In Silico Research of New Potent SARS Cov-2 Main Protease Inhibitors

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Abstract

The covid-19 pandemic caused by SARS-CoV-2 virus has caused more than 5.3 million deaths worldwide since the beginning of 2020. Although there is no specific drug against SARS-CoV-2 until now, studies are daily progressing to find new drugs to eradicate this virus.

In this study we propose new microbial inhibitors of the main protease which represents a therapeutic target of SARS-CoV-2, by the simulation in silico the affinity of 68 compounds extracted from the literature towards the Mpro by using molecular Docking.

This method consists in predicting the protein-ligand interaction mode by Flexx, it's among the most used programs in this approach. We propose new Mpro inhibitors using Molecular Docking by taking as starting structure the ligand N3 of the base complex 6LU7. The Molecular Docking of the 35 microbial compounds selected with regard to the active site of Mpro highlights compounds Ammonificin C (S1), Pityriacitrin E (S2) and Kaempferol compound (S3) as the best inhibitors of this enzyme with respective interaction energies of -36.16 kJ/mol, -28.38 kJ/mol and -27.01 kJ/mol, which exceed that of reference N3 (-15.30 kJ/mol). The predictive study of the physicochemical and pharmacokinetic properties of the compounds (S1), (S2) and (S3), showed that these compounds have good ADME profiles, and they can be proposes as new potentials inhibitors against the Mpro of SARS-CoV-2.

This study reveals the importance of bioinformatics tools in the rapid development of therapeutic molecules and also highlights the antiviral potentialities of microbial metabolites and their ability to be promising drug candidates.

Keywords: ADME, SARS-CoV-2, molecular docking, FlexX, Mpro

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1. Introduction

Since the global pandemic of COVID-19, many researchers around the world are recruiting and exploiting all efforts for the development of therapeutic molecules against SARS-CoV-2, however, to date and with the exception of rehabilitated drugs, there is no specific drug approved for the treatment of this disease. It is therefore essential to search for new molecules with anti-SARS-CoV-2 activity [1],[2]. In this respect, the inhibition of Main protease (Mpro) seems all the more relevant. Mpro or 3CL pro (Main protease or 3 Chymotrypsin-like-protease) encoded by nsp5 cleaves viral poly-proteins at 11 sites at peptide bonds releasing functional proteins for viral replication [3]. Unfortunately, little work has been devoted to this enzyme, which could nevertheless play a crucial role in the treatment of this disease. This enzyme controls replication and is conserved in all coronaviruses including SARS-Cov-2. This is why the use of new Mpro inhibitors can be a good way to treat this disease by blocking the replication of this dangerous virus [4]. The first crystal structure of SARS-CoV-2 Mpro was determined by X-ray diffraction at 2.16 Å resolution, and was deposited at the Protein Data Bank (PDB) by [5], and published on February 5, 2020, under PDB ID 6LU7. Since then, many structures of the protease have been deposited, including the crystallized enzyme with various inhibitors [6]. In our study and in a first place we selected 35 molecules of microbial origin extracted from the literature, to test their mode of interaction towards our target (Mpro). These molecules were selected on the basis of their simple structures and their therapeutic activities against other pathogens, in particular the hepatitis C virus (HCV), whose protease has structural similarities with our target.

In addition, we evaluated the performance of the Molecular Docking program "FlexX" by the RootMean Square Deviation (RMSD) reliability test, and then we performed Molecular Docking calculations on the collection of 35 microbial molecules that can interact with Mpro. The best inhibitors resulting from this Docking have been the subject of a predictive study on their potential physico-chemical and pharmaco-kinetic properties in order to learn about their absorption, distribution, metabolism and excretion profile (ADME).

Materiel and Methods

Assessment of docking quality

The Root Mean Square Deviation (RMSD) test evaluates the ability of a docking program to reproduce the experimental binding modes of a ligand in its protein target. This metric measures average distances between the docking binding mode and the experimental position of a ligand [7]. To perform this test, a ligand is taken out of its X-ray structure and redocked in the binding site using FlexX. Thereafter, the docked binding mode is compared with the experimental conformation, and the RMSD between these two poses is calculated. The prediction is acceptable when the RMSD is less than or equal to 2 Å [8]. In this study, the RMSD test was undertaken on 101 protein-ligand crystal structures from Protein Data Bank (PDB).

2.2. Protein preparation

Molecular docking study was undertaken on the active site of Mpro. For this purpose, the crystal structure of Mpro was retrieved from RCSB Protein Data Bank (PDB ID; 6LU7, resolution =

2.16Å) and prepared for docking using LeadIT 2.1.8 (www.biosolveit.com). First, all hetero atoms, cofactors and water molecules (except those found in the active pocket of Mpro) were removed from the protein structure. Then, all missing atoms were added and formal charges were computed [9]. Thereafter, the active site of Mpro was defined by selecting all residues within a radius of 6.5 Å around the ligand in the crystal structure. This selection was refined by adding every residue beyond 6.5 Å considered as essential for the continuity of the cavity [10]. Finnaly, the protonation state and the side chains orientations of each amino acid from Mpro active site were inspected and the resultant structure was fully minimized [11].

2.3. Ligand preparation

In this study, 35 ligands of microbial origin from different species drawn from the literature were used for virtual screening with Mpro. These molecules were selected based on their therapeutic bio-activities against various diseases, as well as their structural similarities with our target. The 3D structures of the studied compounds were drawn, minimized and exported as mol2 files using Chem3D 16.0 (http://www.cambridgesoft.com).

2.4. Molecular docking calculations

Molecular docking calculations were performed using FlexX 2.1.8 in which the target atoms are fixed and the ligands are flexible [12]. This algorithm is based on an incremental construction of ligands. FlexX scoring function, which gives scores as ΔG in kJ/mol, was used for ranking of molecules. The best docked conformation for each ligand was analyzed using LeadIT 2.1.8 interaction diagram.

2.5. Drug Likeness prediction

The physicochemical and pharmacokinetic properties of the most promising Mpro inhibitors were predicted using Suiss ADME at http://www.swissadme.ch/. These properties consist of Lipinski and Veber's Rule, Water solubility, Synthetic accessibility, Blood Brain Barrier permeability (BBB), Gastro Intestinal absorption (GI) and Cytochrome P450 (CYP) inhibition. The same parameters of N3 compound (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-) co-crystallized from the 6LU7 complex were also predicted for comparison [13].

Results and discussion

1. Reliability test

1.1. RMSD

The reliability assessment by RMSD was performed on 101 complexes (protein-ligand) that we downloaded from the PDB. Figure 1 shows that 70% of the RMSD values obtained following Molecular Docking by FlexX are below 2Å while only 30% are above this threshold value. These results show that this program correctly simulates protein-ligand interactions.

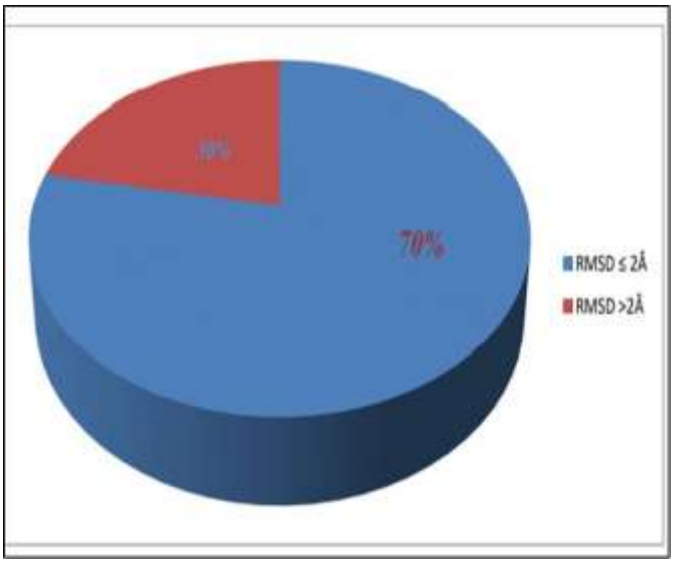


Figure 1: Percentage results of the RMSD test at two intervals

1-2 Visual analysis

Visual analysis is an essential step to confirm the results of the RMSD test and to re-verify the performance of the FlexX program. The visualization of the results through the VMD program allowed us to specify whether the pose of a simulated ligand overlaps with that co-crystallized. In our case, the visual analysis was performed on the Mpro-F3F complex carrying the pdb code 2GZ8. In effect, Figure 2 shows a good superimposition of the pose of the ligand generated by FlexX (colored in green) with respect to that of reference given by X-ray crystallography (colored in red). This further speaks to the reliability of the FlexX program.

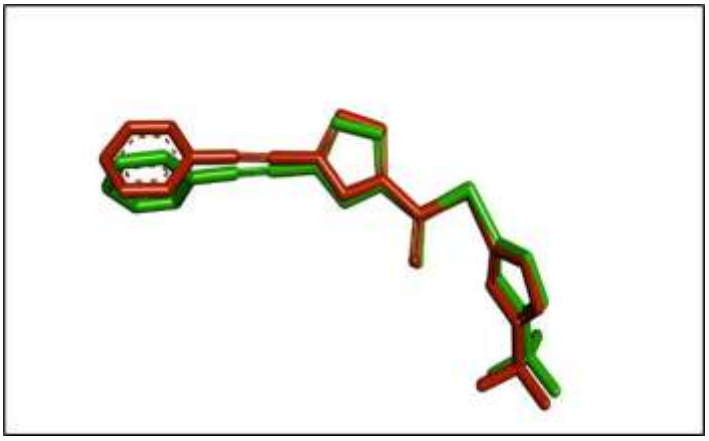


Figure 2: Superposition of the geometry of the F3F ligand of the 2GZ8 complex obtained by X-ray (Colored in red) and that obtained by FlexX (colored in Green).

2. Study of Mpro-ligand interactions

The molecular docking carried out with the 35 microbial compounds towards the Mpro using the FlexX program, allowed us to simulate the intervening interactions in the formation of these complexes and to evaluate their affinities, the latter is calculated and translated by scores whose best score represents the best inhibitor based on interaction energy strength. Among the 35

compounds (Table 1). The three best inhibitors obtained in this work are 1, 2 and 3 with respective Scores of -36.16kJ/mol, -28.38kJ/mol, -27.01kJ/mol.

Tableau 1: The scores of the protein/ligand complexes studied.

Compounds	Compound name	Scores	Microbiol source
1	Ammonificin C	-36,16	*Thermovibrio ammonificans*
2	Pityriacitrin E	-28,38	*Malassezia furfur*
3	Kaempférol	-27,01	*Staphylococcus aureus*
4	Semicochliodinol A	-25,97	*Chrysosporium merdarium*
5	Emodin	-24,88	*Penicillium chrysogenum/Aspergillus ochraceus*
6	Taxifolin	-23,65	*Cercidiphyllum japonicum*
7	Proximicin C	-23,55	*Actinomycete VerrucosisporaMarine*
8	Alarinone	-23,4	*Penicillium chrysogenum*
9	Ω-hydroxyemodin	-23,05	*Penicillium chrysogenum*
10	Eucapsitrione	-22,41	*Cyanobacterium eucapsis*
11	Flavipin	-22,19	*Aspergillus terreus/flavipes*
12	Semicochliodinol	-21,66	*Chrysosporium merdarium*
13	Chrysoginone	-21,5	*Penicillium*
14	Sulochrin	-21,49	*Aspergillus terreus*
15	Chrysophanol	-21,45	*Penicillium islandicum*
16	Coccoquinone A	-21,13	*Staphylotrichum coccosporum*
17	Penimethavone A	-21,05	*Penicillium chrysogenum/Streptomyces sp*
18	Semicochliodinol B	-20,36	*Chrysosporium merdarium*
19	Citriquinochroman	-20,35	*Penicillium citinum*
20	Dihydrocitrinone	-20,14	*Aspergillus terreus*
21	Cordyol C	-19,91	*Aspergillus sp.*
22	Griseoxanthone C	-19,9	*Penicillium patulum*

23	Scequinadoline A	-19,86	*Scedosporium apiospermum/Dichotomomyces cejpii*
24	Holyrine B	-19,36	*Streptococcus mutans*
25	Pyranonigrin A	-19,27	*Aspergillus niger*
26	10-méthoxydihydrofuscin	-18,82	Actinomycètes
27	3A-Hydroxy-3,5-dihydromonacolin L	-18,47	*Aspergillus terreus*
28	2',3'-dihydrosorbicillin	-18,31	*Trichoderma sp*
29	Silybin	-17,93	*Silybum marianum*
30	Piperitol	-17,65	*Oidiodendron griseum*
31	Fuscinarin	-17,18	*Aspergillus sydowii and Penicillium citrinum*
32	Alutenusin	-16,78	*Alternaria*
33	Jbir-90	-16,35	*Streptomyces*
34	Noroquinadolinea	-15,84	*Cladosporium sp.*
35	Purpurester A	-15,74	*Talaromyces purpureogenus*

2-1. Compound 1

The results obtained in this study showed the compound Ammonificin C (1) as the best inhibitor of Mpro with better interaction energy equal to -36.16 kJ/mol. This compound was isolated from the hydrothermal marine bacterium *Thermovibrio ammonificans*, old studies have already approved it as an anticancer molecule [14], [15].

According to the visual analysis carried out by FlexX, this molecule showed perfect stability within the active site of Mpro which was ensured by numerous hydrophobic interactions formed with the residues: Leu167, Thr190, pje5, Glu166, Gln189, Met 165 and Met 49. As well as nine hydrogen bonds, the first two of which are formed between the Glu 166 residue and the inhibitor, and three others with a 166, Gln189, Met 165 and the Met 49 and three others with a pje5 residue. Then the two residues Gln192 and Thr190 bind by two hydrogen bonds in the same place on the inhibitor (Figure 3).

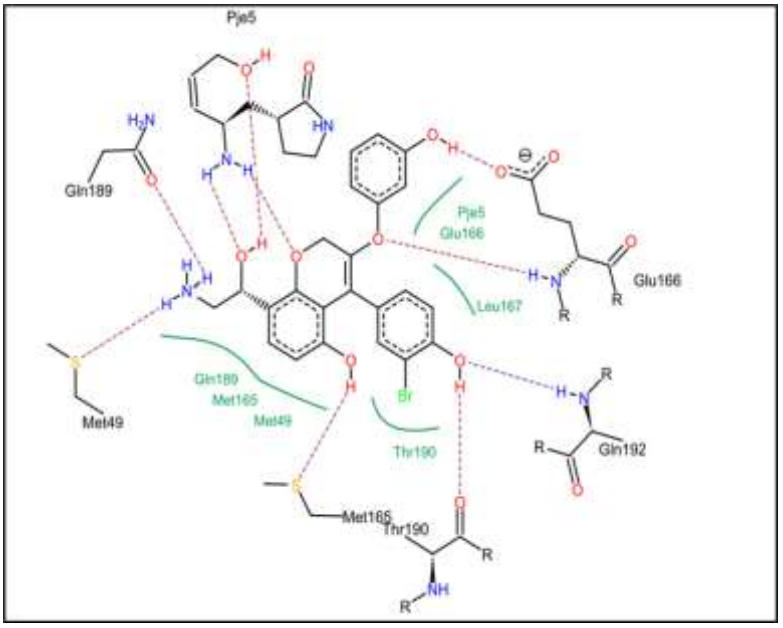
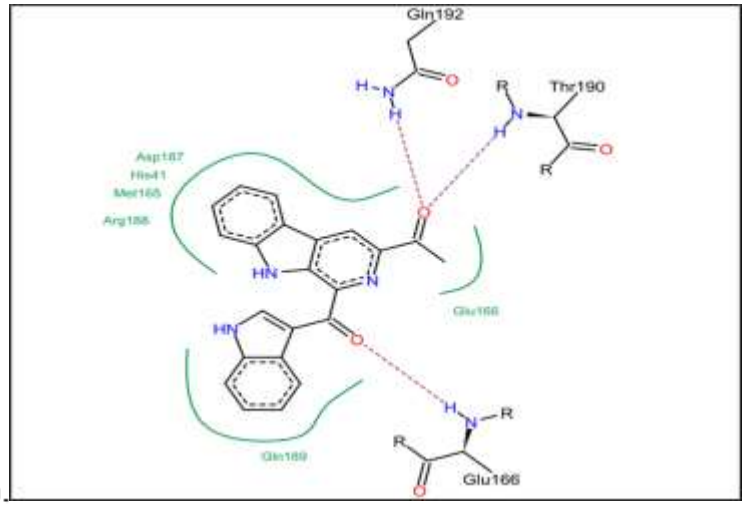


Figure 3: Mode of interaction of compound (1) in the active site of Mpro

2-2. Compound 2

Pityriacitrin E (2) is the second compound which presented a good affinity towards the Mpro of SARS-CoV-2 with an interaction energy of -28.38kJ/mol, this molecule is an alkaloid which has been isolated from the bacterium Malassezia furfur, it has been approved for its antiviral and antifungal activities [15].

Visual analysis of this compound shows that it establishes three hydrogen bonds. The first two are formed between the inhibitor and the amine function of the two residues Gln192, Thr190. The third bond is between the amine function of Glu 166 and the ketone function of the inhibitor. In addition, the Mpro-48 complex is stabilized by numerous hydrophobic interactions formed with residues Asp187, His41, Met165, Arg188,Gln189,Glu166 (Figure



4).

Figure 4: Mode of interaction of compound (2) in the active site of Mpro.

2-3. Compound 3

Kaempferol (3) is also a potent potential Mpro inhibitor extracted from the bacterium Staphylococcus aureus which provided interaction energy equal to -27.01 kJ/mol. This compound inhibits the active site of Mpro by forming six hydrogen bonds. The first two are formed between residue Tyr45 and Asp187, (Figure 5).

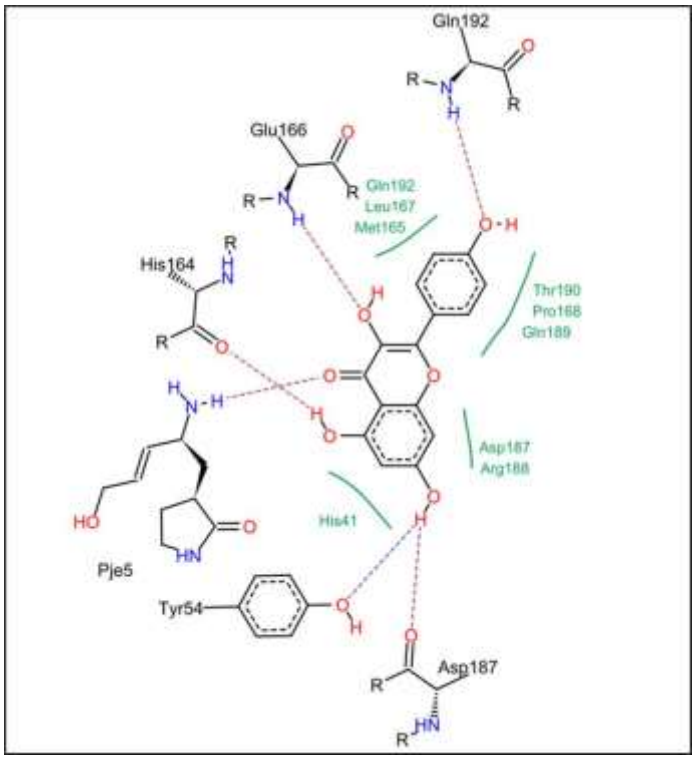


Figure 5: Mode of interaction of compound (3) in the active site of Mpro.

The results obtained by the FexX show that most of the compounds with a better affinity than that of reference towards the Mpro of SARS-CoV-2, are extracted from fungi mainly the genera Penicillium and Aspergillus, which indicates that these two genera have potentialities promising antivirals against SARS-CoV-2.

3. Predictions of ADME parameters

Before proposing compounds 1, 2, 3 as new, more potent inhibitors of the Mpro of SARS-CoV-2, it was essential to complete our work with a predictive study of the physico-chemical and pharmaco-kinetic properties in order to verify their profile ADME. For this, N3 was used as standard.

3.1. Physico-chemical properties

According to (Table 2), the physico-chemical results obtained by the SwissADME server show that compounds 1, 2, 3 respond perfectly to Lipinski's and Veber's rules [17], which indicates that they can be administered by orally without causing any problems . However, compound 3 has a good solubility in water which promotes its permeability in the blood. Compared to the accessibility to synthesis, compounds 1, 2, 3 seem to be chemically synthesizable as the results show with values below 5.

Table 2: Physico-chemical properties of the top three compounds Properties.

Properties	N3	1	2	3
Formula	C19H30N4O5	C23H20BrNO6	C22H15N3O2	C15H10O6
MW (g/mol) Molecular weight	394.47	486.31	353.37	286.24
nLF Number of flexible links	13	5	3	1
nON Number of hydrogen acceptor	6	7	3	6
nOHNH Number of hydrogen donors	3	5	2	4
Log P	1.68	2.79	3.58	1.58
TPSA Å²	130.40	125.40	78.61	111.13
Lipinski's rule	Compliant	Fully Compliant	Fully Compliant	Fully Compliant
Veber's rule	Nor compliant	Fully compliant	Fully compliant	Fully compliant
Water solubility	Medium	Low	Low	Soluble
Accessibility of synthesis	41	4.39	2.70	3.14

3-2. Pharmacokinetic properties

Table 3: Pharmacokinetic properties of the three best compounds

Properties	N3	1	2	3
GI absorption	High	High	High	High
BBB permeability	Low	Low	Low	Low
CYP1A2 inhibition	Negative	Negative	Positive	Positive
CYP2C19 inhibition	Negative	Negative	Positive	Negative
CYP2C9 inhibition	Negative	Negative	Positive	Negative
CYP2D6 inhibition	Negative	Positive	Positive	Positive
CYP3A4 inhibition	Negative	Positive	Positive	Positive

3.2.1. Uptake

Reference ligand (N3) and compounds 1, 2, 3 show good gastrointestinal (GI) permeability, so they can cross the gastrointestinal tract to reach the bloodstream [17].

3.2.2. Distribution

All other compounds including N3 cannot cross the blood-brain barrier (BBB) effects [18].

3.2.3. Metabolism

We find that only N3 does not show any inhibitory effect for CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. However, compounds 1, 2, 3 inhibit the 5 isoforms of cytochromes (CYP), which is at the origin of drug interactions in the liver. Hower this problem can be solved when optimizing these compounds. Furthermore the results obtained from the two tables suggest that our potential molecules have a good pharmacological profile, therefore they can be exploited in the process of developing drugs against SARS-CoV-2.

Conclusion

The major objective of our work is the acquisition of skills in computer simulation, in particular Molecular Docking, in order to contribute to the development of new inhibitors of microbial origin of Mpro, a recent enzymatic target playing a key role in the fight against SARS- CoV-2.

In this study we propose new Mpro inhibitors by Molecular Docking. By taking as starting structure the ligand N3 of the base complex 6LU7. The Molecular Docking of the 35 microbial compounds selected with regard to the active site of Mpro highlights compounds number 1, 2 and 3 as the best inhibitors of this enzyme with respective interaction energies of -36.16 kJ/mol , -28.38 kJ/mol and -27.01 kJ/mol.

On the other hand, the predictive study of the physicochemical and pharmacokinetic properties of the compounds Ammonificin C compound (1), Pityriacitrin E compound (2), Kaempferol compound (3), shows that the latter have a good ADME profile.

This work reveals the importance of bioinformatics tools in the rapid development of therapeutic molecules and also highlights the antiviral potentialities of microbial metabolites and their ability to be promising drug candidates.

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