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Identification of potential SARS-CoV-2 inhibitors from South African medicinal plant extracts using molecular modelling approaches

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ABSTRACT

The coronavirus is a group of viruses found in animals as well as humans and have been detected since the 1960s. However, a newly identified form, SARS-CoV-2, has triggered a recent pandemic of respiratory disease now called COVID-19. There is currently no specific antiviral drug for the treatment of this pandemic, with most treatment strategies focused on symptomatic management and supportive therapy. As such, several drug discovery efforts are ongoing for potent treatment agents, with medicinal plants gradually gaining prominence. Approximately 80% of the South African population use traditional medicines to meet their primary health care needs. The current study aimed to identify potential COVID-19 therapeutic agents from a list of 29 bioactive compounds isolated from commonly used South African medicinal plants using molecular docking and molecular dynamics. Molecular docking identified arabic acid from *Acacia senegal* and L-canavanine found in *Sutherlandia frutescens* as a potential inhibitor of SARS-CoV-2 3C-like main protease. Similarly, hypoxoside isolated from *Hypoxis hemerocallidea* and uzarin from *Xysmalobium undulatum*, were identified as a potential inhibitor of SARS-CoV-2 receptor binding domain and SARS-CoV-2 RNA-dependent polymerase. These four bioactive compounds exhibited favourable binding orientations characterized by strong molecular interactions within respective inhibitors binding pockets of the target enzymes. Molecular dynamics simulations revealed that the binding of the identified inhibitors are characterized by structural perturbations which favour the inhibitory potency of these bioactive compounds. Additionally, *in silico* pharmacokinetic assessment of the compounds demonstrated favourable anti-SARS-CoV-2 properties. Although not conclusive, further experimental exploration of these compounds could serve as a starting point for the discovery of novel SARS-CoV-2 therapeutic.

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1. Introduction

Coronavirus 2 is a novel severe acute respiratory syndrome coronavirus. It was initially isolated from three people with pneumonia related to the pattern of acute respiratory illness cases in Wuhan (Spiteri et al., 2020, Zhou et al., 2020). Bioinformatic analysis of SARS-CoV-2 shows it is closely correlated to the original SARS-CoV (Andersen et al., 2020). Now called SARS-CoV-2, phylogenetic analyses shows 96 % similarity of SARS-CoV-2 to the bat SARS-CoV (BatCov RaTG13), like zoonotic Betacoronavirus, it is expected that bats served as hosts for its precursor (Wu. et al., 2020). Like other respiratory diseases, SARS-CoV-2, causes lung

damage with inception of the disease possibly leading to progressive respiratory failure and even mortality due to alveolar damage (Zhou et al., 2020).

Several biological targets have been explored thus far as possible therapeutic targets for treating SARS-CoV-2, however there is still the absence of specific therapy (Wu et al., 2020, Rodríguez-Morales et al., 2020a). Notably, SARS-CoV-2 RNA-dependent RNA polymerase (SARS-CoV-2 RdRp) (Wu et al., 2020, Kirchdoerfer and Ward, 2019, Subissi et al., 2014, Imbert et al., 2006, Chu et al., 2006), SARS-CoV-2 3C-like main protease (3CL^{Pro}) (Anand et al., 2003, Mamidala et al., 2020, Bacha et al., 2004, Yang et al., 2003) and the SARS-CoV-2 receptor binding domain (RBD) (Zhou et al., 2020, Wu et al., 2020, Ge et al., 2013, Rodríguez-Morales et al., 2020b) have been extensively investigated as viable therapeutic targets. The experimentally resolved X-ray crystal structure of

each of these targets has further allowed for studies into the structural dynamics of these enzymes as well aiding in the design of potential inhibitors. Considering the crucial roles of these enzymes in the life cycle of these SAR-CoV-2, inhibiting any of these could reduce the severity of the infection. Currently, there are no clinically approved antibodies exclusively for coronaviruses, making it even more challenging for treating SARS-CoV-2 (Rodríguez-Morales et al., 2020a, ul Qamar et al., 2019). A recent report by Khaerunnisa et al. 2020, disclosed that some medicinal plant compounds could be effective in treating coronavirus. South Africa is a country that has a strong history of traditional medicinal plants (Van Wyk, 2011). Phytochemical studies of these plants have shown different classes of compounds with numerous biological properties including the treatment of various diseases and life-threatening conditions such as viral diseases (Mehrbod et al., 2018). There has been no report that has explored the possible therapeutic activity of South African medicinal plants against SARS-CoV-2. Therefore, this study is intended to evaluate the potency of compounds found in these plants to treat SARS-CoV-2. While traditional methods of drug discovery could take years, computational techniques aid in accelerating the drug discovery process. As such, this study seeks to employ *in silico* molecular docking models to investigate the possible inhibitory activity of some bioactive compounds isolated from some commonly used South African medicinal plants (Table 1) against SARS-CoV-2 RdRp, 3CL^{pro} and SAR-CoV-2 RBD. The species chosen were based on their use for the common cold, flu, respiratory conditions, antimalarial, antiviral and antioxidant activity (Table 1).

2. Materials and methods

2.1. System preparations and retrieval of X-ray crystal structures of target proteins

For this study, three of the most extensively investigated therapeutic targets in SARS-CoV-2, whose X-ray crystal structures have been experimentally resolved were retrieved from the protein data bank (Berman et al., 2002). The enzymes retrieved include; SARS-CoV-2 RNA-dependent polymerase (PDB:7BTF) (Gao et al., 2020), SARS-CoV-2 main protease (3CL^{pro}) (PDB:6LU7) (Liu et al., 2020) and the receptor binding domain (RBD) of the viral S-protein, (PDB:6M17) (Yan et al., 2020). All chains except chains A of both SARS-CoV-2 RNA dependent RNA polymerase (NSP12) and 3CL^{pro} were deleted to reduce computational cost. Likewise, although both chain E and F of 6LU7 represent the SAR-CoV-2 RBD, only chain E was used for molecular docking to reduce computational cost. All chains retained for molecular docking and further analysis encompassed the active sites of the viral enzymes. Each enzyme was then prepared for molecular docking using UCSF Chimera (Pettersen et al., 2004).

2.2. Ligand acquisition and preparation

The active compounds of the medicinal plants selected for this study (Table 1) were modelled using the MarvinSketch software (ChemAxon, 2013). The modelled structures were then taken through energy minimization using Avogadro 1.2.0 software (Hanwell et al.,

Table 1
Compounds selected from South African medicinal plants

Species	Compound	Activity
<i>Artemisia afra</i> Jacq. ex Willd.	α -thujone, apigenin, chrysoeriol, tamarixetin, acacetin	Respiratory infections as well as gastro-intestinal infections, malaria, measles, cold and flu (Liu et al., 2009, Van Wyk, 2011)
<i>Acacia senegal</i> (L.) Willd.	Arabic acid	Colds, coughs, diarrhea, dysentery, anti-inflammatory, sore throat (Duke, 2012, Van Wyk, 2011)
<i>Aloe ferox</i> Mill.	Aloin (also called Barbaloin)	Anti-inflammatory, analgesic, antiseptic, germicidal, antiviral, antiparasitic (Thrigh and Weitz, 2006, Rezazadeh et al., 2016)
<i>Aspalathus linearis</i> (Burm.f.) R. Dahlgren	Aspalathin, nothofagin, chrysoeriol	Antioxidant, immunomodulating properties, asthma, expectorant in chronic catarrh and pulmonary tuberculosis (Van Wyk and Wink, 2018, Rahmasari et al., 2017)
<i>Centella asiatica</i> (L) Urb.	Asiaticoside, madecassoside, asiatic acid	Tuberculosis, lupus, skin diseases, eye diseases, fever, inflammation, asthma, rheumatism (Brendler, 2010, Roy et al., 2013)
<i>Dicerotheramus rhinocerotis</i> (L.f.) Koekemoer	Rhinocerotinoic acid	Influenza and fever in the flu epidemic of 1918, stomach cancer, anti-inflammatory activity (Dekker et al., 1988, Ticha et al., 2015)
<i>Hypoxis hemerocallidea</i> Fisch. & C.A. Mey.	Hypoxoside	Anti-inflammatory, antineoplastic, immune support for HIV and cancer patients, antioxidant, antiviral (Albrecht et al., 1995, Liebenberg et al., 1997, Hutchings, 1996)
<i>Pelargonium sidoides</i> DC.	Umckalin (7-hydroxy-5,6-dimethoxycoumarin)	Acute respiratory infections including cold, bronchitis, sinusitis, anti-microbial (Kolodziej, 2002, Agbabiaka et al., 2008)
<i>Lippia javanica</i> (Burm. f.) Spreng.	Myrcenone, carvone, piperitenone, ipsenone, linalool	Bronchitis, colds, chest ailments, coughs, fever, asthma, malaria, measles (Okhale et al., 2016, Van Wyk, 2011)
<i>Siphonochilus aethiopicus</i> (Schweinf.) B.L. Burt	α -terpineol	Asthma, colds, coughs, pain relief, inflammation, malaria (Lategan et al., 2009, Astani et al., 2010)
<i>Strelitzia nicotai</i> Regel & Körn.	Bilirubin	Antioxidant, anti-cancer (Dwarka et al., 2017)
<i>Sutherlandia frutescens</i> (L.) R. Br.	L-canavanine, D-pinitol, gamma (γ) aminobutyric acid (GABA), sutherlandioside	Asthma, cancer, chronic bronchitis, colds, cough, diabetes, dysentery, fever, gastritis, heart failure, indigestion, influenza (Stokes, 2002, Faleschini, 2011)
<i>Warburgia salutaris</i> (Bertol.f.) Chiov.	Warburganal, mukaadial, salutarisolid, polygodial, isopolygodial	Colds, coughs, fever, headache, influenza, malaria, rheumatism, lung and venereal diseases (Van Wyk and Gericke, 2000, Leonard and Viljoen, 2015)
<i>Xysmalobium undulatum</i> (L.) Ait.f.	Uzarin, xysmalorin	Diarrhea, wound healing, headache, hysteria, antispasmodic, colds, flu, malaria and other fever, including typhoid (Vermaak et al., 2014, Krishna et al., 2015)

2012). Avogadro was integrated with UFF force field and this force field was used to optimize the molecular geometries of the compounds and a steepest descent algorithm for structural minimization. The pdbqt formats of all the compounds were then generated using AutoDock Vina (Trott and Olson, 2010).

2.3. Active site identification and molecular docking

The active site for SARS-CoV-2 RNA-dependent RNA polymerase and 3CL^{pro} retrieved were mapped out using their experimental reported active site residue. The binding site of SARS-CoV-2 RNA-dependent RNA polymerase employed in this study was consistent with the binding site of Remdesivir as recently reported by Yin et al. (2020). The binding site of SAR-CoV-2 RBD was mapped out using six previously RBD amino acids (L455, F486, Q493, S494, N501 and Y505) which have been shown to play a critical role for binding to ACE2 receptors and for determining the host range of SARS-CoV-like viruses (Andersen et al., 2020). Prior to molecular docking, the retained chains for each of the viral enzyme were prepared on UCSF chimera where all small molecules including DNA, Zn²⁺ and Mg²⁺, water, were removed. Hydrogen ion was then added, and each structure subsequently saved as a pdb file. Using a grid box which encompassed the respective co-crystallised inhibitors, the corresponding coordinates were mapped out with the AutoDock Vina incorporated in UCSF Chimera (Yang et al., 2012). The defined coordinates of the SARS-CoV-2 RNA-dependent binding site region were obtained from the grid box coordinates $x = 144.452$, $y = 148.961$, $z = 163.495$ for the centre and coordinates $x = 10.9051$, $y = 18.7302$, $z = 15.2392$ for the size of the grid box. Grid box coordinates for main protease binding site determined as $x = -13.18$, $y = 11.97$ and $z = 68.70$ for the centre of the box while $x = 9.56$, $y = 16.20$ and $z = 11.99$ were coordinated for the size of the grid box. For the SAR-CoV-2 RBD binding site, grid box coordinates included $x = 28.346$, $y = 22.296$, $z = 25.538$ for the centre and coordinates $x = 178.034$, $y = 122.853$, $z = 244.886$ for the size of the grid box. Molecular docking was subsequently performed using AutoDock Vina. Output of docking was viewed on UCSF Chimera using the integrated ViewDock module after docked complexes were saved for further analysis. Validation of molecular docking protocol was performed by redocking the native ligands, bound ligand in the crystal structure, re-docked and prepared protein keeping the same grid box. Additionally, for further validation, superimposition of docked complexes with the experimentally resolved structure and their accompanying native ligands was performed.

2.4. Molecular dynamics (MD) simulations

This was performed using the GPU version of AMBER 18 with an integrated PMEMD module (Salomon-Ferrer et al., 2013), according to standard simulation protocols, which has been employed extensively in our previous reports (Salomon-Ferrer et al., 2013, Olotu et al., 2018, Oguntade et al., 2017, Agoni et al., 2018a, Agoni et al., 2018b). Parameterization of the inhibitor was carried out using the ANTECHAMBER module wherein atomic partial charges (AM1BCC) gaff, using the bcc charge scheme was generated (Wang et al., 2001). The FF14SB AMBER force field (Maier et al., 2015) was then used to parameterize the protein. Using the LEAP module, hydrogen atoms were also added, while the entire system was neutralized by the addition of counter ions (Na⁺, Cl⁻) followed by a subsequent generation of ligand, protein and complex topologies as well as parameter files. The systems were explicitly solvated with water using the TIP3P orthorhombic box size of 7Å, which enclosed all atoms of the protein (Jorgensen et al., 1983, Case et al., 2005). Before running the LEAP module, the pdb4amber script was executed to protonate the histidine residues at a constant pH (cpH). This was appointed to automatically modify the protein system for use with LEAP. Both complexes were minimized initially for 2000 minimization steps applying a

restraint potential of 500 kcal/mol and then fully minimized for another 1000 steps of steepest descent without restrain. This was followed by the gradual thermalization of the systems with a temperature range of 0–300 K for 50 ps after which each system was equilibrated for 500 ps while the temperature and pressure were kept constant at 300 K and 1bar, respectively using the Berendsen barostat (Berendsen et al., 1984). This was followed by MD production runs of 100 ns for each system during which the SHAKE algorithm (Kräutler et al., 2001) was used to constrict all atomic hydrogen bonds. The MD simulation was initiated using a time step of 1fs and coordinates were saved at 1ps interval followed by subsequent analysis of trajectories using the integrated PTRJ and CPPTRAJ module (Roe and Cheatham, 2013). Visualization of the complexes and data plots were carried out using the graphical user interface of UCSF chimera and Microcal origin analytical software, respectively (Seifert, 2014).

3. Results

3.1. Exploration of binding modes of bioactive compounds towards SARS-CoV-2 RdRp, 3CL^{pro} and SAR-CoV-2 RBD

To investigate the possibility of our selected bioactive compounds acting as potential therapeutic agents in the treatment of SARS-CoV-2 we performed molecular docking of the compounds against SARS-CoV-2 RdRp, 3CL^{pro} and the SAR-CoV-2 RBD. The corresponding docking score of each compound as calculated is presented in Table 2. As highlighted, arabic acid and L-canavanine exhibited the most favourable docking score of -5.2 kcal/mol in binding to 3CL^{pro} amongst all the compounds. Hypoxoside, exhibited the most favourable docking score of -6.9kcal/mol against

Table 2

Docking scores of selected bioactive compounds to 3CL^{pro}, SAR-CoV-2 RBD and SARS-CoV-2 RdRp.

Compound	Main Protease (3CL ^{pro}) (kcal/mol)	Receptor binding domain (RBD) of SARS-CoV-2 (kcal/mol)	SARS-CoV-2 RNA-dependent RNA polymerase (kcal/mol)
Arabic acid	-5.2	-4.1	-1.7
Aloin	24.0	-6.0	-3.3
Acacetin	-3.4	-6.0	-3.1
Nothofagin	1.0	-6.5	-2.9
Apigenin	-3.2	-6.3	-3.2
Asiatic acid	46.0	-5.9	-3.4
Tamarixetin	-3.1	-6.0	-2.9
Ipsenone	-3.9	-4.1	-1.9
Rhinocerotinoic acid	5.9	-5.9	-3.4
Bilirubin	31.9	-6.8	-2.9
Piperitenone	-2.9	-4.4	-2.7
Hypoxoside	7.0	-6.9	-3.0
Carvone	-2.7	-4.0	-2.1
Chrysoeriol	-3.1	-6.3	-3.1
Linalool	-4.2	-4.1	-2.2
Myrcenone	-5.0	-3.8	-2.0
α -terpineol	-2.8	-3.9	-2.1
L-canavanine	-5.2	-4.0	-2.0
α -thujone	-3.8	-4.2	-2.3
Gamma (γ) aminobutyric acid (GABA)	-4.3	-3.2	-1.6
Sutherlandioside	60.0	-6.5	-2.9
Warburganal	6.1	-5.6	-2.6
Mukaadial	6.3	-4.8	-2.8
Salutarisolid	3.2	-5.7	-3.1
Polygodial	4.0	-5.0	-2.8
Isopolygodial	6.6	-4.4	-2.4
Uzarin	43.3	-6.2	-3.5
Xysmalorin	44.9	-4.0	-3.0
Umckalin	-1.7	-5.2	-2.7

SAR-CoV-2 RBD while uzarin exhibited the most favourable docking score of -3.5 kcal/mol towards SARS-CoV-2 RdRp.

3.2. Molecular insights on the interaction profile of hit bioactive compounds within binding pockets SARS-CoV-2 RdRp, 3CL^{Pro} and SAR-CoV-2 RBD

To corroborate the possible binding of the leading docked bioactive compounds as shown in Table 2, the intermolecular interactions between the bioactive compounds and their respective targeting enzymes were further analysed. Using LIGPLOT (Wallace et al., 1995) and Discovery studio (BIOVIA, 2015), two-dimensional as well as three-dimensional interactions were generated to visualize and compare the binding modes of these ligands. Fig. 1 shows both arabic acid and L-canavanine engaged in strong interactions with binding site residues of 3CL^{Pro}. Notable interactions include hydrogen bonds with residues His163, Val308, Ser144, Leu141, Asn142, Gly143 and in Cys145 with arabic acid and residues Ser144, Cys145, Leu141, Asn142 and Glu166 with L-canavanine. The recurrence of residues

Ser144, Cys145, Leu141 and Asn142 suggest they could be essential to inhibitor binding.

It was also observed that both compounds are completely buried in the binding pocket of 3CL^{Pro} forming various interactions including the strong hydrogen bond interactions and hydrophobic interactions. These interactions anchor both compounds tightly to 3CL^{Pro} possibly accounting for the high docking score as observed for these compounds.

Analysis of the ligand interaction profile of hypoxiside, the highest docked score bioactive compound towards SARS-CoV-2 RBD, also revealed an extensive pool of interactions consisting of hydrogen bond interactions with Tyr505, Gly496, Asn501, Tyr495, Tyr449 and Tyr453 in addition to hydrophobic interactions.

Molecular docking also revealed that uzarin exhibited the highest docking score towards SARS-CoV-2 RdRp amongst the 29 bioactive compounds investigated in this study. As shown in Fig. 3, uzarin is anchored at the entrance of the hydrophobic pocket by two strong hydrogen bonds with Arg553 and Arg556. Subsequently, uzarin is also shown to engage in two strong hydrogen bond interactions (Tyr456 and Ser682) in deeper regions of the hydrophobic core.

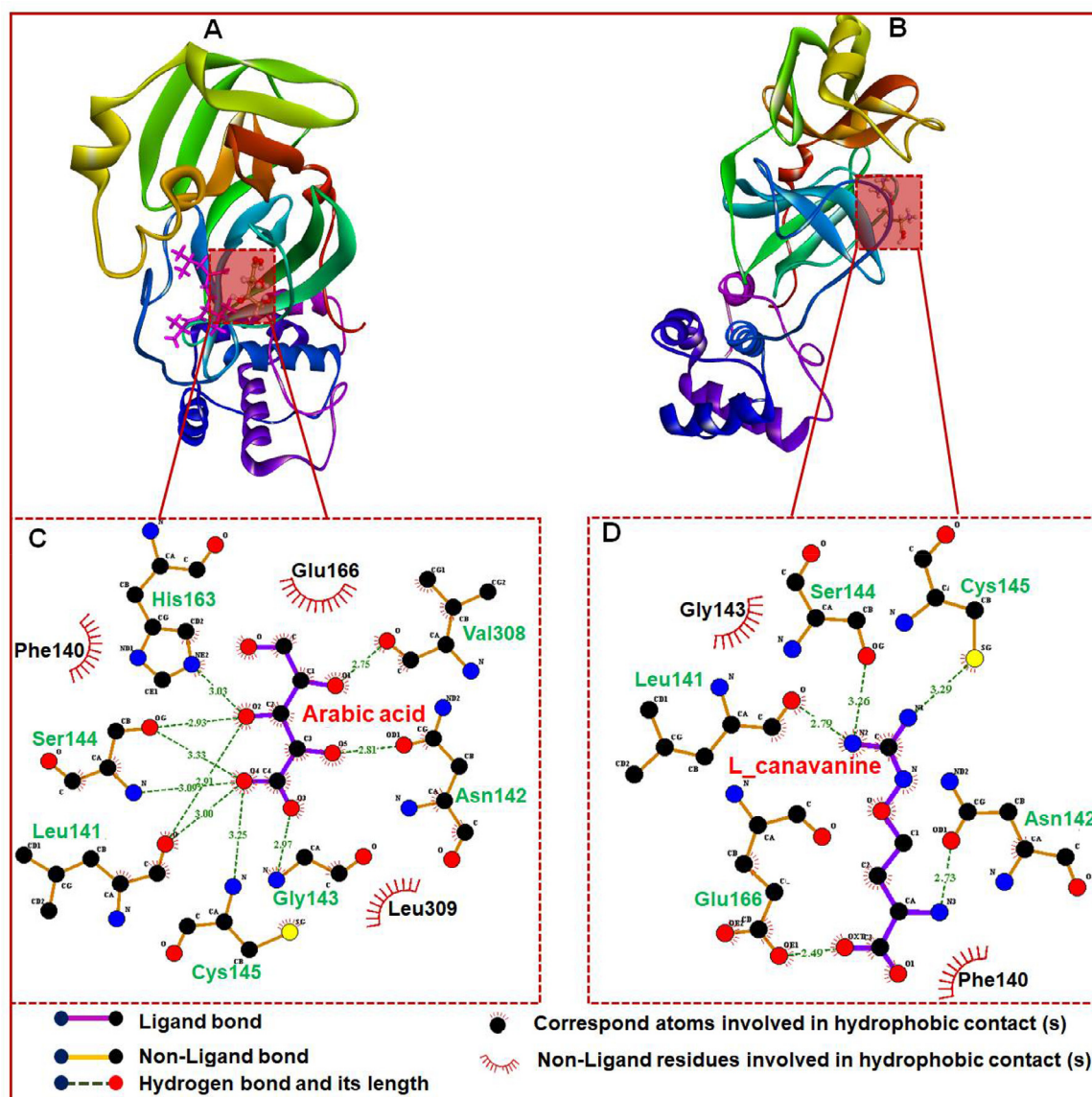


Fig. 1. A) 3D representation of 3CL^{Pro}-arabic acid complex. B) 3D representation of 3CL^{Pro}-L-canavanine complex. C) 2D representation of the ligand interaction plot of the 3CL^{Pro}-arabic acid complex. D) 2D representation of the ligand interaction plot of the 3CL^{Pro}-L-canavanine complex.

4. Discussion

4.1. Molecular docking insights

The docking scores suggest that relative to the other bioactive compounds from the select South African medicinal plants, arabic acid, L-canavanine, hypoxoside and uzarin could bind completely to the respective targets. Advantageous binding modes of ligands or inhibitors to biological targets allows for inhibitor stability and subsequent favourable interactions that could influence the overall function of the biological target (Du et al., 2016). Therefore, the strong interactions observed for the four bioactive hits identified suggest that these compounds could modulate the functions of the respective biological targets that they bind to. Having been experimentally established to possess therapeutic activity in the treatment of the common cold, flus, respiratory conditions and viral infection (Liu et al., 2009, Van Wyk and Wink, 2018, Van Wyk, 2011, Stokes, 2002, Vermaak et al., 2014), the ability of these compounds to exhibit the most favourable docking scores towards SARS-CoV-2 biological targets suggests that they should be further probed as potential SARS-CoV-2 treatment agents.

Exploration of ligand interaction profiles of the identified bioactive compounds reveal insights that corroborate previous reports. A recent report by Hall and Ji, 2020 revealed that His163 is crucial to inhibition of 3CL^{pro} since the mutation of its homologous residue His162 in SARS protease results in enzyme inactivity. Therefore, the observed strong hydrogen bond interaction of arabic acid with His163 further confirms the essentiality of this residue while suggesting a possible 3CL^{pro}-inhibitory potential of arabic acid. Also, arabic acid and L-canavanine are shown to form a hydrogen bond with Cys145, one of the catalytic dyad (Cys145 and His41) (ul Qamar et al.,

2020), similar to previously reported 3CL^{pro} inhibitors, further suggesting their possible inhibitory activity against 3CL^{pro} as well. However, fewer hydrophobic interactions were formed in the L-canavanine complex relative to arabic acid complex, suggesting binding free energy calculations could show a higher binding free energy of arabic acid towards 3CL^{pro} relative to L-canavanine. Previous study by Maeno et al. 1979, showed that L-canavanine inhibits the synthesis of viral RNA in influenza RI/5+ virus I, therefore, the ability to exhibit favourable binding orientation and correspondingly strong binding pocket interactions also suggests it could be considered as a drug candidate to be explored further for COVID-19 treatment.

As shown in Fig. 2, hypoxoside is anchored to the inhibitory binding site of SARS-CoV-2 RBD by an extensive network of strong hydrogen bonds which could favour binding site stability and affinity. This could have also accounted for the high docking score relative to other bioactive compounds as estimated. Having been previously reported to possess antiviral activity (Liebenberg et al., 1997), the favourable binding of hypoxoside towards SARS-CoV-2 RBD further suggests it could also be explored as a potential therapeutic option for SARS-CoV-2 as well by specifically targeting SARS-CoV-2 RBD.

Uzarin is anchored within the SARS-CoV-2 RdRp binding pocket by strong hydrogen bond interactions as shown in Fig. 3. These interactions together with other hydrophobic interactions could account for its most favourable docking orientation relative to other bioactive compounds investigated. A comparison of the docking score of uzarin (−3.5 kcal/mol) to the docking score of Remdesivir (Wang et al., 2020), a known experimentally reported SARS-CoV-2 RdRp inhibitor, within the same gridbox, showed that Remdesivir exhibited a higher docking score of −5.9 kcal/mol. Nonetheless, the strong hydrogen bond interactions suggest some level of ligand stability within the binding pocket and hence warrants further investigation as potential

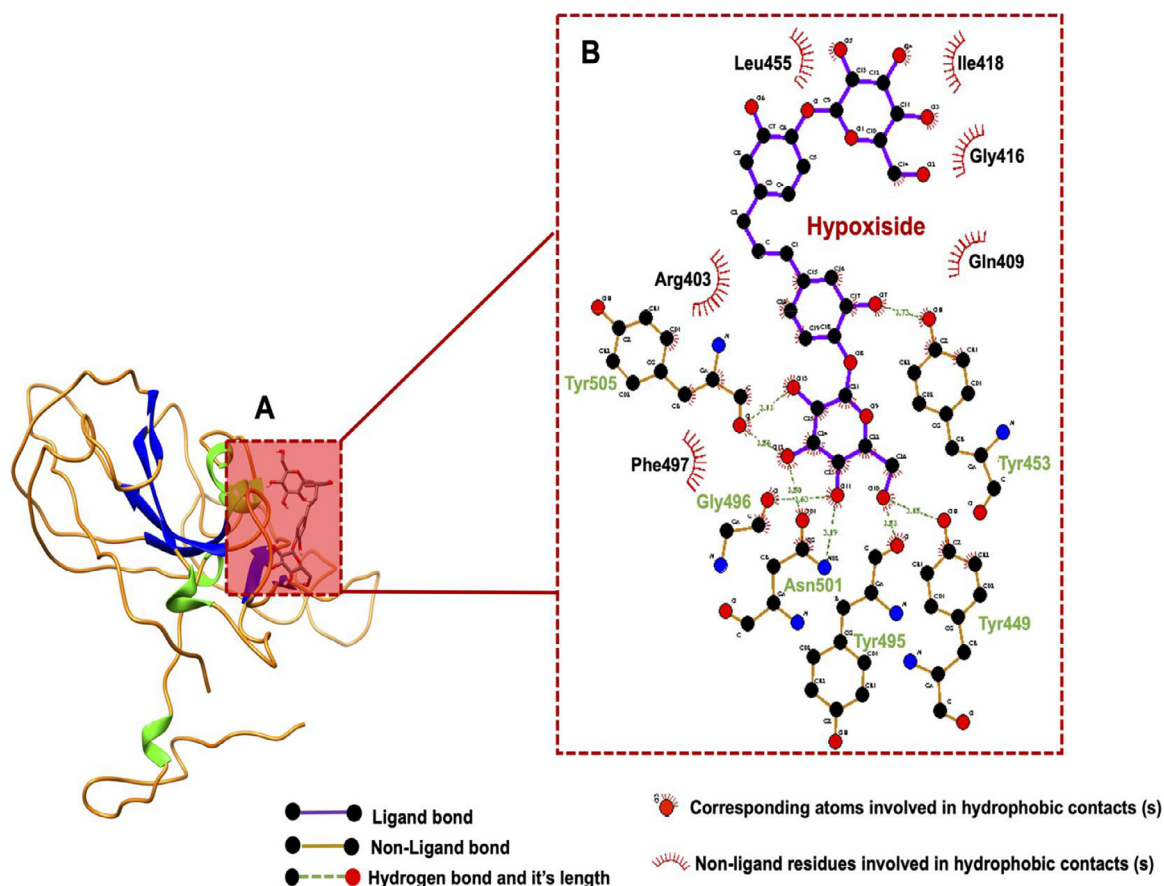


Fig. 2. A) 3D representation of SARS-CoV-2 RBD-hypoxoside complex. B) 2D representation of the ligand interaction plot of the SARS-CoV-2 RBD-hypoxoside complex.

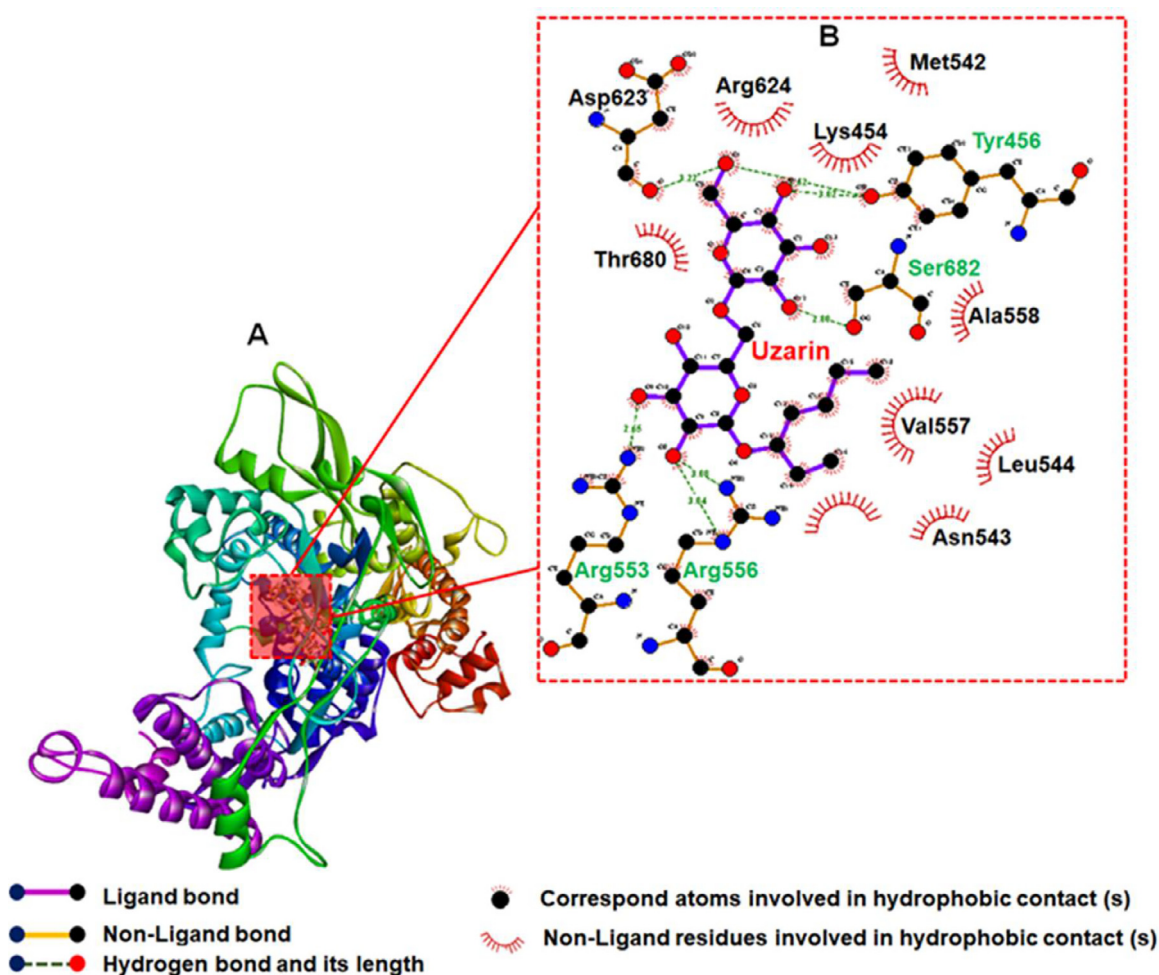


Fig. 3. A) 3D representation of SARS-CoV-2 RdRp-uzarin complex. B) 2D representation of the ligand interaction plot of the RdRp-uzarin complex.

SARS-CoV-2 RdRp inhibitor. Additionally, Uzarin, is the main component of a frequently used over-the-counter drug i.e. Uzara, in Germany ([National Center for Advancing Translational Sciences](#)), therefore, its favourable binding toward SARS-CoV-2 RdRp suggests, it could possibly be repurposed and further explored as a potential COVID-19 therapeutic agent.

4.2. Conformations dynamics of SARS-CoV-2 RdRp, 3CL^{pro} and SARS-CoV-2 RBD upon inhibitor binding using Molecular dynamics simulations

Molecular dynamics simulations are a widely employed computational technique that provides a time-based atomistic analysis of the conformational dynamics of biological molecules such as proteins ([Salmaso and Moro, 2018](#)). MD simulations also allows for a thorough elucidation of the impact of bound ligands on the structural dynamics of these biological molecules. As such, in this report MD simulation was employed to provide essential structural and atomistic insights associated with the binding of the identified bioactive compounds, since these structural implications are crucial to the possible inhibitory potency of the bioactive compounds. A 70ns MD simulation was therefore performed. To ensure a reliability of the MD simulation, an initial assessment of the root mean square deviation (RMSD) of the generated trajectories was performed. As shown in [Figs. 4A, 5A, 6A and 7A](#), all simulated systems achieved convergence between 10ns to 30ns, an evidence of well equilibrated systems, hence insights from the analysed trajectories are reliable.

4.2.1. Conformational insights from SARS-CoV-2 3CL^{pro} complexes

In unravelling the conformational dynamics of 3CL^{pro} upon the binding of arabic acid and L-canavanine, the trajectories generated from the MD simulation were analysed using the post-MD techniques root mean square deviation (RMSD) ([Pitera, 2014, Maiorov and Crippen, 1994](#)), root mean square fluctuation (RMSF) ([Bornot et al., 2011, Sneha, 2016](#)) and radius of gyrations (ROG) ([Labanov, 2008](#)). The stability of a tertiary protein structure is essential in the maintenance of the overall function of that protein. As such, we assessed the inhibitory impact of both arabic acid and L-canavanine upon binding to 3CL^{pro}. As shown in [Fig. 4A](#), arabic acid bound 3CL^{pro} exhibited a relatively lower root mean square deviation of c- α atoms in comparison with the unbound 3CL^{pro} with an average RMSD value of 1.86Å and 3.67Å. This suggested a relatively more stable structural conformation of 3CL^{pro} upon binding of arabic acid, thus a possible indication that the inhibitory mechanism of arabic acid towards 3CL^{pro} is characterized by an ability to decrease the deviation of c- α atoms. To further understand the structural implications of arabic acid binding a calculation of the radius of gyration of c- α atoms of 3CL^{pro} over the simulation period was calculated plotted as shown in [Fig. 4B](#). The unbound 3CL^{pro} conformation exhibited a relatively higher average ROG of 22.16Å while the arabic acid bound conformation exhibited a lower average ROG of 22.13Å. Radius of gyration of the c- α atoms of a protein correlated with protein compactness where a lower ROG is consistent with a compact structural conformation ([Labanov, 2008, Agoni et al., 2019, Agoni et al., 2020](#)). As such, the relatively lower average ROG of arabic acid bound 3CL^{pro} suggests a tight of compact enzyme conformation, consistent with the relatively stable system as

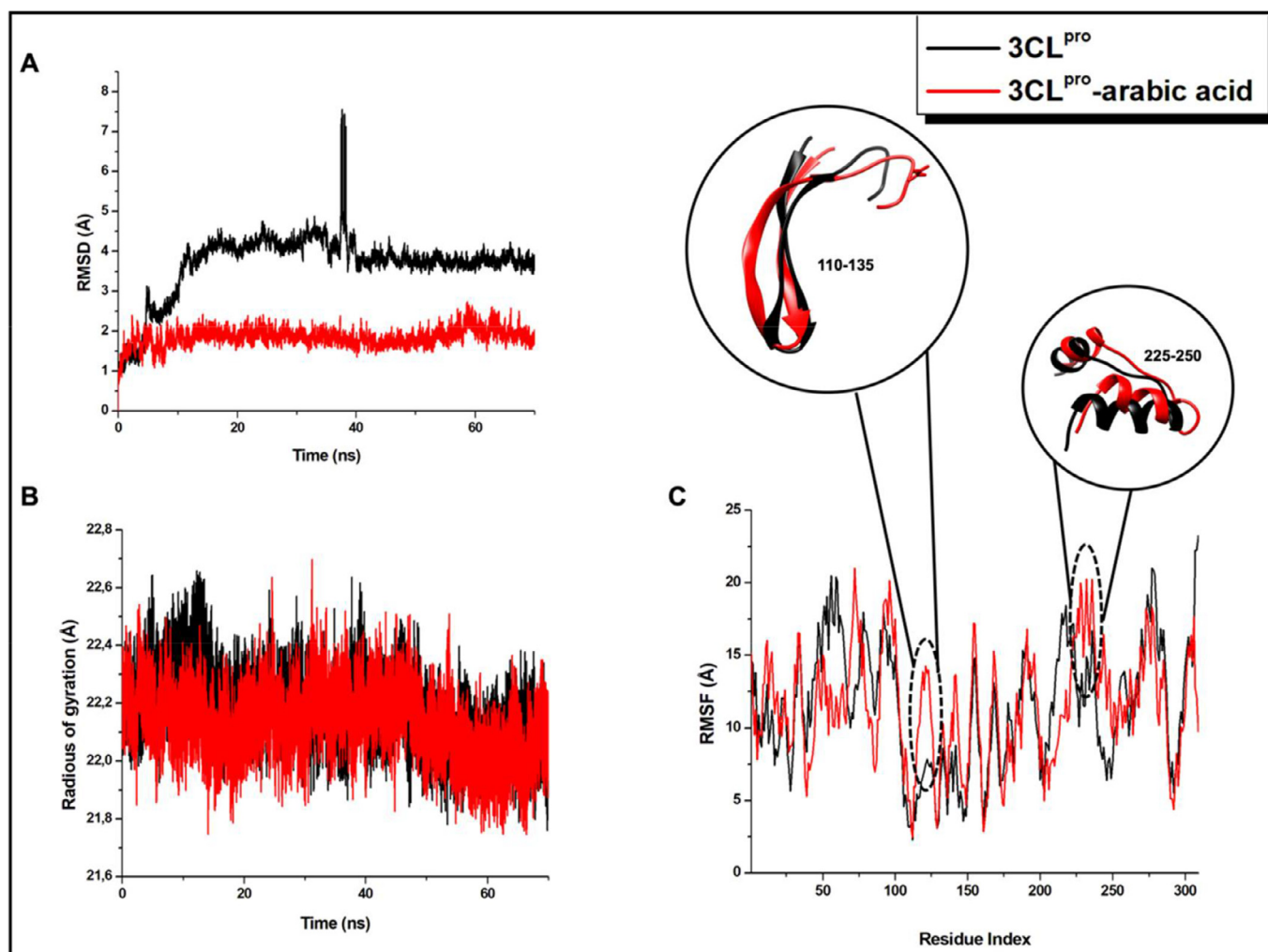


Fig. 4. A) Comparative c- α atom RMSD of the arabic acid bound (red) and unbound 3CL^{pro} (black) providing insights of the stability of 3CL^{pro}. B) Comparative c- α atom ROG of the arabic acid bound (red) and unbound 3CL^{pro} (black) providing insights of the compactness of 3CL^{pro}. C) Comparative c- α atom RMSF of the arabic acid bound (red) and unbound 3CL^{pro} (black) providing insights on residue flexibility of 3CL^{pro}. Insert shows regions of prominent variation in residue flexibility.

observed in the RMSD calculation. Root mean square fluctuation of c- α atoms of 3CL^{pro} was also performed to access the flexibility of the individual residues of 3CL^{pro} as shown in Fig. 4C. Overall, there was both bound and unbound conformations of 3CL^{pro} exhibited an average RMSF of 11.50Å. However, there was obvious variation in residue flexibility of residues within specific regions of the enzyme, notably, residues between “110–135” and “225–250”. Within these regions, flexibility of residues in the arabic acid bound conformation was relatively higher, suggesting the binding of arabic acid, possibly induced the increase flexibility of these regions.

Having exhibited favourable binding properties against 3CL^{pro}, we also explored the possible structural and conformational changes that could accompany the possible therapeutic activity of L-canavanine. As shown in Fig. 5A, the L-canavanine bound conformation exhibited an average RMSD of 4.76Å while the unbound model had an average RMSD of 3.67Å. This suggested that unlike arabic acid, L-canavanine induces an increase in the deviation of the c- α atoms as a possible mechanism of its inhibition. Also, a relatively higher average ROG of 22.27Å was exhibited by the L-canavanine bound 3CL^{pro} in comparison with a lower average ROG in the unbound conformation of 22.16Å. This suggested a less compact 3CL^{pro} upon L-canavanine binding, consistent with structural disorientation as evidenced in the unstable bound enzyme conformation in the RMSD calculation. Interestingly, although some specific residue regions such as “110–135” of the bound conformation exhibited higher residue fluctuation than

the unbound 3CL^{pro}, the overall structural flexibility of 3CL^{pro} was higher in the unbound structure. The unbound exhibited an average RMSF of 11.50Å while the L-canavanine bound structure exhibited an average RMSF of 9.11Å. Thus, L-canavanine inhibition of 3CL^{pro} is characterized by a decrease in protein stability and compactness but with a decrease in overall residue flexibility.

4.2.2. Conformational insights from SARS-CoV-2 Receptor binding domain (RBD) complex

Structural and conformational changes associated with the binding of hypoxoside were also accessed and reported as shown in Fig. 6. From the RMSD assessment as shown in Fig. 6A, hypoxoside binding is characterized by an increase in the deviation of the c- α atoms of SARS-CoV-2 RBD with the bound conformation exhibiting an average RMSD of 6.12Å while the unbound showed an average RMSD of 5.97Å. An average ROG of 20.85Å was also estimated for the bound SARS-CoV-2 RBD while the unbound structure showcased an average ROG of 19.91Å as shown in Fig. 6B. This indicated that the hypoxoside induces a less compact structure of SARS-CoV-2 RBD upon binding. As shown in Fig. 6C, residue flexibility assessment by a calculation of the RMSF of the c- α atoms of each residue of SARS-CoV-2 RBD showed that the binding of hypoxoside decreased the structural flexibility of SARS-CoV-2 RBD with an average RMSF of 11.70Å. Nonetheless, specific residue regions, notably “450–460” and “506–519” in the bound conformation exhibited prominent higher residue fluctuations

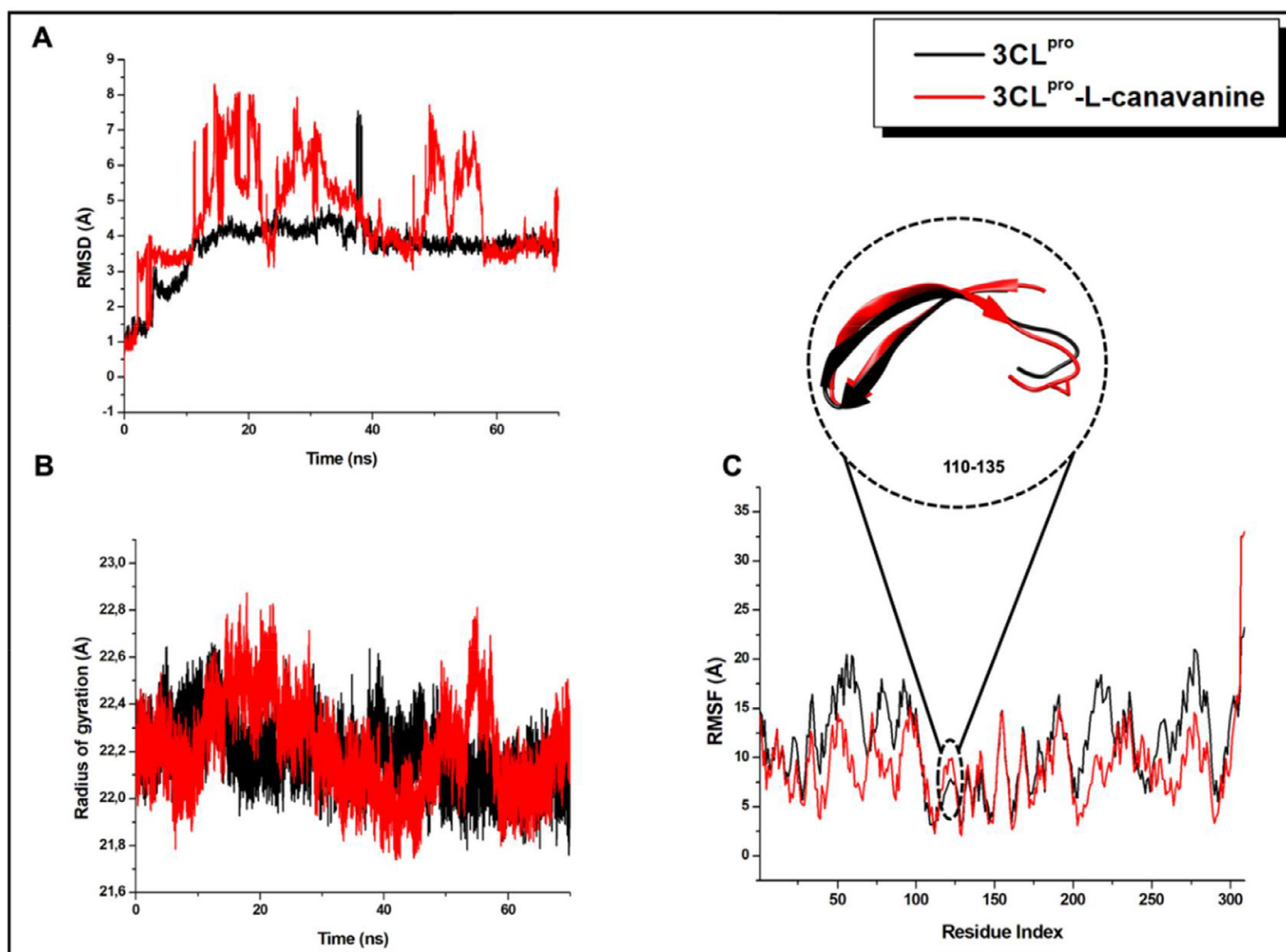


Fig. 5. A) Comparative c-α atom RMSD of L-canavanine bound (red) and unbound 3CL^{pro} (black) providing insights on the stability of 3CL^{pro}. B) Comparative c-α atom ROG of the L-canavanine bound (red) and unbound 3CL^{pro} (black) providing insights of the compactness of 3CL^{pro}. C) Comparative c-α atom RMSF of the L-canavanine bound (red) and unbound 3CL^{pro} (black) providing insights on residue flexibility of 3CL^{pro}. Insert shows regions of prominent variation in residue flexibility

relative to the to the unbound. Interestingly, these regions encompassed the binding site residues of SARS-CoV-2 RBD suggesting that hypoxoside binding disorients the structural integrity of binding site residues hence their increased flexibility. This increased flexibility of binding site residues could in turn favour the inhibitory activity of hypoxoside. The overall average RMSF of the unbound SARS-CoV-2 RBD was estimated to be 11.49Å.

4.2.3. Conformational insights from SARS-CoV-2 RNA-dependent RNA polymerase complex

An analysis of the generated trajectories from the MD simulation of the SARS-CoV-2. RdRP-uzarin was also performed to ascertain the structural and conformational changes associated with the binding of uzarin. Likewise, RMSD, ROG and RMSF assessment of the bound complex was performed in comparison with the unbound conformation as presented in Fig. 7. Binding of uzarin is shown to decrease the stability of SARS-CoV-2 RdRP by inducing an increase in its c-α atoms deviations with an average RMSD of 2.36Å as shown in Fig. 7A. This was in comparison with the unbound conformation which exhibited relatively lower average RMSD of 1.93Å. Also, the binding of uzarin induced a more compact enzyme structure of SARS-CoV-2 RdRP evidenced by a decreased average ROG of 28.67Å. This was in comparison with the unbound conformation which exhibited an average ROG of 28.89Å as shown in Fig. 7B. Uzarin binding was also shown to increase the flexibility of individual residues of SARS-CoV-2 RdRP

evidenced by the relatively higher average RMSF of 8.14Å in comparison with the unbound structure which exhibited an average RMSF of 7.90Å. Specific residue regions that showcased prominently higher RMSF values included regions “317–417” and “760–830” which encompassed some of the active site residues of SARS-CoV-2 RdRP as shown in Fig. 7C. This suggested that the binding of uzarin interfered with the structural integrity of SARS-CoV-2 RdRP, hence the increase in residue flexibility. This could therefore form the bases for its potential inhibitory potency.

4.3. Assessing the Pharmacokinetic properties of arabic acid, L-canavanine, hypoxoside and uzarin

The physicochemical and pharmacokinetics properties of molecular inhibitors are key aspects to their general medicinal effectiveness. Consequently, this study investigated the physicochemical and pharmacokinetic properties of the identified potential SARS-CoV-2 inhibitors (arabic acid, L-canavanine, hypoxoside and uzarin) using the online platform SwissADME (Diana et al., 2017). An *in silico* evaluation of the physiochemical and pharmacokinetics properties, particularly absorption, distribution, metabolism, and excretion provides an understanding of the response of the inhibitor *in vivo* whilst decreasing the risk of late-stage disapproval screening. These properties as reviewed from SwissADME are presented below in Table 3. Based on the brain or intestinal estimated permeation (BOILED-Egg) model,

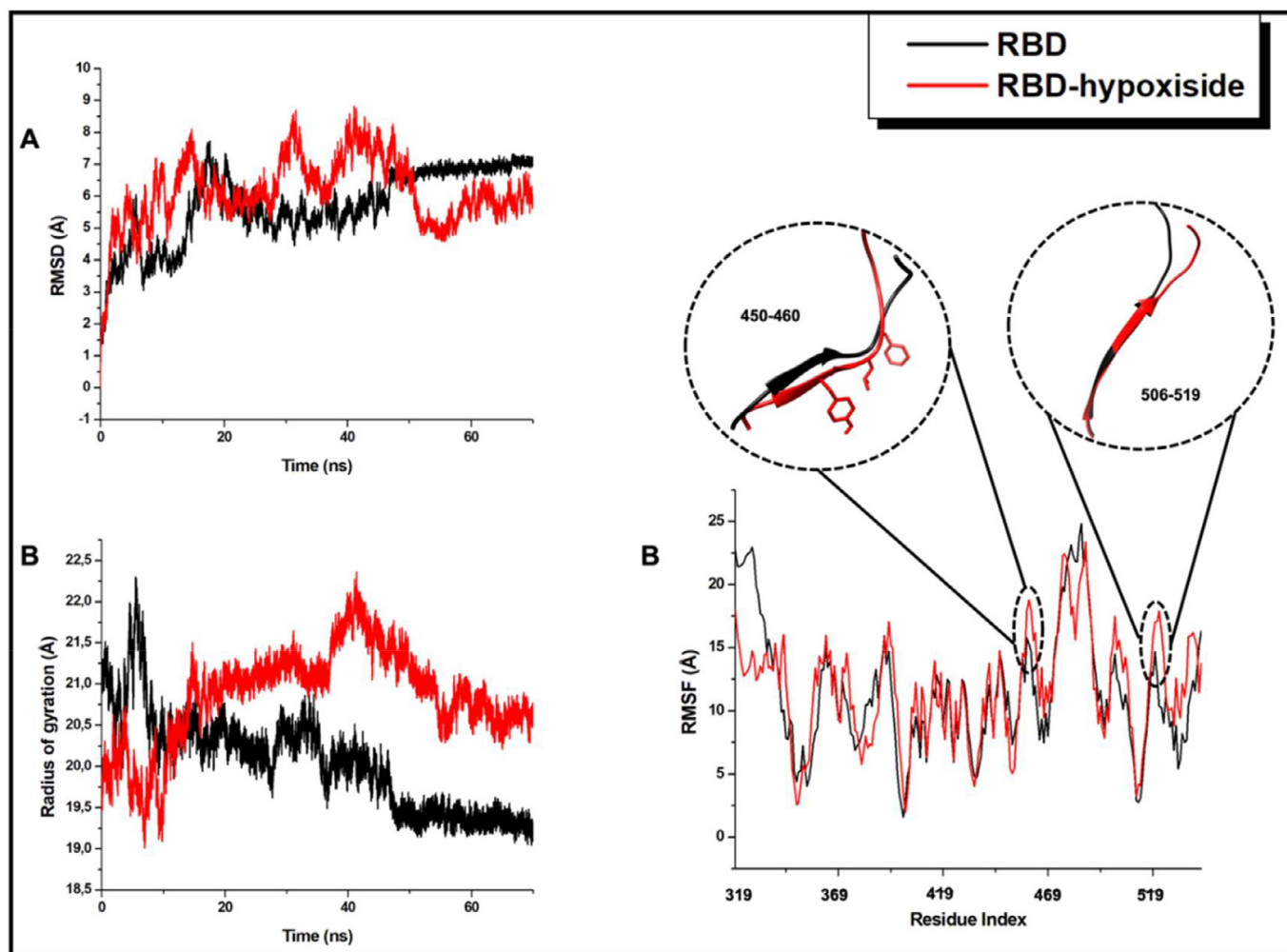


Fig. 6. A) Comparative c- α atom RMSD of the hypoxiside bound (red) and unbound SARS-CoV-2 RBD (black) providing insights on the stability of SARS-CoV-2 RBD. B) Comparative c- α atom ROG of the hypoxiside bound (red) and unbound SARS-CoV-2 RBD (black) providing insights on the compactness of SARS-CoV-2 RBD. C) Comparative c- α atom RMSF of the hypoxiside bound (red) and unbound SARS-CoV-2 RBD (black) providing insights on residue flexibility of SARS-CoV-2 RBD. Insert shows regions of prominent variation in residue flexibility

the SwissADME online platform was used to compute the lipophilicity (logP) and polarity of arabic acid, L-canavanine, hypoxiside and uzarin. LogP of compounds estimates their permeability across cellular membranes and could influence absorption as well as bioavailability of these compounds. According to Lipinski's Rule of 5, the calculated Log P (CLog P) of a compound intended for oral administration should not be greater than 5. As such, higher logP usually correlated with a compound with minimal potential of permeating lipid membrane. With a logP of -2.00, -2.48, -0.68 and -0.04 for arabic acid, L-canavanine, hypoxiside and uzarin, thus suggesting that these compounds will exhibit high membrane permeability. These molecules that can readily cross cell membranes are essential in biological research and medicine. Moreover, these compounds convey high bioavailability, thus increasing absorption. Based on the admetSAR (Yang et al., 2019) platform, arabic acid has an acute oral toxicity of 0.11 kg/mol, L-canavanine has an acute oral toxicity of 1.803 kg/mol, hypoxiside 2.395 kg/mol and uzarin 4.857 kg/mol. This confirms that the toxicity displayed by these compounds are dose dependant. The molecular weight (MW) of arabic acid (166.13 g/mol), L-canavanine (176.17 g/mol), hypoxiside (606.57 g/mol) and uzarin (698.79 g/mol), discloses that arabic acid and L-canavanine will produce minimal to toxicity (<500g/mol). Whereas hypoxiside and uzarin have a MW greater than 500 g/mol, it is important to note that natural products are usually do not adhere to the Lipinski's Rule of 5, thus inferring hypoxiside and uzarin should be further investigated for their

potential anti-SARS-CoV-2 properties. Also, with their large MW, a synthetic fragmentation of their structures to smaller more simpler compounds could increase the bioactivity and decrease toxicity (Olotu et al., 2018). It is also important to note that uzarin, it is currently available as an over the counter drug for gastrointestinal problems. In all, it is evident that regardless if their in compliance with although they many drug-likeness rules, a further experimental evaluation of these compounds could yield very promising therapeutic agents.

5. Conclusion

The aim of this study was to investigate the potential of the active compounds found in South African traditionally used plants against SARS-CoV-2. The compounds were tested *in silico* for the inhibition to 3CL^{Pro}, SARS-CoV-2 RBD and SARS-CoV-2 RdRp. Molecular docking revealed that four bioactive compounds from the 29 selected compounds exhibited potential of being investigated further, as possible therapeutic agents against SARS-CoV-2. The bioactive compounds; arabic acid from *Acacia senegal* and L-canavanine found in *Sutherlandia frutescens* were shown to exhibit favourable binding modes in the active site of 3CL^{Pro} with corresponding strong interactions. Results also revealed that hypoxiside isolated from *Hypoxis hemerocallidea* and uzarin from *Xysmalobium undulatum* exhibited favourable binding orientations

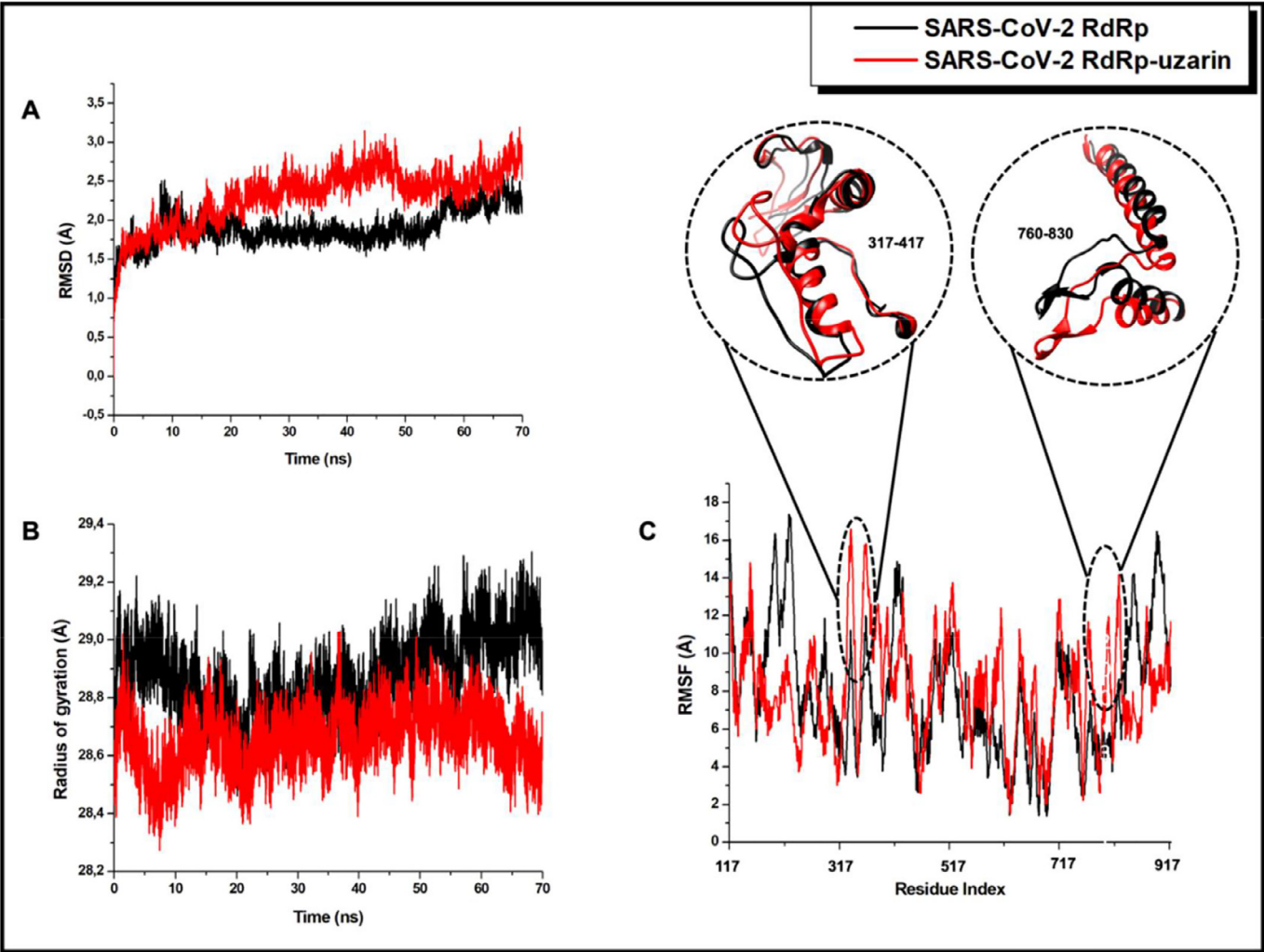


Fig. 7. A) Comparative c-α atom RMSD of the uzarin bound (red) and unbound SARS-CoV-2 RdRp (black) providing insights on the stability of SARS-CoV-2 RdRp. B) Comparative c-α atom ROG of the uzarin bound (red) and unbound SARS-CoV-2 RdRp (black) providing insights of the compactness of SARS-CoV-2 RdRp. C) Comparative c-α atom RMSF of the uzarin bound (red) and unbound SARS-CoV-2 RdRp (black) providing insights on residue flexibility of SARS-CoV-2 RdRp. Insert shows regions of prominent variation in residue flexibility

Table 3
The physicochemical and ADMET properties of arabic acid, L-canavanine, hypoxoside and uzarin

	Arabic acid	L-canavanine	Hypoxoside	Uzarin
Molecular weight (g mol ⁻¹)	166.13 g/mol	176.17 g/mol	606.57 g/mol	698.79 g/mol
Molecular formula	C ₅ H ₁₀ O ₆	C ₅ H ₁₂ N ₄ O ₃	C ₂₉ H ₃₄ O ₁₄	C ₃₅ H ₅₄ O ₁₄
Lipophilicity (logP)	-2.00	-2.48	-0.68	-0.04
Water solubility	Soluble	Soluble	Soluble	Soluble
GIT absorption	Low	Low	Low	Low
BBB permeability	No	No	No	No
Bioavailability score	0.56	0.55	0.17	0.17
Hydrogen bond (donors/acceptors)	5/6	4/5	10/14	8/14
BOILED-Egg representation of lipophilicity and polarity				

within the inhibitor binding pocket of SARS-CoV-2 RBD and SARS-CoV-2 RdRp, respectively, characterized by strong interactions. Molecular dynamics simulations revealed that the binding of the identified inhibitors are characterized by structural perturbations which favour the inhibitory potency of these bioactive compounds. Likewise, *in silico* pharmacokinetic assessment of the compounds revealed favourable properties that warrants a further exploration of their anti-SARS-CoV-2 properties. Although not conclusive and requiring further *in vitro* analysis, these compounds could lead to the disruption of the activity of the respective enzymes that they bind to, and thus could serve as a starting point for the discovery of novel SARS-CoV-2 therapeutic.

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Declaration of Competing interest

All authors declare that there are no conflicting interests.

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