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Research Article

CHARACTERIZATION OF 4-SULFAMOYL-A-PIPERIDINE SULFONES AS POTENT INHIBITORS OF MATRIX METALLOPROTEINASE-2: BIOLOGICAL ACTIVITY PREDICTION AND MOLECULAR DOCKING ANALYSIS

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ABSTRACT

Context: Matrix metalloproteinases (MMP) are zinc dependent extracellular endopeptidases that play vital role in cancer invasion, metastasis and proliferation. Still date, few molecular scaffolds are known to inhibit MMP-2 activity, most of which contain a sulfone group in their side chain.

Objective: This study was designed to identify and evaluate the activity of α-piperidine sulfones as potent inhibitors of MMP-2 by Quantitative Structure Activity Relationship (QSAR) and docking analyses.

Materials and Methods: Half minimal inhibitory concentration (IC50) values of 153 compounds against MMP-2 were retrieved from literature. 2D and 3D Molecular descriptors were calculated using Molecular Operating Environment. Outliers were removed by Principal Component Analysis (PCA) and 3D QSAR model was built by multiple linear regressions (MLR). Molecular docking was carried out using Autodock 4.2.

Results: We evaluated the activity of 12 newly designed compounds using the QSAR model (r^2=0.767). Four compounds were found to be potentially active with IC50 values ranging from 0.06-0.07µmol/L and were subjected to docking analysis against the target (PDB id: 3AYU).

Conclusion: This study has identified novel potential inhibitors of MMP-2. Further virtual and high throughput approaches coupled with toxicogenomics should identify safe and potential inhibitors of MMP-2 and other geno-proteomic targets involved in the complex tumor metastasis pathway.

Keywords: IC50, Metastasis, MMP-2, Piperidine, QSAR, Sulfones.

INTRODUCTION

MMP are zinc dependent extracellular endopeptidases of metazincin superfamily that play a pivotal role in degradation and extracellular matrix remodeling, embryonic development, tissue morphogenesis, wound repair, inflammation and cancer [1-2]. Matrix metalloproteinases have been classified into five distinct subgroups on the basis of structural homology: the Collegenases, Gelatinases, Stromelysins, Matrilysins and membrane type MMPs[3]. Based on the substrate specificity, MMPs are classified into 28 types, that share a common catalytic domain containing two Zn^{2+} and two or three Ca^{2+} ions. The Ca^{2+} ions and one of the Zn^{2+} ions form the structural motif that stabilizes the domain structure whereas the second Zn^{2+} ion participates in the catalytic process[4-5]. Over expression and activity of MMP's have been reported in a spectrum of diseases[6-7]. MMP-2 is a gelatinase crucially involved in cancer invasion, metastasis, cellular transformation and tumor growth. Abnormal levels of MMP-2 have been implicated in tumor metastasis[8]. Located on 16.q.13 chromsomal loci, MMP-2 enzymatically cleaves the following substrates: gelatin, collagen IV, V, VI, X, elastin, fibronectin and Integrins[9-10]. These protein substrates promote cell adhesion to the extracellular matrix, and function in chemotaxis and control of cell proliferation. Enzymatic proteolysis of the substrates by MMP-2 causes cell to lose their surface adhesion and promotes migration leading to tumor metastasis[11-13]. Thus inhibition of MMP-2 activity by small molecules is a rational and promising approach for control of tumor cell metastasis and proliferation[14-15]. Still date, few molecular scaffolds are known to inhibit MMP-2 activity, most of which contain a sulfone group in their side chain. In our current study, novel α-piperidine sulfones were designed and their biological activity was evaluated *insilico* using QSAR models. Interactions of the designed ligands with the active site of MMP-2 were studied using molecular docking analysis.

MATERIALS AND METHODS

Compound Dataset Creation:

IC50 of 153 compounds against MMP-2 was retrieved from the literature cited in the references[16-21]. All the retrieved compounds shared a common sulfone bridge in their structure. The compounds were energy minimized under MMFF94x force field with a RMS gradient of 0.1. 298 2D, i3D and x3D molecular descriptors were calculated for the energy minimized structures using a Molecular Operating Environment.

Principal Component Analysis:

PCA was carried out using MATLAB version 7.10.0. PCA was performed for sample selection prior to model building. Molecular descriptors and log IC50 (pIC50) were used as independent and dependent variables respectively. Data was preprocessed by mean centering and cross-validation was carried out by leave-one out (LOO) method. Significant outliers were removed from sample subset for further processing.

3D- QSAR Model Building:

3D- QSAR model was built by MLR Method. The compounds were divided into training set and validation set. Clustering was done by k-means agglomerative HCA method using three principal components. 10% of the selected samples were segregated as validation set. The training set compounds were used for 3D-QSAR model building using SPSS statistics version 17.0. Various MLR models were built and the model with best correlation coefficient (r^2) was used for predicting the biological activity of designed compounds. The stepwise forward automated algorithm was used for descriptor selection.

Target Preparation:

The three dimensional crystal structure of MMP-2 (PDB id: 3AYU) was retrieved from the Protein Data Bank. The target structure was pre-processed by standard methods before binding site analysis. Energy minimization of the processed target was carried out using the Hamiltonian force field OPLS-AA. Active site analysis was performed using the Site Finder module of Molecular Operating Environment.

Molecular Docking Analysis:

Interactions of the designed compounds with the active site of MMP-2 were studied by molecular docking. Autodock 4.2 tool was used for molecular docking analysis. Both the receptor and ligands were prepared by addition of hydrogen's and gasteiger charges. A grid defining the active site was constructed before running the docking simulation. Genetic algorithm was adopted for conformer search while docking.

RESULTS

Principal Component Analysis:

PCA analysis showed the following compounds to be significant outliers: 147, 149,150, 152, and 153. A PCA model with three principal components was selected for identification of significant outliers within the compound samples considered. The RMSEC and root mean square error of cross-validation (RMSECV) were found to be 83.61 and 457076.7 respectively. The five outlier compounds were removed from the sample data and 148 compounds were chosen for further analysis as shown in Figure 1.

Figure 1: Principal Component Analysis plot of PC1 vs PC2. Compounds 147, 149, 150, 152 and 153 are shown as significant outliers.

Quantitative Structure Activity Relationship:

The 148 selected compounds were segregated into training and validation set by cluster analysis. Compounds were grouped into fifteen clusters (10 per cent of the sample size) and one compound from each cluster was chosen by randomization for validation set. This resulted in a training set with 133 compounds and validation set with 15 compounds. 3D- QSAR was performed with the training set by Multiple Linear Regression Analysis. Thus pIC50 of 133 compounds with 298 descriptors were subjected to MLR analysis. 8 models were built by stepwise MLR with a median correlation coefficient of 0.68 (Q1-Q8 0.38-0.76). The model with r^2 value of 0.767

consisting of eight components was selected for prediction of biological activity. The equation of the selected model is given below:

pIC50 = 23.514 + 16.507 (Log P BCUT 1/3) - 0.003 (Electrostatic Energy) + 4.975 (Molecular Globularity) + 16.194 (PEOE Charge GCUT 0/3) + 0.027 (Total Positive Vander Waal Surface Area 2) – 0.054 (Polar Volume at - 2.0) + 0.517 (Lipinski Donor Count) – 0.197 (Number of Oxygen Atoms)

Model validation was carried out by applying the eight model components of the validation set compounds to the QSAR model. The predicted and experimental pIC50 values of validation compounds are shown in **Table 1.**

Table 1: Predicted and Observed pIC50 Values of Validation Set Compounds

No significant difference was observed between the predicted and observed pIC50 values at a Confidence interval of 95%. The scatter plot of predicted versus observed pIC50 values are shown in **Figure 2.**

Figure 2: Scatter Plot of Predicted vs Observed pIC50 of Validation Set Compounds ($r^2 = 0.767$)

Twelve novel α-Piperidine Sulfones containing diverse substitutions in the benzene ring at 4^{th} position para to the sulfonyl group were designed. The common structure of the designed compounds is shown in **Figure 3.**

Figure 3: Common Molecular Scaffold of Designed Inhibitors of MMP-2

Molecular descriptors were calculated and biological activity of the designed molecules was predicted using the built QSAR model. The substituents at positions R_1 and R_2 and predicted biological activities are shown in **Table 2.**

Table 2: Biological Activity Prediction of Designed Compounds

It was observed that presence of sulfamoyl group at R_2 position confers better biological activity than the conventional compounds. Compounds designed with cyclopropane and halogens at R_1 position and sulfamoyl group at R_4 position displayed significantly increased biological activities with IC50 values at nanomolar concentrations. Thus four compounds containing 4-Sulfamoyl-α-Piperidine Sulfones were found to potentially inhibit MMP-2 at sub-minimal nanomolar concentrations.

Target-Ligand Interaction Studies:

Potential energy of the target as calculated under Hamiltonian OPLS-AA force field was 300.1965 kcal/mol. Post-minimization potential energy as calculated under similar conditions was - 3058.9302 kcal/mol. Active site of the energy minimized target was formed of the following residues: ILE15, TRP10, LYS9, PRO8, LYS7, PHE4, PHE3, ASN2, TYR1, PHE39, TRP42, THR46, PRO47, LEU48, and PHE50.

The results of molecular docking analysis are shown in **Table 3.**

Table 3: Molecular Docking Analysis of Active Compounds

The final docked conformation of compounds with the active site of the target is shown in **Figure 4.**

Figure 4: Docked Conformation of (A) Compound 6; B) Compound 7; C) Compound8; D) Compound 9 with the Active Site Residues of Matrix Metalloproteinase-2

DISCUSSION

The Eigen Value of Cov (X) of the selected model was 6300000 with a percentage variance of 0.01 and a cumulative percentage variance of 100. We determined the RMSEC for the descriptor dataset of 153 compounds to be 83.61 and the RMSCEV to be 457076.7. RMSECV measures differences between the observed and predicted values by a model [22-23]. In leave one out method adopted for cross validation, each sample is left out of the model formulation followed by its prediction. It is therefore a model's ability to predict new samples[24]. RMSECV is generally defined by the following equation:

$$RMSECV = \sum_{i=1}^{n} ¿¿¿¿$$

Where $\hat{y}_{i¿}$ is the predicted value, y_i is the measured value and n is the number of measurements. Outliers from Principal component analyses were removed from further processing.

Biological activity of a molecule is a function of its structure and physico-chemical properties. Hence a logistic regression model was built using the computed 3D- and 2D- descriptors as independent variables. The stepwise forward selection algorithm that commences with addition of each computed descriptor tests the addition of each descriptor using a chosen model comparison criterion by adding descriptors that improve the quality of the model, and repeating this process until none improves the quality[25]. The cross validated model then predicted the biological activity of the novel designed compounds.

The four compounds were individually docked to the active site of MMP-2 by Autodock 4.2. Autodock analyzes the interactions of small molecules with active sites of large peptide assemblies in terms of binding energy (ΔG), hydrogen bonding interactions, π-π interactions, ligand conformation within the active site and root mean square deviation (RMSD) of the active site residues[26]. The free energy of binding is calculated using the empirical formula,

Binding energy (ΔG) = Intermolecular energy + Vanderwaal's hydrogen bond desolvation energy + Electrostatic energy + Total internal energy + Torsional energy – Unbound energy of the system. The similarity of docked structures is measured by computing the root mean square deviation and clusters are created based on the comparison of conformations and estimated RMSD values[27]. The sulfamoyl group at R_2 position in compound 6 interacts with the active site ASN2 through back bone donation of electrons. Two hydrogens in hydroxyamide group of compound 6 interact in a similar way with TRP42 through back bone electron donation whereas the oxygen of the same group interacts with THR46 by acting as a side chain acceptor. In contrast, the sulfamoyl group of compound 9

interacts with TRP10 by acting as a side chain acceptor whereas the hydroxyl hydrogen of hydroxyamide interacts with ASN2 through back bone electron donation. Unlike compounds 6 and 9, the sulfamoyl group did not participate in bonding interactions in compounds 7 and 8. The hydrogen attached to the nitrogen atom of hydroxyamide group interacts with ASN 2 through back bone donation in compound 7 whereas the hydrogens of both nitrogen and oxygen of hydroxyamide group are involved in back bone donation with ASN 2 in compound 8. The R_1 substituent was not found to involve in any active bonding interaction. Thus the sulfamoyl substituent in R_2 and the hydroxyamide group are essential for the designed compounds to exert MMP-2 inhibitory activity. Thus the biological activity of the designed compounds was evaluated using ligand and target based approaches in this study.

CONCLUSION

Tumor metastasis from primary to distant sites is a major crucial cause of mortality associated with cancer. Though tumor metastasis is a complex multi-step process, loss of cell adhesion caused by proteolysis of adhesive proteins by Matrix metalloproteinases remains the primordial cause. The study has identified novel potential inhibitors of MMP-2. Further virtual and high throughput approaches coupled with toxicogenomics should identify safe and potential inhibitors of MMP-2 and other geno-proteomic targets involved in the complex tumor metastasis pathway.

Table 1: Predicted and Observed pIC50 Values of Validation Set Compounds

Compound ID	Observed pIC50	Predicted pIC50
Compound9	3.082066934	2.608680629
Compound16	2.723455672	2.590317034
Compound24	-0.698970004	1.130574997
Compound25	0.113943352	1.514366709
Compound32	-0.698970004	-0.007529939
Compound39	1.51851394	1.116518535
Compound45	1.491361694	1.64127052

Compound67	1.886490725	2.044844945
Compound74	0.531478917	1.05058155
Compound84	-0.397940009	-0.430091078
Compound87	-0.397940009	-0.744945899
Compound99	0.113943352	0.07589681
Compound105	-0.065501549	-0.131684169
Compound133	-1	-0.657799663
Compound141	-0.698970004	-0.539460734

Table 2: Biological Activity Prediction of Designed Compounds

Compound ID	R_1	R_2	Predicted pIC50	IC50
Compound 1	-Cl	-H	1.092802146	12.38232349
Compound 2	-Cl	$-COOCH_3$	0.300884037	1.999327948
Compound 3	-Cl	$-CONH_2$	-0.454919026	0.350817278
Compound 4	-Cl	-CN	0.428695451	2.683462008
Compound 5	-Cyclopropane	$-OCOCH_3$	-0.107898051	0.780013194
Compound 6*	Cyclopropane	$-SO_2NH_2$	-1.159542612	0.069255997
Compound 7*	-Cl	$-SO_2NH_2$	-1.162298113	0.068817975

Compound 8*	-I	$-SO_2NH_2$	-1.12023465	0.075816783
Compound 9*	-Br	$-SO_2NH_2$	-1.144705233	0.071662964
Compound 10	$-NH_2$	$-CH_3$	0.2801286	1.906025032
Compound 11	$-NH_2$	-Pyridine	0.054055116	1.132544084
Compound 12	-Cl	-Pyrimidine	0.93629604	8.635670039

*Active compounds are highlighted in bold

Table 3: Molecular Docking Analysis of Active Compounds

CompoundID	ΔG(kcal/mol)	kI (µM)	Intermolecular Energy	Vanderwaals HB Desolvation Energy	Electrostatic Energy	Total internal Energy	Torsional Energy	Unbound Energy
Compound6	-5.7	66.42	-7.79	-7.94	0.15	-0.77	2.09	-0.77
Compound7	-6.47	18.23	-8.26	-8.24	-0.01	0.49	1.79	0.49
Compound 8	-7.23	5.04	-9.02	-9.01	0.0	0.08	1.79	0.08
Compound 9	-6.34	22.58	-8.13	-8.07	-0.06	-0.22	1.79	-0.22

Figure 1: Principal Component Analysis plot of PC1 vs PC2. Compounds 147, 149, 150, 152 and 153 are shown as significant outliers.

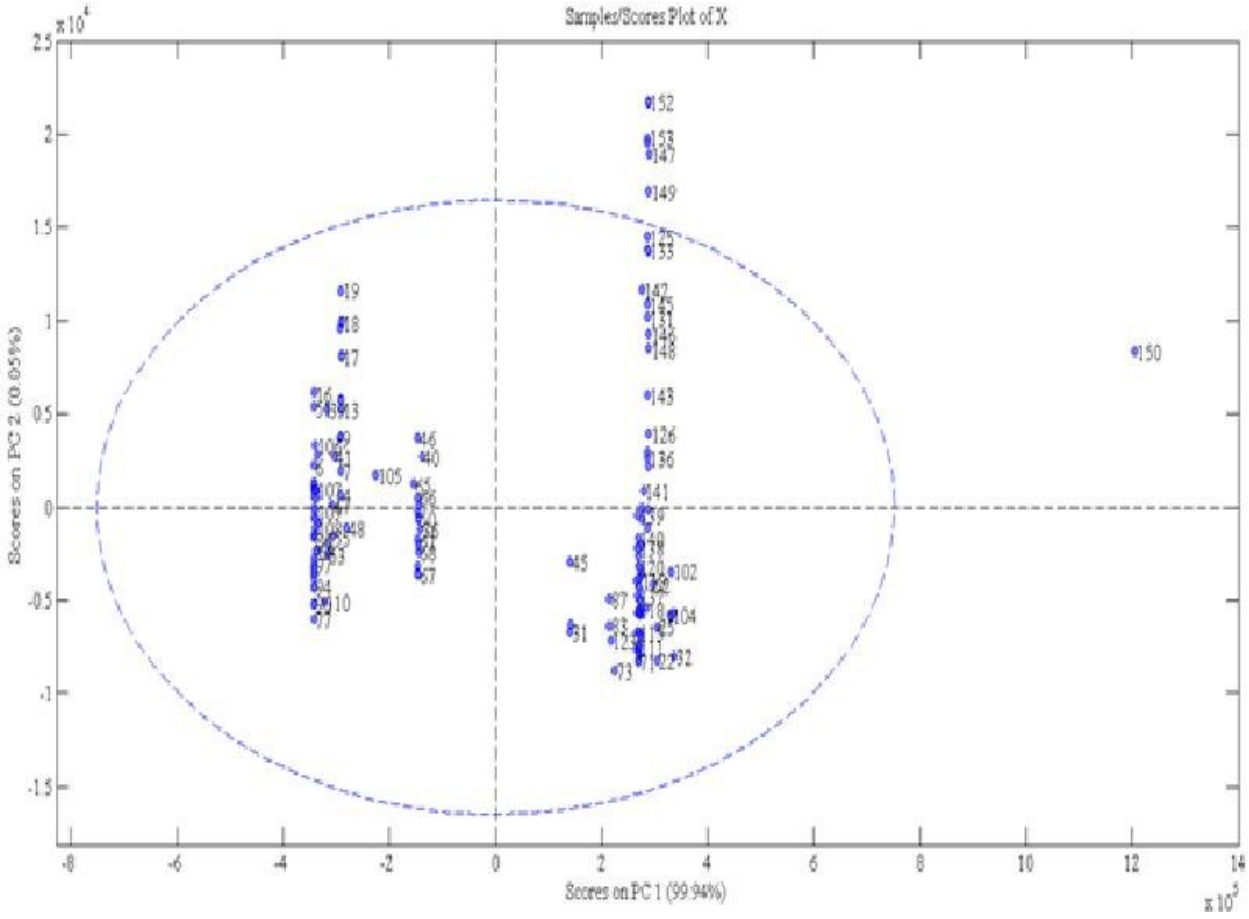


Figure 2: Scatter Plot of Predicted vs Observed pIC50 of Validation Set Compounds (r^2 = 0.767)

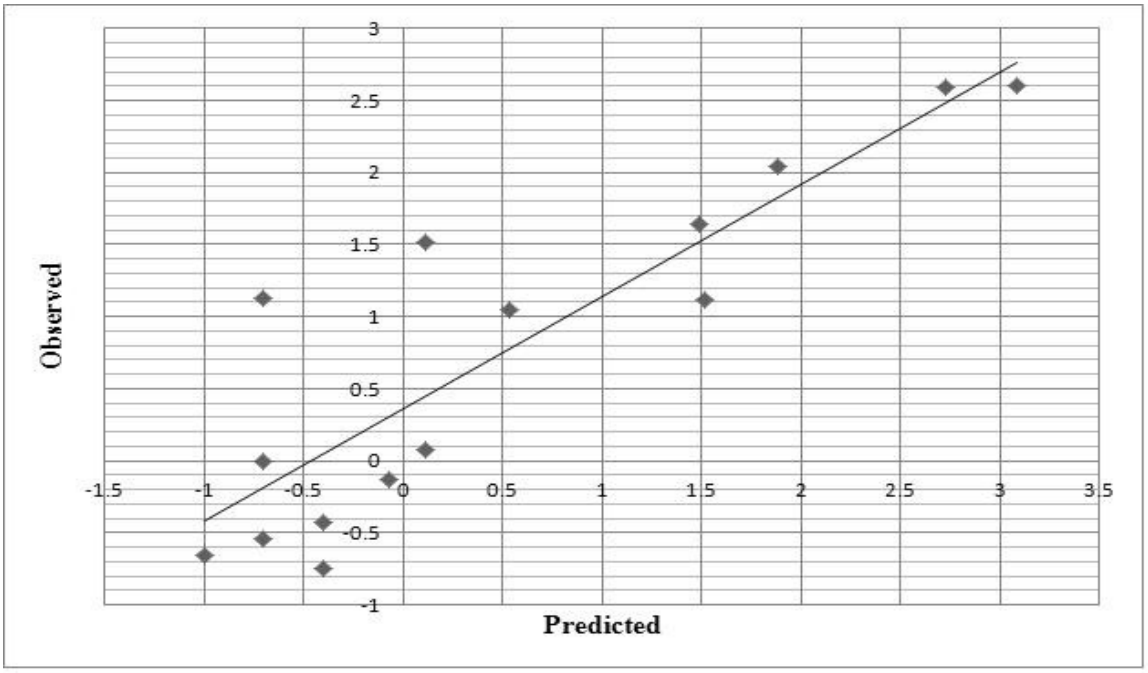


Figure 3: Common Molecular Scaffold of Designed Inhibitors of MMP-2

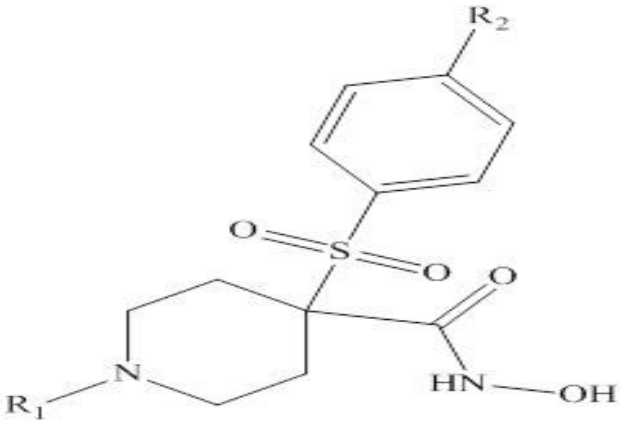
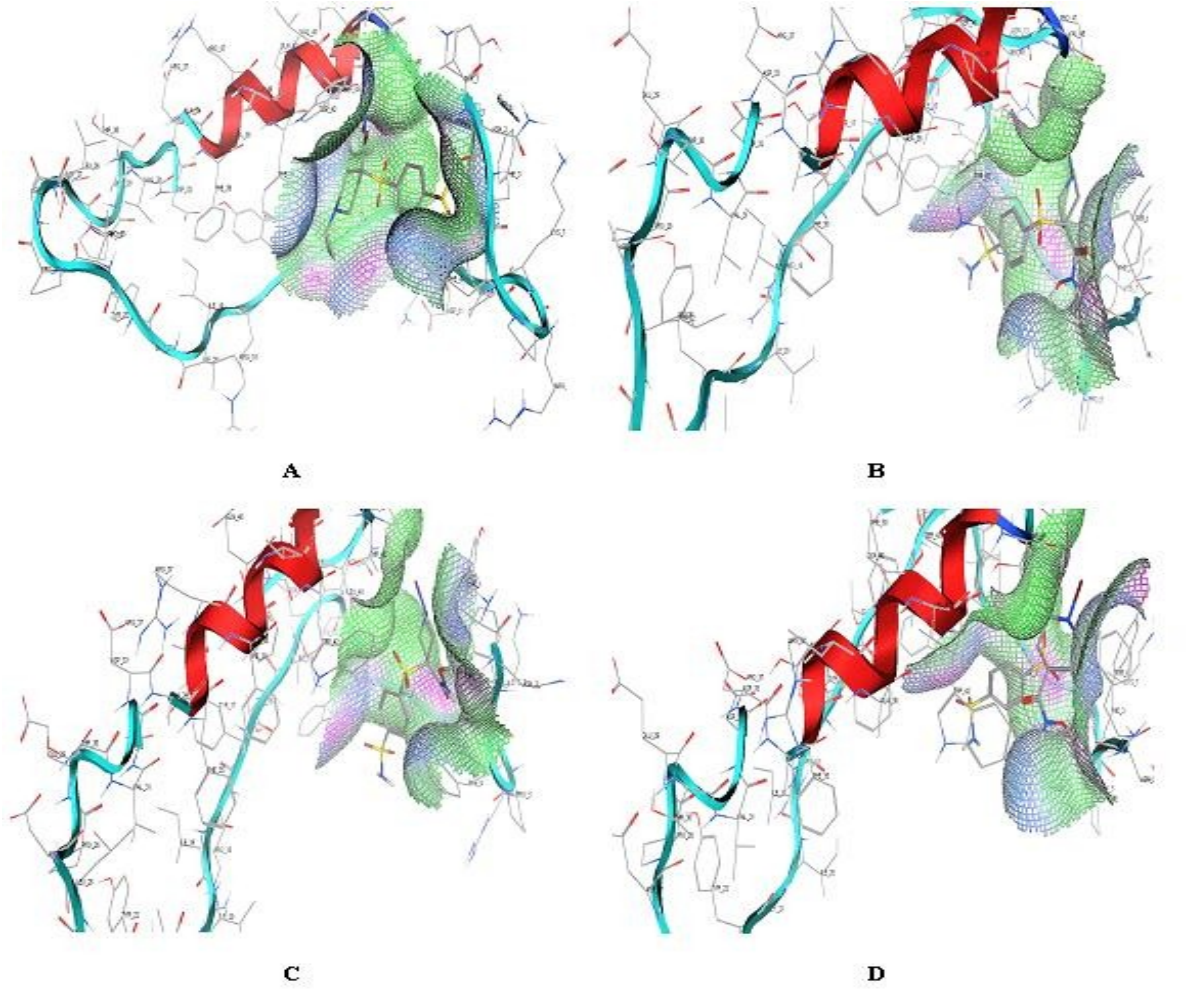


Figure 4: Docked Conformation of (A) Compound 6; B) Compound 7; C) Compound8; D) Compound 9 with the Active Site Residues of Matrix Metalloproteinase-2



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CONFLICT OF INTERESTS

We have no conflict of interest to declare.

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