Abstract

Introduction: With regard to the limited success of antiviral medications against COVID-19, traditional medicines gain increasing attention. Despite less distribution within the plant kingdom, biflavonoids (BFs) are invaluable secondary metabolites with potential applicability for prevention and treatment of several diseases. With regard to scientific indications, BFs are privileged starting points toward anti-COVID-19 agents and some BF structures such as amentoflavone exhibited inhibitory effects on respiratory syndrome viruses. Additional 2/3-phenyl-4H-chromen-4-one moiety of BFs with respect to flavonoids is implicated in further binding interactions to the intended target. The functional importance of 3-chymotrypsin-like protease (3CLpro) in forming viral RNA raises its potential to be targeted in SARS-CoV2.

Method and Material: In this project, 115 privileged biflavonoid structures with biological activity were used for qualitative and quantitative analysis in terms of binding to SARS-CoV-2 3CLpro active site. Druggability properties of selected ligands were examined by the SwissADME website. Biflavonoid derivatives were subjected to molecular docking simulation with Autodock4.2 software to identify top ranked ligands based on the analysis of ligand binding patterns to co-crystallographic site. In order to validate the docking results and evaluate the stability of top ranked complexes, molecular dynamics (MD) simulations were performed for 50 ns in aqueous medium. Analysis of MD results were mediated by using RMSD, RMSF, R_g, intermolecular and intramolecular hydrogen bonds, and SASA criteria. Subsequent to attaining stable ligand-enzyme complexes, molecular binding energy at the ligand-amino acid level was calculated using density functional theory (DTF) method by Gaussian 09 program. Finally, based on obtained structure binding relationship, hypothetical biflavonoid pharmacophores with optimal binding capability to SARS-CoV-2 3CLpro were proposed.

Results: Despite structural resemblance of studied BFs, wide range of binding affinities could be assigned to the interacted conformations (Genkwanol C: ΔG b -7.11 kcal/mol and Garciniaflavone C: ΔG b -12.66 kcal/mol). Glu166 was the sole preserved binding residue within all simulated BF complexes. Hydrophobic contacts played important role in stability of Garciniaflavone C complex. Top ranked biflavonoid structures had interaction with at least one of the catalytic dyads of SARS-CoV-2 3CLpro (His41 and Cys145). Among top ranked ligands, 7,7´´,4´´´-tri-O-methylagathisflavone showed higher stability during molecular dynamic simulation. Glu166 as sole preserved binding residue has negative binding energy in all top ranked ligand. 5´´´,7´´´-dihydroxy-4´´´-oxo-4´´´H-chromen-2-yl ring in Garciniaflavone C participates in all polar interactions. In 7,7´´,4´´´-tri-O-methylagathisflavone Ser46 and His41 (-4.62 and -9.64 Kcal/mole respectively) plays the most important role in stability of complex. In 8,8´´-biapigenil Thr54, His164, and Asp48 (-10.00, -8.37, and -8.23 Kcal/mol respectively) have important role in π-stacking and H-bond interactions respectively. Selectivity index of Garciniaflavone C، 7,7´´,4´´´-tri-O-methylagathisflavone, and 8,8´´-biapigenil were obtained as 1.22, 1.39, and 1.31, respectively . Molecular docking study demonstrated more selectivity to SARS-CoV-2 3CLpro for 7,7´´,4´´´-tri-O-methylagathisflavone in comparison with SARS-CoV-1 3CLpro.

Discussion and conclusion: Results of current study revealed that Garciniaflavone C ، 8,8´´-biapigenil and 7,7´´,4´´´-tri-o-methylagathisflavone were capable of forming stable complexes with SARS-CoV2 3CLpro. Moreover, BFs under study could collectively occupy substantial space of enzyme active site. Such observation indicated the potential of these BF structures for the design of hybrid chemicals with optimum binding features and enzyme inhibition. 7,7´´,4´´´-tri-o-methylagathisflavone showed more selectivity in binding toward SARS-CoV2 3CLpro with regard to SARS-CoV1 3CLpro. Although the biological activity and its mechanism are yet to be explored within BFs, the results of this study emphasize on the potentiality of BFs in binding and probably inhibiting SARS-CoV2 3CLpro

Key words: SARS-CoV-2, 3CLpro, Biflavonoid, Free binding energy, Molecular dynamic, Density functional theory