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Synthesis of novel WAY 100635 derivatives containing a norbornene group and radiofluorination of [^{18}F]AH1.MZ as a serotonin 5-HT$_{1A}$ receptor antagonist for molecular imaging

Matthias M. Herth,* Vasko Kramer, and Frank Rösch

5-HT$_{1A}$ receptors are involved in a variety of psychiatric disorders and *in vivo* molecular imaging of the 5-HT$_{1A}$ status represents an important approach to analyze and treat these disorders. We report herein the synthesis of three new fluoroethylated 5-HT$_{1A}$ ligands (AH1.MZ, AH2.MZ and AH3.MZ) as arylpiperazine derivatives containing a norbornene group. AH1.MZ (K_i = 4.2 nM) and AH2.MZ (K_i = 30 nM) showed reasonable *in vitro* affinities to the 5-HT$_{1A}$ receptor, whereas AH3.MZ appeared to be non-affine toward the 5-HT$_{1A}$ receptor. The receptor profile of AH1.MZ and AH2.MZ showed selectivity within the 5-HT system. ^{18}F-labelling via [^{18}F]FETos to [^{18}F]AH1.MZ was carried out in radiochemical yields of $>$70%. The final formulation of injectable solutions including [^{18}F]FETos synthon synthesis, radiosynthesis and semi-preparative high-performance liquid chromatography (HPLC) separation took no longer than 130 min and provided [^{18}F]AH1.MZ with a purity of $>$98% as indicated by analytical HPLC analyses.

Keywords: radiofluorination; WAY 100635; [^{18}F]AH1.MZ; 5-HT$_{1A}$ antagonist; PET

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter that is involved in physiological and pathophysiological processes in both the peripheral and the central nervous systems. Therefore, the 5-HT system is one of the most important targets for medicinal chemistry.[1–4] Alterations in serotonin neurotransmission have been implicated in a number of human disorders such as migraine, schizophrenia, depression and anxiety as well as in normal human functions such as learning, sleep, sexual activity and appetite. Especially, it is the 5-HT$_{1A}$ receptor that may be involved in these various physiological processes.[5–10] A major task in the clinical routine is depression, because it affects an estimated 121 million people worldwide[11] while the molecular basis for depression is not fully being understood. However, deficits in the activity of serotonin-mediated neurons in the brain are clearly central to the disease.[12] *In vivo* studies to quantitatively determine 5-HT$_{1A}$ receptor availability would provide a significant advance in the understanding of the mentioned disorders and conditions. Positron emission tomography (PET) is an appropriate tool to measure *in vivo* directly, non-invasively and repetitively the binding potential, the receptor availability, uptake kinetics of radio tracers for neuroreceptors as well as to evaluate the efficacy of drugs directed to these molecular targets.

A number of neurotransmitter analogs labelled with β^+ emitter containing radioligands were synthesized as radiopharmaceuticals for imaging the 5-HT$_{1A}$ receptor. To date, *in vivo* studies have been performed with several 5-HT$_{1A}$ selective antagonists such as [^{11}C]WAY 100635[13] and [^{18}F]*p*-MPPF.[14] The chemical structures of WAY 100635 and *p*-MPPF are presented in Scheme 1.

Fiorino *et al.* synthesized new arylpiperazines containing a norbornene group as 5-HT$_{1A}$ receptor antagonists and reported their outstanding *in vitro* selectivity for the 5-HT$_{1A}$ receptor.[15] 4-[3-[4-(*o*-Methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**1**) showed even affinity in the subnanomolar range for 5-HT$_{1A}$ receptors and moderate to no affinity for other relevant receptors (Table 1).

Therefore, we decided to slightly modify promising ligands by replacing a methoxy- by a fluoroethoxy group for labelling purposes, to alter the position of the fluoroethoxy group within the phenylic ring (Scheme 2) and to determine the affinities of the new compounds toward several 5-HT receptors. In addition, we report the optimized labelling and purification procedure of the promising candidate [^{18}F]AH1.MZ.

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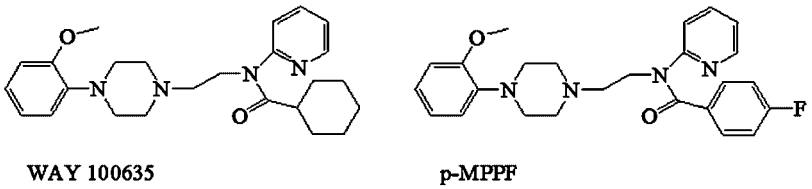
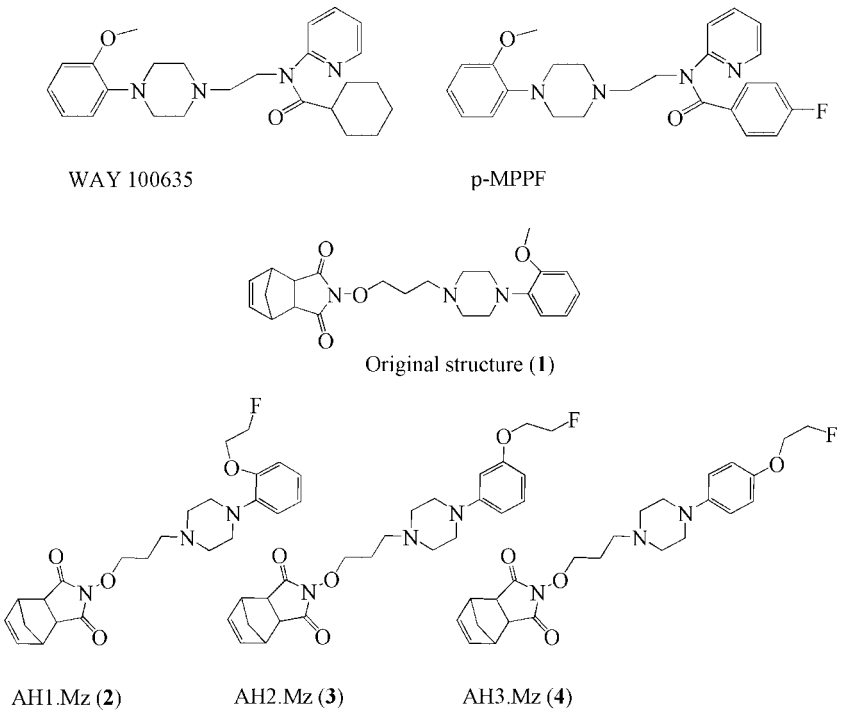


Scheme 1. Chemical structures of WAY 100635 and *p*-MPPF.

Table 1. Affinities of (**1**) for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, D_1, D_2, α_1 and α_2 receptors

	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	D_1	D_2	α_1	α_2
(**1**)	0.021	$>10^4$	$>10^4$	$>10^4$	$>10^4$	75.3	3650

K_i values in nM are based on the means of four experiments.



Scheme 2. Structures of novel compounds (**2**)–(**4**) compared with the original (**1**).

Results and discussion

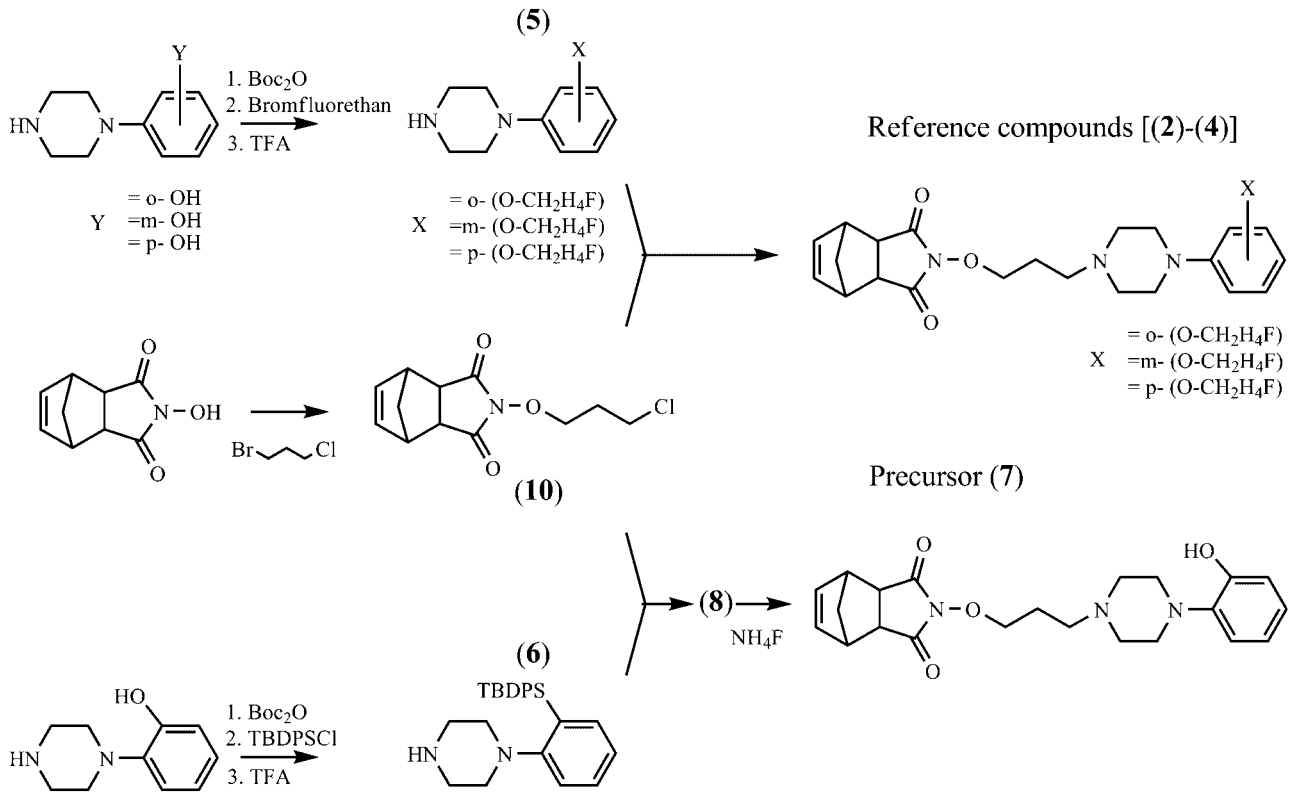
Chemistry

Organic synthesis of WAY 100635 derivatives containing a norbornene group has been described by Fiorino *et al.*[15]. Owing to the necessary structural replacement of a methoxy- by a fluoroethoxy group for labelling purposes, a similar synthesis route was applied, but hydroxyphenylpiperazines were used as starting materials for both precursors and reference compounds. The synthesis strategy, shown in Scheme 3, employs a protection/deprotection approach of the secondary amine and the phenolic hydroxy group followed by alkylation of the key intermediates (**5**) or (**6**) performed in MeCN in the presence of K_2CO_3 and NaI under reflux. By means of Finkelstein exchange, alkylation yields were improved by the increased leaving tendency of iodine. However, (**10**) was synthesized by the starting heterocycle endo-*N*-hydroxy-5-norbornene-2,3-dicarboximide with 1-bromo-3-chloropropane in the presence of NaOH in absolute ethanol as reported by Fiorino *et al.*[15]

Receptor characterization

The potential of new fluoroethylated derivatives related to their affinity and selectivity was examined by determining affinities (K_i's) to 5-HT receptors by radioligand binding assays through NIMH Psychoactive Drug Screening Program (PDSP). The results are summarized in Table 2.

AH1.MZ (**2**) ($K_i = 4.2$ nM) and AH2.MZ (**3**) ($K_i = 30$ nM) reveal a low to moderate nanomolar affinity to the 5-HT_{1A} receptor, whereas AH3.MZ (**4**) shows no affinity toward the 5-HT_{1A} receptors. Medium receptor affinity toward 5-HT_{2B} ($K_i = 144$ nM) could be detected for the *o*-fluoroethylated compound (**2**), whereas the *m*-substituted ligand (**3**) lost this affinity.



Scheme 3. General synthesis strategy.

Table 2. Affinities of novel compounds (**2**)–(**4**) to 5-HT neuroreceptors

	Original structure (**1**)[a]	AH1.MZ (**2**)	AH2.MZ (**3**)	AH3.MZ (**4**)
5-HT_{1A}	0.021	4.2 ± 0	30 ± 6	> 10.000
5-HT_{1B}	—	343 ± 57	> 10.000	> 10.000
5-HT_{1D}	—	> 10.000	n.d.	n.d.
5-HT_{1E}	—	382 ± 59	1738 ± 238	> 10.000
5-HT_{2A}	$> 10^4$	599 ± 60	4406 ± 794	2229 ± 490
5-HT_{2B}	—	144 ± 9	1150 ± 140	3570 ± 333
5-HT_{2C}	$> 10^4$	> 10.000	> 10.000	> 10.000
5-HT_3	—	> 10.000	n.d.	n.d.
5-HT_{5A}	—	1767 ± 279	> 10.000	> 10.000
5-HT_6	—	> 10.000	> 10.000	> 10.000
5-HT_7	—	n.d.	552 ± 53	> 10.000

K_i values in nM ± SEM of human receptors are based on the means of four experiments. n.d., not determined.
[a]K_i values reported by Fiorino *et al.*

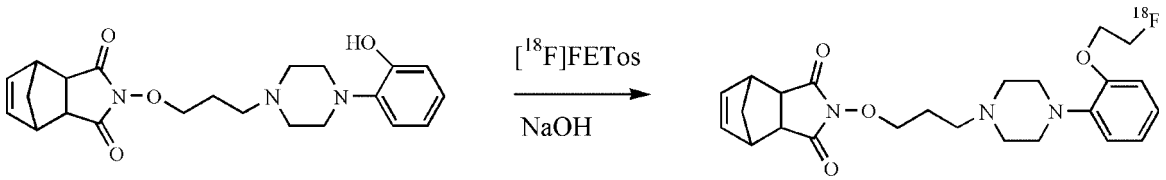
However, AH1.MZ and AH2.MZ showed a reasonable *in vitro* affinity profile, with high to moderate affinity to the 5-HT_{1A} receptor and selectivity to other 5-HT receptors. The outstanding affinity and selectivity of the reference compound 4-[3-[4-(*o*-methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**1**) is lost by introducing a fluoroethyl group (Table 2).

Radiochemistry

The ^{18}F-labelling of the precursor (**7**) was carried out similar to that reported in Herth *et al.*[16] The necessary [^{18}F]FETos synthon production was performed in an automated module according to Bauman *et al.*[17] and used for [^{18}F]fluoroalkylation (Scheme 4).

The [^{18}F]fluoroalkylation of the precursor (**7**) was optimized only due to temperature variation resulting in radiochemical yields of > 70% (Figure 1). Final reaction conditions were 120°C, 7 mmol of precursor (**7**) and 7 mmol of 5 N NaOH dissolved in 1 mL of dry DMSO with a reaction time of 20 min.

The final formulation of injectable solutions including [^{18}F]FETos synthon synthesis, radiosynthesis and a semi-preparative high-performance liquid chromatography (HPLC) separation (μBondapak C_{18} 7.8 × 300 mm column, flow rate 8 mL/min, eluent: MeCN/0.25 M NH_4Ac buffer, pH 4.3 adjusted with



Scheme 4. Radiosynthesis of [^{18}F]AH1.MZ.

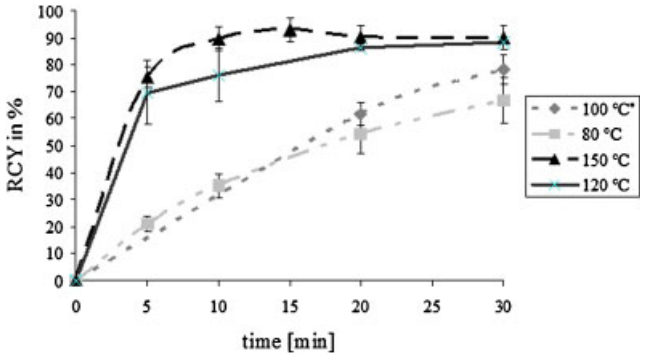


Figure 1. [^{18}F]fluoroalkylation of 7 mmol of precursor (**7**) to [^{18}F]AH1.MZ at different reaction temperatures using DMSO and 7 mmol of 5 N NaOH.

acetic acid (25/75)) took no longer than 130 min and provided [^{18}F]AH1.MZ with a radiochemical purity of >98% as indicated by analytical HPLC analyses (ET 250/8/4 Nucleosil 5 C_{18}, flow rate 1 mL/min, eluent: MeCN/0.05 Na_2HPO_4 buffer, pH 7.4 adjusted with H_3PO_4 (40:60)). Thereby, amounts of ~3 GBq of [^{18}F]fluorine were used as starting radioactivity. A typical specific activity of 5 GBq/μmol could be observed for these low-scale activities.

In conclusion, a new ^{18}F-labelled compound could be obtained as an injectable solution in overall radiochemical yields of about 25% within 130 min.

Experimental

General

Chemicals were purchased from ABX, Acros, Aldrich, Fluka, Merck or Sigma and were used without further purification. Moisture sensitive reactions were carried out under an argon or a nitrogen atmosphere using dry solvents over molecular sieve.

Flash chromatographies were conducted on silica gel 60 (0.040–0.063 mm, Acros) columns. TLCs were run on pre-coated plates of silica gel 60F_{254} (Merck). HPLC was performed on the following systems:

Analytical HPLC: System equipped with a Sykam S 1100 Solvent Delivery System, S 8110 Low Pressure Gradient Mixer, Rheodyne 9725i Inject Valve, Linear UVIS-205 Absorbance Detector, Axxiom Chromatography 900-200 Pyramid, Pyramid 2.07, loop: 20 μL).

Semi-preparative HPLC: HPLC system equipped with a Dionex P 680A pump, software Dionex Chromeleon vers., a Raytest 6.6 NaI scintillation counter (Gabi) and a Linear UVD170U (254 nm) absorbance detector. Reversed-phase HPLC was carried out for analytical separations using an ET 250/8/4 Nucleosil 5 C_{18} column and for semi-preparative applications a μBondapak C_{18} 7.8 × 300 mm column was used. Three hundred and 400 MHz NMR spectra were recorded on a Bruker 300 MHz-FT-NMR-Spectrometer AC 300 or a Bruker-Biospin DRX 400 MHz spectrometer. Chemical shifts were reported in parts per million (ppm). FD mass spectrometry (MS) was performed on a Finnigan MAT90-Spectrometer.

Radio-TLC plates were scanned and detected using a Canberra Packard Instant Imager.

Chemistry

*4-(3-Chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**10**)*

4-(3-Chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**10**) was synthesized as described by Fiorino *et al.*[15]

General procedure for boc-protection

To a solution of 15.7 mmol of *N*-hydroxyphenylpiperazine and 27 mmol of $NaHCO_3$ dissolved in 50 mL of THF/H_2O/dioxane (1:1:1), 18.8 mmol of Boc_2O was added and stirred overnight. Later, the solution was diluted with H_2O, extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated. Purification by column chromatography (PE:EtOAc 7:3) gave the final pure product.

*Tert-butyl 4-(2-hydroxyphenyl)piperazine-1-carboxylate (**11a**)*: A total of 2.8 g of *N*-2-hydroxyphenylpiperazine (15.7 mmol), 2.3 g of $NaHCO_3$ (27 mmol) and 4.1 g of Boc_2O (18.8 mmol) yielded 2.43 g (8.9 mmol, 57%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.135–7.054 (m, 2H), 6.959–6.928 (m, 1H), 6.875–6.19 (m, 1H), 3.597–3.566 (t, 4H), 2.837–2.805 (t, 4H), 1.470 (s, 9H). MS (FD) *m/z* (% rel. int.): 278.2 (100.0 $[M]^+$), 279.2 (17.27 $[M+1]^+$). R_f = 0.6 (PE/EtOAc 7:3).

*Tert-butyl 4-(3-hydroxyphenyl)piperazine-1-carboxylate (**11b**)*: A total of 5 g of *N*-3-hydroxyphenylpiperazine (28.04 mmol), 4.11 g of $NaHCO_3$ (48 mmol) and 7.32 g of Boc_2O (33.6 mmol) yielded 6.98 (25.05 mmol, 89%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.129–7.074 (t, 1H), 6.524–6.401 (m, 3H), 3.606–3.572 (t, 4H), 3.123–3.088 (t, 4H), 1.465 (s, 9H). MS (FD) *m/z* (% rel. int.): 278.1 (100.0 $[M]^+$), 279.1 (10.78 $[M+1]^+$). R_f = 0.52 (PE/EtOAc 7:3).

*Tert-butyl 4-(4-hydroxyphenyl)piperazine-1-carboxylate (**11c**)*: A total of 2.8 g of *N*-4-hydroxyphenylpiperazine (15.7 mmol), 2.3 g of $NaHCO_3$ (27 mmol) and 4.1 g of Boc_2O (18.8 mmol) yielded 4.01 g (14.4 mmol, 92%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.101–6.784 (m, 4H), 3.702–3.669 (t, 4H), 3.111–3.054 (t, 4H), 1.459 (s, 9H). MS (FD) *m/z* (% rel. int.): 278.1 (100.0 $[M]^+$), 279.1 (15.56 $[M+1]^+$). R_f = 0.35 (PE/EtOAc 7:3).

Alkylating procedure for 1,2-bromofluoroethane

NaH (7.19 mmol) was gradually added to the corresponding phenolic derivatives (7.19 mmol) dissolved in 50 mL of dry, cold DMF (0°C) and stirred for 30 min. To the resulting mixture 1,2-bromofluoroethane (7.19 mol) was added slowly and later stirred for 20 h at 60°C. After evaporation of the solvent, the residue was taken up in EtOAc, washed with H_2O and finally extracted 3 × with EtOAc. The combined organic extracts were dried

(Na_2SO_4), filtered and evaporated. Chromatography of the residue gave the pure product.

Tert-butyl 4-(2-(2-fluoroethoxy)phenyl)piperazine-1-carboxylate (***12a***): A total of 2 g of *tert*-butyl 4-(2-hydroxyphenyl)piperazine-1-carboxylate (7.19 mmol), 189.4 mg of NaH (7.19 mmol) and 916 mg of 1,2-bromofluoroethane (0.52 mL; 7.19 mmol) yielded 2.04 g (5.8 mmol, 86%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 6.968–6.842 (m, 4H), 4.852–4.825 (m, 1H), 4.694–4.667 (m, 1H), 4.304–4.276 (m, 1H), 4.209–4.182 (m, 1H), 3.591 (bs, 4H), 3.029 (bs, 4H), 1.459 (s, 9H). MS (FD) *m/z* (% rel. int.): 324.2 (100.0 $[M]^+$), 325.2 (11.16 $[M+1]^+$). R_f = 0.54 (PE/EtOAc 7:3).

Tert-butyl 4-(2-(3-fluoroethoxy)phenyl)piperazine-1-carboxylate (***12b***): A total of 2 g of *tert*-butyl 4-(3-hydroxyphenyl)piperazine-1-carboxylate (7.19 mmol), 189.4 mg of NaH (7.19 mmol) and 916 mg of 1,2-bromofluoroethane (0.52 mL; 7.19 mmol) yielded 1.92 g (5.5 mmol, 81%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.189–7.135 (t, 1H), 6.566–6.403 (m, 3H), 4.815–4.788 (m, 1H), 4.657–4.630 (m, 1H), 4.241–4.212 (m, 1H), 4.148–4.119 (m, 1H), 3.562–3.528 (t, 4H), 3.128–3.094 (t, 4H), 1.461 (s, 9H). MS (FD) *m/z* (% rel. int.): 323.9 (100.0 $[M]^+$), 324.9 (17.82 $[M+1]^+$). R_f = 0.7 (PE/EtOAc 7:3).

Tert-butyl 4-(2-(4-fluoroethoxy)phenyl)piperazine-1-carboxylate (***12c***): A total of 2 g of *tert*-butyl 4-(4-hydroxyphenyl)piperazine-1-carboxylate (7.19 mmol), 189.4 mg of NaH (7.19 mmol) and 916 mg of 1,2-bromofluoroethane (0.52 mL; 7.19 mmol) yielded 1.55 g (4.79 mmol, 67%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 6.942–6.810 (m, 4H), 4.801–4.772 (m, 1H), 4.643–4.614 (m, 1H), 4.210–4.182 (m, 1H), 4.117–4.089 (m, 1H), 3.573–3.540 (t, 4H), 3.012–2.979 (t, 4H), 1.460 (s, 9H). MS (FD) *m/z* (% rel. int.): 323.9 (100.0 $[M]^+$), 324.9 (17.23 $[M+1]^+$). R_f = 0.76 (PE/EtOAc 7:3).

General procedure for TBDPS-protection

Tert-butyldiphenylsilyl chloride was added to a solution of the corresponding phenolic derivative, imidazole, and 30 mL of THF at 0°C. This solution was stirred at 0°C for 30 min, then stirred at 40°C for 1 h, cooled, diluted with brine and extracted with CH_2Cl_2. Standard workup gave crude silyl ether, which was purified by silica gel column chromatography.

4-[2-(Tert-butyl-diphenyl-silanyloxy)-phenyl]-piperazine-1-carboxylic acid tert-butylester (***9a***): A total of 2.27 g of *tert*-butyldiphenylsilyl chloride (8.27 mmol), 2.30 g of 4-(2-hydroxyphenyl)piperazine-1-carboxylate (8.27 mmol) and 1.11 g of imidazole (16.2 mmol) yielded 1.33 g (2.57 mmol, 31.2%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.716–7.694 (d, 4H), 7.432–7.320 (m, 6H), 6.994–6.412 (m, 4H), 3.573 (bs, 4H), 3.043 (bs, 4H), 1.472 (s, 9H), 1.088 (s, 9H). MS (FD) *m/z* (% rel. int.): 516.2 (100.0 $[M]^+$), 517.2 (42.08 $[M+1]^+$). R_f = 0.65 (PE/EtOAc 4:1).

4-[4-(Tert-butyl-diphenyl-silanyloxy)-phenyl]-piperazine-1-carboxylic acid tert-butylester (***9b***): A toal of 2.27 g of *tert*-butyldiphenylsilyl chloride (8.27 mmol), 2.30 g of 4-(4-hydroxyphenyl)piperazine-1-carboxylate (8.27 mmol) and 1.11 g of imidazole (16.2 mmol) yielded 1.1 g (2.12 mmol, 25%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.657–7.631 (d, 4H), 7.466–7.384 (m, 6H), 6.723–6.593 (dd, 4H), 2.955–2.889 (m, 8H), 1.471 (s, 9H), 1.008 (s, 9H). MS (FD) *m/z* (% rel. int.): 415.9 (100.0 $[M]^+$), 416.9 (31.68 $[M+1]^+$). R_f = 0.62 ($CHCL_3$/MeOH 5:1).

General procedure for boc-deprotection

The starting material (2.4 mmol) was carefully and gradually dissolved in trifuoroacetic acid (TFA) (10 mL). After 2 h of stirring at room temperature, the solutions were diluted with 30 mL of ether and carefully neutralized with NH_4OH and ice bath cooling. The layers were separated and the aqueous layer was extracted 3 × with ether. The combined organic extracts were washed with water, dried (Na_2SO_4), filtered and evaporated to afford a viscous oil. Silica gel column chromatography of the residues gave the pure products.

1-(2-(2-Fluoroethoxy)phenyl)piperazine (***5a***): A total of 1 g of *tert*-butyl 4-(2-(2-fluoroethoxy)phenyl)piperazine-1-carboxylate (2.4 mmol) and 10 mL of TFA yielded 435 mg (1.55 mmol, 64%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.073–6.856 (m, 4H), 4.861–4.819 (m, 1H), 4.702–4.661 (m, 1H), 4.313–4.246 (m, 1H), 4.235–4.142 (m, 1H), 3.374 (bs, 8H). MS (FD) *m/z* (% rel. int.): 223.5 (100.0 $[M]^+$), 224.5 (13.32 $[M+1]^+$). R_f = 0.92 (CH_3Cl/MeOH 8:1).

1-(2-(3-Fluoroethoxy)phenyl)piperazine (***5b***): A total of 1 g of *tert*-butyl 4-(2-(2-fluoroethoxy)phenyl)piperazine-1-carboxylate (2.4 mmol) and 10 mL of TFA yielded 653 mg (2.31 mmol, 95%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.183–7.128 (t, 1H), 6.656–6.354 (m, 3H), 4.812–4.4.784 (m, 1H), 4.654–4.626 (m, 1H), 4.237–4.179 (m, 1H), 4.144–4.117 (m, 1H), 3.342 (bs, 1H), 3.181–3.148 (t, 4H), 3.060–3.027 (t, 4H). MS (FD) *m/z* (% rel. int.): 223.6 (100.0 $[M]^+$), 224.6 (10.02 $[M+1]^+$).

1-(2-(4-Fluoroethoxy)phenyl)piperazine (***5c***): A total of 1 g of *tert*-butyl 4-(2-(2-fluoroethoxy)phenyl)piperazine-1-carboxylate (2.4 mmol) and 10 mL of TFA yielded 676 mg (2.39 mmol, 99%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 6.898–6.830 (m, 4H), 4.851–4.739 (m, 1H), 4.640–4.612 (m, 1H), 4.229–4.162 (m, 1H), 4.115–4.086 (m, 1H), 3.039 (s, 8H), 2.306 (bs, 1H). MS (FD) *m/z* (% rel. int.): 224.1 (100.0 $[M]^+$), 225.1 (1.12 $[M+1]^+$).

1-[2-(Tert-butyl-diphenyl-silanyloxy)-phenyl]-piperazine (***6a***): A total of 1.19 g of 4-[2-(*tert*-butyl-diphenyl-silanyloxy)-phenyl]-piperazine-1-carboxylic acid *tert*-butylester (2.31 mmol) and 8.7 mL of TFA yielded 0.91 g (2.2 mmol, 94%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.660–7.629 (d, 4H), 7.466–7.389 (m, 6H), 6.727–6.691 (m, 2H), 6.619–6.593 (m, 2H), 3.213 (bs, 8H), 1.049 (s, 9H). MS (FD) *m/z* (% rel. int.): 416.2 (100.0 $[M]^+$), 417.2 (32.98 $[M+1]^+$).

General procedure for N-alkylation

A 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**10**) (0.006 mol) and NaI (0.009 mol) were stirred in 50 mL of dry MeCN under reflux for 30 min. Then, the appropriate arylpiperazine (0.03 mol) and anhydrous K_2CO_3 (0.009 mol) were added. The reaction mixture was stirred under reflux for 24 h. After cooling, the mixture was filtered, concentrated to dryness and the residue was dissolved in water (50 mL). The solution was extracted several times with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The crude mixtures were purified by silica gel column chromatography.

4-(3-{4-[2-(Tert-butyl-diphenyl-silanyloxy)-phenyl]-piperazin-1-yl}-propoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (***8***): A total of 2.16 g of 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (8.47 mmol), 1.125 g of NaI (7.5 mmol), 0.91 g of 1-[2-(*tert*-butyl-diphenyl-silanyloxy)-phenyl]-piperazine (2.31 mmol) and 5.4 g of K_2CO_3 (7.5 mmol) yielded 1.33 g (2.1 mmol, 90%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.725–7.693 (m, 4H), 7.423–7.304 (m, 6H), 6.941–6.909 (dd, 1H),

6.826–6.771 (m, 1H), 6.598–6.545 (t, 1H), 6.454–6.427 (d, 1H), 6.158–6.152 (m, 2H), 4.073–4.005 (t, 2H), 3.413 (bs, 3H), 3.251–3.105 (m, 6H), 2.693 (bs, 4H), 2.003–1.885 (m, 2H), 1.768–1.732 (d, 1H), 1.508–1.477 (d, 2H), 1.077 (s, 9H). MS (FD) *m/z* (% rel. int.): 635.9 (100.0 $[M]^+$), 635.9 (62.64 $[M+1]^+$). $R_f = 0.45$ ($CHCl_3$/MeOH 8:2).

4-(3-{4-[2-(2-Fluoroethoxy)-phenyl]-piperazin-1-yl}-propoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (**2**): A total of 1.53 g of 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (6.01 mmol), 0.79 g of NaI (5.32 mmol), 0.356 g of 1-(2-(2-fluoroethoxy)phenyl)piperazine (1.64 mmol) and 3.83 g of K_2CO_3 (5.32 mmol) yielded 260 mg (0.58 mmol, 38%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 6.999–6.815 (m, 4H), 6.162–6.123 (t, 2H), 4.838–4.810 (t, 1H), 4.679–4.651 (t, 1H), 4.277–4.249 (t, 1H), 4.182–4.155 (t, 1H), 4.063–4.025 (t, 2H), 3.395 (s, 2H), 3.297 (bs, 4H), 3.192 (s, 2H), 2.951–2.873 (m, 6H), 2.082 (bs, 2H), 1.754–1.723 (d, 1H), 1.495–1.460 (d, 1H). MS (FD) *m/z* (% rel. int.): 443.3 (100.0 $[M]^+$), 444.3 (26.04 $[M+1]^+$). $R_f = 0.4$ ($CHCl_3$/MeOH 12:2).

4-(3-{4-[2-(3-Fluoroethoxy)-phenyl]-piperazin-1-yl}-propoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (**3**): A total of 1.53 g of 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (6.01 mmol), 0.79 g of NaI (5.32 mmol), 0.356 g of 1-(2-(3-fluoroethoxy)phenyl)piperazine (1.64 mmol) and 3.83 g of K_2CO_3 (5.32 mmol) yielded 460 mg (1.02 mmol, 52%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.190–7.035 (m, 1H), 6.585–6.339 (m, 3H), 6.227–6.058 (s, 2H), 4.791–4.776 (t, 1H), 4.647–4.618 (t, 1H), 4.228–4.171 (t, 1H), 4.135–4.107 (t, 1H), 4.029–3.986 (m, 2H), 3.399 (bs, 2H), 3.157 (bs, 6H), 2.566 (bs, 6H), 1.897–1.806 (m, 2H), 1.752–1.722 (d, 1H), 1.490–1.461 (d, 1H). MS (FD) *m/z* (% rel. int.): 443.0 (100.0 $[M]^+$), 444.0 (33.19 $[M+1]^+$). $R_f = 0.44$ ($CHCl_3$/MeOH 8:1).

4-(3-{4-[2-(4-Fluoroethoxy)-phenyl]-piperazin-1-yl}-propoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (**4**): A total of 1.53 g of 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (6.01 mmol), 0.79 g of NaI (5.32 mmol), 0.356 g of 1-(2-(4-fluoroethoxy)phenyl)piperazine (1.64 mmol) and 3.83 g of K_2CO_3 (5.32 mmol) yielded 200 mg (0.44 mmol, 27%) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 6.883–6.815 (m, 4H), 6.141 (s, 2H), 4.792–4.764 (t, 1H), 4.633–4.606 (t, 1H), 4.199–4.171 (t, 1H), 4.106–4.078 (t, 1H), 4.033–3.991 (t, 2H), 3.403 (bs, 2H), 3.159 (bs, 3H), 3.087–3.055 (t, 4H), 2.614–2.531 (m, 6H), 1.909–1.818 (m, 2H), 1.755–1.726 (d, 1H), 1.496–1.466 (d, 1H). MS (FD) *m/z* (% rel. int.): 442.9 (100.0 $[M]^+$), 443.9 (43.73 $[M+1]^+$). $R_f = 0.52$ ($CHCl_3$/MeOH 8:1), 0.6 (EtOH).

4-{3-[4-(2-Hydroxy-phenyl)-piperazin-1-yl]-propoxy}-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (**7**): A solution of 0.68 g of 4-(3-{4-[2-(*tert*-butyl-diphenyl-silanyloxy)-phenyl]-piperazin-1-yl}-propoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (1.14 mmol) and 0.21 g of NH_4F (2.8 mmol) in anhydrous MeOH (30 mL) was stirred for 2 h at 70°C. After evaporation of the solvent, the residue was taken up in NH_4OH and extracted 3 × with $CHCl_3$. The combined organic extracts were washed with brine, dried with Na_2SO_4 and evaporated. Chromatography ($CHCl_3$/MeOH 10:1) of the residue yielded 380 mg (0.96 mmol, 84 %) of the pure product. ^{1}H-NMR (300 MHz, $CDCl_3$), δ [ppm] = 7.154–7.124 (dd, 1H), 7.073–7.018 (m, 1H), 6.937–6.900 (dd, 1H), 6.855–6.793 (m, 1H), 6.154–6.141 (t, 2H), 4.041–3.998 (t, 2H), 3.408 (bs, 2H), 3.175–3.162 (m, 2H), 2.893–2.863 (t, 4H), 2.617 (m, 6H), 1.912–1.820 (m, 2H), 1.762–1.732 (d, 1H), 1.500–1.471 (d, 1H). MS (FD) *m/z* (% rel. int.): 396.8 (100.0 $[M]^+$), 397.8 (40.03 $[M+1]^+$). $R_f = 0.67$ ($CHCl_3$/MeOH 5:1).

Radiochemistry

[^{18}F]FETos synthesis

To a dried Kryptofix®2.2.2./[^{18}F]fluoride complex, 4 mg of ethyleneglycol-1,2-ditosylate in 1 mL of acetonitrile was added and heated under stirring in a sealed vial for 3 min. Purification of the crude product was accomplished using HPLC (Lichrosphere RP18-EC5, 250 × 10 mm, acetonitrile/water 50:50, flow rate 5 mL/min, R_t: 8 min). After diluting the HPLC fraction containing the [^{18}F]FETos with water (HPLC fraction/water 1:4), the product was loaded on a C_{18}-Sepac cartridge, dried with a nitrogen stream and eluted with 1.2 mL of DMSO. The whole preparation time was about 40 min and the overall radiochemical yield was between 60 and 80%.[17]

Radiolabelling of [^{18}F]AH1.MZ

[^{18}F]FETos diluted in 0.8 mL of dry DMSO was added to a solution of 3 mg of 4-{3-[4-(2-hydroxy-phenyl)-piperazin-1-yl]-propoxy}-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (**7**) (7 mmol) and 1.5 μL of 5 N NaOH (7 mmol) dissolved in 0.2 mL of dry DMSO. The solution remained at 120°C for 20 min and was quenched with 1 mL of H_2O. Reactants and by-products were separated from [^{18}F]AH1.MZ by semi-preparative HPLC (μBondapak C_{18} 7.8 × 300 mm column, flow rate 8 mL/min, eluent: MeCN/NH_4Ac buffer, pH 4.3 adjusted with acetic acid (25/75)). The retention times of [^{18}F]AH1.MZ, [^{18}F]FETos and 4-{3-[4-(2-hydroxy-phenyl)-piperazin-1-yl]-propoxy}-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dion (**7**) were 5.02, 13.4 and 9.72 min, respectively. The collected product was diluted with water (4:1), passed through a conditioned strataX-cartridge (1 mL of EtOH, 1 mL of H_2O), washed with 10 mL of H_2O and eluted with at least 1 mL of EtOH. Finally, EtOH was removed *in vacuo* and [^{18}F]AH1.MZ was dissolved in 1 mL of saline.

In vitro receptor and monoamine transporter binding

Binding assays were performed by the NIMH PDSP at the Department of Biochemistry, Case Western Reserve University, Cleveland, OH, USA (Bryan Roth, Director). Compounds AH1.MZ (**2**), AH2.MZ (**3**) and AH3.MZ (**4**) were assayed for their affinities for the 5-HT receptor family. Competitive binding experiments were performed *in vitro* using cloned human receptors. Reported values of the inhibition coefficient (K_i) are mean ± SD of four separate determinations.

Conclusion

Fiorino *et al.*[15] reported about an outstanding high affine ($K_i = 0.021$ nM) and selective compound, 4-[3-[4-(*o*-methoxyphenyl)-piperazin-1-yl]propoxy]-4-azatricyclo-[5.2.1.02,6] dec-8-ene-3,5-dione, for the 5-HT_{1A} receptor subtype. By replacing the methoxy- by a fluoroethoxy group of the parent compound, three different reference compounds (**2**)–(**4**) were obtained enabling a labelling strategy with [^{18}F]FETos. *In vitro* evaluation of these ligands showed high to moderate affinities to the 5-HT_{1A} receptor of AH1.MZ ($K_i = 4.2$ nM) and of AH2.MZ ($K_i = 30$ nM), but not any affinity toward the 5-HT_{1A} receptor of the *p*-substituted fluoroethylated compound (AH3.MZ) ($K_i > 10.000$ nM). The receptor profile of AH1.MZ and AH2.MZ demonstrates selectivity within the 5-HT system. However, the outstanding affinity and selectivity of the literature reference compound 4-[3-[4-(*o*-methoxyphenyl)piperazin-1-yl]propoxy]-4-aza tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**1**) is mainly lost by introducing a fluoroethyl group. Nevertheless, compound

AH1.MZ may provide potential for molecular imaging of the 5-HT_{1A} receptor system. Because of this potential, ^{18}F-labelling via [^{18}F]FETos to [^{18}F]AH1.MZ was carried out and optimized. Radiochemical yields were >70%. The final formulation of injectable solutions including [^{18}F]FETos synthon synthesis, radiosynthesis and a semi-preparative HPLC separation took no longer than 130 min and provided [^{18}F]AH1.MZ with a purity >98%.

In the near future, a broad receptor screening, *in vitro* autoradiography and *in vivo* PET experiments are planned to verify the potential for non-invasive molecular imaging of [^{18}F]AH1.MZ as an ^{18}F-tracer for the 5-HT_{1A} system.

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