Identification of potent COVID-19 main protease (MPRO) inhibitors from flavonoids

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Abstract

COVID-19 epidemic that commenced in Wuhan in December 2019 has spread worldwide within a few months. Currently, there are no targeted therapeutics and effective treatment options remain very limited. Mpro is a key CoV enzyme, which plays a pivotal role in viral replication and transcription, making it an attractive drug target for this virus. This study was conducted to evaluate the efficacy of flavonoids as drug target molecules against COVID-19 Mpro by molecular docking study. COVID-19 Mpro was docked with 14 flavonoid compounds as well as 4 already existing drugs and the binding energies were determined.Based on the results,we have identified two potential flavonoids with high binding capacity and possible drug candidates. This study will pave a way for doing advanced experimental research to evaluate the real medicinal potential of these compounds to cure COVID-19.

Introduction

A new Coronavirus (CoV) identified as COVID–19 virus is the etiological agent responsible for the 2019–2020 pandemic that commenced in Wuhan[ZhuN et al. 2020, Qun Li et al. 2020, Zhou Pet al. 2020, WuF et al. 2020]. So far no drugs have been developed and very limited numbers of therapies areavailable for its treatment. Coronaviruses cause some of the highly prevalent and severe diseases, including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)7 in humans as well as in animals. A study by Zhou et al.(ZhouP. et al. 2020) reported that the COVID–19 virus genome is composed of ~30,000 nucleotides. Its replicase gene encodes two overlapping polyproteins, pp1a and pp1ab which are required for replication of the virus and its transcription. These polyproteins release functional polypeptides after proteolytic cleavage by the main protease(Mpro). The importance of Mpro in the life cycle of COVID–19 identifies the Mpro as an attractive target for antiviral drug design[Pillaiyar Tet al. 2016].

COVID–19 is composed of three domains (Fig. 1a). Domains I (residues 8–101) and II (residues 102–184) have an antiparallel β-barrel structure. Domain III (residues 201–303) contains five α-helices arranged into a largely antiparallel globular cluster that is connected to domain II employing a long loop region (residues 185–200). COVID–19 virus Mpro has a Cys–His catalytic dyad, and the substrate-binding site is located in a cleft between Domain I and II [Jin Z et al. 2020].

In silico based screening has proven to be a very useful tool to meet the challenges of antiviral drug discovery [Murgueitio MSet al. 2012]. Natural compounds turned out to be cheaper and safer drug candidates against several diseases [Shen B2015, Thomford NEet al. 2018].Flavonoids are naturally occurring polyphenolic biomolecules widely found in plants and perform a wide variety of biological functions [Samanta A et al. 2011]. Many of these are known to be effective antiviralsthat can act at different stages of viral infection, particularly at the molecular level to inhibit viral growth. Studies using flavonoids against a wide range of DNA and RNA viruses have been done. In general, flavonoids work by several mechanisms. They can block attachment and entry of viruses into cells, interfere with various stages of viral replication processes or translation and polyprotein processing to prevent the release of

the viruses to infect other cells [Veckenstedt, A. et al.1981; Tao, J.et al. 2007; ShibataC. et al. 2014; SauterD. et al. 2014; SongJ. M. et al. 2005;].

In the current study, we have docked 14 flavonoids against Mpro using AutoDockVina and out of these ligands, threepotential candidates have been identified. We also docked four drugs like azithromycin, remdesivir, favipiravir and hydroxychloroquine with Mpro which have shown potential against COVID–19 [Holshue ML et al. 2020,Wang M et al. 2020, Yao X et al. 2020].

Methodology

Ligand and Receptor molecule Preparation

The crystal structure of Mpro having PDBID 6lu7 essential for virus replication was downloaded from the protein data bank web site (http://www.rcsb.org/pdb) (PDB ID: 6LU7: Resolution 2.16 Å) [Jin Z et al. 2020]. Structures of small molecules investigated for docking studies were obtained from https://pubchem.ncbi.nlm.nih.gov/ as SDF form and in the 3D Conformer.

Molecular docking

To understand the binding interaction of these molecules with COVID19 virus Mpro, AutoDock Vina [Morris GM et al. 2009]was used to predict their binding poses. Docking studies were attempted to explore the binding mode of the suggested protease inhibitors onto the 3D structure of Mpro of COVID-19 using AUTODOCK tools 1.5.6. Before docking, polar-H atoms were added to the Mpro followed by the addition of Kollman charges. The macromolecule file was then saved in pdbqt format and ready to be used for docking. Ligands were also saved in pdbqt format after detecting the torsion angles. Default settings were used for all other parameters. PyMOL4.3.0 [The PyMOL Molecular Graphics System, version2.0Schrondinger, LIC.],LigPlot+[Wallace ACet al. 1995] and Protein-ligand Interaction Profiler [SalentinS et al. 2015]was used to visualize the binding interactions between these ligands and main protease of COVID-19.

Visualization of docking results

To analyze the docking results PyMOL 4.3.0and Ligplot + were used. Binding site residues were identified -Protein-ligand Interaction Profiler was used to analyze the binding site residues of Azithromycin as ligPlot+ could not plot and show the interacting amino acid residues due to the complex structure of azithromycin.

Results

Molecular Docking

14 Flavonoids and 4 existing drugs were used as ligands to bind to the COVID-19 main protease. Structure and their docking scores have been shown in Table 1 and Table 2, respectively. Out of these ligands, flavonoids Procyanidin b2 and Mangiferin showed high binding affinity with the Mpro having Docking affinity -9.4 and -8.5kcal/mol respectively. Azithromycin a semi-synthetic antibiotic showed the affinity -13.4 kcal/mol which took it to be on the top of all the docked ligands. Other previously proposed inhibitors of COVID -19 like flavipiravir, ramdesivir and hydroxychloriquinone were predicted to show binding with Mpro with docking scores -7.2, -8.1, -6.2, respectively.

Analysis and visualization of best docked ligands

The docking poses of all the ligands were visualized using Pymol, LigPlot+ and Protein-ligand Interaction Profiler. Three ligands with the highest binding affinity to Mpro, azithromycin, procyanidin b2 and Mangiferin were visualized and the binding residues of Mpro in the binding pocket were analyzed. Azithromycin was interacting with Tyr54, Asp187, Gly143, Glu166 residues in the binding pocket of Mpro(Fig.1). The residues Gln189, His41, Glu166, Gly143, Asn142 of binding pocket were responsible for the binding of Procyanidin b2 in the binding pocket (Fig.2) and Mangiferin showed interaction with His41, Gln192, Asn 142, Ser144, Cys145, Gly143, Leu141, Thr190, Arg188 residues of Mpro(Fig.3).

Discussion

A novel newly emerged SARS-CoV-2 is presenting major threats to human health nowadays [Zhu Net al. 2020]. Currently, no specific clinical therapeutics are available for the treatment of SARS-CoV-2-mediated infections [Zhou Yet al. 2020]. Thus, there is anurgent need to identify and characterize novel drug candidates to overcome the health losses caused by SARS-CoV-2. To provide natural scaffolds for drug development, we have screened flavonoids against novel drug target, Mpro.

It has been previously reported that flavonoids exert their antiviral effects via blockage of cellular receptors, inhibiting viral antigenic determinants, loss of enzymatic functions, and/or inhibition of particle biosynthesis [Chang LK et al. 2003]. Furthermore, the antiviral activity ofmany flavonoids has been reported previously against various viral strains[Song JMet al.2005, Savi LAet al. 2010]. Antiviral natural product-based medicines have also been used for two previous coronavirus outbreaks of SARS-CoV and MERS-CoV which suggest that nature has tremendous potential to provide treatment for the ongoing pandemic of COVID-19 [Li SYet al.2005, Lin CW et al. 2005, Lau KMet al. 2008]. Azithromycin in our study showed a high affinity of binding to Mpro. While initial studies after the disease outbreak indicated that azithromycin had synergistic effects with other antimalarial drugs in reducing the virus load and bringing about clinical improvement, the concern on its use for the treatment of COVID-19 is now gaining pace [Kelleni M 2020].However, a recent study conducted in France on 11 patients showed no rapid anti-viral clearance or clinical benefits in patients with severe infection [Molina J et al. 2020].
Another study (Jennifer C.E et.al. 2020) showed the addition of azithromycin in combination with antimalarial drugs may induce heart failure and cardiovascular mortality. The use of Natural products as a therapeutic for COVID-19 can provide a safer alternative.

Two highly potential flavonoids in our study that are mangiferin and procyanidin b2 may show a better outcome in COVID- 19.Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside) is a bioactive ingredient predominantly isolated from the mango tree and Procyanidin B2 can be found in Cinchona pubescens, Cinnamomumverum, Crataegusmonogyna, Uncariaguianensis, Vitis vinifera, Litchi chinensis, the apple, and in Ecdysantherautilis.These two flavonoids may be the potentialities to combat the emergence of COVID-19.

Mangiferin showed polar contacts with His41, Gln192, Asn 142, Ser144, Cys145, Gly143, Leu141, Thr190, Arg188 residues in the binding pocket of Mpro (Table 4). Residues Met165, Glu166 and Gln189 were responsible for the hydrophobic binding of the mangiferin with the bending site. Procyanidin bound to the binding site by residues Gln189, His41, Glu166, Gly143, Asn142, Cys145 (Table 5)and there were hydrophobic interactions through Met165 and Gln189 with distance 3.61 and 3.86 respectively. Met165 and Gln189 form a hydrophobic pocket. Both flavonoids form multiple hydrogen bonds with the main chain of the residues in thesubstrate-binding pocket, which also helps lock the inhibitor insidethe substrate-binding pocket (Fig.4). In Azithromycin binding residues were Tyr54, Asp187, Gly143, Glu166. In previous studies, it has been seen thatthe most variable regions were the helical domain III and surface loops,but the substrate-binding pockets located in a cleft between domainsI and II are highly conserved among all Mpros of CoV family, suggesting theantiviral inhibitors targeting this site should have wide-spectrumanti-CoV activity.

Conclusion

Our results propose that flavonoids such as Procyanidin b2 and mangiferin havea better binding affinity to Mpro of COVID-19 than hydroxyquinone, flavipiravir and ramdesivir.Two of the compounds bearing good binding potency are components of dietary foods that suggest the biologically safe profile of these compounds further supporting the potential of these compounds as starting points for therapeutics against COVID-19.However, further studies should be conducted for the validation of these compounds using in vitro and in vivo models to pave a way for these compounds in drug discovery.

Declarations

Statement of competing interest: The authors declare no competing interest.

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Tables

Due to technical limitations, the tables are only available as a download in the supplemental files section.

Figures

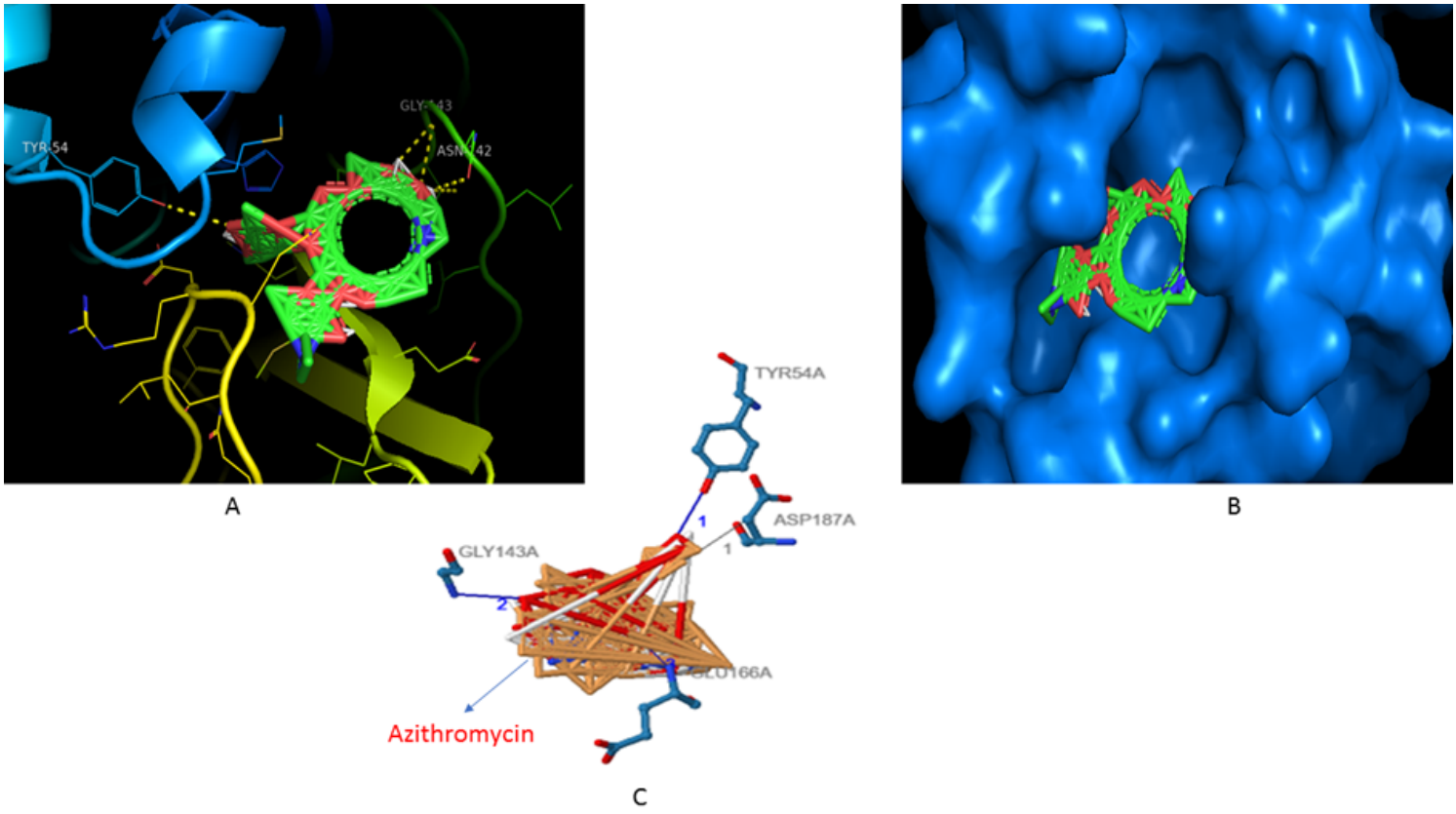

Figure 1

Docking of Azithromycin with the main protease of COVID-19.

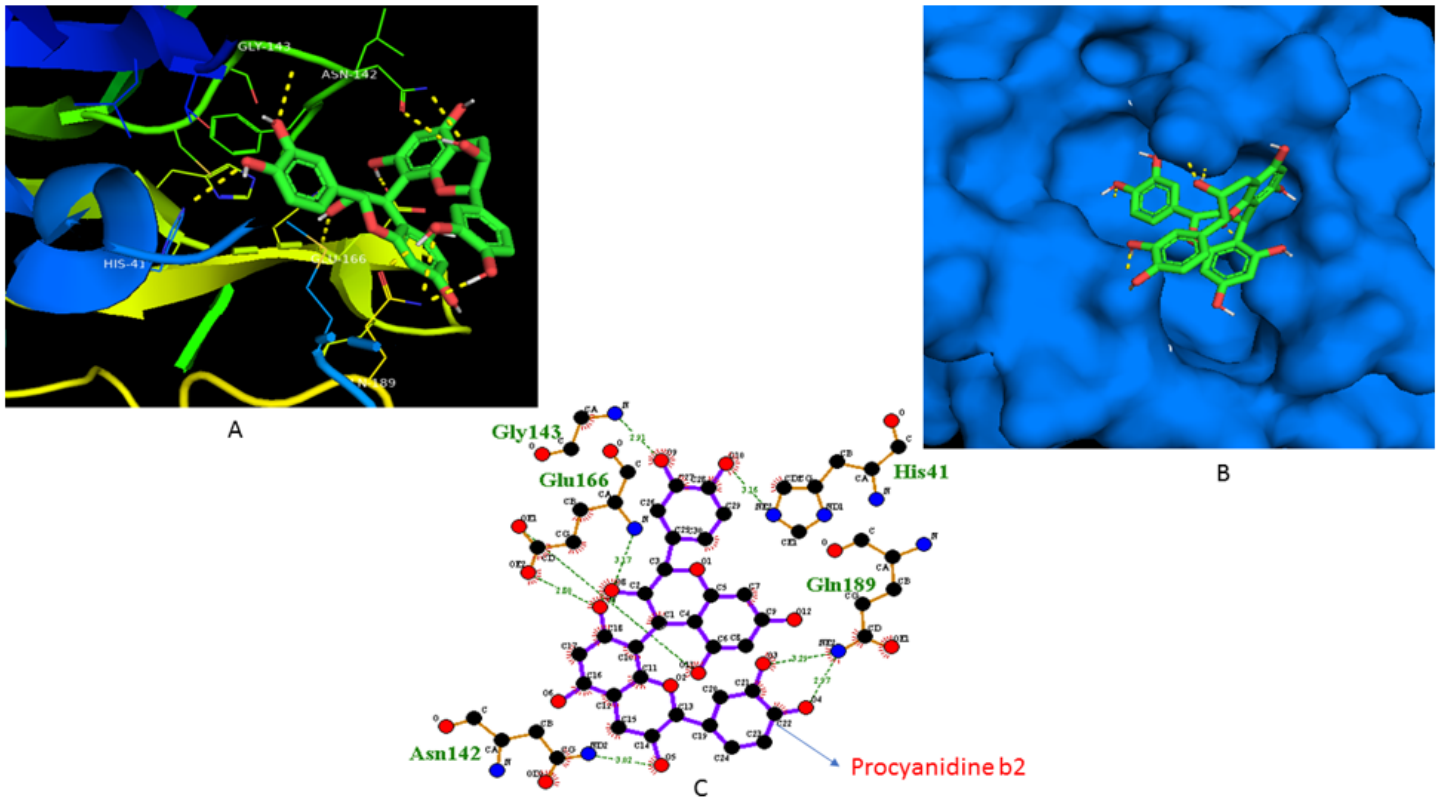

Figure 2

Docking of Procyanidin b2 with the main protease of COVID-19.

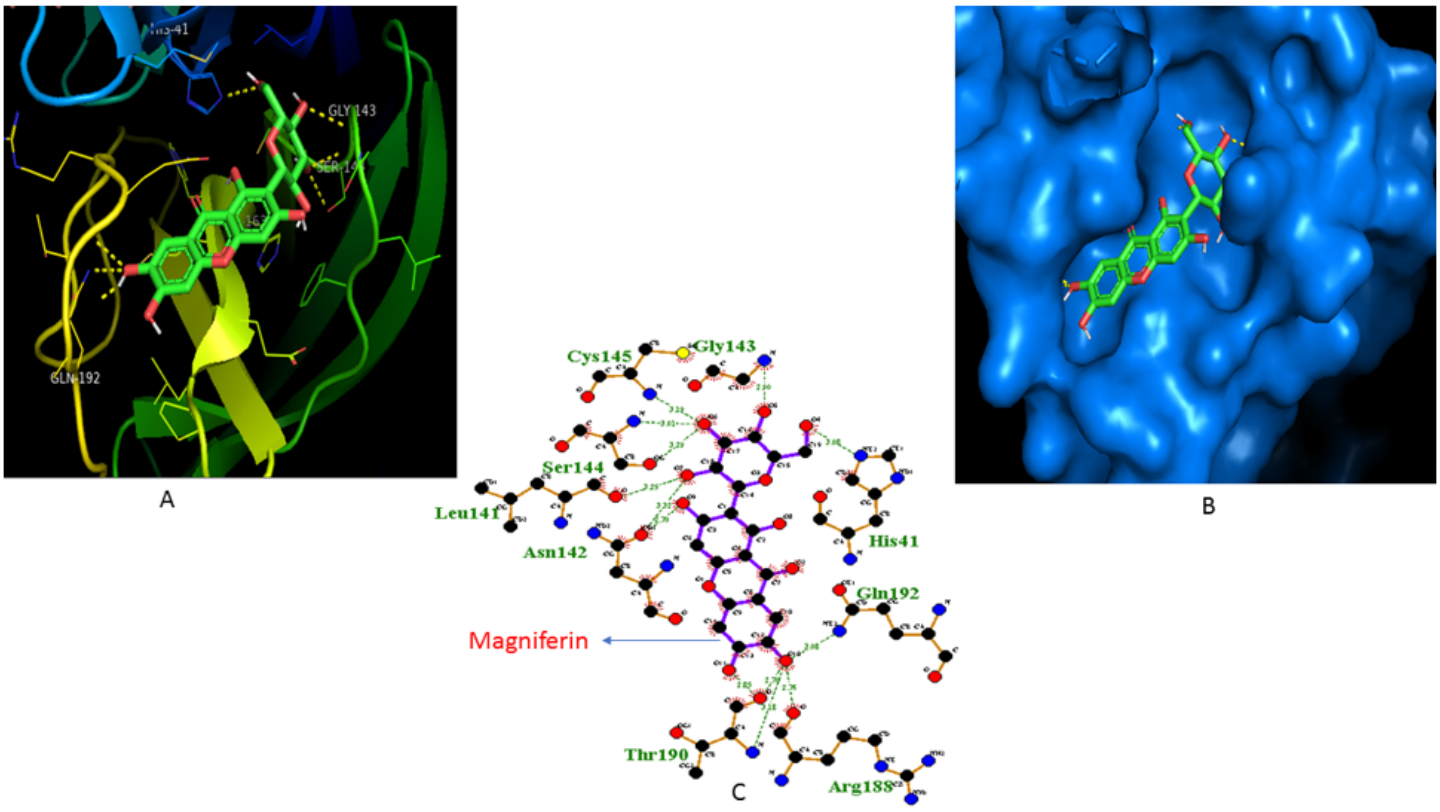

Figure 3

Docking of Mangiferin with the main protease of COVID-19.

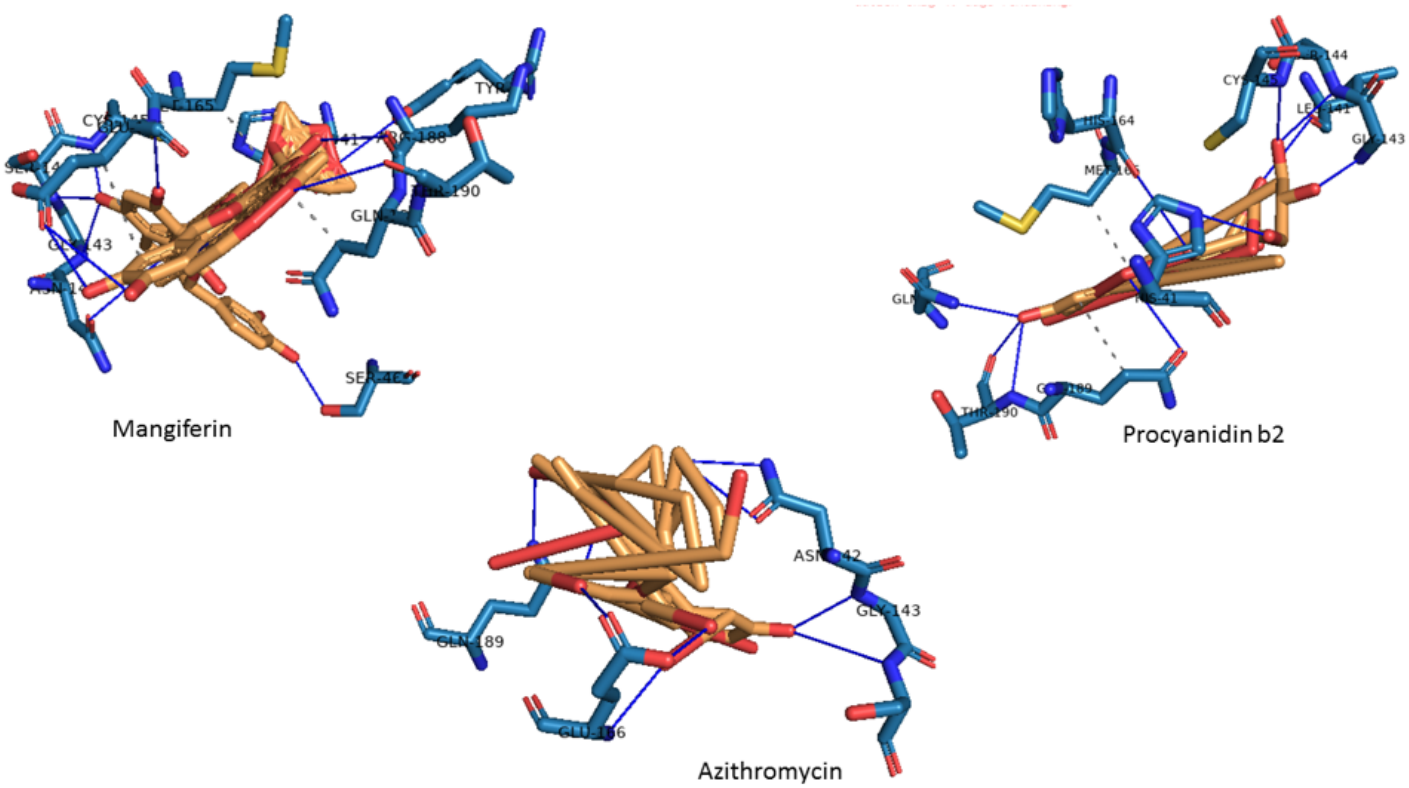

Figure 4

Mangiferin, Procyanidin and Azithromycin showing interaction with Mpro. Blue solid lines indicate H-bonding and black dotted lines show hydrophobic bonding.

Supplementary Files

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