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Research Article

Molecular Docking and Drug Likeness Prediction of New Potent Sars Cov-2 Main Protease Inhibitors

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ABSTRACT

The novel corona virus whose outbreak took place in December 2019 continues to spread at a rapid rate worldwide. The Main protease (M^{pro}) plays critical role in the SARS-CoV-2 life cycle through virus replication and transcription process making it as an attractive drug target. Herein, molecular docking study followed by drug-Likeness prediction, were performed in order to identify new potent M^{pro} inhibitors. Indeed, molecular docking of 1880 compounds into the M^{pro} active site reveals compounds S1 and S2 as promising inhibitors of this enzyme with binding energy of -39,22 KJ/mol, -36.27 KJ/mol respectively. These two compounds were also predicted to have satisfying drug likeness properties, indicating that they might be promising lead compounds for further anti-SARS CoV-2 drug research.

INTRODUCTION

The corona virus disease 2019 (COVID-19) pandemic has left a mark in all countries, with more than 552 million cases worldwide and over 6.27 million deaths as of 15 Mai 2022 [1]. The clinical symptoms of COVID -19 often overlap and can affect any system in the body [2]. They include fever, sore throat dry cough, headache, pneumonia with potentially progressive respiratory failure owing to alveolar damage, and even death [3, 4].

Currently, no specific targets are available for severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). However, a number of proteins are considered essential to the SARS CoV-2 lifecycle and therefore provide a significant number of targets for inhibiting viral host entry and replication [5]. Indeed, during viral replication, the SARS CoV-2 Main protease (M^{pro}) plays crucial role in the viral life cycle through virus replication and transcription process. Hence, this enzyme presents attractive targets for small molecule inhibitors since no human proteases with a similar specified cleavage are characterized [6].

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Thus, the identification of new potent M^{pro} inhibitors with improved pharmacokinetic properties remains important.

Herein, molecular docking approach followed by visual inspection and drug likeness prediction were performed in order to identify new potent SARS CoV-2 M^{pro} inhibitors, which could help in progressive attempts in the therapeutics of COVID-19

MATERIALS AND METHODS

Protein Preparation

The crystal structure of SARS-CoV-2 main protease (M^{pro}) in complex with **S-[5-(TRIFLUOROMETHYL)-4H-1,2,4-TRIAZOL-3-YL] 5-(PHENYLETHYNYL)FURAN-2-CARBOTHIOATE (F3F); a potent inhibitor, was retrieved** from the Protein Data Bank (PDB ID: **2GZ8**) [7]. The structure of the enzyme was prepared for docking, minimized and refined using the Protein Preparation Wizard implemented in Schrödinger suite [8]. This preparation was undertaken to eliminate crystallographic waters, to add missing hydrogen and chain atoms, and to assigne the appropriate charge and protonation state for amino acid residues at pH 7.0±2. The enzyme structure was subjected to an energy minimization using the

OPLS-2005 force-field [9]. The co-crystal inhibitor (**F3E**) was used to identify the active site of M^{pro} by selecting all amino-acids residues within a radius of 6.5 Å. This selection was refined by adding every residue beyond 6.5 Å considered as essential for the continuity of the protein cavity [10].

Ligand Preparation

A chemical library contained 1405 analogs compounds to F3F were retrieved from PubChem database in 3D sdf format. These compounds were prepared for docking using LigPrep module of Schrödinger suite [8] which undertakes hydrogen atom addition, amending realistic bond lengths and angles and generation for each compound a number of structures with various enantiomers (when undefined), protonation states at pH 7.4±1 and tautomers. Partial charges were assigned to the structures using the OPLS-2005 force-field [11]. The final chemical library consisted of 1880 molecules in sdf format was used for docking calculations.

Molecular Docking Calculations

Molecular docking calculations of 1880 compounds were undertaken on M^{pro} binding site using FlexX which was based on an incremental construction of ligands. Docking calculations were done with the default parameters. FlexX scoring function, which gave scores as binding energy in kJ/mol, was used for molecule ranking [12]. FlexX requested to retain 10 poses per molecule although the ranking of a molecule was solely based on its top-ranked pose.

Visual Inspection

The resulting top-ranked 100 compounds from docking calculation were analyzed by visual inspection in order to eliminate false positive ones. In this context, three types of interactions were considered: hydrogen bonds, π-π stacking and hydrophobic interactions. The retained molecules had to be well buried into the M^{pro} cavity. They also had to present a good protein-ligand complementarity and an optimized number of hydrogen bonds and π-π stacking when possible, especially with the dyad catalytic residues (His41 and Cys145) [13,14].

Validation of Docking Protocol

The Root Mean Square Deviation (RMSD) test represents the ability of a docking program to reproduce the experimental binding modes of a ligand. It is a metric, which measures average distances between the docking binding mode and the experimental position of a ligand. The prediction is acceptable when the RMSD is less than or equal to 2 Å beyond which the prediction is considered irrelevant. In our work, the performance of the docking program FlexX was evaluated by calculating RMSD values of 100 protein-ligand crystal structures from the Protein Data Bank (PDB) [15].

ADMET Prediction

The top ranked hits were further filtered by the prediction of their physic-chemical, pharmacokinetics and toxicity properties using ADMET lab version 2.0 (https://admetmesh.scbdd.com). These properties consist of Lipinski and Veber's Rule, Blood-Brain Barrier permeability (BBB), Gastro-Intestinal absorption (GI), Cytochrome P450 (CYP) inhibition, and toxicity (Ames test, hERG inhibition and carcinogenicity). The same parameters of **F3F** were also predicted for comparison.

RESULTS AND DISCUSSIONS

Validation of Docking Protocol

The performance of docking program (FlexX) was evaluated by calculating RMSD values of 100 protein-ligand complexes from the PDB. The predicted binding mode was considered correct when the RMSD was below 2.0 Å. As shown in Table 1, 70% of the RMSD values are less than or equal to 2 Å, thus indicating that the used docking protocol reproduce correctly the experimental conformation of a ligand in its binding site [16]. In the most cases, there was a negligible deviation between the experimental and the docked conformation as shown in Fig. 1 for M^{pro} inhibitor (PDB ID: **2GZ8**).

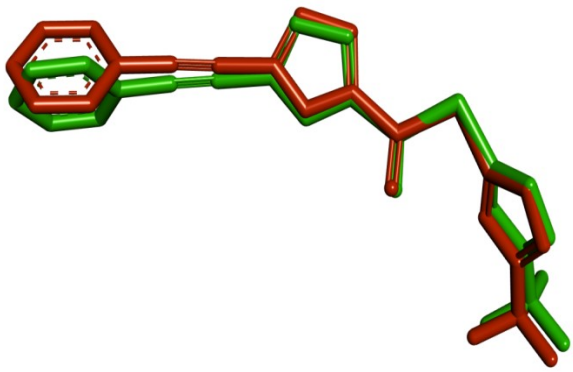


Figure 1: Superposition of **F3F** given by X-ray crystallography (colored in green) and by molecular docking using FlexX (colored in red).

Table 1: List of 100 complexes Protein-Ligand used in RMSD test.

Protéine	Ligand	RMSD (Å)	Protéine	Ligand	RMSD (Å)	Protéine	Ligand	RMSD (Å)
1N7I	SAH	1,73	5I3A	HQE	0,36	2VJ8	HA2	1,51
1AH3	NAP	1,52	5TFT	HEM	1,27	3CCC	7AC	0,76
1AIM	ZYA	1,18	5URS	8LA	9,78	3I28	34N	1,29
1EB2	BPO	1,5	5ZAN	9A6	6,05	3K5F	AYH	1,56
1EKO	184	4,88	6FH5	DD8	0,97	3KOO	24D	1,8
1K1M	FD4	4,41	6MDA	JED	0,59	3LJT	LA3	1,92
1P9S	DIO	4,47	6MDB	JE4	1,49	3OF8	10Y	4,95
1RTI	HEF	1,96	6MDC	JEA	1,97	3RZ3	U94	2,43
1YKR	628	0,74	6MDD	JE7	0,67	3TPP	5HA	1,45
1YZ3	SAH	1,1	6O9X	M0S	5,72	4CGA	QLW	2,42
1ZVX	FIN	1,87	6OA3	M0M	3,7	4EY7	E20	1,78
2G5P	3GP	3,41	6OHS	MJY	1,62	4G9C	0WP	1,49
2G71	SAH	1,74	1OI9	N20	0,6	4KZO	NAP	10,02
2JBJ	G88	5,3	2OPB	SAH	1,32	4LXM	1YU	10,04
2ONZ	TMJ	0,4	2QJR	TB	0,84	4MO8	2VQ	1,94
2R4B	GW7	1,3	3VVG	ZGB	1,99	4OGN	2U5	1,76
2RJP	886	1,48	1C84	761	1,14	4ONC	FMT	2,55
2V35	J54	7,24	1H39	R03	1,73	4ZZ2	3YG	1,12
2XBU	5GP	2,39	1LI9	PO4	1,57	5C28	4XV	2,51
2ZJF	BSU	0,48	1KIM	THM	0,92	5CLU	S8A	1,5
2EW8	SO4	9,06	1N8Q	DHB	1,96	5D0R	B1T	1,18
3N9S	TD4	1,19	1OGQ	NAG	3,75	5EEC	ZXN	1,92
3QQK	HEM	1,21	1PNN	984	1,45	5HVT	NVS	1,15
3QTQ	X35	0,67	1YW8	A75	2,8	5IWC	6EQ	2,31
3VP2	BP0	1,84	2AN5	SAH	1,18	5J9Y	6HL	1,95
3WYM	3K9	1,52	2ANQ	NDP	2,74	5SZ7	72H	1,92
4CDL	LLK	4,22	2BU5	TF1	3,17	6AAH	9T6	1,78
4IU6	FZ1	6,34	2CL5	SAM	1,95	6DND	PLP	0,77
4MIK	JIL	0,97	2F6V	SK2	1,45	6OA3	M0M	3,61
4MQ4	2D5	2,45	2OGZ	U1N	1,97	6Q0Z	P7V	1,91
4NCM	704	1,11	2QDH	M2P	1,76	7TLN	INC	1,97
4YTF	4HZ	5,04	2R3N	SCX	1,88	2V11	C80	1,33
5AFW	EDO	5,58	2RF6	SO4	1,05	2GZ8	F3F	1,70
5FI2	5XX	5,41						

Table 2: PubChem ID and Binding energy of **S1, S2** and **F3F**.

Compound	PubChem ID	Binding energy (Kj/mol)
S1	1627998	-39,22
S2	1892742	-36.27
F3F	2822496	-15.96

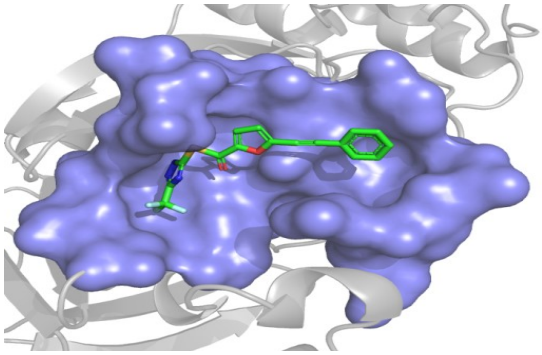
Docking Calculations and Visual Inspection

The co-crystal ligand **F3F** complexed with M^{pro} (PDB ID: **2GZ8**) was chosen as starting structure to search for similar compounds from PubChem. In order to identify new potent M^{pro} inhibitors, 1880 analog compounds to this ligand were prepared and docked into the active site using FlexX. The resulting top-raked 100 compounds were further analyzed by visual inspection in order to eliminate false positive ones which may have high docking score but present a bad surface complementarity or haven't a rational number of interactions with the studied binding sites. Out of these, compounds **S1** and **S2** showed a higher M^{pro} inhibitory potency than that of **F3F**, the reference molecule, whose binding energy is -15.96 KJ/mol. Still more remarkably, these two compounds showed highest negative binding

energy of -39.22 KJ/mol and -36.27 KJ/mol respectively, thus indicating their important inhibitory potency against the enzyme (Table 2).

Poses Analysis

The binding mode of the most promising inhibitors **S1** and **S2** into the M^{pro} binding site was predicted using the poses given by FlexX. As shown in Fig. 2, these two molecules cover the entire M^{pro} binding cavity as in the case of **F3F**, thus leading to a high inhibitory patency.



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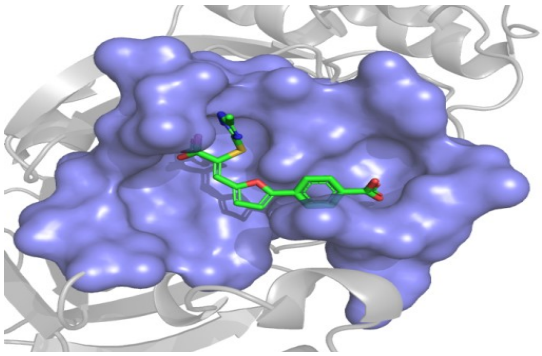

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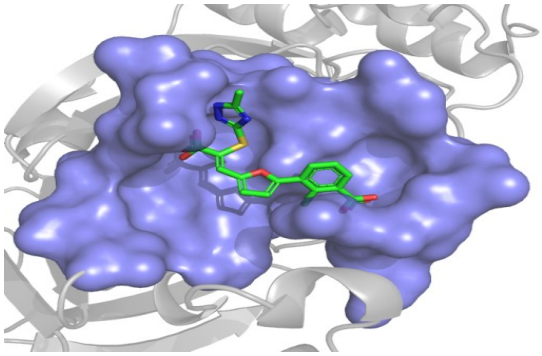


(c)

Figure 2: Positioning of F3F **(a)** S1 **(b)** and S2 **(c)** into the M^{pro} active site. The most plausible pose of each compound is presented as obtained by docking with FlexX. The binding site cavity is represented in blue. The ligand atoms are color-coded as follows: carbon in green, oxygen in red and nitrogen in blue. The images were drawn using PyMol.

The difference of the inhibitory potency between these two promising compounds and **F3F** may be explained by the different number of hydrogen bonds between them and the protein. Indeed, whereas **S1** and **S2** are involved in nine and seven such bonds respectively, **F3F** is involved in only four. In addition, **S1** and **S2** interact with Cys145 in contrary to **F3F** which has a bare contact with this residue. It should be noted that Cys145 was described to play an important role in M^{pro} activity because it is one of the catalytic dyad residues in the active site [14].

Drug Likeness Prediction

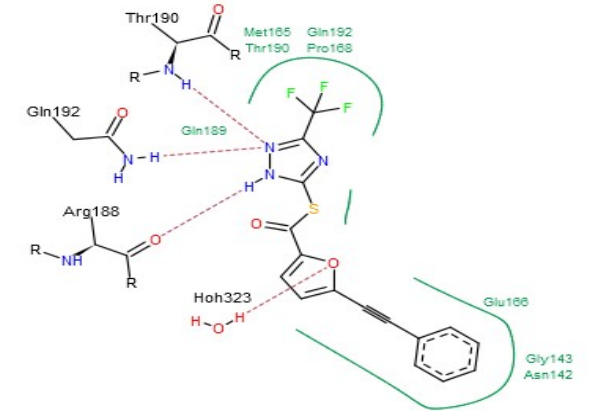
Physicochemical, pharmacokinetic and toxicity parameters of **S1**, **S2** and **F3F** were predicted using pkCSM at http://biosig.unimelb.edu.au/pkcsm/. As shown in Table 3, **S1** and **S2** had low BBB penetration, which might protect the central nervous system from their potential side effects. They also showed a high gastrointestinal absorption and water solubility, which ensure their further *in vivo* usage.

Table 3: The predicted physicochemical, pharmacokinetic and toxicity parameters of **F3F**, **S1** and **S2**.

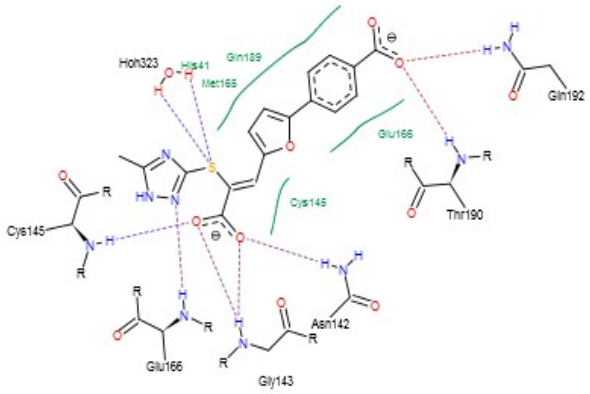
Properties	F3F	S1	S2
Molecular weight	319.87	401.50	360.79
Rotatable bonds	8	8	5
H-bond acceptors	2	5	5
H-bond donors	1	2	1
Log P	4.15	4.10	2.80
TPSA Å^2	28.16	142.61	120.14
Lipinski's rule of 5	Suitable	Suitable	Suitable
Veber's rule	Suitable	Suitable	Suitable
Water solubility	Soluble	Soluble	Soluble
GI[a] absorption	High	High	High
BBB[b] perméabilité	High	Low	Low
CYP[c] 1A2 inhibition	No	No	No
CYP[c] 2C19 inhibition	No	No	No
CYP[c] 2C9 inhibition	No	No	No
CYP[c] 2D6 inhibition	No	No	No
CYP[c] 3A4 inhibition	No	No	No
AMES toxicity	Low	Low	Low
hERG[d] inhibition	Low	Low	Low
Carcinogenicity	Low	Low	Low

[a] GI: Gastro-Intestinal, [b] BBB: Blood-Brain Barrier, [c] CYP: Cytochrome P450, [d] hERG: human ether-ago- go related gene.

Furthermore, they were not found to inhibit CYP (enzymes that should not be inhibited because of their essential role for the metabolism of many drugs in the liver). With no Veber and Lipinski's rule violation, both **S1** and **S2** follow the criteria for orally available drugs. Still more remarkably, they were predicted to be nontoxic according to their negative results for AMES test, hERG inhibition and carcinogenicity.



(a)



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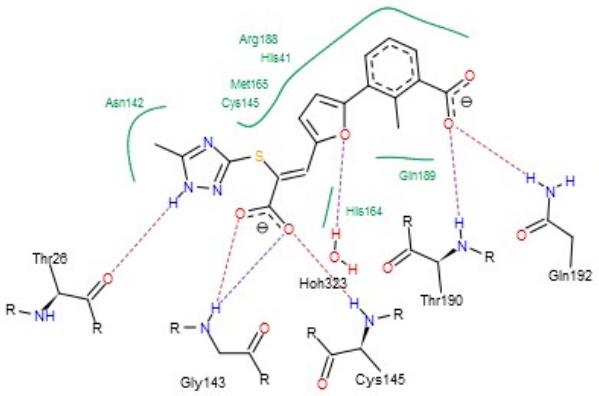

(c)

Figure 3: Binding mode prediction of **F3F** (**a**) **S1** (**b**) and **S2** (**c**) into the M^{pro} active pocket. Purple doted lines represent hydrogen bonds. The images were done with the Ligand Interaction Diagram from LeadIt.

CONCLUSION

In summary, molecular docking approach was used in order to identify new potent SARS CoV-2 M^{pro} inhibitors. After the validation of docking protocol using RMSD test, compounds **S1** and **S2** were reviled as new inhibitors of this enzyme with binding energy of -39,22 KJ/mol and -36.27 KJ/mol respectively. The binding mode analysis showed that these promising hits cover the entire M^{pro} binding site in a rational orientation, where their hydrogen bond with Cys145 seem to play an important role, leading to their high inhibitory potency. Still more remarkably, **S1** and **S2** were predicted to have good drug likeness and toxicity profile indicating that they might be promising lead compounds for further anti SARS CoV-2 drug discovery.

ACKNOWLEDGEMENTS

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HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ABBREVIATIONS

BBB: Blood-Brain Barrier
COVID-19: Coronavirus disease 2019
CYP: Cytochrome P450
GI: Gastro-Intestinal
hERG: human ether-ago- go related gene
M^{pro}: Main protease
RMSD: Root Mean Square Deviation
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

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