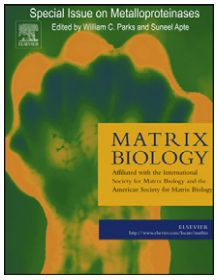
Review



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.

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 Zn^{2+} 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 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 μM [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 muscoskeletal 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].

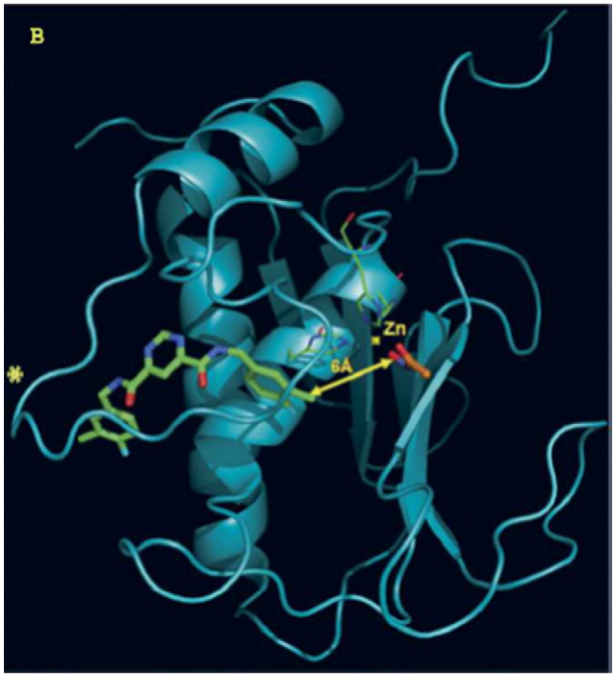

Fig. 1. Docked structure of MMP-13 CAT domain with pyrimidine dicarboxamide (green) and acetohydroxamate (orange). The two docked structures are 6 Å apart. The "selectivity loop" is denoted by an *.
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Although selective MMP-13 inhibitors have been described 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-phenyl-prop-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 μM), 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)-cyclopentapyrimidin-4-one scaffold led to two compounds (2-[(4-methylphenyl)methyl sulfanyl]-1,5,6,7-tetrahydrocyclopenta[d]pyrimidin-4-one; Q1/**20** and methyl 4-[(4-oxo-1,5,6,7-tetrahydrocyclo penta[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 μM 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-