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Synthesis, radiofluorination and first evaluation of (±)-[^{18}F]MDL 100907 as serotonin 5-HT$_{2A}$ receptor antagonist for PET

Ute Mühlhausen,[a]* Johannes Ermert,[a] Matthias M. Herth,[b] and Heinz H. Coenen[a]

In some psychiatric disorders 5-HT$_{2A}$ receptors play an important role. In order to investigate those *in vivo* there is an increasing interest in obtaining a metabolically stable, subtype selective and high affinity radioligand for receptor binding studies using positron emission tomography (PET). Combining the excellent *in vivo* properties of [^{11}C]MDL 100907 for PET imaging of 5-HT$_{2A}$ receptors and the more suitable half-life of fluorine-18, MDL 100907 was radiofluorinated in four steps using 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene as a secondary labelling precursor. The complex reaction required an overall reaction time of 140 min and (±)-[^{18}F]MDL 100907 was obtained with a specific activity of at least 30 GBq/μmol (EOS) and an overall radiochemical yield of 1–2%. In order to verify its binding to 5-HT$_{2A}$ receptors, *in vitro* rat brain autoradiography was conducted showing the typical distribution of 5-HT$_{2A}$ receptors and a very low non-specific binding of about 6% in frontal cortex, using ketanserin or spiperone for blocking. Thus, [^{18}F]MDL 100907 appears to be a promising new 5-HT$_{2A}$ PET ligand.

Keywords: radiofluorination; 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene; (±)-[^{18}F]MDL 100907; 5-HT$_{2A}$ antagonist; PET

Introduction

Serotonergic 5-HT$_{2A}$ receptors are of central interest in the complex pathophysiology of human cerebral disorders such as anxiety, depression, Alzheimer's disease and schizophrenia.[1–3] In order to investigate the role of these receptors there is an increasing interest in obtaining a selective and high affinity radiolabelled ligand for direct *in vivo* receptor binding studies using positron emission tomography (PET). For this purpose several radiotracers have been developed, of which [^{11}C]MDL 100907 and [^{18}F]altanserin proved to be most useful.

Both MDL 100907 and altanserin bind to the 5-HT$_{2A}$ receptor with high affinity. While for MDL 100907 a K_i value of 0.2 nM[4] and a K_D value of 0.56 nM (using [^{3}H]MDL 100907)[5] were reported, for altanserin a K_i of 0.13 nM[6] and a K_D of 0.3 nM[7] were determined. Binding to other 5-HT receptor subtypes is very low for MDL 100907,[5] and for altanserin moderate to low.[7] A further difference between these two tracers is the binding to receptors outside the serotonergic system. Altanserin shows a relatively high affinity for D_2 (62 nM) and especially for adrenergic-α_1 receptors (4.55 nM),[6,8] whereas the affinity of MDL 100907 to these receptors is insignificant.[9]

Another disadvantage of altanserin is the formation of at least four different metabolites in humans, which lower the bioavailability and may cross the blood–brain barrier (BBB).[8] On the other hand, pharmacokinetic studies with MDL 100907 in rats and dogs indicate that the drug undergoes extensive first-pass metabolism that significantly reduces its bioavailability. The major metabolite of MDL 100907 in animals has been found to be 3′-*O*-demethylated MDL 100907 (MDL 105725), which also exhibits a high binding affinity to 5-HT$_{2A}$ receptors (K_i = 0.45–2.2 nM).[10] However, it was shown that the BBB permeability of MDL 100907 is more than four times that of the metabolite MDL 105725, and that MDL 100907 does not undergo significant metabolism to MDL 105725 in the brain.[10] Furthermore, ^{11}C-labelled MDL 100907, with the radiolabel at either the 2′ or the 3′ position, has been prepared and evaluated. Rat and baboon studies have been carried out with the 2′ labelled compound while the 3′ labelled compound has been evaluated in monkey and humans. Data from both animal and human studies indicated that [^{11}C]MDL 100907 labelled either at the 2′ or 3′ position is a useful radioligand for *in vivo* studies using PET.[4,11,12] If 3′-*O*-demethylation would play a major role, than differences should have occurred with the 2′-*O*-radiomethylated analogue.

Nevertheless, a major draw back of [^{11}C]MDL 100907 is the short half-life of carbon-11 (20 min). This not only necessitates the availability of a cyclotron close to the PET scanner but also causes concern to reach a state of reversible binding during the PET scan.[7,11] These problems can be avoided by the use of fluorine-18 with its half-life of 109.7 min. In summary [^{11}C]MDL

[a]*Institute of Neurosciences and Biophysics (INB-4): Nuclear Chemistry, Research Center Jülich GmbH, D-52425 Jülich, Germany*

[b]*Institute of Nuclear Chemistry, University of Mainz, Fritz-Strassmann-Weg 2, D-55128 Mainz, Germany*

**Correspondence to: Ute Mühlhausen, Institute of Neurosciences and Biophysics (INB-4): Nuclear Chemistry, Research Center Jülich GmbH, D-52425 Jülich, Germany. E-mail: u.muehlhausen@fz-juelich.de.*

100907 is a very specific high affinity ligand for 5-HT_{2A} receptors, only hampered by the short half-life of C-11, while the binding of [^{18}F]altanserin lacks specificity.

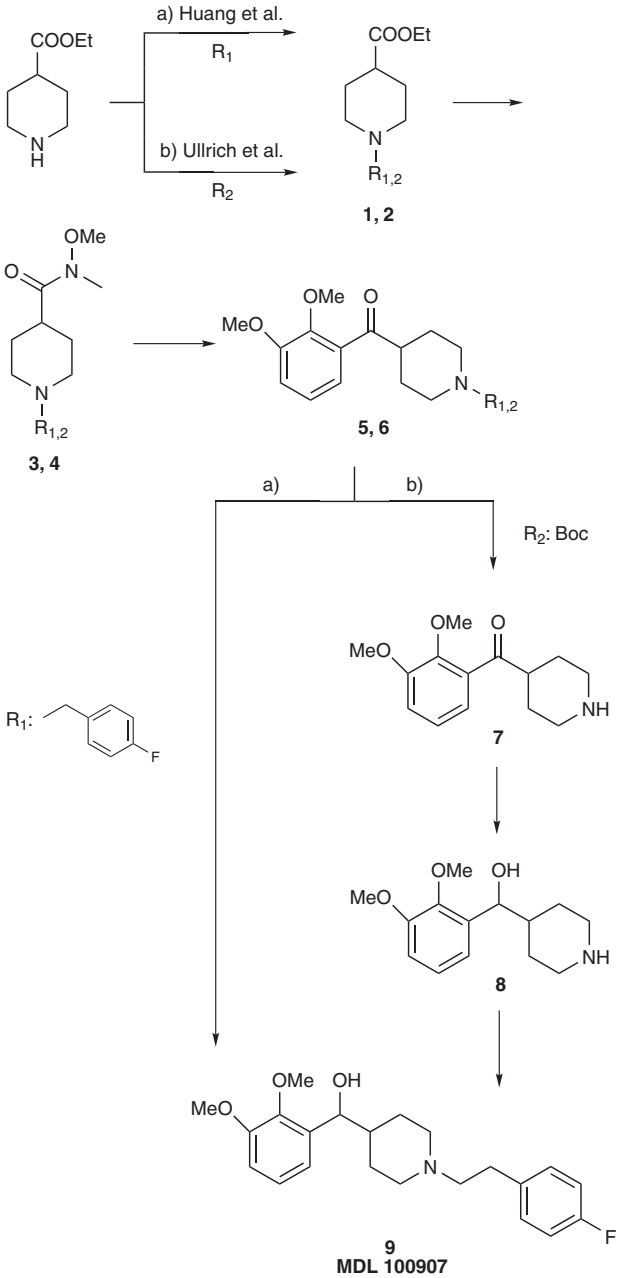
For combining the superior pharmacological and pharmacokinetic properties of MDL 100907 for PET imaging of 5-HT_{2A} receptors and the more suitable half-life of fluorine-18, the isotopically labelled compound (±)-[^{18}F]MDL 100907 was prepared by a four step radiosynthesis. From the known metabolism of MDL 100907 it might be expected that the 3′-*O*-demethylated metabolite [^{18}F]MDL 105725 will be formed *in vivo*. The 2′-labelled [^{11}C]MDL 100907 proved to be useful in rat and baboon even though [^{11}C]MDL 105725 probably occurs *in vivo*.[12] Therefore, it appears worthwhile to find out if the metabolism of ^{18}F-labelled MDL 100907 will interfere in PET measurements of 5-HT_{2A} receptors in the brain. *In vitro* rat brain autoradiography was performed in order to confirm the binding of n.c.a. (±)-[^{18}F]MDL 100907 in analogy to the cerebral pattern of 5-HT_{2A} receptors. In this study, aiming at establishing a radiosynthesis of ^{18}F-labelled MDL 100907, only the racemic compound (±)-[^{18}F]MDL 100907 was prepared but of course the identical labelling procedure can also be performed with the optically resolved precursor to yield (+)-[^{18}F]MDL 100907.

Results and discussion

Chemistry

For the organic synthesis of MDL 100907 principally two different procedures are described in the literature[13–15] (cf. Scheme 1). In the method by Huang *et al.*[14] ethyl 1-(4-fluorophenethyl)piperidine-4-carboxylate (**1**) is initially synthesized by coupling of 2-(4-fluorophenyl)ethyl bromide to ethyl isonipecotate. Then **1** is subsequently coupled to 1,2-dimethoxybenzene and reduced to MDL 100907.[13,14] The method by Ullrich *et al.*[15] changes the reaction sequence using 1-*tert* butyl 4-ethyl piperidine-1,4-dicarboxylate (**2**) for coupling to 1,2-dimethoxybenzene (veratrole). After reduction and deprotection of the resulting piperidine derivative, reaction with 2-(4-fluorophenyl)ethyl bromide was conducted yielding MDL 100907 (**9**). Even though the six step route of Ullrich *et al.*[15] includes one more reaction step, it is better suited with respect to enantiomeric purity of MDL 100907. The intermediate (±)-(2,3-dimethoxyphenyl)-(piperidin-4-yl)methanol (**8**) can optically be resolved[15] and allows the introduction of a variety of N-substituents. Compound **8** was used as the labelling precursor in the work presented here. Both methods use an activated Weinreb amide (**3**, **4**) for coupling of the piperidine moiety to 1,2-dimethoxybenzene.

In order to improve and shorten the method by Ullrich *et al.*[15] a five step synthesis was developed where instead of the activated Weinreb amide **4** the aldehyde *tert*-butyl 4-formylpiperidine-1-carboxylate (**11**) was employed (cf. Scheme 2). For this purpose commercially available piperidin-4-yl-methanol was protected using di-*tert*-butyl dicarbonate. The resulting alcohol **10** was oxidized with oxalylchloride and DMSO in a Swern oxidation to get **11**. Subsequent reaction with 1,2-dimethoxybenzene, which was activated with *sec*-BuLi first, yielded *tert*-butyl 4-((2,3-dimethoxyphenyl)(hydroxy)methyl)piperidine-1-carboxylate (**12**). In contrast to the method by Ullrich *et al.* no reduction of the coupling product is necessary, which saves one reaction step. Deprotection of **12** with TFA yields (±)-(2,3-dimethoxyphenyl)-(piperidin-4-yl)methanol (**8**), which can be

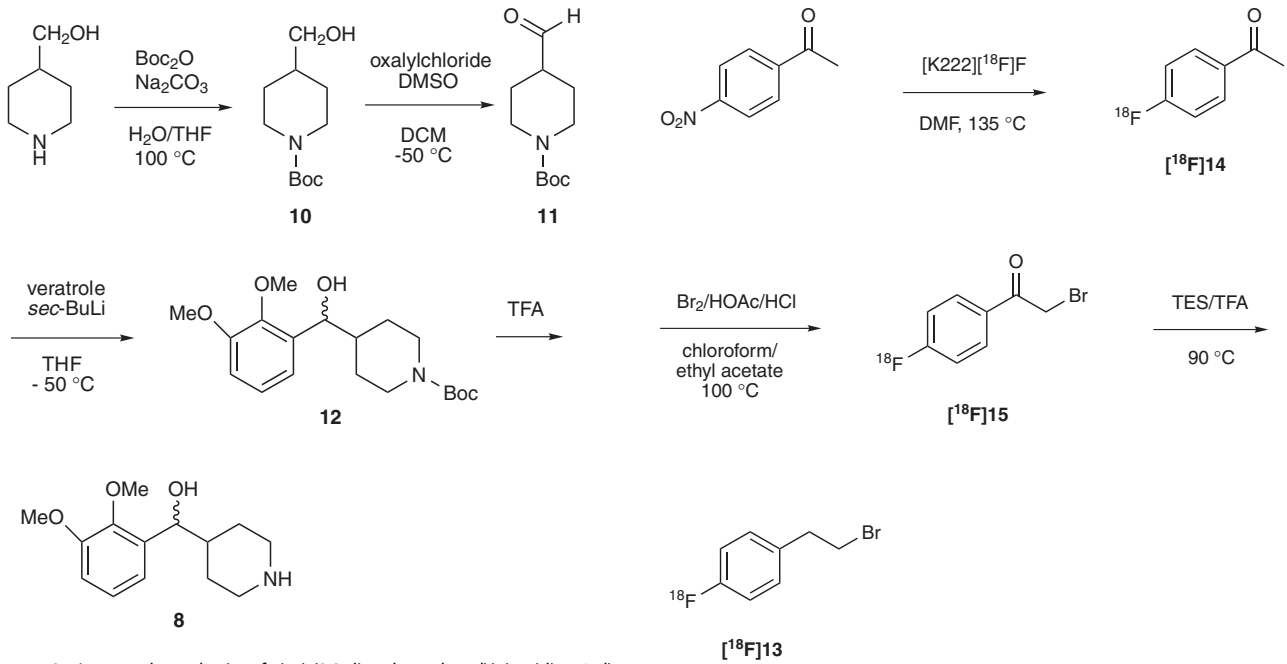


Scheme 1. Synthesis of MDL 100907 by Huang *et al.*[14] (a) and Ullrich *et al.*[15] (b).

alkylated with various phenylethyl bromides like 2-(4-fluorophenyl)ethyl bromide. In order to get (±)-MDL 100907 (**9**) **8** was deprotonated using $NaHCO_3$ and reacted with 2-(4-fluorophenyl)ethyl bromide as described by Ullrich *et al.* Using this procedure the reaction route to **8** was abbreviated by one step and the average overall yield was optimized (54 vs 43%[15] or 48% (own work)).

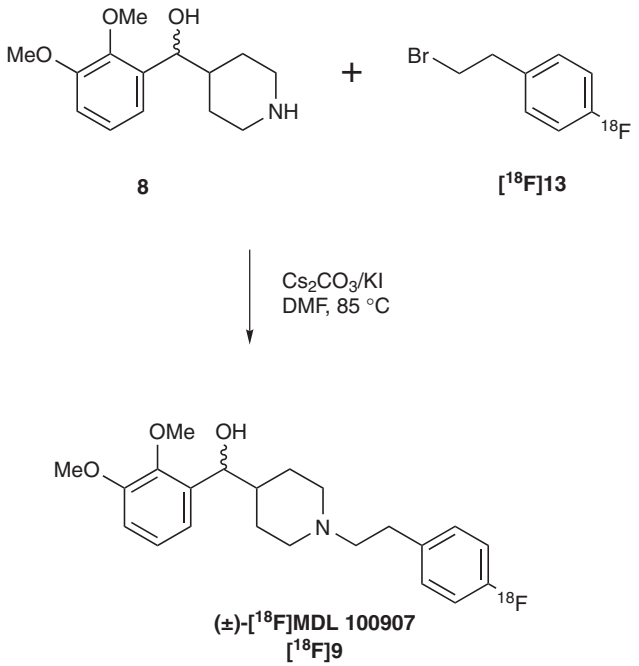
Radiochemistry

So far the synthesis of a suitable labelling precursor for the radiofluorination to no-carrier-added (n.c.a.) [^{18}F]MDL 100907 (**[^{18}F]9**) by direct nucleophilic substitution was not successful. Therefore, a more complex labelling procedure was chosen here. Using 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**) as a



Scheme 2. Improved synthesis of (±)-(2,3-dimethoxyphenyl)(piperidine-4-yl)-methanol (**8**).

Scheme 4. Radiosynthesis of 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**).



Scheme 3. Radiosynthesis of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**).

secondary labelling precursor, the piperidine derivative (±)-(2,3-dimethoxyphenyl)(piperidine-4-yl)methanol (**8**) was alkylated yielding n.c.a. (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) (cf. Scheme 3).

*1-(2-Bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**)*

The preparation of 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**) is described as a high-yield three-step synthesis by Hwang *et al.*[16] For the preparation of 2-bromo-4′-[^{18}F]fluoroacetophenone (**[^{18}F]15**) basically two different methods are described in the literature.[16,17] Both of them use 4-nitroacetophenone as labelling precursor. The difference lies in the bromination reaction of 4-[^{18}F]fluoroacetophenone (**[^{18}F]14**). While Hwang *et al.*[16] use an acidic bromine solution, Dence *et al.*[17] conduct this reaction step with polymer-bound perbromide. However, both reaction procedures could not be reproduced as described. This was also observed in a study by de Vries *et al.*[18] who used the method by Dence *et al.*[17] for the synthesis of 2-bromo-4′-[^{18}F]fluoroacetophenone (**[^{18}F]15**). The labelling reaction was reproducible but despite considerable effort the bromination step failed or gave poor yields. Therefore, they could only produce an overall radiochemical yield (RCY) for **[^{18}F]15** of 3.9±2.7% instead of 65%.[17]

After failure of the bromination reaction using the method by Dence *et al.*[17] the principal reaction sequence of Hwang *et al.*[16] was adopted here with some modifications of the procedure (cf. Scheme 4).

As a first step commercially available 4-nitroacetophenone was labelled with n.c.a. [^{18}F]fluoride using Kryptofix® 222 and potassium carbonate. The labelling was conducted in DMF as solvent with conventional heating at 130°C. 4-[^{18}F]Fluoroacetophenone (**[^{18}F]14**) was obtained with a RCY of 60–70%. To remove the solvent and unreacted [^{18}F]fluoride, solid phase extraction (SPE) was used followed by passing through a drying cartridge to remove water. Removal of water was essential for the next reaction step in order to achieve radical bromination in the side chain and to avoid side reactions.

After removal of the solvent, bromination was carried out in chloroform/ethyl acetate, adding an acidic bromine solution (hydrochloric and acetic acid) at a temperature of 100°C. Analysis of the reaction mixture using reverse-phase high performance liquid chromatography (HPLC) showed two radioactive products. As described in the literature the desired 2-bromo-4′-[^{18}F]fluoroacetophenone (**[^{18}F]15**) as well as the dibrominated 2,2-dibromo-4′-[^{18}F]fluoroacetophenone were formed.[16] The RCY of **[^{18}F]15** was 62–76%, that of the side product 23–37%. An earlier report on a low fraction of <5% of

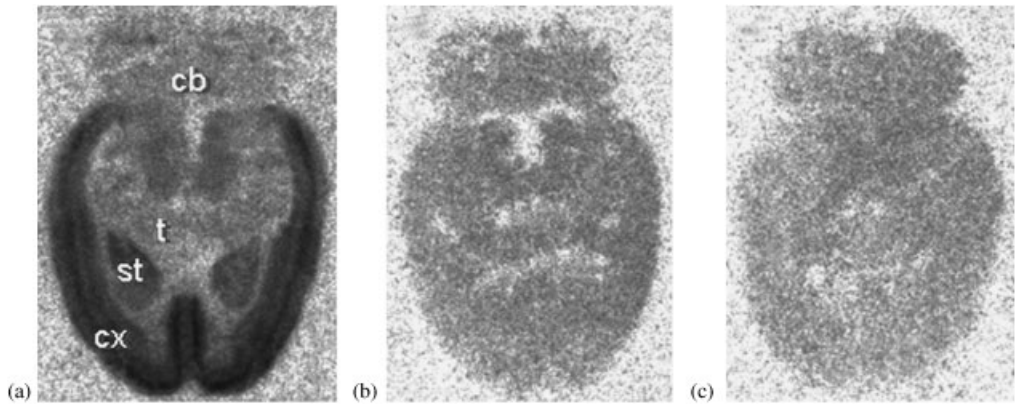


Figure 1. Autoradiography of [^{18}F]MDL 100907 (**[^{18}F]9**) distribution in horizontal rat brain sections: (a) total binding; (b) non-specific binding in the presence of ketanserin (10 μM); (c) non-specific binding in the presence of spiperone (10 μM); cb, cerebellum; cx, cortex; st, striatum; t, thalamus.

dibrominated side product using the same reaction conditions[16,19] could not be reproduced here. For the next reaction step the solvent as well as the remaining acids and traces of water had to be removed. This was accomplished by using an Alumina N cartridge followed by a drying cartridge. Relative to **[^{18}F]14** the RCY of **[^{18}F]15** after work up was 39–48%.

For reduction of **[^{18}F]15** to 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**), triethylsilane and TFA were used. The reaction temperature was varied between 90 and 100°C. It was observed that the RCY at lower temperatures was higher and that formation of a side product, which supposedly is 2-bromo-1-(4′-fluorophenyl)ethanol, was less. Very problematic with this reaction step was a loss of radioactivity, probably due to defluorination via formation of volatile triethylfluorosilane. HPLC analysis of the reaction mixture showed a RCY for 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**) between 52 and 86%, depending on reaction temperature, while the side product was formed in a RCY of 10–37%. Furthermore, no **[^{18}F]15** could be detected anymore. For further reaction **[^{18}F]13** had to be isolated by semi-preparative HPLC. In order to remove water, which is critical to achieve coupling of **[^{18}F]13** to (±)-(2,3-dimethoxyphenyl)(piperidine-4-yl)methanol (**8**) (cf. Scheme 3), elution of purified **[^{18}F]13** after SPE was conducted over a drying cartridge. **[^{18}F]13** was isolated with a RCY of 13–26% relative to **[^{18}F]15**. The overall RCY of **[^{18}F]13** relative to [^{18}F]fluoride was 2.5–7% with a radiochemical purity of > 99% in a preparation time of 110 min.

*(±)-[^{18}F]MDL 100907 (**[^{18}F]9**)*

In order to get (±)-[^{18}F]MDL 100907 (**[^{18}F]9**), the piperidine derivative **8** was deprotonated using caesium carbonate and reacted with **[^{18}F]13** (Scheme 3). In contrast to observations by Hwang *et al.*[16] who tried to couple **[^{18}F]13** to piperidine under various conditions, the formation of the elimation product of **[^{18}F]13**, 4-[^{18}F]fluorostyrol, was moderate (<15%) under the conditions applied here. (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) was isolated by semi-preparative HPLC with a RCY of 40–50%, relative to **[^{18}F]13**. The radiochemical purity was > 99% and the specific activity was at least 30 GBq/μmol (end of synthesis). The preparation time of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) was 140 min and the overall RCY 1–2% relative to [^{18}F]fluoride. There is still room for optimization of the reaction procedure concerning RCY as well as handling. This will become even more attractive if ongoing work on a preferred direct labelling approach is not successful.

In vitro autoradiography

For verifying the cerebral distribution of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) according to the location of 5-HT$_{2A}$ receptors, *in vitro* autoradiography with cryosections of rat brain was conducted. High binding of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) was observed in the cortex with different intensities in various regions (cf. Figure 1(a)). Especially in the frontal part 5-HT$_{2A}$ receptors have been reported to be localized in the rat brain.[20] Moderately high binding was observed in striatum and low but still specific binding was measured in thalamus and cerebellum. This binding pattern is in good accordance with the distribution of 5-HT$_{2A}$ receptors in rat brain[20,21] and reflects the binding of [^{3}H]MDL 100907 previously described.[20] For blocking of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) binding ketanserin (10 μM) as well as spiperone (10 μM) were used (cf. Figure 1 (b) and (c)). With both an excellent displacement of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) was achieved, resulting in a non-specific binding of only about 6% in frontal cortex with both blockers. In relation to the total binding (as average of the brain regions investigated) non-specific binding was only about 15% with ketanserin and 10% with spiperone. This reflects the reported pharmacological characteristics of [^{3}H]MDL 100907[20] as expected, due to authentic labelling of the compound with fluorine-18.

Experimental

General

The chemicals were analytical grade or better and were used without further purification (Sigma-Aldrich, Steinheim, Germany). Alumina N and Oasis HLB 1 cc cartridges were purchased from Waters (Eschborn, Germany), LiChrolut RP-18e cartridges from Merck (Darmstadt, Germany). Analytical TLC was performed on aluminium-backed sheets (Silica gel 60 F_{254}) and normal-phase column chromatography was performed using Silica gel 60, both from Merck (Darmstadt, Germany).

NMR spectra (^{1}H-200 MHz) were obtained on a DPX Avance 200 spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts are reported in parts per million, solvent peaks were referenced appropriately.

HPLC was performed on the following system from Dionex (Idstein, Germany): an Ultimate 3000 LPG-3400A HPLC pump, an Ultimate 3000 VWD-3100 UV/VIS-detector (272 nm), a UCI-50 chromatography interface, an injection valve P/N 8215.

Radioactivity was detected with a Gabi Star NaI(Tl) radioactivity-detector from Raytest (Straubenhardt, Germany) and analysis of HPLC data accomplished with Chromeleon 6.80 software.

Reversed-phase HPLC was carried out using a Gemini 5 μm C18 110A column, for analytical separations with a dimension of 250 mm × 4.6 mm (flow 1 mL/min) and for semi-preparative applications 250 mm × 10 mm (flow 5 mL/min) from Phenomenex (Aschaffenburg, Germany). Analysis or isolation of **[^{18}F]13**, **[^{18}F]14**, and **[^{18}F]15** was done by isocratic elution with acetonitrile and water 65:35 (v/v). For [^{18}F]MDL 100907 (**[^{18}F]9**) isocratic elution with acetonitrile, water and TEA 50:50:0.1 (v/v/v) at a pH of 9.0 (phosphoric acid) was applied. For purpose of identification of all radioactive products the respective non-radioactive standard compounds were co-injected and co-eluted with the radioactive products.

Phosphor imager plates were scanned with a laser phosphor imager BAS 5000 (Fuji, Düsseldorf, Germany) utilizing software from the vendor (Version 3.14, Raytest, Straubenhardt, Germany). The resolution of a phosphor imager scan is 25 μm.

Radiochemical reactions were conducted in 5 mL conical vials (Reactivial) from Wheaton Scientific (Millville, IL, USA), which were closed with a silicon septum.

Chemistry

*tert-Butyl 4-(hydroxymethyl)piperidine-1-carboxylate (**10**)*

Piperidin-4-yl-methanol (5.02 g, 43.4 mmol) and sodium carbonate (4.59 g, 43.2 mmol) were suspended in a mixture of water (29 mL) and THF (11 mL). Di-*tert*-butyl dicarbonate (10.45 g, 51.6 mmol) was added and the resulting mixture stirred at 95°C for 2.5 h. After cooling to room temperature, water (100 mL) was added and the product extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine (2 × 70 mL) and after drying over sodium sulfate the solvent was evaporated under reduced pressure. This yielded the pure product **10** (9.34 g, 43.4 mmol) in 100%. ^{1}H NMR (DMSO-d_6): 3.98 (m, 2H, H2,6), 3.34 (s, 2H, CH_2), 2.62 (m, 2H, H2,6), 1.52 (m, 3H, H3,4,5), 1.45 (s, 9H, Boc), 1.06 (m, 2H, H3,5).

*tert-Butyl 4-formylpiperidine-1-carboxylate (**11**)*

Under an atmosphere of argon a solution of oxalylchloride (2 mL, 22.0 mmol) in absolute dichloromethane (50 mL) was cooled to −50°C. Absolute dimethylsulfoxide (3.5 mL, 44.0 mmol) in absolute dichloromethane (10 mL) was added dropwise. Then **10** (4.30 g, 20.0 mmol) was added. After stirring for 20 min at −50°C triethylamine (14 mL) was added and the suspension was allowed to warm up to room temperature. The reaction was stopped by the addition of water (100 mL) after 2.5 h and the mixture extracted with dichloromethane (4 × 50 mL). The combined organic phases were washed with water (3 × 70 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure to give **11** (4.27 g, 20.0 mmol) in 100% yield. ^{1}H NMR (DMSO-d_6): 9.60 (s, 1H, CHO), 3.84 (m, 2H, H2,6), 2.96 (m, 2H, H2,6), 2.51 (m, 1H, H4), 1.85 (m, 2H, H3,5), 1.44 (s, 9H, Boc), 1.42 (m, 2H, H3,5).

*tert-Butyl 4-((2,3-dimethoxyphenyl)(hydroxy)methyl)piperidine-1-carboxylate (**12**)*

Under an atmosphere of argon, 1,2-dimethoxybenzene (1.30 mL, 10.0 mmol) dissolved in absolute THF (25 mL) was cooled to −50°C and *sec*-butyl lithium (1.3 M in *n*-hexane, 10 mL, 13.0 mmol) was added dropwise. The mixture was stirred at room temperature for 75 min before cooling again to −50°C. Then compound **11** (2.0 g, 9.4 mmol) dissolved in absolute THF (10 mL) was added and the mixture stirred at room temperature for 3 h. The reaction was quenched with water (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic phases were washed with brine (2 × 70 mL), dried over sodium sulfate, and the solvent was evaporated under reduced pressure. Purification by column chromatography (*n*-hexan:ethyl acetate, 2:1) gave **12** (2.0 g, 5.7 mmol) in 61% yield. ^{1}H NMR (DMSO-d_6): 6.98 (m, 3H, ArH), 5.04 (d, 1H, OH), 4.63 (t, 1H, CHOH), 3.92 (s, 3H, OMe), 3.85 (s, 3H, OMe), 2.64 (m, 3H, P2,6), 1.75 (m, 3H, P2,4,6), 1.39 (s, 9H, Boc), 1.26 (m, 4H, P3,5).

*(±)-(2,3-Dimethoxyphenyl)-(piperidin-4-yl)methanol (**8**)*

At 0°C **12** (1.25 g, 3.65 mmol) was carefully dissolved in trifluoroacetic acid (12.5 mL, 161 mmol). The resulting solution was stirred at room temperature for 3 h. After addition of ethyl acetate (50 mL) and water (30 mL) the mixture was adjusted at pH 5 using sodium carbonate. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phases washed with brine (2 × 70 mL). After drying over sodium sulfate, the solvent was evaporated under reduced pressure to give **8** (0.9 g, 3.60 mmol) in 99% yield. ^{1}H NMR (DMSO-d_6): 6.96 (m, 3H, ArH), 4.91 (d, 1H, OH), 4.58 (t, 1H, CHOH), 3.80 (s, 3H, OMe), 3.71 (s, 3H, OMe), 2.87 (m, 2H, P2,6), 2.34 (m, 2H, P2,6), 1.68 (m, 1H, P4), 1.51 (s, 1H, NH), 1.14 (m, 4H, P3,5).

Radiochemistry

N.c.a. [^{18}F]fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by the bombardment of an isotopically enriched [^{18}O]water target with 17 MeV protons at the JSW cyclotron BC 1710 (FZ Jülich).[22] The [^{18}F]fluoride solution was azeotropically dried as described in the literature upon addition of Kryptofix® 222 and potassium carbonate for anion activation.[23]

*4-[^{18}F]fluoroacetophenone (**[^{18}F]14**)*

To the dry [K222][^{18}F]F complex, 4-nitroacetophenone (2–3 mg, ≈10 μmol), dissolved in absolute DMF (0.5 mL), was added and the mixture stirred for 10 min at 130°C, which gave RCY of 60–70%. After dilution with water (20 mL), **[^{18}F]14** was fixed on a conditioned Oasis HLB 1 cc cartridge (Waters), washed with water (5 mL), dried with air and eluted with diethyl ether (4.5 mL) through a glass column (LiChrolut 65 × 10 mm, Merck) filled with 4-Å molecular sieves and sodium sulfate (170 mg). The solvent was evaporated under a stream of argon at 700 mbar.

*2-Bromo-4′-[^{18}F]fluoroacetophenone (**[^{18}F]15**)*

The residue of **[^{18}F]14** was taken up in choroform/ethyl acetate (1:1, 1 mL) and an acidic solution of bromine (3.2 g Br_2 in 25 mL acetic acid and 1.25 mL concentrated hydrochloric acid; 0.1 mL) was added. The mixture was stirred at 100°C for 6 min. In order to destroy the excess bromine at the end of the reaction, a 5% aqueous solution of sodium bisulfite (1.5 mL) was added. After

separating the layers, the organic phase was diluted with diethyl ether (3.5 mL) and passed over a conditioned Alumina N cartridge (Waters) followed by a glass column (LiChrolut 65 × 10 mm, Merck) filled with 4Å molecular sieves and sodium sulfate (170 mg). The RCY of **[^{18}F]15** after work up was 39–48% relative to **[^{18}F]14**. The solvent was evaporated under a stream of argon at 700 mbar.

*1-(2-Bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**)*

For the reduction of **[^{18}F]15**, triethylsilane (0.05 mL) and TFA (0.5 mL) were added to the residue. The mixture was stirred at 90–95°C for 15 min. After dilution with water (15 mL) it was passed over a conditioned Oasis HLB 1 cc cartridge (Waters), washed with water (5 mL), dried with air and eluted with acetonitrile (1 mL). The solution was injected into a semi-preparative reversed-phase HPLC. Elution occurred at $k' = 3.71$. The RCY of isolated **[^{18}F]13** was 13–26% relative to **[^{18}F]15**. After dilution with water (20 mL) the collected fraction was fixed on a conditioned Oasis HLB 1cc cartridge (Waters) again, washed with water (5 mL) and eluted with dry DMF (1 mL) through a glass column (LiChrolut 65 × 10 mm, Merck) filled with 4Å molecular sieves and sodium sulfate (170 mg).

*(±)-[^{18}F]MDL 100907 (**[^{18}F]9**)*

The secondary labelling precursor **[^{18}F]13** (in 0.7 mL DMF) was added to a suspension of (±)-(2,3-dimethoxyphenyl)(piperidine-4-yl)methanol (**8**) (12.6 mg, 50 μmol), caesium carbonate (16.3 mg, 50 μmol) and potassium iodide (8.3 mg, 50 μmol) in dry DMF (50 μL) under an atmosphere of argon. The mixture was stirred at 85°C for 8 min and after dilution with water (15 mL) passed through a conditioned LiChrolut RP18e cartridge (Merck). The cartridge was washed with water (5 mL) and eluted with acetonitrile (1 mL). The solution was injected into a semi-preparative reversed-phase HPLC. Elution occurred at $k' = 3.44$. The RCY of isolated **[^{18}F]9** was 40–50% relative to **[^{18}F]13**. After dilution with water (20 mL) the collected fraction was fixed on a conditioned Oasis HLB 1 cc cartridge (Waters), washed with water (5 mL) and eluted with ethanol (0.7 mL).

***In vitro* autoradiography**

For autoradiography, frozen brains of Wistar rats were cut horizontally at −18°C into 20 μm thick slices (Leica AG Microsystems, Germany), mounted onto gelatin-coated object glasses and stored at −80°C until use. For the binding assays, the brain sections were thawed and dried at 23°C and treated after a literature protocol[20] with some modifications. After preincubation for 10 min at 23°C in 50 mM Tris-HCl, pH 7.4, the cryosections were incubated with 30 pM (±)-[^{18}F]MDL 100907 (**[^{18}F]3**) in buffer for 45 min at 23°C ($n = 6$ cryosections). Adjacent sections were incubated in the presence of 10 μM ketanserin or spiperone. After treatment the sections were washed twice for 5 min in Tris-HCl, dipped in deionized water, dried, and exposed to a phosphor imager plate (Fuji).

Conclusion

Combining the excellent *in vivo* properties of [^{11}C]MDL 100907 with the more suitable half-life of fluorine-18, made the radiosynthesis of (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) attractive, even by a rather complex four step radiosynthesis. For this purpose 1-(2-bromoethyl)-4-[^{18}F]fluorobenzene (**[^{18}F]13**), synthesized as a secondary labelling precursor in three steps, could successfully be coupled to the piperidine-derivative **8**, to prepare (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) with specific activities of at least 30 GBq/μmol in an overall RCY of 1–2% and a preparation time of 140 min. If on-going work on the principally more attractive direct labelling approach to (±)-[^{18}F]MDL 100907 (**[^{18}F]9**) does not succeed, the reaction procedure described here will serve as a basis for optimization studies. Using optically resolved (+)-(2,3-dimethoxyphenyl)(piperidine-4-yl)methanol as labelling precursor in this approach will yield (+)-[^{18}F]MDL 100907. *In vitro* autoradiography using rat brain slices verified the excellent distribution of (±)-[^{18}F]MDL 100907 with high binding to 5-HT$_{2A}$ receptors and very low non-specific binding of about 6% in frontal cortex using ketanserin or spiperone for blocking. In further studies the *in vivo* formation of the 3′-*O*-demethylated metabolite [^{18}F]MDL 105725 has to be evaluated, especially whether it interferes with PET measurements of 5-HT$_{2A}$ receptors in the brain. Thus, it appears of high interest to explore the potential of [^{18}F]MDL 100907 (**[^{18}F]9**) as a new ligand for PET studies of 5-HT$_{2A}$ receptors.

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