

Total synthesis and evaluation of [¹⁸F]MHMZ

Matthias M. Herth,^{a,*} Fabian Debus,^{b,†} Markus Piel,^a Mikael Palner,^c
Gitte M. Knudsen,^c Hartmut Lüddens^b and Frank Rösch^a

^aInstitute of Nuclear Chemistry, University of Mainz, Fritz-Strassmann-Weg 2, 55128 Mainz, Germany

^bDepartment of Psychiatry, Clinical Research Group, Untere Zahlbacher Straße 8, 55131 Mainz, Germany

^cCenter for Integrated Molecular Brain Imaging, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark

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Abstract—Radiochemical labeling of MDL 105725 using the secondary labeling precursor 2-[¹⁸F]fluoroethyltosylate ([¹⁸F]FETos) was carried out in yields of ~90% synthesizing [¹⁸F]MHMZ in a specific activity of ~50 MBq/nmol with a starting activity of ~3 GBq. Overall radiochemical yield including [¹⁸F]FETos synthon synthesis, [¹⁸F]fluoroalkylation and preparing the injectable [¹⁸F]MHMZ solution was 42% within a synthesis time of ~100 min. The novel compound showed excellent specific binding to the 5-HT_{2A} receptor ($K_i = 9.0$ nM) in vitro and promising in vivo characteristics.

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Serotonergic 5-HT_{2A} receptors are of central interest in the pathophysiology of schizophrenia and other diseases, including Alzheimer's disease and personality disorders.¹ The serotonergic system is also implicated in sleep, aging, and pain.² In vivo studies of 5-HT_{2A} receptor occupancy 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 of radio tracers for neuroreceptors.

A number of neurotransmitter analogs labeled with β⁺-emitter containing radioligands were synthesized as radiopharmaceuticals for the imaging of the 5-HT_{2A} receptor. To date, in vivo studies have been performed with several 5-HT_{2A} selective antagonists such as [¹¹C]MDL 100907,³ [¹⁸F]altanserin,⁴ and [¹¹C]SR 46349B⁵.

Within those ligands, [¹⁸F]altanserin and [¹¹C]MDL 100907 represent the radioligands of choice for in vivo 5-HT_{2A} PET imaging because of their high affinity and selectivity for the 5-HT_{2A} receptor {altanserin:

$K_i = 0.13$ nM⁴; (*R*)-MDL 100907: $K_i = 0.57$ nM⁶}. Affinities are more than 100-fold higher for other receptors such as 5-HT_{2C}, α₁, D₁, and D₂. Nevertheless, it was proposed that the selectivity of [¹¹C]MDL 100907 for 5-HT_{2A} receptor is slightly higher than the selectivity for this receptor of [¹⁸F]altanserin.⁸ Both tracers show in vitro and in vivo experiments, high affinity, selectivity, and a good ratio of specific to non-specific binding for 5-HT_{2A} receptors.^{3,7} The advantage of [¹⁸F]altanserin over [¹¹C]MDL 100907 is the possibility to perform equilibrium scans lasting several hours and to transport the tracer to other facilities based on the 110 min half-life of [¹⁸F]fluorine. A drawback of [¹⁸F]altanserin is its rapid and extensive metabolism. Four metabolites are formed in humans that cross the blood–brain-barrier,⁷ whereas metabolites of [¹¹C]MDL 100907 do not enter the brain to any larger extent.⁹

The aim of this study was to develop an ¹⁸F-analog of MDL 100907 (**1**) combining advantages of both ligands, the better selectivity of MDL 100907 and the superior isotopic properties of [¹⁸F]fluorine. For this purpose we decided to replace one of the *O*-methyl groups by an *O*-2-[¹⁸F]fluoroethyl moiety resulting in [¹⁸F]MHMZ ([¹⁸F]FE1-MDL 100907) ((3-[¹⁸F]fluoro-ethoxy-2-methoxy-phenyl)-1-[2-(4-fluoro-phenyl)ethyl]-4-piperidine-methanol, **2**) (Fig. 1).

The methoxy group in the 3-position seemed to be more suitable for labeling because previous [¹¹C]MDL 100907

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* Corresponding author. Tel.: +49 6131 39 25849; e-mail: herthm@uni-mainz.de

† These authors equally contributed to this work.

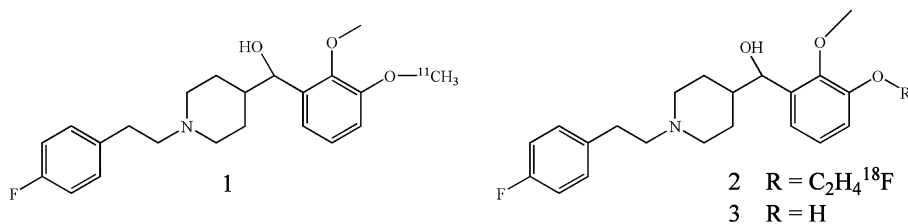


Figure 1. Structures of [^{11}C]MDL 100907 (1), [^{18}F]MHMZ (2), and MDL 105725 (3).

studies showed that metabolism predominantly resulted in the formation of its 3-OH analog MDL 105725 ((3-hydroxy-2-methoxy-phenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine-methanol, **3**). ^{18}F -Labeling in the 2-position would therefore lead to extensive formation of the labeled 3-OH-analog (2- ^{18}F fluoro-ethoxy-3-methoxy-phenyl)-1-[2-(4-fluoro-phenyl)ethyl]-4-piperidine-methanol that may be expected to cross the blood–brain-barrier or to be metabolized within the brain and thus interfere with the interpretation of the labeled tracer uptake.^{10,11}

A useful synthetic route to MDL 100907 and its racemic precursor MDL 105725 has been published by Huang et al.³ The route depended upon a key transformation of an ester to a ketone via an amide intermediate (Fig. 2) and was carried out essentially as published³ with minor modifications.

Finally, MHMZ was synthesized via a fluoroalkylation of the precursor MDL 105725 in dry DMF by addition of sodium hydride and 1-bromo-2-fluoroethane (Fig. 3) in a yield of 40%. A chiral derivatization of the final product MHMZ was not performed.

The purity of MHMZ was examined to be higher than 98% as indicated by HPLC analysis (ET 250/8/4

Nucleosil[®] 5 C₁₈; MeCN/H₂O 40:60, $R_f = 8.68$ min). These results justified further analyses like determination of the affinity and the route for radioactive syntheses, receptor autoradiography, and metabolism studies.

A radioligand competition binding assay was carried out with GF-62 cells, a clonal cell line expressing high amounts (5–7 pmol/mg) of the 5-HT_{2A} receptor, in test tubes containing [^3H]MDL (0.2 nM) and seven different concentrations of test compounds (1 μM –1 pM) in a total of 1 mL assay buffer. Ketanserin (1 μM) was used to determine non-specific binding. The 5-HT_{2A} binding affinities of the racemic MHMZ and the reference compounds altanserin and MDL 100907 are shown in Table 1.

MHMZ showed a 4.5 times lower affinity as compared to the parent compound MDL 100907 but still was in the nanomolar range. The assay was performed $n = 4$ times.

[^{18}F]Fluoroalkylation of the precursor MDL 105725 was carried out using [^{18}F]FETos, which was produced in an automated module.¹² Optimization of the reaction conditions gave radiochemical yields of about 90% at a reaction temperature of 100 °C in a reaction time of

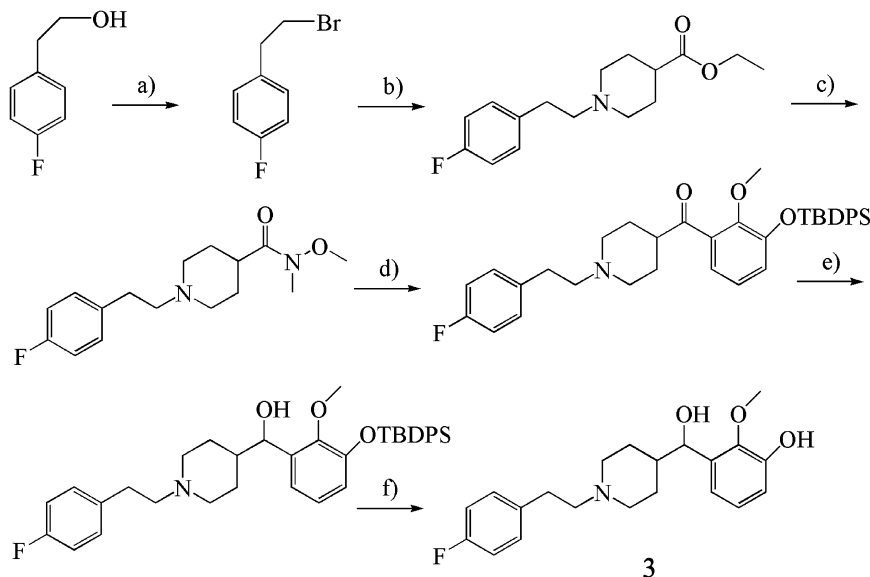


Figure 2. (a) PBr_3 , toluene; (b) K_2CO_3 , DMF; (c) $\text{Me}(\text{MeO})\text{NH}$ HCl, EtMgBr , THF; (d) $n\text{-BuLi}$, THF, TBDPS-guajacol; (e) NaBH_4 , MeOH; (f) K_2CO_3 , MeOH, H_2O .

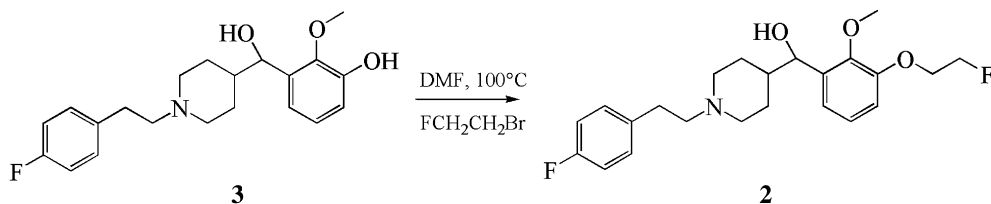


Figure 3. Synthesis of MHMZ.

Table 1. Receptor binding data of MDL 100907 derivatives and altanserine

Compound	K_i (nM)
MHMZ	9.00 ± 0.10
Altanserine	0.74 ± 0.88
MDL 100907	2.10 ± 0.13

10 min using 7 mmol precursor and 7 mmol 5 N NaOH as a base in dry DMF as a solvent.

The optimization procedure of the radiochemical yield of [^{18}F]MHMZ is exemplified for the parameter temperature in Figure 4. The final formulation of the injectable solution including a semipreparative HPLC (ET 250/8/4 Nucleosil[®] 5 C₁₈; MeCN/H₂O 40:60, $R_f = 8.68$ min) took no longer than 100 min and provided [^{18}F]MHMZ (2) with a purity >96% as indicated by analytical HPLC analyses. The specific activity was determined to be ~50 MBq/nmol with a starting amount of radioactivity of 3 GBq of [^{18}F]fluorine.

Autoradiographic images of the 5-HT_{2A} receptor obtained with [^{18}F]MHMZ showed excellent visualization results in rat brain sections (Fig. 5). Images were in complete agreement with the distribution obtained with [^3H]MDL 100907¹³ (also Fig. 6B and C). Highest binding was detected in lamina V of the frontal cortex, the caudate-putamen, the motor trigeminal nucleus, the facial nucleus, and the pontine nuclei. Minor binding was detected in the olfactory system, the mesencephalon, and the hippocampus.

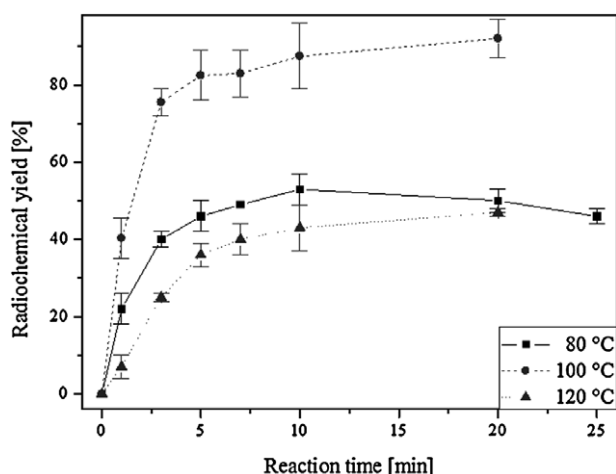


Figure 4. [^{18}F]Fluoroalkylation of 7 mmol MDL 105725 at different reaction temperatures using DMF and 7 mmol 5 N NaOH.

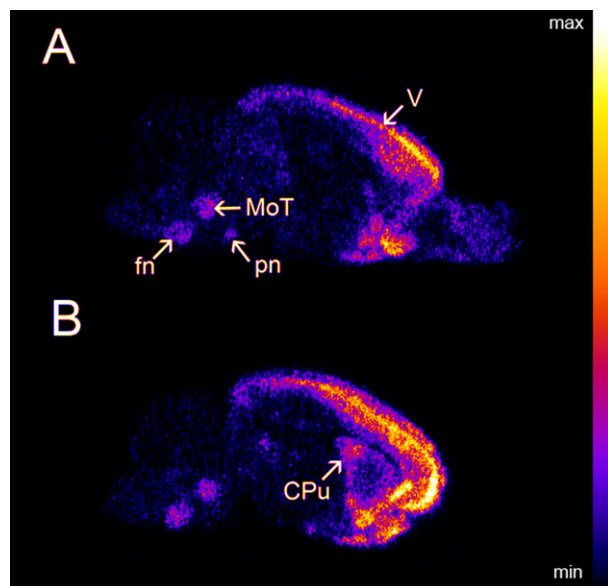


Figure 5. Images of an autoradiography of [^{18}F]MHMZ binding at 14 μm thick rat brain sections; (A and B) total binding at a concentration of 5 nM with (A) lateral 0.9 mm and (B) lateral 2.4 mm from bregma. Major binding was detected in lamina V (V) of the frontal cortex, in the caudate-putamen (CPu), and three regions of the brain stem, the motor trigeminal nucleus (MoT), facial nucleus (fn), and the pontine nuclei (pn). Non-specific binding was determined in the presence of 10 μM ketanserin which led to total inhibition of [^{18}F]MHMZ binding (cf. C' Fig. 6). Specific activity was 1.38 MBq/nmol (at the end of the incubation period).

Competition autoradiography assays (data not shown) with 5 nM [^{18}F]MHMZ and 10 μM of fallypride, WAY 100635, and prazosin showed that [^{18}F]MHMZ is highly specific for 5-HT_{2A} receptors. Displacement could only be detected with fallypride. Here, co-incubation led to a displacement of 30% ($n = 4$, $\pm 6\%$ SEM) of total binding in the frontal cortex as well as in the caudate-putamen, which does not imply that [^{18}F]MHMZ recognizes D2/D3 receptors but might rather be explained by the known cross affinity of fallypride to 5-HT₂ receptors.¹⁴

Binding parameters of [^{18}F]MHMZ of different regions of the rat brain obtained with autoradiography assays at sagittal sections are displayed in Table 2. Binding in the cerebellum was at the level of non-specific binding so levels of binding in different brain regions are also given relative to that.

A comparison of the binding of [^{18}F]altanserine and [^{18}F]MHMZ (Fig. 6) displays that [^{18}F]MHMZ is in

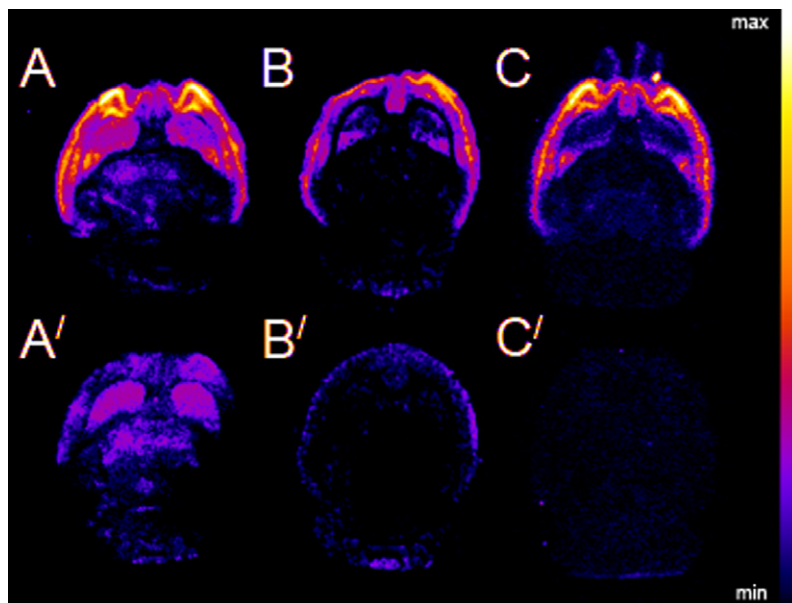


Figure 6. Autoradiographic images of the total binding and non-specific binding, respectively, of (A/A') [^{18}F]altanserin, (B/B') [^3H]MDL 100907 and (C/C') 5 nM [^{18}F]MHMZ at 14 μm rat brain sections. Non-specific binding was determined in the presence of 10 μM ketanserin. Specific activity of [^{18}F]MHMZ and [^{18}F]altanserin was ~ 160 kBq/nmol (at the end of the incubation period). Washing was done 2×2 min at room temperature with (A/A') in ice-cold reaction buffer, 2×2 min at room temperature with (B/B') and 3×2 min at room temperature (4 min with buffer containing 0.01% Triton X-100). Reaction buffer was 50 mM Tris buffer, pH 7.4, containing 120 mM NaCl₂ and 5 mM KCl.

Table 2. Binding parameters obtained with [^{18}F]MHMZ from binding experiments at 14 μm sagittal sections of the rat brain ($x = \text{means} \pm \text{SEM}$)

	<i>n</i>	pmol/mm ³	Region/cerebellum
<i>Frontal cortex</i>			
Laminae I–IV	4	23.30 \pm 1.69	26.9 \pm 0.9
Lamina V	4	51.60 \pm 5.24	59.5 \pm 2.8
Laminae VIa + VIb	4	27.27 \pm 2.76	31.4 \pm 1.3
Caudate-putamen	4	16.80 \pm 2.33	19.2 \pm 1.4

no way inferior to [^{18}F]altanserin in terms of specificity for 5-HT_{2A} receptors. Figure 6 also shows the complete agreement of the binding of [^3H]MDL 100907 and [^{18}F]MHMZ.¹⁵

The metabolite analyses of rat plasma (Fig. 7) showed that [^{18}F]MHMZ underwent fast metabolism. Plasma samples were taken at 5, 10, 30, and 60 min and analyzed by radio-TLC. One polar metabolite was found in rat plasma which is not likely to cross the blood–brain-barrier because of its hydrophilicity. The percentage of unmetabolized fractions was 43%, 32%, 16%, and 7% at 5, 10, 30, and 60 min, respectively.

In conclusion, precursors and reference compounds of [^{18}F]MHMZ were synthesized in high yields. The new ^{18}F -labeled compound could be obtained as an injectable solution in overall radiochemical yields of about 42% within a synthesis time of about 100 min in a purity of >96% and high specific activities. This is very similar to the radiosynthesis of [^{18}F]altanserin, which takes 75–100 min and results in a radiochemical yield between 30% and 50%.⁴

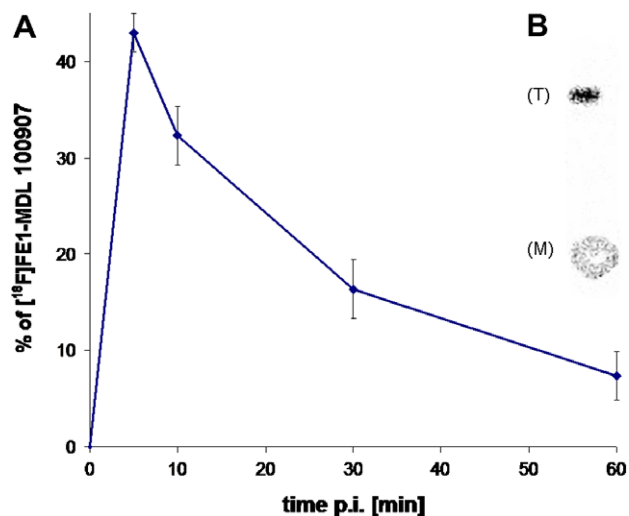


Figure 7. (A) Plasma clearances of [^{18}F]MHMZ at 5, 10, 30, and 60 min ($n = 3$ per time point; means \pm SD shown). (B) Radioactivity in TLC plate of plasma samples at 5 min pi is shown. Spots for [^{18}F]MHMZ (T) ($R_f = 0.76$) and its metabolite (M) ($R_f = 0.16$) were clearly visible.

First autoradiographic studies showed excellent *in vitro* binding with high specificity of [^{18}F]MHMZ for 5-HT_{2A} receptors and very low non-specific binding.

[^{18}F]MHMZ undergoes fast metabolism resulting in one very polar active metabolite.

Except from the slightly decreased affinity the reported *in vitro* data seem to be comparable with those of [^3H]MDL 100907. Our data suggest that the aim of

developing a novel ^{18}F -analog of MDL 100907 (**1**) combining the better selectivity of MDL 100907 as compared to altanserin and the superior isotopic properties for the clinical routine of ^{18}F fluorine as compared to ^{11}C carbon could be achieved.

All together, new auspicious results concerning the synthesis and of the in vitro studies of ^{18}F MHMZ justify further experiments like ex vivo brain regional distribution and in vivo small animal PET studies to verify the potential of this new 5-HT_{2A} imaging ligand.

Acknowledgments

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References and notes

1. Scientific articles: Kristiansen, H.; Elfing, B.; Plenge, P.; Pinborg, L. H.; Gillings, N.; Knudsen, G. M. *Synapse* **2005**, *58*, 249.
2. Lemaire, C.; Cantineau, R.; Guillaune, M.; Plenevaux, A.; Christiaens, L. *J. Nucl. Med.* **1991**, *32*, 2266.
3. Huang, Y.; Mahmood, K.; Mathis, C. A. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 949.
4. Hamacher, K.; Coenen, H. H. *Appl. Radiat. Isot.* **2006**, *64*, 989.
5. Alexoff, D. L.; Shea, C.; Fowler, J. S.; King, P.; Gatley, S. J.; Schleyer, D. J.; Wolf, A. P. *Nucl. Med. Biol.* **1995**, *22*, 892.
6. Heinrich, T.; Boetcher, H.; Pruecher, H.; Gottschlich, R.; Ackermann, K.-A.; van Amsterdam, C. *Chem. Med. Chem.* **2006**, *1*, 245.
7. Tan, P.-Z.; Baldwin, R. M.; Fu, T.; Charney, D. S.; Inis, R. B. *J. Labelled Compd. Radiopharm.* **1998**, *42*, 457.
8. Meltzer, C. C.; Smith, G.; DeKosky, S. T.; Pollock, B. G.; Mathis, A. M.; Moore, R. Y.; Kupfer, D. J.; Reynolds, C. F. *Neuropsychopharmacology* **1998**, *18*, 407.
9. Scot, D.; Heath, T. G. *J. Pharm. Biomed. Anal.* **1998**, *17*, 17.
10. Ullrich, T.; Ice, K. C. *Bioorg. Med. Chem.* **2000**, *8*, 2427.
11. Lundkvist, C.; Halldin, C.; Ginovart, N.; Swahn, C.-G.; Carr, A. A.; Brunner, F.; Farde, L. *Life Sci.* **1996**, *58*, 187.
12. Bauman, A.; Piel, M.; Schirrmacher, R.; Rösch, F. *Tetrahedron Lett.* **2003**, *44*, 9165.
13. Lopez-Gimenez, J. F.; Mengod, D.; Palacios, J. M.; Vilario, M. T. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *356*, 446.
14. Stark, D.; Piel, M.; Hübner, H.; Gmeiner, P.; Gründer, G.; Rösch, F. *Bioorg. Med. Chem.* **2007**, *15*, 6819.
15. Autoradiography experiments were carried out at room temperature in reaction buffer (50 mM Tris/HCl buffer, pH 7.4, containing 120 mM NaCl₂, and 5 mM KCl) with ^3H MDL 100907 and ^{18}F MHMZ and on ice with ^{18}F altanserin. Sections with ^{18}F MHMZ were washed 2 × 2 min in reaction buffer containing 0,01% Triton X-100 and 1 × 2 min in reaction buffer, shortly dipped into deionized water, and quickly dried in a stream of cold air. Sections with ^{18}F altanserin were washed in pure ice-cold reaction buffer 2 × 10 min, sections with ^3H MDL 100907 were washed in pure buffer 2 × 2 min. Sections were exposed to Fuji phosphor screen for 3 h when ^{18}F was used and for 5 days when ^3H was used. Screens were read out with a Fuji FLA-7000 scanner. For ^{18}F quantification was done after calibration by a standard curve which was obtained by a dilution series of the radiotracer. Calibration was repeated for each fresh radiotracer synthesis. Calibration for sections with ^3H was done with Amersham microscale standards. Calibration, quantification and data evaluation was done with Multi Gauge, Fujifilm image analysis software.