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IN SILICO MOLECULAR DOCKING STUDIES ON SOME NOVEL *N*-SUBSTITUTED SULFONAMIDE ANTHRANILATE HYDROXAMIC ACID DERIVATIVES FOR ITS CYTOTOXIC POTENTIAL AGAINST CANCER MARKERS

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ABSTRACT

N-Substituted sulfonamide anthranilate hydroxamic acid derivatives have a better binding affinity towards the Matrix Metalloprotease (MMP) enzyme as per the literature. Based on that we have selected the Matrix Metalloprotease-1 (MMP-1) domain of MMP enzyme and performed the molecular docking studies using the SYBYL X 2.1 software. We have designed fifteen new chemical new entities for the docking studies and among that two chemical entities were found to have better binding affinities towards the MMP-1 target. By studying the total docking scores of all the new chemical entities we have concluded that the groups like benzyl group at N^{th} position, methoxy group at 4^{th} position of phenyl sulfonamide nucleus, dimethylamine group at 3^{rd} position of hydroxyl benzamide nucleus, etc. are responsible for better activities and binding affinities towards MMP-1 target. So, based on that we can proceed for the further synthesis of those molecules which has higher affinities towards the MMP domains. Some of the positions like 4^{th}, 6^{th} position in Hydroxy Benzamide Nucleus and 2^{nd}, 3^{rd}, 5^{th}, 6^{th} positions in Phenyl Sulfamido Nucleus are unsubstituted and still, we are in process of study in the future projects.

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INTRODUCTION

Cancer is a disease that has always been a major threat and has been characterized by the proliferation of abnormal cells which is the cause for a broad group of various diseases. Indian Council of Medical Research (ICMR) reports that India is likely to have over 17.3 lakh new cases of cancer and over 8.8 lakh deaths due to the disease by 2020[1]. The anticancer drugs were discovered mainly by serendipity or inhibiting metabolic pathways crucial to cell division. Their exact mechanism of action was often a subject of retrospective investigation. In recent years, hydroxamic acid derivatives have attracted increasing attention, for their potential as highly efficacious in combating various etiological factors associated with cancer. Also, the sulfonamides constitute an important class of drugs with several types of pharmacological agents possessing antibacterial[2], anti-carbonic anhydrase[3], diuretic[4], anti-inflammatory[5], anti-viral[6], anticancer[7] activities, etc. A large number of structurally novel sulfonamide derivatives have ultimately been reported to show substantial protease inhibitor properties of some metalloprotease inhibitors belonging to this class, which by inhibiting several matrixes metalloprotease (MMPs) shows interesting antitumor properties. Some of these compounds are being evaluated in clinical trials. A large number of sulfonamide MMP inhibitors ultimately reported also lead to the design of effective tumor necrosis factor-α converting enzyme (TACE) inhibitors, potentially useful in the treatment of inflammatory states of various types. Since MMPs contribute synergistically to the pathophysiology of many diseases such as arthritis, bacterial meningitis, tumor invasion, the dual inhibition of these enzymes will emerge as an interesting target for the drug design of anticancer/anti-inflammatory drugs.

The key enzymes responsible for extracellular matrix (ECM) breakdown of the cell are matrix metalloproteinases (MMPs). MMP genes have been identified in humans and many are implicated in cancer. ECM degradation by MMPs not only enhances tumor invasion but also affects tumor cell behavior and leads to cancer progression. The ECM holds cells together and maintains the three-dimensional structure of the body. It also plays critical roles in cell growth, differentiation, survival, and motility. For a tumor cell to metastasize from the primary tumor to other organs, it must locally degrade ECM components that are the physical barriers for cell migration. The key enzymes responsible for ECM breakdown are matrix metalloproteinases. This review highlights recent developments with regard to the cellular and molecular mechanisms of MMPs that influence tumor cell growth, invasion, and metastasis. Matrix metalloproteinase-1 (MMP-1, interstitial collagenase, fibroblastic collagenase) is a member of the MMP family of extracellular proteases. The target of MMP-1 includes collagen, gelatin, entactin, pro-TNF-alpha, and the chemokine SDF-1[8]. MMP-1 is an important target for inhibitor screening due to its involvement in diseases such as cancer.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small-molecule ligands to the appropriate target binding site with overall minimum energy[9]. The small molecule, known as ligand usually fits within a protein's cavity which is predicted by the search algorithm. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets to predict the affinity and activity of the small molecule. The results are analyzed by a statistical scoring function that converts interacting energy into numerical values called the docking score; and also the interacting energy is calculated. Predicting the mode of protein-ligand interaction can assume the active site of the protein molecule and further help in protein annotation. Since docking plays an important role in the rational drug designing and discovery, the present study was focused on designing some novel *N*-substituted sulphonamide anthranilate hydroxamic acid derivatives and identify the compounds with good binding interaction against the selected target metalloprotease (MMP-1) by using molecular docking studies.

MATERIALS AND METHODS

The 3D structures of compounds of *N*-substituted sulphonamide anthranilate hydroxamic acid derivatives were generated using the SYBYL-X 2.1.1 package. The geometry optimization was done with the help of the standard Tripos force field[10] using a distance dependent-dielectric function, energy gradient of 0.001 kcal/ mol, and MMFF94 as the electrostatics. Conformational analysis of *N*-substituted sulphonamide anthranilate hydroxamic acid derivatives was performed using a repeated molecular dynamics-based simulated annealing approach as implemented in SYBYL-X 2.1.1. The molecule was heated up to 1000 K within 2000 fs, held at this temperature for 2000 fs, and annealed to 0 K for 10,000 fs using an exponential annealing function. By employing this procedure, 100 conformations were sampled out during 100 cycles to account for the conformational flexibility to find the most likely conformations occurring most often in the resulting pool. All conformations were minimized with the Tripos force field and atomic charges were calculated using the MMFF94 method.

Molecular docking was used to get the information for further structural optimization by understanding the binding mode of the compounds. Surflex-Dock that adopted an empirical scoring function and a patented searching engine was employed for molecular docking. The crystal structure of the catalytic fragment of human fibroblast collagenase complexed with CGS-27023A, NMR(Nuclear Magnetic Radiation), minimized average structure, PDB ID 3AYK, was extracted from Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). During the process of docking, water molecules were removed and ligand was extracted. The polar hydrogens as well as united atom AMBER7FF02 were assigned for the proteins PDB ID 3AYK. Then, the ligand-based model was adopted to generate the "protomol", leaving the threshold and bloat parameters at their default values of 0.50 and 0 A^o. Compounds of *N*-substituted sulphonamide anthranilate hydroxamic acid derivatives were docked within the prepared proteins. The mode of interaction of the relative ligand in the crystal structure against enzyme PDB 3AYK was used as a standard docked model. The maximum number of poses per ligand was set to 15 with no constraints to perform the molecular docking. For comparative analysis of the designed molecules, D_score[11] , PMF_score[12], G_score[13] and Chem_score[14] were estimated using the C-Score module of the Sybyl-X 2.1.1.

Proposed Pharmacophore

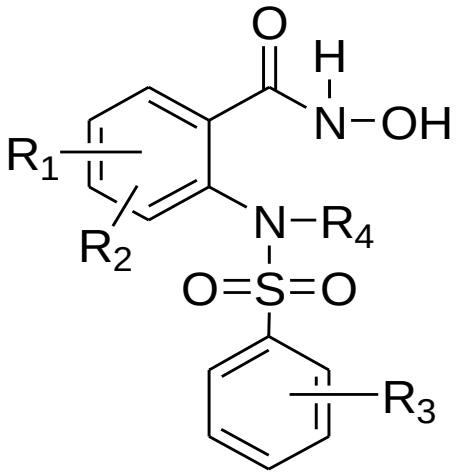
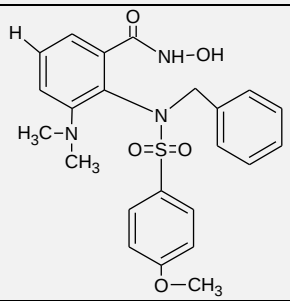
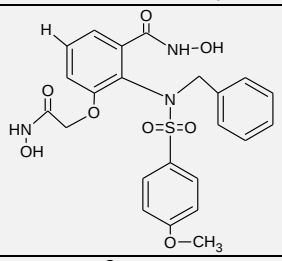
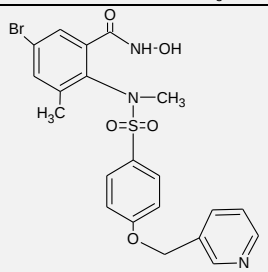
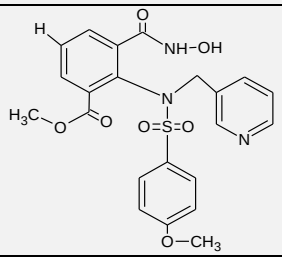
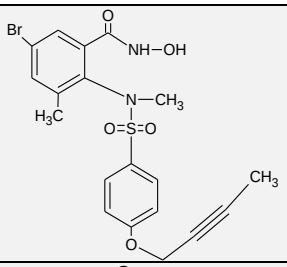
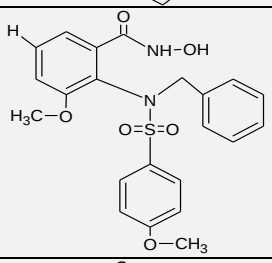
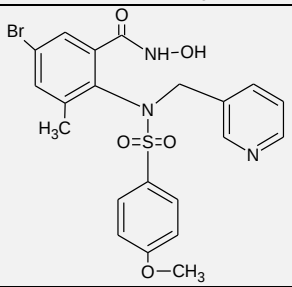
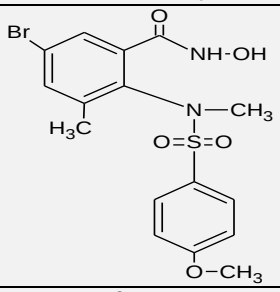
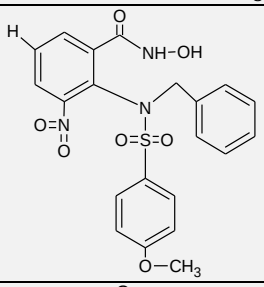
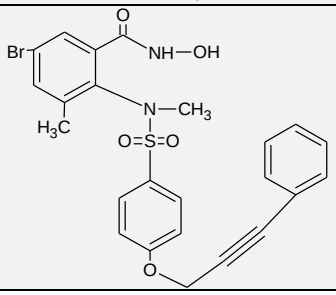
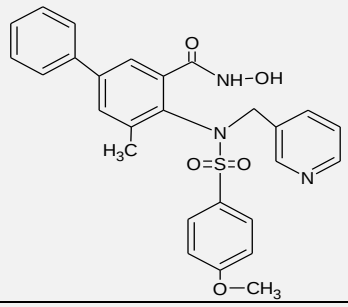
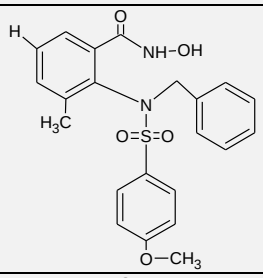
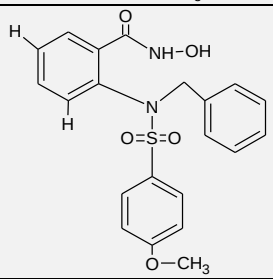
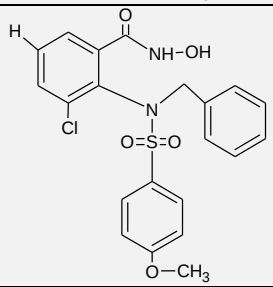
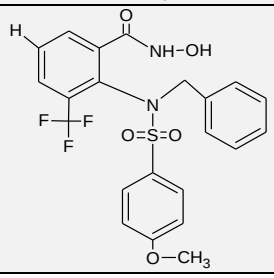


Figure: A.

Table No. 1: Docking scores of N-substituted sulfonamide anthranilate hydroxamic acids derivatives against Metalloprotease-1 target (MMP-1).

S.No.	Name	Structure	Total Score	G Score
1.	2-[(*N*-benzyl-4-methoxyphenyl) sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide		8.7082	-201.8899
2.	2-[(*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(2-(hydroxyamino)-2-oxomethoxy]-*N*-hydroxybenzamide		8.4257	-201.8968
3.	2-[methyl(4-(pyridine-3-yl methoxy) phenyl)sulfonamido]-5-bromo-*N*-hydroxy-3-methylbenzamide		8.3092	-227.8569
4.	Methyl-2-[{(4-methoxyphenyl)sulfonyl} (pyridine-3-ylmethyl)amino]-3-(hydroxy carbamoyl) benzoate		7.9097	-248.0594

5.	2-[4-(but-2-yn-1-yloxy)benzene sulfonamide]-5-bromo-3-methyl-N-hydroxy benzamide		7.89	-234.0791
6.	2-((*N*-benzyl-4-methoxyphenyl) sulfonamido)-3-methoxy-*N*-hydroxybenzamide		7.7403	-190.1758
7.	2-[{(4-methoxyphenyl)sulfonyl} (pyridine-3-ylmethyl)amino]-5-bromo-3-methyl-N-hydroxy Benzamide		7.6369	-229.3181
8.	2-[N-methyl-4-methoxy phenyl sulfamido]-5-bromo-3-methyl-N-hydroxy benzamide		7.5909	-138.0968
9.	2-((*N*-benzyl-4-methoxyphenyl) sulfonamido)-3-nitro-*N*-hydroxy benzamide		7.5287	-190.8948
10.	2-[n-methyl-4-((3-phenyl prop-2-yn-1-yl-)oxy)benzene sulfonamide]-4-bromo-3-methyl-N-hydroxy benzamaide		7.487	-155.3859

11.	2-[{(4-methoxyphenyl)sulfonyl} (pyridine-3-ylmethyl)amino]-5-phenyl-3-methyl-N-hydroxy benzamide		7.3979	-188.6607
12.	2-((*N*-benzyl-4-methoxyphenyl) sulfonamido)-3-methyl-*N*-hydroxy benzamide		7.1078	-141.7344
13.	2-((*N*-benzyl-4-methoxyphenyl) sulfonamido)-*N*-hydroxy benzamide		7.0271	-207.5761
14.	2-((*N*-benzyl-4-methoxyphenyl) sulfonamido)-3-chloro-*N*-hydroxy benzamide		6.8481	-247.7305
15.	2-((*N*-benzyl-4-methoxyphenyl) sulfonamido)-3-trifluoro methyl-*N*-hydroxybenzamide		6.8126	-172.1106

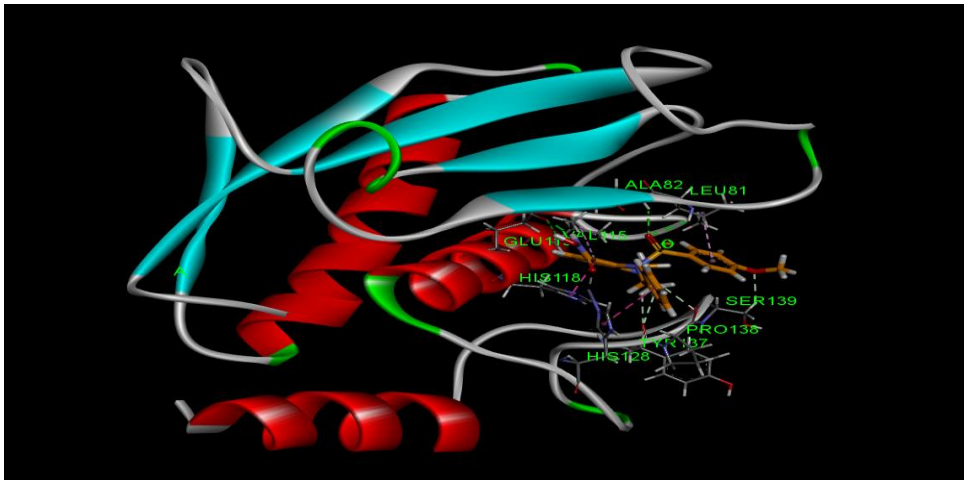


Figure 1: The interaction of the 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethyl amino hydroxybenzamide at the binding pocket of the MMP-1 (PDB ID: 3AYK).

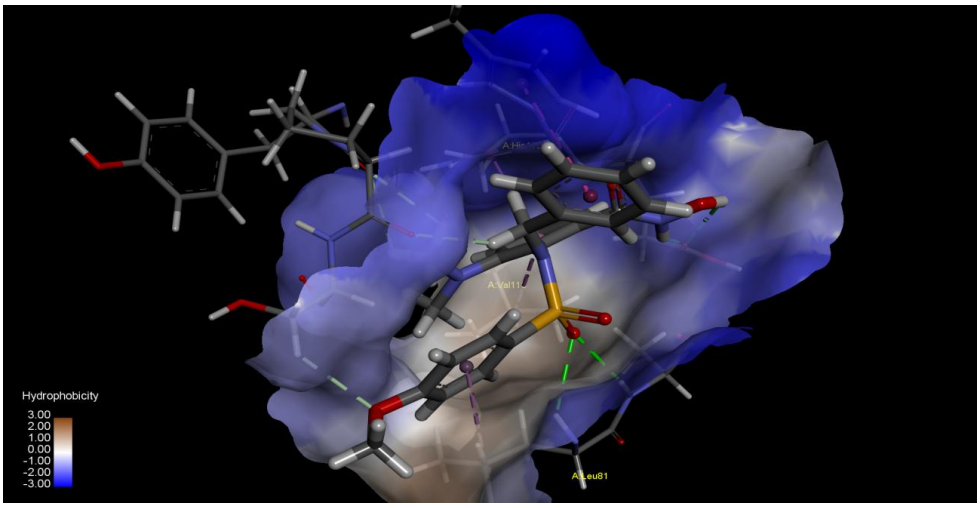


Figure 2: Hydrophobic interactions of 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*- : 3AYK hydroxybenzamide at the binding pocket of the MMP-1 PDB ID.

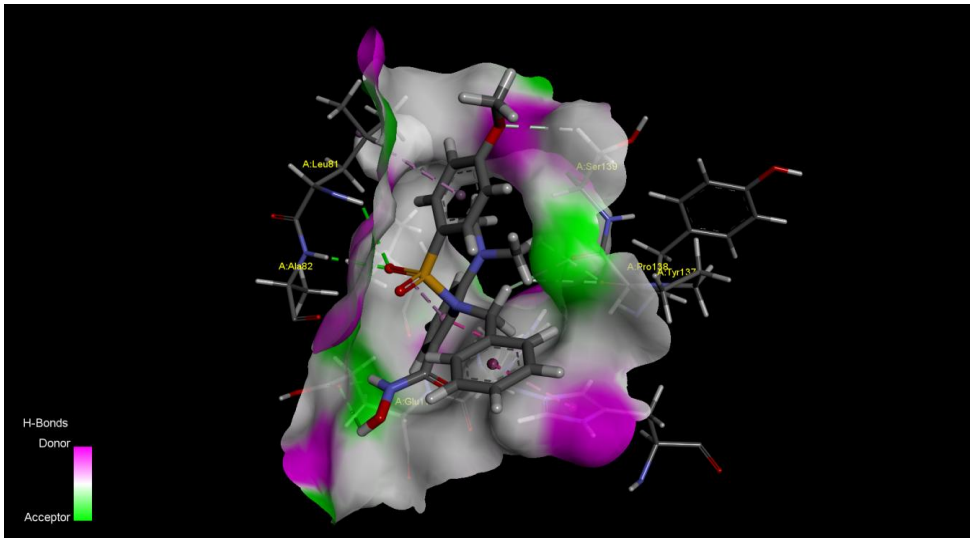


Figure 3: Hydrogen bond interaction 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*- hydroxybenzamide at the binding pocket of the enzyme PDB ID: 3AYK.

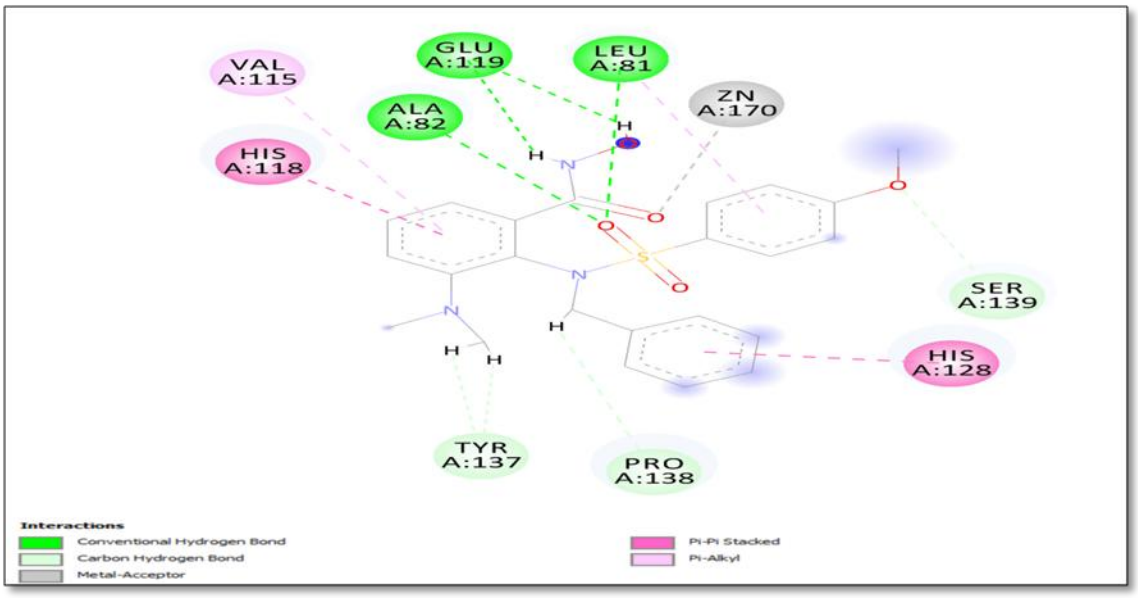


Figure 4: 2D interactions of the 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide at the binding site of the enzyme PDB ID: 3AYK.

RESULT AND DISCUSSION

Molecular docking was conducted in between the cancer target (MMP-1) and ligand, to elucidate their interactions and to obtain additional information in their molecular binding mode with the selected target (PDB ID: 3AYK). The results of molecular docking showing appropriate interactions with the main amino acid residues at the active site of the enzymes are shown in figures (1-4). The docking energy of the 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxy benzamide was found better score (8.7082). The interaction of the 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide with enzyme PDB ID: 3AYK is depicted in (Figure 1). The main interactions are the hydrophobic interactions with HIS118, VAL115, HIS128 (Figure 2). The most important residues in the binding pocket of enzyme PDB ID: 3AYK target was hydrophobic interactions with HIS118, VAL115, HIS128 as depicted in (figure 4). Also, the hydrophobic interaction is shown between hydrogens of -NH-OH of 2-((*N*-benzyl-4-methoxy phenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide with GLU119. The further oxygen atom of sulfonyl group of 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide shows hydrophobic interaction with ALA81 and LEU81.

All the figures indicate that the 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide molecule is embedded in the hydrophobic region of subdomains of selected targets. 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethyl amino)-*N*-hydroxybenzamide is encircled by the hydrophobic amino acid residues as shown in the figure (1-4).

Structural Activity Relationship (SAR) of N-substituted sulfonamide anthranilate hydroxamic acid.

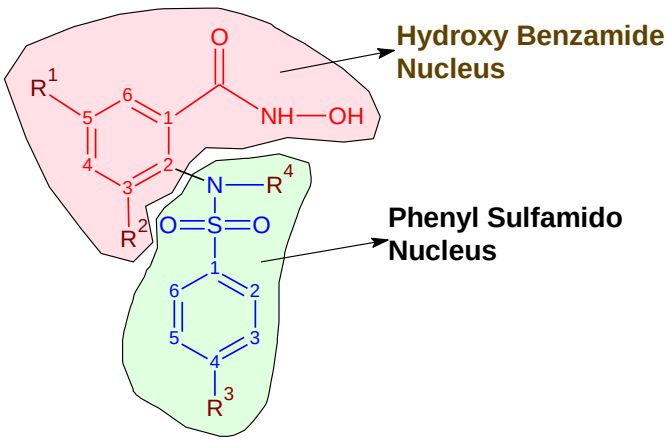


Figure 5: Structure of *N*-sustituted sulfonamide anthranilate hydroxamic acid.

From this study, it is clear that the phenyl sulfamido substitution at the second position of hydroxy benzamide nucleus is essential for the good binding on MMP target. N-methyl or N-benzyl substitution at phenyl sulfamido nucleus (i.e. R_4) increases the binding affinity towards amino acids like Histidine, they form a covalent bond with amino acids of MMP targets protein. Para substitution of methoxy group at Phenyl sulfamido nucleus (i.e. R_3) increases the binding affinity towards MMP targets. Substitution of electron donating species (like $-CH_3$,$-NH_2$, $-N(CH_3)_2$) at third position increases the binding affinity towards amino acids of MMPs targets by formation of strong Hydrogen bond. Substitution of electron with-drawing groups (like Br^-) at the fifth position increase the binding affinity towards amino acids (Histidine,Valine) of MMPs target. Substitution of any electron with-drawing species (eg.-CF_3,-NO_2, Cl^- etc.) at third position decreases the binding affinity towards MMPs target. Unsubstituted positions are 4^{th} and 6^{th} position in hydroxy benzamide nucleus and 2^{nd}, 3^{rd}, 5^{th}, 6^{th} positions in phenyl sulfamido nucleus. So, we can plan for the substitution of electron withdrawing groups as well as electron donating groups in the unsubstituted position for the further molecular docking studies.

CONCLUSIONS

We have selected the metalloprotease target by performing extensive literature studies and came to know that metalloprotease target is better for the binding of the drugs. After the selection of target, target validation was done by molecular docking studies. By performing molecular docking studies we have understood that there are fifteen new chemical entities found to be susceptible for binding with the Matrix metallo protease domain of the target. In that 2-molecules were having the better binding affinities towards MMPs. So, based on that we have derived Structural Activity Relationship (SAR). This SAR will be helpful for the further synthesis of N-substituted sulfonamide anthranilate hydroxamic acid molecules and then proceeding for *invitro* and *invivo* studies. Based on the SAR we have understood that the two new chemical entities 2-((*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(dimethylamino)-*N*-hydroxybenzamide and 2-[(*N*-benzyl-4-methoxyphenyl)sulfonamido)-3-(2-(hydroxyamino)-2-oxomethoxy]-*N*-hydroxybenzamide have the higher total score value of 8.7082 and 8.4257 respectively which indicates better binding affinity towards MMPs. By further funding we can go for the synthesis of better docked and binding molecules. Substitution of certain group like benzyl, amino, dimethylamino, methoxy, bromine, nitro increases the binding affinity either by forming hydrogen bond or by covanlent bond. Substitution of certain groups like but-2-yn-1-yloxy, trifluoro methane, hydroxy carbomyl leads loss in the binding affinity towards the MMPs target proteins. By these understanding we are proceeding for the synthesis. Future work will be planned for the synthesis and analysis of compounds/drugs for heat generation, lead generation,lead optimization and pre-clinical studies. So, *N*-substituted sulfonamide anthranilate hydroxamic acid molecules has a greater impact in the field of pharmaceutical chemistry, predicted to be used as MMPs inhibitors. From the total review, we can conclude that there was a diversified activity profile that occurred due to the changes in the various position of a molecule with the same N-substituted sulfonamide anthanilate hydroxamic acid group on the terminal. So, this single molecule can work furthermore and more with various other compatible groups not only in the field of cancer therapy but also other associated diseases.

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Competing Interest:
The authors declare no conflict of interest.

List of Abbreviations

AMBER .....- Assisted Model Binding with Energy Refinement
D score .....- Docking score
ECM .....- Extracellular Matrix
G score .....- Glide score
ICMR .....- Indian Council of Medical Research
MMFF94.....- Merck Molecular Force Field 94
MMP-1.....- Matrix Metalloprotease-1
NMR .....- Nuclear Magnetic Radiation
PDB.....- Protein Data Bank
PMF .....- Potential of Mean Force
SDF-1.....- Stromal cell derived factor-1
TACE.....- Tumor Necrosis Factor-α Converting Enzyme
TNF.....- Tumor Necrosis Factor

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