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New strategies for targeting matrix metalloproteinases

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Abstract

The development of matrix metalloproteinase (MMP) inhibitors has often been frustrated by a lack of specificity and subsequent off-target effects. More recently, inhibitor design has considered secondary binding sites (exosites) to improve specificity. Small molecules and peptides have been developed that bind exosites in the catalytic (CAT) domain of MMP-13, the CAT or hemopexin-like (HPX) domain of MT1-MMP, and the collagen binding domain (CBD) of MMP-2 and MMP-9. Antibody-based approaches have resulted in selective inhibitors for MMP-9 and MT1-MMP that target CAT domain exosites. Triple-helical “mini-proteins” have taken advantage of collagen binding exosites, producing a family of novel probes. A variety of non-traditional approaches that incorporate exosite binding into the design process has yielded inhibitors with desirable selectivities within the MMP family.

Keywords

Matrix metalloproteinase (MMP); Exosite; Collagen; Inhibitor; Triple-helical peptide

Introduction

Matrix metalloproteinases (MMPs) have long been recognized as potential targets for a variety of pathologies, including tumor angiogenesis and metastasis, osteoarthritis (OA), inflammation, periodontitis, vascular diseases, post-myocardial infarction remodeling, neurodegenerative diseases, and neuropsychiatric disorders [1–7]. The development of MMP inhibitors has typically proceeded along the path of active site Zn²⁺ inhibition. The most common zinc-binding group used for this purpose is hydroxamic acid [8,9]. However, one reason why hydroxamic acid--based inhibitors have not been successful in clinic trials is their lack of selectivity [9,10]. The low selectivity originated from the fact that inhibitors targeting the enzyme active sites face the challenge of very similar chemistry and

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configuration of these sites across the MMPs [11]. In addition, under certain circumstances, hydroxamic acids may chelate zinc in a non-selective fashion [9,10]. An often observed side effect of hydroxamic acid-based MMP inhibitors has been musculoskeletal syndrome (MSS). MSS has been attributed to inhibition of MMP-1 and ADAM17/TACE [12,13]. A pyrimidine-2,4,6-trione derivative that inhibits MT1-MMP, MMP-2, and MMP-9 is not associated with MSS, and thus demonstrates that better selectivity has the potential to create therapeutically useful MMP inhibitors [14]. Similarly, MMP-13 inhibition does not induce MSS in rat models [15].

More recent strategies for developing inhibitors with greater selectivity consider secondary binding sites (exosites) [16–19]. Also referred to as regulatory sites, unique exosites have been proposed to be present in all MMPs [20]. Considerable prior work has utilized phage display or combinatorial peptide libraries to find peptide-based inhibitors of MMPs [21]. Although these inhibitors may target exosites, the actual binding sites have often not been identified. The following discussion focuses on probes that interact with distinct secondary binding sites of MMPs, and in some cases utilize non-traditional zinc-interaction motifs.

MMP-13 specificity pockets within the catalytic domain

Aventis discovered a pyrimidine dicarboxamide that had low micromolar potency for MMP-13 and no activity against other MMPs when tested at 100 μ M [22]. The potency of this compound was further improved to a low nanomolar compound (N4,N6-bis(4-fluoro-3-methylbenzyl)pyrimidine-4,6-dicarboxamide) without losing selectivity [22]. The Aventis molecule binds within a “specificity loop” (subsite S_1') of the MMP-13 catalytic (CAT) domain, which is recognized as an exosite (Fig. 1) [22,23]. Pfizer reported discovery of highly selective nanomolar range MMP-13 inhibitors based on pyrimidinedione and quinazolinone scaffolds acting via binding to the same S_1' exosite [24,25]. Furthermore, pyrimidinedione derivatives were efficacious and safe in rabbit and dog models of OA [25,26] and mouse models of rheumatoid arthritis [27]. Similarly, Alantos Pharmaceuticals identified a new class of highly selective non- Zn^{2+} -binding MMP-13 inhibitors [15,28,29]. ALS 1-0635 provided histologic and clinical efficacy without musculoskeletal toxicity. Binding studies of ALS 1-0635 to the MMP-13 CAT domain indicated non-competitive, reversible MMP-13 inhibition and non-exclusive binding when tested against a non-specific Zn^{2+} chelator. The compound displayed bovine and human articular cartilage protection at sub-micromolar concentrations in vitro. It also provided chondroprotection in the in vivo rat model of acute and chronic OA at reasonable concentrations. Furthermore, no MSS was observed in ALS 1-0635-treated animals, even at a 200-fold greater concentration than that of marimastat known to induce this condition [15].

Although selective MMP-13 inhibitors have been described by Alantos, Aventis, Boehringer, Pfizer, and Wyeth, important pharmacokinetic (PK) and/or other data have not been reported for many of these compounds, and no clinical studies have appeared. For example, no PK or MSS data has been reported for the Aventis and Wyeth compounds [22,30]. The first series of Pfizer compounds, while exhibiting good PK and MSS data, were tested against a limited number of MMPs [31–33]. In similar fashion, the Boehringer compounds exhibited good PK data but were tested against a limited number of MMPs, and

not at all in a MSS model [34,35]. The Alantos compounds exhibited excellent MMP selectivity and good PK data, but were not tested in a MSS model [28,29]. Only the second series of Pfizer compounds were reported to exhibit excellent MMP selectivity and good PK and MSS data [24,25,27]. However, as mentioned above, no clinical studies have been reported for the Pfizer compounds. In our hands, we found the primary Pfizer compound (E)-4-((1-methyl-2,4-dioxo-6-(3-phenylprop-1-enyl)-1,2-dihydroquinazolin-3(4H)-yl)methyl)-benzoic acid (Fig. 2) to have low solubility (it could only be tested at a maximal concentration of 2.5 μ M), and it inhibited cytochrome P450 1A2 [36].

As a result of high-throughput screening and structure–activity relationship studies, we identified a novel, highly selective class of MMP-13 inhibitors (Fig. 2) [37]. Medicinal chemistry characterization of the compound Q/4 2-(arylmethylthio)-cyclopentapyr-imidin-4-one scaffold led to two compounds (2-[(4-methylphenyl)methyl sulfanyl]-1,5,6,7-tetrahydro-clopenta[d]pyrimidin-4-one; Q1/20 and methyl 4-[(4-oxo-1,5,6,7-tetrahydrocyclopenta[d]pyrimidin-2-yl) sulfanylmethyl] benzoate; Q2/24) that demonstrated improved potency (as measured by K_i) and selectivity compared to compound 4 [38]. Most significantly, compound 20 did not inhibit MMP-8, whereas compound 4 did. In addition, compounds 4, 20, and 24 did not inhibit MMP-1 or TACE [38], and MSS has been attributed to inhibition of MMP-1 and ADAM17/TACE [13].

Mechanistic characterization revealed a noncompetitive nature of these inhibitors with binding constants in the low μ M range. Surprisingly, compound Q/4 exhibited non-mutually exclusive binding (positive cooperativity) when co-tested with the Aventis molecule N4,N6-bis(4-fluoro-3-methylbenzyl)pyrimidine-4,6-dicarboxamide (see Fig. 2 for structure) [36]. Similarly, compound Q/4 binding to MMP-13 was synergistic with the Pfizer molecule (Fig. 2). These results indicate that compound Q/4 has the potential to work through a different mechanism than previously identified inhibitors. Crystallographic analyses revealed two binding modes for compound Q1/20 in the MMP-13 S_1' subsite and in a S_1/S_2^* subsite [36]. Type II collagen- and cartilage-protective effects exhibited by compounds Q/4, Q1/20, and Q2/24 suggested that these compounds might be efficacious in future *in vivo* studies. Finally, these compounds were also highly selective when tested against a panel of 30 proteases, which, in combination with a good cytochrome P450 inhibition profile, suggested low off-target toxicity and drug–drug interactions in humans [36].

Compounds Q/4, Q1/20, and Q2/24 have 10–100 times lower affinity than the primary Pfizer and Aventis compounds. However, compounds Q/4, Q1/20, and Q2/24 possess a smaller molecular scaffold than previously described MMP-13 inhibitors (Fig. 2), suggesting that greater affinity can be achieved by increasing the size of future analogs of Q/4, Q1/20, and Q2/24. A similar approach was recently described using a thienol[2,3-d]pyrimidine scaffold and extension into the S_1'' subsite of MMP-13 [39]. Targeting multiple structural water molecules within the S_1' subsite as binding partners has also been suggested as a way to improve the potency of MMP-13 inhibitors [40].

MT-MMP loop in the CAT domain

The need for proper interaction of MT1-MMP with cell surface partners is exemplified in studies of the 163–170 loop region within MT1-MMP CAT domain. This MT (membrane type)-loop region is present in the CAT domain of MT1-MT6-MMPs, but is absent in all other MMPs. Deletion of this loop results in the mislocalization of MT1-MMP relative to the β 1 integrin adhesion complexes and subsequent decrease of activity towards collagen films [41].

Monoclonal antibody (mAb) 9E8 inhibits MT1-MMP activation of proMMP-2, but not other MT1-MMP catalytic activities [42]. Analysis of mAb 9E8 interaction with MT1-MMP determined that the antibody bound to the Pro163 to Gln174 loop in the CAT domain [43]. Binding of mAb 9E8 to the loop interferes with TIMP-2 binding, preventing formation of the MT1-MMP·TIMP-2·proMMP-2 complex required for proMMP-2 activation [43].

An MT1-MMP near infrared probe was designed based on a peptide sequence identified in a phage display substrate library [44]. To enhance selectivity, the phage display library was screened against the MT1-MMP loop sequence (Arg160 to Gln174, designated MT1-160p) rather than the complete sequence of MT1-MMP or its CAT domain. The non-substrate peptide MT1-AF7p (His-Trp-Lys-His-Leu-His-Asn-Thr-Lys-Thr-Phe-Leu) displayed the highest affinity towards MT1-160p ($K_D = 0.075$ nM). MT1-AF7p was labeled with Cy5.5 (Cy5.5-MT1-AF7p) and chosen for further validation in vivo [44]. The evaluation was performed in mice carrying MDA-MB-435 breast cancer xenografts (expressing high levels of MT1-MMP) or A549 xenografts (low MT1-MMP levels). MDA-MB-435 xenografts had significantly higher signal accumulation and better tumor contrast than the A549 xenografts. However, more precise quantitative data on tumor uptake and PK will be needed to determine the further utility of this probe.

The HPX domain

Diversity in the HPX domains among MMPs makes this exodomain a good target in the search for selective MMP inhibitors. A hurdle is the typically low affinity of compounds that disrupt protein–protein interactions.

The outer blade regions of the MT1-MMP HPX domain have non-homologous loop sequences compared to other members of the MMP family. Our laboratory synthesized peptide models of the 5 MT1-MMP HPX domain loops (blade I strand 4, blade II strand 2, blade II strands 3–4, blade III strand 1, and blade IV strand 4), and examined their inhibitory activity for MT1-MMP processing of a triple-helical substrate [21]. Two peptides were micromolar inhibitors of MT1-MMP but did not inhibit MMP-1. Val-Phe-Asp-Glu-Ala-Ser-Leu-Glu-Pro-NH₂, from blade II strands 3–4, had the best IC₅₀ value, 238 μ M. This peptide may directly or indirectly inhibit MT1-MMP dimerization or interaction of MT1-MMP with the substrate triple-helix. Val-Arg-Asn-Asn-Gln-Val-Nle-Asp-Gly-Tyr-Pro-Nle-Pro-NH₂, which is an N-terminal extension and modified version of the blade IV strand 4 peptide, was more effective than the parent peptide for MT1-MMP inhibition of triple-helical peptidase activity (IC₅₀ = 670 μ M).

A small molecule MT1-MMP HPX domain inhibitor was identified using virtual ligand screening of the NCI/NIH Developmental Therapeutics Program ~275,000 compound library [45]. Compound NSC405020 [3,4-dichloro-N-(1-methylbutyl)benzamide] inhibited MT1-MMP homodimerization but not catalytic activity towards a fluorogenic peptide substrate or proMMP-2 activation. NSC405020 was shown to reduce the collagenolytic activity of MCF7- β 3/MT1-MMP cells. The compound was effective in vivo, as intratumoral injections reduced tumor size significantly.

Collagen binding exosites

Using a combinatorial one peptide one bead library, Xu et al. determined that the MMP-2 collagen binding domain (CBD) binds a short segment of the α 1(I) collagen chain [46]. More specifically, Cys-Gly-Ala-Hyp-Gly-Ala-Hyp-Gly-Ser-Gln-Gly-Ala (designated P713, where Hyp = 4-hydroxyproline) was identified as an inhibitor of MMP-2 activity. P713 inhibited 90% of MMP-2 gelatin cleavage (IC_{50} of $\sim 30 \mu\text{M}$), but less than 20% of the MMP-2 activity on a peptide substrate which did not require the CBD for binding. To examine the specificity towards MMP-2, comparative inhibition assays were performed with MMP-8, with no alteration in MMP-8 activities observed upon P713 treatment [46].

Based on the single-stranded peptide model of the α 1(I)715-721 collagen sequence identified above as a ligand for the MMP-2 CBD, our group assembled a triple-helical version of this ligand [α 1(I)715-721 THP; (Gly-Pro-Hyp)₄-Gly-Ala-Hyp-Gly-Ala-Hyp-Gly-Ser-Gln-Gly-Ala-Hyp-(Gly-Pro-Hyp)₃-GPY-NH₂] [47]. α 1(I)715-721 THP inhibited MMP-2 and MMP-9 hydrolysis of a triple-helical peptide model of type V collagen, but did not inhibit MMP-2 or MMP-9 hydrolysis of a short, single-stranded substrate or a triple-helical peptide model of types I-III collagen. To our knowledge, this demonstrated the first use of an exosite binder to selectively inhibit one collagen-based MMP activity (type V) but not another (types I-III).

Incorporation of a zinc binding group within a collagen-model, triple-helical construct would add sequence diversity and eliminate off target interactions with non-collagenolytic proteases. Phosphinic peptides [$\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}$] have been shown to behave as transition state analog inhibitors of MMPs [48]. Phosphinate triple-helical MMP inhibitors have several potential advantages over other inhibitor constructs. These analogs allow incorporation of specificity elements for both the S and S' subsites of the enzyme. Although binding to the non-primed region of the active site is generally weaker than the primed site to prevent product inhibition [49], it does add sequence diversity and potential selectivity. The triple-helical structure allows for interaction with both the active site and exosites [50-52]. Triple-helical probes are less susceptible to general proteolysis than peptides and other folded proteins and thus offer favorable PK [53-56]. We have found that selective, high affinity inhibitors can be developed for MMPs based on triple-helical structure.

We have produced a series of THPIs based on Gly $\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}$ Leu, Gly $\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}$ Val, and Gly $\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}$ Ile transition state analogs (Table 1) [16,47,50,57,58]. The Gly $\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}$ Leu inhibitor [C₆-Gly-Pro-Flp-(Gly-Pro-Hyp)₄-Gly-Pro-Gln-Gly $\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}$ (R,S)Leu-Ala-Gly-Gln-Arg-Gly-Ile-Arg-(Gly-Pro-Hyp)₄-Gly-Pro-Flp-

NH₂], developed based on the MMP cleavage site in types I–III collagen [47], was effective for all collagenolytic MMPs tested, but offered a substantial range of activities (Table 1).

Two mutants of MMP-1, MMP-1(R291A) and MMP-1(I290A,R291A), where Ile290 and Arg291 were identified as two residues within the HPX domain that facilitate interactions between collagenolytic MMPs (MMP-1) and triple-helical structures, were tested with GlyΨ{PO₂H–CH₂}Leu THPI [50]. Low nM K_i values were observed for inhibition of MMP-1 activity by the THPI (Table 1). The K_i values for MMP-1(R291A) and MMP-1(I290A,R291A) were ~ 2-times and ~ 4-times higher, respectively, than wild type MMP-1 (Table 1). Thus, mutation of Ile290 and Arg291 decreased the affinity of MMP-1 for the THPI, indicating interaction of inhibitor with the HPX domain.

The GlyΨ{PO₂H–CH₂}Val inhibitor [C₆-(Gly-Pro-Hyp)₄-Gly-Pro-Pro-GlyΨ{PO₂H–CH₂} (R,S)Val-Val-Gly-Glu-Gln-Gly-Glu-Gln-Gly-Pro-Pro-(Gly-Pro-Hyp)₄-NH₂], based on the cleavage site in type V collagen by MMP-9 [16], was selective for MMP-2 and MMP-9 [16]. As was the case for the GlyΨ{PO₂H–CH₂}Leu THPI, the thermal stability of the GlyΨ{PO₂H–CH₂}Val THPI was greatly reduced compared to the parent substrate (Table 1) [16]. We synthesized a stabilized version of the α1(V)GlyΨ{PO₂H–CH₂}Val THPI, designated α1(V)GlyΨ{PO₂H–CH₂}Val [mep_{14,32},Flp_{15,33}] THPI. This THPI was tested with MMP-2 and MMP-9, and the K_i values were 189.1 and 90.6 nM, respectively (Table 1).

α1(V)GlyΨ{PO₂H–CH₂}Val [mep_{14,32},Flp_{15,33}] THPI has been applied in vivo. Citrate synthase (CS) was identified as an intracellular substrate of MMP-9 in a mice model of post-myocardial infarction (MI) [59]. Increased CS processing was abolished with the inhibition of MMP-9 by α1(V)GlyΨ{PO₂H–CH₂}Val [mep_{14,32},Flp_{15,33}] THPI [59]. Thus, MMP-9 inhibition improved mitochondrial function post-MI.

GlyΨ{PO₂H–CH₂}Ile inhibitors were synthesized based on prior results with single-stranded, hydroxamate-based inhibitors that were selective between MMP-8 and MT1-MMP [60]. Unfortunately, neither of the two GlyΨ{PO₂H–CH₂}Ile inhibitors were selective for MT1-MMP over MMP-8 (Table 1). This result was consistent with our prior study in which selectivity found in single-stranded peptides is not applicable to the same sequences within a triple-helix [61]. Nonetheless, the GlyΨ{PO₂H–CH₂}Ile-Tyr-Phe THPI did offer a promising lead for an MT1-MMP probe.

Antibodies and minibodies

Preparation of mouse mAbs using MMP-9 as antigen resulted in the identification of REGA-3G12, a selective inhibitor of MMP-9 [62]. REGA-3G12 recognizes the Trp116 to Lys214 region of MMP-9, located in CAT domain but not part of the Zn²⁺ binding site [63]. REGA-3G12 bound to MMP-9 with K_D = 2.1 nM and inhibited MMP-9 catabolism of type II gelatin [62]. A single chain variable fragment derived from REGA-3G12 selectively inhibited MMP-9 compared to MMP-2 [64]. Gelatin hydrolysis was inhibited 44% at a single chain variable fragment concentration of 5 μM [64]. REGA-3G12 was effective in vivo, preventing interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys [65].

A selective, fully human MT1-MMP inhibitory antibody (DX-2400; $IC_{50} \sim 1-5$ nM) was developed using a human Fab displaying phage library [66,67]. DX-2400 was found to inhibit tumoral MT1-MMP activity, which in turn inhibited MDA-MB-231 primary tumor growth but not MCF-7 tumor growth in xenograft mouse models [66]. DX-2400 also inhibited metastasis [66]. Unfortunately, antibodies are subject to proteolysis and may be removed from circulation rapidly.

Targeting Abs directly to the metal complex in the MMP active site would be expected to increase binding affinity. In addition, as the enzymes' active site is obligatory, it can rarely develop drug resistance through genetic or epigenetic alternations. Achieving the ultimate goal of Ab superior potency and selectivity will also require additional enzyme surface epitope binding. These proposed novel function blocking Abs targeting the MMP active site (designated metallo bodies) would exhibit superior properties over classical Abs by mimicking molecular recognition aspects of MMPs endogenous inhibitors, TIMPs, while offering higher selectivity [68].

To generate specificity against the Zn^{2+} -protein complex, mice are immunized with rationally designed synthetic organic ligands bound to metal ion (Zinc-Tripod), which mimic structural and chemical motifs of the relatively exposed catalytic Zn^{2+} -His machinery in the active MMP. This stimulates a first immune response against the small synthetic mimicry antigen and is followed by subsequent immunization with the full-length MMP to induce in vivo affinity maturation towards the native conformation of the catalytic site and additional surface epitopes presented in the whole enzyme active form. The Sagi laboratory demonstrated that this immunization procedure yields function blocking metallo bodies directed at the catalytic Zn^{2+} and enzyme surface epitopes in activated MMP-9 [68]. Metallo body SDS4 selectively bound and inhibited MMP-2/-9 with $K_i = 40$ nM, and was shown to have therapeutic potential in an inflammatory bowel disease animal model [68]. Noteworthy, MMPs neutralizing mAbs raised by conventional methods (against native proteins or protein fragments) generally interact with surface loops rather than the zinc ion in the active form of the enzyme [63,66].

Overview

The application of structural biology approaches has yielded detailed information on the secondary binding sites/exosites utilized by MMPs for interaction with their substrates and inhibitors [18,69]. In turn, biomolecules designed for specific exosite targeting have provided selective MMP probes. Thus, exosite-based inhibitors appear to have significant advantages over prior active site-based inhibitors, achieving a level of selectivity ultimately desired for potential MMP therapeutic agents.

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Abbreviations

CAT	catalytic
CBD	collagen-binding domain
HPX	hemopexin-like
mAb	monoclonal antibody
MI	myocardial infarction
MMP	matrix metalloproteinase
MSS	musculoskeletal syndrome
MT	membrane type
OA	osteoarthritis
PK	pharmacokinetic
THP	triple-helical peptide
THPI	triple-helical peptide inhibitor

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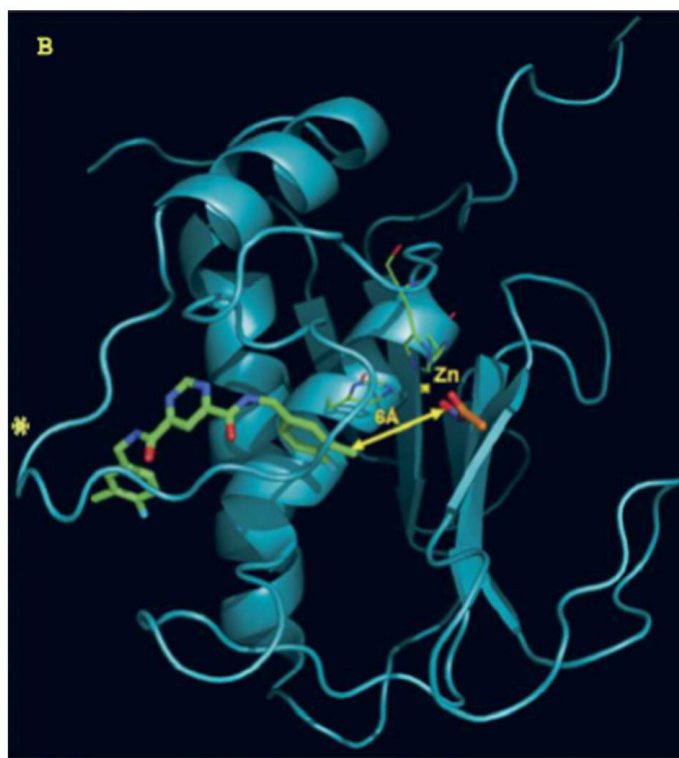


Fig. 1. Docked structure of MMP-13 CAT domain with pyrimidine dicarboxamide (green) and acetohydroxamate (orange). The two docked structures are 6 Å apart. The “selectivity loop” is denoted by an *.

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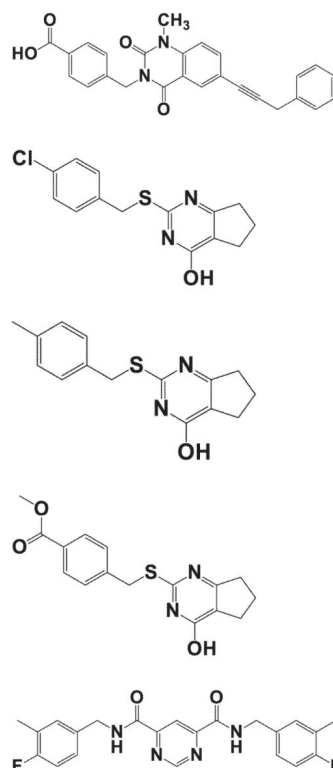


Fig. 2. Structures, from top to bottom, of (E)-4-((1-methyl-2,4-dioxo-6-(3-phenylprop-1-enyl)-1,2-dihydroquinazolin-3(4H)-yl)methyl)benzoic acid, reported by Pfizer as Compound 2 [24], compound Q/4 (2-[(4-chlorobenzyl)thio]-3,5,6,7-tetrahydro-4H-cyclopenta[d]pyrimidin-4-one) [37], compound Q1/20 (2-[(4-methylphenyl)methyl sulfanyl]-1,5,6,7-tetrahydrocyclopenta[d]pyrimidin-4-one) [38], compound Q2/24 (methyl 4-[(4-oxo-1,5,6,7-tetrahydrocyclopenta[d]pyrimidin-2-yl)sulfanylmethyl] benzoate) [38], and N4,N6-bis(4-fluoro-3-methylbenzyl)pyrimidine-4,6-dicarboxamide, reported by Aventis as Compound 4 [22].

Table 1Inhibition of MMPs by GlyΨ{PO₂H-CH₂}X_{xx} THPIs

Enzyme	Inhibitor	Inhibitor T_m (°C)	$K_i^{(app)}$ (nM)
MMP-1	GlyΨ{PO ₂ H-CH ₂ }Leu	30	7.83 ± 1.03 ^a
MMP-1(R291A)	GlyΨ{PO ₂ H-CH ₂ }Leu	30	12.76 ± 1.60 ^a
MMP-1(I290A,R291A)	GlyΨ{PO ₂ H-CH ₂ }Leu	30	23.66 ± 0.03 ^a
MMP-2	GlyΨ{PO ₂ H-CH ₂ }Leu	30	0.18 ± 0.00 ^a
MMP-9	GlyΨ{PO ₂ H-CH ₂ }Leu	30	0.02 ± 0.01 ^a
MT1-MMP	GlyΨ{PO ₂ H-CH ₂ }Leu	30	122.5 ± 22.3 ^a
MMP-2	GlyΨ{PO ₂ H-CH ₂ }Val	25	4.14 ± 0.47 ^a
MMP-9	GlyΨ{PO ₂ H-CH ₂ }Val	25	1.76 ± 0.05 ^a
MMP-2	Stabilized GlyΨ{PO ₂ H-CH ₂ }Val	43	189.1 ± 26.54
MMP-9	Stabilized GlyΨ{PO ₂ H-CH ₂ }Val	43	90.6 ± 6.67
MMP-8	GlyΨ{PO ₂ H-CH ₂ }Ile-His-Lys	<5	124.6 ± 6.9
MT1-MMP	GlyΨ{PO ₂ H-CH ₂ }Ile-His-Lys	<5	4704 ± 708.4
MMP-1	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	110.59 ± 29.8
MMP-2	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	17.82 ± 1.9
MMP-3	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	13600.33 ± 5160.7
MMP-8	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	62.1 ± 2.5
MMP-9	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	0.03 ± 0.02
MMP-13	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	77.13 ± 14.4
MT1-MMP	GlyΨ{PO ₂ H-CH ₂ }Ile-Tyr-Phe	40	46.15 ± 4.7

^a Assay performed at 10 °C.