In-Silico tool analysis with target COVID-19 M_{PRO} inhibition

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Under the Supervision of

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BONAFIDE CERTIFICATE

Certified that this project report **"In-Silico tool analysis with target COVID-19 M_{pro} inhibition"** is the bonafide work of "**SHOAIB KHAN**" who carried out the project work under my supervision.

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Approval Sheet

This thesis/dissertation/report entitled **In-Silico tool analysis with target COVID-19 M_{pro} inhibition** by **Shoaib khan** is approved for the degree of **Bachelor of Pharmacy**.

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Statement of Project Report Preparation

1. Thesis title: **In-Silico tool analysis with target COVID-19 M_{pro} inhibition**

2. Degree for which the report is submitted: **Bachelor of Pharmacy**

3. Project Supervisor was referred to for preparing the report.

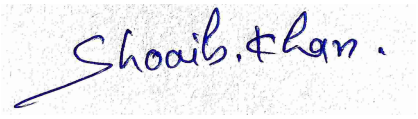
4. Specifications regarding thesis format have been closely followed.

5. The contents of the thesis have been organized based on the guidelines.

6. The report has been prepared without resorting to plagiarism.

7. All sources used have been cited appropriately.

8. The report has not been submitted elsewhere for a degree.



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ABSTRACT

Computational technique approaches all methods and analysis in drug discovery as the best prediction level to target any disease even epidemic or pandemic with gaining popularity and implementation in the research area. Its demonstrate the elaboration of drug molecule on the basis of molecular docking, model generation, simulation and finding pharmacokinetic and pharmacodynamic properties through estimated software's or servers. Although, its composite the various algorithm during running the docking, simulation and even model generation through different kind of Machine learning tools or high computational tools with improved reliability and feasibility in result prediction. It also plays an important role to come into preclinical or even clinical trials after results verification with includes all data pertaining to the docking, simulation, and models confirmation such as QSAR and other properties analysis provides confirmation to the next level of the process continuation.

CHAPTER-1 - INTRODUCTION

The development of new drugs in computational technology is an interdisciplinary complexity that entails a tremendous process that is time consuming, and is rapidly growing popularity, implementation and evaluation. [1] [2] In this field of various terms, computer-aided drag design (CADD) / computer-assisted molecular modeling (CAMM) computer-aided molecular design (CAMD) / computational drag design in the form of this field in the form of a virtual experiment proved by drug design, rational Drug design, rational drug design with computer support. [1]

Virtual experimental drugs use the three-dimensional (3D) structure of the target molecule from the start to provide high affinity and selectivity to the target molecule with a small number of compounds with good PK-PD (pharmacodynamic-pharmacodynamic) properties are more preferable in present This improves knowledge about interactions between ligands and target macromolecules, such as relatively large, systematic use in other computational tools for immediate targeting and molecular docking, and more. It consists of increasing the yield of screening compounds by concentrating to find out more likely bind compounds with the target as virtual high-throughput screening [vHTS] or new potential lead compound suggesting fragment-fragment-based ligand design [FBD].[2] Alternatively, Computational modeling is mainly used to minimize time and resources for rapid growth of chemical synthesis and biological testing, virtual screening to discover new chemicals in various chemical skeletons by finding databases in 3D chemical structures in commercial, public or private realization. By increasing the number of citations of matching keywords, "Virtual Screening" was established from 4 in 1997 to 302 in 2004.

Some of these computer tools in CADDD (Computer-Aided Drug Discovery and Development) is registered to identify hits (active drug candidates), additional evaluations, and optimized leads via transfer into biologically active compounds improving the physicochemical, pharmaceutical, pharmacokinetic properties to convert into suitable drugs form. However, it also includes virtual screening, purpose is to concentrate the precipitation of compounds with selective properties of the active drug, drug-like, and lead-like characteristics, and to eliminate undesirable characteristics such

as inactivity, reactivity, toxic and undesirable ADMET. or pharmacokinetic of drugs. [1] Currently, a global pandemic respiratory disease caused by the SARS-CoV2 virus COVID-19, a new severe acute respiratory syndrome first appeared in Wuhan City, China at the end of 2019 but in short few months it turns pandemic in all continents as globally reported. This highly contagious virus belongs to a family called Coronavirus because the crown-shaped glycoprotein that peaks on its surface can infect other species such as bats, birds, pigs, cows, and other mammals too as well as a mutation that is easy to transmit from animals to humans and from person to person by sneezing, coughing, and fomites.[3]

It is well known that SARS-CoV2 targets antiviral drugs for maturation, which is currently the most attractive component of the major protease (Mpro), which depends almost entirely on the activity of Mpro. For example, viral maturation of important proteins such as RNA-dependent RNA polymerase (RdRp, Nsp12) and helicase (Nsp13) require Mpro to cleave 12 non-structural proteins (Nsp4-Nsp16). Additionally, in several studies, Mpro inhibition has been experimentally demonstrated to prevent viral replication. [4][5] Although, It also nullifies the virus, independent of host proteases (cell membranes) that differ from other cell types of other organ types/ receptors that require the virus to enter into the host cell.[5]

In computational/in silico determines the structural complex in-between of atomic level protein-ligand is pivotal to design the ligand with high specificity and affinity toward the target protein for their desirable action, a searching mechanism which responsible to protein-ligand recognition, binding feasibility to enhance drug development in underlying disease treatment like molecular dynamics simulation guided in the studies of biomolecules [6][7] and makes efficient docking studies.[8]

CHAPTER-2 - LITERATURE REVIEW

1. DOCKING

Molecular docking in the term of computational terminology is tried to predict the structural intermolecular complex between the ligand and the receptor at the binding site (so-called default pose / default binding mode), which entails prediction of the default position, orientation and composition. [9][10] These interactions evaluate the basic understanding between receptor and ligand along with defining affinity estimation to priorities the synthesis and ligand optimization techniques. [9] For e.g., the macromolecule of SARS-CoV2 of 6lu7 PDB with tideglusib ligand complex approaches Mpro inhibitory action.[11] Docking is the most vigorous and useful tool in research area & in silico drug design, among of them, now is the most primary component of various drug discovery program,& fig1 showing a pictorial representation of docking with basic understanding tools & servers.[12-16]

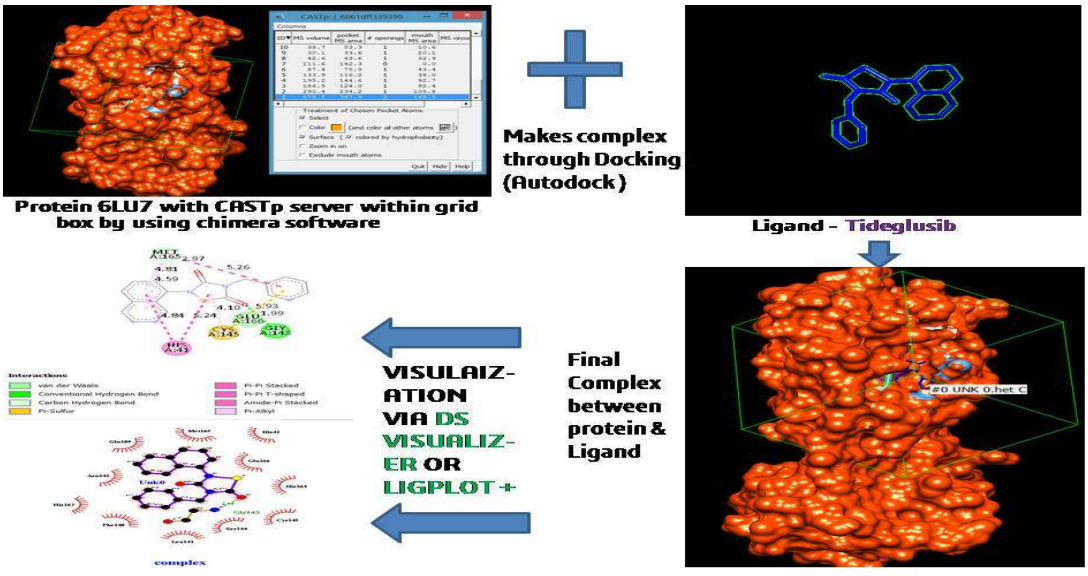


Fig: 1. Protein 6lu7[74] dock with defining pocket site via using CAST p 3.0 [75] server with defined grid box to dock with Tideglusib[76] molecule in Autodock[77] and visualize under DS visualize [78] and Ligplot+ [79] .

In docking tool strategy, encourage to enabling of non-specialists through web-tools within of enlist database set of compounds and still different integrated approaches with non paid attention to maddening details. These docks depend, whether obtained from protein structure, comparative modeling of X-ray crystallography, NMR [17-19], and soon electron microscopy. In proteins, the structure of the binding site may be incomplete, inverted or formed on side chains that are ambiguous, and some residuals may be repeated at various positions in the structure along with the metal-binding site chops parameters and in structural waters.[20] It plays an important role in the perception that needs to be done or extracted or handled by displaceable methods in some alternative way, if possible.[21-22]

2. IN-SILICO TOOLS PROFILING

In-silico tool analysis models have the best accuracy and reliable prediction in a continuous manner while there is no early prediction of PK/PD behavior and drug toxicity profiling to molecular development. Still, non-expert new researchers can access the various freely standalone software tool at a large scale of some specific commercialized modeling companies. Thus, It enhances the development of an open-access tool in the scientific prediction of ADMET profiling in future drug design and discovery. The continuous manner of using a new open-access tool makes the more qualitative and reliable prediction of new chemical entities (NCE) due to inherent the multiple characterizations of previous data in existing molecules or entities. There is few enlist commonly and popular open access In-silico tools have been discussed here with some example of drugs as input. [23]

2.1 ADMETlab

ADMET profiling evaluation through web-based platform was named from Dong et al. gives ADMETlab, that deal ADMET chemical database profiling query comprehensively around 288,967 chemicals compounds and 31 optimized QSAR models. This is highly suitable for rapid screening of ADMET open access profiling with subsequently screening and prioritization in any NCEs. [23] [24]

There are Five In-silico tools in ADMETlab named as – 1. Drug-likeness Evaluation; 2. ADMET prediction; 3. Systemic Evaluation; 4. Application Domain and 5. Aggregation Prediction. Drug-likeness investigation user can carry out under functional modules within 31 ADMET endpoints predictions such as 3 physicochemical properties, 6 absorptions, 3 distribution, 10 metabolisms, 2 elimination, 7 toxicity profiling enlisted with one use prediction & 5-rules-based models along with systematic evaluation and database screening. Predictions based on a prepared model using modeling tools like RF, SVM, RP, PLS, NB, DT, for representation patterns includes 2D, Electrotopological state atom e.g. E-state, & Molecular ACCess System (MACCS) that using database input ADMET entries from Drugbank database, ChEMBL database, EPA database and some literature-based that found in In silico tool documentation record.[24] Although it's taking input data as SMILE or SDF format in the given format with the exported .cvs output file.[25]

2.2 admetSAR

The prediction of ADMET properties in user-friendly, freely accessible web-tool also named as admetSAR with providing input name, SMILES, CAS Registry Number(CASRN), and another similarity search. [54] It also demonstrate50 around important ADMET endpoints with multiple ecotoxicity endpoints like Biodegradation and chemotoxicity in crustaceans, fishes, Tetrahymena pyriformis, and honeybees along with employing QSAR models.[26] On updated admetSAR 2.0, is prepared on the basis of 47 optimized models for predictions of drug discovery and ecotoxicity.[27]
It also includes some Machine learning tools employed in python script through a scikit-learn package like SVM, RF, and kNN models. Although, it has one important feature of prediction CYP450 enzyme inhibition, drug toxicity key, and drug-drug interaction tools. For elaboration, the data collection by using SMILES of Aspirin as input and predict the ADMET properties from predict tab that helps in drug discovery and prediction for ecotoxicity endpoints and fig 2 representation probability data of Aspirin ADMET predicted profile in bar graph format, where values in color forms likes +,-, II, Mitochondria and Non-required values and fig 3 representation for ADMET probability regression.[28-30]

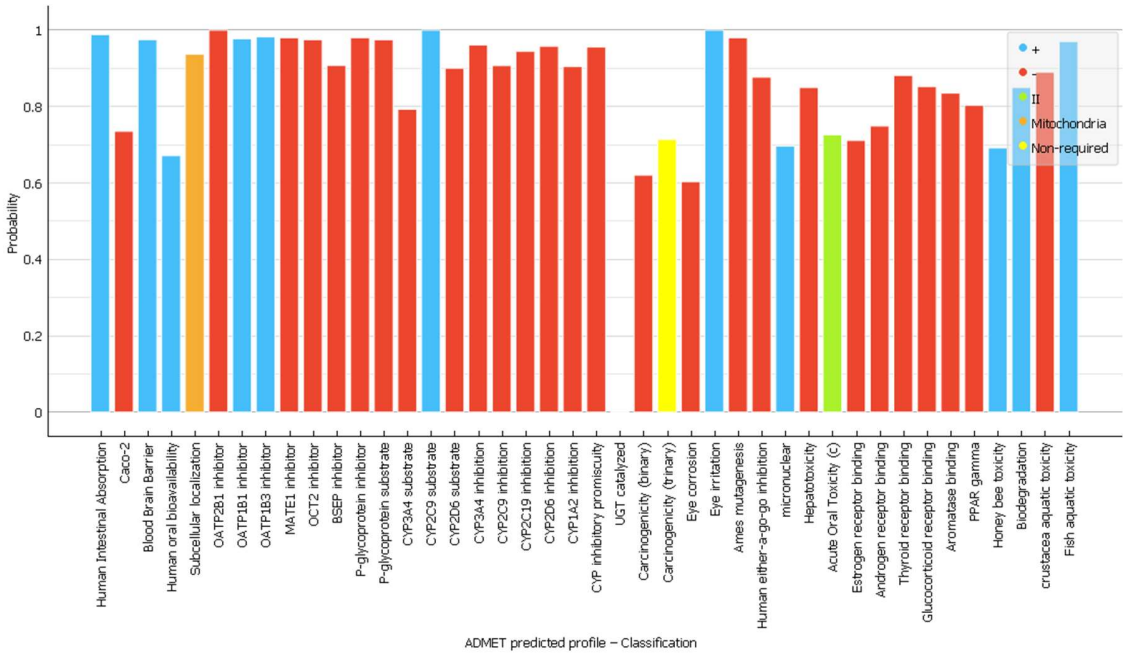


Fig:2. Representation probability data of Aspirin ADMET predicted profile in bar graph format[28]

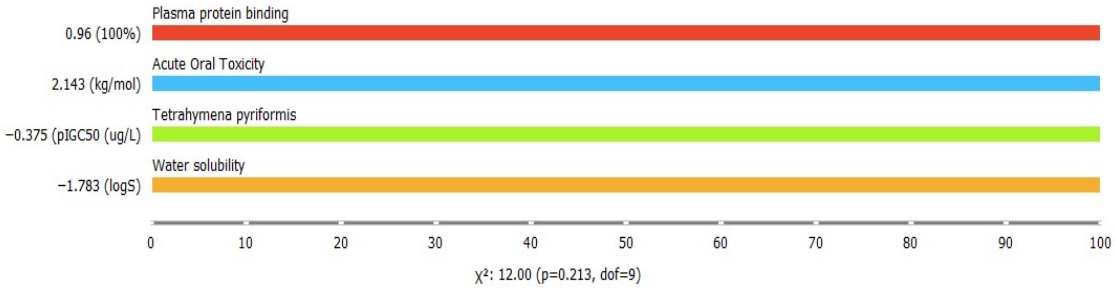


Fig: 3. Representation for ADMET probability regression.[28]

2.3 CypReact

CypReact is the most capable, open access, in-silico tool for predicting enzymatic reaction associated with the CYP450 enzyme.[29][30] It has tools for the prediction of metabolism with some require prediction of explicit drug interaction with one or more metabolism enzymes even also with prediction in individual enzymatic reactions. Its first steps help to predict the reactant whereas the query molecule selects any one of the nine most significant enzymes to enlist CYP450 named as CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. The validation and preparation of the dataset of CypReact consist of 1632 molecules used to show the meritorious outcome.[29] Furthermore, the user can provide the SDF file or SMILES

string input arbitrary molecule and its result of precisely predict the reaction of input compound with any 9 CYPs enlisted above enzymes .[31]

2.4 DrugMint

It is another server that predicting Drug-likeness properties of any new chemical entities (NCE).[32] DrugBank 2.5 developing approved this server experimentally to NCE.[33] It also includes some prepared models using open-source software packages like WEKA, PaDeL, and SVM_Light. DrugMint consist of four modules as- a) Query molecule has to draw the structure with employing Marvin applet; b) Performing virtual screening from diverse chemical libraries; c) To preparation of virtual chemical libraries, analog designing performing for lead optimization & analogs-based drug designing with employed user-specified scaffold, blocks and their linkers; and d) It also contains search database from ZINC and CheEMBL database to finding possible drug candidates. This is performing with using packages like PaDEL software used by Dhanda et al. for computational descriptors and molecular fingerprints to development of models.[33] Although WEKA software also employed for the modeled selection features. For analysis, a user must draw a single structure in input that capable to predict drug-likeness properties in output data with 89.96% accuracy in a developed model.[34]

2.5 SwissADME

This is the most commonly used high-speed web tool to predict physicochemical properties includes molecular weight, hydrogen bonding agent like H-bond donor, molar refractivity and H-bond acceptor, number of heavy atoms, Csp3 fraction. The pharmacokinetics contains the digestive tract absorption CYP enzyme inhibitor, the blood-brain barrier permeable P-gp substrate, Skin permeation, water-solubility, lipophilicity, drug-likeness also includes Lipinski, Ghosh, Verber, Egan factors and Bioavailability score & medicinal chemistry for any NCE friendliness includes Pan-assay interference structure [PAINS] alert, lead likeness, synthetic accessibility.[35]
The SwissADME is the best tool lies in different input possibilities methods with computation of various molecules in a single click to interactive output display in easy way representation and saving results possibilities for further analysis and interpretation.

Swiss ADME has the most important function to integrate with the Swiss Drug Design workspace to collect data of CADD (Computer-Aided Drug Design), tools were developed by the Molecular Modeling Group of the Swiss Institute of Bioinformatics(SIB). It consists of various tools in Swiss Drug Design:

2.5.1 SwissSimilarity

It is performed on the basis of multiple libraries in virtual screening is based on small molecular ligands which consist of existing drugs or bioactive molecules, and readily synthesizable virtual compounds which can available commercially at around 205 million enlisted.[36] They have also carried out the prediction in 2D molecular fingerprints and 3D super positional and fast non-super positional similarities approach.

2.5.2 SwissTargetPrediction

It helps to estimate the target of a bioactive small molecule within of macromolecular pocket site. Achieving a bio-target built from a library of known active molecules enrolls 370,000 from 3000 proteins via combination of 2D and 3D similarities proteins derived from *Homo sapiens* L. ssp.*sapiens, Mus musculus* L., and *Rattus norvegicus Berk.*[37]

2.5.3 SwissDock

By performing an algorithm based on docking software, it estimates the prediction of molecular interactions between organic small molecules and target proteins/macromolecules such as EADock DSS.[38]

2.5.4 SwissBiosostere

It is useful to design a bioisosteric with the replacement of small organic molecules knowledge base molecular approaches.[39]

2.5.5 SwissParam

It enrolls the molecular mechanic's calculation for small organic molecules employing topology and parameters companionable with the CHARMM force field for all atoms using CHARMM and GROMACS.[40]

Although, SwissADME displays interactive Bioavailability Radar plot analysis which offers the instant idea of drug-likeness properties in an input molecule. The plot analysis consists of six physicochemical properties in an optimal range and their size such as MW: 150-500 g / mol; solubility (below log S 6); Lipophilic (indicates between -0.7 and +5.0 on XLOG P3); Polarity (TPSA between 20 and 130 A2); Flexibility (9 or less rotatable bonds), saturation (carbon ratio of 0.25 or more in sp3 hybrid). This also represents a physicochemical range of each defined axis mentioned properties showing in colored as in pink area and query compound must stay within the range of entirely prepared pink zone considering drug-like properties or nature (fig 4 (a); Tideglusib molecule).[41]

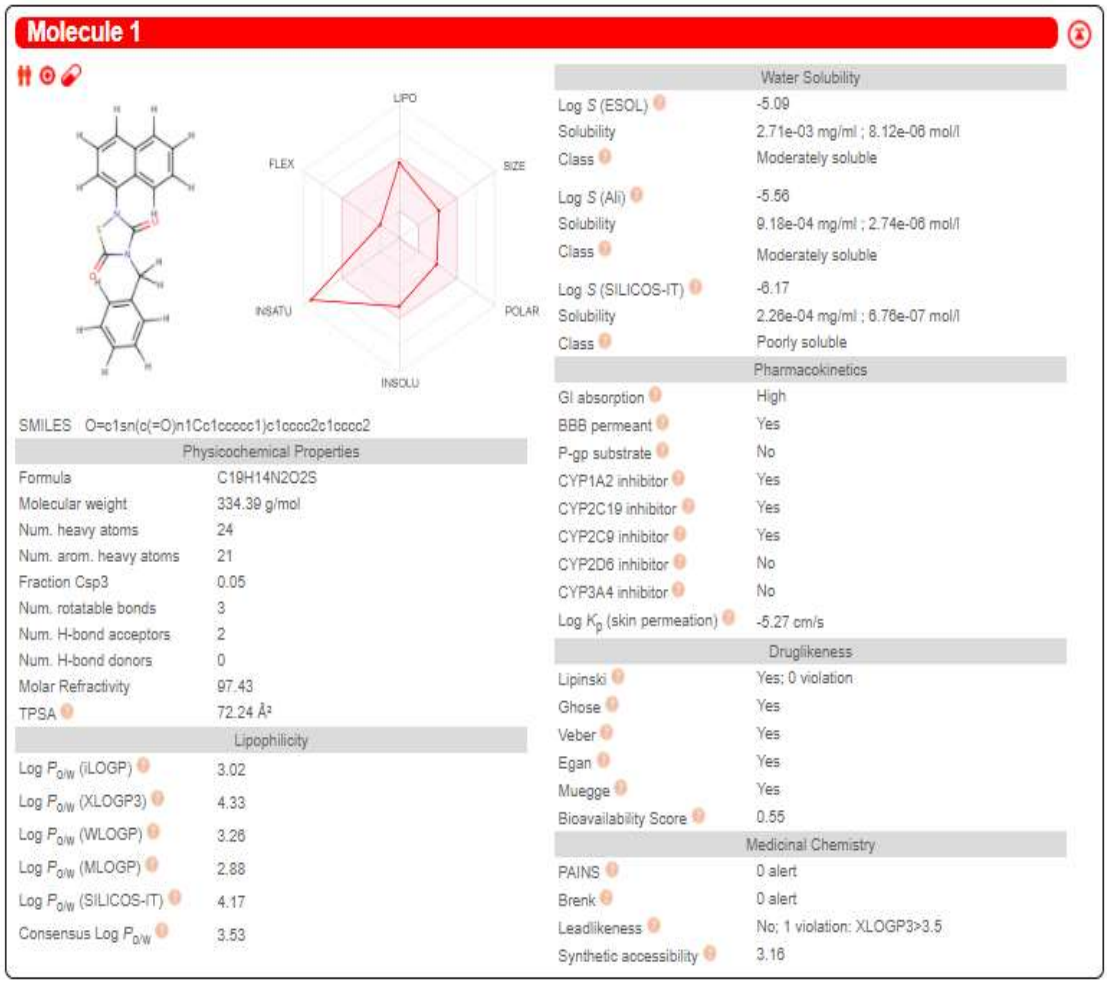


Fig 4 (a): Tideglusib molecule[41][80]

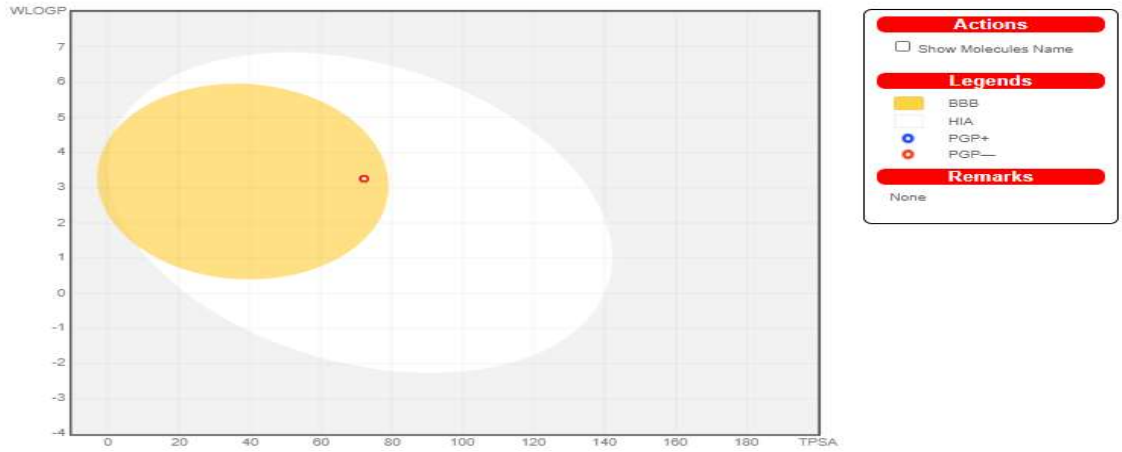


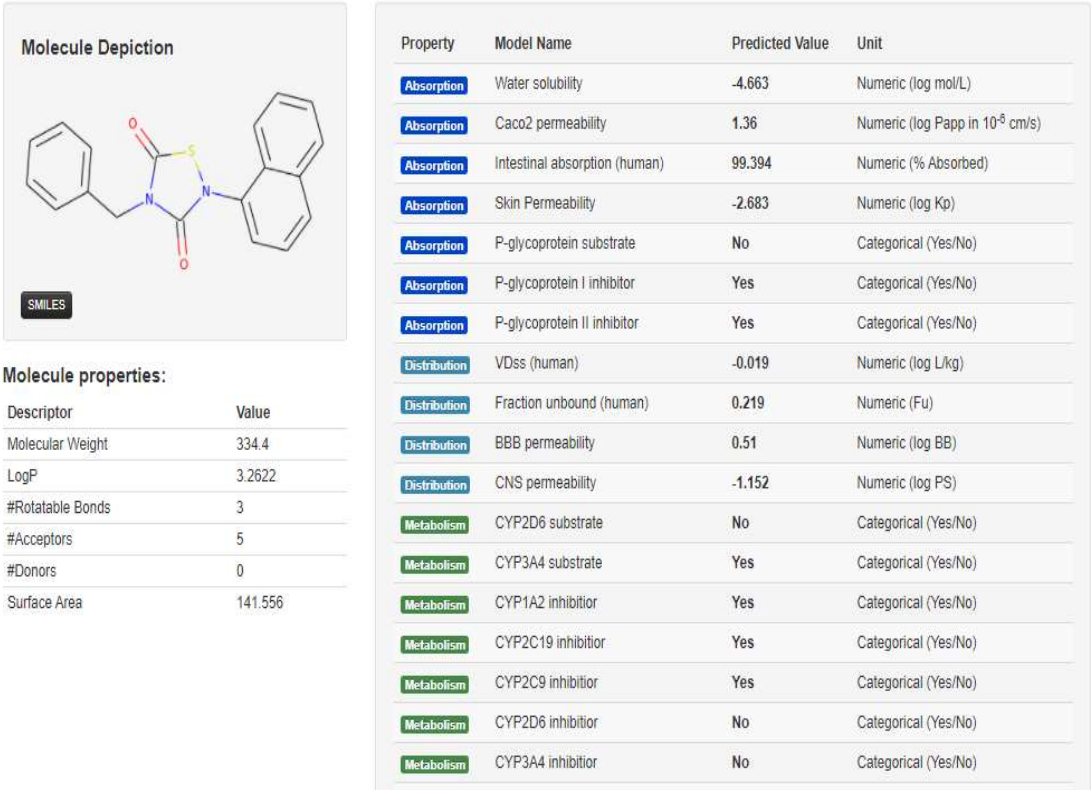
Fig 4(b) BOILED-Egg plot- Tideglusib[42][43][80]

The BOILED-Egg plot can predict simultaneously two key ADME parameters such as accessibility in blood-brain barrier (BBB) and the passive GI absorption in tract (HIA), which employs two physicochemical descriptors E.g TPSA (polarity as well as WLOGP (Lipophilicity); Fig 4(b) represent the tideglusib BOILED-Egg. During analysis of comprising Egg-shaped plot consist Yolk part represent the physicochemical space to extremely permeation of BBB with feasibility and the white part represent the physicochemical zone to the high absorption of HIA.
However, the yolk-white spacing is not mutually exclusive with the external gray areas, still its suggesting prediction of molecular properties in low absorption as well as inadequate brain penetration. In the Input file of user can draw structure with the following conversion SMILES notation to predict the ADMET properties with an output analysis in the file format of .csv as well as visualization plot representations such as Bioavailability Radar plot and BOILED-Egg plot accessibility .[42][43]

2.6 pKCSM

In silico freely accessible tool pKCSM helps to predict the ADMET properties based on the graphic signature to develop the predictive models. [44][45] The analysis of integrated major platforms can rapidly be evaluated in pharmacokinetic and toxicity required for the finding out the drug-likeness and bioavailability just by providing query molecule as an input SMILES format. It also refers to the molecular properties that register the pKCSM signature of the principal component such as toxicophore

fingerprint, Lipophilicity, atomic Pharmacophore frequency calculation, MW, number of the rotatable bond, surface area, etc. as well as the distance-based signature. This platform also predicts the ADMET properties as quantitative analysis by using 14 regression-based models and outcomes categories in 16 classification-based models in the form of binary classes. It also represents in the schematic flow to compute the ADMET properties of Disulfiram in fig. by providing SMILES notation as an input and output can be copied & pasted in an excel or as in text file format .[46]



Molecule Depiction

SMILES

Molecule properties:

Descriptor	Value
Molecular Weight	334.4
LogP	3.2622
#Rotatable Bonds	3
#Acceptors	5
#Donors	0
Surface Area	141.556

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-4.663	Numeric (log mol/L)
Absorption	Caco2 permeability	1.36	Numeric (log Papp in 10^{-5} cm/s)
Absorption	Intestinal absorption (human)	99.394	Numeric (% Absorbed)
Absorption	Skin Permeability	-2.683	Numeric (log Kp)
Absorption	P-glycoprotein substrate	No	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	Yes	Categorical (Yes/No)
Distribution	VDss (human)	-0.019	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0.219	Numeric (Fu)
Distribution	BBB permeability	0.51	Numeric (log BB)
Distribution	CNS permeability	-1.152	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	Yes	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	Yes	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	Yes	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	Yes	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	No	Categorical (Yes/No)

Continue…

Excretion	Total Clearance	0.015	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.351	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	Yes	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.385	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	0.711	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	Yes	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	0.317	Numeric (log ug/L)
Toxicity	Minnow toxicity	-0.068	Numeric (log mM)

Fig 5 : Tideglusib Molecule ADMET properties[46][81]

3. MOLECULAR DYNAMICS

Browse the matter properties of condensates dating back to 10 years by running the first Monte Carlo (MC) and Molecular Dynamics (MD) liquid simulation models represented by Metropolis and others using a computer for 10 years in the 1950s [47] & Alder & Wainwright [48] respectively. Since now, MD simulations grows and applied rapidly in various scientific areas due to the continuous progression of software and hardware in this field. Although there are no more preparatory experts, and many experiments today are using computer simulation as a tool for interpreting or analyzing the measurement data whenever the measurement data is too complicated in the exploration process with simple analytical models. In such case neutron scattering, as in the case of the direct correlation between the characteristics obtained by MD simulation and the experimental observation data, the spatial and time scale data that can be bid for the calculation are very measurable. This is the employment of various features availability together in user-friendly with the reliability of software performance to compute the calculation such as Charmm [49] ,

NAMD [50] , Amber [51] , Gromacs [52] , Gromos [53] , DL_POLY [54] etc. uses and output data visualize and analyze with VMD [55] , gOpenMol [56] , nMoldyn [57] , ..., .This allows many users to use various MD simulation tools on a daily basis.
When expressing a good model, in a system of a particular composition, it represents the atomic force between atoms. Ideally, in order to solve the electronic configurationally structure of a particular nucleus, we can calculate the consequential force of each of the following specific atoms, which employs the first principle.[58
Developing ab initio MD (AIMD) simulations now works steadily with growth when pioneering work was initiated by Car and Parrinello [59] , and more recently uses density functional theory (DFT) [60] employs on system treat for reasonable size e.g. several hundreds of atoms as well as achievement of time scale period up to 100 ps, so this is to allow to solve the various problem of interest. However, these ab initio laws are often forbidden for a large amount of useful space and time scales.
In such cases, we use the empirical force field (FF) -based method, using the duty approximation as at a higher level. This is possible to enable a simulation system containing 100 out of 1000 atoms in a nano or microsecond time.[61][62] In addition, the quality of the force field must be evaluated through experiments. Here too, neutrons play an important role, especially due to the scattering of neutrons useful in this validation procedure as above mentioned complementarity while other many different types of experimental results also runs to validate the some FF parameters .[63]

3.1 MD principle

This method combines the equation of Newton's law of motion to form N particles to create the dynamic trajectory of the system. First of all, you need to establish initial conditions, such the each particle position and their velocities indicate the force acting among the particles in the corresponding model of the determined boundary condition, either in the form of electronic structure calculations or using an empirical force field. . Next, you need to go using the classic motion equation:

$$m_i \frac{d^2 r_i}{dt^2} = f_i = -\frac{\delta}{\delta r_i} U(r_1, r_2, \ldots, r_N), \qquad (3.1)$$

Here, N number of particle coordinates employ the potential energy U (r1, r2, ..., rN) dependency. Since the second-order N coupled system of nonlinear differential equations (3.1) cannot be solved accurately, the appropriate integration algorithm must be solved numerically, step by step.[64]

3.2 Force Field (FF)

This is a formula that defines the system energy dependence on the particle's coordinates. It includes the form of potential energy analysis between atoms (U (r1, r2, ..., rN)), in which you need to enter a set of parametric. In general, parameters can be obtained using semi-empirical quantum or ab initio mechanical calculation or via data fitting into an experiment, Such as - X-ray, infrared, NMR, Raman, and neutron spectroscopy, electron diffraction and neutron diffraction etc. A set of atoms supported by FF defining a general model in the active region with simple elastic forces such as harmonics in molecular form is simulated replacing the true potential. There are various literature mentioned the various force fields with the distinct degrees of complexities and their orientation which treat the un-identical systems. Here, force field expression in typical representation look define as:

$$U = \sum_{bonds} \frac{1}{2} k_b (r - r_0)^2 + \sum_{angles} \frac{1}{2} k_a (\theta - \theta_0)^2 + \sum_{torsions} \frac{V_n}{2} [1 + \cos(1 + \cos(n\varphi - \delta)] + \sum_{improper} V_{imp} + \sum_{LJ} 4\varepsilon_{ij} \left(\frac{\sigma_{ij}^{12}}{r_{ij}^{12}} - \frac{\sigma_{ij}^{6}}{r_{ij}^{6}} \right) + \sum_{elec} \frac{q_i q_j}{r_{ij}} ,$$

Here, the first four terms synthesize the total energy of intramolecular or local contributions, includes bond stretches, dihedral, angular bends, and improper twists with an end of two terms interaction define the repulsive and interactive forces, van der Waals forces (in this case 12 -6 Lennard-Jones), Coulomb force with potential. [65]

3.3 Simulation Set-up

Four steps for running MD simulation can be generalized:

3.3.1 System setting for MD

Design a rational configuration system as a method of interesting workflow, and adopt a method to handle electrostatic interactions while the system contains partial charges, mediating the radius cutoff range including the effective force field of the selected system, and choose the integration algorithm (MD Select the range of time steps identified as (if licensed in code) and work ensembles such as NVE, NVT, NPT, etc.[66]

3.3.2 Equilibration

In general, the default settings do not define conditions for MD simulation to navigate so that other temperatures should be simulated when imported from a previous system. The initial positions of some atoms may have been randomly determined, or they may not be real due to a lack of short-range correlation. However, during NPT simulation is employ the desirable pressure and temperature in a system allows the correspondence to equilibrium density and its start from an ordered configuration with the melted system as well as make sure that long-distance order has completely disappeared. However, the choice of temperature and barometric pressure controllers is not used to calculate non-essential attributes as in the simulation part. To ensure the achievement at equilibrated state then the equilibrium stage, pressure, density, and various energy components must be tracked, where it fluctuates within of average value range without pertaining any kind of drift establishment.[67]

3.3.3 Production

Production perform is ensures the attained desirable temperature and pressure in an equilibrium situation. The pressure when avoiding the use of barostat should oscillate around the average achieved value in equilibrium. Therefore, it is reliable to manipulate fixed cells to avoid the unpleasant handling of volume fluctuations during trajectory analysis. [68][69]If we have to decide how much longer time will be taken during simulation. So you can run a much longer period of time compared to the break of an interesting workflow. However, that simulation period should be long enough to justify the Ergodic hypothesis, such as time average coincides in given property with

an ensemble average.[70] During simulation of standard liquids or crystals, some systems, such as glass, do not erodic under most real-world conditions. The final conclusion will depends on historical background of the system.

3.3.4 Analysis

At this stage, the simulated trajectory is finally analyzed and the desired properties are extracted. We have access to atomic positions, velocities, and statistical characters represented by forces or computational variables of the function of time [71]

VIRTUAL SCREENING FOR 6LU7 PDB BY USING AUTODOCK VINA (COVID -19 , PROTEASE ENZYME)

The latest announcement of the high-resolution of the SARS-CoV-2Mpro, crystal structure complex containing the Co-crystal ligand as an inhibitor, covalently bonded with PDB ID: 6LU7 [82] , available in the scientific community, the latest breakthrough in COVID-19 has been launched.

METHODOLOGY TO PREPARATION OF LIGAND AND MACROMOLECULE

COLLECTION OF PROTEASE INHIBITOR DRUGS

Collection of most suitable drugs molecule for best predictive action target with protease enzyme from PubChem[83] database enlisted as *Tadalafil* (PubChem CID –110635); *Etoposide* (PubChem CID – 36462); *Tideglusib* (PubChem CID –11313622); *Ziprasidone* (PubChem CID – 60854); *Rolapitant* (PubChem CID – 10311306 *); N-(2-((4-Methoxy-2-methylphenyl)amino)-2-oxo-1-(pyridine-3-yl)ethyl)-N-(4-(1 methoxyethyl)phenyl)acrylamide* (PubChem CID – 154703706); *Cinanserin* (PubChem CID – 5475158); *Shikonin* (PubChem CID – 479503); *1-phenyl-3-pyridin-3-ylurea* (PubChem CID – 674807) and *2-(cyanomethoxy)-N-[(1,2-thiazol-4-yl)methyl]benzamide* (PubChem CID – 146037583).

PREPARATION OF DRUG MOLECULES STRUCTURE

The drug molecule was first downloaded from PubChem into a 2D format in SDF form then after it converts into a 3D structure with explicit all hydrogen via using Marvin sketch(Chem Axon/Version 20.11)[84]software & after that minimization carried out through Chem ultra 3D (CambridgeSoft\ChemOffice2004)[85] software with applying MM2 force field & after that it was taken out to final optimization through

AutoDockTools (ADT 4.2)[77] software of chooses ligand to optimized & save it in PDBQT format to further AutoDock Vina run[86].

PREPARATION OF RECEPTOR STRUCTURE

The macromolecule taken out from the PDB file was 6LU7 that contain two chains of chain A & C & where the chain C was deleted as well as their Hetatoms (ligands who were attached with macromolecule) & optimize through run the script of Dockprep[87] from Chimera software[88], where all missing hydrogen, charges automatically added and optimize with following steps and save it in PDB format and further transform into PDBQT format via using ADT 4.2 tool[77].

VIRTUAL SCREENING PROCEDURE

Docking was done using AutoDock vina (version 1.1.2). [86] The center of grid box defined by using DS visualize[78] and CASTp server[75] (pocket site) within the co-crystal ligand of N3[11] in 6LU7 PDB molecule.

RESULTS

Active Binding Amino Acids Are – His41 & Cys145 [89]

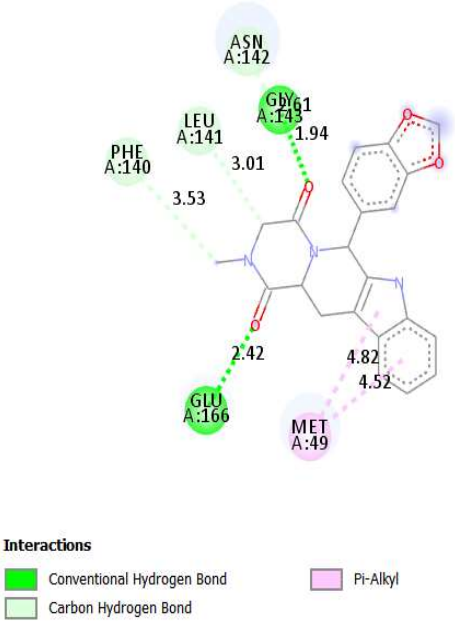
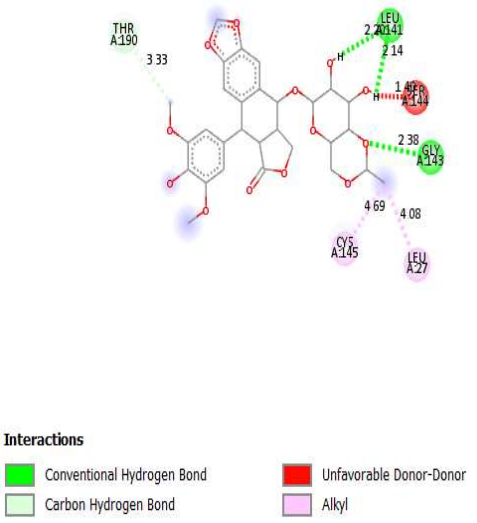
Co-Crystal ligand (N3) inhibitor in SAR-CoV-2 M^{pro} amino acids are – G143,C145, H164,E166, T190,Q189, H163 .[11]

The Drug molecules performed and visualize within pocket site of 6LU7 Macromolecule.

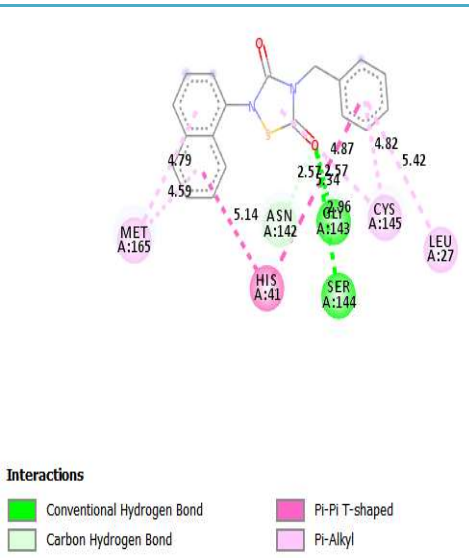
LIGANDS	RMSD VALUE (Kcal/mol)
1. Tadalafil (PubChem CID –110635)	-9.3
2. Etoposide (PubChem CID – 36462)	-8.3
3. Tideglusib (PubChem CID –11313622)	-7.8
4. Ziprasidone (PubChem CID – 60854)	-7.8
5. Rolapitant (PubChem CID – 10311306)	-7.4
6. N-(2-((4-Methoxy-2-methylphenyl)amino)-2-oxo-1-(pyridine- 3-yl)ethyl)-N-(4-(1-methoxyethyl) phenyl) acrylamide (PubChem CID – 154703706)	-7.1
7. Cinanserin (PubChem CID – 5475158)	-7.0
8. Shikonin (PubChem CID – 479503)	-7.0
9. 1-phenyl-3-pyridin-3-ylurea (PubChem CID – 674807)	-6.5
10. 2-(cyanomethoxy)-N-[(1,2-thiazol-4 yl)methyl] benzamide (PubChem CID – 146037583)	-6.1

POST-DOCKING DATA ANALYSIS

The conclusion of the virtual screening experiment were ranked based on the binding energy of the RMSD scoring form that received the highest score. Analysis of protein-ligand interactions was performed at Biovia Discovery Studio Visualizer 2020(DassaultsystèmesBioviacorp). [78]

NAME OF LIGANDS	DOCKING VISULIZATION	LOG FILES
1. Tadalafil (PubChem CID – 110635)		mode | affinity | dist from best mode | (kcal/mol) | rmsd l.b.| rmsd u.b. 1 -9.3 0.000 0.000 2 -9.0 2.030 5.238 3 -8.5 1.950 2.341 4 -8.3 2.907 5.615 5 -8.2 3.271 4.573 6 -7.9 2.604 5.433 7 -7.3 4.638 8.810 8 -7.3 3.726 5.249 9 -7.2 4.110 5.745 Writing output ... done.
2. Etoposide (PubChem CID – 36462)		mode | affinity | dist from best mode | (kcal/mol) | rmsd l.b.| rmsd u.b. 1 -8.3 0.000 0.000 2 -7.7 2.008 3.217 3 -7.5 1.457 8.589 4 -7.5 2.274 7.656 5 -7.4 2.455 4.517 6 -7.4 2.265 7.506 7 -7.2 2.311 8.105 8 -7.1 2.445 3.184 9 -7.0 5.133 9.915 Writing output ... done.

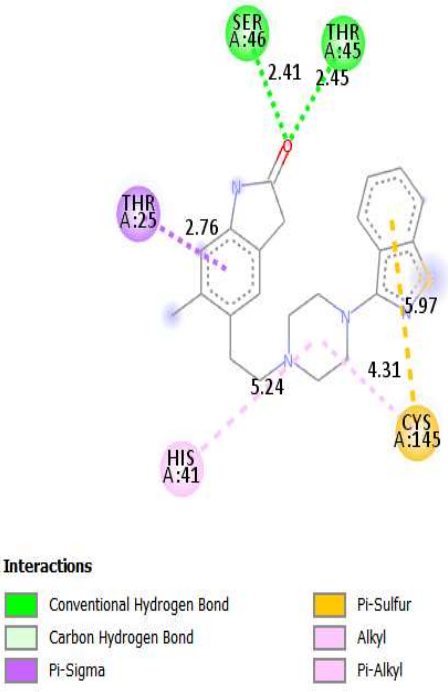
3.
Tideglusib (PubChem CID – 11313622)



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.8	0.000	0.000
2	-7.5	0.815	2.487
3	-7.0	2.448	6.909
4	-6.8	2.508	6.612
5	-6.8	3.071	5.057
6	-6.7	1.727	3.494
7	-6.7	4.711	8.141
8	-6.6	3.221	5.529
9	-6.3	3.353	5.359

Writing output ... done.

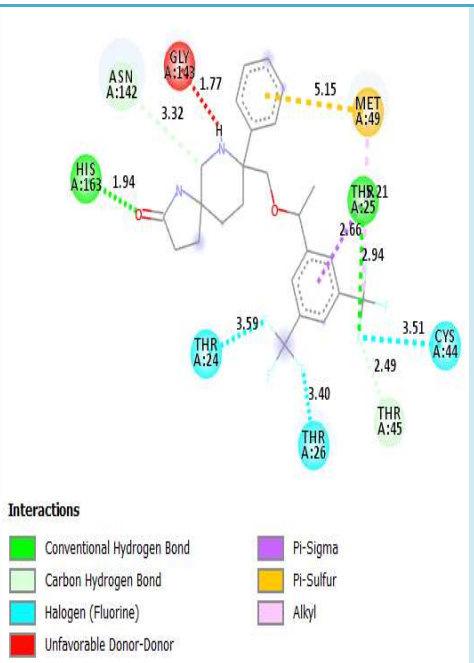
4.
Ziprasidone PubChem CID – 60854)



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.8	0.000	0.000
2	-7.7	2.475	3.954
3	-7.4	4.565	9.146
4	-7.1	4.254	6.373
5	-7.0	2.408	3.129
6	-7.0	7.692	10.407
7	-6.9	1.545	1.881
8	-6.9	8.734	11.191
9	-6.9	4.145	9.161

Writing output ... done.

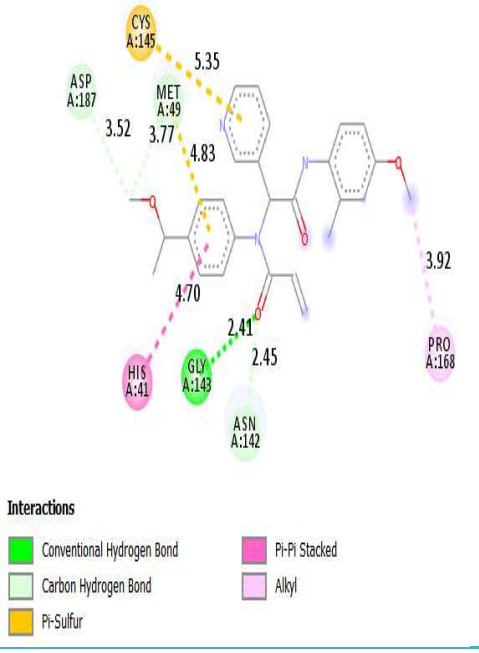
5. Rolapitant (PubChem CID – 10311306)



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.4	0.000	0.000
2	-7.3	2.584	4.647
3	-7.3	0.264	3.068
4	-7.3	5.489	9.235
5	-7.3	2.596	3.993
6	-7.2	3.003	5.415
7	-7.1	2.312	4.428
8	-7.1	4.812	7.848
9	-7.1	2.504	4.085

Writing output ... done.

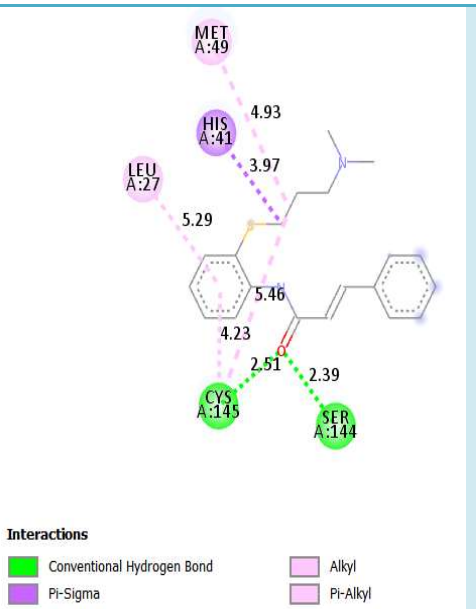
6. N-(2-((4-Methoxy-2-methylphenyl)amino)-2-oxo-1-(pyridine 3-yl)ethyl)-N-(4-(1-methoxyethyl)phenyl)acrylamide (PubChem CID – 154703706)]



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.1	0.000	0.000
2	-7.0	2.096	7.446
3	-7.0	2.691	6.636
4	-6.9	2.848	6.548
5	-6.8	2.227	6.952
6	-6.4	1.745	4.231
7	-6.2	2.153	7.781
8	-6.2	2.534	5.418
9	-6.0	2.351	4.337

Writing output ... done.

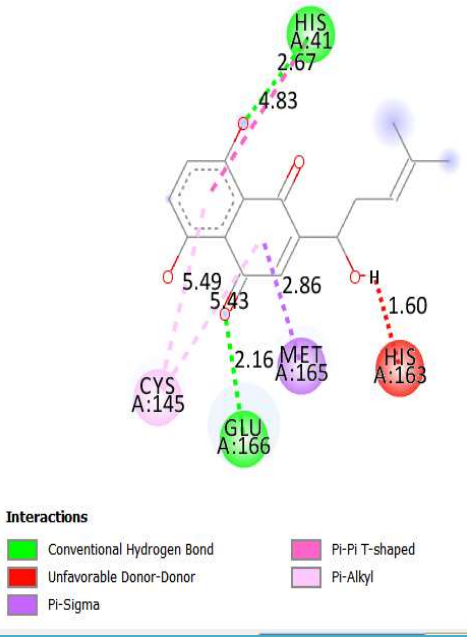
7. Cinanserine (PubChem CID – 5475158)



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.0	0.000	0.000
2	-7.0	2.810	6.524
3	-6.7	2.384	5.555
4	-6.4	1.708	6.402
5	-6.3	2.140	6.277
6	-6.2	2.865	6.948
7	-6.1	3.082	5.934
8	-6.1	3.160	4.997
9	-6.1	4.216	7.103

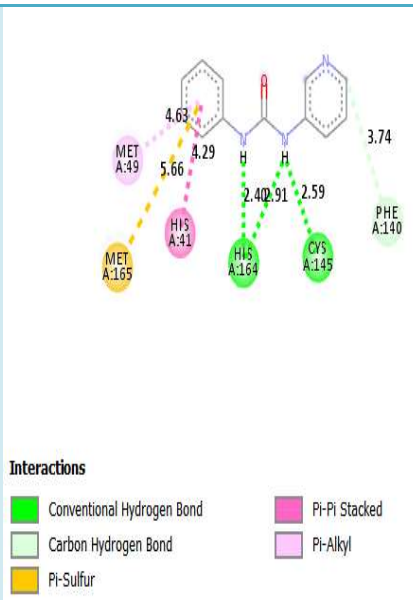
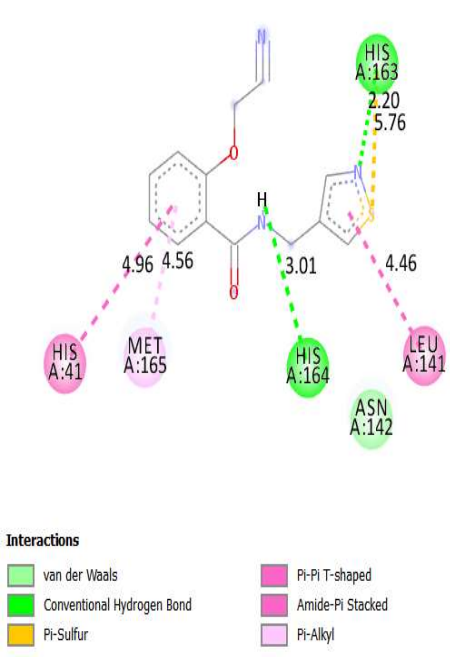
Writing output ... done.

8. Shikonin (PubChem CID – 479503)



mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.0	0.000	0.000
2	-7.0	1.718	6.892
3	-6.7	2.838	5.868
4	-6.7	3.441	6.631
5	-6.4	3.251	6.276
6	-6.4	2.690	3.215
7	-6.4	2.394	4.462
8	-6.3	3.246	6.153
9	-6.2	1.258	3.203

Writing output ... done.

9. **1-phenyl-3-pyridin-3-ylurea (PubChem CID – 674807)**		mode | affinity | dist from best mode | (kcal/mol) | rmsd l.b.| rmsd u.b. 1 -6.5 0.000 0.000 2 -6.4 1.158 6.963 3 -6.0 3.551 4.466 4 -5.9 3.629 4.313 5 -5.6 3.827 6.699 6 -5.4 4.250 4.786 7 -5.4 4.184 7.070 8 -4.9 5.581 6.250 9 -4.9 5.351 6.992 Writing output ... done.
10. **2-(cyanomethoxy)-N-[(1,2-thiazol-4-yl)methyl]benzamide (PubChem CID – 146037583)**		mode | affinity | dist from best mode | (kcal/mol) | rmsd l.b.| rmsd u.b. 1 -6.1 0.000 0.000 2 -6.1 3.750 6.346 3 -5.9 1.937 3.499 4 -5.9 1.515 3.360 5 -5.7 1.762 2.095 6 -5.6 3.283 5.899 7 -5.6 3.115 5.020 8 -5.6 3.173 5.361 9 -5.5 3.468 5.216 Writing output ... done.

CHAPTER-3-CONCLUSION

In silico-tool analysis employ the in silico docking methods to enhance the significant changes as well as improvement the past over the decades. Through freely available web servers designed for specific task in command line programs such as docking tools, increment of ease of use of bench-working biologist. It also presents one of the future challenges in the silico anchoring sector to automate the entire process or make it truly accessible to the public. In docking algorithm still further modification or improvement with enhancement of reliability in results .[72] We have demonstrated molecular docking (Autodock Vina) in a way that finds the best approach to drug collection toward the target of Mpro enzyme inhibition activity of COVID-19. Its analysis results reported the best predictive molecules list which may be in future helpful to overcome the problem in this area and it's will be helpful to further studies or analysis of results with defining with Molecular dynamics and other model generation tools to confirm their data and predict the best results with some improvements.

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