This article reviews the different approaches that can be used to rationalize the selectivity of matrix metalloproteinase inhibitors.

Insight into the structural determinants for selective inhibition of matrix metalloproteinases

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The matrix metalloproteinase (MMP) family has been a pharmaceutical target for over 20 years. Despite massive research and development efforts, only one MMP inhibitor (Periostat) has been approved by the FDA for the treatment of periodontal disease. Possible reasons for the low success rate of MMP inhibitors in the clinic include unwanted side effects caused by their lack of selectivity, poor oral bioavailability and decreased potency *in vivo*. We review how three-dimensional structures (3D) of MMP inhibitor complexes as well as the inhibition profile of compounds screened on MMP can be used to guide the optimization of selectivity of MMP inhibitors. Analysis of MMP 3D structures provides a ranking of their pockets on the basis of opportunities for selective interactions. One can use inhibition data to build pharmacophore or quantitative structure–activity models (QSAR) for virtual screening of libraries of novel MMP inhibitors. Combining protein- and ligand-based approaches, we conclude that targeting a single pocket is not always sufficient to achieve the desired selectivity profile. Finally, we also outline novel series of selective MMP inhibitors that exploit differences in the intrinsic flexibility of the catalytic domain to form selective interactions with a given MMP.

The matrix metalloproteinases (MMPs) are zinc- and calcium-dependent neutral endopeptidases involved in the degradation of the extracellular matrix and in tissue remodeling [1]. Currently, approximately 27 MMPs are known. These have been grouped into subfamilies on the basis of their substrate specificity. Transcriptional regulation, zymogen activation and endogenous inhibitors control MMP activity under normal physiological conditions [2]. Disturbance of this physiological balance may lead to an over-expression of MMPs followed by accelerated matrix degradation. The latter is associated with several pathologies including cancer-cell invasion and metastasis, the loss of cartilage in osteoarthritis, rheumatoid arthritis, cardiovascular diseases, acute lung injury, chronic obstructive pulmonary disease, eye and skin diseases and periodontitis [3]. As a consequence, the MMP family has emerged as an attractive pharmaceutical target. Blocking MMP gene transcription, proenzyme activation or active site-directed inhibitions are possible approaches for therapeutic intervention.

So far, pharmaceutical research has mainly focused on the discovery of small molecule active site-directed inhibitors [4]. Some of these inhibitors showed promising pre-clinical results in various cancer models. However, advanced clinical trials of these compounds failed because of lack of efficacy and musculoskeletal side effects. Some researchers also performed a thorough and critical analysis of this failure [2,5]. Their analysis resulted in the identification of three main causes for this failure: the complex biology of the MMPs, the cross-reactivity of the tested inhibitors, and the design of the clinical trials. More recent studies have also led to a rethinking of the potential roles of the MMPs in several pathologies, including cancer. Therefore, inhibition of the MMPs is still regarded as a valid strategy [3].

In this paper, we will review one particular issue associated with MMP inhibition, namely, selectivity. Knowledge of the three-dimensional (3D) structure of MMPs can provide valuable insights into the structural determinants of selective inhibition of a particular MMP [6]. In the first part of this review, we discuss computational approaches that have been used to identify regions of the MMP catalytic site amenable for selective interactions with inhibitors. The analysis of inhibitor binding data that complement the

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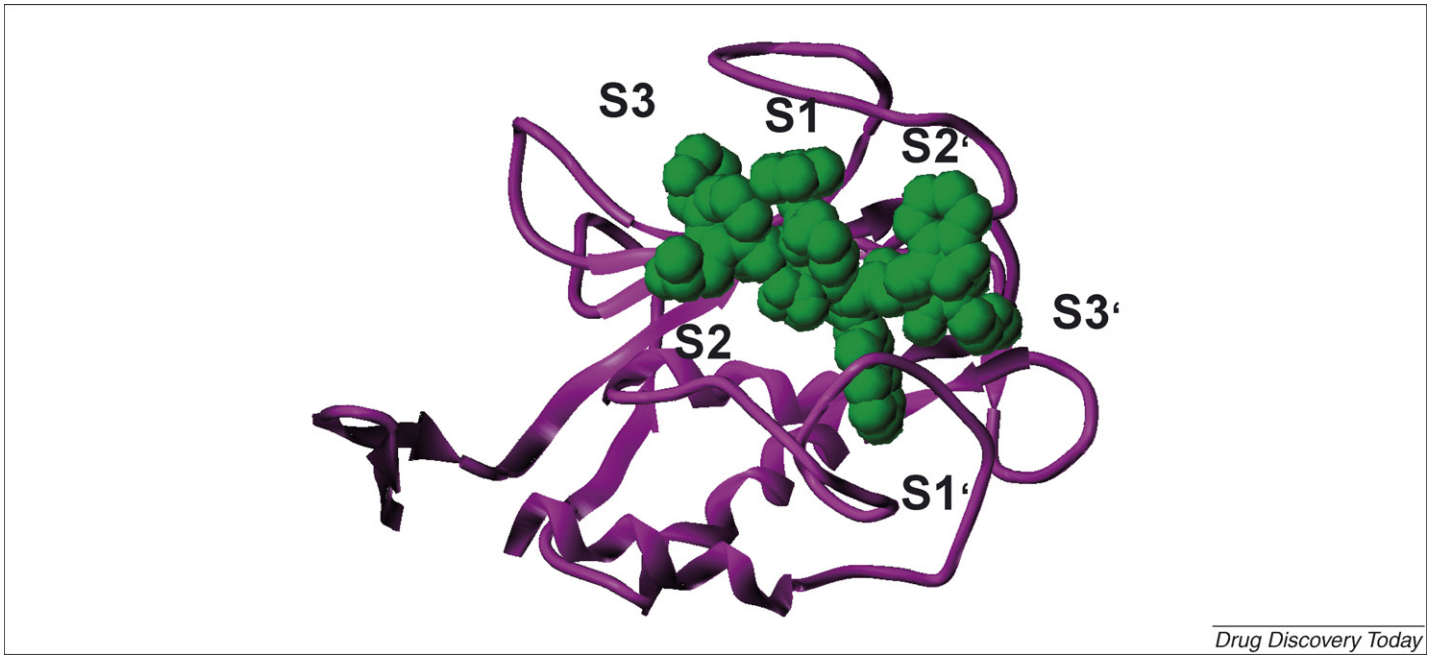


FIGURE 1

Characteristic fold of the MMP catalytic domain indicated by a ribbon-tube encoding secondary structural elements. The six pockets of the MMP-binding site are displayed as space filling models.

analysis of protein structures is reviewed in the second part. Next, we analyze selectivity from the perspective of inhibitors and proteins. Finally, we highlight some recent development in the field of selective MMP inhibitors.

Selectivity from the perspective of proteins

Most members of the MMP family share the same organization into three distinct and well-conserved domains, namely, an amino terminal propeptide, a catalytic domain and a hemopexin-like domain at the carboxy terminal. So far, most of the drug discovery efforts aiming at the treatment of the above mentioned diseases have focused on targeting the catalytic domain. The availability of 3D structures of several MMPs has contributed to guide the design of inhibitors. Currently, the Protein Structure Databank (PDB, http://www.rcsb.org/) contains 3D structures of 102 MMPs and nine related disintegrin and metalloproteases from the ADAM family. These 3D structures have been determined either by X-ray diffraction or by nuclear magnetic resonance (NMR) spectroscopy. All these structures exhibit the characteristic fold of zinc-dependent endopeptidases consisting of a five-stranded β sheet and three α helices. These structural analyses have also confirmed earlier biochemical results that six subsites (Figure 1), three on either side of the cleavage site, are mandatory for the observed proteolytic activity. Over the past couple of years, several authors have published comprehensive reviews on MMP 3D structures and we refer the reader to these [4,7,8]. However, we would like to emphasize that the structure of the catalytic domain is maintained in the full-length protein. This is an important observation as only the structure of the catalytic domain is available for most of the MMPs. The available 3D structures provide some insight into the structural determinants of selectivity.

More specifically, these 3D structures can be aligned in a common reference frame. Then for each of the aligned MMPs, one can use computational techniques like the GRID forcefield (Box 1) to identify hot spots of binding that is those regions where the most favorable non-covalent interactions can be formed [9]. However, this produces too many data points to enable visual inspection of common interaction patterns. Therefore, chemometrical tools like principal component analysis (PCA, Box 2) or consensus principal component analysis (CPCA, Box 2) are utilized for extracting relevant information from these hot spots analyses [10].

Terp *et al.* [11] computed GRID molecular interaction fields (MIFs) for the binding sites of 10 aligned MMPs, including five X-ray structures and five homology models. They, then, applied CPCA on the matrix of GRID MIFs. In addition to the well-known differences in the S1′ pocket, their work highlighted the importance of unprimed pockets, S2 and S3, for selective interactions. Their results also suggest possible discrimination between eight of the ten investigated MMPs.

Another group used force field interaction energies to compare the structures of 24 MMPs, including nine X-ray structures and 15 homology models [12]. As a result, they ranked the MMP pockets in decreasing order of similarity: S1′ (most similar) > S2 > S3′ > S1 ~ S3 > S2 (least similar).

BOX 1

GRID force field

The GRID [31] approach was developed for identifying energetically favorable binding sites for small chemical fragments, referred to as probes, on a macromolecular target. The probe is moved through a regular grid of points around the target and the interaction energy is computed at each point of the grid as a sum of the following terms: Lennard-Jones, electrostatic, hydrogen bonding, as well as entropic in some cases. The results from a GRID calculation can be visualized as iso-energy contours.

BOX 2

Principal component analysis (PCA) and consensus principal component analysis (CPCA)

In PCA [32], a data matrix is approximated as the product of two smaller matrices, the scores and the loadings matrices. The scores matrix gives a simplified picture of the objects, which are described in terms of their projection onto the principal components (PCs). The loadings matrix contains the PCs, which are linear combinations of the original variables. The first PC describes the maximum variance among all possible directions, the second PC the next largest variation among all possible directions orthogonal to first PC and so on. Analysis of the scores plots gives information about the clustering of the objects while the loading plots contain information about the relative contributions of the variables to the PCs (Figure I).

CPCA [10] is a PCA variation to analyze data organized in blocks. CPCA captures the structure of the full data matrix as well as the structure of each block. In other words, CPCA provides global scores and loadings as well as local scores and loadings that express the 'point of view' of each block.

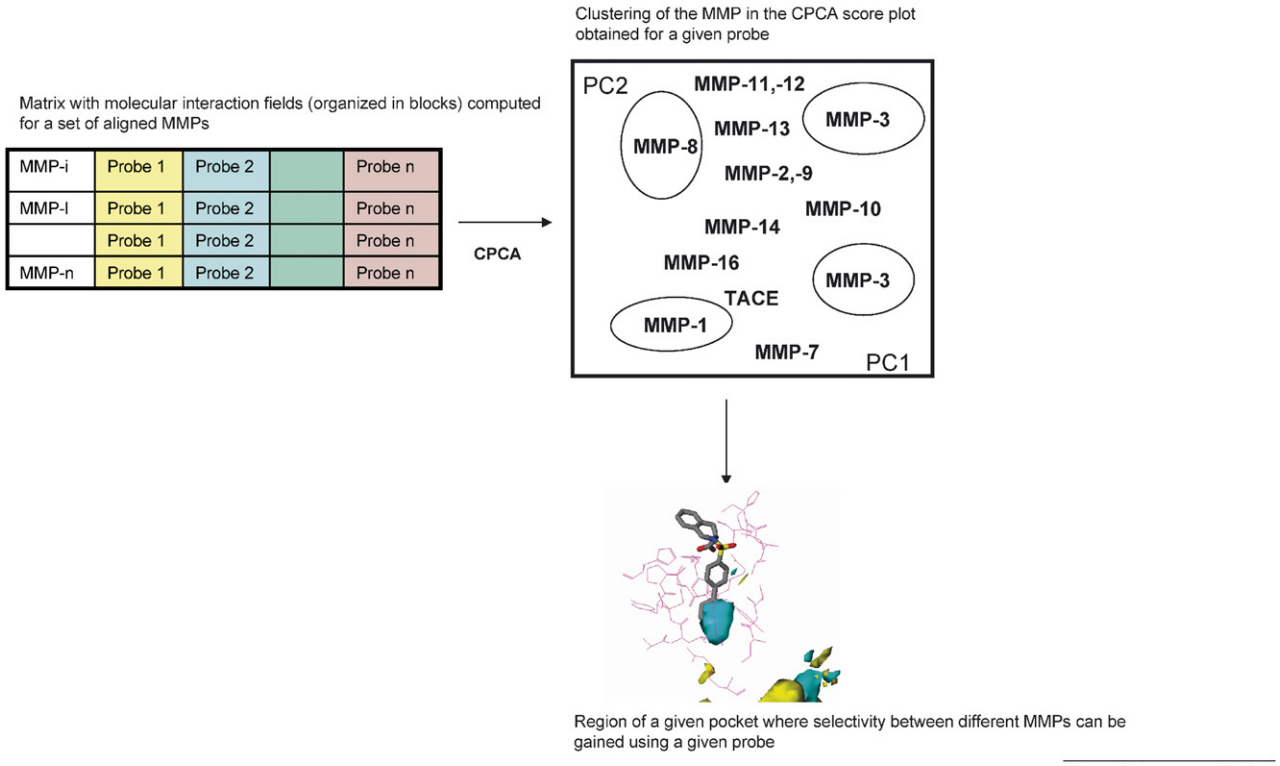


FIGURE I

Overview of the GRID (DrugScore)/CPCA procedure.

We considered a data set of 56 MMP structures (53 X-ray structures and three homology models) and one X-ray structures of the related tumor necrosis factor α converting enzyme (TACE), a member of the ADAM family, to systematically explore similarities and differences in MMP-binding sites [13]. For this purpose, two methods, based on force field interaction energies (GRID) and knowledge-based statistical potentials (DrugScore) (Box 3), were used to characterize the MMP-binding sites. A consensus principal component analysis was performed for all the MMP-binding sites and each of the six subpockets. We found that both approaches, GRID/CPCA and DrugScore/CPCA, capture similar information with respect to the discrimination between different MMPs. This analysis produced a global classification of the MMP family, referred to as 'target family landscape', as well as a ranking of each of the subpockets based on the opportunities for selective interactions: S1′ > S2, S3, S3′ > S1 > S2′. Some general trends for inhibitor design could also be derived from this analysis. In agreement with previous studies, the S1′ pocket appears to be the primary site for achieving selective interactions. Binding of additional substituents to other pockets can also contribute to selectivity towards a particular MMP. Our results also highlight the importance of steric, hydrophobic and non-polar interactions to gain selectivity. Another conclusion is that considering multiple structures from the same MMP adds conformational diversity to the data set. In other words, using a single structure may lead to spurious conclusions.

Therefore, this target family landscape could be refined as new structures become available in the PDB. In particular, members of the ADAM family and other related metalloproteases, which exhibit the same fold as the MMPs, could be included. Some MMP inhibitors may also cross-inhibit the ADAM family and other related metalloproteases, which is believed to account for some

BOX 3

DrugScore

DrugScore [33] is a knowledge-based potential, derived from the analysis of 1376 crystallographic ligand-protein complexes. This information was converted into distance-dependent pair preferences and solvent-dependent singlet preferences for ligand and protein atoms. Applications of DrugScore include the recognition of near-native poses from flexible docking, the prediction of binding affinities, its use as objective function in docking, and the identification of hot spots in binding cavities. In this case, regions of high binding propensities for defined atom types are displayed as isosurfaces.

of the side effects associated with long-term treatments with MMP inhibitors.

Selectivity from the perspective of inhibitors

All the available data on MMP inhibitors provide an invaluable source of knowledge about the structural requirements for blocking a given MMP while sparing other members of the family. These data can be used to build pharmacophore models and to derive (quantitative) structure–activity relationships ((Q)SAR). Kontogioris *et al.* [14] have reviewed the application of pharmacophore mapping and (Q)SAR to MMP inhibitors.

Tsai and Lin compared pharmacophore mapping and 3D QSAR to model the MMP-1 activity of inhibitors [15]. These researchers highlighted the complementarity of both approaches. One possible application of their models for MMP-1 activity is the virtual screening of libraries of potential MMP inhibitors. MMP-1 is considered as an anti-target for most MMP projects as its inhibition is believed to be responsible for the musculoskeletal side effects of the earlier generations of MMP inhibitors.

Selectivity from the perspective of proteins and inhibitors

The protein- and ligand-based approaches reviewed above can be combined to gain further insight into the structural determinants of selectivity for a given MMP.

The GRID/CPCA and DrugScore/CPCA models served to rationalize experimental binding affinity differences for nine series of inhibitors from the medicinal chemistry literature [13]. The binding modes of these inhibitors had been inferred from crystal structures or docking. The results of these analyses were consistent with the differences in experimental binding affinities between several MMP subtypes. We concluded that targeting only one pocket was not always sufficient to achieve the desired selectivity profile.

Other researchers docked several inhibitors in MMP-1 and MMP-2 to rationalize the inhibition profile for these two subtypes [16]. Their results served as a basis for the design of two inhibitors with improved activity and selectivity for MMP-2.

Solomon and colleagues combined inhibitor docking with analysis of protein structures, spectroscopic and kinetic studies to gain insight into the MMP-2/TACE selectivity profile of a mechanism-based MMP inhibitor [17]. This compound, known as SB-3CT, exhibits inhibitory activity on MMP-2 in the nanomolar range and on TACE in the micromolar range. Both enzymes share a substantial sequence and structure homology within their catalytic domain. Solomon and colleagues showed that SB-3CT behaved as a non-competitive inhibitor of TACE by inducing significant conformational changes while it acted as a competitive inhibitor of MMP-2 without changing the MMP-2 conformation. Their structural analysis indicated that subtle differences in the second shell of amino acids surrounding the catalytic zinc might account for the different modes of interactions of SB-3CT with MMP-2 and TACE.

Latest developments in the field of selective MMP inhibitors

A large number of MMP inhibitors have been described in journals or in patents. Most of the MMP inhibitors share three common features: (1) a group binding to the catalytic zinc, (2) side chains binding to the different subpockets, (3) a peptidic backbone or a peptidomimetic scaffold which orients the zinc-binding group (ZBG) and the different side chains for optimal interactions with the protein. Excellent and comprehensive reviews on MMP inhibitors are available [4,7,8,18,19]. In the following paragraph, we focus on the identification of novel ZBGs and the discovery of highly selective inhibitors devoid of ZBGs.

Earlier medicinal chemistry programs have explored a handful of ZBGs, including hydroxamate, reversed hydroxamate, carboxylate, thiolate, phosphinate and phosphonate [7]. Although the hydroxamate function appears to be the ideal ZBG for MMP inhibitors, it has liabilities such as lack of selectivity versus other physiologically important metals, sensitivity to rapid *in vivo* hydrolysis, rapid excretion and low bioavailability [20]. As a consequence, research has focused on the identification of alternative ZBGs [21].

Puerta *et al.* [22] carefully selected 11 potential novel ZBGs for screening on MMP-3. These fragments were between 3.5- and 717-fold more potent on MMP-3 than acetohydroxamic acid. One of them, a pyrone, was subsequently combined with six aryl moieties known to bind to the S1′ pocket of MMP-3 [23]. As a result, three compounds exhibited nanomolar activity on MMP-3 and high selectivity towards MMP-1 and MMP-2 (Figure 2). This selectivity might originate from the pyrone ZBG. More recently [24], the same research group reported MMP-3 inhibition data for seven additional fragments selected for their preference for late transition metals. These fragments showed improved activity on

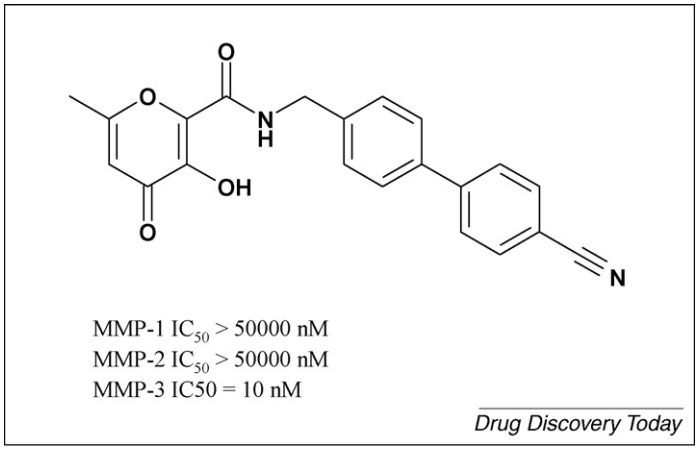
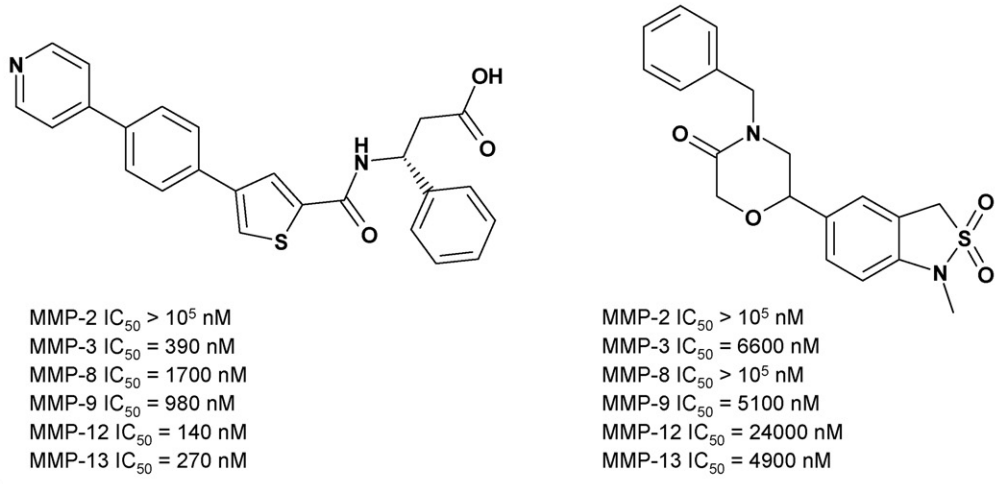


FIGURE 2

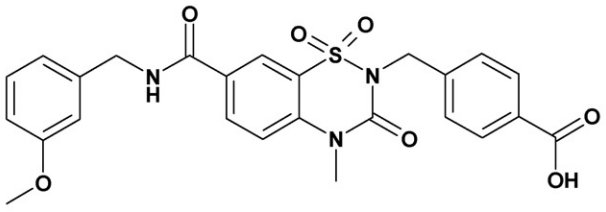
Structure of a potent and selective pyrone-based inhibitor of MMP-3.



MMP-2 IC_{50} > 10^5 nM
MMP-3 IC_{50} = 390 nM
MMP-8 IC_{50} = 1700 nM
MMP-9 IC_{50} = 980 nM
MMP-12 IC_{50} = 140 nM
MMP-13 IC_{50} = 270 nM

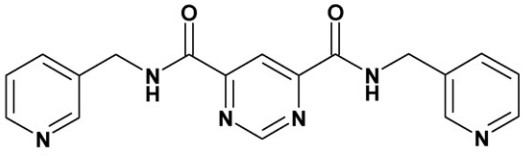
MMP-2 IC_{50} > 10^5 nM
MMP-3 IC_{50} = 6600 nM
MMP-8 IC_{50} > 10^5 nM
MMP-9 IC_{50} = 5100 nM
MMP-12 IC_{50} = 24000 nM
MMP-13 IC_{50} = 4900 nM

a



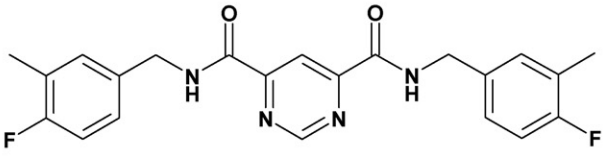
MMP-1 IC_{50} > 10^5 nM
MMP-3 IC_{50} > 3 10^4 nM
MMP-7 IC_{50} > 3 10^4 nM
MMP-9 IC_{50} > 10^5 nM
MMP-13 IC_{50} = 4.85 nM
MMP-14 IC_{50} > 10^5 nM

b



MMP-13 IC_{50} = 6600 nM

c



MMP-13 IC_{50} = 8 nM

d

Drug Discovery Today

FIGURE 3

Structures of MMP inhibitors devoid of ZBGs. (a) Crystal structures of these inhibitors bound to MMP-12 is available. (b) Example of a potent and selective MMP-13 inhibitor disclosed by Pfizer. (c) Pyrimidine dicarboxamide MMP-13 inhibitor identified by HTS of the Aventis corporate collection. (d) Pyrimidine dicarbxamide MMP-13 inhibitor resulting from the structure-based optimization of the screening hit shown in (c).

MMP-3 (from 4 to 185-fold) compared to acetohydroxamic acid. As far as we know, full-length inhibitors with these seven novel ZBGs have not been disclosed yet.

Other researchers described pyrrolidine derivatives with nanomolar activity on MMP-2 and MMP-9, but they did not report any selectivity data [25,26]. Docking in MMP-2 suggested their carboxylic ester as potential ZBGs. On the other hand, efforts to use *N*-hydroxyurea as potential ZBGs resulted in compounds with activity on MMP-8 [27] and MMP-12 [28] in the high micromolar range.

Novel classes of MMP inhibitors that do not bind to the catalytic zinc have also been described. Morales and colleagues published the crystal structures of two non-zinc chelator inhibitors bound to MMP-12 [29]. These inhibitors (Figure 3a) are located halfway down the S1′ pocket. Differences in the size, shape and composition of the S1′ pocket across the MMP family contribute to their selectivity. Pfizer also disclosed several series of highly selective non-zinc chelating MMP-13 inhibitors (Figure 3b) identified by high throughput screening [19]. High throughput screening of the Aventis corporate collection resulted in the discovery of a pyrimidine dicarboxamide (Figure 3c) with micromolar activity on MMP-13 and no detectable inhibition of a panel of MMPs (1,2,3,7,8,9,10,11,12,14,16) [30]. Its good water solubility (500 μM at pH 7.5) and reasonable bioavailability (51% in rat) made this hit a good starting point for optimization. Subsequent determination of its crystal structure in complex with MMP-13 revealed a novel-binding mode characterized by the absence of interaction with the catalytic zinc. One of the pyridyl arms is located toward the entrance of the S1′ pocket while the second pyridyl arm protrudes from the S1′ pocket in a side pocket (referred to as S1′*) that has never been described for other MMPs (Figure 4). A structural alignment of this crystal structure with the catalytic domains of 11 other MMPs suggested the nature of residues at positions 218 and 248 (MMP-13 numbering) as well as the sequence and conformation of the specificity loop at the bottom of the S1′ pocket as critical determinant for the selectivity of this class of inhibitors. Furthermore, this crystal structure enabled the structure-based optimization of this series of inhibitors. These efforts resulted in nanomolar inhibitors with the same selectivity profile and physical and pharmacokinetic properties as the initial hit (Figure 3d).

Conclusion

The quest for selective MMP inhibitors still constitutes one of the main challenges in the search for successful clinical candidates. Three-dimensional structures of MMP inhibitor complexes and inhibition profiles of compounds screened on MMPs provide an invaluable source to gain insight into the structural determinants of selectivity. Analysis of these data enables one to identify and characterize selectivity regions for discrimination of MMP family members. Then, one could use structure-based design techniques to identify and prioritize fragments binding to these selectivity regions. Once these fragments have been selected, structure-based design techniques could be applied to identify linkers connecting these fragments to the core of known MMP inhibitors. Application of high throughput screening has also resulted in the discovery of highly selective inhibitors. The availability of crystallographic structures of these highly selective inhibitors bound to their target has helped to optimize their potency while maintaining their selectivity. These highly selective inhibitors exploit differences in the intrinsic flexibility of the catalytic domain to form selective interactions with a given MMP. A systematic analysis of the flexibility of the different MMPs, using molecular dynamic, might contribute to identifying unique conformational features for a given MMP. These unique features might subsequently be

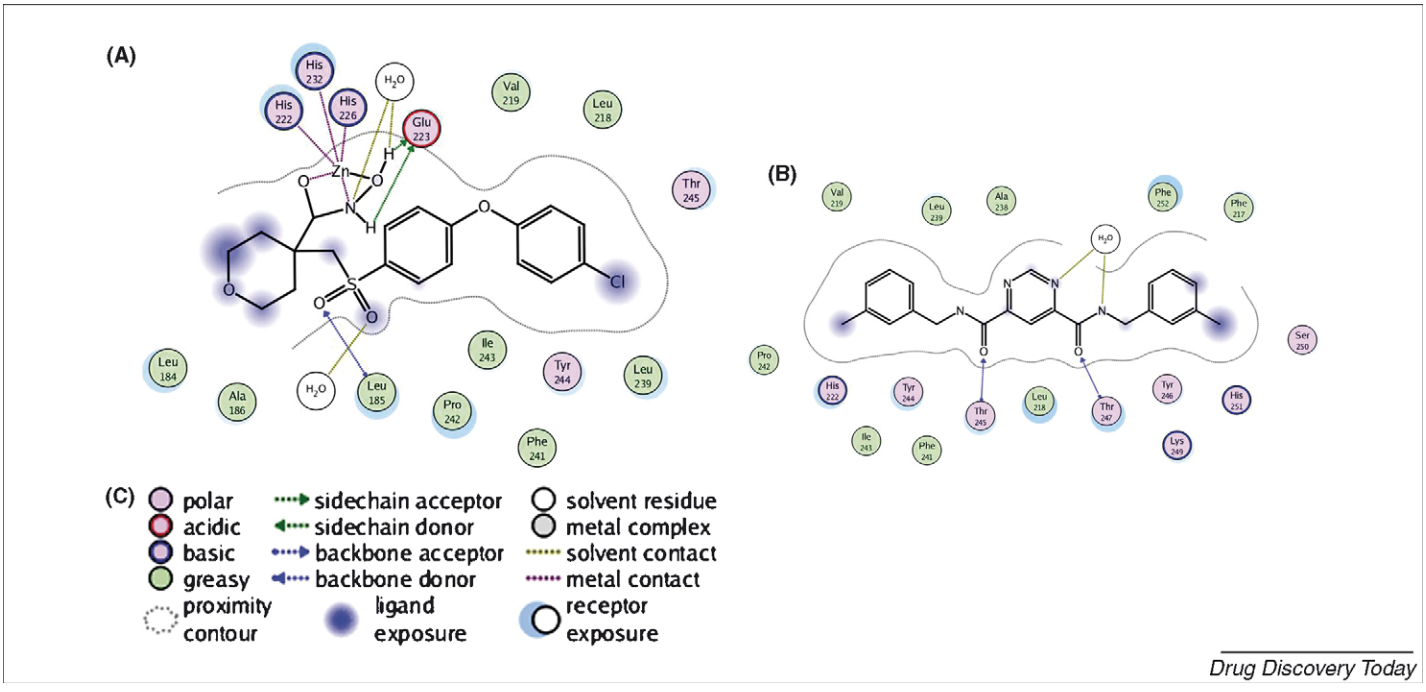


FIGURE 4

Ligand interaction diagrams for (a) an inhibitor with a ZBG bound to MMP-13 (PDB code: 830C), (b) an inhibitor devoid of a ZBG bound to MMP-13 (PDB code: 1XUC). (c) Symbols used in the ligand interaction diagrams.

exploited to design highly selective inhibitors for that particular MMP. These highly selective inhibitors can be utilized as a pharmacological tool to probe the effect of blocking a given MMP, while sparing the other members of the MMP family.

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