Novel matrix metalloproteinase inhibitors: an updated patent review (2014 - 2020)

Submitted version of the published article:
Lenci, E., Cosottini, L., & Trabocchi, A. (2021). Novel matrix metalloproteinase inhibitors: an updated patent review (2014 - 2020). Expert Opinion on Therapeutic Patents, 31(6), 509–523.
https://doi.org/10.1080/13543776.2021.1881481

Novel matrix metalloproteinase inhibitors: an updated patent review (2014-2020)

Abstract

Introduction: Matrix MetalloProteinases **(**MMPs) are key enzymes in several pathophysiological processes connected to the extracellular matrix (ECM) degradation. Earlier clinical trials evaluating broad spectrum MMP inhibitors as cancer therapeutics failed to succeed, and resulted in toxic side effects, such as musculoskeletal pain and inflammation, due to poor selectivity. As it is now recognized that some MMPs are essential for tumor progression and metastasis, but others play host-protective functions, selective MMP inhibitors are needed, and their interest has grown also for therapeutic applications beyond cancer, such as infectious, inflammatory and neurological diseases.

Areas covered: This updated review describes new patents concerning MMP inhibitors published within January 2014 and June 2020, with therapeutic applications spanning from cancer to inflammatory and neurological disorders.

Expert opinion: Although the number of patents has decreased with respect to previous decade, new applications provide selective matrix metalloproteinase inhibitors for therapeutic treatments beyond cancer. For several applications, the need of selective inhibitors resulted in the development of new non-hydroxamate compounds, paving the way towards a renewed interest towards MMPs as therapeutic targets. In particular, inhibitors able to cross the blood-brain barrier have been disclosed and proposed for the treatment of neurological conditions, infections, wound healing and cancer.

Keywords: angiogenesis, articular cartilage, cancer, protease inhibitor, zinc-binding group

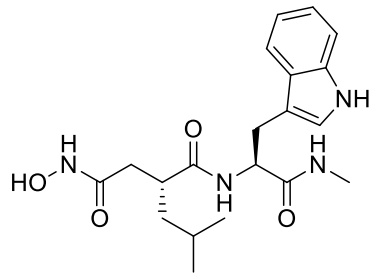
Article highlights

- MMPs are key in several physiological processes connected to the extracellular matrix (ECM) degradation, including growth, wound healing and other functions for tissue reorganization, as well as pathological conditions with increased MMP activity
- New patents on selective inhibitors against gelatinases, collagenases, membrane-type and elastase subgroups, released within January 2014 and July 2020 are herein summarized, reporting general formula structures and representative compounds claimed in each patent.
- Selectivity among closely related MMPs has been achieved by developing inhibitors with the hydroxamate being replaced with heterocyclic scaffolds as the zinc binding groups.
- A promising application of patented MMP inhibitors is the development of a molecular construct containing a chemical probe for molecular imaging studies to follow drug distribution and measure drug efficiency.
- The recent development of MMP inhibitors capable to cross the blood-brain barrier can be used for the treatment of neurological conditions and cancer.

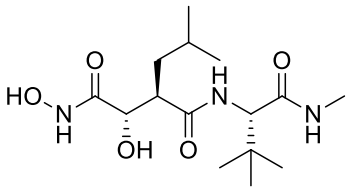
1. Introduction

Due to their key role in the degradation of several extracellular matrix components, matrix metalloproteinase (MMP) are critical for a variety of physiological and pathological processes, and are promising targets for new drugs to combat a variety of different diseases [1]. The interest in developing inhibitors has exploded over three decades ago but experienced a setback at the beginning of this century, due to the failure of several high-profile clinical trials [2]. Even the number of patents on MMPs inhibitors decreased significantly [3-4], despite research is still active to better clarify the involvement of MMPs in different pathologies, spanning from cancer and inflammatory diseases, to neurological and immunological disorders [5-7]. It is now clear that initial MMP inhibitor clinical trials were held too prematurely and mixed conclusions were drawn from the wrong selection of animal model studies [8]. Also, most of the clinical trials were conducted with compounds that

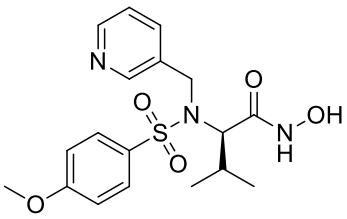
showed low bioavailability and poor metabolic profile, and more importantly showing the inhibition of several MMPs simultaneously, as well as other metalloenzymes [9]. This lack of specificity of first generation of MMPs inhibitors (including Ilomastat, Marimastat and CGS-27023A (Figure 1, up), has been identified as the major responsible for dose-limiting side effects/toxicity issues, specifically with respect to the development of arthralgia, myalgia, tendinitis, musculoskeletal syndrome (MSS) and gastrointestinal disorders [10]. It is now well known that each MMPs has a different role in a given disease, and the role of MMPs may change from detrimental to beneficial, or vice versa, during the course of disease. Most of these first generation inhibitors are hydroxamic acid-based compounds. As this group has poor selectivity for Zn^{2+} over other divalent first row transition metals [11], these compounds often were found to inhibit other metalloenzymes, including those of the ADAM (A Disintegrin And Metalloprotease) family. Also, hydroxamic acids are highly susceptible to human metabolic reactions, that transform them into the less active carboxylic acids or amides, releasing the toxic hydroxylamine.



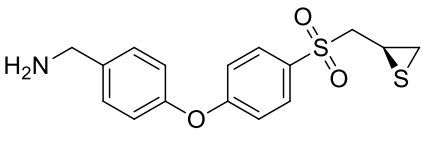
Ilomastat



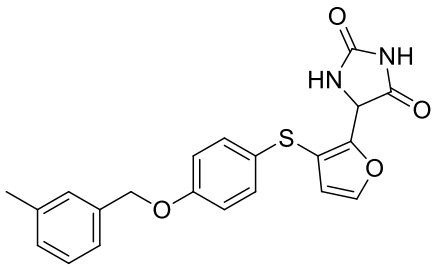
Marimastat



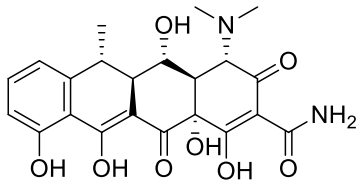
CGS-27023A



ND336
under preclinical trials
for the treatment of diabetic foot ulcers



FP-025
under phase II clinical trials
for the treatment of asthma and COPD



Periostat
approved
for the treatment of
periodontal diseases

Figure 1: (up) First-generation broad spectrum MMP inhibitors that have progressed but failed in preclinical/clinical trials; (down) second-generation MMP inhibitors that are currently under preclinical/clinical trials or have been approved by the FDA as drugs.

Until now, 23 members of the MMP family have been characterized and described. On the basis of their substrate specificity, their primary structures and their cellular localization, MMPs are subdivided into six classes (Table 1) [12]:

- Collagenases (MMP-1, -8 and -13),
- Gelatinases (MMP-2 and -9),
- Stromelysins (MMP-3, -10 and -11),
- Matrilysins (MMP-7 and -26),
- Membrane-type MMPs (MT-MMPs) (MMP-14, -15, -16, -17, -24 and -25)
- Others (MMP-12, -19, -20, -21, -23, -27 and -28).

Subgroup	Name	Tissue Distribution	Substrates
Collagenases	MMP-1 (Collagenase-1)	Fibroblasts, interstitial, endothelial cells, vascular walls, platelets, macrophages	Collagens (I, II, III, VII, VIII, X), aggrecan, casein, nidogen, serpins, perlecan, proteoglycan link protein, α1-antichymotrypsin, α1-antitrypsin, α1-proteinase inhibitor, IGFBP-3, IGFBP-5, IL-1β, L-selectin, ovostatin, recombinant TNF-α peptide, SDF-1
	MMP-8 (Collagenase-2)	Neutrophils, macrophages	Collagens (I, II, III, V, VII, VIII, X), aggrecan, laminin, nidogen α2-antiplasmin, pro-MMP-8
	MMP-13 (Collagenase-3)	Vascular walls, smooth muscle cells, macrophages	Collagens (I, II, III, IV), gelatin, aggrecan, versican, fibronectin, laminin, perlecan, tenascin-C, osteonectin, plasminogen activator 2, pro-TGF-β3, pro-MMP-9, pro-MMP-13, SDF-1
Gelatinases	MMP-2 (Gelatinase-A)	Endothelial cells, vascular walls, adventitia, leukocytes, dermal fibroblasts, platelets	Collagens (I, II, III, IV, V, VII, X, XI), aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein, versican, active MMP-9, active MMP-13, FGF R1, IGF-BP3, IGF-BP5, IL-1β, recombinant TNF-α peptide, TGF-β, β-amiloid protein precursor
	MMP-9 (Gelatinase-B)	Endothelial cells, vascular walls, adventitia, macrophages	Collagens (IV, V, VII, X, XIV), fibronectin, laminin, nidogen, proteoglycan link protein, versican, CXCL5, IL-1β, IL2-R, plasminogen, pro-TNFα, SDF-1, TGF-β
Stromelysins	MMP-3 (Stromelysin-1)	Endothelial cells, Vascular smooth muscle cells, platelets	Collagens (II, III, IV, IX, X, XI), aggrecan, casein, decorin, elastin, fibronectin, laminin, nidogen, perlecan, proteoglycan, proteoglycan link protein,

			versican, α1-antichymotrypsin, α1-proteinase inhibitor, antithrombin III, E-cadherin, fibrinogen, IGF-BP3, L-selectin, ovostatin, pro-HB-EGF, pro-IL-1β, pro-MMP-1, pro-MMP-8, pro-MMP-9, pro-TNFα, SDF-1
	MMP-10 (Stromelysin-2)	Uterus, preeclampsia	Collagens (III, IV, V), fibronectin, laminin, nidogen, pro-MMP-1, pro-MMP-8, pro-MMP-10
	MMP-11 (Stromelysin-3)	Brain, uterus	Laminin, α1-antitrypsin, α1-proteinase inhibitor, IGFBP-1
Matrilysins	MMP-7 (Matrilysin-1)	Endothelial cells, vascular walls, uterus	Collagens (IV, X), aggrecan, casein, elastin, enactin, laminin, proteoglycan link protein β4 integrin, decorin, defensin, E-cadherin, Fas-L, plasminogen, pro-MMP-2, pro-MMP-7, pro-MMP-8, pro-TNFα, transferrin, and syndecan α2-antiplasmin
	MMP-26 (Matrilysin-2, endometase)	Breast cancer cells	Collagen (IV), gelatin, casein, fibrinogen, fibronectin, fibrin, fibronectin, pro-MMP-2, β1-proteinase inhibitor
Membrane-Type MMPs	MMP-14 (MT1-MMP)	Human fibroblasts, Vascular smooth muscle cells, uterine, vascular walls	Collagens (I, II, III), aggrecan, dermatan, fibrin, fibronectin, laminin, nidogen, perlecan, tenascin, vitronectin, $\alpha_v\beta_3$ integrin, CD44, gC1qR, pro-MMP-2, pro-MMP-13, pro-TNFα, SDF-1
	MMP-15 (MT2-MMP)	Human fibroblasts, leukocytes	Collagen (I), aggrecan, fibronectin, laminin, nidogen, perlecan, tenascin, vitronectin, pro-MMP-2, pro-MMP-13, tissue transglutaminase
	MMP-16 (MT3-MMP)	Human leukocytes, vascular walls	Collagen (I), aggrecan, casein, fibronectin, laminin, perlecan, vitronectin, pro-MMP-2, pro-MMP-13
	MMP-17 (MT4-MMP)	Brain	Fibrinogen, fibrin, pro-MMP-2
	MMP-24 (MT5-MMP)	Leukocytes, lung, pancreas, kidney, brain	Chondroitin sulfate, dermatin sulfate, fibronectin, pro-MMP2, pro-MMP-13
	MMP-25 (Leukolysin, MT6-MMP)	Leukocytes	Collagen (IV), gelatin, fibrin, fibronectin, pro-MMP-2
Others	MMP-12 (metalloelastase)	Macrophages	Collagen (IV), elastin, fibronectin, laminin, vitronectin, plasminogen
	MMP-19 (RASI-1)	Liver	Collagens (I, IV), gelatin, aggrecan, casein, fibronectin, laminin, nidogen, tenascin
	MMP-20 (Enamelysin)	Tooth enamel	Aggrecan, amelogenin, cartilage oligomeric protein
	MMP-21 (XMMP)	Macrophages, fibroblasts, human placenta	α1-antitrypsin, aggrecan
	MMP-23 (CA-MMP)	Ovary, testis, prostate	Gelatin, casein, fibronectin
	MMP-27 (CMMP)	Endometrium, macrophages, breast cancer cells	Gelatin
	MMP-28 (Epilysin)	Skin, keratinocytes	Casein

Table 1: The family of matrix metalloproteinases (MMPs) with their tissue distribution and substrates. Highlighted in red are the MMPs discussed in this patent review.

Differences in MMP enzymes are the specificity of their preferred substrates, which can be either constituents of the extracellular matrix, or constituents of the basement-membrane, including aggrecan, collagen, elastin, fibronectin, gelatin and laminin. Also, they can be secreted or membrane-associated and distributed in different area of the body, expressed in the connective tissue or secreted by pro-inflammatory cells, including fibroblasts, osteoblasts, macrophages, neutrophils and lymphocytes. Despite these functional differences, members of the MMP family are highly conserved, with a high degree of sequence similarity in the catalytic domain, which consists of a Zn^{2+} ion coordinated by a tris(histidine) motif. However, it is now well known that the active site within the various MMPs presents subsites which differ for the depth, length and the amino acid composition. Thanks to the advance in the X-ray crystallography, NMR analysis and homology modeling studies, it has been possible over last years to develop a more selective second generation of inhibitors against specific MMPs believed to be involved in a given pathology. These compounds, with desirable selectivity, do not show high toxicity, especially those directed against MMP-2, MMP-9, MMP-13, and MT1-MMP, as these enzymes are not involved in musculoskeletal syndrome [13]. Also, most of these compounds do not possess the hydroxamic group as a zinc chelating motif, thus resulting to improved pharmacokinetic profile. Several MMP inhibitors are now under clinic trials (see ND336 [14] and FP-025 [15], Figure 1), and one of them (Periostat, doxycycline) has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of periodontal disease [16]. All these features reveal the renewed interest in the development of selective MMP inhibitors. This is especially true considering that MMPs inhibitors are important not only for the development of antitumor compounds [17-19], but also for blocking the degradation of articular cartilage in arthritis [20], or lessening the effects of neurological disorders [21]. In this updated review, we summarize new patents on the development of selective inhibitors against different classes of the MMP family, released within January 2014 and July 2020, following the precedent publication [3-4], reporting general formula structures and representative compounds that are claimed in each patent. Also, the efficacy of these representative compounds are provided with biological data available from

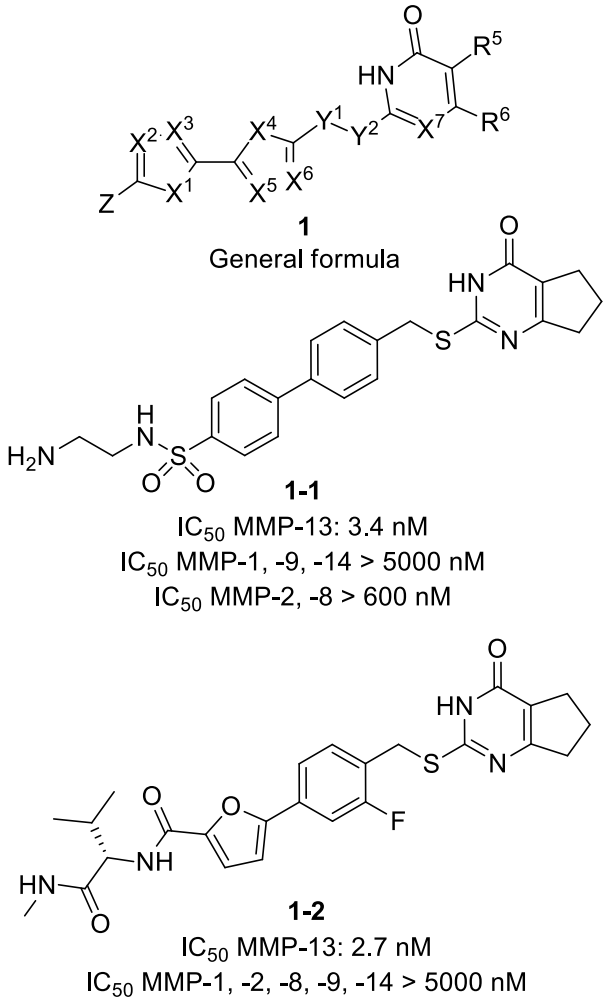
the patents. The inventions reported in the patents are mostly compounds that show binding affinity to MMPs with high selectivity. Although most of them are directed against gelatinases, due to the involvement of these targets in angiogenesis and metastasis [22], some examples have been reported also against MMPs of the collagenases, membrane-type and elastase subgroups. Another promising application of such compounds is the development of a molecular construct where the MMP ligand is conjugated to a chemical probe for molecular imaging studies to follow drug distribution and measure drug efficiency [23]. In this context, some patents have been released in recent years and described in this patent review.

2. Patents evaluation

2.1 Collagenases inhibitors

Collagenase-1 (MMP-1), collagenase-2 (MMP-8), and collagenase-3 (MMP-13) belong to the family of matrix metalloproteinases responsible for cleaving native fibrillar collagens [24]. Each collagenase shows some preferences for the substrate. In particular, MMP-1 cleaves more efficiently collagen of type III, MMP-8 type I- and MMP-13 type II-collagen [25]. In addition to fibrillar collagens, collagenases can cleave and activate various non-matrix proteins, including cytokines, chemokines and growth factors, thus regulating many pathophysiological processes involved in cell growth and survival, organ development and tissue regeneration. Among the three enzymes, MMP-13 is of particular interest, as it has a broad substrate specificity, cleaving not only collagen, but also gelatin, fibronectin, large tenascin C, aggrecan, versican, fibrillin and osteonectin [26] Also, it appears to be involved in the activation of TGF-β_3 and in the inactivation of different chemokines [27]. Considering its broad proteolytic capacity, MMP-13 is strictly controlled, and high levels of MMP-13 expression are found only in few physiological conditions, related to skeletal development [28]. On the contrary, many pathological conditions, including osteoarthritic cartilage, rheumatoid synovium, chronic cutaneous ulcers, chronic periodontitis, atherosclerosis, intestinal ulcerations and aortic aneurysms, are characterized by an over-expression of this collagenase [29-30]. For example, MMP-13 is

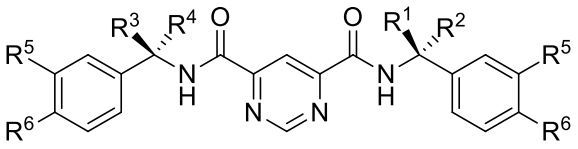
expressed in the cartilage of human osteoarthritis patients and not in the normal adult cartilage. For this reason, much research is devoted to the development of selective inhibitors able to target this enzyme, with potential applications in the treatment of these conditions, as well as cancer. Despite several MMP-13 inhibitors have been reported in the literature so far [31-33], no MMP-13 inhibitors have received FDA approval yet, typically because most of them are not selective, or show poor solubility or bioavailability. This is the reason why the search for new MMP-13 inhibitors is still active. In this context, researchers from Florida Atlantic University in 2017 reported the development of 1,2,4-triazole derivatives of general formula **1** as potent and selective MMP-13 inhibitors (Figure 2) [34]. In particular, compounds **1-1** and **1-2** (Figure 2) were found possessing high potency and selectivity as inhibitors of the MMP-13 enzyme, as revealed by IC50 values determined using a fluorescence resonance energy transfer assay with triple-helical peptides as substrates [35-36]. These compounds were found interacting with the catalytic active site without chelating the Zn^{2+} ion, and showing selectivity among closely related MMP enzymes, such as MMP-1, -2, -8, -9 and -14. The authors claim the application of these compounds in the treatment of osteoarthritis (OA), as MMP-13 is upregulated in the cartilage of patients affected by this pathology [37-38], as well as in inflammatory bowel diseases, melanoma cell invasion and breast cancer metastasis. The authors have also advanced in the development of MMP-13 inhibitors, by developing exosite-binding compounds that modulate the activity of MMP-13 by binding to the S1′ specificity pocket and not to the catalytic zinc ion, thus achieving better specificity among metalloproteinase subtypes and less toxicity [39-40].



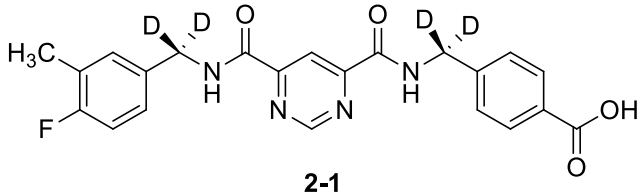
Z is $S(O)_2NR^{1A}R^{1B}$ or $C(=O)NHCH(R^{2A})C(=O)NHR^{2B}$;
R^{1A} is H, R^{1B} is variously substituted $(C_1\text{-}C_4)$-alkyl, $(C_3\text{-}C_7)$-cycloalkyl, 5- to 7-membered heterocycloalkyl or heteroaryl group; R^{2A} is $(C_1\text{-}C_4)$-alkyl, $(C_3\text{-}C_7)$-cycloalkyl, $(C_6\text{-}C_{10})$-aryl, 5- to 7-membered heterocycloalkyl or heteroaryl group; R^{2B} is H, $(C_1\text{-}C_4)$-alkyl, $(C_3\text{-}C_7)$-cycloalkyl, $(C_6\text{-}C_{10})$-aryl, 5- to 7-membered heterocycloalkyl or heteroaryl group; X^1 and X^4 are CH, O, S, $C(R^3)=C(R^3)$, NR^3 or $N=C(R^3)$; X^2, X^3, X^5 and X^6 are each independently O, S, N or CR^3 such that the resulting rings are aryl or heteroaryl; R^3 is independently at eacj occurrence H, $(C_1\text{-}C_4)$-alkyl or halo;
Y^1 and Y^2 are CHR, S, O, NR or a bond; X^7 is N or CR; R is H or $(C_1\text{-}C_4)$-alkyl; R^5 and R^6 are each independently H, $(C_1\text{-}C_4)$-alkyl, or halo, or form together with the ring carbon atoms to which they are bonded a 5- to 7-membered cycloalkyl or heterocyclyl ring.

Figure 2: Formula structure and representative compounds from Florida Atlantic University MMP-13 inhibitor patent.

Another entry to MMP-13 inhibitors is represented by the patent filed by researchers from the Aquilus Pharmaceuticals [41], describing inhibitors that can be used in the treatment of osteoarthritis and for other MMP-13 mediated symptoms characterized by ECM degradation or remodeling, in particular pain and inflammation. These compounds are characterized by a pyrimidinyl bis-amide group of general formula **2** (Figure 3). Among this class of compounds, **2-1** (in either diastereomeric and enantiomeric mixture), showed potent MMP-13 inhibiting activity, with an IC50 value of less than 100 nM for MMP-13 and high selectivity over other MMP enzymes [42].



2
General formula



2-1
IC_{50} MMP-13 < 100 nM

R^1, R^2, R^3 and R^4 are a hydrogen or a deuterium or an optionally substituted alkyl, a fluoroalkyl, cycloalkyl, alkenyl, alkynyl, heteroaryl group, or $COOR^{10}$, $CONR^{10}R^{11}$, SO_2R^{10} and $SO_2NR^{10}R^{11}$ wherein R^{10} and R^{11} are independently a deuterium or an optionally substituted alkyl, a fluoroalkyl, cycloalkyl, alkenyl, alkynyl, heteroaryl group or form together with the nitrogen atom a optionally substituted 3- to 8-membered ring; R^5 and R^6 are optionally substituted alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, aryl or heteroaryl group. The substiturents when taken together with the nitrogen or carbon atoms to which they are attached can form a optionally substituted 3- to 8-membered ring.

Figure 3: Formula structure and representative compound from Aquilus Pharmaceuticals MMP-13 inhibitor patent.

2.2 Gelatinases inhibitors

Gelatinases (MMP-2 and MMP-9) are, to best of our knowledge, the most studied subgroup of MMPs, especially because of their well-established role in cancer pathologies. In fact, high levels of these two enzymes are reported in many human tumors, including those affecting breast, brain, pancreas, colon-rectum, lung, bladder, skin and prostate [43]. Also, as they play a primary role in the angiogenic switch and cell invasiveness [44-46], the over-expression of these enzymes is often correlated with tumor aggressiveness and poor prognosis. In fact, in addition to the degradation of collagens and other extracellular matrix components, gelatinases are able to release several pro-angiogenic factors, such as the transforming growth factor beta (TGF-β) [47]. However, different roles have been observed for these two enzymes in the angiogenic switch, in platelet function, and in cell migration, as they activate diverse signaling pathways [48]. For example, MMP-2 interacts with integrin $\alpha_v\beta_3$ that activates hypoxia-induced transcription factor-1α (HIF-1α) and the expression of the vascular endothelial growth factor VEGF [49]. Differently, MMP-9 interacts with CD44 and tyrosine kinase epidermal growth factor receptor (EGFR), inducing the downstream cell signaling cascade that favors

cancer cell migration and invasion [50]. Also, it has been demonstrated how MMP-2 and MMP-9 inhibition can show beneficial or detrimental effects depending on the stage of tumor progression [9,51,52]. Thus, the selective inhibition of gelatinases is of relevance for a successful clinical trial, which has to be achieved despite the high structural similarity of the two gelatinases [53]. An exhaustive overview of patents reporting the development of gelatinase inhibitors has been reported in 2017 [54], however many other advances in this field have been reported both in the literature [55-59] and in patents recently. In fact, gelatinases inhibitors have a variety of different therapeutic applications that go beyond the development of antitumoral drugs. For example, MMP-2 inhibitors can be used in the treatment of heart injury, strokes and ischemic events [60], whereas MMP-9 inhibitors can find application in treating a variety of inflammatory and neurodegenerative disorders [61].

For example, in the international patent WO 2018/175670 [62], Ojima, Alford and coworkers reported the development of small molecule inhibitors of general formula **3** that can selectively target the hemopexin domain of MMP-9, without inhibiting its proteolytic function (Figure 4). These compounds can have applications as cancer treatment drugs. In fact, it is known that in proMMP-9, the hemopexin domain is required for the interaction with cell surface proteins, including CD44 and $\alpha_4\beta_1$ integrins, that are involved in cancer cell migration by activating the EGFR-MAP kinase-dependent signaling pathway [50]. The assessment of inhibitory activity of test compounds for proMMP9-mediated migration revealed that compounds such as **3-1** are able to disrupt the MMP-9 homodimerization, preventing the association of proMMP-9 with both $\alpha_4\beta_1$ integrin and CD44, resulting in the reduction of EGFR signaling pathways that normally induce cancer cell invasion and angiogenesis [63].

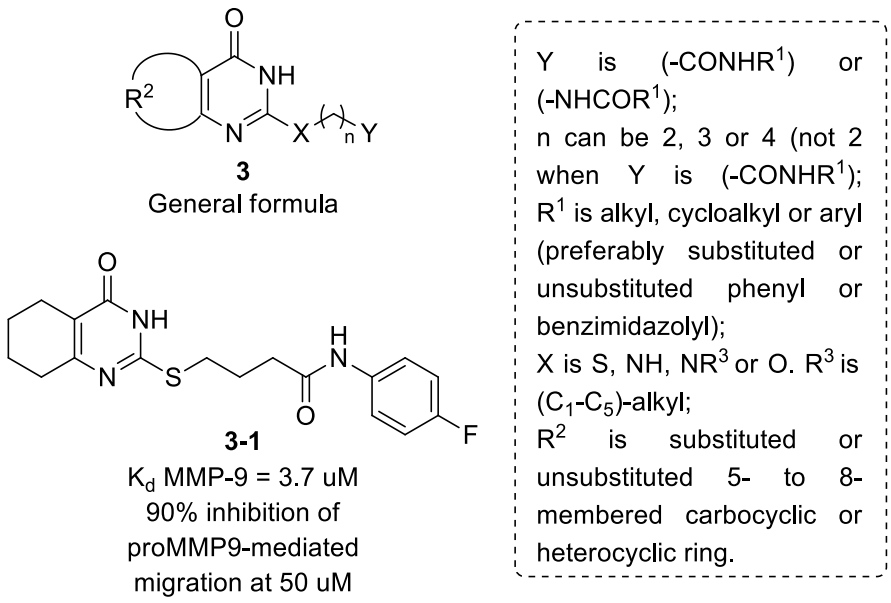
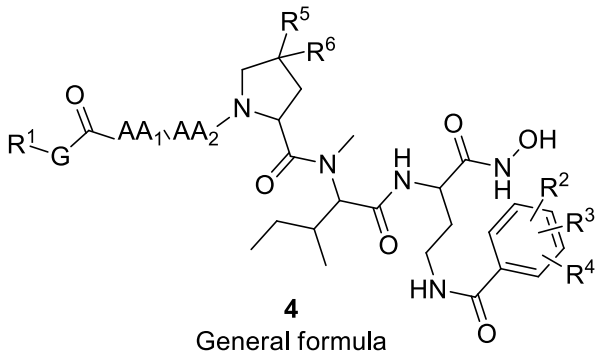


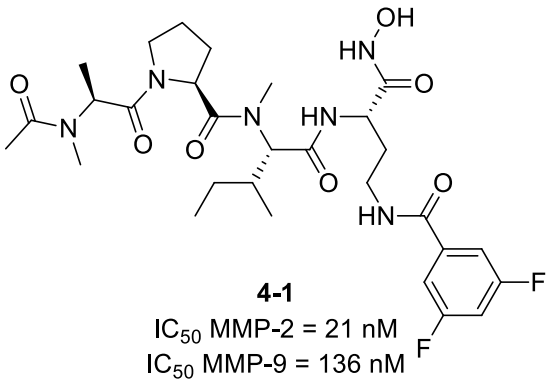
Figure 4: Formula structure and a representative compound from the State University of New York MMP-9 inhibitor patent.

Among MMP family members, MMP-2 and MMP-9 are the most abundant in the brain [64]. Under physiological conditions, these MMPs are involved in the regulation of the glutamatergic neuro-transmission, modulating physiological and morphological synaptic plasticity, as well as the hippocampal and prefrontal cortical function [65]. Gelatinases dysregulation has been linked to a number of neurological and psychiatric diseases, such as epilepsy [66], schizophrenia [67], Alzheimer disease [68], autism (in particular associated to fragile X syndrome), mental retardation, bipolar disorders, depression and drug addiction [69]. For this reason, it is particularly important to develop gelatinase inhibitors that are capable of crossing the blood-brain barrier. In this context, in the international patent WO 2017/085034 [70] spanish researchers of the company Iproteos developed new gelatinases inhibitors with general formula **4** (Figure 5) that are capable of inhibiting MMP-2 and MMP-9 with high potency and selectivity versus collagenases (MMP-1), stromelysins (MMP-3) and matrilysins (MMP-7). These compounds, in particular compound **4-1** and **4-2** [71], have the capacity to cross the blood-brain barrier and may be of particular interest for development of drugs targeting the CNS.

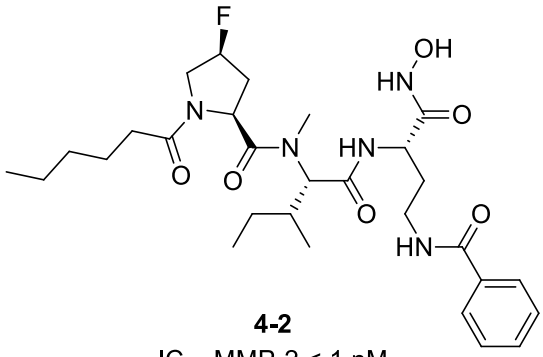


4
General formula

AA_1 is either absent or N-methyl-phenylalanine, N-methyl-tryptophan, N-methyl-tyrosine, N-methyl-iso leucine; AA_2 is either absent or N-methyl-phenylalanine, N-methyl-alanine, N-methyl -P-alanine and N-methyl-leucine; G is a linear or branched (C_1-C_{10})-alkylene, wherein one or more non-vicinal methylene moieties may be replaced by oxygen atoms; R^1 is H or phenyl; R^2, R^3 and R^4 are independently H or F; R^5 and R^6 are independently H or F or a salt thereof.



4-1
IC_{50} MMP-2 = 21 nM
IC_{50} MMP-9 = 136 nM



4-2
IC_{50} MMP-2 < 1 nM
IC_{50} MMP-9 = 10 nM

Figure 5: Formula structure and representative compounds from Iproteos gelatinase inhibitor patent.

2.3 Membrane-type MMP inhibitors

Membrane-Type MMPs (MT-MMPs) include four transmembrane MMPs: MMP-14 (MT1-MMP), MMP-15 (MT2-MMP), MMP-16 (MT3-MMP) and MMP-24 (MT5-MMP); as well as two glycosyl-phosphatidylinositol-anchored MMPs: MMP-17 (MT4-MMP) and MMP-25 (MT6-MMP).

The membrane type-1 MMP (MMP14) is expressed in most tissues and has wide substrate specificity, digesting the triple helical portions of interstitial collagen types I, II, and III and other ECM components, including aggrecan, dermatan, fibrin, fibronectin, laminin, nidogen and several cytokines (Table 1). A dysregulation of the MT1-MMP gene has been found in dysfunctional connective tissue metabolism and abnormal development of teeth and lungs, and an elevated expression of MMP-14 in various carcinomas [72]. MMP-14 is known to activate other MMPs, in particular gelatinases. The activation of pro-MMP-2 by MMP-14 is considered to contribute to local degradation of extracellular matrix during cell migration and proliferation, especially in the case of CNS disorders. For example, in cerebral ischemia, activation of MMP-2 by MMP-14 leads to the disruption of the BBB, followed by a second wave of damage mediated by MMP-9 [73]. Accordingly, some recent patents focused the attention on the simultaneous inhibition of either gelatinases and MMP-14 enzymes, by developing compounds that are capable of crossing the BBB. For example, Dr. Chang and Dr. Mobashery from the University of Notre Dame Du Lac (US) developed selective slow-binding MMP-2, MMP-9, and/or MMP-14 inhibitors that are water-soluble and able to cross the blood-brain-barrier [74]. These compounds, with general formula **5** (Figure 6), are believed to possess a key role in the control of neurological disorders, as they can reach therapeutic concentrations in the brain and be cleared without provoking CNS side effects due to their accumulation. In particular, compound **5-1** (ND-336) is able to inhibit simultaneously MMP-2, MMP-9 and MMP-14. On the contrary, compound **5-2** (ND-378), containing a *p*-acetamidomethyl substituent instead of the *p*-aminomethyl group, is a nanomolar inhibitor of MMP-2, with a high selectivity versus MMP-9 and MMP-14 (with a residence time bound to MMP-2 of 18.2 min, it results in a sustained inhibition of MMP-2 even at concentrations below the K_d values). The authors claim that compound **5-1** (ND-336), as well as some related analogues, can be used to treat cancer or a neurological condition, including epilepsy, amyotrophic lateral sclerosis (ALS), Alzheimer's Disease, Parkinson's disease or spinal cord injury. Also, such compounds can be used to accelerate the epithelialization of tissues adjacent to a wound, especially when it is caused by diabetes [14,75].

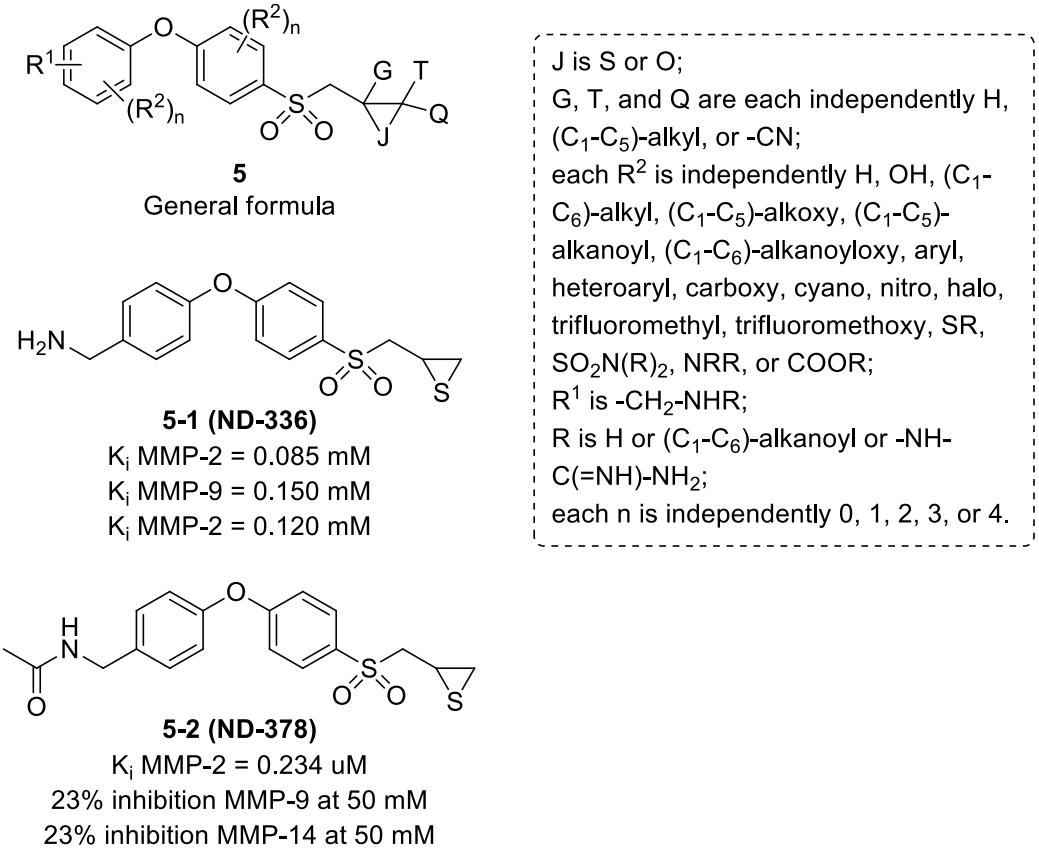
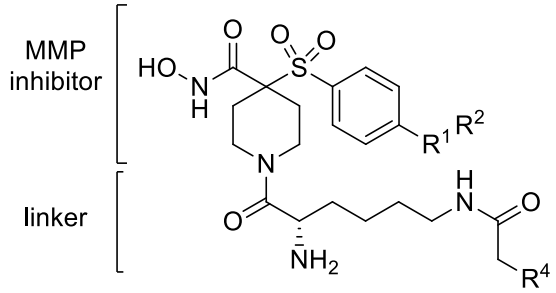


Figure 6: Formula structure and representative compounds from Iproteos MMP-2, MMP-9 and MMP-14 inhibitor patent.

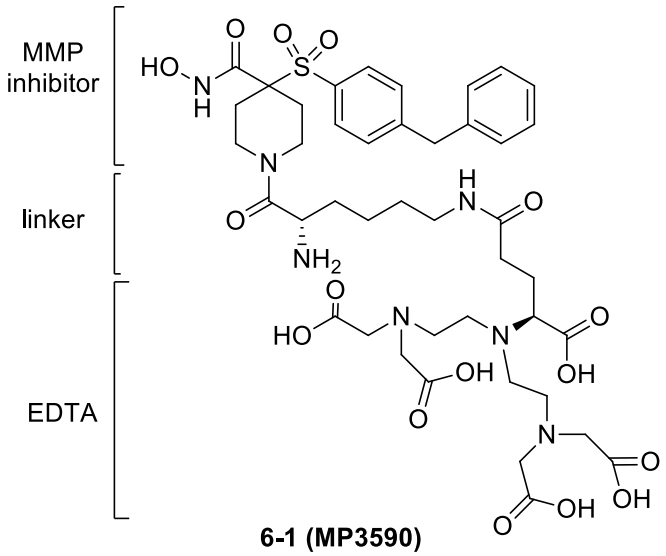
As the role of gelatinases and MMP-14 in angiogenesis and tumor growth still need to be completely characterized and studied, there is a need for inhibitors that can be used as targeting agents for molecular imaging studies. In this context, Freskos and coworkers from the Mallinckrodt company developed compounds that target MMP-14 (MT1-MMP) and MMP-2 with high affinity and conjugated them to a chelating agent for the complexation of ^{111}In, ^{67}Ga or ^{99m}Tc [76]. Such conjugate agent can be used as imaging probes to acquire contrast images in patients. The images are reconstructed to determine the concentration of MMP enzyme and to enable a diagnosis of the presence of a solid tumor, indicated by a locally high concentration of MMP enzymes (especially MMP-2 and MMP-14). These compounds have the general structure **6** (Figure 7), where the piperazine α-sulfone hydroxamate derivative is linked to a bifunctional amide linker and to a metal

chelator, such as ethylenediaminetetraacetic acid (EDTA), 4-[(1,4,8,11-tetraazacyclotetradec-1-yl)methyl]benzoic acid (CPTA), cyclohexanediaminetetraacetic acid (CDTA), ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), diethylenetriaminepentaacetic acid (DTPA) or 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA). For example, compounds **6-1** (MP3590) and **6-2** (MP3647) have been complexed with a radionuclide and used to diagnose the presence of solid tumors in patients by determining the concentration of MMP enzymes in the acquired contrast images. The same authors also conjugated these inhibitors to a series of dyes with different absorption/emission lengths that have been used to visualize tumors [77]. Moreover a fluorescent reporter prototype was developed by inserting the compound linked to DSPE-carboxyfluorescein moiety into a lipid bilayer via a PEG(5000) linker [78].

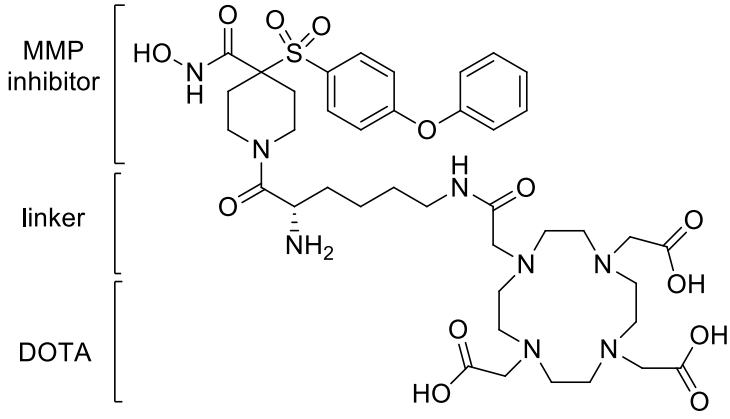


6
General formula

R^1 is O or S;
R^2 is pyridyl or optionally substituted phenyl with 1 to 5 substituents selected from the group consisting of OH, OCH_3, OCF_3 and CH_3;
R^3 is the linked metal chelator portion.



6-1 (MP3590)
IC_{50} MMP-2 = 0.8 nM
IC_{50} MMP-14 = 118 nM



6-2 (MP 3647)
IC_{50} MMP-2 = 0.7 nM
IC_{50} MMP-14 = 25.2 nM

Figure 7: Formula structure and representative compounds developed by the Mallinckrodt company that inhibit MMP-2 and MMP-14 with use as imaging agents.

Similarly, Becker and Lutz from the Loyola University of Chicago developed novel agents of general formula **7** where the same piperazine α-sulfone hydroxamate derivative is linked to a boron-rich carborane cluster [79,80]. These boron-containing moieties can be used in the treatment of different diseases, including various type of cancers and rheumatoid arthritis, through the so-called 'boron neutron capture therapy' (BCNT) [81]. BNCT is a cancer treatment that damages a tumor in a very precise manner. In fact, the drug containing ^{10}B atoms is selectively transported into tumor cells and after the irradiation with thermal neutrons, ^{10}B nuclei adsorb neutrons to form excited ^{11}B nuclei and emit α-particles and $^{7}Li^{3+}$ ions that damage the surrounding tissue, under a range of only 5-9 μm. These molecules, such as **7-1** and **7-2** (Figure 8) are able to inhibit MMP-2 and MMP-9, while being selective over the MMP-1, and can be used for the treatment of arthritic tissue by using the 'boron neutron capture synovectomy' (BNCS) with the same irradiation approach as described for the BNCT technique [82].

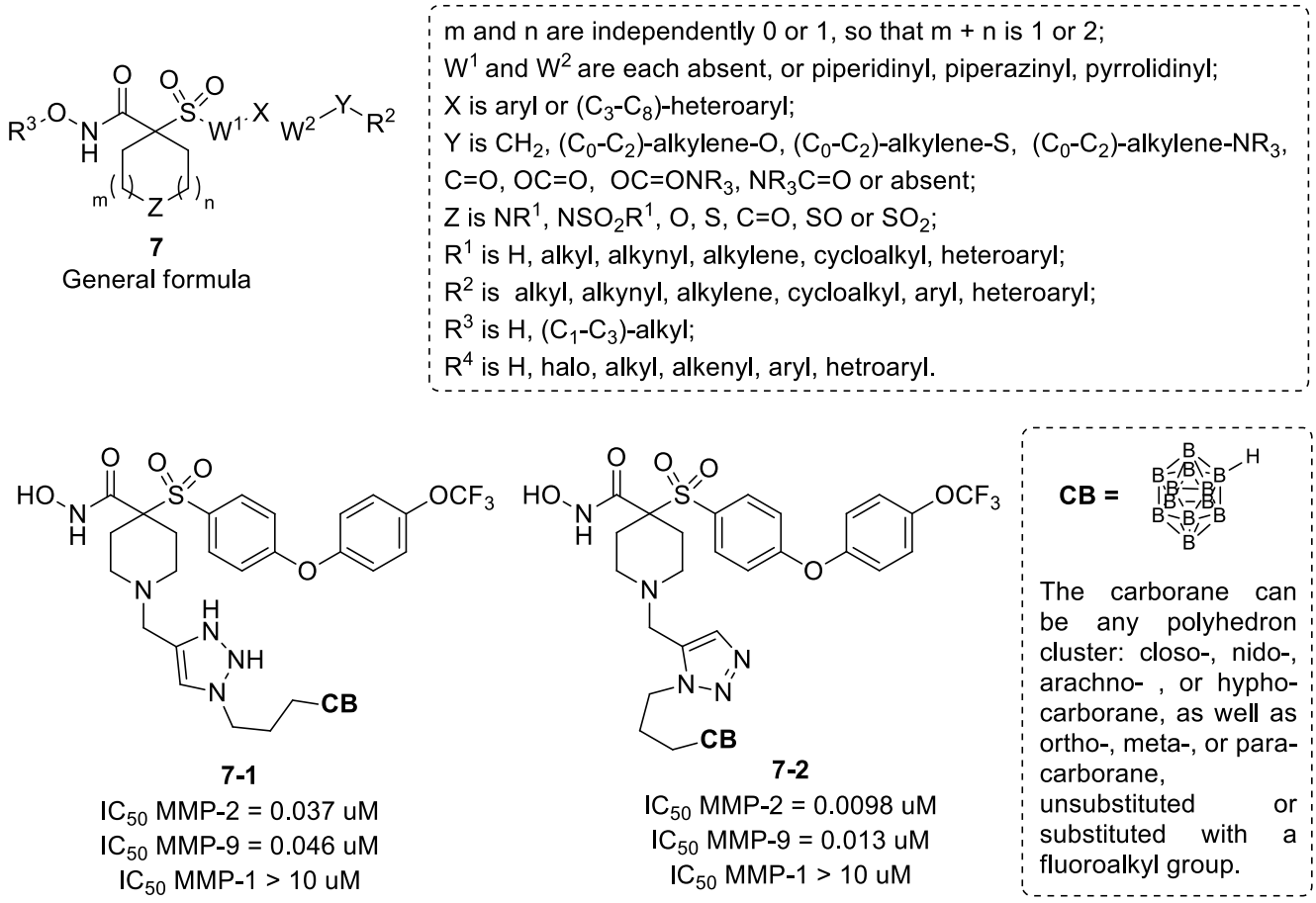


Figure 8: Formula structure and representative compounds developed by the Loyola University of Chicago that inhibit MMP-2 and MMP-9 can be used for 'boron neutron capture therapy' (BCNT) and 'boron neutron capture synovectomy' (BNCS).

Another membrane-type MMP that has been reported in several papers and patents is MMP-17 (MT4-MMP), as it is involved in the pathogenesis of many different diseases, including arthritis, cardiovascular disease, and cancer progression [83]. This MMP is a glycosylphosphatidylinositol-anchored proteinase and presents unique characteristics as compared to the other MMPs in terms of sequence homology, substrate specificity, and internalization mode. For example, it lacks the cytoplasmic tail, suggesting it may have different functional capabilities, and has been reported to be unable to activate pro-MMP-2 [84,85]. However, though the mechanisms for many pathophysiological actions of MMP-17 still have to be fully understood, this enzyme has been

considered as the target for the development of selective inhibitors. In particular, in a patent that also reports the discovery of one biological function of MMP-17, researchers from the Centro Nacional de Investigaciones Cardiovascular Carlos III reported a monoclonal antibody able to inhibit MMP-17 proteolytic activity. This monocolonal antibody (LEM-3/10) binds to a precise epitope within MMP-17 and can be used to treat MMP-17-related diseases by increasing the activity of patrolling monocytes, such as infections (e. g. *L. monocytogenes* infection), neurological diseases (e. g. Alzheimer's disease) and cancer [86]. In fact, as it has been demonstrated that MMP-17 cleaves αM integrin (Itgam), the inhibition of MMP-17 activity leads to enhanced αM integrin-dependent crawling of patrolling monocytes and its adhesion on the inflamed endothelium.

2.4 Human macrophage elastase inhibitors

Macrophage elastase (MMP-12) is a particular type of MMP. It exhibits all the characteristics of other MMPs, but it is preferentially produced by macrophages infiltrating into tissues where injury or remodeling is occurring [87]. MMP-12 has a broad substrate range. In addition to its activity against elastin, MMP-12 has been shown to digest collagen type IV, fibronectin, laminin, vitronectin and plasminogen. Although it seems to be mainly involved in chronic obstructive pulmonary disorder (COPD) [88], MMP-12 has a role also in the malignant progression of several types of cancer [89], as well as other diseases including asthma, emphysema [90], acute lung injury. Thus, MMP-12 is a target of great interest in medicinal chemistry and several inhibitors have been developed over the years [91]. Recently, researchers from the Foresee Pharmaceuticals reported the synthesis and assessment of hydantoin derivatives with general formula **8** (Figure 9) that have high activity and specificity for MMP-12 (see for example compound **8-1**) [92].

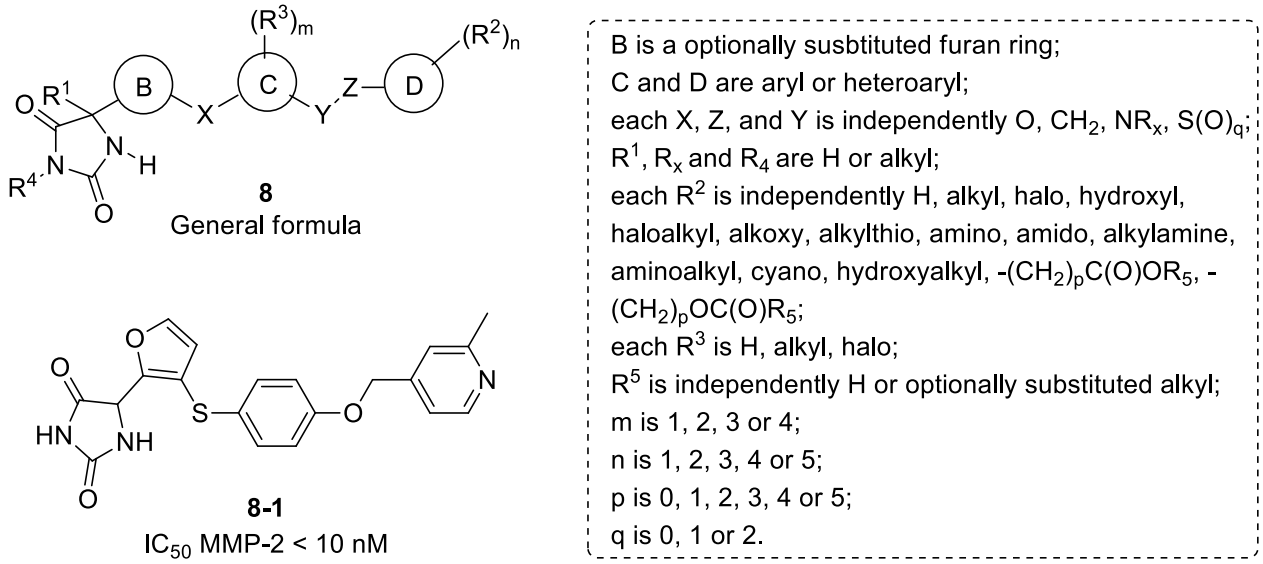


Figure 9: Formula structure and representative compound developed by researchers from Foresee Pharmaceuticals that inhibit MMP-12.

Also, researchers from the DSM IP Assets B. V. developed phosphinic peptide derivatives with general formula **9** (Figure 10) that are selective MMP-12 inhibitors [93]. These compounds can be used to prevent and treat age-associated fragmentation of the dermal matrix, that is usually due to the degradation of elastin and collagen by MMP-12. These compounds, in particular the most active **9-1**, can be used to prepare cosmetic compositions, such as cream, waxes, pastes, lotions, and so on, which are used to treat or improve the appearance of the skin and/or the scalp.

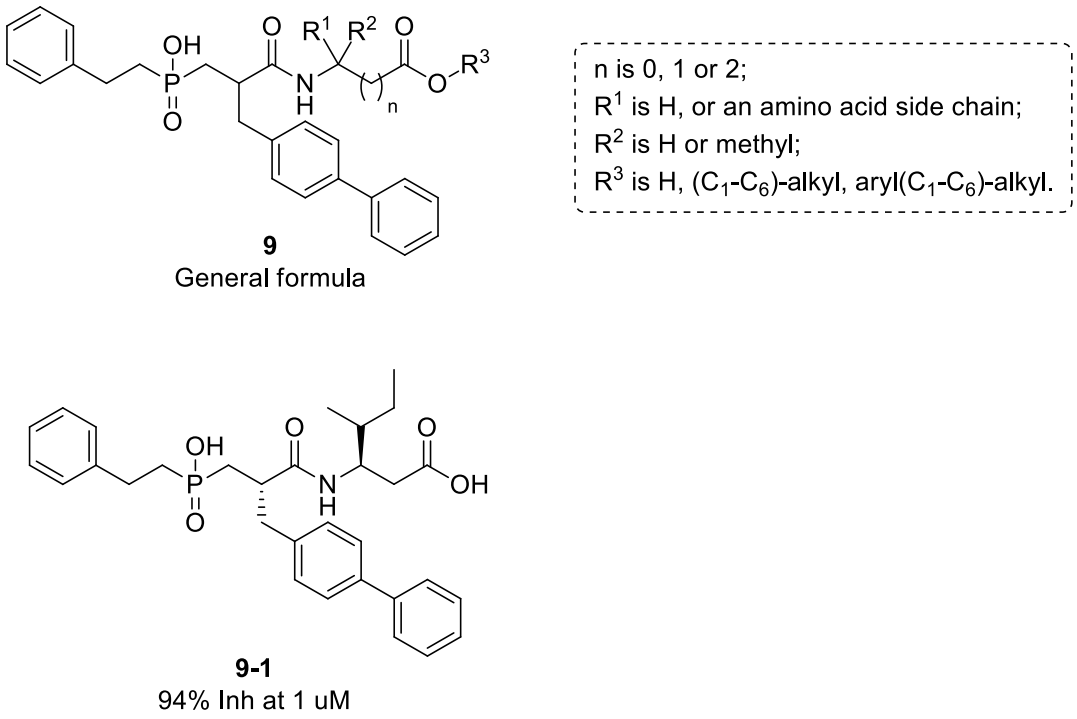
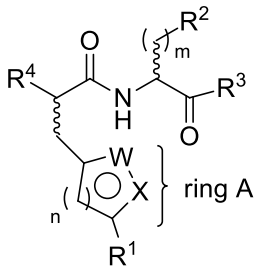


Figure 10: Formula structure and representative compounds developed by researchers from DSM IP Assets B. V. that inhibit MMP-12.

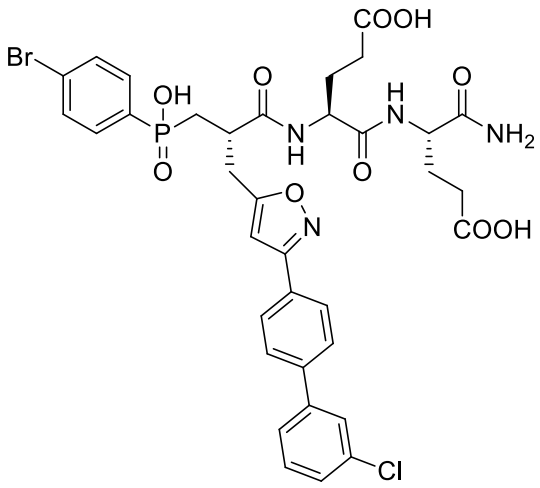
As an entry within the field of infectious diseases, researchers from the Commissariat a l'Energie Atomique et aux Energie Alternatives and the University of British Columbia developed selective MMP-12 inhibitors with general formula **10** (Figure 11) that can be used as antiviral agents, specifically acting on extracellular MMP-12 [94]. In fact, while MMP-12 promotes secretion of IFNα at the intracellular level, extracellular MMP-12 induces the clearance of secreted IFNα, thus decreasing the antiviral infection [95]. Application of these compounds can be found in the treatment of hepatitis B, C or D, or the infection of HIV, herpes viruses or rotaviruses, as well as other chronic respiratory tract infections. In order to be selective only for the extracellular MMP-12, these inhibitors should show membrane-impermeability, possessing at least 1 or 2 negative charges at a physiological pH. Indeed, compound **10-1** (RXP470.1) is membrane impermeable, thanks to its double negative charge, and once tested in model mice infected with coxsackievirus type B3, it showed a reduced morbidity and a significant retention of body weight compared to the control, by the inhibition of

MMP-12, which results in a highly elevated plasma IFNα levels (normally reduced by extracellular MMP-12) and in the blockade of the viral replication after 7 days of treatment.



10
General formula

n is 1 or 2;
m is 1, 2, 3 or 4;
R^1 is H or optionally substituted phenyl, biphenyl, pyrimidine, pyrazole, oxadiazole, thiadiazole, pyrrole, thiazole, thiophene;
R^2 is COOH, carboxamide, amino, 4-hydroxyphenyl, 1H-imidazole, hydroxyl, isopropyl, methyl;
R^3 is amino, carboxymethylpiperidine, carboxymethyl-3-aminophenyl, or a glutamate, homoglutamate, aspartate, glutamine, alanine, lysine, arginine, tyrosine, histidine, serine or leucine residue of L or D configuration;
R^4 is H, carboxymethyl, -CH_2COOH, benzylphosphinate.

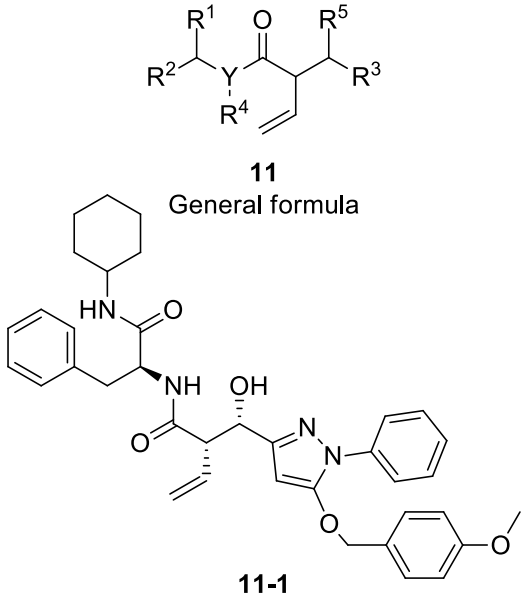


10-1 (RXP470.1)
inhibition of MMP12 with enhancement of IFNα plasma level and reduction of viral load and morbidity

Figure 11: Formula structure and representative compounds developed by researchers from Commissariat a l'Energie Atomique et aux Energie Alternatives and the University of British Columbia that inhibit MMP-12.

Finally, researchers from the Centre National de la Recherche Scientifique and the University de Reims Champagne-Ardenne developed α-vinyl carbonylated compounds with general formula **11** (Figure 12), that selectively inhibit MMP-12 and/or MMP-9 and are particularly useful for the prevention and treatment of chronic obstructive pulmonary disease (COPD), with reported

application for emphysema induced by cigarette smoke [96]. The inhibition of MMP-9 can assists the effect on MMP-12 inhibition, by increasing the resistance to inflammation and cartilage degradation. The hydrophobic portions of these compounds have an important role on the selectivity, as the long hydrophobic portion in place of the R^1 group, such as the biphenyl group of compound **11-1** (Figure 14) can fit better within the S1' subsite of the enzyme catalytic site of MMP-12.



R^1 is -CO-R^8, where R^8 is OH, NHR^{10}, $NR^{10}R^{11}$ with R^{10} and R^{11} are H, alkyl, alkynyl, OH, or optionally substituted phenyl, cyclohexyl, cyclopentyl;
R^2 and R^3 are alkyl, alkynyl, cycloalkyl, aryl, -(C_1-C_6)-R^{12}, where R^{12} is OH, phenyl, biphenyl, benzyl, cyclohexyl, cyclopentyl, COOH, $COOR^9$, SR^9 with R^9 = H, alkyl, alkynyl;
Y is N or O;
R^4 is H, alkyl, alkynyl, aryl, -(C_1-C_6)-R^{12}, where R^{12} as before;
R^5 is XR^6R^7, where X is O, N or S, R^6 and R^7 are H, alkyl, SO_2R^{13} with R^{13} = halogen, alkyl, alkynyl, aryl, -(C_1-C_6)-R^{12}, where R^{12} as before.

Figure 12: Formula structure and representative compounds from Centre National de la Recherche Scientifique and the Universite de Reims Champagne-Ardenne MMP-9 and MMP-12 inhibitor patent.

3. Conclusion

In this report the patents describing new matrix metalloproteinase inhibitors, published between 2014 and 2020 are described. Eleven patents were found and classified in four specific grouped targets comprising collagenases, gelatinases, membrane-type MMP and elastase. The compounds reported in the patents are small molecule entities that are used to treat, diagnose or prevent some MMP-induced-diseases as tumors, neurological condition or inflammatory diseases. The targets of these

compounds are few MMPs if compared with the whole class, and the patented inventions specifically refer to MMP-13, -2, -9, -12, -14 (MT1-MMP), -17 (MT4-MMP). These enzymes are of major importance for their implication in various pathological conditions beyond cancer that are described in the patents, including inflammatory and neurological disorders, chronic obstructive pulmonary diseases, development of atherosclerotic plaque and more. New chemical entities with selective inhibition profile have been identified and claimed, taking advantage of new chemical entities in place of the hydroxamate group as a zinc binding group.

4. Expert opinion

MMPs are key in several physiological processes connected to the extracellular matrix (ECM) degradation, including growth, wound healing and other functions for tissue reorganization, as well as pathological conditions with increased MMP activity. MMP inhibition has great therapeutic potential for the treatment of inflammatory disorders, although requiring better knowledge of MMP functions and the discovery of more potent and selective MMP inhibitors. Despite functional differences and roles of these enzymes in various pathological diseases, members of the MMP family are very much conserved, with a high degree of sequence similarity in the catalytic domain. This is nowadays the challenge in developing new molecular entities capable of showing selectivity among specific MMPs, in order to address the need of selective chemical probes, methods for diagnosis and small molecule drugs.

Although the number of patents on MMP inhibitors decreased over last years, recent applications have been filed to claim new compounds as selective inhibitors against gelatinases, collagenases, membrane-type and elastase subgroups, particularly addressing the development of antitumor compounds, but also compounds useful for blocking the degradation of articular cartilage in arthritis, or reducing the effects of neurological disorders. An innovative application on the use of MMP inhibitors is the development of molecular constructs where the MMP ligand is conjugated to a chemical probe for molecular imaging studies to follow drug distribution and measure drug

efficiency. Also, the recent development of MMP inhibitors capable to cross the blood-brain barrier may open the way towards new research addressing the need of therapeutic strategies for neurological conditions and brain cancer.

Concerning which MMPs have been recently taken into account, research towards MMP-13 inhibitors is active, as this collagenase is of special interest in pathological inflammatory conditions and no MMP-13 inhibitors have received FDA approval, yet, due to lack of selectivity and poor bioavailability. Also, the two gelatinases, MMP-2 and MMP-9, are still most studied due to their well-established role in cancer pathologies, being key players in the angiogenic switch and cell invasiveness. Actually, interest to gelatinases is related to the link between their dysregulation and a number of neurological and psychiatric diseases, and suitable inhibitors that are capable of crossing the blood-brain barrier have been patented, accordingly. Finally, phosphinic-based MMP-12 inhibitors have been claimed as antiviral agents, specifically acting on extracellular MMP-12 due to their membrane-impermeability provided by the presence of negative charges at a physiological pH. The patent evaluation over last seven years showed a decrease in the number of patents. Nevertheless, what has been disclosed is markedly different from prior art, with new non-hydroxamate compounds being developed as selective MMP inhibitors, and other compounds claimed for their capability in crossing the blood-brain barrier, with a direct effect on CNS diseases and brain tumors. We expect that in the coming years the interest towards MMPs as therapeutic targets will increase, given the validated role of such enzymes in a wide range of pathologies beyond cancer, that is still of primary importance, spanning from inflammatory to infectious and neurological diseases. Indeed, the quality of the inhibitors in terms of drug candidates for MMP-related diseases is key to give access to a range of clinical trials for the treatment of pathologies claimed in recent patents.

Acknowledgment

The authors acknowledge MIUR-Italy ("Progetto Dipartimenti di Eccellenza 2018-2022" allocated to Department of Chemistry "Ugo Schiff") and Fondazione Cassa di Risparmio di Pistoia e Pescia (Bando Giovani@Ricerca scientifica 2018) for financial support.

Declaration of interest

None to declare.

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers

[1] Fingleton B. Matrix metalloproteinases as valid clinical targets. Curr Pharm Design 2007;13: 333–346

[2] Dove A. MMP inhibitors: Glimmers of hope amidst clinical failures. Nat Med 2002;8:95 ****This article reviews the importance of MMPs in other clinical settings beyond cancer and arthritis, where evidence for MMP function contributing to the pathophysiology of disease is strong**

[3] Beckett RP, Whittaker M. Matrix metalloproteinase inhibitors 1998. Exp Opin Ther Patents 1998;8: 259–282

[4] Li N-G, Tang Y-P, Duan J-A, Shi Z-H. Matrix metalloproteinase inhibitors: a patent review (2011 – 2013). Exp Opin Ther Patents 2004;24:1039-1052

[5] Hu J, Van den Steen PE, Sang Q.-XA, Opdenakker G. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat Rev Drug Discov 2007;6:480–498 ***This review outlines the major classes of MMP inhibitors and gives advice about the differential roles of specific MMPs in pathophysiology obtained with animal models for drug discovery applications**

[6] Murphy G, Nagase H. Progress in matrix metalloproteinase research. Mol Aspects Med 2008;29:290–308

[7] Khokha R, Murthy A, Weiss A. Metalloproteinases and their natural inhibitors in inflammation and immunity. Nat Rev Immunol 2013;13:649–665 ***This work describes the role of metalloproteinases, including MMPs, in regulating in immune cell development and function, beyond their importance for degrading the extracellular matrix components**

[8] Fields GB. The Rebirth of Matrix Metalloproteinase Inhibitors: Moving Beyond the Dogma. Cells 2019;8: 984 ****This review is critical for understanding the failure of first generation MMP inhibitors and discussing new trends of their development**

[9] Vandenbroucke RC, Libert C. Is there new hope for therapeutic matrix metalloproteinase inhibition? Nat Rev Drug Discov 2014;13:904–927 ***Thorough account of the role of MMPs in inflammatory disorders and insight on their inhibition as a therapeutic approach for the treatment of such diseases**

[10] Rudek MA, Venitz J, Figg WD. Matrix Metalloproteinase Inhibitors: Do They Have A Place in Anticancer Therapy? Pharmacotherapy 2002;22:705–720

[11] Puerta DT, Cohen SM. A bioinorganic perspective on matrix metalloproteinase inhibition. Curr Topics Med Chem 2004;4:1551–1573

[12] Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Prog Mol Biol Transl Sci. 2017;147:1-73. ***This review provides an updated overview of the structural and functional information of the various MMP isoforms, as well as their involvement in different diseases**

[13] Devy L, Dransfield DT. New strategies for the next generation of matrix-metalloproteinase inhibitors: Selectively targeting membrane-anchored MMPs with therapeutic antibodies. Biochem Res Int 2011;2011: 191670

[14] Nguyen TT, Ding D, Wolter WR, et al. Validation of Matrix Metalloproteinase-9 (MMP-9) as a Novel Target for Treatment of Diabetic Foot Ulcers in Humans and Discovery of a Potent and Selective Small-Molecule MMP-9 Inhibitor That Accelerates Healing. J Med Chem 2018;61:8825–8837

[15] Amar S, Fields GB. Potential clinical implications of recent matrix metalloproteinase inhibitor design strategies. Expert Rev Proteomics 2015;12(5):445-447

[16] Boelen GJ, Boute L, d'Hoop J, et al. Matrix metalloproteinases and inhibitors in dentistry. Clin Oral Investig 2019;23:2823–2835

[17] Fields GB. Mechanisms of Action of Novel Drugs Targeting Angiogenesis-Promoting Matrix Metalloproteinases. Front Immun 2019;10:1278

[18] Lia N-G, Shib Z-H, Tang Y-P, Duan J-A. Selective matrix metallo proteinase inhibitors for cancer. Curr Med Chem 2009;16:3805-3827 ***This review focuses on selective MMP inhibitors with specific zinc-binding groups developed as antitumor agents since the late 90s, particularly addressing the structure of the compound classes in tabular view**

[19] Tauro M, McGuire J, Lynch CC. New approaches to selectively target cancer-associated matrix metalloproteinase activity. Cancer Metastasis Rev 2014;33:1043–1057

[20] Martel-Pelletier J, Welsch DJ, Pelletier JP. Metalloproteases and Inhibitors in Arthritic Diseases. Best Pract Res Clin Rheumatol 2001;15:805-829

[21] Chopra S, Overall CM, Dufour A. Matrix metalloproteinases in the CNS: Interferons get nervous. Cell Mol Life Sci 2019;76:3083–3095 ****This article reviews beneficial and detrimental roles of MMPs in CNS pathologies**

[22] Zheng H, Takahashi H, Murai Y, et al. Expressions of MMP-2, MMP-9 and VEGF are closely linked to growth, invasion, metastasis and angiogenesis of gastric carcinoma. Anticancer Res 2006;26:3579–3583

[23] Fields GB. Protease-activated delivery and imaging systems. In The Cancer Degradome—Proteases in Cancer Biology. Edwards D, Hoyer-Hansen G, Blasi F, Sloane B, Eds.; Springer: New York, NY, USA, 2008; 827–851

[24] Ala-aho R, Kähäri V-M. Collagenases in cancer. Biochimie 2005;87(3):273-286

[25] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression, Nat Rev Cancer 2002;2:161–174

[26] Knäuper V, López-Otín C, Smith B, Knight G, Murphy G. Biochemical characterization of human collagenase-3. J Biol Chem 1996;271:1544–1550

[27] Deng SJ, Bickett DM, Mitchell JL, et al. Substrate specificity of human collagenase 3 assessed using a phage-displayed peptide library. J Biol Chem 2000;275:31422-31427

[28] Inada M, Wang Y, Byrne MH, et al. Critical roles for collagenase-3 (MMP13) in development of growth plate cartilage and in endochondral ossification. Proc Natl Acad Sci USA 2004;101:17192-17197

[29] Alipour H, Raz A, Zakeri S, Djadid ND. Therapeutic applications of collagenase (metalloproteases): A review, Asian Pac J Trop Biomed 2016;6(11):975-981

[30] Morrison C, Overall C. Microarray and Proteomic Analysis of Breast Cancer Cell and Osteoblast Co-Cultures: Role of Osteoblast Matrix Metalloproteinase (MMP)-13 in Bone Metastasis. J Biol Chem 2011;286:34271-34285

[31] Nara H, Kori M. Discovery of novel, highly potent and selective matrix metalloproteinase (MMP-13) inhibitors with a 1,2,4-triazol-3-yl moiety as a zinc binding group using a structure-based design approach. J Med Chem 2017;60:608-626 ***This article reports an example of the discovery of compounds possessing a novel non-hydroxamate ZBG for highly potent and selective MMP-13 inhibition**

[32] Lia N-G, Shib Z-H, Tang Y-P, Duan J-A. New selective inhibitors of MMP-13 for inflammatory diseases: a patent evaluation (W02012151158). Expert Opin Ther Pat 2013;23:669-675

[33] Nara H, Sato K, Kaieda A, et al. Design, synthesis, and biological activity of novel, potent, and highly selective fused pyrimidine-2-carboxamide-4-one-based matrix metalloproteinase (MMP)-13 zinc-binding inhibitors. Bioorg Med Chem 2016;24:6149-6165

[34] Florida Atlantic University Board of Trustees. Selective matrix metalloproteinase-13 inhibitors. WO2018226837 (2018)

[35] Lauer-Fields JL, Fields GB. Triple-helical peptide analysis of collagenolytic protease activity. Biol Chem 2002;383:1095-1105

[36] Lauer-Fields JL, Broder T, Sritharan T, Chung L, Nagase H, Fields GB. Kinetic analysis of matrix metalloproteinase activity using fluorogenic triple-helical substrates. Biochemistry 2001;40: 5795-5803

[37] Takaishi H, Kimura T, Dalal S, Okada Y, D'Armiento J. Joint diseases and matrix metalloproteinases: A role for MMP-13. Curr Pharm Biotechnol 2008;9:47-54

[38] Neuhold LA, Killar L, Zhao WG, et al. Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. J Clin Invest 2001;107:35-44

[39] Roth J, Minond D, Darout E, et al. Identification of novel, exosite-binding matrix metalloproteinase-13 inhibitor scaffolds. Bioorg Med Chem Lett 2011;21(23):7180-7184

[40] Spicer TP, Jiang J, Taylor AB, et al. Characterization of selective exosite-binding inhibitors of matrix metalloproteinase 13 that prevent articular cartilage degradation in vitro. J Med Chem 2014;57:9598-9611

[41] Aquilus Pharmaceuticals, Inc. Matrix metalloproteinase inhibitors and methods for the treatment of pain and other diseases. WO2014062204 (2014)

[42] Baragi VM, Becher G, Bendele AM, et al. A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: Evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models. Arthritis Rheumatol 2009;60:2008-2018

[43] Bauvois B. New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: Outside-in signaling and relationship to tumor progression. BBA Rev Cancer 2012;1825:29-36 ****This review accounts for the significance of gelatinase inhibitors as anticancer agents and discuss new data useful for the design and development of novel gelatinase-targeting inhibitors**

[44] Vihinen P, Ala-aho R, Kahari VM. Matrix metalloproteinases as therapeutic targets in cancer. Curr Cancer Drug Targets 2005;5:203-220

[45] Fingleton B. Matrix metalloproteinase inhibitors for cancer therapy: the current situation and future prospects. Expert Opin Ther Tar 2003;7:385-397

[46] Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401-410

[47] Mu D, Cambier S, Fjellbirkeland L, et al. The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. J Cell Biol 2002;157:493-507

[48] Bergers G, Brekken R, Mcmahon G, Vu TH, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000;2:737–744

[49] Chetty C, Lakka SS, Bhoopathi P, Rao JS. MMP-2 Alters VEGF Expression via αVβ3 Integrin-Mediated PI3K/AKT Signaling in A549 Lung Cancer Cells. Int J Cancer 2010;127:1081–1095

[50] Dufour A, Zucker S, Sampson NS, Kuscu C, Cao J. Role of Matrix Metalloproteinase-9 Dimers in Cell Migration. J Biol Chem 2010;285:35944–35956

[51] Higashi S, Miyazaki K. Identification of amino acid residues of the matrix metalloproteinase-2 essential for its selective inhibition by beta-amyloid precursor protein-derived inhibitor. Biol Chem 2008;283:10068-10078

[52] Roomi MW, Monterrey JC, Kalinovsky T, Rath M, Niedzwiecki A. Patterns of MMP-2 and MMP-9 expression in human cancer cell lines. Oncol Rep 2009;21:1323-1333

[53] Deryugina, E. I.; Quigley, J. P. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 2006;25:9-34

[54] Xun L. Gelatinase inhibitors: a patent review (2011-2017). Expert Opin Ther Patents 2018;28:31-46

[55] Adhikari N, Amin SA, Jha T. Collagenases and gelatinases and their inhibitors as anticancer agents. In Cancer-Leading Proteases: Structures, Functions, and Inhibition. Gupta SP Ed.; Elsevier: New York, NY, USA, 2020; 265-294

[56] Beutel B, Song J, Konken CP, et al. New in Vivo Compatible Matrix Metalloproteinase (MMP)-2 and MMP-9 Inhibitors. Bioconjug Chem 2018;29(11):3715-3725

[57] Lenci E, Contini A, Trabocchi A. Discovery of a D-pro-lys peptidomimetic inhibitor of MMP9: Addressing the gelatinase selectivity beyond S1′ subsite. Bioorg Med Chem Lett 2020;30(20):127467

[58] Lenci E, Innocenti R, Di Francescantonio T, et al. Identification of highly potent and selective MMP2 inhibitors addressing the S1′ subsite with D-proline-based compounds. Bioorg Med Chem 2019;27(9):1891-1902

[59] Mangiatordi GF, Guzzo T, Rossano EC, et al. Design, Synthesis, and Biological Evaluation of Tetrahydro-β-carboline Derivatives as Selective Sub-Nanomolar Gelatinase Inhibitors. ChemMedChem 2018;13(13):1343-1352

[60] Rempe RG, Hartz AM, Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab 2016;36(9):1481–1507 ****This paper reviews current understanding and knowledge of MMPs in brain disorders, and address them as potential therapeutic targets**

[61] Brkic M, Balusu S, Libert C, Vandenbroucke RE. Friends or Foes: Matrix Metalloproteinases and Their Multifaceted Roles in Neurodegenerative Diseases. Mediators Inflamm 2015;2015:620581

[62] The Research Foundation for the State University of New York. Matrix metalloproteinase-9 hemopexin domain inhibitors and methods of treatment using same. WO2018175670 (2018)

[63] Alford VM, Kamath A, Ren X, Kumar K, et al. Targeting the Hemopexin-like Domain of Latent Matrix Metalloproteinase-9 (proMMP-9) with a Small Molecule Inhibitor Prevents the Formation of Focal Adhesion Junctions. ACS Chem Biol 2017;12(11):2788–2803

[64] Yong VW. Metalloproteinases: Mediators of pathology and regeneration in the CNS. Nat Rev Neurosci 2005;6:931-944

[65] Nagy V. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci 2006;26:1923-1934

[66] Konopka A, Grajkowska W, Ziemiańska K, Roszkowski M, et al. Matrix metalloproteinase-9 (MMP-9) in human intractable epilepsy caused by focal cortical dysplasia. Epilepsy Res 2013; 1(2):45-58

[67] Yamamori H, Hashimoto R, Ishima T, Kishi F, et al, Plasma levels of mature brain-derived neurotrophic factor (BDNF) and matrix metalloproteinase-9 (MMP-9) in treatment-resistant schizophrenia treated with clozapine. Neurosci Lett 2013;556:37-41

[68] Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, et al, Enhanced neuronal plasticity and elevated endogenous sAPPα levels in mice over-expressing MMP9. J Neurochem 2012;121:239-251

[69] Bienkowski P, Samochowiec J, Pelka-Wysiecka J, Grzywacz A, et al. Functional polymorphism of matrix metalloproteinase-9 (MMP9). Pharmacol Rep 2015;67:442–445

[70] Iproteos S.L. Gelatinase inhibitors and use thereof. WO2017085034 (2017)

[71] Bertran A, Khomiak D, Konopka A, Tarragó T, Prades R. Design and synthesis of selective and blood-brain barrier-permeable hydroxamate-based gelatinase inhibitors. Bioorg Chem 2020;94:103365

[72] Bartolome RA, Ferreiro S, Miquilena-Colina ME, Martinez-Prats L, et al. The Chemokine Receptor CXCR4 and the Metalloproteinase MT1-MMP Are Mutually Required During Melanoma Metastasis to Lungs. Am J Pathol 2009;174:602-612.

[73] Lakhan SE, Kirchgessner A, Tepper D, Leonard A. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. Front Neurol 2013;4:32

[74] University of Notre Dame Du Lac. Selective matrix metalloproteinase inhibitors. WO2015127302 (2015)

[75] Jones JI, Nguyen TT, Peng Z, Chang M. Targeting MMP-9 in Diabetic Foot Ulcers. Pharmaceuticals 2019;12:79

[76] Mallinckrodt LLC. Matrix metalloproteinase (MMP) targeted agents for imaging and therapy. WO2014151988 (2014)

[77] Freskos JN, Asmelash B, Gaston KR, Karwa A, et al. Design and synthesis of MMP inhibitors with appended fluorescent tags for imaging and visualization of matrix metalloproteinase enzymes. Bioorg Med Chem Lett. 2013;23:5566-5570

[78] Remacle AG, Shiryaev SA, Golubkov VS, Freskos JN, et al. Non-destructive and selective imaging of the functionally active, pro-invasive membrane type-1 matrix metalloproteinase (MT1-MMP) enzyme in cancer cells. J Biol Chem 2013;288:20568-20580

[79] Loyola University of Chicago. Carborane hydroxamic acid matrix metalloproteinase inhibitors and agents for boron neutron capture therapy. WO2020006384 (2020)

[80] Lutz MR, Flieger S, Colorina A, Wozny J, et al. Carborane-Containing Matrix Metalloprotease (MMP) Ligands as Candidates for Boron Neutron-Capture Therapy (BNCT). ChemMedChem2020;15(20):1897-1908

[81] Valliant J, Guenther KJ, King AS, Morel PSP, et al. The medicinal chemistry of carboranes. Coord Chem 2002;232:173-230

[82] Trivillin VA, Bruno LJ, Gatti DA, Stur M, et al. Boron neutron capture synovectomy (BNCS) as a potential therapy for rheumatoid arthritis: radiobiological studies at RA-1 Nuclear Reactor in a model of antigen-induced arthritis in rabbits. Radiat Environ Biophys 2016;55:467-475

[83] Yip C, Foidart P, Noël A, Sounni NE MT4-MMP: The GPI-Anchored Membrane-Type Matrix Metalloprotease with Multiple Functions in Diseases. Int J Mol Sci 2019;20:354

[84] English WR, Puente XS, Freije JM, Knauper V, et al. Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor-alpha convertase activity but does not activate pro-MMP2. J Biol Chem 2000;275(19):14046-14055

[85] Itoh Y, Kajita M, Kinoh H, Mori H, et al. Membrane type 4 matrix metalloproteinase (MT4-MMP, MMP-17) is a glycosylphosphatidylinositol-anchored proteinase. J Biol Chem 1999;274(48):34260-34266

[86] Centro Nacional de Investigaciones Cardiovascular Carlos III. Inhibitors of Membrane-Type 4 matrix metalloproteinase and its use in the treatment of diseases benefiting from an increase in the activity of patrolling monocytes. WO 2019086683 (2019)

[87] Lagente V, Le Quement C, Boichot E. Macrophage metalloelastase (MMP-12) as a target for inflammatory respiratory diseases. Exp Opin Ther Targets 2009;13:287-295. ***This review discusses the involvement of MMP-12 in different inflammatory and respiratory disorders**

[88] Demedts IK, Morel-Montero A, Lebecque S, et al. Elevated MMP-12 protein levels in induced sputum from patients with COPD. Thorax 2006;61(3):196-201

[89] He MK, Le Y, Zhang YF, et al. Matrix metalloproteinase 12 expression is associated with tumor FOXP3+ regulatory T cell infiltration and poor prognosis in hepatocellular carcinoma. Oncol Lett 2018;16:475-482

[90] Churg A, Wang R, Wang X, et al. Effect of an MMP-9/MMP-12 inhibitor on smoke-induced emphysema and airway remodelling in guinea pigs. Thorax 2007;62(8):706-711

[91] Norman P. Selective MMP-12 inhibitors: WO-2008057254. Exp Opin Ther Pat 2009;19(7):1029-1034

[92] Foresee Pharmaceuticals USA Inc. Matrix Metalloproteinase (MMP) Inhibitors and methods of use thereof. WO2019222157 (2019)

[93] DSM IP Assets B.V. Phosphinic peptide derivatives for use as MMP-12 inhibitors. WO2017093093 (2017)

[94] Commissariat a l'Energie Atomique et aux Energie Alternatives and the University of British Columbia. Inhibitors of MMP-12 as antiviral Agents. EP2907512 (2014)

[95] Devel L, Rogakos V, David A, et al. Development of selective inhibitors and substrate of matrix metalloproteinase-12. J Biol Chem 2006;281:11152-11160

[96] Centre National de la Recherche Scientifique – CNRS and the University de Reims Champagne-Ardenne. Metalloproteinase inhibitors, methods for producing same, and therapeutic uses thereof. WO2015028766 (2015)