

Review

Molecular Imaging Probes Based on Matrix Metalloproteinase Inhibitors (MMPIs)

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Abstract: Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases which are secreted or anchored in the cell membrane and are capable of degrading the multiple components of the extracellular matrix (ECM). MMPs are frequently overexpressed or highly activated in numerous human diseases. Owing to the important role of MMPs in human diseases, many MMP inhibitors (MMPIs) have been developed as novel therapeutics, and some of them have entered clinical trials. However, so far, only one MMPI (doxycycline) has been approved by the FDA. Therefore, the evaluation of the activity of a specific subset of MMPs in human diseases using clinically relevant imaging techniques would be a powerful tool for the early diagnosis and assessment of the efficacy of therapy. In recent years, numerous MMPIs labeled imaging agents have emerged. This article begins by providing an overview of the MMP subfamily and its structure and function. The latest advances in the design of subtype selective MMPIs and their biological evaluation are then summarized. Subsequently, the potential use of MMPI-labeled diagnostic agents in clinical imaging techniques are discussed, including positron emission tomography (PET), single-photon emission computed tomography (SPECT) and optical imaging (OI). Finally, this article concludes with future perspectives and clinical utility.

Keywords: Matrix metalloproteinases (MMPs); positron emission tomography (PET); single-photon emission computed tomography (SPECT) and optical imaging (OI)

1. Introduction

Molecular imaging is a rapidly emerging multidisciplinary field that can visualize physiological or pathological processes inside the body at the cellular or molecular level. Molecular imaging plays an important role in noninvasive earlier diagnosis, the accurate detection of diseases or dysfunctions, treatment follow-ups, personalized treatments, and it is also useful in drug development and discovery processes [1]. There are several modalities available for clinical imaging purposes, including positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), X-ray computed tomography imaging (CT), optical imaging (OI) by either bioluminescence or fluorescence, and ultrasound (US). Each imaging modality has advantages and disadvantages in aspects, such as spatial resolution, penetration depth and sensitivity which are collected in Table 1 [2]. In recent years, many efforts have been made to improve the imaging instruments and advanced image reconstruction techniques to obtain high resolution images that disclose even tiny lesions and gather accurate quantification of pathological processes. Both academia and the pharmaceutical industry are making a great effort to develop novel molecular imaging probes. The ideal molecular imaging probe must bind to its target with enough affinity, have optimal circulation time inside the body, rapidly

clear from the tissues and present high metabolic stability. There are numerous molecular imaging agents available both for preclinical and clinical applications, based on small molecules, peptides, affibodies, aptamers, antibodies and nanoparticles [2]. Among these agents, small molecule-based probes remain promising candidates for clinical applications, because they have favorable pharmacokinetic and pharmacodynamic profiles, generally have short circulation times, and are rapidly cleared from the body, thereby reducing the possibility of off-target toxicity. The design and development of molecular imaging probes are unlikely to be successful unless the molecular target is well defined, and its biological role is well characterized. According to this requirement, matrix metalloproteinases (MMPs) are highly validated molecular targets for the development of both therapeutics and diagnostic agents [3–6]. MMPs are frequently found to be overexpressed or highly activated in numerous human diseases, including cancer, atherosclerosis, stroke, arthritis, cardiovascular diseases, periodontal disease, respiratory tract disorders, glomerulonephritis, abdominal aortic aneurysm expansion, inflammatory bowel disease, neurodegeneration, chronic obstructive pulmonary disease, multiple sclerosis and liver fibrosis [7–11]. MMPs represent valuable targets for the development of new therapeutic and diagnostic tools. Owing to the important role of MMPs in many human diseases, several MMP inhibitors (MMPIs) have been developed as novel therapeutics and a few of them namely, Marimastat, BB-1101, Rebimastat, Prinomastat, RO-32-3555, Ilomastat, CGS-27023A, Batimastat, Tanomastat, among others have entered in advanced stages of clinical trials. However, many MMPIs have failed in the last phases of clinical trials mainly due to musculoskeletal toxicity, joint pain and swelling, as well as bone marrow suppression and venous thromboembolism. These side-effects could be due to the high concentrations of inhibitors required for the therapy [3–6]. However, they may be useful for the development of imaging probes because even low concentrations of tracers would be enough to image the activated MMPs in preclinical and clinical use (e.g., in a preclinical model).

Table 1. A comparison of various imaging modalities available for clinical imaging purposes.

	PET	SPECT	MRI	CT	US	OI
Spatial Resolution	1–2 mm	1–2 mm	25–100 μ m	50–200 μ m	50–500 μ m	2–3 mm
Penetration Depth	limitless	limitless	limitless	limitless	mm to cm	cm
Time Resolution	sec–min	min	min–hr.	min	sec–min	sec–min
Principle	γ -rays	γ -rays	Radio waves	x-rays	High frequency Sound waves	Visible and Near infrared light
Sensitivity	high	high	poor	high	poor	poor
Probe Quantity	ng	ng	μ g–mg	-	μ g–mg	μ g–mg
Probes	^{18}F , ^{11}C , ^{68}Ga , ^{64}Cu	$^{99\text{m}}\text{Tc}$, ^{123}I , ^{125}I , ^{111}In , ^{177}Lu	Gd, SPIO, Fe_3O_4 , MnO	I_2 , Ba, Xe, Kr	microbubbles	Fluorescent dye and NIR dyes
Risk	Ionizing Radiation	Ionizing Radiation	No radiation	Ionizing Radiation	No radiation	No radiation

Hence, radio-/fluorophore- labeled small molecule MMPIs could be useful for the detection of activated MMPs by means of molecular imaging techniques, such as SPECT, PET and OI. In recent years, numerous labelled MMPIs imaging agents have emerged. This review summarizes the MMP-based imaging probes for diseases listed above. The labelled MMPIs imaging agents based on the specific disease application and on their structure have been organized. An intensive broad discussion has been made on the synthetic methods including the yield, purity, overall synthesis time and specific activity of the probes, in vitro MMP isoform selective binding affinity, in vitro imaging, in vivo biodistribution, metabolic stability assessment, and in vivo preclinical to human clinical imaging applications. This review specifically covers the development of small-molecule PET, SPECT and OI probes but does not include

MRI imaging probes, which have been reviewed elsewhere [3–6]. The experimental details on the radiosynthesis of MMPi in Table 2 have been summarized. This review does not cover the MMP activatable probes. This work widens and updates previous reviews [6] reported in the literature which focused only on one disease [4,5] or one enzyme [3].

Table 2. The experimental details in the radiosynthesis of matrix metalloproteinases inhibitors (MMPi) based imaging probes.

Probe	Probe Type	Total Synthesis Time	Radio Chemical Yield	Radio Chemical Purity	Activity at the End of the Synthesis	log P/log D	In Vitro Assay Results (Cold or Hot Compound)
1	¹¹ C	20–25 min	40–60%	-	-	-	-
2	¹⁸ F						
3	¹¹ C						
4–8	¹¹ C	20–25 min	40–60%	92–98%	0.6–0.8 Ci/μmol	-	-
9	¹¹ C	-	20–30%	>99%	0.6–0.8 Ci/μmol	-	-
10	¹¹ C		15–25%	>95%	0.6–0.8 Ci/μmol		
11	¹²³ I	-	6.1%	>95%	237.3 Ci/μmol	-	Unlabelled Inhibitor MMP-2 = 2.5 nM MMP-9 = 4.6 nM
12	¹²⁵ I		22.8%	>95%	2.1 Ci/μmol		
13	¹²³ I		41.7%	>95%	237.2 Ci/μmol		
14	¹²⁵ I		65.9%	>95%	2.2 Ci/μmol		
15	¹⁸ F	113 min	12.4%	>97%	32–72 GBq/μmol	clog P = 4.04 clog D = 4.03	MMP-2 = 4 nM MMP-8 = 2 nM MMP-9 = 50 nM MMP-13 = 11 nM
16	¹⁸ F	115 min	45.6%	>95.5%	42–97 GBq/μmol	clog P = 2.54 clog D = 2.53	MMP-2 = 4 nM MMP-8 = 2 nM MMP-9 = 50 nM MMP-13 = 11 nM
17	¹⁸ F	160–180 min	12.1%	98.8%	39 GBq/μmol	-	MMP-2 = 8 nM MMP-8 = 0.9 nM MMP-9 = 0.5 nM MMP-13 = 0.9 nM
18	¹⁸ F	95–11 min	31–46%	98%	3.5 GBq/μmol	18-(R) clog D = 2.23	18-(R) MMP-2 = 10.4 nM MMP-9 = 0.5 nM
						18-(S) log D = 1.16	18-(S) MMP-2 = 0.3 nM MMP-9 = 0.1 nM
19	¹⁸ F	110–120 min	30%	98%	14–57 GBq/μmol	log D = 0.60 clog D = 1.53	MMP-2 = 0.13 nM MMP-8 = 0.02 nM MMP-9 = 0.03 nM MMP-13 = 0.006 nM
20	¹⁸ F	97 min	47%	98%	9–46 GBq/μmol	log D = 2.25 clog D = 1.25	MMP-2 = 0.7 nM MMP-8 = 0.4 nM MMP-9 = 0.07 nM MMP-13 = 0.05 nM
26	¹¹ C	15–20 min	40–55%	>99%	0.6–0.8 Ci/μmol	-	-
27	¹²³ I	-	55–65%	>98%	> 50 Ci/μmol	-	Unlabelled Inhibitor MMP-2 = 9.3 nM MMP-9 = 201 nM
28	¹²³ I		65–75%	>98%	> 58 Ci/μmol		
29	¹²³ I		65–75%	>98%	> 50 Ci/μmol		
30	¹²³ I	-	55–65%	>98%	> 58 Ci/μmol	-	Unlabelled Inhibitor MMP-2 = 23 nM MMP-9 = 429 nM MMP-2 = 48 nM MMP-9 = 740 nM
31–37	¹¹ C	20–25 min	40–60%	>95%	0.6–0.8 Ci/μmol	-	Parent inhibitor MMP-1 = 1.5 μM MMP-2 = 0.003 μM MMP-3 = 0.008 μM MMP-7 = 7.2 μM MMP-9 = 2.2 μM MMP-13 = 0.006 μM

Table 2. Cont.

Probe	Probe Type	Total Synthesis Time	Radio Chemical Yield	Radio Chemical Purity	Activity at the End of the Synthesis	log P/log D	In Vitro Assay Results (Cold or Hot Compound)
38	¹¹ C	20–25 min	35–55%	>99%	0.6–0.8 Ci/μmol	-	-
39	¹¹ C	40 min	50–60%		11–26 GBq/μmol	-	MMP-2 = 110 nM MMP-9 = 200 nM
40	¹⁸ F	70 min	10	98	5 GBq/μmol	clog P = 4.44 clog D = 1.46	MMP-1 = 18,500 nM MMP-2 = 16 nM MMP-3 = 7100 nM MMP-9 = 107 nM MMP-12 = 44 nM MMP-13 = 22 nM MMP-12 = 1600 nM MMP-1 = 6100 nM MMP-2 = 61 nM MMP-3 = 6800 nM MMP-9 = 1500 nM MMP-12 = 500 nM MMP-13 = 220 nM MMP-14 = 4600 nM
41	¹⁸ F	60 min	3%	100	12 GBq/μmol	clog P = 3.76 clog D = 0.67	MMP-2 = 7 nM MMP-9 = 2 nM
42	¹²⁵ I		49%	> 90%	0.146 GBq/μmol (2.176 Ci/μmol)	3.68	MMP-2 = 23 nM MMP-8 = 138 nM MMP-9 = 7 nM MMP-13 = 207 nM
43	¹⁸ F	142 mi	11.3%	98	23 GBq/μmol	clog D = 2.88	MMP-2 = 58 nM MMP-8 = 58 nM MMP-9 = 27 nM MMP-13 = 51 nM
44	¹⁸ F	140 min	43%	>97%	11–20 GBq/μmol	-	MMP-2 = 29 nM MMP-8 = 1170 nM MMP-9 = 1.3 nM MMP-13 = 362 nM
45	⁶⁸ Ga	-	87 % Post-labelling 66 % Pre-labelling	97 % Post-labelling 76 % Pre-labelling	-	-	-
46	¹²³ I		28%	95	0.2–6.3 GBq/μmol	clog P = 1.40	Precursor FB-ML5 MMP-2 = 12.5 nM MMP-9 = 31.5 nM MMP-12 = 138.0 nM
47	¹²⁴ I		44%	93	0.4–14.0 GBq/μmol	clog D = 0.92	MMP-2 = 4.4 nM MMP-9 = 8.1 nM MMP-12 = 1.5 nM MMP-13 = 4.1 nM
51	¹⁸ F	3 h	13–16%	95%	41–66 GBq/μmol	-	Precursor Ki, MMP-2 = 103 nM MMP-12 = 221 nM MMP-9 = 2376 nM MMP-13 = 530 nM
53	¹²³ I	-	41.7%	95%	16.2 GBq/μmol	-	MMP-2 = 1.8 nM MMP-9 = 7.2 nM
67	¹⁸ F	150 min	1.3%	99%	1.5 TBq/μmol	-	MMP-1 = 910 nM MMP-2 = 0.87 nM MMP-9 = 1.95 nM
52	¹⁸ F	119 min	37	98	3–33 GBq/μmol	log D 1.65	MMP-2 = 5 nM MMP-8 = 21 nM MMP-9 = 108 nM MMP-13 = 0.07 nM
60	¹⁸ F	-	10–20	95	>90 GBq/μmol (>2.5 Ci/μmol)	-	
62	¹²³ I	-	38–63%	98	-	-	
63	¹⁸ F	105 min	46.3	99	0.7–33.2 GBq/μmol	log D = 2.50 clog D = 1.18	

Table 2. Cont.

Probe	Probe Type	Total Synthesis Time	Radio Chemical Yield	Radio Chemical Purity	Activity at the End of the Synthesis	log P/log D	In Vitro Assay Results (Cold or Hot Compound)
64		29 min	36–50%	98%		log D = 3.35	MMP-2 = >100 nM MMP-8 = >100 nM MMP-9 = >100 nM MMP-13 = 56 nM MMP-14 = >100 nM
65	¹¹ C	75 min	20–23%	98%	122–330 GBq/μmol 18–35 GBq/μmol 18–20 GBq/μmol	log D = 2.77	MMP-2 = >100 nM MMP-8 = >100 nM MMP-9 = >100 nM MMP-13 = 26 nM MMP-14 = >100 nM
66		74 min	73%	99		log D = −1.12	MMP-2 = >100 nM MMP-8 = >100 nM MMP-9 = >100 nM MMP-13 = 1.8 nM MMP-14 = >100 nM
75	¹²⁵ I	-	91%	98%	5.1 GBq/μmol	-	MMP-12 = 8.5 nM MMP-2 = 81 nM MMP-8 = 19 nM
76		126 min	37%	99	3–59 GBq/μmol	log D = 0.48	MMP-9 = 2230 nM MMP-12 = 0.19 nM MMP-13 = 288 nM MMP-2 = 35 nM MMP-8 = 2 nM
77		140 min	43	99	7–57 GBq/μmol	log D = 0.00	MMP-9 = >10,000 nM MMP-12 = 0.001 nM MMP-13 = 16 nM MMP-2 = 0.84 nM MMP-8 = 0.18 nM
78		161 min	22	99	3–68 GBq/μmol	log D = 0.63	MMP-9 = 15.1 nM MMP-12 = 0.0004 nM MMP-13 = 3.2 nM

1.1. Classifications and Structures of Matrix Metalloproteinases

During the last 40 years, MMPs have become an attractive pharmacological target due to their relationship with several diseases, especially in cancer invasion and metastasis [12]. The main role of these zinc-dependent endopeptidases lies not only in their ability to remodel the extracellular matrix (ECM) by degrading the connective tissue, but also in their involvement in contact cell regulation [13]. The normal function of these enzymes is related to tissue homeostasis by maintaining the complex ECM and preserving its biochemical and mechanical properties. Therefore, a dysregulation in MMP activity could lead to both the development and cancer progression, as well as other diseases such as atherosclerosis, osteoarthritis, pulmonary and cardiovascular diseases [14]. The entire MMP genome includes 24 distinct genes encoding for more than 26 members. These have been classified based on the substrate type (collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others) or by their structure [15]. Structurally distinct MMPs such as MMP-2, -7, -8, -9, -12 and -13 have been targeted with MMPs labelled as diagnostic agents [6]. MMPs are synthesized and secreted as proenzymes (zymogens) apart from MMP-8 and MMP-9 that are present in neutrophils and located inside granulomas. The activation process typically occurs extracellularly by proteolytic cleavage by serine proteinases or even by other MMPs. Commonly, MMPs are multi-domain proteins, which contain a signal sequence that targets the peptide for secretion; a propeptide responsible for the inactivation; a catalytic domain; a linker domain (also called a hinge region); and a hemopexin-like domain [16]. The propeptide is made up by the PRCGXPD motif that can inhibit the enzyme by a Cys-Zn²⁺ chelation that occludes the active site. Apart from the auto inhibition by tissue inhibitors of matrix metalloproteinases (TIMPs) and the general plasma proteinase inhibitor, α2-macroglobulin can also regulate MMPs activity [17]. The MMP catalytic domain consists of a globular domain formed by approximately 170 amino acids where five central β-sheets are wrapped around four α-helices. These metalloenzymes contain two zinc ions: One is part of the catalytic site and is coordinated by three

histidines contained in the well conserved zinc-binding motif (HEXXHXXGXXH); the second exerts structural functions and its absence is linked to an autoinhibition allosteric process [18]. Furthermore, at least 2 or 3 calcium ions having structural functions are present depending on the 3D-crystal structure. Furthermore, the general structure presents five β -sheets (β I– β V) and three α -helices (α A, α B and α C) within the N-terminal subdomain. The catalytic site is located in the α B (Figure 1). By linking α B and α C, a notable Ω -loop is found, presenting a variety of lengths and amino acid compositions depending on the type of MMP. The Ω -loop has become one of the paradigms in MMP drug discovery, as it is the least conserved fragment within the catalytic domain that can offer different ligand selectivity [19]. There are several hydrophobic pockets described in the catalytic domain of MMPs which are designated by a primed or unprimed S subsite. As described by Babine and Bender [20,21], the subsites on the left side of the Zn^{2+} ion are designated without a prime S (S1, S2, S3...), while those present on the right side are primed (S1', S2', S3' ...) and the numbers refer to the residue numbers present on the substrate that occupy the pockets being the first ones cleaved by the MMP and 2 and 3 the adjacent.

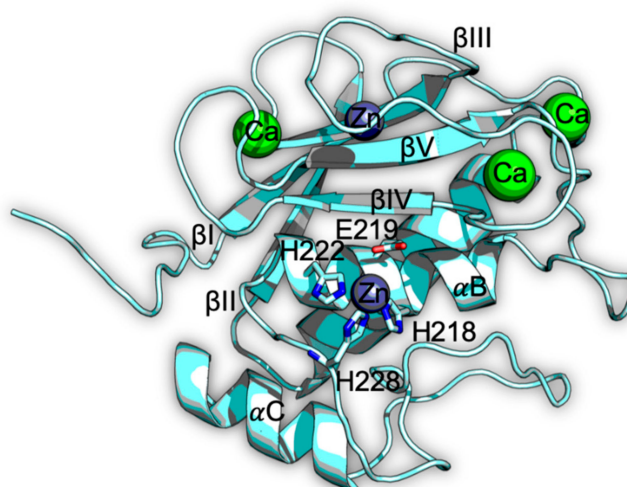


Figure 1. MMP Catalytic subunit. A cartoon representation of the MMP-1 (PDB code 1CGF) showing the different α -helices and β -sheets with the corresponding coordinated metal ions. The sticks highlight the active site with the three histidines and the catalytic glutamic acid.

The S1' pocket is a highly hydrophobic cavity formed by the Ω -loop. Although this subsite is present in all MMPs, its volume and plasticity varies depending on the nature of the Ω -loop, giving rise to a MMP classification according to the size of the S1' pocket: Small (MMP-1, MMP-7, MMP-20, MMP-22), medium (MMP-2, MMP-8, MMP-9, MMP-12, MMP-14, MMP-16), and large (MMP-3, MMP-10, MMP-13) [22]. The S1' pocket is the major hydrophobic cavity near the active site and has attracted major attention in the design of selective MMP inhibitors because of its variation in size and depth [23]. Furthermore, another side pocket has been described, the S1'* , which extends beyond the S1' pocket, and its accessibility depends also on the position and conformation of the Ω -loop. The presence of this extra pocket has been only mentioned in some MMPs, such as MMP-8 and MMP-13 [24,25]. In addition to the S1' and S1'* pockets, the S1, S2, S3 and S2', S3' subsites can also offer some degree of selectivity [21].

1.2. MMPs as Biomarkers in Cancer, Atherosclerosis, Osteoarthritis, Pulmonary and Cardiovascular Diseases

Although the knowledge that MMP overexpression has an influence in the risks and prognosis of several types of cancer and other diseases is not new, the application of MMPs as biomarkers is relatively recent. An ideal biomarker presents the capacity to be measured and evaluated as an indicator of the biological process of the target. Therefore, a biomarker should be accurate, non-invasive, sensitive, non-expensive and easy to perform [26]. The recently developed biomarkers are deficient in specificity

and sensibility when they are applied in a single combination, so nowadays biomarker combination is one of the strategies to overcome these weaknesses [27].

Currently, MMPs are being explored as biomarkers in several fields such as diagnosis, monitoring and treatment efficacy in different diseases. In this line, their application in cancer, atherosclerosis, osteoarthritis, and pulmonary and cardiovascular diseases has been remarkable. This novel approach comes mainly because the overexpression of MMPs in these diseases is specific and elevated. Moreover, these enzymes are available in the extracellular media, which means the biomarker molecule does not need to cross the cell membrane. An extensive review made by Huang et al. [28] summarized how MMP-9 can be explored as a potential biomarker, and also how this MMP is overexpressed in several types of tumors such as colorectal carcinoma, breast, pancreatic, ovarian, cervical, osteosarcoma non-small cell lung cancer (NSCLC) and giant cell tumor of bone (GCTB). This makes MMP-9 a preferential candidate for the early detection of these types of tumors. The elevated MMP-9 expression in those tumors compared with healthy controls can be detected due to the recent development of experimental analytical techniques such as proteomic analysis, immunohistochemical studies, immunochemistry assays and tandem mass spectrometry [29–31]. The augmented levels of MMP-9 can be detected in plasma or blood samples, showing triple protein levels compared to healthy patients. The study carried out by Zajkowska et al. [29] showed that patients with cervical cancer in stages III and IV present MMP-9 plasma levels of 3344.44 ng/mL and 325.80 ng/mL, respectively, with almost three times more compared to healthy patients which present with normal levels of 166.00 ng/mL. De Chiara et al. [32] measured the MMP-9 levels in blood samples and found them to be triple (302.92 ng/mL) for NSCLC patients than in healthy controls (136.14 ng/mL). By tissue sampling, the overexpression of MMP-9 has also been detected by immunohistochemical assays using micro-RNA and labelled IgG in different types of cancer such as colorectal carcinoma, and breast cancer. Moreover, this overexpression has been linked to metastatic processes [30,31]. In the case of GCTB, the resection after surgery was the only available option to get the samples in order to measure the MMP-9 mRNA expression in peripheral tissue, confirming the overexpression in this type of cancer. These published diagnostic screenings in MMP-9 present as the main drawback the lack of tumor localization for blood samples and the inconvenience of the biopsy sampling. This gives an idea of the potential of MMP-9 as a biomarker.

Further, MMP-2, -7, -11, and -12, have been reported to be involved in cell growth and metastasis processes. In this line, MMP-12 overexpression has been linked to the grade and stage of esophageal squamous cell carcinoma in tissue sampling [33]. This protease has also been connected with the exposure to tobacco smoke and with gefitinib-resistant NSCLC that leads to a poor prognosis [34,35]. Related to its implication in the effects of the exposure to cigarette smoke, MMP-12 is a key enzyme in diseases such as chronic obstructive pulmonary disease (COPD) and has been proposed as an early biomarker due to its elevated levels in tissue sampling [36,37].

MMP-11 has promoted the occurrence and development of malignant tumors such as breast [38], gastric [39], colorectal (CRC) [40], and lung cancer [41]. Similarly, MMP-7 overexpression has been correlated with the incidence of gastric cancer. The growth/differentiation factor 15 overexpression was also linked with this type of cancer, showing almost more than ten times the protein level compared with healthy patients [42]. MMP-7 has been described to play critical roles in the development and progression of other malignant tumors including CRC [43,44] and triple-negative breast cancer [45], amongst others. In conclusion, MMP-7 has been designated as a tumor suppressor. MMP-2 has been involved in the migration and invasion of ovarian, colorectal, bladder and breast cancer, where the increased activity and expression of these enzymes have been described [46–49]. Additionally, a remarkable synergic effect between Cullin1 and MMP-2 in CRC prompt diagnosis has been proposed by Deng et al. An extensive study with 470 patients showed that protein levels were significantly upregulated in CRC tissues compared to the healthy ones [50]. However, the overexpression of MMP-2 has been linked with poor prognosis in patients with epithelial ovarian and lung cancers [51,52].

On the other hand, MMPs have also been involved in the development and progression of atherosclerosis, which is correlated with cardiovascular diseases, such as a stroke and ischemic heart

diseases, two of the top worldwide causes of death. The activity of these proteases promotes the loss of collagen, elastin, and other ECM proteins, inducing the necrotic core of atherosclerotic plaque, which finally could end in myocardial infarction or a stroke [10]. It has been shown that increased levels of MMP-9 are present in specific risk groups, like obese people that present a profile for early atherosclerosis development [53]. Furthermore, Rohde et al. demonstrated that MMP-9 serum levels were increased in patients with definite carotid atherosclerotic disease compared with normal patients; and also, that only MMP-9 levels were increased in contrast with other proposed biomarkers such as MMP-1 and MMP-3 [54]. A study carried out by Nilsson et al. [55] using a cohort of 1500 patients, and measuring the plasma levels of MMP-1, -3, -7, -10, and -12, demonstrated that MMP-7 and MMP-12 were elevated in type 2 diabetes, which is related to atherosclerosis and coronary events. Another meta-study carried out by Zhong et al. [56] in which the serum levels of MMP-1 and TIMP were evaluated in patients with atrial fibrillation (AF), demonstrated that elevated mRNA levels of MMP-1 and decreased circulating levels of TIMP2 were significantly associated with AF risk.

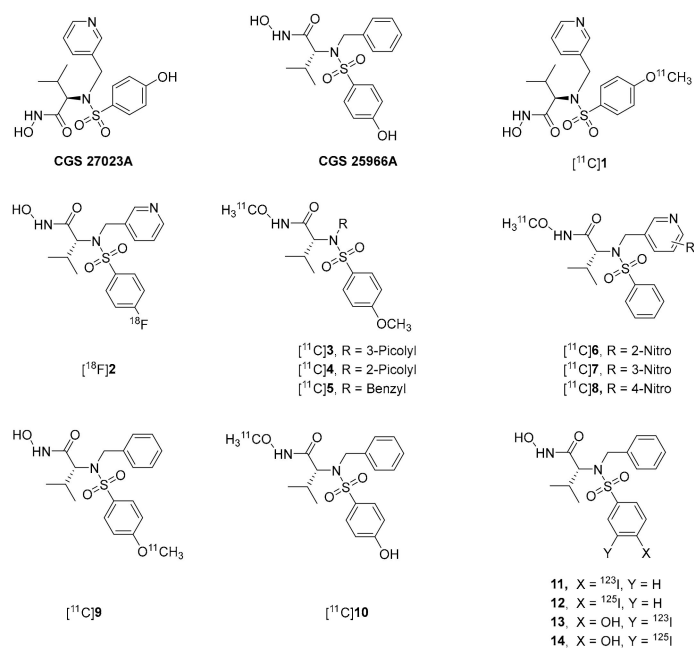
Osteoarthritis (OA) is also one of the diseases linked to MMP overexpression due to their implication in the breakdown of articular cartilage in synovial joint tissues. Many publications have linked OA to the overexpression of MMPs in the affected tissue, especially MMP-1, -2, -9 and -13 [57,58]. MMP-1 is proposed as an early OA biomarker due to its increased concentrations in the synovial fluid in the early stage of OA compared to decreased levels when OA continues progressing [59]. Remarkably, MMP-13 has raised interest in clinical studies, as it is overexpressed in the cartilage of patients with OA and is identified as a target protein in the signaling pathways implicated in the regulation of cartilage breakdown [60,61]. MMP-3 has also been proposed as an OA biomarker together with other pro-inflammatory enzymes due to its markedly up-regulation in this disease [62].

2. MMPs Labelled Imaging Agents for Cancer

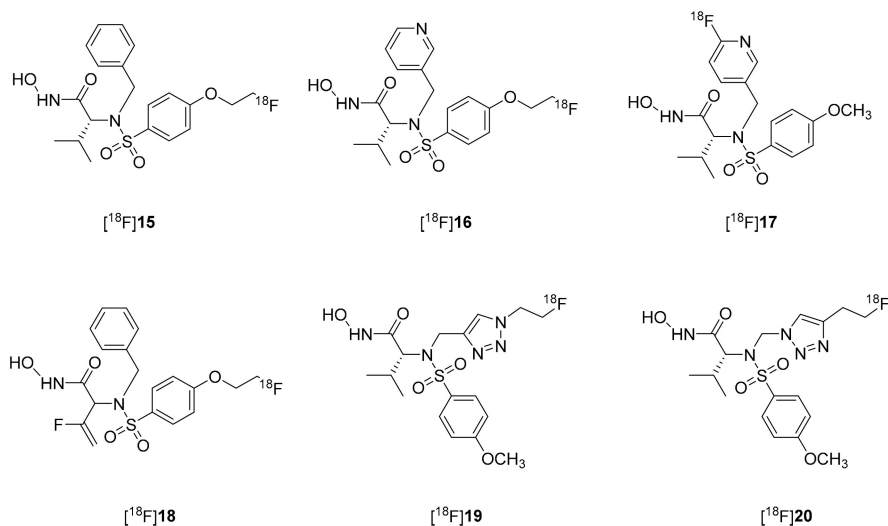
2.1. CGS 27023 and CGS 29566 Based MMPs Labelled Imaging Probes

Several MMPs such as Batimastat, Marimastat, Tanomastat, Prinomastat (AG-3340), Rebimastat, CGS 25966A, and CGS 27023A have entered human trials. Among these, CGS 27023A presents both good pharmacokinetics and IC₅₀ in the nanomolar range for several MMP isoforms.

Inspired by the MMP inhibitory activity of CGS 27023A, Zheng et al. [63] designed a number of ¹¹C or ¹⁸F labeled analogs such as [¹¹C]1, [¹⁸F]2 and [¹¹C]3. These are the first MMP radiotracers described in the literature [63]. Later on, the same research group reported analogs [¹¹C]4–[¹¹C]8, as radiolabeled MMPs [64]. However, no reports of in vivo applications of [¹¹C]4–[¹¹C]8 have been found. Further, based on CGS 25966A, Zheng et al. [65] reported simple synthetic methods for [¹¹C]9 and its isomer [¹¹C]10. The novel no-carrier-added radioiodinated MMPs [¹²³I]11, [¹²⁵I]12, [¹²³I]13 and [¹²⁵I]14 were prepared by radioiodination of their precursors with [¹²³I]NaI or [¹²⁵I]NaI (Scheme 1) [66]. Preliminary in vitro data showed affinities towards MMP-2 and MMP-9 in the lower nanomolar range comparable to those of the parent compound CGS 27023A. In vivo biodistribution using [¹²⁵I]14 in CL57 B/6 mice for 65 min p.i. showed rapid blood and plasma clearance and low retention in normal tissues. This is indicative of the suitability of these radiotracers for imaging MMP activity in vivo. However, in vivo studies using animal models that represent diseases with known MMP overexpression (e.g., tumor: Lewis lung carcinoma bearing mice; atherosclerotic plaques: apolipoprotein E-deficient mice) are needed to support their observation. Wagner et al. described a series of [¹⁸F]-labeled compounds ([¹⁸F]15–20) (Scheme 2) [67]. Compound [¹⁸F]16 presented 43-fold increased potency compared to the parent compound CGS 27023A. Neither of these two compounds presented a tissue-specific accumulation in wild type (WT) mice, which can be considered an advantage in the study of MMP dysregulation. Therefore, compounds [¹⁸F]15 and [¹⁸F]16 could be useful for the in vivo imaging of activated MMPs in inflammation, cancer or atherosclerosis.



Scheme 1. Chemical structure for compounds CGS 25966A, CGS 27023A and 1–14.



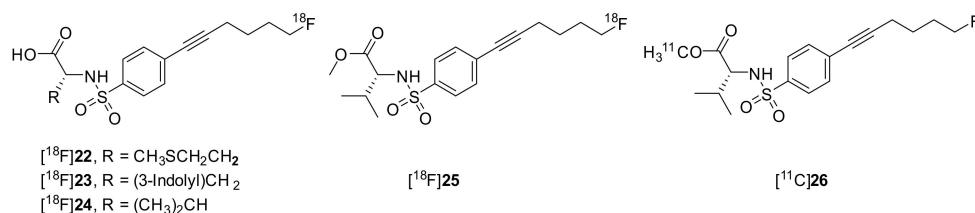
Scheme 2. Chemical structure for compounds 15–20.

The same group reported a fully automated robust and reproducible radiosynthesis for [¹⁸F]15 with reasonable yields, whereas compound [¹⁸F]17 was synthesized in a one-step radiosynthesis with moderate radiochemical yield. Despite the latter presenting a high lipophilicity (experimental log D = 1.85), the initial PET in vivo studies in WT mice (C57/BL6) showed neither unspecific accumulation of [¹⁸F]17 in the heart or carotid regions, nor accumulation of radioactivity in the bones due to tracer decomposition. Compound [¹⁸F]18 was obtained in its two enantiomerically pure (*R*)- and (*S*)-isomers, and both demonstrated to be potent fluorinated inhibitors of MMP-2 and MMP-9 [68]. Remarkably, the (*S*)-enantiomer was even more active than the (*R*)-isomer in contrast to CGS 25966. Unfortunately, (*S*)-[¹⁸F]18 is metabolically unstable in WT mice, which hinders further clinical development of this tracer. Triazole-containing unlabeled precursors of compounds [¹⁸F]19 and [¹⁸F]20 [69], possess increased hydrophilicity compared to CGS 25966A (clog D values ranging from 0.60 to 2.25) and nanomolar inhibition potencies for MMP-2, MMP-8, MMP-9, and MMP-13 (see Table 2) [70].

This non-peptidic low-molecular-weight fluorescent probe may be clinically translated for endoscopic or surgical procedures.

2.3. MMP Inhibitor Imaging Probes with Carboxylic Acid as Zing Binding Group (ZBG)

Furumoto et al. [73] designed and developed ^{18}F -labeled carboxylic acid-based MMPis [^{18}F]22–24 for PET cancer imaging. The precursors were synthesized in four steps starting from the D-isomer of the corresponding amino acid. Although compound [^{18}F]22 was chemically unstable and thus unsuitable for biological applications, compounds [^{18}F]23 and [^{18}F]24 (Scheme 4) were isolated in moderate radiochemical yields, and their biological evaluation was reported by the same authors [74].



Scheme 4. Chemical structure for compounds 22–26.

A biodistribution study in the Ehrlich tumor-bearing mice showed that the accumulation of [^{18}F]24 in tumor was higher than in other organs, except for the liver, small intestine, and bone. When [^{18}F]25, a methyl ester of [^{18}F]24, was used as a prodrug, the uptake in liver at 30 min p.i. decreased by half and the tumor uptake increased by 2.4 times, compared with [^{18}F]24. A radio-thin-layer chromatographic analysis of [^{18}F]25 demonstrated that it can be easily converted to the parent drug in vivo and thus accumulated in the tumor tissue. Therefore, [^{18}F]25 could be considered a prodrug of [^{18}F]24. Whole body autoradiography using [^{18}F]25 showed tumor-specific accumulation of radioactivity, with higher accumulation in the bone and intestine. However, the increase of the uptake in the bone was time-dependent, suggesting the accumulation of free $^{18}\text{F}^-$ anion, released from the terminal alkyl chain of [^{18}F]25. These results suggest that the prodrug strategy could be useful, however the release of free [$^{18}\text{F}^-$] should be avoided.

Structurally related compound [^{11}C]26 has been studied as a potential PET cancer tracer [75]. The biodistribution and micro-PET studies in breast cancer animal models, such as MCF-7 transfected with IL-1 α implanted athymic mice, and MDA-MB-435 implanted athymic mice confirmed that the localization of [^{11}C]26 in the tumor was mediated by non-specific processes (Figure 2).

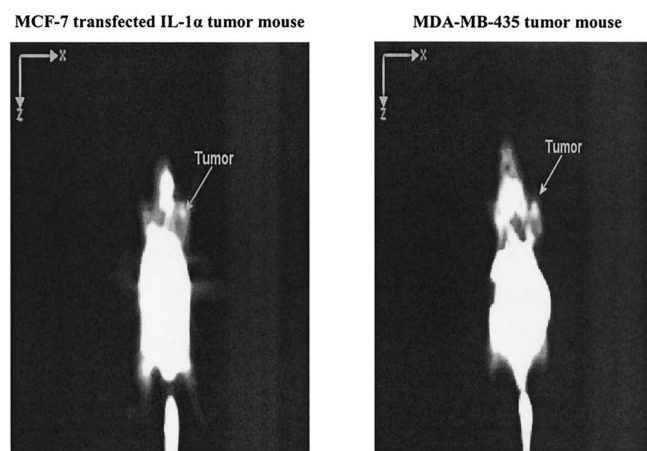
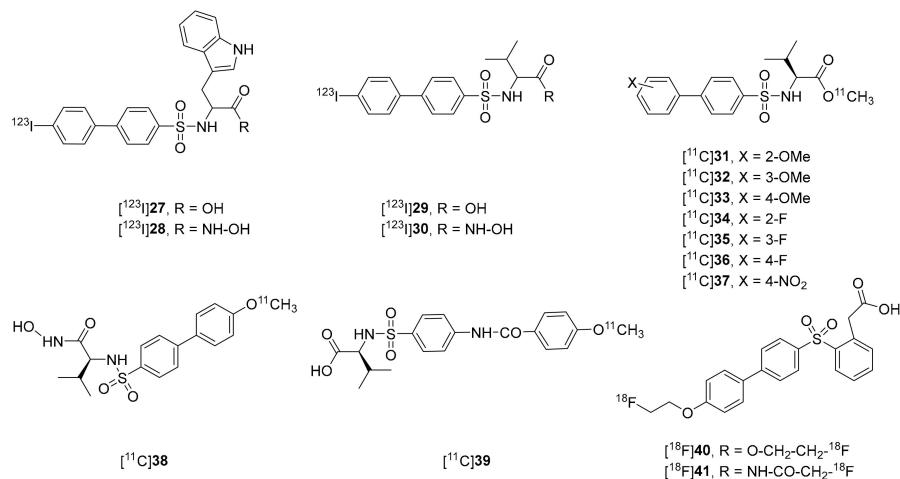


Figure 2. Micro-PET images of [^{11}C]26 in a MCF-7 transfected with IL-1 α implanted athymic mouse, and a MDA-MB-435 implanted mouse, after an initial 30 min uptake period. Reprinted with permission from [75] Copyright © 2003 Elsevier Inc. All rights reserved.

2.4. Biphenyl Sulfonamide Based MMPis Labelled Imaging Probes

Oltenfreiter et al. developed a series of radioiodinated carboxylic and hydroxamic acids [^{123}I]27–30 (Scheme 5) [76–78].



Scheme 5. Chemical structure for compounds 27–41.

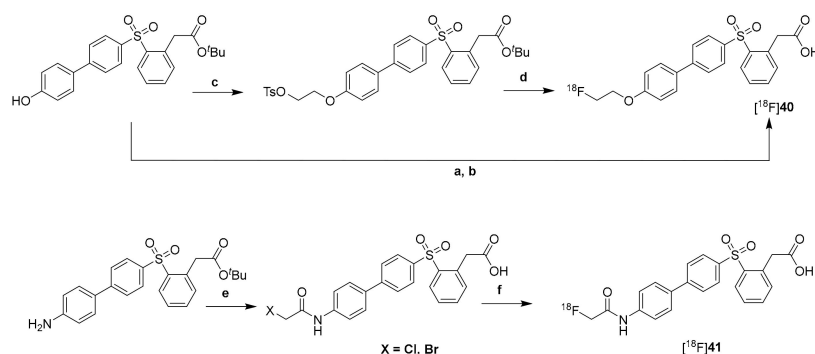
The *in vitro* zymography and enzyme assays showed IC₅₀ in the nanomolar range on gelatinases and high selectivity for MMP-2 (see Table 2). The *in vivo* biodistribution in NMRI white mice and in A549 lung carcinoma bearing athymic mice [78] showed that [^{123}I]27 and [^{123}I]28 have the potential to be used as SPECT tumor imaging agents.

The biodistribution evaluation in A549-tumor bearing athymic mice showed good tumor uptake for compound [^{123}I]29, but not so good for compound [^{123}I]30 [79]. A metabolite analysis by plasma HPLC after 2h revealed no degradation of [^{123}I]29, but compound [^{123}I]30 presented 30% of the metabolites, and 65.4% of the intact compound. However, 91.8% of the accumulated activity in the tumor was produced by the intact compound and only 8.1% by the metabolite. The *in vivo* evaluation revealed that these MMPis could have potential as tumor imaging agents, but further research is necessary.

Qi-Huang Zheng et al. reported the synthesis of a series of biphenyl sulfonamide based MMPi radiotracers, [^{11}C]31–37, for the evaluation as potential PET cancer imaging agents [80]. Despite exhibiting strong inhibitory activity on MMP-13, no *in vivo* imaging data have been reported for these compounds.

The same authors reported the synthesis of [^{11}C] labeled MMP inhibitors [^{11}C]38 and [^{11}C]39 for the evaluation as PET cancer imaging agents [81]. Compound S-38 is a potent MMPi for several MMP subtypes with IC₅₀ ranging from 0.003 to 7.2 μM. The *in vitro* biological data showed that both [^{11}C]38 and structurally related compound [^{11}C]9 possessed favorable pharmacokinetics making them eligible for *in vivo* studies. However, even though [^{11}C]38 had good biodistribution in breast cancer animal models, the tumors were invisible in micro PET imaging studies.

Kuhnast et al. reported the synthesis and labelling by methylation with [^{11}C]CH₃I of a N-sulfonylamino acid derivative to obtain compound [^{11}C]39 [82]. This compound is a selective and highly potent MMP-2 and MMP-9 inhibitor. Compound [^{11}C]39 exhibited a strong inhibitory effectiveness for gelatinases (110 nM for MMP-2 and 200 nM for MMP-9). *In vivo* studies in normal mice demonstrated favorable pharmacokinetics and [^{11}C]39 was found to be rapidly excreted and stable up to 30 min post injection. Thus, compound [^{11}C]39 seems to be a suitable compound for PET imaging of MMPs activity. However, the evaluation of this derivative in tumor bearing mice is needed to further validate its applicability. Digilio et al. reported the synthesis of ^{18}F labelled arylsulfones [^{18}F]40 and [^{18}F]41 based on the nanomolar activity and selectivity of their non-radioactive counterparts (Scheme 6) [83].



Scheme 6. Synthetic route for $[^{18}\text{F}]40$ – 41 . Reagents and conditions: (a) (1) $\text{C}_2\text{H}_4(\text{OTs})_2$, $[^{18}\text{F}]\text{K}(\text{K} 222)\text{F}$, $100\text{ }^\circ\text{C}$; (2) MeONa , $120\text{ }^\circ\text{C}$. (b) TFA , CH_3CN , rt. (c) $\text{C}_2\text{H}_4(\text{OTs})_2$, K_2CO_3 , CH_3CN , $82\text{ }^\circ\text{C}$. (d) (1) $[^{18}\text{F}]\text{K}(\text{K} 222)\text{F}$, $105\text{ }^\circ\text{C}$; (2) TFA , CH_3CN , rt. (e) Chloroacetylchloride or bromoacetyl bromide, DIPEA , DMF . (f) (1) $[^{18}\text{F}]\text{K}(\text{K} 222)\text{F}$, $130\text{ }^\circ\text{C}$; (2) TFA , CH_3CN , rt.

Tracer $[^{18}\text{F}]40$ was selected to study the uptake of radioactivity in a human glioblastoma mouse model as this compound presented the best MMP inhibition profile and efficiency of radiosynthesis. The biodistribution of $[^{18}\text{F}]40$ was dominated by liver uptake, gastrointestinal tract uptake and hepatobiliary clearance. When compared to $[^{18}\text{F}]\text{fluorodeoxyglucose}$ (FDG), compound $[^{18}\text{F}]40$ exhibited an excellent tumor uptake and demonstrated to be specific (Figure 3).

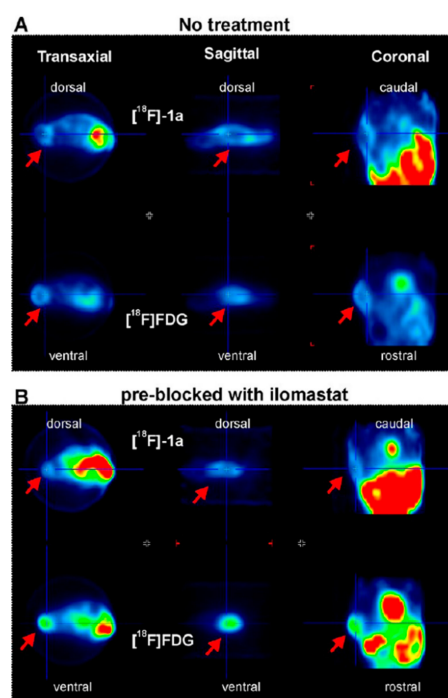
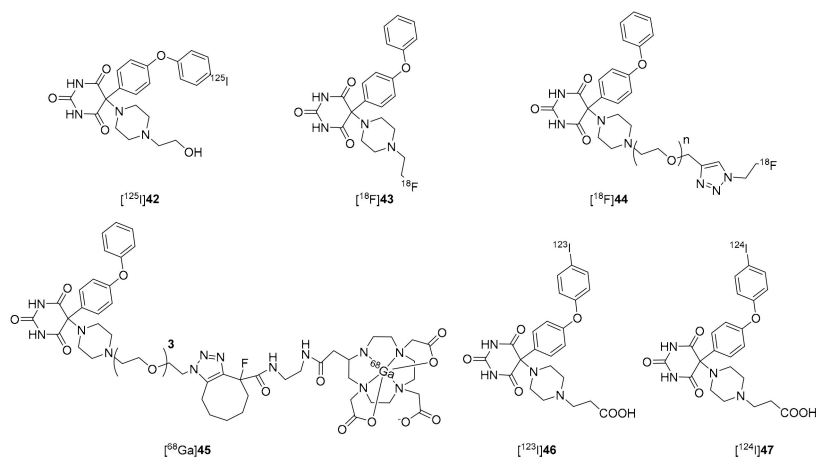


Figure 3. ^{18}F PET images of the abdomen of nude mice subcutaneously grafted with a human U-87 MG glioblastoma. Red arrows show the tumor in different slices. (A) PET images obtained by sequential administration of $[^{18}\text{F}]40$ (**1a**) (upper row) and $[^{18}\text{F}]\text{FDG}$ (bottom row), showing colocalization of tracer uptake. The $[^{18}\text{F}]40$ (**1a**) scan was acquired 100 min post-tracer injection; $[^{18}\text{F}]\text{FDG}$ was injected immediately after completion of the $[^{18}\text{F}]40$ (**1a**) scan and the image was acquired 45 min post- $[^{18}\text{F}]\text{FDG}$ injection. Both scans were static acquisitions (injected dose 7.4 MBq , acquisition time 30 min). (B) PET images obtained by sequential administration of $[^{18}\text{F}]40$ (**1a**) (upper row) and $[^{18}\text{F}]\text{FDG}$ (bottom row), in a mouse that received 40 mg/kg of the broad-spectrum MMPI Ilomastat 30 min before injection of $[^{18}\text{F}]40$ (**1a**). The timing scheme and injected doses are the same as in panel A. Reprinted with permission from [83] Copyright © 2013 American Chemical Society.

2.5. Barbiturate Based MMPs Labelled Imaging Probes

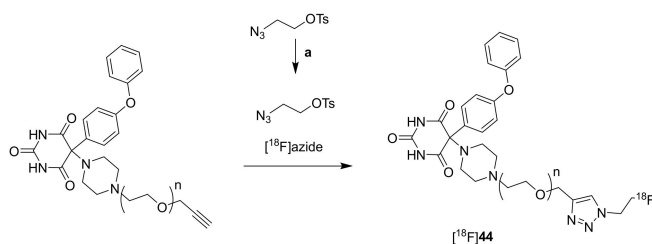
Breyholz et al. described a barbiturate-based MMPi radiotracer [^{125}I]42 (Scheme 7) for the noninvasive in vivo visualization of activated MMPs using SPECT or PET imaging modalities [84]. The corresponding non-labeled compound showed some selectivity towards MMP-2 and -9 (IC_{50} MMP-2 = 7 nM; MMP-9 = 2 nM).



Scheme 7. Chemical structure for compounds 42–47.

The same group reported the structurally related [^{18}F]43 radiotracer [85]. The unlabeled analogous showed nanomolar MMP inhibition potencies with higher selectivity for MMP-9 against MMP-2 (MMP-2, IC_{50} = 23 nM; MMP-9, IC_{50} = 7 nM). Preliminary biodistribution studies indicated that there was no tissue specific accumulation in WT C57/BL6 mice and demonstrated favorable pharmacokinetic behavior corresponding to the physical half-life of the positron emitter. Small-animal PET images of the coronal whole-body showed that the tracer uptake in non-target organs was low over all the time points and showed rapid clearance through the renal and hepatobiliary route. Thus, these barbiturate-based MMP-targeted radiotracers may be applicable in noninvasive in vivo imaging of MMP-2 and MMP-9-associated diseases by means of PET.

A second generation of radio fluorinated barbiturate-based MMP tracers containing a triazole linker was described (Scheme 8) [86].

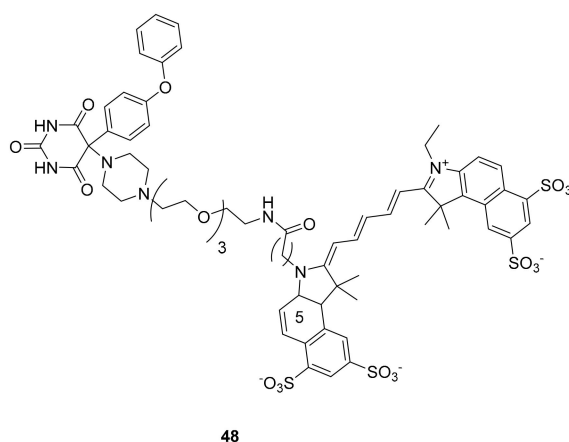


Scheme 8. Synthetic route for [^{18}F]44. Reaction conditions: (a) [^{18}F]fluoride (potassium 222 cryptate), K_2CO_3 , CH_3CN , 84 °C, 15 min; (b) $\text{CuSO}_4 \cdot \text{H}_2\text{O}$, sodium ascorbate, DMF, rt, 8 min.

The in vivo biodistribution studies of [^{18}F]44 in WT mice showed a high level of radioactivity in the liver, and a slower liver clearance and faster kidney clearance than the more hydrophobic [^{18}F]43. Hence, the alteration of the chemical structure by introducing a mini-PEG and a triazole unit in [^{18}F]44 led to a favorable pharmacokinetic behavior for in vivo PET-imaging. However, preclinical PET/CT studies in disease models with known MMP up-regulation (e.g., tumor, Lewis lung carcinoma bearing mice; atherosclerotic plaques, apolipoprotein E-deficient mice) should be assessed [87]. There are also examples of barbiturate MMP inhibitors labelled with [^{68}Ga]45 [88].

More recently Wagner et al. developed the more hydrophilic radioiodinated barbiturate-based MMP tracers [^{123}I]46-[^{124}I]47. These compounds presented high inhibitory potency (IC_{50} (MMP-2) = 29 nM, IC_{50} (MMP-9) = 1.3 nM) [88].

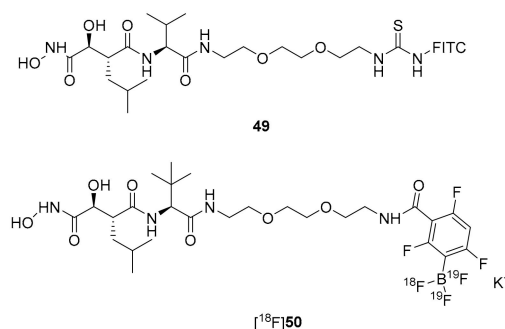
In 2008, Faust et al. reported Cy5.5 labeled barbiturate based MMPis as fluorescence photoprobes for imaging MMPs [89]. They designed a convergent 10 step synthetic route to obtain non-hydroxamate MMP inhibitor **48**, which showed high affinity (IC_{50} value: 48 nM for MMP-2) measured by a fluorogenic assay using commercially available MMP-substrates and the purified enzyme. Fluorescence microscopy studies using highly (A-673, rhabdomyosarcoma) and moderate (HT-1080, fibrosarcoma) MMP-2 expressing cell lines showed efficient binding of **48** to the MMP-2 positive cells while no binding to MMP-2 negative cells (MCF-7, breast cancer cells). Pre-dosing experiments using 50 μM of the unlabeled barbiturate successfully blocked the binding of the Cy 5.5-labeled ligand **48** and showed specific uptake of the tracer. Therefore Cy 5.5 labeled tracer **48** (Scheme 9) may be a promising candidate for sensitive MMP detection in vivo.



Scheme 9. Chemical structure for compound **48**.

2.6. Marimastat Based MMPis Labelled Imaging Probes

Overall, a series of labelled Marimastat derivatives for in vivo PET imaging of MMP in cancer [90,91] have been described. First, FITC conjugated Marimastat (**49**) (Scheme 10) was reported to image MDA-MB-231 breast cancer cells stably transfected with MMP-14 by fluorescence.



Scheme 10. Chemical structure for compounds **49–50**.

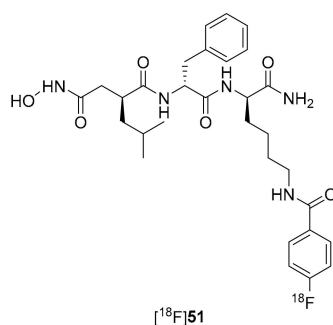
Later, a one-step synthesis of stable [^{18}F]aryltrifluoroborate-**50** under mild conditions by the use of a stable arylboronic acid as a captor of aqueous [^{18}F]fluoride was reported. This facile method avoids tedious multistep syntheses or harsh fluorination conditions.

Syngeneic 67NR murine mammary carcinoma or 67NR/CMV luciferase derived primary tumor in Balb/C mice were used for in vivo PET imaging. After the injection of [^{18}F]50 to tumor-bearing mice and PET image acquisition, a low but detectable and specific uptake in the primary tumor was

observed. However, in mice injected with higher concentration of [^{18}F]50 or control- ArBF_3 lacking the Marimastat moiety, no tumor localization was observed, with clearance only to the liver, bladder, and submaxillary salivary gland. As a specificity control, tumor-bearing mice were blocked with an unlabeled marimastat prior to the tracer injection. The pre-blocked mice had clearly reduced uptake with similarly sized tumors. The time-activity curve analysis revealed that [^{18}F]50 accumulated in the tumor after 60 min. Overall, the PET imaging and the time-activity curve analyses showed specific ^{18}F -labeling of the tumor as well as the bladder, liver, stomach, and gut.

2.7. Tripeptide Hydroxamic Acid Labelled with ^{18}F as Imaging Probes for MMPs

Elsinga and colleagues synthesized [^{18}F]51 (Scheme 11) by the direct acylation of the corresponding unlabeled inhibitor with *N*-succinimidyl-4- ^{18}F fluorobenzoate [92].



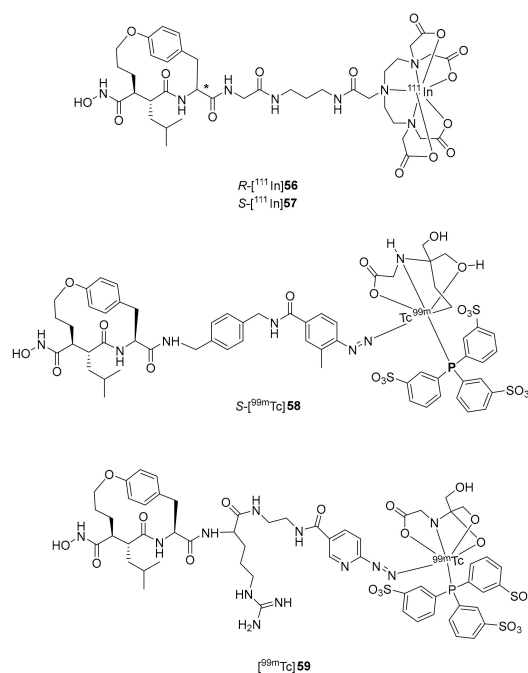
Scheme 11. Chemical structure for compound 51.

The resulting radiotracer [^{18}F]51 was evaluated in human bronchial epithelium 16HBE cells and breast cancer MCF-7 cells showing rather low binding to these cell lines. Specificity studies showed that cellular binding was reduced by 36.6% and 27.5% in MCF-7 and 16HBE cells, respectively, after co-incubation with the unlabeled counterpart.

The *in vivo* kinetics of [^{18}F]51 were examined in a HT1080 fibrosarcoma tumor bearing mice. In microPET scans, HT1080 tumors exhibited a low and homogeneous uptake of the tracer, suggesting that tracer binding was not only to membrane-bound disintegrin and metalloproteinase (ADAMs) but also to the extracellular MMPs. The tumors of mice injected with [^{18}F]51 showed specific uptake confirmed by blocking studies. The *ex vivo* biodistribution showed a rapid excretion through the kidneys and the liver. The metabolite assays indicated that the parent tracer represented $23.2 \pm 7.3\%$ ($n = 2$) of the total radioactivity in plasma after one and half hours after treatment. The autoradiography of a tumor slice confirmed the regular uptake on the tumor. A high kidney uptake was observed in the microPET/CT images. The change of the tumor to plasma ratio was statistically significant in contrast to the change of the tumor to muscle ratio. Therefore, the binding of [^{18}F]51 in the HT1080 xenograft mice was target mediated. Compound [^{18}F]51 may be suitable for the visualization/quantification of diseases overexpressing simultaneously MMPs and ADAMs.

2.8. Thiirane Based MMPis Labelled Imaging Probes

Wagner et al. reported ^{18}F -fluorine labeled radiotracers based on SB-3CT, a slow-binding and mechanism-based MMP-2 and -9 inhibitor [93]. Inspired by the selectivity and activity of SB-3CT, they synthesized non-radiolabeled analogues and evaluated their MMP inhibition profile showing K_i values ranging from 20.9 nM to 815 nM. These values are comparable to SB-3CT but they were non-selective inhibitors, with mixed kinetics and attractive residence times for MMP-2. However, this compound was unstable in human and mouse serum. The *in vivo* biodistribution studies revealed a rapid hepatobiliary elimination of the thiirane [^{18}F]52 (Scheme 12) and its metabolites in C57BL/6 mice and therefore, it is not suitable for PET imaging and further *in vivo* evaluations in MMP associated mouse models of disease.



Scheme 13. Chemical structure for compounds 56–59.

$[^{111}\text{In}]56$ showed specific uptake by microautoradiography in surgically induced MI in C57BL/6 mice, using $[^{111}\text{In}]57$ as a negative control. Further, gamma well counting demonstrated a 3- to 4-fold increase uptake in the same animal model. The same authors demonstrated, by using a model of carotid aneurysm in apolipoprotein E deficient mice, that $R-[^{111}\text{In}]56$ is a useful radiotracer to noninvasively predict the propensity of an aneurysm to expand (Figure 5) [98].

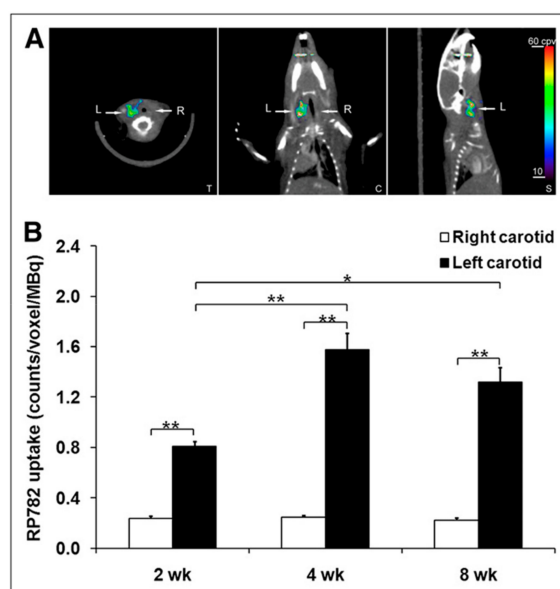


Figure 5. In vivo imaging of MMP activation in aneurysm. (A) An example of fused micro SPECT/CT images of mouse at 4 weeks after surgery to induce carotid aneurysm. The arrows point to aneurysmal left (L) and control right (R) carotid arteries. (B) The image-derived quantitative analysis of background-corrected $[^{111}\text{In}]56$ (RP782) carotid uptake. The background-corrected tracer uptake in left carotid artery peaked at 4 weeks after surgery and was significantly higher than uptake in right carotid artery at every time point studied. $n = 5$ –18 in each group. * $p = 0.01$. ** $p < 0.001$. C 5 coronal slice; S 5 sagittal slice; T 5 transverse slice. Reprinted with permission from [98] copyright © 2010 by the Society of Nuclear Medicine, Inc.

The micro-SPECT/CT imaging studies with analogous [^{99m}Tc]58 radiotracer demonstrated a 5-fold increase of myocardial uptake. Additionally, tracer [^{99m}Tc]58 showed specific uptake in atherosclerotic lesions in apolipoprotein E (ApoE $^{-/-}$) mice deficient in the low-density-lipoprotein receptor (LDLR $^{-/-}$) (Figure 6). After treating abdominal aortic aneurysm (AAA) mouse models with [^{99m}Tc]59, the SPECT/CT images showed that [^{99m}Tc]59 displayed a maximum tracer uptake (Figure 7). Although further validation is required, [^{99m}Tc]59-based molecular imaging might improve patient AAA management, as well as other disorders associated with MMP activity dysregulation.

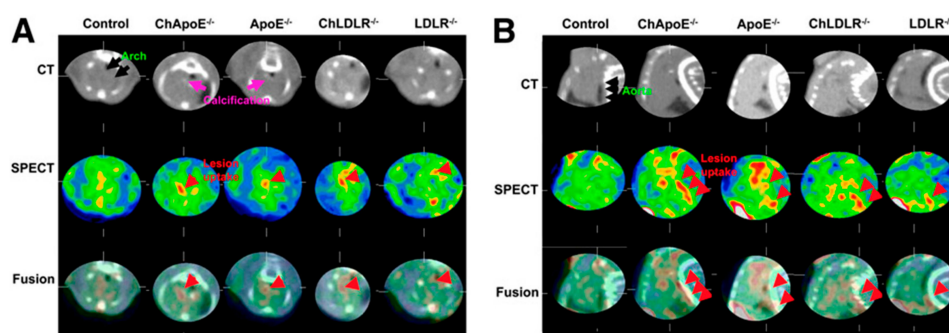


Figure 6. In vivo micro-SPECT/ micro-CT transverse (A) and sagittal (B) zoomed and masked images of the 5 study groups. Compound [^{99m}Tc]58 uptake in atherosclerotic aortic lesions was identified noninvasively in arch and abdominal aorta by micro-SPECT aided by micro-CT; aortic arch calcification was detected by micro-CT. Ch 5 high-cholesterol-fed. Reprinted with permission from [97] copyright © 2009 by the Society of Nuclear Medicine, Inc.

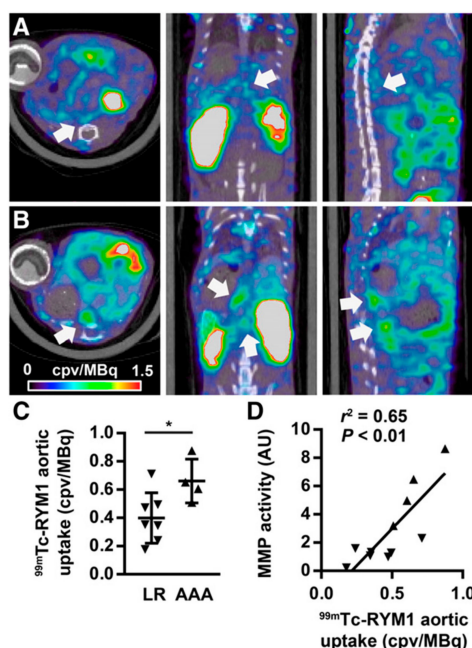


Figure 7. Compound [^{99m}Tc]59 (RYM1) imaging of AAA. (A,B) Examples of fused [^{99m}Tc]59 (RYM1) SPECT/CT images of animals from the low remodeling (A) and aneurysm (B) groups, classified on the basis of a visual in situ analysis of abdominal aorta. Transversal (left), coronal (middle), and sagittal (right) views are shown. Arrows point to areas of maximal tracer uptake in abdominal aorta. (C) Quantification of [^{99m}Tc]59 (RYM1) signal in area of maximal tracer uptake in suprarenal abdominal aorta in low remodeling and AAA groups. * $p < 0.05$. (D) Correlation between [^{99m}Tc]59 (RYM1) signal in vivo and MMP activity quantified by zymography ex vivo. AU 5 arbitrary units; cpv 5 counts per voxel; LR 5 low remodeling. Reprinted with permission from [99] copyright © 2017 by the Society of Nuclear Medicine and Molecular Imaging.

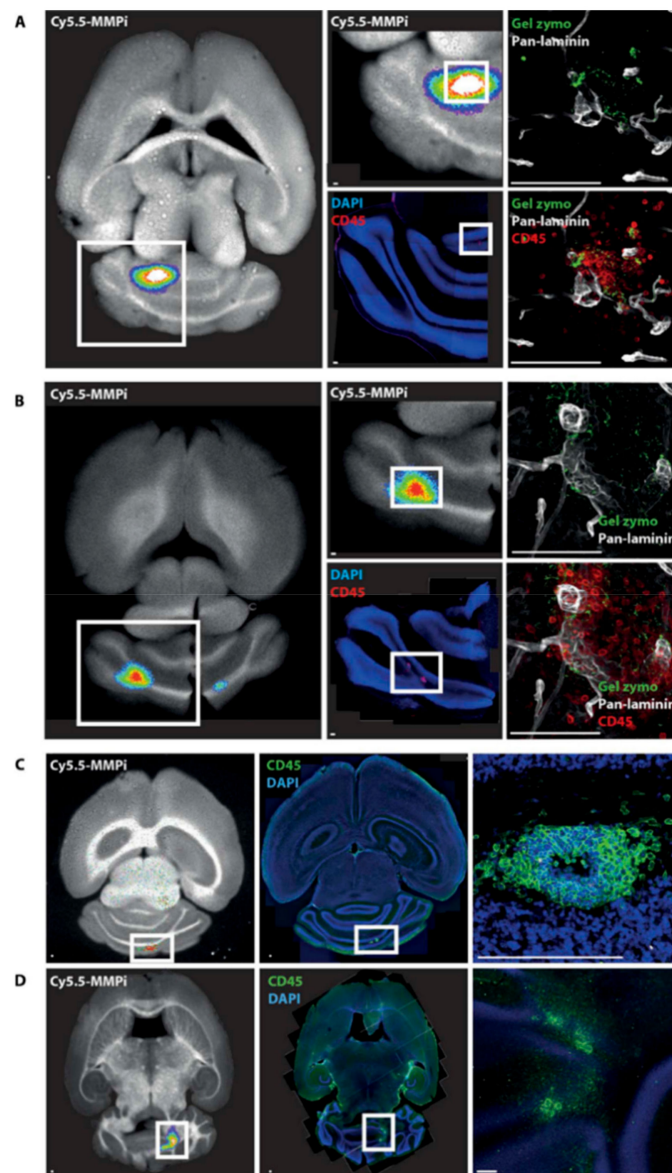


Figure 8. Cy5.5-MMPI 21 tracer signal localizes at sites of perivascular cuffs and gelatinase activity in vivo. (A,B) Five hours after intravenous injection of 21 (4 nmol) in two score 3 EAE mice, the brains were sliced into thick sections (200 μ m) and imaged by FRI; color-encoded near infrared fluorescence and white-light images were captured and matched (left images). The same brain slices were then analyzed by in situ zymography for detection of activated gelatinases and subsequently stained with pan-laminin to define vessel borders and with CD45 antibodies to visualize inflammatory infiltrates (right images). Scale bars, 100 μ m. (C,D) FRI of thick EAE brain sections (200 μ m) at 5 h after intravenous injection of (4 nmol). Overlay of color-encoded near-infrared fluorescence image with significant MMP activity (red) projected onto the white-light image. The same brain slice was subsequently immunofluorescently stained with CD45 antibody; blue signal. 4',6-diamidino-2-phenylindole (DAPI) staining of nuclei. Two separate brains are shown in (C,D). Scale bars, 100 μ m. Reprinted with permission from [103] Copyright © 2016, American Association for the Advancement of Science.

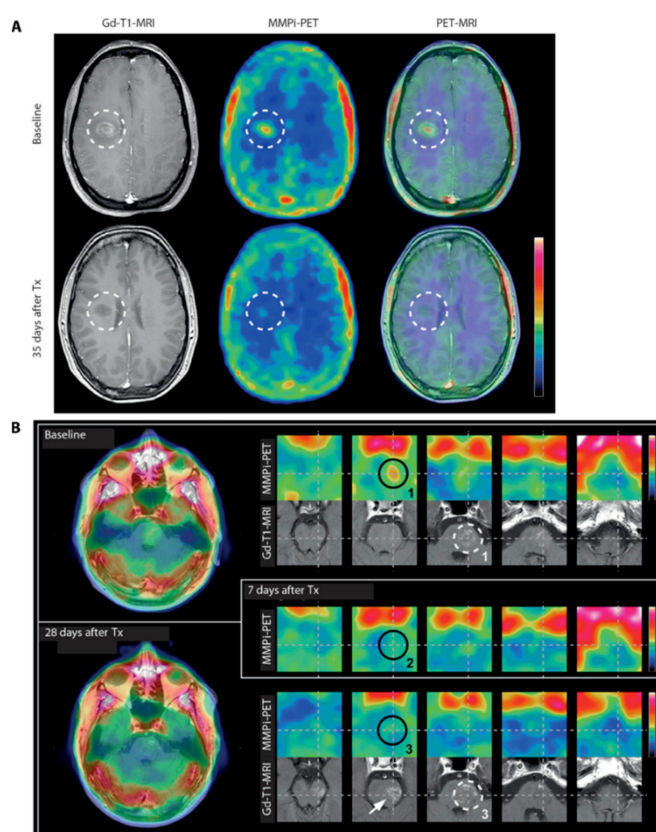
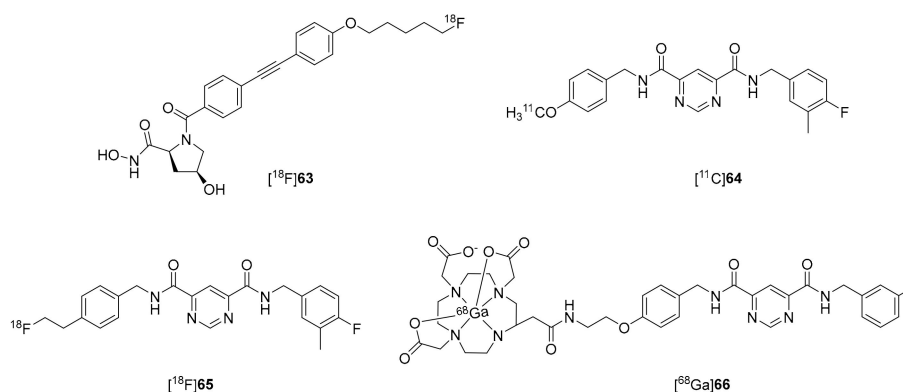


Figure 9. MMPI-PET [^{18}F]15 and Gd-T1-MRI in MS patients before and after anti-inflammatory treatment. (A) [^{18}F]15 and Gd-T1-MRI of patient #6 representing an acute attack of RRMS (EDSS 2.0) at baseline (top) and at 35 days after treatment (Tx) (methylprednisolone) (bottom); white dashed circles mark colocalization of the MMPI-PET signal and contrast enhancement in the MRI scan, which resolves by 35 days after treatment, correlating with a residual MMPI-PET signal. Left: Gd-T1-MRI. Middle: [^{18}F]15 scan 30 to 60 min after injection co-registered to the Gd-T1-MRI scan. Right: An overlay of Gd-T1-MRI and (PET-MRI). (B) Patient #2 represents an acute attack of RRMS. Left: Single co-registered [^{18}F]15 (30 to 60 min after injection) and Gd-T1-MRI slice at baseline (top) and 28 days after treatment (1 g of methylprednisolone, three times per day) (bottom). Right: Five consecutive PET (upper row) and corresponding MRI (bottom row) slices at baseline (top panel), 7 days after therapy (middle panel, PET only), and 28 days after therapy (bottom panel). Note the intense contrast enhancement in Gd-T1-MRI (maximum, white dashed circle #1) with spatially adjacent but not identical MP activity (black circle #1) at baseline. Reduced MMPI-PET signal at 7 days after therapy (black circle #2), and by 28 days after therapy, the MMPI-PET signal remains low (black circle #3), but the Gd-T1-MRI signal now colocalizes to the original MMP active area from the baseline scan. Reprinted with permission from [103] Copyright © 2016, American Association for the Advancement of Science.

5. MMPIs Labelled Imaging Agents for Rheumatoid Arthritis (RA), and Osteo Arthritis (OA)

MMP-13 is overexpressed in the cartilage of patients with OA. The unlabeled Compound 63 (Scheme 15) is a picomolar MMP-13 inhibitor ($\text{IC}_{50} = 0.07 \text{ nM}$) with high selectivity towards other MMPs and ADAMs [105]. Interestingly, [^{18}F]63 exhibited excellent serum stability, and favorable in vivo biodistribution, which may be useful for PET imaging in RA and OA.



Scheme 15. Chemical structure for compounds 63–66.

Selective and potent MMP-13 inhibitors **64–66** were synthesized by Hugenberg et al. and successful radiolabeling was performed with ^{11}C , ^{18}F , and ^{68}Ga , respectively [106]. Although $[^{11}\text{C}]\mathbf{64}$ and $[^{18}\text{F}]\mathbf{65}$ showed moderate metabolic stability, the more hydrophilic $[^{68}\text{Ga}]\mathbf{66}$ showed an enhanced blood retention time in biodistribution studies. Nearly no elimination of the $[^{68}\text{Ga}]\mathbf{66}$ tracer-associated activity from the liver was detected, which lead the authors to assume that the tracer somehow accumulates in the liver, due to the metabolism. They concluded that radiolabeled selective MMP-13 inhibitors-based PET tracers could enable earlier and more specific diagnosis of several MMP-13 related diseases, such as OA and RA.

6. MMP-13 Labelled Imaging Agents for Chronic Obstructive Pulmonary Disease (COPD) and Lung Inflammation

The synthesis and application of $[^{18}\text{F}]\mathbf{45}$ in cancer was described by Elsinga and colleagues as described before [92]. In 2015, they studied its utility in COPD [107]. Dynamic microPET studies in air or cigarette smoke (CS) exposed BALB/c mice showed a 2-fold increased the uptake in mice exposed to CS compared to air exposed mice (Figure 10).

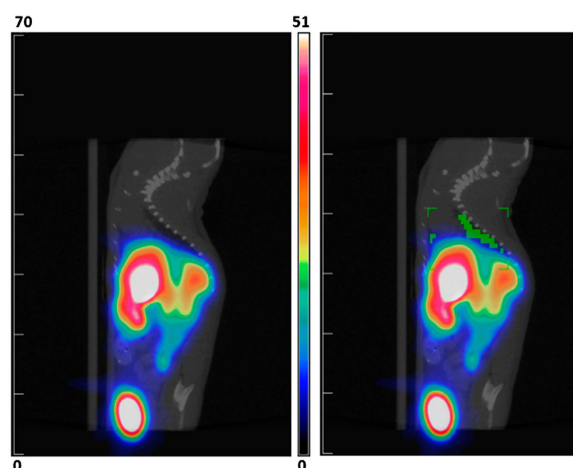
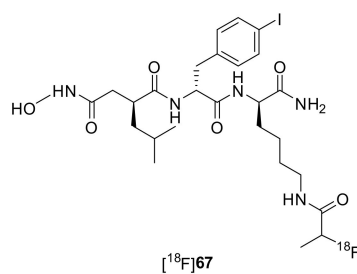


Figure 10. In vivo $[^{18}\text{F}]\mathbf{45}$ microPET/CT images of a mouse exposed to cigarette smoke (CS) shown in a sagittal view. The right view indicates the ROIs (Regions Of Interest) drawn in the lung. The microPET images correspond to the sum of all the frames from 12 to 90 min p.i. of $[^{18}\text{F}]\mathbf{45}$. Reprinted with permission from [107] Copyright © World Molecular Imaging Society 2015.

Temma et al. developed $[^{18}\text{F}]\mathbf{67}$ (Scheme 16) for MMPs-targeted PET [108]. Non-radiolabeled **67** exhibited IC_{50} in the nanomolar range (MMP-2 = 4.4, MMP-9 = 8.1, MMP-12 = 1.5, MMP-13 = 4.1 nM). The in vivo and ex vivo PET confirmed a 2-fold increased accumulation in the CS exposed lungs compared to normal mice (Figure 11).



Scheme 16. Chemical structure 67.

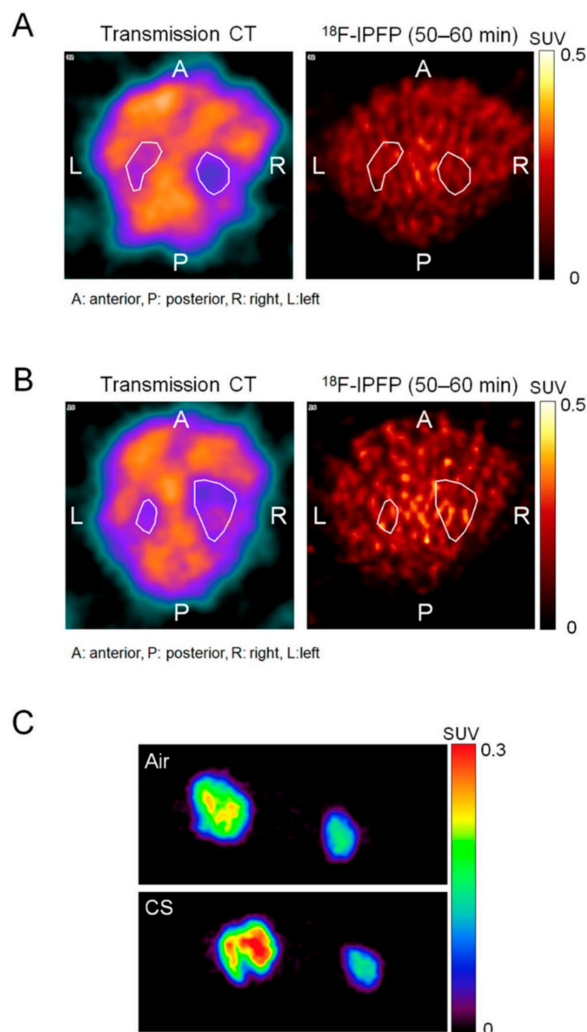
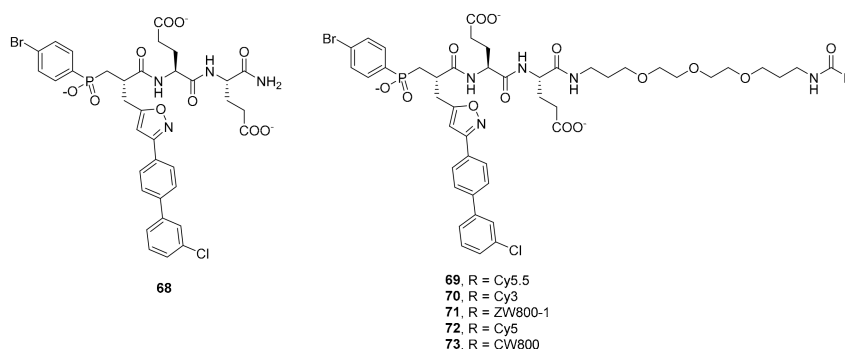


Figure 11. Transverse view of transmission CT and PET images at 50–60 min after injection of [¹⁸F]67 IPFP in the (A) Air and (B) CS groups. (C) Maximum intensity projection images of removed lungs (left) and heart (right) from the Air (upper) and CS (lower) groups. Reprinted with permission from [108] copyright © 2018, Springer Nature.

A macrocyclic hydroxamate based radiotracer [^{99m}Tc]59 was mentioned before for its utility in early vascular imaging and AAA [109,110]. Further, in vivo and ex vivo studies of [^{99m}Tc]59 in murine models of COPD demonstrated its higher uptake in lung tissue.

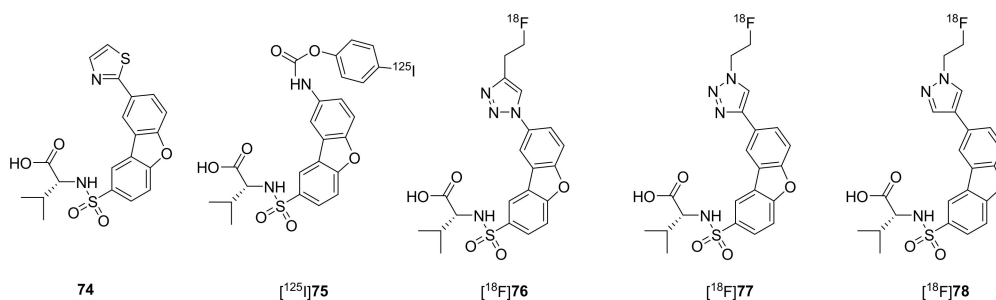
Sadeghi et al. [111] developed fluorescent probes (69–73) (Scheme 17) to image active MMP-12 in inflammation and aneurysm. Among these probes, 70 ($K_i = 6.1$ nM) and 71 ($K_i = 3.4$ nM) presented the higher potency and selectivity towards MMP-12. Animals injected with 71 showed the highest

signal in previously implanted sterile polyvinyl material (sponges) harvested for 60 min and a specific aneurysmal carotid artery when compared to a non-specific (scramble) control probe in WT mice.



Scheme 17. Chemical structure for compounds 68–73.

Mukai and col. [112] described **74** (Scheme 18), a dibenzofuran sulfonamide-based MMPI. Interestingly, due to the specific interactions with the S1' pocket, compound **74** exhibited more than a 150-fold activity for MMP-12 over MMP-8 and -13. Moreover, it prevented lung inflammation induced by recombinant human MMP-12 in C57BL/6 mice [113]. Inspired by these results, Mukai and colleagues designed and synthesized novel MMP-12-targeted dibenzofuran radiolabelled compounds with a variety of linker structures such as carbamate, amide, and sulfonamide. Among these, the best results were obtained for the carbamate linker present in compound [¹²⁵I]**75** (IC₅₀ = 8.5 nM).



Scheme 18. Chemical structure for compounds 74–78.

Wagner and colleagues designed and synthesized a series of dibenzofuran unlabeled sulfonamides **76–78** [36]. These compounds revealed excellent inhibitory activities (IC₅₀ values: 0.0004–0.19 nM) and high selectivity (≥25 fold) for MMP-12 and to date, they are the most potent MMP-12 inhibitors described in the literature.

Preliminary biodistribution and PET studies in C57BL/6 mice showed that the corresponding radiotracers [¹⁸F]**76–78** exhibited no specific uptake in non-excretion organs, indicating them as promising MMP-12 based PET imaging agents.

7. Summary and Future Perspectives

In summary, in last two decades, several fluorescent-labelled or radio-labelled MMPIs have been developed as imaging agents for different diseases. Remarkably, pilot-clinical studies of [¹⁸F]**15** in MS patients described by Wagner et al. form an excellent basis for the feasibility of MMPI probes toward clinical trials [104]. A combined in vitro and in vivo evaluation revealed that molecular imaging probes based on MMPIs could have potential as validated imaging agents, but further research is necessary to reach clinical application. Although biodistribution studies of certain MMPI radiotracers in wild type mice have been described, further extensive SPECT/PET preclinical studies with proper controls in

murine disease models (at least 5 mice per group), metabolite formation, stability studies, and toxicity studies are essential to confirm their clinical utility.

The clinical translation of MMPs for PET or SPECT imaging as well as other prognostic and diagnostic biomarkers, requires time and is very costly. Therefore, it is crucial to select the candidates that have the highest possibilities of success to be translated into the clinic, based not only in the abovementioned preclinical models and pharmacokinetic studies, but also on economic perspectives.

8. Conclusions

The overexpression of different MMPs has been associated with a number of relevant diseases such as cancer, atherosclerosis, stroke, arthritis, cardiovascular diseases, periodontal disease, respiratory tract disorders, glomerulonephritis, abdominal aortic aneurysm expansion, inflammatory bowel disease, neurodegeneration, chronic obstructive pulmonary disease, multiple sclerosis and liver fibrosis. In recent years, many MMPIs have been developed as novel therapeutics for these disorders, with some of them having entered clinical trials. More recently, numerous MMPIs labeled imaging agents have emerged as powerful tools for the early diagnosis and assessment of the efficacy of currently approved therapies. The structure-based design of highly selective MMPIs broadens the possibilities of succeeding in the development of clinically useful imaging tools. This knowledge, gathered by a wide number of research groups, would undoubtedly allow the validation of specific MMPs as relevant biomarkers in different diseases.

This review summarizes the main achievements in the field of MMPIs imaging probes and constitutes an important contribution to support further research in this area. Our research group has designed, in the last years, highly potent and selective inhibitors of MMP-2, while avoiding other MMPs, including the highly homologous gelatinase MMP-9 [114–118]. The structure based drug design on MMP-13 carried out by the authors has also provided very interesting and highly selective inhibitors [119]. All these compounds constitute an important starting point for the development of imaging probes. Based on this experience, the authors are currently outlining the design strategies to obtain ^{18}F marked analogues for PET and fluorochrome derivatives for fluorescent detection.

As highlighted in this review, a combination of medicinal chemistry and chemical biology tools has become essential for the detection of diseases in the early stages remarkably improving the treatments. MMPs have demonstrated to be relevant biomarkers for several of the most prevalent diseases. Although many contributions have been reported to date, a deeper knowledge of the involvement of these metalloenzymes will afford, in the near future, small molecule candidates as molecular imaging probes which can be useful in the clinical practice.

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