

***In-silico* pharmacophoric and molecular docking-based drug discovery against the Main Protease (M^{Pro}) of SARS-CoV-2, a causative agent COVID-19**

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Abstract: COVID-19 (Coronavirus Disease 2019) caused by a novel ‘SARS-CoV-2’ virus resulted in public health emergencies across the world. An effective vaccine to cure this virus is not yet available, thus requires concerted efforts at various scales. In this study, we employed Computer-Aided Drug Design (CADD) based approach to identify the drug-like compounds - inhibiting the replication of the main protease (M^{Pro}) of SARS-CoV-2. Our database search using an online tool “ZINC pharmer” retrieved ~1500 compounds based on pharmacophore features. Lipinski’s rule was applied to further evaluate the drug-like compounds, followed by molecular docking-based screening, and the selection of screening ligand complex with M^{Pro} based on S-score (higher than reference inhibitor) and root-mean-square deviation (RMSD) value (less than reference inhibitor) using AutoDock 4.2. Resultantly, ~200 compounds were identified having strong interaction with M^{Pro} of SARS-CoV-2. After evaluating their binding energy using the AutoDock 4.2 software, three compounds (ZINC20291569, ZINC90403206, ZINC95480156) were identified that showed highest binding energy with M^{Pro} of SARS-CoV-2 and strong inhibition effect than the N3 (reference inhibitor). A good binding energy, drug likeness and effective pharmacokinetic parameters suggest that these candidates have greater potential to stop the replication of SARS-CoV-2, hence might lead to the cure of COVID-19.

Keywords: SARS-CoV-2, N3 Inhibitor, ZINC database, molecular docking, virtual screening, drug design.

INTRODUCTION

The recent outbreak of pandemic (Coronavirus Disease COVID-19) caused by the SARS-CoV-2 virus, formerly called 2019-nCoV, erupted from the meat market in Wuhan, China (Xu *et al.* 2020). This pandemic has not only created health problems for humans in almost all countries, but it has also created virtual boundaries that isolated each affected country from rest of the world. This pandemic created medical emergency for everybody at international level that badly affected the international relations, travelling, import export, tourism as well as religious gatherings. The efficient and quick responses were adapted by centers for disease control as a preventive measure to restrict and overcome this novel disease (Ji *et al.* 2020). Corona viruses that cause disease in different organisms has large viral genome consisting of single-stranded positive-sense RNA strand. The full genome sequence of this novel strain was published immediately in a month after onset of this disease in January 2020. This timely work helped the scientists to understand and quickly screen the viral strain of this disease at genomic level (de Wilde *et al.* 2017; Zhu *et al.* 2020). On February 12, 2020, World Health Organization

(WHO) released the official name of 2019-nCoV as SARS-CoV-2 (Imai *et al.* 2020). According to Situation Report-147 of WHO, till 15, June 2020, COVID-19 has been reported throughout the world (Chen, Liu and Guo 2020), reporting more than 7.8 million confirmed cases and 431,541 casualties (Lai *et al.* 2020; Stoeklin *et al.* 2020). Wu *et al.* (2020) revealed that SARS-CoV-2 has similar sequence with beta coronavirus, at 5’ UTR and contains Orflab (main protein), nsp (non-structural protein), S protein (spike protein), glycoprotein, while at 3’ UTR, it confined several undefined non-structural protein properties. SARS-CoV-2 consists of the family of beta coronavirus, but it has a diverse genome sequence than SARS-CoV and MERS-CoV. SARS-CoV-2 has been reported to show ~80% sequence identity and about 89 % similarity with previously identified SARS-CoV (Simmons *et al.* 2011; Qamar *et al.* 2020; Wu *et al.* 2020). SARS-CoV-2 consists of polypeptide of about 800 kDa that is usually cleaved by different proteases *viz.* papain-like proteases and chymotrypsin-like proteases leading to formation of several non-structural proteins (nsp), which are indispensable for the viral replication (Chang *et al.* 2014; Qamar *et al.* 2020). These nsp are good possible target sites for the antiviral drug to treat SARS-CoV-2 effectively.

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Previously, N3 inhibitor was identified and reported in protein databank (PDB) with M^{pro} of the SARS-CoV-2 in form of crystal structure complex (PDB Entry: 6LU7; Jin *et al.* 2020). For ligand screening, N3 inhibitor was selected from the well-known ZINC database using a pharmacophore-based online tool ZINC-pharmer. Computational processes and virtual screening play major role in drug designing. Virtual high throughput screening (vHTS) is considered as an fundamental part for designing drug (Lyne 2002).

In this article, novel bioinformatic techniques such as pharmacophore-based ligands screening from databases, structure-based screening, molecular docking, were used to screen the databases containing varieties of drug like compounds against catalytic site i.e. M^{pro} of SARS-CoV-2. The drug like compounds with strong interaction, greater binding energy and strong inhibition effect with M^{pro} of SARS-CoV-2 were identified. These candidates of drug-like compounds could render great potential to stop the replication of SARS-CoV-2 and ultimately lead to development of COVID-19 cure. Therefore, the discovered virtual hits could serve as a starting point to develop future drug candidates. Furthermore, main protease (M^{pro}) inhibitors identified in this study warrant further *in vitro* and *in vivo* evaluations for the inhibition of COVID-19.

MATERIALS AND METHODS

Computational analyses

Preparation of coordinate file for SARS-CoV2 M^{pro}

The X-ray crystal structure of SARS-CoV2 M^{pro} (PDB ID: 6LU7, resolution = 2.16 Å) was taken from PDB database. Protein structure was optimized by using Discovery-Studio-Visualizer (DSV, ver. 3.1) and Auto Dock Tools (ADT, version 1.5.4). The script was then saved for further review in file format pdbqt.

Pharmacophore-based virtual-screening of M^{pro} inhibitor

An online tool “ZINC-pharmer” (<http://zincpharmer.csb.pitt.edu/pharmer.html>) from ZINC database was used for pharmacophore-based virtual screening of drug-like compounds (Sterling and Irwin 2015). The three-dimensional (3D) structure of the M^{pro} of COVID-19 (<https://pdb101.rcsb.org/>) and “SDF” extension file of inhibitor N3 (PDB id “6LU7”) were uploaded into the ZINC-pharmer to generate pharmacophore features of the ligand within the distance cut-off range of receptors (Koes and Camacho 2012). This type of screening is important for the identification of appropriate inhibitors against the target, the prediction of novel drug-like compounds and facilitate further assessment (Lu *et al.* 2018). Lipinski’s rule was applied to find out drug-like properties of the selected compounds obtained through molecular docking (Lipinski 2004). The rule explains the ideal and important molecular properties of drug’s pharmacokinetics’ (Yang

et al. 2018). “Prepare Ligands” in AutoDock 4.2 was used to design structures using PDB format of top hit compounds obtained from previous step. Versatile torsions were allocated, and free rotation was permitted for the acyclic dihedral angles. For further analysis, pdbqt file format was used.

Molecular docking

The retrieved drug-like compounds were docked with M^{pro} of SARS-CoV-2. Simulations for molecular docking were performed as operating system using AutoDock 4.2 based on MGL tools 1.5.4, which uses the languages Python 2.5 and 2.7 (Morris *et al.* 2009). These molecules were visualized inside the active site with DSV 3.1 and PyMol 9.19 (Inwood *et al.* 2009; Studio 2008). A reference was also prepared for molecular docking by eliminating ligand, the addition of hydrogens and energy minimization of M^{pro} by using AutoDock 4.2 software (Cosconati *et al.* 2010). The energy minimized structure was further used as a receptor for molecular docking. The catalytic site of M^{pro} (VAL3, LEU4, THR24 – THR26, HIS41, CYS44, THR45, SER46, MET49, and GLN189) was obtained using active site prediction server of DoGSite (Volkamer *et al.* 2012). Using AutoGrid 4.2 the grid maps of the interaction energies of different types of atoms is precalculated. In each docking for SARS CoV-2 M^{pro}, a grid box was created using a grid map of 45 × 45 × 45 points, 60 × 60 × 60 grid spacing points respectively 0.375 Å and 0.420 Å. The grid maps within the protein structure is based on the respective ligand binding site. The molecular docking by using AutoDock 4.2 was carried out with selected parameters: the number of GA runs was 150; the maximum number of assessments (short) was 250000; the maximum number of generations was 27000; the number of GA runs was 10; and the rate of gene mutation was 0.02 to find and evaluate the interaction between ligands and M^{pro} (Mujwar and Pardasani 2015). The most efficient hits were selected based on the S-score of N3 inhibitor and RMSD (root mean square deviation) values. The S value is a scoring value that measures the affinity of the ligand with the receptor and it is calculated by default scoring-built function in AutoDock 4.2. While RMSD values are used to compare conformation of docked against reference conformation. The retrieved compounds having lower S-score and RMSD value than the reference substrate can work as a inhibitor (Qamar *et al.* 2016). For further analysis, the binding energy of these hits was identified. Binding affinity showed the hydrophobic and polar interaction of the ligand with the active site of receptor. It ranges between 5 to 15 kcal/mol that is regarded as a strong interaction between the ligand and receptor (Mujwar and Pardasani 2015). The compound that has equal or higher binding affinity than the reference inhibitor is considered for further analysis of absorption, distribution metabolism excretion, and toxicity profiling. AdmetSAR server was used to evaluate the physical and chemical properties of drug-like compounds.

Validation and ADMET analysis of hits compounds

The best selected compounds were evaluated by drug likeness properties. The drug likeness properties were done by using an online tool the Molinspiration server (<https://www.molinspiration.com/>). Further the ligand property evaluating through using an online tool known as “Admet SAR” (<http://lmm.d.ecust.edu.cn/admetSar2/>) was used to check the molecular properties and effectiveness of the drug (Monteiro *et al.*, 2019). The ADMET parameter’s calculation i.e. absorption, distribution, metabolism, excretion, and toxicity are the significant determination of drug candidate’s fate, behavior and toxicity level in human body (Hussain *et al.* 2019).

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RESULTS**Pharmacophore-based virtual screening and database preparation**

CADD-based virtual screening renders an efficient and quick method to discover novel drug compounds. In this study, best hit compounds with a similar feature of reference complex of receptor M^{pro} with N3 inhibitor were screened from ZINC database with the pharmacophore model which was achieved by ZINC pharmacophore. The pharmacophore key features with one hydrophobic, one aromatic, one H-acceptor, and one H-donor were selected to generate a pharmacophore model and screened the ZINC database containing ~250 million compounds in 3D format. As a result, 700 out of 1500 hit compounds were chosen through Lipinski’s thumb rule as explained in methodology section and further processed through molecular docking.

Molecular docking

The significance of molecular docking to discover drugs against multiple fatal diseases is widely recognized. Herein, the M^{pro} of COVID-19 was docked along with N3 inhibitor having most favorable active site(s) (VAL3, LEU4, THR24 - THR26, HIS41, CYS44, THR45, SER46, MET49 and GLN189) of M^{pro} determined by active site prediction server of DoGSite (fig. 1).

Subsequently, S-score based best docking model was selected as a reference and all of the hits were docked against that most active site (P1) of M^{pro} by using the Auto Dock 4.2 docking algorithm. Consequently, 200 compounds were identified with a lower S-score (docking score) than the reference inhibitor. The best docked hit compounds having lower S-score and RMSD value were selected for further evaluation. The binding interaction of these 200 hits compounds with the M^{pro} was determined by using the AutoDock 4.2 software. In the specified order of choice, the best poses were defined based on lowest binding energy in the largest cluster, number of hydrogen bonds with active site residues and conservation of interactions with those from control docking. This was performed to ensure that the hits docked exactly into the correct binding place of interest. The hits that showed the significant interaction with active site (VAL3, LEU4, THR24 - THR26, HIS41, CYS44, THR45, SER46, MET49, and GLN189) of M^{pro} of SARS-CoV-2 were considered as a good inhibitor (fig. 1). The compounds were selected that showed the greater interaction than reference inhibitor with the active site of M^{pro} of SARS-CoV-2 as shown in table 1. Three out of 200 hits were selected for the ADMET profiling analysis.

Validation and ADMET analysis of hit compounds

The Molinspiration server was used to predict the drug like properties of proposed inhibitors against SARS CoV-2 M^{pro}. All the selected compounds showed a zero violation to Pfizer’s rule of five and accepted the druglike properties i.e. molecular weight (table 1). All the selected compounds were subjected to the AdmetSAR server to predict the physical and chemical properties of drug likeness in order to further validate the drug like properties (table 2).

Binding interaction analysis of selected drug-like compounds

S-score measures the strength of the interaction between the receptor and ligands. Based on the S-score, the compounds can be selected for a good drug compound. The compounds with the ZINC IDs “ZINC20291569”, “ZINC90403206” and “ZINC95480156” showed strong interaction against the active site (p1) (VAL3, LEU4, THR24-THR26, HIS41, CYS44, THR45, SER46, MET49, and GLN189) of the M^{pro} of SARS-CoV-2. The docking conformations of selected compounds are shown in fig. 3.

DISCUSSION

Recently, several studies have been carried out to find the therapeutic vaccine against SARS-CoV-2, but with little success (Yu *et al.* 2020). Considering the widespread of virus and the consequent human fatalities reported across the globe, there is an urgent need to find a cost-effective antiviral drug / vaccine for the control of this menace.

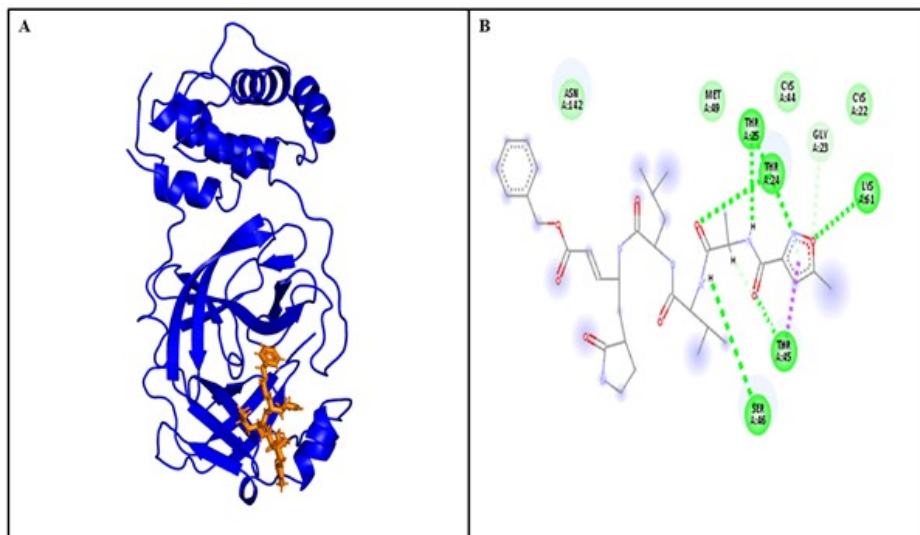


Fig. 1: (A) A 3-D complex of SARS-Cov2 m^{pro} (Blue color) interacts with N3 inhibitor (orange color) (B) Docked N3 [N-[(5-methylisoxazol-3-yl) carbonyl] alanyl-l-valyl-n~1~((1r,2z)-4-(benzyloxy)-4-oxo-1- { [(3r)-2-oxopyrrolidin-3-yl] methyl} but-2-enyl)-l-leucinamide] complex with SARS-Cov-2 M^{pro}, atoms of side chain such as THR-A25, THR-A24,LYS-A61, THR- A45, and SER-A46 are making hydrogen bond are shown in green line.

Table 1: Docking scoring, binding energy, and Lipinski's rule scan results of the selected compounds

Name (ZINC ID)	S-Score	RMSD	Binding energy kcal/mol	Lipinski's drug-likeness Score				
				MW	LogP	H-bond donor	H-bond acceptor	tPSA
ZINC20291569	-10.1489	1.6258	-21.74	333.348	2.85	2	6	89
ZINC90403206	-12.6313	1.675258	-26.330	331.485	3.831	2	3	58
ZINC95480156	-10.9725	1.4352	-22.901	488.5	4.397	3	8	145
N3 (Reference)	-8.1190	4.0853	-21.60					

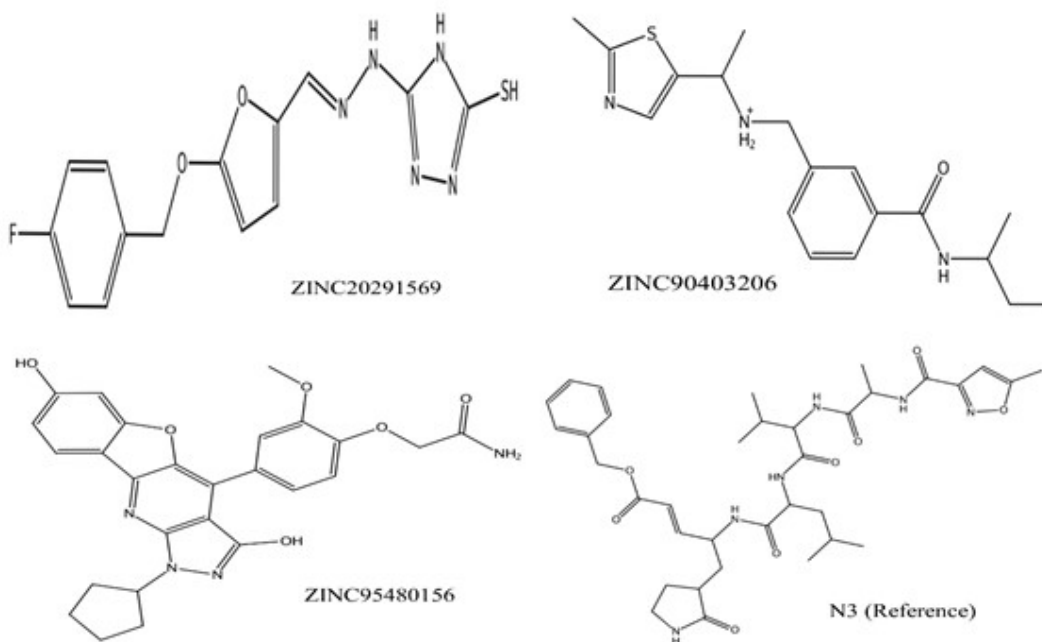


Fig. 2: 2D structures of finally selected drug-like compounds with ZINC IDs and reference N3 inhibitor

Table 2: ADMET profiling for selected hit of drug-like compounds

Properties	ZINC-ID		
	ZINC20291569	ZINC90403206	ZINC95480156
Absorption			
BBB (Blood Brain Barrier)	BBB ^{+ve}	BBB ^{+ve}	BBB ^{+ve}
HIA (human Intestinal Absorption)	HIA ^{+ve}	HIA ^{+ve}	HIA ^{+ve}
CaCO ₂ permeability	CaCO ₂ ⁻	CaCO ₂ ⁺	CaCO ₂ ⁻
PGI (p-glycoprotein inhibitor)	Non-inhibitor	Non-inhibitor	Inhibitor
PGI (p-glycoprotein substrate)	Non-substrate	Non-substrate	Substrate
ROCT (Renal Organic Cation Transporter)	Non-inhibitor	Non-inhibitor	Non-inhibitor
Aqueous solubility (LogS)	-3.4069	-2.5307	-2.5150
Metabolism			
CYP450 2C9 substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 substrate	Non-substrate	Non-substrate	Substrate
CYP450 1A2 inhibitor	Inhibitor	Inhibitor	Non-Inhibitor
CYP450 2C9 inhibitor	Non-Inhibitor	Inhibitor	Inhibitor
CYP450 2D6 inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP2450 C19 inhibitor	Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP450 3A4 inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP inhibitory promiscuity	High	High	Low
UGT catalyzed inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
Toxicity			
AMES toxicity	Non-toxic	Non-toxic	Non-toxic
Carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic

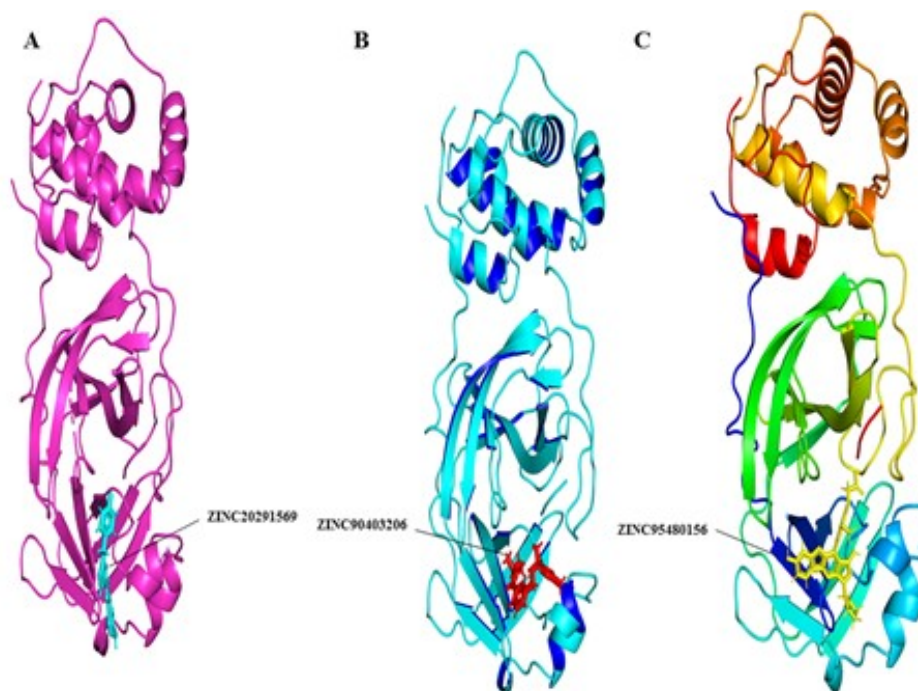
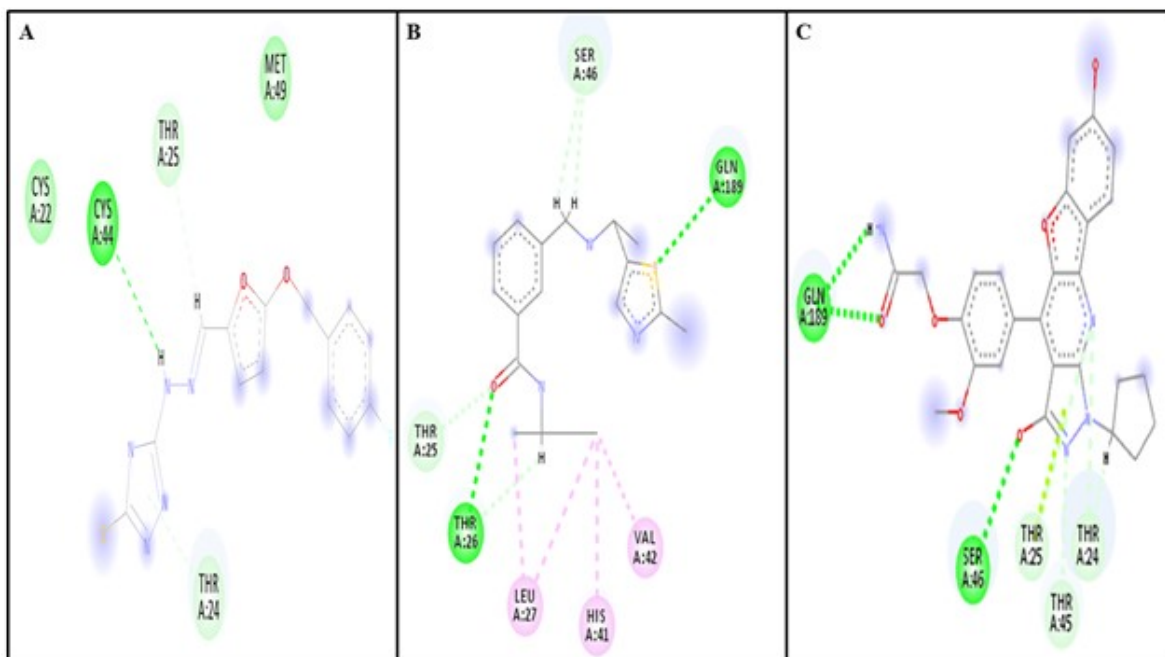
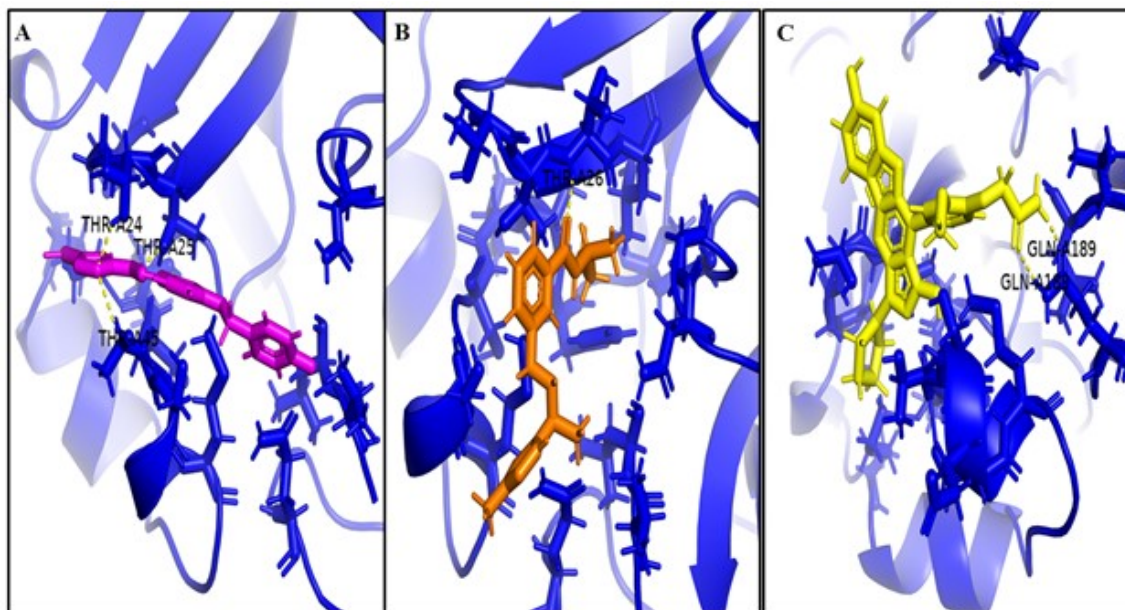


Fig. 3: Receptor-ligands (ZINC IDs: ZINC20291569, ZINC90403206, ZINC95480156) binding interaction analysis. (A) shows the 3D computationally predicted structure of SARS-Cov-2 M^{pro} (cartoon representation with pink color) complex with novel inhibitor ZINC20291569, (B) shows the 3D computationally predicted structure of SARS-Cov-2 M^{pro} (cartoon representation with cyans color) complex with novel inhibitor ZINC90403206 (C) shows the 3D computationally predicted structure of SARS-Cov-2 M^{pro} (cartoon representation with rainbow color) complex with novel inhibitor ZINC95480156. PyMol version 9.19 was used for these receptor-ligand complex.



Supplementary file 1: (A) 2D representation of ligand “ZINC20291569” binding mode with receptor active site of SARS-Cov-2 M^{Pro}. Sidechain residue CYS-A44 is making hydrogen bond with ligand “ZINC20291569”. (B) 2D representation of ligand “ZINC90403206” binding mode with receptor active site of SARS-Cov-2 M^{Pro}. Sidechain residue GLN-A189 and THR-A26 are making hydrogen bonding with a ligand “ZINC90403206”. (C) 2D representation of ligand “ZINC95480156” binding mode with receptor active site of SARS-Cov-2 M^{Pro}. Sidechain residues GLN-A189, THR-A25 and SER-A46 are making hydrogen bonding with a ligand “ZINC95480156”. Discovery Studio Visualizer version 3.1 was used for these assessments.



Supplementary file 2: (A) 3D representative docked pose and polar interactions between the ligand “ZINC20291569” (magenta color) and most active binding pocket’s residues (Blue color) of SARS-Cov-2 M^{Pro}. (B) 3D representative docked pose and polar interactions between the ligand “ZINC90403206” (orange color) and most active binding pocket’s residues (Blue ribbon color) of SARS-Cov-2 M^{Pro}. (C) 3D representative docked pose and polar interactions between the ligand “ZINC95480156” (yellow color) and most active binding pocket’s residues (Blue color) of SARS-Cov-2 M^{Pro}. PyMol version 9.19 has been used for these assessments.

An efficient technique for drug discovery is to test whether existing drug-like compounds are effective against viral infections. However, the conventional drug discovery techniques are time-taking and less efficient (Hui *et al.* 2020). At present, the broad-spectrum antiviral drugs chloroquine and remdesivir are being used to treat the SARS-CoV-2 (Forni *et al.* 2017; Wang *et al.* 2020). *In silico* study revealed that N3 inhibitor stopped the biological function of HCoV-NL-63 by blocking its active catalytic site (To *et al.* 2013; Wang *et al.* 2016). Considering above-mentioned discussion, the present study suggested compounds which strongly bound with the M^{pro} of SARS-CoV-2 catalytic site by inhibiting its proteolytic activity and can be considered as drug compounds.

The three drug-like compounds were further evaluated with AdmetSAR server to screen ADMET-properties (Cheng *et al.* 2012). Blood-Brain-Barrier (BBB) is the resistor present within endothelial cells and stop the brain from up taking any pharmaceutical. BBB is an important factor in the field of drug discovery (Alavijeh *et al.* 2005). Oral bioavailability is considered an important factor for the selection of active drug for patients (Thomas *et al.* 2006). ADMET characteristics of effective drug-like compounds such as P-G substrate (P-Glycoprotein substrate), BBB penetration, HIP (Human intestinal preparation), ROCT (Renal Organic cation transporter), CaCO₂ permeability, represented the positive results for the likeness of an effective drug. CYP (Cytochrome P450) is a group of isoenzymes involved in the catabolism of various chemicals such as steroids, drugs, carcinogens, and bile acids, etc. A profile of ADMET test for a good and effective drug compound consisted following parameters: (a) it must pass the BBB, (b) absorb in the human intestine, (c) absorb the CaCO₂ permeability, (d) non-toxic, (e) non-carcinogenic, and (f) non-inhibitor to CYP enzyme. The three (ZINC20291569, ZINC90403206, ZINC95480156) compounds significantly accepted these parameters of ADMET (table 2). The selected compounds for drug i.e. ZINC ID, docking score, RMSD value, binding energy value, the binding residue of the M^{pro} of COVID-19, Lipinski's results (table 1) and ADMET analysis (table 2). The selected compounds are capable to serve as the novel, structurally different and potentially active inhibitors against the M^{pro} of SARS-CoV-2 (fig. 2).

Our *in-silico* study found three inhibitors with strong inhibition potential effect of drug lead may be one of therapeutic inhibitor against M^{pro} of SARS-CoV-2 by efficiently targeting the active site of M^{pro} of SARS-CoV-2. Therefore, our finding of three druglike compounds i.e. "ZINC20291569", "ZINC90403206", and "ZINC95480156" require further *in-vitro* and *in-vivo* work for structure-based optimization before commercialization.

CONCLUSION

The main objective of current study was the pharmacophore-based virtual screening using the ZINC database, the molecular docking of selected compounds, and finally the evaluation of binding interaction against the M^{pro} of SARS-CoV-2. Three compounds from the ZINC database (ZINC20291569, ZINC90403206, and ZINC95480156) showed the strong interaction against the active site of the M^{pro} of SARS-CoV-2. Results show that these compounds could be potentially used as a drug against SARS-CoV-2. To validate these results, *in-vitro* as well as *in-vivo* analyses are required to turn these possible inhibitors into therapeutic medicines. We anticipate that the insights gained in current study may prove useful for further exploration and production of novel natural therapeutic agents against COVID-19.

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