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Total synthesis and evaluation of [^{18}F]MHMZ

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Abstract—Radiochemical labeling of MDL 105725 using the secondary labeling precursor 2-[^{18}F]fluoroethyltosylate ([^{18}F]FETos) was carried out in yields of ~90% synthesizing [^{18}F]MHMZ in a specific activity of ~50 MBq/nmol with a starting activity of ~3 GBq. Overall radiochemical yield including [^{18}F]FETos synthon synthesis, [^{18}F]fluoroalkylation and preparing the injectable [^{18}F]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.

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 [^{11}C]MDL 100907,[3] [^{18}F]altanserin,[4] and [^{11}C]SR 46349B[5].

Within those ligands, [^{18}F]altanserin and [^{11}C]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 nM[4]; (*R*)-MDL 100907: K_i = 0.57 nM[6]}. Affinities are more than 100-fold higher for other receptors such as 5-HT$_{2C}$, α_1, D_1, and D_2. Nevertheless, it was proposed that the selectivity of [^{11}C]MDL 100907 for 5-HT$_{2A}$ receptor is slightly higher than the selectivity for this receptor of [^{18}F]altanserin.[8] Both tracers show in in vitro and in 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 [^{18}F]altanserin over [^{11}C]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 [^{18}F]fluorine. A drawback of [^{18}F]altanserin is its rapid and extensive metabolism. Four metabolites are formed in humans that cross the blood–brain-barrier,[7] whereas metabolites of [^{11}C]MDL 100907 do not enter the brain to any larger extent.[9]

The aim of this study was to develop an ^{18}F-analog of MDL 100907 (**1**) combining advantages of both ligands, the better selectivity of MDL 100907 and the superior isotopic properties of [^{18}F]fluorine. For this purpose we decided to replace one of the *O*-methyl groups by an *O*-2-[^{18}F]fluoroethyl moiety resulting in [^{18}F]MHMZ ([^{18}F]FE1-MDL 100907) ((3-[^{18}F]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 [^{11}C]MDL 100907

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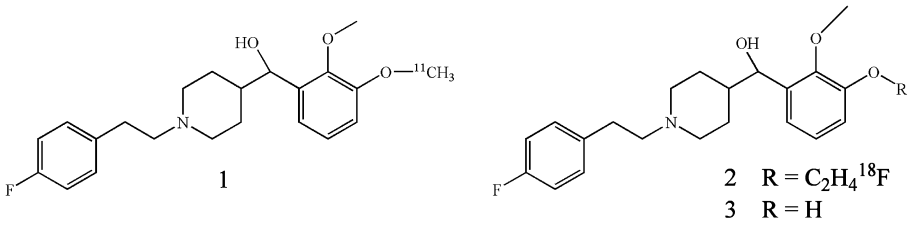


Figure 1. Structures of [^{11}C]MDL 100907 (1), [^{18}F]MHMZ (2), and MDL 105725 (3).

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 published[3] 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 C_{18}; MeCN/H_2O 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 [^{3}H]MDL (0.2 nM) and seven different concentrations of test compounds (1 μM–1 pM) in a total of 1mL 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

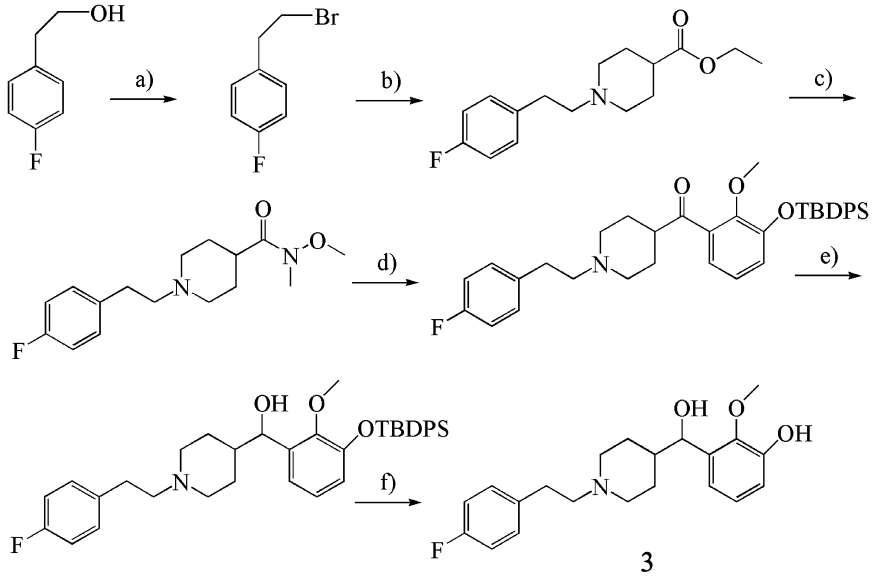
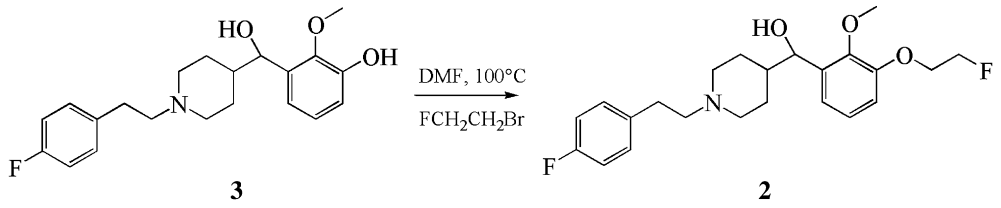


Figure 2. (a) PBr_3, toluene; (b) K_2CO_3, DMF; (c) Me(MeO)NH HCl, EtMgBr, THF; (d) *n*-BuLi, THF, TBDPS-guajacol; (e) $NaBH_4$, MeOH; (f) K_2CO_3, MeOH, H_2O.



3 2

Figure 3. Synthesis of MHMZ.

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

Compound	K_i (nM)
MHMZ	9.00 ± 0.10
Altanserin	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 C_{18}; MeCN/H_2O 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 [^{3}H]MDL 100907[13] (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.

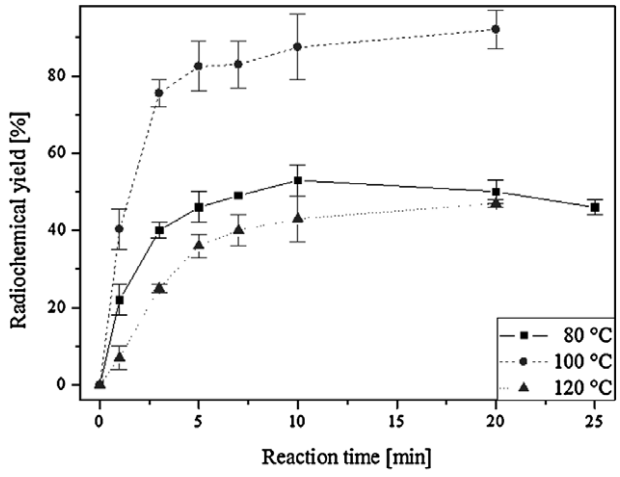


Figure 4. [^{18}F]Fluoroalkylation of 7 mmol MDL 105725 at different reaction temperatures using DMF and 7 mmol 5 N NaOH.

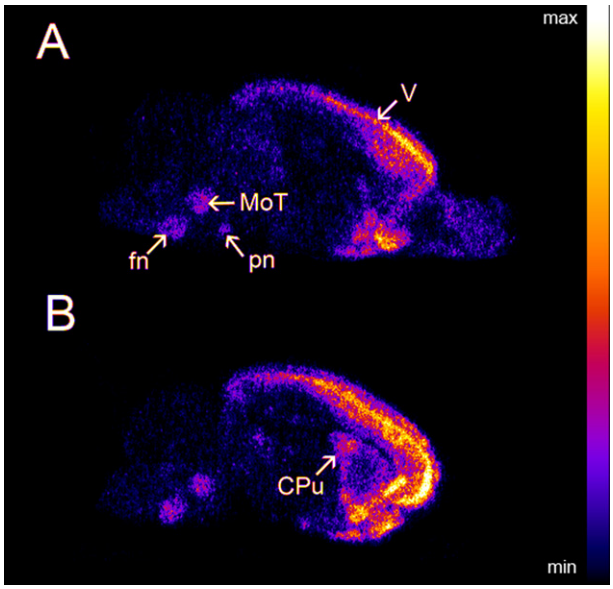


Figure 5. Images of an autoradiography of [^{18}F]MHMZ binding at 14 µm thick rat brain sections; (A and B) total binding at a concentration of 5 nM with (A) lateral 0.9 mm and (B) lateral 2.4 mm from bregma. Major binding was detected in lamina V (**V**) of the frontal cortex, in the caudate-putamen (**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, ±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-HT_2 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]altanserin and [^{18}F]MHMZ (Fig. 6) displays that [^{18}F]MHMZ is in

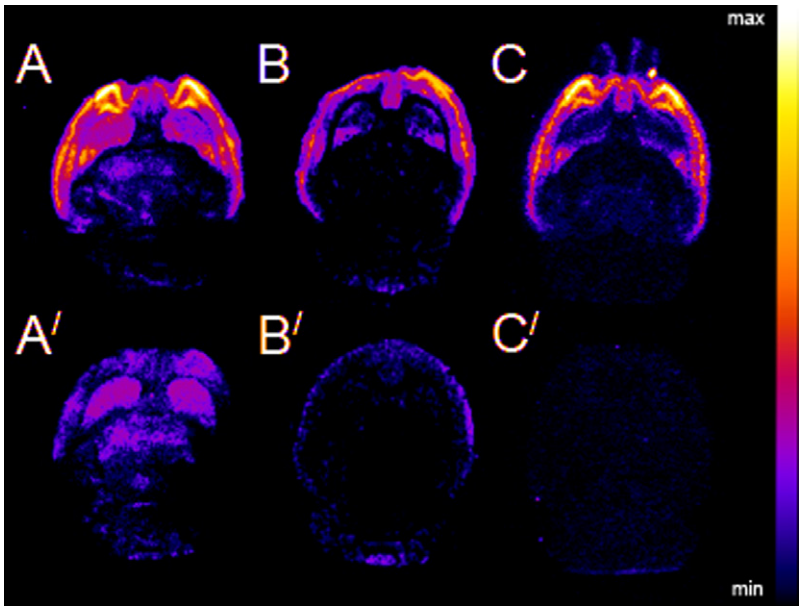


Figure 6. Autoradiographic images of the total binding and non-specific binding, respectively, of (A/A′) [^{18}F]altanserin, (B/B′) [^{3}H]MDL 100907 and (C/C′) 5 nM [^{18}F]MHMZ at 14 μm rat brain sections. Non-specific binding was determined in the presence of 10 μM ketanserin. Specific activity of [^{18}F]MHMZ and [^{18}F]altanserin was ~160 kBq/nmol (at the end of the incubation period). Washing was done 2 × 10 min for (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 $NaCl_2$ 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 = means ± SEM)

	n	pmol/mm^3	Region/cerebellum
Frontal cortex			
Laminae I–IV	4	23.30 ± 1.69	26.9 ± 0.9
Lamina V	4	51.60 ± 5.24	59.5 ± 2.8
Laminae VIa + VIb	4	27.27 ± 2.76	31.4 ± 1.3
Caudate-putamen	4	16.80 ± 2.33	19.2 ± 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 [^{3}H]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]

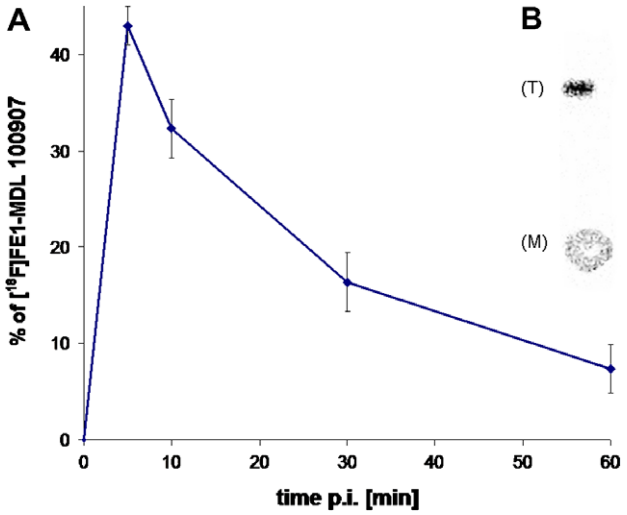


Figure 7. (A) Plasma clearances of [^{18}F]MHMZ at 5, 10, 30, and 60 min (n = 3 per time point; means ± SD shown). (B) Radioactivity in TLC plate of plasma samples at 5 min pi is shown. Spots for [^{18}F]MHMZ (**T**) (R_f = 0.76) and its metabolite (**M**) (R_f = 0.16) were clearly visible.

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 [^{3}H]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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15. Autoradiography experiments were carried out at room temperature in reaction buffer (50 mM Tris/HCl buffer, pH 7.4, containing 120 mM $NaCl_2$, and 5 mM KCl) with [^{3}H]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 [^{3}H]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 ^{3}H 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 ^{3}H was done with Amersham microscale standards. Calibration, quantification and data evaluation was done with Multi Gauge, Fujifilm image analysis software.