A New Class of Highly Potent Matrix Metalloproteinase Inhibitors Based on Triazole-Substituted Hydroxamates: (Radio)Synthesis and in Vitro and First in Vivo Evaluation

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Supporting Information

ABSTRACT: In vivo imaging of MMPs is of great (pre)clinical interest and can potentially be realized with modern three-dimensional and noninvasive in vivo molecular imaging techniques such as positron emission tomography (PET). Consequently, MMP inhibitors (MMPIs) radiolabeled with positron emitting nuclides (e.g., ¹⁸F) represent a suitable tool for the visualization of activated MMPs with PET. On the basis of our previous work and results regarding radiolabeled and unlabeled derivatives of the nonselective MMPIs, we discovered a new class of fluorinated MMPIs with a triazole-substituted hydroxamate substructure. These novel MMPIs are characterized by an increased hydrophilicity compared with the lead structures and excellent MMP inhibition potencies for MMP-2, MMP-8, MMP-9, and MMP-13 (IC₅₀ = 0.006−107 nM). Therefore, one promising fluorinated triazole-substituted hydroxamate (30b) was selected and resynthesised as its ¹⁸F-labeled version to yield the potential PET radioligand [¹⁸F]30b. The biodistribution behavior of this novel compound was investigated with small animal PET.

INTRODUCTION

In 1962 Jerome Gross and Charles M. Lapière published their studies about the collagenolytic activity in amphibian tissues. They investigated the metamorphosis in tadpole tissues and observed the degradation of collagen by a proteinase that operated at neutral pH and physiological temperature.¹ Six years later, a corresponding enzyme was isolated in its proform from human skin by Eisen et al.² The proteinase was initially called interstitial collagenase and was later renamed as matrix metalloproteinase 1 (MMP-1) representing the first member of the matrix metalloproteinase (MMP) enzyme family. Similar to a disintegrin and metalloproteinases (ADAMs) and the ADAMs with a thrombospondin motif (ADAMTs), MMPs are a subfamily of the ubiquitously expressed metzincins enzyme superfamily.³−⁵ MMPs are multidomain Zn²⁺-dependent endopeptidases with a catalytic domain containing two Zn²⁺ and two or three Ca²⁺ ions. The Ca²⁺ ions and one of the Zn²⁺ ions are called structural and are responsible for the stabilization of the domain structure.⁶−⁸ The second Zn²⁺ ion participates in the catalytic process and is located directly in the active site of MMPs. It is coordinated by three histidine residues that are part of the Zn²⁺-binding consensus sequence HExxHxxGxxH (where x represents a variable amino acid) which is characteristic for proteolytically active metzincins.⁹ To date, 23 members of the human MMP family are described that are in the majority of cases excreted as inactive proenzymes characterized by an N-terminal prodomain that shields the ...
catalytic site from potential substrate molecules. After activation, namely, delocalization of the prodomain, via the so-called cysteine-switch mechanism, MMPs normally possess a broad substrate specificity.\textsuperscript{10–12} They degrade on the one hand extracellular matrix (ECM) and basement membrane (BM) proteins and are on the other hand involved in the processing of nonconventional substrates such as cytokines, chemokines, growth factors, serine proteinase inhibitors, cell receptors, and other MMPs.\textsuperscript{13} According to their substrate recognition and cleavage mechanism, MMPs can be grouped in collagenases, gelatinases, stromelysins, matrilysins, membrane-associated MMPs, and MMPs with no group designation.\textsuperscript{12} MMPs are inhibited and regulated by the common protease inhibitor α2-macroglobulin, the angiogenesis inhibitor thrombospondin 1 (TSP-1), and the four tissue inhibitors of metalloproteinases (TIMPs).\textsuperscript{14} The enzymes are involved in physiological processes such as wound healing,\textsuperscript{15} ovulation,\textsuperscript{16} muscle homeostasis,\textsuperscript{17} bone remodeling,\textsuperscript{18} apoptosis,\textsuperscript{19} and neurogenesis.\textsuperscript{20} In contrast, overexpression and overactivity of MMPs are observed in pathological situations including cancer,\textsuperscript{21} rheumatoid arthritis,\textsuperscript{22} osteoarthritis,\textsuperscript{23} multiple sclerosis, chronic obstructive pulmonary disease,\textsuperscript{24} and cardiovascular disease.\textsuperscript{25}

Consequently, the molecular imaging of activated MMPs in vivo, preferably with noninvasive approaches in the field of nuclear medicine such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) as well as the optical techniques fluorescence reflectance imaging (FRI) and fluorescence mediated tomography (FMT), is of great (pre)clinical interest because it would represent a powerful tool for the diagnosis and therapy control of MMP associated diseases. A potential approach to achieve this aim is the application of radiolabeled or fluorescence dye-labeled MMP inhibitors (MMPIs) functioning as molecular imaging probes for the visualization of activated MMPs.\textsuperscript{26}

In this context two slightly different concepts are followed in our group:

1. The first strategy is aimed at the specific imaging of discrete subclasses of MMPs. In this case we utilize a pyrimidine-2,4,6-trione (barbiturate) derivative (RO 28-2653) as lead structure for labeled analogues. This compound is described as an MMPI selective for the gelatinases (MMP-2, MMP-9), neutrophil collagenase (MMP-8), and two of the membrane-associated MMPs (MMP-14, MMP-16).\textsuperscript{27} Different labeled analogues of this lead structure were synthesized and evaluated partly in vitro and in vivo. Representative barbiturate-based MMPIs were labeled with the γ-emitters \(^{125}\)I and the SPECT-compatible \(^{123}\)I, with the positron emitting \(^{124}\)I, \(^{18}\)F, and \(^{68}\)Ga and with the fluorescence cyanine dye Cy5.5, resulting in a series of tracers with fine-tuned pharmacokinetics for different imaging modalities.\textsuperscript{28–32}

2. The second approach is based on the development of labeled small-molecule non-peptide MMPIs bearing broad-spectrum inhibition potency without explicit specificity for MMP-subgroups. Here, the hydroxamate-based MMPIs \textit{1a} (CGS 27023A) and \textit{1b} (CGS 25966) were chosen as lead compounds for the design of labeled derivatives (Figure 1). In contrast to the barbiturate analogues, chelating the \(\text{Zn}^{2+}\) ion of the enzyme active site in a tridentate manner, the CGS analogues coordinate to the \(\text{Zn}^{2+}\) ion in a bidentate mode using the hydroxamate moiety.\textsuperscript{33–35} In addition to the Cy5.5-labeled photoprobe \textit{1b} that shows specific MMP binding in vitro and ex vivo,\textsuperscript{36} the \(^{125}\)I-labeled hydroxamate \(\text{[HO}^{125}\text{I}]\text{1c}\) (HO\(1^{125}\text{I})\text{-CGS 27023A}) was synthesized and successfully used for specific scintigraphic imaging of MMP activity in the arterial wall of mice in vivo.\textsuperscript{37,38} In our subsequent work we focused on the development of a second radiotracer generation characterized by the introduction of the most prominent positron emitter \(^{18}\)F. Three different analogues \(\text{[18F]1d} (\text{[18F]EtO-CGS 27023A}), \text{[18F]1e} (\text{[18F]EtO-CGS 25966}), \text{[18F]1f} (\text{[18F]EtO-CGS 27023A})\) were radiosynthesized and evaluated in vitro and in vivo.\textsuperscript{39–42}

The most promising and accessible candidate \(\text{[18F]1e}\) that represents a hydrophilic member of the series shown in Figure 1 (calculated log \(\text{D}\) (clog\(\text{D}\)) of 4.03; experimental log \(\text{D}\) (log\(\text{D}\)) of 2.02) was selected for a good manufacturing practices (GMP) compliant fully automated radiosynthesis aiming at first-in-man studies.

To further improve the pharmacokinetics of these hydroxamate-based radiotracers, we aimed to shift the main clearance route from the hepatobiliary system to the kidneys by increasing the hydrophilicity of the new radiotracer class compared to \(\text{[18F]1e}\).\textsuperscript{44} A faster excretion of radiotracer that is not specifically bound to the target tissue results in a desired high signal-to-noise ratio of detected target tissues within a shorter time frame. Therefore, a new class of MMPIs was developed that consists of additional typical hydrophilic moieties,\textsuperscript{45–48} such as minipolyethylene glycol (mini-PEG) units and/or triazole subcores, at ring B (Figure 1) or that contains these hydrophilic structural elements instead of ring B. Ring B was chosen for these modifications because this residue is located in the S\(_{4}^\prime\) enzyme pocket that is solvent-exposed and should tolerate limited structural variations.\textsuperscript{49} The work presented here describes the (radio)synthesis and in vitro and first in vivo evaluation of a new class of triazole-substituted hydroxamate-based MMPIs displaying remarkable in vitro properties with elevated inhibition potencies and moderate to high hydrophilicities, respectively.

**RESULTS AND DISCUSSION**

**Chemistry.** To evaluate the MMP inhibition potencies of the new mini-PEG and/or triazole-substituted hydroxamates, the corresponding nonradioactive fluorinated target compounds were synthesized and tested by in vitro fluorgenic MMP assays.

Building blocks used for the synthesis of the MMPIs are represented by the mini-PEG alkyne derivatives \textit{7} and \textit{8} (Scheme 1). Monosubstitution of tetracyethylene glycol with
propargyl bromide and subsequent mesylation gave the PEGylated alkyne 3. Nucleophilic substitution of the mesyl moiety with 4-hydroxybenzaldehyde and reduction with sodium borohydride yielded the PEGylated benzyl alcohol 5, which was converted to the benzyl bromide 7 in two steps. Compound 7 was obtained in overall chemical yield of 21%.

Derivative 8 was obtained with a chemical yield of 37% over both steps by nucleophilic substitution of the monosubstituted mini-PEG alkyne intermediate 2 with 2-fluoroethyl 4-methylbenzenesulfonate,

\[ \text{Reactions} \] 

**Scheme 1. Syntheses of the Mini-PEG Compounds 7 and 8**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Conditions</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>propargyl bromide, NaH, THF</td>
<td>41%</td>
</tr>
<tr>
<td>b</td>
<td>MsCl, Et,N, CH₂Cl₂</td>
<td>93%</td>
</tr>
<tr>
<td>c</td>
<td>4-hydroxybenzaldehyde, Cs₂CO₃, DMF</td>
<td>59%</td>
</tr>
<tr>
<td>d</td>
<td>NaBH₄, MeOH</td>
<td>93%</td>
</tr>
<tr>
<td>e</td>
<td>(TFA)₂O, THF</td>
<td>100%</td>
</tr>
<tr>
<td>f</td>
<td>LiBr, THF</td>
<td>100%</td>
</tr>
<tr>
<td>g</td>
<td>2-fluoroethyl 4-methylbenzenesulfonate, NaH, THF</td>
<td>90%</td>
</tr>
</tbody>
</table>

**Scheme 2. Syntheses of the MMPIs 16a–c**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Conditions</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>4-methoxybenzene-1-sulfonyl chloride, pyridine</td>
<td>75%</td>
</tr>
<tr>
<td>b</td>
<td>K₂CO₃, DMF, 7</td>
<td>79%</td>
</tr>
<tr>
<td>c</td>
<td>HCl (gas), CH₂Cl₂</td>
<td>95%</td>
</tr>
<tr>
<td>d</td>
<td>O-(tetrahydro-2H-pyran-2-yl)hydroxylamine, EDC, NMM, HOBT, DMF</td>
<td>84%</td>
</tr>
<tr>
<td>e</td>
<td>4 N HCl in dioxane, methanol/dioxane, 1:1</td>
<td>89%</td>
</tr>
<tr>
<td>f</td>
<td>CuSO₄·5H₂O, sodium ascorbate, DMF, H₂O, 1-azido-2-fluoroethane (15a), 1-azido-2-(2-(2-fluoroethoxy)ethoxy)ethane (15b) or 2-deoxy-2-fluoro-( \beta )-glucopyranosylazide (15c)</td>
<td>21% (16a), 27% (16b), 6% (16c)</td>
</tr>
</tbody>
</table>
sulfonamide 10 was derived from the commercially available tert-butyl ester of D-valine hydrochloride 9 and 4-methoxybenzene-1-sulfonyl chloride in the presence of pyridine. N-Alkylation of 10 with benzyl bromide 7 gave the carboxylic acid ester 11, which was transformed to the carboxylic acid 12 using gaseous hydrochloric acid in dichloromethane. Conversion of 12 into the corresponding hydroxamic acid ester 13 was achieved by O-THP hydroxylamine, EDC, NMM, HOBT, DMF (92%); (f) 4 N HCl in dioxane, dioxane/MeOH 1:1 (86%); (g) CuSO4·5H2O, sodium ascorbate, DMF, H2O, 8 (6%).

Scheme 3. Synthesis of the MMPI 24a

“Reaction conditions: (a) NaBH(OAc)3, C2H4Cl2 (90%); (b) 4-methoxybenzene-1-sulfonyl chloride, pyridine (92%); (c) K2CO3, DMF, 7 (66%); (d) HCl (gas), CH2Cl2 (73%); (e) O-THP hydroxylamine, EDC, NMM, HOBT, DMF (92%); (f) 4 N HCl in dioxane, dioxane/MeOH 1:1 (86%); (g) CuSO4·5H2O, sodium ascorbate, DMF, H2O, 8 (6%).

The azido key intermediate 23 was previously synthesized according to a literature procedure published by our group starting from the sulfonamide 10 and the benzaldehyde 17 (Scheme 3). This synthetic approach was improved in this work because an alternative and shorter synthesis sequence utilizing the azidobenzaldehyde 17 instead of 19 as mini-PEG building block was developed (Scheme 3). Compared to the synthesis of 19, the preparation of 17 saved at least three reaction steps. Starting from the tert-butyl ester of D-valine 9 and the benzaldehyde 17, reductive amination with sodium triacetoxyborohydride in dichloroethane provided the N-benzylated D-valine tert-butyl ester 18. Substitution reaction with 4-methoxybenzene-1-sulfonyl chloride in pyridine at 60 °C gave the carboxylic acid ester 20. After acidic removal of the ester protective group, conversion of the corresponding carboxylic acid 21 into the THP-protected hydroxamic acid ester 22, and cleavage of the THP protecting group with HCl in dioxane, the key intermediate 23 was obtained in 48% overall yield. Copper(1) catalyzed click reaction with the mini-PEGylated fluoroalkyne 8 provided the double mini-PEGylated 1,2,3-triazole substituted hydroxamic acid 24.

Compared to the lead structures, hydroxamates 16a–c and 24 are characterized by the additionally introduced mini-PEG and 1,2,3-triazole units. An advanced approach for the modification of the pharmacokinetics of the lead structure deals with the replacement of ring B by a 1,2,3-triazole unit (Figure 1). The 1,2,3-triazole ring system is endowed with unique chemical and biological stability. In general 1,4-disubstituted 1,2,3-triazoles are putatively metabolically inert and contain H-bond acceptor sites capable of replacing the H-
The synthetic route to a variety of 1,2,3-triazole substituted and mini-PEGylated hydroxamic acid derivatives is depicted in Scheme 4.

N-Alkylation of the sulfonamide with propargyl bromide under basic conditions, acidic hydrolysis of the ester with gaseous hydrochloric acid, and conversion of the carboxylic acid with tert-butylhydroxylamine, EDC, HOBt, and NMM gave the hydroxamic acid ester. After the cleavage of the tert-butyl group with hydrochloric acid gas in dichloroethane the propargylic hydroxamic acid was obtained with a high overall yield of 44%. Copper(I) catalyzed click reactions of with different azidoalkyl and azido mini-PEGylated compounds.

Table 1. MMP Inhibition Potencies, clogD, and logD(exp) of Novel Hydroxamic Acids

<table>
<thead>
<tr>
<th>compd</th>
<th>MMP-2 (nM)</th>
<th>MMP-8 (nM)</th>
<th>MMP-9 (nM)</th>
<th>MMP-13 (nM)</th>
<th>clogD</th>
<th>logD(exp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3 ± 1</td>
<td>2 ± 0.4</td>
<td>6 ± 3</td>
<td>34 ± 12</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>11 ± 2</td>
<td>23 ± 4</td>
<td>27 ± 2</td>
<td></td>
<td>3.81</td>
<td></td>
</tr>
<tr>
<td>1c</td>
<td>4 ± 3</td>
<td>2 ± 1</td>
<td>50 ± 27</td>
<td>11 ± 0.3</td>
<td>4.03</td>
<td>2.02 ± 0.03</td>
</tr>
<tr>
<td>1d</td>
<td>0.35 ± 0.1</td>
<td>0.11 ± 0.01</td>
<td>0.37 ± 0.03</td>
<td>0.09 ± 0.03</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td>1e</td>
<td>2 ± 1</td>
<td>0.2 ± 0.08</td>
<td>0.6 ± 0.2</td>
<td>6 ± 3</td>
<td>2.09</td>
<td></td>
</tr>
<tr>
<td>1f</td>
<td>4 ± 0.5</td>
<td>5 ± 0.8</td>
<td>5 ± 1</td>
<td>4 ± 1</td>
<td>2.09</td>
<td></td>
</tr>
<tr>
<td>1g</td>
<td>20 ± 30</td>
<td>58 ± 6</td>
<td>14 ± 3</td>
<td>18 ± 2</td>
<td>2.09</td>
<td></td>
</tr>
<tr>
<td>1h</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
<td>3 ± 0.3</td>
<td>1 ± 0.2</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>1i</td>
<td>5 ± 0.5</td>
<td>14 ± 2</td>
<td>2 ± 0.4</td>
<td>10 ± 3</td>
<td>2.60</td>
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<tr>
<td>1j</td>
<td>107 ± 42</td>
<td>8 ± 0.7</td>
<td>7 ± 0.2</td>
<td>5 ± 0.02</td>
<td>2.58</td>
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<tr>
<td>1k</td>
<td>0.13 ± 0.07</td>
<td>0.02 ± 0.04</td>
<td>0.03 ± 0.003</td>
<td>0.006 ± 0.003</td>
<td>1.53</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td>1l</td>
<td>0.07 ± 0.03</td>
<td>0.03 ± 0.001</td>
<td>0.06 ± 0.02</td>
<td>0.02 ± 0.0002</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>1m</td>
<td>0.1 ± 0.03</td>
<td>0.04 ± 0.006</td>
<td>0.05 ± 0.007</td>
<td>0.04 ± 0.007</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>1n</td>
<td>1.2 ± 0.3</td>
<td>9 ± 1</td>
<td>0.9 ± 0.05</td>
<td>6 ± 1</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>1o</td>
<td>3 ± 0.3</td>
<td>5 ± 0.7</td>
<td>4 ± 0.9</td>
<td>1 ± 0.3</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>1p</td>
<td>0.5 ± 0.9</td>
<td>0.5 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>1q</td>
<td>&gt;100 μM</td>
<td>&gt;100 μM</td>
<td>&gt;100 μM</td>
<td>&gt;100 μM</td>
<td>1.76</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± SD of three experiments. b clogD values were calculated by ACD/Chemsketch, version ACD/Labs 6.00 (log D = log P at physiological pH 7.4). d log D value was determined for compound [18F]30b. e K values, where SDs are not denoted.
Scheme 5. Radiosynthesis of $^{18}$F-Labeled $[^{18}$F$]30b$\textsuperscript{a}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\includegraphics[width=0.8\textwidth]{Scheme_5.png}};
\end{tikzpicture}
\end{center}

\textsuperscript{a}Reaction conditions: (a) K(K$_2$CO$_3$)$[^{18}$F$]F$, K$_2$CO$_3$, CH$_3$CN, 110 °C, 100 s (58%); (b) CuSO$_4$:SH$_2$O, sodium ascorbate, DMF, rt, 10 min (55%).

were accomplished with varying yields (18–88%), resulting in a small series of 1,2,3-triazole containing hydrophilic hydroxamic acid derivatives 30a–g. The 1,2,3-triazoles 30a–g were prepared using copper(II) sulfate and sodium ascorbate in DMF. A second 1,3-dipolar cycloaddition of compound 30e with the fluoro-PEG alkyne 8 yielded the desired click reaction product, but purification was impossible because of decomposition of the target compound during the purification procedures.

In summary, a novel class of triazole and/or mini-PEG containing hydroxamate MMPIs were prepared from the alkyne and azido substituted key intermediates 14, 23, and 28 by 1,3-dipolar cycloaddition. This reaction strategy yielded fluorinated nonradioactive reference compounds of potential MMP radioligands 16a–c, 24, 30b, 30d, 30f, and 30g, whose MMP inhibition potencies can be measured by in vitro fluorogenic assays, as well as precursors for $^{18}$F-labeling 14, 23, 28, 30a, and 30c. Precursors 14, 23, and 28 can potentially be $^{18}$F-labeled via two-step procedures using radiosynths, i.e., $[^{18}$F$]15a$, $[^{18}$F$]15b$, or $[^{18}$F$]15c$ for the alkyne precursors 14 and 28 and $[^{18}$F$]8$ for the azido compound 23. Nucleophilic substitution of the tosylate precursors 30a and 30c with $[^{18}$F$]$fluoride can potentially yield the radiolabeled analogues $[^{18}$F$]30b$ and $[^{18}$F$]30d$ in one step.

In Vitro Enzyme Assays and clogD Values. The MMP inhibition potencies of the hydroxamic acids 14, 16a–c, 24, 28, and 30a–h against activated MMP-2, -8, -9, and -13 were measured by fluorogenic in vitro inhibition assays following the procedure previously described.\textsuperscript{58} The resulting IC$_{50}$ values for the investigated MMPs were compared to those of the parent compounds 1a, 1b, and 1e.

As displayed in Table 1, the new generation of mini-PEG and/or 1,2,3-triazole substituted hydroxamic acids revealed excellent MMP inhibition potencies with IC$_{50}$ values in the nanomolar to picomolar range (0.006–107 nM). In comparison to the potent MMPI 1e (IC$_{50}$ values of 2–50 nM) the inhibition potencies of 30b–g were significantly increased with IC$_{50}$ values in the picomolar to low nanomolar range (0.006–5 nM). The 1,2,3-triazole ring appears to play an important role regarding the enzyme binding potencies, and also its position in the molecule seems to be of significance. In particular, compounds 30b–d and 30g, with the 1,2,3-triazole moiety positioned close to the hydroxamate group that chelates the Zn$^{2+}$ ion of the enzyme active site in a bidentate manner, show considerably higher inhibition potencies (IC$_{50}$ = 0.006–0.6 nM) than compounds 16a–c and 24 with a benzyl polyethylene glycol spacer between the backbone of the molecule and the 1,2,3-triazole unit that still represent potent MMPIs (IC$_{50}$ = 0.2–58 nM).

The aforementioned excellent binding potencies for 30b–d and 30g could be due to additional attractive interactions between the hydrophilic triazole nitrogen atoms and the Zn$^{2+}$ ion of the enzyme active site or other functional groups in the enzyme. In contrast, compound 30h with two triazole substituted hydroxamates linked via a mini-PEG chain (side product of the reaction of 28 with 29c, formal click product of 30e and 28, structure not shown in Scheme 4) was completely inactive in the in vitro assay.

The lead structures based on 1a–b and 1e were modified by the introduction of hydrophilic groups such as mini-PEG units and 1,2,3-triazole moieties in combination with fluorinated building blocks. Therefore, an evaluation of the hydrophilic properties of the target compounds is required. Table 1 also displays the calculated log D values (clogD) of the synthesized hydroxamic acids to indicate the changes of the lipophilicities caused by the structural modifications of the lead compounds 1a,b and the fluorinated analogue 1e. Compared to 1e (clogD = 4.03), the new polyethylene glycol and/or 1,2,3-triazole substituted hydroxamic acid derivatives (clogD = 0.58–2.86) show a considerably increased hydrophilicity, as desired.

Additionally, the log D value of the radiofluorinated analogue $[^{18}$F$]30b$ (see section Radiochemistry) was determined experimentally (logD(exp) = 0.60 ± 0.01). The logD(exp) differs from the clogD (clogD(30b) = 1.53) by 1 unit. Compared to the radiofluorinated analogue $[^{18}$F$]1e$ (logD(exp) = 2.02 ± 0.03), the triazole substituted hydroxamic acid $[^{18}$F$]30b$ is approximately 26 times more hydrophilic.

The new MMPI class of mini-PEG and/or 1,2,3-triazole substituted hydroxamates is characterized by at least one major improvement. All new MMPis showed an increased hydrophilicity compared to the lead structure 1b. Furthermore backbone triazole substituted MMPIs represented by compounds 30b–d and 30g turned out to be the most potent MMPI class developed by our group so far. These results encouraged us to radiosynthesize a representative $^{18}$F-labeled isotope for further in vitro and initial in vivo evaluation.

Radiochemistry. Because of the excellent MMPI potency and high hydrophilicity of compound 30b, this derivative was chosen for the radiosynthesis of its $^{18}$F-labeled analogue $[^{18}$F$]30b$. For this purpose a semiautomated two-step procedure was developed and optimized (Scheme 5). The preparation of the $[^{18}$F$]$fluoroethyl-1,2,3-triazole substituted inhibitor $[^{18}$F$]30b$ consisted of the nucleophilic radiofluorination of 2-azidoethyl-4-methylbenzenesulfonylate and subsequent copper(I) catalyzed cycloaddition with the alkyne precursor 28.
According to a procedure previously described by Glaser and Åstad, the radiosynthesis of 1-azido-2-[\(^{18}\text{F}\)]fluoroethane (\([^{18}\text{F}]15\text{a}\)) was achieved.\(^{31}\) An improvement of the literature procedure could be realized by direct separation of the immediately formed \([^{18}\text{F}]15\text{a}\) from the reaction mixture by distillation. By this we gained a savings of time of at least 15 min compared to the literature procedure. 1-Azido-2-[\(^{18}\text{F}\)]fluoroethane could be isolated after 20 min in an average radiochemical yield of 58 ± 4% (decay corrected, \(n = 6\)). The click labeling was performed outside the automated radiosynthesizer. The optimal reaction conditions proved to be a reaction time of 10 min under stirring at room temperature, using aqueous solutions of copper(II) sulfate pentahydrate (1.4 equiv relative to the alkyne precursor 28) and sodium ascorbate (1.8 equiv relative to 28). This reaction step successfully provided the 1,2,3-triazole \([^{18}\text{F}]30\text{b}\) in an average radiochemical yield of 55 ± 7% (decay corrected, \(n = 5\)). After purification by semipreparative HPLC and concentration by rotary evaporation and formulation, the two-step radiosynthesis of \([^{18}\text{F}]30\text{b}\) was accomplished with an overall radiochemical yield of 30 ± 3% (decay corrected, \(n = 5\)) in 110 ± 10 min. \([^{18}\text{F}]30\text{b}\) was isolated in radiochemical purities of >98% with specific activities in the range of 14–57 GBq/\(\mu\)mol at the end of the synthesis. The radioligand was formulated in phosphate-buffered saline (PBS) to determine the log \(D\) values and to study its in vitro stability in human serum at 37 °C.

**In Vitro Stability.** By use of human blood serum, an in vitro stability study was carried out. During long-term incubation for up to 120 min at 37 °C, \([^{18}\text{F}]30\text{b}\) revealed excellent stability. As shown in Figure 2, only the parent compound \([^{18}\text{F}]30\text{b}\) was observed by radio-HPLC. Significant decomposition products or radiometabolites could not be detected. Because of the fact that first in vivo PET experiments were carried out with mice, the blood serum stability measurement of \([^{18}\text{F}]30\text{b}\) was additionally performed in mouse blood serum. Likewise, no significant decomposition of products or radiometabolites was detected (see Supporting Information).

**In Vivo Biodistribution Study.** Representative coronal whole body images 0–1, 1–5, 5–10, and 90–120 min after tracer injection in C57/BL6 mice are shown in Figure 3.

Overall, \([^{18}\text{F}]30\text{b}\) is cleared quickly and efficiently from the body through hepatic and renal elimination with no significant tracer remaining in nonexcretion organs 90–120 min pi. Immediately upon injection of \([^{18}\text{F}]30\text{b}\) high levels of radioactivity were observed in the liver and the kidneys. While the activity in the kidney decreased \((T_{\text{max}} = 30\text{ s}, T_{1/2} = 13\text{ min pi})\) in parallel to the activity in the blood, the liver first showed a further accumulation of \([^{18}\text{F}]30\text{b}\) \((T_{\text{max}} = 4\text{ min}, T_{1/2} = 18\text{ min pi})\) before clearance into the gallbladder and finally into the intestine (Figure 4).

Defluorination of the radioligand in vivo potentially impairing image interpretation (indicated by bone uptake of \([^{18}\text{F}]\)fluoride ions) was not observed in the entire dynamic imaging study. Furthermore, accumulation of \([^{18}\text{F}]30\text{b}\) in organs/tissues such as the brain, myocardium, lung, and muscles, indicating unspecific binding, was not observed.

Interestingly, in comparison to \([^{18}\text{F}]1\text{e}\), compound \([^{18}\text{F}]30\text{b}\) does not show any significant difference in the biodistribution characteristics. The overall shapes of the time–activity curves of \([^{18}\text{F}]30\text{b}\) reveal similar radiotracer dynamics as observed in studies using \([^{18}\text{F}]1\text{e}\) (see Figure 4). Only the clearance of \([^{18}\text{F}]30\text{b}\) from kidneys and liver is slightly delayed compared to \([^{18}\text{F}]1\text{e}\) (for kidney, \(T_{\text{max}} = 1.5\text{ min}\) and \(T_{1/2} = 3\text{ min pi}\); for liver, \(T_{\text{max}} = 2.5\text{ min}\) and \(T_{1/2} = 7\text{ min pi}\)).
Obviously, the structural modification of $[^{18}\text{F}]\text{Ie}$ directed toward a higher hydrophilicity via the substitution of ring B by a triazole moiety in variant $[^{18}\text{F}]\text{30b}$ did not significantly influence the biodistribution behavior. In future experiments radiolabeled triazole-substituted hydroxamates with an additional hydrophilic subunit (e.g., $[^{18}\text{F}]\text{30d}$ with a mini-PEG chain) will be investigated to examine the influence of an additional shift to higher tracer hydrophilicity on the biodistribution characteristics. Potentially, by the additional introduction of a mini-PEG chain, this structural modification would lead to a radiotracer with the desired increased renal clearance characteristics.

**Biostability and Metabolism of $[^{18}\text{F}]\text{30b}$**. Three 10-month-old female ICR (CD1) wild type mice were examined at 30 min pi to determine the biostability of $[^{18}\text{F}]\text{30b}$. Representative radio-HPLC traces are shown in Figure 5.

The % ID/g values of the total organs are listed in Table 2 for 30 min time points. The retention time of unchanged $[^{18}\text{F}]\text{30b}$ tracer was between 14.1 and 14.3 min. A total of four metabolites were detected, with retention times of 3.8 min (metabolite 1), 11.1 min (metabolite 2), 13.1 min (metabolite 3), and 16.1 min (metabolite 4).

The muscle % ID/g of $[^{18}\text{F}]\text{30b}$ at 30 min was 0.21% with no other metabolites present. In plasma, at 30 min, the radioactivity was partitioned between 63% (0.29% ID/g) of the parent tracer and 37% (0.17% ID/g) of metabolite 1. No other metabolites were found in plasma. In the liver sample at 30 min, 26% parent (0.78% ID/g) was present while the remaining 74% of activity was distributed between three metabolites, 4% metabolite 1 (0.12% ID/g), 44% metabolite 3 (1.32% ID/g), and 26% metabolite 4 (0.78% ID/g). In the kidney homogenate, the parent tracer was present at higher amounts compared to other tissues at 89% (4.6% ID/g) along with three metabolites: 5% metabolite 1 (0.26% ID/g), 44% metabolite 3 (0.21% ID/g), 2% metabolite 4 (0.1% ID/g). The tracer and the metabolites are predominantly cleared through the kidneys and through urine.

As shown in Table 2, although $[^{18}\text{F}]\text{30b}$ metabolizes to about 37% to a more polar compound in vivo in mice, in plasma no other metabolites were detected. It appears that the tracer clears through urine via kidneys. Though there is some activity remaining in muscle, this might be due to the experimental limitation to exclude blood from muscle.
CONCLUSION

On the basis of our previous results with radiolabeled and unlabeled derivatives of the lead MMPIs 1a and 1b, the synthesis and in vitro characterization of a new MMPI series of fluorinated triazole-substituted hydroxamates are described. The novel compounds possess moderate to high hydrophilicities with clogD values ranging from 0.58 to 2.86 and represent potent inhibitors of MMP-2, -8, -9, and -13 with IC₅₀ values between 0.006 and 107 nM. In particular, inhibitors 30b−d and 30g that are characterized by the substitution of the phenyl or pyridyl group by a triazol unit at the ring B position (compare Figure 1) displayed excellent inhibition potencies with IC₅₀ values in the picomolar range (0.006−0.6 nM). Compounds 30b−d and 30g turned out to be the most potent MMPI class developed so far by our group. The radiosynthesis of the ¹⁸F-labeled counterpart ¹⁸F]30b of the promising derivative 30b was successfully realized in a two-step procedure with an overall radiochemical yield of 30 ± 3% (decay corrected). The radiofluorinated triazole-substituted hydroxamate MMPI ¹⁸F]30b showed an excellent serum stability in vitro and a rapid clearance, as shown by in vivo biodistribution studies in wild type mice. Furthermore, undesired unspecific binding of the radiotracer in nonexcretion organs was not observed, and compared to the more lipophilic analogue ¹⁸F]1e, compound ¹⁸F]30b does not possess any significant difference concerning its biodistribution pattern. In any case ¹⁸F]30b exhibits a promising MMP-targeted radiotracer for the noninvasive PET imaging of activated MMPs in vivo. In future steps compound ¹⁸F]30b will be evaluated in preclinical PET/CT studies using murine disease models that are characterized by up-regulated levels of activated MMPs (e.g., for atherosclerotic plaques, apolipoprotein E-deficient mice; for tumor, Lewis lung carcinoma bearing mice). In parallel, the radiosynthesis of ¹⁸F]30d will be established to examine the influence of an additional shift to higher tracer hydrophilicity in vivo.

EXPERIMENTAL SECTION

General. All chemicals, reagents, and solvents for the synthesis of the compounds were analytical grade, purchased from commercial sources, and used without further purification unless otherwise specified. All air- and moisture-sensitive reactions were performed under argon atmosphere. Solvents were purified and dried by literature methods where necessary. The melting points are uncorrected and were determined in capillary tubes on a Stuart Scientific SMP3.

Table 2. % ID/g of the Metabolites of ¹⁸F]30b in Muscle, Kidney, Liver, Plasma, and Urine

<table>
<thead>
<tr>
<th>metabolite</th>
<th>muscle</th>
<th>kidney</th>
<th>liver</th>
<th>plasma</th>
<th>urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁸F]30b: t_R ≈ 14.2 min</td>
<td>0.21</td>
<td>4.60</td>
<td>0.78</td>
<td>0.29</td>
<td>27.86</td>
</tr>
<tr>
<td>metabolite 1: t_R ≈ 3.8 min</td>
<td>0.00</td>
<td>0.26</td>
<td>0.12</td>
<td>0.17</td>
<td>2.32</td>
</tr>
<tr>
<td>metabolite 2: t_R ≈ 11.1 min</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td>metabolite 3: t_R ≈ 13.1 min</td>
<td>0.00</td>
<td>0.21</td>
<td>1.32</td>
<td>0.00</td>
<td>2.32</td>
</tr>
<tr>
<td>metabolite 4: t_R ≈ 16.1 min</td>
<td>0.00</td>
<td>0.10</td>
<td>0.78</td>
<td>0.00</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Figure 5. Representative radio-HPLC traces for 30 min pi of the metabolism study of ¹⁸F]30b. The radiochemical purity of ¹⁸F]30b was >98% before injection. The samples were analyzed by HPLC, using a γ-detector (Raytest GmbH/Agilent). The HPLC was done on a Phenomenex C18 column (250 mm × 4.6 mm) using a gradient method with acetonitrile and water (both having 0.05% TFA).
capillary melting point apparatus. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Thin layer chromatography (TLC) was carried out on silica gel coated polyester backed TLC plates (Polygram, SIL G/UV254 Macherey-Nagel) using solvent mixtures of cyclohexane (CH), ethyl acetate (EA), and methanol (MeOH). Compounds were visualized under UV light (254, 365 nm). NMR spectra were recorded in CDCl3, CD3OD, or DMSO-d6 on Bruker ARX300, Bruker DPX300 (293 MHz, 300 MHz, 13C NMR, 75 MHz), 19F NMR, 282 MHz, 2 bum) a Bruker AMX 400 (1H NMR, 400 MHz, 13C NMR, 100 MHz), and Varian Unity Plus 600 (1H NMR, 600 MHz; 13C NMR, 151 MHz) spectrometers. TMS (δ 0), CDCl3, DMSO-d6, CD3OD (δ 3.31), and CFCl3 (δ 7.17) were used as internal standards, and all chemical shifts were recorded in ppm (δ). Exact mass analyses were conducted on a Bruker MicroToF apparatus. The chemical purities of each new nonradioactive compound were ≥95% and assessed by analytical gradient reversed-phase HPLC system A or B (λ = 254 nm). HPLC system A consisted of two Smartline 1000 pumps and a Smartline UV detector 2500 (Herbert Knauer GmbH), a GabiStar γ-detecter (Raytest Isotopenmessgeräte GmbH), and a Nucleosil 100-5 C-18 column (250 mm × 4.6 mm). The recorded data were processed by the GINA Star software (Raytest Isotopenmessgeräte GmbH). The HPLC method A1 started with a linear gradient from 10% to 90% CH3CN in water (0.1% TFA) over 9 min, followed by a linear gradient from 90% to 100% CH3CN in water (0.1% TFA) over 6 min, with a flow rate of 1 mL min–1 (unless otherwise specified). HPLC method A2 started with 10% CH3CN in water (0.1% TFA) for 15 min, followed by a linear gradient from 30% to 90% CH3CN in water (0.1% TFA) over 3 min, followed by a linear gradient from 90% to 100% CH3CN in water (0.1% TFA) over 2 min with a flow rate of 1 mL min–1. HPLC system B consisted of two K-1800 pumps and an S-2500 UV detector (Herbert Knauer GmbH) and a GabiStar γ-detecter (Raytest Isotopenmessgeräte GmbH). The recorded data were processed by the ChromGate HPLC software (Herbert Knauer GmbH). HPLC method B1 using a Nucleosil 100-5 C18 column (250 mm × 4.6 mm) started with a linear gradient from 10% to 80% CH3CN in water (0.1% TFA) over 18 min, holding for 20 min and followed by a linear gradient from 80% to 100% CH3CN in water (0.1% TFA) over 2 min, with a flow rate of 1.5 mL min–1. HPLC method B2 using an ACE 5 AQ column (250 mm × 10 mm) started with a linear gradient from 10% to 80% CH3CN in water (0.1% TFA) over 18 min, holding for 20 min and followed by a linear gradient from 80% to 100% CH3CN in water (0.1% TFA) over 2 min, with a flow rate of 1.5 mL min–1. HPLC method A1: tR = 7.32 min (δ 417.18 ppm, 100%).

General Procedure for the Preparation of Triazoles 16a–c, 24, and 30a–h. To a solution of the alkyne compound (0.032–2.00 mmol, 1.0 equiv) in DMF (8 mL/1 mmol) and H2O (2 mL/mmol) were added CuSO4·5H2O (50 mol %), sodium ascorbate (60 mol %), and the corresponding azide (0.032–2.00 mmol, 1.0–1.2 equiv) in sequence. After being stirred at room temperature, the reaction mixture was diluted with water and extracted with dichloromethane (3 × 200 mL). The combined organic phases were washed with brine, dried over magnesium sulfate, and concentrated to give 27 as a colorless wax (17.4 g, 43.80 mmol, 88%). [2]3/4 + 107.8 (δ 0.98, CHCl3). MS-ES-EM m/z = 419.1614 [(M + Na)+] calcd for C19H28N2O5SNa+: 419.1611.

2-(4-Methoxyphenylsulfonamido-N-(prop-2-yn-1-yl))-3-methylbutanoic Acid (26). A stirred solution of the carboxylic acid ester 25 (22.0 g, 57.67 mmol) in dichloromethane (100 mL) was cooled to 0 °C. Hydrochloric acid gas was bubbled through the solution. TLC was used to monitor the reaction progress (EA). After complete conversion the solvent was removed under reduced pressure to give 26 as a colorless wax (18.6 g, 57.23 mmol, 99%). MS-ES-EM m/z = 348.0877 [(M + Na)+] calcd for C21H22N2O5SNa+: 348.0876.
(R)-2-[(1-2-18F)Fluoroethyl]-1H-1,2,3-triazol-4-yl-methyl-4-methylisoxazolylsulfonamido)-N-hydroxy-3-methylbutanamide ([18F]30b). In a computer controlled TRACERlab FaPEGc, synthesizer aqueous [18F]fluoride ions (0.1–5.5 GBq) from the cyclotron target were passed through an anion exchange resin (Sep-Pak Light Waters Accell Plus QMA cartridge, preconditioned with 5 mL of 1 M K₂CO₃, and 10 mL of water for injection). [18F]Fluoride ions were eluted from the resin with a mixture of 40 μL of 1 M K₂CO₃, 200 μL of water for injection, and 800 μL of DNA-grade CH₃CN containing 18 mg (48 μmol) of Kryptofix 2.2.2 (K222) in the reactor. Subsequently, the aqueous K(K222) [18F]F solution was carefully evaporated to dryness in vacuo. An amount of 12 μL (14 mg, 60 μmol) of precursor 2-azidoethyl 4-methylbenzenesulfonate was added to the reactor in a 5 mL flask that contained 400 μL of dry DMF and was mixed and heated at 110 °C for 100 s. Meanwhile, the labeled product [18F]15a was distilled from the reactor and killed to ~10 °C. The radiosynthetic [18F]15a was collected with a radiochemical yield of 58 ± 4% in 20 min (80 ± 5% SD, decay corrected, n = 6). Then 60 μL of 0.4 M CuSO₄·H₂O, 40 μL of water, and 6 mg (30 μmol) of sodium carbonate in 100 μL of water, and 6 mg (17.6 μmol) of alkyne 28 in 100 μL of DMF were added. After 10 min at ambient temperature, the mixture was passed through a Waters Sep-Pak Light cartridge filled with quartz wool. The cartridge was rinsed with DMF (0.2 M). The eluate was diluted with 500 μL of water, and the resulting mixture was purified by gradient-radio-HPLC system C flow = 5.5 mL/min; eluents, A, CH₃CN/TFA, 1000/1, B, H₂O/TFA, 1000/1, isocratic, A/B 18/82 (v/v); λ = 254 nm; column, ACE 5 AQ (250 mm × 10 mm)). The product fraction of compound [18F]30b (retention time t₁/₂([18F]30b) = 37 min) was evaporated to dryness in vacuo and redissolved in 1 mL of 0.9% NaCl/EtOH (9/1 v/v). Product compound [18F]30b was obtained in an overall radiochemical yield of 30 ± 3% (decay-corrected, based on cyclopropane-derived [18F]fluoride ions, n = 5) in ≥10 min from the end of radionuclide production. [18F]30b was isolated in radioactive purities of >98% with specific activities in the range of 14–57 GBq/μmol at the end of the synthesis.

In Vitro Enzyme Inhibition Assays (Table 1). The inhibition potencies of hydroxamic acid derivatives 14, 16a–c, 24, 28, and 30a–h against activated MMP-2, -8, -9, and -13 were assayed using the synthetic fluorogenic substrate (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[(2,4-dinitrophenyl)oxy]-2,3-diaminopropionyl)Ala-Arg-NH₂ (R&D Systems) as described previously. Briefly, MMP-2, -8, -9, or -13 (each at 2 nM) and test compounds at varying concentrations (10 pM to 1 mM) in Tris-HCl (50 mM), pH 7.5, containing NaCl (0.2 M), CaCl₂ (5 mM), ZnSO₄ (20 μM), and 0.05% Brij 35 were preincubated at 37 °C for 30 min. An aliquot of substrate (10 μL of a 50 μM solution) was added to the enzyme–inhibitor mixture (90 μL), and the fluorescence changes were monitored using a Fusion Universal microplate analyzer (Packard Bioscience) with excitation and emission wavelengths of 330 and 390 nm, respectively. Reaction rates were measured from the initial 10 min and plotted as a function of inhibitor concentration. From the resulting inhibition curves, the IC₅₀ values were calculated by nonlinear regression analysis using the Grace 5.1.8 software (Linux).

Determination of the Partition Coefficient (log D₂₀). The lipophilicity of radioligand [18F]30b was assessed by determination of the water–octanol partition coefficient following a published procedure. In brief, an amount of approximately 20 kBq [18F]30b was mixed with equal amounts (0.5 mL) of PBS (pH 7.4) and 1-octanol, and the resulting biphasic system was mixed vigorously for 1 min at room temperature. The tubes were centrifuged (3000 rpm, 2 min), and three samples of 100 μL of each layer were counted in a γ counter (Wallac Wizard, Perkin-Elmer Life Sciences). The partition coefficient was determined by calibrating the ratio cpm(octanol)/cpm(PBS) and expressed as log D₂₀ (log(cpm_octanol/ cpm_PBS)). Two independent experiments were performed in triplicate, and data were provided as mean values ± standard deviation.

Stability in Human Serum. The serum stability of radioligand [18F]30b was evaluated by incubation in human serum at 37 °C for up to 120 min. An aliquot of the PBS-formulated 18F-labeled compound (20 μL, 5 MBq) was added to a sample of human serum (200 μL), and the mixture was incubated at 37 °C. Samples of 20 μL each were taken after periods of 10, 20, 30, 60, 90, and 120 min and quenched in methanol/CH₃Cl (1:1 v/v), 100 μL following centrifugation for 2 min. The organic layer was analyzed by analytical radio-HPLC (tᵣ = 8.70 min; analytical HPLC system A, method A2, starting with 30% CH₃CN in water (0.1% TFA) for 15 min, followed by a linear gradient from 30% to 90% CH₃CN in water (0.1% TFA) over 3 min, followed by a linear gradient from 90% to 30% CH₃CN in water (0.1% TFA) over 2 min with a flow rate of 1 mL/min).

Biostability and Metabolism Study. Approximately 11.1 MBq [18F]30b (in a maximum volume of 200 μL) was injected into three mice via tail vein injection. The animals were sacrificed at 30 min pi. Whole blood was obtained, weighed, and centrifuged at 3000 rpm (3 min) to isolate plasma. Urine was also collected. The muscle, kidneys, and liver were harvested, weighed, and homogenized in lysis buffer (1% SDS in PBS buffer). An aliquot of each sample (400 μL) was subsequently removed, mixed with 400 μL of acetonitrile and 100 μL of 3% acetic acid in acetonitrile, vigorously mixed, and placed on dry ice for 3 min. After thawing, the samples were centrifuged at 13000 rpm (8 min) to allow for the separation of supernatant from the pellet. The supernatant was then removed and assayed for radioactivity in a PerkinElmer Wizard γ-counter (20 s). A 2 μL aliquot from the dose sample was counted along with the samples and was used to calculate the % ID/g. The samples were analyzed by HPLC, using a γ-detector (Raytest GmbH/Aglent). HPLC was done on a Phenomenex C18 column (250 mm × 4.6 mm) using a gradient method with acetonitrile and water (both having 0.05% TFA).

Animals. Adult C57/B16 mice (male, 21–24 g) were anesthetized by isoflurane/O₂, and one lateral tail vein was cannulated using a 27 Ga needle connected to 15 cm polyethylene catheter tubing. [18F]1e or [18F]30b (250 kBq/g bodyweight) was injected as a bolus (100 μL of compound flushed with 100 μL of saline) via the tail vein, and subsequent PET scanning was performed. Experiments were conducted according to Canadian animal welfare guidelines.

Small Animal PET Scanning. PET experiments were carried out using a small-animal high-resolution (0.7 mm full width at half-maximum) small animal scanner (32 module quad-HIDAC, Oxford Positron Systems Ltd, Oxford, U.K.) with uniform spatial resolution (<1 mm) over a large cylindrical field (165 mm diameter, 280 mm axial length). List-mode data were acquired for 120 min and reconstructed into dynamic time frames using an iterative reconstruction algorithm. Subsequently, the scanning bed was transferred to the computed tomography (CT) scanner (Inveon, Siemens Medical Solutions, U.S.) and a CT acquisition with a spatial resolution of 80 μm was performed for each mouse. Reconstructed image data sets were co-registered based on extrinsic markers attached to the multimodal scanning bed for each mouse. Reconstructed image data sets were transferred to the co-registered PET data, and analyzed quantitatively. Regional uptake was calculated as percentage of injected dose by dividing counts per milliliter in the VOI by total counts in the mouse multiplied by 100 (% ID/mL).
Experimental procedures and analytical data for compounds 1−8, 10−14, 18, 20−23 and NMR data of 25−28. This material is available free of charge via the Internet at http://pubs.acs.org.

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Affiliations

1 Both authors contributed equally to this work and share senior authorship.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ADAM, a disintegrin and metalloprotease; ADAMT, a disintegrin and metalloproteinase with a thrombospondin motif; BM, basement membrane; CH, cyclohexane; ECM, extracellular matrix; EDTC, N'-ethyl-N'- (3-dimethylaminopropyl) carbodiimide hydrochloride; EA, ethyl acetate; EM, exact mass; FMT, fluorescence mediated tomography; FRIL, fluorescence reflectance imaging; GMP, good manufacturing practices; HOBT, 1-hydroxybenzotriazole; ICR, imprinting control region; K222, Kryptofix 2.2.2; log D, log P at physiological pH 7.4; MMP, matrix metalloprotease; MMPi, matrix metalloprotease inhibitor; NMM, 4-methylmorpholine; PEG, polyethylene glycol; PET, positron emission tomography; pi, postinjection; ROI, region of interest; % ID, percentage injected dose; SD, standard deviation; SPECT, single photon emission computed tomography; THP, tetraydropryranyl; TIMP, tissue inhibitor of metalloprotease

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