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Original Article: Inhibitory Activity Analysis of Anti-HCV Coumarins Against Main Protease of SARS-COV2 via Molecular Docking

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Abstract

The inhibition effect of two dimeric coumarinswas validated against the main protease of severe acute respiratory syndrome -coronavirus 2 (SARS-COV2) via Molecular docking approach. The phytochemicals *5, 5′-bi (6, 7-dihydroxycoumarin)* and *6, 6′, 7, 7′- tetrahydroxy-5,8′- bicoumarin* from *Viola philippica* are inhibitor activity of NS3/4Aprotease of hepatitis C virus (HCV). The inhibition for SARS-COV2 main protease is estimated by evaluation of binding energy and conformation. The value of predicted binding energy equals -8.07 for *5, 5′-bi (6, 7-dihydroxycoumarin)* conform its ability for SARS-COV2 main protease inhibition. This in vitro analysis identified the activity of an anti-HCV natural inhibitor for SARS-COV2 therapy.

Keywords: SARS-COV-2, Molecular Docking, Protease Inhibitor, Coumarin, *Viola philippica.*

Introduction

The Pandemic of severe acute respiratory syndrome -coronavirus 2 (SARS-COV2) that is known as coronavirus disease 2019 (COVID-19) worldwide led to the worldwide attempt for repurposing or the design vaccines and drugs(Adhikari, Meng *et al.*, 2020, Lai, Shih *et al*., 2020). The case of the novel pandemic is a member of the coronavirus large family, which are enveloped, positive-sense, and single-stranded RNA (+ssRNA) viral(Moreno-Eutimio, López-Macías *et al.*, 2020, Pal, Berhanu *et al.*, 2020).A positive-sense RNA can act as messenger RNA (mRNA) whichis directly translated into viral proteins by the ribosomes of host cells(Ahlquist 2002). Other important examples of +ssRNA viruses include pathogens such as the Middle East respiratory syndrome (MERS), severe acute respiratory syndrome -coronavirus 1 (SARS-COV-1) (Moreno-Eutimio, López-Macías *et al.*, 2020), and hepatitis C virus (HCV)(Fraser, Hershey *et al*., 2009).

Application of naturalcompounds for antiviral therapy appeared as phytochemicalsprotease inhibitors(Ogungbe, Crouch *et al.*, 2010, Aberoumand 2012, Kim, Seo *et al.*, 2014, Zakaryan, Arabyan *et al.*, 2017)thatprevent viral replication by selective binding to their (Patick and Potts 1998). Coumarincompounds are animportant class of phytochemicals that naturally occur in plant kingdom(Matos, Santana *et al.*, 2015). Phytochemical term generally was used to refer the natural chemical that may have the biological effects, but not all identified as essential nutrients.

Coumarins are a group of benzopyrones chemicals that are phytochemicalor modified substances of the natural originwhich in some cases wereshownanti-inflammatory and anti-viral activities(Matos, Santana *et al*., 2015, Menezes and Diederich 2019). Dimerics coumarin was derived feature the C-C or C-O-C biaryl as well as terpene sidechain linkages or cyclobutane ring that their key structural similarities as well as pharmacological effects were reviewed in terms of inhibitors of α-glucosidase or

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viruses(Menezes and Diederich 2019). The*5, 5′-bi (6, 7- dihydroxycoumarin)* and *6,6′,7,7′- tetrahydroxy-5,8′- bicoumarin* (Wang, Zhang *et al.*, 2019) are C-C linked natural dimeric coumarins from *Viola philippica* inhibit activities of NS3/4Aprotease of HCV.The *Viola philippica Cav*. is synonymized as *Viola yedoensis Makino (Violaceae)*and is a perennial herb which has beendistributed throughout China and its dried whole is a traditional medicine for treatment of boils, furuncles, carbuncles,and infections such as hepatitis.The NS3/4A protease is a crucial enzyme inthe maturation process of HCV and biosynthesis of the virus surface glycoprotein, α-glucosidase.These bicoumarins play an essential role in the HCV infection of host cells thatare widely applied for treatment of liver diseases.

Experimental inhibitory effect determination of proposed drugs for emergence SARS-COV2therapy is cost and time-consuming, therefore, application of a computational approach for investigation of molecular docking interaction to SARS-COV2 main protease with repurposing or design drugsof is necessary.Molecular docking simulation is a key method for drug proposition which estimates the energy and conformation ofcandidate chemicals opposedto targetreceptor protein(Kotha, Adimulam *et al.*, 2015, Torres, Sodero *et al*., 2019). This strategy was applied for In-vitro evaluation of the phytochemicals inhibitory effect opposed HCV (Akher, Farrokhzadeh *et al.*, 2019)and Ebolaviral(Veljkovic, Loiseau *et al.*, 2015, Raj and Varadwaj 2016, Dhama, Karthik *et al.*, 2018, Nasution, Alkaff *et al.*, 2018).Previously, investigation of molecular docking interaction of the SARS-CoV-2 main protease was studied with some natural and novel synthetic coumarin analogues (Chidambaram, Ali *et al*., 2020, Maurya and Mishra 2020, Milenković, Dimić *et al.*, 2020, Yañez, Osorio *et al.*, 2020, Abdelmohsen, Albohy *et al.*, 2021, Chidambaram, El-Sheikh *et al.*, 2021).In other hands, molecular docking approach collaborated with In-vitro assays analysis was used for evaluation the protease inhibitory effect of the ((3-(1-(phenylamino) ethylidene) -chroman-2,4-dione)), ((3-(1-((3-chlorophenyl)amino) ethylidene) -chroman-2,4-dione)), ((3-(1-((4-chlorophenyl) amino) ethylidene)-chroman-2,4-dione)), and their palladium (II) complexes opposed main protease of SARS-COV2 (Milenković, Dimić *et al.*, 2020). Hence, this work attempt to evaluate the inhibitory effect of two anti-HCVcoumarins for emergence SARS-COV2therapy.Molecular docking approach was applied for analysis of in hibitionactivity of these phytochemicals opposed main protease of the SARS-COV2.

Molecular Docking Study

The structures of bothcoumarins*5, 5′-bi (6, 7-dihydroxycoumarin)* and *6,6′,7,7′- tetrahydroxy-5,8′-bicoumarin*were drawn and then were optimized by the semi-empirical AM1 method using Hyperchem program (version 7) (HyperChem 2002) software. In the next step, the crystallography structure of the main protease of SARS-COV2(PDB ID: 6LU7) was adapted from the protein data bank (PDB) which was introduced as a target in searching for anti-SARS-COV2 agents.Firstly, the molecular docking approach was done using auto dock 4.(http://mgltools.scripps.edu) software with application of the Lamarckiangenetic algorithm (LGA) search.With the aim of estimating on the binding energy, a grid box with 60 × 60 × 60 points was defined as the inhibitory site of SARS-COV2.The rigid spacing of the cube was equal to 0.375 Å while the offset values from the center of the main protease were equal to X = 0.944, Y = 6.111 and Z = 7.528 in order to cover the active site for the inhibitory study. Theco-crystallized inhibitor alpha-ketoamide (N3) was assisted for the evaluation of inhibitory effect of two proposedcoumarins based on an estimated binding energy.The chemical name of alpha- ketoamideligand is n- [(5-methylisoxazol-3-yl) carbonyl] alanyl -l-valyl-n~1~-((1r,2z)-4-(benzyloxy) -4- oxo-1-{[(3r)-2- oxopyrrolidin-3-yl] methyl} Sbut-2-enyl)-l- leucinamide (Jin, Du *et al.*, 2020). The second molecular dockingapproach was performed using Molegro Virtual Docker (MVD) softwarewith the application of a search algorithm, MolDock SE (simplex evolution)(Thomsen and Christensen 2006).The search space with a radius of 27 Å was defined inthe inhibitory site of SARS-COV2for a similarity screeningtoreferencealpha-ketoamide.The reference ligandis implemented as a scoring function rewarding poses similar to the

specified pattern(Thomsen and Christensen 2006, Chauhan and Shakya 2009, Muppalaneni and Rao 2012). The referencealpha-ketoamideis considered as a collection of template groups which represent chemical features with a number of centers.The steric group matches all atoms and was applies for shape matching without taking any chemical groups into account. Matching of each atom with defined groups is rewarded using a Gaussian formula,in which the weight of template groups in this equation is set up by a strength parameter.Hydrogen donor and acceptors, negative and positive charges, and ring groups with an equal strength 1 as well as steric criteria with strength 0.5 wereconsidered for similarity screening. The steric group that checks the matching of all atoms and is employed formatchingthe shape of candidate ligand with native compoundswithout taking into account any chemical groups.

Result and Discussion

A molecular docking strategy was applied for estimation of energy and conformation of two phytochemical inhibitorsto the SARS-COV2 main protease. The inhibitory effect of two anti-HCV coumarins from *Viola philippica*was investigated for aninvitro evaluation.

The estimated binding energy for *5, 5′-bi (6, 7-dihydroxycoumarin)* and *6, 6,7,7′- tetrahydroxy-5,8′-bicoumarin*respectively was -8.07and -6.47 (Kcal/Mol). In the studied situation, the predicted binding energy for co-crystallized inhibitor alpha-ketoamide was -7.67(Kcal/Mol). Accordingly,*5, 5′-bi (6, 7- dihydroxycoumarin)* has a higher affinity forSARS-COV2, the main protease to native inhibitor alpha-ketoamide.For this phytochemical, the value of inhibit constant (K_i) and ligand efficiency (LE) respectivelyis equal to 1.22 uM and -0.31. The value of LE definition of binding energy per non-hydrogen atom of a proposedchemical to its receptorassists in narrowing focus to lead drugs with optimal combinations of physicochemical and pharmacological properties (Reynolds, Tounge *et al.*, 2008).

The molecular docking study was done for evaluation of 10 natural antiviral coumarin analogues to the main protease of SARS-CoV-2(Chidambaram, El-Sheikh *et al*., 2021). The obtained result using Autodock Vina was shown that the prescribed anti-HIV cumarins respectively, were Inophyllum A (−8.4 Kcal/Mol), Mesuol (−7.6 Kcal/Mol), Calanolide A (1b) (−7.5 Kcal/Mol), Pteryxin (−7.3 Kcal/Mol), Isomesuol (−7.2 Kcal/Mol), Suksdorfin (−7.0 Kcal/Mol), Calanolide A (1a) (−6.8 Kcal/Mol), Seselin (−6.6 Kcal/Mol) as well as Rutamarin (−7.0 Kcal/Mol) which is a herpus simplex virus inhibitor.While for Collinin (−6.1 Kcal/Mol),natural coumarin as anti-HBV, a lower affinity was obtained as opposed to thealpha-ketoamide (−6.6 Kcal/Mol) inhibitor. Also, Autodock Vina was applied for theSARS-CoV-2 main protease inhibitory analysisfor 9 coumarin based derivatives and their novel synthetic benzopyran-connected pyrimidine (Chidambaram, Ali *et al*., 2020). The result was shown that todda-coumaquinone (−7.8 Kcal/Mol), synthetic compound (−7.1 Kcal/Mol), heraclenol (−7.0 Kcal/Mol), imperatorin (−6.8 Kcal/Mol), oxepeucedanin (−6.8 Kcal/Mol) and heraclenin (−6.8 Kcal/Mol) displayed a remarkable inhibitory activity in intricate with the alpha-ketoamide (−6.6 Kcal/Mol) while the computed binding energy was not notablefor other derivatives contains angelicin, psoralen, hydroxychloroquine bergapten, aesculetin, saxalin.

Then, the MolDock score based on molecular docking was done for a similarity screening for thealpha-ketoamidetemplate. It was confidence, native inhibitor reference contain 6 hydrogen acceptors, 9 hydrogen donors, 49 steric criteria and 16 ring groups.The optimal conformation of this phytochemical, with most MolDock scores selected with the aim of estimating the similarity. The values of the MolDock score and similarity score for this phytochemical respectively were -88.50 and -195. It was mentioned that the values of similarity score for 6,6′,7,7′- tetrahydroxy-5,8′- bicoumarin was -188. The selected conformation of *5, 5′-bi (6, 7-dihydroxycoumarin)* in the inhibitory site of SARS-COV2. The template groups of alpha-ketoamide are shown in Figure 1.

The process of molecular docking was validated through re-docking the native inhibitor with the main proteaseof SARS-COV2. In other words, the alpha-

ketoamide molecule was removed and re-dockedinto the crystallographic structure of the SARS-COV2 main protease. Superimposed structures of native and docked alpha-ketoamide inhibitor with the lowest binding energy were shown in Figure 2.

Also, ligand map probing of native and optimal conformation of re-docked alpha-ketoamide was shownin figure 3 that can be seen the hydrogen interactions with Gly 143 (A), Glu 166 (A), Gln 189 (A) and Thr 190 (A) amino acids of active site were seenfor both ligands. Furthermore, commonly Thr 26 (A), Cys 145 (A) andAsn 142 (A) amino acids were in the steric interaction with the main protease of SARS-COV2 while there was not obviously any electrostatic interaction.

In figure 4, the hydrogen bonds and steric interactions were shown that determine the interlock for *5, 5'-bi (6, 7- dihydroxycoumarin)*. It can be seen that His 41 (A), His 164 (A), Cys 145 (A), Glu 166 (A) and Met 165 (A) amino acids of the main protease of SARS-COV2 were in the hydrogen interactions with this phytochemical, while the steric interactions were Leu 141 (A), Phe 140 (A), Glu 166 (A) and Met 165 (A) amino acids.

This paper offers alogical insight into theinhibitory effect of anti-HCVbicoumarinsfrom *Viola philippica*opposing the main protease of SARS-COV2. Overall, *5, 5'-bi (6, 7- dihydroxycoumarin)* were introduced for SARS-COV2 treatment.

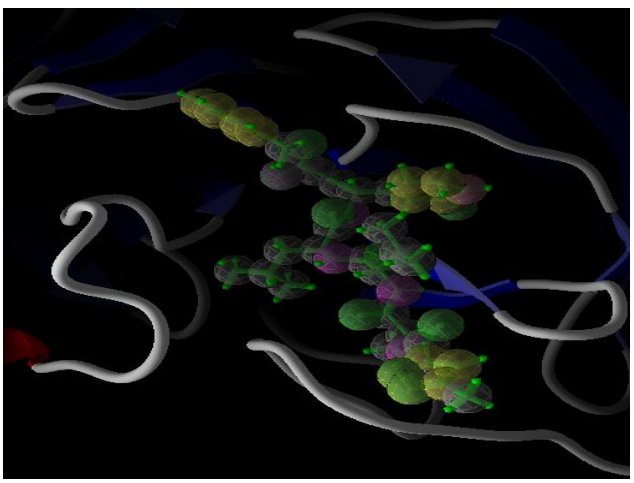


Fig. 1 Visualization of template groups of alpha-ketoamide (green);16 ring groups (yellow),6 hydrogen acceptor (green), 9 hydrogen donor (purple), and 49 steric criteria (gray).

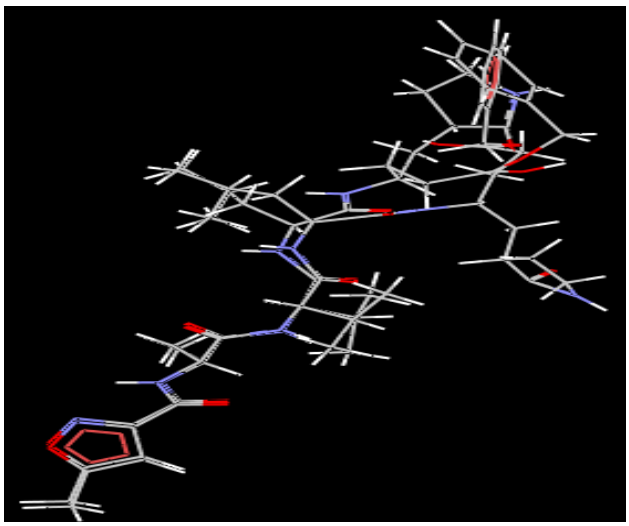
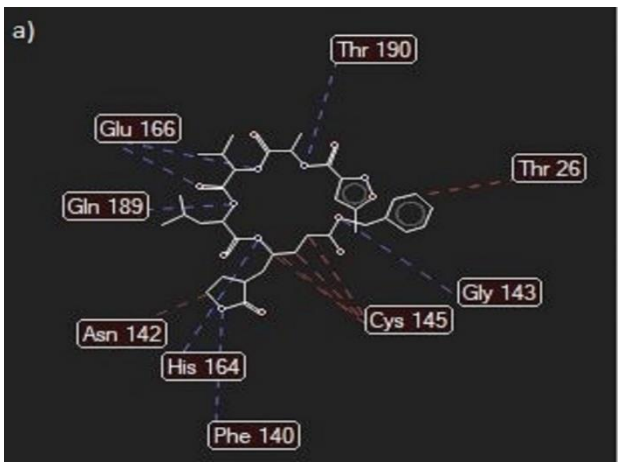


Fig. 2 Superimposed structures of native and docked alpha-ketoamide.



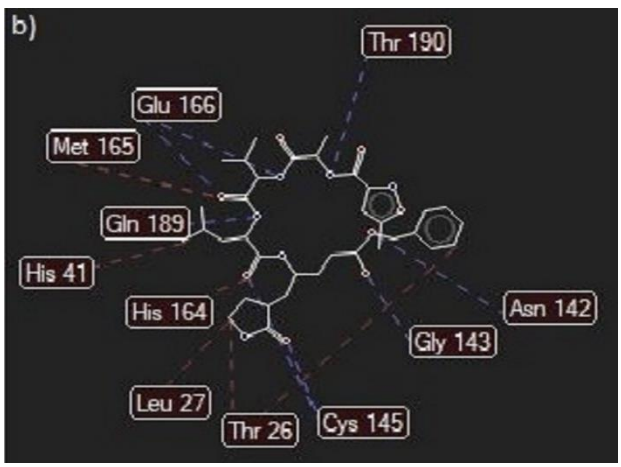


Fig. 3 Hydrogen bonds (red dash) and steric interactions (blue dash) of native and optimal conformation of alpha-ketoamide.

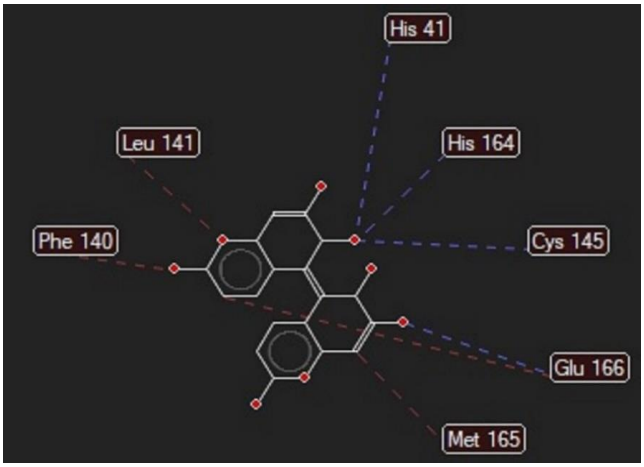


Fig. 4 Hydrogen bonds (red dash) and steric interactions (blue dash) of optimal conformation of *5, 5′-bi (6, 7-dihydroxycoumarin)*.

Conclusion

The inhibition effect of *5, 5′-bi (6, 7-dihydroxycoumarin)* and *6,6′,7,7′- tetrahydroxy-5,8′-bicoumarin*was validatedas opposed to the main protease of the SARS-COV2through Molecular docking method. The inhibitor activity of theseanti-hepatitis coumarins is estimated by evaluation of their binding energy and conformation.The values of predicted binding energy indicated the ability of *5, 5′-bi (6, 7- dihydroxycoumarin)* for SARS-COV2 therapy. This value was equal-8.07 (Kcal/Mol). The optimal conformation of the phytochemical in the inhibitory site of SARS-COV2 was selected by MolDockscore. A collection of template groups includeshydrogen donor and acceptor, negative and positive charge, ring groups and steric were involved for estimation of the best conformation. The phytochemical inhibitor *5, 5′-bi (6, 7-dihydroxycoumarin)* from *Viola philippica*was discovered for SARS-COV2 therapy.

Reference

Abdelmohsen, U.R. and *et al.* 2021. "Natural coumarins as potential anti-SARS-CoV-2 agents supported by docking analysis." RSC Advances, 11(28): 16970-16979.

Aberoumand, A. 2012. "Screening of phytochemical compounds and toxic proteinaceous protease inhibitor in some lesser-known food based plants and their effects and potential applications in food." International Journal Food Science and Nutrition Engineering, 2(3): 16-20.

Adhikari, S.P. and *et al.* 2020. "Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review." Infectious Diseases of Poverty, 9(1): 1-12.

Ahlquist, P. 2002. "RNA-dependent RNA polymerases, viruses, and RNA silencing." Science, 296(5571): 1270-1273.

Akher F.B. and *et al.* 2019. "Discovery of novel natural flavonoids as potent antiviral candidates against hepatitis C virus NS5B polymerase." Medical hypotheses, 132: 109359.

Chauhan, P, M. Shakya. 2009. "Model based design of inhibitors for c-jun." Bioinformation, 4(6): 223.

Chidambaram, S. and *et al.* 2021. "Synthesis of novel coumarin analogues: Investigation of molecular docking interaction of SARS-CoV-2 proteins with natural and synthetic coumarin analogues and their pharmacokinetics studies." Saudi Journal biological sciences, 28(1): 1100.

Chidambaram, S.K. and *et al*. 2020. "In silico molecular docking: Evaluation of coumarin based derivatives against SARS-CoV-2." Journal of infection and public health, 13(11): 1671-1677.

Dhama, K. and *et al*. 2018. "Advances in designing and developing vaccines, drugs, and therapies to counter Ebola virus." Frontiers in immunology, 9: 1803.

Fraser, C.S. and *et al*. 2009. "The pathway of hepatitis C virus mRNA recruitment to the human ribosome." Nature structural & molecular biology, 16(4): 397.

http://mgltools.scripps.edu.

HyperChem, H. 2002. "Release 7 for windows." HyperCube, Ed.

Jin, Z. and *et al.* 2020. "Structure of Mpro from COVID-19 virus and discovery of its inhibitors." bioRxiv.

Kim, D.W. and *et al.* 2014. "Phenolic phytochemical displaying SARS-CoV papain-like protease inhibition from the seeds of Psoralea corylifolia." Journal of enzyme inhibition and medicinal chemistry, 29(1): 59-63.

Kotha, S. and *et al.* 2015. "Identification of Novel CDK Inhibitors by Molecular Docking and Consensus Scoring Approach." International Journal Computer Applications, 975: 8887.

Lai, C.C. and *et al.* 2020. "Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges." International Journal antimicrobial agents, 105924.

Matos, M.J. and *et al*. 2015. "Coumarins—an important class of phytochemicals." Phytochemicals-isolation, characterisation and role in human health, 113-140.

Maurya, A.K. and N. Mishra. 2020. "In silico validation of coumarin derivatives as potential inhibitors against Main Protease, NSP10/NSP16-Methyltransferase, Phosphatase and Endoribonuclease of SARS CoV-2." Journal Biomolecular Structure and Dynamics, 1-16.

Menezes, J.C. and M.F, Diederich. 2019. "Natural dimers of coumarin, chalcones, and resveratrol and the link between structure and pharmacology." European Journal medicinal chemistry, 182: 111637.

Milenković, D.A. and *et al*. 2020. "Several coumarin derivatives and their Pd (II) complexes as potential inhibitors of the main protease of SARS-CoV-2, an in silico approach." RSC Advances, 10(58): 35099-35108.

Moreno-Eutimio, M.A. and *et al*. 2020. "Bioinformatic analysis and identification of single-stranded RNA sequences recognized by TLR7/8 in the SARS-CoV-2, SARS-CoV, and MERS-CoV genomes." Microbes and infection, 22(4-5): 226-229.

Muppalaneni, N.B. and A.A, Rao. 2012. "Virtual screening of plant derived compounds for aldose reductase inhibition using molecular docking." Bioinformation 8(20): 980.

Nasution, M. and *et al*. 2018. Discovery of Indonesian natural products as potential inhibitor of Ebola virus VP40 through molecular docking simulation. AIP Conference Proceedings, AIP Publishing LLC.

Ogungbe, I.V. and *et al*. 2010. "Phytochemical investigation of Verbesina turbacensis Kunth: Trypanosome cysteine protease inhibition by (–)-bornyl esters." Natural product communications, 5(8): 1934578X1000500801.

Pal, M. and *et al*. 2020. "Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update." Cureus, 12(3).

Patick, A,K. Potts. 1998. "Protease inhibitors as antiviral agents." Clinical microbiology reviews 11(4): 614-627.

Raj, U. and P.K. Varadwaj. 2016. "Flavonoids as multi-target inhibitors for proteins associated with Ebola virus: In silico discovery using virtual screening and molecular docking studies." Interdisciplinary Sciences: Computational Life Sciences, 8(2): 132-141.

Reynolds, C.H. and *et al*. 2008. "Ligand binding efficiency: trends, physical basis, and implications." Journal medicinal chemistry, 51(8): 2432-2438.

Thomsen, R. and M.H. Christensen. 2006. "MolDock: a new technique for high-accuracy molecular docking." Journal of medicinal chemistry, 49(11): 3315-3321.

Torres, P.H. and *et al.* 2019. "Key topics in molecular docking for drug design." International Journal molecular sciences, 20(18): 4574.

Veljkovic, V. and *et al*. 2015. "Virtual screen for repurposing approved and experimental drugs for candidate inhibitors of EBOLA virus infection." F1000Research 4.

Wang, Y.L. and *et al*. 2019. "Lignans, flavonoids and coumarins from Viola philippica and their α-glucosidase and HCV protease inhibitory activities." Natural product research, 33(11): 1550-1555.

Yañez, O. and *et al*. 2020. "In Silico Study of Coumarins and Quinolines Derivatives as Potent Inhibitors of SARS-CoV-2 Main Protease." Frontiers in Chemistry 8.

Zakaryan, H. and *et al*. 2017. "Flavonoids: promising natural compounds against viral infections." Archives of virology, 162(9): 2539-2551.