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Design, Synthesis, Radiolabeling, and in Vitro and in Vivo Evaluation of Bridgehead Iodinated Analogues of *N*-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexanecarboxamide (WAY-100635) as Potential SPECT Ligands for the 5-HT$_{1A}$ Receptor

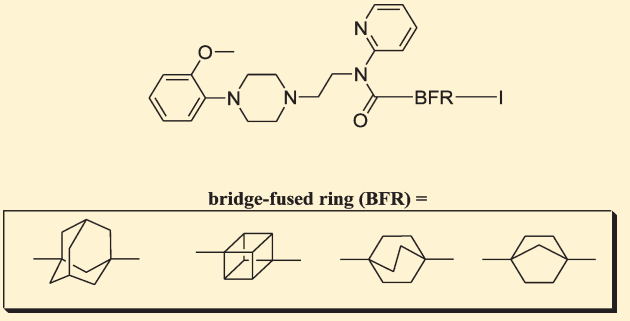
Rana Al Hussainy,*[,†] Joost Verbeek,[†] Dion van der Born,[†] Anton H. Braker,[†] Josée E. Leysen,[†] Remco J. Knol,[‡] Jan Booij,[‡] and J. (Koos) D. M. Herscheid[†]

[†]Department of Nuclear Medicine and PET Research, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

[‡]Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Supporting Information

ABSTRACT: Here we describe the design, synthesis, and pharmacological profile of 5-HT$_{1A}$ receptor ligands related to **1** (WAY-100635). The cyclohexyl moiety in **1** and its O-desmethylated analogue **3** were replaced by the bridgehead iodinated bridge-fused rings: adamantyl, cubyl, bicyclo[2.2.2]octyl, or bicyclo[2.2.1]heptyl. All analogues displayed a (sub)nanomolar affinity for the 5-HT$_{1A}$ receptor in vitro. Compounds **6b** and **7b** appeared to be selective for this receptor over other relevant receptors and could easily be iodinated with radioactive iodine-123. In humane hepatocytes, [^{123}I]**6b** showed a low propensity for amide hydrolysis and a stable carbon−iodine bond. The biodistribution of [^{123}I]**6b** and [^{123}I]**7b** in rats revealed that the carbon−iodine bond was also stable in vivo. Unfortunately, the brain uptake and the specificity for both radioligands were significantly lower than those of the parent molecule **1**. In conclusion, the designed tracers are not suitable for SPECT imaging.



INTRODUCTION

5-Hydroxytryptamine-1A (5-HT$_{1A}$) receptors belong to the G-protein-coupled receptors. They couple to the G$_i$-protein to inhibit the production of the second messenger cyclic adenosine monophosphate.[1,2] These receptors are expressed on the cell bodies of 5-HT neurons as somatodendritic autoreceptors and mediate the inhibition of 5-HT cell firing and 5-HT release. They are also expressed as postsynaptic receptors in the 5-HT neuron terminal fields. High 5-HT$_{1A}$ receptor concentrations are found in the brainstem raphe nuclei, and high densities of postsynaptic 5-HT$_{1A}$ receptors are found in the limbic forebrain (hippocampus, entorhinal cortex, and septum). Low densities are observed in the basal ganglia and in the adult cerebellum.[3]

5-HT$_{1A}$ receptors mediate various serotonergic functions[4,5] and may be implicated in various pathologies including depressive[6,7] and anxiety[8] disorders, Alzheimer's disease,[9,10] and schizophrenia.[11−13] Studies using partial 5-HT$_{1A}$ agonists such as the anxiolytic agent buspirone suggested that the 5-HT$_{1A}$ receptor is involved in the pathogenesis and treatment of depression.[3,8] These data may implicate the importance of the 5-HT$_{1A}$ receptor as a target for drug therapy and/or as a marker to study the underlying pathophysiology of neuropsychiatric disorders.

Molecular imaging techniques using positron emission tomography (PET) or single photon emission computerized tomography (SPECT) are valuable to investigate changes of 5-HT$_{1A}$ receptor densities. Radioligands structurally similar to the 5-HT$_{1A}$ antagonist *N*-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)cyclohexanecarboxamide (**1**, WAY-100635,[14] Figure 1), were proven to be the most promising for imaging purposes.[7,9,11,13] Compound **1** itself has been labeled with carbon-11 in position A or position B. Since the main metabolic pathway of **1** is the hydrolysis of the amide bond, a label in the cyclohexanecarbonyl moiety is favored. Otherwise, the lipophilic metabolite **2** (WAY-100634)[15] carries the label, and as it also enters the brain and binds to α_1 adrenoceptors, it interferes with the scintigraphic measurements.[15−17]

PET studies using the tracer [carbonyl-^{11}C]**1** showed that 5-HT$_{1A}$ receptor binding was reduced by 42% in the brainstem

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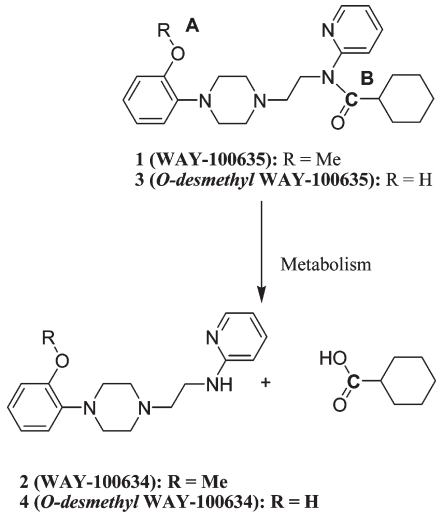

Figure 1. Main metabolic pathway of compound **1**.

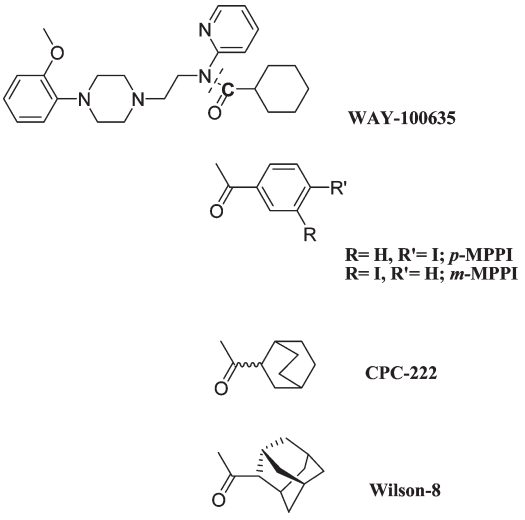

Figure 2. WAY 100635, WAY 100635 SPECT analogues *p*-MPPI and *m*-MPPI, WAY 100635 analogues containing a bulky substituent CPC-222 and Wilson-8.

raphe nuclei and by 27% in mesiotemporal cortex (hippocampus–amygdala) in patients suffering from major depressive disorder, compared to controls.[7] Although this tracer produces superior images of 5-HT_{1A} receptors in human brain, it has a major drawback because it is labeled with carbon-11 ($t_{1/2}$ = 20 min) and thus can only be used where both a cyclotron and a PET-camera are in proximity. Alternatively, for SPECT, longer lived isotopes, like iodine-123 ($t_{1/2}$ = 13.2 h), can be used. SPECT studies can be better scheduled than PET studies, and SPECT cameras are available in almost all hospitals. Therefore, it is highly desirable to have iodine-123 labeled radiopharmaceuticals to visualize the 5-HT_{1A} receptor density in the human brain. As such, iodine-123 analogues of **1** like 4-iodo-*N*-{2-[1-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide (*p*-MPPI), and 3-iodo-*N*-{2-[1-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide (*m*-MPPI) were developed (Figure 2). To enhance the in vivo stability of these compounds, the iodine was attached to an aromatic moiety instead of the

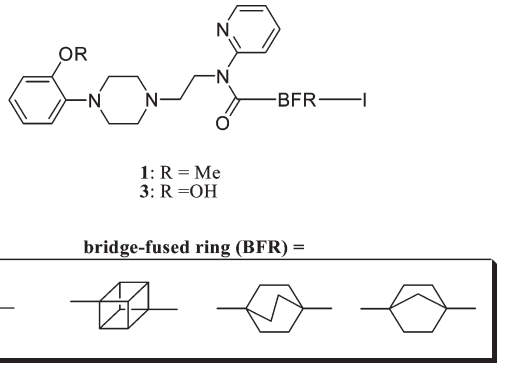

Figure 3. Novel analogues of **1** and **3**.

cyclohexyl ring. However, as found for all analogues of **1** with an aromatic substituent, both [^{123}I]*p*-MPPI and [^{123}I]*m*-MPPI showed low binding potentials and rapid metabolism in human subjects.[3,18–20]

A potential radioiodinated ligand for labeling of brain 5-HT_{1A} receptors in vivo should comply with the following criteria:[10] (i) high affinity and selectivity for the 5-HT_{1A} receptor, (ii) moderate lipophilicity (to foster crossing of the blood–brain barrier (BBB)), (iii) low tendency to lead to racemic mixtures during the radiolabeling and during metabolism, (iv) stable carbon–iodine bond and low metabolism (or metabolism to noninterfering BBB-permeable radioactive metabolites), (v) amenability for labeling with iodine-123, and (vi) low toxicity (acceptability for intravenous injection at low doses into human subjects).

The aim of this study was to evaluate bridgehead iodinated analogues of **1** and **3** (O-desmethylated analogue of **1**) with a bridge-fused ring (BFR) system attached to the carboxamide such as adamantane, cubane, bicyclo[2.2.2]octane, and bicyclo-[2.2.1]heptane (depicted in Figure 3) as possible SPECT ligands, since they might fulfill the above set of requirements. The synthesis, receptor binding affinity, and stability of these compounds are reported and discussed. The binding selectivity of **6b** and **7b** is reported. A no carrier added (nca) synthesis of [^{123}I]**6b** and [^{123}I]**7b** is described, as well as the biodistribution of these two compounds in rats.

RESULTS

Chemistry. The chemical structures of both **1** and its metabolite **2** are shown in Figure 1. Compound **2** and its *O*-desmethyl analogue **4** (Figure 1) were used as basic structural features to synthesize the bridgehead iodinated analogues **6a–d** and **7a–d**, respectively. A general route for the desired analogues of **1** and **3** is depicted in Scheme 1. Compound **2** was prepared according to procedures described by Pike and Cliffe. For the synthesis of **4** the same route has been used.[16,21] However, **4** was also be prepared by demethylation of **2**.[22]

3-Iodoadamatane-1-carboxylic acid (**5a**) was synthesized from the commercially available 3-hydroxyadamatane-1-carboxylic acid with the aid of KI/P_2O_5 in phosphoric acid.[23,24] Synthesis of **5b** started with the commercially available dimethyl 1,4-cubanedicarboxylate which after monosaponification was treated with I_2/iodobenzene diacetate (IBDA) to undergo a Moriarty iodo-decarboxylation.[25–27]

4-(Ethoxycarbonyl)bicyclo[2.2.2]octane-1-carboxylic acid (**12**), the precursor of **5c**, was synthesized from **8** in four steps as

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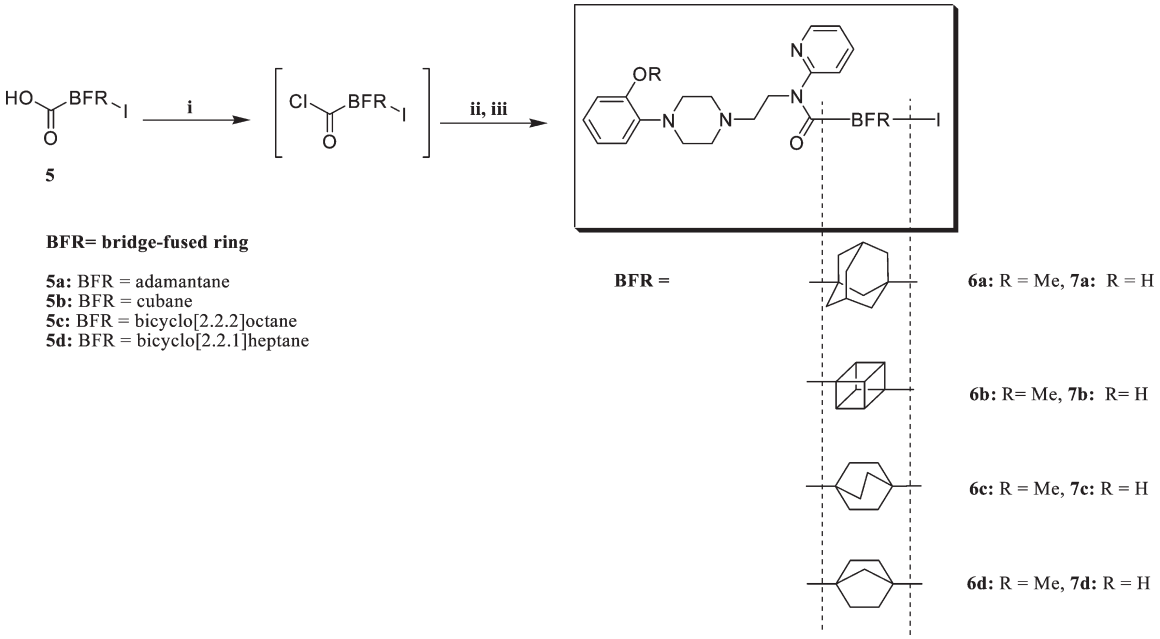
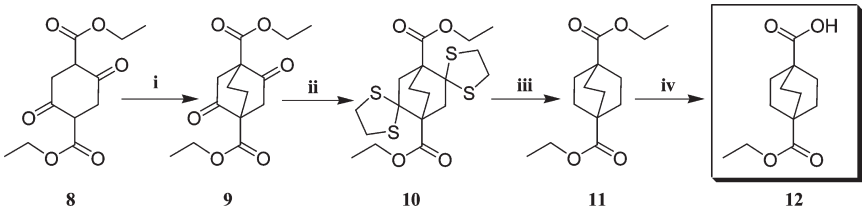
Scheme 1. General Pathway for the Synthesis of the Analogues of 1 and 3[a]



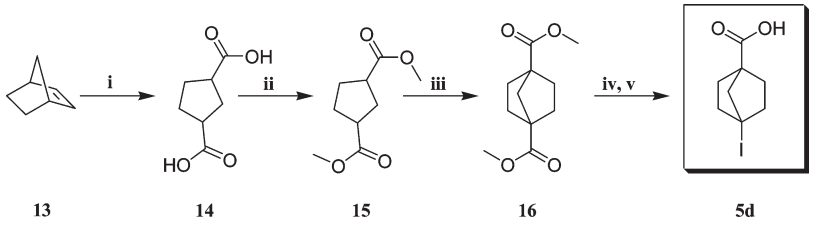
[a] Reagents and conditions: (i) thionyl chloride or dichloromethyl methyl ether, MeCN, Δ; (ii) **2** or **4**, NEt_3, $CHCl_3$, room temp. (iii) For analogues of **3**: methylamine, dioxane, room temp.

Scheme 2. Synthetic Pathway for Compound 12[a]



[a] Reagents and conditions: (i) NaH, 1,2-dibromoethane, dimethoxyethane, 110 °C; (ii) 1,2-ethanedithiol, HCl, $CHCl_3$, 0 °C; (iii) Raney Ni, EtOH, reflux; (iv) KOH, EtOH.

Scheme 3. Synthetic Pathway for Compound 5d[a]



[a] (i) $RuCl_3$ in a mixture of EtOAc and MeCN, $NaIO_4$ in H_2O, room temp; (ii) MeOH, H_2SO_4, reflux; (iii) LDA, HMPA, THF, 1-bromo-2-chloroethane; (iv) NaOH in MeOH, (v) (a) IBDA/I_2, benzene, reflux, (b) NaOH in a mixture of MeOH and water, THF.

described by Boulerice and Frazer (Scheme 2),[28−31] a route that was found to be more efficient than the bridge-forming alkylation of diethyl 1,4-cyclohexanedicarboxylate with 1-bromo-2-chloroethane.[32]

Iodo-decarboxylation of **12** was performed by a Hunsdiecker reaction,[33,34] which was followed by saponification to give **5c**.

Unfortunately, synthesis of **16** via a bridge-forming alkylation of **8** with dibromomethane instead of dibromoethane was not possible. Therefore, as outlined in Scheme 3, compound **5d** was synthesized starting from an oxidation of norbornene (**13**).[35,36] Esterification of **14** followed by a bridge-forming alkylation of **15** with 1-bromo-2-chloroethane gave the diester **16**.[32] Monosaponification and a subsequent Moriarty iododecarboxylation afforded **5d**.[25−27]

Formation of the acid chlorides of iodocubane (**5b**) and 4-iodobicyclo[2.2.1]heptane (**5d**) could be achieved with thionyl chloride in MeCN.[16] In the case of 3-iodoadamantane (**5a**) and 4-iodobicyclo[2.2.2]octane (**5c**), the reaction mixture turned

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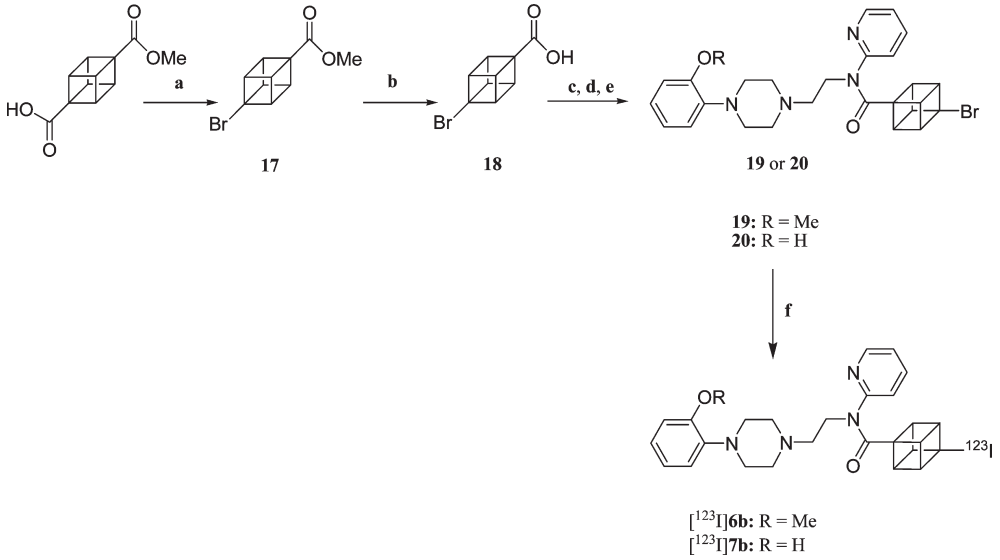
Scheme 4. Synthetic Pathway of the Precursors (19 and 20) and Radiosynthesis of [^{123}I]6b and [^{123}I]7b[a]



[a] Reagents and conditions: (a) HgO/Br_2, CH_2Cl_2, 80−90 °C; (b) NaOH in MeOH and water; (c) thionyl chloride, MeCN, 140 °C; (d) WAY-100634 or *O*-desmethyl WAY-100634, NEt_3, $CHCl_3$, room temp. (e) For *O*-desmethyl WAY-100634 analogue: NEt_3, H_2O/MeCN (1:5), 50 °C. (f) [^{123}I]Iodide, Cu(II) triflate, MeCN, 140 °C.

dark brown because of decomposition of the starting material. This could be circumvented by using 1,1-dichlorodimethyl ether in $CHCl_3$ under continuous removal of the volatile products methyl formate and HCl.[37] Acylation of **2** with the above acid chlorides of **5a**−**d** gave the four analogues **6a**−**d** in moderate to high yields (26−80%). However, upon reaction with **4** a competition was always observed between N- and O-acylation. Therefore, this compound was first double acylated, after which the *O*-acyl bond was broken with methylamine to afford the analogues **7a**−**d**, albeit in somewhat lower yields (28−54%).

All synthesized analogues were stable in solvents like dimethylsulfoxide, ethanol, and water for at least several days. Even after 6 months in cold (−20 °C) ethanol no decomposition was detected, both by HPLC and by ^{1}H NMR analysis.

Radiolabeling. Radiolabeling of the analogues **6a**−**d** was attempted by an isotopic exchange reaction using iodine-123 in anhydrous MeCN with Cu(II) triflate as a catalyst in a closed vial at 140 °C.[38] Unfortunately, under these conditions it was only possible to obtain [^{123}I]**6a** and [^{123}I]**6b** in 40% and 85% yields, respectively. For **6c** and **6d** only starting material and free iodide-123 were recovered.

The nca radioligands [^{123}I]**6b** and [^{123}I]**7b** that are needed for the biodistribution studies could be obtained from the corresponding bromo derivatives **19** and **20** in an overall radiochemical yield of 40% and 35%, respectively (Scheme 4). Their radiochemical purity was >99%, even after 24 h, and the specific activity (SA) was calculated to be >1 TBq (37 Ci)/μmol at the end of synthesis (EOS).

Lipophilicity of the Investigated Compounds. The lipophilicity (log $D_{7.4}$ values) of all compounds was calculated by using MarvinSketch (http://www.chemaxon.com), and the retention times (t_R) on HPLC were measured. Data are shown in Table 1. It appeared that the calculated log $D_{7.4}$ values for **6b** and **7b** did not correctly reflect the lipophilicity, probably because of the cubane structure. Therefore, the log $D_{7.4}$ of these compounds and of compound **1** for comparison was determined experimentally. Both compounds showed a high log $D_{7.4}$ (4.14 ± 0.13 and 4.03 ± 0.07, respectively) compared to **1** (3.03 ± 0.04). With exclusion of the cubyl analogues (**6b** and **7b**), a striking correlation was observed between the calculated log $D_{7.4}$ values and the t_R on HPLC (r = 0.9237, Pearson correlation test) for all compounds. By use of this correlation, the log $D_{7.4}$ values of **6b** and **7b** can be estimated to be 4.64 and 4.35.

5-HT$_{1A}$ Receptor Affinity. Competitive binding experiments were performed using the 5-HT$_{1A}$ agonist [^{3}H]8-hydroxy-2-(di-*N*-propylamino)tetralin ([^{3}H]8-OH-DPAT) and a membrane suspension from cloned human 5-HT$_{1A}$ receptor expressed in HEK-293 EBNA cells. Inhibition curves were measured for the eight compounds. IC_{50} values were derived from nonlinear regression curve fitting, and K_i values were calculated according to the Cheng−Prusoff equation.[39] Data are shown in Table 1.

All compounds revealed affinity for the 5-HT$_{1A}$ receptor in the low nanomolar range. Within the rank of these analogues the t_R on HPLC was also correlated with the K_i for the 5-HT$_{1A}$ receptor (analogues of **1**, r = 0.9708; analogues of **3**, r = 0.8541).

Receptor Binding Profile. For the cubyl analogues **6b** and **7b**, the selectivity for the 5-HT$_{1A}$ receptor over a variety of other relevant receptors was determined by the NIMH/Psychoactive Drug Screening Program (PDSP). The results are shown in Table 2.

Metabolic Stability. To have an indication on the metabolic stability of the new BFR analogues, the iodine-123 labeled cubyl analogue [^{123}I]**6b** was incubated with human hepatocytes. Formation of metabolic fragments was measured over time using HPLC. As a reference, we used the in-house available fluorinated analogue 4-[^{18}F]fluoro-*N*-{2-[1-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide ([^{18}F]MPPF),[41] a compound with an in vivo hydrolysis rate comparable to that of **1** itself.[42,43] Results are depicted in Table 3.

Biodistribution of [^{123}I]6b and [^{123}I]7b in Rat. Distribution of radioactivity was measured in blood and peripheral organs, including the thyroid and brain regions. At 45 min after intravenous injection of [^{123}I]**6b**, radioactivity was determined

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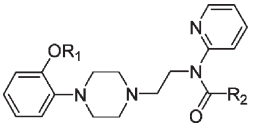
Table 1. Affinity of BFR Analogues for the Human 5-HT$_{1A}$ Receptor, Their t_R on Reversed Phase HPLC, and Calculated log $D_{7.4}$



compd	R_1	R_2	t_R (min)[a]	log $D_{7.4}$ [b]	IC_{50} (nM) (n = 3)[c]	K_i (nM)
1	CH_3	cyclohexyl	21.3	4.08	0.91 ± 0.43	0.39
6a	CH_3	iodoadamantyl	83.4	5.02	5.04 ± 0.02	2.15
6b	CH_3	iodocubyl	43.4	2.81[d]	2.59 ± 0.33	1.11
6c	CH_3	iodobicyclo[2.2.2]octyl	58.6	4.91	2.61 ± 0.35	1.11
6d	CH_3	iodobicyclo[2.2.1]heptyl	35.5	4.46	2.02 ± 0.40	0.87
3	H	cyclohexyl	15.4	3.93	0.77 ± 0.36	0.33
7a	H	iodoadamantyl	57.0	4.87	3.95 ± 1.75	1.68
7b	H	iodocubyl	30.2	2.66[d]	1.50 ± 0.35	0.64
7c	H	iodobicyclo[2.2.2]octyl	39.9	4.76	4.57 ± 0.86	1.95
7d	H	iodobicyclo[2.2.1]heptyl	24.5	4.34	1.19 ± 0.01	0.47

[a] Analytical HPLC was performed on a C18, 10 μm column (Kromasil, 4.6 mm × 250 mm). Eluent: MeOH/H_2O/DIPA (65/35/0.02). Products were detected at λ = 254 nm at flow rate of 1 mL min^{-1}. [b] Calculated log $D_{7.4}$ using MarvinSketch (http://www.chemaxon.com). [c] Results are presented as concentration producing 50% inhibition (IC_{50}) in nM and the mean of three experiments per drug ± SD. [d] Calculated values appeared not to reflect lipophilicity correctly due to the presence of the cubyl moiety.

Table 2. Affinity (K_i of Iodocubyl Analogues (6b and 7b) for Selected Receptors[a]

compd	5-HT$_{1A}$	5-HT$_{2B}$	5-HT$_7$	α_{1A}-A	α_{1B}-A	α_{1D}-A	D_4
1[b]	2.2	24	10000	20	322	ND	16
6b	2.2 ± 0.1	121 ± 7	161 ± 14	78 ± 2.5	185 ± 10.9	98 ± 3.8	329 ± 20
7b	1.4 ± 0.1	272 ± 23	50 ± 4	176 ± 6.2	25 ± 1.15	181 ± 8.63	890 ± 66

[a] The primary assays on the tested receptors: serotonin receptor subtypes 5-HT$_{1E}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_6$; adrenoceptor subtypes α_{2A}, α_{2B}. Serotonin transporter (SERT) σ receptors σ1R and σ2R showed <50% inhibition at 10 μM. For receptors that showed >50% inhibition (serotonin receptor subtypes 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{5A}$; adrenoceptor subtypes α_{2C}, β_1, β_2, β_3; dopamine receptor subtype D_2), the K_i values were at least a factor 100 greater than those for the 5-HT$_{1A}$ receptor. Results are presented as apparent equilibrium inhibition constant (K_i) in nM and the mean of three experiments ± SEM. ND = not determined. [b] Data from experiments performed by NIMH-PDSP.[40]

Table 3. Stability of [^{123}I]6b in a Medium with Human Hepatocytes

	stability (%)			
compd	t = 0 min	t = 15 min	t = 60 min	t = 150 min
[^{123}I]**6b**	99.9	90.7	60.1	24.9
[^{123}I]iodocubanecarboxylic acid		9.0	39.7	74.9
free [^{123}I]iodide	0.1	0.3	0.2	0.2
[^{18}F]MPPF	99.2	45.2	22.7	8.4
[^{18}F]fluorobenzoic acid		24.8	41.8	50.1
free [^{18}F]fluoride		19.6	31.2	40.6
unidentified compd	0.8	11.4	4.3	0.9

in several tissues. Uptake of the radioactivity in the 5-HT$_{1A}$ receptor rich region hippocampus was found to be higher than in other brain tissues (for frontal cortex, $P < 0.05$; for occipital cortex, rest cortex, striatum, cerebellum, $P < 0.01$ for each; data were analyzed by ANOVA (one-way analysis of variance) followed by Dunnett's test to correct for multiple comparison; $P \leq 0.05$ indicates significant difference). In vivo specificity of this radiolabeled compound was examined by preinjection of the nonlabeled compound **1**. Uptake in thyroid, as an indication of free iodide-123, was low. The results are shown in Table 4.

The results of the biodistribution of the O-desmethylated analogue [^{123}I]**7b** and its uptake in the brain at 15, 45, and 120 min are shown in Table 5.

The binding of both radioligands [^{123}I]**6b** and [^{123}I]**7b** is expressed by the ratios of radioactivity uptake at 45 min in the hippocampus, frontal cortex, occipital cortex, and striatum over the cerebellum as a reference tissue.

DISCUSSION

[Carbonyl-^{11}C]**1** is widely used as the "gold standard" to image the 5-HT$_{1A}$ receptor using PET. For SPECT, however, there is still need for an effective radiopharmaceutical labeled with iodine-123. Analogues containing the moiety **2** have a good chance to display high 5-HT$_{1A}$ receptor binding affinity. However, this moiety should not contain the radiolabel because it also enters the brain, interfering with the scintigraphic measurements.[15−17] For an iodinated analogue of **1**, it is also essential to have the radiolabel (iodine-123) in the other part of the molecule. Observations suggest that this part is preferentially nonaromatic. Evaluation studies of the iodinated aromatic analogues of **1**, *p*-MPPI and *m*-MPPI (Figure 2), showed that both SPECT ligands metabolized rapidly and have low binding

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Table 4. Tissue Distribution of [^{123}I]6b at 45 min after Injection[a]

	45 min no pretreatment[b]	45 min with pretreatment[b,c]
blood	0.24 ± 0.03[e]	0.33 ± 0.02
heart	0.16 ± 0.01	0.20 ± 0.01
lung	0.28 ± 0.03	0.31 ± 0.11
liver	2.34 ± 0.25	2.77 ± 0.05
kidney	1.37 ± 0.05	1.32 ± 0.14
thyroid[d]	0.01 ± 0.01	0.01 ± 0.00
frontal cortex	0.06 ± 0.01	0.05 ± 0.01
occipital cortex	0.06 ± 0.01	0.05 ± 0.01
rest cortex	0.05 ± 0.01	0.04 ± 0.00
striatum	0.04 ± 0.00	0.04 ± 0.00
hippocampus	0.10 ± 0.02[e]	0.06 ± 0.01
cerebellum	0.05 ± 0.00	0.05 ± 0.00

[a] Values are averaged (n = 4) % ID/g ± SD and decay corrected. [b] Rats were intravenously injected with 7 MBq [^{123}I]**6b**. [c] Intravenous pre-injection at t − 5 min of **1** (2 mg/kg). [d] Radioactivity presented as % ID/organ. [e] $P \leq 0.05$ indicates significant difference between "no pretreatment" and "with pretreatment" values. Data were analyzed using the paired t test (two-tailed analysis).

Table 5. Tissue Distribution of [^{123}I]7b at 15, 45, and 120 min after Injection[a]

	15 min	45 min	120 min
blood	0.18 ± 0.01	0.20 ± 0.02	0.22 ± 0.01
heart	0.22 ± 0.00	0.14 ± 0.01	0.12 ± 0.01
lung	0.59 ± 0.03	0.30 ± 0.02	0.19 ± 0.00
liver	2.38 ± 0.06	2.08 ± 0.16	1.97 ± 0.10
kidney	5.54 ± 0.07	5.47 ± 0.18	3.12 ± 0.17
thyroid[b]	0.28 ± 0.01	0.21 ± 0.01	0.25 ± 0.03
frontal cortex	0.34 ± 0.02	0.11 ± 0.00	0.04 ± 0.00
occipital cortex	0.37 ± 0.03	0.13 ± 0.02	0.04 ± 0.00
rest cortex	0.28 ± 0.00	0.09 ± 0.00	0.03 ± 0.00
striatum	0.23 ± 0.02	0.07 ± 0.00	0.03 ± 0.00
hippocampus	0.44 ± 0.03	0.18 ± 0.01	0.05 ± 0.00
cerebellum	0.25 ± 0.04	0.08 ± 0.00	0.04 ± 0.00

[a] Rats were intravenously injected with 7 MBq [^{123}I]**7b**. Values are averaged (n = 4) % ID/g ± SD and decay corrected. [b] Radioactivity presented as % ID/organ.

potentials in vivo and relative high affinities for the α_1 adrenoceptor and/or the dopamine receptor subtype D_2.[3,18−20] Unfortunately, aliphatic iodine compounds are prone to fast deiodination in vivo, either by nucleophilic substitution or by β-elimination. It was anticipated that the above discrepancy might be solved by placing the iodine atom on a bridgehead position of a BFR system. Shielding by the ring structure prevents a backside S_N2 attack, and elimination will lead to a highly strained ring system. Also, hydrolysis of the carbon−iodine bond (S_N1 substitution) seems unlikely because the formation of an intermediate cation is expected to be very slow, comparable to what has been found for bridgehead tosylated BFRs[44] (Table 7).

The use of a BFR as a substituent for cyclohexane has two additional advantages. The first is that both the iodine atom and the moiety **2** are attached to a bridgehead by which these compounds remain achiral. Second, because of steric hindrance of the carboxamide bond, the rate of metabolic hydrolysis might be decreased. It has been depicted that analogues of **1** containing a bulky group instead of the cyclohexyl moiety such as CPC-222 and Wilson-8 (Figure 2) were more metabolically resistant than compound **1** itself.[45] Since the introduction of an iodine atom is known to increase the lipophilicity, which might result in compounds that will not cross the BBB or have a high degree of nonspecific binding,[46] we also wanted to prepare the corresponding *O*-desmethyl analogues to compensate for this effect.

Table 6. Ratio of Uptake of [^{123}I]6b and [^{123}I]7b in Brain Regions over Cerebellum[a]

tissue of interest	[^{123}I]**6b**	[^{123}I]**7b**
hippocampus	2.51 ± 0.41*	2.38 ± 0.08*
occipital cortex	1.21 ± 0.11	1.73 ± 0.21**
frontal cortex	1.16 ± 0.10	1.44 ± 0.02**
striatum	0.91 ± 0.01	0.93 ± 0.02**

[a] Results are presented as ratio ± SD (n = 4) of uptake in brain region over cerebellum at 45 min after injection: (*) $P \leq 0.01$; (**) $P \leq 0.05$. Data were analyzed by ANOVA (one-way analysis of variance) followed by Dunnett's test to correct for multiple comparison. $P \leq 0.05$ indicates significant difference.

Table 7. Relative Rate of Hydrolysis of Tertiary Tosylated BFR Systems

R tosylate	calcd[a]	exptl[a]
R = *tert*-butyl	1	1
R = adamantyl	10^{-4}	10^{-3}
R = bicyclo[2.2.2]octyl	10^{-8}	10^{-7}
R = bicyclo[2.2.1]heptyl	10^{-14}	10^{-13}
R = cubyl	10^{-25}	10^{-10}

[a] Calculated and experimental values as published in Eaton et al.[44]

Although some difficulties were encountered in the synthesis of the iodinated bridgehead carboxylic acids **5c** and **5d**, all eight analogues **6a**−**d** and **7a**−**d** were successfully obtained in fair to moderate yields. All compounds showed high affinity (low K_i) for the 5-HT_{1A} receptor, although their affinities were 2- to 5-fold lower than that of the parent compounds **1** and **3**. A highly significant correlation between the t_R on HPLC and the calculated log $D_{7.4}$ was observed for all compounds, with exception of the cubyl analogues. By use of this correlation, the log $D_{7.4}$ of **6b** and **7b** could be estimated to be 4.64 and 4.35, respectively. These extrapolated values are closer to the measured log $D_{7.4}$ values (4.14 and 4.03, respectively) than to the calculated values of 2.81 and 2.61, respectively. Interestingly, when **2** was replaced by **4**, the t_R values on HPLC of all four analogues (**7a**−**d**) were considerably reduced, as well as almost all the K_i values (reduced K_i values increased affinities). This indicates that lipophilicity not only is an important factor for crossing the BBB but also affects 5-HT_{1A} receptor binding affinity. On the other hand, lipophilicity increases with the size of the BFR: adamantane > bicyclo[2.2.2]octane > cubane > bicyclo[2.2.1]heptane > cyclohexane. Hence, the reduction of affinity might also be explained by steric factors; a too bulky substituent could hinder the interaction with the receptor or result in a less stable ligand−receptor complex.

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Preliminary experiments revealed that radiolabeling by an isotopic exchange of iodine-127 for iodine-123 was only effective in the adamantyl and cubyl analogues **6a** and **6b**. For **6a** this was more or less expected, since there is little strain in the adamantyl moiety, but the ease of labeling of **6b** over the other analogues **6c** and **6d** is rather surprising. This is probably due to the fact that the exocyclic bridgehead bond of cubane has more S-character. The calculated hybridization for cubane is $sp^{2.2}$ compared to $sp^{2.83}$ for bicyclo[2.2.1]heptane and $sp^{3.19}$ for bicyclo-[2.2.2]octane.[47,48] As the lipophilicity of the adamantyl analogues **6a** and **7a** might be too high for in vivo application, we further focused our attention only on the cubyl analogues **6b** and **7b**. Since for the biodistribution studies a high SA is needed, the corresponding precursors **19** and **20** were synthesized in order to perform a nonisotopic halogen exchange followed by HPLC separation. This is possible because products and precursors have a large difference in retention time: 63 min for **6b** versus 37 min for **19**, and 47 min for **7b** versus 31 min for **20**. The nonisotopic halogen exchange reaction was also successful, albeit a somewhat lower yield was obtained (bromine/iodine-123 60−70% versus iodine/iodine-123 85%). This reaction seems to be highly sensitive for water. For both radioiodinated cubane analogues good yields were obtained only under the required anhydrous conditions. Addition of water up to 10% of the reaction mixture of compound **19** reduced the radiolabeling yield from 60% to only 16%.

Compounds **6b** and **7b** showed a high degree of selectivity for the 5-HT_{1A} receptor over all other relevant receptors. Both **6b** and **7b** have less affinity for the 5-HT_{2B} receptor, the α_{1A} adrenoceptor, and especially the dopamine receptor subtype D_4 than compound **1** itself.

As anticipated for all synthesized analogues, the carbon−iodine bond was found to be stable in solution. Incubation of [^{123}I]**6b** with human hepatocytes also demonstrated good metabolic stability. With up to 150 min of incubation no formation of free iodide-123 could be detected. At 15 min of incubation more than 90% of the parent compound [^{123}I]**6b** was still present, whereas under the same conditions [^{18}F]MPPF, taken as a reference compound, showed a rapid metabolism (>55%). This better metabolic stability of [^{123}I]**6b** compared to [^{18}F]MPPF must for a large part be due to the expected decrease in rate of the amide hydrolysis.

After intravenous injection of [^{123}I]**6b** in rats, accumulation of the radioactivity in the thyroid was low at 45 min after injection (0.01% of the injected dose), which indicates that also in vivo there is no deiodination. Following intravenous injection of [^{123}I]**7b** at 45 min after injection, radioactivity in the thyroid was also low (0.21% of the injected dose). Importantly, it remained constant over time, confirming the stability of the cubyl carbon−iodine bond.

The uptake of [^{123}I]**6b** upon 45 min after injection showed a higher tissue distribution profile in 5-HT_{1A} receptor-rich brain region hippocampus (0.10% of the injected doses) compared to frontal cortex or occipital cortex (0.06% of the injected dose each; $P < 0.05$ each). As expected, the uptake in the hippocampus was also higher compared to other brain regions where low densities of 5-HT_{1A} receptors are anticipated such as cerebellum and striatum (0.05% and 0.04% of the injected dose, respectively; $P < 0.01$, each). In another experiment, "specific" binding was determined by pretreating animals with nonradioactive **1**. In this study, the [^{123}I]**6b** brain uptake was decreased to the background levels in the 5-HT_{1A} receptor-rich brain region hippocampus ($P \leq 0.05$), while it stayed almost exactly the same in those with lower densities ($P \geq 0.05$) as shown in Table 4.

[^{123}I]**7b** was rapidly cleared from the brain after 15 min after injection (hippocampus, 0.44%, 0.18%, and 0.05% of the injected dose at 15, 45, and 120 min, respectively), as shown in Table 5.

The extent of specific binding of [^{123}I]**6b** and [^{123}I]**7b** in the brain area can be expressed by the ratios of radioactivity uptake in the brain regions over the cerebellum, taken as a reference tissue as shown in Table 6. Unfortunately, these ratios are disappointingly low compared to those of [carbonyl-^{11}C]**1** (hippocampus, 14.3; frontal cortex, 7.7; occipital cortex, 8.6; striatum, 1.6).[3,49] Despite the fact that these two compounds showed a high binding affinity and a promising binding selectivity in vitro, in vivo overall brain uptake and binding specificity were lower than those of most PET/SPECT 5-HT_{1A} tracers. This might be due to the relatively high log $D_{7.4}$ of **6b** and **7b** (4.14 and 4.03, respectively) compared to that of molecule **1** (log $D_{7.4}$ = 3.03), preventing these compounds from penetrating the BBB.

CONCLUSIONS

Eight novel iodinated analogues of **1** were synthesized. They all bind to the 5-HT_{1A} receptor with K_i in the (sub)nanomolar range. The cubyl analogues **6b** and **7b** were easily radiolabeled and showed a favorable receptor profile and a relatively good metabolic stability. The cubyl carbon−iodine bond was found to be stable both in vitro and in vivo. The biodistribution experiments of [^{123}I]**6b** and [^{123}I]**7b** in rats showed poor brain uptake of both radiotracers and low ratios of uptake in 5-HT_{1A} receptor rich areas (e.g., hippocampus) over cerebellum. This indicates that the designed tracers are not suitable for SPECT because of a too high lipophilicity imaging.

EXPERIMENTAL SECTION

Chemistry: General Procedures. The solvents were dried according to standard procedures. All reactions involving moisture sensitive compounds were performed under an anhydrous atmosphere of dry argon, unless otherwise indicated. Reactions were monitored by using thin-layer chromatography (TLC) on silica-coated plastic sheets (Merck silica gel 60 F_{254}) with the indicated eluent. The compounds were visualized by UV light (254 nm), I_2, ceric ammonium molybdate (CAM), bromocrysol, or potassium permanganate staining, followed by charring at 130 °C. Flash chromatography refers to column purification using the indicated eluent and Acros silica gel (0.030−0.075 mm). All solvents were purchased from Fluka, Sigma-Aldrich, or Acros and used without further purification unless stated otherwise. Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were determined in the indicated solvent using a Bruker AC 200 MHz (200.13 and 50.32 MHz, respectively), a Bruker Avance 250 MHz (250.13 and 62.90 MHz, respectively), or a Bruker MSL 400 MHz (400.13 and 100.61 MHz, respectively) instrument. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane (^{1}H, ^{13}C) and coupling constants J in Hz. Mass spectra were recorded on a Finnigan MAT-90 mass spectrometer. Analytical HPLC was performed on a C18, 10 μm column (Kromasil, 4.6 mm × 250 mm), using MeOH/H_2O/DIPA (65/35/0.02) as eluent and a flow rate of 1 mL·min^{-1}. Products were detected at λ = 254 nm. All tested compounds have a purity of at least 95% according to NMR and HPLC analyses.

General Procedure for Synthesis of Compounds 6a and 6c. Target compounds were synthesized in a two-step reaction using 1,1-dichlorodimethyl ether (1 equiv). In a dried closed reaction vial and under argon atmosphere, the reaction mixture of the corresponding

starting material and 1,1-dichlorodimethyl ether was heated for 3 days at 50 °C in $CDCl_3$. The volatile products (HCl and methyl formate) were removed. Then **2** (1 equiv) in $CDCl_3$ and NEt_3 (2 equiv) were added to the corresponding acid chloride. The new reaction mixture was stirred at room temperature, and the reaction was complete within 5−10 min.

*3-Iodo-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl) adamantane-1-carboxamide (**6a**).* Starting with **5a** (307 g, 1 mmol) and **2** (312 mg, 1 mmol) gave 212 mg (35%) of **6a** as a colorless glass after column chromatography ($MeOH/CH_2Cl_2/NEt_3$, 1:1:0.01). ^1H NMR (200.13 MHz, $CDCl_3$): δ 1.60 (t, J = 15.7 Hz, 2H, 2 × CH), 1.80 (s, 4H, 2 × CH_2), 1.88 (bs, 2H, CH_2), 2.42 (q, J = 11 Hz, 4H, 2 × CH_2), 2.55−2.7 (m, 8H, $N(CH_2)_3$ and CH_2), 2.83−2.90 (m, 4H, $N(CH_2)_2$), 3.72−3.92 (m, 5H, CH_3 and NCH_2), 6.75−6.98 (m, 4H, 2 × CH_2), 7.18−7.32 (m, 2H, 2 × CH), 7.75 (dt, J = 7.6 Hz, 1H, CH), 8.45 (dd, J = 4.9 Hz, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 32.29, 34.28, 37.87, 48.10, 48.19, 48.95, 50.44, 50.94, 53.34, 53.70, 55.18, 55.52, 110.98, 117.94, 120.75, 122.69, 122.86, 138.33, 141.10, 148.77, 152.04, 156.43, 176.03. HRMS (EI) m/z calcd for $C_{29}H_{37}IN_4O_2$, 600.1961; found, 601.2028 (M + H).

*4-Iodo-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl) bicyclo[2.2.2]octane-1-carboxamide (**6c**).* Starting with **5c** (280 mg, 1 mmol) and **2** (312 mg, 1 mmol) gave 151 mg (26%) of **6c** as a colorless glass after column chromatography ($MeOH/CH_2Cl_2/NEt_3$, 1:1:0.01). ^1H NMR (250.13 MHz, $CDCl_3$): δ 1.75−1.81 (m, 6H, 3 × CH_2), 2.28−2.34 (m, 6H, 3 × CH_2), 2.55−2.68 (m, 6H, $N(CH_2)_3$), 2.90−3.09 (m, 4H, $N(CH_2)_2$), 3.72−3.90 (m, 5H, CH_3 and NCH_2), 6.77−7.04 (m, 4H, 4 × CH), 7.19−7.32 (m, 2H, 2 × CH), 7.74 (dt, J = 7.6 Hz, 1H, CH), 8.51 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (62.90 MHz, $CDCl_3$): δ 32.70, 38.90, 40.19, 43.93, 48.90, 50.51, 53.39, 55.25, 55.59, 111.19, 117.98, 120.85, 122.71, 122.99, 125.25, 138.25, 141.24, 148.90, 152.15, 156.61, 177.31. HRMS (EI) m/z calcd for $C_{27}H_{35}IN_4O_2$, 574.1805; found, 575.1887 (M + H).

General Procedure for Synthesis of Compounds 6b and 6d. Target compounds were synthesized in a two-step reaction using thionyl chloride (1 equiv). The reaction mixture was refluxed at 70 °C in dry MeCN (4 mL) under argon atmosphere for 45 min to 1 h, and the excess thionyl chloride was totally evaporated under reduced pressure, then coevaporated with MeCN (3 × 1 mL). Compound **2** (1 equiv) in dry MeCN and NEt_3 (2 equiv) was added to the corresponding acid chloride. The new reaction mixture was stirred at room temperature overnight. The residue was dissolved in water and extracted with CH_2Cl_2. The organic phase was collected, dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure.

*4-Iodo-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl)cubyl-1-carboxamide (**6b**).* Starting with **5b** (300 mg, 1.09 mmol) and **2** (346 mg, 1.11 mmol) gave 441 mg (70%) of **6b** as a colorless glass after column chromatography ($AcOEt/NEt_3$, 1:0.01). ^1H NMR (200.13 MHz, $CDCl_3$): δ 2.60−2.90 (m, 6H, $N(CH_2)_3$), 2.95−3.07 (m, 4H, $N(CH_2)_2$), 3.81 (s, 3H, CH_3), 3.97−4.20 (m, 8H, 6 × CH and NCH_2), 6.79−7.05 (m, 4H, 4 × CH), 7.15−7.30 (m, 2H, 2 × CH), 7.77 (dt, J = 7.6 Hz, 1H, CH), 8.4 (dd, J = 4.9, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 35.62, 44.62, 49.81, 51.08, 53.07, 54.42, 55.20, 55.58, 59.09, 110.98, 118.04, 119.35, 120.79, 121.87, 123.00, 138.31, 140.62, 148.83, 151.97, 154.85, 171.17. HRMS (EI) m/z calcd for $C_{27}H_{29}IN_4O_2$, 568.1335; found, 569.1412 (M + H).

*4-Iodo-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl)bicyclo[2.2.1]heptane-1-carboxamide (**6d**).* Starting with **5d** (50 mg, 0.19 mmol) and **2** (65 mg, 0.21 mmol) gave 94 mg (80%) of **6d** as a colorless glass after column chromatography ($MeOH/CH_2Cl_2/NEt_3$, 1:1:0.01). ^1H NMR (200.13 MHz, $CDCl_3$): δ1.00−1.35 (m, 2H, CH_2), 1.62−2.25 (m, 8H, 4 × CH_2), 2.4−2.79 (m, 6H, $N(CH_2)_3$), 2.80−3.15 (m, 4H, $N(CH_2)_2$), 3.69−4.00 (m, 5H, CH_3 and NCH_2), 6.75−7.05 (m, 4H, 4 × CH), 7.18−7.39 (m, 2H, 2 × CH), 7.75 (dt, J = 7.6 Hz, 1H, CH), 8.5 (dd, J = 4.9 Hz, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 34.95, 36.37, 43.46, 47.10, 50.36, 52.82, 53.22, 54.58, 55.18, 55.43, 110.98, 117.92, 120.75, 122.74, 123.06, 123.25, 138.15, 141.02, 149.03, 152.03, 155.30, 173.25. HRMS (EI) m/z calcd for $C_{26}H_{33}IN_4O_2$, 560.1648; found, 561.1720 (M + H).

General Procedure for Synthesis of Compounds 7a, 7b, 7c, 7d. The acid chlorides of **5a**, **5b**, **5c**, and **5d** were synthesized as mentioned above. Then **4** (0.5 equiv) and NEt_3 (2 equiv) were dissolved in $CDCl_3$ or MeCN and added to the corresponding acid chloride. This new mixture was stirred at room temperature for 10 min followed by aminolysis in dioxane with 40% methylamine, unless otherwise indicated. This reaction mixture was stirred at room temperature overnight.

*N-(2-(4-(2-Hydroxyphenyl)piperazin-1-yl)ethyl)-4-iodo-N-(pyridin-2-yl)adamantane-1-carboxamide (**7a**).* Starting with **5a** (612 mg, 2 mmol) and **4** (298 mg, 1 mmol) in $CDCl_3$ (8 mL) gave a double substituted compound. Then 40% methylamine (1 mL) in dioxane (5 mL) was added, and this reaction mixture was stirred at room temperature overnight to give 164 mg (28%) of **7a** as a colorless glass after column chromatography ($CH_2Cl_2/AcOEt/NEt_3$, 2:1:0.01). ^1H NMR (250.13 MHz, $CDCl_3$): δ 1.60 (t, J = 15.7 Hz, 2H, 2 × CH), 1.80 (s, 4H, 2 × CH_2), 1.88 (bs, 2H, CH), 2.42 (q, J = 11 Hz, 4H, 2 × NCH_2), 2.55−2.70 (m, 8H, $N(CH_2)_3$ and CH_2), 2.85−2.90 (m, 4H, $N(CH_2)_2$), 3.85 (t, J = 7 Hz, 2H, NCH_2), 6.80−6.98 (m, 2H, 2 × CH), 7.02−7.18 (m, 2H, 2 × CH), 7.26−7.37 (m, 2H, 2 × CH), 7.81 (dt, J = 7.6 Hz, 1H, CH), 8.54 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ 32.28, 34.27, 37.88, 48.03, 48.21, 48.94, 50.93, 52.32, 53.77, 55.47, 113.86, 119.82, 121.19, 122.55, 122.91, 126.20, 138.40, 138.78, 148.84, 151.27, 156.39, 176.26. HRMS (EI) m/z calcd for $C_{28}H_{35}IN_4O_2$, 586.1805; found, 587.1870 (M + H).

*N-(2-(4-(2-Hydroxyphenyl)piperazin-1-yl)ethyl)-4-iodo-N-(pyridin-2-yl)cubyl-1-carboxamide (**7b**).* Starting with **5b** (200 mg, 0.730 mmol) and **4** (238 mg, 0.798 mmol) in MeCN (8 mL) gave a double substituted **7b**. This compound was then dissolved in water and basified MeCN (4 mL) with NEt_3 (1 equiv) and heated for 2 h at 50 °C. Then MeCN was evaporated and the water layer was extracted with CH_2Cl_2 (3 × 1 mL). The collected organic layers were dried over Na_2SO_4, and the solvent was evaporated in vacuo. Column chromatography ($AcOEt/NEt_3$, 99%, 0.01) gave 238 mg (54%) of **7b** as a colorless glass. ^1H NMR (200.13 MHz, $CDCl_3$): δ 2.20−3.00 (m, 10H, $N(CH_2)_3$ and $N(CH_2)_2$), 3.6−4.10 (m, 8H, 6 × CH and NCH_2), 6.69−7.29 (m, 6H, 6 × CH), 7.7 (dt, J = 7.4, 1H, CH), 8.4 (dd, J = 4.9, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 36.10, 45.16, 51.10, 52.32, 53.65, 54.43, 55.89, 60.02, 113.87, 119.24, 119.82, 121.14, 121.74, 126.27, 138.16, 138.72, 148.91, 151.28, 154.60, 171.15. HRMS (EI) m/z calcd for $C_{26}H_{27}IN_4O_2$, 554.1179; found, 555.1243 (M + H).

*N-(2-(4-(2-Hydroxyphenyl)piperazin-1-yl)ethyl)-4-iodo-N-(pyridin-2-yl)bicyclo[2.2.2]octane-1-carboxamide (**7c**).* Starting with **5c** (440 mg, 1.57 mmol) and **4** (234 mg, 0.785 mmol) in $CDCl_3$ (8 mL) gave a double substituted compound. Then 40% methylamine (2.5 mL) in dioxane (5 mL) was added, and this reaction mixture was stirred at room temperature overnight to give 167 mg (38%) of **7c** as a colorless glass after column chromatography ($CH_2Cl_2/AcOEt/NEt_3$, 5:1:0.01). ^1H NMR (250.13 MHz, $CDCl_3$): δ 1.70−1.85 (m, 6H, 3 × CH_2), 2.22−2.38 (m, 6H, 3 × CH_2), 2.59−2.66 (m, 6H, $N(CH_2)_3$), 2.75−2.86 (m, 4H, $N(CH_2)_2$), 3.72−3.82 (m, 2H, NCH_2), 6.79−6.96 (m, 2H, 2 × CH), 7.01−7.16 (m, 2H, 2 × CH), 7.20−7.33 (m, 2H, 2 × CH), 7.78 (dt, J = 7.6 Hz, 1H, CH), 8.53 (d, J = 4.9 Hz, 1H, CH). ^{13}C NMR (62.90 MHz, $CDCl_3$): δ 32.70, 38.93, 40,16, 43.76, 48.93, 52.39, 53.82, 55.49, 113.94, 119.86, 121.17, 122.78, 122.86, 126.29, 138.34, 138.82, 148.98, 151.36, 156.54, 177.40. HRMS (EI) m/z calcd for $C_{26}H_{33}IN_4O_2$, 560.1648; found, 561.1717 (M + H).

*N-(2-(4-(2-Hydroxyphenyl)piperazin-1-yl)ethyl)-4-iodo-N-(pyridin-2-yl)bicyclo[2.2.1]heptane-1-carboxamide (**7d**).* Starting with **5d** (50 mg, 0.188 mmol) and **4** (28 mg, 0.094 mmol) in dry MeCN (5 mL) gave a double acylated **7d**. The double acylated product was then dissolved in a mixture of MeOH (4 mL), saturated $NaHCO_3$ (1 mL), and water (1 mL). The reaction mixture was heated for 2 h at 50 °C. Then MeOH

was evaporated and the water layer was extracted with CH_2Cl_2 (3 × 1 mL). The combined CH_2Cl_2 layers were dried with Na_2SO_4, filtered, and evaporated in vacuo. Column chromatography of the crude product was required using 2.5% MeOH in CH_2Cl_2 to give **7d** (20.4 mg, 40%). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.12−1.24 (m, 2H, CH_2), 1.80−1.97 (m, 6H, 3 × CH_2), 2.01−2.16 (m, 2H, CH_2), 2.53−2.71 (m, 6H, $N(CH_2)_3$), 2.75−2.87 (m, 4H, $N(CH_2)_2$), 3.90 (t, J = 7 Hz, 2H, NCH_2), 6.80−6.88 (m, 1H, CH), 6.90−6.96 (m, 1H, CH), 7.02−7.15 (m, 2H, 2 × CH_2), 7.27−7.35 (m, 2H, 2 × CH), 7.79 (dt, J = 7.6 Hz, 1H, CH), 8.53 (dd, J = 4.9 Hz, 1H, CH). ^{13}C NMR (50.32 MHz, $CDCl_3$) δ 35.02, 36.63, 43.67, 47.41, 52.58, 53.06, 53.92, 54.82, 55.68, 114.07, 120.00, 121.32, 123.20, 123.25, 126.45, 138.31, 138.96, 149.19, 151.52, 155.61, 173.54. HRMS (EI) m/z calcd for $C_{25}H_{31}IN_4O_2$, 546.1497; found, 547.1556 (M + H).

Radiolabeling: Water-free Iodide. To sodium [^{123}I]iodide, for labeling (SA > 7.5 TBq/μmol, obtained from BV Cyclotron VU), was added 0.01 N NaOH (~1 mL) followed by 0.1 M H_2SO_4 (0.1 mL). The radioiodide was trapped on a platinum column under nitrogen atmosphere.[38] The column was washed with H_2O (5 mL) and subsequently MeCN (5 mL) under nitrogen atmosphere and finally dried with nitrogen. ^{123}I was eluted with MeCN and hydrogen gas, resulting in >90% recovery of the activity in less than 0.3 mL of MeCN.

Radiolabeling by Isotopic Exchange. Water-free nca radioiodide (~1 GBq) dissolved in dry MeCN (100 μL) was added[38] to a mixture of the precursor **6a**−**d** (2 mg) and Cu(II) triflate (0.1 mg) dissolved in dry MeCN (200 μL). This reaction mixture was heated in a closed vial for 30 min at 140 °C. Labeling yields were determined with analytical HPLC (Kromasil C18, MeOH/H_2O/DIPA (65/35/0.02)).

Radioligands for Biodistribution Experiments. *[^{123}I]4-Iodo-N-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl) cubyl-1-carboxamide ([^{123}I]**6b**).* Water-free nca radioiodide (2.3 GBq) concentrated in dry MeCN (100 μL) was added to a mixture of the precursor **19** (2 mg, 3.8 μmol) and Cu(II) triflate (0.1 mg) as a catalyst in dry MeCN (200 μL) in a closed vial. This reaction mixture was heated for 30 min at 140 °C. Product [^{123}I]**6b** was isolated by HPLC using a C18, 10 μm column (Kromasil, 4.6 mm × 250 mm) and 35% EtOH/H_2O, acidified with AcOH to pH 4.3) as eluent with a flow rate of 1 mL/min (t_R = 63 min for **6b** and t_R = 37 min for **19**). The radioactive fraction (7 mL) was collected and diluted with water to 10% EtOH. Product was trapped on a classic SepPak C18 cartridge. The SepPak was rinsed with water (20 mL) and inversely eluted with EtOH (0.5 mL). The ethanolic fraction was further formulated with 4.5 mL of isotonic citrate buffer (pH 5.4) and sterilized by filtration over a 0.22 μm filter to give [^{123}I]**6b** in a concentration of 185 MBq/mL. The total synthesis time was about 2.5 h.

HPLC of a 50 μL sample (9.2 MBq) revealed a radiochemical purity of 99.7% and no detectable UV signal corresponding to product and precursor. Since the limit of detection in our system is about 5 ng (8.8 × 10^{-6} μmol), the SA at EOS can be calculated to be >1 TBq/ μmol. After 24 h at room temperature (time point of injection in rats) the purity was still >99%.

*[^{123}I]4-Iodo-N-(2-(4-(2-hydroxyphenyl)piperazin-1-yl)ethyl)-N-(pyridin-2-yl)cubyl-1-carboxamide ([^{123}I]**7b**).* Water-free radioiodide (2.6 GBq) in dry MeCN (100 μL) was added to a mixture of the precursor **20** (2 mg, 3.9 μmol) and Cu(II) triflate (0.1 mg) as a catalyst in dry MeCN (200 μL) in a closed vial. This reaction mixture was heated for 40 min at 140 °C. HPLC purification (MeCN/H_2O/TFA, 30:70:0.15) was done by use of μBondpak C18 column (17.8 mm × 300 mm) and flow 1 mL/min (t_R = 47 min for **7b** and t_R = 31 min for **20**). The radioactive fraction (6 mL) was collected and diluted with water to 15% MeCN. Labeled compound was trapped on Sep-Pak C8 plus, which was then rinsed with water (20 mL), inversely eluted with 0.5 mL of EtOH, and further formulated with 4.5 mL of isotonic citrate buffer (pH 5.4) and sterilized by filtration over a 0.22 μm filter to give [^{123}I]**7b** in a concentration of 186 MBq/mL. The total synthesis time was about 2.5 h.

HPLC of a 50 μL sample (9.3 MBq) revealed a radiochemical purity of 99.4% and no detectable UV signal corresponding to product and precursor. Since the limit of detection in our system is about 5 ng (9 × 10^{-6} μmol), the SA at EOS can be calculated to be >1 TBq/μmol. After 24 h at room temperature (time point of injection in rats) the purity was still >99%.

Receptor Binding in Vitro: Materials. [^{3}H]8-Hydroxy-*N*,*N*-dipropyl-2-aminotetralin ([^{3}H]8-OH-DPAT) (154.2 Ci/mmol, 5.71 TBq/mmol), cloned human serotonin 5-HT_{1A} receptor (HEK-293 EBNA cells, 1 unit = 2.5 μL), the 96-well microplates, and a Uni-Filter-96 GF/C filter plate were obtained from Perkin-Elmer Life Sciences (Wellseley, MA). Tris(hydroxymethyl)aminomethane was purchased from AppliChem (Darmstadt, Germany). HCl and $MgSO_4$ were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The filtration was carried out with a MicroBeta FilterMate-96 harvester (PerkinElmer), and the radioactivity was quantified using a Wallac MicroBeta TriLux counter (PerkinElmer).

5-HT_{1A} Receptor Binding. Binding experiments were performed using the 5-HT_{1A} agonist [^{3}H]8-OH-DPAT and a membrane suspension containing the human 5-HT_{1A} receptor. A quality control of the radioligand and the membrane preparation was done by performing radioligand saturation binding experiments. The membrane suspension was diluted (factor 1:40) in binding buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgSO_4$) and incubated in the dark on 96-well microplates for 1 h at 37 °C with the following radioligand concentrations: 0.25, 0.5, 0.75, 1.00, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, and 10.00 nM in a volume of 200 μL. Nonspecific binding was measured at each radioligand concentration in the presence of 10 μM **1**. Incubation was rapidly terminated by filtration over a UniFilter-96 GF/C filter plate presoaked in 0.3% polyethyleneimine (PEI), followed by three rapid washes with ice-cold washing buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgSO_4$) using a MicroBeta FilterMate-96 harvester. Filter plates were dried for 2 h in an oven at 54 °C. Radioactivity was quantified with 10 μL of liquid scintillation fluid using a Wallac MicroBeta TriLux counter. The measured K_D for the 5-HT_{1A} receptor in this membrane preparation was comparable to the one provided by the manufacturer (1.48 and 1.86 nM, respectively).

For the competitive binding experiments, an assay volume of 200 μL per well was used composed of 100 μL of the diluted membrane suspension, 50 μL of 8 nM of [^{3}H]8-OH-DPAT radioligand dissolved in binding buffer (final concentration in the assay 2 nM), 10 μL of each novel analogue in increasing concentrations (range 10^{-11}−10^{-6} M), and 40 μL of the binding buffer. Incubation in the 96-well microplates was carried out in the dark for 2 h at 37 °C. Hereafter, incubation was rapidly terminated (vide supra). Filter plates were dried overnight in an oven at 54 °C. Radioactivity was quantified (vide supra). IC_{50} values were derived from nonlinear regression curve fitting using the computer program Graphpad Prism (version 4.00), and K_i values were calculated according to the Cheng−Prusoff equation using the measured K_D of [^{3}H]8-OH-DPAT radioligand.

Receptor Binding Profile. The receptor binding profile of compounds **6b** and **7b** was investigated by radioligand binding by NIMH/Psychoactive Drug Screening Program (PDSP). For assay conditions and assay protocol see http://pdsp.med.unc.edu/pdspw/binding.php.

Stability toward Human Hepatocytes. Approximately 4 million of cryopreserved human liver cells (In Vitro Technologies, Leipzig, Germany) were thawed rapidly and suspended in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were maintained at 5% CO_2 humidified atmosphere and at 37 °C and washed with phosphate buffered saline (PBS). Viability was determined by trypan blue exclusion. After centrifugation, cells were washed and dissolved in the same medium as above. The viable cell concentration was adjusted to 1.0 million per mL. To 1 mL of this cell

suspension 75 μL of [^{123}I]**6b** (50 MBq) or 4-[^{18}F]fluoro-*N*-{2-[1-(2-methoxyphenyl)piperazine-1-yl]ethyl}-*N*-(pyridine-2-yl)benzamide ([^{18}F]MPPF) (50 MBq) was added. After incubation for 15, 60, and 150 min at 37 °C, a 200 μL sample was taken, added to 200 μL of MeOH, sonicated, and centrifuged. The composition of the supernatant was determined using HPLC combined with online radioactivity detection.

Lipophilicity of the Investigated Compounds. The lipophilicity of **6b** and **7b** was determined by measuring the partition of the compounds between 1-octanol and water (buffer) and is expressed as the log $D_{7.4}$ value. The investigated compounds were dissolved in 1-octanol (3 mL) in a concentration of 1 mg/mL. The phosphate buffer (25 mM, pH 7.4) was made of 1.160 g of NaH_2PO_4 and 2.175 g of $NaHPO_4$ in 1 L of water. The HPLC eluent consisted of methanol/phosphate buffer (80:20), which was used for further dilutions.

The HPLC samples were made as follows: the same amounts of the buffer and the 1-octanol stock (250 μL) were added to each other in a mixing vial, and the mixture was shaken on a vortex for 1 min. After resting for 30 min, a sample was taken from the 1-octanol layer (50 μL) and diluted with 450 μL of the HPLC eluent in a new vial. Another 50 μL was taken from this new mixture and diluted again with 450 μL of the HPLC eluent. This mixture was again diluted in the same manner, and 50 μL of this sample was injected into the HPLC. A 50 μL sample of the buffer layer was also injected into the HPLC. This whole procedure was repeated five times.

As a reference, the partition of **1** between 1-octanol and water (buffer) was measured in a similar way. The log $D_{7.4}$ was calculated according to the formula $\log D_{7.4} = \log_{10}(A_{oct}/A_{buffer})$, where A_{oct} is the average concentration of the five octanol samples and A_{buffer} is the average concentration of the five buffer samples.

Biodistribution Studies of [^{123}I]6b and [^{123}I]7b in Rat: General Procedure. Animal experiments were performed on male Wistar rats (Harlan CPB, Zeist) weighing approximately 230 g. Approval for the applied animal protocol used was obtained from the National Council on Animal Care and the in-house Ethics Committee according to the guidelines of the law on animal experiments of The Netherlands. The solution of the radiolabeled compounds was prepared 1 day prior to these studies. Rats were anesthetized with 2% isoflurane and received an intravenous injection of 200 μL of the radiolabeled compounds in the tail vein. After different time intervals, the rats were sacrificed, under anesthesia, by cervical dislocation, and blood was collected from the decapitated body. Tissues of interest were removed, weighed, and measured for radioactivity using a LKB Wallac 1282 CompuGamma CS. For calculation of the injected dose, five aliquots of the injected solution were weighed and counted for radioactivity. Results were decay corrected and expressed as percentage of injected dose per gram of tissue ± standard deviation (% ID/g ± SD).

*[^{123}I]**6b***. Two groups of four rats were used. The first group received 200 μL (7 MBq, <20 ng) of the radiolabeled compound intravenously and was sacrificed at 45 min after injection. In the second group, rats received an intravenous administration of nonlabeled compound **1** (2 mg/kg) 5 min prior to the intravenous administration of [^{123}I]**6b** (7 MBq). Rats were also sacrificed and dissected at 45 min after injection.

*[^{123}I]**7b***. Three groups of four rats were used. Each rat received an intravenous injection of 200 μL (7 MBq, <20 ng) of the radiolabeled compound. They were sacrificed at 15, 45, and 120 min after injection and processed as above.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details and characterization data of nonkey compounds **2**, **4**, **5a**−**d**, **9**−**12**, and **14**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +31 20 4449704. Fax: +31 20 4449121. E-mail: r.alhussainy@rnc.vu.nl.

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■ ABBREVIATIONS USED

5-HT, 5-hydroxytryptamine; PET, positron emission tomography; SPECT, single photon emission computed tomography; *p*-MPPI, 4-iodo-*N*-{2-[1-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide; *m*-MPPI, 3-iodo-*N*-{2-[1-(2-methoxyphenyl)piperazin-1-yl]ethyl}-*N*-(pyridin-2-yl)benzamide; BFR, bridge-fused ring; nca, no carrier added; BBB, blood−brain barrier; SA, specific activity; EOS, end of synthesis; IC_{50}, concentration producing 50% inhibition; K_D, apparent equilibrium dissociation constant; K_i, apparent equilibrium inhibition constant; t_R, retention time; log $D_{7.4}$, log to the base 10 of the partition coefficient at pH 7.4; *r*, Pearson correlation coefficient; IBDA, iodobenzene diacetate; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DIPA, diisopropylamine

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