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Artículo original

Are adrenomedullin positive modulators novel matrix metalloproteinase inhibitors?

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MARIO A. GARCÍA, SONSOLES MARTÍN-SANTAMARÍA, ANA RAMOS, BEATRIZ DE PASCUAL-TERESA [*]

Departamento de Química. Facultad de Farmacia, Universidad San Pablo CEU, Urbanización Montepríncipe, 28668-Boadilla del Monte, Madrid (Spain)

ABSTRACT

Matrix metalloproteinases (MMPs), are a family of structurally related zinc containing enzymes that play a major role in the breakdown of connective tissue and therefore, are targets for therapeutic inhibitors in many inflammatory, malignant, and degenerative diseases. On the other hand, it has been recently demonstrated that one of these enzymes, MMP-2, a type IV collagenase, termed gelatinase A, cleaves the angiogenic peptide adrenomedullin (AM) (1). AM is a peptide hormone that plays a critical role in several diseases such as diabetes, hypertension and cancer. In a High Throughput Screening (HTS) carried out at the National Cancer Institute (NCI), a series of AM modulators were identified, with an interesting hypotensive activity (2). In order to shed light into the mechanism of action of these interesting compounds, we have hypothesized that they may be

[*] Beatriz de Pascual-Teresa.
Facultad de Farmacia. Universidad San Pablo CEU.
Ctra. Boadilla del Monte, km. 5,300. Boadilla del Monte. Madrid.
Phone: +34913724796. Fax: +34913724009 bpaster@ceu.es
Sonsoles Martín-Santamaría.
Facultad de Farmacia. Universidad San Pablo CEU.
Ctra. Boadilla del Monte, km. 5,300. Boadilla del Monte. Madrid.
Phone: +34913724798 Fax: +34913724009 smar.fcex@ceu.es

Abbreviations: MMPs: Matrix metalloproteinases; AM: adrenomedullin; HTS: High Throughput Screening; NCI: National Cancer Institute.

affecting the biodisponibility of AM in the blood stream by inhibiting the MMP-2 protease activity. In the present work, we present a theoretical study, making use of molecular mechanics, docking and virtual screening techniques, with the aim of demonstrating this hypothesis. Biological evaluation of MMP-2 inhibition by some selected compounds, followed the computational work, leading us to propose a structurally new type of MMP-2 inhibitors, with possible interest as anticancer and antiangiogenic agents.

Key words: Zinc-binding-group (ZBG).—Docking.—Virtual screening.

RESUMEN

¿Son los moduladores positivos de adrenomedulina nuevos inhibidores de metaloproteasas de la matriz?

Las metaloproteasas de la matriz (MMPs) pertenecen a la familia de enzimas que contienen zinc y juegan un papel predominante en la degradación del tejido conectivo. Por ello se consideran dianas terapéuticas para procesos de inflamación y enfermedades malignas y degenerativas. Por otro lado, se ha demostrado recientemente que un miembro de esta familia, MMP-2, una colagenasa de tipo IV también conocida como gelatinasa A, es capaz de degradar un péptido angiogénico denominado adrenomedulina (AM) (1). AM es una hormona peptídica que desarrolla un papel importante en diversas patologías como diabetes, hipertensión y cáncer. Se ha identificado mediante un cribado de alto rendimiento (HTS) de la colección de compuestos del Instituto Nacional del Cáncer (NCI), una serie de moduladores con interesante actividad hipotensora (2). El mecanismo de acción de estos moduladores es desconocido y nosotros proponemos que pueden afectar a la biodisponibilidad de la AM en el torrente sanguíneo por medio de la inhibición de la actividad de la MMP-2. En este trabajo presentamos un estudio teórico que hace uso de técnicas como mecánica molecular, *docking* y Cribado Virtual con el objetivo de demostrar esa hipótesis. A continuación del estudio computacional se llevó a cabo la evaluación biológica de algunos compuestos, permitiéndonos proponer un nuevo tipo de ZBG que puede ser interesante para el diseño de nuevos inhibidores de MMPS, con interés como agentes anticancerosos y antiangiogénicos.

Palabras clave: Grupo de unión al zinc.—Docking.—Cribado virtual.

INTRODUCTION

Matrix Metalloproteinases Classification

Matrix metalloproteinases (MMPs), also called matrixins, are a family of structurally related zinc-containing enzymes that mediate the breakdown of connective tissue and are therefore targets for therapeutic inhibitors in many inflammatory, malignant, and degenerative diseases (3).

The mammalian MMP family is now known to include at least 24 enzymes. The most studied are three collagenases: interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13). These enzymes can degrade the fibrillar collagens, which are generally resistant to proteolysis. The MMP family also includes two type IV collagenases, termed gelatinase A (MMP-2) and gelatinase B (MMP-9), which can degrade type IV collagen of basal laminae, as well as other non helical collagen domains and proteins, such as fibronectin and laminin. Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) degrade various proteoglycan components of the extracellular matrix as well as fibronectin and laminin.

Several subsites have been described (S1, S2, S3, S1', S2', and S3') being responsible for different roles affecting activity and selectivity, depending on the MMP considered (4). These requirements can be satisfied by a variety of different structural classes of MMP inhibitors (MMPIs), which have been discovered by a number of methods, including structure-based design (5, 6) and combinatorial chemistry (7).

Proteolytic Reaction Mechanism

The proteolytic reaction of MMPs has been rationalized on the basis of structural information (Figure 1) (8). It has been proposed that the scissile amide carbonyl coordinates to the active site Zn^{2+} ion. This carbonyl is attacked by a water molecule that is simultaneously hydrogen bonded to a conserved glutamic acid

(Glu198 in MMP-8) and coordinated to the Zn^{2+} ion. The water donates a proton to the Glu residue that transfers it to the nitrogen of the scissile amide. This is followed by the Glu residue shuttling the remaining proton from the water molecule to the nitrogen of the scissile amide with resultant peptide bond cleavage. During this process, the positively charged Zn^{2+} ion helps to stabilize negative charge at the carbon of the scissile amide and a conserved Ala (Ala161 in MMP-8) residue helps to stabilize the positive charge at the nitrogen of the scissile amide.

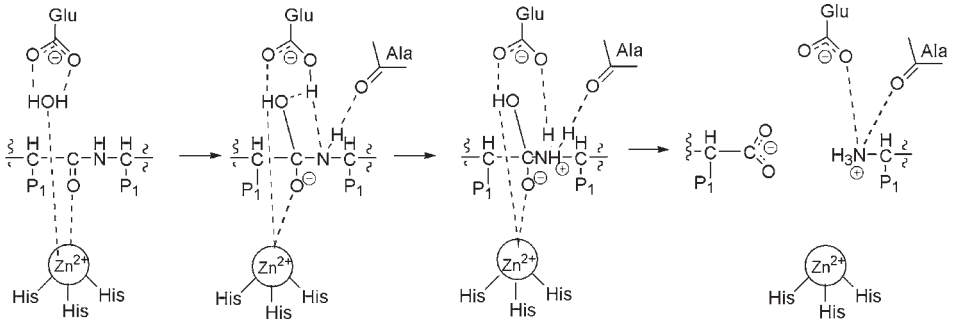


FIGURE 1. ***Mechanism of proteolysis carried out by MMPs****, as reported by Lovejoy et al.*

Biological Implications of MMPs

Matrix metalloproteinases are responsible of remodelling and degrading extracellular matrix, and are implicated in a wide variety of biological processes such as embryonic development, blastocyst implantation, nerve growth, ovulation, morphogenesis, angiogenesis, tissue resorption and remodelling, bone remodelling, apoptosis, cancer invasion and metastasis, arthritis (osteo- and rheumatoid-), atherosclerotic plaque rupturing, pulmonary emphysema, aortic aneurysms, breakdown of blood-brain barrier, periodontal disease, skin ulceration, corneal ulceration, gastric ulcer, multiple sclerosis, Crohn's disease, psoriasis, dermatitis, congestive heart failure and Alzheimer's disease (9).

Matrix Metalloproteinases Inhibitors (MMPIs)

Many groups of compounds are used as MMPs inhibitors. The requirements for a molecule to be an effective MMP inhibitor are: a) a functional group (e.g. carboxylic acid, hydroxamic acid, sulfhydryl, etc.) capable of chelating the catalytic Zn^{2+} ion (this will be referred as zinc binding group or ZBG); b) at least one functional group which provides a hydrogen bond interaction with the enzyme backbone; and c) one or more side chains that undergo effective van der Waals interactions with the enzyme subsites. These requirements can be satisfied by a variety of different structural classes of MMP inhibitors, which have been discovered by a number of methods, including structure-based design (5) and combinatorial chemistry (7).

Zn^{2+}-chelating hydroxamates have been the most frequently used ZBG in MMPI design, because of their higher ΔG values, but as shown below, a number of other groups are possible. However, strong Zn^{2+}-chelating moieties disproportionately drive binding mode and make the contributions from the rest of the compound smaller, reducing other opportunities for improved specificity. Indeed, it has been demonstrated that some hydroxamate derivatives bind many off-target metalloproteinases (MPs) that are not MMPs (10). This may be related to the lack of selectivity of hydroxamic acid for zinc over other divalent transition metals. The use of weaker zinc chelating groups would reduce the strength of zinc binding, and the optimization on the rest of the molecule should lead to nanomolar inhibitors. This means, that the design of MMPIs should be addressed individually in order to achieve selectivity.

The reported types of MMPIs include hydroxamates (succinyl hydroxamates, sulfonamide hydroxamates, reverse hydroxamates, naphthyl hydroxamates), non-hydroxamates (carboxylates and N-carboxyalkyl ZBGs, thiol ZBGs, phosphorus-based ZBGs), and miscellaneous natural products (7, 11).

Novel Zinc Binding Groups

In spite of the time and the money invested, and the numerous groups interested on the development of MMPIs based on the

described ZBGs (Table 1 summarises the most important ZBGs described in the literature), no MMPI has been licensed for treatment of cancer until date. This is mainly due to low activity, low selectivity, high toxicity, low bioavailability and/or low solubility. Only one MMPI, Periostat™ (CollaGenex Pharmaceuticals, Inc.), a low-dose tetracycline derivative, is licensed for use in periodontitis and it is under clinical trials for other indications (12). Thus, research focused on the search of new ZBG becomes necessary.

TABLE 1. *Summary of the most important Zinc Binding Groups (ZBGs) described in the literature (11). Atoms implicated in zinc coordination are displayed in bold format. ZBGs with unknown binding mode are displayed without bold atoms*

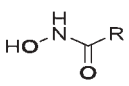
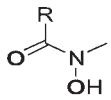
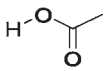
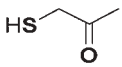
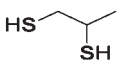
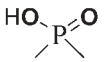
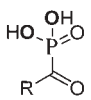
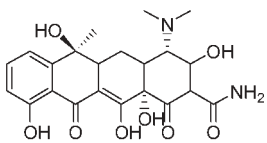
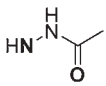
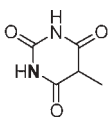
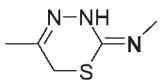
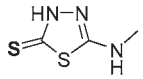
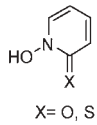
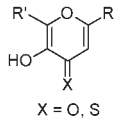
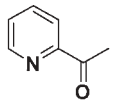
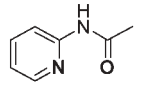
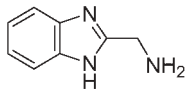
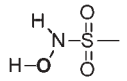
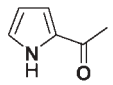
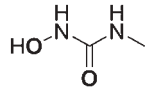
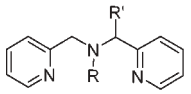
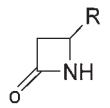
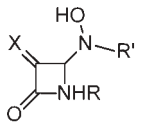
Name	*Representative Structure*	*Name*	*Representative Structure*
Hydroxamate		Reverse Hydroxamate	
Carboxylate		Thiol	
Thiol carbonyl		Dithiol	
Phosphate		Phosphinate	
Carbamoyl Phosphonate		Tetracycline	
Hydrazide		Thiirane	
Barbiturate		Thiadiazine	

TABLE 1. *Summary of the most important Zinc Binding Groups (ZBGs) described in the literature (11). Atoms implicated in zinc coordination are displayed in bold format. ZBGs with unknown binding mode are displayed without bold atoms* (cont.)

Name	*Representative Structure*	*Name*	*Representative Structure*
Thiadiazole thione		Sulfodiimine	
Pyridone and Pyridinetione		Hydroxipiranone and Hydroxipiranthinone	
Pyrimidin-carbonil		N-pyrimidin-amide	
Aminomethyl benzimidazole		Hydroxysulfonamide	
Pirrol carbonyl		Hydroxyurea	
Dipicolylamine		β-Lactam	
Squaric Acid (X=O, S)			

Several new ZBGs have been recently described (11). The hydrazide scaffold has also been described as a good ZBG, and exhibits potent selectivity for MMP-2 versus MMP-1. Brown et al. described a novel ZBG based on a thiirane moiety, which behaves similarly to TIMP-1 and TIMP-2, the natural tissue inhibitors of MMPs (13). Barbiturates have also been cocrystalized (PDB code: 1g4k) in complex with MMP-3 but no information on the activity as

MMPI has been reported to date. A selective inhibitor of MMP-9 has been obtained using a thiadiazine scaffold, and a similar ZBG, the thiadiazole thione, has been analyzed in the catalytic site of MMP-3. Browner et al. investigated the differences among three identical compounds bearing different ZBGs (hydroxamate, carboxylate and sulfodiimines, crystalized in complex with MMP-7), concluding that the binding potency differences among them were consequence of the geometry adopted by the ZBG chelating the zinc atom (14). Another theoretical study showed that pyridone analogues can behave as ZBGs as well. Also, the aminomethyl benzimidazole group has been described as a ZBG with micromolar activity.

Others ZBGs not described by Rao et al. include hydroxysulfonamides (15), hydroxyureas (16), dipicolylamines (17), β-lactams (18) and squaric acids (18). Additionally, 3D structures of many MMP-Inhibitor complexes, solved either by X-ray diffraction or NMR techniques, have shown that the different chelating ZBGs coordinate catalytic zinc ion most frequently in a pentacoordinated way (19).

Binding of MMPIs is not only explained by coordination of the ligand ZBG to the catalytic zinc ion. Binding and, more importantly, selectivity, have been addressed by interactions with key residues belonging to different subsites, specially the S1' region. Thus, extensive hydrophobic interactions between the lipophilic moieties of the ligands and the S1' pocket can be largely responsible for the binding potencies (20).

Since a combination of factors, including the nature of ZBG, scaffolds and side chains that occupy different subsites, influence the selectivity and that the structural differences between MMPs take place mainly in the S1' sub-site, all these features will have to be taken into account in order to design compounds with the desired selectivity.

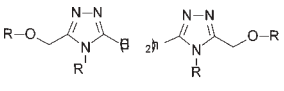
Working Hypothesis: Can AM positive modulators be MMP2 inhibitors?

Matrix metalloproteinases are responsible of degrading several components of the extracellular matrix (ECM), among other proteins

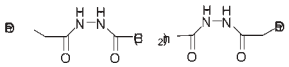
and peptides of the extracellular medium as mentioned before. Adrenomedullin (AM), a 52 amino acid peptide isolated from a human pheochromocytoma (adrenal tumour) (21), is an interesting example of MMP substrate. Martínez *et al.,* have reported how the adrenomedullin is cleaved by MMP-2 (1). Interestingly they also report that AM is not cleaved by MMP-9, so MMP-2 may be considerered as a specific protease of AM.

Our group has identified by a HTS a series of compounds that modulate the activity of adrenomedullin (Table 2) (22). These compounds have demonstrated to increase the production of cAMP, the second messenger released upon binding of AM to its receptor, but, interestingly, only in presence of the AM peptide. Furthermore, they are able to bind to AM but there is no direct relationship between the affinity to AM and the amount of cAMP production. These facts led us to the hypothesis that the mechanism of action of these compounds may involve affecting the availability of AM. That is, modulators of AM could inhibit the MMP-2 protease activity, so, the AM levels would be increased and consequently the cAMP production would be elevated. This work tries to shed light into the possibility that AM modulators and some derivatives could act as matrix metalloproteinases inhibitors. This hypothesis could drive, not only to an explanation of the mechanism of action of the modulators, but also to the discovery of novel ZBGs which could be exploited for the design of potent and selective inhibitors for MMP-2.

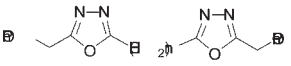
TABLE 2. *Positive AM modulators and related compounds, that were used in the computational study*



Series 1



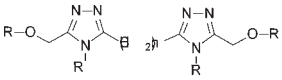
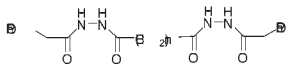
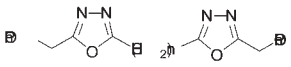
Series 2



Series 3

compd	*n*	R	R'	compd	*n*	compd	*N*
1a	8	Ph	NH-Ph	**2j**	4	**3j**	4
1b	8	*p*-Cl-phenyl	NH_2	**2k**	5	**3k**	5

TABLE 2. *Positive AM modulators and related compounds, that were used in the computational study* (cont.)

Series 1				Series 2		Series 3	
compd	*n*	R	R'	compd	*n*	compd	*N*
1c	8	*o*, *p*-diCl-phenyl	NH_2	**2l**	6	**3l**	6
1d	8	1-hydroxi-2-naphtyl	NH_2	**2m**	7	**3m**	7
1e	8	2-hydroxi-1-naphtyl	NH_2	**2n**	8	**3n**	8
1f	8	*p*-Cl-phenyl	NH-Ph				
1g	8	*o*, *p*-diCl-phenyl	NH-Ph				
1h	8	1-hydroxi-2-naphtyl	NH-Ph				
1i	8	2-hydroxi-1-naphtyl	NH-Ph				
1j	4	Ph	CH_2Ph				
1k	5	Ph	CH_2Ph				
1l	6	Ph	CH_2Ph				
1m	7	Ph	CH_2Ph				
1n	8	Ph	CH_2Ph				

MATERIALS AND METHODS

Macromolecule Selection

The 3D superimposition of the eleven different models of the 1hov against 1ck7 and 1qib (see Table 3) was carried out using Sybyl7.2 (23).

TABLE 3. *Different entries of the MMP-2 deposited in the Protein Data Bank*

Pdb Code	Information	Experimental Method and Resolution	Reference
1ck7	Full length protein	X-Ray (2.80 Å)	(35)
1eak	Catalytic domain (inactive and mutant)	X-Ray (2.66 Å)	(36)
1qib	Catalytic domain with the inhibitor Batimastat (not resolved)	X-Ray (2.80 Å)	(37)
1gxd	ProMMP-2/TIMP-2 complex (inactive)	X-Ray (3.10 Å)	(38)
1hov	Catalytic domain with inhibitor SC-74020 (i52) complex	NMR (11 models)	(32)

All models were selected for docking procedures. For docking purposes, the protonation state of histidines and glutamates in the binding site was maintained as it was in the NMR structure. Both zinc and calcium heteroatoms were kept throughout the docking study.

Ligand Processing

All the ligands (series 1, 2 and 3, together with compound i52; Figure 2 and Table 2) were used in its neutral protonation state. Assignment of the atom types and charge calculations were performed by using Sybyl 7.2. A conformational analysis was first performed to all compounds to be docked by use of the program Macromodel (24) and the Monte Carlo methodology. The parameters given to Macromodel were set to default with some exceptions. The force field selected was OPLS-AA. GS/SA solvation model was selected. The program was set to explore trough 1000 steps modifying three torsional angles each step. The limit of acceptance was set to 50 kJ/mol above the instant minimum found. The minimization method selected was conjugated gradient. The convergence RMS was set to a limit of 2 Å. The criteria of minimization convergence was set to 0.05 kJ/ Å-mol.

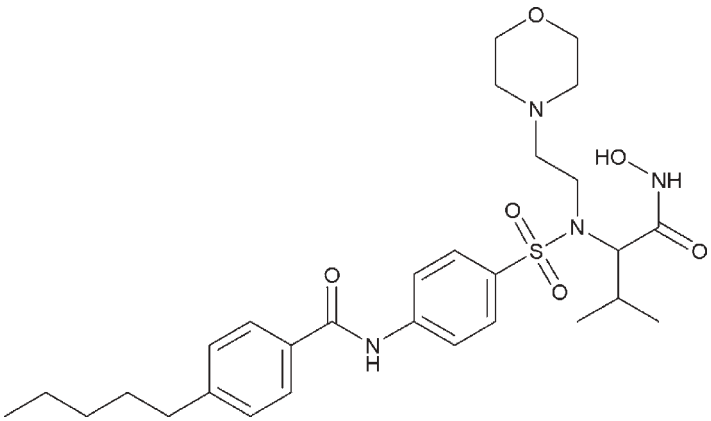


FIGURE 2. ***Chemical structure of compound i52.***

Superimposition

All the conformers resulting from the conformational search were then superimposed to the ligand i52 (Figure 2) present in the 1hov structure. This ligand was previously submitted to the same conformational search analysis and followed the same superimposition procedure in order to validate the methodology. The criteria selected for the superimposition was the maximum number of atoms fitted using the CSR program (25). Only the conformers with the higher number of atoms fitted were selected for the docking studies. The RMSD average of the selected ligands by superimposition to i52 was 0.9345 ± 0.2543.

Docking

The docking studies were carried out using AutoDock 3.0 program (26). Two different parameter sets for the zinc ion were used: the implicit parameters for the zinc inside AutoDock program, and the Stote and colleagues (27) which means r = 1.1 Å, ε = 0.25 kcal/mol and a formal charge of +2e. Very slight differences were observed within both protocols (data not shown).

The Lamarckian Genetic Algorithm (LGA) was used as the search engine. The active site was defined using AutoGrid. The grid size was set to 128 x 60 x 60 points with a grid spacing of 0.375 Å centered on the catalytic zinc of each 1hov model. Step sizes of 2 Å for translation and 60º for rotation were chosen. The maximum number of energy evaluations was set to 250000. For each of the 100 independent runs, a maximum number of 27000 LGA operations were generated on a single population of 100 individuals. The compounds were studied with no restraints between the ZBG and the zinc atom. Other variables were set by using AutoDock Tools (ADT) (26).

Perl-based Filter

A perl program was developed in order to classify the compounds into three different categories depending on the coordination with the zinc ion: trigonal bypiramide coordination, square-based pyramide coordination and no coordinating compound using the mean atom distance and angles displacement published by Alberts and colleagues (Figure 3) (28).

Biological Assays

The MMP-2 Colorimetric Drug Discovery Kit (purchased from Biomol International, Lp., Exeter, UK) was selected for the biological evaluation of MMP-2 inhibition by the selected compounds 1n, 2m and 2n. Inhibition assays were carried out in duplicate at four different concentrations (100 μM, 25 μM, 6.5 μM and 3.25 μM). The inhibitor NNGH (29) was also included as a prototypic control inhibitor. A commercially available negative control, with reported no MMP inhibitory activity, was treated similarly (30).

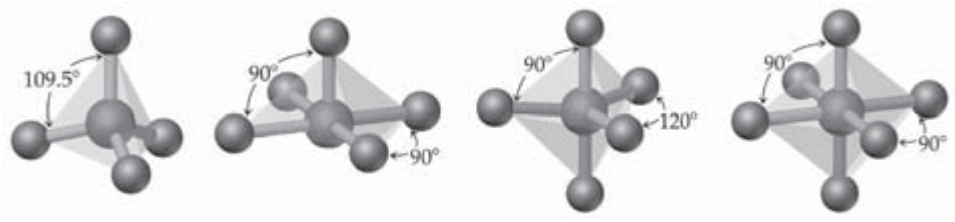


FIGURE 3. ***Different geometries for the coordination of the Zn^{2+} ion found in the bibliography for proteases zinc-dependent.*** *MMPIs are found to coordinate by square-based pyramidal or trigonal bipyramidal geometry (19, 28). Zinc is the center ion and the donnor atoms are in coordinating position. Coordinating atoms in MMP are 3 His and one or two atoms of the ligand.*

RESULTS

MMP-2 Selection

Five structures corresponding to MMP-2 subtype metalloproteinase are found in the Brookhaven Protein Data Bank (31), as shown in Table 3. No X-ray structures of MMP-2-Inhibitor complexes have been reported so far, and the PDB codes 1ck7 and 1qib corresponding to active MMP-2 exhibit low resolution values (2.80 Å). Only the NMR structure with PDB code 1hov corresponds to a complex with a hydroxamic acid inhibitor (i52, see Figure 2). For this reason it was considered as the host structure for docking purposes. The eleven models that contain the PDB 1hov (M1, M2,... M11) were considered in the subsequent docking studies, as it would allow to reflect the dynamic nature of MMP-2 in an aqueous environment.

Conformational Search and Docking

The series of compounds is characterized by the hydrocarbonated chain that joins the two potential ZBG, which means, that all compounds have at least 10 rotatable bonds. A torsional problem has been reported finding good docked hits when the rotatable

bonds of the ligands were more than 10 (19). Trying to minimize this difficulty, all compounds (1a to 3n) were submitted to a conformational search using Macromodel in order to get the maximum conformational diversity. The conformational search of all compounds led to very similar conformers in terms of energy and, in general, non-extended conformations as the most stable ones.

AutoDock was selected as the program to be used in the docking procedures. The selection was based on its wide use in similar cases described in the literature. In order to validate AutoDock as a docking tool, the first ligand that followed this protocol (conformational search, superimposition and docking) was compound i52 (32), the ligand present in 1hov structure in the complex with MMP-2. AutoDock was able to reproduce the NMR results and to predict the conformation in which i52 is bound inside the MMP-2 active site.

Data Analysis

A perl-based program was developed to filter the vasta amount of data generated. Distances and the angles between the ZBG, the three catalytic histidines and the zinc ion, were calculated from the docking results. Further analysis of the data allowed the identification of the docked geometries with the adequate coordination. For the MMP family, the pentacoordinated zinc has been shown to be the most frequent coordination state (19), with two possible geometries: trigonal bypiramide or square-based pyramide. Both possibilities were scanned by the perl filter. The analysis was set to a first limit of 2.53 Å of distance, and 0º of variation from the angles described in the literature (28). The distance of 2.53 Å is the maximum reported distance between a coordinating atom and the Zn^{2+} reported for MMPs.

Following this protocol, 98 conformers were found to be able to coordinate Zn ion inside any of the 11 NMR models. Table 4 shows the ability of these compounds to bind the MMP-2 active site. Complex MMP-2:i52 was submitted to the same perl filter leading to only two positive hits. A detailed analysis of the 11 NMR complex models, led to the observation that only models 1 and 3 fullfilled the requiered distance and angle restrictions found in the literature for

zinc coordination. A positive hit for one compound is considered when at least one of its conformers is able to coordinate the zinc ion, for one of the NMR models, taking into account the distances and angles restrictions described above. Eleven is then the maximum of possible positive hits for each compound. Using this procedure, we found 59 positive hits within a maximun of 264 (24 compounds * 11 models). AutoDock predicted that all compounds were able to coordinate the zinc atom at least in one of the eleven NMR models and at least in one conformation, except compound 1g.

TABLE 4. *Number of positive hits through the eleven NMR models resolved for 1hov in Protein Data Bank for each docked compound*

Compound	*Number of positive hits*	*Compound*	*Number of positive hits*	*Compound*	*Number of positive hits*	*Compound*	*Number of positive hits*
1a	1	**1j**	3	**2j**	5	**3j**	5
1b	2	**1k**	2	**2k**	3	**3k**	3
1c	1	**1l**	3	**2l**	2	**3l**	2
1d	2	**1m**	3	**2m**	5	**3m**	1
1e	2	**1n**	4	**2n**	2	**3n**	3
1f	1						
1g	0					**i52**	2
1h	2						
1i	2						

MMP-2 inhibitors should not only be good zinc coordinating agents, but also they should interact with key amino acids which are crucial for the ligand binding (7, 33, 34). Taking into account this information, the 98 positive docking hits were visually inspected displaying the residues within 4 Å around the ligand. Thus, residues belonging to S1, S2, S3, S1', S2', and S3' subsites were analyzed looking for putative interactions with each of the 98 conformers (data not shown). Compound i52 was included in this analysis to provide a reference for contacts with the residues belonging to the different subsites. Interactions with S1' residues were used as a filter to select 27 docking conformers, belonging to 15 different

compounds, as those which better fulfilled the interactions' pattern exhibited by i52. These results are displayed in Figure 4.

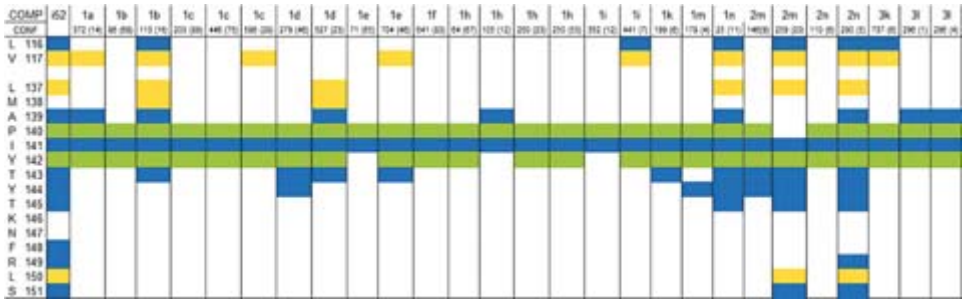


FIGURE 4. ***Representation of the pattern of interaction between the 27 docking conformers analyzed and the amino acids of S1' subpocket of MMP-2*** *(position in the energy ranking in Autodock is given in parenthesis). Conserved amino acids are colored yellow. No conserved amino acids are colored blue. Amino acids that are reported to be able to establish hydrogen bonds are colored green. Compound* ***i52*** *from the PDB 1hov is also included as a reference.*

Compounds 1n, 2m and 2n were the most promising candidates to perform MMP-2 inhibition assays, because they showed a similar S1' sub-sites contacts pattern to that of i52. These interactions included unique residues to MMP-2, as Ala139, Thr 143, Tyr144, and Thr145, and were not observed for the other docking conformers. Figure 5 shows the resulting docking for 1n (blue) superimposed to i52 (orange).

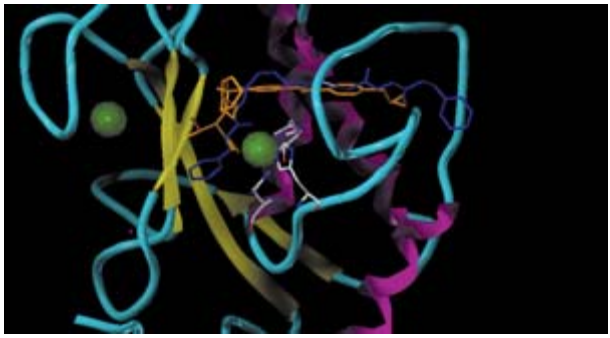


FIGURE 5. ***Docking result for 1n (blue) in comparison with i52 (orange) resolved by NMR. 1n*** *is able to coordinate Zn^{2+} occupying the same subistes as* ***i52****.*

Biological Assays

According to the computational work, preliminary MMP-2 inhibition assays were carried out in duplicate for compounds 1n, 2m and 2n. Only 1n showed an interesting MMP-2 inhibitory activity. Preliminary data show the decrease of the MMP-2 activity in the presence of 1n, suggesting that this compound may be a promising MMP-2 inhibitor. Unexpectedly, compounds 2m and 2n did not show detectable MMP-2 inhibition. It has to be taken into account that these two compounds are synthesis intermediates, so their character as positive/negative AM modulators has not been determined yet. AM affinity studies are currently in progress.

CONCLUSION

In this work we have carried out a theoretical study making use of molecular mechanics, docking and molecular dynamics techniques with the aim of demonstrating that positive modulators of AM, may be acting as MMP-2 inhibitors as a possible mechanism of action. This hypothesis has proven to be correct, according to our theoretical studies, since almost all compounds evaluated are able to bind MMP-2 in some of the conformations considered, by chelating the zinc ion and interacting with key aminoacids in the binding site of this enzyme. This ability and versatility to coordinate Zn^{2+} ion seems to be a prominent feature of this family of compounds not described before, to the best of our knowledge. Additionally, the moderated electron donor character of the aryloxi oxygen and the heterocyclic nitrogen, makes this family of compounds an excellent alternative ZBG to hydroxamic acids, where the pronounced zinc chelating properties may cause lack of specificity. Biological evaluation of MMP-2 inhibition by some selected compounds, selected according to the previous theoretical work, has shown that compound **1n** possesses an interesting inhibitory activity.

Interestingly, the ZBG contained in this compound, does not appear in any of the many MMP2 inhibitors reported in the bibliography. This finding opens up an unexplored structural field

for the search of MMP-2 inhibitors with interest for the treatment of many inflammatory, malignant, and degenerative diseases.

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