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Antimalarial phytochemicals as potential inhibitors of SARS-CoV-2 guanine N7-methyltransferase (nsp 14): an integrated computational approach

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ABSTRACT

The inhibition of capping enzymes such as guanine-N7-methyltransferase (GMT) is an attractive target for regulating viral replication, transcription, virulence, and pathogenesis. Thus, compounds that target the Severe Acute Respiratory Syndrome Corona Virus 2 GMT (S2GMT) will enhance drug development against COVID-19. In this study, an in-house library of 249 phytochemicals from African medicinal plants was screened using computational approaches including homology modeling, molecular docking, molecular dynamic simulations, binding free energy calculations based on molecular mechanics/Poisson-Boltzmann surface area (MMPBSA) and Absorption-Distribution-Metabolism-Excretion-Toxicity (ADMET) analysis for inhibitors of S2GMT. The top-ten ranked phytochemicals (TTRP) obtained from the docking analysis to S2GMT were further docked to SARS-COV N7-MTase. Among the TTRP, the top-four ranked phytochemicals (TFRP) viz: 3 alkaloids (Isocryptolepine, 10'-Hydroxyusambarensine and Isostrychnopentamine) and a flavonoid (Mulberrofuran F) interacted strongly with critical catalytic residues whose interference either reduce or completely abolish N7-MTase activity, indicating their potential as capping machinery disruptors. The interactions of TFRP with the catalytic residues of S2GMT were preserved in a 100 ns simulated dynamic environment, thereby, demonstrating high degree of structural stability. The MMPBSA binding free energy calculations corroborated the docking scores with isocryptolepine having the highest binding free energy to S2GMT. The TFRP showed favourable drug-likeness and ADMET properties over a wide range of molecular descriptors. Therefore, the TFRP can be further explored as potential S2GMT inhibitors in *in vitro* and *in vivo* experiments.

Abbreviations: COVID-19: The Coronavirus disease 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Corona Virus 2; N7-MTase: Guanine-N7 methyl transferase; S2GMT: Severe Acute Respiratory Syndrome Corona Virus 2 guanine-N7 methyl transferase; SGMT: Severe Acute Respiratory Syndrome Corona Virus guanine-N7 methyl transferase; THP: Ten-hit phytochemical; FLP: Four-lead phytochemical; ADMET: Absorption, Distribution, Metabolism, Excretion and toxicity; RMSF: Root mean square fluctuation; RMSD: Root mean square deviation

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1. Introduction

Coronaviruses are typical RNA viruses which cause respiratory diseases in humans (Zhu et al., 2020). These diseases are rather mild, typical of upper respiratory tract infections. However, the outbreak of the severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) introduced a new paradigm to the infectious nature of these viruses. It has been described as the first major pandemic of the millennium, and

novel, since the first reported instance of infecting humans (Peiris, Chu et al., 2003; Peiris, Lai et al., 2003; Peiris, Yuen et al., 2003). The origins and epidemiology of SARS-CoV have been extensively reported (Cheng et al., 2007). Studies indicate that these viruses were initially restricted to such animals as civets, camels and bats, before the 'jumping' of the viruses to man. This has been attributed to the dearth of bio-safety measures in the wet market (Webster, 2004; Woo

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et al., 2006). The virus is highly infectious, with over 8,000 persons infected in 2003 when the epidemic first broke out, with a 10% fatality, spread to over 30 countries, spanning 5 continents (Cheng et al., 2007; Decroly et al., 2011). In 2012, another coronavirus (CoV) outbreak, the Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in Saudi Arabia. The mortality rate of MERS has been shown to be about 35% higher than SARS. Though human to human transmission of the MERS-CoV was not as efficient as the SARS-CoV, the large healthcare-associated outbreaks in the Middle-East and South Korea in 2015, points to the possibility of sustained human transmission of the virus. The Wuhan outbreak of SARS-CoV-2 in December 2019 and its impact on global public health has introduced a new paradigm to the scientific study of coronaviruses, and the need for detailed understanding of their pathogenicity and host-virus interaction, to afford insights for potential therapeutics.

Coronaviruses infect cells when S proteins from protruding spikes fuse with certain receptors on the host cell (Haagmans et al., 2014; Hoffmann et al., 2020; Li et al., 2003; Raj et al., 2013). Upon entering the cell, CoV shed their coat and release the viral RNA genome into the host cell. The CoV RNA genome is the largest viral RNA genome, with a size of 25-32 kilobases (Sola et al., 2005), and has been demonstrated to be highly conserved across different CoV strains. It is arranged into two main regions. The 5' non-structural protein coding region consists of the replicase genes, which make up two-thirds of the entire genome. The second region is the 3' structural and non-essential accessory protein coding regions (Masters, 2006; Yang & Leibowitz, 2015). The CoV genomic RNA is central to the life, and thus the infectious nature of the virus. It initiates the viral infectious cycle, offers a template for replication and transcription in the host cell and functions as a substrate for packaging into the daughter virus (Lim et al., 2016). In all CoV, the replicase gene represents two-thirds of the 5' region of the genome, and is made up of two overlapping open reading frames (ORFs), ORF1a and ORF1b. Upon the release and shedding of viral nucleocapsid into the host cell cytoplasm, CoV replication is initiated with the translation of ORF 1a and 1b, to yield polyprotein pp1a (Yang & Leibowitz, 2015). Post-translation modification ensures the cleavage of the polyproteins into about 15 non-structural proteins (Nsp) (Lim et al., 2016; Luo et al., 2005; Sola et al., 2005). Nsps are central to the infectious life and replication of CoV in the host cell. The role of Nsp14 is central to the life of CoV, and is the subject of many studies. Much like the proof-reading role of exonucleases (ExoN) in DNA viruses, Nsp14 functions to maintain replication fidelity in CoV (Eckerle et al., 2010). Therefore, unlike the observed variations in other RNA viruses, Nsp14 has been implicated in the low mutation rate of SARS-CoV, a function tied to its ExoN activity. Other than proofreading ventures, Nsp14 also participates in viral replication and transcription, as well as in viral virulence and pathogenesis (Eckerle et al., 2010). It also functions as an S-adenosylmethionine (SAM)-dependent guanine-N7 methyl transferase (GMT) (Chen et al., 2009). To evade host defense and aid in

translation, the cap1 structure at the 5' region of CoV mRNA is critical (Ma et al., 2015). Therefore, the production of this structure is significant to the survival of the virus in the host, and it is formed via a set of four reactions (Ivanov et al., 2004). Initially, Nsp13 RNA triphosphatase (RTPase) hydrolyzes nascent RNA, yielding pp-RNA. Secondly, a yet unidentified guanylyltransferase (GTase) hydrolyzes GTP, transfers the resulting GMP to pp-RNA, and forms Gppp-RNA. In the final stage, Nsp14 methylates the 5'guanine of the Gppp-RNA at the N7 position, while Nsp16 methylates the ribose of the first nucleotide at the 2'-O position (Bouvet et al., 2010; Chen et al., 2009). The significance of Nsp10 to these reactions have been demonstrated (Chen et al., 2009; Decroly et al., 2011). Nsp14 is a highly conserved protein in CoV, while its significance across different strains has been shown (Ma et al., 2015). The proofreading function provided by Nsp14 that allows for fidelity reproduction of RNA, its methylation capping activity, and the protection it offers against hosts innate immune system qualify Nsp14 as an ideal drug target. Plant bioactive compounds with potentials against such targets are therefore of therapeutic value (Saramago et al., 2021; Tahir, 2021). Phytochemicals are particularly promising candidates for CoV, since their ability to inhibit methyltransferases have been demonstrated (Maurya & Mishra, 2021; Saldívar-González et al., 2018).

Malaria is a life-threatening parasitic disease, caused by parasites *Plasmodium spp.* with highest mortality record from African (World Health Organization 2019). Comparatively, malaria and COVID-19 shares common presentation of symptoms such as fever, nausea, vomiting, arthralgia headaches etc (O'Brien et al., 2001), among several ties, the associated syndemics has been reported (Hussein et al., 2020). The spread of COVID-19 in African especially in malarial endemic countries is conceived to be lower than anticipated (Napoli & Nioi, 2020), the inverse relationship between COVID-19 and malaria prevalence in this region has given rise to several hypothesis, among which, the preventive and curative effect of known antimalarial has been described (Zhou et al., 2020). Also malaria is known to induce anti-GPI antibodies which could identify SARS-CoV-2 glycoproteins, thereby exacting protective role against the virus or causing a milder disease pattern (Hussein et al., 2020). Several antimalarial drugs have been studied at different level of experimentation as potential agents against viruses and COVID-19 with varying level of success (Gendrot et al., 2020; Hosseini & Amanlou, 2020; Liu et al., 2020; Rodrigues-Diez et al., 2020; Savarino et al., 2003; Voss et al., 2020; Wang et al., 2020; Yao et al., 2020). Antimalarial compounds are therefore potential candidates to be repurposed for both COVID-19 prophylaxis and therapy (Schlagenhauf et al., 2020). Thus, the aim of this study was to screen an in-house library of phytocompound derived from African medicinal plants with documented *in vitro* or *in vivo* antimalarial activities, using comprehensive computational approaches for possible therapeutic candidates for CoV, while exploiting the inhibitory potentials of these phytocompounds against SARS CoV-2 GMT.

2. Materials and methods

2.1. Sequence dynamics simulation

The FASTA format of the protein sequence (protein ID: YP_009725309.1) of SARS CoV-2 Nsp14 on National Center for Biotechnology Information (NCBI) nucleotide database (NCBI Reference Sequence: NC_045512.2) was obtained. The simulation of the protein sequences was performed on the DynaMine server (<http://dynamine.ibsquare.be/submission/>) using default setting (Cilia et al., 2014). This simulation tool provides a rapid prediction for fast backbone movements of proteins that is established based on the amino acid sequence by employing statistical analysis of chemical shift data from nuclear magnetic resonance (Cilia et al., 2014).

2.2. Homology modeling of Nsp14 and evaluation of model quality

The unavailability of the deposited 3D crystal structure of the SARS CoV-2 Nsp14 that contains both the exon and guanine-N7-methyltransferase component as at the time this work was undertaken informed the reason for the modeling of the Nsp14 protein from its deposited protein sequence. The homology model of Nsp14 was built using the SWISS-MODEL server (<https://swissmodel.expasy.org/>) (Biasini et al., 2014). The fully automated protein structure homology modelling server accessible via ExPASy web server, was used to develop a model (Waterhouse et al., 2018). The FASTA format of the protein sequence (protein ID: YP_009725309.1) of SARS-CoV-2 Nsp14 on National Center for Biotechnology Information (NCBI) nucleotide database (NCBI Reference Sequence: NC_045512.2) was fetched onto the SWISS-MODEL server. From the SWISS-MODEL template library (SMTL version 2020-07-15, PDB release 2020-07-10) a search for evolutionary related structures matching the target sequence as suitable template was performed. A similarity search using the BLAST (Camacho et al., 2009) and HHblits (Remmert et al., 2011) databases simultaneously generated homologues of known PDB structures that were ranked based on QMEAN scoring function (Waterhouse et al., 2018). The top-ranked structures were selected and used as templates to build Nsp14 models. The best model was refined by GalaxyRefine (Heo et al., 2013), a web-tool available on GalaxyWEB server (<http://galaxy.seoklab.org/>) and further refined by 3DRefine server (<http://sysbio.rnet.missouri.edu/3Drefine/>) (Bhattacharya et al., 2016). The qualities of the refined models were then analyzed using ERRAT (Colovos & Yeates, 1993) ProSA (Wiederstein & Sippl, 2007) and by generating Ramachandran plots on PROCHECK (Laskowski et al., 1993).

2.3. Binding site prediction

3DLigandSite server (<http://www.sbg.bio.ic.ac.uk/3dligandsite/advanced.cgi>), was used to predict the active site amino acid residues of the modeled protein targets.

Table 1. Binding site coordinates of Coronaviruses Nsp14.

	center_x	center_y	center_z	Size_x	Size_y	Size_z
Modeled SARS-CoV-2 GMT (Å)	2.68	−26.5	12.00	25.0	28.42	35.56
SARS-CoV GMT (Å)	−42.66	−50.13	5.95	23.86	21.16	23.40

2.4. Retrieval of protein structure

The three-dimensional (3D) structure of SARS-CoV Nsp14 comprised of the Exon and guanine-N7-methyltransferase (PDB ID: 5C8S) (Ma et al., 2015) that was used as template for modeling and for docking analysis was retrieved from the Protein Data Bank (<http://www.rcsb.org>).

2.5. Protein preparation

The crystal structure of the of SARS-CoV Nsp14 was processed by removing existing ligands and water molecules, together with the modeled protein (S2GMT) missing hydrogen atoms were added according to the amino acid protonation state at pH 7.0 utilizing Autodock version 4.2 program (Scripps Research Institute, La Jolla, CA). Thereafter, non-polar hydrogens were merged while polar hydrogens were added to each protein. The process was repeated for each protein and subsequently saved into a dockable pdbqt format for molecular docking.

2.6. Ligand preparation

Antimalarial phytochemicals (249) from African medicinal plants of different classes (alkaloids, terpenoids, flavonoids, chalcones phenolics, polyacetlenes, quinine, coumarins, xanthone, steroids and lignans) were compiled from literature search. The Structure Data Format (SDF) structures of the GMT reference inhibitor (Sinefungin) and substrate SAM, and some of the compounds were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov), while other compounds not present on the database were drawn with ChemDraw version 19. All the compounds were converted to mol2 chemical format using Open babel (O'Boyle et al., 2011).

2.7. Active site targeted molecular docking of phytochemicals

An active site targeted virtual screening of the phytochemicals and reference inhibitors against modeled S2GMT was initially performed using AutoDock Vina (Trott & Olson, 2010) in PyRx (Python prescription) 0.8 (Dallakyan & Olson, 2015). Based on the docking scores, and binding poses, a list of the top docked compounds with binding affinities higher than the reference compounds was generated. The top docked compounds were further docked into the active regions of the SGMT. For each of the docking steps, the ligands were imported via the OpenBabel (O'Boyle et al., 2011), plug-in tool in PyRx 0.8 the Universal Force Field (UFF) was used as the energy minimization parameter and conjugate gradient descent as the optimization algorithm. The active regions of the 2 proteins as defined by the grid boxes used for docking studies are presented in Table 1. All the other parameters

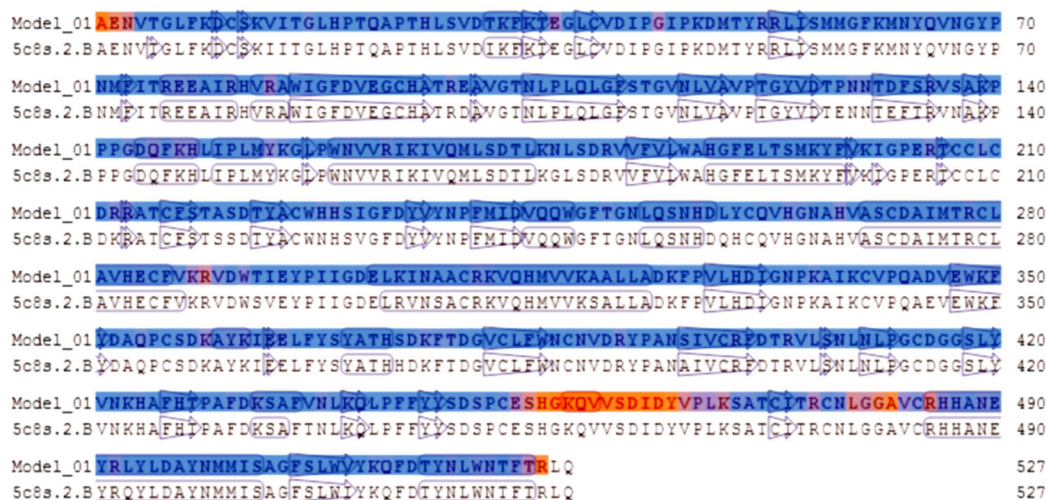


Figure 1. Model-template sequence alignment of SARS-CoV-2 and SARS-CoV Nsp14.

Table 2. Summary of quality check and validation scores of modeled SARS-CoV-2 Nsp14.

		Modeled protein	5C8S
SWISS-MODEL	QMEAN	−1.99	
ProSA-web	Z-score	−9.3	−3.9
PROCHECK (Ramachandran plot)	Most favored regions	93.4%	92.2%
	Additional allowed regions	6.4%	7.8%
	Disallowed regions	0.0%	0.0%
ERRAT plot	Overall quality factor	82.271	65.3968
PyMOL (Model-Template alignment)	RMSD	0.385	

were kept as default. The molecular interactions were viewed with Discovery Studio Visualizer version 16.

2.8. Molecular dynamics simulation and MM/GBSA analysis

The stability, structural/conformational fluctuations that occurred in the docked phytochemicals-modeled SARS-CoV-2 Nsp14 systems was monitored by performing 100 ns molecular dynamics simulation MDS studies. Desmond module of Schrodinger 2019-4 was used to study the complexes in the explicit solvent system with OPLS3 force field. Under the orthorhombic periodic boundary conditions for 10 Å buffer region, the molecular system was solvated with water (TIP3P) molecule (Jorgensen et al., 1983) while the system was neutralized by adding Na^+ as counter ions. An ensemble (NPT) of Nose-Hoover thermostat (Martyna et al., 1992) and barostat was applied to maintain the constant temperature (300 K) and pressure (1 bar) of the systems, respectively. A hybrid energy minimization algorithm with 1000 steps of steepest descent followed by conjugate gradient algorithms was utilized. Another algorithm, limited memory Broyden-Fletcher-Goldfarb-Shanno (LBFGS) algorithm with convergence threshold gradient of 1 Kcal/mol/Å was also employed for energy minimization. A Smooth Particle Mesh Ewald method for calculating long range electrostatic interactions with a cut-off radius of 9 Å for short range van der Waals and Coulomb interactions was used. Multiple time step RESPA integration (reference system propagator algorithms) was used in the dynamics study for bonded, near and far-bonded interactions with 2, 2 and 6 fs,

respectively. The data were collected for every 100 ps, and the obtained trajectory was analyzed with Maestro graphical interface. Various structural parameters, like Root Mean Square Deviation (RMSD), Root Mean Square Fluctuations (RMSF), Radius of Gyration (rGyr), Intramolecular Hydrogen Bonds (intraHB), Molecular Surface Area (MolSA), Solvent Accessible Surface Area (SASA) and Polar Surface Area (PSA) were calculated as a function of time to explore the structural behaviour of the SARS-CoV-2 Nsp14, top hit phytochemicals and S2GMT-top hit phytochemicals complexes. In order to estimate the free energy change that describes the binding of the top-hit phytochemicals to the S2GMT from the docking studies through the MDS trajectories, MM-GBSA (MM-GBSA (Molecular Mechanics, the Generalized Born model and Solvent Accessibility) calculations were carried out, and free energy estimations were computed for 11 snapshots (one every 10 ns).

2.9. ADMET study

The top hits from the docking studies were subjected to various druglikeness and ADMET filtering analysis. The druglikeness analysis which includes Lipinski, Veber, Ghose, Egan and Muegge were performed on the SwissADME (<http://www.swissadme.ch/index.php>) webserver (Daina et al., 2017), while the predicted Absorption, Distribution, Metabolism, Excretion and toxicity (ADMET) study was analysed using the SuperPred webserver (<http://lmmd.ecust.edu.cn/admetar1/predict/>) (Cheng & Ivanov, 2012). The SDF file and canonical SMILES of the compounds were downloaded from PubChem Database or copied from ChemDraw to calculate ADMET properties using default parameters.

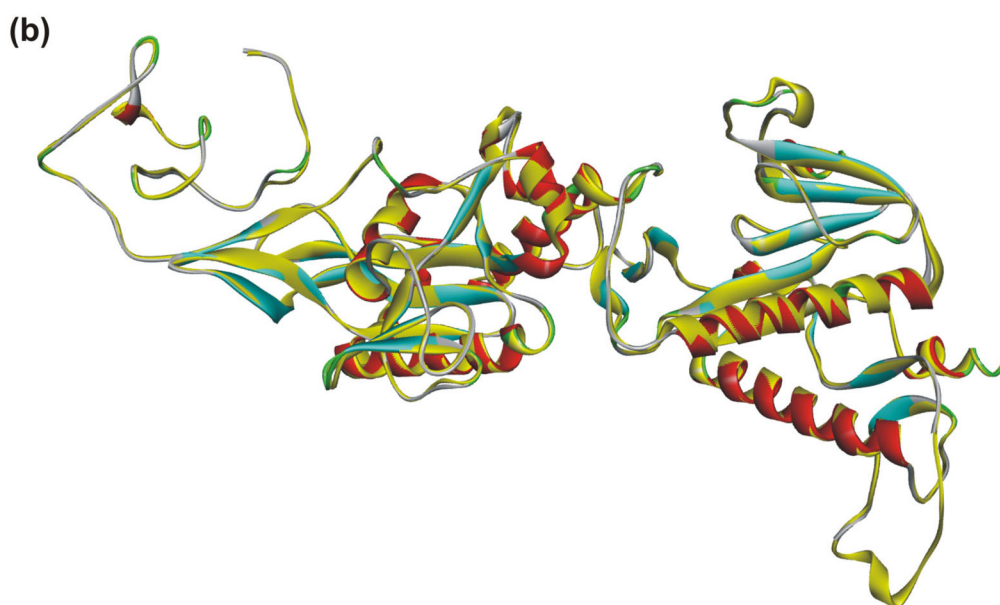
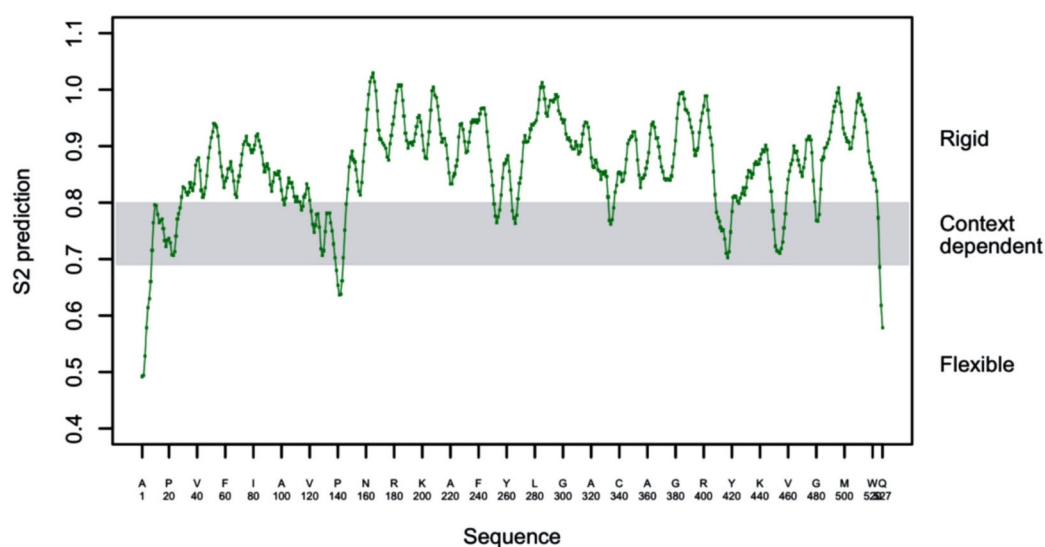


Figure 2. (a) Sequence dynamics of model protein sequence (YP_009725309.1) (b) Model-Template structural alignment of SARS-CoV-2 Nsp14 (Yellow) and SARS-CoV Nsp14: 5C8S (Red and blue).

3. Results and discussion

3.1. Homology modeling and evaluation of model quality

The search for suitable template from the SWISS-MODEL template library (SMTL) and sequence alignment identified a total of 38 templates that were found to match the target sequence. This list was filtered by a heuristic down to 16. A hit list of five templates was further selected for modeling. Finally a SARS-CoV Nsp14 with PDB ID: 5C8S (Ma et al., 2015) was selected as the best template. The Global Model Quality Estimate (GMQE) score is an estimated quality score of expected output model, using a particular template. The selected template has a GMQE score of 0.98, sequence coverage of 1.00, sequence identity of 95.07%, and sequence similarity of 0.65 with the template (Figure 1). A template with sequence similarity of more than 30% (65% in this case) with the sequence of interest is generally considered suitable and model obtained therewith will be

expected to be reliable and plausible (Xiang, 2006). The QMEAN Z-score of the model generated was -1.99 (Table 2). The QMEAN score gives an estimate of the degree of native-likeness of the model, and scores close to 0 and not lower than -4.0 indicate models having satisfactory agreement with experimental structures of similar size (Benkert et al., 2011).

The modeled SARS-CoV-2 Nsp14 was refined using 3DRefine. Analysis of the refined models with PROCHECK and Protein Sequence Analysis tool (ProSA) suggested that the 3DRefined-model had higher improvement in quality and was therefore selected for subsequent steps. The ProSA tool calculates the overall quality score as a measure of its Z-score in relation to the Z-scores of PDB experimentally determined native structures. The Z-score of our model from ProSA-web analysis was -9.3 (Figure 2A and Table 2), this falls within the range of those of the PDB native structures. In addition, the amino acids local energy profile plot showed that all the residues across the sequence have negative

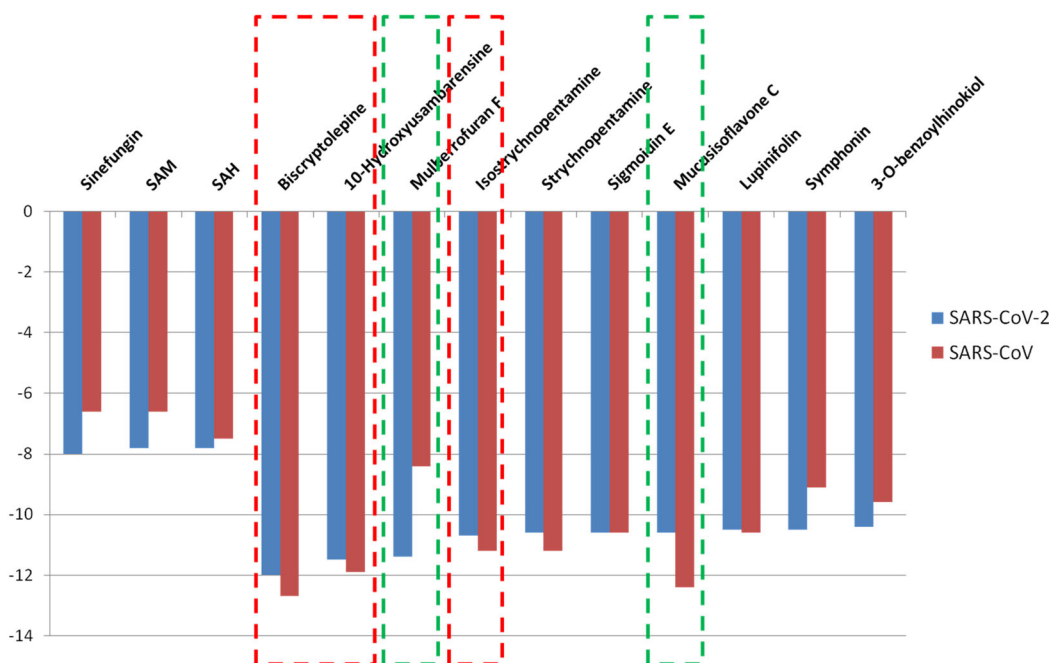


Figure 3. Binding energies reference inhibitor (sinefungin), reference substrates and ten hit phytochemicals to the catalytic residues of SAR-CoV-2 and SAR-CoV guanine N7- methyltransferase. The red dotted boxes show the top 3 docked alkaloids while green dotted boxes show the top docked flavonoids.

values (Figure S1b) indicating favorable amino acid energy profile, high accuracy and reliability of the model. From the Ramachandran phi/psi torsion angles plot as generated from PROCHECK analysis, 100% of the residues in the modeled protein structure are in the allowed regions with 93.4% in the most favored regions, 6.4% in additional allowed regions and only 0.0% in disallowed regions (Figure S1c and Table 2). This indicates that the model has good stereochemical quality and energetically favored geometry. The overall quality factor (OQF) of 82.271% from ERRAT plot analysis of the model falls above the generally accepted score of >50% while the OQF for the template used was 65.3968%. ERRAT error plot is a statistical analysis of the non-bonded atomic interaction of protein structures with the quality level calculated as OQF, and higher OQF scores implying higher model quality. The SARS-CoV-2 Nsp14 model ERRAT plot (Figure S1d) showed that most of the residues are properly folded with very low error rate, hence the high OQF. The refined Guanine-N7 methyltransferase model structure was aligned and superimposed with the 5C85 template pdb structure using PyMOL software. The structural alignment showed an RMSD of 0.385 Å (Figure 2c and Table 2) indicating that the model had a very high structural similarity with the template and thus reliable and suitable for the study.

3.2. Ligand modeling

Ligands present in the template structure were transferred by swiss-model to the modeled protein to analyses the interaction between the native ligand of the template and the model protein. The ligands (Guanosine-P3-Adenosine-5' and 5'-Triphosphate, S-Adenosyl-L-Homocysteine), interacted in similar manner with the model as the template. Also 3 of the 5 metal complexes (Zn3-5) interacted in similar manner as the template.

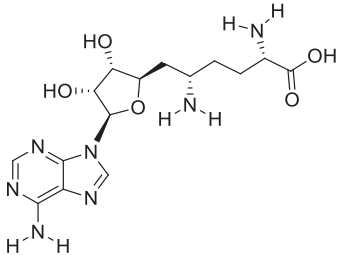
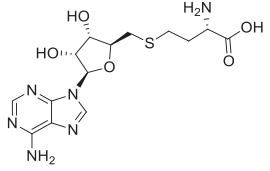
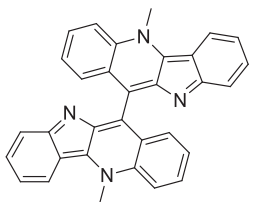
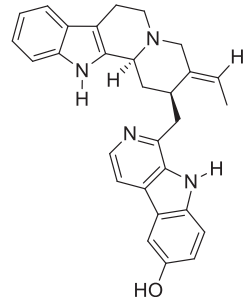
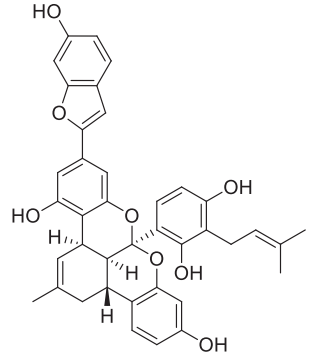
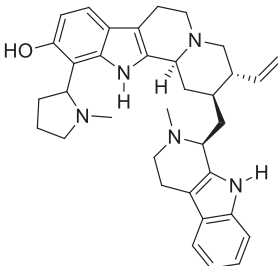
This shows that the binding region for the ligands in the template is conserved in the modeled protein.

3.2. Molecular docking of compounds with the target protein

The results of the docking analysis of the 249 bioactive compounds alongside the reference inhibitors (sinefungin and S-adenosyl-L-homocysteine (SAH) used as positive controls) against modeled S2GMT are presented in Table S1 (supplementary material). From the result, a hit-list of 21 phytochemicals with binding affinities higher than -10.0 Kcal/mol was defined. These 21 phytochemicals exhibited various orientations at the catalytic site, and had binding affinities higher than the reference inhibitors (sinefungin: -8.1 , and SAH: $-7.8 - 10.0$ Kcal/mol) for S2GMT (Table S1). The selected compounds were further docked to the active site region of SGMT. The ten top-ranked phytochemicals (THP) to the modeled S2GMT and the corresponding binding affinities to the SGMT are represented in Figure 3. From these ten, the four lead phytocompound (FLP) with the highest binding affinity to modeled S2GMT were further selected for molecular dynamics simulation analysis. These include are three alkaloids (Isocryptolepine, 10'-Hydroxyusambarensine, Isostrychnopentamine), and a flavonoid (Mulberrofuran F) with binding energies of -12.0 , -11.5 , -11.4 and -10.7 Kcal/mol to SGMT and -12.7 , -11.9 , -11.2 and -8.4 Kcal/mol to SGMT respectively. The interactions of the top 4 phytochemicals to both viral GMT are presented in Table 3.

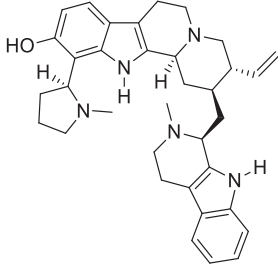
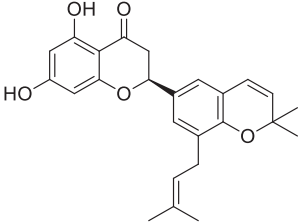
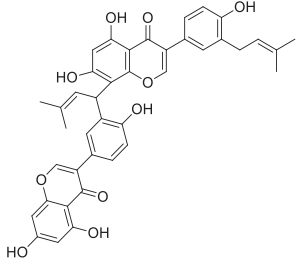
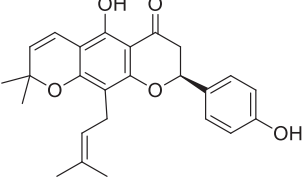
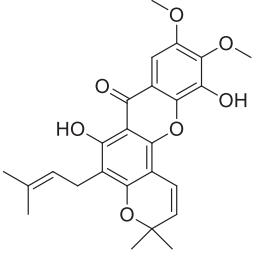
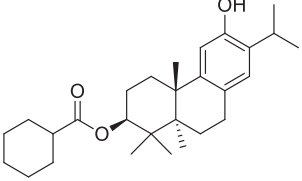
From the FLP that were docked to modeled S2GMT, biscryptolepine and 10-hydroxyusambarensine expressed higher binding affinity to SGMT compared to that of SARS-CoV-2. While mulberrofuran F had a binding affinity of -11.4 Kcal/mol to the modeled S2GMT, a lower binding affinity of -8.4 was observed. In view of the later, Mucisoflavone C an isoflavone was selected as the 2nd top docked compound to SGMT.

Table 3. The structures of top-ten docked phytochemicals and reference compounds to the catalytic residues of SAR CoV-2 guanine-N7 methyltransferase.

S/No	Bioactive Compounds		Class of compound	Plant species (Family)
S1	Sinefungin			
S2	S-adenosyl-L-homocysteine(SAH)		Nucleoside	
1	(60)_Biscryptolepine		Cryptolepines	<i>Cryptolepis sanguinolenta</i> (Periplocaceae)
2	10 -Hydroxyusambarensine		Indole alkaloids	<i>Strychnos usambarensis</i> (Loganiaceae)
3	Mulberrofuran F		2-arylbenzofuran flavonoids	<i>Morus mesozygia</i> (Moraceae)
4	Isostrychnopentamine		Indole alkaloids	<i>Strychnos usambarensis</i> (Loganiaceae)

(continued)

Table 3. Continued.

S/No	Bioactive Compounds		Class of compound	Plant species (Family)
5	Strychnopentamine		Indole alkaloids	<i>Strychnos usambarensis</i> (Loganiaceae)
6	Sigmoidin E		Flavanones	<i>Erythrina abyssinica</i> (Leguminosae)
7	Mucisoflavone C		Isoflavones	<i>Morus mesozygia</i> (Moraceae)
8	Lupinifolin		Flavanones	<i>Derris trifoliata</i> (Leguminosae)
9	Symphonin		Xanthenes	<i>Allanblackia monticola</i> (Guttiferae)
10	3-O-benzoylhinokiol		Quinones	<i>Hoslundia opposita</i> (Lamiaceae)

Mulberrofuran F was more selective for S2GMT S2GMT, while Mucisoflavone C was more specific for SGMT (Figure 3).

3.3. Molecular interactions between the selected phytochemicals and viral guanine N7-methyltransferase

The amino acid interactions of the FLP with viral GMT revealed that, the phytochemicals majorly interacted with

the residues through hydrogen bonding and hydrophobic interactions, electrostatic interactions, with few other interactions (Table 4). A comparative analysis of the binding pattern of the reference inhibitor (sinefungin) to both the modeled S2GMT and the the earlier strain SGMT was studied. Since the template has a high sequence similarity with the model enzyme, we expected a similar binding mode but our result shows that though sinefungin was docked in substrate

Table 4. Interactions of top docked phytochemicals with active site residues of SARS-CoV-2 and SARS-CoV guanine N7- methyltransferase.

Compounds	Coronavirus	Numbers	Hydrogen bonds Interacting residues (Bond distance)	Numbers	Electrostatic bond Interacting residues (Bond distance)	Numbers	Other Interacting residues
Sinefungin	SARS-CoV-2	11	VAL ²⁹⁰ (3.32) TYR ³⁶⁸ (3.00, 2.26,) ASN ³⁸⁶ (2.47, 2.82) VAL ³⁸⁷ (2.09, 2.19) ASN ³⁸⁸ (3.90) PHE ²⁸⁶ (3.90) ASP ³⁵² (3.31) CYS ³⁸⁷ (4.18,3.93) HIS ⁴²⁷ (2.95, 2.91) TYR ³⁶⁸ (1.98) ASN ³⁸⁸ (2.57) ASP ³⁵² (2.06, 2.68) VAL ²⁸⁷ (2.13) LYS ²⁸⁸ (2.58)	4	PHE ³⁶⁷ (4.46,5.52) ALA ³⁵³ (5.144.46)		none
SAM					none		none
SAH		8	HIS ⁴²⁷ (2.95) TYR ³⁶⁸ (2.59) ASN ³⁸⁸ (2.22, 2.55) ASP ³⁵² (2.57, 2.68) VAL ²⁸⁷ (2.13) LYS ²⁸⁸ (2.98)		none		none
Biscryptolepine			TRP ³⁸⁵ (2.59) ASN ³⁸⁶ (2.63, 2.47, 3.59) GLN ³¹³ (3.90)		PHE ⁴²⁶ (3) (3.89, 3.91, 4.92) TRP ³⁸⁵ (2) (5.17, 4.96)	2	LYS ³³⁶ (2) (4.08, 3.82)
10-Hydroxyusambarensine		4	ASN ³⁸⁶ (2) (3.03, 2.26), ASP ³⁵² (2) (1.97, 3.06)	8	PHE ⁴²⁶ (4) (4.75, 3.65, 5.46, 3.65), PRO ³³⁵ (4.79), CYS ³⁸⁷ (2) (5.26, 4.79)		noe
Mulberrofuran F		6	GLN ³¹³ (2.98) LYS ³³⁶ (2) (2.74, 2.85) ASN ³⁰⁶ (2) (2.59, 2.19) ASN ³⁸⁶ (3.74)	6	PHE ⁴²⁶ (2) (3.92, 3.94) CYS ³⁴⁰ LEU ³⁸³ (4.39) ARG ³¹⁰ (5.49607)	1	CYS ³⁰⁹ (5.94)
Isostrychnopentamine		6	GLY ³³³ (2.98, 2.00) ASN ³⁸⁶ (2.84, 2.15) ASN ³⁰⁶ (2.50, 2.99)	5	CYS ³⁰⁹ (5.24) PHE ⁴²⁶ (4.51), TRP ²⁹² (5.05) ARG ²⁸⁹ (5.23)		none
Sinefungin	SARS-CoV	11	ALA ³⁵³ (2.36) TRP ³⁶⁸ (1.93) ASP ³⁵² (2.11) TRP ³⁸⁵ (2.77, 2.59) ASN ³⁸⁶ (2.19, 2.52) GLY ³³³ (3.77) ASN ³⁸⁶ (2.39) CYC ³⁸⁷ (3.87,3.90)	3	PHE ³⁶⁷ (4.64) ALA ³⁵³ (4.00, 4.35)		none
SAM		5	ASP ³⁵² (2.47, 2.31, 3.18) TRP ³⁶⁸ (2.47) ASP ³⁵² (2.11)		none		none
SAH		8	ALA ³⁵³ (3.03) ASN ³⁸⁸ (2.58) ASP ³⁵² (2.44, 2.16, 2.95) ASN ³⁸⁶ (2.39) HIS ⁴²⁷ (2.30) PHE ³⁶⁷ (2.82)		none	2	TRP ³⁹² (5.96, 5.01)
Biscryptolepine		2	TRP ³⁸⁵ (3.01) ASN ³⁸⁶ (2.81)	7	PHE ⁴²⁶ (4.25, 4.06, 5.66, 3.86) TRP ²⁹² (5.78) VAL ²⁹⁰ (5.09) PRO ³³⁵ (5.30)	2	ARG ³¹⁰ (4.51, 4.47)
Mucisoflavone C		6	ARG ³¹⁰ (2.64) LYS ³³⁶ (2.21) ASN ³⁸⁶ (2.64) ASN ³⁸⁸ (1.93) TRP ³⁸⁵ (2.61) HIS ⁴²⁷ (2.81)	14	VAL ²⁹⁰ (3.95) TRP ²⁹² (4.51, 5.03) PHE ⁴²⁶ (4.38, 3.96, 5.04) HIS ⁴²⁴ (4.65) ALA ³⁵³ (4.28, 3.76) PHE ³⁶⁷ (4.54) CYS ³⁸⁷ (4.56, 5.09) ILE ³³² (5.28) CYS ³⁰⁹ (4.29)	3	ARG ³¹⁰ (4.18) HIS ⁴²⁴ (4.07) ASP ³⁰⁶ (3.90),
10 -Hydroxyusambarensine			ASP ³⁵² (3.54)		PHE ⁴²⁶ (4.94, 3.85, 3.60) PRO ³³⁵ (5.24, 4.46, 5.28) VAL ²⁹⁰ (4.97) CYS ³⁸⁷ (4.78, 5.00)		ASP ³⁵² (3.22)
Isostrychnopentamine			ASN ³⁸⁶ (2.87) GLY ³³³ (3.26)		ILE ³³⁸ (3.47, 5.34) PHE ⁴²⁶ (4.76, 3.73, 3.76) ARG ³¹⁰ (4.81, 5.18)		none

binding cavity but it interacted differently to the catalytic residues of both protein. A previous study that compared the interaction of G3A the major activator of the enzyme to both model and SGMT report a different binding pattern of G3A to both enzymes (Selvaraj et al., 2021). In our study sinefungin was docked above the central β 1 in the loops connecting the β strands of SGMT in a hydrophobic environment known to be created by amino acids such as Ile332, Phe367, and Val389 (Ma et al., 2015). Sinefungin made 7 hydrogen bonds with ALA³⁵³, TRP³⁶⁸, ASP³⁵², TRP³⁸⁵, ASN³⁸⁶, ASN³⁸⁶ of the SGMT, while it made 7 hydrogen bonds VAL²⁹⁰, TYR³⁶⁸, ASN³⁸⁶, VAL³⁸⁷, ASN³⁸⁸, PHE²⁸⁶, ASP³⁵². The purine ring of sinefungin formed 2 hydrogen bonds with ALA⁵³⁵ and TYR³⁶⁸ and a Pi-

Alkyl and Pi-Pi-stacked interactions with ALA⁵³⁵ and PHE³⁶⁷ of SGMT respectively, while the same ring formed 2 hydrogen bonds TYR³⁶⁸ and ASN³⁸⁸ of modeled S2GMT. The hydrophobic interactions (Pi-Alkyl and Pi-Pi-stacked) between the ring and residue ALA⁵³⁵ and PHE³⁶⁷ of modeled S2GMT was similar to those observed in SGMT. The 2,5-diamino moiety which was responsible for the majority of the hydrogen bonds with TRP³⁸⁵, ASN³⁸⁶ and ASP³⁵² of SGMT formed 4 hydrogen bonds with VAL²⁸⁷, ASN³⁸⁶ and PHE²⁸⁶ of modeled S2GMT. In both cases, the two amino groups integrated with different amino acids (Figures 4a and 5a). This most have been responsible for the differences in the binding energies for the binding of sinefungin to the modeled S2GMT and SGMT.

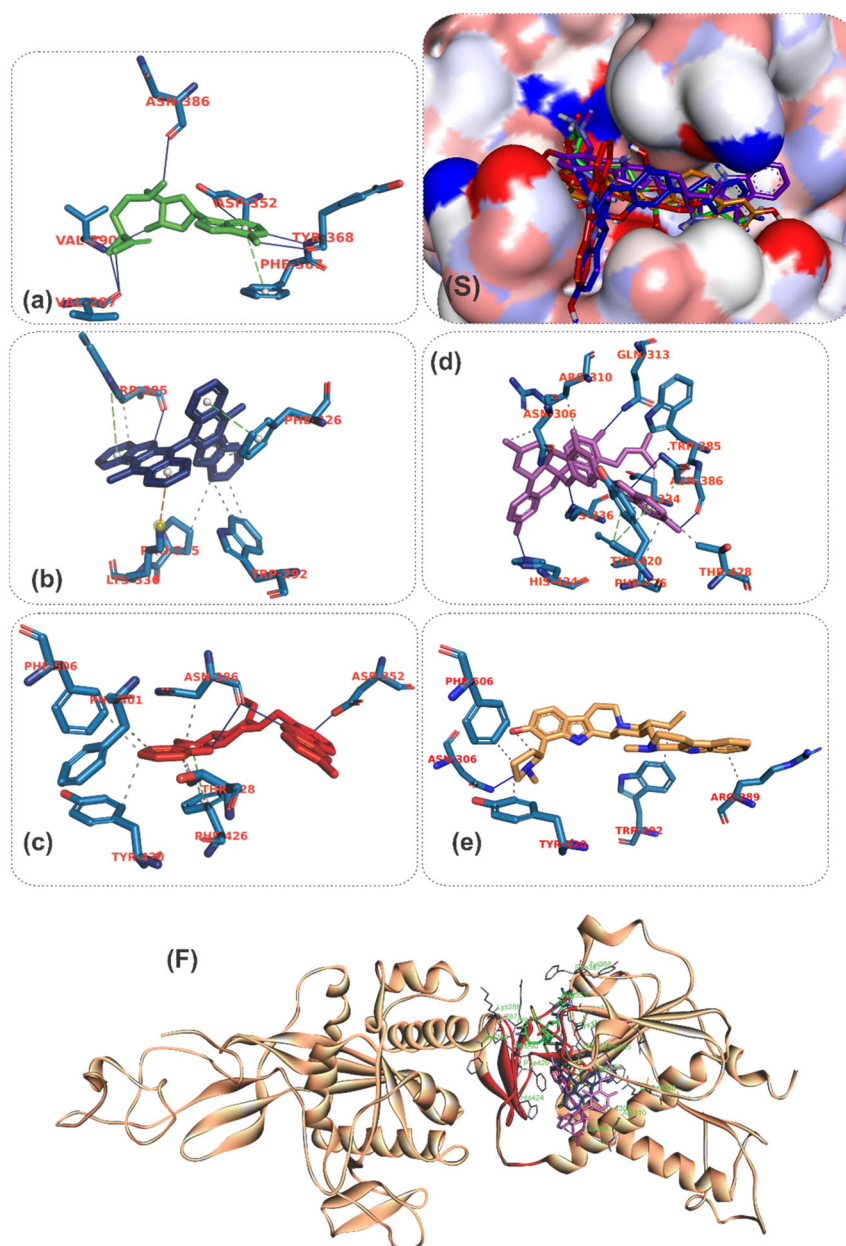


Figure 4. (S) Surface view representation of interacting ligands in binding cavity of modeled guanine N7- methyltransferase of SARS-CoV-2 Nsp14. Ligands in sticks representation are represented by colours: (a) Sinefungin- green (b) Biscryptolepine- blue (c) 10'-hydroxyusambarensine -red, (d) Mulberrofuran F- purple and (e) Isostrychnopentamine-gold. Types of interactions are represented by Green- dotted lines: H-bond interactions, light purple-dotted line: hydrophobic interactions (Pi-Alkyl, Alkyl & pi-stacking) purple-dotted line: Pi-Pi T Shaped, yellow-dotted lines: Pi-sulphur interactions, pi-stacking interactions. Three-letter amino acids are in red colour.

SAM and SAH exhibited similar binding pattern to the two viral GMT. They were docked in the SAH-GpppA binding pocket. They interacted mostly via conventional hydrogen bonds to same amino acid residue. Biscryptolepine, a cryptolepine alkaloid isolated from *Cryptolepis sanguinolenta* (Osafo et al., 2017), with documented antimalarial, antibacterial and antifungal activities (Cimanga et al., 1998; Van Miert et al., 2005) was the top docked compounds to both viral GMT. It demonstrated strong interactions with the amino acid residue responsible for the binding of SAM, SAH and Sinefungin. A conventional hydrogen bond to TRP³⁸⁵ was observed while 2 carbon hydrogen bonds were observed between the 5-methyl attached to the quindoline moiety and ASN³⁸⁶ residue. Two Pi-Cation with LYS³³⁶, 3 Pi-Pi stacking and 2 Pi-Pi T

shaped interaction were observed with PHE⁴²⁶ and TRP³⁸⁵ of S2GMT respectively. Earlier *in vitro* studies have associated certain alkaloids from *Cryptolepis sanguinolenta* with antiviral activities (Cimanga et al., 1996). Recent studies also suggested that several alkaloid cryptolepins from *Cryptolepis sanguinolenta* may inhibit SARS-CoV-2 proteins (Borquaye et al., 2020).. 10'-Hydroxyusambarensin, an indilo alkaloid isolated from *Strychnos sambarensis* (Onguéné et al., 2013), the 2nd top compound to the modeled S2GMT exhibited a higher binding affinity to SGMT. It interacted via conventional hydrogen bond to ASN³⁸⁶ and ASP³⁵², 2 Pi-Pi stacking, and a Pi-Alkyl and Alky interaction to PHE⁴²⁶. An alkyl interaction to PRO³³⁵ and 2 Pi-Alkyl interactions to CYS³⁸⁷ were also observed with S2GMT. In the same vein a carbon hydrogen

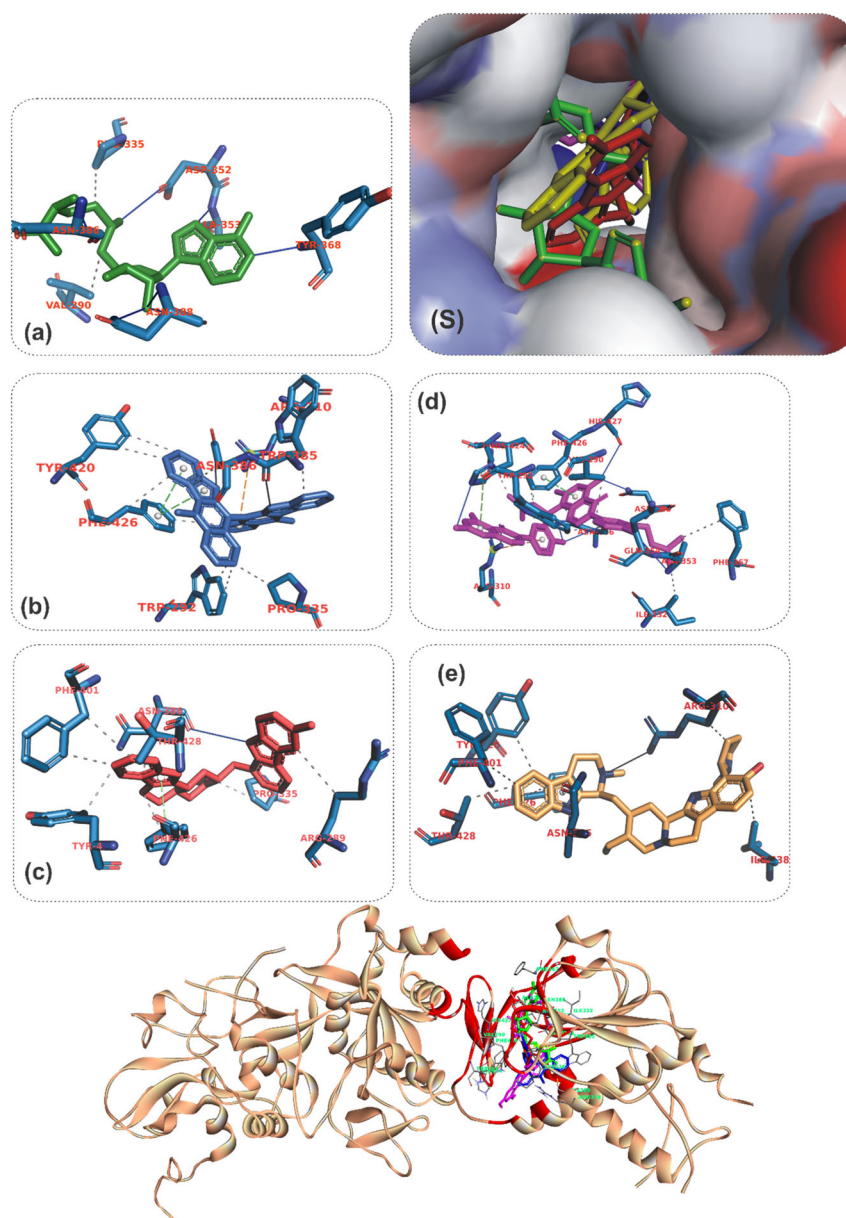


Figure 5. Cartoon representation of interacting ligands in binding cavity of guanine N7- methyltransferase of SARS-CoV Nsp14. Ligands in sticks representation are represented by colours: (a) Sinefungin-green; (b) Biscryptolepine - blue (c) 10' - hydroxysambarensine-red, (d) Mucisoflavone C - purple and (e) Isostrychnopentamine - gold.

bond, Pi-Anion, Pi-Pi, Alkyl and Pi-Alkyl interaction was observed between 10'-Hydroxysambarensin and the modeled SGMT protein. Mulberrofuran F isolated from *Morus alba*, has been used to treat hypotension (Fukai et al., 1985). Its interaction to modeled S2GMT involved conventional H-bond to 7 amino acid residues, while the hydrophobic interaction includes: Pi-Sulfur to CYS³⁰⁹, Pi-Pi stacking to PHE⁴²⁶ and Pi-Alkyl interaction to LEU³⁸³ and ARG³¹⁰. On the other hand mucisoflavone C interacted via hydrogen bond, Pi-Cation and Pi-Anion to ARG³¹⁰ HIS⁴²⁴ and ASP³⁰⁶ respectively. The various hydrophobic interactions observed includes: Pi-Sigma, Pi-Pi, Stacking, Pi-Pi T shaped Alkyl and P-Alkyl to SGMT. Six carbon hydrogen bonds to 4 amino acid residues were observed between Isostrychnopentamine an indole alkaloid and S2GMT. An Alkyl interaction to CYS³⁰⁹ and CYS³⁸⁷ a Pi- Alkyl to PHE⁴²⁶, TRP²⁹² and ARG²⁸⁹ were the

hydrophobic interaction that were observed. A similar pattern of interaction was further observed to the SARS-CoV N7-MTase. Among the several amino acid residues: ARG³¹⁰, ASP³³¹, GLY³³³, PRO³³⁵, TYR³⁶⁸, CYS⁴¹⁴, and CYS⁶¹⁶ that are essential for the methyltransferase activity (Fabrega et al., 2004; Jin et al., 2013). interfering with the binding to ASP³³¹ and GLY³³³ have been reported to completely abolish the GMT activity (Fabrega et al., 2004). Also, the disruption of the interaction of TRP²⁹² that stacks against the ribose for optimal positioning of SAM has been shown to reduce the GMT activity by more than 50%. Among the amino acid residues that hold the purine moiety of guanosine in position, PHE⁴²⁶ demonstrated the largest influence on the GMT activity (Fabrega et al., 2004). In the same vain the importance of ASN³⁸⁶ which is located in proximity to the atoms involved in methyl transfer, has been reported. LYS³³⁶

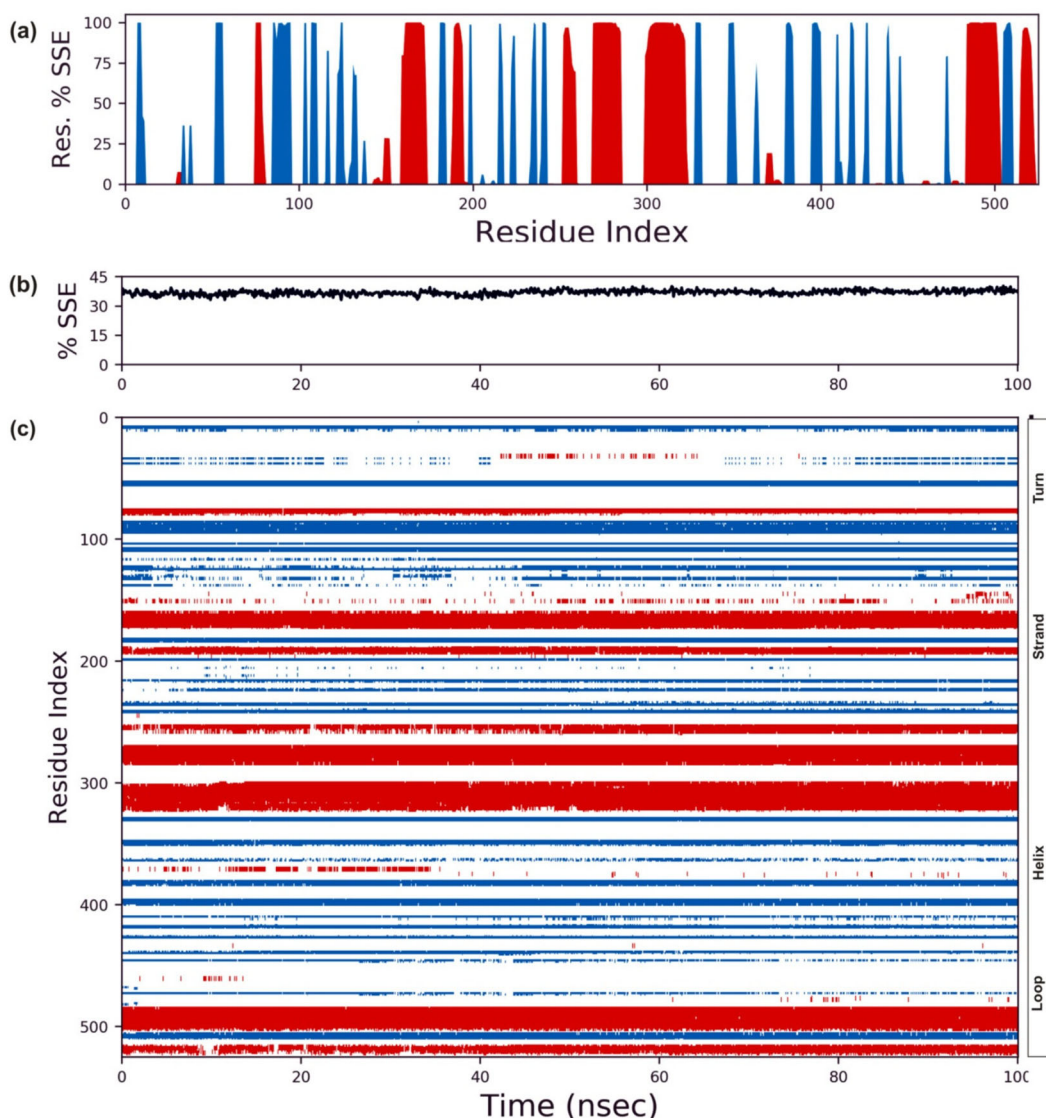


Figure 6. Modeled SARS-CoV-2 Nsp14 secondary structural analysis during 100 ns MD simulation (a) SSE distribution by residue index throughout the protein structure (b) summary of the SSE composition for each trajectory frame (c) residue and its SSE assignment over time.

contributes the positive potential for binding the triphosphate moiety of SAM. These amino acids help the ligands to be oriented in a highly constricted pocket, ensuring that the methyl group of SAM is brought in close proximity to the N7 of guanine to realize the methyl transfer using an in-line mechanism (Fabrega et al., 2004). Thus, compounds that bind in similar orientation as the substrates will inhibit the viral GMT activity. Biscryptolepine, 10'-Hydroxyusambarensine and Isostrychnopentamine have been reported to have strong binding tendencies to various SARS-CoV-2 non-structural proteins such as 3-chymotrypsin-like protease, RNA-dependent RNA polymerase and spike glycoprotein (Borquaye et al., 2020; Gyebi et al., 2022).

3.4. Result from molecular dynamic analysis

A 100-ns all-atom molecular dynamics simulation was performed on the complexes of the FLP with the modeled SARS-CoV-2 Nsp14. The MDS trajectories were analysed for:

3.4.1. Ligand properties

The FLP were analysed relative to their reference conformation. The plot for the various parameters includes, a stable RMSD fluctuation ($< 3.0 \text{ \AA}$) observed for all the ligands with respect to the reference conformation, showing no immense dynamical alteration (Cheng et al., 2012). FLP in the complexes showed a stable Radius of Gyration (rGyr) profile, suggesting that no conformational alterations (Figure S2b–S5b supplementary data) (Sinha & Wang, 2020). Biscryptolepine and 10'-hydroxyusambarensine displayed no intramolecular hydrogen bond (intraHB) distortion during the simulation (Figure S2b–S5b supplementary data) while Mulberrofuran F and Isostrychnopentamine expressed minimal intraHB fluctuation. The MolSA, SASA and PSA for the top four phytochemicals were in acceptable ranges, indicating that they were imposingly stable in the complexes during the simulation run. The conformational evolution of every rotatable bond (RB) in the top four phytochemicals was analysed using the Ligand Torsion Profile (LTP). The plots for these parameters are presented in Figure S6–S9 (supplementary data).

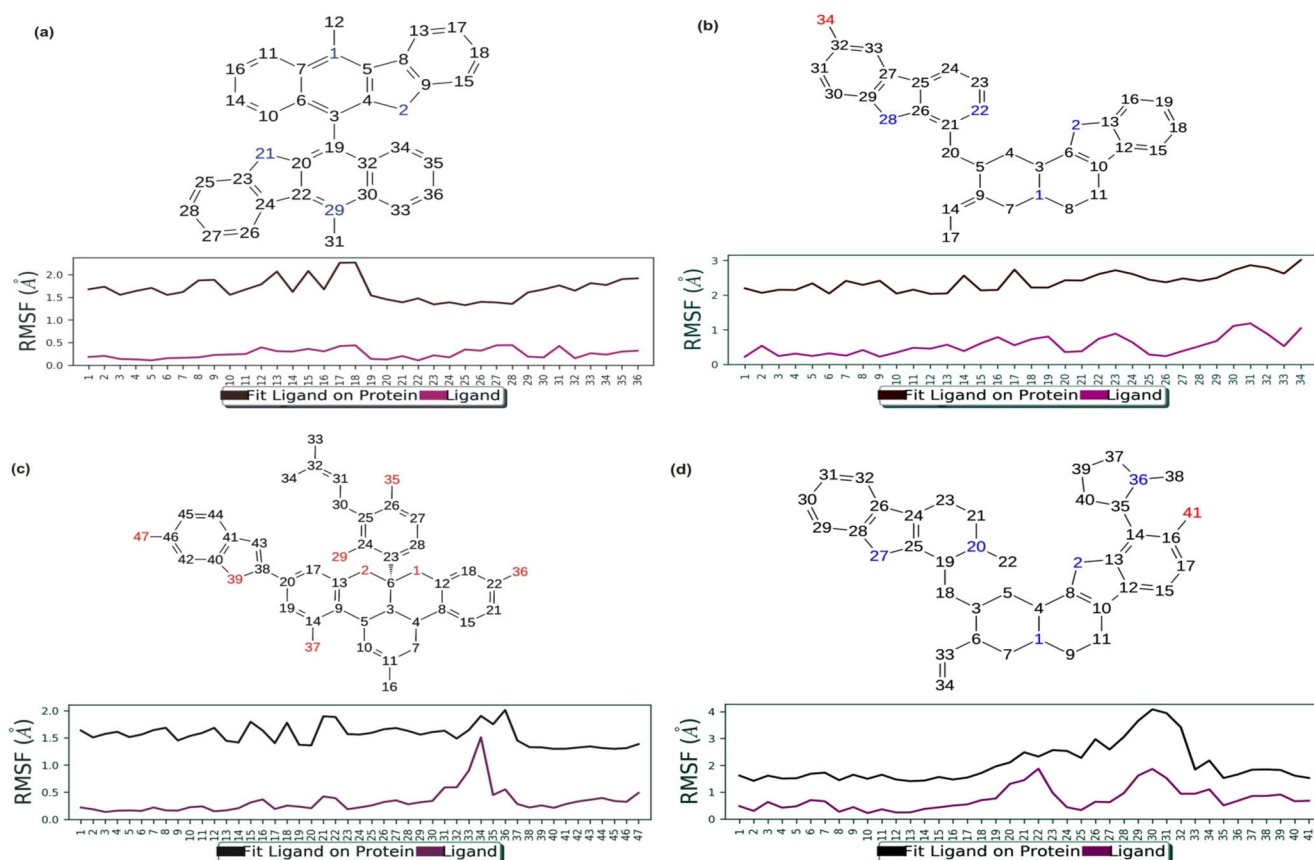


Figure 7. Analysis of MD Simulation results: Ligand Root Mean Square Fluctuation (L-RMSF) (Å) of (a) Biscryptolepine F (b) 10-Hydroxyusambarensine (c) Mulberrofuran F (d) Isostrychnopentamine complexed with modeled SARS-CoV-2 guanine-N7 methyltransferase during 100 ns MD simulation.

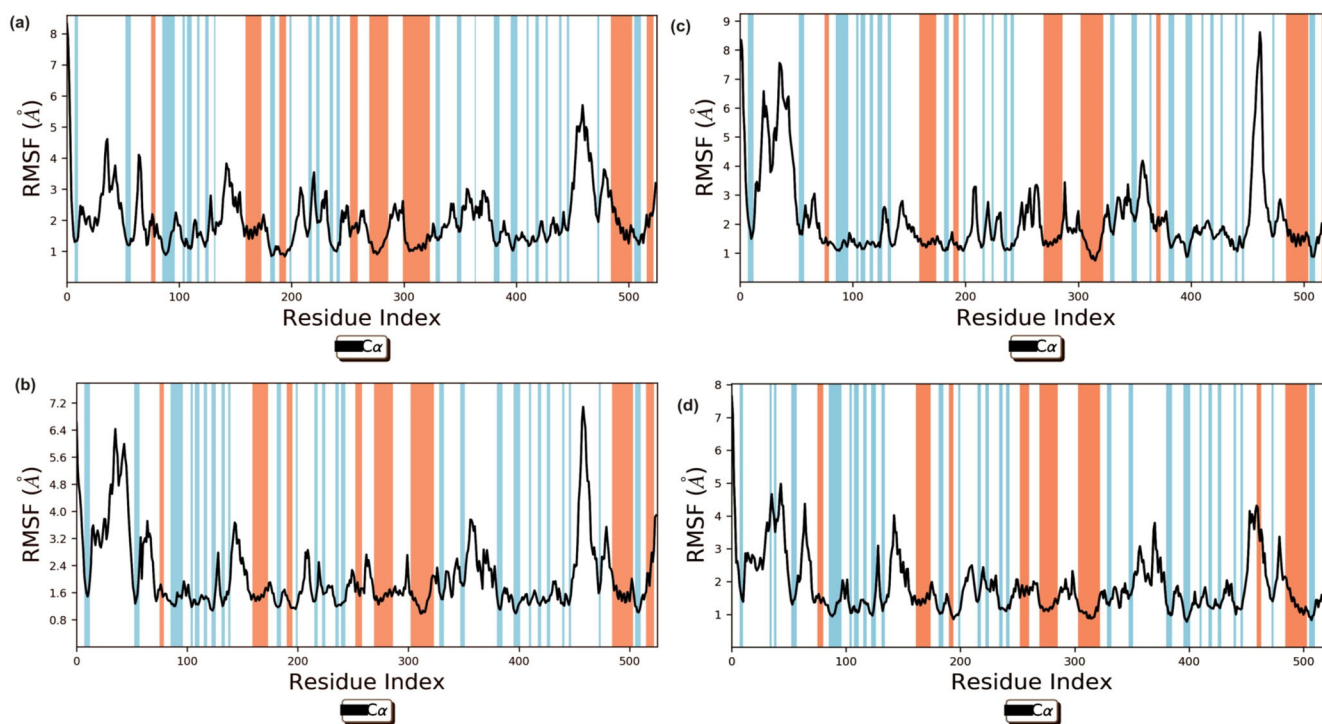


Figure 8. RMSF (Å) Analysis of modeled SARS-CoV-2 Nsp14 complexed with (a) Biscryptolepine (b) 10-Hydroxyusambarensine (c) Mulberrofuran F (d) Isostrychnopentamine during 100 ns MD simulation. Secondary Structure Elements: alpha-helical and beta-strand regions are highlighted in red and blue backgrounds, respectively.

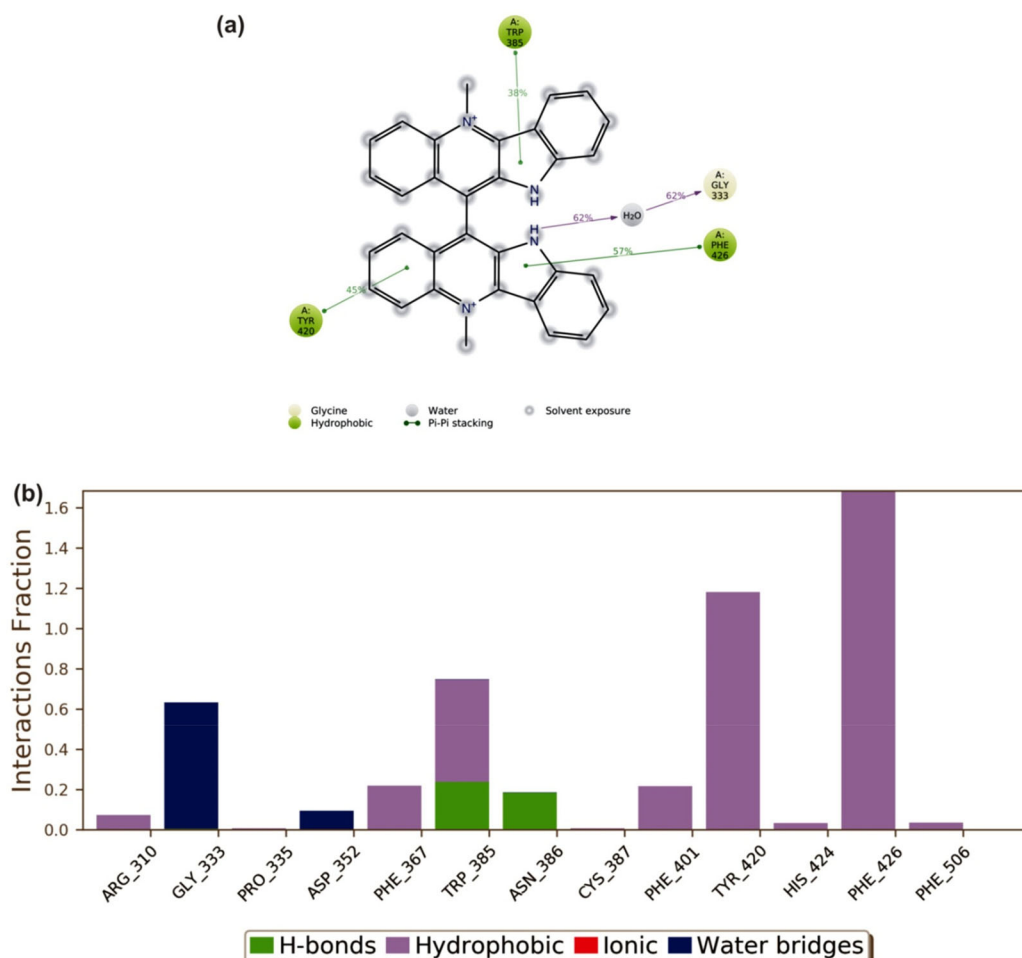


Figure 9. (a) A schematic details of biscriptolepine atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown (b) simulation interactions plot showing categorized modeled SARS-Cov-2 guanine-N7 methyltransferase-biscriptolepine interactions.

3.4.2. Protein secondary structure

The secondary structure elements (SSE) of the modeled SARS-CoV-2 Nsp14 such as alpha-helices and beta-strands were observed throughout the simulation. Figure 6a shows the SSE distribution by residue index throughout the protein structure. Figure 6b summarizes the SSE composition for each trajectory frame, and Figure 4c monitors each residue and its SSE assignment over time. The analysis showed that 19.23% was Helix, 17.63% was strands, while the Total SSE was 36.05%.

3.4.3. Ligand Ligand root mean square fluctuation

The Ligand Root Mean Square Fluctuation (L-RMSF) is useful for characterizing changes in the ligand atom positions. Figure 7a–d shows the fluctuations of the FLP broken down by atom, corresponding to the 2D chemical structure in the top panel. The 'Fit Ligand on Protein' line shows the phytochemicals fluctuations, with respect to the protein. Some degree of fluctuation (though within the acceptable range of $<2\text{Å}$) was observed in biscriptolepine atoms especially around atoms number 17 and 18. The internal atoms of the ligands experienced fewer fluctuations at that atom no (Figure 7a). For 10-hydroxyusambarensine the atoms involved in the interactions were stable with a lesser degree

of fluctuations with respect to the protein (Figure 7b). Atoms around number 34 demonstrated the highest internal fluctuations, but the hydrophobic interactions with CYS³⁴⁰ and LEU³⁸³ minimized the fluctuations (Figures 7c and d). Isostrychnopentamine presented some degree of internal fluctuations around atoms number 22 and 30. The fluctuation around the former atoms was reduced with their interactions with ASN³⁸⁶ while the ones around atom number 30 were still maintained in the bonded atoms (Figures 7d).

3.4.4. Protein root mean square fluctuation

Figure 8a–d shows the RMSF of modeled SARS-CoV-2 Nsp14 complexed with the FLP. The peaks indicate areas of the protein with the most fluctuations. In all the graphs, the SSE (alpha-helical and beta-strand) were more rigid than the unstructured parts while the N and C terminal fluctuated the most. For the 4 complexes the highest fluctuation was observed with the amino acid residues around residue number 40 and 450. The fluctuations in these unstructured parts were minimized by the binding of biscriptolepine and isostrychnopentamine. The catalytic and substrate binding residue were stable throughout the simulation period indicating that the binding of the FLP did not compromise the structural integrity of the protein (Cheng et al., 2012).

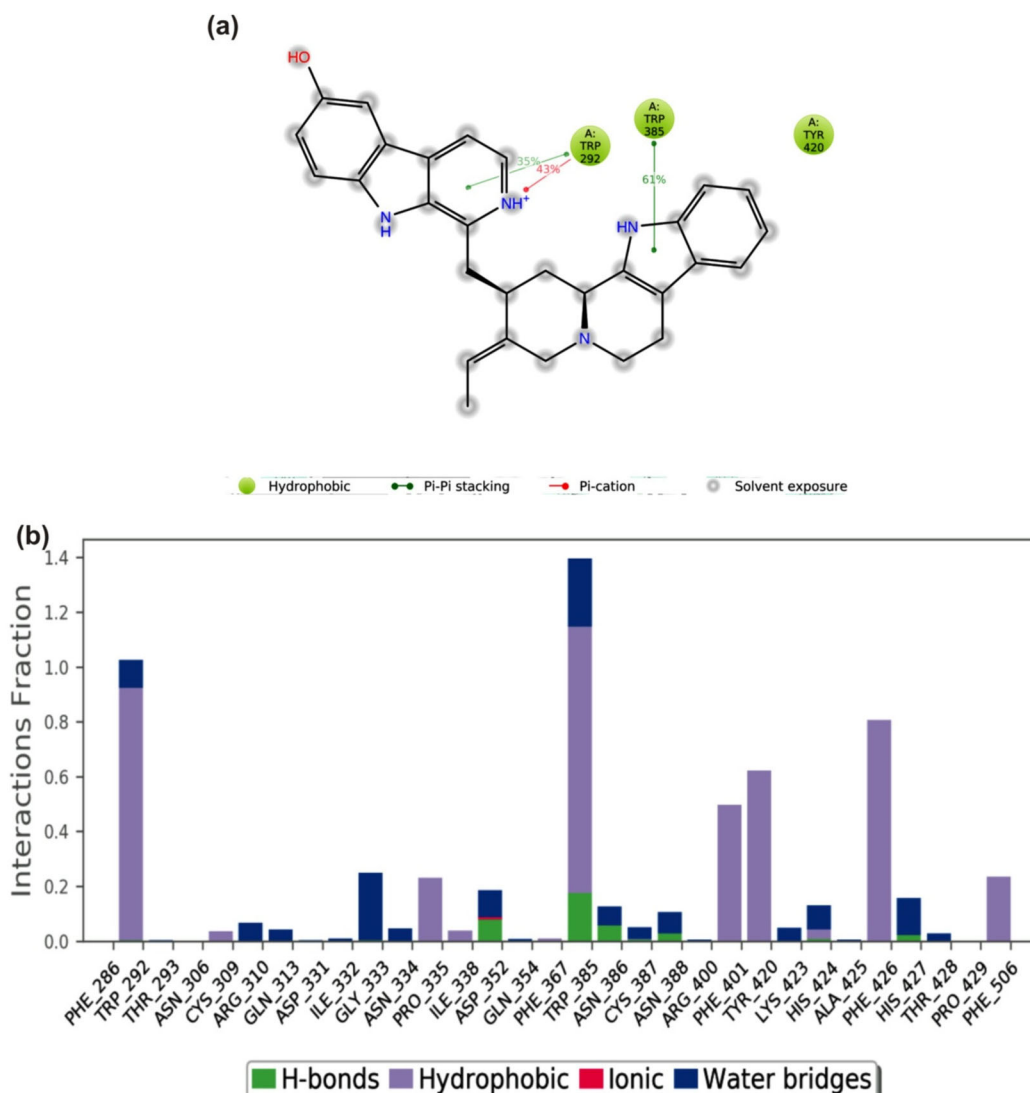


Figure 10. (a) A schematic details of 10-hydroxyusambarensine atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown (b) simulation interactions plot showing categorized modeled SARS-Cov-2 guanine-N7 methyltransferase - 10-hydroxyusambarensine interactions.

3.4.5. Protein-Ligand contacts

The 2D-trajectory interaction diagram (Figures 9a–12a) and the stacked bar interactions plots were categorized by type (Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges) and summarized in Figures 9–12. Biscryptolepine made 13 contacts with various residues of S2GMT, from which TYR⁴²⁰, TRP³⁸⁵ and PHE⁴²⁶ maintained contact for about 45%, 38% and 57% of the simulation time via hydrophobic interactions respectively; while TRP³⁸⁵ and ASP³⁵² maintained the highest hydrogen bond contacts (Figures 9). From the 31 contact that was established with 10-hydroxyusambarensine, TYR²⁹² and TRP³⁸⁵ also maintained contact for about 35% and 61% of the simulation time via hydrophobic interactions respectively, while TRP³⁸⁵ and ASP³⁵² maintain the highest hydrogen bond contacts (Figure 9). During the simulation mulberrofuran F made 27 contacts with the various residues, TYR⁴²⁰ and PHE⁴²⁶ maintained the highest hydrophobic contacts while ASN³⁰⁶ and THR⁴²⁸ maintained the highest hydrogen bond contacts for 53% and 32% respectively (Figure 11). Isostrychnopentamine also made 27 contacts of which

TRP²⁹² and TRP³⁸⁵ maintained the highest hydrophobic contact of 46% and 61% respectively. Ionic interactions within 3.7 Å of each other that do not involve a hydrogen bond were maintained for 32% and 46% to GLN³¹³ and HIS²⁴² (Figure 12).

A timeline presentation of the interactions and contacts (hydrogen bonds, hydrophobic, ionic, and water bridges) is summarized in (Figures S6–S9 supplementary data). The top panel shows the total number of specific contacts the protein made with the ligand over the course of the trajectory. The bottom panels indicate which residues interacted with the ligand in each trajectory frame.

3.4.6. Root mean square deviation analysis

Figures 13–16 shows the RMSD of modeled SARS-CoV-2 Nsp14 complexed with the FLP. C α shows the RMSD evolution of the modeled protein (left Y-axis). All protein frames were first aligned on the reference frame backbone, and then the RMSD was calculated based on the atom selection. Ligand RMSD (right Y-axis) indicates how stable the ligand is with respect to

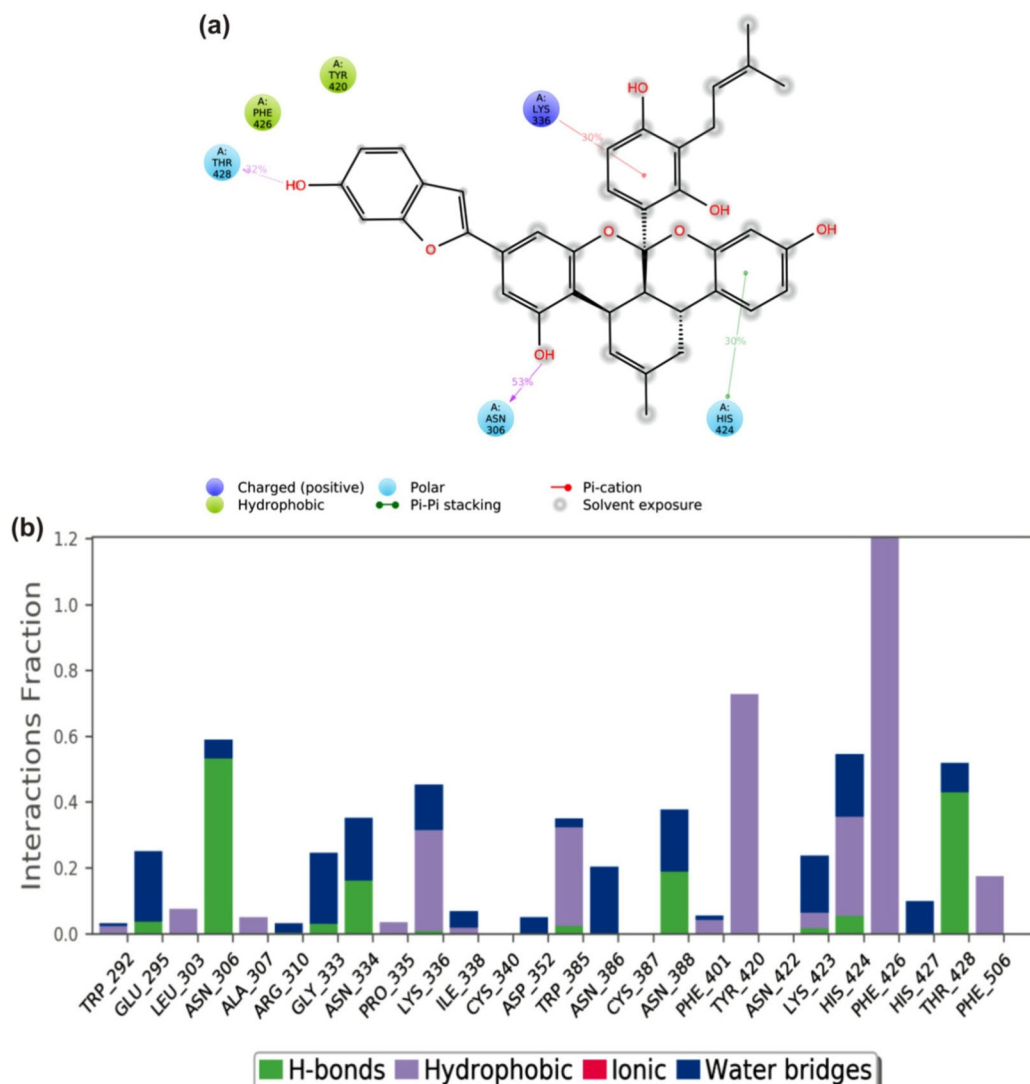


Figure 11. (a) A schematic details of Mulberrofuran F atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown (b) simulation interactions plot showing categorized modeled SARS-CoV-2 guanine-N7 methyltransferase-Mulberrofuran F interactions.

the protein and its binding pocket. ‘Lig fit Prot’ shows the RMSD of a ligand when the protein-ligand complex is first aligned on the protein backbone of the reference and then the RMSD of the ligand heavy atoms is measured.

For biscriptolepine-protein complex, the proteins $C\alpha$ and ligand bond protein were stabilized after 10 ns this was maintained during the simulation run with minimal fluctuating a convergence was also reached toward 100 ns (Figure 13). Both the $C\alpha$ protein and the modeled S2GMT complexed with 10-hydroxyusambarensine gained stability around 20 ns. The complex displayed various fluctuations around 20, 40 and 70 ns. These recorded fluctuations were less than 3.0 Å (Figure 14). Mulberrofuran F complex and the $C\alpha$ protein attained stability at the beginning of the simulation and a convergence was also reached toward 100 ns. The complex demonstrated more stability throughout the simulation than the modeled SARS-CoV-2 Nsp14 frames aligned on its reference frame backbone (Figure 15). Both Isostrychnopentamine complex and its $C\alpha$ protein achieved stability at the beginning of the simulation just

after 0 ns, this stability was maintained throughout the simulation with a little deep around 20 and 50 ns. The range of fluctuation was between 2.4–4.8 and 2.4–4.0 respectively (Figure 16). The average RMSD are 4.811, 3.912, 3.343 and 3.613 Å for (biscriptolepine 10-hydroxyusambarensine, mulberrofuran F and isostrychnopentamine)-protein complexes respectively.

The several thermodynamics parameters that were analyzed from the 100 ns full atomistic MD trajectory files of the FLP-SARS-CoV-2 Nsp14 complexes revealed that the interactions phytochemicals to the various catalytic residues were appreciably maintained during the period of the 100 ns run. All the four top phytochemicals with strong binding affinities to the modeled SARS-CoV-2 Nsp14 expressed considerable degree of structural stability, compactness, and flexibility (Cheng et al., 2012). This is a positive indication that could be well adapted to experimental models. The binding patterns from the docking analysis were also maintained in the simulated dynamic environment.

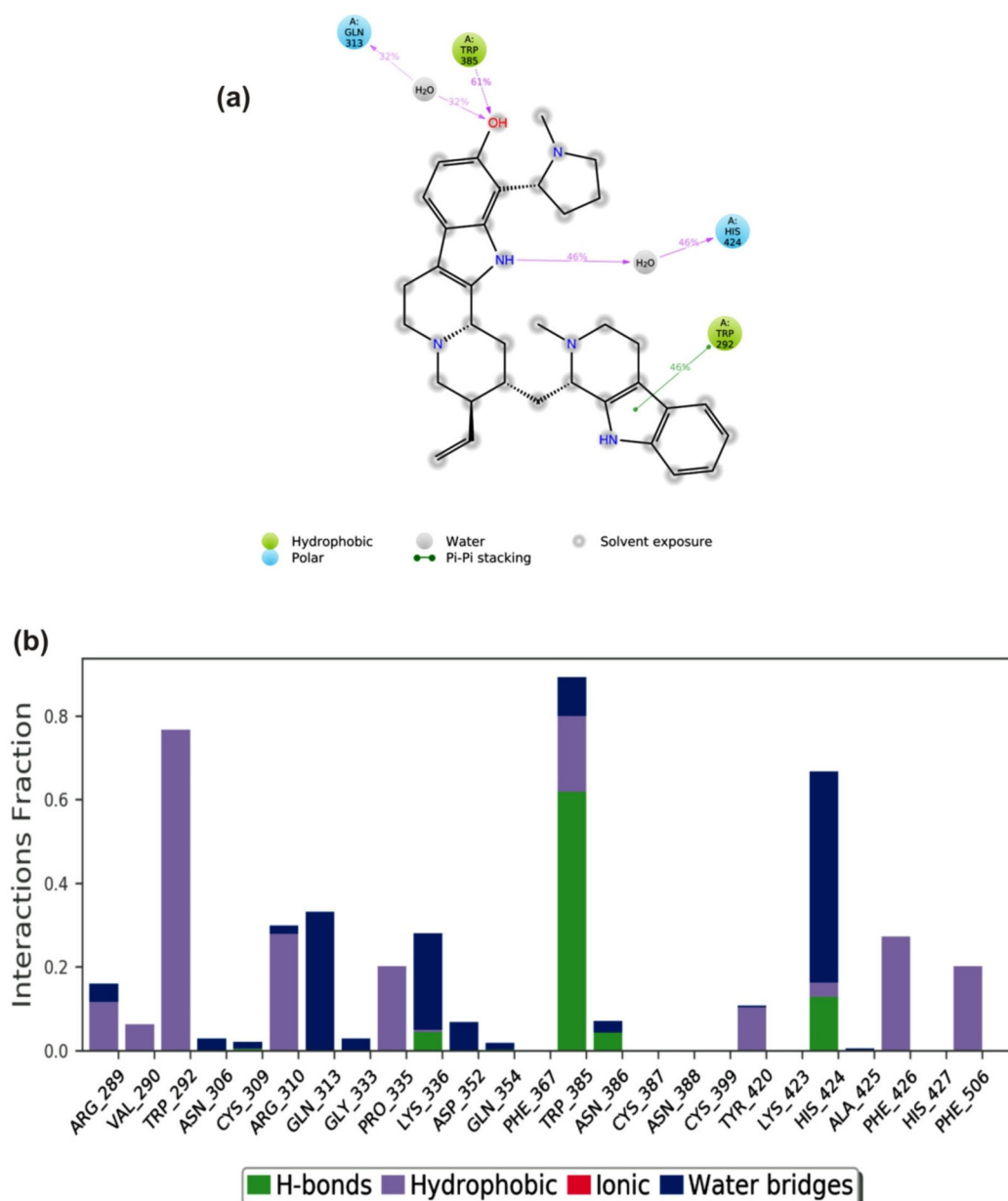


Figure 12. (a) A schematic details of Isostrychnopentamine atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown (b) simulation interactions plot showing categorized modeled SARS-Cov-2 guanine-N7 methyltransferase - Isostrychnopentamine interactions.

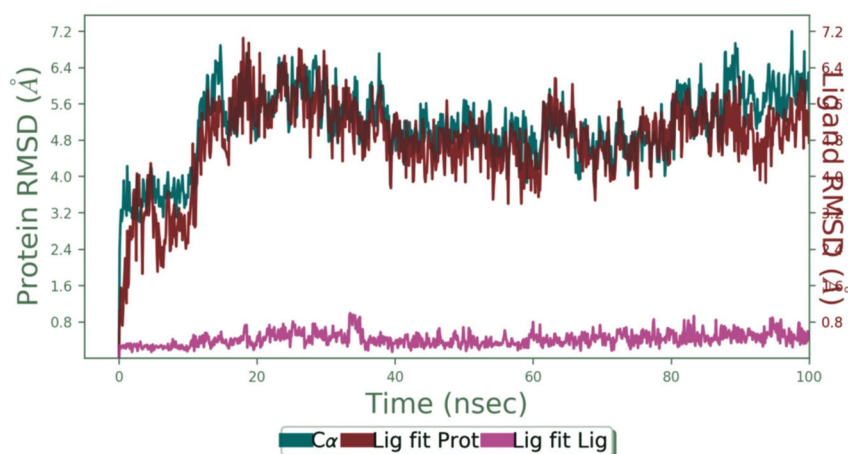


Figure 13. The RMSD (Å) of modeled SARS-CoV-2 Nsp14 complexed with biscriptolepine in the guanine-N7-methyltransferase region. Blue line (Cα): RMSD of the protein alone; Red line (Lig fit prot): RMSD protein ligand complex and Purple line (Lig fit Lig): ligand alone.

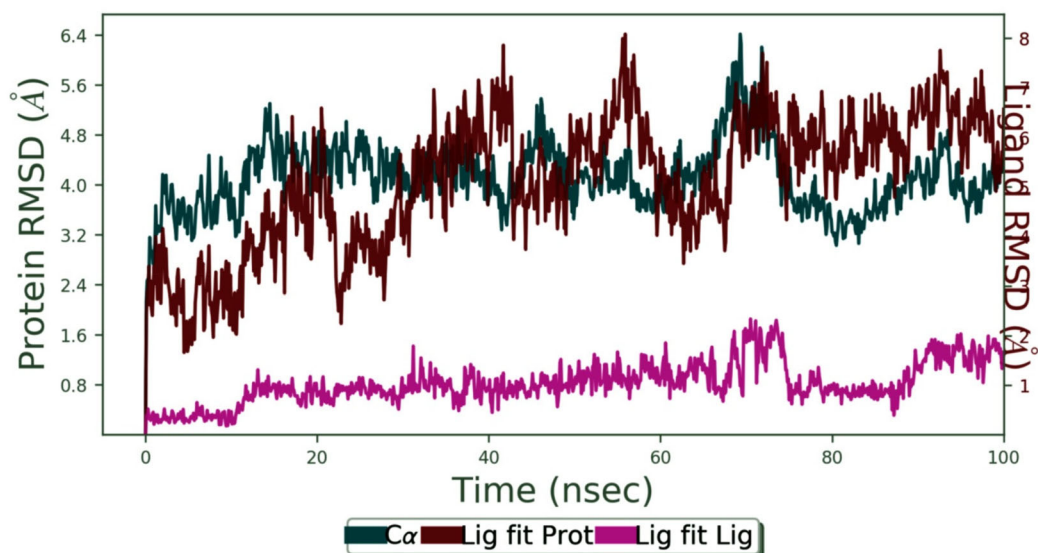


Figure 14. The RMSD (Å) of modeled SARS-CoV-Nsp14 complexed with 10-hydroxyusambarensine in the guanine-N7-methyltransferase region. Blue line ($C\alpha$): RMSD of the protein alone; Red line (Lig fit prot): RMSD protein ligand complex and Purple line (Lig fit Lig): ligand alone.

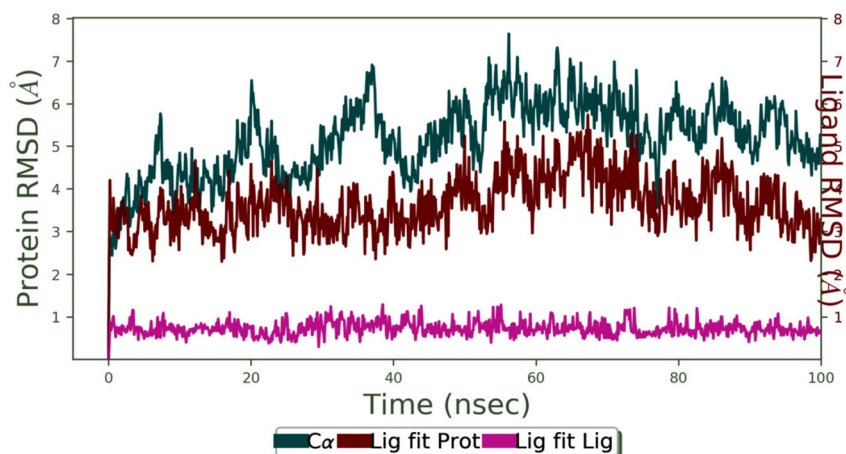


Figure 15. The RMSD (Å) of modeled SARS-CoV-2 Nsp14 complexed with mulberrofuran F in the guanine-N7-methyltransferase region. Blue line ($C\alpha$): RMSD of the protein alone; Red line (Lig fit prot): RMSD protein ligand complex and Purple line (Lig fit Lig): ligand alone.

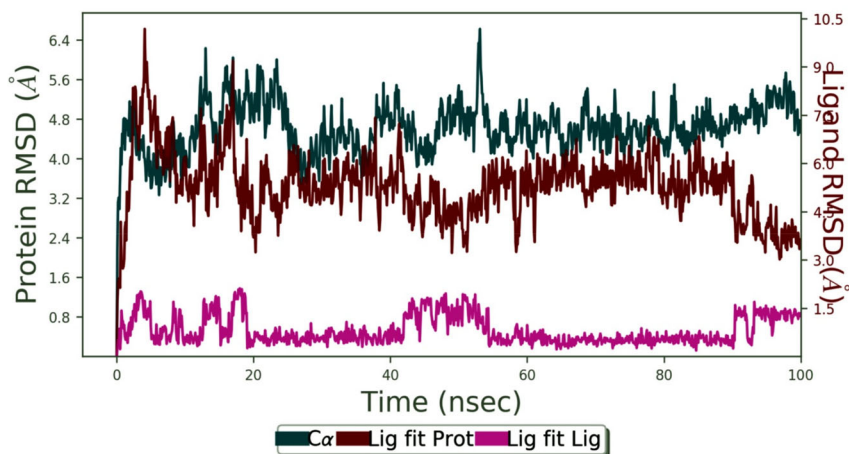


Figure 16. The RMSD (Å) of modeled SARS-CoV-2 guanine-N7-methyltransferase complexed with isostrychnopentamine. Blue line ($C\alpha$): RMSD of the protein alone; Red line (Lig fit prot): RMSD protein ligand complex and Purple line (Lig fit Lig): ligand alone.

Table 5. MM-GBSA binding free energies and their standard deviation for the top four ranked phytochemicals in complex with the modeled SARS-CoV-2 Nsp14 guanine-N7-methyltransferase.

Modeled SARS-CoV-2 Systems with	ΔG_{TOTAL} (kcal/mol)	$\Delta E_{Coulomb}$ (kcal/mol)	$\Delta E_{Covalent}$ (kcal/mol)	ΔE_{vdW} (kcal/mol)	ΔE_{Hbond} (kcal/mol)	ΔE_{Lipo} (kcal/mol)	$\Delta E_{Packing}$ (kcal/mol)	$\Delta E_{SelfCont}$ (kcal/mol)	$\Delta E_{Solv-GB}$ (kcal/mol)
Hydroxyusambarensine	-62.22 ± 25.42	3.06 ± 56.40	-6.81 ± 12.82	-41.20 ± 14.20	0.84 ± 2.81	-25.03 ± 8.70	-11.20 ± 6.77	-1.03 ± 1.39	12.94 ± 41.12
Isostrychnopentamine	-56.36 ± 17.08	-67.96 ± 67.31	1.51 ± 20.21	-49.48 ± 15.66	-1.25 ± 3.31	-29.25 ± 7.05	-7.10 ± 2.06	0.69 ± 1.55	90.84 ± 56.31
Biscryptolepine	-87.19 ± 22.16	4.65 ± 36.50	-15.17 ± 23.92	-43.65 ± 21.01	1.61 ± 3.94	-22.00 ± 6.35	-15.63 ± 3.62	-0.68 ± 1.50	-5.04 ± 26.60
Mulberroofuran F	-63.46 ± 37.78	6.78 ± 41.79	2.88 ± 22.92	-49.71 ± 25.85	0.15 ± 5.79	-26.08 ± 8.81	-10.57 ± 4.13	0.18 ± 1.98	6.56 ± 24.98

Table 6. *In silico* physicochemical and ADMET^a properties of the top-docked phytochemicals to guanine N7- methyltransferase of the modeled SARS-CoV-2 Nsp14.

	Biscryptolepine	Mulberroofuran F	Isostrychnopentamine
a) Physicochemical properties			
Molecular weight (g/mol)	462.54	630.68	549.75
Num. heavy atoms	36	47	41
Num. arom. Heavy atoms	34	27	18
Num. rotatable bonds	1	4	4
Num. H-bond acceptors	4	8	4
Hydrogen bond donor	0	5	3
cLogP	5.89	4.03	3.51
Molar Refractivity	151.01	179.51	180.14
TPSA (Å ²)	35.64	132.75	61.53
Drug-likeness			
Lipinski	Yes	Yes	Yes
Veber	Yes	Yes	Yes
Bioavailability Score	0.55	0.55	0.55
(b) ADMET			
Absorption (Probability)			
HIA	HIA+ (0.92)	HIA+ (0.984)	HIA+ (0.708)
Caco-2 Permeability Cm/s	Caco2+ (-0.577)	Caco2+ (-5.84)	Caco2+ (-5.343)
P-glycoprotein Substrate	Neg. (0.727)	Neg. (0.778)	Pos. (0.662)
P-glycoprotein Inhibitor	Neg. (0.656)	Neg. (0.007)	Neg. (0.778)
Distribution (Probability)			
Blood-Brain Barrier	BBB+ (0.988)	BBB+ (0.565)	BBB+ (0.864)
PPB %	72.493	80.43	82.962
VD L/kg	0.625	-0.39	-1.375
Metabolism (Probability)			
CYP450 1A2 Inhibitor	Pos. (0.976)	Neg. (0.184)	Neg. (0.136)
CYP450 1A2 Substrate	Pos. (0.828)	Neg. (0.362)	Neg. (0.468)
CYP450 3A4 Inhibitor	Neg. (0.186)	Neg. (0.551)	Neg. (0.66)
CYP450 3A4 Substrate	Neg. (0.634)	Pos. (0.586)	Neg. (0.678)
CYP4502C9 Inhibitor	Neg. (0.12)	Pos. (0.819)	Neg. (0.183)
CYP450 2C9 Substrate	Pos. (0.503)	Neg. (0.780)	Neg. (0.356)
CYP4502C19 Inhibitor	Pos. (0.5)	Pos. (0.651)	Neg. (0.17)
CYP450 2C19 Substrate	Pos. (0.745)	Pos. (0.594)	Pos. (0.501)
CYP4502D6 Inhibitor	Pos. (0.576)	Neg. (0.493)	Neg. (0.62)
CYP450 2D6 Substrate	Neg. (0.41)	Neg. (0.455)	Neg. (0.597)
Elimination			
T _{1/2} (Half Life Time)	2.246 h	2.373 h	2.451 h
CL (Clearance Rate)	1.588	1.339	1.716
Toxicity			
hERG Blockers	Pos. (0.764)	Neg. (0.674)	Neg. (0.301)
H-HT	Neg. (0.11)	Neg. (0.236)	Neg. (0.292)
AMES	Neg. (0.416)	Neg. (0.499)	Neg. (0.386)
SkinSen	Neg (0.285)	Neg (0.34)	Neg (0.323)
LD ₅₀ (LD ₅₀ of acute toxicity)	2.803 -log mol/kg (728.055 mg/kg)	3.38 -log mol/kg (261.17 mg/kg)	3.59 -logmol/kg (138.02 mg/kg)
DILI	Pos. 0.712	Neg. (0.644)	Neg. (0.904)
Pharmacokinetics			
GI absorption	High	High	High
Log K _p (skin permeation) cm/s	-4.61	-4.53	-6.93

^aADMET: Absorption, distribution, metabolism, elimination, and toxicity; GI: Gastro-intestinal; BBB: Blood Brain Barrier; P-gp: permeability glycoprotein; CYP: cytochrome P450; hERG: human Ether-à-go-go-Related Gene; HIA: Human Intestinal Absorption; H-HT: Human Hepatotoxicity AMES: Ames Mutagenicity; DILI: Drug Induced Liver Injury; VD: Volume Distribution; PPB: Plasma Protein Binding.

3.5. MM-GBSA method for estimating phytocompound binding free energy

The computed free energy estimations for 11 snapshots (one every 10ns) are summarized in average values and their standard deviation in Figure S10 (supplementary data) and Table 5.

The binding free energy of the FLP to the modeled SARS-CoV-2 Nsp14 yielded dG values that collaborates the docking analysis. Biscryptolepine had the highest binding free energy to the S2GMT. Just like the static docking analysis, 10-hydroxyusambarensine and mulberroofuran F had close

binding free energy of 62.2 and 63.3 kcal/mol, while isostrychnopentamine had the fourth binding free energy to S2GMT.

3.6. *In silico* drug-likeness and ADMET properties of top docked phytochemicals to the guanine-N7-methyltransferase of the modeled SARS-CoV-2 Nsp14

One of the challenges in the drug design and development processes is the potential toxicity of the drug candidates, it was therefore necessary to check the toxicity and druggability of the FLP using the more rapid, simple and economic feasible computational technique (Raies & Bajic, 2016). The FLP (biscryptolepine, 10-hydroxyusambarensine, mulberrofuran F and isostrychnopentamine) from the docking analysis were subjected to the predictive drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) filtering analyses. The results of the predictive filtering analysis for biscryptolepine, Mulberrofuran F, Isostrychnopentamine is represented (Table 6). The physicochemical and ADMET predictive analysis for 10-hydroxyusambarensine have been reported in our previous work (Gyebi et al., 2022). The three phytochemicals fulfilled the requirement for the physicochemical analysis (Lipinski and Veber), thereby suggesting favourable physicochemical/druggable properties (Table 6) (Daina et al., 2017; Lipinski, 2000). A favourable Lipinski and Veber properties indicates good absorption or permeation and good oral bioavailability respectively (Veber et al., 2002). All the three phytochemicals expressed positive and high probability of human intestinal absorption and non-inhibitor to the permeability-glycoprotein (P-gp). Mulberrofuran F and isostrychnopentamine were non-substrate to the (P-gp). These phytochemicals are suggested to be well absorbed into the blood stream subverting the capability of P-gp to pump them back into the intestinal lumen, bile ducts, urine-conducting ducts and capillaries respectively (Lin & Yamazaki, 2003). The *in silico* blood brain barrier (BBB) test predicts the blood brain barrier penetration of a molecule. SARS-CoV-2 has been reported to infect the brain, thus indicating its ability to cross the blood brain barrier (BBB) (Zanin et al., 2020). The three phytochemicals displayed properties that suggest their ability to cross the BBB, hence their potential to ensure overall viral clearance. The estimated half-life time (less than 2 hours) and clearance rate fall within the moderate range. The three phytochemicals presented a tolerable LD₅₀ between (51 ~ 500 mg/kg), Among the various various *in silico* toxicity descriptors, hERG channel plays a vital role in the repolarization and termination stages of action potential in cardiac cells (Raschi et al., 2008), compounds that block the hERG channel have the potential to cause cardiotoxicity (Kratz et al., 2017). Mulberrofuran F and isostrychnopentamine did not exhibit the potential of being hERG channel blockers, suggesting that they may not cause hERG channel-related cardiotoxicity. The three phytochemicals did not exhibit mutagenicity *in silico*, hence may not cause genetic mutations, which do initiate the pathophysiology of other diseases such as cancer. The impact of the compounds on phase I drug metabolism in the liver was also analysed using the various cytochrome P450 descriptors. The phytochemicals displayed less inhibitory potential for the

various cytochrome P450, thus may not adversely affect phase I drug metabolism in the liver to a large extent. The three phytochemicals demonstrated high probability of absorption, subcellular distribution, and low toxicity. The ADME/tox analysis indicated high aqueous solubility, ability to pass the high human intestinal absorption, low acute oral toxicity with a good bioavailability score (Table 6). Result from the previous analysis showed the druggability of 10-hydroxyusambarensine (Gyebi et al., 2022).

4. Conclusion

This study evaluated the potential of 249 phytochemicals obtained from an in-house library of phytochemicals from African medicinal plants against a modeled SARS-CoV-2 N7-MTase. From the list of phytochemicals with higher binding energies than the reference inhibitors, the top four ranked phytochemicals (FLP) (biscryptolepine 10-hydroxyusambarensine, mulberrofuran F and isostrychnopentamine) displayed the highest binding energies, best conformations and similar binding mode as the reference inhibitors, to both SARS-CoV-2 and SARS-CoV N7-MTase. These FLP interacted with important catalytic residues of both viral N7-MTase in a similar binding pattern as the reference inhibitors. In consequence they may disrupt the RNA capping machinery, the replication and survival of the viruses. The MM-GBSA based binding free energy calculations corroborated the results from the docking analysis, further indicating biscryptolepine with the highest binding affinity to the SARS-CoV N7-MTase. The FLP demonstrated favourable druggable and *in silico* ADMET properties over a wide range of descriptors. These potential inhibitors (FLP) of SARS-CoV-2 N7-MTase demonstrated high degree of structural stability, compactness, and flexibility in a simulated dynamic environment. Thus, they are well adaptable and suggested for further *in vitro* and *in vivo* experimental studies as anti-COVID-19 agents.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its [supplementary materials](#).

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