Total synthesis and evaluation of [18F]MHMZ

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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-HT2A receptor (Ki = 9.0 nM) in vitro and promising in vivo characteristics.

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Serotonergic 5-HT2A 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-HT2A 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-HT2A receptor. To date, in vivo studies have been performed with several 5-HT2A 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-HT2A PET imaging because of their high affinity and selectivity for the 5-HT2A receptor (altanserin: Ki = 0.13 nM; (R)-MDL 100907: Ki = 0.57 nM6). Affinities are more than 100-fold higher for other receptors such as 5-HT2C, α1, D1, and D2. Nevertheless, it was proposed that the selectivity of [11C]MDL 100907 for 5-HT2A receptor is slightly lower than the selectivity for this receptor of [18F]altanserin.8 Both tracers show in vitro and in in vivo experiments, high affinity, selectivity, and a good ratio of specific to non-specific binding for 5-HT2A 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)-[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...
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). 18F-Labeling in the 2-position would therefore lead to extensive formation of the labeled 3-OH-analog (2-[18F]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, Rf = 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-HT2A 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 1mL assay buffer. Ketanserin (1 µM) was used to determine non-specific binding. The 5-HT2A 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.

[18F]Fluoroalkylation of the precursor MDL 105725 was carried out using [18F]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 15 min.

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

Figure 2. (a) PBr3, toluene; (b) K2CO3, DMF; (c) Me(MeO)NH HCl, EtMgBr, THF; (d) n-BuLi, THF, TBDPS-guaiacol; (e) NaBH4, MeOH; (f) K2CO3, MeOH, H2O.
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}\text{F}]\text{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\textsuperscript{18} 5 C\textsubscript{18}: MeCN/H\textsubscript{2}O 40:60, \(R_f = 8.68\) min) took no longer than 100 min and provided \[^{18}\text{F}]\text{MHMZ}\) (2) with a purity >96% as indicated by analytical HPLC analyses. The specific activity was determined to be \(\sim 50\) MBq/nmol with a starting amount of radioactivity of 3 GBq of \[^{18}\text{F}]\text{fluorine}\).

Autoradiographic images of the 5-HT\textsubscript{2A} receptor obtained with \[^{18}\text{F}]\text{MHMZ}\) showed excellent visualization results in rat brain sections (Fig. 5). Images were in complete agreement with the distribution obtained with [\(^{3}\text{H}\)]MDL 100907\textsuperscript{15} (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.

Competition autoradiography assays (data not shown) with 5 nM \[^{18}\text{F}]\text{MHMZ}\) and 10 \(\mu\)M of fallypride, WAY 100635, and prazosin showed that \[^{18}\text{F}]\text{MHMZ}\) is highly specific for 5-HT\textsubscript{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}\text{F}]\text{MHMZ}\) recognizes D2/D3 receptors but might rather be explained by the known cross affinity of fallypride to 5-HT\textsubscript{2} receptors.\textsuperscript{14}

Binding parameters of \[^{18}\text{F}]\text{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}\text{F}]\text{altanserin}\) and \[^{18}\text{F}]\text{MHMZ}\) (Fig. 6) displays that \[^{18}\text{F}]\text{MHMZ}\) is in

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

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<thead>
<tr>
<th>Compound</th>
<th>(K_i) (nM)</th>
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<tr>
<td>[^{18}\text{F}]\text{MHMZ})</td>
<td>9.00 ± 0.10</td>
</tr>
<tr>
<td>Altanserin</td>
<td>0.74 ± 0.88</td>
</tr>
<tr>
<td>MDL 100907</td>
<td>2.10 ± 0.13</td>
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**Figure 4.** \[^{18}\text{F}]\text{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}\text{F}]\text{MHMZ}\) binding at 14 \(\mu\)m 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 \(\mu\)M ketanserin which led to total inhibition of \[^{18}\text{F}]\text{MHMZ}\) binding (cf. C of Fig. 6). Specific activity was 1.38 MBq/nmol (at the end of the incubation period).
no way inferior to $^{[18\text{F}]}$altanserin in terms of specificity for 5-HT$_{2A}$ receptors. Figure 6 also shows the complete agreement of the binding of $^{[3\text{H}]}$MDL 100907 and $^{[18\text{F}]}$MHMZ.$^{15}$

The metabolite analyses of rat plasma (Fig. 7) showed that $^{[18\text{F}]}$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.

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

<table>
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<tr>
<th>Region</th>
<th>n pmol/mm$^3$</th>
<th>Region/cerebellum</th>
</tr>
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<tr>
<td>Laminae I–IV</td>
<td>23.30 ± 1.69</td>
<td>26.9 ± 0.9</td>
</tr>
<tr>
<td>Lamina V</td>
<td>51.60 ± 5.24</td>
<td>59.5 ± 2.8</td>
</tr>
<tr>
<td>Laminae V Ia + V Ib</td>
<td>27.27 ± 2.76</td>
<td>31.4 ± 1.3</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>16.80 ± 2.33</td>
<td>19.2 ± 1.4</td>
</tr>
</tbody>
</table>

In conclusion, precursors and reference compounds of $^{[18\text{F}]}$MHMZ were synthesized in high yields. The new $^{18\text{F}}$-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 $^{[18\text{F}]}$altanserin, which takes 75–100 min and results in a radiochemical yield between 30% and 50%.$^{4}$

Figure 6. Autoradiographic images of the total binding and non-specific binding, respectively, of (A/A’) $^{[18\text{F}]}$altanserin, (B/B’) $^{[3\text{H}]}$MDL 100907 and (C/C’) 5 nM $^{[18\text{F}]}$MHMZ at 14 µm rat brain sections. Non-specific binding was determined in the presence of 10 µM ketanserin. Specific activity of $^{[18\text{F}]}$MHMZ and $^{[18\text{F}]}$altanserin was $\sim 160$ 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 and 5 mM KCl.

Figure 7. (A) Plasma clearances of $^{[18\text{F}]}$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 $^{[18\text{F}]}$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 $^{[18\text{F}]}$MHMZ for 5-HT$_{2A}$ receptors and very low non-specific binding.

$^{[18\text{F}]}$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 $^{[3\text{H}]}$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-MHZM 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$_2$A imaging ligand.

Acknowledgments

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References and notes

15. Autoradiography experiments were carried out at room temperature in reaction buffer (50 mM Tris/HCl buffer, pH 7.4, containing 120 mM NaCl, 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$]$MHMZ 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.