

Molecular Docking of Fragment Library and Triazoles against Protease Protein of *Mycobacterium fortuitum*

Project thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of
Technology

in

Bioinformatics

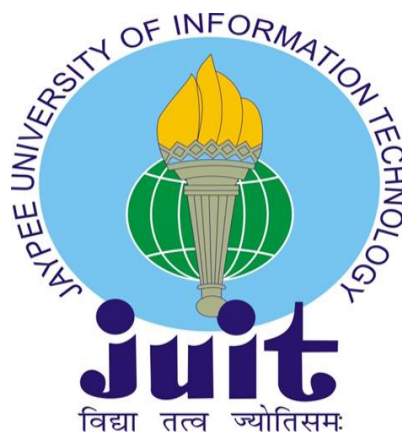
By

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Under the supervision of

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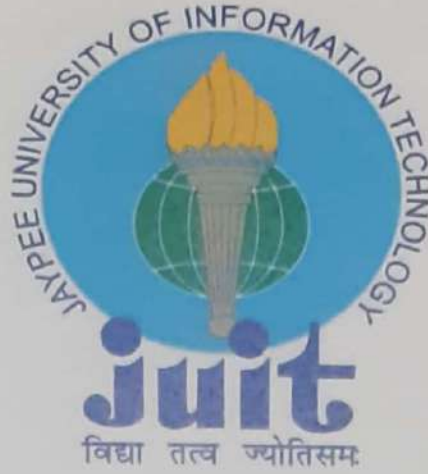
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CERTIFICATE

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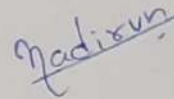
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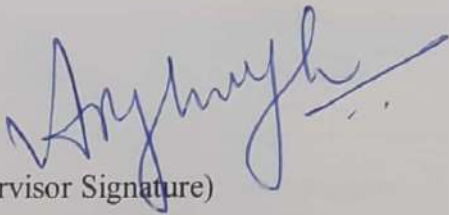
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LIST OF ABBREVIATIONS

1. MTBC – *Mycobacterium tuberculosis* complex
2. NTM – Non-tuberculous Mycobacteria
3. EPS – Eexopolysaccharide
4. IND – Investigational New Drug Process
5. CADD – Computer Aided Drug design
6. SBDD – Structure Based Drug Design (SBDD)
7. LBDD – Ligand Based Drug Design (LBDD)
8. FBDD – Fragment Based Drug Design (FBDD)
9. HTS – High Throughput Screening
10. NMR – Nuclear Magnetic Resonance
11. SPR – Surface Plasmon Resonance
12. DS – Discovery Studio
13. USCF – University of California, San Francisco
14. HBD – Hydrogen Bond Donors
15. HBA – Hydrogen Bond Acceptors
16. cLogP/cLogD – Computed Logarithm of The Partition or Distribution Coefficient
17. PSA – Polar Surface Area
18. PDBQT – Protein Data Bank, Partial Charge (Q) &, Atom Type (T)

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ABSTRACT

Mycobacterium fortuitum is a nontuberculous species of the phylum Actinomycetota. *M. fortuitum* is an important human pathogenic NTM, which resists stress conditions inside macrophages by exploitation of virulence specific genes. A significant part of the host organism's infection is caused by biofilms. One of the biggest functional groups of proteins is the protease family. Proteases are enzymes that break down proteins into smaller peptides or individual amino acids. Since proteases are frequently secreted into the microbial environment, they make excellent markers for the detection of bacterial infections. Protease protein is one of the proteins that *M. fortuitum* upregulates during the biofilm growth phase.

Fragment-based drug design (FBDD) is a typical approach in the field of drug development, and it entails screening tiny organic molecules against a target protein to identify binding fragments that can be further refined into lead compounds. The core advantage of FBDD is that it focuses on the critical interactions between the ligand and the protein, which lowers chemical complexity and library size while raising hit rate and optimising efficiency. FBDD can provide a powerful and efficient strategy for discovering innovative therapeutics against *M. fortuitum* by concentrating on the active region of the protease protein of that organism with small fragments.

The triazole nucleus is one of the most significant and well-known heterocycles, and it is a common and essential component of a variety of natural products and therapeutic agents, according to numerous studies. Triazoles work by blocking an enzyme involved in building the bacteria's cell membrane, which prevents its growth.

Using the Bioinformatic tools we can find the potential drug like components to combat the disease of *M. fortuitum*. A Library of Fragments & Triazoles and their derivatives will therefore be subjected to molecular docking against the protease protein of *M. fortuitum* in order to suggest suitable ligand molecules with low binding energies.

CHAPTER-1 INTRODUCTION

1.1 Introduction

The Mycobacteriaceae family includes the genus Mycobacterium, which is distinguished by a thick, hydrophobic cell wall that contains mycolic acids. There are 188 species in this genus [1], which is broadly divided into the *Mycobacterium tuberculosis* complex (MTBC) and Nontuberculous Mycobacteria (NTM) based on their pathogenesis. Due to their rising infection rates and diverse varieties of virulence among diverse hosts, including humans, NTM have attracted a lot of attention in recent years[2], [3] . Based on their generation time, NTM can be divided into rapid-growers and slow-growers. NTM have a variable geographic distribution, species spectrum, set of clinical manifestations, and antibiotic susceptibility profile, which makes it difficult to recognise and diagnose them [4]. In accordance to the NTM species, different treatment regimens, drug resistance patterns, and treatment outcomes are currently advised. This results in a time-consuming and difficult management of these infections with few therapeutic options.

Mycobacteria, numerous saprophytes, and known pathogens are all members of the Mycobacterium genus. These latter are pervasive in nature and can be found in soil and water. The gastrointestinal tracts of some humans and animals contain some of them. Actinomycetota's *M. fortuitum* is a non-tuberculous species. One of the most widespread and quickly spreading pathogenic NTMs in the world, *M. fortuitum* causes a variety of infections. Despite convincing evidence to the contrary, *M. fortuitum* is frequently considered to be a saprophytic living organism only. The formation of biofilms is a characteristic trait of Mycobacteria. Mycobacteria have many advantages over planktonic growth patterns due to their capacity to live in biofilms. For instance, Mycobacteria that grow in biofilms and are resistant to environmental attack seem to have environmental sources.

The prevalence of *M. fortuitum* infections is rising, but there is very little information available about its pathogenesis and virulence factors. In order to identify novel drug targets and potential inhibiting sites/residues of the targets, as well as to gain a better understanding of the molecular mechanisms underlying *M. fortuitum* pathogenesis, it is necessary to examine the various interactions of *M. fortuitum* proteins that play a significant role in its pathogenesis. This will pave the way for the development of new drugs as therapeutics.

A study by one of our University's PhD scholars stated that Protease Protein was upregulated in the *M. fortuitum* biofilm formation process. Protease protein has been chosen as the target protein in order to perform molecular docking with two libraries, one a library of fragments having molecular weight less than 300 Daltons and another a library of triazoles & its derivatives taken as the ligand molecules.

All the methodologies and resources adopted to perform molecular docking and analyse the results are well explained in this thesis. The thesis highlights about the genus Mycobacteria, the infections that are generally caused by *M. fortuitum*, role of biofilms and proteases in biofilms, and the base crust that our project is built on i.e., drug discovery and importance of molecular docking which is a part of computer aided drug designing compared to the traditional approach.

1.2 Problem Statement

A significant part of the host organism's infection is caused by biofilms. Our project focuses on finding molecules and potential target sites residues with the least amount of binding energy that can prevent protease protein regulation and, as a result, prevent the development of biofilms.

1.3 Objectives

The aim of our project is to perform Molecular Docking of a Library of Fragments, and a library of Triazoles and its derivatives against Protease Protein of *M. fortuitum* so as to propose suitable ligand molecules with least binding energies.

1.4 Methodology

- Study on the target molecule – Protease Protein
- Ligand Preparation
- Blind Docking
- Comparing Binding Energies of the ligands output files generated
- View residual interactions in both 2D and 3D

1.5 Organization

The Thesis consists of the following chapters –

Chapter 1: A brief summary of the project is provided in this chapter. Additionally, it provides an overview of the bacterium *M. fortuitum* and the infectious function of its own protein taken as target protein, whose structure was created using the idea of homology modelling. This chapter also covers the project's overall problem statement and objectives. A brief summary of the project's methodology is also provided in this chapter, along with a description of the steps involved in molecular docking of the target molecule with the library of ligands.

Chapter 2: Information on *M. fortuitum* and various types of infections it causes, *M. tuberculosis* complex, non-tuberculous Mycobacteria, and genus Mycobacteria in general are all covered in this chapter. It includes a thorough explanation of biofilms, their stages of formation, and specifically the biofilms produced by Mycobacterium, as well as details on how they contribute to infection. The significance of proteases in biofilms is also covered in this chapter. The importance of drug discovery, its process and comparison of computer aided drug design with traditional methods is well explained. Additionally, software products for docking like Autodock, Autodock Vina, and Pyrx are covered in literature.

Chapter 3: This chapter provided information on the steps we would take to advance the project's goal methodically. The preparation of ligands and molecular docking are both well covered. Details on the features to be chosen at each stage of ligand preparation and molecular docking are provided in this chapter. The chapter also contains all information about softwares that are used besides those for docking.

Chapter 4: In this chapter we are providing with the binding energies of the docked molecules and have proposed the molecule with the least binding energy among the whole. This chapter details the result of best-chosen ligands taking in account the residual interactions. The results at different levels desired i.e., in 2D and 3D format are also given. The docked poses of ligands (both from fragment library and triazole derivatives) with the lowest binding energies among the molecules for which docking has been done are presented throughout the entire chapter.

Chapter 5: The entire conclusion of the work included in this project thesis is contained in this chapter. It provides information on how to enhance the project and what we can do going forward in line with the research objectives and its enhancement. In this chapter we have conclude least binding energy molecule to be the potential drug that can be used in the future for the treatment of disease related to *M. fortuitum*.

CHAPTER-2 LITERATURE SURVEY

2.1 Genus Mycobacterium

The only genus in both the Actinomycetales order and the Mycobacteriaceae family is Mycobacteria. The NCBI genome, a database has the genome sequences of nearly 150 different members of this genus [5]. Mycobacteria are rod-shaped, Gram-positive, catalase-positive bacteria measuring 0.2 to 0.6 mm in width and 1.0 to 10 mm in length. Additionally, they do not generate spores and are not mobile. Different species of Mycobacteria have distinct colonial structures, with some forming tough or homogeneous colonies. A colony may be white, orange, or pink in colour. Even while certain Mycobacterial species are microaerophilic, majority of them belong to aerobic organisms [6].

Mycobacteria have four layers of cell walls that are extraordinarily thick. The innermost layer is made of peptidoglycan, whereas the outer layers are made of lipids. Since lipid is present, the bacterium can thrive both in acidic and alkaline conditions. Lipid also makes the bacteria's cells somewhat resistant to different basic dyes. Mycobacteria tend to clump together and stay on the surface of liquid substances due to their cell walls being hydrophobic. They typically form fungus-like pellicles on fluid culture media as a result of their hydrophobic nature: "Fungus bacteria" are referred to as Mycobacterium[6].

Mycobacteria may be important in the degradation of organic matter, particularly in sphagnum marshes, despite the fact that there are no instances of their making food go bad. They don't significantly produce dangerous chemicals or contaminate food. Pathogenic strains are aggressive because they can defeat immune defences, which results in recurrent infections [6].

The slow rate of reproduction of these bacteria, with a generation time of about 20 hours, means that it may take up to six weeks to isolate and identify them. However, in some cases, certain species may grow in as little as 5 to 7 days. These bacteria are acid-alcohol fast, so even when exposed to powerful mineral acids or acidified alcohol after staining, they do not lose their colour. It is essential to recognise Mycobacteria by their characterization of acid-fastness, which would be caused by waxy elements in the cell walls. Other Gram-positive bacteria require careful staining of their cell walls [6].

Infections from infected individuals are not considered a significant risk factor because many of the non-mycobacteria present are saprophytes, and some of them are commensal bacteria commonly found in animals [6].

2.1.1 Classification of genus Mycobacteria

2.1.1.1 *M. tuberculosis* complex

For countless generations, a group of bacterial subspecies closely related to each other, called the *M. tuberculosis* complex (MTBC), have affected both human and animal populations [7].

The closely related bacteria that cause tuberculosis in humans as well as animals make up the *M. tuberculosis* complex (MTBC). One of the most prevalent causes of mortality and morbidity in the world today is still this illness. The mycobacteria enter the body of the host through the air, and once inside the lungs, macrophages phagocytate them [8].

The main constituents of the MTBC (*M. tuberculosis* complex) include *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canettii*, *Mycobacterium microti*, and other similar species [9].

Other MTBC group members, with the exception of *M. tuberculosis*, are related genetically and have about 90% nucleotide resemblance. *M. tuberculosis*, *M. africanum*, and *M. bovis* primarily infect animals, whereas *M. microti* and *M. bovis* mainly infect people, despite the fact that all members of the MTBC are genetically linked [10].

2.1.1.2 Non-tuberculous Mycobacteria

Non-tuberculous Mycobacteria, or NTM, are Mycobacteria that are not a member of the *M. tuberculosis* complex and do not cause leprosy [11]. Microorganisms known as non-tuberculosis Mycobacteria are found in soil, dust, and water, including both regulated and unregulated sources (the water that people drink or shower in such as reservoirs, streams, and rivers). These sources contain water sources that are both natural and man-made [12]. Biofilms, microbial assemblages that adhere to surfaces in moist environments, such as the sewage in buildings, can be created by non-tuberculosis Mycobacteria [13]. Chronic bone and joint

infection are known to be brought on by Mycobacteria, whether tuberculous and nontuberculous [14].

2.2 *Mycobacterium fortuitum*

Actinomycetota's *M. fortuitum* is a non-tuberculous species. Despite convincing evidence to the contrary, *M. fortuitum* is generally considered to be a saprophytic live organism solely. It is a widespread bacterium which can be found in wastewater, soil, and both untreated and treated water [15].

It is a microorganism that quickly multiplies and spreads disease easily. Although it can also cause localised skin disorders, osteomyelitis (bone inflammation), bone infections, and eye infections following trauma, *M. fortuitum* seldom causes pneumonia [16].

Nosocomial *M. fortuitum* infection is possible. After the damage is either directly or indirectly related to tainted tap water from any tainted source. Implanted devices including catheters, site of injection abscesses, and infected endoscopes are further possible sources of *M. fortuitum* infection [15].

2.2.1 Types of infections caused by *M. fortuitum*

M. fortuitum cause a broad range of infections. Infections due to *M. fortuitum* includes both pulmonary as well as extrapulmonary infections, among which few are mentioned.

2.2.1.1 Skin and soft tissue infection

M. fortuitum has established a reputation as one of the pathogens connected to skin as well as soft tissue infection. It results in cutaneous infections following surgery or trauma that can spread to the lungs, lymph nodes, bones, joints, and meninges, often fatally, in people with compromised immune systems [17]. Due to *M. fortuitum*, post-breast implant breast infections are common [17], [18]. *M. fortuitum* enters through puncture wounds, and the results can range from ulceration to cellulitis to abscesses to even draining sinus tracts [19] [20].

2.2.1.2 Blood stream infections

M. fortuitum is a major source of blood-borne infections due to its high propensity to colonise and infect intravascular catheters and other intravenous devices. Early identification of species in bloodstream and testing for antibiotic susceptibility can be used to treat *M. fortuitum* blood-borne infections [21].

2.2.1.3 Bone infections

Variety of bone infections like osteomyelitis, otitis media, etc are reported to be caused by *M. fortuitum*. following medical treatments such as surgery, stem cell transplant, and trauma. It has also been reported that *M. fortuitum* causes spinal osteomyelitis in drug users who inject their drugs [22], [23]. Bone infections caused by *M. fortuitum* can sometimes be treated with surgery and last for six weeks; however, treating a dual infection with *M. tuberculosis* and *M. fortuitum* takes about nine months of medication.

2.2.1.4 Disseminated infections

Disseminated infection is the medical term for the spread of an infection from one body organ to another. Patients with immunosuppressed have reported varying occurrences of *M. fortuitum* disseminated infections in the form of folliculitis and nodular lesions [24], [25]. Disseminating infections have started to occur in patients with immunocompetent status in recent years as well [26], [27].

2.2.1.5 Pulmonary infections

Most *M. fortuitum* lung infections affect people who already have pre-existing lung conditions like cystic fibrosis or tuberculosis [28]. Another cause of pulmonary infections caused by *M. fortuitum* is gastro-oesophageal infection [29].

2.2.1.6 Cardiac Infections

There have been reports of sporadic *M. fortuitum* cardiac infections in medical facilities. Endocarditis in adults typically results in death [30], but reports of some researchers also show that *M. fortuitum* endocarditis in children recovered [31]. *M. fortuitum* was identified as the most common NTM to cause infection in patients who had cardiac device implantations [32] [33].

2.3 Biofilm

The concept of microscopic organisms existing within a local environment rather than primarily as autonomous substances is one that is quickly gaining acceptance. Communities of organisms called "biofilms" exist in the extracellular matrix [34].

They are capable of growing on both abiotic and biotic surfaces, causing a number of diseases. A dynamic biofilm formation process on surfaces includes adhesion, growth, movement, and the generation of extracellular polysaccharides. The biological and metabolic state of the microorganisms within the biofilm impart a high rate of resistant bacteria [34].

2.3.1 Biofilm Formation Stages

Just like other communities, biofilms grow gradually over time. A biofilm has a universal five-stage growth cycle with traits which are independent of the phenotypic of the organisms [34]. Step 1 is the attach phase, which can begin to function in just a few seconds and is probably prompted by environmental signals. These signals, which differ from organism to organism, include change in pH, temperature, oxygen concentration, osmolality. Because of their higher surface area and reduced shear stresses, rough surfaces seem to be more likely to support the growth of biofilms. In stage I some cells get detach have the subtraction, the original binding can be reversed. the bacterial cell growth rate at this step is logarithmic. Step II is known as irreversible binding and it starts minutes after step 1. Following attachment to epithelial surface of the microorganism the release of signals which are chemical signals that communicate within the bacterial cells, the bacteria start to grow [34].

The hereditary mechanisms hidden exopolysaccharide (EPS) production are activated when the sign force exceeds a particular edge level, that can trap planktonic and supplements microbes. When cell aggregates gradually layer with a thickness more than 10mm, the biofilm is in step III, also referred as maturation I, during which time cell aggregates are created and mobility is reduced. Step IV or maturations-2 refers to the point at which biofilms attain their maximum thickness, which is typically greater than 100mm. Cell dispersion is shown in step V. A small percentage of the bacteria change into planktonic phenotypes and depart the biofilm [34].

2.3.2 Mycobacterium Biofilms

Mycobacterial biofilms that are planktonic are more sensitive to the environment's irritants and disinfectants. Numerous environmental investigations, especially those that concentrate on water sources, have confirmed the presence of Mycobacteria that form biofilms. NTM can cause respiratory issues in persons with chronic diseases such as bronchiectasis and old tuberculosis scars. Infections linked to biomaterials represent a significant group of biofilm-related infections, and in this context, rapidly proliferating Mycobacteria are the most often identified organisms. The development of biofilms is a significant contributor to antimicrobial resistance because they provide defence against drugs that would typically be effective against the identical bacteria when they are in a planktonic condition. The biofilm-forming microbes' antibiotic resistance may make treatment ineffective, and biofilms must be manually removed in order to cure the infection. Understanding biofilm is crucial for managing patients with a variety of NTM diseases. [35]

2.4 Proteases

Proteases are crucial components for all life forms including prokaryotic organisms, fungi, plants, and animals. However, they prefer proteolytic compounds derived from microbial sources. Compared to those obtained from plants and animals because they essentially possess all the qualities required for their uses of biotechnology. Illnesses linked to biomaterials are a significant group of biofilm-related infections, and in this context, one of the most commonly isolated organisms are quickly developing [36].

One of the most significant categories of enzymes are bacterial proteases, which are employed in many industrial processes including those in the food, silver recovery, pharmaceutical, and detergent sectors, as well as in the production of leather, textile, and wool, among other things. A further area of potential application is in the management of domestic and industrial waste and medical usage [36].

A large group of enzymes known as bacterial proteases play crucial roles in cell viability, response, and pathogenicity. Bacterial proteases are particularly intriguing candidates for diagnostic and therapeutic uses because of their significance in bacterial viability and pathogenicity [37].

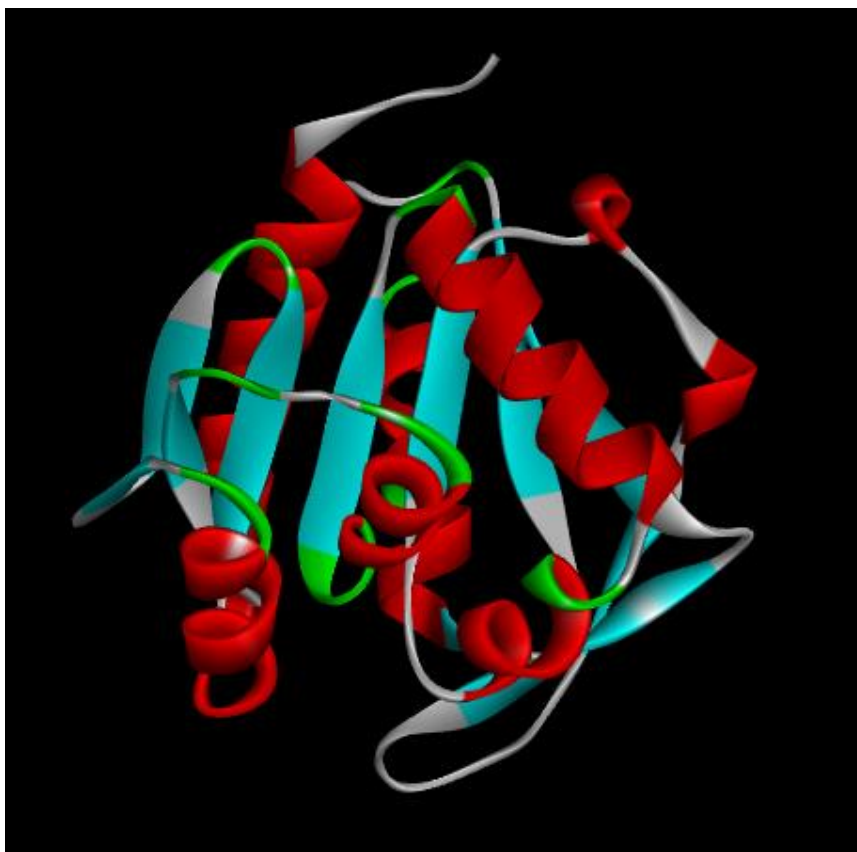


Figure1: 3D model of Protease Protein Receptor used for docking.

2.5 Drug Discovery

Drug discovery is a process which utilises computational, experimental, translational, and clinical models to identify potentially new therapeutic entities. Despite improvements in biotechnology and our understanding of biological systems, the process of discovering new medicines is still time-consuming, expensive, difficult, and ineffective [38]. The creation of a new drug involves a protracted, pricey, and complicated procedure that can take 12 to 15 years to complete and cost in excess of one billion dollars [39].

Understanding of a biological target and finding new drugs based on it is called drug design [38]. The idea for a target can come from a variety of sources, including academic and medical research, in addition to the private sector. Before selecting a target for a cost-prohibitive drug discovery programme, it might be necessary to gather a body that offers supporting evidence

over a prolonged amount of time. The pharmaceutical company and, lately, some academic institutions, have streamlined a number of preliminary steps to find molecules with the necessary properties to create legal drugs after deciding on a target [39].

Drug design, in its most basic form, entails creating molecules that are complementary in charge and shape to the molecular target with which they interact and bind. In the big data era, the development of drugs frequently but not always relies on computer modelling methods and bioinformatics strategies. The discovery of small, potent compounds with high affinities for the target of interest is the key step in an effective drug discovery pipeline [38].

2.5.1 Drug discovery process

The process of discovering new drugs can be extremely difficult. This implies that the time it takes to come up with a new drug may exceed ten years. The length of time required can be explained by the fact that an active ingredient must pass through a number of stages before being approved, from discovery to approval. Only after thorough investigation and extensive research is an active ingredient declared to be an effective drug [40].

Preclinical studies using animal and cell-based models, human clinical trials, and regulatory approval are all steps in the development and discovery of new drugs. The latter two steps are necessary to market the drug. Modern drug discovery requires the identification of screening hits, medicinal chemistry, and optimisation of those hits to increase their affinity, selectivity (to decrease the likelihood of side effects), efficacy/potency, metabolic stability (to lengthen the half-life), and oral bioavailability. Once a compound has been found that satisfies all of these criteria, the process of developing the drug will start before clinical trials [38].

The process of discovering new drugs involves many phases and steps. It typically goes through the following stages:

2.5.1.1 Early Drug Discovery

New understandings of a disease's mechanism that enable researchers to create a treatment that interrupts or reverses its effects.[41]

The early stages of drug discovery involve numerous procedures and examinations. Researchers collaborate to identify and enhance potential leads to a specific target. The sub-

processes that are involved in this stage include Target Identification and Validation, High Throughput Screening or High Content Screening, Hit Identification, Assay Development and Screening, Hit-To-Lead, Lead Generation and Optimisation, and In Vivo and In Vitro Assays [40].

2.5.1.2 Pre-Clinical Phase

The substances discovered throughout Early Drug Discovery are extensively tested both in a lab setting and using alternative or animal models. during the Pre-Clinical Phase. Before clinical trials for humans can start, the objective is to provide enough proof of safety and efficacy. Once this goal has been accomplished, it is also helpful to determine the right dosages to test in humans [40]. Researchers must ascertain a drug's potential for toxicity, also known as serious harm, prior to testing it on humans. Preclinical research is of two types: in vitro and in vivo [42].

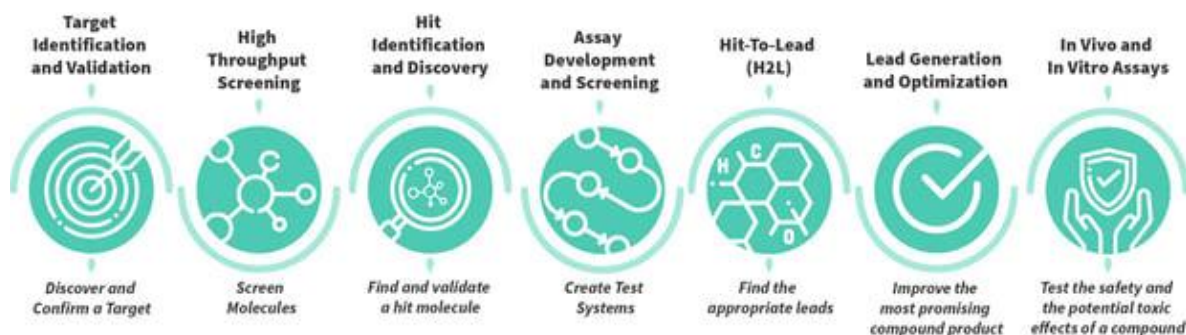


Figure2: Steps involved in 1st and 2nd phases of Drug Discovery Process [40].

2.5.1.3 Clinical Phases

Preclinical research can provide basic answers about the safety of a drug, but it cannot replace studies on how the drug will interact with the human body. Studies or trials conducted on humans are referred to as "clinical research". The developers will think about what they want to achieve for each of the various Clinical Research Phases as they design the clinical study and start the Investigational New Drug Process (IND), a pre-clinical research subprocess [43].

Clinical trials have four phases: Phase I, Phase II, Phase III, and Phase IV. Typically, 20 to 80 healthy volunteers will be used in the initial stage to test the drug candidate's safety and toleration. After tolerance and efficacy have been tested in a small group, Phases IIa and IIb

are launched to examine the effectiveness, tolerability, and dosage in a larger group. For this, the dosage form is first created. While phase IIa studies are primarily concerned with the therapy concept (proof of concept), phase IIb studies aim to determine the appropriate dose. Phase II studies typically enrol 100–500 adult study participants [40].

Prior to a drug's approval, doctors test it on hundreds of thousands of patients to determine whether its safety and efficacy can be established across a broad patient population. Drug interactions are also looked at. The majority of the time, phase II and phase III studies are referred to as controlled investigations in which a few individuals receive the novel medication while a second group receives the currently available standard medication [40].

2.5.1.4 Regulatory Approval

After a drug has successfully finished its clinical trials, data is collected and analysed. The appropriate authorities can then review it. Before a drug or vaccine can be distributed, a national regulatory agency or centralised process has to authorise the sale of the product. In the end, only one out of the many tested compounds makes it through the regulatory testing and clinical study phases. Therefore, only one compound can be used to make a drug or vaccine [40], [44].

2.5.1.5 Post-Market Monitoring

After receiving regulatory approval for marketing, phase IV studies—also known as post-marketing surveillance trials—are carried out. More complete information can be gathered regarding the safety and effectiveness of the new drug. a larger patient base gathering information from the drug and contrasting it with currently recommended treatments. These investigations are designed to assess a medication's long-term effects. In this way, negative outcomes can be pointed out and avoided [40], [44].

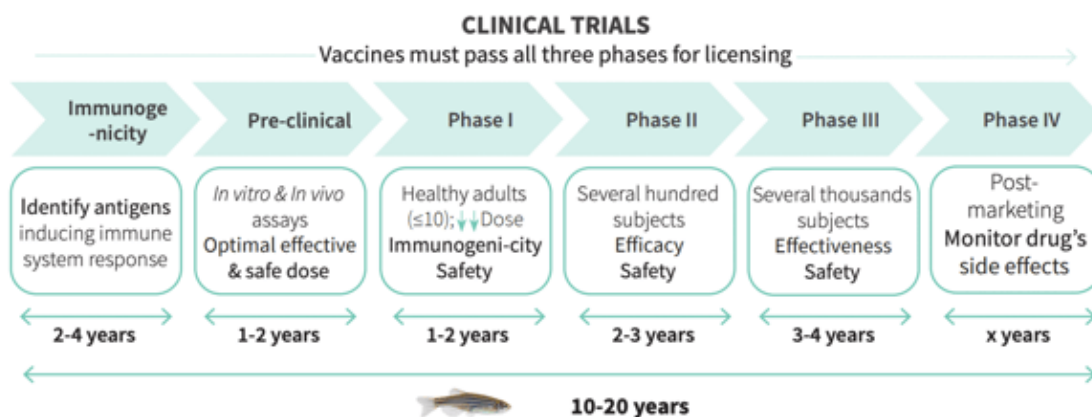


Figure 3: Steps involved in 3rd, 4th and 5th phases of Drug Discovery Process [40].

2.6 Computer Aided Drug design (CADD)

The creation of new drug compounds now heavily relies on computer-aided drug design, which has become a potent technology. CADD's role in a drug discovery process is to expedite the identification of new lead compounds including the structural optimisation of those compounds in preparation for pharmacological testing. The main orientations in CADD are based on the availability of the empirically derived three-dimensional structure of the target macromolecule [45]. In the process of developing new drugs, lead compounds are efficiently identified and improved using chemical biology and computational drug design techniques. To help find potential candidate drugs, computer-aided drug design employs structural data about the drug's target or structured data about recognised ligands with bioactivity [46]. Two techniques that are frequently utilised in computer-assisted drug development are ligand-based drug design and structure-based drug design [45]. Both of these approaches can be used in conjunction with molecular docking for virtual lead optimisation and identification [47].

A new drug's introduction to the market can be described by a number of titles, but the term "development chain" or "pipeline" is most frequently used to describe the process. Discovering the proteins that may serve as pharmacological targets in pathogenesis is the initial step in rational drug design. A quick overview of CADD's history, DNA as a target, receptor theory, structure optimisation, structure-based development of drugs, , and graph machines is given in the study "Computer-Aided Drug Design: An Innovative Tool for Modeling" [48].

2.6.1 Structure Based Drug Design (SBDD)

The three-dimensional structure of the compound of interest (enzyme/receptor) is used in the structure-based approach to generate or screen candidate ligands (modulators), which are then synthesised, biologically tested, and optimised [46]. The most effective use of structure-based drug development is when it is integrated into the entire process of finding new therapeutic leads. It's also critical to remember that structure-based drug discovery guides the development of a drug lead, which is a chemical with a minimum micromolar affinity for the target but is not a therapeutic product [49]. In SBDD, the target protein's structure is known, and interactions or bioaffinities for every substance that has been tested calculate the results of the docking process; to create a new medication molecule that demonstrates improved interaction with specific protein.

Several cycles of SBDD are completed before the optimised lead enters clinical trials. The target protein is isolated, purified, and its structure is determined using some computational methods in the first cycle. Placing chemicals into a protein's (active site) using simulated screening of various databases. The top compounds are examined using biological tests. Once the structure of target molecule is known after that we need to identify the potential drug candidate that could interact with our target molecule and modify its structure. The most promising lead from the first cycle — the one with the least degree of in-vitro micro-molar inhibition — is used to determine the protein's structure in the second cycle, which also identifies the active regions of the chemical that can be enhanced for further potency growth. The lead compound is further improved utilising a protein structures after going through numerous further cycles, such as lead synthesis, and the optimised compounds often show a considerable improvement in target selectivity and binding affinity [49].

2.6.2 Ligand Based Drug Design (LBDD)

If the three-dimensional structure of a target protein is unknown, a set of ligands that affect the target can be analysed to identify important molecular descriptors, including structural and physiochemical properties, that are responsible for the observed biological activity. This information can help to determine the crucial features required for a ligand to interact effectively with the target protein. [46]. Two typical ligand-based techniques are

pharmacophore-based approaches and quantitative-structure activity relationships (QSARs). In LBDD, it is assumed that compounds with similar structures also have comparable biological characteristics and interact similarly with the target proteins.

A quantitative structure-activity relationship (QSAR) is a connection between a molecule's expected properties and its actual biological activity. In modern drug development, target fishing, also referred to as target identification, is a critical first step that comprises identifying the interactions between the protein of bioactive small compounds in order to investigate the mechanism of action. The application of reverse or inverse docking is increasing the effectiveness of drug repositioning and drug rescue. It involves the hypothesised binding pores of a small-molecule drug or ligands into a wide range of clinically relevant macromolecular targets [50].

2.7 Fragment Based Drug Design (FBDD)

In the past fifteen years, fragment-based drug design has become a viable alternative to high throughput screening (HTS) in drug discovery for the identification of lead compounds. With approved drugs and a large number of compounds in clinical trials, fragment-based screening and optimisation techniques have had significant success in numerous drug discovery projects.

Effective library design is necessary to find high-quality FBDD hits. To ensure optimal pharmacophore and chemical diversity, physicochemical properties and molecular complexity, good library design is crucial. With 20 fragment to lead publications released in 2019 and 21 publications in 2020, the method is now frequently used in pharmaceutical, biotechnology, and academic institutions around the world [51], [52].

Contrary to fragments, which are more likely to form atom-efficient binding interactions, complex molecules have a higher chance of forming ineffective interactions and/or collisions with the desired target [53], [54]. Additionally, fragment hit rates can be used to evaluate a target's potential druggability [55] and to pinpoint challenging binding regions, such as allosteric sites or tiny "hot spot" binding pockets that are frequently associated with protein-protein interactions [56].

The first step in the fragment-based drug design process is the discovery of fragments or low molecular weight compounds that typically bind to the target of interest with weak affinity.

The fragments that form outstanding interactions are then further optimised to produce compounds with high affinity and selectivity. Because fragments have a low affinity for their target, hits must be detected using biophysical methods like nuclear magnetic resonance, X-ray crystallography, or surface plasmon resonance. These methods are extremely sensitive, and some of them offer thorough information on protein fragment interactions that is crucial for lead to fragment optimization [57].

Despite the enormous technological advancements of recent years, experimental fragment screening methods face a number of difficulties, including low throughput, expensive equipment and experiment requirements, and high protein and fragment concentration needs. Computational methods that are crucial for fragment library design, fragment screening, and initial fragment hit optimization were created to address problems with experimental screening approaches. The combination of computational fragment screening and optimisation methods with experimental methods yields the best results. The application of fragment-based drug design to significant biological targets has been facilitated by the use of virtual fragment-based screening in conjunction with experimental techniques. [58]

2.7.1 FBDD V/S HTS

While large complex libraries of drug-like molecules are screened in HTS, smaller and fewer complex molecules are used in FBDD screening because they exhibit greater 'atom-efficient' binding interactions than larger molecules, despite having a lower affinity for protein targets. Therefore, fragment hits can act as a better starting point for subsequent optimisation, especially for targets that are challenging to drug. Small fragment libraries enable a proportionately greater coverage of their respective 'chemical space' compared to larger HTS libraries made up of larger molecules because the number of possible molecules increases exponentially with molecular size [57].

Fragment libraries can sample a much larger chemical space than HTS libraries with a lot a lesser number of compounds. In contrast to HTS hits, which typically have stronger affinities within the nM – μ M range, fragment hits typically have weaker affinities with dissociation constant (K_d) values in the μ M–mM range. As a result, they frequently require more involved chemical processes to arrive at a compound that resembles lead, which can be especially challenging in an academic setting. Additionally, because of their lower affinities, biochemical

assays, which are frequently used for HTS screens, cannot provide an accurate assessment of fragment binding. Instead, binding is typically examined using biophysical methods like nuclear magnetic resonance (NMR), surface plasmon resonance (SPR), X-ray crystallography, and thermal shift assays, with two orthogonal methods frequently used to validate any hits [57].

Advantages of screening low molecular weight fragment-based libraries over traditional higher molecular weight chemical libraries are several. These include:

- More hydrophilic hits, where enthalpically driven binding is more probable to contribute to affinity. Entropically driven binding makes it much simpler to increase affinity by introducing hydrophobic groups; beginning with a hydrophilic ligand improves the likelihood that the final, optimised ligand won't be overly hydrophobic (log P 5).
- Greater ligand efficiency, which increases the likelihood that the final, optimised ligand will have a low molecular weight (MW 500).
- Fragment library screening of N compounds is equivalent to screening N²–N³ compounds in a conventional library because, in theory, two to three fragments can be combined to form an optimised ligand.

A fragment library has an even greater combinatorial advantage because fragments are less probable to contain sterically blocking groups that interfere with a ligand-protein interaction that would otherwise be beneficial [59].

2.8 Molecular Docking

Using a technique called docking, it is possible to foretell how two molecules will orient themselves when joined to create a stable complex. The "lock and key" concept can be used to describe molecular docking. Here, the ligand can indeed be thought of as the key and the protein as the lock. It describes the ligand's optimal orientation for attaching to a specific protein. A protein molecule might be needed first in order to accomplish a docking. The sources for docking are the ligands and protein structure [60].

The capacity of a protein and nucleic acid to bind with small molecules to form a supramolecular complex has a significant impact on the dynamics of a protein, which can either

enhance or inhibit their biological function. Small molecules' behaviour in target proteins' binding sites can be explained by molecular docking. The method aims to predict the affinities of ligands and their proper locations within a protein's binding pocket.

The search algorithm affects docking performance. The creation of a composition comprising every potential conformation and orientation of the protein and ligand combination is the first stage in docking. The scoring function accepts input and outputs a number that indicates a favourable interaction in the second phase.

2.8.1 Importance of Docking in drug discovery

Docking is an essential step in the process of drug discovery. Docking is a computational method that helps scientists understand how small molecule drugs bind to their target proteins, which is crucial for the design of new drugs with high potency and selectivity.

During the drug discovery process researchers must identify molecules that have the potential to bind to a specific target protein associated with a disease. Docking allows them to stimulate the interaction between the small molecules and the target protein and predict the strength and location of the binding.

By using docking simulations to prioritise the most promising compounds, researchers are screening a large number of potential drug candidates. It can reduce the cost and time required for experimental testing and increase the likelihood of success in developing a new drug.

2.8.2 Broad categories of Docking

2.8.2.1 Local docking – It is also known as site specific docking. In the receptor the binding site is known and docking refers to finding the position of the ligand in that binding site.

2.8.2.2 Global docking – The binding site is unknown and the search for the binding site as well as the position of the ligand in the binding site can then be performed sequentially or simultaneously. This type of docking is also called blind docking.

2.8.3 Docking Tools

2.8.3.1 Autodock

AutoDock is a software program that allows users to perform docking simulations of small molecule ligands to a set of grids that represent a target protein. This enables the prediction of ligand behaviour and interaction within the protein's active site. The software uses a Monte Carlo simulation technique in conjunction with a rapid energy assessment that utilizes grid-based molecular affinity potentials. After docking is complete, the results can be visualized in a three-dimensional format. AutoDock 4 is available for use under the General Public License at no cost. The method generates a generally unbiased docking when given a volume surrounding the protein. Different uses of autodock are that it can be used for lead optimization, it can be used for protein-protein docking, structure-based drug design and used for combination of library design [60].

2.8.3.2 Autodock Vina

An open-source tool for molecular docking is called AutoDock Vina. The average binding mode prediction accuracy is markedly increased by AutoDock Vina. It works particularly well for protein-ligand docking [61].

2.8.3.3 Pyrx

Pyrx is a software application that facilitates virtual screening for the identification of potential drug candidates by enabling the screening of large libraries of compounds against potential therapeutic targets. The software uses a technique called docking, which involves the prediction of the binding orientation and strength of a small molecule ligand to the active site of a protein [62].

CHAPTER 3 – MATERIALS & METHODS

3.1 Databases, Software programs and Tools Used

3.1.1 Databases

Selleck Chemicals is an acknowledged supplier of therapeutic antibodies, compound screening libraries, and small molecule inhibitors of the highest quality. More than 120,000 inhibitors are provided by Selleck Chemicals for research of cell signalling pathways. Over 8,000 small molecules with verified biological and pharmacological activities are included in Selleck's Bioactive Compound Libraries. Preclinical and clinical studies have shown the safety and efficacy of these molecules, and many of them have been FDA-approved drugs [63]. ChemDiv offers comprehensive drug discovery and initial clinical development outcomes by gaining more value from possible therapeutic candidates through quick, efficient results and efficient capital allocation [64].

3.1.2 Discovery Studio Visualizer

DS Visualizer is a free, functionality molecular modelling tool for viewing, sharing, and analysing data on proteins and small molecules is the Discovery Studio Visualizer. Software for modelling molecules and macromolecule systems is available under the name Discovery Studio. Researchers and their coworkers can exchange results quickly and effectively, with no loss of time or scientific knowledge [65].

3.1.3 UCSF Chimera application

UCSF Chimera is a software program that enables interactive visualization and analysis of molecular structures and related data, such as density maps, docking result trajectories, supramolecular assemblies, sequence alignments, and conformational ensembles. The application provides tools for creating high-quality movies and images. Users can manipulate the visual representation of the molecular structures to facilitate interpretation and analysis of the data [66].

3.1.4 Open Babel

The main purpose of the computer programme Open Babel, a chemical expert system, is to convert between different chemical file formats. A remedy for the overpopulation of different chemical file formats is offered by Open Babel. Additionally, it offers a wide range of helpful features, including filtering, batch conversion, substructure and similarity searching, conformer searching, and 2D depiction [60].

3.1.5 Perl Script Used for Docking

```
#!/usr/bin/perl
print"ligand file name:\t";
$ligandfile=<STDIN>;
chomp $ligandfile;
open (FH, $ligandfile) || die "file can't open\n";
@file=<FH>;

for($i=0;$i<@file;$i++)
{
print"@file[$i]\n";
@name=split(/\./,@file[$i]);
}
for($i=0;$i<@file;$i++)
{
chomp @file[$i];
print"@file[$i]\n";
system("vina.exe --receptor PROT_model.pdbqt --config conf.txt --ligand @file[$i] --log
@file[$i]_log.log --out @file[$i]_out.pdbqt");
}
```

Figure 4: Perl script for performing molecular docking.

PERL SCRIPT Pseudocode

- 1-Ask user for the name of the ligand file and store it in the variable "\$ligandfile".
- 2-Remove any newline character from the user input using "chomp".
- 3-Open the file with the name stored in "\$ligandfile" using "open" command, and assign the file handle to "FH". If the file cannot be opened, print an error message and exit the program using "die".
- 4-Read the contents of the file stored in "FH" and store it in an array "@file".
- 5-For each element in the "@file" array, print the element.
- 6-For each element in the "@file" array, split the element at the period (".") and store the result in an array "@name".
- 7-For each element in the "@file" array, remove any newline character using "chomp", print the element and execute a system command to run "vina.exe" with the following parameters:
 - 8-receptor file: "PROT_model.pdbqt" .
 - 9-configuration file: "conf.txt".
 - 10-ligand file: the current element in the "@file" array.
 - 11-log file: a file with the same name as the current element in "@file" array, with "_log.log" appended to it.
 - 12-output file: a file with the same name as the current element in "@file" array, with "_out.pdbqt" appended to it.
- 13-End of program.

3.2 Target Molecule Selection – *M. fortuitum* Protease Protein

Proteome *M. fortuitum* was examined during its planktonic and biofilm development phases. According to the study, the protein protease is elevated throughout the biofilm generation phase. It's been suggested that it would be a good pharmacological target. In pathogenicity, bacterial proteases are important. As a result, *M. fortuitum* was used as a drug development target.

3.3 Ligand Selection

The two libraries (Fragment Library & Triazoles and Triazole Derivatives) for performing molecular docking against our target molecule were downloaded from the Selleckchem site and ChemDiv site respectively.

3.3.1. Fragment Library

Fragment-based drug discovery (FBDD) is a method used for finding lead compounds as part of the drug discovery process. Small, low-molecular-weight organic molecules known as fragments have these characteristics. In order to create a lead with greater affinity, it is based on the identification of small chemical-based fragments, which may only weakly bind to the biological target [59]. The "rule of three" (Ro3), which has come to be synonymous with Lipinski's rules in the fragment world, was used as a general guideline for focusing on physicochemical properties when designing fragment libraries [67]. These are: hydrogen bond donors (HBD) ≤ 3 , hydrogen bond acceptors (HBA) ≤ 3 , computed logarithm of the partition or distribution coefficient (cLogP/cLogD) ≤ 3 and most importantly molecular weight ≤ 300 Da. In addition, polar surface area (PSA) ≤ 60 and freely rotatable bonds ≤ 3 are often considered Ro3 criteria [57]. However, these are not 'hard and fast' guidelines, and the selection standards have changed over time. At least one of these rules will frequently be broken by successful fragments [68], with a higher HBA count being the most typical example [57].

Since the fragments have relatively low affinity for their targets, they must have high water solubility so that they can be screened at higher concentrations [59]. In order to keep the desired pharmacokinetic properties and exclude compounds with known toxic structures (toxicophores), commercially available collections are typically examined and filtered before the design and growth of a fragment set can begin. Most effective FBDD campaigns use libraries with between 1000 and 2000 compounds [57], [69].

3.3.2 Triazoles and its derivatives

The triazole nucleus is a highly significant and widely recognized heterocyclic compound, frequently appearing as a fundamental element in numerous natural products and medicinal substances. An important category of heterocyclic compounds with a variety of pharmacological properties is the triazole. There are two probable isomeric forms of it, 1,2,3 triazoles and 1,2,4 triazoles, and it is also known as pyrroldiazoles. It is a five-membered diunsaturated ring structure with tri nitrogen atoms containing in a heterocyclic core [70].

Triazoles is white pale crystal with a 120°C melting point that are soluble in both water and alcohol. Triazole is a crucial heterocyclic structure found in many medications, including anticonvulsants, antimalarial, antimicrobial, antitumor, antiviral, antiproliferative, anticancer, antioxidants, analgesics, antifungal, antiplasmodial, antibacterial, immunostimulants, and antidiabetic drugs. This wide range of applications demonstrates the significant pharmacological importance of triazole [70].

Organic compounds with the triazole aromatic ring are extremely helpful in treating fungus infections and are also used as insecticides in agricultural systems [70]. Triazoles like fluconazoles are used to treat various fungal infections in people. It is frequently used to aid in the prevention of candidiasis in those who are more susceptible to infection, such as following an organ transplant for infants with low birth weight [71].

3.4. Preparations of Ligands

Fragment Library of 1015 molecules and library of 4042 molecules of Triazole derivatives were individually visualized, and saved separately using Discovery Studio Visualizers. Before performing blind docking, the software UCSF Chimera was used to prepare the ligands that were then again saved individually. Finally, the software Open Babel was used to generated ligand file in pdbqt format in order to proceed with docking.

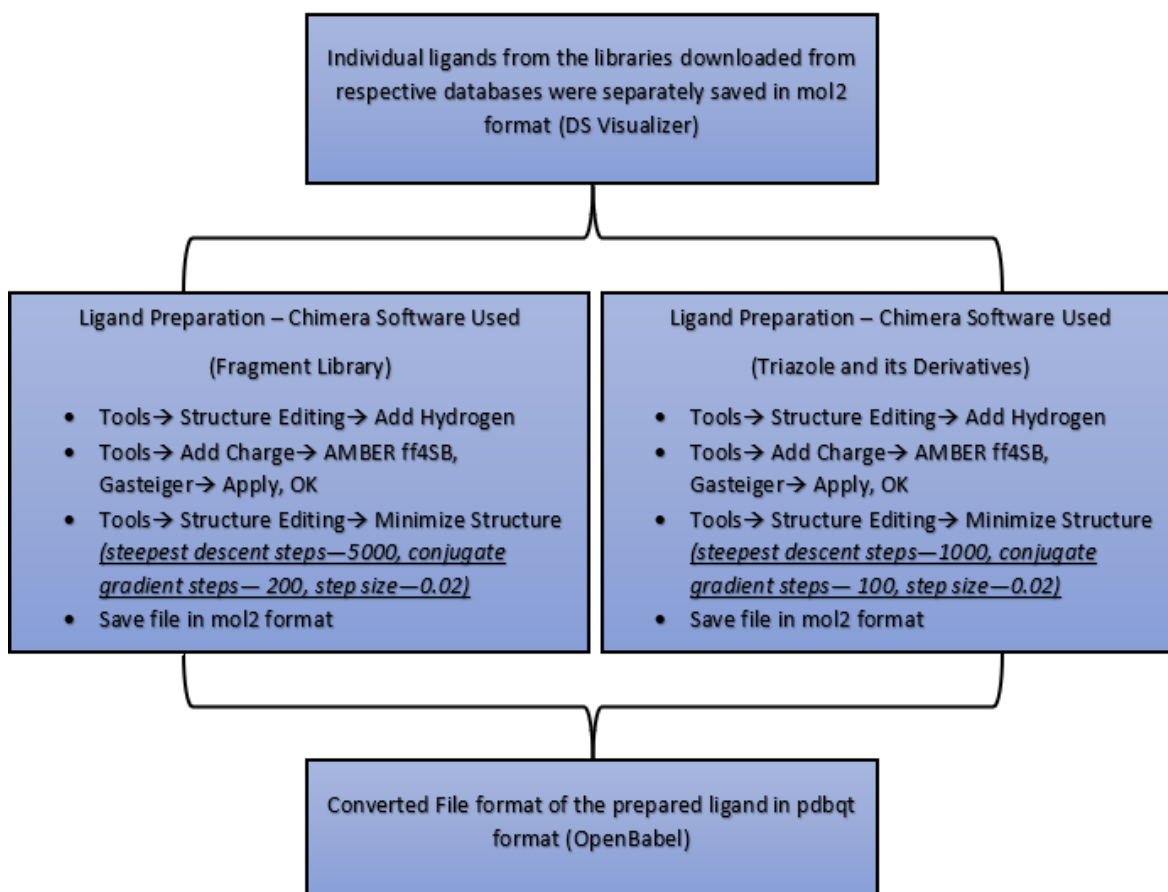


Figure 5: Steps involved in ligand preparation.

3.5 Docking using Autodock Vina

1-Prepared the receptor: The first step is to prepare the receptor for docking, involves cleaning the protein structure, removing any water molecules or unwanted atoms, and assigning charges and protonation states. Used software programs such as Chimera or PyMOL for this purpose.

2-Prepared the ligands: The next step we did was to prepare the ligands for docking, this involves generating a 3D structure of the ligand, assigning charges and protonation states, and then saving the file in the appropriate format, such as PDB or mol2. You can use software programs such as OpenBabel or Avogadro for this purpose.

3-Configuration file was created: In this step, a configuration was created file that specifies the docking parameters of the protein, such as the search space, the exhaustiveness, and the scoring function.

4-Wrote the Perl script and stored that in the same folder where configuration file and ligands were saved.

5-Started docking simulation: Once the receptor, ligands, and configuration file have been prepared, we can run the docking simulation. We can use a batch file or a scripting language such as Perl to automate the process for multiple ligands. With the following steps and using autodock vina multiple ligands could be docked in one go.

- 1-A folder named VINA was made.
- 2-In that folder all ligands were saved in the pdbqt format which we want to dock.
- 3- In the same folder VINA stored configuration of the protein, saved it with name conf and saved the protein molecule in the pdbqt format.
- 4-Wrote a perl script to dock multiple ligands and saved that script in VINA folder.
- 5-Again saved all the name of all the ligands in the file ligandfile which we wanted to dock.
- 6- Command prompt was opened, wrote the command: cd with the directory of VINA i.e, cd VINA
- 7-Then wrote the command: perl script.pl. This command will be used to run the script file.
- 8-Command prompt displayed 'ligand file name', then wrote the command ligandfile.txt. Docking simulations will start after that.

3.6 Docking using Pyrx (another approach to dock multiple ligands in one go)

- 1- Software Pyrx was used in the process of docking.
- 2- File → protein was selected → Protein molecule first loaded loaded in pdb format.
- 3- File → ligands were selected one by one in sdf format.
- 4- Selected the protein → right clicked → selected autodock option □ macromolecules. These steps converted protein in pdbqt format.
- 5- Selected ligand → then energy minimization option was selected.
- 6- Selected ligands one by one → right clicked → selected autodock option → macromolecules. These steps converted ligands in pdbqt format.
- 7- ctrl key was clicked and then selected all the ligands.
- 8- ctrl key was again clicked and then selected the protein.

- 9- Vina wizard was opened for the further process, selected molecule and then clicked on forward, grid box will cover the entire protein for its configuration.
- 10- Visualization using pymol was done.
- 11- All the result saved automatically on C drive> users > HP > mgltools >pyrx

3.7 Analysis of the Protein–Ligand Complex

After the docking simulation is complete, you need to analyse the results to identify the best binding poses using Binding energy. The protein–ligand complex’s visualization and analysis were performed using Discovery Studio Visualizer software, Pymol could also be used for analysis. The hydrogen bonding, polar, and hydrophobic interaction between the protein and ligands were analysed and visualized in both 3D and 2D in the same software mentioned above.

CHAPTER 4: RESULTS & DISCUSSION

The best binding modes or conformations of the ligand with the active residues of a protein target are provided by in silico molecular docking. Additionally, the evaluation of the docking output is based on the extremely low binding energy (lowest binding affinity), which represents the best conformation of the ligand in the active pockets of the target. As previously mentioned, a library of fragments and a library of triazoles were molecularly docked with the homology-modelled protease protein as the receptor.

The binding affinity scores of the best ligand's binding modes of fragment library ranged from -6.2 to -2.9 kcal/mol and of library of triazoles ranged from -8.47 to -3.42.

Thus, ligand numbers 25, 736, 793, 733, 916 (fragment library) & ligand numbers 3934, 3760, 3906, 2625 (triazole library) were chosen as the best to evaluate their residual interactions using the Discovery Studio software because they had higher binding affinity values than the other submissions.

According to rule of three (RO3) the selected compounds from fragment library based on the binding energies possessed the desirable Molecular properties and drug-likeness as shown in Table 1. Table 2 depicts Molecular properties of the selected ligands from the library of Triazoles and its derivatives.

Table 3. and Table 4. depict 5 best binding affinity scores along with the residues of the protease protein involved in receptor ligand interactions.

Molecule Id in the library	SMILES Format	Molecular weight	AlogP	H-bond acceptors	H-bond donors	PSA
25	<chem>NC1=CC=CC=C1C1=NC2=C(N1)C=CC=C2</chem>	209.247	2.524	2	2	0.269
736	<chem>CC1=NOC(NS(=O)(=O)C2=CC=C(N)C=C2)=C1C</chem>	267.304	0.918	4	2	0.425
793	<chem>N1C2=CC=CC=C2N=C1C1=CC=CC=N1</chem>	195.22	2.548	2	1	0.223
733	<chem>N1C2=C(C=CC=C2)N=C1C1=CN=CC=C1</chem>	195.22	2.12	2	1	0.222
916	<chem>OC1=CC=CC=C1C(=O)NC1=CC=CC=C1</chem>	213.232	2.372	2	2	49.33

Table1. Molecular properties and drug-likeness of the selected ligands (fragment library)

Molecule Id in the library	Similarity ID	molregno	ID number	SMILES Format
3934_C549-0683	778,718	1,211,916	C549-0683	<chem>CCOc1ccc(Cn2nnc(C(=O)Nc3ccc(Cl)cc3)c2N)cc1</chem>
3760_C337-0010	74,048	942,389	C337-0010	<chem>COC(=O)c1nnn(CC(=O)Nc2cccc(OC)c2)c1C(=O)OC</chem>
3906_8007-1892	741,284	1,347,078	8007-1892	<chem>CCOc1ccc(/C=N/NC(=O)c2nnn(-c3nonc3N)c2CN2CCCC2)cc1</chem>
2625_C368-0480	1,124,396	1,089,026	C368-0480	<chem>CCc1ccc(NC(=O)Cn2nnc(C(=O)NCc3ccc4c(c3)OCO4)c2N)cc1</chem>

Table2. Molecular properties of the selected ligands (Triazole Library) with Protease protein.

Molecule Id in the library	Binding Affinity (kcal/mol)	Residues involved in receptor ligand Interactions
25	-6.2	LYS70, VAL71, PHE98, ASP101.
736	-6.2	GLU19, GLU22, THR83, VAL84, HIS114, PRO164.
793	-6	LYS70, VAL71, ASP101
733	-5.9	LYS70, VAL71, ASP101
916	-5.9	LYS70, VAL71, ASP72, ASP101.

Table 3. Free binding energies and residues of a protein target interactions with the selected ligands from fragment library.

Molecule Id in the library	Binding Affinity (kcal/mol)	Residues involved in receptor ligand Interactions
3934_C549-0683	-8.47	SER A: 105, LYS A:107, PRO A:108, SER A:181, GLY A:106, PRO A:182
3760_C337-0010	-8.4	HIS 1:52, HIS A: 114, PRO A:164, TYR A:133, GLU A:19, THR A: 83, ASP A:51, GLY A:17, ARG A:50, ASP A:51
3906_8007-1892	-8.35	ALA A:163, PRO A:164, TYR A:133, GLU A:19, CYS A:133, ARG A:15, GLY A:17
2625_C368-0480	-8.3	PRO A :164, ALA A:163, THR A:83, GLU A:19, ASP A:51, HIS A:51

Table 4. Free binding energies and residues of a protein target interactions with the selected ligands from Triazole library.

4.1 Discussion on top five ligands having the best binding affinity in the Fragment Library

Ligand 25 formed one conventional H-bond with ASP101 amino acid residue of the receptor. Residues PHE98 and LYS70 showed pi-pi T shaped and pi-alkyl interactions with ligand 25 respectively. Furthermore, ligand 25 also formed pi donor H-bond and pi sigma interaction with residue VAL71.

Ligand 736 formed two conventional H-bond with GLU19 and HIS114 amino acid residues of the receptor. One pi-anion interaction with GLU22 residue, one pi-sulphur interaction with HIS114 and one pi donor H-bond with THR83 residue were formed. Furthermore, one pi-alkyl interaction with PRO164 amino acid and one alkyl interaction with VAL84 amino acid were also observed.

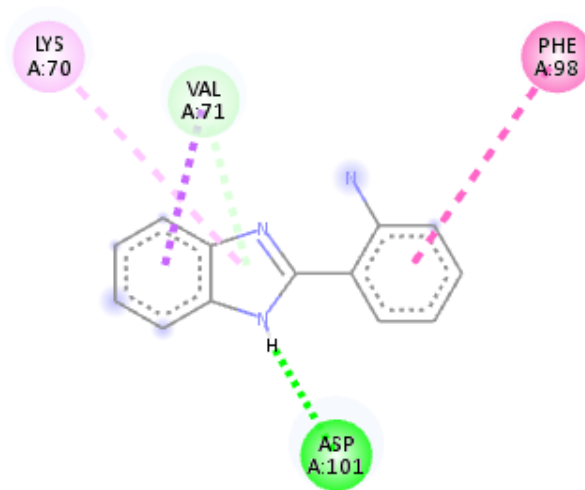
Despite having -0.1 kcal/mol difference in binding affinities the ligand 733 and ligand 793 showed same interactions with the receptor. Both ligand 733 and ligand 793 formed one conventional H-bond with ASP101 residue, pi donor H-bond and pi sigma interactions with VAL71 residue, and one pi alkyl interaction with LYS70 residue of the receptor.

Ligand 916 formed four conventional H-bond with the residues VAL71, ASP72, ASP101. Furthermore, the ligand formed one carbon hydrogen bond with LYS70 amino acid residue and one pi alkyl interaction with VAL71 residue.






Figure 5A, 6A, 7A, 8A, and 9A gives a clear view on different types of receptor ligand interactions involved.

Furthermore, to gain knowledge about the inner workings of the Protease protein modelled receptor, receptor surfaces (hydrophobicity and hydrogen bonds) were developed. The hydrophobicity index of the ligand protein was calculated using the hydrophobicity surfaces, which in Figures 5B, 6B, 7B, 8B, and 9B demonstrate the hydrophilic or hydrophobic characteristics of the amino acid side chains. Additionally, the surfaces of the amino acid residues are coloured according to how hydrophobic they are, ranging from blue for hydrophilic to brown for hydrophobic. Figures 5C, 6C, 7C, 8C, and 9C illustrate how H-bond surfaces are coloured, with H-bond donors being magenta and H-bond acceptors being green.

A



Interactions

- | | |
|--|--|
|  Conventional Hydrogen Bond |  Pi-Pi T-shaped |
|  Pi-Donor Hydrogen Bond |  Pi-Alkyl |
|  Pi-Sigma | |

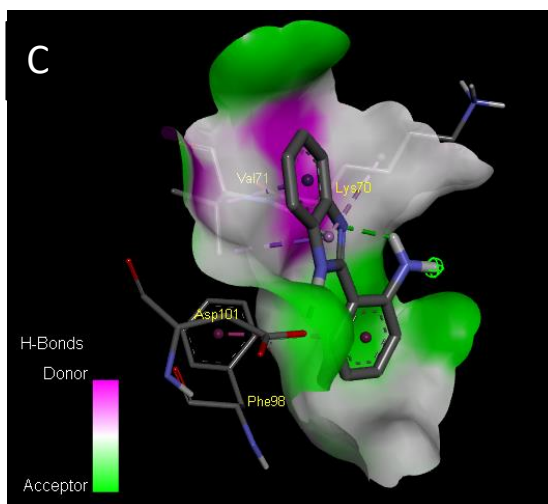
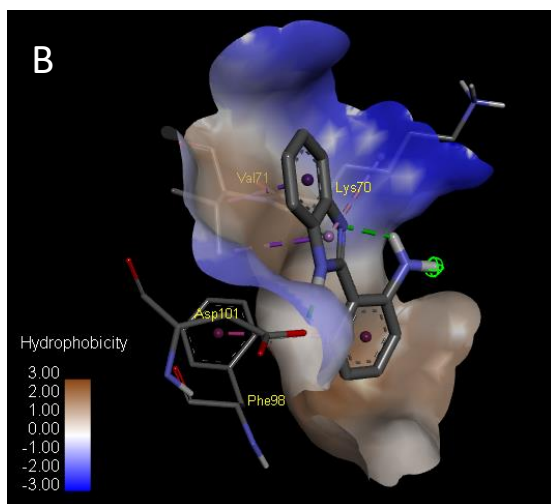
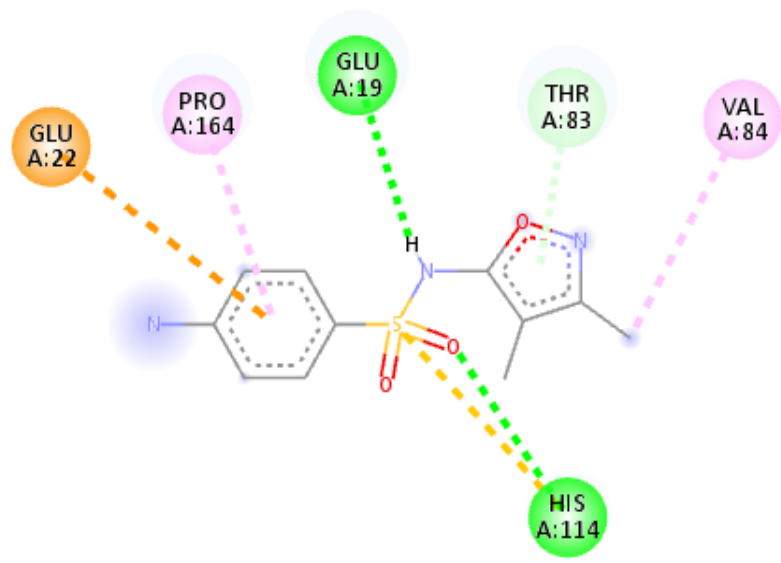








Figure 6: Interaction of complex 25 during docking. A) 2D docked view of complex 25. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

- | | |
|--|---|
|  Conventional Hydrogen Bond |  Pi-Sulfur |
|  Pi-Anion |  Alkyl |
|  Pi-Donor Hydrogen Bond |  Pi-Alkyl |

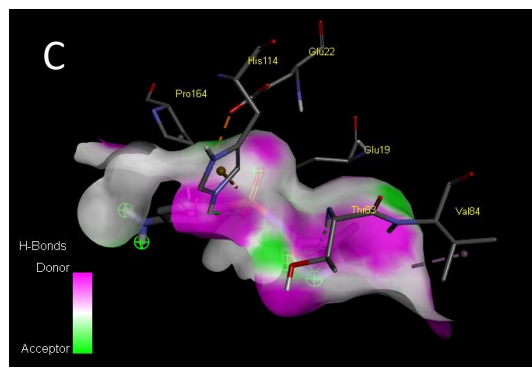
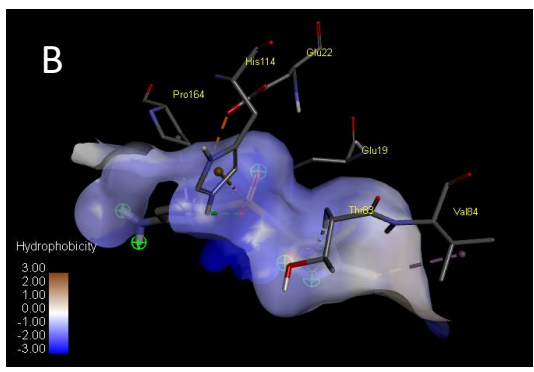
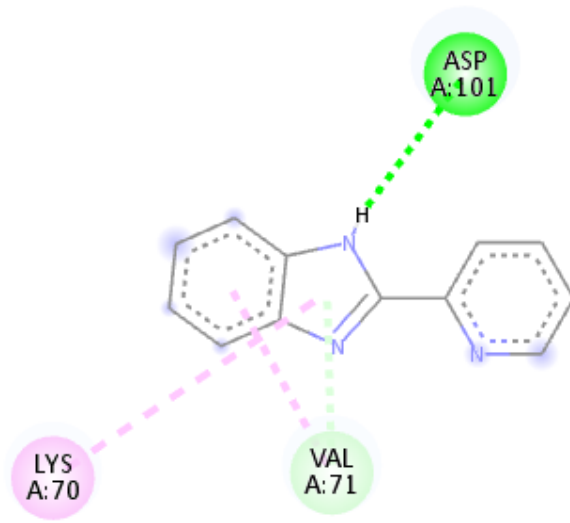






Figure 7: Interaction of complex 736 during docking. A) 2D docked view of complex 736. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

 Conventional Hydrogen Bond
 Pi-Donor Hydrogen Bond

 Pi-Sigma
 Pi-Alkyl

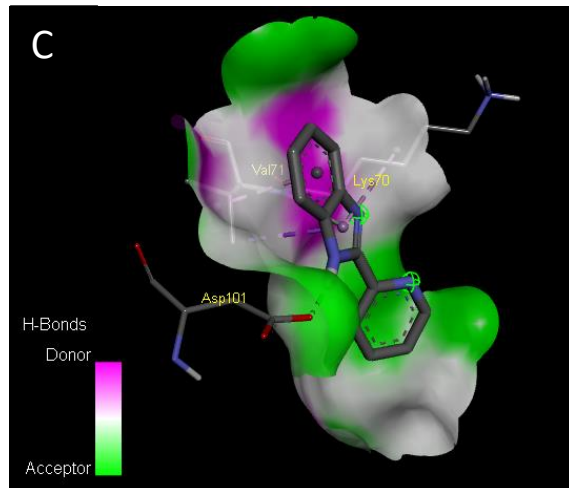
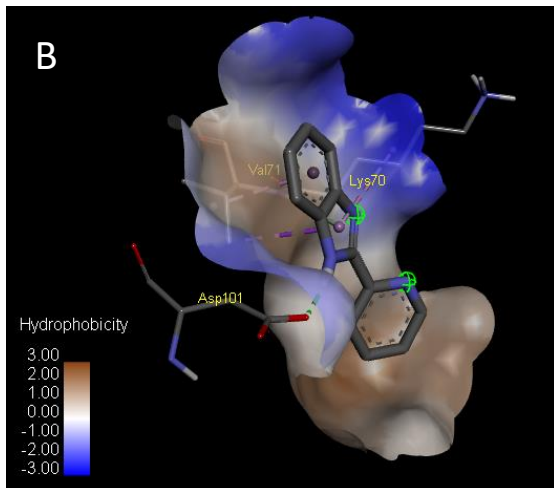
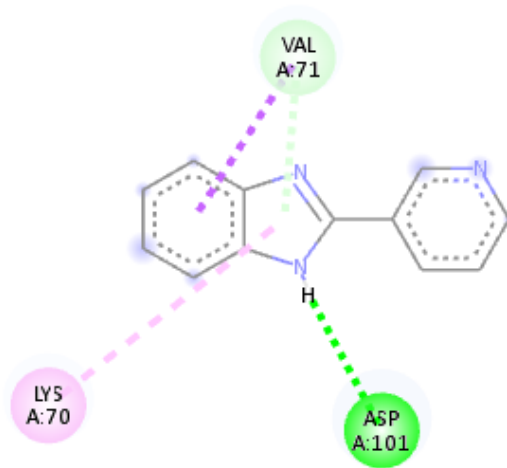






Figure 8: Interaction of complex 793 during docking. A) 2D docked view of complex 793. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

 Conventional Hydrogen Bond	 Pi-Sigma
 Pi-Donor Hydrogen Bond	 Pi-Alkyl

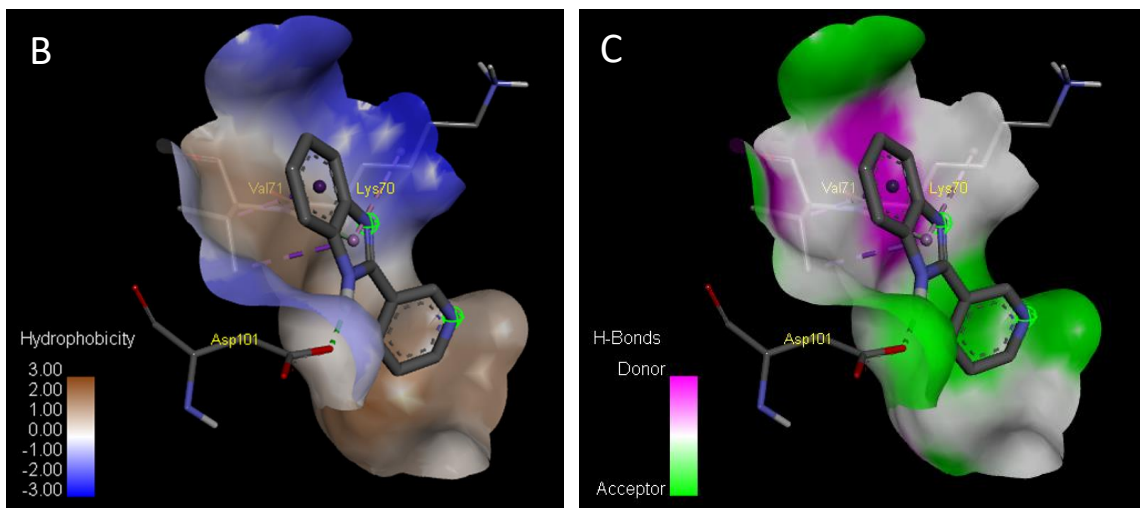
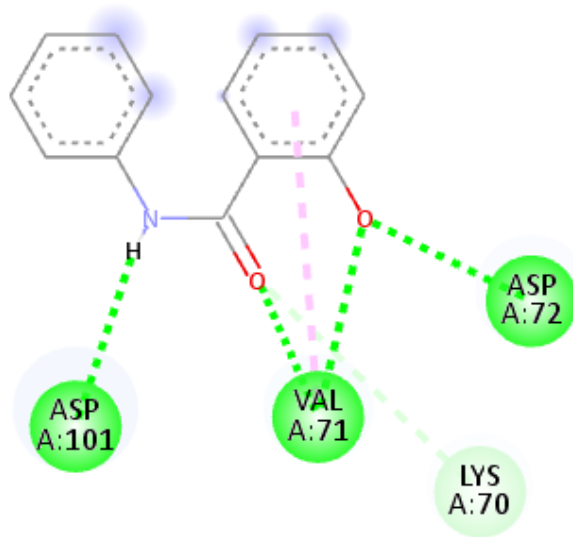


Figure 9: Interaction of complex 733 during docking. A) 2D docked view of complex 733. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

- Conventional Hydrogen Bond
- Carbon Hydrogen Bond

- Pi-Alkyl

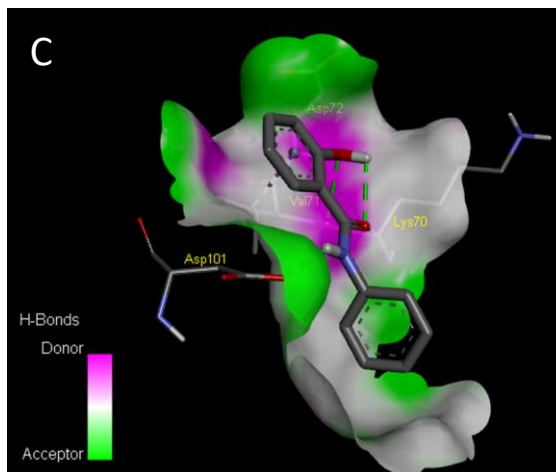
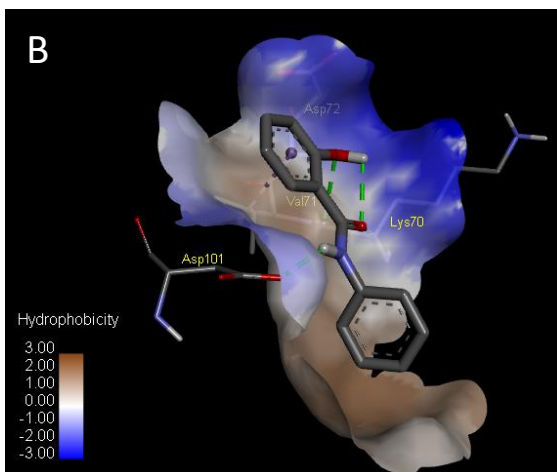


Figure 10: Interaction of complex 916 during docking. A) 2D docked view of complex 916. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

4.2 Discussion on top five ligands having the best binding affinity in the Library of Triazoles

Ligand 3760_C337-0010 three conventional H-bond with ASP A:51, GLU A:19, THR A:83 amino acid residues of the receptor. Receptor HIS A:52, ARG A:50, GLY A:17 shows Vander Waals interactions with C337-0010 respectively. Furthermore, C337-0010 also formed pi-Alkyl bond interactions with residue A:114HIS.

Ligand 3934_C549-0683 formed two conventional H-bond with SER-A:181, GLY A:106 amino acid residues of the receptor. Residues LYS A:107 and PRO A:182 showed pi-alkyl interactions with C549-0683 respectively. Furthermore, C549-0683 also formed Vander Waals with residues SER A:105, PRO A:108.

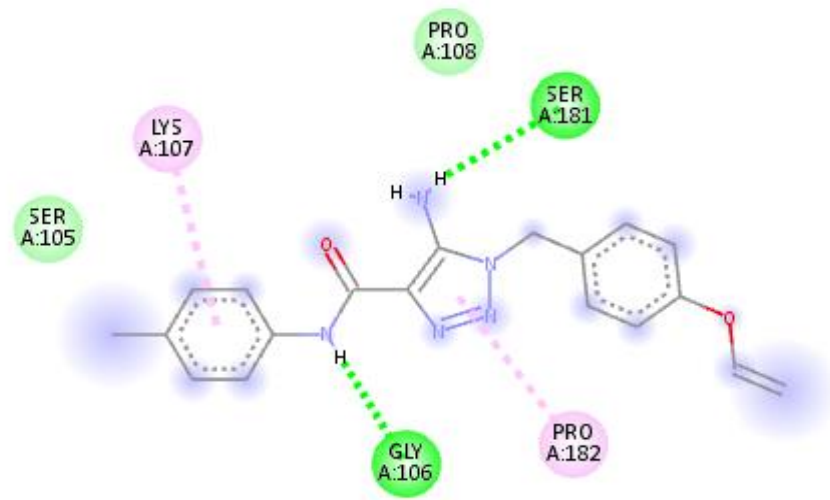
Ligand 3906_8007-1892 formed one conventional H- bond with GLY A:17 amino acid residues of the receptor. Residues ALA A:163, TYR A:133, PRO A:164, CYS A:113, ARG A:50 showed Pi-alkyl interactions with the 8007-1892 respectively. Furthermore 8007-1892 also formed Pi-Anion interactions with residues GLY A:19.

Ligand 2625_C368-0480 formed Three conventional H-bond with ASP A:51, GLU A:19, THR A:83 amino acid residue of the receptor. Residues HIS a:52 showed Pi-Sigma interactions with C368-0480 respectively. Furthermore, C368-0480 also formed Pi – Alkyl interactions with residues ALA A:163, PRO A:164.

Figure 10A, 11A, 12A, and 13A gives a clear view on different types of receptor ligand interactions involved.

Furthermore, to gain knowledge about the inner workings of the Protease protein modelled receptor, receptor surfaces (hydrophobicity and hydrogen bonds) were developed. The hydropathy index of the ligand protein was calculated using the hydrophobicity surfaces, which in Figures 10B, 11B, 12B, and 13B demonstrate the hydrophilic or hydrophobic characteristics of the amino acid side chains. Additionally, the surfaces of the amino acid residues are coloured according to how hydrophobic they are, ranging from blue for hydrophilic to brown for hydrophobic. Figures 10C, 11C, 12C, and 13C illustrate how H-bond surfaces are coloured, with H-bond donors being magenta and H-bond acceptors being green.

A



Interactions

- van der Waals
- Conventional Hydrogen Bond
- Pi-Alkyl

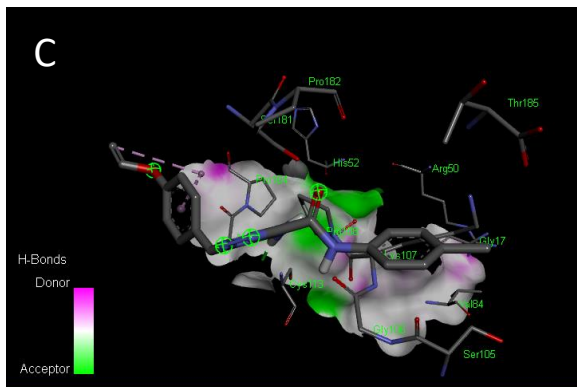
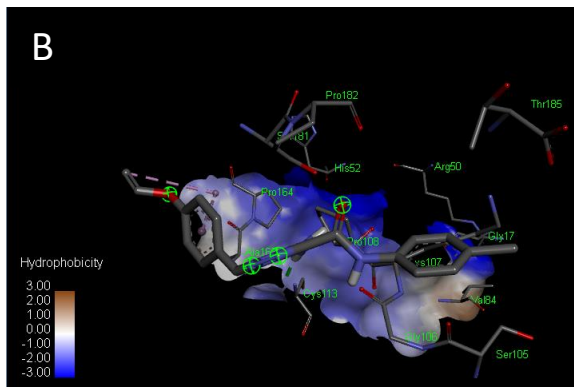
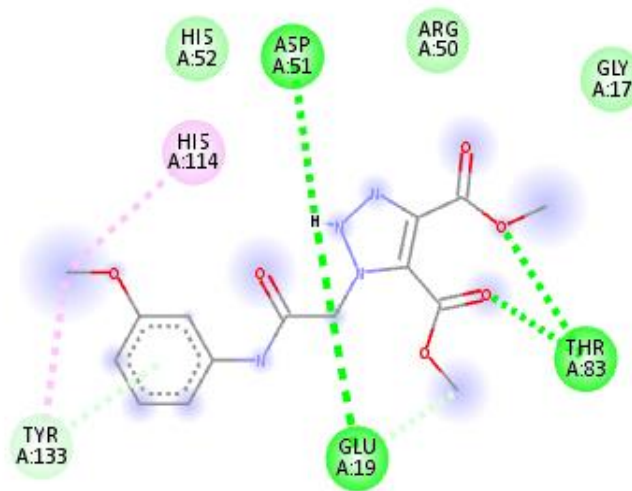


Figure 11: Interaction of complex 3934 during docking. A) 2D docked view of complex 3934. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

- | | |
|----------------------------|------------------------|
| van der Waals | Pi-Donor Hydrogen Bond |
| Conventional Hydrogen Bond | Pi-Pi Stacked |
| Carbon Hydrogen Bond | Pi-Alkyl |

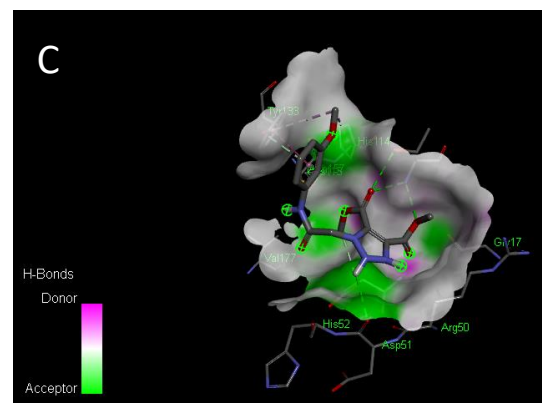
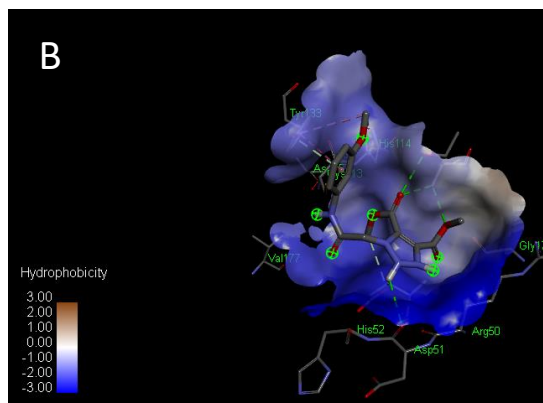
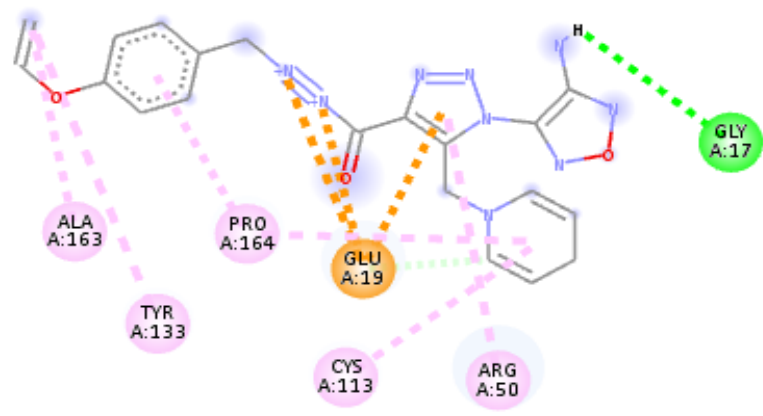








Figure 12: Interaction of complex 3760 during docking. A) 2D docked view of complex 3760. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

- | | |
|--|--|
|  Attractive Charge |  Pi-Anion |
|  Conventional Hydrogen Bond |  Alkyl |
|  Carbon Hydrogen Bond |  Pi-Alkyl |

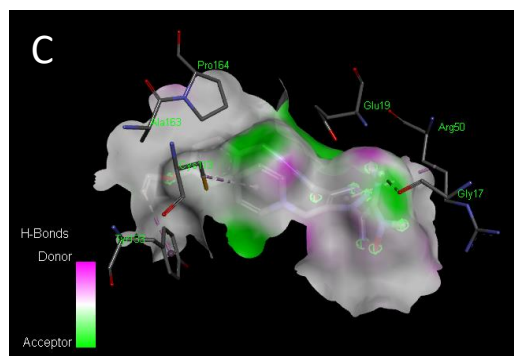
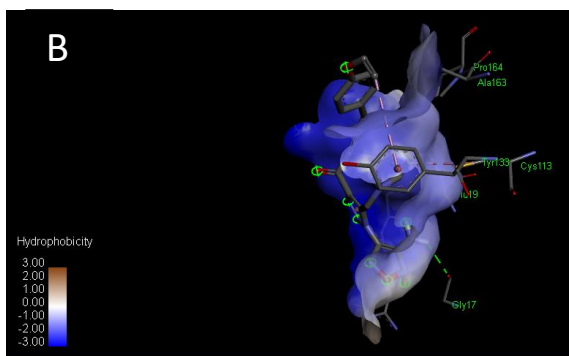
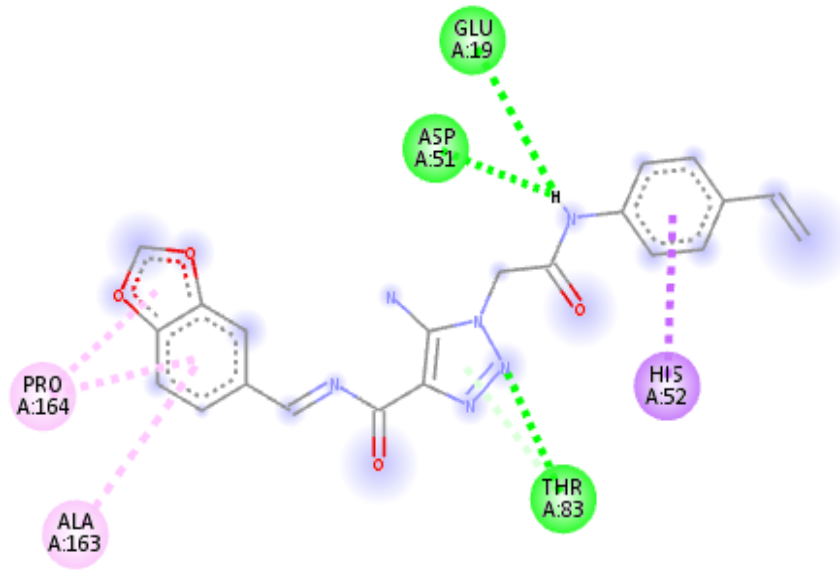


Figure 13: Interaction of complex 3906 during docking. A) 2D docked view of complex 3906. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

A



Interactions

- Conventional Hydrogen Bond (Green)
- Pi-Donor Hydrogen Bond (Light Green)
- Pi-Sigma (Purple)
- Pi-Alkyl (Pink)

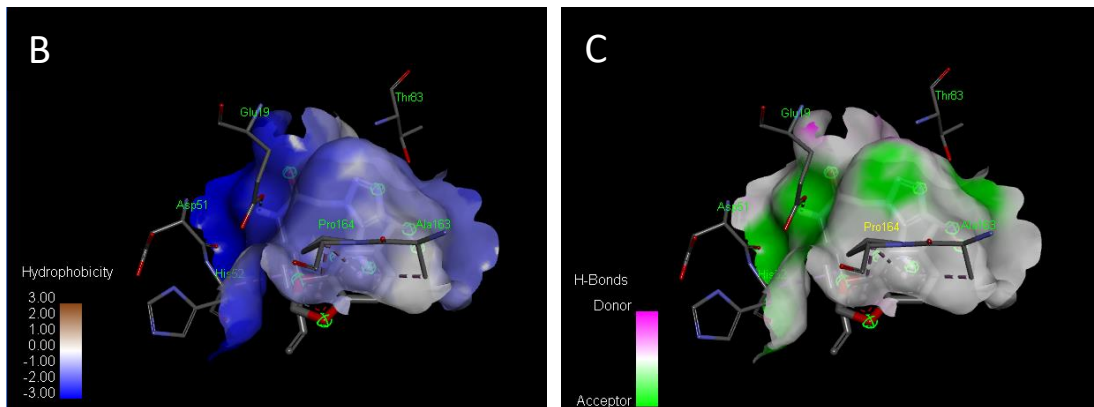


Figure 14: Interaction of complex 2625 during docking. A) 2D docked view of complex 2625. B) Surface view showing its hydrophobicity. C) Surface view showing its hydrogen bonding.

5. CONCLUSION

M. fortuitum causes various diseases, the process of molecular docking helps revive drug molecules for use against different protein targets, ultimately facilitating drug repurposing and discovery. This method forecasts the ideal conformation between the ligand and receptor, the best binding affinity, and a model of the atomic-level relationship between small molecules and proteins.

In this project, we have taken two libraries for docking analysis to determine whether they can become potential drugs or contribute to the lead druggable compound. Our triazole library has 4042 molecules, and we have done docking. On the basis of least binding affinity, we conclude that four molecules of the triazole library are potential drugs. After further analysis of the interactions in 2D and 3D format, we conclude that C368-0480, 8007-1892, C549-0683, and C337-0010 are the least binding molecules proposed as potential inhibitors of the upregulation of protease protein. In the fragment library, we have 1015 molecules of molecular weight less than or equal to 300 Da. After docking and analysis of the binding site residues, on the basis of least binding affinity, we have concluded that five molecules i.e. complex number 736, 793, 733, 25 and 913 can act as potential fragments which can be together linked or merged and become crucial part of the lead drug compounds. The identified compounds can be further improved to enhance their efficacy, specificity, and safety, potentially serving as a starting point for new drug development.

Result and analysis of this project will be helpful for researchers who are involved in the drug design for *M. fortuitum*. This project has provided valuable insights into the molecular interactions between a library of fragments and triazole compounds with the protease protein. These molecules with low binding energies could be used as one of the core molecules to be included while designing a drug against the protease protein of *M. fortuitum*.

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