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Original Research

Computational hunting of natural active compounds as an alternative for Remdesivir to target RNA-dependent polymerase

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Abstract: The hunt for potential lead/drug molecules from different resources, especially from natural resources, for possible treatment of COVID-19 is ongoing. Several compounds have already been identified, but only a few are good enough to show potential against the virus. Among the identified druggable target proteins of SARS-CoV-2, this study focuses on non-structural RNA-dependent RNA polymerase protein (RdRp), a well-known enzyme for both viral genome replication and viral mRNA synthesis, and is therefore considered to be the primary target. In this study, the virtual screening followed by an in-depth docking study of the Compounds Library found that natural compound Cyclocurcumin and Silybin B have strong interaction with RdRp and much better than the remdesivir with free binding energy and inhibition constant value as '-6.29 kcal/mol and 58.39 µM', and '-7.93kcal/mol and 45.3 µM', respectively. The finding indicated that the selected hits (Cyclocurcumin and Silybin B) could act as non-nucleotide anti-polymerase agents, and can be further optimized as a potential inhibitor of RdRp by benchwork experiments.

Key words: Virtual screening; Remdesivir, COVID-19; Cyclocurcumin.

Introduction

The threat of the COVID-19 (2019 novel coronavirus disease) is expeditiously increasing and leading to cause a global health and economic issue, causing more than 26 million cases leading to ~873,000 deaths all-inclusive, with a probable mortality rate of ~3.3 % (1). The first patient detected to have fallen from infection caused by COVID-19 was on December 01, 2019 (2-4). On March 11, 2020, the World Health Organization announced COVID-19 as a worldwide pandemic because of the rapid SARS-CoV-2 propagation globally. Now there are no effective pharmacological therapies that can evoke viral confinement and removing SARS-CoV-2 infections effectively and easily, as well as no large-spectrum medications for other pathogenic coronaviruses, except the analog nucleotide remdesivir. Several other potential drugs are currently being considered as an alternative that involves antiviral drugs (e.g. immunoglobulin, favipiravir, azvudine, and danoprevir), antimalarial drugs (hydroxychloroquine), antibiotics and antiparasitics (e.g. carrimycin, and ivermectin), kinase inhibitors, monoclonal antibodies, hormonal preparations, cardiovascular drugs and viamins (5, 6). Even to date there is no approved vaccine against this virus, although there are many in various phases of the clinical trial. A total of 182 vaccine candidates for which 122 are in pre-clinical and 58 are in human trials (https://www.who.int/). Various platforms exist for the design of candidate vaccines for COVID-19 which includes nucleic-acid vaccines, non-replicating viral vectors vaccines, virus vaccines, and protein-based vaccines (7).

The sequenced whole-genome of causative pathogen (SARS-CoV-2) determined that it is closely associated with severe acute respiratory syndrome coronavirus (SARS-CoV) and is the 5th strain of beta coronaviruses (8, 9). The prevalence of contact-to-contact transmission of coronavirus is probably due to the high virus spike protein interaction with the host receptor (10-12), leading to a rapid increase of infection globally.

The viral RNA synthesis is catalyzed by the RNA-dependent RNA polymerase (RdRp, also named as nsp12), a vital element of coronavirus replication or transcription machinery, and therefore consider as the important therapeutic target for antiviral drugs (13). RdRp has minimal activity alone (14), but its polymerase activity is greatly enhanced by the association of nsp7 and nsp8 cofactors. NSP7 and NSP8 maximize RdRp binding and assassination as accessory factors. Like other polymerases, RdRp has a polymerase domain whose structure resembles a cupped "right hand". A fingers domain, a palm domain, and a thumb domain are also present in the polymerase domain (15, 16). RdRp is important for viral genome replication as well as viral mRNA synthesis (13, 17). RdRp is therefore regarded as the main target for analog antiviral nucleotide inhibitors i.e. remdesivir which have an active drug within the cells and display a possible application of COVID-19 therapy.

Remdesivir was originally established for the control of Ebola and Marburg viruses. Studies have shown that remdesivir lowers viral titers and viral RNA in both

SARS-CoV-1 and MERS-CoV infections of primary human epithelial airway cell cultures *in vitro*. Currently, 44 global trials to examine its efficacy in SARS-CoV-2 infection have been recorded (https://clinicaltrials.gov).

The challenges continue with battles against this novel coronavirus disease in several countries and search for effective remedy is need of time. In the present work, using the *in silico* art of techniques, we have anticipated the possible inhibitors (phytochemicals) against SARS-CoV2 RdRp.

Materials and Methods

Database collection and refinement

Natural compounds were obtained from the ZINC database (https://zinc.docking.org) by narrowing the results as selecting "nautral_products" as a subset and then filter the results by choosing "in Trials" under the 'Bioactive And Drugs' category. A total of 452 compounds were resulted and were downloaded in .sdf format. These compounds were imported in Discovery Studio (DS) 2020 and processed by using the ligand preparation tool. The prepared compounds library was screened against the active site of RdRp using the DS Libdock tool and the AutoDock Vina (version 1.1.2) program inside the PyRx (version 0.8) software to detect possible lead compounds.

Protein structure preparation

The RdRp protein 3D structure [PDB ID: 7BV2] was obtained from the protein data bank. Co-crystalized ligands and water molecules were removed and protein was prepared in monomer form by DS 2020 using protein preparation wizard and saved in .pdb format.

Receptor-based virtual screening

The prepared compounds library was screened against the active site of RdRp using LibDock tool of DS and AutoDock Vina (version 1.1.2) program within PyRx (version 0.8) software. The compound library was imported into PyRx workspace in sdf format and translated into pdbqt format by Open Babel in PyRx software. Finally, the top-ranked compounds were further processed for in-depth docking analysis.

Molecular docking

Two best screened natural compounds were further docked to the RdRp catalytic site using the 'Autodock4.2' program. The energy of ligands was minimized by the MMFF94 force field. The size of grid box parameters were set to 24×22×28 Å. The x, y, and z values were kept as 91.758, 92.459, and 103.771, respectively. All other parameters have been set to standard. The results of the docking simulations have been visualized and analyzed by the DS 2020 program.

LIGPLOT+ analysis

After the docking, LIGPLOT+ Version v.2.1 analyzed the phytochemical-RdRp complexes to classify hydrogen and hydrophobic interactions between the essential important amino acid residues of RdRp and phytochemicals. The 3-dimensional structures of 'phytochemicals-RdRp' interaction generated were transformed into 2-dimensional figures using the LIGPLOT algorithm.

ADME prediction

The identified top-ranking potential hits were subjected to ADMET (absorption, distribution, metabolism, elimination, toxicity) predictions using the PreADMET web tool, which includes caco-2, MDCK, BBB, HIA, plasma protein binding, and skin permeability data. PreADMET is an online platform for estimating ADME data and creating a drug-like library using the *in silico* approach.

Results

Structure-based virtual screening, computational docking, and Ligplot analysis

First, we conducted high-throughput computational screening of various natural compounds retrieved from the ZINC database against the SARS-CoV-2 RdRp active site, followed by computational docking and Ligplot experiments. In this study, Cyclocurcumin and Silybin B have demonstrated effective interactions with RdRp compared to other compounds. Cyclocurcumin has shown to interact with RdRp across 11 amino acid residues, namely Trp617, Asp618, Tyr619, Pro620, Lys621, Cys622, Asp623, Asp760, Asp761, Cys813, and Ser814. (Figure 1A); while 13 amino acid residues, namely Val588, Ile589, Gly590, Thr591, Ser592, Lys593, Leu758, Ser759, Asp760, Asp761, Cys813, Ser814 and Gln815 of RdRp was found to interact with Silybin B (Figure 1B).

The binding energy for Cyclocurcumin–RdRp, and Silybin B–RdRp catalytic domain interactions was found to be -6.29 kcal/mol, and -7.93 kcal/mol, respectively, while the inhibition constant for the same

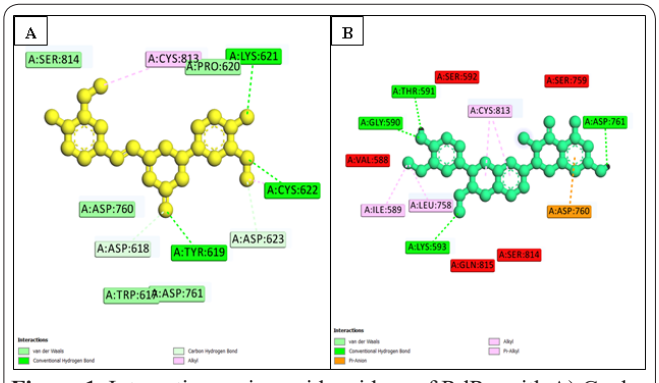

Figure 1. Interacting amino acid residues of RdRp with A) Cyclocurcumin, and B) Silybin B.

Table 1. Binding energy and inhibition constant of phytochemicals with RdRp.

Compound	Binding Energy (kcal/mol)	Inhibition Constant (μM)
Remdesivir	-5.26	748.83
Cyclocurcumin	-6.29	58.39
Silybin B	-7.93	45.3

Table 2. ADME properties of top screened compounds Silybin B and Cyclocurcumin.

ID	Value	
	Silybin B	**Cyclocurcumin**
BBB	0.0608989	0.09951
Buffer_solubility_mg_L	9.00919	344.138
Caco2	4.84461	26.8009
CYP_2C19_inhibition	Inhibitor	Inhibitor
CYP_2C9_inhibition	Inhibitor	Inhibitor
CYP_2D6_inhibition	Non	Non
CYP_2D6_substrate	Non	Non
CYP_3A4_inhibition	Inhibitor	Inhibitor
CYP_3A4_substrate	Weakly	Weakly
HIA	78.550647	94.138899
MDCK	0.0556942	0.125313
Pgp_inhibition	Non	Inhibitor
Plasma_Protein_Binding	87.754608	90.515082
Pure_water_solubility_mg_L	1.09166	11.4956
Skin_Permeability	-4.23602	-2.9169
SKlogD_value	2.37608	3.5204
SKlogP_value	2.37608	3.5204
SKlogS_buffer	-4.72876	-3.02957
SKlogS_pure	-5.64536	-4.50577

was 58.39 µM, and 37.35 µM, respectively (Table 1). Amino acid residues Gly590, Thr591, and Leu758 of RdRp were found to make hydrogen bond with Silybin B (Figure 2A); while Asp760 and Cys813 are the common hydrophobic interacting amino acid residues of RdRp with Cyclocurcumin and Silybin B (Figure 2B). The identified compounds (Cyclocurcumin and Silybin B) exhibited promising ADMET properties, none of which were found to contain any high-risk chemical group (Table 2). Figure 3 indicates the surfaces of the receptors, such as interpolated charge, H-bond, hydrophilicity and solvent-accessible surface (SAS). The hydrophobicity surface suggested that the receptor inside the molecule has more hydrophilic properties. SAS is the surface area of a protein that can be covered by a solvent. Here, in both receptor ligand complexes, the SAS surface revealed that 7BV2 residues had a relatively high SAS propensity.

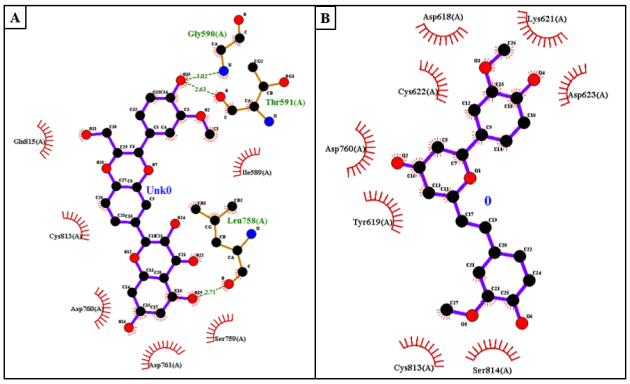

Figure 2. Ligplot analysis of RdRp showing the hydrophobic interacting amino acid residues (red arcs) and hydrogen bond (green dashed line) with **A)** Silybin B, and **B)** Cyclocurcumin.

Discussion

Phytochemicals contain a range of molecules with various curative potentials and have been recognized as a significant resource for the development of new drug therapies (18). Natural product-derived phytochemicals can be used to create more active drugs based on natural compound structures (19, 20). RdRp is a conserved enzyme that is essential for the replication and transcription of coronavirus genomes (15, 21).

The degree of interaction between ligand and protein is determined by binding energy and the high (negative) energy implies the successful binding of the inhibitor with its target protein (22, 23). Consequently, the detachment rate of such a ligand with its target protein is lower and this ligand may have an increased half-life (24). Accordingly, Cyclocurcumin and Silybin B have successful binding to RdRp compared to other compounds and are much stronger than the remdesivir in terms of binding capacity in this study (Table 1). Remdesivir is the only authorized treatment for the person suffering from serious COVID-19 infection (25). Curcumin derivatives such as dihydrocurcumin glucuronide and curcumin sulfate have shown effective RdRp binding in a recent study (26). Computational experiments targeting RdRp's active site investigated many natural compounds including hesperidin, glycyrrhizin, quercetagetin, myricetin to be an effective inhibitor (27, 28).

Ligplot/2D interaction analysis shows that Cyclocurcumin and Silybin B make hydrogen and hydrophobic interaction with the important amino acid residue of RdRp. It has been revealed that both hydrogen and hydrophobic interaction are vital in the stability of inhibitors to its corresponding protein (29, 30).

The RdRp active site comprises of seven conserved motifs (A to G). Motifs A, B, C, and D are from the palm subdomain, with the amino residues Ser759, Asp760, and Asp761 in motif C form the active site center (31).

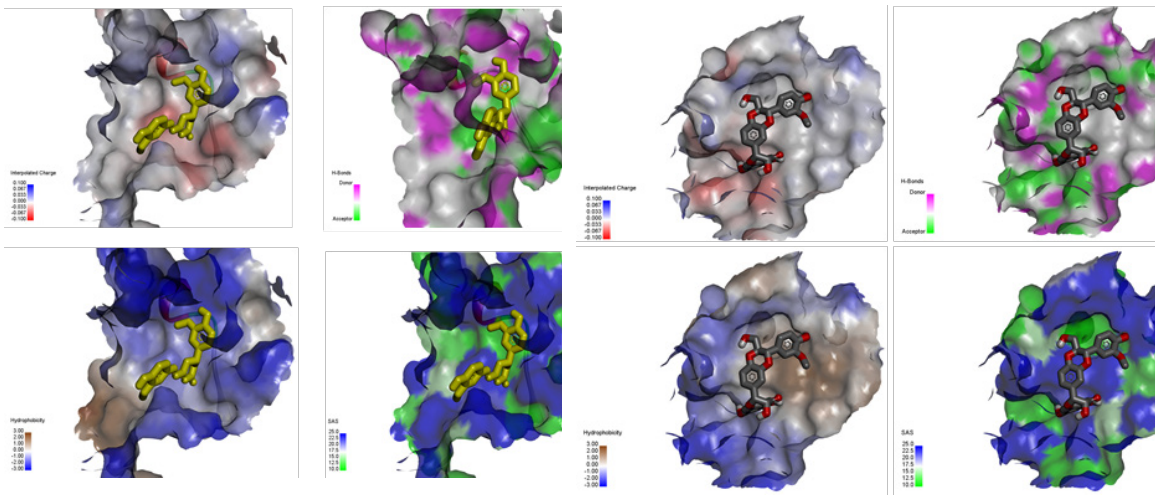

Figure 3. The schematic representation of interpolated charge, H-bond, hydrophobicity, and SAS of 7BV2 around Silybin B, and Cyclocurcumin.

Interestingly, RdRp's amino residue Ser759, Asp760, and Asp761 have been shown to interact with Silybin B in this study. Besides, Cyclocurcumin has also been found to bind with amino residue Asp760 of RdRp. The result indicated that the selected hits (Cyclocurcumin and Silybin B) could act as non-nucleotide anti-polymerase agents. The uniqueness of this study is that these natural compounds can be further tested for antiviral efficacy, as our computation study indicates that these compounds have a better binding efficacy to the RdRp active site than remdesivir and are naturally less toxic.

Various techniques (*in silico*, *in vitro,* and *in vivo*) are being used to find out the cure for the COVID-19 global pandemic. In this research, *in silico* screening technique was used to identify a possible anti-viral agent for a novel infection with COVID-19. Compounds obtained from virtual screening were subjected to molecular docking experiments to assess their interactive stability at the RdRp catalytic site and finally was found that Cyclocurcumin and Silybin B efficiently bind with RdRp and can be further studied (*in vitro* and *in vivo* assessments) for their optimization as a potential inhibitor of RdRp.

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Conflict of Interest

The authors declare no conflict of interest.

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