Understanding the variability of the S1' pocket to improve matrix metalloproteinase inhibitor selectivity profiles

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Abstract

Matrix metalloproteinases (MMPs) are a family of proteins involved in a wide range of pathologies. Because MMP broad-spectrum inhibition is associated with severe side effects, selectivity has become a priority in the design of MMP inhibitors, and it is often achieved by targeting the variable S1' pocket. However, the specific characteristics of the S1' pocket that determine inhibitor selectivity are often not described and, in many cases, challenging to identify. In this review we have inspected the variability of the S1' pocket across the MMP family, and we propose explanations for the selectivity of previously described inhibitors. These analyses provide valuable insights into how to design novel inhibitors selective for a given MMP.

Keywords:

Matrix metalloproteinases; selectivity; extracellular matrix degradation; drug design; proteases.

Teaser:

Inspecting the structures of selective MMP inhibitors and the nature and variability of the S1' pocket across the MMP family has provided valuable information on selective MMP inhibition.

1. INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of proteases that degrade various components of the extracellular matrix (ECM) [1]. MMPs are involved in the development of a wide range of diseases, so MMP inhibitors are of interest in various areas of clinical therapy (see Table 1) [2–6]:

- MMP-1 is involved in diseases such as rheumatoid arthritis, atherosclerosis, pulmonary emphysema, fibrosis and cancer [7–9]. Even though the precise cause of the musculoskeletal syndrome (MSS) generated as a side-effect of broad-spectrum MMP inhibitors is not known, it was initially hypothesized to be related to MMP-1 inhibition. As a result, there has been increasing interest in MMP inhibitors sparing MMP-1 [10] (see Table 2) and only a few MMP-1 inhibitors have been reported since 2010 (see Table S1).

- MMP-2 is involved in many diseases, such as heart failure, asthma and liver fibrosis, but it is best known for its role in cancer progression. MMP-2 has been validated as a key target for several types of cancer and this has been the major reason why many MMP-2 inhibitors have been developed over the last decade [11–28]. MMP-2 has been implicated in tumor invasion, metastasis and angiogenesis due to the degradation of type IV collagen, which is a major component of basement membranes. It has also been reported that mice knockouts (KO) of MMP-2 gained significantly less weight on a high fat diet than their respective control groups [29] and that the plasma levels of MMP-2 in obese patients are significantly higher than in controls [30]. To date MMP-2 is one of the most studied MMPs and since 2010 a total of 18 manuscripts have been published on MMP-2 inhibitors (see Tables 2 and S2).

- MMP-3 has been shown to play a role in the spread of metastatic tumors associated with melanoma and breast cancer [31], and the fact that it acts as a precursor of other MMPs made it an attractive early target for cancer therapy. Nevertheless, subsequently it was also recognized as an anti-tumoral MMP [32] and no new MMP-3 inhibitors have been reported in the bibliography since 2010 (see Table 2). It has also been reported that MMP-3 KO mice fed with a high fat diet not only showed hyperphagy but also gained more weight and the weights of their isolated subcutaneous and gonadal fat deposits were significantly higher than those of the controls [33].

- MMP-7 has been reported to have a potential role in tumor metastasis and inflammatory processes [34]. However, few MMP-7 inhibitors have been reported in recent years and MMP-7 is generally treated as an off-target when developing MMP inhibitors directed at other MMPs (see Tables 2 and S3).

- MMP-8 is associated with pathologies such as rheumatoid arthritis, asthma and periodontitis. It cleaves the insulin receptor, which leads to insulin resistance [35], and the serum levels of MMP-8 are significantly higher in obese individuals than normal weight individuals [35]. However, due to its antitumour properties it is regarded as an anti-target in cancer therapy [32] and therefore MMP-inhibitor selectivity over MMP-8 is often sought (see Table 2). Consequently, since 2010, only one paper has reported the design of new MMP-8 inhibitors (see Table S4).

- MMP-9 is involved in many cardiovascular diseases [36]. MMP-9 inhibition could have positive implications for ischemic stroke, heart disease and inflammation [37]. It has also been reported that the plasma levels of MMP-9 are significantly higher in obese patients than in controls [30]. Although there is some controversy about using MMP-9 as a target for cancer due to its reported anti-angiogenic and anti-tumorigenic functions [16], its involvement in the degradation of basement membrane type IV collagen and its leading role in cancer progression make MMP-9 an attractive target and nowadays MMP-9 inhibitors are still being developed (see Tables 2 and S5).

- MMP-10 plays an important role in pathologies such as arthritis, fibrosis and cancer. It is also able to activate pro-collagenases [38]. However, not many MMP-10 inhibitors have been reported since 2010 (see Tables 2 and S6).

- MMP-12 is involved in chronic obstructive pulmonary disease, atherosclerosis, emphysema and lung cancer [39]. Moreover, it has been confirmed that MMP-12 expression is up-regulated at mRNA, protein and activity levels in visceral and subcutaneous white adipose tissue from obese mice and humans [40]. The number of MMP-12 inhibitors that have been developed during the last decade is a reflection of the interest in MMP-12 as a key target in these diseases (see Tables 2 and S7).

- MMP-13 has been identified as a key target in osteoarthritis due to its role in catalyzing the hydrolysis of type II collagen, which is the main structural component of the cartilage matrix. MMP-13 is also involved in rheumathoid arthritis and cancer. Interestingly, it has recently been reported that the inhibition of MMP-13 prevents diet-induced obesity in mice and suppresses adipogenesis in 3T3-L1 preadipocytes [41]. On this basis, it has been proposed that the inhibition of MMP-13 could be a strategy for preventing obesity [41]. In the last decade, the field of MMP-13 inhibitor development has expanded considerably after the discovery of selective MMP-13 inhibitors that bind to the larger S1’ pocket in MMP-13 without the presence of a zinc-binding group (ZBG; see Tables 2 and S8).

- MMP-14 has been reported to direct obesogenic collagen turnover and it has been linked to human obesity traits [42]. However, MMP-14 is regarded as an anti-target for cancer therapy [18]. Moreover, as with MMP-1, inhibition of MMP-14 has been postulated as a possible cause of the MSS [43] observed as a side-effect of most broad-spectrum MMP inhibitors. Therefore, selectivity over MMP-14 is often sought by MMP inhibitors (see Table 2). Consequently, only two manuscripts describing the design of new MMP-14 inhibitors have been reported since 2010 (see Table S9).

Insert Table 1 here

Insert Table 2 here

Given the involvement of MMP inhibitors in a wide range of pathologies, there have been many attempts to design safe MMP inhibitors in the past. The first approach to the design of MMP inhibitors was the development of peptidomimetic inhibitors (*e.g.*, batimastat and marimastat; see Figure 2) designed to block the cleavage of collagen by chelating the catalytic zinc ion (see Figure 1A) using a hydroxamic acid moiety as the ZBG [2–4]. Next, small-molecule inhibitors with different ZBGs were developed (*e.g.*, hydroxamates, carboxylates, thiols and phosphorous-based ZBGs) [2], but the administration of these inhibitors resulted in the development of MSS and failure in clinical trials. Although none of the explanations for the occurrence of MSS proposed over the years have been confirmed, this side effect is believed to be a result of broad-spectrum MMP inhibition [64,65]. In light of these facts, other types of inhibitor have been developed for the purpose of occupying the S1’ pocket, a less conserved region adjacent to the zinc-binding site (see Figures 1 and S1). The S1’ pocket is accessed through a tunnel created by the wall-forming segment (*i.e.*, residues Pro242, Ile243 and Tyr244; MMP-13 numbering) and is delimited by a loop of variable length and flexibility called the Ω-loop, which is made up of different residues in different MMPs (see Figure 1B). Another feature of the S1’ pocket is the residue in position 218 (MMP-13 numbering), which is different in some MMPs (Arg in MMP-1, Tyr in MMP-7 and Leu in MMPs -2, -3, -8, -9, -10, -12, -13 and -14). This variability has revealed that some inhibitors take advantage of the differences in size and shape of the S1’ pocket among the members of the MMP family to achieve selectivity [66]. Furthermore, several inhibitors that bind to the S1’ pocket but which lack a ZBG have been reported for MMP-13 [43,67–69], MMP-8 [70] and MMP-12 [71]. Although the presence of a ZBG plays a role in determining the potency of MMP inhibitors [72,73], inhibitors devoid of a ZBG are able to expand to a side pocket adjacent to the S1’ pocket and known as the S1’’ pocket [43] (see Figure 1A). This pocket is present in MMPs that have a long or flexible Ω-loop, such as MMP-13 or MMP-8, but not in MMPs with a short or rigid Ω-loop, so it can be targeted to achieve inhibitor selectivity over these MMPs [43,67–70]. In fact, although most of the MMP inhibitors reported to date contain hydroxamate as a ZBG

[37], most of the inhibitors analyzed in this review —because of their selectivity profiles— do not belong to this class of inhibitors (see Figure 2).

Insert Figure 1 here

Overall, since the S1' pocket is a clear focus of variability among the members of the MMP family, the current general belief is that this pocket has great potential for the design of selective inhibitors and that, given the success of recent inhibitors in achieving selectivity, future generations of MMP inhibitors should target this pocket [5,66,73,76]. Therefore, the aim of this review is to analyze the differences in the S1' pocket among the different members of the MMP family and shed light on unreported mechanisms of MMP inhibitor selectivity, so that they can be rationally exploited in the future to develop novel selective inhibitors for specific MMPs. In order to achieve this goal we have: **(a)** classified the S1' pockets of the MMP experimental X-ray structures deposited in the Protein Data Bank (PDB) [74,75]; **(b)** performed protein-ligand docking simulations to determine whether the differences in selectivity can be attributed to steric hindrances resulting from differences in the size and shape of the S1' pocket; and **(c)** compared the electrostatic properties and the hydrophobicity of various S1' pockets in order to explain the selectivity of previously reported MMP inhibitors. As the examination of the recent literature revealed that most MMP inhibitors have been developed for MMP-2, MMP-9, MMP-12 and MMP-13 (see Table 2 and Tables S1 to S9), these MMPs are the main focus of this review.

Insert Figure 2 here

2. CALCULATION DETAILS

The X-ray structures of human MMP-1, -2, -3, -7, -8, -9, -10, -12, -13 and -14 released before the 25th of April of 2019 were obtained from the PDB [74,75]. Of the 425 subunits obtained, 23 subunits were discarded as they did not contain the catalytic domain of the enzyme. Then, the remaining subunits were superimposed onto the A subunit of the structure with PDB code 1ROS [71] by using the "Align to" option of the Protein Preparation Wizard [92]. Next, the conformation of the Ω-loop of the remaining 402 subunits was studied and the side chains on that loop facing the S1' pocket were identified and kept for further analysis (*i.e.*, side chains facing the outside of the S1' pocket do not have a direct influence on the size and shape of the S1' pocket and, therefore, they were omitted for the purpose of classification). With this information, for each MMP, these subunits were classified according to the conformation of their Ω-loop and a representative subunit was chosen in each case.

The protein structures used for docking were prepared with Protein Preparation Wizard [92] using the following procedure: **(a)** the original hydrogen atoms were removed and hydrogen atoms were added to the structures; **(b)** the *cap termini* option was set to on; **(c)** the ionization and tautomeric states of the ligand were generated with Epik [93] (here the state with the lowest energy was selected for each ligand; **(d)** the hydrogen bonds at pH 7 were assigned with PROPKA; **(e)** the force field OPLS_2005 was used to minimize the structure at 0.30 Å; and **(f)** all water molecules were removed from the structure. Then, the resulting protein structures were used to generate the corresponding grids for the protein-ligand docking with Maestro [94] by using default parameter values except for the following settings: **(a)** the center coordinates of the grids were (49.0, 81.0, 1.0); **(b)** halogens were included as acceptors; **(c)** the inner box sizes were (15, 15, 15); and **(d)** the outer box sizes were (35, 35, 35). With these features, the resulting grids encompassed the zinc-binding site and the S1' pocket of the corresponding MMP. The ligands used for docking were obtained from their corresponding crystal structures at the PDB [74,75] and were prepared with Epik [93] using the Protein Preparation Wizard [92] interface. Protein-ligand dockings shown in Figures S3, S5, S6, S8, S10, S12, S14, S17 and S20 were performed with Glide [95] by using default parameter values except for the following settings: **(a)** SP precision; **(b)** halogens were included as acceptors; **(c)** at most 1000 poses per ligand were written out; and **(d)** 5000 poses per ligand were included in post-docking minimization. Finally all the resulting poses were kept irrespective of their protein-ligand docking scores and they were shown in the corresponding figures. Protein-ligand dockings for Table 3 were performed with Glide [95] by using default parameter values except for the following settings: **(a)** XP precision; **(b)** ligand sampling: none (refine only); **(c)** XP descriptor information was written.

Electrostatic, hydrophobicity and electrostatic complementarity [96] comparisons were performed with Flare [97] by using the superimposed X-ray structures**.**

3. S1' POCKET CLASSIFICATION AND DOCKING SIMULATIONS TO EXPLAIN SELECTIVITY

The S1' pockets of the different members of the MMP family are generally classified into three categories based on their depth [3,66]: **(a)** shallow (MMP-1 and MMP-7); **(b)** intermediate (MMP-2, MMP-8, MMP-9, MMP-12 and MMP-14); and **(c)** deep (MMP-3, MMP-10 and MMP-13). Examining the experimental structures available in the PDB [74,75] for the human structures reveals that, in many cases,the Ω-loop adopts several conformations for each MMP (see Figure 3). For a better understanding of the variability of the S1' pocket, we have classified the different human MMPs according to the conformations of their Ω-

loop. In the paragraphs below we discuss, for each MMP, the different Ω-loop conformations that resulted from this classification.

Insert Figure 3 here

MMP-1

Of the 11 X-ray structures deposited in the PDB [74,75] for human MMP-1, 10 have coordinates for the catalytic domain and have been analyzed. Of the 18 subunits analyzed, 17 present the same conformation of the Ω-loop (conformation A, see Table S10) in which the side chain of the residue Arg214 faces the catalytic zinc ion (see Figures 3A and S2). Only one subunit shows a different conformation of Arg214 in which its side chain extends to the S1' pocket (see Figures 3A and S2) but, according to Fabre *et al.* [66], this should be considered an exception. Considering that in most cases residue Arg214 closes the S1' pocket and prevents the interaction of the ligands with the residues of the Ω-loop, the specific conformation of the Ω-loop of MMP-1 may not be relevant to the design of MMP inhibitors because Arg214 should be a steric hindrance for those MMP inhibitors that extend to the S1' pocket. This would explain the little MMP-1 inhibitory activity observed in MMP inhibitors from **1** to **17** [10,43,67,69,70,77–83], **21** [85], **22** [39], **24** [87] and **34** [68] (see Figure 2). To illustrate this, protein-ligand docking was performed using MMP inhibitors of different sizes that show little or no activity on MMP-1 (see Figure S3). In their respective co-crystallized MMPs, some of these inhibitors occupy the S1'' pocket interacting with the Ω-loop (see Figure S3A), others partly occupy the S1' pocket without interacting with many of the residues in the Ω-loop (see Figure S3B), and others just reach the region that is occupied by Arg214 in MMP-1 (see Figure S3C). Despite their differences, in all cases, the docked poses of these compounds in MMP-1 could not simultaneously occupy the zinc-binding region and the S1' pocket due to the blockade by Arg214, resulting in a different predicted binding mode, which would explain their little or no bioactivity for MMP-1. This supports the idea that selectivity over MMP-1 can be achieved simply by occupying the S1' pocket [66], preferably by seeking an interaction with the Ω-loop deep in the S1' pocket to ensure that MMP-1 will not be able to accommodate the compound.

MMP-2

Of the 5 X-ray structures deposited in the PDB [74,75] for human MMP-2, all their 9 subunits show the same conformation of the Ω-loop (see Table S11 and Figures 3B and S4). Nuclear magnetic resonance (NMR) structures reveal some flexibility of the Ω-loop of MMP-2 [98] and molecular dynamics (MD) simulations indicate that two states are possible, but one of them appears only rarely [98]. The Ω-loop of MMP-2 is much shorter than that of MMP-8 and MMP-13 (see Figure 1B) and this has allowed inhibitors

of these MMPs to achieve selectivity over MMP-2 by aiming to occupy the S1'' pocket. This is the case of the MMP-8 inhibitor **4** [70], and the MMP-13 inhibitors **1** [67], **2** [67] and **5** [43], which show little activity on MMP-2 (see Figure 2). To illustrate this, protein-ligand docking with MMP-2 was performed with these four compounds (see Figure S5). The docked poses were unable to occupy the S1'' pocket as in their original experimental structures, resulting in a different predicted binding mode of the inhibitor and explaining their selectivity over MMP-2. Therefore, the design of MMP-13 and MMP-8 inhibitors with bulky groups in the S1'' pocket should give them selectivity over MMP-2. Although MMP-8 inhibitor **3** [70] (see Figure 2) does not reach the S1'' pocket in MMP-8, its docked poses on MMP-2 reveal a binding mode different from that observed in MMP-8 (see Figure S6). The different binding mode of this compound in MMP-8 and MMP-2 can be attributed to the difference in length of the Ω-loop between these two MMPs (see Figure 1B) and it provides a plausible explanation for the selectivity of this compound.

MMP-3

For human MMP-3, the 31 X-ray structures deposited in the PDB [75,99] have been analyzed (see Table S12). The 14 residues that constitute the Ω-loop of human MMP-3 (see Figure 1B) make it highly flexible as indicated by NMR spectroscopy [100,101] and the large number of conformations that this loop adopts in the 56 subunits of these 31 X-ray structures (see Table S12 and Figures 3C and S7). Owing to the sequence length of the Ω-loop in MMP-3, the S1' pocket of MMP-3 is frequently classified as large, in the same category as MMP-13 [66]. However, despite the similarity between these two proteins regarding the number of residues that constitute their respective Ω-loops (see Figure 1B), the residue differences between them allow specific residue conformations to be adopted in each MMP that play an important role in the definition of the shape and size of the S1' pocket. While in most of the conformations of the MMP-3 Ω-loop the side-chain of residue Leu229 (and in some conformations the side chains of residues Leu226 and Thr227) hinders access to the bottom of the S1' pocket, this is not the case for MMP-13, in which no residue side-chains face the S1' pocket with the exception of the small side chain of Thr247 (see Figure 3H). As a result, the S1'' pocket of MMP-13 is larger than that of MMP-3, enabling it to accommodate bigger ligands. Many MMP-13 inhibitors, including **1** [67], **2** [67], **5** [43] and **6** [69], have taken advantage of this larger size to achieve selectivity over MMP-3 (see Figures 2 and S8), and we propose following this strategy to obtain MMP-13 inhibitors that are selective over MMP-3. The S1' pocket of MMP-8 may also prove to be larger than that of MMP-3 in some cases, despite being classified as intermediate [66]. This is because the Ω-loop of MMP-8 is able to adopt a particular conformation (conformation B shown in Figure S11) in which the conformation of the residues from Arg222 to Tyr227 is different, and the different orientations of the side chains of residues Thr224 and Tyr227 allow for a larger S1' pocket cavity (see Figures 3E and S11). Triggering this conformational change may be a useful

strategy in developing MMP-8 inhibitors selective over MMP-3, as is the case of MMP-8 inhibitor **4** [70] (see Figure 2).

MMP-7

For human MMP-7, the 5 X-ray structures deposited in the PDB [75,99] have been analyzed (see Table S13) and the Ω-loop of the 6 subunits they contain can be classified as two different conformations (see Figures 3D and S9). In MMP-7, the residue equivalent to Leu218 of MMP-13 is Tyr215. Because of this particular residue, the S1' pocket of MMP-7 is typically classified as shallow, in the same category as MMP-1 [66]. Similar to Arg214 in MMP-1, in MMP-7, Tyr215 adopts a conformation that faces the zinc-binding region and prevents the binding of ligands with the S1' pocket (see Figures 3A and 3D). Therefore, this residue should constitute a steric hindrance for those ligands which interact with the Ω-loop, and it provides an explanation for the selectivity observed in the majority of ligands that perform these type of interactions. This is illustrated by the docking on MMP-7 of several MMP inhibitors that show selectivity over MMP-7 (*i.e.*, **2** [67], **3** [70], **7** [77], **10** [78], **11** [78], **16** [82], and **23** [86]; see Figure 2), whose docked poses present a binding mode other than that observed in their original MMP (Figure S10 shows the docking of inhibitor **23** [86]). However, an experimental structure of MMP-7 (*i.e.*, the A subunit at 2Y6D) with a large ligand has been reported to show an alternate conformation of residue Tyr215, opening the access to the S1' pocket in order to accommodate the ligand [34] (see Figures 3D and S9C). Nevertheless, this experimental structure lacks the coordinates of the residues from Gly242 to Asn248, and it could be argued that the size of the S1' pocket of MMP-7 in this particular conformation may still be significantly smaller than that of other MMPs, such as MMP-8 or MMP-13. Thus, the targeting of the S1' pocket by MMP inhibitors directed to these MMPs should provide their selectivity over MMP-7. Actually, inhibitors that target the S1'' pocket of MMP-8 and MMP-13 have been shown to present high selectivity over MMP-7, supporting the previous idea. This is the case of MMP-8 inhibitor **4** [70] and MMP-13 inhibitors **1** [67], **2** [67], **5** [43] and **6** [69] (see Figure 2).

MMP-8

For human MMP-8, 25 X-ray structures (containing 30 subunits) have been deposited in the PDB [75,99] and analyzed (see Table S14). In the most common conformation of the Ω-loop for MMP-8 (conformation A), the side chain of residue Tyr227 faces the inside of the S1' pocket (see Figures 3E and S11B). Although both the unbound and bound forms of the enzyme present this Ω-loop conformation (see Table S14), the ligands of those structures do not reach the bottom of the S1' pocket. Conformation B is

observed in structures with large ligands which are able to establish contacts with the Ω-loop. In this conformation, the overall arrangement of the residues of the Ω-loop is altered to better accommodate the ligand (*i.e.*, the side chains of Arg222 and Tyr227 face slightly outwards, thus granting the ligand access to the S1' pocket, and the side chain of Thr224, which faced the outside of the S1' pocket in conformation A, faces the inside of the S1' pocket, allowing it to interact with the ligand; see Figures 3E and S11C). Yet another conformation has been observed for the Ω-loop of MMP-8 (conformation C) in which Tyr227 has an open conformation (see Figures 3E and S11D). However, only one structure has been determined with this conformation, in which the 2-(N-morpholino)-ethanesulfonic acid moiety of the ligand is in close proximity to Tyr227, which therefore could be the cause of this conformation. Therefore, although the S1' pocket of MMP-8 is often classified as medium-sized [66], the Ω-loop of MMP-8 demonstrates a certain degree of flexibility by adopting a different conformation (conformation B) and being able to accommodate large ligands in the S1' pocket. This B conformation should then be considered when discussing selectivity over MMP-8 since, in terms of size, the S1' pocket of MMP-8 in this conformation is more similar to the S1' pocket of MMP-13 than to that of other MMPs with the same Ω-loop length, such as MMP-12 and MMP-14 (see Figure 1B). Nevertheless, as the S1' pocket of MMP-13 is still larger than that of MMP-8, it is possible for MMP-13 inhibitors that bind to the S1' pocket to achieve selectivity over MMP-8 by targeting the S1'' pocket. This is the case for the MMP-13 inhibitors **2** [67] and **5** [43] (see Figure 2) where protein-ligand docking on MMP-8 predicts that neither of these two compounds is able to bind as they do in MMP-13 (see Figure S12). Thus, although some of their docked poses reach the S1'' pocket of MMP-8, none of them are able to place the carboxylic group at the S1'' pocket and at the same time extend to the zinc-binding region (see Figure S12 for the docking of **2** on the three Ω-loop conformations for MMP-8). This could be attributed to the difference in size, shape and flexibility between the S1' pockets of these MMPs. Actually, MD simulations show that the Ω-loop of MMP-13 is much more flexible than that of MMP-8 [102]. This may provide an explanation for the selectivity observed in these inhibitors.

MMP-9

Of the 24 X-ray structures deposited in the PDB [74,75] that contain the catalytic domain of human MMP-9, their corresponding 49 subunits have been analyzed (see Table S15). The Ω-loop in MMP-9 is special in the sense that it presents two consecutive proline residues (Pro254 and Pro255, see Figure 1B). Given the exceptional conformational rigidity of proline it can be inferred that the presence of these two proline residues significantly reduces the flexibility of the MMP-9 Ω-loop (see Figure 3F). In fact, most of the structures analyzed present the same conformation, regardless of the presence of a ligand (see Table S15 and Figure S13B), and this is in agreement with previous reports by Fabre *et al.* [66]. Only in

two subunits of one experimental structure did we observe a slightly different arrangement of the Ω-loop (conformation B; see Figures 3F and S13C). This rigidness of the Ω-loop of MMP-9 together with the small size of its S1' pocket allow the identification of inhibitors of MMPs with a long and more flexible Ω-loop that are selective over MMP-9 by introducing a bulky substituent in the S1' pocket which cannot be accommodated by MMP-9. Protein-ligand docking on MMP-9 of several MMP inhibitors that are selective over MMP-9 (*i.e.*, **1-6** [43,67,69,70], **16** [82] and **34** [68]; see Figure 2) has been conducted in order to illustrate this fact (Figure S14 shows the docking of inhibitor **4** [70]). Some of these inhibitors occupy the larger S1' pocket in their original experimental structure (sometimes reaching the S1'' pocket, which is not present in MMP-9), but are not able to do so in MMP-9. Because of the difference in size and shape of the S1' pocket between MMP-9 and the original MMP of these inhibitors, their docked poses fail to reproduce the binding mode observed in the original MMP, which offers an explanation for their selectivity.

MMP-10

For human MMP-10, only 3 X-ray structures (containing 6 subunits) have been deposited in the PDB [74,75] and have been analyzed here (see Table S16). MMP-10 has a very flexible Ω-loop, showing variability among different experimental structures and often low electron density for some of its Ω-loop residues (see Figure S15). For example, the coordinates of residue Phe242 have not been obtained for any of the experimental structures analyzed here due to the lack of electron density, probably indicating the flexibility of that region of the loop. However, despite this flexibility, the S1' pocket of MMP-10 may present differences in size, shape or electrostatic properties when compared to the S1' pockets of MMPs which also present a long Ω-loop, which would explain the high selectivity values of the MMP-13 inhibitors **5** [43] and **6** [69] over MMP-10 (see Figure 2). Unfortunately, the lack of electron density for some residues makes it difficult to relate this selectivity to size, shape or electrostatic differences between MMP-10 and MMP-13.

MMP-12

For human MMP-12, 70 X-ray structures (containing 119 subunits) have been deposited in the PDB [74,75] and have been analyzed here. All of them show the same conformation of the Ω-loop (see Table S17 and Figure S16) except for one particular structure (*i.e.*, the B conformation), whose main characteristics are the conformations adopted by residues Tyr242 and Met236 (*i.e.*, Tyr242 faces out of the S1' pocket and Met236 blocks the access to the S1' pocket; see Figures 3G and S16C). Despite the reasonably large amount of residues that conform the Ω-loop in MMP-12 and define an S1' pocket

classified as medium-sized compared to other MMPs [66], the low variability of the Ω-loop observed among the experimental structures of MMP-12 indicates that this loop has limited flexibility. Apart from residue Lys241, which seems to adopt a wide range of conformations, the other residues of the Ω-loop do not present alternate conformations. Assuming that conformation A is the only conformation of the MMP-12 Ω-loop that allows the ligand to reach the S1' pocket, inhibitors directed to MMPs with a larger and more flexible Ω-loop should be able to achieve selectivity over MMP-12 by targeting the S1'' pocket, which is blocked by residue Ile245 in MMP-12. To illustrate this, protein-ligand docking was performed using ligands selective over MMP-12 (*i.e.*, **1** [67], **2** [67], **4** [70] **6** [69] and **34** [68]; see Figure 2; Figure S17 shows the docking of inhibitor **6** [69]). In all cases, the docked poses of these compounds in MMP-12 could not occupy the S1' pocket due to its smaller size, therefore resulting in a different hypothetical binding mode of the inhibitors than that observed in their experimental structures, which would explain their selectivity over MMP-12.

MMP-13

Of the 43 X-ray structures deposited in the PDB [75,99] that contain the catalytic domain for human MMP-13, their corresponding 104 subunits have been analyzed (see Table S18). The Ω-loop of MMP-13 is composed of 13 residues (see Figure 1B) and its S1' pocket is classified as deep [66]. The large Ω-loop of MMP-13 has a high degree of flexibility and it adopts several conformations (see Figures 3H and S18). This flexibility has also been confirmed by NMR spectroscopy [100]. From the structures classified here, 2 subunits corresponded to unbound forms of the enzyme and 102 subunits corresponded to bound forms. We found that all the bound forms in which the ligand does not bind to the zinc-binding site and/or the S1' pocket present the same Ω-loop conformation (conformation A). In contrast, when the ligand binds to the zinc-binding site and/or the S1' pocket, then the Ω-loop shows different conformations depending on the type of ligand that binds to MMP-13. In structures where the ligand reaches only the S1' pocket, we observed the conformations from B to F and J. In the cases where the ligands occupy the S1'' pocket, however, most of the structues presented different conformations of the Ω-loop (conformations from G to I), meaning that the high flexibility of the Ω-loop of MMP-13 allows it to adapt in order to accomodate ligands in the S1'' pocket.

MMP-14

Of the 4 X-ray structures deposited in the PDB [74,75] that contain the catalytic domain for human MMP-14, their corresponding 5 subunits have been analyzed (see Table S19). The S1' pocket of MMP-14

is classified as intermediate and its Ω-loop has the same length as those of MMP-13 and MMP-8 (see Figure 1B). However, recent MD simulation analyses have shown that the Ω-loop in MMP-14 is less flexible than that of MMP-13 or MMP-8 [102]. A closer look at the side chains of the Ω-loop in MMP-14 that are oriented towards the S1' pocket reveals that the side chain of residue Met264 occupies part of the pocket (see Figures 3I and S19). The low flexibility of the loop might not allow the adoption of a different conformation for the side-chain of this residue and, therefore, seeking a steric clash with this particular residue may provide an anchor for identifying inhibitors selective over MMP-14. When performing docking simulations on MMP-14 with co-crystallized inhibitors selective towards this MMP, (*i.e.*, **1-6** [43,67,69,70], **11** [78], **15** [81], **16** [82], **24** [87], **27** [88], **28** [69] and **34** [68]; see Figure 2), residue Met264 represented a steric hindrance for these inhibitors that did not allow them to bind to the S1' pocket as they would in their original MMPs, or at least not in the same fashion (Figure S20 shows the docking of inhibitor **15** [81]). This would provide an explanation for the selectivity observed in these inhibitors. Based on these observations, inhibitors that reach the S1' pocket are expected to have selectivity over MMP-14.

4. DIFFERENT SEQUENCE, DIFFERENT POCKET

While the previous section illustrated how the size and shape of the S1' pocket are crucial determining factors in the selectivity of many MMP inhibitors, the residue differences in different Ω-loop sequences may also translate into different hydrogen bond interactions and Van der Waals contacts with the ligand among the different MMPs, as well as different electrostatic and hydrophobic properties of the S1' pocket. These type of differences also constitute an opportunity to achieve inhibitor selectivity. Here we will examine the characteristics of several MMP-2, MMP-9, MMP-12 and MMP-13 inhibitors and propose the possible causes of their selectivity over other MMPs based on the the characteristics of the S1' pocket.

4.1. Selectivity towards MMP-2

A critical aspect in the design of MMP-2 inhibitors for cancer therapy is avoiding anti-targets such as MMP-3, MMP-8, MMP-9 and MMP-14, whose inhibition could be detrimental to cancer prognoses [7,16,32]. In the previous section we have proposed that targeting the S1' pocket would lead to MMP-2 inhibitors that are selective over MMP-14. Here we will focus on how MMP-2 inhibitors have been able to achieve selectivity over MMP-8 and MMP-9.

Compound **29** [89], which is co-crystallized with MMP-8, was found to be more selective for MMP-2 than for MMP-8 (see Figure 2). Although the authors attribute the increase in MMP-2 affinity to the possible

release of the torsional strain of the two rings of the biphenyl group in the wider S1' pocket of MMP-2 [89], this increased activity could also be related to the different electrostatic natures of the two S1' pockets. In this regard, Figure 4A shows that the cyano group of the ligand has better electrostatic complementarity with the S1' pocket of MMP-2 than with the S1' pocket of MMP-8. Therefore, the inclusion of negative groups in MMP-2 inhibitors targeting this region of the S1' pocket should provide them selectivity over MMP-8.

Insert Figure 4 here

Due to its reported anti-angiogenic and anti-tumorigenic properties [16], MMP-9 is another clear anti-target that is usually considered in the development of MMP-2 inhibitors. Interestingly, Tochowicz *et al.* [83] reported two inhibitors co-crystallized with MMP-9, **18** and **30,** which were more active for MMP-2 than for MMP-9 (see Figure 2). Although Arg249 extends away from the S1' cavity of MMP-9, the authors hypothesize that the mobility of its side-chain is responsible for this difference in activity [83]. This residue is replaced with the less bulky residue Thr143 in MMP-2, which would result in much weaker hindering by its side-chain [83]. Amin *et al.* [103] also propose this mechanism to obtain MMP-2 inhibitors that are selective over MMP-9. Nevertheless, considering that MD simulations show that the Ω-loop of MMP-2 is more flexible than that of MMP-9 [104], another possible explanation for the selectivity of compounds **18** and **30** could be that residue Phe148 in MMP-2 may be able to get closer to the ligand and establish better hydrophobic interactions than residue Pro255 in MMP-9 (see Figure 5).

Insert Figure 5 here

4.2. Selectivity towards MMP-9

Interestingly, while searching for MMP-12 inhibitors, Morales *et al.* [71] obtained the high-throughput screening hit **31**, which is more active for MMP-9 than for MMP-12 (see Figure 2). While we could not relate this difference in activity to electrostatics or hydrophobics, given the smaller size of this inhibitor compared to the other ones obtained and the narrower shape of the S1' pocket of MMP-9 compared to that of MMP-12, the authors hypothesized that the higher activity of this inhibitor for MMP-9 could be explained by its better accommodation in the MMP-9 S1' pocket [71].

4.3. Selectivity towards MMP-12

The S1’ pocket of MMP-12 is mostly characterized by its high hydrophobicity [71]. The combination of a series of residues (*i.e.*, Ala234, Val235, Phe237, Lys241, Val243 and Phe248) provide the S1’ pocket with a more hydrophobic environment compared to other MMPs [71]. In fact, the selectivity of many MMP-12 inhibitors has already been attributed to this hydrophobic nature. Morales *et al.* [71] identified the MMP-12 inhibitors **32** and **33**, which are highly selective over MMP-2 (see Figure 2). The authors claim that these ligands are less stabilized by hydrophobic interactions in the more open MMP-2 S1’ pocket than in the S1’ pocket of MMP-12 [71]. These inhibitors were also more active for MMP-12 than for MMP-3, which the authors attribute to the fact that MMP-3 has a larger S1’ pocket and the ligands are not able to occupy its volume to the same degree as in MMP-12 [71]. These hypotheses are in agreement with our docking simulations showing that lipophilic interactions of these two ligands with the protein are stronger for MMP-12 than for MMP-2 and MMP-3 (see Table 3). Likewise, our docking simulations of compounds **15** [81] and **25** [81] show that the lipophilic interactions of these ligands with the protein are significantly stronger for MMP-12 than for MMP-2, -3, -8, -9 and -13, MMPs over which these inhibitors are selective (see Table 3 and Figure 2). A similar situation may occur in the case of compounds **7**-**9** [77] and **24** [87] (see Table 3 and Figure 2), since these inhibitors introduce hydrophobic moieties in the S1’ pocket and are also selective over other MMPs. Overall, the predominance of hydrophobic residues in the S1’ pocket of MMP-12 makes its hydrophobic environment an attractive characteristic to target in the design of selective inhibitors.

Insert Table 3 here

Furthermore, electrostatics can also play a part in determining selectivity for MMP-12. For instance, in the case of compound **32** [71], the ethoxy group of the ligand (see Figure 2) places a negative electrostatic surface in the S1’ pocket of MMP-12 that results in a better electrostatic complementarity with MMP-12 relative to MMP-8 (see Figure 4B). This higher electrostatic complementarity would explain why this ligand displays a higher bioactivity for MMP-12 than for MMP-8. A similar situation occurs in the case of the MMP-12 inhibitors **15** [81] and **25** [81] (see Figures 2, 4C and 4D), as the π system of the phenyl ring of these ligands projects a negative electrostatic surface to both sides of the ring resulting in a better electrostatic complementarity with MMP-12 than with MMP-8.

Differences in the electrostatics of the S1’ pocket can also be exploited to achieve selectivity over MMP-3. In the case of the MMP-12 inhibitor **7** [77] (see Figure 2), the negative electrostatic surface of the π system of the ligand's phenyl ring makes the ligand more suitable to interact with the S1’ pocket of MMP-12 than with the S1’ pocket of MMP-3 (see Figure 6A). This is in agreement with the lower affinity of this compound for MMP-3 than for MMP-12 (see Figure 2). Similarly, the MMP-12 inhibitors **15** [81] and **25** [81], which are also selective over MMP-3 (see Figure 2), present a negative electrostatic surface projecting from their thiophene and phenyl rings towards the S1’ pocket of this protein (see Figures 6B

and 6C). In all these cases, the negative electrostatic surface of the ligand shows better electrostatic complementarity with MMP-12 than with MMP-3, and this could explain the selectivity of these ligands over MMP-3 (see Figure 2).

Insert Figure 6 here

4.4. Selectivity towards MMP-13

Although, as discussed in the previous section, the more secure approach to selectively inhibiting MMP-13 is considered to be the targeting of the S1'' pocket, some MMP-13 inhibitors have shown different levels of selectivity over other MMPs without reaching this subpocket. This is, for example, the case of inhibitor **13** [10], which is more active for MMP-13 than for MMP-3 and MMP-8 (see Figure 2). The analysis of the electrostatic potential interactions between the ligand and these three targets reveals that if the binding mode observed by the inhibitor in MMP-13 were conserved in the binding sites of MMP-3 and MMP-8, the electrostatic complementarity of this compound and the corresponding binding site would be worse for MMP-3 and MMP-8 than for MMP-13 (see Figures 6D and 4E, respectively). Therefore, both of these situations would result in a decrease in bioactivity for either MMP relative to MMP-13. These observations correlate with the observed selectivity of this MMP-13 inhibitor over both of these MMPs (see Figure 2). MMP-13 inhibitor **26** [81] (see Figure 2) also places a negative electrostatic surface in the S1' pocket of MMP-8 through the π system of its last phenyl ring, therefore decreasing the affinity of the ligand for this MMP (see Figure 4F). Interestingly, though, the MMP-8 inhibitor **35** [81] can in its turn take advantage of its higher electrostatic complementarity with the S1' pocket of MMP-8 relative to the S1' pockets of MMP-13, MMP-2 and MMP-3 to achieve selectivity over these MMPs (see Figure 2) by placing a methyl group bearing a positive electrostatic surface in this region of the S1' pocket (see Figure 7).

Insert Figure 7 here

Nevertheless, targeting the S1'' pocket in MMP-13 offers the advantage that selectivity over MMP-1, -2, -3, -7, -8, -9, -12 and -14 is generally achieved, as these proteins either lack this subpocket or have a smaller one and this constitutes a steric hindrance for MMP-13 inhibitors to bind to them. By contrast, given the large size of the MMP-8 S1' pocket, the selectivity of some MMP-13 inhibitors may not be a consequence of steric hindrances, as these inhibitors should fit well in the MMP-8 binding site. In these cases, MMP-13 selectivity may be governed by other characteristics of the MMP-13 binding site. This is, for instance, the case of the MMP-13 inhibitor **6** [69] (see Figure 2). The electrostatic analysis of this inhibitor in the S1' pocket of MMP-13 reveals that its negatively-charged carboxylic acid group located at the S1'' pocket shows a high electrostatic complementarity with that region of the protein, which has a positive electrostatic potential caused partly by the basic residue Lys140 in MMP-13 that is not present in

MMP-8 (see Figure 8). Thus, the presence of this negative group of the ligand boosts the activity of this inhibitor for MMP-13. Moreover, in MMP-8, an acidic residue (*i.e.*, Asp115) is present in this region of the pocket, making the electrostatic environment of MMP-8 less suitable for a negatively charged group (see Figure 8). Therefore, this feature of the ligand may also be responsible for the selectivity observed over MMP-8 (see Figure 2). This feature is also present in other co-crystallized MMP-13 inhibitors that extend deeply in the S1'' pocket, such as inhibitors **2** [67], **5** [43] and **34** [68] (see Figure 2). All of these inhibitors have been reported to be highly potent and selective over all the other members of the MMP family for which bioactivities have been measured.

Insert Figure 8 here

Based on this analysis, we can propose two mechanisms for obtaining potency and selectivity for MMP-13 given two characteristic features of this target: **(a)** extension to the S1'' pocket, as MMP-13 is the MMP with the deepest S1' pocket; and **(b)** incorporation of a negatively charged group in the compound able to establish an electrostatic interaction with the residue Lys140, not present in other MMPs.

4.5. Selectivity over MMP-3 and MMP-8

Overall, in this section, apart from establishing several criteria for identifying inhibitors able to attain higher potency for a certain MMP compared to others, we have also acquired novel valuable information on how to avoid the preference of specific targets by inhibitors of other MMPs. In order to avoid the inhibition of MMP-3 and MMP-8, the inhibitor should be able to project a negative electrostatic surface to the S1' pocket of both proteins, so that a repulsive interaction can occur due to their negative potential. In the case of MMP-3, its S1' pocket has been shown to have a more negative electrostatic region than MMP-12 and MMP-13 (see Figure 6). In the case of MMP-8, its S1' pocket has been shown to have a more negative electrostatic character than MMP-2, MMP-12 and MMP-13 (see Figure 4). Therefore, based on these observations, it should be possible to take advantage of the electrostatic characteristics of the S1' pockets of these MMPs by designing MMP inhibitors which incorporate functional groups that would generate repulsive electrostatic interactions in these environments while maintaining a high affinity for the targeted MMP.

5. CONCLUSIONS

In this review we have proposed several mechanisms for MMP inhibitors to achieve selectivity over other MMPs. These mechanisms have been summarized in Figure 9. We have shown how to take advantage of differences in the size and shape of the S1' pocket, as is the case for the shallow pockets of MMP-1 and MMP-7, and the deeper pockets of MMP-13 and MMP-8, as well as how to exploit the differences in residue composition between S1' pockets. More importantly, we have shown that the variability in the S1' pocket characterizes each MMP in terms of hydrophobicity and electrostatic properties and that this variability can be rationally exploited to achieve MMP inhibitor selectivity: we have shown that hydrophobic interactions are relevant to the selectivity of MMP-12 inhibitors, that adding a negative charge to the S1'' pocket increases the selectivity of MMP-13 inhibitors and that the presence of a negative electrostatic environment in the S1' pocket contributes to inhibitor selectivity over MMP-3 and MMP-8. Therefore, the hydrophobicity and electrostatic properties of the S1' pocket of MMPs need to be considered in the design of new MMP inhibitors as they offer an opportunity to achieve improved selectivity profiles.

Insert Figure 9 here

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TABLE CAPTIONS

Table 1*.* MMPs, their alternative names, functional classification, main substrates and some of the pathologies in which they are involved

This information has been obtained from the BRENDA [60], KEGG [61], ExPASy [62] and MEROPS [63] databases.

Table 2. Summary of manuscripts reporting MMP inhibitors since 2010

Table 3*.* *LipophilicEVdW:* lipophilic term obtained from protein-ligand docking using GlideXP [95,105] for different compounds against different MMPs.

LipophilicEVdW is a term from the GlideXP scoring function which is derived from the hydrophobic grid potential at the hydrophobic ligand atoms. The MMP-2, -3, -8, -9 and -13 structures used for docking were the structures with PDB [74,75] codes 3AYU (A) [106], 1HFS (A) [107], 1I73 (A) [108], 4WZV (A) [84], and 2YIG (A) [88], respectively. The MMP-12 structure used for docking in each case corresponded to the structure with which the compound was co-crystallized (*i.e.*, structures with the PDB IDs 2WO8 (A) [77], 2WO9 (A) [77], 2WOA (C) [77], 3TS4 (A) [81], 3LIK (A) [87], 4EFS (A) [81], 1ROS (A) [71] and 1UTZ (A) [71] for inhibitors **7** [77], **8** [77], **9** [77], **15** [81], **24** [87], **25** [81], **32** [71] and **33** [71], respectively). [a, b, c] Statistically significant outlier values determined by Dixon's Q test at 99%, 90% and 80% confidence, respectively.

FIGURE LEGENDS

Figure 1. Binding site of MMPs. Panel A shows the binding site of MMP-13 (structure with PDB [74,75] ID 3WV1 [43]), with the different pockets of the binding site. The residues of the S1, S2, S3, S1', S2', S3' and S1'' pockets are in pink, orange, salmon, green, blue, cyan and violet, respectively. The ligand and the catalytic zinc ion are represented in spacefill format. The zinc ion is colored black. Panel B shows the Ω-loop length and residue sequence for MMP-1, -2, -3, -7, -8, -9, -10, -12, -13 and -14. The numbers before and after each sequence segment indicate the location of the first and last segment residue for each MMP. Residues with side chains facing towards the S1' pocket in at least one representative subunit of the corresponding MMP are highlighted in the Clustal X color scheme.

Figure 2. Structures and activities of MMP inhibitors discussed in the review. The information provided about each compound is: **(a)** the name used to refer to it in the text; **(b)** its original name in the manuscript in which it was described for the first time; **(c)** its 2D structure; and **(d)** the bioactivities measured for the different MMPs. Marvin [90] was used to draw the 2D structures of the inhibitors. The bioactivities of batimastat and marimastat were obtained from Reaxys [91].

Figure 3. The different Ω-loop conformations observed in each MMP. Panels A, C, D, E, F, G, and H show the superposition of representative structures for MMP-1, -3, -7, -8, -9, -12 and -13, respectively. Panels B and I show the single available conformation for the Ω-loop in MMP-2 and MMP-14. These structures correspond to the representative subunits from Tables S11 to S19. All panels have the same orientation to facilitate comparison. This figure was obtained with Maestro [94].

Figure 4. Inhibitors selective over MMP-8. The five screen captions that form each panel have the same orientation to facilitate comparison and, from left to right, show: ***(i)*** the electrostatic potential around the ligand; ***(ii)*** the electrostatic potential around the binding site of the MMP that is compared with MMP-8; ***(iii)*** the electrostatic potential around the binding site of MMP-8; ***(iv)*** the electrostatic complementarity between the ligand and the MMP that is compared with MMP-8; and ***(v)*** the electrostatic complementarity between the ligand and MMP-8. In the first three screen captions of each panel, the positive and negative electrostatic potentials are shown in blue and red, respectively. In the last two screen captions of each panel, the molecular surface of the protein is colored by the electrostatic complementarity between the ligand and the protein: green and red represent the areas of good and bad complementarity, respectively. The protein backbone of the MMP that has not been crystallized with the inhibitor (in purple) has been superposed to the experimental complex (in orange) to determine its relative position to the co-crystallized ligand (in green). The co-crystallized inhibitors shown in panels from A to F are **29** [89], **32**

[71], **25** [81], **15** [81], **13** [10] and **26** [81] (which were co-crystallized with MMP-8, MMP-12, MMP-12, MMP-12, MMP-13 and MMP-13, respectively). A circle indicates the region (either from the corresponding MMP or from the ligand) involved in differences in the bioactivity of the same ligand relative to the two MMPs that are compared in each panel. This figure was obtained with Flare [97].

Figure 5. Inhibitors selective over MMP-9. The three screen captions that form each panel have the same orientation to facilitate comparison and, from left to right, show: **(a)** the hydrophobic area around the ligand; **(b)** the hydrophobicity of the MMP-2 binding site; and **(c)** the hydrophobicity of the MMP-9 binding site. In the first screen caption of each panel, the hydrophobic surface of the ligand is shown in beige. In the next two screen captions of each panel, the molecular surface of the protein is colored by its polarity: while the hydrophilic areas are shown in blue, the hydrophobic areas are in beige. The inhibitors shown in panels A and B are **30** [83] and **18** [83], respectively, which were both co-crystallized with MMP-9. A circle indicates the region (either from the corresponding MMP or from the ligand) involved in differences in the bioactivity of the same ligand relative to the two MMPs that are compared in each panel.

Figure 6. Inhibitors selective over MMP-3. This figure has been prepared using the same guidelines as Figure 4. The co-crystallized inhibitors shown in panels from A to D are **7** [77], **25** [81], **15** [81], **13** [10] (which were co-crystallized with MMP-12, MMP-12, MMP-12 and MMP-13, respectively).

Figure 7. Inhibitor **35** [81], selective for MMP-8. This figure has been prepared using the same guidelines as Figure 4. Panels A, B and C show the comparison between the binding site of MMP-8 and the binding sites of MMP-13, MMP-2 and MMP-3, respectively.

Figure 8. MMP-13 inhibitor **6** [69], selective over MMP-8. This figure has been prepared using the same guidelines as Figure 4. It shows the comparison between the binding site of MMP-13 and the binding site of MMP-8.

Figure 9. Summary of ways to achieve selectivity for MMP-2, MMP-9, MMP-12 and MMP-13 over other MMPs obtained from SAR analyses. Each cell contains the ways by which MMP inhibitors of the MMP in the corresponding column are able to achieve selectivity over the MMP in the corresponding row. The figures and tables that were used to reach the conclusions summarized in each cell are indicated in parentheses. The same color refers to the same mechanism of achieving selectivity.

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