Exploration of Baicalin analogues against main Protease involved in Covid-19 as potential inhibitors involving in silico approaches

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Abstract. The proteolytic enzymesinvolved in the processing and replication of coronavirus serve a promising drug targetsfor coronavirus. Although a few numbers of vaccines have been made available, there is a great need for effective treatment to manageit. The current study involved the exploration of baicalin analogues as potential inhibitor of viral proteolytic enzyme. Utilizing various computational tools (scaffold morphing, molecular docking and pharmacokinetic studies), the **B74** and **B86** analogueswere screened as potential inhibitors of main protease. Accordingly, these analogues can be explored further in the search of promising therapy against the coronavirus infection.

1 Introduction

In the mid of years 2019 and 2020, the severe acute respiratory pneumonia type of cases arose globally. The coronavirus disease-2019 (COVID-19), also known as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been designated as a global pandemic. A total of 772,138,818 confirmed cases and 6,985,964 deaths have been reported by WHO as on 6December 2023 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). In order to develop the safe and effective treatment, it is important to look into the pathophysiology of this virus. It primarily affects the respiratory system leading to severe consequences. We have briefly discussed the key molecular events involved in SARS-CoV-2 infection herein (Figure 1). The coronavirus binds with the cellular receptors (ACE2) via viral spike proteins (SP) inside the host [1]. Upon entry into the host cell, SARS-CoV-2 complexes with the angiotensin converting enzyme 2 (ACE2). In this process the viral genome endocytoses into the host cell, ACE2. Further, transmembrane proteolytic enzymes (furin and TMPRSS2) initiate the proteolytic activation of SP by priming [2-4]. Next to priming process, virus is processed by another proteolytic enzymes; 3-chymotrypsin like protease (3CLPro) and papain like proteases. This leads to the formation of sixteen non-structural proteins (NSPs) that are involved in viral replication. Among these two proteases, the 3CLPro is called as the main protease and responsible for producing 11 NSPs alone. Therefore, the main protease is an important target for the development of anti-SARS-CoV-2 agents.

Several natural compounds such as flavones, flavonols, aurones, chalcones etc. are marked potential antiviral agents. Their potential benefit against the coronavirus is being explored in recent studies [5-9]. Baicalin, a flavone compound, has been well reported with antiviral properties against a wide range of viruses [10-14]. The current study involves the scaffold morphing and structure based drug designing of some Baicalin analogues against the main protease of SARS-CoV-2.
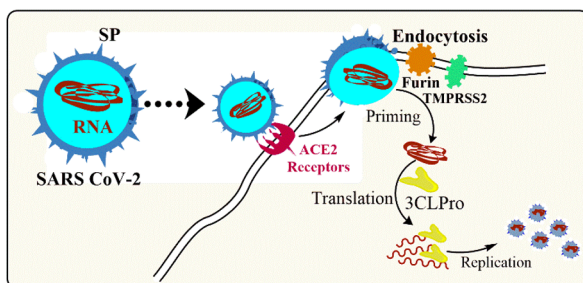

Fig. 1 Molecular events involved in the interaction of SARS-CoV-2 with in the host cell

2 Materials and Methods

2.1 Scaffold morphing

Scaffold morphing is a rationalized drug designing tool in medicinal chemistry that offers the improved potency and contributes overall therapeutics of a molecule [15]. The bioisosteric replacement is a scaffold morphing method that involves the exchange of functional groups of a molecule with their bioisosteres and improve the potency as well as the pharmacokinetic profile of that particular molecule [16]. The scaffold morphing has been carried out with a web server MolOpt for the bioisosteric transformation in baicalin [17]. MolOpt is a recently developed web tool for scaffold morphing. With the data mining, an inbuilt protocol of MolOpt, the ten replaceable sites of baicalin were explored. The generated analogues of baicalin were sorted on the basis of synthetic accessibility and the leading molecules were submitted to the molecular docking studies.

2.2 Molecular docking

The Molecular docking experiments were executed with the Maestro Schrodinger software. The SMILES notations of baicalin analogues were taken for the 3D chemical structures of the ligands. Ligands were prepared with the 'LigPrep' module. The 3D X-ray crystal structure of main protease 3CLPro (PDB ID: 6LU7, resolution 2.16 Å) was retrieved from the Protein Data Bank (https://www.rcsb.org/) to the 'protein preparation wizard' module. In the protein preparation, addition of hydrogens, allocation of bond order, missing loops as well as side chains improvement, and removal of water molecules within 5Å of het groups were considered and followed by the receptor grids generation around the co-crystallized ligands using 'receptor grid generation' option. The docking analysis was performed using extra precision (XP), Glide XP G-score and the Prime Molecular Mechanics Generalized Born Surface Area MM-GBSA values were calculated for free binding energies. The selection of best poses was based on the good G-scores and optimum binding orientations. The molecular interactions of docked poses were analyzed.

2.3. Prediction ADME properties

The pharmacokinetic profile of baicalin analogues i.e. ADME (Absorption, Distribution, Metabolism, and Excretion) properties were predicted using the QikProp program of the Schrödinger software. Various parameters such as solvent accessible surface area (SASA), QPPCaco (predicted apparent Caco-2 cell permeability in nm/s, QPlogBB (predicted brain/blood partition coefficient), QPPMDCK (predicted apparent MDCK cell permeability in nm/s), QPlogS (predicted aqueous solubility), QPlogKhsa (prediction of binding to human serum albumin), and percent human oral absorption (predicted human oral absorption on 0–100% scale) were calculated. Among these parameters, Caco-2 cells and MDCK cells are taken as the representing models for the gut blood barrier and the blood-brain barrier (BBB), respectively. The acceptability of the compounds to be orally bioavailable was estimated on the basis of Lipinski's rule of five [18].

3 Results and Discussion

3.1 Scaffold morphing through bioisosteric replacement

The chemical structure of baicalin was processed with the MolOpt webserver to generate different analogues with improved pharmacokinetic and pharmacodynamic profiles. Ten potential bioisosteric replacement sites were retrieved from the server. A total of 902 molecules were generated by the bioisosteric replacement at these ten sites (Figure 2). These molecules were then ranked on the basis of synthetic feasibility, which led to a total of 100 molecules. Then the top two molecules were investigated further for the molecular docking studies.
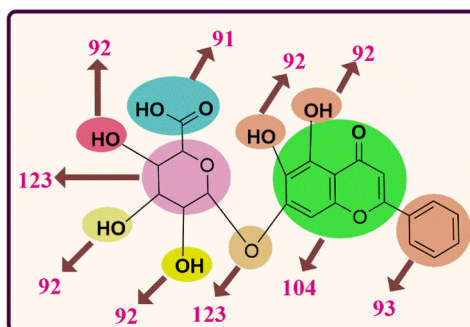

Fig. 2 Different bioisosteric replacement sites of baicalin

## 3.2 Molecular docking analysis and MMGBSA calculation

Docking analysis of baicalin analogues was carried out to investigate the multi-targeting potential against the targets of SARS-CoV-2. Both the molecules demonstrated good docking score (G score) and displayed crucial interactions with binding site amino acid residues. B74 and B86 have shown good binding affinities in terms of MM-GBSA scores (Table 1).

Table. 1 Glide score, binding energy, and key interactions of Baicalin analogues

Compound ID	3CLPro(PDB ID:6LU7)		
	Glide score (kcal/mol)	ΔG MMGBSA (kcal/mol)	Key Interactions
B74	-7.865	-51.27	Hie41, Hie164, Glu166, Gln189
B86	-7.266	-45.336	Hie163, Glu166

3.2.1. Binding mode and interactions of ligands with main protease (3CLPro)

The main protease (3CLPro) of SARS-CoV-2 is essentially involved in the processing of polyproteins which are translated from viral RNA. 3CLPro acts on the cleavage site of polyproteins [19]. Therefore, both the baicalin analogues were docked within the catalytic site of 3CLPro. A catalytic triad (His41, Cys145 and Ala285) present in 3CLPro is important for the enzymatic activity and N-terminal residue Glu166 keeps the S1 domain of this enzyme in an active conformation.[20] The inhibitors which interact with the His 41 residue of the catalytic triad and Glu166 are considered to be very good inhibitors of 3CLPro.In our study both the analogues were found to interact with these residues via H-bond.[21] These analogues also shown H-bond interactions with Gln189, and His164 residues (Figure 3).

(A)   (B)
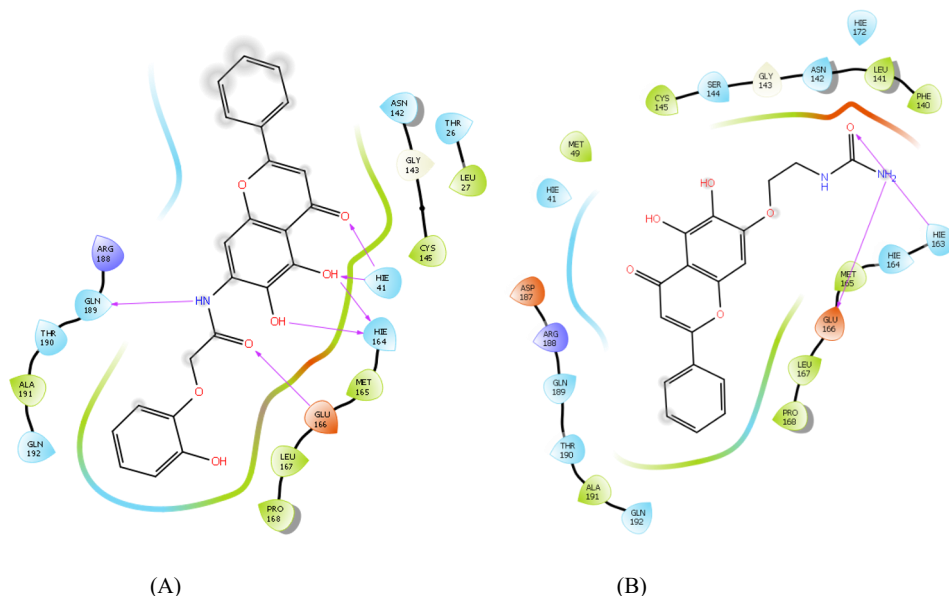
Fig. 3 The interactions of (A) B74 and (B) B86 within the active site of main protease (3CLPro)

3.3 ADME Predictions

The ADME properties of both the analogues were investigated to analyze their drug-like properties. Both the compounds were in agreement with Lipinski's rule of five. Physiochemical features including molecular weight, hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), lipophilicity (LogP), etc. are generally critical for crossing lipophilic barriers like BBB. On the basis of predicted values of these parameters, both the compounds might be able to penetrate into such barriers.[22] The QPlogKhsa descriptor indicates the predicted value of plasma protein binding amount of drugs which is an important factor and should be under a prescribed range. The predicted values of

QPlogKhsa showed optimum binding of the compounds with the plasma protein.[23] Other calculated parameters, QPPCaco, QPlogBB, QPLogKhsa, and QPPMDCK primarily indicate the capability of the compound's distribution inside the body. These compounds exhibited moderate to significant penetrability in case of in vitro MDCK cells and in vitro Caco-2 cells. Moreover, all compounds exhibited significant oral absorption.[24] The predicted ADME properties revealed that these compounds have drug-like properties and could be considered as good drug candidates (Table 2).

Table. 2 ADME properties of baicalin analogues

Molecule	Mol MW	HBD	HBA	QP log $P_{o/w}$	SASA	Rule of 5	QPP MDCK	QPP Caco	QP logBB	QP logK hSa	QP logS	% human oral absorption
B74	419.5	3	7	2.87	713.5	0	30.86	76.77	-2.2	0.25	-6.14	77.53
B86	356.3	4	5.7	1.06	615	0	67.94	31.38	-2.2	-0.34	-4.25	59.97

4 Conclusion

The combination of structure-based drug designing and a scaffold morphing approach was successfully utilized to identify putative analogues of baicalin against main protease (3CLPro) to manage Covid-19. Initially, the bio-isosteric replacement was done on ten suggested sites of baicalin in order to generate a library of its analogues. From a library of 902 analogues, 100 were selected on the basis of synthetic possibility and submitted for docking analysis against main protease (3CLPro). Then the top two molecules were investigated further for the molecular docking studies. The binding affinity and ADME properties of these molecules were also determined. The *in-silico* ADME prediction advised the drug-like properties of these analogues. The most active molecules B74 and B86 have strong binding interactions with the active site of main protease. However, further experimental validation is required to confirm their inhibitory activities against 3CLPro. The protocol implemented in this study may become useful framework in the future for the development of novel plant based small molecules against the COVID-19.

5 Acknowledgement

6 References

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