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COMPUTATIONAL UNDERSTANDING OF SELECTIVE MMP INHIBITORS IN COPD

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ABSTRACT

Chronic Obstructive Pulmonary Diseases (COPD) refers to multiple lung diseases, is the occurrence of chronic bronchitis and emphysema, a pair of commonly co-existing diseases of the lungs in which the airways become narrowed because of inflammatory cells like neutrophils, CD_8 cells and macrophages. These cells produce the Matrix metalloproteinases (MMPs), cathapsins and other proteinase enzymes. In humans there are 26 MMPs are present, which are classified in to six categories based on the structure of their substrate, sequence similarity and characteristics of structural domain. Structurally, MMPs are similar, but functionally different - but the general function of MMP is to degrade the extracellular matrix according their specific substrates. From the literature, three MMPs [MMP-09, MMP-12 & MMP-14] which are implicated in COPD were selected. Our aim is was to achieve a meaningful understanding of selectivity of individual ligands towards their enzymes, while our interest also is to find explanations for selective and non-selective inhibitors.

INTRODUCTION: Principally, lung diseases are of two types, obstructive lung diseases and restrictive lung diseases. Chronic Obstructive Pulmonary Disease (COPD) comes under the obstructive lung disease. COPD is also known as chronic obstructive lung disease (COLD), chronic obstructive airway disease (COAD), chronic airflow limitation (CAL) and chronic obstructive respiratory disease (CORD). COPD refers to multiple lung diseases, is the occurrence of chronic bronchitis and emphysema, a pair of commonly co-existing diseases of the lungs in which the airways become narrowed. Some times COPD co-exist with Asthma but there are some differences between COPD and Asthma. Chronic bronchitis is a chronic inflammation of the bronchi (medium-size airways) in the lungs. Chronic bronchitis refers to an inflammatory process in the walls of bronchioles with excessive production of mucus and sputum into the tubes with tissue swelling that may narrow or close off bronchial tubes, which obstructs the air flow.

Emphysema is a long-term progressive disease of the lungs that primarily causes shortness of breath due to over-inflation of the alveoli (air sacs in the lung); it is defined as enlargement of the air spaces distal to the terminal bronchioles with destruction of the walls of alveoli, results in less O_2 and CO_2 exchange.

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The emphysematous lung loses its "springy-ness" or elasticity. Asthma, an airway disease, causing breathing problem that makes it more difficult to get air in and out of the lungs. When a person has asthma, the breathing tubes are inflamed. They may react to smoke, pollen, dust, air pollution, allergies, or other triggers. In a person with asthma, the breathing tubes may tighten, become inflamed and swollen.

Sometimes asthma co-exist with COPD. Both diseases (chronic obstructive pulmonary disease & asthma) are characterized by airflow obstruction and a chronic persistent inflammatory process, but the nature of the inflammation differs markedly between these diseases.

Airway inflammation in asthma is characterized by an eosinophilic inflammation, with an increase in activated and degranulating eosinophils in bronchial biopsies, BAL, and in induced sputum [1, 2]. There is also an increase in CD41 T lymphocytes (T-helper type 2 cells) that appear to orchestrate the eosinophilic inflammation and degranulated mast cells that underlie the rapid and episodic bronchoconstrictor responses that are so characteristic of asthma. Inflammation affects all of the airways in asthma and does not involve the lung parenchyma.

The pathology of COPD differs markedly from that of asthma. In larger airways, there is evidence of neutrophil rather than an eosinophilic inflammation, as judged by increased numbers of neutrophils in BAL. Induced sputum shows a characteristic increase in the proportion of neutrophils that is much greater in patients with COPD.

Cigarette smoke (and other irritants) activates macrophages in the respiratory tract that releases neutrophil chemotactic factors, including IL-8 and LTB4. These cells then release proteases that break down connective tissue in the lung parenchyma, resulting in emphysema, and also stimulate mucus hypersecretion.

These enzymes are normally counteracted by protease inhibitors, including a1-antitrypsin (a1-At), secretory leukoprotease inhibitor (SLPI), and tissue inhibitor of MMPs (TIMPs). Cytotoxic T cells (CD81) may also be involved in the inflammatory cascade [3]. In this mechanism so many proteases are involved in tissue degradation but MMPs (Matrix Metalloproteinases) actively participate in extracellular degradation.

If the proteinases hypothesis for the development of emphysema is true, one might hypothesize that the macrophage is responsible [4, 5].

Studies have demonstrated the release of metalloproteinase activity by macrophages, although the lack of adequate technology long delayed a full understanding of the exact nature of this activity and its contribution to disease processes.

Recent studies showed that the secretion of some MMPs was increased in COPD patients, which accelerated the degradation of ECM and thereby resulted in the extensive damage of lung tissues. MMPs play an important part in the development of smoking-related COPD. The present study was designed to achieve a meaningful understanding of selectivity of individual ligands towards their enzymes, while our interest also is to find explanations for selective and non-selective inhibitors.

MATERIALS AND METHODS

Drug Target Structures: The protein data bank (PDB) *http://www.rcsb.org/pdb/home/home.do* archive contains information about experimentally determined structures of proteins, nucleic acids and complex assemblies with small molecules. Crystal structures of target molecules have been downloaded from this site (**Table 1, Table 2**).

Ligand sources: In order to study the interaction pattern and active site differences of MMP-9, MMP-12 & MMP-14, reported selective inhibitors were considered (**shown in fig. 1**). These reported selective inhibitors were docked in to selective protein and non selective proteins.

TABLE 1: CRYSTAL STRUCTURES TAKEN FOR STUDY

Protein name	Uniprot ID	PDB ID	Resolution (Å)
MMP-9	P14780	2OVX	2.0
MMP-12	P39900	1ROS	2.0
MMP-14	P50281	3MA2	2.05

TABLE 2: PROTEIN SEQUENCE INFORMATION FROM UNIPROT

SEQUENCE INFORMATION	MMP-09	MMP-12	MMP-14
Total length	707 (Amino acids)	470 (Amino acids)	582 (Amino acids)
Signal peptide	1-19	1-16	1-20
Pro peptide	20-93	17-105	21-111
Cysteine switch	97-104	90-97	91-98
Catalytic domain	105-224 and 391-520	106-287	112-322
Hemopexin domain	521-707	288-470	323-508
Fibronectin repeats	225-390	---	---
Transmembrane	---	---	542-562
Topological domain	---	---	563-582

List of inhibitors:

a) 9-01 - (R)-3-(4-Benzoylphenyl)-2-(5-((4-butylphenyl)ethynyl) thiophene-2- sulfonamido)-N-hydroxypropanamide [6]

b) 9-02 - Sulfonamide derivative [9]

c) 12-01 - 3-hydroxy-4-arylsulfonyltetrahydropyranyl-3-hydroxamic acid analog [11]

d) 12-02 - keto acids derivative [7]

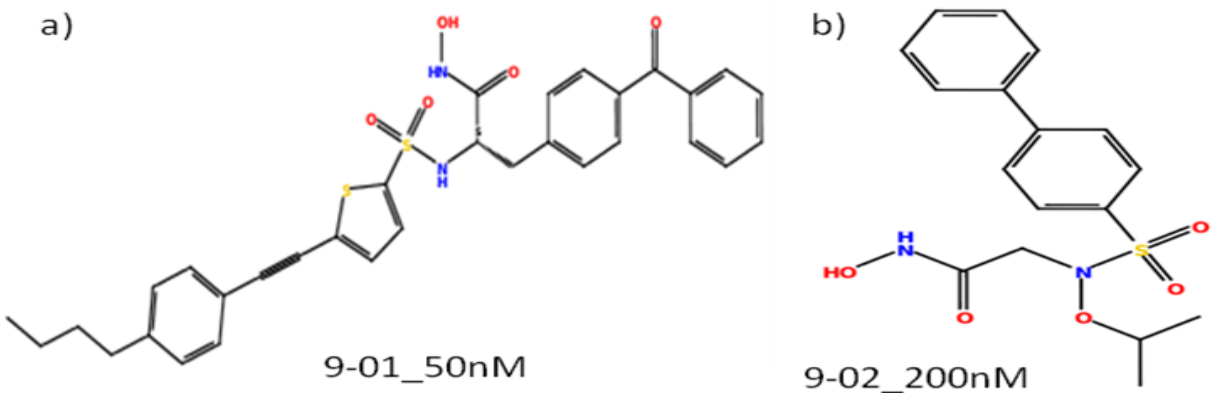
e) 12-03 - INCB8765 [8]

f) 12-04 - INCB3619 [8]

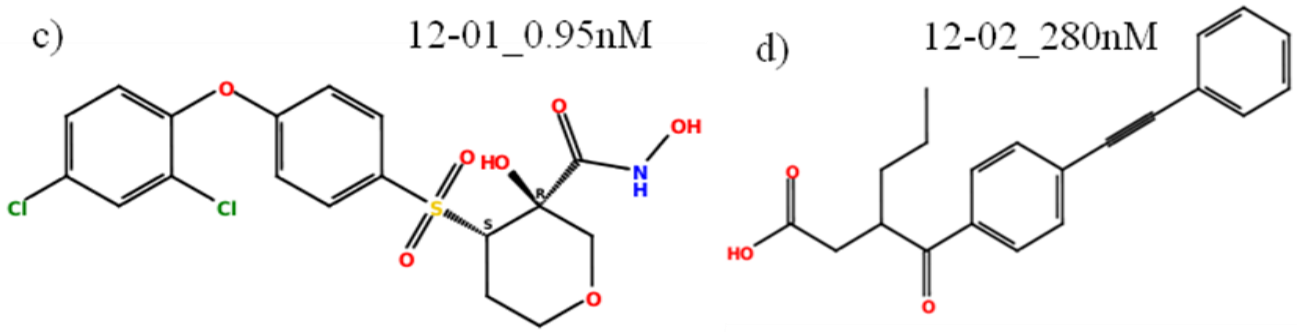
g) 12-05 - {2-[4-(4-Methoxy-phenoxy) benzenesulfonyl]phenyl}aceticacid [10]

h) 12-06 - 3-[4-(4-Chlorophenylethynyl) benzoyl]nonaicacid [7]

Selective Inhibitors of MMP-9:



Selective inhibitors of MMP-12



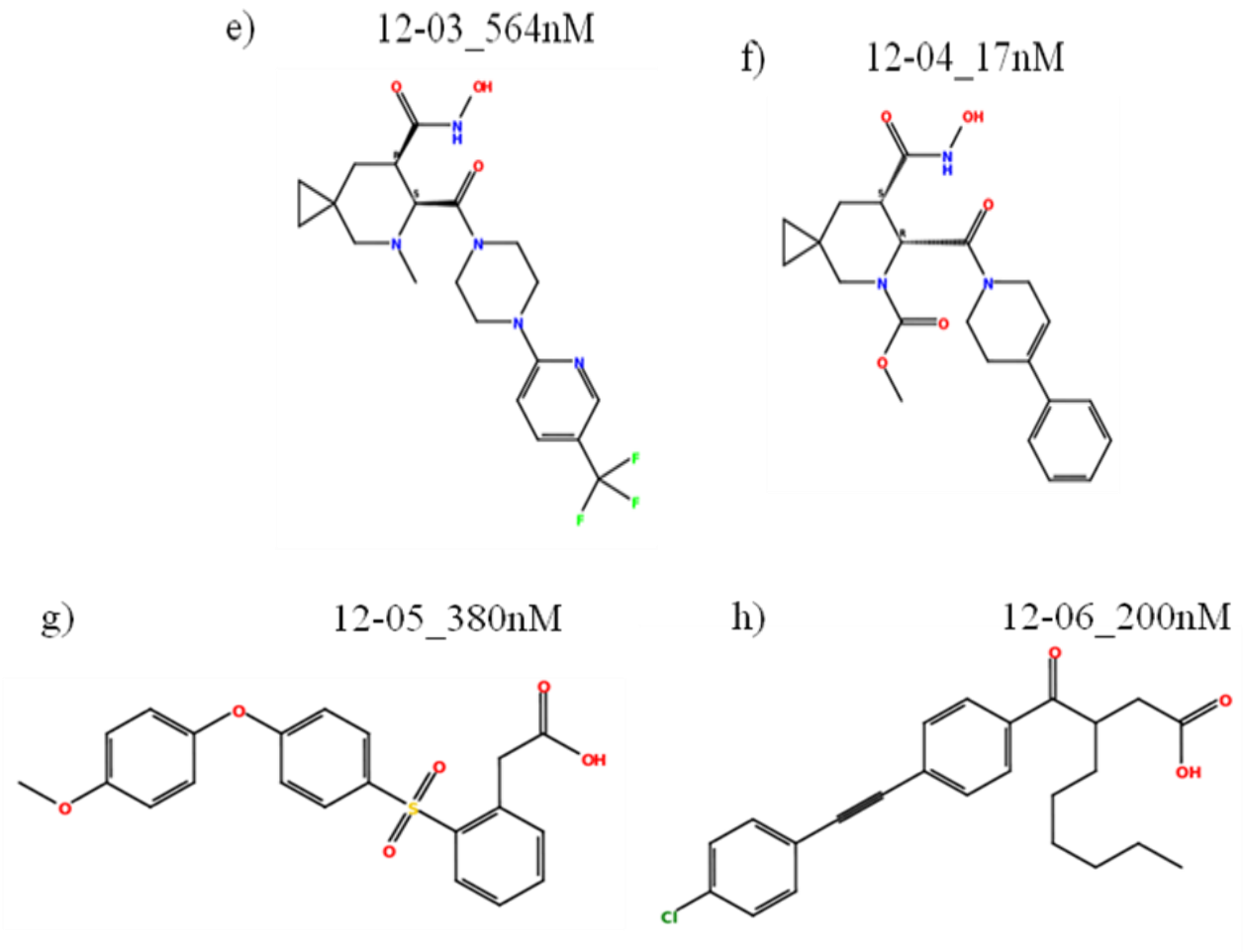


FIGURE 1: SELECTIVE INHIBITORS OF MMP-9 AND MMP-12 INHIBITORS ALONG WITH THE IC_{50} VALUES

Nomenclature of inhibitors: Ex: 9-01_50nM in this 9-01 represents the inhibitor for MMP-09, and remaining part 50nM represents the potency of inhibitor.

Nomenclature of inhibitors: Ex: 9-01_50nM in this 9-01 represents the inhibitor for MMP-09, and remaining part 50nM represents the potency of inhibitor. In **table 3**, inhibitor names and their activity profiles are given.

TABLE 3: SELECTED INHIBITORS FOR CURRENT STUDY

Inhibitors	Inhibitory Potency IC_{50} (nM)		
	MMP-9	MMP-12	MMP-14
9-01	50	--	6600
9-02	200	--	>10,000
12-01	5.3	0.95	150
12-02	3600	480	30600
12-03	>5000	564	>5000
12-04	304	17	772
12-05	420	380	8100
12-06	1510	200	--

SOFTWARE USED: All the calculations were performed on a workstation Operating system RHEL5WS (Red Hat Enterprise Linux 5 Work Station) with processor E5345 XEON 2.33GHZ and 4 GB RAM, 3×300GB (Each) hard disk capacity. Molecular modeling tasks were performed with Schrödinger 9.2v and Sybyl-X 2.0 softwares. Default settings were replaced with customized settings for required conditions during all calculations.

PROTEIN PREPARATION: Protein structures must be prepared to make the structure compatible with the forcefields. An unprepared or poorly-prepared protein structure will lead to poor results or may even prevent modeling jobs from running. It consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions and cofactors. Some structures are multimeric, and may need to be reduced to a single unit. Because of the limited resolution of X-ray experiments, it can be difficult to distinguish between NH and O, and the placement of these groups must be checked. PDB structures may be missing information on connectivity, which must be assigned, along with bond orders and formal charges.

Prepwiz: Prepwiz is a module from Schrödinger 9.2v (**Protein Preparation Wizard; Epik version 2.2, Schrödinger, LLC, New York, NY, 2011**). The preparation of a protein by prep wiz involves a number of steps. The procedure assumes that the initial protein structure is in a PDB-format file, includes a cocrystallized ligand, and does not include explicit hydrogens. Therefore it adds hydrogens, convert selenomethionines to methionines, treat disulphides, found overlapping regions, cap the termini, delete water which are not essential, assign H-bonds and do whole protein refinement.

Biopolymer preparation: The Biopolymer module is an integral part of the Sybyl-x 2.0v molecular modeling environment which delivers an extensive set of tools for building, visualizing, manipulating, and predicting the 3D structure of biological molecules, including proteins, peptides, nucleic acids, and polysaccharides. It uses Random Tweak Algorithm, allowing seamless transition from small molecules to macromolecular structures, without the problems of disparate force fields or formats for small ligands vs. biomolecules artificially imposed by other softwares. This seamless integration smooths the path to the effective use of biological receptors in drug design, allowing docking simulations to proceed without lengthy "setups" of the protein-ligand complex.

ACTIVE SITE PREDICTION: The location of a binding site for protein-ligand or protein-protein interactions is not known in advance, even though the protein structures are available. Here, computational studies can help to suggest likely binding sites, and even to predict whether a given protein is likely to bind ligands tightly. Many such approaches have been explored. The modules used for active site prediction are explained below.

Site map: Site map is an integral part of Schrödinger 9.2v (**Sitemap, version 2.5, Schrödinger, LLC, New York, NY, 2011**) for active site prediction. In some cases, however, the location of a binding site for protein-ligand or protein-protein interactions is not known in advance, even though the protein structures are available. Efforts to design better ligands for these receptors can profit from an understanding of how well the known ligands complement the receptor and how extension of the ligands into adjacent regions could promote binding. Determining whether there are nearby sites that might be useful for allosteric binding can also be important.

Molcad: MOLCAD is a module from Sybyl-x 2.0v for active site prediction. It creates and displays molecule surfaces in the protein in which it maps key properties, including lipophilicity, electrostatic potential and hydrogen bonding sites.

DOCKING: Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Docking is done by using following tools.

GLIDE DOCK:

Glide (**Grid-based ligand docking with energetics**) is the molecular docking application module in the Schrödinger 9.2v molecular modeling suite with the MASTERO graphical user interface. The docking studies are done and the ligands are ranked in an ascending order of glide score. (**Glide, version 5.7, Schrödinger, LLC, New York, NY, 2011).**

Ligand Preparation: LigPrep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or Maestro format. The resulting structures can be saved in either SD or Maestro format. The LigPrep module is used to produce a single, low-energy, 3D structure with correct chiralities for each successfully processed input structure. It is also used to produce a number of structures from each input structure with various ionization states, tautomers, stereochemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present.

Grid Generation: Grid file is the creation of a site on the protein where the ligand is subjected to docking. It is performed in any of the three ways, One can go for an option of selecting an already cocrystal ligand and a grid may be generated at its position and existing ligand will be removed. Secondly, we may also mention the residues nearby active site in a case where there is no ligand and we want a grid to be generated big enough to accommodate the space near the active site or the third option is to simply mention the X, Y, Z coordinates on the protein.

Principle behind glide: Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active site regions of the receptor. The shape and the properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses.

Schrödinger's Glide Score rewards favorable lipophilic, hydrogen bonding, and metal ligation contacts and penalizes frozen rotatable bonds and steric clashes. In addition, Glide Score incorporates a term proportional to the Coulomb-vdW interactions, as well as a small number of potential energy terms that reward hydrogen bond donors found in the active sites hydrophilic regions and penalize hydrogen bond donors and acceptors found in the hydrophobic regions.

GScore = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

GlideScore is based on ChemScore, but includes a steric clash term, buried polar terms devised by Schrodinger to penalize electrostatic mismatches, and has modifications in other terms.

SEQUENCE AND STRUCTURE ALIGNMENT TOOLS:

Multiple Sequence Viewer: The Multiple Sequence Viewer panel is an alignment, visualization, and manipulation toolkit for multiple sequences. The multiple sequence viewer (MSV) has its own projects, which contain all the sequences in the project along with associated data. The project is stored in a single file with a .msv extension, and by default is stored inside the Maestro project.

Superposition: The superposition scheme works by aligning the first selected atom in each structure, then attempting to align the second selected atom, and so on. The atoms are aligned to the first entry in the Project Table that is being used in the superposition (the Reference entry). After superimposing atoms, the results of the operation are displayed in the RMSD text box near the bottom of the panel.

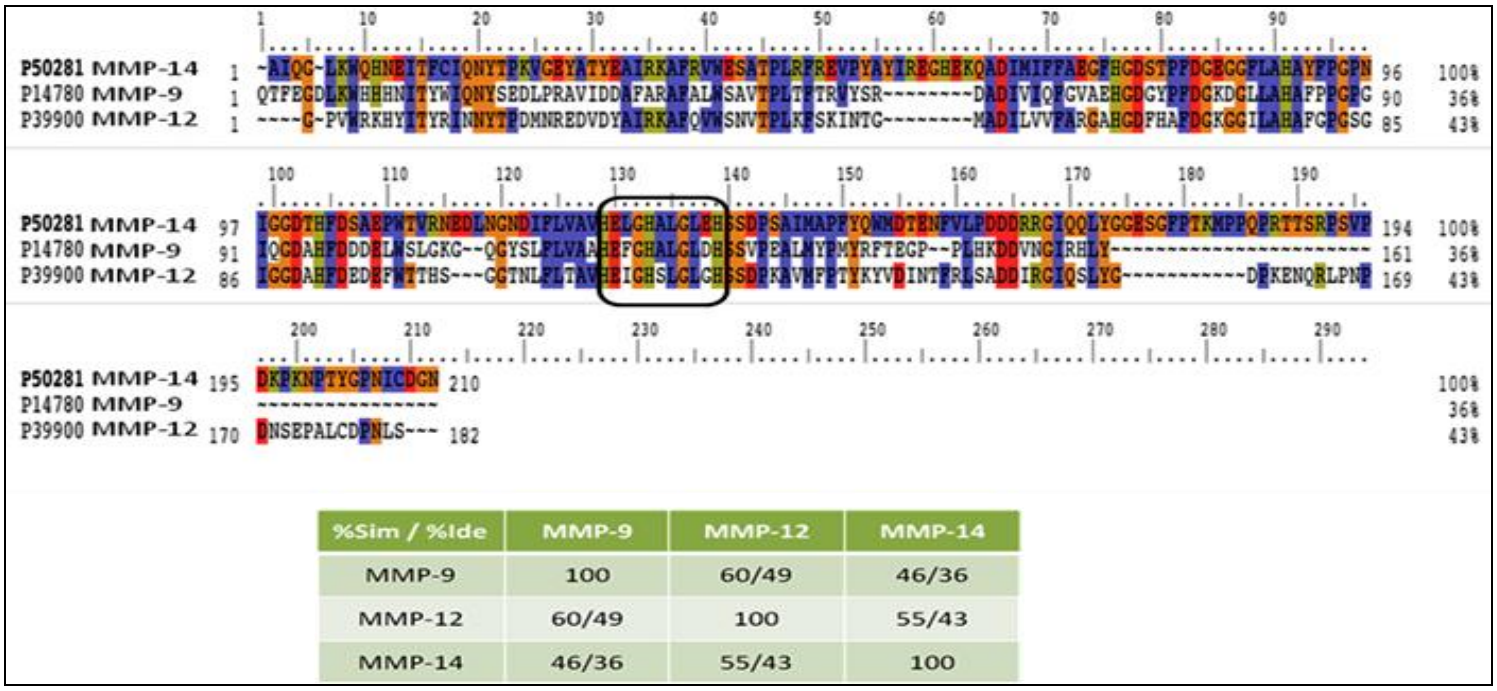
The results include the molecule numbers, the RMS deviation of the atoms from those of the first structure, and the maximum difference between superimposed atom positions.

RESULTS & DISCUSSION:

Sequence alignment of MMPs: Our primary aim was to understand the extent of identity between MMP-9 (2OVX), MMP-12 (1ROS) and MMP-14 (3MA2), which was accomplished through sequence alignment methodology in maestro using multiple sequence viewer tool. Between these three human MMPs (-09, -12, and -14), percentage of sequence similarity and percentage of sequence identity were observed. Aligned sequences of amino acid residues along with the percentage of similarity are shown in the below **fig. 2,** In percentage table MMP-9 and MMP-12 are highly similar, identical to compare MMP-14.

In sequence alignment highlighted part is zinc binding motif, in this part three histidines are present this are same in all MMPs and glutamic acid residue are same and remaining residues are different. The three histamines are used for zinc stabilization and glutamic acid is used for catalysis process.

Comparison of MMP-9, MMP-12 & MMP-14 Active Sites Volumes and Surfaces: The below table describes the active site differences like volume, surface area and energy minimization values of selected MMPs (**Table 4**). MMP-9 & MMP-12 volumes are bigger than the surface area that means depth of the active site channel is higher. In the case of MMP-14 surface area is more than volume, which means depth of active site channel is smaller. This information describes the how MMPs are differ from one to another



%Sim / %Ide	MMP-9	MMP-12	MMP-14
MMP-9	100	60/49	46/36
MMP-12	60/49	100	55/43
MMP-14	46/36	55/43	100

FIGURE 2: CATALYTIC SEQUENCE ALIGNMENT OF MMPS

TABLE 4: ACTIVE SITE DETAILS, ENERGY MINIMIZED DETAILS OF MMP-09, -12, AND -14

MMP	MMP-09	MMP-12	MMP-14
PDB ID	(2OVX)	(1ROS)	(3MA2)
ENERGY VALUES (Kcal/mol)	-1562.281	-1411.193	-1326.407
Surface area ($Å^2$)	419.284	333.488	242.907
Volume ($Å^3$)	475.361	388.501	237.384

Superposition of three selected MMPs: By superimposing the structures of the three MMPs the arrangement of α-helices and β-sheets in all the three MMPs can be observed and it also explains the variation in the S1 loop in selected MMPs. It was observed that all the helices and sheets were exactly superimposed but there is difference in S1 loop (Selectivity loop) of these MMPs. Figure-3 depicts the overlap of all three MMPs considered in the present study.

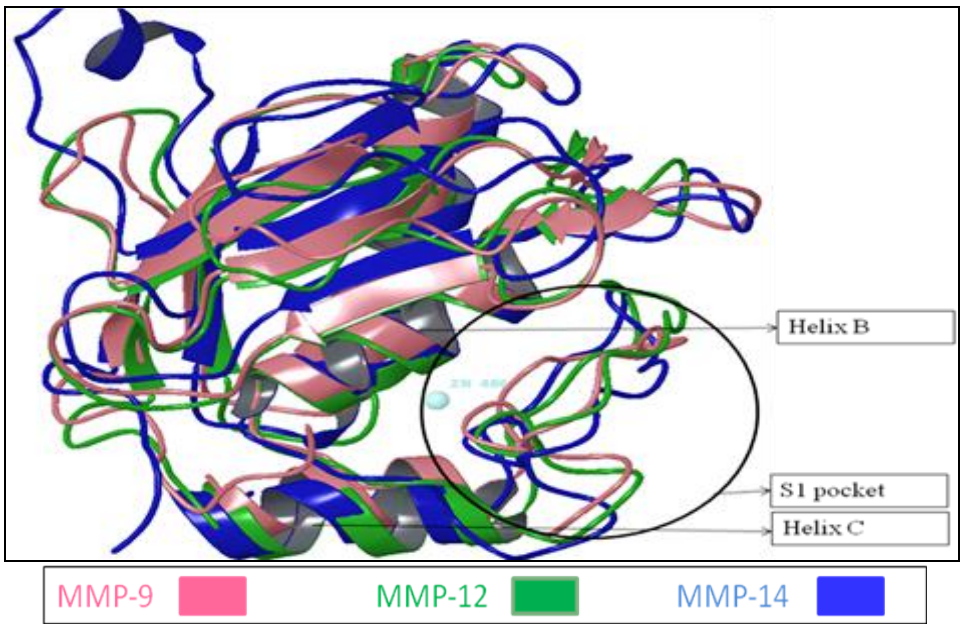


FIGURE 3: SUPERPOSITION OF MMP-09, -12, AND -14 ENZYMES. THE KEY AREAS ARE DENOTED ALONG WITH THE CATALYTIC ZINC. MOST PARTS LIKE HELIX-B, HELIX-C, S1 POCKET ARE FAIRLY CONSERVED STRUCTURALLY

Superposition of three selected MMPs active sites within 8Å of catalytic zinc: The selected MMPs were superimposed by using Zn^{+2} ions and three histidines. The superimposed structure of MMPs gave a maximum RMSD value of 0.5.

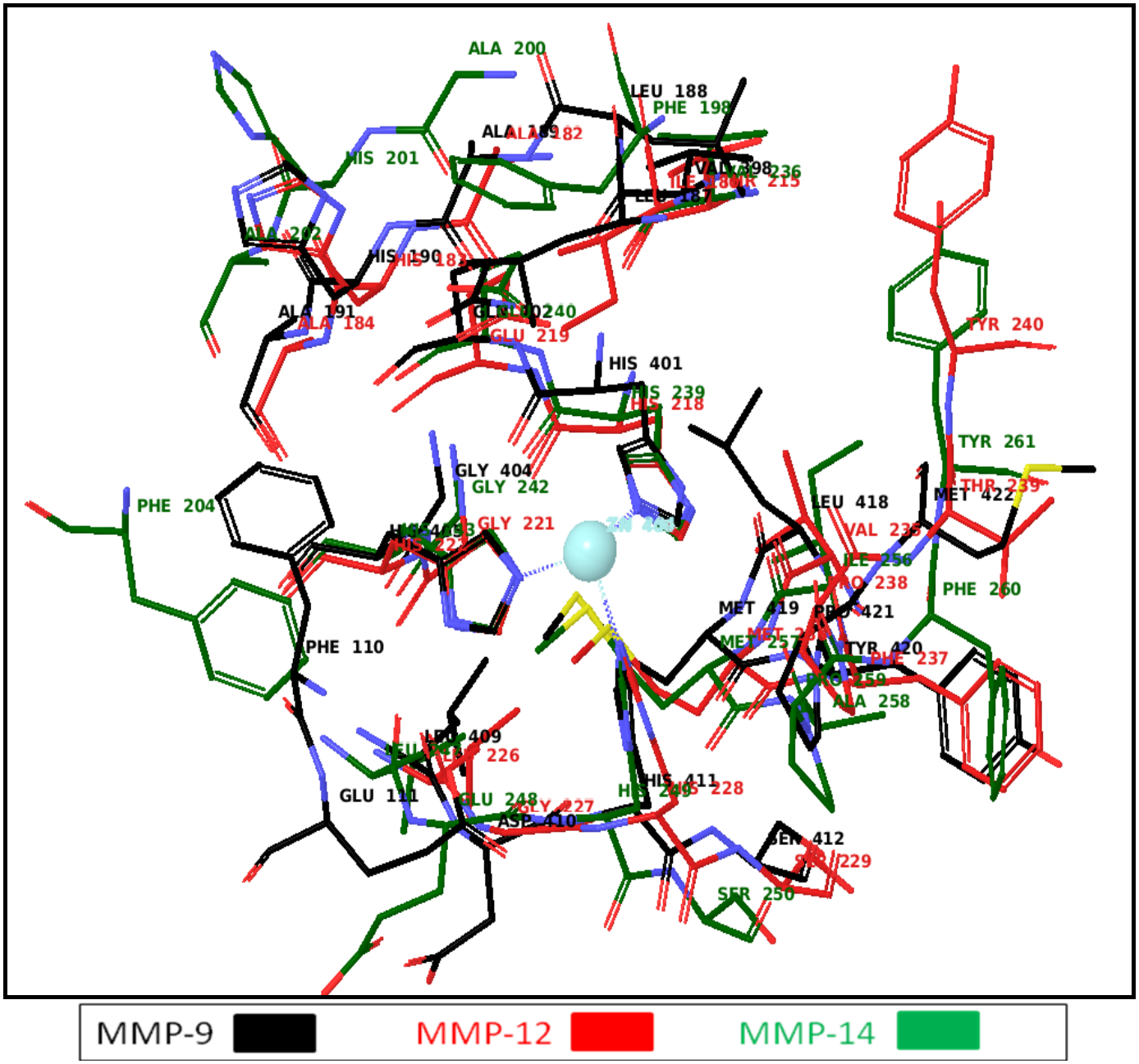


FIGURE 4: SUPERPOSITION OF ACTIVE SITE RESIDUES LIE WITHIN THE CATALYTIC ZINC. Zinc and the three histidine residues that coordinate with Zinc are the key for alignment. All residues that form selectivity pockets could be recognized

Similarities & Differences between the Active Site Amino acid Residues: A comparison of active site residues of these enzymes is given in **Table 5** below. Table 5 explains the active site amino acid residues of MMP-9, MMP-12 & MMP-14. Most of amino acid residues are identical but remaining amino acids are identical or different. In this table amino acid residues properties like aliphatic, aromatic, hydrophobic other properties decreased from MMP-9 to MMP-14.

In active site groove nearer to Zn^{+2} binding site all MMPs channel are same but S1 loop side active site is varies. This variation occurred by some amino acids, these residues mainly cause the selectivity. That residues information explains below;

TABLE 5: A COMPARISON OF ACTIVE SITE RESIDUES OF THESE ENZYMES

MMP-09	MMP-12	MMP-14
L187	I180	F198
A189	A182	A200
H190	H183	H201
A191	A184	A202
F396	F213	F234
L387	L214	L235
V398	T215	V236
A399	A216	A237
A400	V217	V238
H401	H218	H239
E402	E219	E240
F403	I220	L241
G404	G221	G242
H405	H222	H243
A406	S223	A244
L407	L224	L245
G408	G225	G246
L409	L226	L247
D410	G227	E248
H411	H228	H249
S412	S229	S250
S413	S230	S251
V414	D231	D252
P415	P232	P253
E416	K233	S254
A417	A234	A255
L418	V235	I256
M419	M236	M257
Y420	F237	A258
P421	P238	P259
M422	T239	F260
Y423	Y240	Y261
R424	K241	Q262
F425	Y242	W263
T426	V 243	M264
E427	D244	D265
G428	I245	T266

MMP-9 active site channel & S1 loop: Figure 5 explains the (A) S1 loop and active site channel of MMP-9, and (B) explains the S1 loop amino acid residues like E416, Y420, M422, R424, T426 are may be changes the active site volume and size because this amino acid side orientation towards the active site channel. This is the total S1 loop sequence, that starts with residue F396 and ends with the residue G428 "FLVAAHEFGHALGLDHSSVPEALMYPMYRFTEG".

In the overall sequence of S1 loop, color coding sequence are active site determining amino acids, red color coding amino acid are towards facing into the active site and green color coding amino acids are facing outwards the active site. Hence, these residues are probable causes for the active site surface and volume and the shape.

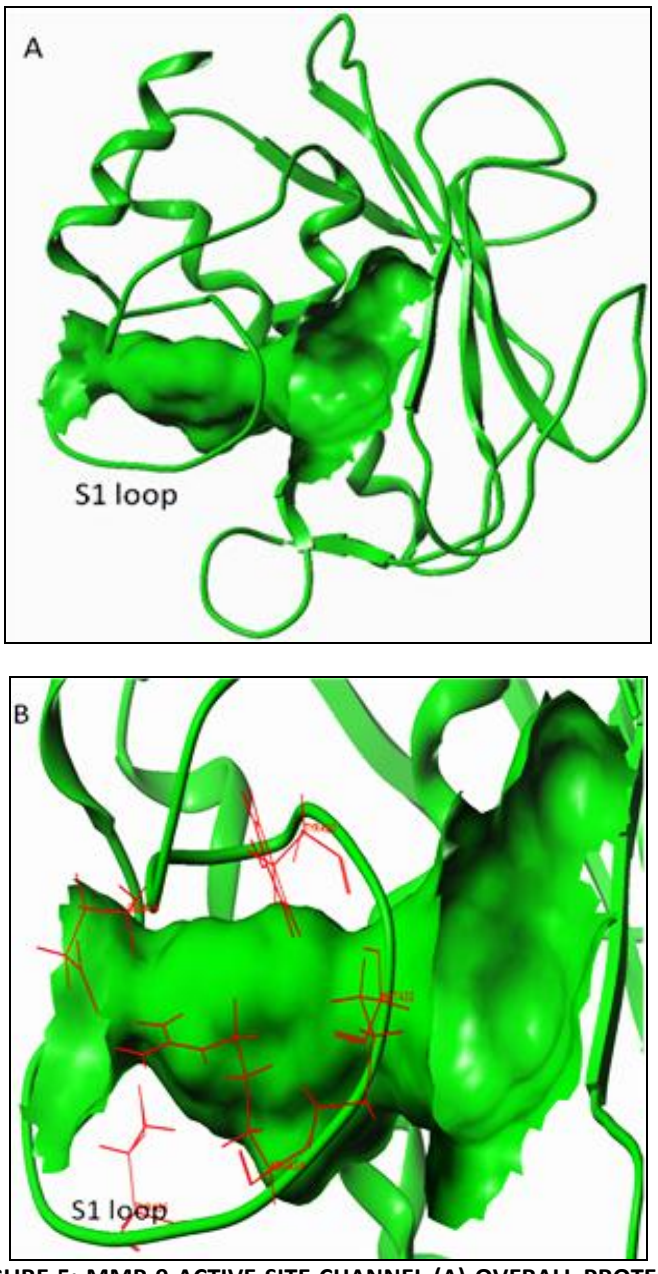


FIGURE 5: MMP-9 ACTIVE SITE CHANNEL (A) OVERALL PROTEIN, AND (B) ACTIVE SITE RESIDUES IN S1-LOOP

MMP-12 Active Site Channel & S1 Loop: Figure 6 explains (A) S1 loop and active site channel of MMP-12, while (B) explains the S1 loop amino acid residues like K233, F237, T239, K241, V243 are may be changes the active site volume and size because this amino acid side orientation towards the active site channel.

S1 loop sequence starts with the residue F213 and ends with residue I245 "FLTAVHEIGHSLGLGHSS DPKAVMFPTYKYVDI". Color coding sequence in S1-loop determines the key residues within the active site of MMP-12, while the green color coding amino acid are towards the active site and red color coding amino acids face outwards the active site.

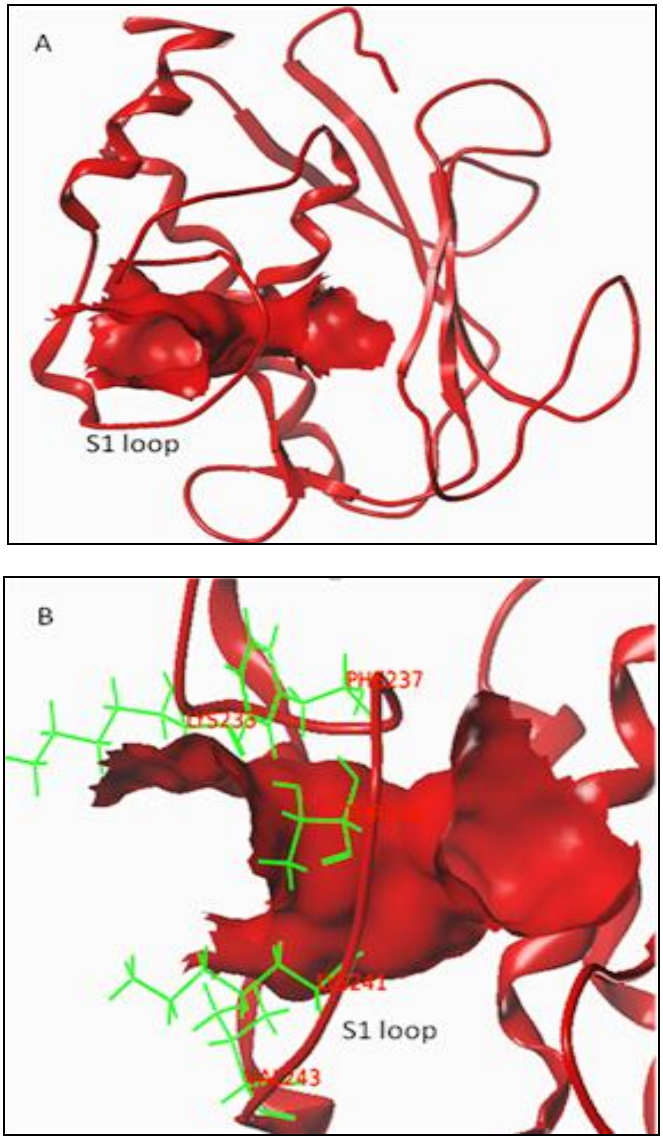


FIGURE 6: (A) ACTIVE SITE CHANNEL OF MMP-12 AND (B) KEY RESIDUES OF S1 POCKET

MMP-14 Active Site Channel: Figure 7 explains (A) S1 loop and active site channel of MMP-12, while (B) explains the S1 loop amino acid residues like S254, A258, F260, Q262, M264 are may be changes the active site volume and size because this amino acid side orientation towards the active site channel. S1 loop sequence starts with the residue F213 and ends with residue T245 "FLVAVHELGHALGLEHSSD PSAIMAPFYQWMDT".

Color coding sequence in S1-loop determines the key residues within the active site of MMP-12, while the green color coding amino acid are towards the active site and red color coding amino acids face outwards the active site.

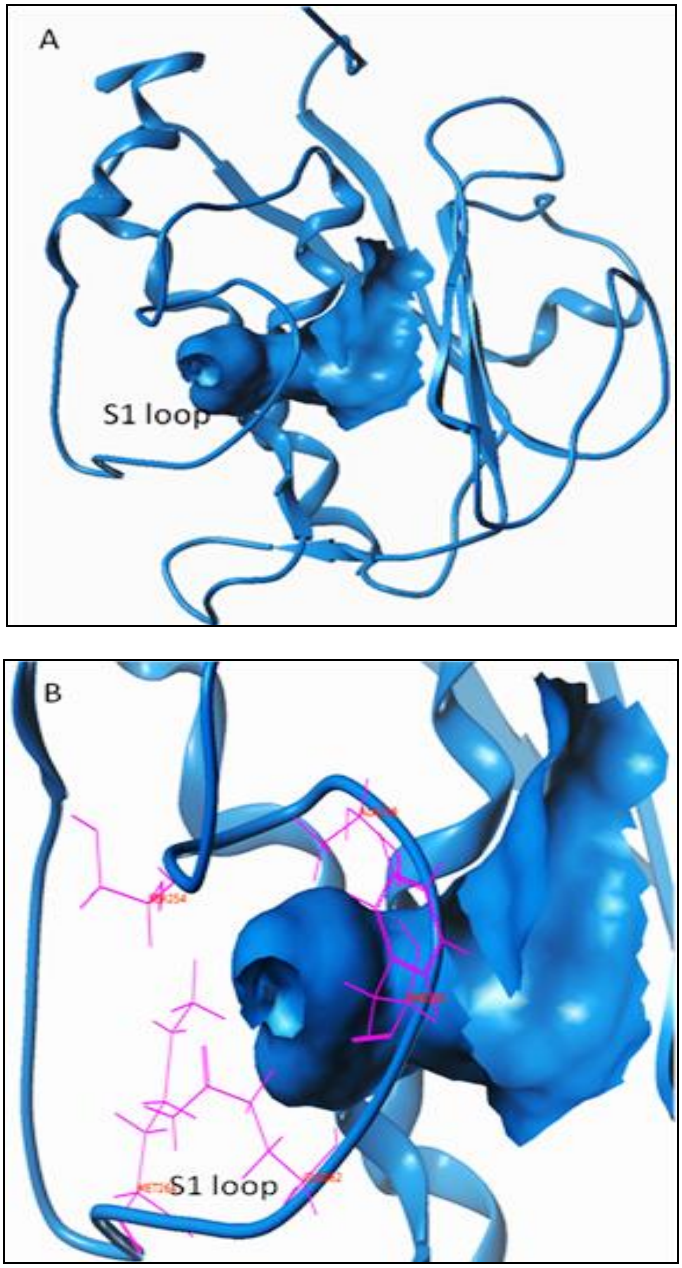


FIGURE 7: (A) ACTIVE SITE CHANNEL OF MMP-14 AND (B) KEY RESIDUES OF S1 POCKET

Superposition of MMP-09, MMP-12 & MMP-14 Channels and Ribbons: A parallel comparison of MMP-09, MMP-12, and MMP-14 enzymes can only be made when the active site residues could be seen together. In order to accomplish this, not only the proteins were superposed, but also the residues were compared along side.

The overall structures of the S1-pocket were looked at deeply to understand the size and shapes of the active sites of MMP-09, -12, and -14 enzymes. Secondly, the key residues along the S1-pocket have been identified to resolve the structural differences leading to active site shape differences. **Figure 8** gives a crisp analysis of above exercise. Even though the sequence variations exist within the S1 loop, a overall structural alignment was achieved. This leads to an observation that the active sites may have similar shape. Reasonably small molecules may have non-selectivity in inhibiting these enzymes. But, key residues along S1 pocket may determine the selectivity. Panel B of Figure-8 explains the S1-loop amino acid residues R424, T426 in MMP-09, K421, V243 in MMP-12 and Q262, M264 in MMP-14 are some of the key residues which could be responsible for active site volume and shape variations within these particular enzymes.

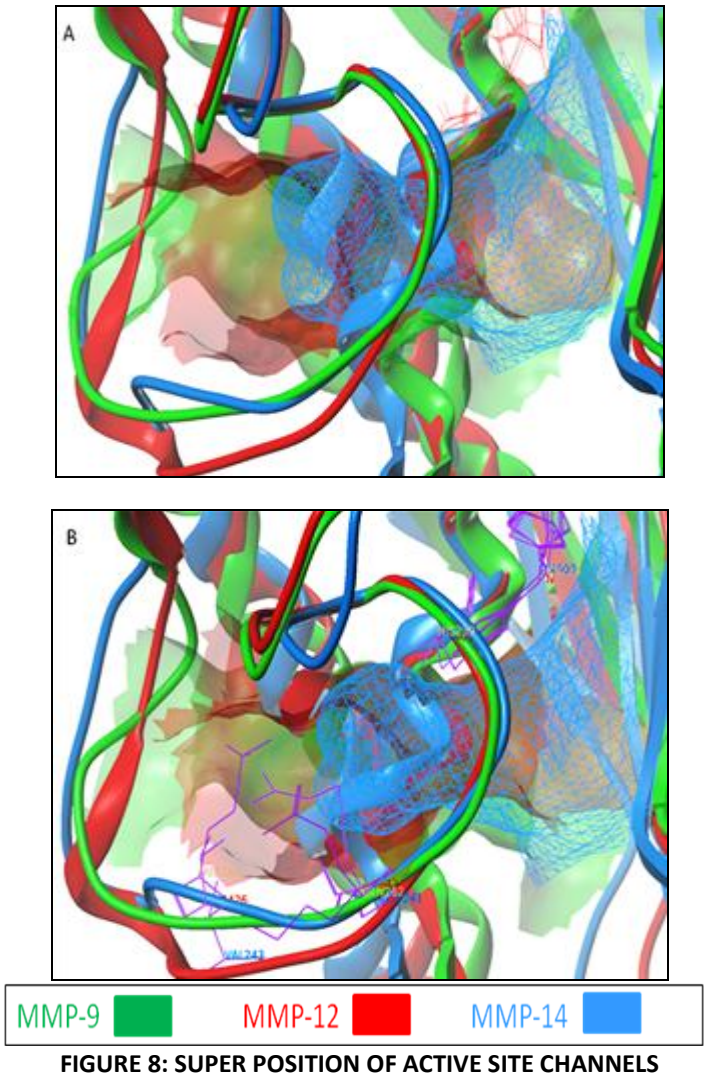


FIGURE 8: SUPER POSITION OF ACTIVE SITE CHANNELS

Docking Results: In order to understand these selectivity variations within these enzymes by the selected inhibitors, we took molecular docking as a tool for analysis. For each inhibitor, all stereochemically possible isomers were evaluated in docking. Standard precision docking parameters were employed, while 10 conformations for each structure were generated. A large consensus value means majority of conformations align with each other within the active site and produce similar interactions with the residues.

MMP-9 SP Docking Results: Initially, all selected inhibitors as shown in figure 1 were docked into MMP-09. Following **Table 6** describes the docking results of these inhibitors, dock score, consensus percentage, their IC_{50}, and the interacting amino acid residues within the active site. The S-isomer of 9-01 ligand has greater docking score than the corresponding R-isomer with almost similar consensus (60% vs 95%). While 9-02 has no isomers and has a lesser dock score. The IC50s of 9-01 and 9-02 are 50 and 200, respectively, and correlate very well with the dock scores (-7.5 and -7.1). Although this may not directly explain the experimental observation, we understand that a general correlation should exist between dock score and inhibitory activity. Similarly some of the non-selective MMP-12 inhibitors that have inhibitory activity towards MMP-09 have demonstrated similar observation (such as a dock score of -7.9 for an IC0 of 420nM towards MMP-09 for the inhibitor 12-05). **Figure 9** below shows the docking results of various conformations of selective MMP-09 inhibitors within the active site of MMP-09. The interacting residues within the active site are presented in the above table. These ligands interact with the Zinc atom to bring sufficient inhibitory activity.

MMP-12 SP Docking Results: Interactions of the selected inhibitors in the study were also analyzed with MMP-12 using docking approach. MMP-12 selective ligands were expected to behave very well within MMP-12 and a general rank-order of activities with dock scores were expected. It was achieved in some cases, while a detailed analysis and rigorous docking studies were yet to be made to ascertain the selectivity profile of inhibitors of MMP-12 within MMP-12 and cross docking with MMP-09 expected to give some underlying message toward selectivity.

TABLE 6: RESULTS OF DOCKING STUDIES OF MMP-09 INHIBITORS IN MMP-09

Ligand title	MMP-09			
	Dock score	Consensus	Activity	Interacting A.A
9-01-R	-4.3	95%	--	F110,E111,A189,Q402
9-02	-7.1	60%	200	G186,Q402
12-01-SR	-6.1	40%	5.3	P421
12-02	-10.9	100%	3600	L188,A189
12-03-SS	-6.9	95%	--	Y423
12-04-SS	-7.6	100%	--	A189,Q402
12-05	-7.9	80%	420	I188,A189,Q402
12-06	-5.3	50%	1510	L188,A189
9-01-S	-7.5	60%	50	I188,A189,Q402
12-01-RR	-6.4	10%	--	Q402
12-01-RS	-5.8	20%	--	--
12-01-SS	-5.3	10%	--	--
12-03-RR	-5.5	40%	--	L188,A189
12-03-RS	-4.9	100%	>5000	A189
12-03-SR	-6.2	80%	--	L188,A189,R424
12-04-RR	-7.4	40%	--	L188
12-04-RS	-7.5	40%	304	L188
12-04-SR	-6.8	60%	--	L188

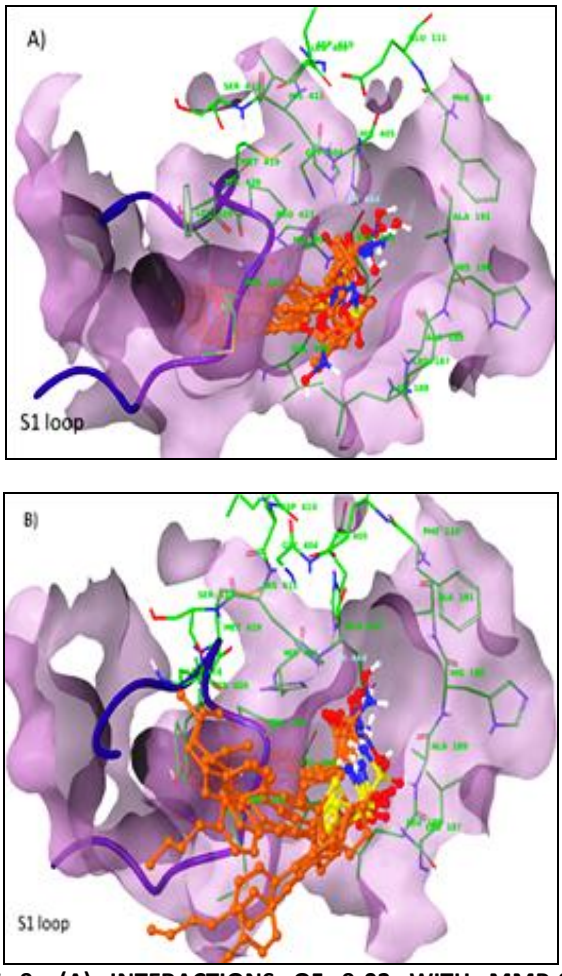
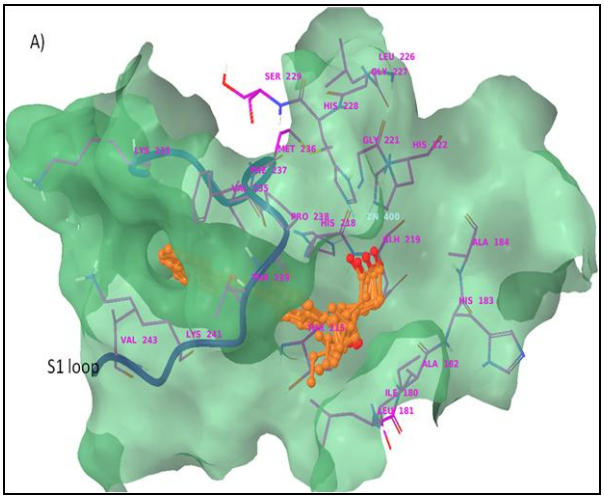


FIGURE 9: (A) INTERACTIONS OF 9-02 WITH MMP-09. (B) INTERACTION OF 9-01 WITH MMP-09

Interestingly, the SS isomer of 12-04 has the highest dock score (-7.8) with a greater consensus which is predicted to be the same diastereomer that was described as the potent inhibitor. Similarly, the SR and SS isomers of 12-01 showed poor consensus, while exhibited greater potency (0.95nM) toward MMP-12 inhibition.

Figure 10 below shows the docking results of various conformations of selective MMP-12 inhibitors within the active site of MMP-12. The interacting residues within the active site are presented in the below **table 7**. These ligands interact with the Zinc atom to bring sufficient inhibitory activity.



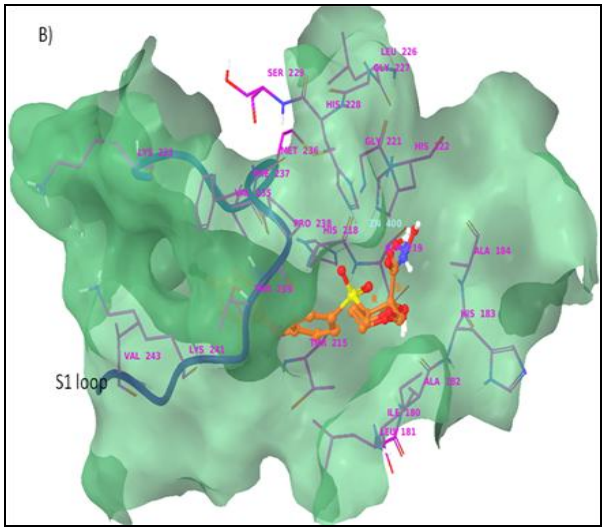


FIGURE 10: (A) INTERACTIONS OF 12-02 WITH MMP-12. (B) INTERACTION OF 12-03 WITH MMP-12

MMP-14 SP Docking Results: It is interesting to observe how the MMP-09 and MMP-12 inhibitors fare with MMP-14 inhibition. There are no small molecule super potent inhibitors of MMP-14 were found in literature, while 9-01 and 12-01 are the only inhibitors that have potent inhibition (50 and 150nM, respectively) towards MMP-14. 9-02 and 12-02 molecules have virtually no inhibition against MMP-14. This leads to interesting calculations one could try as following (a) Either to observe the non-selectivity towards MMP-14, or (b) selectivity towards MMP-14. To our surprise, majority of ligands excepting 12-02, have usually low docking scores towards MMP-14 suggesting the selectivity towards MMP-14.

Although this is a preliminary observation, stronger and robust docking studies should be made to strengthen the argument. Nevertheless, in an attempt to see how MMP inhibitors fare towards selectivity and non-selectivity across three MMPs, we believe, with proper modifications, one can guide molecules for desired inhibitory potential.

Figure 11 depicts the example interactions of 9-02 and 12-02 with MMP-14 upon docking. **Table 8** gives the details of docking of various inhibitors into MMP-14. Even though all docked confirmations were presented, a general interaction profile can be understood from the above figure.

TABLE 7: RESULTS OF DOCKING STUDIES OF MMP-12 INHIBITORS IN MMP-12

Ligand title	MMP-12			
	Dock score	Consensus	Activity	Interacting A.A
9-01-R	-8.2	80%	--	L181,A182,E219,P238
9-02	-7.9	100%	--	L181,A182,E219,P238
12-01-SR	-7.9	20%	0.95	A182,E219,P238
12-02	-12.4	100%	480	E219
12-03-SS	-7.6	100%	--	E219,Y240
12-04-SS	-7.8	95%	17	L181,A182,E219
12-05	-8.6	95%	380	L181,A182,E219
12-06	-9.1	60%	200	L181,A182,E219
9-01-S	-8.2	20%	--	L181,A182,E219
12-01-RR	-4.4	70%	--	Ll181,A182,E219,P238
12-01-RS	-7.6	20%	--	A182,E219
12-01-SS	-7.9	20%	--	L181,A182,E219
12-03-RR	-7.9	100%	--	L181,A182,E219
12-03-RS	-6.6	10%	564	L181,A182,H218,P238
12-03-SR	-7.4	100%	--	E219,K241
12-04-RR	-7.1	20%	--	L181,A182,E219
12-04-RS	-6.9	50%	17	L181,E219
12-04-SR	-5.9	20%	--	E219

TABLE 8: RESULTS OF DOCKING STUDIES OF MMP-14 INHIBITORS IN MMP-14

Ligand title	MMP-14			
	Dock score	Consensus	Activity	Interacting A.A
9-01-R	-4.3	40%	**50**	E240
9-02	-4.8	20%	>10000	L199,A200,E240
12-01-SR	-3.4	20%	150	--
12-02	-6.9	40%	30600	
12-03-SS	-4.1	40%	>5000	E240
12-04-SS	-4.4	40%	772	E240
12-05	-5.2	20%	8100	N231,P259
12-06	-3.5	40%	NA	P259
9-01-S	-3.8	20%	6600	
12-01-RR	-4.0	10%	--	
12-01-RS	-3.9	30%	--	E240
12-01-SS	-4.2	10%	--	E240
12-03-RR	-3.8	20%	--	
12-03-RS	-4.1	50%	>5000	E240
12-03-SR	-3.8	20%	--	
12-04-RR	-4.9	70%	--	
12-04-RS	-5.3	20%	772	
12-04-SR	-5.3	10%	--	A200

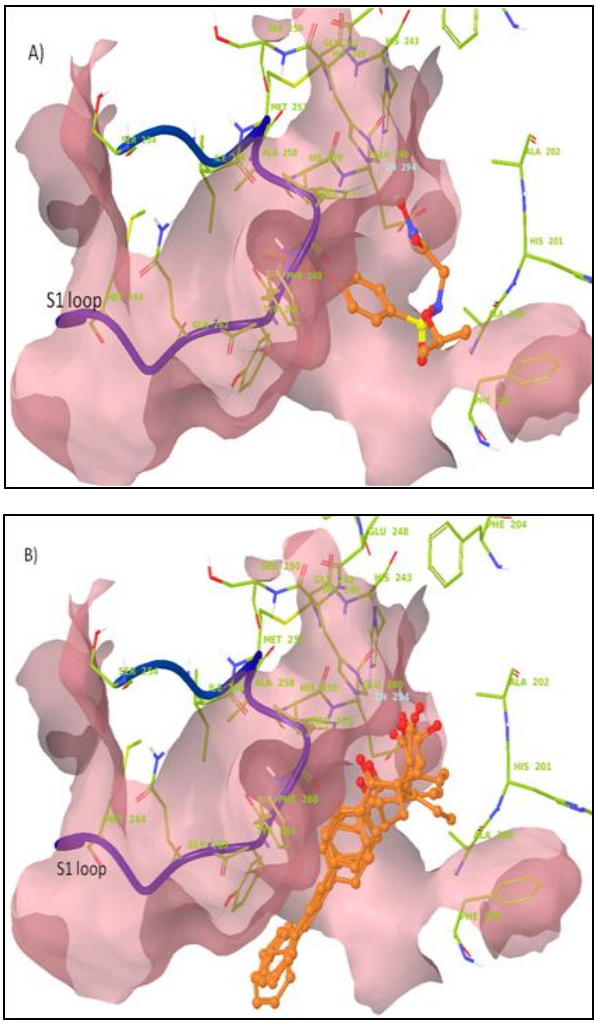
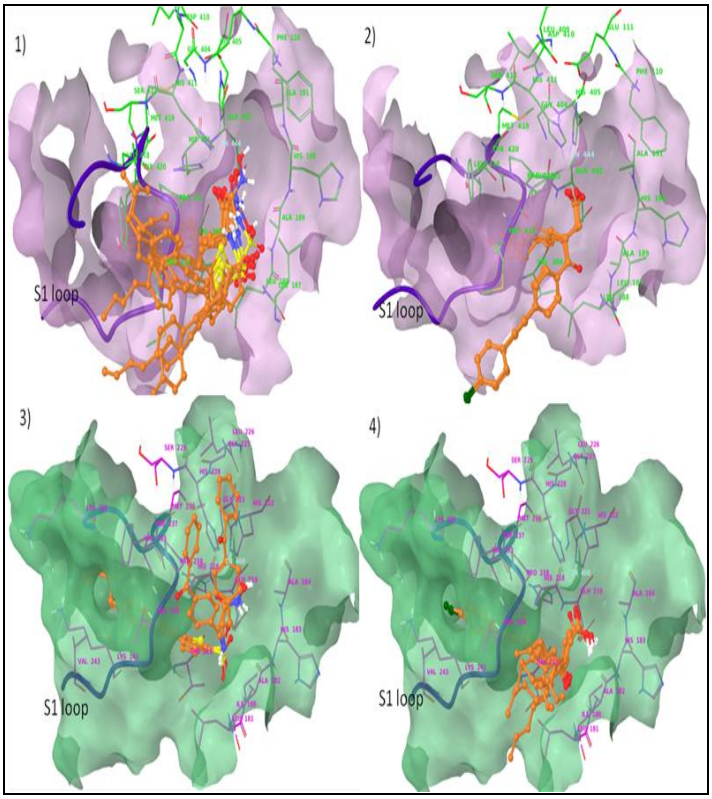


FIGURE 11: (A) INTERACTION OF 9-02 WITH MMP-14 (B) INTERACTION OF 12-02 WITH MMP-14

Selectivity of MMPs: The reported ligands docked in to selective protein or another protein, the docking score, and the ligand interaction give a explanation of selectivity of MMPs. **Figure 12** shows explanation for the selectivity and non-selectivity for enzyme inhibition for the selected inhibitors.



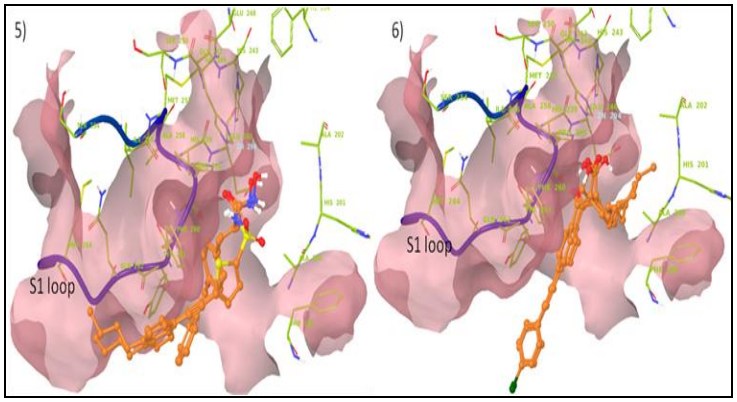


FIGURE 12: ALL SELECTED MMPS ARE DOCKED BY SELECTIVE MMP INHIBITORS AND NON SELECTIVE MMP INHIBITORS. 1) Explains 9-01 inhibitor selective to MMP-9 it can be docked in MMP-9, Dock score is -7.5, IC_{50} is 50, and consensus is 60%. **2)** Explains 12-06 is selective inhibitor of MMP-12 it can be inhibitor docked in MMP-9, Dock score is -5.3, IC_{50} is 1510, and consensus is 50%. **3)** Explains 9-01 inhibitor selective to MMP-9 it can be docked in MMP-12, Dock score is -8.2, and consensus is 20%. **4)** Explains 12-06 is selective inhibitor of MMP-12 it can be inhibitor docked in MMP-12, Dock score is -9.1, IC_{50} is 200, and consensus is 60%. **5)** Explains 9-01 inhibitor selective to MMP-9 it can be docked in MMP-14, Dock score is -3.8, IC_{50} is 6600, and consensus is 20%. **6)** Explains 12-06 inhibitor selective to MMP-12 it can be docked in MMP-14, Dock score is -3.5, IC_{50} is NA(not announced), and consensus is 40%.

CONCLUSIONS: The aim of this work is to find out how selective inhibitors of MMP-09 and MMP-12 interact with respect to their intended enzymes. Our another aim is also to understand the similarities and differences within the active sites of three MMP enzymes involved in COPD. We tried to understand these three MMPs, their structures, and structure-function similarity through computational methods. Using in silico technologies such as molecular docking and scoring we tried to understand the cross functional activities of the inhibitors and active site comparison of MMP.

Some of the observations are presented here in

1. MMP-9(2OVX), MMP-12(1ROS) and MMP-14(3MA2) have similar architecture within the active site.

2. Difference causing residues are

   A. MMP-9 is E416, Y420, M422, R424, and T426.

   B. MMP-12 is K233, F237, T239, K241, and V243.

   C. MMP-14 IS S254, A258, F260, Q262, and M264.

The last two amino acid residues are mainly structure differencing residues

Some conclusions drawn from above and docking experiments are the following:

1. The interacting residues of 9-02 inhibitor are G186, Q402, P241 and 12-03 inhibitor interacting residues are E219, K241 with MMP-09.

2. The interacting residues of 9-02 inhibitor are L181, A182, E219, P238 and 12-03 interacts with L188, A189 in MMP-12.

3. L119, A200, and E240 interact with 9-02 and 12-03 in MMP-14

Analysis the above docking results MMP-9, MMP-12 are more selective over MMP-14 because using selective inhibitors its give differences of selected MMPs. Means above residues in active site comparison residues are S1 loop amino acid residues are checked by docking in docking results near to zinc atom all residues of all MMPs are same but in S1 loop residues are P241 in MMP-9, P238 in MMP-12 are same but this residue or nearer residue are not interact with MMP-14.

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