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Original Research

Combinatorial approach of vitamin C derivative and anti-HIV drug-darunavir against SARS-CoV-2

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Abstract

Background: Coronavirus disease-2019 (COVID-19) has become a pandemic around the globe due to the Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2), a new variant of the Coronavirus (CoV) family. The rapid transmission of the infectious disease, 135,646,617 positive cases from which 2,930,732 mortality cases were recorded until 11 April 2021. In an emergency, several existing anti-viral, anti-malarial, and anti-HIV drugs have been used on a repurposing basis. However, without proper clinical evidence, it may create several side effects for the patient. Thus, recommending potential and less-toxic regimens at this emergency stage is the most crucial aspect for any physician. **Methods**: We have hypothesized a combinatorial drug approach against COVID-19 and to select potential combinations from ten anti-HIV drugs and ten vitamin C derivatives were systematically validated using advanced bioinformatic tools. Initially, the chemical structures used as ligands from PubChem and the target protein, SARS-CoV-2 main protease (PDB ID: 6Y84) from the protein data bank were retrieved for this study. Further, assess the potency, toxicity, drug-ability, and pharmacokinetics profiles using several bioinformatics tools, viz., molecular docking by the AutoDock 4.1 software with predicting activity spectra for substances, Molsoft, ProTox, and SwissADME tools. Molecular dynamics simulation was also employed for most potential candidates to assess their binding stability using GROMACS 5.1.4 software. **Results**: The above computational investigation indicated that 'darunavir with L-ascorbyl-2,6-dibutyrate or ascorbic acid-2-sulfate' combinations strongly inhibit the SARS-CoV-2-main protease as a potential treatment option against COVID-19. Mostly, vitamin C derivatives enhanced the anti-COVID activity and might reduce the post-treatment side effects of darunavir in combination. **Conclusions**: Overall, the present work suggests that bioinformatics tools are suitable for recognizing potential candidates in an emergency, and herein the selected 'anti-HIV-drug-vitamin c derivatives' cocktails may potential-cum-fewer toxic regimens against COVID-19 treatment.

Keywords: Coronavirus; COVID-19; Pandemic; Primary health care; Compute-aided drug design; Combinatorial drug approach

1. Introduction

The emerging Coronavirus disease-2019 (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has continuously added a higher number of morbidity and mortality cases, globally [1–3]. Under this unrestrained grievous health endemic situation, the World Health Organization (WHO) has declared it as a Public Health Emergency of International Concern [1,4]. The outbreak and quick transmission of the COVID-19 is terrible than the previously emerged SARS-CoV and any other viral diseases [2,5]. The symptoms exhibited by the patients under COVID-19 infection are similar to that of the previously SARS-CoVs infection, including fever-cough, complicity during the breath, muscle pain, sore throat and sputum production. However, severe pneumonia and multi-organ dysfunction under COVID-19 infection have been found to enhance the mortality rate, predominantly in immune-compromised patients, i.e., in the elderly age group (>60 years) and patients with co-morbidities [4,6].

The food and drug administration (FDA) has not recommended any drug(s) rather re-purposed a few medications to treat this harmful infectious disease to tackle the pandemic in an emergency. However, fighting against the emerging aggressive positive-sense single-strand RNA or (+) ssRNA viral infection without effective medicine is the most significant panic situation for protecting the global health system [7,8]. Indeed, experts or physicians used several alternative combinations of existing anti-viral, anti-malarial, anti-HIV (human immunodeficiency virus), and anti-inflammatory drugs on a non-random trial basis without much clinical experience to control the situation [6,9,10]. Mainly, approved anti-viral drugs such as baloxavir marboxil, darunavir, favipiravir, lopinavir, oseltamivir, remdesivir, ritonavir, etc., along with several immune-modulatory, anti-inflammatory remedies such as Fingolimod, Sarilumab and Tocilizumab are used in

multiple combinations against COVID-19 [9–11]. Even the combined treatment with obsolete anti-malarial quinine derivatives such as hydroxyl-chloroquine with the macrolide (azithromycin) and tetracycline (doxycycline) class of antibiotics is continuously used as an alternative against COVID-19 [11,12]. The COVID-19 is an aggregated form of previously recognized SARS and the Middle East respiratory syndrome (MERS) virus and its genome was found to have >70% genomic similarity with the previous variants. Thus, previously recommended drug combinations for SARS and MARS on a repurposing basis, now used against SARS-CoV-2. Strategically applied drug combinations or combinatorial formulae seem to yield a positive response against SARS-CoV-2. Nevertheless, several issues also exist with drug dose combination and its post-treatment side effects [13,14].

In parallel, some claims for using certain Traditional Chinese Medicines (TMC) to control COVID-19 [15,16]. In collaboration with the State Administration of Traditional Chinese Medicine, the Hubei Hospital of Traditional Chinese Medicine filed a trial application to start the phase-I clinical trial with a health-boosting TMC formulation against COVID-19 [17]. Similarly, few also claim for the use of Ayurvedic and Homeopathic formulations from the Indian Unani system and other Western traditional medicines for the treatment of COVID-19 [18,19]. As per previous reports, homeopathic remedies also successfully prevented Cholera, Spanish Influenza, Yellow fever, and Typhoid diseases [20–22]. Thus, locating an active and non-toxic/less toxic combinatorial approach is essential in this emergency.

Therefore, the present work also proposed an alternative health-boosting and immune-modulating 'mainstream-natural combined formula' against COVID-19. High throughput screening approaches were employed in selecting a specific active anti-HIV drug and a vitamin C derivative (VCD) for their possible practice in combination/synergistic for the treatment of COVID-19.

2. Material and methods

2.1 Preparation of ligand and target protein structures for molecular docking study

Based on previous literature and research findings, ten anti-HIV protease inhibitor drugs namely, amprenavir (PubChem ID: 65016), ASC09 (PubChem ID: 53361968), atazanavir (PubChem ID: 148192), darunavir (PubChem ID: 213039), indinavir (PubChem ID: 5362440), lopinavir (PubChem ID: 92727), nelfinavir (PubChem ID: 64143), ritonavir (PubChem ID: 392622), saquinavir (PubChem ID: 441243), tipranavir (PubChem ID: 54682461) and vitamin C or L-ascorbic acid (PubChem ID: 54670067) as a code, VCD-1 with another nine VCDs, VCD-2 (2-O-α-D-glucopyranosyl-l-ascorbic acid with a PubChem ID: 54713602), VCD-3 (L-ascorbyl 2,6-dibutyrate with a PubChem ID: 54693078), VCD-4 (glyceryl ascorbate with a PubChem ID: 67266814), VCD-5 (3-O-ethyl-l-ascorbic acid with a PubChem ID: 150736, VCD-6 (ascorbic acid 2-sulfate with a PubChem ID: 54675759), VCD-7 (6-deoxy-l-ascorbic acid with a PubChem ID: 54679898), VCD-8 (5,6-diacetoxy-l-ascorbic acid with a PubChem ID: 54682503), VCD-9 (3-O-methyl-l-ascorbic acid with a PubChem ID: 11805516), VCD-10 (3,4-dihydroxy-5-(1-hydroxy-2-acetoxyethyl)furan-2(5H)-one with a PubChem ID: 54718197) were retrieved from PubChem data base (https://pubchem.ncbi.nlm.nih.gov/) as ligands for docking against the retrieved three-dimensional protein structure of SARS-CoV-2-protease. Later on, the target enzyme (main protease of SARS-CoV-2 with PDB ID: 6Y84) was retrieved from the protein data bank. The software Avogadro 1.2 was used for the energy minimization of each ligand before the docking study.

Initially, each ligand chemical structure was converted to dot PDB (.pdb) data format, as a required format according to the used docking software, AutoDock 4.1. Later on, based on the binding pattern or molecular interactions from high-throughput molecular docking study against SARS-CoV-2 protease, the lead anti-HIV drugs and VCDs were selected [23,24]. The molecular docking was performed, followed by a multi-step AutoDock tutorial. Again, a double docking study was conducted to assess the combinatorial action of both leading anti-HIV drugs and ascorbic acid derivatives against the same target SARS-CoV-2 protease [25–27]. The molecular interaction and binding pattern of protein-ligand docking complexes were visualized by BIOVIA DSV v 4.5 software [28].

2.2 Possible biological activity prediction and drug-likeness analysis

Furthermore, the physicochemical properties such as molecular weight (MW), number of hydrogen acceptors (H-BA) and donors (H-BD), topological surface area (tPSA), partition or distribution coefficient (XLogP) value, number of rotatable-bonds (RB), molar refractivity (MR), collectively known as standardized Lipinski rules of five (RO5) parameters were recorded from PubChem database. The overall drug-likeness score of individual anti-HIV drugs and VCDs properties was recorded using the tool, MolSoft (https://www.molsoft.com/).

Again, to predict the possible therapeutic potencies such as vaso-protective, respiratory analeptic, immunostimulant, antioxidant, anti-inflammatory and anti-viral properties of ascorbic acid and their derivatives through the advanced bioinformatics tool, the Perdition of Activity Spectra for Substances (PASS) (http://www.pharmaexpert.ru/passonline/) was used. The specific biological activities of each candidate as per the present objectives, probable activity (Pa) and probable inactivity (Pi) values were recorded through data mining processes against the training data set presented in the PASS data library.

Table 1. Recorded docking scores and ligand efficacy against protease of SARS-CoV-2 virus (PDB ID: 6Y84) and overall drug-likeness scores, lethal doses and bioavailability scores of VCDs based on chemical structures.

VCDs	Docking score in kcal/mol (type of interaction and their bond length)	Drug likeness score	Lethal dose (mg/kg)	Bioavailability score
VCD-1	−4.94 (**Lys5**: Conventional-H-bond 2.50 Å; **Trp207**: Conventional-H-bond 2.04 Å; **Leu282**: Conventional-H-bonds 1.97 Å & 2.19 Å; **Ser284**: Conventional-H-bond 2.03 Å; **Glu288**: Conventional-H-bonds 1.81 Å & 2.23 Å)	0.74	3367	0.56
VCD-2	−2.87 (**Pro52**: Conventional-H-bonds 1.86 & 1.90 Å; **Asn53**: Conventional-H-bond 1.97 Å; **Tyr54**: Conventional-H-bond 2.0 Å; **Glu55**: Conventional-H-bonds 1.92, 2.68 Å)	0.53	25000	0.11
VCD-3	−6.19 (**Lys5**: Conventional-H-bonds 2.03 Å, 2.31 Å & 2.50 Å; **Gly127**: Conventional-H-bonds 2.27 Å & 2.91 Å)	0.34	25000	0.56
VCD-4	−4.56 (**Arg4**: Conventional-H-bond 2.13 Å-Carbon-H-bond 3.03 Å; **Lys5**: Conventional-H-bond 2.32 Å; **Trp207**: Conventional-H-bond 2.07 Å; **Leu282**: Conventional-H-bond 2.20 Å; **Ser284**: Conventional-H-bonds 1.90, 2.09 & 2.30 Å)	0.80	5000	0.56
VCD-5	−4.78 (**Phe3**: Carbon-H-bond 3.46 Å; **Lys5**: Conventional-H-bond 2.0 Å; **Trp207**: Conventional-H-bond 2.39 Å; **Leu282**: Conventional-H-bonds 1.72 Å & 1.89 Å; **Ser284**: Conventional-H-bond 2.11 Å; **Glu288**: Conventional-H-bond 2.07 Å)	0.27	5000	0.56
VCD-6	−6.75 (**Phe3**: Conventional-H-bond 2.18 Å; **Arg4**: Conventional-H-bond 2.10 Å; **Lys5**: Conventional-H-bonds-1.68, 1.70, 2.18, 2.83, 3.10 Å; **Glu288**: Conventional-H-bond 2.24 Å)	0.24	10000	0.11
VCD-7	−5.61 (**Phe3**: Conventional-H-bond-2.17 Å; **Arg4**: Conventional-H-bond-2.95 Å- Carbon-H-bond 3.70 Å; **Trp207**: Conventional-H-bond 2.15 Å; **Leu282**: Conventional-H-bonds 1.81 Å & 2.25 Å; **Ser284**: Conventional-H-bond 2.34 Å)	0.11	5000	0.56
VCD-8	−5.98 (**Lys5**: conventional-H-bond 2.05, 2.16, 2.56 & 2.61 Å; **Gln127**: Conventional-H-bonds 2.19, 2.23 & 2.45 Å)	0.12	5000	0.56
VCD-9	−4.72 (**Phe3**: Carbon-H-bond 3.71 Å; **Lys5**: Conventional-H-bonds 2.55, 2.53, 2.58 & 2.71 Å; **Leu282**: Conventional-H-bonds 1.78 & 2.18 Å; Ser284: Conventional-H-bond 1.78 Å-Carbon-H-bond 3.63 Å)	0.45	5000	0.56
VCD-10	−5.79 (**Lys5**: Conventional-H-bonds 1.90, 1.93, 2.42 & 2.57 Å; **Van125**: Conventional-H-bond 2.09 Å; Gln127: Conventional-H-bonds 2.03 & 2.36 Å)	0.41	5000	0.56

VCD-1, L-ascorbic acid; VCD-2, 2-O-α-D-glucopyranosyl-l-ascorbic acid; VCD-3, L-ascorbyl 2,6-dibutyrate; VCD-4, glycerylascorbate; VCD-5, 3-O-ethyl-l-ascorbic acid; VCD-6, ascorbic acid 2-sulfate; VCD-7, 6-deoxy-l-ascorbic acid; VCD-8, 5,6-diacetoxy-l-ascorbic acid; VCD-9, 3-O-methyl-l-ascorbic acid; VCD-10, ascorbic acid-6-acetate.

2.3 Toxicity and pharmacokinetics profile analysis

The tool, ProTox (http://tox.charite.de/protox_II/), was employed to predict the probable toxicity profiles such as hepatotoxicity, carcinogenicity, immunotoxicity toxicity, mutagenicity, cytotoxicity, toxicity class and lethal dose (LD_{50}) values of the selected anti-HIV drugs as well as VCDs. Simultaneously, the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties were assessed using the tool, SwissADME (http://www.swissadme.ch/) for each candidate.

2.4 Molecular-dynamics simulation of selected protein-ligand complexes

To check the stability/flexibility of docking complexes, selected two docking complexes (based on the effective docking score), from each side (one from anti-HIV drugs, i.e., 'main protease-darunavir' and one from VCDs, namely, 'main-protease- ascorbic acid-2-sulfate or VCD-6' were selected for MD simulation with the software GROMACS 5.1.4 (Groningen Machine for Chemical Simulations) in the GROMOS force field in 50 ns time scale [25–27]. In the first step, we have generated topologies files for each complex using the PRODRG server (http://prod

Table 2. Recorded docking scores and ligand efficacy against protease of SARS-CoV-2 virus (PDB ID: 6Y84) and overall drug-likeness scores, lethal doses and bioavailability scores of individual anti-HIV drugs based on chemical structures.

Anti-HIV drugs	Docking score in kcal/mol (type of interaction and their bond length)	Drug likeness score	Lethal dose (kg/mg)	Bioavailability score
Amprenavir	−8.43 (**Lys5**: Pi-alkyl 5.1 Å-Pi-cation 4.1 Å; Ala7: Pi-alkyl-3.98 Å; **Gln127**: Conventional-H-bond 1.66 Å-Carbon-H-bond 3.35 Å; Glu290: Pi-anion 3.50 Å)	1.14	300	0.55
ASC09	−9.56 (**Lys5**: Conventional-H-bonds 1.96 & 2.01 Å-Pi-alkyls 4.76 & 4.82 Å; **Ala7**: Conventional-H-bond 1.86 Å-Pi-alkyls 4.45 Å & 4.76 Å; **Ala116**: Pi-alkyl 5.17 Å; **Gly124**: Pi-sigma-3.84 Å; **Val125**: Conventional-H-bond 1.72 Å-Carbon-H-bond 2.87 Å; **Tyr126**: Pi-alkyl 5.01 Å-Pi-sigma 3.52 Å)	1.09	150	0.17
Atazanavir	−7.37 (**Arg4**: Conventional-H-bond-2.42 Å; **Lys5**: Conventional-H-bond 2.28 Å; **Ala7**: Pi-alkyl 5.30 Å; **Gln127**: Carbon-H-bonds 3.28 & 3.44 Å; **Lys137**: Pi-alkyl 5.30 Å-Pi-cation 4.70 Å; **Asp289**: Pi-anion 3.69 Å)	0.11	200	0.17
Darunavir	−10.25 (**Arg4**: Pi-alkyl 4.85 Å-Pi-loan pair 2.75 Å; **Lys5**: Conventional-H-bonds 2.70 & 2.63 Å-Pi-loan pair 3.34 Å-Pi-alkyl 4.43 Å; **Tyr126**: Pi-alkyls 4.34 & 4.43 Å; **Leu282**: Conventional-H-bond 2.85 Å; **Glu288**: Pi-anions 4.17 & 4.20 Å)	0.60	245	0.55
Indinavir	−7.80 (**Arg4**: Conventional-H-bond 2.14 Å-Carbon-H-bond 3.23 Å; **Lys137**: Pi-alkyls 3.97 & 5.10 Å; **Leu286**: Pi-alkyl 5.42 Å; **Glu288**: Pi-anions 3.44 & 3.99 Å)	1.86	5000	0.55
Lopinavir	−9.00 (**Arg4**: Pi-alkyls 5.31 & 5.48 Å; **Lys5**: Conventional-H-bond-2.20 & 2.66 Å; sulfurs 2.88 & 2.94 Å; **Ser284**: Conventional-H-bond 2.14 Å; **Glu288**: Conventional-H-bond-2.07 Å)	1.10	5000	0.55
Nelfinavir	−9.97 (**Asn133**: Conventional H-bond 1.73 Å; **Gly195**: Conventional H-bond 1.94 Å; **Thr198**: Pi-sigma 3.67 Å)	1.41	600	0.55
Ritonavir	−8.75 (**Arg4**: Conventional-H-bonds 1.71 & 2.0 Å-Carbon-H-bond 3.76; **Lys5**: Conventional-H-bonds 2.51 & 2.56 Å; **Lys137**: Pi-alkyl 4.43 Å; **Val171**: Pi-alkyl 5.38 Å; **Leu286**: Pi-alkyl 4.93 Å; **Glu288**: Pi-alkyl 4.93 Å-Pi-anion 3.41 Å)	0.11	1000	0.17
Saquinavir	−8.97 (**Lys5**: Pi-alkyl 4.48 Å; **Ala7**: Pi-alkyl 4.10 Å; **Gln127**: conventional-H-bond 2.44 & 3.04 Å; **Lys137**: Conventional-H-bond 2.23 Å; **Leu286**: Pi-alkyl 4.64 & 5.11 Å)	0.69	500	0.17
Tipranavir	−9.98 (**Lys5**: Pi-alkyl 4.62 Å-Pi-cation 4.64 Å; **Ala7**: Pi-alkyl 4.46 Å; **Tyr126**: Pi-alkyl 3.93 Å; **Gln127**: Conventional-H-bond 1.77 Å; **Cys128**: Pi-alkyl 4.76 Å; **Glu288**: Pi-anion 3.13 & 3.44 Å)	0.72	333	0.56

rg1.dyndns.org/submit.html). Selected docking complexes with the SPC-E water-cubic box model (whose volume was about 976.40, 976.50, 977.48, 977.48 nm^3, respectively) were simulated and then added a total of 30576, 30564, 30573 and 30563 numbers of solvent molecules to each system of docking complexes. Concurrently, neutralized the system by adding 4 Na+ ions and the energy minimization using 50,000 steepest descent steps for each docking complex. After minimizing the system, the NVT (number of particles, volume and temperature) and NPT (number of particles, pressure, temperature) equilibrations were performed for equilibrating the system for the 100 ps time scale and a 50 ns time scale of MD simulation was performed [25–27].

3. Results

3.1 Preparation of ligand and target protein structures for molecular docking study

As per the research hypothesis, the docking scores (kcal/mol) were used as a standard parameter for selecting the most efficient combination of any vitamin C derivatives with a potent anti-HIV drug against deadly SARS-CoV-2 (Tables 1,2). The docking score of L-ascorbic acid and derivatives was within -7.00 kcal/mol. Among all derivatives, the VCD-6 (ascorbic acid 2-sulfate) with docking score -6.75 (Fig. 1), and the VCD-3 (L-ascorbyl 2,6-dibutyrate) with docking score -6.19 kcal/mol (Fig. 2) were chosen for further studies (Table 1). On the other hand, all the selected ten anti-HIV drugs had docking scores within -11.00 kcal/mol (Table 2). Darunavir was chosen from all anti-viral drugs as the most active drug against SARS-CoV-2 protease, based on the docking a score, -10.25 kcal/mol (Fig. 3). Therefore, based on the overall docking score and molecular interactions, the VCD-6 and VCD-3 among vitamin C derivatives, and darunavir among all ten anti-viral drugs were selected for further studies.

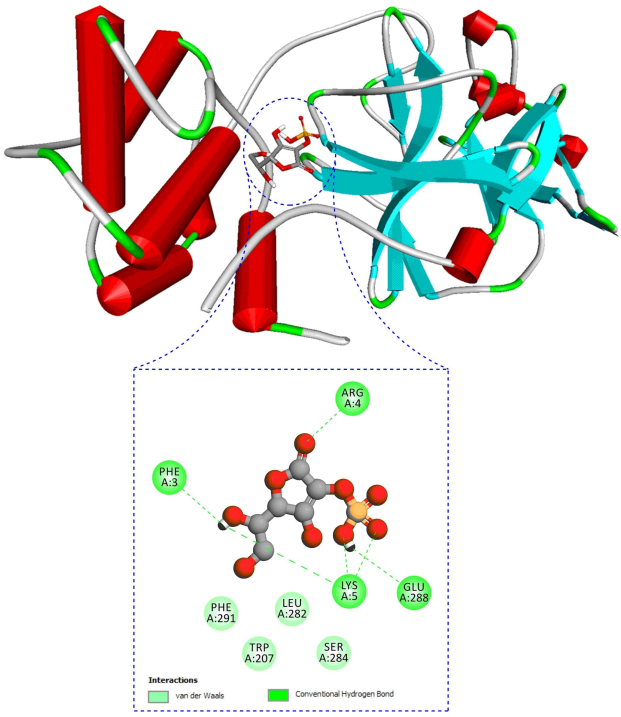

Fig. 1. Molecular interaction of most effective VCD, VCD-6 (ascorbic acid 2-sulfate) with docking score -6.75 kcal/mol against SARS-CoV-2 protease from docking study.

Likewise, a double docking study between darunavir and VCD-6 and VCD-3 against SARS-CoV-2 main protease (VCD-6 and VCD-3 were re-docked against main protease-darunavir docking complex, individually) was performed. It was discernable that the docking score between darunavir and VCD-6 (ligand-ligand) was only -2.05 kcal/mol, but the score was elevated to -10.76 kcal/mol when dual ligands (protein – ligand + ligand) were used in docking studies. Similarly, the docking score between darunavir and VCD-3 alone was -2.45 kcal/mol and the score was found to be enhanced to -10.39 kcal/mol when dual/second ligands were used in docking studies. As per the present hypothesis, a total docking score of both ligand-ligand and dual docking score of -12.81 and -12.84 kcal/mol were anticipated (Fig. 4). Thus, both VCD-6 and VCD-3 are proposed to be used with darunavir in the combinatorial approach against SARS-CoV-2. According to the structural activity relationship (SAR), VCD-6 was comparatively more active as more hydroxy (-OH) groups support establishing strong-H-bond interaction and sulfate groups support enhancing the biological activity.

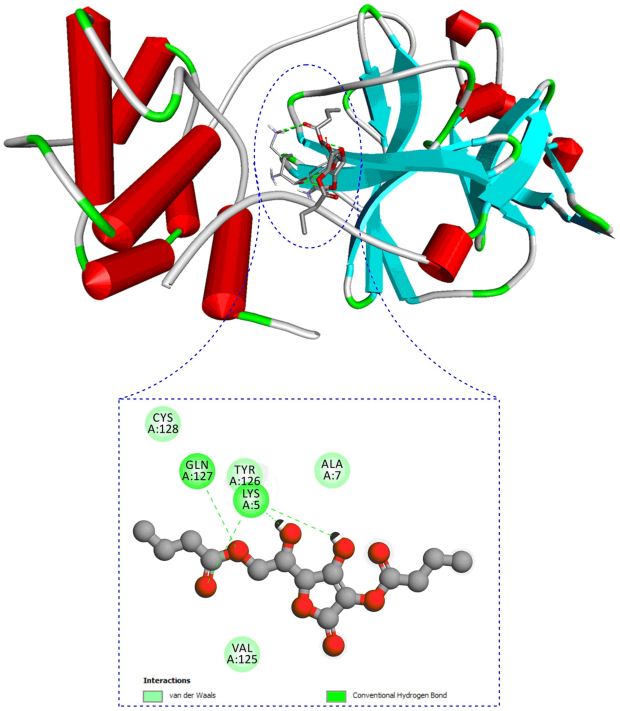

Fig. 2. Molecular interaction of most effective vitamin C derivative, VCD-3 (L-ascorbyl 2,6-dibutyrate) with docking score -6.19 kcal/mol against SARS-CoV-2 protease from docking study.

3.2 Possible biological activity prediction and drug-likeness analysis

Several strategies have been used to screen/filter the potential lead drug candidates passing through a specific standard parameter at the early stage of drug discovery. Herein, after docking interaction, we took several ideal drug candidate selection parameters into account for more evidence of drug-ability at the primary stage based on their chemical composition. During the selection of most combinatorial candidates from vitamin

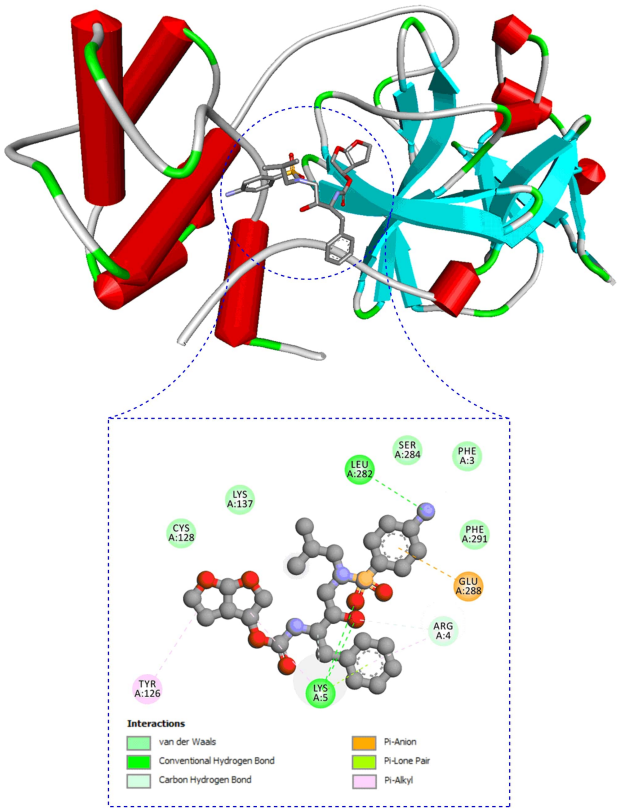

Fig. 3. Molecular interaction of second most effective most anti-HIV drug, darunavir with docking score, -10.25 kcal/mol against SARS-CoV-2 protease from docking study.

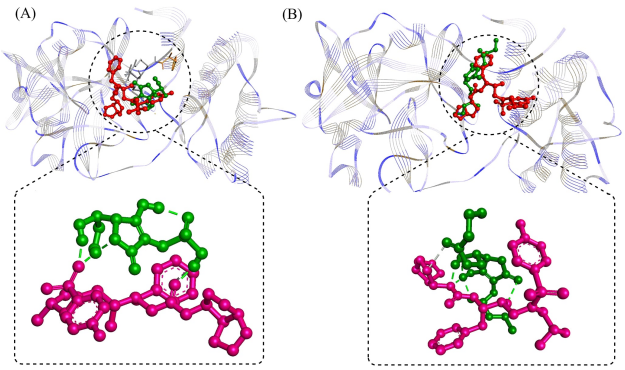

Fig. 4. Combinatorial approach of anti-HIV drug with VCD. (A) Darunavir (in red color) with the VCD-6 (in green color). (B) Darunavir (in red color) with the VCD-3 (in green color) at a time against SARS-CoV-2 protease.

C derivatives, all possible biological activities such as anti-oxidant, anti-inflammatory, immune-stimulant, vaso-protective, anti-viral and respiratory analeptic properties were carefully considered and analyzed by the PASS tool (Table 3). From recorded anti-viral values, only VCD-6 and VCD-2 PASS scores, $0.715 > 0.005$ and $0.715 > 0.002$, respectively, were found to be the most potent and the rest of the derivatives showed moderate anti-viral activity. As per the PASS program, a score $\geq$0.700 is considered as a standard score for any biological activity, and scores within 0.700 to 0.500 are considered as average scores, while a score $<$0.500 is considered as a below average score for any biological activity. Similarly, except for VCD-4, VCD-5, VCD-7 and VCD-10, the rest derivatives were bearing potent antiinflammatory activity (Table 3). However, the most effective derivative based on the docking score, i.e., VCD-6 had moderate immune-stimulant, respiratory analeptic activity. On the other hand, the second-most derivative, i.e., VCD-3 was active in all biological systems with moderate anti-viral ($0.599 > 0.006$) activity. Overall, both VCDs are proposed to be ideal for use in a combinatorial approach with the selected anti-HIV drug against SARS-CoV-2.

Furthermore, based on physicochemical and biological properties, the overall drug-likens score or drug-ability scores of VCDs were recorded (Fig. 5; Table 1). All VCDs were found to possess a favorable positive rating of a drug-ability score to be used as a druggable agent. Notably, VCD-1 and VCD-4 showed comparatively higher drug-ability scores, 0.74 and 0.80, respectively, were recorded. The drug-ability score of VCD-6 and VCD-3 was 0.24 and 0.34, respectively. On the other hand, the drug-likeness score of the most effective anti-HIV drugs, darunavir and tipranavir were recorded to be 0.60 and 0.72, respectively, as per the tool MolSoft (Table 2). The drug-likeness score depends on the chemical composition and characteristics within an acceptable range, such as molecular weight, XlogP value, the number of rotatable bonds, molar refractivity, and the number of hydrogen bond-acceptors and hydrogen bond-donors, topological surface area, etc. As per the principle of MolSoft, the drug-likeness scores range within a 0 to 2 considered in the acceptable drug-likeness score, and less than < 0 and > 2 is considered as a non-drug drug-likeness score for a drug candidate.

3.3 Toxicity and pharmacokinetics profile analysis

The pharmacokinetics and toxicity profile also play a crucial role in the late stage of drug development modules or clinical validation stage before the human recommendation. Thus, the computational tools can predict the possible pharmacokinetic and toxicity profiles of any proposed drug molecule. Accordingly, the used ligands toxicity and the pharmacokinetic profiles were recorded (Tables 4,5). Results indicate that the VCD-anti-HIV drug combination formulation will make it safer and balance the toxicity during administration as VCDs showed potential antioxidative and anti-inflammatory activity. The higher toxicity class-VI for VCD-6 and VCD-3 also indicated that both are non-toxic and safer among all VCDs (Table 4). Additionally, the predicted LD_{50} values (mg/kg) can be considered another conclusive factor in the approval of VCDs in higher concentrations than the selective anti-HIV drug in the combinatorial approach. The documented pharmacokinetics profiles found that the proposed VCDs in the present study are suit-

Table 3. Recorded possible biological activity according to objectives of the study of VCDs from the tool, prediction of activity spectra for substances (PASS).

VCDs	Vaso-protective	Respiratory analeptic	Immuno-stimulant	Anti-oxidant	Anti-inflammatory	Anti-viral
VCD-1	0.948 > 0.002	0.339 > 0.092	0.557 > 0.029	0.928 > 0.003	0.779 > 0.008	0.567 > 0.009
VCD-2	0.979 > 0.001	0.980 > 0.003	0.873 > 0.005	0.929 > 0.003	0.742 > 0.011	0.715 > 0.005
VCD-3	0.934 > 0.002	0.916 > 0.004	0.719 > 0.013	0.797 > 0.003	0.804 > 0.006	0.599 > 0.006
VCD-4	0.949 > 0.002	0.879 > 0.005	0.628 > 0.020	0.875 > 0.003	0.595 > 0.033	0.691 > 0.003
VCD-5	0.944 > 0.002	0.951 > 0.003	0.560 > 0.028	0.897 > 0.003	0.603 > 0.031	0.504 > 0.022
VCD-6	0.917 > 0.002	0.523 > 0.033	0.583 > 0.025	0.821 > 0.003	0.703 > 0.015	0.715 > 0.002
VCD-7	0.880 > 0.003	0.890 > 0.004	0.602 > 0.023	0.918 > 0.003	0.603 > 0.031	0.552 > 0.012
VCD-8	0.878 > 0.003	0.876 > 0.005	0.713 > 0.014	0.868 > 0.003	0.931 > 0.004	0.573 > 0.009
VCD-9	0.919 > 0.002	0.914 > 0.004	0.546 > 0.030	0.911 > 0.003	0.615 > 0.028	0.547 > 0.013
VCD-10	0.905 > 0.003	0.964 > 0.003	0.710 > 0.014	0.915 > 0.003	0.860 > 0.005	0.575 > 0.009

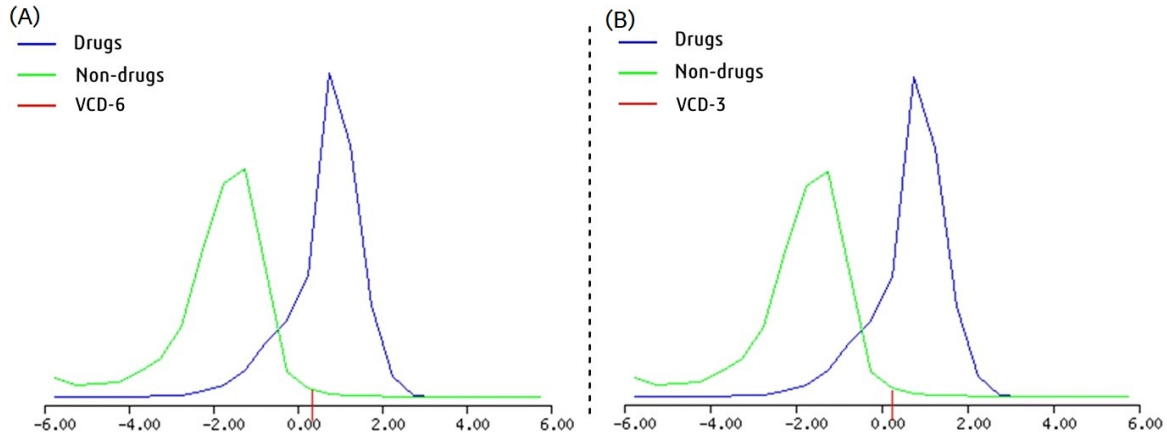

Fig. 5. Overall drug likeness score of two effective most VCDs. (A) VCD-6 (0.24). (B) VCD-3 (0.34), respectively.

able to be used in combination with mainstream medicine (Table 6; Fig. 6). Further, the blood-brain barrier, gastrointestinal absorption, skin permeation profiles are also satisfied also satisfied as promising drug candidates.

3.4 Molecular dynamic simulation of selected protein-ligand complexes

The structural stability of selected two docking complexes was analyzed through the root mean square deviation (RMSD)-protein backbone, root mean square fluctuation (RMSF)-C-alpha and Radius of gyration (Rg) of protein by MD simulation in 50 ns time scale (Figs. 7,8). The elucidated RMSD plot for both 'protease-darunavir' and 'protease-VSD-6' complexes showed a continuous deviation in the backbone protein during 50 ns. Mainly, darunavir holds some stability in stating within 20 ns, while both are in the same fluctuation place at the end, after 45–50 ns (Fig. 7A). At the same from each ligand flexibility study, VCD-6 maintained more stability than darunavir throughout 50 ns (Fig. 7B). Overall, VCD-6 showed comparatively more stability than darunavir from RMSD analysis. Rg-plots and RMSF-plots also showed that both complexes were similar fluctuation with 50 ns MD simulation. Additionally, both ligands could therapeutically potential towards block the viral function through solid H-bond interactions with SARS-CoV-main protease (PDB ID: 6Y84) from the MD simulation (Fig. 8).

4. Discussion

The advanced computation program presently plays an ideal platform to accelerate the drug discovery process with minimal resources and time during the selection of lead drug candidates [26,28,29]. Indeed, advanced computational intelligence is able to mimic the entire human living system through coding/programming languages. Molecular docking is one of the efficient computational platforms used for locating active therapeutic agents, based on the interaction between the targeted enzyme and ligand complexes [25,28]. Molecular docking has also been applied since the last decade to efficiently screen out several potential drug molecules than traditional wet-laboratory approaches. Theoretically, docking score is the sum of the individual molecular interaction of the ligand with the target protein, where types of bonds (conventional H-bond is stronger than other bond formation), bond-length (lesser in the distance (Å)

Table 4. Recorded toxicity profiles with color indication along with toxicity class of VCDs from the tool, ProTox.

VCDs	Hepatotoxicity	Carcinogenicity	Immuno-toxicity	Mutagenicity	Cyto-toxicity	Toxicity class
VCD-1	Safest	Safest	Safest	Safest	Moderately safe	V
VCD-2	Safest	Safest	Safest	Safest	Moderately safe	VI
VCD-3	Safest	Safest	Safest	Safest	Moderately safe	VI
VCD-4	Safest	Safest	Safest	Safest	Moderately safe	V
VCD-5	Safest	Safest	Safest	Moderately safe	Moderately safe	V
VCD-6	Safest	Safest	Safest	Moderately safe	Safest	VI
VCD-7	Safest	Moderately safe	Safest	Safest	Moderately safe	V
VCD-8	Safest	Safest	Safest	Moderately safe	Moderately safe	V
VCD-9	Safest	Safest	Safest	Safest	Moderately safe	V
VCD-10	Safest	Safest	Safest	Moderately safe	Moderately safe	V

The drugs are classified based on the levels of toxicity as Safest and moderately safe.

Table 5. Recorded toxicity profiles with color indication along with toxicity class of anti-HIV drug from the tool, ProTox.

Anti-HIV drugs	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Toxicity class
Amprenavir	Safest	Moderately safe	Safest	Safest	Moderately safe	III
ASC09	Moderately safe	Moderately safe	Toxic/Hazardous	Safest	Moderately safe	III
Atazanavir	Moderately safe	Moderately safe	Safest	Moderately safe	Moderately safe	III
Darunavir	Toxic/Hazardous	Moderately safe	Moderately safe	Moderately safe	Moderately safe	III
Lopinavir	Safest	Safest	Safest	Safest	Safest	V
Indinavir	Toxic/Hazardous	Moderately safe	Toxic/Hazardous	Safest	Moderately safe	V
Nelfinavir	Moderately safe	Moderately safe	Toxic/Hazardous	Safest	Moderately safe	IV
Ritonavir	Toxic/Hazardous	Moderately safe	Safest	Safest	Moderately safe	IV
Saquinavir	Moderately safe	Moderately safe	Safest	Safest	Safest	IV
Tipranavir	Toxic/Hazardous	Moderately safe	Moderately safe	Safest	Moderately safe	IV

The drugs are classified based on the levels of toxicity as Safest, moderately safe and toxic/hazardous.

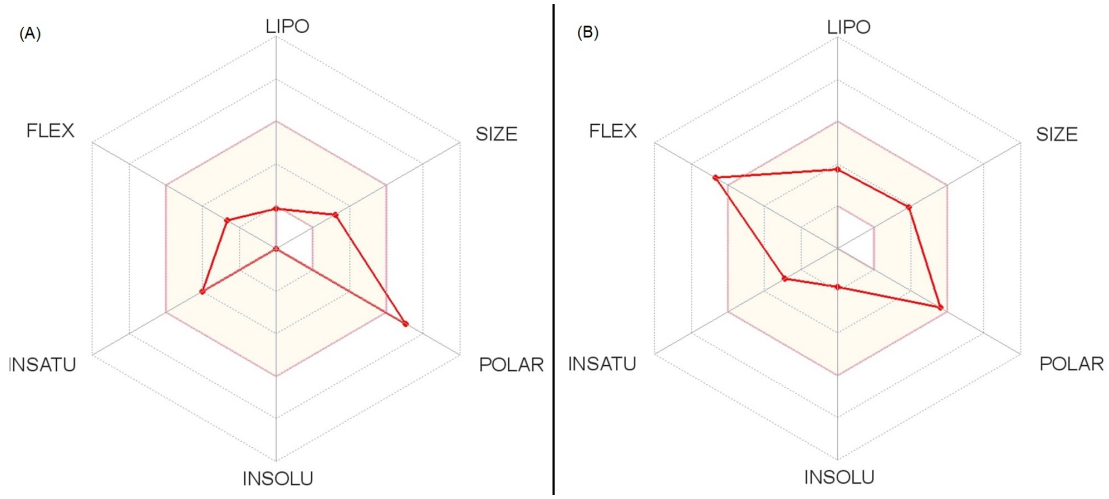

Fig. 6. Overall predicted pharmacokinetics reports of two effective most VCDs. (A) VCD-6. (B) VCD-3.

consider as more in strength) [25–27]. For example, VCD-6 showed an effective docking score as most of the interactions in their complex formed with H-bonds with minimum bond-length (Å) (Table 1). Thus, it will continue to play an essential role in early drug discovery before experimentation [28–30]. Several pharmaceutical industries and exclusive drug research laboratories/stations are also using this advanced technique in novel drug development cascades [29,31].

On the other hand, natural products are always vital for nutraceuticals, pharmaceutical supplements, especially against infectious diseases [32,33]. Identified several

Table 6. Recorded possible pharmacokinetics profiles of VCDs based on its individual chemical structure against a training set drug molecule using the tool, SwissADME.

VCDs	GI-abs.	BBB permit	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p (cm/s)
VCD-1	High	No	No	No	No	No	No	No	−8.54
VCD-2	Low	No	Yes	No	No	No	No	No	−10.55
VCD-3	High	No	Yes	No	No	No	No	No	−7.48
VCD-4	Low	No	No	No	No	No	No	No	−9.69
VCD-5	High	No	No	No	No	No	No	No	−8.61
VCD-6	Low	No	No	No	No	No	No	No	−9.45
VCD-7	High	No	No	No	No	No	No	No	−7.70
VCD-8	High	No	No	No	No	No	No	No	−8.63
VCD-9	High	No	No	No	No	No	No	No	−8.78
VCD-10	High	No	No	No	No	No	No	No	−8.39

BBB, blood-brain barrier; GI-abs., gastrointestinal absorption Log K_p (skin permeation).

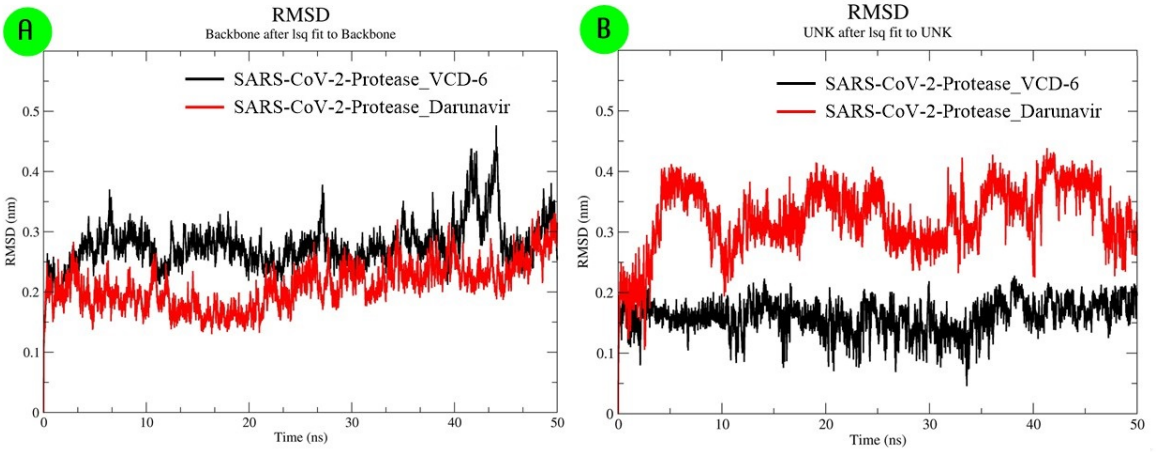

Fig. 7. Conformational stability in the form of RMSD-score. (A) Stability of protease-darunavir' and protease-VCD-6' docking complexes. (B) Individual ligand stability in the docked complexes within 0–50 ns in the overlayed figure.

unique secondary metabolites from different medicinal and aromatic plants are continuously used in different ways for control of several human health ailments [20,32,33]. Indeed, natural products have been in use in several developed western countries as Complementary and Alternative Medicine (CAM) [20,34,35]. Approximately 48% of the population in Australia, 70% in Canada, 42% in the USA, 38% in Belgium and 75% in France communities use phytoextract as CAM in day-to-day life [36]. Indeed, WHO also emphasizes developing or locating natural-product(s) based drug candidates for mainstream use. Simultaneously, ascorbic acid or vitamin C derivatives, having a wide range of biological activity against respiratory tract infection, with anti-bacterial, anti-viral and potent immune-stimulant activities [37–40]. Recently, some non-clinical reports also indicate that vitamin C has potent anti-SARS-CoV-2 activity and is now under clinical validation too (trail no. NCT04323514). On the other hand, anti-HIV drugs are the most suitable therapeutic choice against SARS-CoV-2, as previously exhibited positive response against SARS and MARS on a repurposing basis with severe side effects from long-term practice. Thus, the present attempt with a suitable anti-oxidative and immune-stimulant natural regimen based-combinatorial approach towards control of SARS-CoV-2 may minimize the host toxicity.

In the present scenario, finding a newer drug candidate is a challenge. Most pharmaceutical companies face the same problem as selected lead candidates disappointment in clinical trials due to lack of drug-likeness property, even reported active in preliminary *in vitro* and *in vivo* testing [33,36,41]. Thus, biological activity is a primary parameter for to establishing a lead candidate and several other standard parameters should be followed during clinical trial investigation for recognition in mainstream medicine. The advanced artificial inelegance platform analyzed this parameter for any desired or unknown chemical through a data mining procedure. Hence, the drug-ability investigation based on high throughput computational screening is

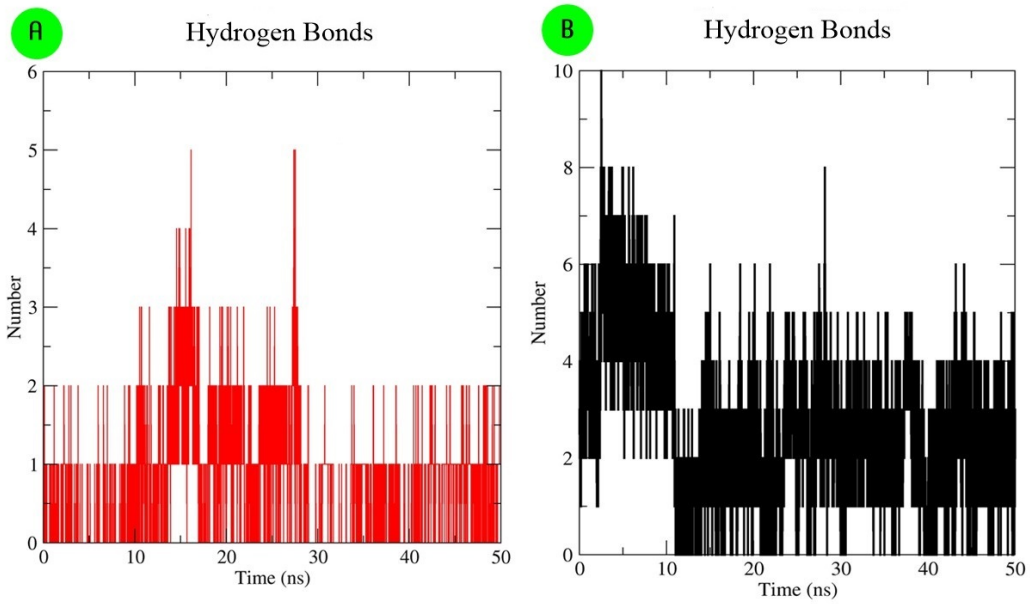

Fig. 8. Interaction stability analyses in the form of H-bond. (A) H-bond interaction of 'protease-darunavir' docking complex. (B) H-bond interaction of 'protease-VCD-6' docking complex during 50 ns.

more beneficial to keep a suitable drug lead candidate at the early stage [23–28].

Several mainstream medicines come from natural resources or contain active biological scaffolds/constituents and require continuous exploration of more natural products to utilize in mainstream medicine to fulfill the unavailability of drugs in an emergency [32,42]. The current bioassay-guided plant extraction and characterization methods have isolated many active secondary metabolites for therapeutic uses. At the same time, advanced medicinal chemistry and bioinformatics tools currently help locate active candidates targeting any specific pathogen/disease to accelerate and give enough reports for early drug discovery [41,42]. Under the emergency, this selected combination could be the right option than any non-clinical hit-and-trial prescription to treat SARS-CoV-2 infected patients. Notably, using two anti-HIV drugs might be associated with a higher risk of hepatic and liver injury to patients. Thus, potent vitamin C could be the right candidate with an anti-HIV drug to manage side effects for its anti-oxidative, anti-inflammatory potency, immune-stimulant, with anti-viral efficacy.

Today, COVID-19 is the most significant pandemic in the 21st century caused by the aggressive mutant SASR-CoV-2 strains in a different interval that the world has been observing since the end of 2019. The lockdown and social distancing as preventive actions to break/pause the viral cycle within the community. However, potential and exclusively vaccine/therapeutic is essential to control the gruesome SARS-CoV-2 strain [43–45]. The combinatorial drug therapy as projected herein using existing medicines and the knowledge of traditional Indian medicine/Ayurveda and traditional Chinese medicine is ingenious and motivated attempts towards control of SARS-CoV-2 and reduces adverse effects from repurposing drugs.

5. Conclusions

From the management and treatment points of view, physicians used several alternative combinations of existing anti-viral, anti-inflammatory and anti-malarial drugs with less clinical experience to tackle the situation of SASR-CoV-2 infection on a case-by-case basis. Still, the post-treatment side effects of two mainstream medicines used in combination drug therapy are under debate. Thus, the present study intends to find a possible potent combinatorial approach with an existing anti-HIV drug, darunavir with a vitamin C derivative against SARS-CoV-2. The potent combination was verified in a cost-effective drug development platform and suggested based on advanced PASS prediction, molecular docking, toxicity-pharmacokinetic profile prediction and MD simulation analyses. It could be a dominant synergistic formula of a natural derivative with an anti-HIV drug to control COVID-19 infection and is expected to produce negligible toxicity due to the presence of anti-oxidative, anti-inflammatory and immune-stimulant natural vitamin C derivative with darunavir. In an emergency, the computer-aided drug design approach utilizing present clinical evidence and genomic information on SARS-CoV-2 gives hints to investigate natural products for its treatment. Finally, verifications of the essential features of prospective drugs loom large ordinarily to a traditional pharmacologist, who would blithely accept the results of bioinformatics analyses for promotion towards drug development screening.

Abbreviations

ADMET, absorption, distribution, metabolism, excretion and toxicity; CAM, complementary and alternative medicine; COVID-19, coronavirus-2019 disease; FDA, food and the drug administration; H-bond, hydrogen bond; HIV, human immunodeficiency virus; LD_{50}, lethal dose; MERS, middle East respiratory syndrome; MD simulation, molecular dynamics simulation; MR, molar refractivity; MW, molecular weight; NPT, number of particles, pressure, temperature; NVT, number of particles, volume and temperature Pa, probable activity; PASS, perdition of activity spectra for substances; Pi, probable inactivity; RB, rotatable-bonds; Rg, Radius of gyration; RMSD, root mean square deviation; RMSF, root mean square fluctuation; RO5, Lipinski rules of five; SAR, structural activity relationship; SARS-CoV-2, severe acute respiratory syndrome corona virus-2; TMC, traditional Chinese medicines; tPSA, topological surface area; VCD, vitamin C derivative; WHO, World Health Organization.

Author contributions

SSS and AS designed and hypothesized the concept. AS carried out the computational work under the guidance of SSS. SSS and AS drafted the MS. BP and MP carefully reviewed and commented on the final MS. All authors contributed to manuscript revision, read, and approved the submitted version.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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