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MOLECULAR DOCKING STUDIES OF CARPROFEN ANALOGUES AGAINST SARSCOV-2 MAIN PROTEASE (Mpro) FOR NOVEL COVID-19 DRUG DISCOVERY

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Abstract

MSARSCoV-2 main protease is a homodimer protein that cleaves polyproteins into several non-structural proteins, thus facilitates viral replication and transcription. Viral replication can be blocked potentially when the activity of this enzyme is inhibited. This benefit makes the enzyme an important site for antiviral drug against COVID-19. There are several inhibitors of main protease (Mpro) which have been approved as antiviral drugs but their application is associated with adverse side effects. These side effects are primarily attributed to the less selectivity of the drugs for the main protease (Mpro). Therefore, the development of selective SARSCoV-2 Mpro inhibitors could be a useful technique for finding new viral drugs that are clinically safer. In this study we have shown molecular docking of 272 similar structures of carprofen with the Mpro. Carprofen is a non-steroidal medication with antiviral properties against SARSCoV-2 Mpro. The library of carprofen analogs was screened for effective inhibitors of Mpro and top 100 best scoring ligands were selected to dock with Mpro. Molecular studies showed that carprofen analogs have selective affinity for Mpro. Three such ligands with mseq 95, 94, 45 were selected which had high binding affinity as compared to control ligands (11b).The docking scores of compounds with mseq 95, 94, 45 in Mpro were –6.9380,-6.8877 and -6.6768 respectively. Further analysis of the ligand-protein interactions revealed that these compounds are involved in a number of interactions with Mpro which gives them better binding affinity with Mpro. The compounds are linked with different amino acid residues of Mpro by different types of intermolecular forces. The specific inhibitory activity of these compounds can be confirmed further by invivo and invitro methods leading to the development of safer anti-viral drugs.

INTRODUCTION

Coronaviruses are a diverse group of enveloped, positive-sense single-stranded RNA viruses belonging to the family *Coronaviridae* and the order *Nidovirales*. These viruses infect a wide range of hosts, including humans and animals, and are responsible for respiratory, gastrointestinal, hepatic, and neurological diseases (Fehr et al., 2015; Wang et al., 2020). Coronaviruses possess one of the largest genomes among RNA viruses and exhibit significant genetic variability, which contributes to their ability to adapt to new hosts and environments.

Historically, several human coronaviruses have caused mild respiratory infections; however, the emergence of highly pathogenic strains such as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome
Coronavirus (MERS-CoV) demonstrated their potential to cause severe disease and global outbreaks (Song et al., 2019).

In December 2019, a cluster of pneumonia cases of unknown etiology was reported in Wuhan, China, which was later identified as being caused by a novel coronavirus named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The disease caused by this virus was subsequently designated as Coronavirus Disease 2019 (COVID-19) (Zhu et al., 2020). The virus spread rapidly across continents due to its high transmissibility and efficient human-to-human transmission. Within a short period, COVID-19 evolved into a global public health emergency and was declared a pandemic by the World Health Organization. Epidemiological and genomic investigations indicated that SARS-CoV-2 is closely related to bat-derived coronaviruses, suggesting a zoonotic origin of the virus (Zhou et al., 2020; Lu et al., 2020). Additional genomic characterization of early viral isolates also revealed important insights into viral evolution, transmission dynamics, and receptor-binding mechanisms (Chan et al., 2020).

The pathogenesis of SARS-CoV-2 infection is closely associated with the interaction between viral surface proteins and host cell receptors. The spike (S) glycoprotein located on the viral envelope plays a critical role in mediating viral entry into host cells. This protein binds specifically to the angiotensin-converting enzyme 2 (ACE2) receptor expressed on the surface of host cells, facilitating viral attachment and membrane fusion (Hoffmann et al., 2020). After receptor binding, host proteases such as transmembrane protease serine 2 (TMPRSS2) activate the spike protein through proteolytic cleavage, enabling the fusion of viral and host membranes (Tang et al., 2020). The cleavage and activation of the spike protein are therefore essential steps in viral entry and replication, making them important targets for antiviral drug development (Asan et al., 2020).

Structurally, the coronavirus genome encodes several structural and non-structural proteins that are essential for viral replication and assembly. Among these proteins, the main protease (3CLpro or Mpro) plays a crucial role in processing viral polyproteins into functional components required for replication and transcription. This enzyme is responsible for cleaving the large viral polyprotein at multiple conserved sites, thereby generating mature non-structural proteins necessary for viral replication (Anand et al., 2003). Due to its indispensable role in the viral life cycle and the absence of closely related homologs in humans, the main protease has become an attractive target for antiviral drug discovery.

Recent structural studies have provided detailed insights into the molecular architecture of SARS-CoV-2 main protease and its active site configuration. These studies have facilitated the rational design of potential inhibitors targeting the protease enzyme. For instance, crystallographic analysis of the SARS-CoV-2 main protease revealed important structural features that can be exploited for the development of α-ketoamide inhibitors and other small-molecule compounds (Zhang et al., 2020). Similarly, investigations into structural variations within the protease enzyme suggest that certain mutations may enhance enzymatic activity and potentially contribute to viral infectivity (Dang et al., 2020).

With the rapid progression of the COVID-19 pandemic, there has been an urgent need to identify effective therapeutic strategies against SARS-CoV-2. Computational approaches such as molecular docking, virtual screening, and artificial intelligence-based drug discovery have been extensively applied to accelerate the identification of potential antiviral compounds. These methods enable researchers to analyze large chemical libraries and predict compounds capable of inhibiting viral targets such as the main protease and RNA-dependent RNA polymerase (RdRp) (Ton et al., 2020; Beck et al., 2020; Ahmad et al., 2020). Computational screening has therefore emerged as a powerful

strategy for the rapid discovery of potential therapeutic candidates against emerging viral pathogens.

In addition to computational drug discovery, several studies have focused on drug repurposing as a strategy to identify existing pharmaceuticals with potential antiviral activity against SARS-CoV-2. Repurposing approved drugs can significantly reduce the time and cost associated with the development of new therapeutics. Large-scale protein interaction studies have also revealed host–virus interaction networks that may serve as potential targets for therapeutic intervention (Gordon et al., 2020). Furthermore, pharmacological reviews have summarized the therapeutic potential of various antiviral and immunomodulatory agents for the treatment of COVID-19 (Sanders et al., 2020).

Some early clinical investigations explored the potential use of drugs such as hydroxychloroquine in combination with azithromycin for the treatment of COVID-19 patients, although the effectiveness and safety of these therapies remain controversial and require further investigation (Gautret et al., 2020). Consequently, there is an ongoing need to identify and develop novel antiviral compounds that can effectively inhibit key viral enzymes involved in SARS-CoV-2 replication.

Overall, understanding the structural and functional characteristics of SARS-CoV-2 proteins is essential for the development of effective antiviral strategies. In particular, targeting essential viral enzymes such as the main protease provides a promising approach for the discovery of novel therapeutic agents against COVID-19. Continuous research involving structural biology, computational drug discovery, and pharmacological evaluation is therefore crucial to combat current and future coronavirus outbreaks.

MATERIALS AND METHODOLOGY

For the docking of ligands at the binding site of Mpro ,following steps were followed;

SELECTION OF PROTEIN STRUCTURE

First the structure of main protease of SARSCoV-2 was searched on PDB databank.The only structure in complex with a ligand (11b) was then selected from PDB (6M0K) of all the available structures of SARSCoV-2 Mpro because this structure contains the co-crystallized ligand molecule at its binding pocket, which will serve as a control ligand during docking process.

PREPARATION OF PROTEIN STRUCTURE

From PDB database, the selected protein was downloaded in 3D structure.

SEARCHING FOR THE SIMILAR STRUCTURES OF CARPROFEN

The available similar structures were searched and the structure of carprofen was drawn on pubchem drugbank.A total of 272 similar structures of carprofen were available on pubchem and were downloaded as a single SDF file.

PREPARATION OF LIGANDS LIBRARY FOR DOCKING

Both the test and control ligands were then imported MOE software.We then washed the structure to remove inorganic salts and ions during crystallization process.Next,we added Hydrogens to the ligands,the partial chargres were calculated and their energy was minimized by the use of quickprep functinality of the software.

DOCKING OF SARSCOV-2 MAIN PROTEASE

The structure of Mprowas imported to molecular operating environment (MOE) software for docking.Then the quickprep command was applied through which enzyme was prepared for docking with the addition partial charges and Hydrogens.Then we solvate the protien for the energy minimization.

Then the redocking of SARSCoV-2Mpro with the cocrytallized ligand was done, which was placed at the binding site with the same conformation by the software. Then by using flexible docking method ,the entire library of

ligands was screened.The active site 11b (control ligand) was used for docking of all other ligands.

SELECTION OF TOP SCORING LIGANDS FOR DOCKING IN SARSCOV-2 MAIN PROTEASE

Once we are done with docking of Mpro, then on the basis of docking results,we selected top 100 scoring ligands and removed the duplicate ligands.Then we docked the total ligands with Mpro to determine the selective ligands (inhibitors) of Mpro.

SELETION OF THE SPECIFIC INHIBITORS OF SARSCOV-2 MAIN PROTEASE

Once we are done with the docking Mpro,then the results were searched for the ligands which shows high binding affinity towards Mpro. Those ligands were selected showing high docking scores S in Mpro.Along with these, the control ligand was also analyzed to determine its interactions.

RESULTS AND DISCUSSION

The different derivatives of carprofen have been shown to exhibit antiviral activity against SARSCoV-2 main protease.Already many research work has been done on it however, no such work regarding complete screening of all its drug like analogs have reported till date.Here our work was to dock all the known drug like analogs of carprofen with SARSCoV-2 Mpro to find any specific inhibitors of main protease and to find such residues interacting with the ligands which are specific to SARSCoV-2 Mpro.By identifying such residues more specific ligands for main protease can be prepared.For this purpose, the similar structures of carprofen were downloaded from pubchem as a single SDF file.There were a total of 272 similar analogs of carprofen,present on pubchem and then we docked these similar analogs with SARSCoV-2 main protease.The top 100 best scoring ligands were selected and the repeated poses were deleted.Thus we obtained total of 12 unique ligands which shows good binding affinity with SARSCoV-2 Mpro along with control ligand.At the end of docking,results were analyzed and those top 3 inhibitors (mseq 95,94,45) having high docking scores with Mpro were selected.Apart from these 3 inhibitors,the control ligand (mseq 1),which show high docking score from all other ligands in Mpro,was also selected for further analysis.All these inhibitors interactions were studied individually with main protease of SARSCoV-2.

The table below shows the detail of selected ligands with respect to their docking score and E-refine values in SARSCoV-2 main protease.

Table 1 Top four best scoring ligands from docking studies of SARSCoV-2 Mpro

S.No	Mol	Mseq	S	E-refine
1	6M0K.A	1	-7.9932	-47.1881
2	129318819	95	-6.9380	-37.1278
3	129318818	94	-6.8877	-39.5108
4	68796220	45	-6.6768	-34.3594

Further detail analysis of interactions between ligands and SARSCoV-2 main protease are discussed below one by one:

CONTROL LIGAND (MSEQ 1)

The ligand with mseq 1(11b) has a chemical formula,$C_{25}H_{25}F\ N_4O_4$ with a molecular weight of 464.49g/mol .This ligand was used as control during docking of SARSCoV-2 Mpro.It is the top 1st best scoring ligand in SARSCoV-2 main protease.The docking of 11b with main protease gives S value of -7.79485.The 2D structure of 11b is given below:

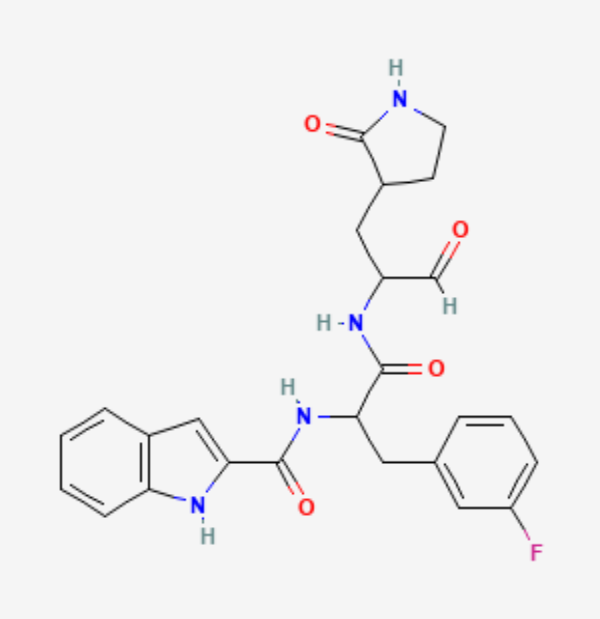

Figure 1The 2D structure of control ligand (11b)

Interaction analysis of control ligand (11b) with SARSCoV-2 Mpro

The 3D structure of SARSCoV-2 main protease with control ligand is stabilized by forming different interactions, which is shown below:

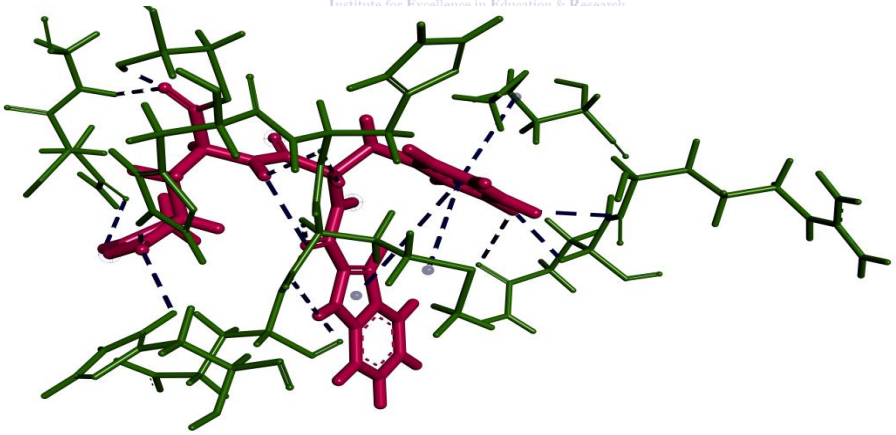

Figure 2 The 3D structure of 11b binded at the active pocket of SARSCoV-2 Mpro by forming different types of interactions with different aminoacid residues. The ligand molecule is shown in dark green color and protein residues in pink color sticks.

The detail analysis of 11b interactions shown that it forms total 12 number of interactions with different aminoacid residues of SARSCoV-2 main protease. Among them, His164 forms one carbon hydrogen bond and one conventional hydrogen bond.Glu166 forms two conventional

hydrogen bonds whereas Asn142, Gln149, His172 and Met165 forms carbon hydrogen bonds. Besides these bonds, there are other several non-bonded interactions that include pi alkyl interactions, Vander Waals forces and covalent bond interactions.

Table 2 List of all types of interactions formed between control ligand and SARSCoV-2 Mpro.

Sr NO	Residue Name	Distance	Category	Types
1	A:CYS145:H - A:FJC405:O33	1.96112	Hydrogen Bond	Conventional Hydrogen Bond
2	A:FJC405:H11 - A:GLU166:O	1.93099	Hydrogen Bond	Conventional Hydrogen Bond
3	A:FJC405:H13 - A:HIS164:O	2.63888	Hydrogen Bond	Carbon Hydrogen Bond
4	A:FJC405:H23 - A:HIS164:O	2.4943	Hydrogen Bond	Conventional Hydrogen Bond
5	A:FJC405:H28 - A:ASN142:OD	2.87615	Hydrogen Bond	Carbon Hydrogen Bond
6	A:GLN189:HA - A:FJC405:F20	2.57637	Hydrogen Bond	Carbon Hydrogen Bond
7	A:GLU166:H - A:FJC405:O01	1.88899	Hydrogen Bond	Conventional Hydrogen Bond
8	A:GLY143:H - A:FJC405:O33	2.64489	Hydrogen Bond	Conventional Hydrogen Bond
9	A:HIS163:HE2 - A:FJC405:O31	1.8381	Hydrogen Bond	Conventional Hydrogen Bond
10	A:HIS172:HD2 - A:FJC405:O31	2.87885	Hydrogen Bond	Carbon Hydrogen Bond
11	A:MET165:HA - A:FJC405:O01	2.4185	Hydrogen Bond	Carbon Hydrogen Bond

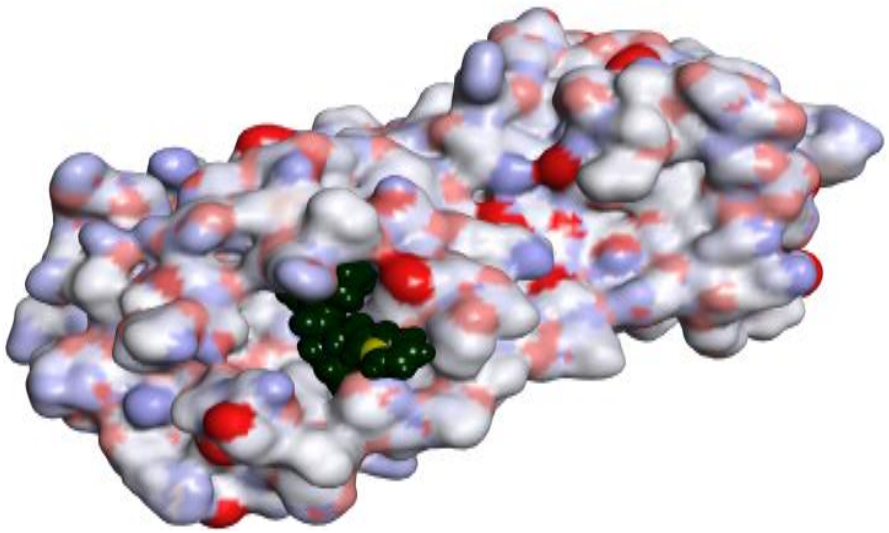

Figure 3 The image depicts the CPK model of control ligand (11b) that actively fits at the binding pocket of Mpro.

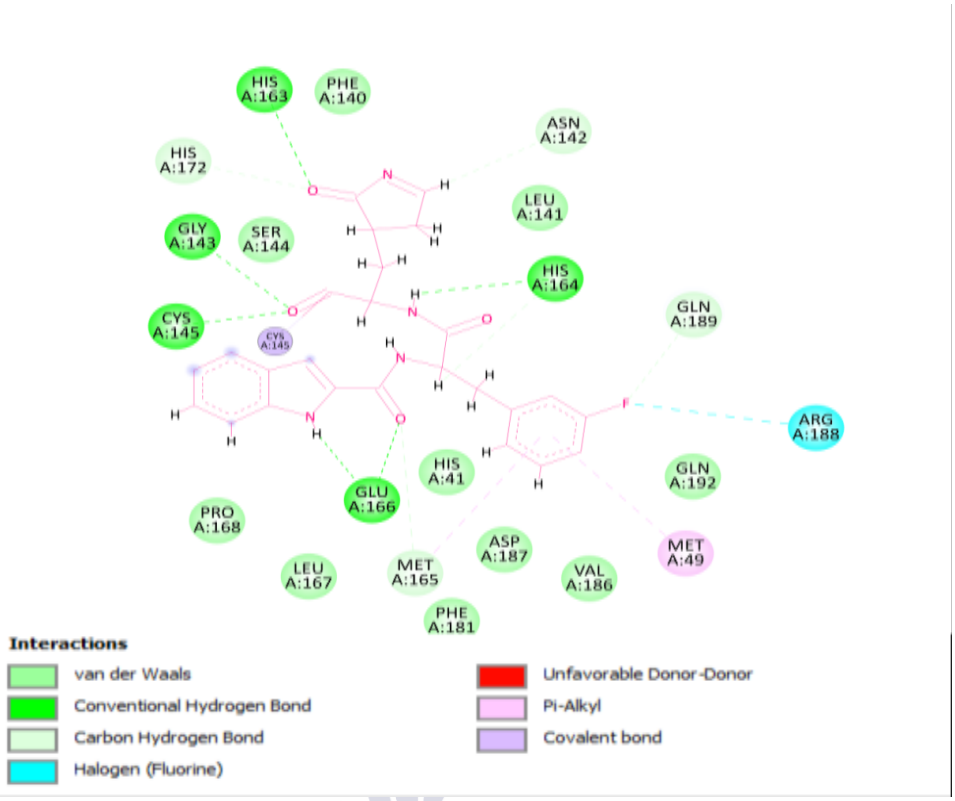

Figure 4 The 2D diagram of ligand (11b) interactions with main protease residues. Each type of interaction is shown with a different color of splitted line.

LIGAND NUMBER 2 (MSEQ 95)

Ligand number two is one of the derivatives of carprofen whose IUAC name is [2, 3Dihydrooxypropyl 2-(6-chloro-9H-Carbazol-2-yl) propanoate]. There are three hydrogen bond donors and four hydrogen bond acceptors in this molecule.The details of all its physical properties are given below in the table.

Table 4 The list of Computed physical properties of ligand number 2(mseq 95)

Sr.No	Property Name	Property Value
1	Molecular Mass	347.8
2	XLogP3-AA	3.1
3	Donor H-bonds	3
4	Acceptor H-bonds	4
5	Rotatable bonds	6
6	Exact weight	347.0924357
7	Monoisotopic Mass	347.0924357
8	Polar Surface Area	82.6 Å²
9	Heavy atoms	24
10	Formal Charge	0
11	Complexity	449
12	Isotope Atom	0
13	Defined Atom	0
14	Undefined Atom	2
15	Defined Bond	0
16	Undefined Bond	0
17	Covalently-Bonded unit	1
18	Compound Is Canonicalized	Yes

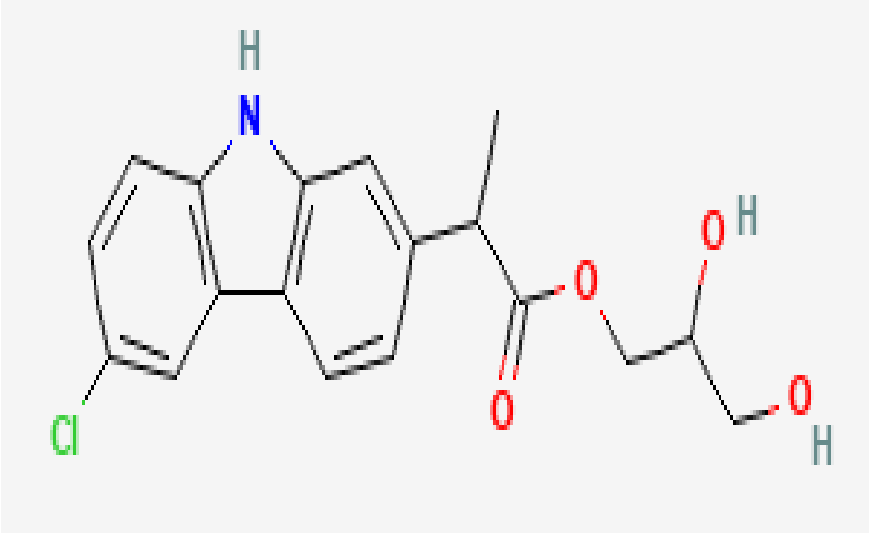

Figure 7 The 2D structure of Ligand number 2 (mseq 95)

It is the second top scoring ligand which has an S value of -6.9380 with the main protease of SARSCoV-2 while the control ligand has S value of -7.9932 with Mpro. To further explain the preferential binding affinity of ligand with main protease, the interactions of the compound were

analyzed using 2D visualizer which are discussed below;

Interaction analysis of the ligand (mseq 95) with SARSCoV-2 main protease

The detail analysis of mseq 95 shown that it forms total of 25 number of interactions with the aminoacid residues of SARSCoV-2 main protease. Among them, Glu166 forms two carbon hydrogen bonds.Met165 makes two pi-alkyl interactions and one pi-sulfur bond.Cys145 undergoes alkyl interaction and makes one pi-sulfur bond.Phe140 form one conventional hydrogen bond, Asn142 form one carbon hydrogen bond.His41 makes pi-pi T shaped interaction and His163 makes pi-alkyl interaction.

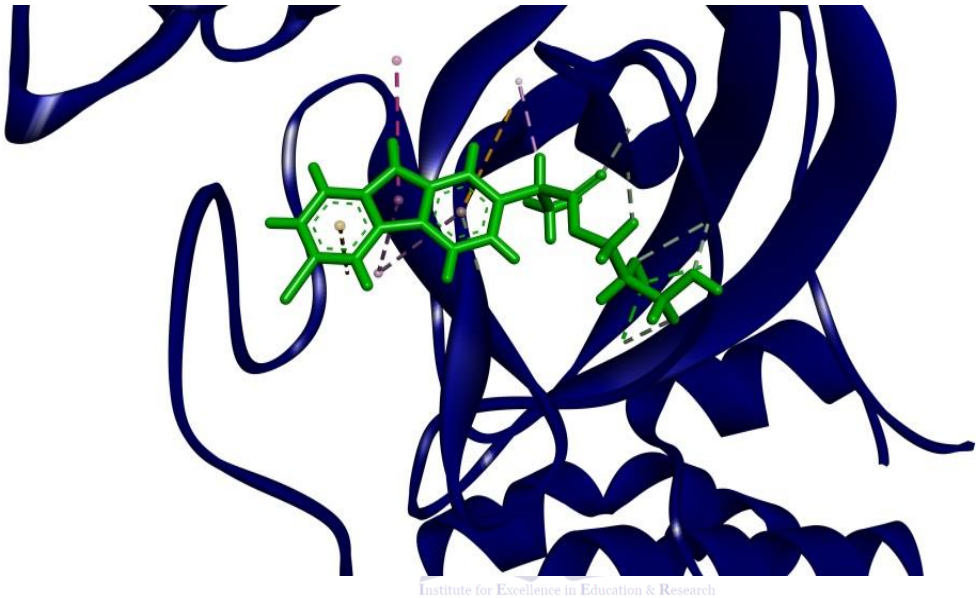

Figure 8 The 3D diagram of ligand molecule (mseq 95) binded at the active pocket of SARSCoV-2 main protease. The ligand molecule is shown in green color sticks and the protein molecule in dark blue ribbon.

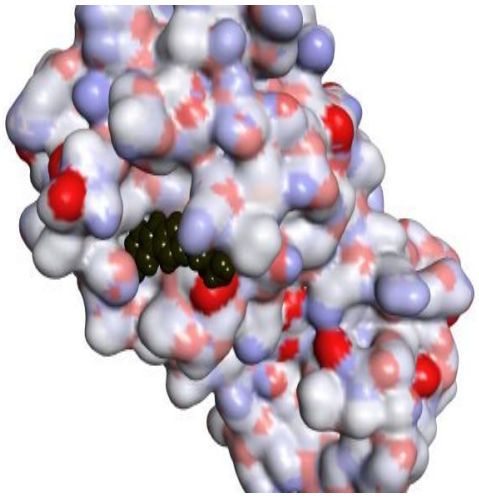

Figure 9 The image above shown depicts the CPK model of ligand number 2 that actively fits at the binding pocket of main protease.The ligand molecule is shown in dark green color balls.

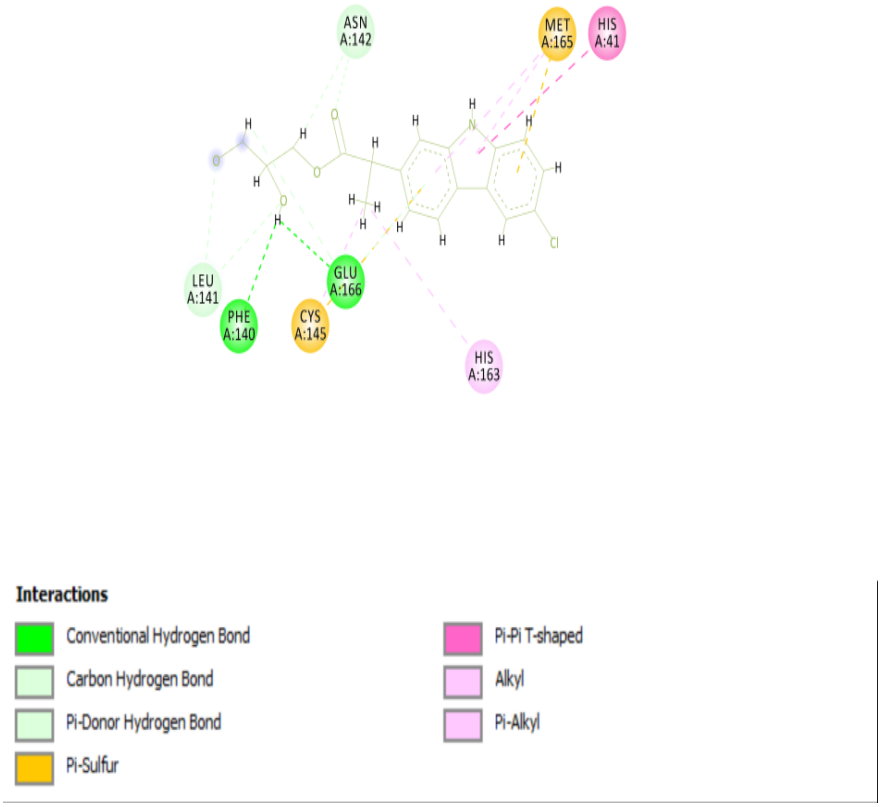

Figure 10 The 2D diagram showing different types of interactions of ligand molecule (mseq 95) with aminoacid residues of main protease .Each type of interaction is shown with splitted lines of different colors.

Table 5 The list of all interactions formed by ligand number 2 (mseq 95) with SARSCoV-2 Mpro.

Sr.N	Residue Name	Distance	Category	Types
1	:*0:H - A:PHE140:O	2.48548	Hydrogen Bond	Conventional Hydrogen Bond
2	:*0:H - A:GLU166:OE2	2.33781	Hydrogen Bond	Conventional Hydrogen Bond
3	A:LEU141:HA - :*0:O	2.94119	Hydrogen Bond	Carbon Hydrogen Bond
4	A:LEU141:HA - :*0:O	2.66507	Hydrogen Bond	Carbon Hydrogen Bond
5		2.19494	Hydrogen Bond	Carbon Hydrogen Bond
6	:*0:H - A:ASN142:OD1	2.41335	Hydrogen Bond	Carbon Hydrogen Bond
7	:*0:H - A:GLU166:OE2	2.78197	Hydrogen Bond	Carbon Hydrogen Bond
8	:*0:H - :*0:O	2.7914	Hydrogen Bond	Carbon Hydrogen Bond
9	A:GLU166:HN - :*0	2.76057	Hydrogen Bond	Pi-Donor Hydrogen Bond
10	A:CYS145:SG - :*0	5.40233	Other	Pi-Sulfur
11	A:MET165:SD - :*0	4.43661	Other	Pi-Sulfur
12	A:FJC0:O - :*0	2.66374	Other	Pi-Lone Pair
13	A:HIS41 - :*0	5.67934	Hydrophobic	Pi-Pi T-shaped
14	:*0:C - A:CYS145	5.02704	Hydrophobic	Alkyl
15	:*0:C - A:FJC405	3.10678	Hydrophobic	Alkyl
16	:*0:C - A:FJC0	2.70323	Hydrophobic	Alkyl
17	A:HIS163 - :*0:C	4.14905	Hydrophobic	Pi-Alkyl
18	:*0 - A:MET165	4.48827	Hydrophobic	Pi-Alkyl
19	:*0 - A:MET165	5.19215	Hydrophobic	Pi-Alkyl
20	:*0 - A:FJC405	4.66778	Hydrophobic	Pi-Alkyl
21	:*0 - A:FJC0	5.02933	Hydrophobic	Pi-Alkyl
22	:*0 - :*0:C	4.77672	Hydrophobic	Pi-Alkyl
23	:*0 - :*0:C	4.42901	Hydrophobic	Pi-Alkyl
24	:*0 - :*0:C	3.59762	Hydrophobic	Pi-Alkyl
25	:*0 - :*0:C	5.31329	Hydrophobic	Pi-Alkyl

3 LIGAND NUMBER 3 (MSEQ 94)

Ligand number three is one of the derivatives of carprofen whose IUPAC name is 1,3-Dihydroxypropan-2-yl-2-(6-chloro-9H-carbazol-2-yl)propanoate.Its molecular weight is 347.8 g/mol.There are three hydrogen bond donors and four hydrogen bond acceptors in this molecule.The detail of all its physical properties are given below in the table;

Table 7 The list of computed physical properties of ligand number 3 (mseq 94).

Sr.No	Property Name	Property Value
1	Molecular Mass	347.8
2	XLogP3-AA	3.1
3	Donor H-bonds	3
4	Acceptor H-bonds	4
5	Rotatable bonds	6
6	Exact weight	347.0924357
7	Monoisotopic Mass	347.0924357
8	Polar Surface Area	82.6 Å²
9	Heavy Atoms	24
10	Formal Charge	0
11	Complexity	445
12	Isotope Atom	0
13	Defined Atom	0
14	Undefined Atom	1
15	Defined bond	0
16	Undefined bond	0
17	Covalently-Bonded Unit	1
18	Compound Is Canonicalized	Yes

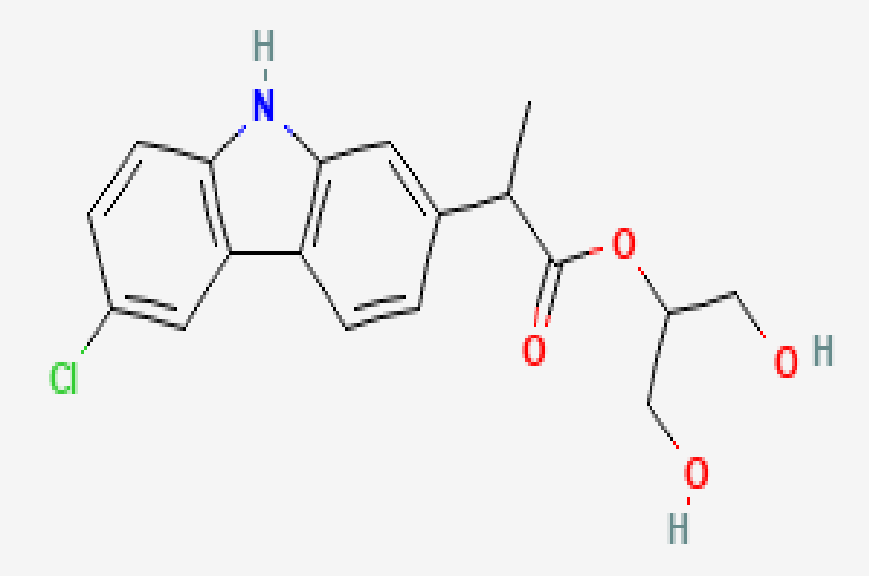

Figure 14 The 2D structure of Ligand number 3 (mseq 94)

It is the third top scoring ligand among 4 selected ligands docked with SARSCoV-2 Mpro.It has S value of -6.8877 with Mpro while control ligand has S value of -7.9932.To further explain the preferential binding affinity of ligand with Mpro,the interactions of the compound were analyzed using 2D visualizer which are discussed below;

Interaction analysis of the ligand (mseq 94) with the Mpro

The analysis of the compound interactions with SARSCoV-2 Mpro revealed that it forms a total of 24 interactions with aminoacid residues at its binding site. It is connected to aminoacids Ser144 with two conventional hydrogen bonds, Met165 with one pi-sulfur bond, one carbon hydrogen bond and also undergoes pi-alkyl interactions.Leu141 forms two carbon hydrogen bonds, Gly143,Asn142 forms one conventional hydrogen bond.

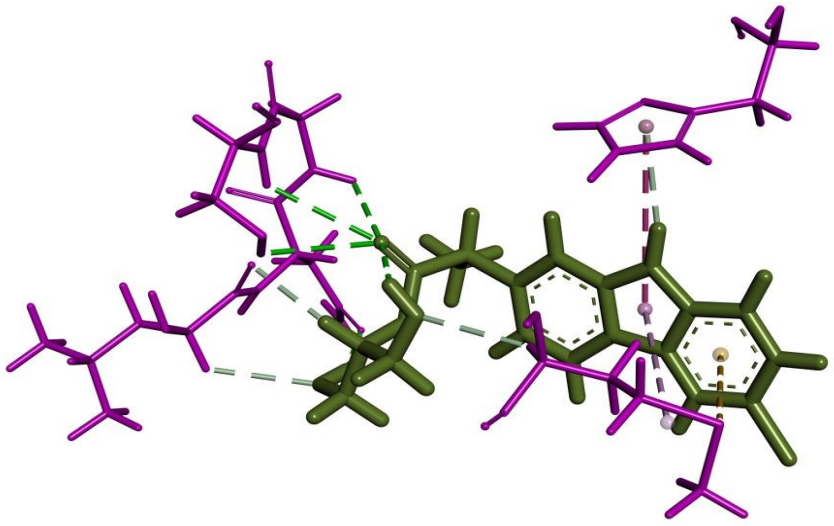

Figure 15 The 3D diagram of ligand (mseq 94) binded at the active site of Mpro by forming different interactions with its aminoacid residues. The ligand molecule is shown in green color sticks and the protein molecule is shown in purple color sticks.

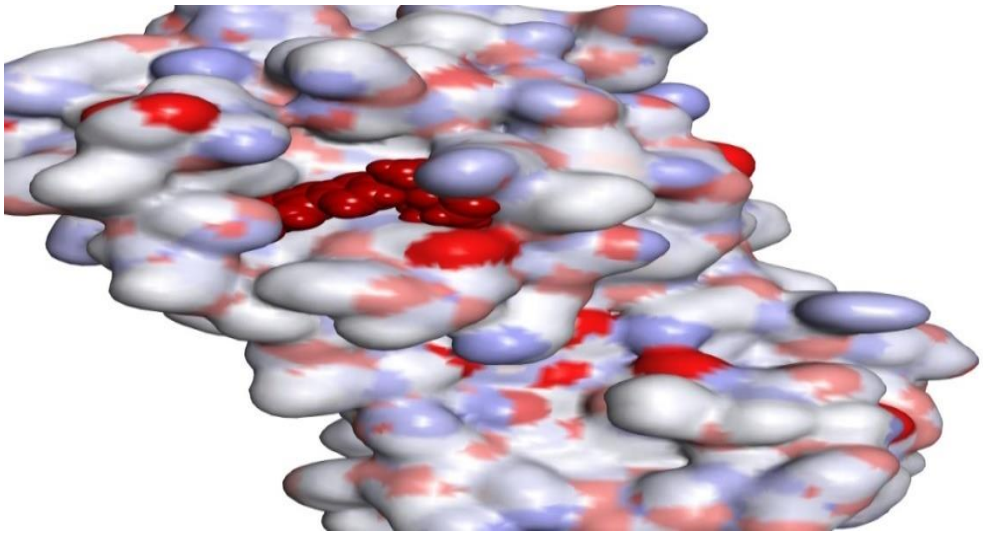

Figure 16 The CPK model of the ligand molecule (mseq 94) properly binded at the active site of Mpro. The ligand molecule is shown in red color balls.

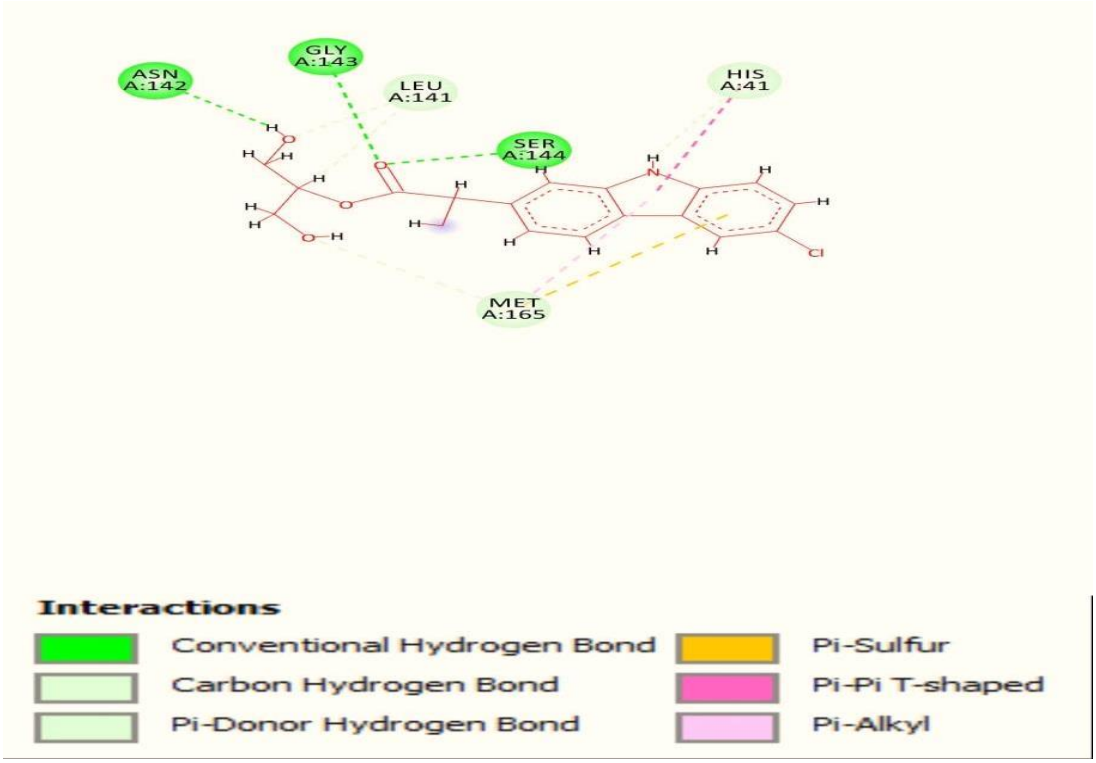

Figure 17 The 2D diagram of ligand (mseq 94) interactions with protein residues of SARSCoV-2 Mpro.Each type of interactions is shown with a different color of splitted lines.

Table 3.8 List of all interactions formed between ligand 3 (mseq 94) and Mpro

Sr. No	Residue Name	Distance	Category	Types
1	A:GLY143:HN - :*0:O	2.84595	Hydrogen Bond	Conventional Hydrogen Bond
2	A:SER144:HN - :*0:O	3.08894	Hydrogen Bond	Conventional Hydrogen Bond
3	A:SER144:HG - :*0:O	3.05657	Hydrogen Bond	Conventional Hydrogen Bond
4	A:FJC405:H23 - :*0:O	2.12187	Hydrogen Bond	Conventional Hydrogen Bond
5	A:FJC0:H - :*0:O	2.24839	Hydrogen Bond	Conventional Hydrogen Bond
6	:*0:H - A:FJC0:O	2.46847	Hydrogen Bond	Conventional Hydrogen Bond
7	:*0:H - :*0:O	2.4496	Hydrogen Bond	Conventional Hydrogen Bond
8	:*0:H - A:ASN142:OD1	2.07584	Hydrogen Bond	Conventional Hydrogen Bond
9	A:LEU141:HA - :*0:O	2.80294	Hydrogen Bond	Carbon Hydrogen Bond
10	A:MET165:HA - :*0:O	2.82085	Hydrogen Bond	Carbon Hydrogen Bond
11	:*0:H - A:LEU141:O	2.49646	Hydrogen Bond	Carbon Hydrogen Bond
12	:*0:H - A:FJC0:O	2.59159	Hydrogen Bond	Carbon Hydrogen Bond
13	:*0:H - :*0:O	2.7914	Hydrogen Bond	Carbon Hydrogen Bond
14	:*0:H - A:FJC405:O01	2.82729	Hydrogen Bond	Carbon Hydrogen Bond
15	:*0:H - A:HIS41	2.64332	Hydrogen Bond	Pi-Donor Hydrogen Bond
16	A:MET165:SD - :*0	4.37808	Other	Pi-Sulfur
17	A:HIS41 - :*0	4.80969	Hydrophobic	Pi-Pi T-shaped
18	:*0:C - A:FJC405	4.15687	Hydrophobic	Alkyl
19	:*0 - :*0:C	4.77672	Hydrophobic	Pi-Alkyl
20	:*0 - A:MET165	4.9686	Hydrophobic	Pi-Alkyl
21	:*0 - A:FJC405	5.08085	Hydrophobic	Pi-Alkyl
22	:*0 - :*0:C	4.42901	Hydrophobic	Pi-Alkyl
23	:*0 - :*0:C	4.20923	Hydrophobic	Pi-Alkyl
24	:*0 - :*0:C	3.65001	Hydrophobic	Pi-Alkyl

LIGAND NUMBER 4 (MSEQ 45)

Ligand number four is one of the derivatives of carprofen whose IUPAC name is 2-[6-chloro-1-[1-diethylamino) ethyl]-9H-carbazol-2-yl] acetic acid. Its molecular weight is 358.9g/mol.There are two hydrogen bond donors and three hydrogen bond acceptors in this molecule.The details of all its physical properties are given below in the table;

Table 4 The list of computed physical properties of ligand number 4 (mseq 45)

Sr.No	Property Name	Property Value
1	Molecular Mass	358.9
2	XLogP3-AA	2.2
3	Donor H-bonds	2
4	Acceptor H-bonds	3
5	Rotatable bonds	6
6	Exact weight	358.1448057
7	Monoisotopic Mass	358.1448057
8	Polar Surface Area	56.3 Å²
9	Heavy Atom	25
10	Formal Charge	0
11	Complexity	471
12	Isotope Atom	0
13	Defined Atom	0
14	Undefined Atom	1
15	Defined Bond	0
16	Undefined Bond	0
17	Covalently-Bonded Unit	1
18	Compound Is Canonicalized	Yes

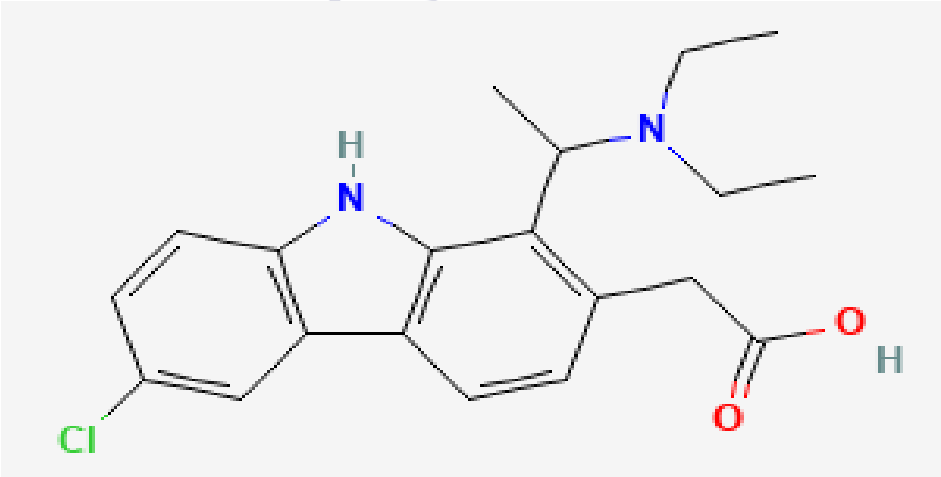

Figure 21 The 2D structure of Ligand number 4 (mseq 45)

It is the top fourth best scoring ligand among selected ligands docked with Mpro.It has S value of -6.6768 with main protease while control ligand has S value of -7.9932.To further explain the preferential binding affinity of ligand with SARSCoV-2 Mpro,the interactions of the compound were analyzed using 2D visualizer which are discussed below;

Interaction analysis of ligand number 4 (mseq 45) with SARSCov-2 Mpro

The detail analysis of mseq 25 interactions shown that it forms total number of interactions with the different aminoacid residues of Mpro. Among them, Gln192 form one conventional hydrogen bond,His141 forms one carbon hydrogen bond,Glu166 forms one donor hydrogen bond, Met165 and Cys145 undergoes pi-alkyl interactions. Besides, there occurs several other non-bonded interactions.

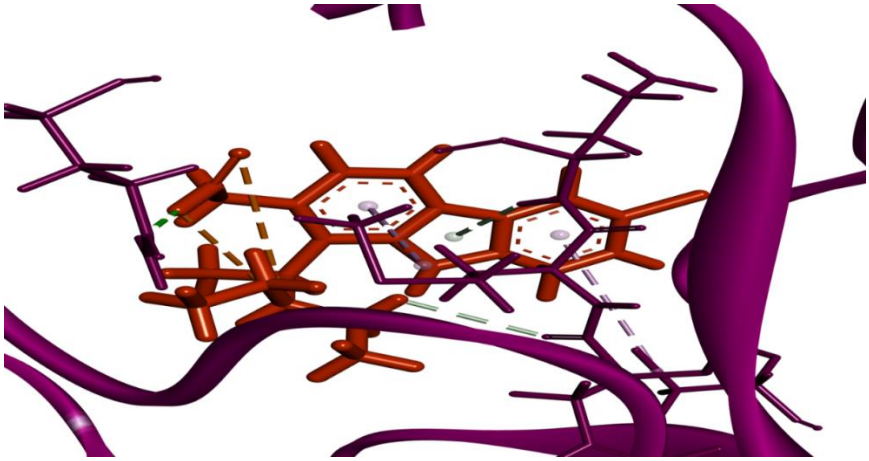

Figure 22 The 3D diagram of ligand molecule (mseq 45) binded at the active site of Mpro, forming different types of interactions with the main protease.The ligand molecule is shown in orange color sticks and protein molecule is shown in purple color sticks.

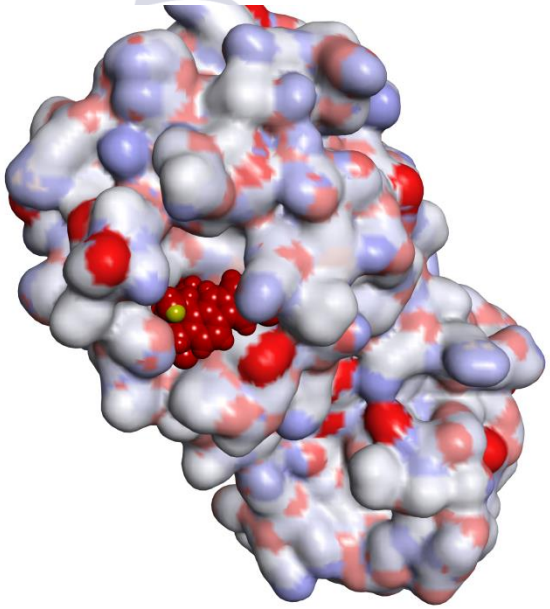

Figure 23 The CPK model of ligand molecule (mseq 45) properly binded at the active site of Mpro. The ligand molecule is shown in red color balls.

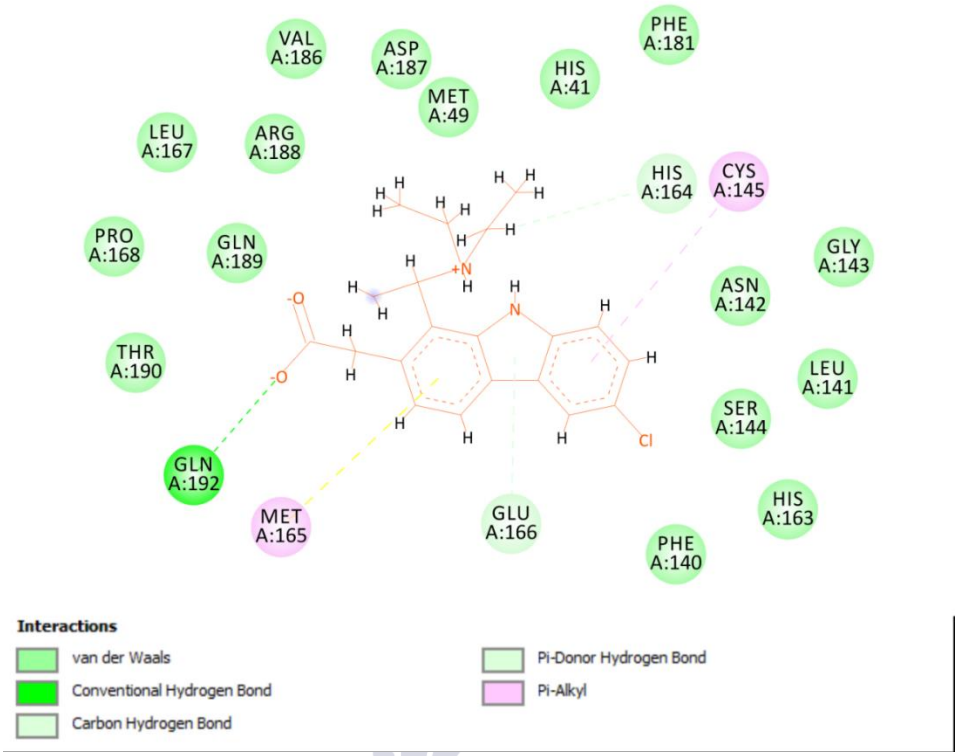

Figure 24 The 2D diagram of ligand (mseq 45) interactions with different protein residues of SARSCoV-2 Mpro.Each type of interactions is shown with a different color of splitted lines.

Table 11 List of all interactions formed between ligand (mseq 45) and Mpro

Sr.No	Residue Name	Distance	Category	Types
1	:*0:N - :*0:O	5.53556	Electrostatic	Attractive Charge
2	:*0:N - :*0:O	4.34057	Electrostatic	Attractive Charge
3	A:GLN192:HE21 - :*0:O	2.4986	Hydrogen Bond	Conventional Hydrogen Bond
4	:*0:H - A:HIS164:O	2.99703	Hydrogen Bond	Carbon Hydrogen Bond
5	:*0:O - A:FJC405	2.76827	Electrostatic	Pi-Anion
6	:*0:O - A:FJC405	3.00275	Electrostatic	Pi-Anion
7	:*0:O - A:FJC405	4.42917	Electrostatic	Pi-Anion
8	:*0:O - A:FJC405	4.1569	Electrostatic	Pi-Anion
9	A:GLU166:HN - :*0	3.08583	Hydrogen Bond	Pi-Donor Hydrogen Bond
10	A:FJC405 - :*0	4.68229	Hydrophobic	Pi-Pi T-shaped
11	:*0 - A:FJC405	4.14749	Hydrophobic	Pi-Alkyl
12	:*0 - A:FJC0	4.6727	Hydrophobic	Pi-Alkyl
13	:*0 - :*0:C	3.59762	Hydrophobic	Pi-Alkyl
14	:*0 - :*0:C	4.20923	Hydrophobic	Pi-Alkyl
15	:*0 - A:MET165	5.30791	Hydrophobic	Pi-Alkyl
16	:*0 - :*0:C	5.31329	Hydrophobic	Pi-Alkyl
17	:*0 - A:CYS145	5.34356	Hydrophobic	Pi-Alkyl
18	:*0 - :*0:C	3.65001	Hydrophobic	Pi-Alkyl

Discussion.

The rapid global spread of Coronavirus Disease 2019 (COVID-19) has intensified the search for effective antiviral agents targeting essential proteins involved in the replication cycle of SARS-CoV-2. Among the viral proteins, the main protease (Mpro), also known as 3-chymotrypsin-like protease (3CLpro), plays a pivotal role in viral replication by cleaving viral polyproteins into functional non-structural proteins required for viral transcription and replication (Anand et al., 2003; Zhang et al., 2020). Due to the absence of closely related homologs in humans and its essential role in the viral life cycle, Mpro has emerged as an attractive target for antiviral drug development. In recent years, computational approaches such as molecular docking and virtual screening have been extensively used to identify potential inhibitors of SARS-CoV-2 Mpro, enabling the rapid screening of large chemical libraries for promising therapeutic candidates (Ton et al., 2020; Beck et al., 2020).

In the present study, molecular docking analysis was performed to evaluate the inhibitory potential of carprofen analogues against SARS-CoV-2 main protease (Mpro). A total of 272 similar structures of carprofen were retrieved from the PubChem database and screened through molecular docking against the active site of Mpro. The docking analysis revealed several ligands exhibiting favorable binding affinity toward the catalytic pocket of the enzyme. Among these compounds, three ligands with mseq numbers 95, 94, and 45 demonstrated the highest binding affinities with docking scores of –6.9380, –6.8877, and –6.6768, respectively. These values were slightly lower than the control ligand (11b), which showed a docking score of –7.9932, but the selected ligands demonstrated strong interactions with key residues located within the catalytic pocket of the enzyme.

Detailed interaction analysis indicated that the selected ligands form multiple interactions with

amino acid residues located at the active site of Mpro, including His41, Cys145, Met165, Glu166, Asn142, and Phe140. These residues are well known to participate in substrate binding and catalytic activity of the protease. Previous structural studies have demonstrated that the catalytic dyad composed of His41 and Cys145 plays a crucial role in the proteolytic mechanism of SARS-CoV-2 Mpro (Zhang et al., 2020). In the current study, ligand **mseq 95** exhibited extensive interactions with residues such as Glu166, Met165, Cys145, His41, and Phe140, suggesting that this compound may effectively occupy the substrate binding pocket and potentially inhibit enzymatic activity. The presence of multiple interaction types including conventional hydrogen bonds, carbon hydrogen bonds, π-alkyl interactions, and π-sulfur bonds further contributes to the stability of the ligand–protein complex.

Similarly, ligand mseq 94 also demonstrated strong binding interactions with several important residues such as Ser144, Met165, Leu141, Gly143, and Asn142, indicating that this compound may also effectively bind within the catalytic pocket of the protease. The formation of hydrogen bonds with residues located near the catalytic region enhances the stability of the complex and may contribute to stronger inhibitory activity. These observations are consistent with previous docking studies that reported strong interactions between potential inhibitors and residues located within the substrate binding site of Mpro (Ahmad et al., 2020).

The third promising ligand identified in this study, mseq 45, also showed significant interactions with residues including Gln192, Glu166, His41, Met165, and Cys145. Although its docking score was slightly lower compared with the other two ligands, the presence of interactions with critical residues within the catalytic pocket suggests that it may also act as a potential inhibitor of SARS-CoV-2 Mpro. Previous computational investigations have highlighted the importance of residues such as **Glu166 and Met165** in stabilizing inhibitor binding within the active site of the protease (Zhang et al., 2020).

The findings of the present study are consistent with previous research demonstrating that small-molecule inhibitors targeting SARS-CoV-2 Mpro can effectively block viral replication. For example, Ton et al. (2020) performed large-scale virtual screening of billions of compounds and identified several molecules capable of binding strongly to the catalytic pocket of the protease. Similarly, Beck et al. (2020) applied deep learning-based drug–target interaction models to identify potential antiviral drugs targeting SARS-CoV-2 proteins. These studies highlight the growing importance of computational approaches in accelerating antiviral drug discovery.

Furthermore, recent studies have suggested that non-steroidal anti-inflammatory drugs (NSAIDs) and their derivatives may possess antiviral properties due to their ability to interact with viral enzymes and host inflammatory pathways. Carprofen, a widely used NSAID, has structural features that enable it to interact with hydrophobic pockets within protein active sites. The identification of carprofen analogues with strong binding affinity toward SARS-CoV-2 Mpro in this study therefore supports the potential repurposing of such compounds for antiviral drug development.

Another important observation in this study is the large number of intermolecular interactions formed between the selected ligands and the protease enzyme. These interactions contribute significantly to the stability of the ligand–protein complex and may enhance inhibitory potential. Molecular docking studies often emphasize the importance of hydrogen bonding networks and hydrophobic interactions in stabilizing inhibitor binding within enzyme active sites. In the present work, the presence of multiple hydrogen bonds and hydrophobic interactions suggests that the selected ligands may effectively occupy the substrate binding pocket and disrupt enzymatic activity.

Despite the promising results obtained from molecular docking, it is important to note that computational predictions alone are not sufficient to confirm biological activity. Docking

studies primarily provide theoretical insights into binding affinity and molecular interactions between ligands and target proteins. Therefore, the inhibitory activity of the identified compounds must be validated through **in** vitro enzymatic assays and in vivo experimental studies. Such experimental validation is essential to determine the pharmacological properties, safety profile, and therapeutic potential of these compounds.

Overall, the present study highlights the potential of carprofen analogues as promising inhibitors of SARS-CoV-2 main protease. The molecular docking analysis identified several compounds capable of forming stable interactions with key catalytic residues within the active site of the enzyme. Among the screened compounds, ligands mseq 95, 94, and 45 demonstrated the most favorable binding interactions and may serve as promising lead molecules for further drug development. Future studies involving molecular dynamics simulations, in vitro enzymatic assays, and in vivo studies are required to further validate the antiviral potential of these compounds and facilitate the development of effective therapeutic agents against

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