Design, (radio)synthesis and applications of radiolabelled matrix metalloproteinase inhibitors for PET
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Summary
Matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinases (ADAMs) are two families of zinc endopeptidases which are secreted in the extracellular matrix (ECM) or attached on the extracellular side of the plasma membrane [1-3]. The diverse implications of MMPs and ADAMs in the regulation of the tissue microenvironment and in the remodeling of the ECM are of a crucial relevance for several types of cancer or inflammation [4, 5]. Therefore, the MMP/ADAM activity profile can be considered as a valuable tool for the staging of a tumor with respect to its invasiveness or for the detection of an inflammatory lesion.

The main focus of this thesis was the design, (radio)synthesis and evaluation of radiolabelled matrix metalloproteinase inhibitors (MMPIs), principally by positron emission tomography (PET), to visualize/quantify the levels of MMPs and ADAMs in vivo.

Chapter 1 gives a general introduction and an outline of the thesis.

Chapter 2 provides an overview of radiolabelled MMPIs and MMP peptide substrates for PET and single photon emission computed tomography (SPECT). This review concludes that, despite some hydroxamate-based tracers aimed at specific

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Figure 1: Structure of [18F]FB-ML5, [18F]-1A, [18F]-2 and [18F]-1B
identification of the proteolytic activity of MMPs and ADAMs in animal models of diverse diseases, most studies are at a preliminary stage. As a result, efforts to develop and assess novel probes targeting MMPs/ADAMs are needed.

Chapter 3 describes the preparation and evaluation of the radiolabelled MMP/ADAM inhibitor \([^{18/19}F]FB-ML5\) [Fig 1]. \([^{18/19}F]FB-ML5\) was successfully prepared by direct acylation of the hydroxamate-based inhibitor ML5 with \(N\)-succinimidyl-4-\([^{18/19}F]\)fluorobenzoate (\([^{18/19}F]SFB\)) [Table 1]. ML5 and FB-ML5 showed high affinity for MMP-2, -9, -12 and ADAM-17 [Table 2]. The incorporation of the fluorobenzoyl moiety did not result in a substantial modification of the affinity for MMP-2, -9 and ADAM-17. However, FB-ML5 displayed an almost 100-fold loss of affinity for MMP-12 compared to ML5. \([^{18F}]FB-ML5\) showed rather low binding to ADAM-17 overexpressing cell lines. Binding of \([^{18F}]FB-ML5\) was reduced by 36.6% and 27.5% in MCF-7 and 16HBE cells, respectively, after co-incubation with 10 µM of ML5. \([^{18F}]FB-ML5\) was evaluated in a HT1080 tumor-bearing mouse model. This PET

<table>
<thead>
<tr>
<th>IC_{50}</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>MMP-12</th>
<th>ADAM-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML5</td>
<td>7.4 ± 2.0 nM</td>
<td>19.5 ± 2.8 nM</td>
<td>2.0 ± 0.2 nM</td>
<td>5.7 ± 2.2 nM</td>
</tr>
<tr>
<td>FB-ML5</td>
<td>12.5 ± 3.1 nM</td>
<td>31.5 ± 13.7 nM</td>
<td>138.0 ± 10.9 nM</td>
<td>24.7 ± 2.8 nM</td>
</tr>
<tr>
<td>1A</td>
<td>nd</td>
<td>14.5 ± 2.57 nM</td>
<td>19.3 ± 4.96 nM</td>
<td>620 ± 89.1 nM</td>
</tr>
<tr>
<td>2</td>
<td>nd</td>
<td>9.19 ± 2.07 nM</td>
<td>1.12 ± 1.08 nM</td>
<td>10.6 ± 0.91 nM</td>
</tr>
<tr>
<td>1B</td>
<td>4.67 ± 0.85 nM</td>
<td>3.67 ± 0.49 nM</td>
<td>nd</td>
<td>43.4 ± 7.74 nM</td>
</tr>
</tbody>
</table>

Table 2: IC_{50} values of ML5, FB-ML5, 1A, 2 and 1B

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Block (or treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT1080 tumor</td>
<td>PET-SUV\text{mean}([^{18F}]FB-ML5)</td>
</tr>
<tr>
<td>COPD model</td>
<td>PET-SUV\text{mean}([^{18F}]FB-ML5)</td>
</tr>
<tr>
<td>HT1080 tumor</td>
<td>SUV\text{mean}([^{18F}]FB-ML5)-1A</td>
</tr>
<tr>
<td>HT1080 tumor</td>
<td>SUV\text{mean}([^{18F}]FB-ML5)-2</td>
</tr>
<tr>
<td>HT1080 tumor</td>
<td>PET-SUV\text{mean}([^{18F}]FB-ML5)-1B</td>
</tr>
</tbody>
</table>

Table 3: PET-SUV\text{mean}/SUV\text{mean} of \([^{18F}]FB-ML5\), \([^{18F}]1A\), \([^{18F}]2\) and \([^{18F}]1B\)
radioligand showed accumulation in the tumor, which was significantly reduced upon blocking with cold ML5 (2.5 mg/kg). The average PET-SUV\textsubscript{mean} was 0.13 ± 0.09 in the baseline group and 0.04 ± 0.03 in the block group [Table 3], at 90 min p.i.

Considering the encouraging results of \[^{18}\text{F}]\text{FB-ML5}\) in the HT1080 xenograft mouse model, chapter 4 describes the evaluation of this peptidomimetic MMP/ADAM inhibitor in a mouse model of pulmonary inflammation. Mice were exposed for four days to cigarette smoke (CS) or air and a dynamic microPET scan was performed on the fifth day. The pulmonary uptake in both groups was relatively low. PET-SUV\textsubscript{mean} were 0.19 ± 0.06 in the lungs of CS-exposed mice compared to 0.11 ± 0.03 in air-exposed mice [Table 3], at 90 min p.i. MMP-9 levels in bronchoalveolar lavage fluid (BALF) were increased from undetectable level to 4615 ± 1963 pg.mL\(^{-1}\) by CS exposure. CS exposure resulted in an upregulation of neutrophils and eosinophils (to a lesser extent) but not of monocytes, as the nature of the inflammatory process in chronic obstructive pulmonary disease (COPD) is primarily neutrophilic. To conclude, the increased MMP expression in a COPD mouse model was associated with an increased uptake of \[^{18}\text{F}]\text{FB-ML5}. Although the probe seems to work, its absolute uptake in target tissues is quite low, thus the sensitivity of imaging will be limited. Better probes are definitely required.

As nonpeptidomimetic MMPIs may exhibit greater specificity compared to peptidomimetic compounds [6], chapter 5 describes the design, (radio)synthesis and evaluation of two piperazine-based MMP/ADAM inhibitors \[^{18}\text{F}]\text{-1A and }[^{18}\text{F}]\text{-2 [Fig 1], with different lipophilicities. The non-radioactive hydroxamate-based inhibitors 1A and 2 were successfully prepared, as well as their nitro-analogues for }[^{18}\text{F}]\text{fluorination by homoaromatic nucleophilic substitution. 1A and 2 demonstrated good in vitro affinities against MMP-9, -12 and ADAM-17 [Table 2]. The synthesis of }[^{18}\text{F}]\text{-1A and }[^{18}\text{F}]\text{-2 led to low and variable radiochemical yields (RCYs) [Table 1]. }[^{18}\text{F}]\text{-1A and }[^{18}\text{F}]\text{-2 demonstrated low target-to-non-target ratios in a HT1080 tumor-bearing mouse model [Table 3] and therefore cannot be considered as suitable imaging agents.}

Finally, chapter 6 reports the development and assessment of another piperazine-based MMP/ADAM inhibitor \[^{18}\text{F}]\text{-1B [Fig 1]. The non-radioactive analogue 1B was successfully prepared by click chemistry and exhibited nanomolar affinities against MMP-2, -9 and ADAM-17 [Table 2]. }[^{18}\text{F}]\text{-1B was synthesized with a satisfactory RCY [Table 1]. Retention of }[^{18}\text{F}]\text{-1B was shown to be MMP/ADAM-mediated in the HT1080 xenograft mouse model [Table 3]. }[^{18}\text{F}]\text{-1B provides a novel lead structure which should be further optimized in view of MMP/ADAM-specific tumor imaging by PET.
References
