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Review

Specific targeting of metzincin family members with small-molecule inhibitors: Progress toward a multifarious challenge

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ABSTRACT

Zn-metalloproteinases are an important class of hydrolytic enzymes that are characterized by the presence of a catalytic zinc(II) atom in their active center which is fundamental for proteolytic activity. Metzincins, a superfamily of Zn-metalloproteinases with many structural and functional commonalities among its members, are responsible for the fine tuning of key physiological functions in mammals and the deregulation of their activity is directly connected to numerous inflammatory and degenerative diseases such as arthritis or cancer. Development of small-molecule exogenous inhibitors of metzincins able to re-establish normal proteolytic activity in pathological conditions has been a field of intense research effort for many years but applications in the clinic were not always successful. One of the main reasons for this failure is the uncontrolled action of these inhibitors on target as well as anti-target metzincin family members. Current medicinal efforts have been shifted to the discovery of target-specific inhibitors that will help to improve our understanding of metzincins biological function and provide the basis for the development of safer pharmaceutical agents. This review focuses on the cases of certain medicinally important metzincins [matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs), ADAMs with thrombospondin motifs (ADAMTSs), and procollagen C-proteinase (PCP)] and summarizes the latest advances on the discovery of inhibitors of these enzymes that display improved selectivity profiles.

Contents

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1. Introduction

The metzincin superfamily of enzymes comprises a large number of Zn-metalloproteases that are characterized by particular sequence similarities which are highly conserved among its members. In 1993, Bode and co-workers proposed the designation 'metzincins' to group together four distinct families of metalloproteases (astacins, adamalysins, matrixins, and bacterial serralysins) based on (a) a common zinc(II)-binding motif of the generic sequence HEXXHXXGXXH(/D) that contains three of the zinc ligands and (b) a conserved methionine residue downstream of the third zinc(II)-binding histidine that participates in a β-turn of similar conformation among the metzincins, called the 'Met-turn'.[1] Furthermore, a distinction between the four aforementioned families has been proposed based on the residue that follows the third zinc(II)-binding histidine which is highly conserved and typical for each of the four families.[2] Despite the low sequence similarity between the members of different metzincin families, a comparable overall topology has been demonstrated by comparison of the three-dimensional structures of representative members of each family.[3] Structural similarities are more striking among the active-site environments of the different metzincin families although notable differences in the substrate-binding regions that extent away from the zinc(II) ion are also of special interest. These differences are more profound in the primed part of the substrate-binding region and especially in the loop that connects the Met-turn with the α-helix part of the catalytic domain, namely the S_1' loop. The length and the aminoacid composition of this loop varies significantly between the members of different metzincin families and less so between the members of the same family.[3,4] For instance, in matrixins and adamalysins this protein segment forms a tube-like pocket which is quite voluminous and, in general, larger than required to accommodate side chains of natural aminoacids while in astacin this region is bordered thus forming a shallow cleft with specificity for short aliphatic residues. Undoubtedly, the overall variability of the S_1' domain of metzincins has been the main selectivity determinant of synthetic active-site directed inhibitors for more than one decade of drug design and development.[5,6]

According to the public MEROPS Peptidase Database (http://merops.sanger.ac.uk), more than 70 metzincin genes have been identified in the human genome and ~60 of them encode zymogens of active (or putatively active) proteases (Table 1). Metzincin proteases are capable of cleaving a diverse array of cellular, extracellular and extracellular matrix substrates including collagens, procollagens, proteoglycans, cytokines and their ligands, chemokines and elastin, thereby modulating tissue structure and function in physiological and pathological states. Over the last 20 years, the revelation of the central role of many metzincins in disease states has been the stimulus for several ambitious medicinal programs aiming to the regulation of their proteolytic activity by small-molecule inhibitors. During early efforts, researchers were unaware of the overwhelming complexity that governs the biological processes mediated by these enzymes and developed broad-spectrum inhibitors that failed to pass advanced clinical trials.[7,8] For example, during degradation of extracellular matrix in cancer, multiple metzincins are participating in complex protease networks while, in addition, other metzincins are generally pro-survival.[9] The need for selective active-site directed inhibitors that will help to probe the function of each metzincin independently has initiated a new wave of research that has resulted so far to next-generation inhibitors with outstanding selectivity profiles in a few cases. In this review, we discuss the most important achievements in the development of small-molecule inhibitors able to target specific metzincins.

2. Matrix metalloproteinases inhibitors (MMPIs)

2.1. Introduction—selectivity issues

Matrix metalloproteinases (MMPs) is a large group of zinc(II)-dependent neutral endopeptidases with high structural and functional similarities known to degrade the components of extracellular matrix and to promote tissue remodelling.[9–11] They belong to the matrixin family of proteases and they are divided according to their structural characteristics into eight distinct groups. Five of them are secreted and three are membrane-type MMPs.[12] Their overexpression has been long associated with the degradation of basement-membrane components during metastasis and angiogenesis in several types of cancer and therefore their exogenous regulation by small-molecule inhibitors (MMPIs) rendered an attractive anti-cancer target during the past two decades.[9] In addition, MMPs are involved in the pathophysiology of numerous other diseases such as arthritis, atherosclerosis, myocardial infarction, and periodontal diseases.[13,14] Despite the intense efforts toward the discovery of effective MMPIs able to reach the clinic, only one compound (Periostat) has been approved by FDA for the treatment of periodontal diseases.[15] Unfortunately, the encouraging early clinical trials of several hydroxamate and non-hydroxamate-type inhibitors during the past decade were followed by unsuccessful advanced clinical trials mainly because of severe musculoskeletal side-effects.[16] This failure is mainly attributed to the extremely complex biology of MMPs which was unexplored during the early period of MMPIs discovery. For example, while some MMPs have promoting effects in tumor progression, other isoforms seem to have a protective role in angiogenetic and metastatic events.[17] Therefore, inhibition of MMP anti-targets might counterbalance the beneficial effects of target-MMPs inhibition. MMPIs that failed clinical trials for cancer treatment were in their majority broad-spectrum inhibitors which means that crossreactivity between target MMPs, anti-target MMPs and possibly other pro-survival related enzymes such as ADAMs and ADAMTSs might caused that failure.[18] Today, the discovery of target-specific MMPIs is an issue of primary importance in order to comprehend the physiological and pathological role of each MMP in health and disease and to eventually develop drugs with improved clinical profile.[19] The most recent advances in the discovery of MMP inhibitors with improved selectivity properties are discussed below.

2.2. Zn-chelating MMPIs

Undoubtedly, the most popular approach to date to obtain potent inhibitors of MMPs is the introduction of a group that is able to chelate the zinc(II) ion in an optimized peptidic or non-peptidic scaffold. The majority of this type of MMPIs bear a hydroxamate group as a zinc(II)-binding group (ZBG) which in its anionic form acts as a bidentate chelator of the metal ion.[5,15] When little were known about the exact biological role of MMPs in the pathophysiology of cancer and inflammation, the development of hydroxamate-type inhibitors of MMPs with potential therapeutic value dominated the medicinal research. Unfortunately, the clinical failure of hydroxamate-type inhibitors, such as batimastat and marimastat, forced the scientific community to re-examine the suitability of this type of molecules for further development.[16] The reasons of this massive failure rely on both the lack of reliable clinical trials and the poor pharmacological profile of MMPIs and have been well-documented in many comprehensive reports.[7,16,20]

As we have already mentioned, one of the main challenges today in the field of next-generation MMPIs is the selectivity index. The failure of early hydroxamate-type inhibitors to exhibit im-

Table 1
Metzincin-encoding genes in human[a]

	Proteolytic activity status		
	Active	Putatively active	Inactive
Matrixins	*MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP19, MMP20, MMP21, MMP23A(MMP23B), MMP24, MMP25, MMP26, MMP28*	*MMP27*	
Astacins	*MEP1A, MEP1B, BMP1, TLL1, TLL2, ASTL*		
Adamalysins	*ADAM8, ADAM9, ADAM10, ADAM12, ADAM15, ADAM17, ADAM19, ADAM28, ADAM33, ADAMTS1, ADAMTS2, ADAMTS3, ADAMTS4, ADAMTS5, ADAMTS7, ADAMTS8, ADAMTS9, ADAMTS12, ADAMTS13, ADAMTS14, ADAMTS15, ADAMTS16, ADAMTS20*	*ADAM20, ADAM21, ADAM30, ADAMDEC1, ADAMTS6, ADAMTS10, ADAMTS17, ADAMTS18, ADAMTS19*	*ADAM2, ADAM3A, ADAM3B, ADAM7, ADAM11, ADAM18, ADAM22, ADAM23, ADAM29, ADAM32*

[a] Data were acquired from MEROPS Peptidase Database (http://merops.sanger.ac.uk) and UniProt database (www.uniprot.org).

proved selectivity properties is mainly due to the strong zinc(II)-binding capacity of hydroxamic acid group that suppresses the overall effect of weaker but more specific non-covalent interactions of the inhibitor backbone with the substrate specificity regions of the enzyme.[4,6] Nevertheless, many research groups have focused on the rational structural optimization of hydroxamate-type MMPIs in order to exploit differences of MMPs specificity pockets and obtain improved selectivity. Interestingly, despite the intense efforts to this direction this approach has only led to partially selective MMPIs for small subsets of MMPs. Some selected examples of partially selective hydroxamate-type inhibitors are shown in Figure 1.

For example, sulfonamide hydroxamate **1** is a potent inhibitor of MMP-1 that spares collagenases MMP-8 and -13, gelatinase MMP-2 and stromelysin MMP-3.[21] Inhibition of MMP-1 was considered to be one of the main reasons for the musculoskeletal syndrome associated with broad-spectrum MMPIs administration in patients.[22,23] Therefore, substantial research effort has focused to optimized inhibitors that spare MMP-1 and target other MMPs such as gelatinases (MMP-2 and -9) and collagenase 3 (MMP-13). To this respect, sulfonamide hydroxamate **2** shows a strong preference for MMP-2 over MMP-1, -3, -7, and -9 while the anthralinate hydroxamic MMPI **3** targets MMP-13 and spares MMP-1, -9 and TACE (Fig. 1).[24,25] From another series of sulfonamide hydroxamates, compound **4** was identified that exhibited significant specificity for MMP-3 over MMP-1, -2, -9, and -14, an enzyme implicated in the pathology of chronic non-healing wounds.[26] Finally, the peptide-like succinyl hydroxamate **5** displays modest selectivity for MMP-3 which has been attributed to specific interactions with the S_1' and S_3' enzyme subsites after extensive SAR studies.[27]

The optimization of MMPIs properties in terms of selectivity and in vivo activity led researchers to the evaluation of a plethora of other ZBGs in lieu of the hydroxamic acid moiety. Carboxylic MMPIs is a well-studied category of alternative zinc(II)-binding inhibitors mainly because of the availability of these compounds since they are synthetic precursors of the more popular hydroxamates. Carboxylates are weaker zinc(II) ligands than hydroxamates, therefore carboxylic MMPIs generally exhibit lower inhibitory potency than the corresponding hydroxamates. This weak zinc(II) affinity of carboxylates can be exploited for improving selectivity properties, as it has been aptly described in the past by Fray et al. en route to the discovery of MMP-3-specific inhibitors.[28] By comparing the inhibition profiles of compounds **6** and **7**, Fray observed that substitution of a carboxylate by a hydroxamate on the same scaffold causes a 10-fold increase in potency of the inhibitor toward MMP-3 but, in addition, decreases the selectivity against MMP-1, -2, -9, and -14 by almost two orders of magnitude (Fig. 2). This effect is attributed to the fact that the strong zinc(II) affinity of hydroxamic acid group is the main determinant of the overall binding energy, in contrast with carboxylates where the binding energy relies to a bigger extent on specific interactions with the specificity pockets. Moreover, **6** also displays negligible affinity for the related metzincins TACE and procollagen C-proteinase (PCP). Other representative studies on carboxylic MMPIs with improved selectivity profile include the macrocyclic peptidomimetic **8** which targets MMP-8 and spares MMP-1, -2, -3, and -9,[29] and the sulfonamide carboxylic derivative **9** that bears a substituted benzofuran at the far end of the P_1' position and displays excellent oral bioavailability and decent selectivity for MMP-13 over seven other isozymes and TACE (Fig. 2).[30–32]

MMPIs bearing a phosphinic acid as a ZBG offer many advantages on the quest of enhanced inhibitor selectivity. In addition to their weak zinc(II)-binding ability which can contribute to efficient enzyme discrimination, phosphinic MMPIs are able to interact with both primed and unprimed subsites of the protease active site. This is due to the advantageous placement of the ZBG in the middle of a pseudopeptidic scaffold and not at its N- or C-terminus, as in the cases of hydroxamate and carboxylate inhibitors. Therefore, specific, non-covalent interactions between a phosphinic peptide and a protease can be significantly increased which can result to enhanced selectivity if these interactions are properly tuned.[33] Recent advances in the convergent chemical synthesis of phosphinic peptide isosters has allowed the screening of diverse chemical libraries leading to the identification of inhibitors of MMP-11 and MMP-12 with unique selectivity profile.[34,35] Thus, compound **10** displays a $K_i = 0.23\ \mu M$ for MMP-11, an enzyme with a deep S_1' cavity, is inactive against MMP-7 that has a shallow S_1' pocket and, most importantly, is at least two orders of magnitude less potent toward six other MMPs that are also characterized by deep S_1' subsites (Fig. 3).[34] This enhanced selectivity is attributed to the *ortho*-substituent of the aryl group in P_1' position which presumably interacts with residues located at the entrance of MMPs S_1' cavity. Indeed, this part of the specificity loop is characterized by

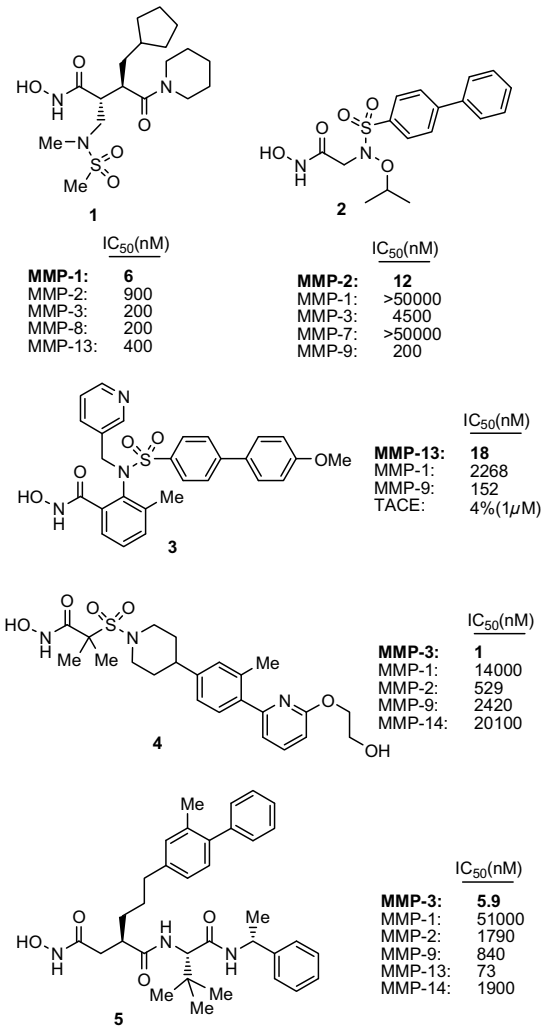


Figure 1. Examples of hydroxamate-type MMP inhibitors.

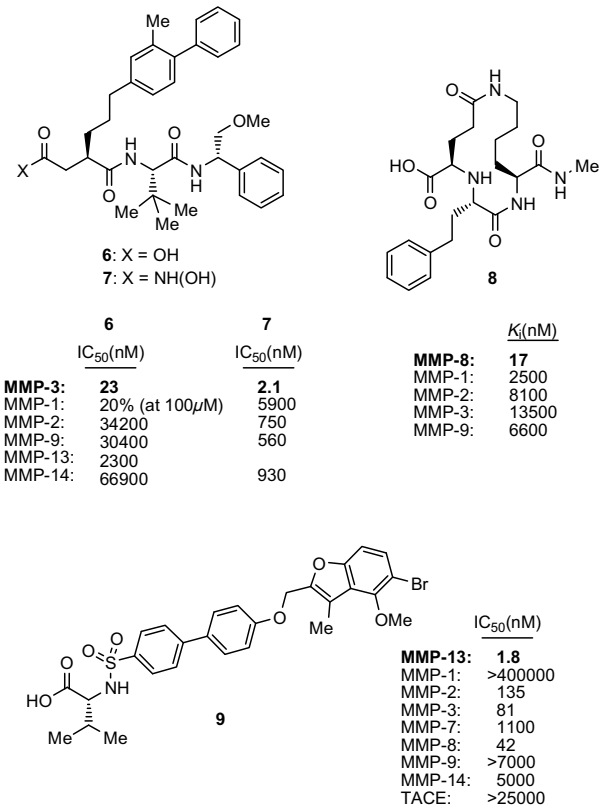


Figure 2. Carboxylic-type MMP inhibitors.

significant aminoacid variability among the different isozymes. In the most interesting case of a phosphinic MMP inhibitor ever reported, the isoxazole-containing phosphinic tetrapeptide **11** blocks potently MMP-12 activity while it spares ten other MMPs, TACE, NEP, and ACE with an impressive degree of selectivity, as shown in Figure 3.[35] The remarkable selectivity of inhibitor **11** for MMP-12 might be explained by the fact that most MMPs (but not MMP-12) cannot tolerate the presence of two glutamate residues in P_2' and P_3' positions of their binding substrate. It is noteworthy that phosphinate **11** of Figure 3 is the only *truly* selective MMP inhibitor ever reported that includes a zinc(II)-chelating group in its structure. Another interesting application of phosphinic peptides in this field was recently reported by Fields and co-workers.[36] Their study was based on the observation that the Gly_{439}-Val_{440} bond of the α1(V) collagen is hydrolyzed selectively by MMP-2 and MMP-9. Consequently, replacement of the Gly-Val amide bond of the α1(V) 436–450 region by a phosphinic acid surrogate resulted to a novel triple-helical transition state analog that displayed nanomolar affinity for gelatinases and little or no activity against other collagenolytic MMPs or MMP-3. Although the thermal instability of this triple-helical molecule does not allow further in vivo applications, the preparation of more stable collagen-like constructs in the future is expected to validate this concept as a new approach for the development of selective MMPIs.

Although a large number of other known ZBGs have been evaluated as alternatives for hydroxamates during the past years, only in a very few cases noteworthy selectivities have been observed. In such a case, a pyrimidinetrione was used as a ZBG and after extensive optimization by SAR studies a subnanomolar MMP-13 inhibitor (**12**) was identified with greater than 100-fold selectivity against MMP-1, -2, -3, -8, -9, -12, and -14 (Fig. 4).[37] According to the authors, the length of the hydrophobic side chain in combination with the optimized setting of heteroatoms are the key elements of the observed selectivity. Some other examples of MMPIs with non-hydroxamate ZBGs that display some preference for a particular MMP are thiol diketopiperazine **13** (selective for MMP-1 over MMP-3 and MMP-9),[38] carbamoyl phosphonate **14** (selective for MMP-2 over MMP-1, -3, -8, -9, -12, -13, and TACE)[39] and sulfone *N*-formylhydroxylamine **15** (retrohydroxamate, selective for MMP-2 over MMP-1, -3, -7, and -9).[40] Substantial effort has been also made for the discovery of novel ZBGs with improved in vivo stability and toxicity profile.[6,41] Although the effect on selectivity of MMPIs bearing such novel ZBGs has not been thoroughly studied,[42] two literature cases are worth-mentioning. In the first one, Cohen and co-workers used an innovative chelator-driven approach to develop pyrone analog **16** that displayed a marked selectivity for MMP-3 over MMP-1 and -2 (Fig. 5).[43,44] Finally, inhibitor **17** bearing the 6*H*-1,3,4-thiadiazine scaffold interacts with both primed and unprimed subsites of MMPs and exhibits good selectivity for MMP-12 over four other MMPs but it is unable to discriminate between MMP-12 and MMP-14 (Fig. 5).[45] We must emphasize that there are no reports that deal with the selectivity profile of inhibitors with such novel ZBGs against a wide panel of MMPs, therefore more detailful studies are expected to validate this approach.

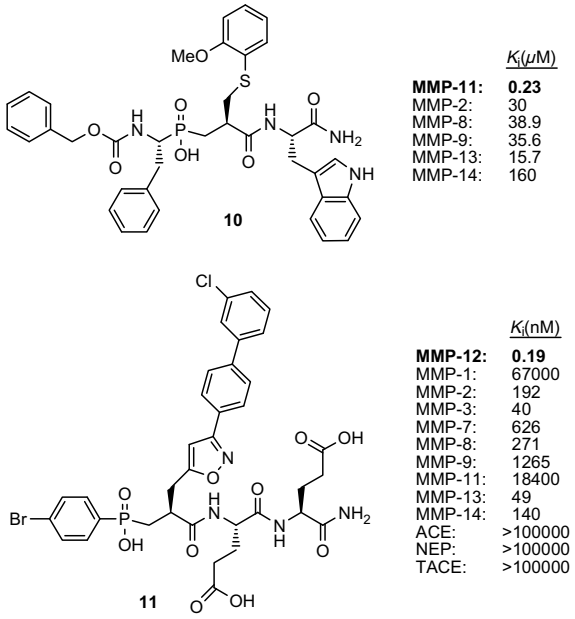


Figure 3. Phosphinic-type MMP inhibitors.

2.3. Mechanism-based MMPIs

In 2000, Mobashery and co-workers reported a novel type of MMPI that blocks gelatinases with a unique mechanistic mode in the field of Zn-metalloproteases.[46] Thiirane **18** was found to behave as a mechanism-based, slow-binding inhibitor for MMP-2 and MMP-9 (with a higher preference for MMP-9) as it was proved by analysis of inhibition kinetic parameters. In contrast, K_i values for MMP-1, -3, and -7 are much higher and furthermore inhibitor **18** does not display a similar slow-binding mechanism-based inhibition profile for these enzymes (Fig. 6). Comparison of data from X-ray absorption spectroscopy (XAS) analysis of latent proMMP-2, active MMP-2, and thiirane-inhibited MMP-2 showed clearly a monodentate binding mode of the sulfur atom of thiirane which is believed to open upon binding by nucleophilic attack from a glutamate residue of the active site. In this process, the zinc(II) ion plays the role of a Lewis-acid activator by coordinating the thiirane sulfur.[47] This covalent binding of **18** causes a conformational change in the local catalytic environment that re-establishes the proenzyme structural motifs by mimicking the binding of the propeptide of MMP-2 zymogen with the catalytic zinc (known as 'cysteine switch'). Study of various modified thiirane analogs revealed that modifications on the distal phenyl ring are able to affect the binding mode of the inhibitor and tune the selectivity toward certain MMPs.[48] Thus, although thiiranes **19** and **20** display similar dissociation constants for most MMPs, sulfonamide thiirane **19** behaves as a mechanism-based inhibitor only for gelatinases and MMP-14 while acetamide thiirane **20** behaves as a mechanism-based, slow-binding inhibitor only for MMP-2 and a competitive inhibitor for MMP-9 and -14, as it was confirmed by dialysis experiments (Fig. 6). This difference in the binding mode of inhibitor **20** with MMP-2, -9, and -14 introduces a new concept for the development of inhibitors that exploit slow-binding inhibition properties only for a selected target-MMP. Moreover, it has been shown that compound **18** is a weak non-competitive inhibitor of TACE that does not undergo thiirane opening upon zinc coordination with the sulfur atom.[49] The overall different binding mode of **18** with TACE as compared to the structurally similar MMP-2 suggests that these enzymes are significantly diverse in their electronic and chemical properties within their active sites.

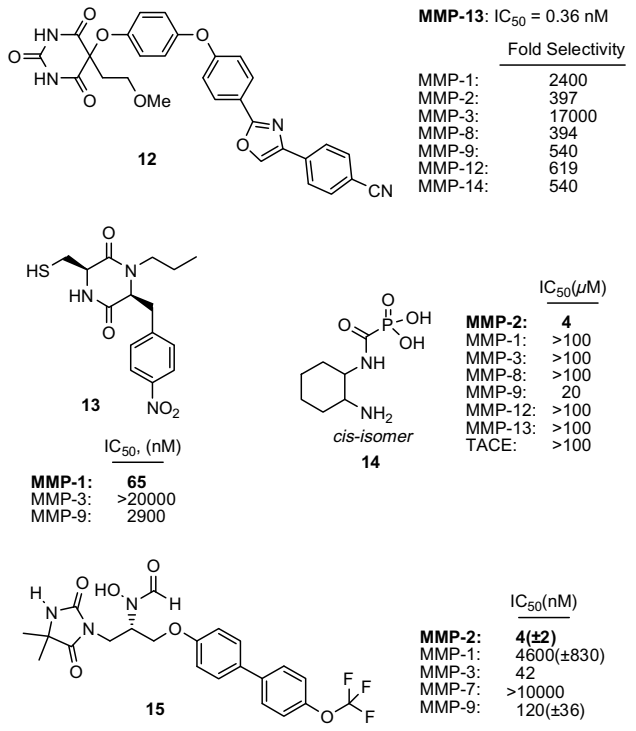


Figure 4. Non-hydroxamate MMP inhibitors.

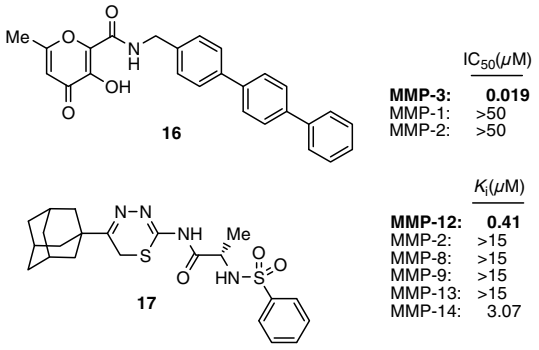


Figure 5. MMP inhibitors bearing novel heterocyclic ZBGs.

The same research group studied also the inhibition profile of similar dithiane molecules that are readily obtained by the hydrolysis of the corresponding thiiranes (although dithianes are not mehanism-based inhibitors they are discussed in this paragraph for the sake of clarity). Even though inhibitor **21** (Fig. 6) exhibits comparable K_i values for gelatinases and MMP-14 and less so for MMP-3, a slow-binding profile is observed only for gelatinases. In all other cases, compound **21** behaves as a competitive inhibitor.[50] These results can be rationalized by the ability of the inhibitor to induce a conformational change to the catalytic zinc microenvironment upon binding to gelatinases that is implicit for the observed slow-binding behavior, as well as alterations of the coordination number of zinc.[51] These changes strongly stabilize the enzyme–inhibitor complex which does not reverse itself readily.

2.4. Non Zn-chelating MMPIs

In 2000, Chen and co-workers observed that compound **22** is a weak inhibitor of MMP-13 (89% at 10 μg/mL) with no activity against MMP-1, -9 or TACE (Fig. 7).[52] The unique characteristic of

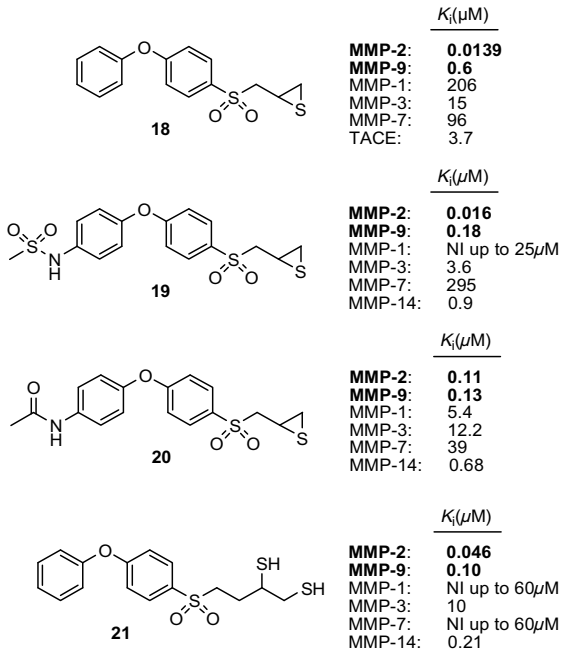


Figure 6. Mechanism-based gelatinase inhibitors.

this compound is that it contains no obvious ZBG which until that time was considered to be a prerequisite for MMP inhibition. Its affinity for MMP-13 was attributed exclusively to specific interactions with the deep S_1' channel of MMP-13 suggesting a novel binding mode that could afford selective MMPIs after optimization. A few years later, a group from Pfizer identified thiophene amide **23** which inhibited potently MMP-12 and -13 with modest selectivity for MMP-2, -3, -8, and -9 (Fig. 7).[53,54] The crystal structure of this compound with MMP-12 revealed the absence of any zinc(II)-chelating interaction, despite the fact that compound **23** possesses a carboxylic group in its structure that could act as a zinc(II) ligand.[54] Inhibitor **23** partially occupies the deep S_1' pocket of MMP-12 participating in three direct and one water-mediated hydrogen bonds and numerous specific hydrophobic interactions that account for its high affinity.

The newly introduced concept of non zinc-chelating MMPIs found a tremendous success in the following years in the case of selective inhibition of MMP-13. In 2005, a group from Aventis reported that pyrimidine dicarboxamide **24** exhibited good potency against MMP-13 (IC_{50} = 6.6 μM) and no detectable activity for MMP-1, -2, -3, -7, -8, -9, -10, -12, -14, and -16 at 100 μM of inhibitor concentration (Fig. 8).[55] The affinity of this novel scaffold for MMP-13 dramatically improved when the pyridyl rings were replaced by substituted phenyl rings. Crystal structure analysis of a MMP-13/**24** complex confirmed the non zinc-chelating binding mode and revealed a unique side pocket of S_1' cavity for MMP-13, referred to as $S_1'^*$ pocket. The inhibitor adopts a bent conformation upon binding and projects the one pyridyl group to the entrance of the S_1' cavity while the second pyridyl group protrudes from the S_1' pocket into the adjacent $S_1'^*$ pocket. This particular conformation of the inhibitor is critical for efficient binding since it determines the shape and size of the unique $S_1'^*$ pocket and fixes the exact geometry of polar interactions between the specificity loop and the inhibitor.[56]

Very recently, high throughput screening of Pfizer compound collection led to thiazolopyrimidinedione **25** which exhibited an impressive degree of selectivity for MMP-13 versus nine other MMP isoforms.[57] Since compound **25** is an ester, metabolizing readily to the corresponding inactive acid form, Pfizer researchers proceeded to the optimization of this lead scaffold by using computer-aided drug design and SAR studies. To this respect, quinazolinone **26** and pyrido[3,4-*d*]pyrimidin-4-one **27** displayed selectivity profiles similar to that of parent compound **25** and nanomolar affinity for MMP-13 (Fig. 8).[57,58] Moreover, compound **27** possesses favorable pharmacokinetic properties and excellent safety profile while it also offers efficient cartilage protection in a rabbit animal model of osteoarthritis.[58] All the above inhibitors are non zinc-chelating ligands as it was revealed by structural and kinetic studies and are believed to confer an ordered structure to the S_1' specificity loop of MMP-13 that is otherwise flexible. This induced rigidity is stabilized by interactions with residues along the S_1' deep channel which are highly specific for MMP-13. It must be also emphasized that the beneficial pharmacology of these MMP-13 inhibitors makes them ideal candidates for the treatment of osteoarthritis without any musculoskeletal syndrome side-effects.

3. ADAMs and ADAMTSs inhibitors

3.1. Introduction—selectivity issues

ADAMs (A Disintegrin And Metalloproteinases) is a large family of proteins (~40 members) and less than 30 of these proteins are expressed in humans while almost half of those have established proteolytic activity.[59] ADAMs are type I integral membrane proteins and several members of this family have been involved in the ectodomain shedding of membrane proteins.[60,61] Cytokines, cytokine receptors, growth factors, and growth factor receptors as well as other substrates such as amyloid precursor protein (APP) and Notch ligand delta are some of the proteins that are processed by ADAMs.[59,61] The most well-studied member of this family is ADAM-17, a protease that releases the proinflammatory cytokine tumor necrosis factor-α (TNF-α) from its membrane-bound precursor.[62,63] There is compelling evidence that ADAM-17 is the major TNF-α convertase (TACE) and since elevated TNF-α levels are associated with inflammatory diseases, such as arthritis and Chron's disease, the development of small-molecule TACE inhibitors emerged as an important pharmaceutical target during the last decade.[64–67] Prompted by the success of anti-TNF treatment of rheumatoid arthritis (RA) by clinically approved TNF-specific antibodies, the majority of clinical trials with TACE inhibitors have been focused to this type of disease.[68] Unfortunately, problems with hepatotoxicity and/or lack of efficacy forced discontinuation of clinical trials with the only two inhibitors that managed to reach Phase II.[69,70] The exact reasons for the development of such adverse effects are still not clear (for a discussion, see Ref. 68). However, the clinical evaluation of highly selective TACE inhibitors with drug-like properties is expected to answer in the future whether targeting TACE is inefficient for treating RA or not.

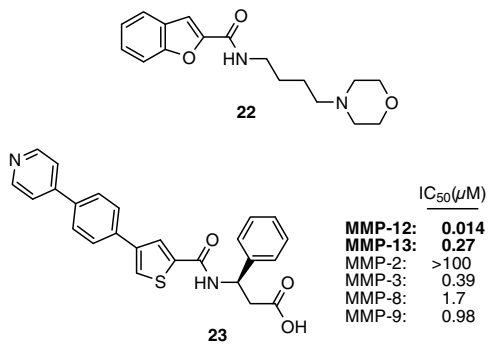
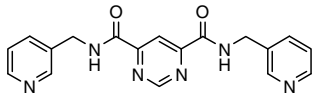


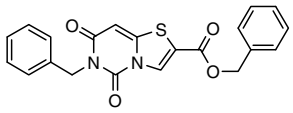
Figure 7. Examples of early non zinc-chelating MMP inhibitors.



24

MMP-13: IC_{50} = 6.6μM

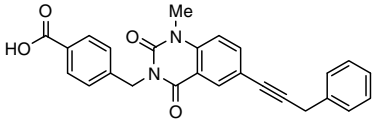
MMP-1, -2, -3, -7, -8, -9, 10, -12, -14, 16: not inhibited up to 100μM



25

MMP-13: IC_{50} = 30nM

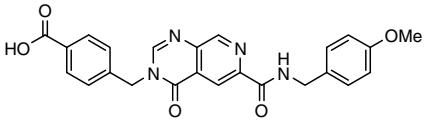
MMP-1, -2, -3, -7, -8, -9, -12, -14, 17: not inhibited up to 100μM



26

MMP-13: IC_{50} = 0.67nM

MMP-1, -2, -3, -7, -8, -9, -12, -14, 17: not inhibited up to 100μM



27

MMP-13: IC_{50} = 6.72nM

MMP-1, -2, -3, -7, -8, -9, -12, -14, 17: not inhibited

Figure 8. Non zinc-chelating MMP-13 selective inhibitors.

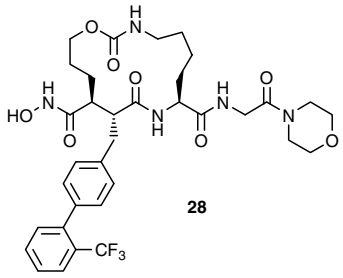
It must be emphasized that drug discovery efforts were mainly directed to TACE inhibitors that are selective over MMPs because of concerns over possible MMPI-associated side-effects. Limited attention has been paid to the discrimination between TACE and other ADAMs or the relevant family of ADAMTS metalloproteases (vide infra). It is still not clear whether the fate of clinical trials so far is dependent on pleiotropic effects of partially selective TACE inhibitors or by other parallel events (i.e., shedding activity of TACE on other membrane substrates such as TNF receptor II).[68] Moreover, novel therapeutic avenues for TACE inhibitors are opening associated with the potential application of these molecules to the inhibition of pathogenic epidermal growth factor receptor (EGFR) signaling in epithelial tumors.[71,72] Recently, it was announced that INCB7839, a dual TACE/ADAM-10 selective inhibitor developed by Incyte, advanced to Phase II clinical trials in refractory cancer and breast cancer patients and the results are eagerly awaited.[73] Furthermore, many members of the ADAMs family have been associated with disease states such as cardiac hypertrophy (ADAM-12)[74] and asthma (ADAM-33).[75] Development of specific inhibitors for these enzymes would be valuable tools for the biochemical and clinical validation of these targets.

Another family of zinc(II)-dependent metalloproteinases that share many common features with MMPs and ADAMs is the ADAMTS (A Disintegrin And Metalloproteinase with Thrombo-Spondin motifs) family.[76,77] Unlike the transmembrane proteins ADAMs, ADAMTSs are secreted molecules, some of which bind to the extracellular matrix (ECM) through their thrombospondin motifs.[76] Understanding ADAMTSs biology is still in its infancy and the development of specific inhibitors for ADAMTSs is expected to play a pivotal role for future advances.[11,72,77] From a therapeutic perspective, aggrecanase activity of certain ADAMTSs has gained the biggest share of attention.[78] Aggrecan is an important proteoglycan in cartilage that is responsible for the plasticity and endurance of joint tissues. Degradation of aggrecan is one of the main processes that cause functional disability of cartilage during arthritis. Taken together that aggrecan appears to have an additional role in collagen protection from degradation,[79] inhibition of aggrecanases has evolved as a potential medicinal target for treatment of osteoarthritis (OA). ADAMTS-4 (aggrecanase-1) and -5 (aggrecanase-2) have been identified as the aggrecanases responsible for aggrecan degradation in OA after the development of the first aggrecanase inhibitors which were evaluated by assays measuring total aggrecanase activity.[80] Recently, AGG-523, an ADAMTS-4/-5 inhibitor developed by Wyeth, has advanced to Phase I clinical trials for the treatment of OA. Furthermore, recent evidence that ADAMTS-5 knockout mice are protected from cartilage destruction suggest that ADAMTS-5 may have a central role in human osteoarthritis as well.[81,82] Efforts toward the development of selective ADAMTS-5 inhibitors have already been reported and future research in this area is expected to be greatly accelerated by the recently published crystal structure of ADAMTS-5 catalytic domain bound to a hydroxamate-type inhibitor.[83]

3.2. TACE inhibitors

Successful early work toward the discovery of selective TACE inhibitors was reported in 2001 by DuPont researchers. Thus, based on the broad-spectrum MMPI marimastat (**29**, Fig. 10), a proper linker between P_1 and P_2' groups was introduced to form a macrocyclic peptidomimetic scaffold that improved the physical properties of the inhibitor without disturbing the necessary extended conformation of the parent peptide backbone.[84] The desired selectivity for porcine TACE (pTACE) over MMPs was obtained after SAR studies on the P_1' side chain of the inhibitor which showed that long biphenyl moieties are better fitted in the S_1' pocket of TACE than that of the MMPs.[84,85] For example, profiling of compound **28** (Fig. 9) revealed that >100-fold selectivity is attainable over a broad panel of MMPs (with the exception of MMP-8 where a 45-fold selectivity is observed). Furthermore, compound **28** displayed potent cellular activity in the inhibition of TNF-α release.

In 2002, scientists from Bristol-Myers Squibb reported the discovery of a γ-lactam hydroxamic acid (IK682, **32**) that was able to inhibit pTACE potently and selectively from 10 MMP isoforms (Fig. 10).[86] The main γ-lactam scaffold of **32** was designed based on the key interactions of known broad-spectrum MMPIs marimastat (**29**) and CGS27023A (**30**) with MMP-3 which are (a) the hydroxamate zinc(II) ligand and (b) a hydrogen bond between the succinyl carbonyl oxygen of marimastat or the sulfone oxygen of CGS27023A and MMP-3 (in particular with Leu164) that suitably projects the P_1' group toward the S_1' subsite. From an homology model of TACE based on the crystal structure of atrolysin (a snake venom protein that belongs to the family of adamalysins, like TACE) and comparison with several crystal structures of MMPs, a uniquely bend-shaped S_1' pocket was identified that was ideally exploited for inhibitor selectivity.[87] Thus, introduction of a (2-methyl-quinolin-4-yl)methoxy group in the *para* position of the phenyl group of **31** was sufficient to switch the inhibition profile of lead **31** from a weak, broad-spectrum MMP/TACE inhibitor to a highly selective pTACE inhibitor, thus confirming the initial hypothesis (Fig. 10). In addition, IK682 behaves as a tight-binding, extremely potent inhibitor for human TACE,[88] is a weak inhibitor of ADAM-33,[89] inhibits potently TNF-α production in a LPS-stimulated whole blood assay (WBA) with an IC_{50} of 0.35 nM and displays good oral availability in rats and dogs. Further optimization



	K_i(nM)
pTACE:	**2.8**
MMP-1:	>2000
MMP-2:	>2000
MMP-3:	>2000
MMP-7:	834
MMP-8:	126
MMP-9:	>2000
MMP-10:	>2000
MMP-12:	>2000
MMP-13:	653
MMP-14:	>2000
MMP-15:	>2000
MMP-16:	>2000

Figure 9. A macrocyclic peptidomimetic selective TACE inhibitor.

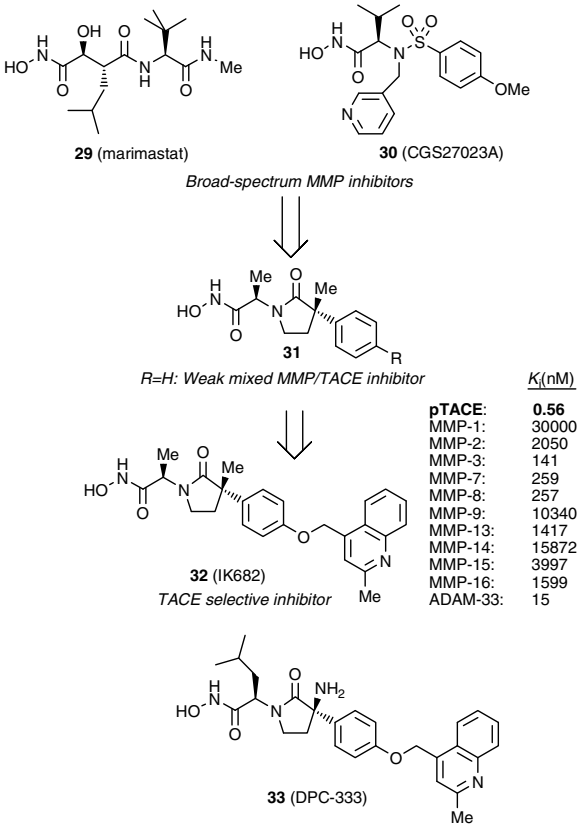
of IK682 structure resulted to analog **33** which is one of the two TACE inhibitors (partially selective) that managed to reach Phase II clincal trials for the treatment of rheumatoid arthritis.[68]

Rational modification of the main structural scaffold of MMPI CGS27023A (**30**) in combination with (2-methyl-quinolin-4-yl)-methoxy group in P_1' position as a selectivity determinant for TACE over MMPs led to the development of several additional novel types of hydroxamates that in some cases showed notable selectivity.[90–92] In such a case, a cyclic succinate derivative named IM491 (piperidine dicarboxamide analog **34**, (Fig. 11)) was identified which exhibited comparable nanomolar potency for pTACE in in vitro and WBA assays, decent cell permeability and high selectivity against MMP-1, -2, and -9.[92] In an effort to solve the problem of toxic metabolites of IM491 derived from hydrolysis of the anilinyl amide bond in vivo, several series of analogs with more stable anilide surrogates were tested.[93,94] Among these, inhibitors **35**–**37** of Figure 11, where the succinyl hydroxamic backbone has been replaced by a β-aminohydroxamic acid, stood out for their selectivity for TACE and their behavior in cellular and pharmacokinetic assays. Thus, the β,β-tetrahydropyran analog **35** displayed subnanomolar affinity for pTACE and significant selectivity against a wide panel of MMPs (modest selectivity for MMP-3 and -7) and three ADAM-TSs.[94] Selective TACE inhibition is also obtained with the very similar piperidinyl analog **36**.[95] Notably, this minor structural modification is sufficient to alter significantly the selectivity profile of the inhibitor (drop of selectivity of **36** for TACE over MMP-14 and -15 as compared to **35**). High in vitro and WBA potency for pTACE as well as exceptional selectivity profile was also observed for the α,β-tetrahydropyran derivative **37** and, in addition, cell permeability and oral bioavailability were enhanced, as compared to **35**.[96] Moreover, suitably substituted five-membered rings instead of the six-membered tetrahydropyran ring of **37** are generally well-tolerated,[97,98] although selectivity factors against some MMPs are slightly inferior.[97] Concerns on the metabolic instability of the P_1' group phenolic bond and on the low but not negligible affinity of **37** for MMP-3, -7, -8, and -12, prompted efforts for further optimization of the P_1' side chain.[99–101] Compounds **38**[100] and **39**[101] are the most interesting inhibitors of this study with excellent overall inhibition profiles (Fig. 12) and pharmacokinetic properties. Compound **38** is currently in pre-clinical trials for RA treatment.[68]

Since hydroxamates often suffer from metabolic liabilities, non-hydroxamate TACE inhibitors have been pursued that included in their structure the (2-methyl-quinolin-4-yl)methoxy P_1' group and various heterocyclic, enolizable Zn ligands.[102,103] For example, SAR on pyrimidine-2,4,6-trione derivatives provided compound **40** which was very selective for TACE over MMP-2, -3, -7, -12, and aggrecanase but, most importantly, extremely potent for a non-hydroxamate inhibitor of this type (Fig. 13).[103] Unfortunately, compound **40** proved incapable of inhibiting TNF-α production in a WBA assay. High potency and selectivity for TACE was also observed for hydantoin[104] and imidazolone[105] derivatives, as in the case of compound **41** which displayed low nanomolar potency for TACE (Fig. 13). Interestingly, a molecular modeling analysis study revealed that the aforementioned non-hydroxamate ZBGs participate in monodentate metal interactions as compared to the bidentate hydroxamates but this shortcoming is compensated by additional hydrogen bonds in the vicinity of the active site.[106] In sharp contrast, pyrimidinetriones are likely to perform bidendate interactions with MMPs as it was previously revealed by analysis of a MMP-8/pyrimidinetrione crystal structure.[107]

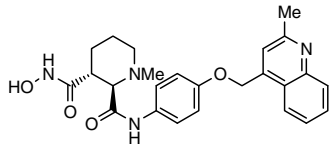
In a parallel research program by Wyeth, several sulfonamide and sulfone hydroxamates were profiled toward TACE and MMPs. Early SAR studies on sulfonamide scaffolds demonstrated that selectivity for TACE over MMPs is greatly enhanced by alkynyl groups in P_1' position such as butynyl groups attached to a phenolic hydroxyl.[108,109] Structural analysis showed that this group is able to extent toward the S_3' subsite through the S_1' pocket by adopting a favorable bent conformation which cannot be accommodated by MMPs with short (MMP-1) or longer (MMP-13) S_1' channels.[110] A representative example of this type of TACE inhibitors is TMI-2 (compound **42**, Fig. 14) which displays modest to good selectivity for 6 MMPs and ADAM-10 and has been used in pre-clinical studies for the treatment of rheumatoid arthritis.[110,111] Further optimization of the backbone as well as the P_1 side chain of parent alkynyl hydroxamic structures was described in a number of following reports and, in some cases, notable selectivity was reported over certain subsets of MMPs.[112–116] To that end, sulfone hydroxamates with various types of aryl (**43**),[112] heteroaryl (**44**),[114] sulfonyl (**45**)[113] or acyl (**46**)[115] N-substituted piperidine rings at P_1 position displayed a high preference for TACE over some MMPs, a property attributed to the more spacious S_1 pocket of TACE as compared to MMPs (Fig. 14).[114]

Other recent reports have focused on the substitution of the metabolically unstable hydroxamic acid group by alternative



	K_i(nM)
pTACE:	**0.56**
MMP-1:	30000
MMP-2:	2050
MMP-3:	141
MMP-7:	259
MMP-8:	257
MMP-9:	10340
MMP-13:	1417
MMP-14:	15872
MMP-15:	3997
MMP-16:	1599
ADAM-33:	15

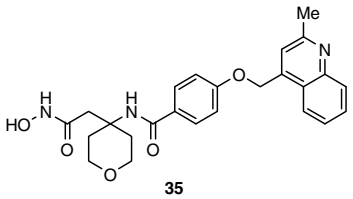
Figure 10. Design of selective TACE inhibitors IK682 and DPC-333.



34 (IM491)

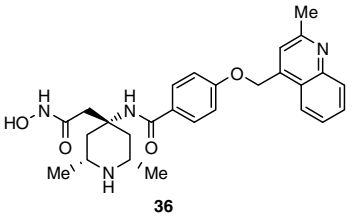
pTACE: IC_{50}=6.2nM

	K_i(nM)
MMP-1:	>4949
MMP-2:	>3333
MMP-9:	>2128



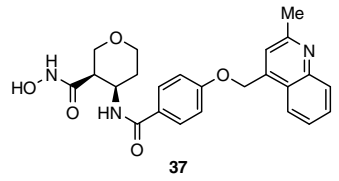
35

	K_i(nM)
pTACE:	**0.35**
MMP-1:	>4949
MMP-2:	>3333
MMP-3:	82
MMP-7:	25
MMP-8:	>3100
MMP-9:	>2128
MMP-13:	>5025
MMP-14:	>5290
MMP-15:	>7088
MMP-16:	>5554
ADAMTS-1:	7800
ADAMTS-4:	4600
ADAMTS-5:	1900



36

	K_i(nM)
pTACE:	**2.8**
MMP-1:	>5000
MMP-2:	>3000
MMP-3:	762
MMP-8:	2050
MMP-9:	>3000
MMP-10:	1650
MMP-13:	>5000
MMP-14:	163
MMP-15:	1700
ADAM-10:	>3000
ADAMTS-1:	7800
ADAMTS-4:	4600
ADAMTS-5:	1900

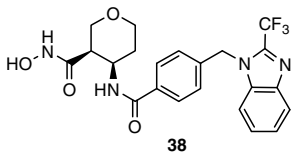


37

pTACE: K_i=0.15nM

	Fold Selectivity
MMP-1:	>30000
MMP-2:	>30000
MMP-3:	427
MMP-7:	200
MMP-8:	5500
MMP-9:	>14000
MMP-10:	320
MMP-12:	93
MMP-13:	>30000
MMP-14:	>30000
MMP-15:	>40000
MMP-16:	>30000
ADAM-9:	>30000
ADAM:10	>50000
ADAMTS-4	20000
ADAMTS-5	2267

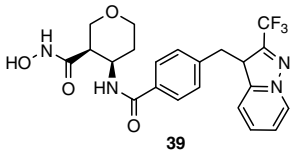
Figure 11. Selective TACE inhibitors by Bristol-Myers Squibb.



38

pTACE: IC_{50}=1.4nM

	K_i(nM)
MMP-1:	>10000
MMP-2:	>10000
MMP-3:	>10000
MMP-7:	>10000
MMP-8:	>10000
MMP-9:	>10000
MMP-10 (IC_{50}):	>10000
MMP-12 (IC_{50}):	8000
MMP-13:	>10000
MMP-14:	>10000
MMP-15:	>10000
MMP-16:	>10000
ADAMTS-1:	>1000
ADAMTS-4:	6700
ADAMTS-5:	1000
ADAM-10:	>10000

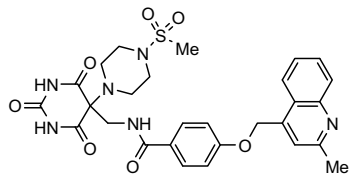


39

pTACE: IC_{50}=1.0nM

	K_i(nM)
MMP-1:	>4946
MMP-2:	>3333
MMP-3:	>4501
MMP-7:	>6368
MMP-8:	>3058
MMP-9:	>2128
MMP-10:	>5346
MMP-12:	>6023
MMP-13:	>5025
MMP-14:	>5290
MMP-15:	>7088

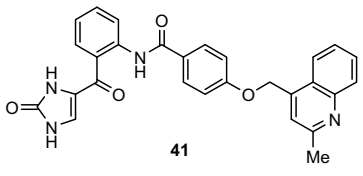
Figure 12. Selective TACE inhibitors by Bristol-Myers Squibb.



40

pTACE: IC_{50}=2nM

	K_i(μM)
MMP-2:	2.17
MMP-3:	>4.50
MMP-7:	>6.37
MMP-12:	1.02
aggrecanase:	11% (at 1μM)



41

pTACE: IC_{50}=9nM

	K_i(nM)
MMP-2:	>3333
MMP-3:	>4501
MMP-7:	>636
MMP-12:	>6023
MMP-13:	4314

Figure 13. Non-hydroxamate TACE inhibitors.

zinc(II) ligands and in some cases the selectivity of these inhibitors for TACE over a limited number of MMPs has been evaluated. For example, reverse hydroxamate **47** is a low nanomolar inhibitor of TACE with excellent selectivity over 8 MMP isoforms (Fig. 15).[117] In addition to the presence of a (2-methyl-quinolin-4-yl)methoxy group in P_1' position of **47** which is a well-established selectivity determinant for TACE over MMPs, selectivity was also attenuated by the existence of an exocyclic double bond on the piperidine ring but the exact role of this structural feature is not clarified. Finally, greater than 100-fold selectivity for TACE over MMP-2, -7, -8, -9, and -13 has been reported for the thiol-based inhibitor **48** which is rather impressive for a molecule of such small size (Fig. 15).[118] In accordance to the related butynyloxy inhibitors of Figure 14, an X-ray crystal structure of **48** in complex with TACE revealed that the phenyl group fills the S_1' pocket in a way that butynyloxy group is properly projected toward the S_3' pocket of the enzyme.

Recently, a study of Zhu et al. raised important issues concerning the use of existing pharmacophore models for the discovery of TACE inhibitors and the evaluation of results derived from SAR studies.[119] In particular, hydroxamate-type inhibitors **49** and **50**, that differ mainly in the stereochemistry of cyclopropyl ring, display both good to excellent selectivity over five MMPs and BMP-1 (PCP) but only **49** is inactive toward ADAM-10 (Fig. 16). Interestingly, X-ray crystal structures of complexes of **49** and **50** with TACE revealed an unexpected switch of the binding mode between these two inhibitors. While hydroxamate **49** projects the quinoline group toward S_3' subsite through the S_1' shallow pocket, hydroxamate **50** is extended in the opposite direction with the carbomethoxy and quinoline groups pointing toward S_1 and S_3 subsites, respectively. This difference in binding mode is reflected to the high variations on the selectivity profile of inhibitors **49** and **50** for TACE and provides a solid basis to the hypothesis that previously described TACE inhibitors might had already taken advantage of both primed and unprimed site binding pockets to achieve selectivity. Notably, the unprimed site binding mode appears more efficient in terms of inhibitor selectivity over MMPs. This could be possibly attributed to the shape of S_3 pocket of TACE which differs significantly from that of MMPs and has been recently recognized as a hot spot for the development of TACE-specific inhibitors.[120] However, this strategy does not seem to discriminate TACE from ADAM-10. Of special interest is also the case of thiirane **18** (Fig. 6) which is suggested to occupy a binding site in TACE almost entirely non-overlapping as compared to the corresponding site in MMP-2, a property that could account for the different kinetic behavior of **18** upon binding with these two enzymes.[49] This

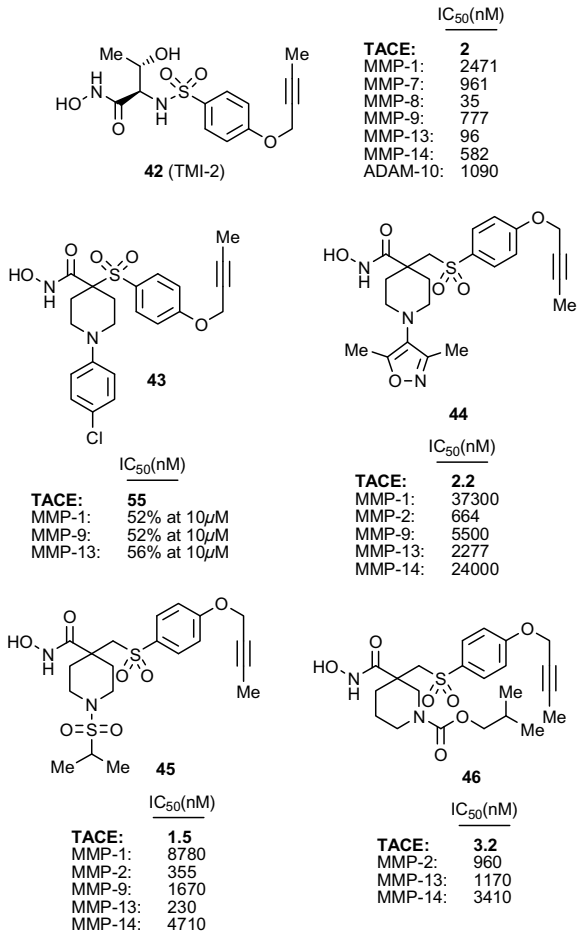


Figure 14. Alkynyl-based selective hydroxamate TACE inhibitors.

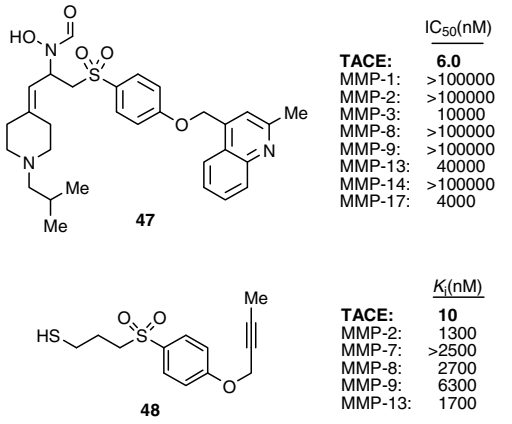


Figure 15. Selective TACE inhibitors **47** and **48**.

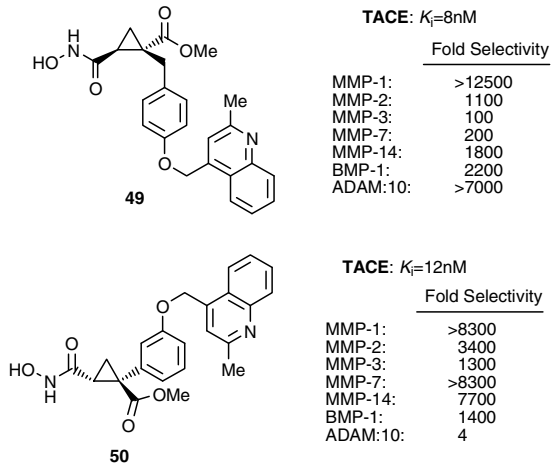


Figure 16. Cyclopropyl hydroxamate-type TACE inhibitors.

unanticipated binding of **18** with TACE is accompanied by profound conformational changes that are suggested to prevent TACE activity. Based on these observations, it has been recently proposed that targeting alternative sites different from the catalytic zinc(II) site which induce perturbations in the structural and electronic balance of TACE catalytic microenvironment can evolve in the future as a potential new approach for the development of TACE selective inhibitors.[121] Finally, it is emphasized that all 'selective' TACE inhibitors that have been described in the literature are only truly selective over MMPs and they have not been evaluated for their selectivity against a wide panel of other proteolytically active ADAMs. This important aspect of TACE inhibitor specificity is still an unexplored issue that undoubtedly needs to be addressed in the future.

3.3. Other ADAMs inhibitors

Among the other members of ADAMs protease family, only ADAM-10 has drawn significant attention with respect to the development of specific inhibitors as anti-cancer agents.[122] Recently, researchers from Incyte Corporation initiated a program toward the identification of hydroxamate-based inhibitors that spare MMPs and are specific for ADAM-10 or for both ADAM-10 and TACE. These efforts were encouraged by evidence demonstrating that ADAM-10 is a major sheddase of human epidermal growth factor receptor-2 (HER-2) which is overexpressed in various types of cancer and is associated with decreased responsiveness to conventional chemotherapeutics.[123] In addition, ADAM-10 and -17 are implicated in the shedding of multiple ErbB ligands which trigger signaling cascades upon binding to ErbB receptors and promote tumorigenesis.[71] The design of these compounds by Incyte researchers relied on the optimization of a cyclic succinate scaffold that had been used in the past to a series of potent TACE inhibitors (see IM491, Fig. 11).[124] This optimization included rigidification of the piperidine ring by the installation of a cyclopropyl group in order to better define the spatial arrangement of P_1' side chain and proper selection of P_1' bicyclic system in order to achieve a non-coplanar orientation between the two rings which proved to be the major determinant of inhibitor selectivity. Furthermore, the selectivity profile is dependent on the type of substitution of piperidine nitrogen (P_2' position) which seems to enhance P_1'–S_1' specific interactions by affecting favorably the overall conformation of the inhibitor.[125] Compound **51** is a typical example of these inhibitors that is highly specific for ADAM-10 over 3 ADAMs and 8 MMPs (Fig. 17).[123,126] On the other hand, compound **52** (INCB3619) is an ADAM-10/-17 specific inhibitor with good selectivity over 3 ADAMs and MMP-1, -3, and -7.[124,126,127] INCB7839, a related dual ADAM-10/-17 inhibitor with improved selectivity over MMP-2, -9, and -14 as compared to INCB3619, has advanced to clinical trials but its structure has not been announced yet.[73] Finally, improved selectivity for ADAM-10 over gelatinases was achieved with inhibitor **53** which is based on a different core scaffold but retains similar structural characteristics in P_1' position with INCB3619 that account for the observed ADAM-10 selectivity.[128] No selectivity data for other ADAMs has been reported for this inhibitor.

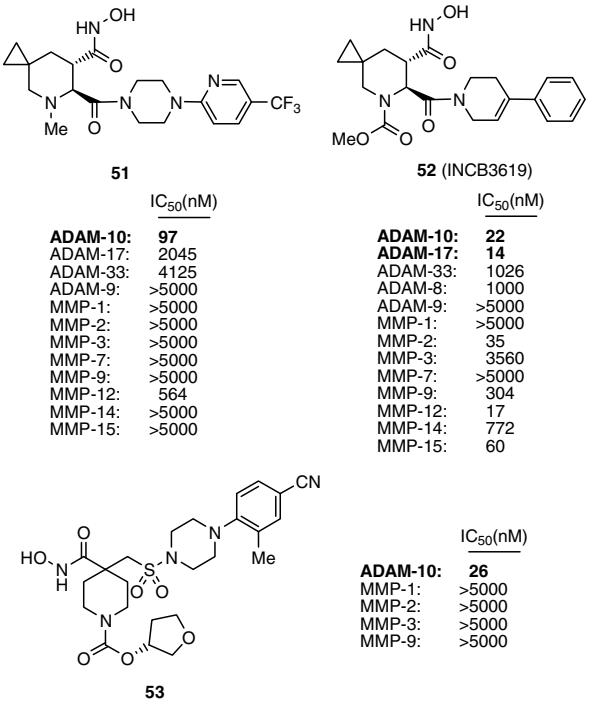


Figure 17. ADAM-10 inhibitors.

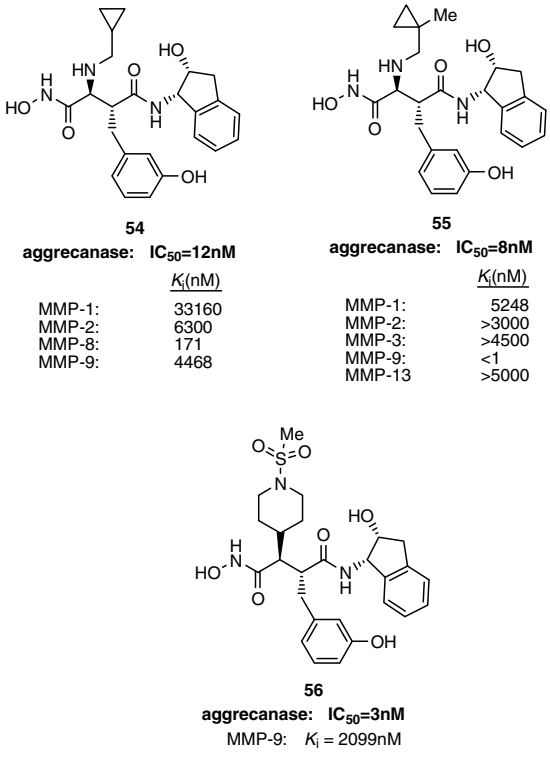


Figure 18. Aggrecanase inhibitors.

3.4. ADAMTSs inhibitors

As it was mentioned above, the contribution of ADAMTS-4 and -5 in osteoarthritis initiated efforts for the discovery of small-molecule synthetic inhibitors that would control their aggrecanolytic activity. Since ADAMTS-4 and -5 had not been isolated during the development of the first inhibitors, their in vitro potency was evaluated using a bovine derived aggrecanase assay measuring the total aggrecanase activity. In 2001, Yao et al. reported the design and development of aggrecanase hydroxamate-type inhibitors by using a structure-based approach.[129] Initial leads were taken from substrate specificity data concerning MMP-8, an enzyme that also exhibits aggrecanolytic activity. To this respect, a tyrosine residue in P_1' position of a peptidic hydroxamate-type scaffold provided a hybrid able to inhibit aggrecan and MMP-8 while sparing MMP-1, -2, and -9. Rigidification of P_2' position, exploitation of P_1/S_1 interactions and shift of pseudotyrosine hydroxyl group from *para* to *meta* position improved inhibitor potency as well as selectivity over MMP-8. Interestingly, as it depicted in Figure 18, minor alterations in P_1 side chain has a great impact in the selectivity of the inhibitor. Thus, compound **54** is selective for aggrecanase over MMP-9 while compound **55** cannot discriminate between these two enzymes.[79,129] A subsequent SAR study of the P_1 position of these molecules further validated the importance of P_1 substitution in the selectivity profile. Indeed, affinity as well as selectivity over MMP-9 was significantly enhanced by rational P_1 optimization leading to analogs such as **56** which also displays >100-fold selectivity for MMP-1 and -2 (Fig. 18).[130]

Recently, researchers from Wyeth reported the first study aiming to the development of non-hydroxamate ADAMTS-5 inhibitors that do not block ADAMTS-4, based on scaffolds identified from high throughput screening.[131,132] Despite the modest affinity of these inhibitors for ADAMTS-5, they succeed to spare ADAMTS-4 and some MMPs. The structures and in vitro properties of two ADAMTS-5 inhibitors derived from Wyeth studies are shown in Figure 19.

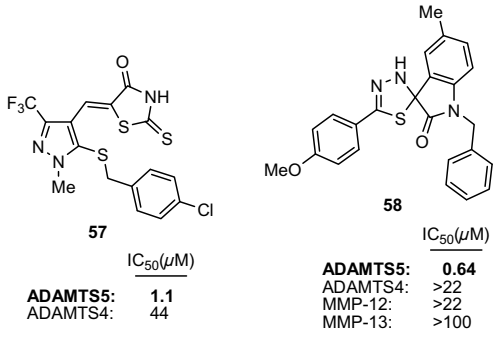
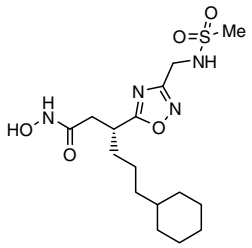


Figure 19. ADAMTS-5 inhibitors.

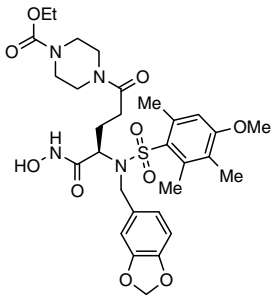
4. Procollagen C-proteinase selective inhibitors

Procollagen C-proteinase (PCP), also known as bone morphogenetic protein-1 (BMP-1), belongs to the astacin family of metzincins and cleaves the C-terminal propeptide of types I, II, and III procollagens resulting in the formation of insoluble, fibrillar collagens.[133] PCP has been proposed as an attractive medicinal target for disrupting the collagen deposition pathway which leads to the formation of scar tissue during dermal wound healing.[134] While PCP inhibitors are known for over a decade, only a few recent reports dealt with the issue of specific inhibition of this enzyme over other ECM-processing metalloproteases. To this respect, Pfizer researchers, developed selective PCP inhibitors, for topical application as anti-scarring agents, that spare MMP-1, -2, -9, and -14 which are important for normal wound healing.[135] Compound **59** is an hydroxamate-type inhibitor derived from this study that shows excellent selectivity over 6 MMPs and favorable physicochemical properties for efficient skin penetration upon topical application (Fig. 19). It is suggested that the (cyclohexyl)propyl side chain plays an important role in the regulation of selectivity



59

	K_i(nM)
PCP:	11
MMP-1:	>100000
MMP-2:	>100000
MMP-3:	91000
MMP-9:	>100000
MMP-13:	>100000
MMP-14:	>100000



60

	K_i(nM)
PCP:	43
MMP-1:	32000
MMP-8:	30000

Figure 20. PCP hydroxamate-type inhibitors.

profile while the oxadiazole heterocycle also contributes significantly to inhibitor specificity as compared to related acyclic succinyl derivatives. In another report, hydroxamate **60** was identified from SAR of combinatorial libraries of hydroxamate-type inhibitors prepared by solid-phase synthetic protocols.[136] As shown in Figure 20, compound **60** shows a preference for PCP over MMP-1 and MMP-8. It is proposed that the 4-methoxy-2,3,6-trimethylbenzenesulfonyl group of inhibitor **60** is the major determinant for the observed PCP selectivity. Interestingly, removal of methyl substituents from arylsulfonyl group of **60** dramatically attenuates affinity for MMP-1 and -8.

5. Summary and conclusions

The family of metzincins has emerged over the past 20 years as an extremely challenging field of medicinal research since deregulation of their physiological activity status is directly connected with diseases of major pharmaceutical interest such as cancer or inflammation. The greatest merit of attention has been undoubtedly given to the discovery of small-molecule active-site directed MMP inhibitors and their clinical evaluation in cancer patients. Unfortunately, all advanced clinical trials with such molecules have been discontinued mainly due to unwanted side-effects such as tendonitis and fibroplasia. Advances toward the understanding of the exact biological function of medicinally relevant metzincins as well as the identification of new metzincins in human has contributed to the revelation of an extremely complex protease network that is finely regulated and highly balanced from both spatial and temporal aspects. In this context, the failure of the first-generation MMP inhibitors does not come as a surprise since only three MMPs were known when the first medicinal projects launched. For instance, several MMPs mediate the release of signaling molecules that where shown to inhibit angiogenesis and vasculogenesis in mice, such as angiostatin and endostain. It is now clear that broad-spectrum metzincin inhibition is inefficient for effective pharmaceutical intervention in cancer since proteolytic products of MMPs or other metzincins are not exclusive contributors to tumor growth and progression, as it was initially thought, but they also seem to mediate host defense. Together with the validation of target and anti-target metzincins in disease states, the bulk of inhibitor design efforts needs to focus on the improvement of inhibitor selectivity index in order to obtain specific tools for probing metzincin function and develop drug-candidates with better chances in clinical applications. The design of such inhibitors is a difficult task given the conserved active-site topology and aminoacid sequence among metzincins as well as the inherent flexibility of metzincin catalytic domain parts that determine substrate specificity. The recent development of truly selective inhibitors for MMP-12 and MMP-13 suggest that specific-targeting of metzincins may not be an elusive goal.[35,57] Future advances in this field are expected to increase the number of metzincins that can be selectively blocked and give a new impetus to the therapeutic prospect of metzincin inhibition.

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