INVESTIGATIONS ON IN SILICO MOLECULAR MODELLING AGAINST FALCIPAIN-2 IN SEARCH OF POTENT ANTI-MALARIAL AGENTS

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Semester VIII

(PROJECT WORK BP812PW)

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DECLARATION

We, **PRIT SAVALIYA (20BPH077), PRIYANSH RASTOGI (20BPH078), VIDHISHA SAVALIYA (20BPH109),** hereby declare that B.Pharm. project work (BP812PW) entitled **"INVESTIGATIONS ON IN SILICO MOLECULAR MODELLING AGAINST FALCIPAIN-2 IN SEARCH OF POTENT ANTI-MALARIAL AGENTS"** being submitted to Institute of Pharmacy, Nirma University for the award of degree of B.Pharm was carried by us under the supervision of **Dr. TEJAS M. DHAMELIYA**, Institute of Pharmacy, Nirma University. The content of this project work, in full or in parts, has not been submitted to any other University for the award of any degree. We also declare that all the information collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.

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Date: 14/05/2024

CERTIFICATE

This is to certify that B.Pharm. Project Work (BP812PW) entitled **"INVESTIGATIONS ON IN SILICO MOLECULAR MODELLING AGAINST FALCIPAIN-2 IN SEARCH OF POTENT ANTI-MALARIAL AGENTS"** being submitted by **PRIT SAVALIYA** (20BPH077), **PRIYANSH RASTOGI** (20BPH078), **VIDHISHA SAVALIYA** (20BPH109), for the award of degree in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy under my direct supervision to my full satisfaction. The content of thesis in full or in parts, has not been submitted to any other University for the award of any degree.

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CERTIFICATE FOR SIMILARITY OF WORK

This is to undertake the B.Pharm Project work (BP812PW) entitled "**INVESTIGATIONS ON IN SILICO MOLECULAR MODELLING AGAINST FALCIPAIN-2 IN SEARCH OF POTENT ANTI-MALARIAL AGENTS**". Submitted by **Prit Savaliya (20BPH077), Priyansh Rastogi (20BPH078), Vidhisha Savaliya (20BPH109),** B.Pharm. Semester VIII is a bonafide research work carried out by us at the Institute of Pharmacy, Nirma University under the guidance of **Dr. Tejas M. Dhameliya**. We are aware about the rules and regulation of Plagiarism policy of Nirma University, Ahmedabad.

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List of Abbreviations

ADMET	Absorption, distribution, metabolism, excretion and toxicity
AMES toxicity	Salmonella/microsome mutagenicity assay
CADD	Computer assisted drug design
CHARMM	Chemistry at HARvard Molecular Mechanics
COVID-19	Coronavirus Disease – 19
GROMACS	GROningen MAchine for Chemical Simulations
HB	Hydrogen Bond
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
hERG	Human ether-a-go-go related gene
LD 50	Lethal dose 50
LogP	Partition Coefficient
MD simulation	Molecular dynamic simulation
MR	Molecular refractivity
MW	Molecular weight
OCT2	Organic cation transporter 2
PDB	Protein Data Bank
RB	No. of rotational bonds
RoG	Radius of gyration
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
SASA	Solvent accessible surface area
TPSA	Topological polar surface area
V_d	Volume of distribution
WHO	World Health Organization

Abstract

Malaria has been a deadly illness transmitted to people by Anopheles mosquitoes, and it can be prevented in two ways, like averting mosquito bites and using medication to prevent the malaria. The development of resistance to anti-malarial medications has caused the poor patient compliance for the treatment of malaria. Hence, there has been a strong need for the design and identification of new chemical entities acting against novel promising malarial targets. Falcipain-2, a cysteine protease, has been such novel target for the investigation of anti-malarial drugs being involved in heme metabolism during the erythrocytic stage. The current state of resistance to malaria treatments has made it imperative to find new anti-malarial drugs using *in silico* tools driven by computer aided drug design. Using AutoDock Vina, 91,001 ligands from the Asinex Elite Synergy 2021-01 library were docked in search of falcipain-2 (PDB ID: 3BPF) inhibitors, which confirmed the discovery of hits (**1**-**20**) with superior binding energy than the natural ligand, E64. Moreover, studies for bioavailability and ADMET for better oral bioavailability and druggability have been employed. Further, the dynamics simulation test of the top two hits (**1** and **2**) were performed through GROMACS 2023.4 for 100 ns, revealing their stability in the docked complex. These findings represent an important start-up in the design and identification of hits against falcipain-2 as anti-malarial agents.

CHAPTER 1: Introduction

Malaria, means bad air and caused by *Plasmodium falciparum*, has afflicted people for ages as these little intruders manage a complicated existence cycle, moving with amazing plasticity between female Anopheles mosquitoes and human hosts. There is a chance that 50% of the world's population might be infected with plasmodium species. According to estimates from the World Health Organization (WHO), there has been 249 million cases in 2022 and 0.61 million deaths, however due to corona virus disease 2019 (COVID-19), these numbers may be hampered or not be reported accurately. Approximately 76% of malaria fatalities occurred in children younger than five. The goals of ongoing research are to comprehend the processes underlying resistance, create novel antimalarial medications, and determine the most effective means of avoiding mosquito bites. As a result, it has been making matters worse, which has exacerbated the worldwide crisis.
(https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021)

Malaria can cause a number of symptoms, such as fever, chills, sweating, headache, malaise, weakness, gastrointestinal trouble, dizziness, confusion, and disorientation (Siqueira-Neto et al. 807). The life cycle of a parasite consists of two phases: the endogenous or asexual phase in the human host and the exogenous sexual phase in the mosquito (Figure 1.1). The female Anopheles mosquito carrying malarial parasites bites healthy human beings, suspending sporozoites during the blood meal. Sporozoites enter the circulation, go to the liver, and infect hepatocytes there. Repetitive asexual fission of sporozoites (pre-erythrocytic /sporozoite /liver stage) within the hepatocyte gives rise to schizonts, which in turn create thousands of merozoites. Sporozoites in *P. vivax* and *P. ovale* infections mature into hypnozoites in hepatocytes, which lie latent for weeks or months before reawakening to cause malaria relapses. Merozoites are released by schizonts as they break open and penetrate red blood cells. Here, after ingesting hemoglobin, merozoites develop and undergo many asexual phases of self-replication (erythrocytic stage). The parasite develops into a trophozoite and then a schizont. The clinical signs of malaria are caused by newly released merozoites that infect additional erythrocytes in the circulation when the erythrocytic schizont ruptures.

Through the process of gametocytogenesis, certain merozoites develop into sexually dimorphic gametocytes. In uninuclear versions of the parasite that enter the circulation, gametocytes have undergone specialization. Male and female gametes are formed after they consume the mosquito blood meal. During the sexual cycle, both male and female gametes produce a zygote that matures into an ookinete and then in the oocyst's shape. The nucleus of the oocyst splits often, producing a large number of sporozoites that go to the mosquito's salivary glands where they infect more hosts and begin the subsequent cycle.(Aly et al. 195) The failure of currently used anti-malarial drugs and the rise of resistant malaria have led to the design and development of new anti-malarial agents acting against novel promising drug targets.

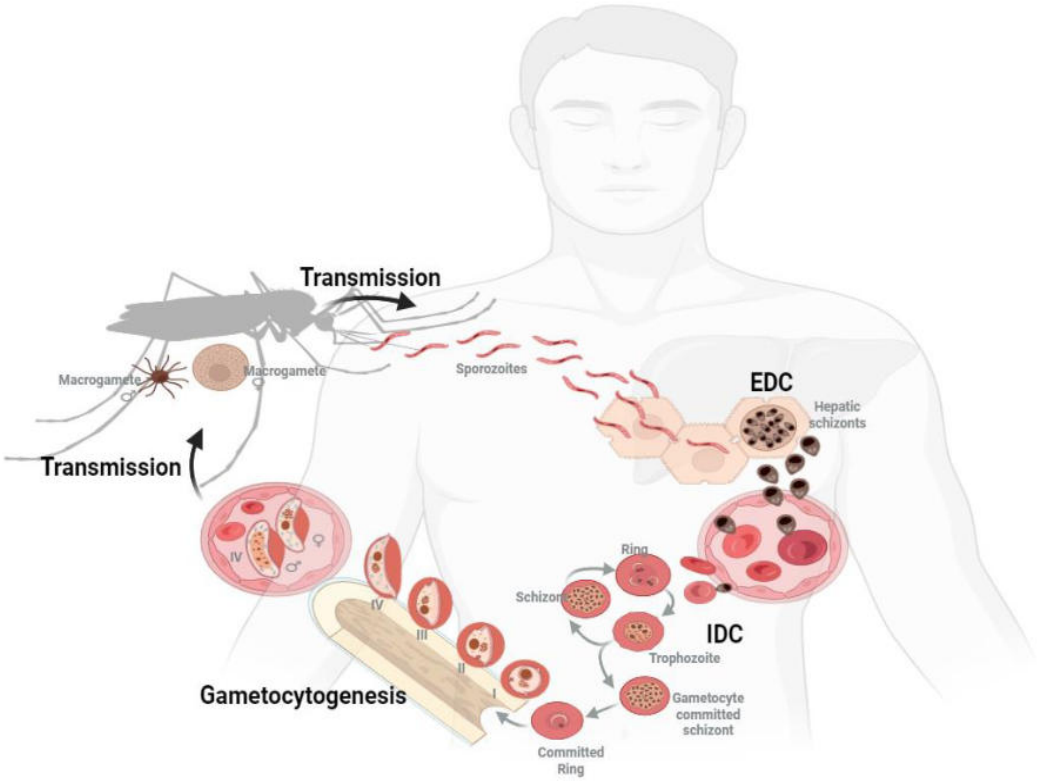


Figure 1.1 The life cycle of malaria parasites.

In an acidic food vacuole, malaria parasites hydrolyze hemoglobin in the free amino acids needed for protein synthesis and perhaps to preserve osmotic stability. Proteases that hydrolyze hemoglobin have been among the possible targets for anti-malarial agents. In this context, falcipain-2 has been the desirable target in search of anti-malarial agents, as they

have been the class of cysteine proteases that play a role in the metabolism of heme during the erythrocytic stage. Since 1988, cysteine protease inhibitors have demonstrated anti-malarial efficacy. The inhibitors of falcipain-2 are crucial to plasmodium because they block the breakdown of hemoglobin. Because of their crucial involvement in the pathophysiology of malaria, the existence of druggable pockets, and the availability of crystal structures for structure-aided drug design, they have been promising drug targets.(Patra et al. 115299)

The only way to control and prevent malarial illness in the absence of a viable vaccination is to utilize antimalarial drugs therapeutically. Numerous investigations revealed that the development of drug-resistant Plasmodium species hampered the effectiveness of most antimalarial drugs. The fact that resistance has been documented for almost all antimalarial drugs in use highlights the urgent need to both find new targets and create new antimalarial drugs that can target validated ones now in use. It is critically necessary to create a new antimalarial drug that targets intraerythrocytic proliferative asexual parasites, particularly those of resistant species, as well as transmissible gametocyte stages. Promising novel targets for the development of new antimalarial drugs against rapidly mutating malarial parasites include a number of enzymes, ion channels, transporters, interacting molecules in red blood cell (RBC) invasion, and molecules responsible for oxidative stress in the parasite, lipid metabolism, and haemoglobin degradation. A new antimalarial agent's potential is evaluated based on a number of criteria, including its ability to treat malaria in single doses, have unique mechanisms of action that do not cross-resipate with existing antimalarial drugs, and be effective against both the gametocytes and asexual blood stages that transmit malaria. In addition, the novel antimalarial drug ought to be effective in preventing infection (chemoprotective compounds) and eliminating Plasmodium vivax hypnozoites from the liver.

There are several methods used in traditional drug development to find a novel anti-malarial medication to fight malaria. These strategies include improving on current dosage schedules and formulations, changing the antimalarial medications already in use, screening natural products, identifying compounds that reverse resistance, combining chemotherapy with other treatments, and taking use of medications that are prescribed for different purposes. The knowledge of Plasmodium cell biology and genome has proven to be a powerful tool for identifying mechanisms of resistance and has great potential to design novel

drugs with both high antimalarial activity and transmission-blocking potential to end malaria forever, in addition to the traditional drug discovery methods for the identification of new ant-malarial agents.

Globally, the Greater Mekong subregion and other African countries, including Eritrea, Rwanda, and Uganda, have been found to have parasite resistance to artemisinin. Resistance to both artemisinin and the partner medication in ACT medication regimens can result in high rates of treatment failure, but resistance to artemisinin alone is rarely the cause of treatment failure. This has been observed in recent years in several areas of the Greater Mekong subregion. Africa has not yet shown evidence of ACT partner medication resistance, and the treatment is still very effective. There are, however, some concerning signs: statistics are missing for a number of nations, and conflicting conclusions regarding the effectiveness of ACT should be carefully investigated. Considering how heavily ACTs are used in Africa, a full-blown treatment failure could have dire repercussions. Dr. Dorothy Achu, the newly appointed Team Lead for Tropical and Vector Borne Diseases for the WHO African Region, observes, "We don't have that many options for malaria drugs." For simple malaria, the only available treatments are combination medicines based on artemisinin. We certainly want to prevent many cases and deaths, so any danger to these medications might have that effect," she continued. In 2016, Imperial College London researchers used a model to simulate the possible effects of widespread resistance in Africa to both artemisinin and a companion medication. An estimated 16 million extra cases of malaria and over 360 000 more severe cases needing hospitalization would occur annually under this scenario.

In the search for effective therapeutic agents, computational methods are being rapidly explored in the design, discovery, and development of drugs. The introduction of any drug to the market has been a labor-intensive, expensive, and time-consuming process with a lot of risks. The process of finding and developing new drugs typically takes 10 to 14 years and requires more than $1 billion in funding overall. Therefore, the computer-assisted drug design (CADD) technique has been a revolutionary drug design strategy being extensively employed to reduce time, cost, and risk-borne elements. The adoption of these techniques cut down the cost of drug discovery and makes it cheaper and easily available.(Surabhi and Singh 504; Talele et al 127; Ooms 141)

Towards the on-going research endeavour from this laboratory using computational tools (Salaria et al. 99; Pande et al. 8; Vegad et al. 221; Dhameliya et al. 1847; Dhameliya et al. 1361; Sureja et al. e202202069; Bhakhar et al. e202202069; Dhameliya at al. c–2792) for the discovery of anti-malarial agents,(Dhameliya and Patel, et al. e202303982; Dhameliya and Vekariya, et al. e202303982; Dhameliya et al. 753) we have performed the virtual screening of 91,001 ligands from Asinex Elite Synergy 2021-01 against falcipain-2 in the hunt of anti-malarial agents followed by the assessment of the *in silico* pharmacokinetic profile and evaluation of the stability of identified hits using molecular dynamics (MD) simulations for 100 ns in the active site of falcipain-2.

CHAPTER 2

2.0 Literature Review

Significant progress has been made in recent years to alleviate the extreme suffering that malaria causes all around the world. Notably, there has been a significant impact from the use of artemisinin-based combination treatment (ACT) for treating malaria and insecticide-treated mosquito nets for malaria prevention. However, the emergence of resistance to both current and historical anti-malarial medications emphasize the necessity of ongoing research to stay ahead of the game. There is a need for new medications, especially ones with novel modes of action. Numerous more natural and artificial chemicals have been created since quinine, the first chemically pure and successful therapy for malaria, was isolated in 1820. However, the parasite strains developed resistance to these medications over time, which decreased their efficacy. As a result, they are no longer used or are only used in specific circumstances.(Tse et al. 93) Malaria infection is a leading cause of sickness and death in Sub-Saharan Africa, especially in Nigeria. In cases when a parasitological test is not possible, the World Health Organization (WHO) has explicitly approved presumptive diagnosis as the first-line therapy for uncomplicated malaria. This program reduces treatment delays, particularly for people who live far from formal healthcare facilities, by allowing village health professionals, merchants, and family members to treat simple malaria illnesses in the patient's home. Over 70% of people with symptomatic malaria in most African regions do not seek medical attention from healthcare facilities; instead, they self-diagnose and treat their illness at home with conventional medications or antimalarial drugs obtained from local pharmacies or drug stores. Such symptomatic malaria patients only seek medical attention after self-medication with conventional or traditional treatments fails, reducing the efficacy of different malaria diagnostic and therapeutic procedures. A cross-sectional randomized study was conducted with 1000 voluntary outpatients from a tertiary hospital in Nigeria to evaluate the validity of clinical malaria diagnosis in contrast to microscopy and rapid diagnostic test kits (RDTs).(Wogu 4)

Almost half of the world's population lives in areas at danger from malaria, which claims the lives of over 400,000 people annually. The necessity for creating innovative treatments is highlighted by the recent stalling in the fight against malaria. The parasite

hemoglobin degradation pathway is a well-known treatment target, as it is active throughout the blood stage of the disease, when death and malaria symptoms occur. The papain-type protease falcipain-2 is an important enzyme in this pathway.(Machin et al. 1) Given their considerable potential as anti-malarial drugs, we have screened alkaloids in this investigation to find putative inhibitors against FP-2. Through the use of many computational pipelines, 340 alkaloids in total were taken into consideration for the study. Initially, screen chemicals were subjected to toxicity risk assessment criteria and pharmacokinetics. Molecular docking techniques were then employed to comprehend the alkaloids' binding effectiveness against FP-2. Additionally, the pkCSM tool was used to predict oral toxicity, and the PharmaGist server was used to analyze 3D pharmacophore characteristics.(Nema et al. 1; Pandey and Dixit 345195)

Although computer-aided drug development (CADD) has been a practice for many years, academics and pharmaceutical companies have recently experienced a seismic change in their use of computational tools. Ample computer power, a wealth of information on ligand characteristics and binding to therapeutic targets and their three-dimensional structures, and the development of virtual libraries of drug-like small compounds available on demand in billions are the main factors defining this change. To fully utilize these resources, efficient ligand screening necessitates quick computational techniques. Fast iterative screening technologies further assist structure-based virtual screening of gigascale chemical regions. Advances in deep learning predictions of target activities and ligand characteristics instead of receptor structure are highly synergistic. In this article, we examine the most recent developments in ligand discovery technologies, their potential to completely transform the drug discovery and development process, and the difficulties they face. We also talk about how the drug discovery process may be made more accessible and affordable by quickly identifying highly powerful, varied, target-selective, and drug-like ligands to protein targets. This will open up new possibilities for the creation of safer and more efficient small-molecule therapeutics.(Sadybekov and Katritch 673; Yu and Mackerell 85)

2.1 Problem Statement

Malaria remains one of the most devastating infectious diseases, with millions of new cases annually leading to significant morbidity and mortality worldwide. Despite ongoing efforts, the emergence of drug-resistant strains of the malaria parasite, Plasmodium falciparum, poses a continuous challenge to existing therapeutic strategies. A critical enzyme implicated in the parasite's lifecycle is Falcipain-2, a cysteine protease that plays a pivotal role in hemoglobin degradation, an essential process for the parasite's survival and growth.

Recent advances in silico molecular modeling offer promising avenues for the rapid and cost-effective screening of potential inhibitors that target key biological pathways, such as those mediated by Falcipain-2. However, the complexity of accurately modeling interactions at the molecular level and predicting the efficacy and safety of these inhibitors remains a significant barrier. There is a pressing need to enhance computational models that predict how potential drug molecules interact with Falcipain-2, to identify novel compounds that can be developed into effective anti-malarial agents.

This research aims to address these challenges by:

1. Developing and refining computational models that can accurately simulate and predict the binding affinities of potential drug candidates to Falcipain-2.
2. Evaluating the effectiveness of newly identified compounds in silico, thus paving the way for further empirical testing and development.

This thesis will focus on the integration of computational biology and pharmacological insights to discover and optimize novel inhibitors of Falcipain-2, potentially offering a new class of anti-malarial drugs.

2.2 Aim

The aim of this research is to use *in-silico* molecular modelling method against Falcipain-2 in search of potent anti-malarial agents.

2.3 Objective

1. Virtual screening of 91,001 ligands of Asinex Elite Synergy 2021-02 against Falcipain-2 using AutoDock Vina in search of anti-malarial agents.
2. Analysis of the interactions of top 20 identified ligands with falcipain-2.
3. To characterize ADMET profile of the top 20 identified hits and to study their bioavailability using rules of bioavailability.
4. Molecular dynamic simulations to check the stability of ligand at the binding site in ligand-receptor complex and to study the energies of complex of top 2 compounds with falcipain-2.

CHAPTER 3

3.0 Materials and Methods

3.1 Data Collection and Preparation

After downloading the 3-D structure of falcipain-2 (PDB: 3BPF)(Kerr et al. 852) from RCSB protein data bank,(*Protein Data Bank*) the co-crystallized ligand (E64) was removed from the protein and essential chain A of interest was extracted with the help of PyMOL.(*DeLano WL (2002). The PyMOL Molecular Genetics Graphics System, DeLano Scientific LLC, San Carlos, CA.*) The protein for molecular docking was prepared by removing water, adding the polar hydrogens, Kollman and Gasteiger-Hückel charges and was saved in .pdbqt format using AutoDock Vina.(Trott and Olson 455) The 91,001 ligands from Asinex Elite Synergy 2021-01 library(*Asinex*) were downloaded and were subjected to optimization using Open Babel by their conversion to .pdbqt files.(O'Boyle et al. 33)

3.2 Molecular Docking

The anchor point of the 3BPF for the resulting receiver grid box was set to size_x = 22.0, size_y = 22.0, and size_z = 22.0 for the ligand using the trial-and-error method with the help of AutoDock Vina. After finding the grid parameters and coordinates for the grid box for the docking and binding sites of E64 molecular docking using AutoDock Vina, the validity of docking protocol was attained through the comparison of the docking pose of the docked E64 with that of the co-crystallized E64. It was found to be co-aligned with the pose of co-crystallized E64. Next, 91,001 optimized ligands of Asinex Elite Synergy 2021-01 including natural product and synthetic molecules were virtually screened against the predicted binding site using AutoDock Vina. The top twenty compounds with highest binding energies (**1**-**20**) ranging from -10.0 to -9.7 kcal/mol were shortlisted for further studies.

3.3 Assessment of Physicochemical Parameters, Drug-likeliness, and ADMET Characteristics

The numerous physicochemical characteristics, drug-likeliness and ADMET profile of the identified hits (**1**-**20**) were calculated *in silico* via pkCSM (Pires et al. 4066) and SwissADME using the online web tools.(Daina et al. 42717)

3.4 MD Simulation

Using the GROMACS version-2023.4 package, MD simulations were run on an NVIDIA Corporation RTX A2000 graphics card running Ubuntu 22.04.3 to verify the stability of the chosen ligand-protein complexes. The CHARMM27 all-atom force field was used to build the protein topology. To build the ligand topologies, the SwissParam server was used. The protein and ligand-protein complexes were solved within a cubic box of 10 Å with SPC216 water model. Using the gmx genion tool, twelve sodium ions were added to the solvated system to neutralize the charged protein. The MD simulation was carried out in three phases, all heavy atoms restrained to keep the original protein folding by utilizing a force constant of 1,000 kJ/mol.nm^2. Using the steepest descent algorithm, energy is minimized in the first step. The system proceeded through two phases of equilibration in the next step, each of which was conditioned for 100 ps. During the first stage of equilibration, the temperature inside the three-dimensional cubic box was controlled using the Berendsen thermostat coupling method at constant number of particles, volume, and temperature (NVT) ensemble. The second equilibration stage was performed at a constant number of particles, pressure, and temperature (NPT) ensemble at 1 atm and 300 K under the guidance of the Parrinello-Rahman barostat. Finally, long-range electrostatic interactions predicted using the Particle Mesh Ewald approach were used in 100 ns MD simulations. Further, the MD trajectories was analyzed using GROMACS tools to compare the data, including root mean square deviation (RMSD), radius of gyration (RoG), solvent accessible surface area (SASA), root mean square fluctuation (RMSF), and number of hydrogen bonds (H-bond).

CHAPTER 4

4.0 Results and Discussion

4.1 Preparation of Protein

Protein was prepared before docking with the help of AutoDock tools.(Morris et al. 2785; Trott and Olson 455) The crystal structure of 3BPF (falcipain-2) protein obtained from RCSB protein data bank.(*Protein Data Bank*) Protein molecules are generated by appropriate extraction, deleting molecules of water, reduction of energy and unnecessary chains, and in this study, the chain A was determined to be essential. By identifying essential amino acid residues, a receptor grid is created around the cavity of the unenergized protein molecule. Co-crystal ligand (E64) of the corresponding protein molecule is selected to identify amino acid residues used to predict the binding site.

4.2 Virtual screening

Virtual screening has been recognized as a computer-based method used in drug discovery which is used to screen large libraries of small molecules to find the structures that have the highest probability of binding to a target of the drug, usually an enzyme or protein receptor. It is highly useful to identify the ligands that would be most effective without having to test them all out in a lab setting. The drug discovery phase may be expedited and cost-effectively lowered with this technique.(Kaczor et al. 73) A total of 91,001 ligands from the Asinex Elite Synergy 2021-01 library have been selected for docking against falcipain-2.(*Asinex*) These downloaded ligands were subjected to optimization using Open Babel for geometrical bond angle, distance or tetrahedral angle.(O'Boyle et al. 33)

SBVS makes use of the target protein's three-dimensional structure, which can be found via techniques like NMR spectroscopy or X-ray crystallography. Using a variety of scoring methods, molecules are docked into the target protein's binding site to predict their interaction and binding affinity. When a target's crystal structure is known, SBVS is frequently employed to enable accurate ligand-receptor interaction investigations. LBVS can be used when the biological target's three-dimensional structure is unclear. This approach depends on your understanding of other molecules that bind to the desired target. Methods

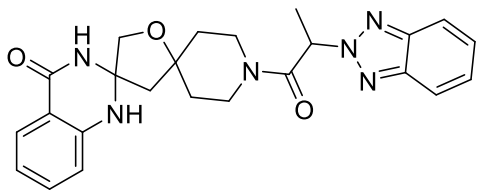
like pharmacophore modeling and quantitative structure-activity relationships (QSAR) are frequently used.

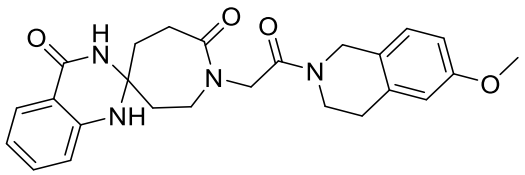
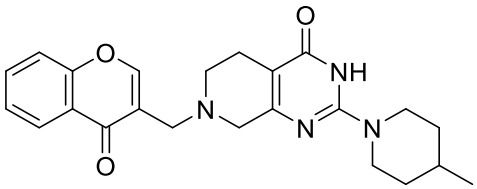
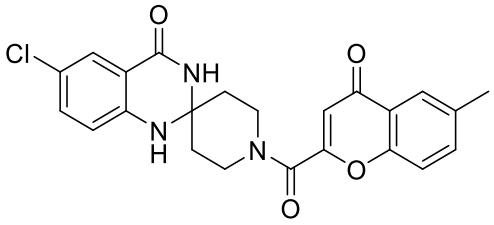
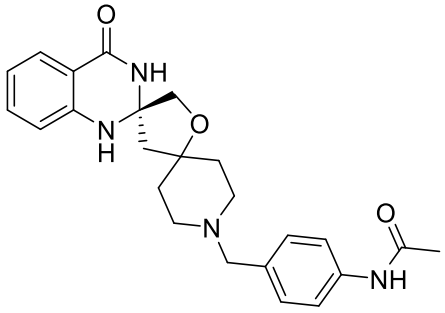
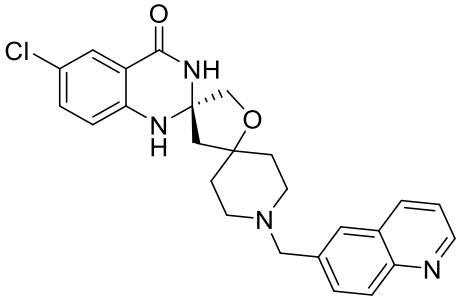
Based on the characteristics and actions of existing binders, LBVS is helpful in the identification of novel ligands. The drug discovery phase may be expedited and cost-effectively lowered with this technique.(Kaczor et al. 73) A total of 91,001 ligands from the Asinex Elite Synergy 2021-01 library have been selected for docking against falcipain-2.(*Asinex*) These downloaded ligands were subjected to optimization using Open Babel for geometrical bond angle, distance or tetrahedral angle.(O'Boyle et al. 33) The steps which are involved in Virtual screening, First Preparation of the desired protein which is prepared by aligning the charges states of the amino acids, remove water molecules, and add hydrogen atoms to prepare the protein structure and compilation of the ligand data by preparing ligand libraries by machining sure their geometries are optimized and charges are assigned, among other correct formats. The 2nd step, which is Docking, the fit and binding affinity of the small molecules, or ligands, against the proteins active site are evaluated computationally. After the docking process we evaluate the Rank and Score of the possibility for each docked position to interact with the target protein that is assigned a score. There are three types of scoring function: empirical, knowledge-based, or based on force field calculations. Scoring functions are the heart of the virtual screening according to which the complexes are assigned a score, regression models generated from empirically measured binding affinities of protein-ligand complexes serve as the foundation for Empirical Scoring Functions. The binding affinity is determined using empirical scoring functions as the sum of weighted terms representing several interaction types, including ionic interactions, hydrophobic effects, hydrogen bonding, and entropic contributions. A coefficient that is tuned against a set of known protein-ligand complexes is assigned to each of these interaction types. SCORE, ChemScore, and X-Score are a few examples. Knowledge-based, the statistical analysis of known protein-ligand complexes from structural databases such as the Protein Data Bank (PDB) is the source of Scoring Functions. These functions operate under the assumption that interactions between specific sorts of atoms in proteins and ligands, which are frequently observed, are energetically beneficial. Usually, the frequency of these interactions in relation to a reference state determines the score. Examples are DrugScore and Potential of Mean Force (PMF). In-depth mathematical models are used by Force Field-Based Scoring Function to explain how the complex of protein and ligand interacts. Potential energies are usually

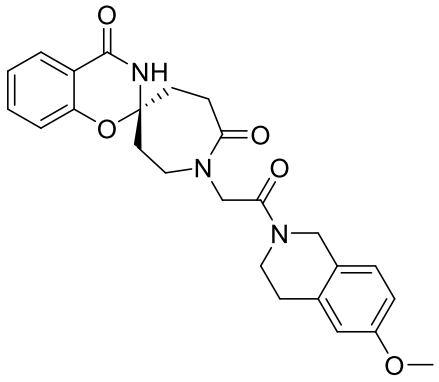
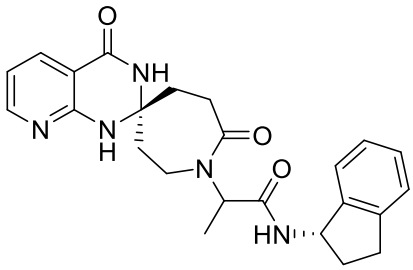
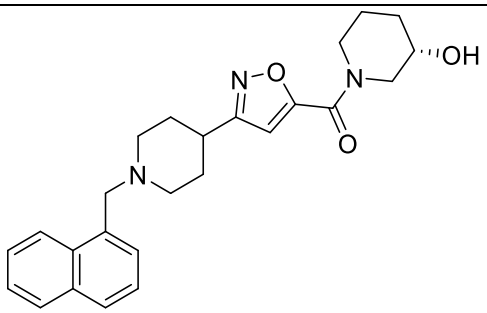
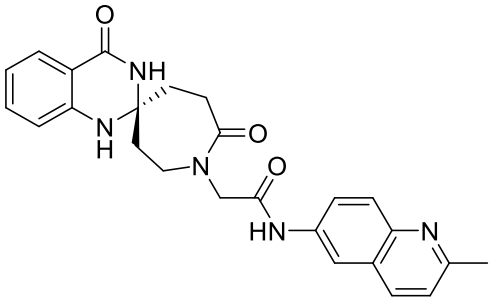
computed using molecular mechanics force fields, which contain terms for electrostatic interactions, bond stretching, torsional strain, angle bending, and van der Waals forces.MM-PBSA (Molecular Mechanics Poisson-Boltzmann Surface Area), CHARMM, and GROMOS are a few examples, now at last the compounds with the highest scores are potentially reassessed or put through additional testing and verification processes like MD or experimental validation. Some benefits of virtual screening are that it allows you to assess huge compound libraries fast, which minimizes the number of candidates that require laboratory testing and synthesis, less costly than high-throughput screening since fewer physical chemicals and related laboratory supplies are required and depending on the data available, can be applied using various methodologies (ligand-based vs. structure-based).

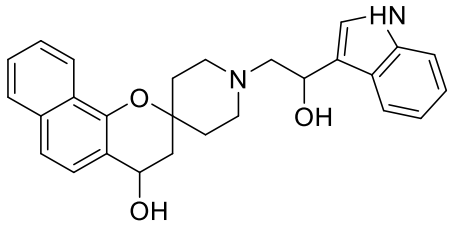
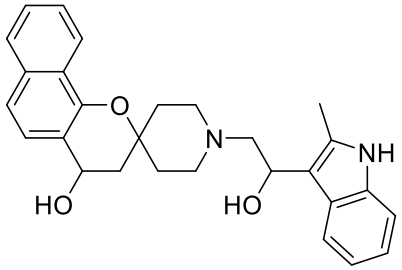
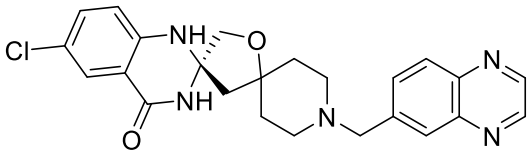
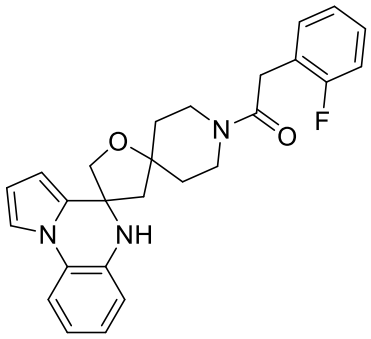
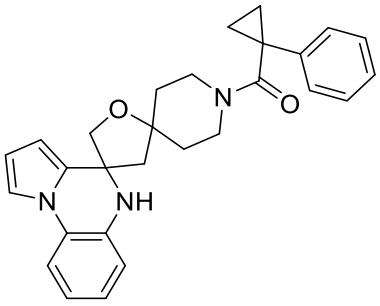
The docking was performed using AutoDock vina, which is a software tool for molecular docking, a process that predicts how small molecules will bind to receptors. The top 20 molecules with the greatest receptor-ligand complex binding affinity have been determined via scoring and evaluated the docked postures based on their RMSD values (Table). The binding energy of the ligands was compared with the docking results of the co-crystal ligand. The highest binding energy was found to be -10.0 kcal/mol with the quinazolinone derivative (**1**). With considerable variance, the binding affinities of the top twenty compounds were quite comparable and next, we also examined the ligand-protein interactions in detail.

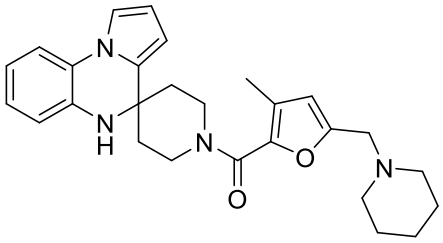
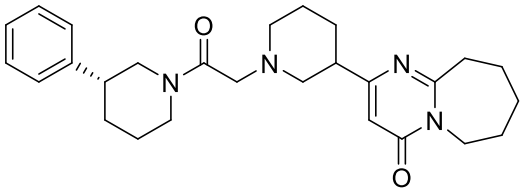
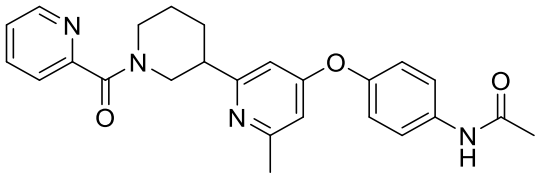
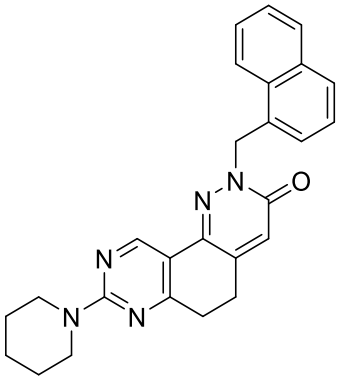
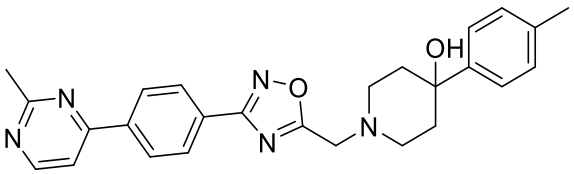
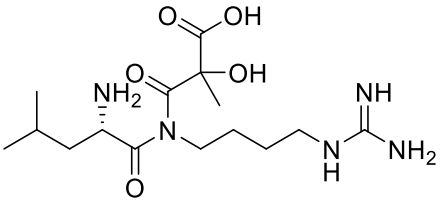
Table 4.1 Top 20 ligands identified from the virtual screening.

Comp. code	**Mol ID**	**Structure**	**Binding energy (kcal/mol)**
1	Mol2250		-10.0

2	Mol2560		-9.9
3	Mol7633		-9.9
4	Mol45370		-9.9
5	Mol2273		-9.8
6	Mol2333		-9.8

7	Mol2628		-9.8
8	Mol2720		-9.8
9	Mol39117		-9.8
10	Mol45403		-9.8

11	Mol47626		-9.8
12	Mol47630		-9.8
13	Mol2348		-9.7
14	Mol3378		-9.7
15	Mol3380		-9.7

16	Mol18215		-9.7
17	Mol31549		-9.7
18	Mol33789		-9.7
19	Mol59032		-9.7
20	Mol23317		-9.7
21	**E64**		-5.8

Compound **1** was determined to have the highest docking score of -10.0, exhibiting conventional hydrogen bond (HB) interaction with His174, carbon hydrogen bond with Leu172 and Ser149 (

Figure). It also shows Pi-anion, Pi-sigma, Pi-Pi T-shaped and Pi-alkyl interaction with Asp234, Leu84, Tyr78 and Ile85, respectively. Compound **2** was found to interact with Leu84, Ile85 and Ala175 through Pi-alkyl interactions and Pi-Sulfur with Cys42, conventional H-bond with Trp206. Compound **3** was found to be involved in π-π interactions with Leu84, Ile85, Phe236 and Asn173 and hydrogen bond interactions with Gln171, His174 and Gln36 and van der Waals non-polar interactions with Ala175. Compound **4** showed π-anion and π-sigma interaction with Asp234 and Leu84, respectively and π-alkyl interaction with Cys42, hydrogen bond interaction with Ile85 and Asn173. Compound s**5** interacted with His174, Gln36 and Trp206 through hydrogen bond, π-anion, and π-sigma interactions with Asp234 and Leu84, respectively.

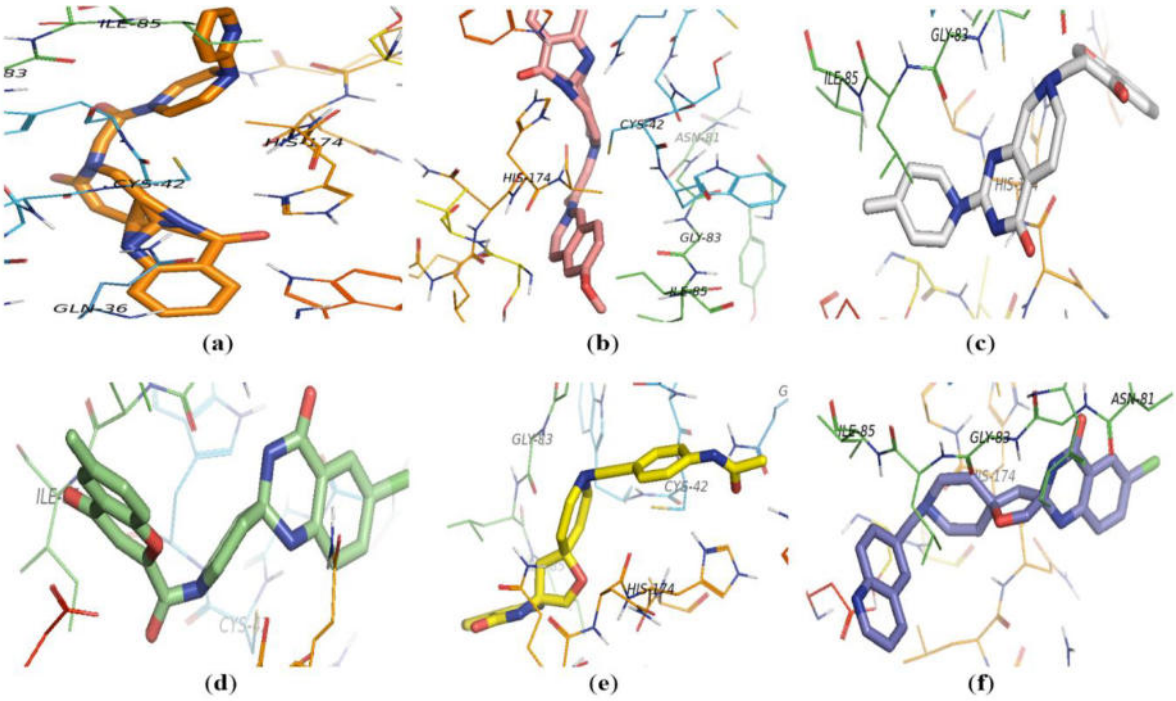
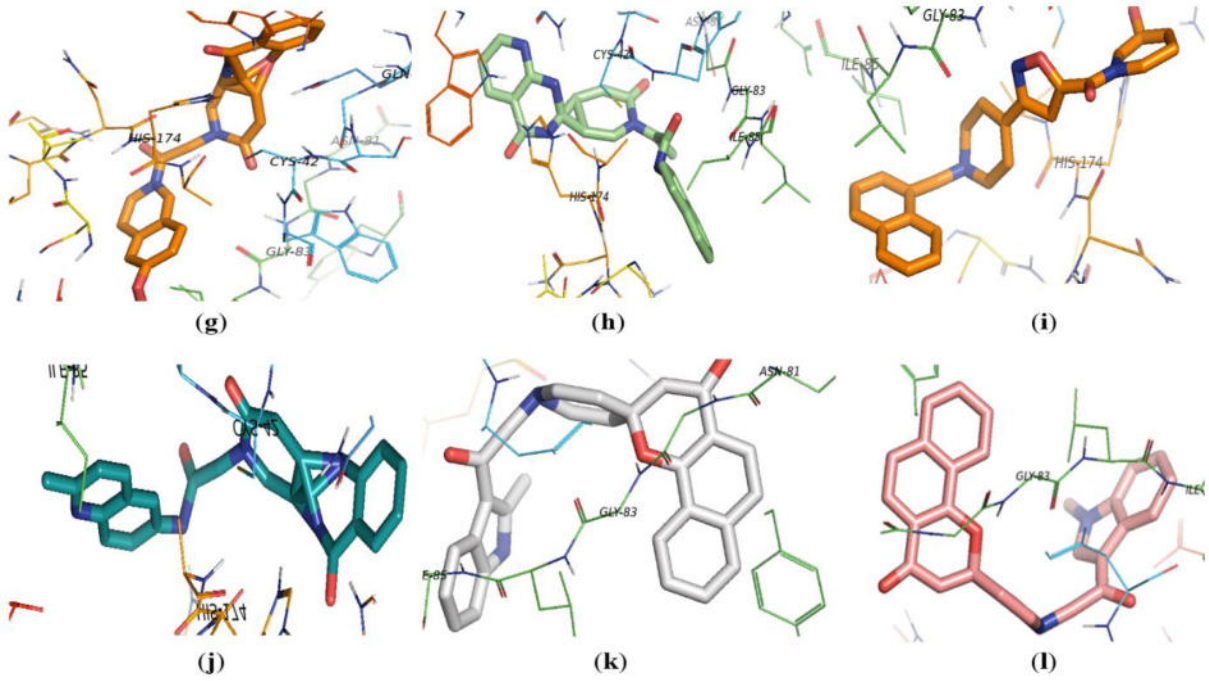


Figure 4.1. The poses representing the identified hits **1** (a), **2** (b), **3** (c), **4** (d), **5** (e), and **6** (f), through AutoDock Vina. The poses for the ligands with the protein have been generated and visualized with PyMOL.

Compounds **6** and **7** have been found interacting with Asn86, Leu84, Asp234 and Ile85 through conventional hydrogen bonds and π interaction, for compound **7** conventional hydrogen bonding with Gly83, Gln171 and His174, and π-sigma and alkyl interaction with Leu84, Ile85, Phe236 and Ala175. Compound **8** has been reported to interact with Gly83 through conventional hydrogen bond and different types of π interaction with Trp43, Ala175, Cys42, Trp206. Isoxazole derivative, **9** has been found to interact with various amino acids like Asp234, Cys42, Trp43, Leu84, Ile85, Gly82 and Gln36, wherein Gly82 and Gln36 formed the hydrogen bonds with the oxygen atoms of isoxazole and piperidinol. Quinoline derivative, compound **10** formed two hydrogen bonds with Gly83 and Trp43 with the carbonyl oxygen of spiro heterocyclic ring, rest all interaction is seen with different π bonds with amino acids like Leu84, Ile85 and Phe236. Compound **11,** being an indole derivative, exhibited the unique interaction with Tyr78 through π-π stacking and all almost similar interactions as seen in the case of the above compounds. Compound **12** showed the hydrogen interaction with Gly83, Ser149 and π interaction with Ile85, Leu84, Asp234 with the aromatic rings of indoles.



(g) (h) (i)

(j) (k) (l)

Figure 4.2 The poses representing the identified hits **7** (g), **8** (h), **9** (i), **10** (j), **11** (k), and **12** (l), through AutoDock vina. The poses for the ligands with the protein have been generated and visualized with PyMOL.

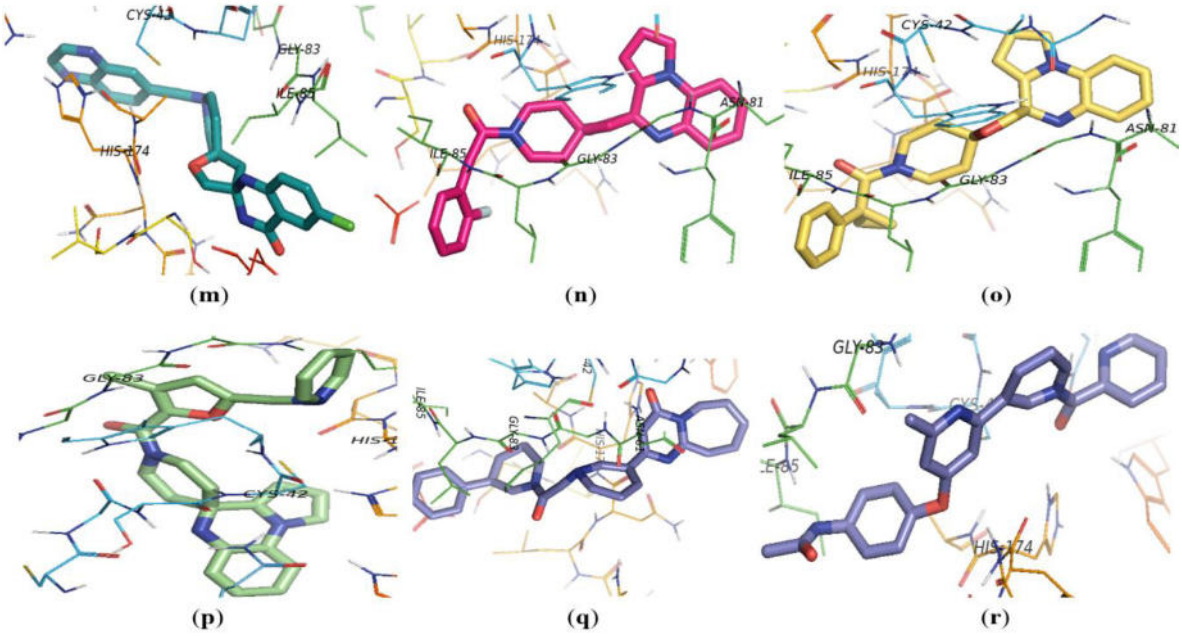


Figure 4.3 The poses representing the identified hits **13** (m), **14** (n), **15** (o), **16** (p), **17** (q), and **18** (r), through AutoDock vina. The poses for the ligands with the protein have been generated and visualized with PyMOL.

For identified hit **13**, it was found to interact with His174 and Gln36 via conventional hydrogen bond with the nitrogen of quinoxaline, and along with the π interactions with Cys42, Asp234, and Leu84. Piperidine derivative compound **14** interacted with Asp234 through π-anion, Ile85 and Cys42 through π-alkyl, Leu84 with π-sigma interactions. Ligand **15** with binding energy of -9.7 kcal/mol interacted with Cys42, Ile85, Asp234 and Leu84 through, π-anion, π-sigma, π-sulfur and alkyl interactions. Further, the oxygen of tetrahydro furan of **15** formed the conventional hydrogen bond with Gyl83. Piperidine ring of the compound **16** formed π-π stacked interactions with Ile85 and Ala175 of chain A and both 1,2,3,4-tetrahydroquinoxaline and spiro piperidine ring interacted through alkyl interactions. In compound **17**, the oxygen of pyrimidone derivative formed three conventional hydrogen bonding interactions with Gln36, Ser41, Cys42 and also the pyrimidone ring interacted via π-π T-shaped interactions with His174. Further, the nitrogen of piperidine ring in **17** formed a similar conventional hydrogen bonding interaction with Gly83. Now, the phenylpiperidine part interacts with Asp234, Leu84, Ile85, Ala175 through π-anion, π-sigma, alkyl and π-alkyl interactions. For compound **18** the oxygen and nitrogen of the piperidin-1-yl(pyridin-2-yl) methanone interacted with His174 and Gln36, respectively via conventional hydrogen bonds, the rest half of the molecule i.e. *N*-phenyl acetamide derivative interacts with Cys42, Leu84, Ala175 through π-sulfur and π-alkyl interactions.

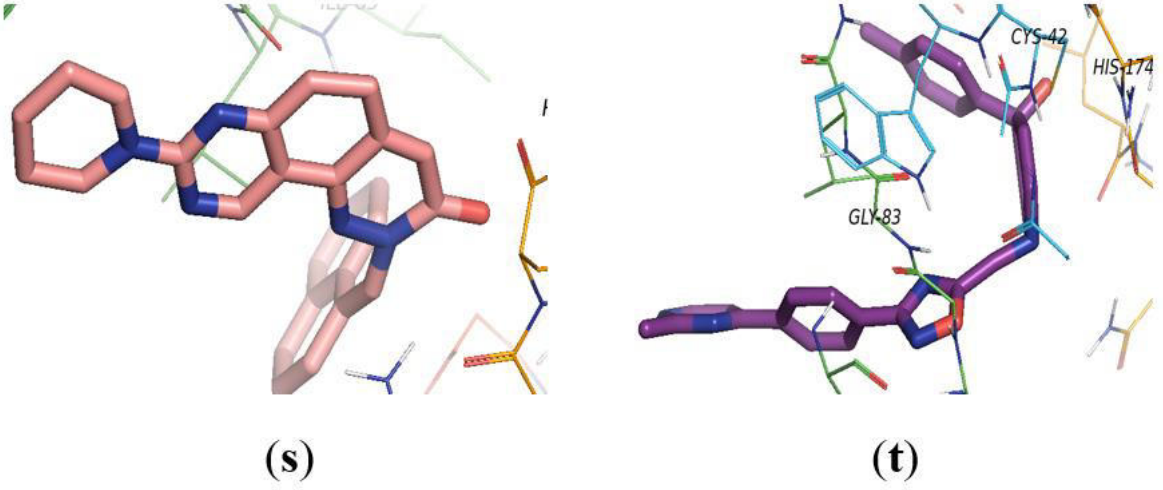


Figure 4.4 The poses representing the identified hits **19** (s), and **20** (t), through AutoDock Vina. The poses for the ligands with the protein have been generated and visualized with PyMOL.

The oxygen of pyridazin-3(2H)-one of 19 interacted with His174 through conventional hydrogen bond, the other three interactions of compound 19 such as π-anion, π-sigma, π-alkyl were observed with Asp234, Leu84 and Ile85. The last most active compound 20 with a binding energy of -9.7 kcal/mol was found to interact through different π-π interactions like π-anion with Asp234, π- π T-shaped with Tyr78, π-alkyl with Lys76, Phe236 and Ile85 and π-sigma interaction with Leu84. The conventional hydrogen bond interaction was observed with His174 between hydroxyl oxygen and His174. In summary, most of the molecules have shown conventional hydrogen bond with His174, Gln36, and Asp234. Cys42 has been the most common amino acid residue at the active site of protein which was found to interact with most of the identified hits.

4.3 Evaluation of ADMET Parameters and Physicochemical Properties

The hits that were subjected to evaluation of physicochemical parameters (Table 4.2), including molecular weight (MW) refers to the mass of a molecule is known as its molecular weight (MW), and it is commonly given in atomic mass units (amu) or daltons (Da). It is computed as the total of each atom's atomic weight within a molecule. A molecule's molecular weight is a key feature that influences its distribution, metabolism, and excretion in biological systems. Compounds with extremely high or low molecular weights may have

poor bioavailability and absorption in medication design., hydrogen bond donors (HBD) are Atoms in a molecule that are capable of donating a hydrogen atom to form a hydrogen bond are known as hydrogen bond donors. These are usually nitrogen or oxygen atoms bound to hydrogen atoms in biological situations. For molecular interactions to occur, hydrogen bonds are essential, especially when it comes to the stability of protein structures and the binding of medications to their target proteins. The solubility and penetration of a molecule through cell membranes can be affected by the quantity of hydrogen bond donors present in it, hydrogen bond acceptors (HBA) are atoms or groups in a molecule that have the ability to receive a hydrogen bond from a hydrogen bond donor are known as hydrogen bond acceptors. These are typically atoms that are electronegative, like nitrogen, fluorine, or oxygen. The quantity of acceptors affects the molecule's solubility and membrane permeability in a similar way as hydrogen bond donors do. When assessing the pharmacokinetic characteristics of medication candidates, donors and acceptors are taken into account., number of rotatable bonds (RB) are any single bonds between two non-terminal atoms, with the exception of amide bonds, which have a partial double-bond nature because to resonance. Stated differently, these bonds permit unrestricted rotation around the bond axis. One measure of a molecule's flexibility is the number of rotatable bonds in it. Higher flexibility is often associated with more rotatable bonds, and this might affect a molecule's capacity to bind to an enzyme or receptor site. However, because the molecule may adopt different conformations, increasing flexibility may also result in less predictable features related to absorption and metabolism., and partition coefficient (LogP) A measure of a substance's hydrophobicity, the partition coefficient, or LogP, shows how well a chemical distributes itself between a hydrophilic phase (water) and a hydrophobic phase (such lipids or fats). The ratio of a compound's concentrations in a combination of two immiscible phases at equilibrium is how it is expressed logarithmically. This ratio is usually found between water and an organic solvent (octanol). Given that it influences a medication's absorption, distribution, and capacity to cross cell membranes, logP is a crucial metric in drug design. Drugs that have a LogP that is too high could be excessively lipophilic, which could result in poor solubility and possible buildup in fatty tissues. Conversely, drugs that have a LogP that is too low could be poorly absorbed due to inadequate interaction using SwissADME (Daina et al. 42717) and pkCSM (Pires et al. 4066). According to the rule of five, the compound can be considered a more promising candidate for drug development if it possesses certain

desirable features.(Lipinski et al. 3) All the compounds **1**-**20** have a molecular weight less than 500 Daltons, a logarithm of the partition coefficient (LogP) less than 5, rotatable bonds (RB) ≤ 5, a maximum of 5 hydrogen bond donors (HBD), and a maximum of 10 hydrogen bond acceptors (HBA). Hence, all the compounds satisfy the minimum requirements of Lipinski rule. The Lipinski criterion was not broken by any hits, indicating their high oral bioavailability. Thus, it can be speculated that these compounds can be suitable drug candidates with respect to their bioavailability.

In pharmaceutical research, the notion of "drug-likeness" is crucial, especially in the initial phases of drug development. It describes the characteristics of a chemical molecule, such as its pharmacokinetics, bioavailability, and capacity to reach its biological target, that render it appropriate for use as a medication. A number of scholars have put out criteria and recommendations to aid in determining drug-likeness; the ones created by Ghose, Veber, Egan, and Muegge are among the most often cited. When sorting through vast libraries of chemicals to find the ones that have the best chance of becoming oral medications, these guidelines are especially helpful.

Table 4.2 The essential properties of identified hits (**1-20**) as per Lipinski's rule of five.

Comp No.	**Molecular Weight (Da)**	**HB Donors**	**HB Acceptors**	**Rotational Bonds**	**Log P**	**No. of violations**
1	446.5	2	5	3	3.95	0
2	448.51	2	4	4	2.99	0
3	406.48	1	5	3	3.69	0
4	437.88	2	4	2	2.9	0
5	420.5	3	4	4	3.07	0
6	448.94	2	4	2	3.24	0
7	449.5	1	5	4	3.3	0
8	433.5	3	4	4	2.57	0

9	419.52	1	5	5	3.53	0
10	443.5	3	4	4	2.57	0
11	442.55	3	4	3	3.5	0
12	442.55	2	4	3	3.07	0
13	449.93	2	5	2	3.04	0
14	431.5	1	3	3	3.82	0
15	439.55	1	2	3	4.02	0
16	444.57	1	3	4	4.28	0
17	448.6	0	4	5	4.02	0
18	430.5	1	5	7	3.65	0
19	423.51	0	4	3	3.74	0
20	441.52	**1**	7	5	3.34	0
Ideal Value	≤500	≤5	≤10	≤10	≤5	≤1

To ensure the drug-likeliness of compounds **1**–**20**, the additional drug-likeness rules proposed by Ghose, Veber, Egan, and Muegge have been examined for these identified hits. The Ghose filter, created in 1999 by Ajay K. Ghose and associates, comprises standards derived from the distribution of physicochemical characteristics noted in recognized medications. The guidelines established by Ghose are weight in molecules: 160–480 Daltons, the range of the log P (octanol-water partition coefficient) is -0.4 to +5.6, molar refractivity: a molecule's electronic polarizability measured in the range of 40 to 130. Between 20 and 70 atoms total (including hydrogen atoms) and these criteria aim to maximize the chance of adequate oral bioavailability and were generated from a dataset of 678 known medicines. Daniel Veber and colleagues (2002) determined the characteristics that impact oral bioavailability, concentrating on polar surface area and molecular flexibility. Veber has the following rules: Ten or fewer bonds are rotatable. Because the molecule is less flexible, fewer

rotatable bonds are often correlated with improved membrane permeability, area of the polar surface: 140 Å² or less. All atoms (apart from hydrogen) that have the ability to establish hydrogen bonds fall under this category; permeability is often improved by smaller regions. These guidelines were created by analyzing 1100 molecules from drug development initiatives, which revealed a direct link between these characteristics and high oral bioavailability. The Egan rule, developed by Pharmacia Corporation's Joseph P. Egan, aims to forecast a compound's likelihood of being well-absorbed in humans. It combines characteristics of polar surface area and lipophilicity:
The polar surface area should be 75 Å² or fewer, the total number of atoms that can form hydrogen bonds (both donors and acceptors) should not exceed 10, and the log P (octanol-water partition coefficient) should be between -1.0 and +5.5. This guideline was developed as a result of research on 805 substances having established oral bioavailability in humans. B. Muegge modified Ghose's criteria based on his research for Pfizer, although they were nonetheless comparable to those of Ghose. Muegge's criteria cover the following: molecular weight: 200–500 Daltons; log P: -2–+5; number of donors and acceptors of hydrogen bonds: 0–5; topological polar surface area: less than 75 Å² or 140 Å² (two thresholds taken into consideration); and number of rotatable bonds: less than 8. Muegge's criteria are often employed in virtual screening to enhance the efficacy of the drug development procedure.

While each set of guidelines has advantages and disadvantages, they are all typically useful as heuristics when evaluating compound libraries. When combined, these guidelines can aid in the ranking of compounds that strike a balance between the essential drug-like qualities and enough novelty to support additional research. All of the hits complied with the minimum requirements proposed by Veber, (Veber et al. 2615) Egan, (Egan et al. 3867) Muegge,(Muegge et al. 1841) The hits (**3**, **4**, **5**, **8**, **9**, **14**, **18**, and **20**) met the criterion of Ghose(Ghose et al. 55) and other could not. Therefore, only a subset of compounds violated the Ghose rule. In summary, these compounds that were identified as hits may have the potential to be excellent candidates for drug development in the future, as they adhere to the criteria for being suitable for drug development and resembling existing drugs.

Next, we studied different parameters for absorption and distribution using pKCSM (Table 4.2) It is an advanced computational platform that forecasts the pharmacokinetics and toxicity of small molecules. pkCSM is a useful tool during the early stages of the

development of drugs since it uses machine learning techniques to forecast a wide range of pharmacokinetic parameters, pharmacological efficacy, and potential toxicity. It was developed by researchers at the University of Cambridge, through a web-based interface, users can immediately acquire predictions for various attributes by inputting chemical structures (in formats like SMILES or uploading files like SDF) into pkCSM. This approach is designed to be user-friendly so that even people without a strong experience in computational chemistry can take advantage of this useful tool. All the compounds have met the requirements for solubility (Ali log S) compound's solubility is determined by how well it dissolves in a given solution. Aqueous solubility is a key factor in pharmacokinetics because it influences the body's ability to absorb drugs when taken orally. A prediction methodology for calculating the logarithm of a compound's solubility in water is called Ali log S. It is based on an approach by Ali et al. that uses computer algorithms to forecast a chemical's ability to dissolve in water based on its structure. A medicine's bioavailability is influenced by its solubility; in order to be absorbed efficiently, a drug must be sufficiently soluble in the digestive tract, molar refractivity (MR) i.e. molecule's polarizability, or its capacity to adjust its electron density in reaction to outside electric fields, is measured by its molar refractivity. It is derived from the molar volume and molecular refractive index and is associated with molecular electronic characteristics. Molar Refractivity (MR) describes how light interacts with a molecule's electrons by taking into consideration the contribution of each atom within the molecule. Understanding molecular characteristics like size, shape, and electron distribution—which influence a drug's interaction with its biological target—is useful in the drug development process, topological polar surface area (tPSA) it is a descriptor used to calculate the area of a polar molecule. It is specifically the surface total of all polar atoms, mostly nitrogen and oxygen, along with any hydrogen atoms that are bonded. Drug transport characteristics, such as the capacity to pass the blood-brain barrier or absorb drugs through the intestines, can be effectively predicted by tPSA. Better permeability of the membrane is often indicated by a lower tPSA, which is especially important when determining a drug's likelihood of penetrating the brain or spinal cord., and Caco2 cell permeability A type of epithelial cells called Caco-2 cells is derived from colon cancer in humans. They are often employed in modelling the intestinal epithelium, a portion of the human gut, in pharmacological research. In Caco-2 cell permeability testing, a compound's capacity to pass through the monolayer of the cell is measured. An established in vitro

method for forecasting intestinal absorption of oral medications is this test. In the human digestive tract, high permeability in Caco-2 cells often implies good absorption while low permeability indicates poor absorption. Most compounds that were shown to have adequate absorption in the human intestine also demonstrated improved oral absorption. The requirements for V_d were satisfied by all the compounds and inhibition of P-glycoprotein substrates was found not possible by compounds (**1, 8** and **17**). In addition, the predicted properties pertaining to toxicity, excretion, and metabolism have been summarized in Table 1. With the exception of compounds **6**, **11**, **12**, and **15**, most of the compounds had medium renal clearance and inhibited CYP2D6 but not CYP3A4. Except **1** and **4**, all compounds had a moderate renal clearance. All of the hits-aside from hits **9**, **11**, **12**, **14**, and **20** were discovered to inhibit uptake transporter substrates in the proximal convoluted tubule (OCT2). Based on acute and chronic toxicity (LD_{50}) estimates in rats experiments are intended to evaluate a substance's effects following extended exposure, which might last anywhere from a few weeks to the whole life of the experimenter. These tests assist in identifying the lowest dose at which detectable side effects are present (LOAEL - Lowest seen Adverse Effect Level) and the dose at which no adverse effects are seen (NOAEL - No Observed Adverse Effect Level), all of the discovered compounds were anticipated to be safe if administered orally and had no cytotoxic effects on the hERG cell line. Additionally, AMES toxicity testing revealed that none of the compounds were mutagenic, and none of them caused skin sensitivity. As a result, these compounds (**1**–**20**) could prove to be effective treatment candidates in the future.

Table 4.3 Absorption and distribution profile of compounds (**1**-**20**).

Comp. Code	**Ali log S**[a]	**MR**[b]	**tPSA (Å²)** [c]	**log P_{app}**[d] **(10^{-6} cm/s)**	**Intestinal absorption**[e] **(%)**	**VD_{ss}**[f] **(log L/kg)**	**Fraction unbound**[g]	**P-gp inhibition**[h]
1	-3.079	132.56	101.38	0.844	86.689	0.176	0.016	no
2	-3.121	137.71	90.98	1.224	96.775	0.427	0.059	yes
3	-3.627	123.89	82.44	0.915	97.121	0.935	0.209	yes

4	-3.819	128.37	91.65	0.829	93.993	0.234	0.055	yes
5	-3.064	129.67	82.7	1.15	88.824	0.862	0.22	yes
6	-4.113	135.67	66.49	0.968	93.642	1.219	0.079	yes
7	-3.993	131.96	88.18	1.259	96.722	-0.097	0.011	yes
8	-3.172	129.81	103.43	1.165	80.25	-0.166	0.187	no
9	-3.772	127.48	69.81	0.991	89.101	1.069	0.055	yes
10	-3.48	136.55	103.43	0.926	87.111	0.454	0.038	yes
11	-3.839	135.31	68.72	0.893	91.626	0.93	0.111	yes
12	-4.371	135.24	57.86	1.11	91.683	0.744	0.086	yes
13	-4.162	133.47	79.38	0.842	93.882	0.754	0.103	yes
14	-4.49	127.55	46.5	1.185	94.566	0.218	0.001	yes
15	-4.14	134.98	46.5	1.187	95.687	0.728	0.032	yes
16	-4.225	140.51	53.65	1.039	94.182	1.079	0.22	yes
17	-4.602	138.96	58.44	1.085	96.155	1.179	1.179	no
18	-4.009	126.43	84.42	1.198	92.56	0.028	0.079	yes
19	-5.123	130.04	63.91	0.586	100	0.277	0.262	yes
20	-3.115	129.92	88.17	1.376	93.758	0.857	0.099	yes
Optimum values	≤0	≤155	≤150	>0.09	>30	low (<−0.15), high (>0.45)	-	-

[a]solubility in water, [b]molar refractivity, [c]topological polar surface area, [d]Caco-2 cell permeability, [e]absorption, [f]volume of distribusstion (human), [g]fraction unbound, and [h]ability to inhibit the P-glycoprotein.

Table 1.4 Metabolism, excretion and safety profile of compounds (**1-20**).

Comp. Code	**CYP2D6[a]**	**CYP3A4[b]**	**CL_T[c] (mL/min/kg)**	**OCT2 substrate[d]**	**AMES[e]**	**hERG I[f]**	**LD_{50}[g]**	**LOAEL[h]**	**Dermal toxicity[i]**
1	no	Yes	0.018	no	no	no	2.186	2.267	no
2	no	Yes	0.41	no	o	no	2.092	1.839	no
3	no	Yes	0.877	no	yes	no	2.858	1.327	no
4	no	Yes	-0.086	no	no	no	2.17	1.688	no
5	no	Yes	0.959	no	no	no	2.423	1.7	no
6	yes	Yes	1.02	no	no	no	2.867	1.397	no
7	no	Yes	0.453	no	no	no	2.188	1.758	no
8	no	Yes	0.479	no	no	no	2.181	0.837	no
9	no	Yes	o.74	yes	no	no	2.811	0.263	no
10	no	No	0.314	no	no	no	2.387	2.23	no
11	yes	Yes	0.895	yes	yes	no	2.53	1.653	no
12	yes	Yes	0.761	yes	yes	no	2.693	0.779	no
13	no	Yes	0.965	no	no	no	2.809	1.185	no
14	no	Yes	0.239	yes	no	no	2.872	0.933	no
15	yes	Yes	0.195	no	no	no	3.064	0.759	no
16	no	Yes	0.954	no	no	no	3.003	-0.408	no
17	no	Yes	0.849	no	no	no	2.57	0.134	no
18	no	Yes	0.636	no	no	no	2.911	1.04	no
19	no	Yes	0.556	no	yes	no	2.861	0.644	no
20	no	Yes	0.5	yes	no	no	3.116	1.297	no
Optimum values	-	-	high (>1), medium	-	-	-	-	-	-

			(>0.1 to <1), low (≤0.1)						

Inhibition of [a]CYP2D6 and [b]CYP3A4, [c]total renal clearance, [d]inhibition of renal OCT2 substrate; [e]AMES and [f]hERG I toxicity; [g]acute and [h]chronic toxicity in rats; [i]sensitivity to skin.

Next, Brain Or Intestinal Estimated Permeation technique (BOILED-Egg) has been a computational approach used to estimate the ability of compounds to permeate through the gastrointestinal tract or blood-brain barrier(Daina and Zoete 1117). Therefore, we speculated the permeation profile using SwissADME (Daina et al. 42717) which demonstrated that all the identified compounds exhibited adequate gastrointestinal absorption and effective inhibition of the P-glycoprotein (Figure). A few hits (**6**, **9**, **11**, **12**, **14**, **15**, **16**, **17**, and **19**) were discovered to have the lowest likelihood of causing CNS neurotoxicity and the highest likelihood of penetrating the BBB.

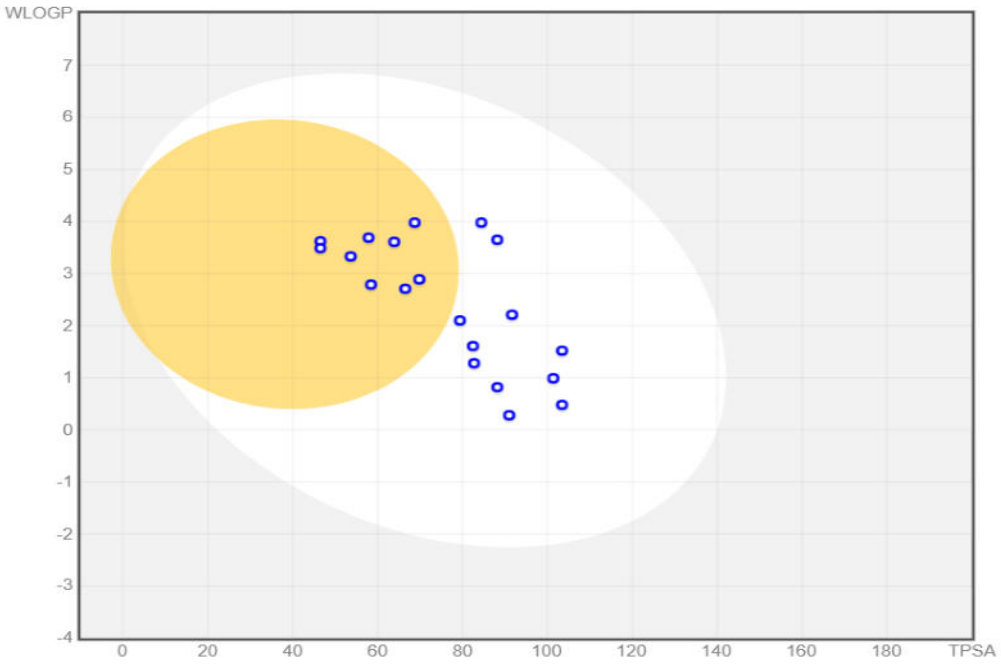


Figure 4.5 BOILED-Egg model of hits (**1-20**) retrieved using SwissADME. The yellow and white portions of egg designate the permeation capability to blood-brain barrier and enteric systems, respectively. The blue spheres speculate the inhibition of P-glycoprotein.

4.4 MD Simulation

Molecular dynamics (MD) simulation have has been the simulation process that involve the examination of the atomic and molecular motions for the transient period of time, which have been extremely useful in the proteomics and drug discovery.(van Gunsteren and Berendsen 992; Hollingsworth and Dror 1129; Maricarmen et al. 3909) Recently, we have also provided the coverage of the applications of molecular dynamics simulations as an important tool for drug discovery in search of anti-malarial agents.(Dhameliya et al. e202302471) In MD simulation, the interactions between the atoms and molecules have been permitted for a while, providing an insight into the system's dynamic development. The measurement of root mean square deviation (RMSD) may reveal the information about the structure and conformation during stimulation, indicating that the stimulation has reached equilibrium and that any fluctuations during the stimulations have been centered around the thermal average. In molecular dynamics simulations, the paths of individual atoms are predicted using Newton's equations of motion. The potential energy, which is obtained from the molecular interactions represented by a selected force field, provides the basis for computing the forces exerted on each atom and their ensuing accelerations. The energies of bonds, angles, dihedrals, and non-bonded interactions (van der Waals and electrostatic forces) within the molecule are all described by mathematical functions that make up the force field. The steps involved in MD are: First, that is system setup in which a simulation box is filled with the starting structure of the molecule or molecules of interest, it is possible to add ions and water molecules to simulate physiological circumstances. Then the second step minimization in order to eliminate any undesirable connections and guarantee that the system begins with a stable configuration, the system is energetically minimized. Now equilibration using carefully calibrated simulations, the system is progressively brought to the required temperature and pressure which is the third step. In order to stabilize the system under study-specific conditions, this step is essential. 5^{th} i.e. production run this is the real simulation phase in which data is gathered and the system is left to change over time. Depending on the biological process under study, this phase may last anywhere from picoseconds to microseconds or longer and the last is to investigate molecular dynamics, interactions, and structural changes over time, the resulting trajectory data is examined. Uses for MD Understanding how proteins fold, function, and interact with other molecules is known as protein dynamics. Drug design, on the other hand, focuses on examining how drugs

interact with their targets to maximize binding affinity and specificity. Finally, molecular properties of materials are studied. and investigating the dynamic mechanisms underlying the catalysis of enzymes.

For small proteins, the changes in the range of 1 to 3 Å are quite normal and well accepted, but the parameters more than 3 Å suggested that the protein is experiencing a significant conformational change during the simulation run. The stability of the ligand in relation to the protein binding pocket is shown by the ligand RMSD. The root mean square fluctuations (RMSF) has been used to characterize local fluctuations of the protein-ligand complex.

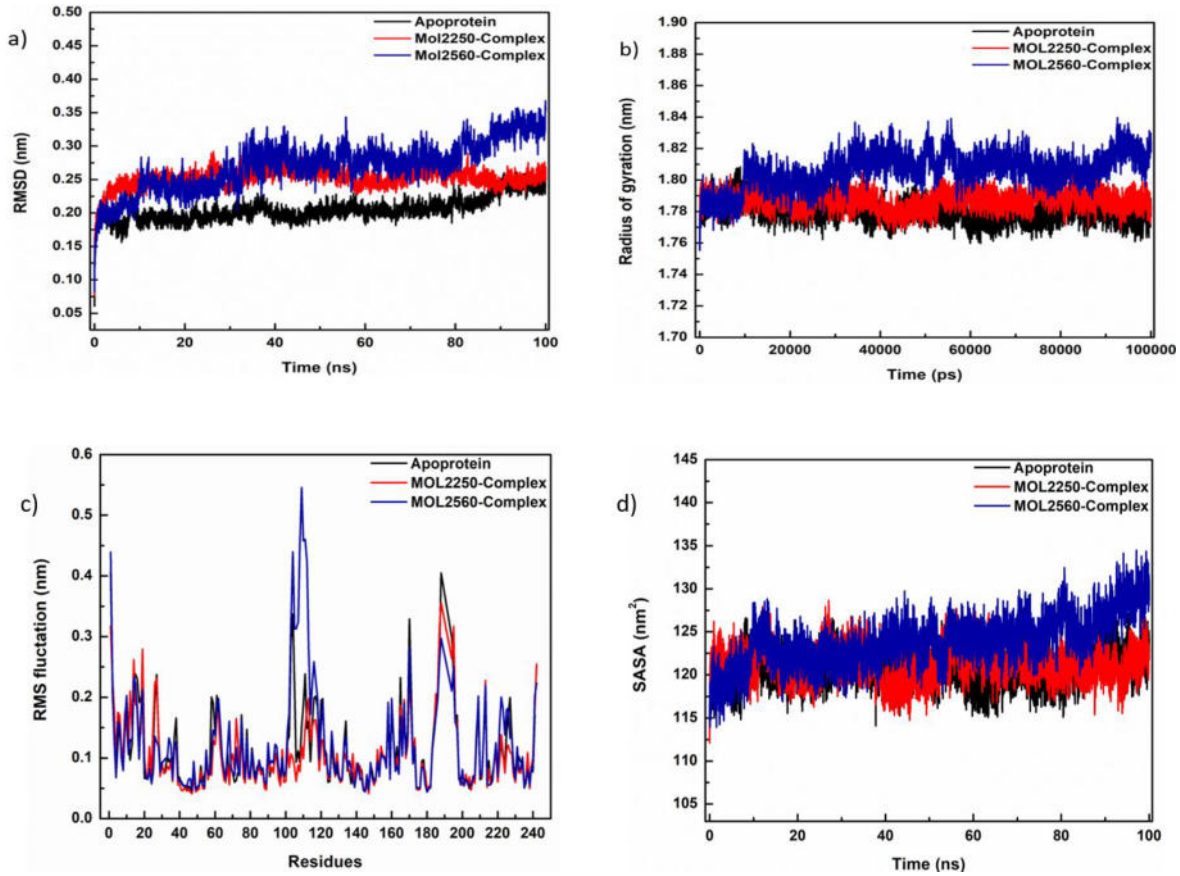


Figure 4.6 The schematic plots of MD simulations having RMSD (a), RoG (b), SASA (c) for MD run of compounds **1** (mol2250) and **2** (mol2560) with the complex of falcipain-2.

The plots of stability of ligand-receptor complex **1** with falcipain-2 has been presented in the Figure indicating no discernible variation in the position of ligand **1** inside the complex. For protein, there is a slight fluctuation in RMSD at the end of the final 80 to 100 ns (0.16 nm to 0.225 nm, average of 0.22 nm). The range of 1.77 nm to 1.81 nm, 1.79 nm as the average has been represented as the least variation in the radius of gyration (RoG, Figure b), supporting the compactness of the structure of the complex without appreciable variations. This may indicate that falcipain-2 might have undergone the change in the conformation to better accommodate compound **1**. The integrity of the structure and atomic flexibility of the complex are denoted by the RMSF (less than 0.35 nm) versus stimulation time plot in Figure 4.5c. The range of 112.5–128 nm^2 has been sufficiently covered by the surface area (120.25 nm^2 as the average, Figure d) and a maximum of four hydrogen bonds of ligand **1** with the protein over the simulation time of 100 ns (Figure 4.6a). In summary, these results indicate the stability of ligand **1** at the protein binding site of falcipain-2.

Next, we analyzed the stability of ligand **2** complexed with falcipain-2 stability, we discovered that the ligand RMSD ranged from 0.08 to 0.55 nm (Figure 4.7a) and it can be speculated from these findings that the current complex of ligand **2** with falcipain-2 has been stable with very little recent change observed over the course of the 100 ns. An average RMSF value of 0.25 nm was obtained for the complex with the least changes in atomic flexibility and its structural stability (Figure c). With an average of 1.82 nm, the RoG has gone as high as 1.84 nm for the complex of ligand **2** with falcipain-**2**. An average of 124 nm^2 (114-134 nm^2) was discovered to be the surface area within the site of action that solvents or molecules of water can reach vs the running time (Figure 4.7d). During the dynamics simulations of 100 ns, the ligand produced up to five HBs within the active region of the protein (Figure 4.7b).

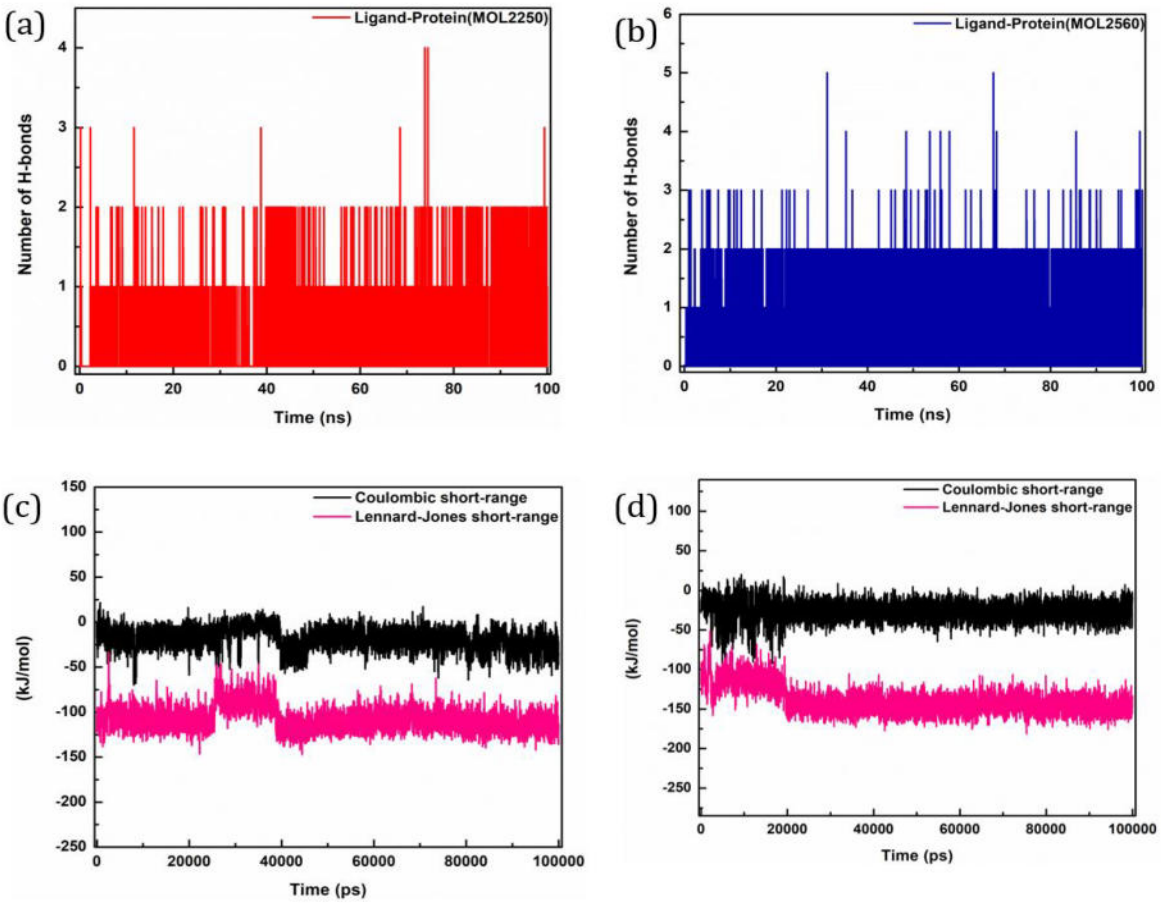


Figure 4.7 The plots of number of hydrogen bonds (a and b) and protein-ligand interaction energy (c and d) for the compounds **1** and **2**, respectively with the complex of falcipain-2 during the MD simulation run.

With this stability endeavor, we also studied the Coulombic short-range and Lennard-Jones short-range interactions of the complexes of **1** and **2** with the falciapin-2. For the ligand **1** complexed with falcipain-2, the energy of Coulombic short-range interactions was found in the range of -75 KJ/mol to 25 KJ/mol and that of Lennard-Jones short-range interactions was found ranging from -130 KJ/mol to 25 KJ/mol. For identified hit **2** (mol2560), these energies were found in the range from -175 KJ/mol to 25 KJ/mol and -175 KJ/mol to -50 KJ/mol. The higher energies of Lennard-Jones short-range interactions have suggested that the complexes of **1** and **2** with falciapin-2 have been well stabilized using hydrophobic van der Waals interactions rather than electrostatic interactions.

CHAPTER 5: Summary

Malaria, a deadly disease transmitted by Anopheles mosquitoes, can be prevented through averting mosquito bites and using medication. However, resistance to anti-malarial medications has led to poor patient compliance. This has led to the need for new chemical entities acting against promising malarial targets, such as falcipain-2, a cysteine protease involved in heme metabolism during the erythrocytic stage. To address this issue, in silico tools driven by computer-aided drug design have been used. Using AutoDock Vina, 91,001 ligands from the Asinex Elite Synergy 2021-01 library were docked to search for falcipain-2 inhibitors. The top 20 compounds with superior binding energies were found to be potential inhibitors of falcipain-2. The hits were examined for their ADMET profile and compliance with Lipinski's rule of 5, revealing their drug-likeness without breaches of the rule of five. MD simulation confirmed the stability of the top two hits in the active site of falcipain-2, speculating the identification of anti-malarial agents against falcipain-2. This research represents an important start-up in the design and identification of hits against falcipain-2 as anti-malarial agents.

Conclusion

In fine, the *in silico* molecular modelling guided search of anti-malarial agents as falcipain-2 inhibitor was performed with the help of virtual screening of 91,001 ligands from Asinex Biodesign library 2021-02 at the binding site of co-crystallized ligand, E64. The top 20 compounds (**1**-**20**) with the superior binding energies (ranging from -10.0 to -9.7 Kcal/mol) were found to be potential inhibitors of Falcipain-2., The hits were examined further for their ADMET profile and compliance with the Lipinski's rule of 5, which revealed their drug-likeness without any breaches of the Lipinski's rule of five. Finally, the investigations using MD simulation confirmed the stability of the top two hits (**1** and **2**) in the active site of falcipain-2 speculating the identification of anti-malarial agents against falcipain-2.

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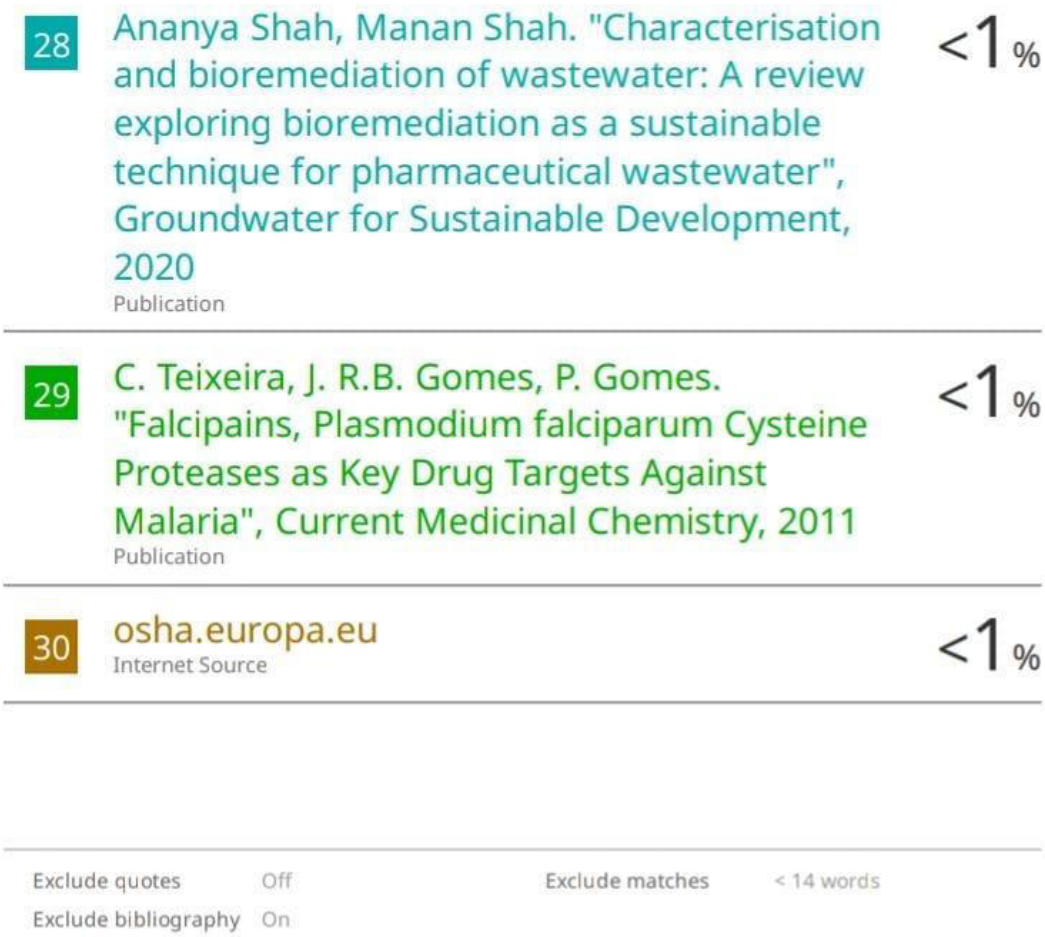
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