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Recent advances in MMP inhibitor design

Jed F. Fisher · Shahriar Mobashery

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Abstract The search for an MMP inhibitor with anticancer efficacy is a nearly three-decade endeavor. This inhibitor is yet to be found. The reasons for this failure include shortcomings in the chemistry of these compounds (including broad MMP sub-type selectivity, metabolic lability, and toxicity) as well as the emerging, and arguably extraordinary, complexity of MMP cell (and cancer) biology. Together these suggest that the successful anticancer inhibitor must possess MMP selectivity against the MMP subtype whose involvement is critical, yet highly temporally (with respect to metastatic progression) and mechanistically (with respect to matrix degradation) regulated. This review summarizes the progression of chemical structure and mechanistic thinking toward these objectives, with emphasis on the disappointment, the perseverance, and the resilient optimism that such an inhibitor is there to be discovered.

Keywords Matrix metalloprotease · Metalloproteinase gelatinase · Collagenase

1. Introduction

The challenge of bringing a matrix metalloproteinase inhibitor to the point of clinical value was exquisitely summarized by Breuer et al. [1] as the juxtaposition of two quotes:

> "Matrix metalloproteinase inhibitors will be approved as drugs, probably this year . . ." [2] (1995)
>
> "Prediction is very difficult, especially about the future"
> (Niels Bohr)

J. F. Fisher · S. Mobashery (✉)
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670
e-mail: mobashery@nd.edu

The *basis* for this challenge can now be understood to reflect the extraordinary difficulty of identifying an MMP (matrix metalloproteinase, or matrix metalloprotease) inhibitor that combines MMP sub-type selectivity with effective modulation of MMP homeostasis during disease progression. While recent chemistry efforts have sustained measurable progress toward the former objective, progress toward the understanding of the latter has served primarily to reveal that the processes determining MMP enzymatic activity are characterized by incredible complexity and interdependency. Nonetheless, these same complexities have affirmed the role of the MMP enzymes as contributors to the pathophysiology of a host of diseases, and have justified the perseverance of the medicinal chemist with respect to MMP inhibitor design. MMP inhibitor design over the past decade is not simply that of evolutionary structural progression toward sub-type selective inhibitors, but arguably as well revolutionary improvements in the design of structures that target the zinc catalytic center of these enzymes. The focus of this review is the *recent* structural progression of MMP inhibitor design, as evidenced by the inhibitors themselves. A critical measure of the importance of this area is the review literature. Within the past year *every* area of MMP research has been critically evaluated by review, including MMP inhibition in cancer chemotherapy [1, 3–8] and advances in inhibitor design [9–18]. This review is organized by chemical structure. We use the customary division between hydroxamate peptidomimetic and non-hydroxamate (peptidomimetic and non-peptidomimetic) based MMP inhibitors, with an emphasis on the trends (as demonstrated by the inhibitor structure) in recent medicinal chemistry design. It will be evident that not only is there significant progress toward the transition from broadly inhibitory hydroxamate to subtype-selective hydroxamate and non-hydroxamate inhibitor design, but that this transition is occurring with an optimism

undiminished by the clinical disappointment of earlier generation MMP inhibitors. While the primary focus is anticancer MMP inhibitors, the substantial promise that MMP inhibitors offer in other disease states [19–28] will be noted in connection with the subtype focus of new MMP inhibitors. The sub-type selective inhibitor designed today with (for example) an osteoarthritis therapeutic endpoint, may prove its value in cancer tomorrow. The inhibitors presented in this review are identified in boldface by either their compound code (where the code is the primary identifier in the literature) or by their 9-digit Chemical Abstracts Registry number (where the compound is exploratory, and the code numbers are either unavailable or not yet assigned). Our use of the CAS Registry Number to identify compounds is due to the uniqueness of this number to compound structure, and to enable facile literature searching of the compound beyond the timeframe of this review. The structure schemes also summarize the MMP sub-type selectivity of the new inhibitors, *insofar as the literature data allow.* The therapeutic MMP objective, and the MMP sub-type screening panel and assay method, differ among research groups. MMP sub-type selectivity, IC_{50} or K_i data are given where these are known. No inference as to the selectivity of an inhibitor should be made where these data are absent.

2. Pioneering hydroxamate structures

Notwithstanding the perspicacity, effort, and expense of their creation, the many early hydroxamate-based MMP inhibitor structures (Fig. 1) must now be regarded as pioneering. This class of structures—whose structural interrelationship as collagen-based peptidomimetic MMP inhibitors is evident by inspection—displays excellent anticancer activity in tumor cell and animal models. The clinical performances of these compounds, while not those of failure, are frequently described (and correctly so) as disappointing. Many of the factors contributing to this disappointment have been identified in the decade since Hodgson's overly optimistic prediction [2]. The first of these relate to the compounds themselves. All of the structures are based on a hydroxamate zinc-binding group, subsequently determined to be metabolically labile. As a class, these are broad-spectrum inhibitors of many MMP sub-types (the profile provided for Ilomastat in Fig. 1 is representative), and in many cases also inhibit members of the ADAMs (TACE) protease family. Only upon clinical patient exposure did it become evident that these compounds (as a class) induced dose-limiting muscular and skeletal pain in a substantial number of patients. With the unraveling of the complexity of MMP physiology—Fingleton [8] enumerates metastasis, tumor growth, angiogenesis, apoptosis, and immune modulation as the five fundamental processes thus far identified—the realization has grown that optimal clinical performance of an MMP must structurally and temporally coincide with intervention at the critical MMP enzymatic event in the particular cancer. The accomplishment of this goal requires a far better grasp of what constitutes appropriate clinical biomarkers and diagnostics [29–35] than was appreciated during the early years of MMP inhibitor creation. Compelling evidence exists that MMP inhibitors may most favorably act earlier, rather than later, in the cancer metastatic process [8, 13]. Last, there is increased appreciation that the

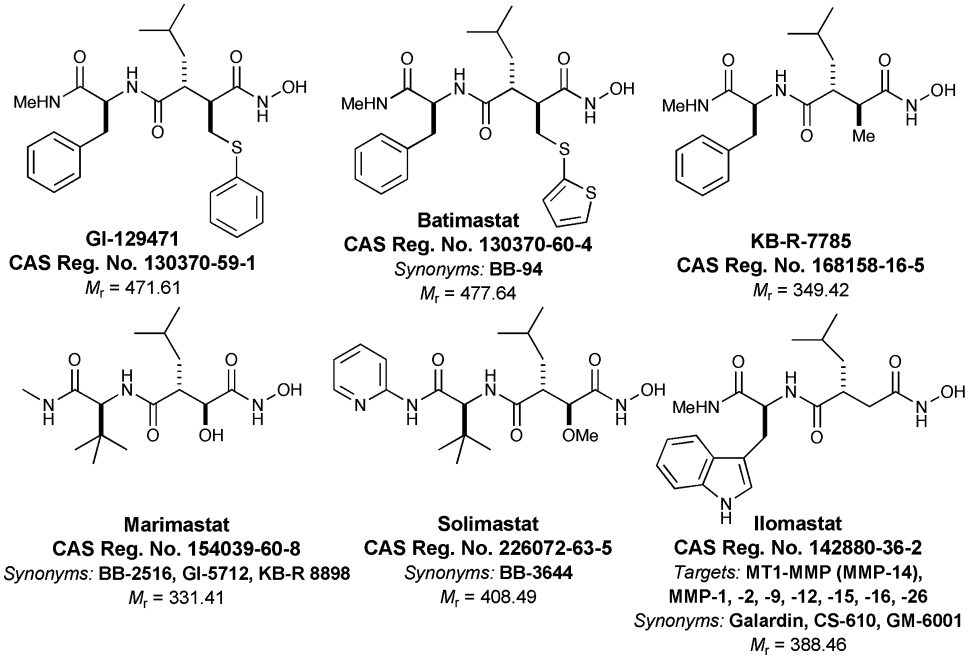


Fig. 1 Collagen-based, peptidomimetic hydroxamates

clinical design of a cytostatic and anti-angiogenic anticancer must be distinctly different than that for a cytotoxic anticancer [13, 36–38].

The situation with the earlier generation MMP inhibitors is well exemplified by the recent clinical reports for Marimastat (an inhibitor of MMPs-1, -2, -3, -7, -9, and -12). In a randomized (versus placebo) Phase III trial for post-first line metastatic breast cancer, it was ineffective at doses (10 mg bid, oral) associated with Grade II or III musculoskeletal toxicity (MST; also referred to as MSS, musculoskeletal syndrome) [39]. While MST was also an issue in a Phase I combination (with carboplatin and paclitaxel) therapy against advanced non-small cell lung cancer, partial response were seen in 12 (of 21 patients) and disease stabilization occurred in another 5 patients [40]. In exploratory anti-angiogenesis combination therapy (with low molecular mass heparin and captopril) in advanced cancer, Marimastat (again 10 mg bid, oral) was well tolerated and the chemotherapy resulted in partial response (1 of 10 patients) or disease stabilization (3 patients) [41]. A phase I/II study with a cohort of relapsed prostate cancer patients (as evaluated by their PSA level), Marimistat gave evidence of efficacy in delaying disease progression, but significance could not be established due to dose-limiting toxicity [42]. The sense from these (and earlier and concurrent clinical studies, as fully summarized by Mannello et al. [13], Rao [15], and Vihinen et al. [17]) is that if only the structure could be adjusted to impart selectivity and abolish toxicity, an MMP inhibitor would achieve clinical impact in cancer chemotherapy. Notwithstanding the reality that this refrain has been heard innumerable times in cancer chemotherapeutic design, the belief in this hope sustains a vibrant effort toward these objectives.

3. Design objectives of new MMP inhibitors

The chemical objectives that coincide with this hope comprise several structural classes. New generation peptidomimetic hydroxamate structures are still being disclosed. However, three new trends in compound design are seen. The first is the use of non-hydroxamate inhibitors [1]. The hydroxamate is regarded as extremely effective, but unless the structure is highly optimized, the hydroxamate is prone to metabolic transformation, and moreover may non-productively chelate the metals of other metalloproteins. Alternative, newer generation inhibitors that use different zinc-binding groups (within both peptidomimetic and non-peptidomimetic motifs), or are based on new design principles (exemplified by the tetracycline and bisphosphonate derivatives, and the endogenous MMP inhibitors), represent an expanding portion of the MMP inhibitor literature.

The second trend is the design of sub-type selective MMP inhibitors in order to achieve improved anticancer therapy, or to specifically address the MMP subtype involved in other MMP-dependent pathophysiology (such as MMP-13 in osteoarthritis). An additional objective, also explicit in sub-type selective inhibitor design, is the elimination of the MTS toxicity that presents in the clinical evaluation of many MMP inhibitors (typically after several weeks of administration at the higher dose levels). The origin of the MTS toxicity is not known. An early hypothesis was a direct relationship to non-specific sub-type inhibition, especially inhibition of MMP-1 (human fibroblast collagenase, responsible for normal turnover of the extracellular matrix in connective tissue). While MMP-1 inhibition has not been directly excluded as the basis for MTS, the recent disclosure by Reiter et al. [43] of the appearance of MTS during the clinical trial of an MMP-1-sparing hydroxamate inhibitor (*vide infra*) does bring this hypothesis into question. An alternative hypothesis suggests non-specific inhibition of the related ADAM (which includes TACE or ADAM-17, TNF-α coverting enzyme) and ADAMTS (which includes aggrecanase) "sheddase" zinc metalloproteinases. Although the MMP inhibitor ensemble certainly includes structures with differential TACE and MMP affinity (see Zask et al. [44] and Duan et al. [45] for recent discussions), the increasing recognition of the structural and biochemical breadth of the zinc metalloprotease family [46] *and* the uncertain basis for this phenomenon, have challenged the design of an inhibitor that lacks this property. The difficulty in removing from the inhibitor structure the basis for this toxicity is exemplified by Solimastat, a broad-spectrum MMP and TACE inhibitor. Pre-clinical study implied that Solimastat had diminished toxicity compared to the structurally-related inhibitor Marimastat. Nonetheless, clinical evaluation of Solimastat was terminated prematurely due to the appearance of MTS toxicity [47].

The third trend is the use of MMP structure as a basis for inhibitor design [15]. While verification that selective MMP inhibition will coincide with efficacy *and* minimal toxicity will remain firmly an experimental determination, the creation of selective inhibitors with the properties that will enable the experiment is already occurring. Indeed, *no* new MMP inhibitor is now prepared without the guidance of structure-based design. At this time the number and breadth of MMP structure (determined by both X-ray and NMR, and including both pro-enzyme [48] and numerous inhibitor complexes [10, 14, 15]) is substantial. These structures have been used for pharmacophore mapping for QSAR analysis [12, 49], for pharmacophore mapping for virtual screening [50], for MMP NMR-validation of pharmacophore hypotheses [51, 52], and for the computational determination of the points of binding pocket similarity and difference [53–57]. These latter analyses by Balaz et al. and Pirard and Matter confirm the predominant contribution of the S1′ and S2 pockets to achieving selective interactions (see also the discussions by

Wasserman et al. [18, 58]). Not surprisingly, MMP domain involvement in endogenous substrate recognition and proteolysis is considerably more complex (see Xu et al. [59], Toth et al. [60], and Pelman et al. [61] for further discussion).

The following sections give a synopsis of the new MMP inhibitor structures. The particular structural features of these inhibitors coincide with particular hypotheses as to MMP selectivity, many of which have yet to receive full experimental evaluation. Nonetheless, there exists for all of these inhibitors the decisive opinion that the concurrent refinement of hypothesis, structure, and pharmacological attribute is providing fresh opportunity for MMP intervention in cancer and other diseases.

4. New generation hydroxamate-based MMP inhibitors

Arguably the key transitional structure from this early generation structures is MMI-270 (Fig. 2). This molecule features oral availability, water solubility, and low molecular mass in a broad-spectrum inhibitor. While the salient features of this molecule have precedent in the structures of Fig. 1, its relative simplicity emphasizes the structural attributes that confer MMP inhibition. These include (examining the structure from left to right) a substituted aryl and an acyl hydrogen bond acceptor (here, a sulfonamide) that is separated by two atoms from the hydroxamate zinc-binding group. There is also an amino acid sidechain-type substitutent on the carbon alpha to the hydroxamate, and a second sidechain substitution on the sulfonamide nitrogen (later shown as unnecessary). The *N*-arylsulfonyl-α-amino acid hydroxamate of MMI-270 mimics the so-called "marimistat" succinate or butanedioate motif (substituted as half hydroxamate and half amide). The limitations of this prototype (metabolic lability, clinical appearance of MST) [62] are addressed to some degree in the remaining structures of Figs. 2 and 3. Cipemastat, which also has this succinate motif, was developed as an MMP-1, -3 and -9 collagenase inhibitor for the treatment of rheumatoid and osteo arthritis. Despite animal efficacy, its clinical trial was terminated prematurely [63]. MMI-166 has an *N*-arylsulfonyl-α-aminocarboxylate zinc-binding group. It is an MMP-2, -9 and -14 selective inhibitor that spares MMP-1, -3 and -7. While it has shown anticancer activity in numerous animal models of human cancer (including implanted lung cancer, melanoma, squamous carcinoma, colon and cervical) [64, 65], there are no data as to its clinical performance. The key structural feature exemplified by MMI-166 is the "deep" aryl (here, a triaryl) substitution. This feature, which appeared virtually simultaneously in all of the concurrent MMP SAR efforts, is immediately recognizable in the remaining structures of Fig. 2. In MMI-166 two phenyl rings are connected by a tetrazole ring; in ABT-770 and PD-166793, the two phenyl rings are directly connected; and in Prinomastat, an oxygen atom connects the two phenyl rings. All three permutations direct the SAR away from MMP-1 (a "shallow pocket" MMP) and toward the "deep pocket" MMPs such as the gelatinases, consistent with

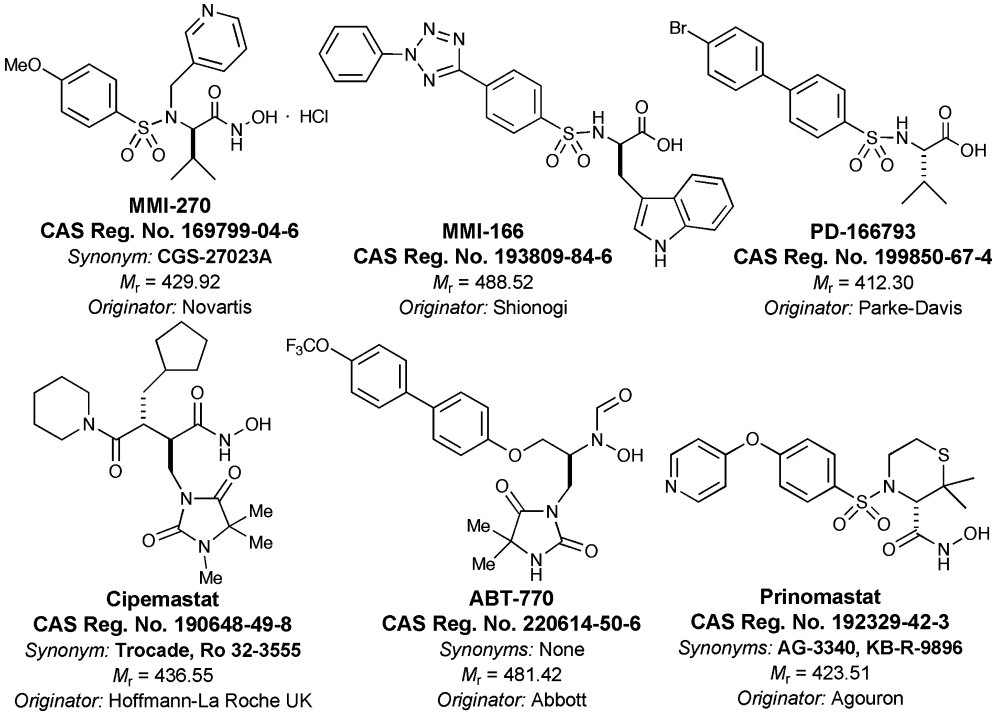
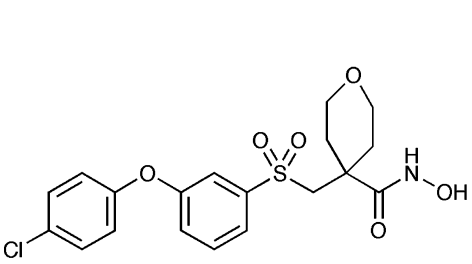
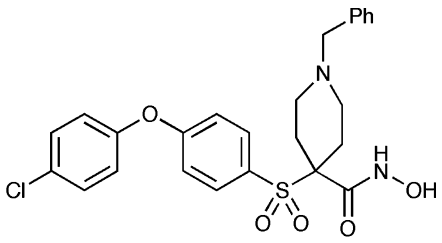


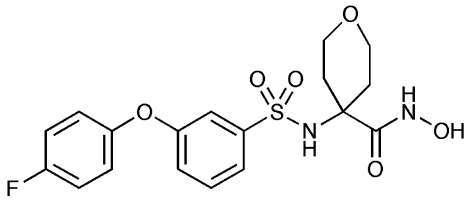
Fig. 2 Peptidomimetic hydroxamates & carboxylates



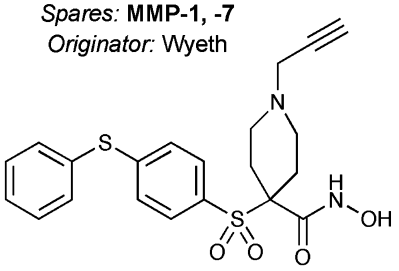
RS-130830
CAS Reg. No. 193022-04-7
M_r = 425.88
Target: **MMP-2, -9, -8, -13**
Spares: **MMP-1, -7**
Originator: Hoffmann-La Roche



CAS Reg. No. 239796-97-5
M_r = 501.00
CAS Reg. No. 239796-78-2, HCl salt
Target: **MT1-MMP, MMP-2, -9, -3, -13**
Spares: **MMP-1, -7**
Originator: Wyeth



CAS Reg. No. 230954-09-3
M_r = 410.42
Target: **MT1-MMP, MMP-2, -9, -8, -3, -12**
Spares: **MMP-1**
Originator: Pfizer



SC-276
CAS Reg. No. 226393-68-6
M_r = 430.54
CAS Reg. No. 226388-60-9, HCl salt
Target: **MMP-2, -9, -8, -3, -13**
Spares: **MMP-1**
Originator: Searle

Fig. 3 New diaryl ether hydroxamates

the belief at that time that MMP-1 sparing structures would not show MST. PD-166793 has efficacy in hypertensive heart failure (see [66, 67] and references cited therein). ABT-770 (and its related structure ABT-518) is a retrohydroxamate that is optimized for MMP-2 and -9 activity over MMP-1. A key structural change contributing to the optimized selectivity was the replacement the biphenyl of ABT-770 with a diphenylether (as is also present in Prinomastat) [68]. Both ABT-518 and ABT-770 have significant anticancer activity in animal models, but are easily human metabolized (and in the case of ABT-770, to an amine metabolite that caused phospholipidosis) [69].

Prinomastat (also known as AG3340) is one of the best-studied MMP inhibitors [70]. It has the *N*-arylsulfonyl-α-amino acid hydroxamate motif that is also found in MMI-166 and MMI-270, but with the cyclization of the sidechain back to the nitrogen (hence an α,β-substitution with respect to the hydroxamate). In general tolerance to substitution (whether $\alpha-$, $\alpha,\alpha-$, $\alpha,\beta-$, or $\beta,\beta-$) within the succinate-type motif is high (also as exemplified by the $\alpha,\alpha-$ and $\beta,\beta-$rings of Fig. 3), although optimization of the substitution for selectivity is very difficult. Although the potency of Prinomastat against MMP-2 is excellent (sub-nM), and with some MMP-1 selectivity (10 nM), under clinical dosing it is probable that Prinomastat inhibits MT1-MMP, MMP-2, -3, -9, 13 and -14 as well as MMP-1. Prinomastat showed excellent preclinical animal anticancer efficacy, including synergy with paclitaxel and carboplatin, and provided evidence that increased MMP-9 gelatinase specificity correlated with MMP inhibitor efficacy [71]. It also has been used to indicate that the relationship between MMP inhibition and metastasis is complex, wherein Prinomastat inhibition of MT1-MMP proteolysis of vitronectin promoted metastasis [72] (see also [73]). More recently Prinomastat was efficacious in a type I diabetes animal model [74] and was successful as an adjuvant in a model of photodynamic therapy [75]. A Phase I study [76] describes moderate (but reversible) arthralgia, myalgia, and MST at high doses, beginning 2 months after therapy initiation. Prinomastat in Phase III evaluation (evaluated in a gemcitabine and cisplatin drug regimen) against non-small cell lung cancer was ineffective [77].

A recurring limitation to these hydroxamates, and Prinomastat in particular, is drug metabolism including loss of the hydroxamate zinc-binding group by its reduction to the amide, its hydrolysis to the carboxylate, and its conjugation as a glucuronide. The foci of the ensuing generation of hydroxamate structures were suppression of metabolism, minimization of MMP-1 inhibitory activity, and control

of subtype selectivity (especially toward the MMP-2 and −9 gelatinases, the MMP-13 collagenase, and TACE) by structure-based design [58, 78]. Testament to the confluence of the chemical strategies toward this objective is provided by the astonishing similarity—with nuanced yet significant differences—among the four structures of Fig. 3. All four contain a linked biaryl (specific for MMPs with the long, narrow S1′ pocket) that achieves MMP-1 collagenase sparing (but not necessarily TACE sparing) selectivity [58]. The first compound of this set, RS-130830, entered into clinical evaluation as an MMP-13 collagenase specific inhibitor for the treatment of osteoarthritis (the outcome of this evaluation is not yet disclosed). Its annulated tetrahydropyran, located alpha to the hydroxamate, introduces a steric block that suppresses metabolism while acheiving the desired MMP selectivity. Extension of this concept to the *N*-sulfonyl-α-aminohydroxamate motif yielded 230954-09-3 [43] notable (by comparison to many closely related compounds) for its metabolic stability. On the basis of a short-term rat fibroplasias model as a surrogate indicator of clinical MST [79], 230954-09-3 and a cognate bicyclo tetrahydropyran were advanced to clinical evaluation for osteoarthritis. The appearance of MST during these trials is important evidence that MST may not correlate to MMP-1 inhibition [43]. While both RS-130830 and 230954-09-3 are orally available, the shorter chain β-sulfonylhydroxamate class (exemplified by 239796-97-5 and SC-276) has improved ADME (especially metabolism) and MMP-1 selectivity properties. The phenoxyphenyl α-sulfonylpiperidinehydroxamate 239796-97-5 is an excellent MMP-9 and -13 inhibitor ($IC_{50} = 1$ nM) that has a good TACE ($IC_{50} = 300$ nM) sparing, and an excellent MMP-1 ($IC_{50} = 800$ nM) sparing, profile [80, 81]. It has excellent oral efficacy in an animal model of osteoarthritis (equal to or superior to RS-130830) [80, 81]. The thiophenoxyphenyl structure SC-276 has nearly identical sub-nanomolar K_i values for MMP-2 and -13 inhibition as compared to its phenoxyphenyl cognate, and greatly improved MMP-1 sparing (SC-276 MMP-1 $K_i = 8700$ nM, phenoxyphenyl cognate MMP-1 $K_i = 270$ nM) [82]. SC-276 was orally active as an anti-angiogenic (mouse bFGF-stimulated corneal neovascularization) and as an anticancer (synergistic with paclitaxel in a mouse MX-1 carcinoma implant) [82].

The pharmacological direction of these MMP inhibitors is evident from the supporting biology that is given. While the structures of Fig. 3 certainly appear to possess the requisite subtype potency and improved ADME properties for anticancer activity, their anticipated utility is not for the treatment of cancer. This choice is a consequence of the uneven anticancer clinical performance of the preceding generation of structures, and an increasing focus on diseases that are believed to coincide with excessive activity of a single MMP subtype (such as MMP-13 in arthritis). Many of the newer hydroxamate and carboxylate MMP inhibitors confirm this trend.

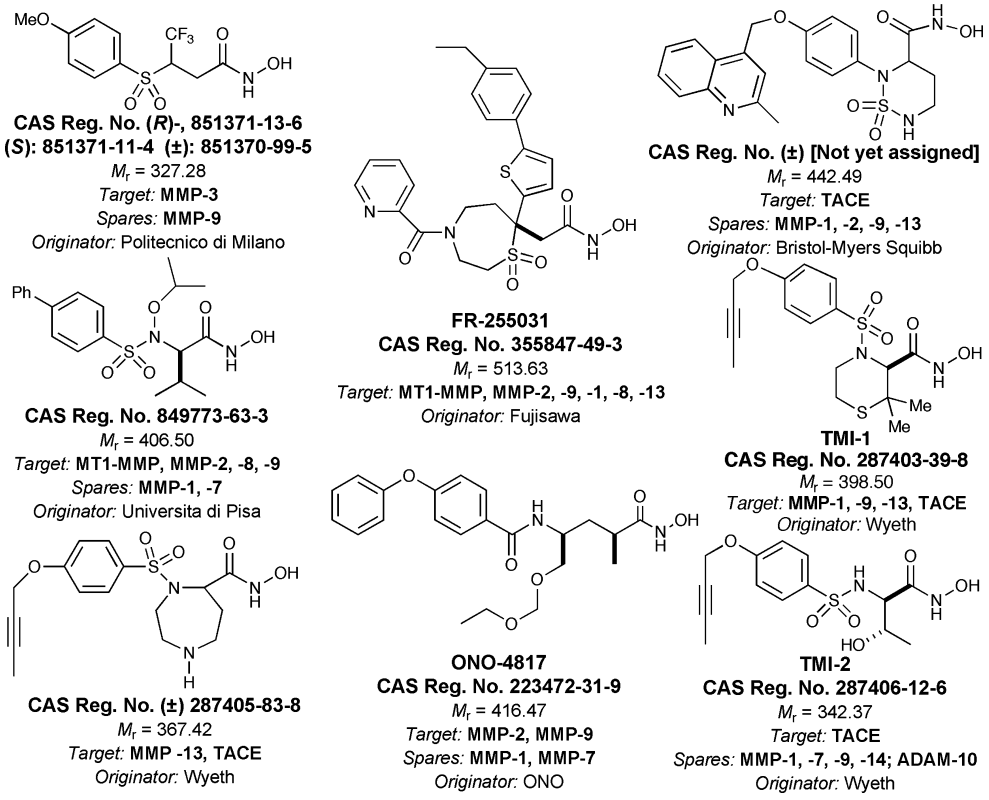


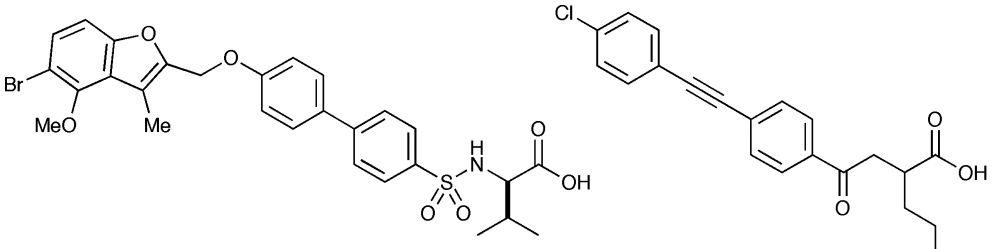
Fig. 4 Newer peptidomimetic hydroxamates

The compounds of Fig. 4 include two with evident structural precedent. Both enantiomers of the β-trifluoromethyl sulfone (851371-13-6 and 851371-11-4) are potent MMP-3 inhibitors with good selectivity (65-fold) against MMP-9 [83]. Given the structures of Fig. 3, the observation that substitution (such as by the trifluoromethyl) to both faces of this β-sulfonylhydroxamate is acceptable is not surprising. (That both enantiomers bind more tightly than the racemate is, however, a conundrum that Sani et al. are addressing.) The *N*-isopropoxy substituted compound 849773-63-3 [84] incorporates recognizable MMI-270 and PD-166793 features and is MMP-1 sparing (MT1-MMP, 15-fold selective and MMP-2, 1500-fold selective against MMP-1). Each of the remaining structures presents a new structural advance in MMP inhibitor design, albeit at the cost of increased structural and stereochemical complexity. FR-255031 is a broad spectrum MMP inhibitor with efficacy in a collagen-induced arthritis animal model [85]. As noted previously, its ability to also inhibit MMP-1 would previously have been regarded as a clinical liability. The data of Reiter et al. appear to preclude such simple judgment [43]. ONO-4817, an orally-available and soluble broad spectrum MMP inhibitor, is MMP-1 and MMP-7 sparing and has efficacy in several animal models of disease. These models include inhibition of cancer [86–90], arthritis [91], restenosis [92], periodontal [93], hypercholesterolemia [94], and inflammatory cytokine colitis [95] activities. The transition to structures with narrower MMP or ADAMS selectivity characterizes the remaining inhibitors. In many cases this objective is accomplished by combining iterative evaluation of the biaryl S1′ space with new cyclic (the dioxothiadiazine from Bristol-Myers, the *N*-sulfonyldiazepane 287405-83-8, the thiomorpholine TMI-1) and acyclic (the D-threonine TMI-2) substituted hydroxamates. The Wyeth 4-butynoxyphenylsulfonamides 287405-83-8 and TMI-1 are dual MMP-TACE inhibitors [44, 96], efficacious in mouse models of LPS TNF-α release and collagen-induced arthritis [97]. Transfer of this segment to the D-threonine derived TMI-2 gives a TACE-specific inhibitor for arthritis [98]. The Bristol-Myers dioxothiadiazine is a second example of the structural transformation of an MMP to TACE-selective inhibitor [99, 100]. Clearly, while building upon the substantial medicinal chemistry knowledge that has accrued for MMP design (as is also evident in the synthesis of anthrax lethal factor inhibitors [101–104]), the therapeutic objectives for these inhibitors is not cancer.

The carboxylate MMP inhibitors of Fig. 5 follow this same trend. By optimization of the benzoxazole substitution, selective MMP-13 (exemplified by 857081-53-9) [105] and aggrecanase ADAMTS [106] nM inhibitors were made. The γ-ketocarboxylate (second structure of this figure) is an MMP-2, -9 and -12 selective inhibitor (although only with μM potency) with efficacy in a hamster PPE-induced emphysema model [107].

5. New generation thiol-based MMP inhibitors

Rebimastat (BMS-275291, Fig. 6) represents one of the earliest chemistry departures from the hydroxamate ZBG. This compound has a thiol zinc-binding group, is orally available, and has broad spectrum MMP inhibitor (IC_{50} values: MMP-1, 9 nM; MMP-2, 40 nM; MMP-3, 160 nM; MMP-7, 25 nM; MMP-9, 30 nM; MMP-14, 40 nM) [108]. The structural scaffold for its thiol is a deep-pocket collagen nonpeptide mimetic (in the same class as the peptidomimetic Prinomastat). It further incorporates, as a design objective, selectivity. It does not inhibit the metalloproteases that release the TNF-α, TNF-RII, L-selectin, IL-1-RII, and IL-6-R cytokines [108]. While the absence of "sheddase" activity continues as a design objective in MMP inhibitors, the appreciation of the utter complexity between matrix degradation— including the MMP proteolytic release of angiogenic and antiangiogenic mediators—and the regulation of MMP activity is substantially greater than even at the time of Rebimastat entry into clinical evaluation (for example [4, 109–121]). Hence the definition of what constitutes



CAS Reg. No. 857081-53-9
M_r = 602.49
Target: **MMP-13, Aggrecanase**
Spares: **MMP-2, -3, -8 (moderate); -1, -7, -9, -14, TACE (excellent)**
Originator: Wyeth

CAS Reg. No. (±) [Not Yet Assigned]
M_r = 396.91
Target: **MMP-2, -9, -12**
Spares: **MMP-1, -7, 14, 15, -16, 26**
Originator: Chinese Academy of Sciences

Fig. 5 New peptidomimetic carboxylates

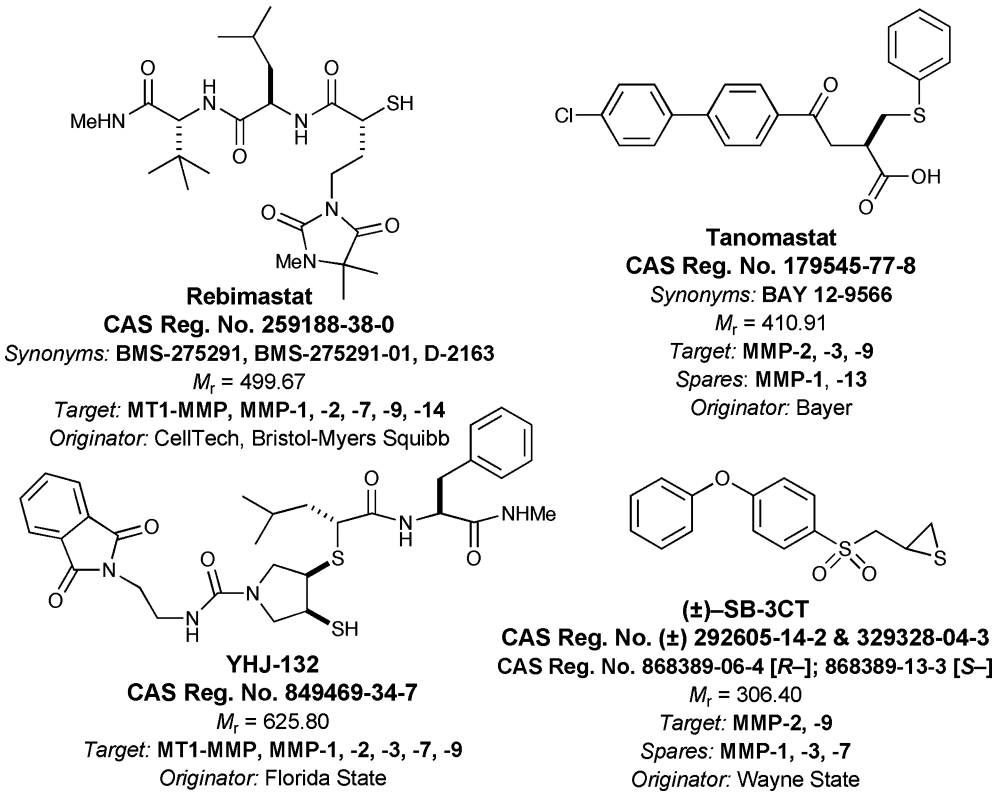


Fig. 6 New thiol-based MMP inhibitors

clinically relevant sheddase-sparing activity remains uncertain [13]. Following the demonstration of anti-metastatic and anti-angiogenic activity in pre-clinical evaluation [108] clinical evaluation of Rebimastat ensued. The outcome from Phase I (patients were primarily colorectal and non-small cell lung) indicated the absence of sheddase-dependent release of TNF-α and TNF-RII, the absence of dose-limiting joint toxicity, and disease stabilization within a portion (approx. 25%) of the patient population [122]. The inclusion of Rebimastat in the drug regimen for a Phase II early stage breast cancer trial resulted, however, in a patient anthralgia (albeit not at statistical significance) interpreted as consistent with MMP inhibitor toxicity [123]. A Phase III trial (using Rebimastat in a paclitaxel/carboplatin regimen in chemotherapy naïve NSCLC patients) confirmed a higher incidence of adverse reaction without survival benefit [124], resulting in termination of the study. Similarly uneven clinical outcomes were reported recently for Tanomastat, a contemporaneous structure with a thioether ZBG (an α-[(phenylthio)methyl]carboxylate). Tanomastat has the immediately recognizable biphenyl deep-pocket segment, and is a better inhibitor of MMP-2 (K_i = 10 nM), MMP-3 (K_i = 130 nM), and MMP-9 (K_i = 300 nM) than it is of MMP-1 ($K_i > 5$ μM) or MMP-13 (K_i = 1.5 μM) [125]. In an early Phase I solid tumor study it showed good tolerance and disease stability (but without response) [126]. In subsequent Phase I studies (advanced cancer, variety of tumor types) Tanomastat gave a well tolerated—but not efficacious—drug combination with etoposide. Its inclusion in a three drug regimen (with etoposide and cisplatin) showed significant hematological toxicity [127]. In contrast, its inclusion with doxorubicin in a Phase I (solid tumor) study gave evidence of good tolerance [128], and with 5-fluorouracil and leucovorin in a three drug regimen in a Phase I advanced solid tumor study it also showed lowered toxicity (with the exception of high dose—at 400 mg m^2 d^{-1}—Tanomastat induced thrombocytopenia that required a 50% dose decrease between cohorts) [129]. Last, Tanomastat was evaluated in a rat model of chronic allograft nephropathy for the purpose of exploratory evaluation of MMP inhibitors in organ transplant rejection. The use of this MMPI *after* kidney transplant (0–12 weeks) was effective in preserving organ function, but was *deleterious* when given after 12 weeks [130]. This outcome parallels that observed in cancer models and emphasizes the challenge to effective MMP clinical design—likely for all MMP-responsive disease states—with respect to dosing and timing in relation to disease progression [131].

The use of thiol zinc-binding groups in MMP inhibitor design continues. Sang et al. [132] describe a new class of broad-spectrum peptidomimetic inhibitors (exemplified by YHJ-132, Fig. 6). Many compounds of this class have good water solubility and are remarkably air-stable in plasma. YHJ-132 is particularly effective against MT1-MMP (K_i = 1 nM) and other MMPs (MMP-1, K_i = 9 nM; MMP-2,

$K_i = 1$ nM; MMP-3, $K_i = 6$ nM; MMP-7, $K_i = 7$ nM; MMP-9, $K_i = 1$ nM). The SAR for this template indicates that appropriate substitution will spare MMP-1 activity, and will reduce inhibitor affinity for MMP-7, with modest cost to MT1-MMP affinity. The K_i values for MMP-2 and MMP-9 correlate well to (and are in some cases better than) those for MT1-MMP. This class inhibits activation of proMMP-2 by endogenous MT1-MMP, and MT1-MMP-dependent fibronectin degradation, in tumor cell lines. Accordingly, the class is described as having promise for potent, selective, stable, and soluble MT1-MMP inhibitor design.

Fragai et al. [133] demonstrated the power of structure-based design in their synthesis of a thiol-substituted saccharide that inhibits MMP-12. NMR evaluation of the inhibitor-enzyme complex verified the basis for their inhibitor design, where specific contacts in the wider S1′ pocket of this MMP are used. Although these inhibitors are not potent, they forcefully exemplify the emergence of the ability to design and create new MMP inhibitors.

An imaginative use of the thiol for MMP zinc-binding recognition is the thiirane SB-3CT. The diphenylether deep-pocket motif is immediately recognizable, as is the likelihood—now verified by extensive experiment—that the sulfur of the thiirane juxtaposes to the catalytic zinc of the MMP. SB-3CT has remarkable MMP selectivity, inhibiting MMP-2 ($K_i = 30$ nM) and MMP-9 ($K_i = 400$ nM) in preference to MMP-1 ($K_i = 210\ \mu$M), MMP-3 ($K_i = 15\ \mu$M), and MMP-7 ($K_i = 100\ \mu$M) [134]. Moreover, the basis for MMP inhibition by this compound is kinetically and mechanistically complex. The MMP zinc engages (as a Lewis acid) the thiirane and activates the thiirane to ring-open, resulting in long–lived inhibitory states of the MMP. As it is sub-type selective and mechanism-based inhibitor, SB-3CT is fundamentally different from all other reported MMP inhibitors. Several publications within the past year have explored the structure-activity (SAR) development, mechanism, and utility of SB-3CT in disease models for cancer and for stroke. A most surprising SAR observation was the determination that both enantiomers of SB-3CT (a racemate) were equally effective for MMP-2 and MMP-9 inhibition [134]. While stereochemistry was always an important design aspect of MMP inhibitor creation, the tolerance with respect to the stereochemistry of this zinc-interacting functional group is remarkable. Evidence for this tolerance was first observed by Parker et al. [135] with an *N*-[(biphenyl)sulfonyl]-α-aminohydroxamate MMP inhibitor template (*R*-valine-derived hydroxamate, $K_d = 0.7\ \mu$M; *S*-valine-derived, $K_d = 0.08\ \mu$M) and by O'Brien et al. [136] in their PD-166793 structure-activity studies. The arguably underappreciated (in terms of its significance to MMP inhibitor design) observation of dual enantiomer recognition (now also observed with SB-3CT) may be direct evidence of the plasticity of the MMP active site with respect to substrate and inhibitor binding [18]. Complementary perspectives on this stereochemical issue are presented by Sani et al. [83] (see also [80]) with their γ-trifluoromethyl-γ-sulfone MMP-3 inhibitors and by Pochetti et al. [137] (*vide infra*, Fig. 10). The MMP inhibition profile for the SB-3CT template responds to distal substitution on the diphenylether segment, either improving MMP-9, or MMP-9 together with MT1-MMP (MMP-14), affinity while retaining that for MMP-2 [138, 139]. Binding does not, however, directly correlate to mechanism-based inhibition. Thus an SB-3CT derivative was shown in tumor cell culture not to inhibit MT1-MMP activation of pro-MMP-2, but to effectively block tumor cell migration and invasion [139]. SB-3CT binds to the active site zincs of both MMP-2 and TACE, but interacts very differently with the two despite the similarity of the active sites [140]. Krueger et al. showed that SB-3CT (5–50 mg kg^{-1} d^{-1}) was a potent inhibitor of liver metastasis in a mouse model of aggressive T-cell lymphoma [141], while Bonfil et al. showed that it was also effective in a mouse model of prostate cancer metastasis to the bone [142]. Last, SB-3CT blocked MMP-9-dependent degradation of the extracellular matrix protein laminin, thus rescuing neurons from apoptotic cell death in a mouse transient focal cerebral ischemia model [143]. These studies demonstrate the potential of a sub-type selective MMP inhibitor to validate the role of MMP-2 and MMP-9 (gelatinase) in disease states.

6. Pyrimidine-based inhibitors

Among the most interesting classes of non-hydroxamate and sub-type selective MMP inhibitors is the pyrimidinetrione (or barbiturate) class (Fig. 7), of which Ro 28-2653 is the foremost example [144]. This compound inhibits the MT1-MMP, MT-3-MMP, MMP-2, MMP-8 and MMP-9 subtypes (IC_{50} values of 10–20 nM) while sparing the MMP-1 activity. It has been shown to reduce tumor growth in chronic arachlor exposure-induced nasal cancer in rats [145], in prostate cancer cell culture and xenograft models [146–148], and in a breast cancer xenograft model [149]. In this latter assay Ro 28-2653 reduced tumor vascularization, inhibited the tumor-promoting effects of fibroblasts, and did not upregulate MMP-9 angiogenic activity (as was observed with Batimastat). It does not inhibit the zinc endoprotease meprin (which is generally regarded as sensitive to hydroxamate-based MMP inhibitors) [150] and has only moderate effects in mice on adipose tissue (2- to 3-fold upregulation of MMP-2 and MT1-MMP, and without upregulation of MMP-9) with no discernible histopathological alteration to joints [151]. On these bases Maquoi et al. [149] conclude that this orally-available class of MMP inhibitors is less likely to exhibit the adverse neuromuscular effects of earlier generation MMP inhibitors. The basis for MMP inhibition was shown, by a high resolution crystal structure

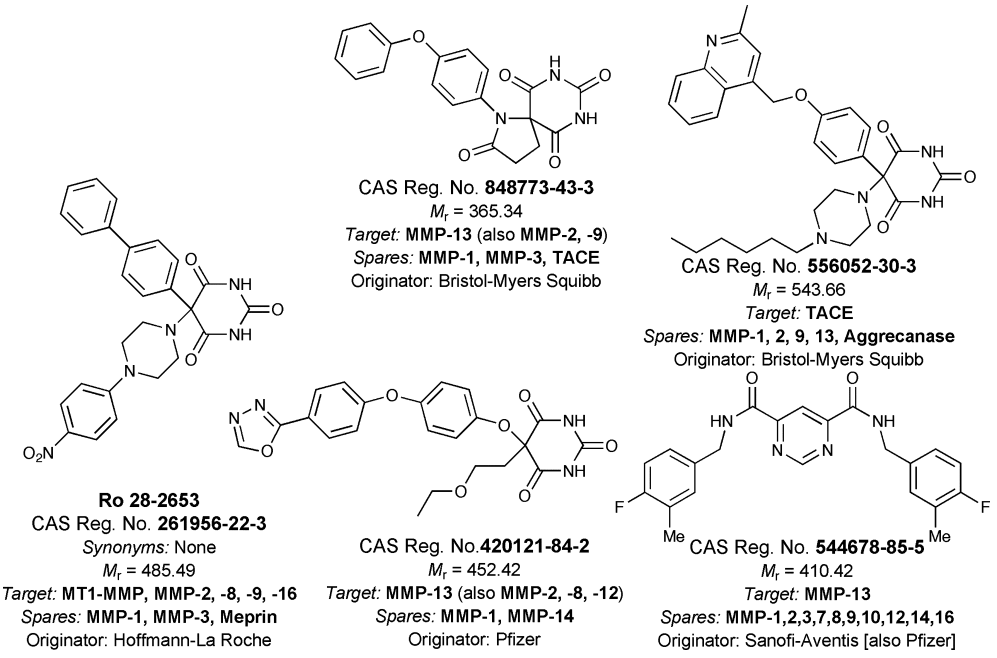


Fig. 7 Pyrimidine-based, MMP inhibitors

(with MMP-8: PDB 1JJ9) with a structural analog of Ro 28-2653, to involve pyrimidinetrione chelation of the active site zinc [152]. The phenyl and piperidinyl segments of the inhibitor occupy respectively the S1′ and S2′ binding pockets of the MMP-8. A significant aspect of this structure is a substantial loop (Ala206–Asn218) movement, originating from a repulsive interaction between the C-4-carbonyl of the bound inhibitor and the Pro217 amide. (The relationship between active site loop motion and pro-enzyme activation, collagen recognition, and collagen hydrolysis are further discussed with respect to the recently determined structures of proMMP-1 [48], MMP-8 mutants [61], MMP-12 with hydroxamate inhibitors [153], and MMP-13 with a non-chelating 4,6-pyrimidinedicarboxamide inhibitor [18, 154].) That the pyrimidinetriones may have general value in zinc protease inhibitor design—selectivity and potency comparable to the hydroxamates—is proven by several independent SAR efforts. Compound 556052-30-3 [45] is remarkably similar in structure to Ro 28-2653, but is TACE selective over MMP as it incorporates the 4-[(2-methylquinolin-4-yl)methoxy]phenyl sidechain previously demonstrated by the Bristol-Myers MMP group to confer (in a hydroxamate inhibitor) TACE selectivity. The TACE IC_{50} value for this compound is 80 nM as compared to IC_{50} values $> 2\ \mu$M for the MMPs evaluated (MMP-1, -2, -9, -13). Replacement of its hexyl (alkyl) sidechain with an arylalkyl sidechain preserved TACE inhibition, and restored MMP-2, -9, and -13 (but not MMP-1) inhibition, emphasizing the sensitivity of the SAR to small structural change. This conclusion is confirmed by two additional pyrimidinetrione efforts directed toward optimization of MMP-13 selectivity (for osteoarthritis). Kim et al. [155] at Bristol-Myers Squibb disclosed the remarkably small ($M_r = 365$) 5-(spiropyrrolidin-5-yl)pyrimidinetrione 848773-43-3, originating from structure-based re-design of a hydroxamate congener. It achieves a $K_i < 5$ nM against MMP-2, -9 and -13 while sparing MMP-1 and TACE (no data are given for MMP-14). Computational evaluation of the Zn(II)–pyrimidinetrione complex indicates that *monodentate* contact between the nitrogen of the pyrimidinetrione *anion* and the zinc (the pK_a for dissociation of the pyrimidinetrione NH proton is 6.4 ± 0.2) [155], consistent also with crystallographic study of the pyrimidinetrione–MMP-3 complex [156]. The question whether MMP-13 over MT-1 MMP (MMP-14) selectivity was achievable within the pyrimidinetrione ZBG was resolved by the Pfizer MMP group [157]. In a commendable example of medicinal chemistry SAR tenacity, substitution of a 1,3,4-oxadiazol-2-yl heteroaryl at C-4′ of the now familiar diphenylether segment gave the pyrimidinetrione 420121-84-2. This compound has an MMP-13 IC_{50} value of 1.0 nM as compared to 220 nM for MMP-14. Other heteroaryl groups gave less favorable selectivity. The IC_{50} values observed for 420121-84-2 in an MMP panel were MMP-1 (13 μM), MMP-2 (20 nM), MMP-8 (30 nM), and MMP-12 (30 nM). Moreover, the compound was orally available in rats ($F = 100\%$) with a moderate $t_{1/2}$ (4 h) and low clearance and low volume of distribution [157] Lastly, Breyholz et al. [158] have made a series of ^{125}I-radiolabled pyrimidinetriones that are similar in structure to 420121-84-2 having MMP-2 and -9 IC_{50} values of <10 nM. These will be used for non-invasive MMP imaging in elevated

MMP-9 atherosclerosis and elevated MMP-2 and -9 cancers, similar to the previous studies reported by this group with radiolabeled hydroxamate MMP inhibitors [159, 160].

The promise to this class of inhibitors appears substantial. Many are not large (as measured by molecular mass), they are easy to synthesize, and they attain the nM potency necessary for pharmacological scrutiny and eventual clinical evaluation. At this time the most exciting aspects of the pyrimidinetriones is their drug likeness (best evidenced by their oral availability) *and* the high probability that many of the design concepts perfected for the hydroxamates will carry forward to the pyrimidinetriones. While the execution within a new class of a design concept is not a trivial undertaking, once the boundary conditions—points of commonality and of difference—between the hydroxamates and the pyrimidinetriones are determined, there is the likelihood that potent, drug-like, and sub-type selective MMP pyrimidinetrione inhibitors will follow quickly.

The final pyrimidine-based inhibitor is the structurally unprecedented, and MMP-13 selective and potent (IC_{50} = 8 nM), pyrimidine-4,6-dicarboxamide inhibitor 544678-85-5 disclosed by the Aventis-Sanofi MMP group [154]. (As noted by Breuer et al. [1] there is a substantial and independent Pfizer [Warner-Lambert] pyrimidinedicarboxamide patent estate, yet to be disclosed.) Moreover, the compound has extraordinary MMP-13 selectivity (IC_{50} values for MMP-1, -2, -3, -7, -8, -9, -10, -12, -14, -16 are greater than 0.1 mM). Examination of the MMP-13 complex with 544678-85-5 indicates an extensive hydrogen-bonding interaction involving the Tyr244–Leu255 "specificity" loop of the MMP-13 active site, but without direct contact with the catalytic zinc. The inhibitor binds within the S1′ pocket as if stabilizing one of several (in the absence of bound inhibitor) conformations available to the specificity loop [18, 154]. The inhibitor is itself bent, with its two distal 3-methyl-4-fluorophenyl segments occupying opposing faces of the Leu218 sidechain. Its 3-methyl-4-fluoro substituents are sufficiently proximal to the zinc as to alter the water ensemble found as a zinc ligand (displacing two water molecules and moving two others, with concomitant improvement of the IC_{50}). These characteristics—and good oral availability (F = 50% in rats)—support the opinion by Engel et al. [154] that this class may for the first time give a definitive evaluation of an MMP subtype inhibitor in MMP-13 disease pathology (osteoarthritis and breast cancer). More recent Aventis disclosures in the patent literature (discussed in [161]) expand the SAR of this class against MMP-13 with the disclosure of more highly substituted and non-symmetric pyrimidinedicarboxamides, albeit without improvement in potency (selectivity data are not given). While the intrinsic potency and structural simplicity of this class compels attention, further ADME and cell biology data are necessary to better define their promise.

A more complete summary of the heterocyclic structures used for MMP inhibitor design is provided by Breuer et al. [1].

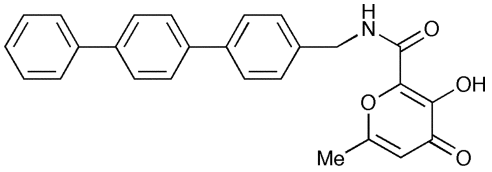
7. Hydroxypyrone-based MMP inhibitors

A recent example of a new ZBG with structural parallel to the pyrimidinetriones is the 3-hydroxypyran-4-one 868368-30-3 (Fig. 8). The compound was designed by computational optimization of zinc-binding fragments [162], based on earlier MMP inhibitor [163, 164] design, by the Cohen and McCammon group. It has excellent selectivity for MMP-3 (IC_{50} = 20 nM) compared to MMP-1 and MMP-2 ($IC_{50} > 50\ \mu$M for both). The structural basis for MMP recognition is proposed as *O,O*-bidentate chelation of the zinc by the conjugate base of the acidic (calcd pK_a of 4.5 ± 1.0) 3-hydroxy-4-pyrone enediolate. Likewise, for both MMP-2 and MMP-3 the terphenyl sidechain is proposed to occupy the S1′ pocket. The remarkable difference in binding affinity between the two is suggested to arise from the greater acidity of the pyrone (compared to a hydroxamate) that improves MMP-3 zinc chelate stability (with reference to the lower optimal pH for MMP-3 activity of 6.0, compared to other MMP enzymes of approx. 7.5). Compound 868368-30-3 inhibited neonatal rat cardiac fibroblast invasion of a collagen matrix with an IC_{50} of 250 nM [162].

A conceptually similar ZPG is the 3, 4-diaminocyclo butenonethione ring. Incorporation of this ring into peptidomimetic structures μM MMP-1 inhibitors [165]. Sieburth et al. have demonstrated that another transition-state type insert, the silanediol, gives effective peptidomimetic inhibitors of zinc (and other) proteases [166, 167]. This insert has not yet been applied to MMP inhibition.

8. Phosphorous-based MMP inhibitors

Structural mimics of the tetrahedral transition state of amide hydrolysis continue to be discovered, and creatively trans-

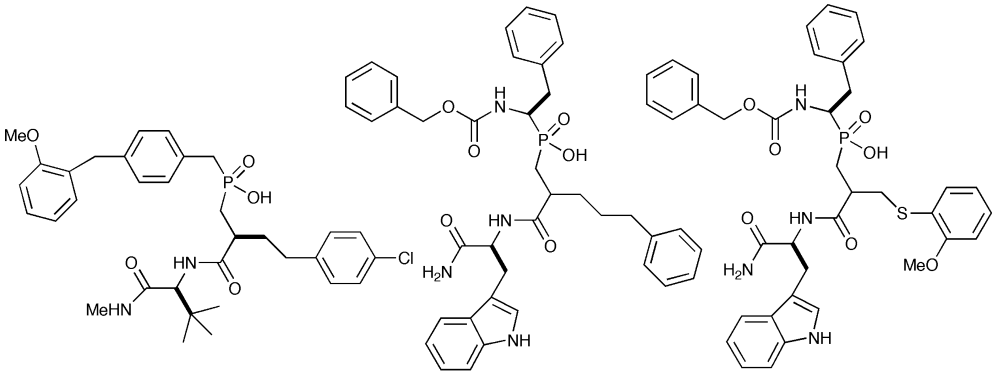


CAS Reg. No. 868368-30-3
M_r = 411.45
Target: **MMP-3**
Spares: **MMP-2**
Originator: Univ of CA San Diego

Fig. 8 Pyrone MMP inhibitor

formed into MMP inhibitors. An example of heteroatom-based tetrahedral mimicry, with a substantial history of design as MMP inhibitors, is the phosphinic ($R_1R_2P(O)OH$) class (reviewed by Dive et al. [168]). The phosphinic group, as its phosphinate conjugate base, binds as a zinc ligand. Appropriate optimization of the R_1 and R_2 substituents on the phosphorous attains nM level inhibition potency. The primary MMP subtype targets for the inhibitors that have been reported are MMP-11 and MMP-13 (Fig. 9). Driven by data supporting a role of specific MMP-13 involvement in cartilage degradation in osteoarthritis, Reiter et al. at Pfizer [169] disclosed a series of MMP-13 phosphinates exemplified by structure 582311-81-7 (compound 28 of this publication). The IC_{50} value of this compound against MMP-13 is 5 nM. It inhibits as well MMP-2 (7 nM), MMP-8 (2 nM), and MMP-12 (5.0 nM) but neither MMP-1 (1200 nM) nor MMP-3 (1600 nM). Its [(methoxyphenyl)methyl]phenyl segment occupies the S2 MMP subsite, and the (4-chlorophenyl)ethyl segment the S1′ subsite. The combination of the 2-methoxy and 4-chloro phenyl substituents is decisive to imparting the MMP-13 to MMP-1 selectivity. In an IL-1 induced nasal cartilage degradation assay the MMP-13 selective compounds of this series were more potent than the broad-spectrum MMP hydroxamate inhibitor MMI-270 (IC_{50} MMP-13, 3 nM; MMP-1, 18 nM) supporting the hypothesis that this degradation is an MMP-13 dependent event.

An independent yet structurally related phosphinate anticancer effort is the substantial design effort of Yiotakis et al. [10, 168, 170, 171] to attain an MMP-11 selective phosphinate inhibitor for MMP-11 breast cancer [172, 173]. Among the exploratory compounds from this effort are RXP-03 and 656823-07-3. RXP-03 is a relatively broad-spectrum inhibitor (K_i values: MMP-2, 55 nM; MMP-9, 40 nM; MMP-8, 4 nM; MMP-10, 45 nM; MMP-11, 6 nM; MMP-13, 16 nM) that spares MMP-1 ($K_i > 5\ \mu$M) and MMP-7 ($K_i > 20\ \mu$M). RXP-03 is synthesized as a diastereomeric mixture with respect to the stereogenic β-carbon (to the phosphorous atom) that bears the phenylpropyl segment. These K_i values pertain to the more active diastereomer of as yet unknown absolute configuration at this carbon. In mice, RXP-03 is metabolically stable and sustains excellent plasma concentration (greater than the MMP-8, -11 and -13 K_i value) for 24 h following 0.1 mg d^{-1} i.p. dosing [172]. The effect of RXP-03 on tumor growth (following s.c. injection of C26 colon carcinoma cells) in mice was used to assess efficacy. Optimal dosing (once daily 5 mg kg^{-1} i.p.) was estimated as substantially less than the dose required for the hydroxamate inhibitor KB-R7785 (twice daily 100 mg kg^{-1} i.p) used in a comparable study. Radioimaging indicated RXP-03 to accumulate (but in heterogeneous fashion) beneath the tumor (suggested as the possible site of dense tumor vascularization) [172]. Remarkably, the efficacy of RXP-03 was found to be dose *and* treatment schedule dependent. Its highest efficacy occurred during the first third of the treatment course, with optimal inhibitor administration coinciding to days 3 to 7 following tumor inoculation. Inhibitor dosing beyond this time window stimulated tumor growth. From these data Dive et al. conclude that "it appears essential to define carefully the spatiotemporal function of each MMP at the various stages of cancer progression" for optimal clinical design [172]. This conclusion aligns with the emerging realization that MMP inhibitors may exert differential roles in inhibition *and* promotion of both tumor growth and metastasis [174–176]. The structural redesign of RSP-03 to attain greater MMP-11 selectivity has yielded 656823-07-3, having very good MMP-11 selectivity (>100 fold K_i difference against MMP-2, -9, -14, -13, and -8) but at a cost in potency (MMP-11, $K_i = 230$ nM) [171]. Modestly potent (μM)



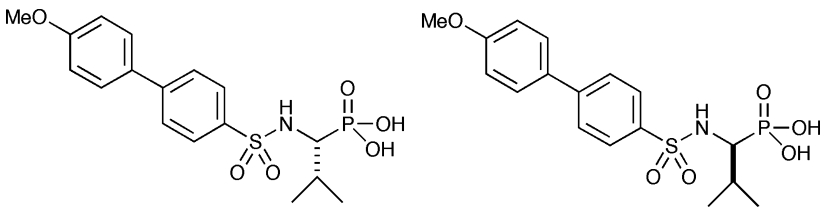
CAS Reg. No. 582311-81-7
Synonyms: None
M_r = 613.12
Target: **MMP-13, MMP-2, -8, -12**
Spares: **MMP-1, MMP-3**

RXP-03
CAS Reg. No. 851069-49-3
Synonyms: CAS Reg. No. 237394-67-1
M_r = 694.76
Target: **MMP-11& MMP-2, -8, -9, -13, -14**
Spares: **MMP-1, MMP-7**

CAS Reg. No. 656823-07-3
Synonyms: None
M_r = 728.79
Target: **MMP-11**
Spares: **MT1 MMP, MMP-2, -8, -9, -13**

Fig. 9 Phosphinic MMP inhibitors

Fig. 10 N-Sulfonyl aminophosphonate MMP Inhibitors



***R*-Sulfonyl Aminophosphonate**
Target: **MMP-2, -3, -8**

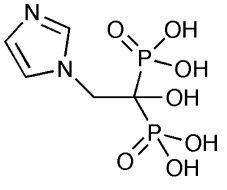
***S*-Sulfonyl Aminophosphonate**

CAS Reg. Nos. *R:* **873586-67-5**; *S:* **873586-68-6**
M_r =399.40
Originator: Universita di L'Aqila, G. d'Annunzio, La Sapienza, Bielefeld

MMP-1 and MMP-2 inhibitors were reported by Breuer et al. [177, 178], and MMP-2 and MMP-8 phosphinate and phosphonate inhibitors by Mazza et al. [179, 180].

More recently Mazza et al. [137] compared the MMP-8 structures of phosphonate inhibitors related to the carboxylate inhibitor PD-166793 (see Fig. 2). The *R-N*-sulfonylaminophosphonate enantiomer (left structure of Fig. 10) inhibits MMP-2 (K_i = 5 nM), MMP-3 (K_i = 40 nM), MMP-8 (K_i = 0.6 nM), and aggrecanase (K_i "nM"). These values establish this compound as the most active phosphonate MMP inhibitor yet made. Its *S*-enantionmer (right structure of Fig. 10) binds poorly to these enzymes (MMP-2: K_i = 1200 nM; MMP-3: K_i > 1000 nM; MMP-8: K_i = 700 nM). Crystal structures of each enantiomer with MMP-8 show nearly identical phosphonate coordination to the zinc and to glutamate-198, similar biphenyl occupancy of the deep S1′ pocket, and similar positions for the isopropyl sidechain in the S1 pocket. The structural accommodation that enables both enantiomers to bind occurs by rotation of the sulfonamide, and also results in a different torsional angle for the biphenyl in the S1′ pocket. Four differences between the MMP-8 bound *R* and *S*-aminophosphonates account for the 1000-fold difference in the MMP-8 K_i values. The more tightly bound *R*-enantiomer has better hydrophobic interactions with the MMP-8 for both the isopropyl and the biphenyl, has an additional hydrogen bond between the sulfonamide nitrogen and the Ala160 carbonyl, and has better hydrogen bond between the sulfonamide carbonyl and the Ala160 amide nitrogen. Mazza et al. emphasize the stereochemical subtleties of inhibitor recognition by MMPs by noting that the absolute configuration of the better bound PD-166793 enantiomer is the same as for these aminophosphonates, whereas the better bound hydroxamate cognate inhibitor has the opposite absolute configuration. The mutual ability of both the inhibitor and MMP to adjust, so as to attain optimal zinc-binding group interaction, emphasizes the requirement for careful evaluation of stereochemistry in MMP inhibitor SAR development. Likewise, Sani et al. suggest that the MMP binding of both of their trifluoromethyl-substituted hydroxamate enantiomers (see Fig. 4) may occur by interchange of the trifluoromethyl and sulfone groups between two binding pockets.

An interesting development with respect to MMP inhibition is the recognition that the amino-bisphosphonates developed for inhibition of osteoclast-mediated bone resorption, exemplified by Zoledronic acid (Fig. 11), have intrinsic anticancer activity (reviewed [181–189]). Early experiments by Boissier et al. [190] indicated the bone anti-metastatic mechanism to be MMP inhibition *via* zinc chelation. Subsequent studies indicate that zinc chelation only partly contributes to the broad-spectrum MMP inhibitory activity of these compounds. Additionally, there are anti-metastatic and anti-invasive mechanisms with the bisphosphonate acting directly on the tumor cell [191–194]. In clinical evaluation of metastatic breast cancer patients Zoledronic infusion resulted in the transient (48 h) reduction in circulating MMP-2 and growth factor (VEGF, bFGF) levels [195]. Moreover, Giraudo et al. [196] have shown, in a mouse model of cervical cancer, that Zoledronic suppresses the MMP-9 dependent appearance of VEGF by reduction in macrophage MMP-9 expression and activity. MMP-2 expression was constitutive in all stages. The efficacy of Zoledronic, as measured by tumor incidence and volume, was comparable to that attained by Batimastat. Hence the aminophosphonates may represent a compound class with excellent safety and tolerance, and



Zoledronic Acid
CAS Reg. No. 118072-93-8
M_r = 272.09
Synonyms: **Zoledronate**, **Zometa**
Target: **MMP-2, -9**
Originator: Ciba-Geigy

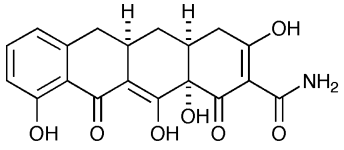
Fig. 11 Bisphosphonate MMP inhibitor

capable of attaining meaningful anticancer activity *via* an MMP inhibition mechanism.

9. Tetracycline-based MMP inhibitors

A comparison between the aminophosphonates and the tetracyclines reveals similarities. For both, there is evidence of direct MMP inhibition as well as other biological activities. The tetracycline analog that has received the largest evaluation as an MMP inhibitor is the chemically-modified tetracycline (CMT) Metastat [197, 198]. While derived from the antibacterial tetracyclines, Metastat (also called COL-3, Fig. 12) is optimized for anti-collagenase activity, resulting in the loss of its antibacterial activity. The tetracyclines, as a class, are found to have innate MMP inhibitory ability. Indeed, doxycycline (an antibacterial tetracycline) is the only compound approved as an MMP inhibitor, for the inhibition of the MMP-7 and -8 activity involved in dental periodontitis [199]. Beyond this activity, there is an emerging literature for the parent tetracyclines, minocycline and doxocycline. This is driven not only by their MMP inhibitory activity, but as well their intriguing efficacy in many (but not all) models of neurological disease (reviewed [200–205]).

Garcia et al. examined doxycycline binding to MMP-7 [206]. The complex is not strong (K_d = 70 μM). Two molecules of doxycycline are bound, in a manner that is proposed to involve its interaction with both the zinc and calcium metals of the enzyme. Two reasons may account for the ability of these compounds to achieve a level of *in vivo* MMP inhibition apparently inconsistent with this weak binding. These compounds may accumulate at the matrix, and act in catalytic fashion by binding to both pro- and active MMP so as to disrupt the enzyme conformation, resulting in autocleavage and loss of enzymatic activity [13, 198]. While a definitive answer as to whether MMP inhibition is the primary tetracycline mechanism, or results from another activity, reduction of MMP activity occurs upon tetracycline administration in several animal models of disease. Recent pharmacological evaluation of the tetracycline mechanism



Metastat
CAS Reg. No. 15866-90-7
M_r = 371.34
Synonyms: **Incyclinide, COL-3, CMT-3**
Target: **MMP**
Originator: SUNY & CollaGenex

Fig. 12 Chemically modified tetracycline MMP inhibitor

is consistent with diminution of MMP-9 expression and activity, and also inhibition of downstream caspase, growth factor, and kinase activities, in both cancer *and* neurological models [207–212]. Tetracycline-dependent reduction in reactive oxygen may contribute to the lowered MMP expression [213, 214]. In a porcine model of acute respiratory distress and septic shock, Metastat was efficacious with significant reduction in cytokine levels, neutrophil elastase activity, and MMP-2 (by 64%) and -9 (by 34%) activity in the bronchoalveolar lavage fluid [215]. Kaliski et al. [216] evaluated the effect of Metastat on ionizing radiation-induced melanoma invasiveness in both cell culture and in an s.c. mouse tumor model. The ionizing radiation increased MMP-2 activity, VEGF receptor levels, and induced VEGF secretion. Concurrent Metastat administration with the ionizing radiation inhibited tumor growth, and correlated with suppression of MMP-2 activity, VEGF levels, and VEGFR expression. Kaliski et al. conclude that combining MMP inhibition with ionizing radiation may reduce radiation-induced invasion and angiogenesis [216].

Clinical trials for Metastat have been undertaken for both cancer and dermatological indications. In the published Phase I and II cancer studies (see Syed et al. [217] and references cited) disease stabilization, but not response, was observed. Both response and stabilization are reported in its clinical evaluation against AIDS-related Kaposi's syndrome [17]. The clinical benefit of Doxycycline in knee osteoarthritis is consistent with MMP inhibition [218].

10. Endogenous MMP inhibitors

Neovastat (AE-941, CAS Reg. No. 305838-77-1) is an anti-angiogenic proteinaceous mixture derived from the ultrafiltration of liquefied fish cartilage [219–221]. It possesses a plethora of activities including MMP inhibition, inhibition of VEGF signaling [222–224], activation of TPA-dependent plasmin formation resulting from the presence of immunoglobulin kappa light chain proteins [225], and induction of endothelial cell apoptosis [226]. There are few recent reports that focus on its MMP inhibition (initial data suggest that this is the result of TIMP-type proteins [227] in the Neovastat mixture). Lee et al. [228] have shown recently that Neovastat was effective in the standard ovalbumin-induced airway inflammation mouse model of asthma, and that this efficacy correlated with reduced MMP-9 activity in the lung lavage fluid.

Several Neovastat anticancer clinical trials have been undertaken. Neovastat treatment is well tolerated, and results in disease stabilization (and survival benefit) in renal cell carcinoma and non-small cell lung cancer [229]. A combination chemotherapy and radiation therapy trial for Stage III

NSCLC remains in the recruiting stage (NCT00005838). An interim report [230] of this placebo-controlled trial observed no difference in toxicity between the Neovastat and placebo arms.

11. Concluding remarks

Within the past year critical assessments of the criteria for successful therapeutic MMP inhibition—based on our present inchoate comprehension of the central role(s) for these proteases in disease—are provided by Breuer, Frant and Reich [1], Fingleton [8], Mannello, Tonti and Page [13], and Vihinen, Ala-aho, and Kahari [17]. In large measure these closing remarks reiterate their wisdom. Moreover, these remarks are underscored by the palpable imperfections of the molecules described in this review.

There can be no doubt whatsoever of the potential contribution of an MMP inhibitor to cancer therapy. The inhibitor will be conceived from an understanding of optimal zinc-binding interactions, and will be refined by MMP structure-based design. It will be selective for the target MMP and will spare the MMP(s) involved in the joint toxicity. Its selectivity may permit a lower *in vitro* potency than is seen in many present inhibitors, minimizing undesired interaction with other metalloenzymes. It will be soluble, and perhaps will possess structure features that will facilitate its localization to the matrix. It will be administered at the point in the cancer progression where the role of the MMP is critical, such as at the time of the early events in metastasis. Its dosage will be guided by biomarkers, and not by dose-limiting toxicity. Its entry into cancer chemotherapy may occur after therapeutic success of the inhibitor in diseases other than cancer.

These are extraordinarily demanding criteria. But the progression of chemical structure to the point of efficacy in disease has always been the zenith of human ingenuity. We have been to this pinnacle with other structures, other targets, and other diseases. Ingenuity is drawn to seemingly intractable opportunity. There is no evidence whatsoever that the opportunity of MMP inhibition is other than magnificent.

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