Design, (radio)synthesis and applications of radiolabelled matrix metalloproteinase inhibitors for PET
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General introduction
In the past decades, matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinases (ADAMs) attracted considerable interest due to their significant role in numerous diseases [1, 2]. Indeed, their powerful proteolytic activity is implicated in the remodelling of the extracellular matrix [3, 4], tissue destruction (e.g. in chronic obstructive pulmonary disease [5, 6] or rheumatoid arthritis [7, 8]), cancer [9-13], atherosclerotic plaque stability [14, 15], immunomodulation [16], neuronal development [17] and in regulatory events related to the liberation of adhesion molecules, growth factors and cytokines [18].

MMPs and ADAMs are therefore attractive targets for therapy and may be useful as biomarkers or targets for in vivo imaging to monitor disease progression and the efficacy of therapeutic intervention. Measuring only active enzyme is crucial, since measuring the overall amount of a certain MMP or ADAM (by, for instance, an immunoassay), does not provide a correct picture of the enzyme activity involved in the disease process nor of the enzyme localization within cells, tissues or the organism. Such information is pivotal for gaining a better understanding of the role of these enzymes in disease mechanisms and for validating them as targets for drug development.

The control of MMP/ADAM activity by inhibitors has therefore gained considerable interest as a possible therapeutic target [19].

MMPs and ADAMs are inhibited by nonspecific protease inhibitors such as α₂-macroglobulin and α₁-antiprotease, and by a small family of specific natural inhibitors towards metalloproteinase activity: tissue inhibitors of metalloproteinases (TIMPs). These endogeneous inhibitors have affinities for MMPs in the $10^{-10}$ to $10^{-16}$ M range and seem the most suitable candidates for labelling and therapy but they lack selectivity and have other biological functions [20-22]. Therefore, synthetic and more specific MMP inhibitors (MMPIs) were prepared. In order to design an MMPI, the following structural features are needed:

(i) at least one functional group that affords a hydrogen bond interaction with the enzyme backbone,
(ii) one or more side chains, which are able of van der Waals interactions with the enzyme subsites and
(iii) a functional group (e.g., hydroxamate, phosphonate, carboxylate, thiol, barbiturate, etc.) which chelates the active-site zinc(II) ion (referred to as zinc binding group) [23-25].
Positron emission tomography (PET) and single photon emission computed tomography (SPECT) [26] are non-invasive nuclear imaging techniques, which have the ability to monitor molecular events in vivo and in real time. They result in a detailed picture of fundamental biochemical and physiological processes in living organisms. In contrast to magnetic resonance imaging, X-rays, or ultrasound, PET and SPECT allow the monitoring of metabolic processes in living subjects. Those nuclear imaging techniques require an exogenous radioactive probe, injected in very low mass amounts, which provides a detectable signal of the biological processes under investigation.

An MMP inhibitor-based radiotracer would allow the non-invasive visualization of MMPs/ADAMs in vivo which represents an important clinical parameter for physicians. PET is more sensitive, has a better spatial resolution and allows a more quantitative measurement than SPECT. As a result, a MMP inhibitor labelled with a positron-emitting radionuclide would be of great interest for the visualization/quantification of active MMPs/ADAMs in vivo. On the other hand, SPECT is available in a greater number of hospitals and imaging centers than PET, therefore a MMP inhibitor radiolabelled for SPECT imaging would be of a high value as well. The overall goal of this thesis was the design, (radio)synthesis and evaluation of radio-labelled MMP inhibitors, mainly by PET, to profile the levels of MMPs and ADAMs in vivo.

Chapter 2 gives an overview of several radiolabelled PET/SPECT probes for MMP/ADAM imaging. The radiosynthesis and their in vitro/in vivo evaluation are reported. For a better overview, the radiotracers are first of all classified according to the nature of their biomolecules: either based on an inhibitor or a peptide substrate. Thereafter, the huge amount of synthetic inhibitors is described according to the structure of their zinc binding group: hydroxamate, carboxylate and barbiturate.

Chapter 3 describes a radiolabelled derivative of the peptidic MMP/ADAM inhibitor ML5: [18F]FB-ML5 for PET. The binding of the radiolabelled MMP/ADAM inhibitor is evaluated in vitro, using 16HBE and MCF-7 cells. The inhibitory action of ML5 and FB-ML5 was also evaluated in vitro, using the recombinant enzymes MMP-2, -9, -12 and ADAM-17. The nanomolar affinity inhibitor [18F]FB-ML5 is subsequently evaluated in a HT1080 xenograft mouse model.

Chapter 4 deals with the microPET evaluation of the hydroxamate-based MMP/ADAM inhibitor [18F]FB-ML5 in an in vivo mouse model of cigarette smoke-induced acute airway inflammation. Following the microPET scan, a bronchoalveolar lavage
(BAL) assay and a cell differentiation assay are carried out to quantify MMP-9 (gelatinase B) levels and the amounts of mononuclear cells, eosinophils and neutrophils in BAL fluid.

Chapter 5 describes the preparation of two non-peptidic hydroxamate inhibitors with different lipophilicities: 1-((4-[18F]fluorophenyl)sulfonyl)-N-hydroxy-4-(methylsulfonyl)piperazine-2-carboxamide or [18F]-1A and 4-([1,1’-biphenyl]-4-carbonyl)-1-((4-[18F]fluorophenyl)sulfonyl)-N-hydroxypiperazine-2-carboxamide or [18F]-2. The design, synthesis, radiosynthesis, in vitro and in vivo evaluation of these piperazine-based inhibitors are reported. The radiolabelling procedure employed for these inhibitors is the homoaromatic nucleophilic substitution with [18F]fluorine. A fluorogenic inhibition assays against MMP-2, -9 and ADAM-17 is performed. A HT1080 xenograft mouse model is employed for the in vivo evaluation of [18F]-1A and [18F]-2.

Chapter 6 reports a new piperazine-based MMP/ADAM inhibitor, 4-(4-(1-(4-(2-(2-(2-[18F]fluoroethoxy)ethoxy)ethoxy)butyl)-1H-1,2,3-triazol-4-yl)benzoyl)-N-hydroxy-1-((4-methoxyphenyl)sulfonyl)piperazine-2-carboxamide [18F]-1B, prepared by copper-catalyzed azide-alkyne cycloaddition. The incorporation of a PEG chain is also performed in order to optimize the target affinity and pharmacokinetic properties of this tracer. [18F]-1B is evaluated in vitro by employing the recombinant enzymes MMP-2, -9 and ADAM-17. Thereafter, [18F]-1B is also evaluated in vivo in mice bearing HT1080 tumors.

Chapter 7 summarizes all experimental results of this thesis.

Chapter 8 contains a general discussion with some perspectives for future research.
References


