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**Original Article**

# Comparative docking of natural and synthetic inhibitors against the MMP-2

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## ABSTRACT

As important gelatinase, matrix Metallo-Protienase-2 is responsible for the cancer metastasis and invasion when overexpressed. So to inhibit the MMP-2 activity several inhibitors are available without satisfactory results. These available inhibitors are either natural or synthetic in terms of the resources. Here we compared their activity with docking against the MMP-2 on the basis of binding energy (BE). Data mining used to screen the inhibitors from available literature and data.

**Key-words:** MMP-2, MMP Inhibitors, Docking, Natural Inhibitors, Synthetic Inhibitors.

## INTRODUCTION

Matrix Metallo-Protienase (MMP) is a major class of proteolytic enzymes that plays a dominant role in ECM degradation. MMPs are regulated by sets of activators and inhibitors so that the integrity of the connective tissue is never compromised[1,2]. The extracellular matrix (ECM) is a complex structure that influences the behavior of its resident cells and migrating cells by providing specific contextual information. The ECM remains in a constant state of remodelling i.e, the breakdown of existing and synthesis of new ECM proteins. Thus ECM remodelliing can alter the interaction between the matrix and the cells. There are many factors which control the degradation of ECM. Literature survey showed that MMPs playing important role in the metastasis and the invasion and inhibition of their activity might be helpful in treatment of the cancer. Surveys showed that most of the patients are dying due to the invasion and metastasis of the cancer in distal organs.

On the basis of substrate specificity and primary sequence similarities, MMPs can be grouped into five subfamilies: collagenases (MMP-1, -8, and -13), stromelysins (MMP-3, -10, and -11), gelatinases (MMP-2, and -9), membrane-type MMPs (MMP-14–17), and others (MMP-7). Out these MMP-2 is reported as taking part in the metastasis and invasion of prostate cancer. While prostate cancer is second leading major cause of death in developed countries and also going to be in developing countries also.

There is no data available on the clinical evaluation of compound to inhibit the MMP-2 up-regulation completely. Although first generation MMPIs have shown promising pre-clinical and early clinical trial data, but they have failed to succeed in phase II and phase III trials[1]. However, more recent studies have led to a rethinking of the potential roles of MMP-2 in cancer progression. Therefore, it is needed to determine the role of MMP-2 in specific stages of tumor progression more precisely.

Mainly the regulation of MMP-2 performed by the tissue inhibitors of metalloproteinages (TIMPs), secreted by cell itself. Sometimes the TIMPs failed in regulation of the MMP-2 leading over expression of MMP-2. This over expression of MMP-2 in tumor or cancer results in invasion and metastasis and finally death of patient. In this condition the inhibitors are taken as drugs. These inhibitors on the basis of their sources divided as natural and synthetic inhibitors. Natural inhibitors are extracted from the natural resources and synthetic inhibitors are synthesized in laboratory. This is a comparative docking study of natural and synthetic inhibitors against the MMP-2.

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## METHODOLOGY

This study was performed with use of all the required facilities at Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, India. With aim to use important data about MMPs and their inhibitors docking

**Data Mining:** The advent of high-performance computing has benefited various disciplines in finding practical solutions to their problems, and our health care is no exception to this. Signal processing, image processing, and data mining tools have been developed for effective analysis of medical information, in order to help clinicians in making better diagnosis for treatment purposes. Data mining has become a fundamental methodology for computing applications in medical informatics. Progress in data mining applications and its implications are manifested in the areas of information management in healthcare organizations, health informatics, epidemiology, patient care and monitoring systems, assistive technology, large-scale image analysis to information extraction and automatic identification of unknown classes. Various algorithms associated with data mining have significantly helped to understand medical data more clearly, by distinguishing pathological data from normal data, for supporting decision-making as well as visualization and identification of hidden complex relationships between diagnostic features of different diseases. In recent years, data mining has been used widely in the areas of science and engineering, such as bioinformatics, genetics, medicine, education. In the study of human genetics, sequence mining helps address the important goal of understanding the mapping relationship between the inter-individual variations in human DNA sequence and the variability in disease susceptibility[2-5]. The Data mining techniques that are used in developing of new drugs are clustering, classification and neural networks. Total 34 Natural inhibitors and 49 synthetic inhibitors were found by data mining against MMP-2. These all the inhibitors were used inhibit the MMP-2 activities without specificity.

**Molecular Docking:** Pharmaceutical research has successfully incorporated a wealth of molecular modeling methods, within a variety of drug discovery programs, to study complex biological and chemical systems. The integration of computational and experimental strategies has been of great value in the identification and development of novel promising compounds. Broadly used in modern drug design, molecular docking methods explore the ligand conformations adopted within the binding sites of macromolecular targets. This approach also estimates the ligand-receptor binding free energy by evaluating critical phenomena involved in the intermolecular recognition process. Today, as a variety of docking algorithms are available, an understanding of the advantages and limitations of each method is of fundamental importance in the development of effective strategies and the generation of relevant results[6-9].

Parameter Optimization of Data Sets and Docking in view of better accuracy and extent of processing speed, Autodock Vina was used for performing the flexible docking of different data sets. Among different features, Autodock Vina offers around two orders more of processing speed in comparison to Autodock 4, which is critical for screening large collection of datasets. Among different advantages Autodock Vina offers include better binding model predicting ability, capability to function on parallelism and multithreading on multicore machines[10-12]. The accuracy and performance optimization were executed prior to *in silico* screening of different datasets under investigation. For making comparison between the orientations of docked and crystal bound ligands, native ligands of crystal structure were removed and docked into the binding cavity. The performance of Autodock Vina is robustly controlled by num modes and exhaustiveness and to enhance both the performance and precision in study three different combinations of num modes (10, 20 and 50) and exhaustiveness (10, 20 and 50) were evaluated. A combination of 50:10 of num_mode and exhaustiveness respectively were found to render optimal accuracy and speed which further was used for performing calculations[8-11]. To estimate the selectivity of activated and ground states towards agonists, antagonists and negative data sets, we docked the data sets in both activated and ground states. The ligand protein complexes were minimized using 200 Chimeras.

**Evaluation of Binding Energetics:** Molecular docking programs use scoring functions to estimate the binding energetics of the predicted ligand-receptor complexes. The energy variation, due to the formation of the ligand-receptor structure, is given by the binding constant (*K*d) and the Gibbs free energy ($\Delta GL$). Prediction of the binding energy is performed by evaluating the most important physical-chemical phenomena involved in ligand-receptor binding, including intermolecular interactions, desolvation and entropic effects. Therefore, the greater the number of physical-chemical parameters evaluated, the greater the accuracy of the scoring function[13-15]. Force-field-based scoring functions estimate the binding energy by summing the contributions of bonded (bond stretching, angle bending, and

dihedral variation) and non-bonded terms (electrostatic and van der Waals interactions) in a general master function[13-15].

## RESULTS

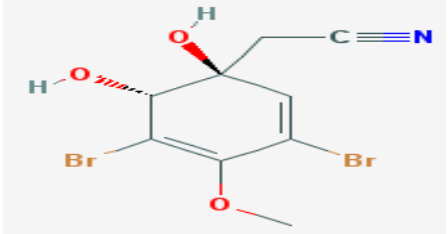
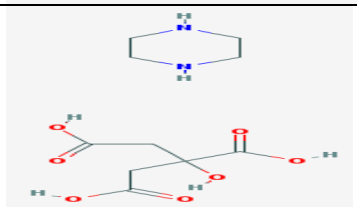
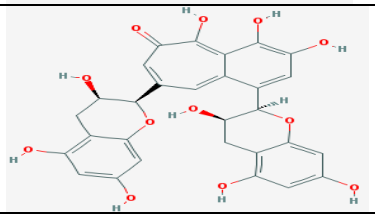
With the help of data mining, we have screened 34 natural and 49 synthetic inhibitors. Before performing the docking study with drug molecules it is necessary to understand the binding mechanism and binding patterns of known selected inhibitors as ligand. Docked ligands were arranged according to their binding energy and top five of each natural as well as synthetic inhibitors. Residues Ala136, Leu137 Ala139, His130, His124, His120, Pro140 Tyr142 and Ile141 identified very important for ligand binding which mainly involve in H-binding and hydrophobic interactions.

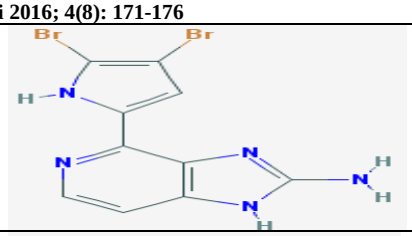
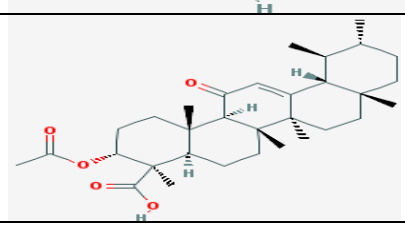
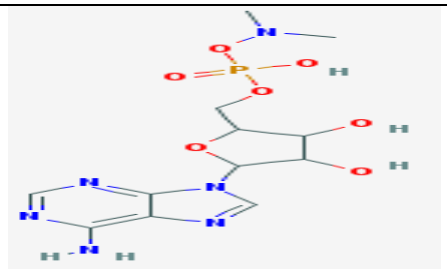
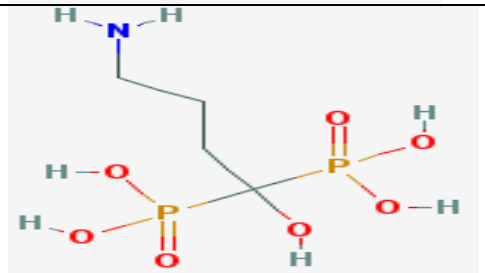
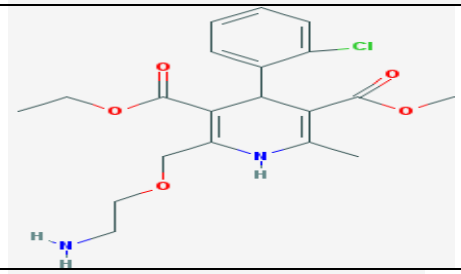
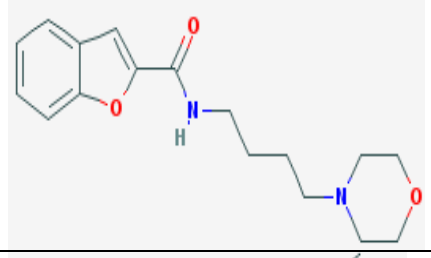
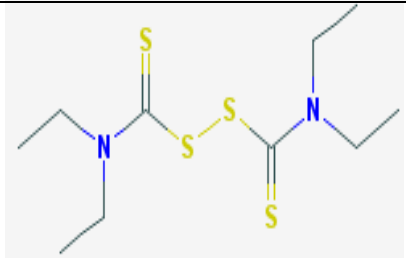
A protein-ligand docking program consists of two essential components, sampling and scoring. Sampling refers to the generation of putative ligand binding orientations/conformations near a binding site of a protein and can be further divided into two aspects, ligand sampling and protein flexibility. Scoring is the prediction of the binding tightness for individual ligand orientations/conformations with a physical or empirical energy function. The top orientation/conformation, namely the one with the lowest energy score, is predicted as the binding mode. Therefore, determination of the assessment of docking procedure in terms of accuracy and performance exhibited a low RMSD value (0.54 Å) which shows that the performed method was reasonable enough to screen the large data set with accuracy and speed.

Comparative Binding Energy of Natural inhibitors, synthetic inhibitors against MMP-2 are represented in Table 1. The analysis of the binding pattern demonstrated that all compounds fit comfortably in the binding pocket of MMP-2 and adopted bonded and non-bonded interactions very well. Mainly the H-bonding with conserved active site residues were found to be dominating.

**Comparative Binding Energy:** With objective to compare the docking results of natural inhibitor, synthetic inhibitors as well as the approved drug molecules of Drug Bank database PubChem, the comparative docking results are shown below in the Table 1. Docking results are promoting the repurposing of drugs rather than the exclusive drug discovered for the individual target protein.

**Table 1: Comparative BE of Inhibitors screened against MMP-2**

| **Natural Inhibitors** | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Name** | **BE (Kcal/Mole)** | **Molecular Formula** | **Chemical Structure** |
| **1.** | Aeroplysinin | **-8.2** | **$C_9H_9Br_2NO_3$** |  |
| **2.** | Piperazine citrate | **-8.0** | **$C_{10}H_{18}N_2O_7$** |  |
| **3.** | Theaflavin | **-7.4** | **$C_{29}H_{24}O_{12}$** |  |

| | | | | |
|---|---|---|---|---|
| **4.** | AGELADINE A | **-7.1** | **$C_{10}H_7Br_2N_5$** |  |
| **5** | 3-O-Acetyl-11-keto-beta-Boswellic Acid | **-6.2** | **$C_{32}H_{48}O_5$** |  |
| **Synthetic Inhibitors** | | | | |
| **Sr. No.** | **Name** | **BE (Kcal/Mole)** | **Molecular Formula** | **Chemical Structure** |
| **1.** | AC1L19BG | **-8.0** | **$C_{12}H_{19}N_6O_7P$** |  |
| **2.** | Alendronate | **-7.8** | **$C_4H_{13}NO_7P_2$** |  |
| **3.** | Amlodipine | **-7.4** | **$C_{20}H_{25}ClN_2O_5$** |  |
| **4.** | cl-82198 | **-7.0** | **$C_{17}H_{22}N_2O_3$** |  |
| **5** | Disulfiram | **-6.8** | **$C_{10}H_{20}N_2S_4$** |  |

Best docked conformation of Natural inhibitor **Aeroplysinin** showed -8.2 kcal/mole BE and showed high affinity towards protein residues Try142 involved in H-binding. While remaining residues Ala136, Leu137 Ala139, His130, His124, His120, Pro140 and Ile141 mainly contributing in weak interaction to stabilize the conformation (Figure 1). MMP-2 is represented by space fill model and wire form while ligand is represented by the ball and stick model in both the figures of individual ligand.

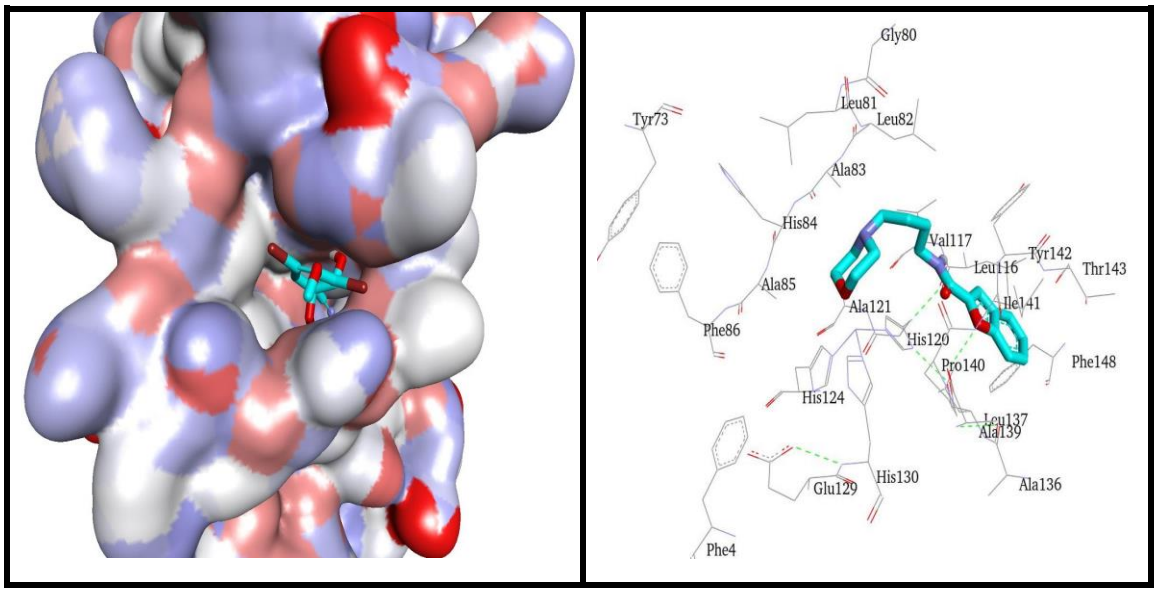



**Fig. 1: Docked conformation of Natural inhibitor Aeroplysinin**

## DISCUSSION

Prostate cancer is the most common cancer in North American men[16]**.** Although it is the second leading cause of death in men older than 60 years, only 2.9% of the new patients will die of prostate cancer. Among many factor responsible for the death of prostate cancer patients, matrix metalloproteinases are one of them. They are mainly usefull enzymes participating in several physicochemical activities of the call. They are produced by body itself for that useful purpose. When produces in excess they participated in several pathetic conditions leading to the death. Gelatinases are distinguished by their fibronectin-like gelatin-binding domain, which allows them to degrade nonfibrillar and enatured collagen. MMP-2 overexpression has been reported in many neoplasms including prostate, ovarian, rothelial, cutaneous, gastric, breast, and cervical cancers. MMP-2 may not only play important role in increased tumor aggressiveness but also be important in the activation of other proteases that are directly involved in tumor angiogenesis.

Das et al., reported higher expression of MMPs-2 upon exposure of human breast cancer and prostate cancer cells to fibronectin[17], and Meng et al., reported increased invasion of lung cancer cells through MMP-2 activity[18]. Similar results were obtained by Pentyala et al., who showed that the extracellular matrix plays a dominant role in gene expression[19], another protease system involved in ECM degradation[20]. Malik et al. also reported that fibronectin has been shown to support cell proliferation and invasion, which are major events for metastasis and to protect tumoral cells from the cytotoxic action of natural killer cells[21-22].

That imbalance of MMPs can be maintained by the tissue inhibitors of metalloproteinases (TIMPs). TIMPs act as the MMP inhibitors and secreted by the cell itself. The role of MMP-2 is important in prostate cancer metastasis, this causes death of the patient suffering from the prostate cancer. To maintain the balance it is a need to inhibit the activity of the MMP-2. Sometime the natural inhibitors TIMPs failed to maintain the balance, so it required providing some MMP-2 inhibitors from outside of the body as drugs. Several drugs are undergoing in trial but there is not even a single drug molecule to provide the satisfactory results after wastage of money as well as the precious time of the researchers. We have used docking studies in our work due to its faster development times and reduced risk attempts to pharmaceutical research and development timelines are often associated with increasing risk. However, the results showed

that the natural inhibitors which are extract or derivatives of the natural resources binds in better manner than the synthetic inhibitors (Laboratory designed), with low binding energy. Compared binding energy of all the natural and synthetic inhibitors, the best ligand Aeroplysinin showed the lowest binding energy (-8.2 kcal/mole) than other inhibitors. Even analysis also showed that the all the top five inhibitors of both groups, natural are better than synthetic inhibitors.

## CONCLUSION

Bioinformatics made revolutionary changes in the field of drug discovery process because we can predict the activity of any drug or compound against any protein computationally both the help of the programs available. This process saves money and time as well. Our study is also showed one fact that the natural inhibitors showed better results than the synthetic inhibitors indicating the benefits of the natural products. So the natural derivatives can be helpful in treatment of disease in better manner rather than the synthetic. Activities can be predicted computationally in short time and money, without any experiment using model organism with more use of bioinformatics technologies.

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