrifuged (5 min, 3000 g). To the whole brain (300 – 400 mg) 1500 μL of acetonitrile was added. The brain was homogenized with a polytron mixer (10 s) and centrifuged (5 min, 3000 g). 750 μL supernatant of this last centrifugation of plasma or brain sample, was processed on a HPLC system (Econosil C18 (250 x 10 mm, 10μm), CH_3CN/NaOAc (0.1M, pH=6.5): 65/35 (pH adjusted to 5 with H_3PO_4). The flow was set at 3 mL/min and the eluate was collected in fractions of 0.5 min during 22.5 min. The retention times of the intact tracer 1.^{123}I, 4-[^{123}I]I-benzoate and [^{123}I]I^- was determined to be respectively ± 14 min, 6.5 min and 3.5 min by HPLC analysis of the non radioactive analogs. To validate the extraction

procedure, plasma and brain samples of non injected mice (n=3) were spiked with 1.^{123}I (± 37 kBq (1 μCi), 200 μL, max 10 % EtOH) and analyzed as described above. The results are expressed as percentages of the total radioactivity eluating of the HPLC system. Averages and standard deviations (s_d) were calculated. The extraction yield was expressed as the percentage of radioactivity present in the supernatant relative to the sum of the radioactivity present in supernatant and pellet of the last centrifugation.

CONCLUSIONS

4-[^{123}I]Iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl]-1-piperazinyl]ethyl}benzamide is a highly selective radioligand for the 5-HT_{1A} receptor and could potentially be the first SPECT tracer for this receptor subtype. It could be prepared in a straightforward way requiring only common conditions. Radiochemical yield averaged around 60 % (s_d=8, n=22). After HPLC purification, chemical purity was higher than 95 % and radiochemical purity higher than 99 %. Specific activity was always higher than 222 GBq/μmol (6 Ci/μmol). Overall yields of 40 % were obtained within 70 min.
4-[^{123}I]Iodo-N-{2-[4-(6-trifluoromethyl-2-pyridinyl]-1-piperazinyl]ethyl}benzamide enters the brain quickly and sufficiently (1.16 % ID, 2 min) and is in fact present as the originally injected tracer (96.48 %, 60 min). The tracer is slowly metabolized as shown by plasma values (75.15 %, 60 min).
Further investigations are in progress to check the selectivity and the specificity of the binding of the tracer in the brain.

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