

Research Article

Designing and Development of Potent Drug Inhibitor to MMP12 in COPD Disease

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ABSTRACT:-

Chronic obstructive pulmonary disease (COPD) is the occurrence of chronic bronchitis or emphysema, a pair of commonly co-existing diseases of the lungs in which the airways become narrowed. This leads to a limitation of the flow of air to and from the lungs, causing shortness of breath (dyspnea). According to the World Health Organisation, COPD kills more people than HIV-AIDS, Malaria and Tuberculosis all put together in the South East Asian region. COPD was classified in two broad categories Chronic bronchitis and Emphysema. Although cigarette smoking is the main environmental risk factor for developing COPD, only about 15% of smokers develop clinically significant disease, suggesting that there are other influences on disease expression. Subsequent work has suggested other important proteases, such as the matrix metalloproteinases (MMP's), cathepsin B and collagenases may also play a role, perhaps as part of a protease/anti-protease cascade. MMPs are thought to be responsible for the turnover and degradation of connective tissue proteins, a function that is clearly performed by several family members. The mode of action of MMP12 protein plays a critical role for causing the COPD disease. The main objective of this paper is to design a small potent drug to block the MMP12 protein through 'Molecular Docking approach' by using the *In silico* tools.

Keywords:- COPD, MMP12, Docking, *In silico*

INTRODUCTION

In clinical practice, COPD is defined by its characteristically low airflow on lung function tests [1]. In contrast to asthma, this limitation is poorly reversible and usually gets progressively worse over time. COPD is caused by noxious particles or gas, most commonly from tobacco smoking, which triggers an abnormal inflammatory response in the lung [2]. The diagnosis of COPD requires lung function tests.

Important management strategies are smoking cessation, vaccinations, rehabilitation, and drug therapy (often using inhalers). Some patients go on to require long-term oxygen therapy or lung transplantation [3]. Chronic Obstructive Pulmonary Disease (COPD) kills more than 3 million people every year, making it the 4th largest cause of death in the world [4]. It has been estimated that by the year 2030, COPD

will become the third biggest cause of death. According to the World Health Organization, COPD kills more people than HIV-AIDS, Malaria and Tuberculosis all put together in the South East Asian region [5].

Worldwide, COPD ranked as the sixth leading cause of death in 1990. It is projected to be the fourth leading cause of death worldwide by 2030 due to an increase in smoking rates and demographic changes in many countries [6]. Although cigarette smoking is the main environmental risk factor for developing COPD, only about 15% of smokers develop clinically significant disease [6] suggesting that there are other influences on disease expression. Previous studies estimated that smoking contributes 15% to the variability of lung function, while genetic factors account for a further 40 % [7]. Thus the two influences together confer a different level of risk than that expected by simply adding them. In a complex disease such as COPD there are likely to be many genes contributing to the overall phenotype, which may have additive or synergistic effects; these are known as epistatic interactions. When interpreting the results of genetic studies in complex diseases it is important to take such effects into account, lest a disease causing locus be missed. There are a variety of statistical methods that can allow for, detect or control for the presence of epistasis [8]. The origin of this was the observation that patients with α 1-antitrypsin (an anti-protease) deficiency (AATD) develop early onset emphysema implicating a role for its target enzymes (neutrophil elastase and proteinase, which can induce many of the features of COPD in animal models [9]. Subsequent work has suggested other important proteases, such as the matrix metalloproteinase's (MMP's) , cathepsin B and collagenases may also play a role, perhaps as part of a protease/anti-protease cascade. The oxidant-antioxidant theory states that disparity between levels of harmful oxidants and protective antioxidants leads to oxidative stress, which in turn influences the

actions of anti-proteases, and expression of proinflammatory mediators. Both of these theories link to the third observation: the importance of inflammation in the pathogenesis of COPD [10]. These concepts are illustrated in

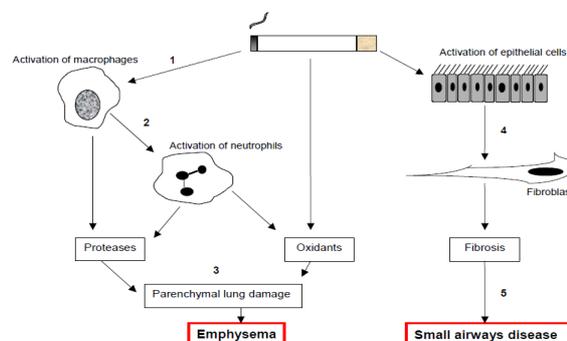


Fig:1. The pathogenesis of COPD

MMPs comprise a large family of extracellular enzymes that share common structural features, particularly those regions involved in the regulation of proteolytic activity. MMPs, or matrixins, are a subgroup of the much larger metalloproteinase superfamily, which also includes astacin, ADAM, and ADAMTS proteinases, among others [11]. Twenty-three different vertebrate MMPs have been cloned to date, and additional members continue to be identified. As their name suggests, MMPs are thought to be responsible for the turnover and degradation of connective tissue proteins, a function that is clearly performed by several family members. Indeed, cancer patients (including some with lung carcinomas) enrolled in clinical studies to assess the chemotherapeutic effects of a broad-acting synthetic metalloproteinase inhibitor developed reversible skin thickening and joint contractures [12]. These sclerotic side effects have been interpreted to indicate that some MMPs, directly or indirectly, are required for the normal catalysis of connective tissue. Although numerous biochemical studies have demonstrated that almost all MMPs can cleave or degrade some protein component(s) of the extracellular matrix, an ability to act on connective tissue protein is not a requirement for membership into the *matrix*

metalloproteinase family. MMPs are the part of large metalloendopeptidase superfamily. During an inflammatory response, leukocyte traffic through tissue barriers, including basement membranes, is only possible if these cells are equipped with enzymes that can remodel the ECM. MMPs are therefore crucial effector molecules of inflammatory cells. However, MMPs can also modify cytokines and chemokines. MMPs can act as switches or as delicate tuners in acute and chronic inflammation, during autoimmune diseases, when triggered in vascular diseases and in their regenerative phase after inflammation [13]. Thus, MMP biology is important in the initiation, execution and resolution phases of acute and chronic inflammatory and ischemic processes and consequently, MMP inhibitors might interfere with these. Both macromolecular inhibitors (natural TIMPs and monoclonal antibodies) and small molecules (synthetic and natural products) have been considered as potential therapies for diseases in which excess MMP activity has been implicated. However, technical difficulties with the biotechnological production of macromolecular proteins and limited patient compliance because of parenteral administration have often been cited as limitations for their development. Nevertheless, monoclonal-antibody derivatives are promising drugs to be used as therapeutics, especially if a high MMP specificity is required [14].

NATURAL MMP INHIBITORS

The negative results obtained in human clinical trial analyzing synthetic MMP inhibitors stirred up the impellent need for compounds that could be more effective in treatment and this search found its answer in the field of natural compounds [15]. In Chinese medicine, tea is a fundamental element and it is considered one of the most potent substances that can help maintain health and prolong life, and these health benefits have been scientifically established and are related to the presence of polyphenols called “*flavonoids*”. Tea

(*Camelliasinensis*) is available in three forms: black, green and oolong, (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG). Away by which EGCG may decrease tumor size is through the binding to urokinase, which is a proteolytic enzyme, often over expressed in human cancer, necessary for invasion and metastasis of cancer cells [16].

The potential medical benefits of consuming green tea have received a great deal of attention over the past few years, most of which has been directed at a group of polyphenolic compounds called catechins. These are condensed into tannins in black tea, and are found in sources other than green tea, such as grape skins and seeds. The catechins are antioxidants, and are probably largely responsible for the reported antioxidant effects of green tea. They have also been found to have anti-inflammatory properties, which may be due to their ability to inhibit tumor necrosis factor (TNF) synthesis, possibly by the inhibition of kinase(s) in signaling cascades, leading to activation of certain transcription factors. They are also inhibitors of matrix metalloproteinases. The reported beneficial effects on a number of clinical conditions, including stroke and cerebral hemorrhage, cardiovascular and liver diseases, bacterial infections, stomach ulcers and cancer may be related to their antioxidant, anti-inflammatory and antiproteinase properties [17]. In COPD studies, use of high-throughput techniques for gene and protein expression profiling and of computerized databases has become a mainstay of biomedical research. There is a need to perform omics studies on patients with COPD, describing the association with the disease in terms of specificity, severity, progress and prognosis and monitoring the efficacy of therapies. These omics analysis highlight the ways to investigate protein profiles of cells, biopsies and fluids, explore protein-based mechanisms of human diseases, identify novel biomarkers for diagnosis, therapy and prognosis of multiple

diseases and discover new targets for drug development [17].

MATERIALS AND METHODS

The protein Matrix metalloproteinase 12(MMP12) was responsible for the disease. The MMP12 sequence was retrieved from the NCBI(National centre for Biotechnology Information) database with accession number NP_002417, which is having length of 470 amino acids.

Structural Analysis Prediction

Primary, secondary and Tertiary structure analysis of MMP12 protein to know about the physico-chemical properties, helix and coils and pdb ids on the basis of the least e-value and identities by the tools and databases.

Prediction of Protein Tertiary Structure

In first step tertiary structure of MMP 12 protein was predicted through CPH Model, HH Pred and Geno 3D. Identification of common PDB ID through these results. CPH Model (Comparative Protein Homology Modelling) is a protein homology modelling server. It was used to identify the common PDB ID on the basis of its score and it's E-value (<http://www.cbs.dtu.dk/services/CPHmodels/>). HH Pred is a free protein function and protein structure prediction server that is based on the HH search and HH bits (<http://toolkit.tuebingen.mpg.de/hhpred>). Geno3D is an automated web server for protein molecular modeling(http://geno3dpbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=/GENO3D/geno3d_home.html). By comparing these results 3BA0 was the common PDB ID for the MMP12 in COPD Disease.

Preparation of Drugs

The drugs were prepared from the natural compounds. Hence there are various sources for selecting the natural compound such as Natural Products, Plant sources, Antimicrobial sources and Marine sources. among all these catechins was selected from clinical conditions,

including stroke and cerebral hemorrhage , cardiovascular and liver diseases , bacterial infections , stomach ulcers and cancer may be related to their antioxidant, anti-inflammatory and antiproteinase properties. The CID files of the Ligands were downloaded from PUBCHEM. Pubchem is a Database of chemical molecules and their activities against biological assays. 50 ligands were prepared in CHEMSKETCH software.

Screening of Drugs

From the library 50 drugs were screened and obtained the molecular properties such as Molecular weight, Molecular formula, Hydrogen acceptors, Hydrogen Donors and Aromatic Rings with the help of Accelrys Discovery Studio in which all properties should be follow the Lipinski's Rule of 5.

The drugs were screened on the following basis

1. Lipinski rule of 5
2. ADME/TOPKAT
3. Energy Minimization

50 Drugs were selected to inhibit the matrix metalloproteinases, all the drugs were passed in molecular property test according to the Lipinski's rule of 5. High throughput screenings which are noncarcinogenic and these are passed through ADMET. From these 4 potential drugs were selected as non-toxic according to their TOPKAT and ADMET parameters.

Molecular Minimization

The molecular minimization studies were carried out both 3BA0 receptor and 4 successful drugs. This step was mainly for the stability of the receptor and ligand molecules. The molecular minimization step was done in ADS (Accelrys Discovery Studio) software.

Docking studies

In the present study the nature of interactions, binding mode and selectivity of 3BA0 protein with the ligands docking was carried out with ADS, HEX and Ligand scout software.

ADS 2.5

Discovery Studio is a well-known suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Accelrys.

It is typically used in the development of novel therapeutic medicines, including small molecule drugs, therapeutic antibodies, vaccines, synthetic enzymes, and areas such as consumer products. It consists of 17-33 software. Molecular minimization studies, High through screening studies and docking studies of 3BA0 was carried out by this software.

Hex 6.3

HEX is an interactive protein docking and molecular superposition program. Hex understands 3BA0 protein and DNA structures in PDB format, and it can also read small molecule SDF files.

Ligand Scout 2.0

LIGANDSCOUT is a software tool that allows to model 3D pharmacophore models from structural data of macromolecule/ligand complexes or from training and test sets of organic molecules. It incorporates a complete definition of three-dimensional chemical features (such as hydrogen bond donors, acceptors, lipophilic areas, positively and negatively ionizable chemical groups) that describe the interaction of a bound small organic molecule (ligand) and the surrounding binding site of the macromolecule.

These pharmacophores can be overlaid and superimposed using a pattern- matching based alignment algorithm that is solely based on pharmacophoric feature points instead of chemical structure. From such on overlay, shared features can be interpolated to create a so-called shared-feature pharmacophore that shares all common interactions of several binding sites/ligands or extended to create a so called merged-feature pharmacophore. The software has been successfully used to predict new lead structures in drug design.

RESULTS

Prediction of Protein Tertiary Structure

The results were obtained from CPH model, Geno3D and HHpred is shown as follows in [Table-1].

Name of Server	PDB ID	E-Value	Score	Identities
CPH Model	3BA0	0.00	994.5	99%
Geno3D	Pdb3ba0A	e-171	99%	99%
HHPred	3ba0_A	3.7E-93	726.1	99%

Table: 1. CPH Model, Geno3D and HHpred Results.

RASMOL

Rasmol is a computer program written for molecular graphics visualization intended and used primarily for the depiction and exploration of biological macromolecular structures, such as those found in the Protein Data Bank, by given command in the command window. To identify the regions of Helix, sheets, various amino acids. 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. Docking is frequently used to predict the binding orientation of drug candidates to our protein target (receptor) in order to in turn predict the affinity and activity of the drug molecule.

DOCKING IN ACCELERYS DISCOVERY STUDIO

After the minimization of all drugs and receptor, docked TOPKAT and ADMET filtered drugs with our receptor- 3BA0 to find orientation with Receptor Binding Pocket with the help of Discovery Studio. Two poses were obtained and found the lowest Cdocker energy shows in [Table-2] and Docking between 3BA0 and ligand shows in [Figure-2] and C docker complex with shows in [Figure-3].

Pose	Name	Absol.Energy	Conf.Num	Mole.Num	Rel.Energy	Pose Num	LibDock Score
1	Mole.1	42.2543	22	1	10.0946	1	58.8811
2	Mole.2	42.2831	21	1	10.1233	2	56.8434

Table: 2.Lowest C Docker Energy.

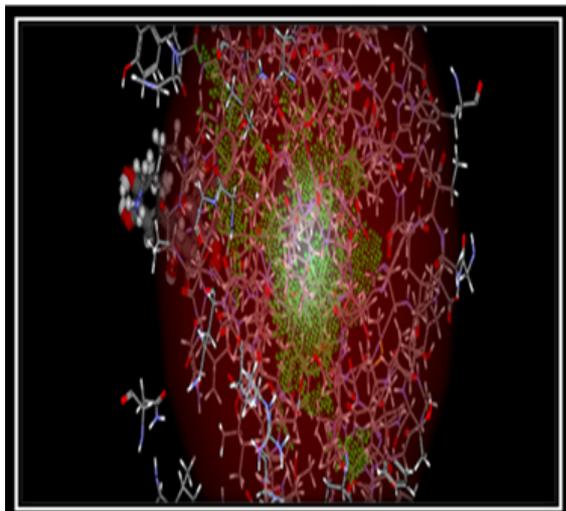
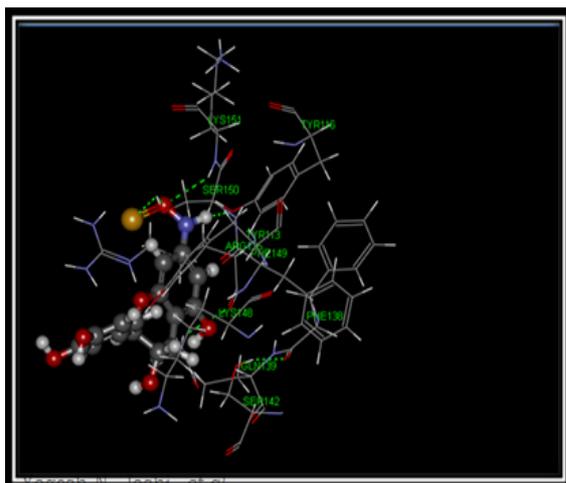


Fig: 2. Docking between 3BA0 recetor and Ligand



DOCKING IN HEX

Docked the best drug (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-3,4-dihydro-2*H*-chromen-7-yl]amino(thioxo), which having a least Cdocker energy in Hex, to study the Pharmacophore Analysis. In Hex, E.min and E.max energy was found. The best molecule was identified and further evaluated by molecular dynamics simulation of protein-ligand complex. Minimized energy of receptor is 56.8434. The docked ligand with the receptor and obtained 2 poses and their Cdocker energy, the drug (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-3,4-dihydro-2*H*-chromen-7-

yl]amino(thioxo) was found which have lowest Cdocker energy -208.44 Kcal/mol at 2th pose, then other drugs. The Docking between the 3BA0 and ligand is shows in [Figure-4]

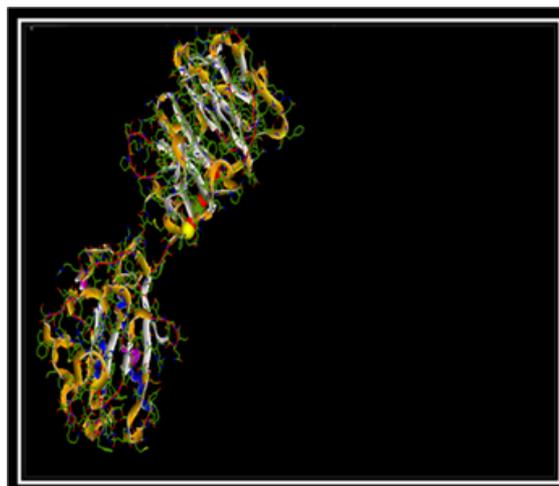


Fig: 4. Hex docking between the 3BA0 and Ligand

Pharmacophore analysis

From the docked complex of pharmacophore the hydrophobic, aromatic,hydrogen bond receptor, a hydrogen donor, a cation, or an anion. On the basis of assays, best molecule was found, having a lowest binding energy in

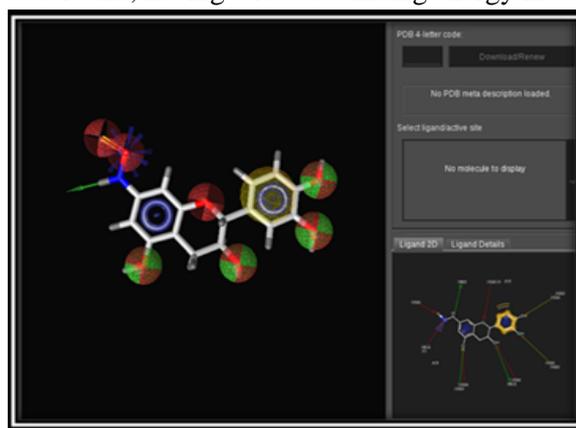


Fig.5: Pharmcophore analysis of ligand

CONCLUSION

The studies on the causes for the disease were conducted though docking and pharmacophore studies on MMP-12(Matrix metalloproteinase-12) protein. Ligand for the protein was the daughter molecule designed fromreferring

natural ligand i.e (2*R*,3*S*)- 2- (3,4-dihydroxy phenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol. Docking studies were successfully performed by binding the ligand at the binding site of the receptor and thus inhibiting the protein to express that causes COPD.

The drug was constructed by modifying it to have less toxic effect and more efficient binding. Daughter Molecule passed through all the necessary requirements such as ADMET and TOPKAT tests. The IUPAC name of designed drug was (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-3,4-dihydro-2*H*-chromen-7-yl]amino(thioxo). It can be used as the potential drug helpful in curing COPD. This drug can be made available commercially only after passing through different phases of clinical tests and FDA approval.

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