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RESEARCH ARTICLE

Repurposing Mitragynine as Anti-SARS-CoV-2 Agent Evidenced by *In Silico* Predictive Approach

Fatahiya Mohamed Tap[a], Nor Hafizah Zakaria[b], Fadzilah Adibah Abdul Majid[b*], Moyeenul Huq AKM[c], Jamia Azdina Jamal[d]

[a] Faculty of Chemical Engineering, Universiti Teknologi Mara, Bukit Besi Campus, 23200 Dungun, Terengganu, Malaysia; [b] Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia; [c] Department of Pharmacy, University of Asia Pacific, Dhaka-1205, Bangladesh; [d] Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

Abstract The outbreak of a new coronavirus strain (SARS-CoV-2) calls for the development of treatment approaches to address the disease. Therefore, an *in silico* study was conducted to evaluate druggability capacity of mitragynine, a natural indole alkaloid compound, using adsorption, distribution, metabolism, and excretion (ADME) prediction and molecular docking simulation to the region binding domain of severe acute respiratory coronavirus 2 (SARS-CoV-2 RBD). The pharmacodynamics of mitragynine were evaluated for its druggability using SwissADME software, and molecular docking simulation was performed using AutoDock software, using SARS-CoV-2 RBD (PDB ID: 6M0J) as the protein target retrieved from Protein Data Bank (PDB). ADME predicted that this compound has excellent druggability, transport properties, and pharmacokinetics, following Lipinski's rule of five. Mitragynine was also nonmutagenic based on the AMES toxicity test. No PAINS alert was observed and the synthetic acceptability score was 4.49, suggesting a moderately synthesised compound. Through the molecular docking approach, mitragynine successfully docked the binding site of SARS-CoV-2 RBD with a binding energy of -6.3kcal/mol and formed hydrogen bonds with the residue N501, which is one of the residues at the binding site of RBD. These findings, together with other therapeutic qualities of mitragynine warrant more research into molecular dynamics, *in vitro*, and *in vivo* studies in COVID-19 therapy.

Keywords: ADME, COVID-19, mitragynine, molecular docking, SARS-CoV-2 RBD.

***For correspondence:**
f.adibah@umt.edu.my

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Introduction

The COVID-2019 pandemic has remained to this day, after the first cases were reported in Wuhan, China, in December 2019 with about 524,878,064 confirmed cases, including 6,283,119 fatalities in May 2022 [1]. The SARS-CoV-2 virus causes the disease, which manifests several signs and symptoms including acute respiratory distress, such as cough, fever, and dyspnea; gastrointestinal issues, such as diarrhoea and vomiting [2]. The WHO experts recommended several treatments for non-critical and severe patients, such as casirivimab and imdevimab (neutralizing monoclonal antibodies), tocilizumab (TOCI), or sarilumab (IL-6 receptor blockers), remdesivir (RDV) and corticosteroids [3]. These treatments are widely debated due to conflicting findings and possible side effects, mainly affecting the hematopoietic and cardiovascular systems

[4]. Clinical studies of casirivimab and imdevimab (CAS/IMD) demonstrated encouraging outcomes in preventing the symptomatic development of COVID-19 infection. However, for severe COVID patients who need supplemental oxygen, CAS/IMD is not approved for the treatment. The report also stated the lack of data on the treatment of CAS/IMD against several variants of concerns (VOC) such as B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.427/B.1.429 (Epsilon), B.1.526 (Iota) and B.1.1.529 (Omicron)[5]. In November 2020, WHO advised against using RDV since the Guideline Developmental Group (GDG) panel discovered a lack of evidence suggesting RDV improved patients' health state [3]. Another setback is that these antivirals are costly. For example, an 800-mg dosage of TOCI costs around $5000 [5]. Other medications, such as hydroxychloroquine, lopinavir/ritonavir, and ivermectin, are not allowed by the WHO in treating the patients, despite of the severity or duration of illness [3]. Other than severe side effects, it is reported that these drugs did not improve the clinical status of the patients [6].

Mitragyna speciosa Korth. (Rubiaceae), commonly called ketum, is a South-East Asian plant used in folk medicine to cure psychological illnesses (chronic depression and anxiety) and severe pain [7]. Mitragynine, an alkaloid from *M. speciosa* has been studied widely in chemistry, pharmacology, and clinical aspects. Several studies have documented the benefit of mitragynine as an anti-analgesic, suggesting a potential treatment for managing pain [8-10]. According to Metastasio *et al*. [7], mitragynine improved the health condition of COVID-2019 patients, including muscle pain and lethargy, and did not appear to cause side effects when *M. speciosa* was stopped after a brief duration of administration. Mitragynine has been shown in previous pre-clinical trials of COVID-19 to have anti-inflammatory properties by lowering cyclooxygenase (COX)-2 expression in lipopolysaccharide (LPS)-treated macrophage cells [11].

In silico methodologies refer to prediction methods using computational approaches to understand and predict druggability in drug design and discovery. Adsorption, distribution, metabolism, and excretion (ADME) evaluation is essential in drug development, as this will accurately predict several properties such as physicochemical properties, pharmacokinetics, and drug-likeness [12]. Moreover, molecular docking is a predictive model that estimates the atomic interaction of small molecules with the protein of interest. This model can be used for subsequent verification using *in vitro* and *in vivo* trials and as a result, the drug discovery process will take less time and cost [13]. Keeping this in view, mitragynine from *M. speciosa* was studied as a SARS-CoV-2 RBD inhibitor utilising an ADME prediction and molecular docking technique.

Materials and Methods

Prediction of ADME by computational tool

The druggability of the molecule including drug-likeness, pharmacokinetics and bioavailability was evaluated by SwissADME from free online tool (http://www.swissadme.ch/)[14]. These predictions are essential to be investigated because they could propose a molecule that could be used as a drug. The SwissADME tool included the standard simplified molecular input line entry system (SMILES) for computational simulation of the molecule. SwissADME allows the user to upload the SMILES data of mitragynine downloaded from the PubChem database. The SDF file for the drug was sent to the ProTox-II online server for computational evaluation of potentially toxic fragments (https://tox-new.charite.de/protox II/index.php?site=home). The service computes acute oral toxicity by oral dosage (LD50), with toxic classifications ranging from I (fatal if consumed) to VI (non-toxic) and AMES mutagenic. To predict cardiotoxic activity, measured activity coefficients (pAct) were determined using ChemAxon's online graphic tool Playground (https://disco.chemaxon.com/calculators/demo/playground/), with pAct values greater than 6.00 indicating inhibitory activity of human ether-ago-go related gene (hERG) channels [15]. Mitragynine was further assessed using Pan-Assay Interference Structures (PAINS) and the chemical-medicinal characteristics based on the synthetic accessibility score were examined using SwissADME server' (http://www.swissadme.ch/) under the "Medicinal Chemistry" section [14].

Target prediction of small molecule

The objective of this approach is to identify the most possible macromolecule receptors for a ligand. A web server, namely SwissTargetPrediction (https://www.swisstargetprediction.ch) was used to predict the targeted protein family for mitragynine which identifies active substance targets using a mix of 2D and 3D homology scores with specific ligands [16].

Molecular docking simulation

The protein target was SARS-CoV-2 RBD (PDB ID: 6M0J), which was retrieved in 3D structure from the Protein Data Bank (PDB) [17]. With the use of bioinformatics programs like AutoDock Tools 4.2 (ADT) and UCSF-Chimera, the inhibitor was removed and the receptor was constructed. The hydrogen bond was generated employing AutoDock Tools 1.5.7, and the grid dimension was computed using the x, y, and z coordinates of the selected binding site.

Ligand preparation

Mitragynine's 3D ligand structure was retrieved from the PubMed Database (https://www.ncbi.mlm.nih.gov/pubchme/)[18]. LigPrep and ADT were used for ligand minimization and preparation. The simulation of molecular docking was conducted using AutoDock 4.2 programme. This programme is designed to identify the ligand posture with the lowest binding energy. The results were then visualised and depicted using Discovery Studio Visualizer, pymol, and UCSF Chimera to visualise and display the molecule's interaction and binding region [19].

Results and Discussion

In this recent pandemic, the treatments for the SARS-CoV-2 virus is critical and various small molecules, such as remdesivir and hydrochloroquine, are utilised to give a remedy for this disease [20]. These two compounds may be effective at inhibiting SARS-CoV-2, but with the increasing applications, adverse side effects are detected and this issue became a concern among the clinicians. The clinical outcome of remdesivir for COVID-19 patients showed that some patients experienced adverse effects on gastrointestinal problems, respiratory toxicity, and cardiovascular toxicity [21]. Thus, there is a need to bring more potent inhibitors derived from plants because they are natural and effective [22]. Several natural compounds, including silvestrol, ouabain, lycorine, homoharringtonine, tylophorine, and 7-methoxycryptopleurine, have exhibited antiviral efficacy against SARS-CoV [23]. Clinical investigations of several plant compounds against SARS-CoV-2 have given a promise to natural therapeutics since the US FDA recently authorised 3CL protease inhibitor NLC-001 as a dietary supplement to boost immune function against the virus [24].

ADME and toxicity studies

The discovery of a drug is important but it is insufficient since the drug may have limits; it may be toxic or have poor ADME qualities [25]. As a result, predicting ADME qualities is viewed as an important step in reducing potential issues later in clinical trials. Hence, SwissADME is the online tool used to predict the mitragynine's ADME properties [14]. Drug-likeness is provisionally examined based on the rule of thumb according to Lipinski *et al*. which stated that for oral administration of the substance, the absorption or permeation is efficient if the substance meets the requirements: molecular weight (MW) ≤ 500 Da, log P ≤ 5, H-bond donors (HBD) ≤ 5 and H-bond acceptors (HBA) [26,27]. The data on physicochemical, druglikeness, pharmacokinetics, toxicity and docking score are summarized in Table 2. In this work, mitragynine satisfied Lipinski's rule of five with MW of 398.50, log $P_{o/w}$ of 3.21, five hydrogen bond acceptors, and one bond donor.

Lipophilicity is described as a chemical compound's ability to dissolve in fat, oil, or any non-polar solvent such as hexane [28]. The lipophilicity of mitragynine showed that iLOGP was 3.78, XLOGP was 3.41, WLOGP was 3.12, MLOGP was 2.02, SILICOS-IT was 3.72, and Consensus log $P_{o/w}$ was +3.21. These values suggest that this compound is moderately soluble in the water and can be used in oral administration and further analysed in the clinical trial [12,29]. The log Po/w values for the proposed compounds were in the range of +2.29 to +4.25 in the investigation of chromone derivatives as COVID inhibitors [30]. These positive scores indicate each chemical is lipophilic and meets the requirements for the drug design. Water solubility properties are calculated based on three criteria; 1) ESOL -4.29, solubility 2.03e-01 mg/ml, and classed as moderate soluble; 2) Ali -4.43, the solubility of 1.48e-02 mg/ml and classed as moderate soluble and 3) SILICOS-IT -5.00, solubility as 3.95 mg/ml and classed as moderate soluble. These values are in accordance with the study by Sepay *et al*. [30] who reported the log *S* values of chromone compounds were in the range of −3.19 to −4.85 using ESOL log *S* method, −2.9 to −4.41 using Ali log *S* method and −4.25 to −6.38 for SILICOS-IT method.

Pharmacokinetic properties were analysed following the prediction of lipophilicity and solubility values. The topological polar surface area (TPSA) is an excellent measure of gastrointestinal drug

uptake (TPSA less than 140 Å^2) and blood-brain barrier permeability (TPSA less than 70 Å^2)[31]. Mitragynine exhibited computational TPSA of 63.76 Å^2 which satisfies the indicator of good intestinal (GI) absorption. The GI absorption plays role in defining the drugs at the intestinal level. High rate of GI absorption results in a rapid rate of drug action [32]. Mitragynine also has adequate blood-brain barrier (BBB) penetration. BBB is an important criterion in drug design. The BBB allows important components to cross the central nervous system, such as oxygen and carbon dioxide [14]. When analysing active efflux across the cell membranes, such as from the gastrointestinal lining to the lumen or from the brain, understanding whether the compounds are substrates or non-substrates of the permeability glycoprotein (P-gp) is critical [14]. The data revealed that mitragynine did not behave as a P-gp substrate. The compound that acts as a P-gp substrate can limit the rate of uptake by the BBB and this is a major problem in drug delivery [33]. Meanwhile, the skin permeability coefficient (Kp) was -6.31 cm/s. The high negative value of the skin permeability coefficient implies less skin permeant of the compound, which is crucial in transdermal drug delivery [14]. This result is supported by the findings of Sepay *et al*. who found Kp values ranging from -4.85 to -5.95 cm/s in nine chromone molecules designed for inhibition of COVID-19 virus replication [30].

It is also necessary to understand how chemicals interact with cytochromes P450 (CYP). This isoenzyme superfamily plays an important role in drug elimination via metabolic biotransformation [34]. The pharmacokinetic study predicted the ability of mitragynine to inhibit CYP2D6 and CYP3A4 but acted as a non-inhibitor toward CYP1A2, CYP2C19, and CYP2C9. Although there are various cytochrome P450 enzymes, the most important ones are, CYP2C9, CYP1A2, CYP2D6, CYP2C19, and CYP3A5, which are involved in metabolising 90% of drugs. The liver is the primary site of expression for these enzymes [35]. Mitragynine did not inhibit CYP1A2, which means that it did not inhibit the liver metabolism. This compound also did not inhibit CYP2C19, which is the enzyme responsible for metabolising anti-ulcer, anti-fungal, anti-malarial, and sedative drugs [36]. It also did not inhibit CYP2C9 which is responsible for metabolising non-steroidal anti-inflammatory drugs (NSAIDs), anti-diabetic agents, and angiotensin II receptor blockers (ARBs) [35]. However, this compound might be contraindicated with anti-hypertensive, antidepressive, antihistamine, and anti-arrhythmic drugs because it inhibited CYP2D6 and CYP3A4 cytochrome enzymes [37]. A previous study also highlighted the potential herb-drug interactions of mitragynine [38]. Therefore, this compound is not advisable for patients who have comorbidities such as heart disease, hypertension, depression, and severe allergic reaction. In terms of bioavailability, drug-likeness indicates the possibility of a molecule becoming an oral drug. The bioavailability score indicates whether a molecule will have at least 10% oral bioavailability in rats [44]. With a bioavailability score of 0.85, we conclude that mitragynine does not contradict the Lipinski, Ghose, Veber, Egan, and Muegge drug-likeness standards.

The objective of medicinal chemistry results is to aid in drug development efforts [39]. Thus, the SwissADME programme provides two complimentary pattern recognition filters (PAINS and Brenk) that detect the possible problematic segments in the examined compounds [16]. If any of the indicated fragments are detected in the molecule under assessment, the programme raises a warning. Taking these parameters into account, mitragynine does not have this sort of fragment, since no alert type was detected. PAINS are chemical components that, rather than binding to a particular target, it responds non-specifically to a broad range of biological targets, resulting in false-positive results. Brenk's structural warning is based solely on the information of a group of compounds that are chemically reactive, toxic, metabolically volatile, or have qualities that cause poor pharmacokinetics [40].

To identify mutagenicity of a substance, the AMES toxicity test uses multiple strains of *Salmonella typhimurium* bacteria with a gene mutation in the production of histidine [41]. Mitragynine tested negative in the AMES toxicity test, suggesting that it is nonmutagenic. The maximum tolerated dose for humans was -0.45 log mg/kg/day. Mitragynine had a score of 4.49 for synthetic accessibility, indicating that it is a moderately synthesised compound. This score is based on a short analysis at the structures of over 13 million molecules, with the idea that the more frequent a structural fragment is, the easier it is to make the molecule [42]. The scale runs from 1 (easy synthesis) to 10 (very difficult synthesis). Meanwhile, the no-observed-adverse-effect level (LOAEL) for oral rat acute toxicity (LD50) was determined to be 3.15 mL/kg, and the no-observed-adverse-effect level (LOAEL) for oral rat chronic toxicity was 0.311 log mg/kg/bw/day.

The potassium ion channel of the human ether-a-go-go-related gene (hERG) is frequently blocked by drug candidates. The obstruction causes long QT syndrome (LQTS), a potentially fatal cardiac adverse effect. As a result, predicting medication-induced hERG-related cardiotoxicity may improve the drug discovery process by screening out potentially toxic new drug targets [43].

In the present study, mitragynine was found to inhibit hERG1 but did not have any inhibitory action against hERG II. SwissTargetPrediction works on the notion that biomolecules with similar characteristics will have similar targets [45]. The top 25 members of the target protein family were observed in the prediction analysis, which was shown on the web page. Based on Figure 1, 46.0% of the target protein for this molecule comes from the group Family A G protein-coupled receptor. The second-highest percentage (14%) is protease protein and the third (10.0%) is protein kinase.

Cortegiani *et al*. [46] performed a thorough review of the data on chloroquine and hydroxychloroquine for COVID-19 treatment. Chloroquine was shown to be effective at inhibiting SARS-CoV-2 growth *in vitro*. However, in a separate study, hydroxychloroquine was found to cause liver toxicity, most likely related to the quinoline group of the drug. Inhibition of hERG channels, irritation of the respiratory tract (ethanolamine), carcinogenicity, and ocular toxicity (derived from 4-Aminoquinoline) all implied that the studied compound was toxic [47]. In their investigation, Hage-Melim *et al*. [47] screened 100 compounds from the SARS-CoV-2-Target library; 33 had no human toxicity alerts, 17 had one alarm, 17 had two alerts, and 15 molecules had three alerts. In the SARS-CoV-2-ML collection, 21 molecules displayed no warning signals, 24 displayed only one alarm, 20 displayed two warnings, and 16 molecules displayed three alerts.

Molecular docking

Molecular docking simulation was used to investigate the binding affinity and binding pose of the ligand molecules at the SARS-CoV-2 RBD binding site. Molecular docking is critical, particularly for screening possible novel therapeutic molecules. This method successfully docked mitragynine against the protein target of SARS-CoV-2 RBD with the docking score of -6.3kcal/mol. According to Kondo *et al*. [48], the binding energy has positive and negative values, with the positive value indicating complex binding destabilisation and the negative value indicating complex binding stabilisation. Based on the molecular docking result, the predicted binding affinity for mitragynine was negative, indicating that complex binding had been stabilised and might serve as a basis for the establishment of better therapies that target SARS-CoV-2 proteins. Binding energy is produced as a result of the simulation to determine the intensity and affinity of the ligand-receptor interaction. The stronger the interaction, the weaker the binding energy, and vice versa [49].

A receptor-binding domain (RBD) is a critical component of a virus that is positioned on the spike domain and helps it to connect to body receptors to gain access into cells and cause infection. These are also the major objectives for SARS-CoV-2 prevention and treatment [50]. The score showed a reasonable binding value when compared to remdesivir (-4.9 kcal/mol), hydroxychloroquine (4.2 kcal/mol), favipiravir (-5.1 kcal/mol), lopinavir (-6.9 kcal/mol) and ritonavir (-6.4 kcal/mol) [51]. In other reports, merimepodib and dexamethaxone, with binding affinities of -7.2 and -7.1 kcal/mol, respectively, showed strong inhibitory effects against the SARS-CoV-2 papain-like protease (PLpro) [52]. They also reported the binding affinities for lopinavir (-5.6 kcal/mol), favipiravir (-5.5 kcal/mol), remdesivir (-5.9 kcal/mol), and hydroxychloroquine (-4.7 kcal/mol).

In a study of four compounds produced from *N. macrophylla*, the ligands had binding energies ranging from -7.1 to -6.3 kcal/mol against the three SARS-CoV-2 target proteins (catechin, catechin-3-rhamnoside, epicatechin, and quercetin) [53]. Norquinadoline A, deoxytryptoquivaline, and deoxynortryptoquivaline showed significant binding to the targets, SARS-CoV-2 main protease, in a screening of quinoline and quinazoline alkaloids against COVID-19, with binding affinities of -8.75, -9.34, and -9.64 kcal/mol, respectively [54]. Mitragynine binds specifically to the SARS-CoV-2 RBD, revealing H-bond interactions with key residues Gly502, Asn501, and Gly496 (two H-bonds), as well as Ser494 (Table 1). It is worth noting that these residues were recently discovered in an inhibitor-bound SARS-CoV-2 protease [55]. The hydrogen bond interactions were further investigated for binding length and hydrogen bond formation as shown in Figure 2. Two hydrogen bonds from the residues hydrogen atom of Gly496 were formed with the aromatic rings from this small molecule. When compared to the previous study by Khelfaoui and colleagues, the antiviral drugs such as piperaquine, lisinopril, delapril, and hydroxychloroquine also formed hydrogen bonds with 6-ring components [56]. One of the hydrogen bonds formed at N501 residues is the residue of RBD. Furthermore, because muscle pain is among the signs of COVID-19 illness, mitragynine is a good candidate for research. Mitragynine has an indoloquinolizidine moiety with a methoxy group at the C-9 position, and the antinociceptive effect of the leaves was induced by μ- and δ -opiate receptors, which work like morphine [57].

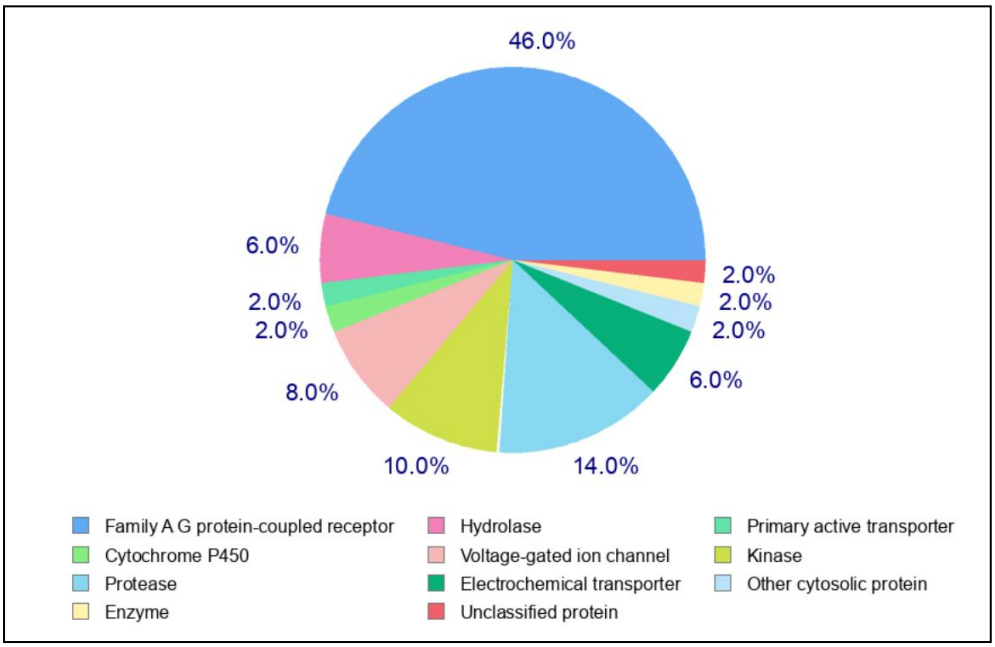

Figure 1. The most favorable target protein family for mitragynine

Table 1. Molecular interactions of mitragynine towards SARS-CoV-2 RBD

Compound	Receptor	Bonds	Residues	Ligand (atom)	Bond length (Å)
Mitragynine	SARS-CoV-2 RBD	5 Hydrogen bonds	Gly502 (H)	O_2	1.8
			Asn 501 (C)	O_2	3.3
			Gly496 (H)	6-Ring	3.2
			Gly496 (H)	6-Ring	2.7
			Ser494	O_2	3.7

Table 2. The data on physicochemical, docking score, solubility, druglikeness, and pharmacokinetic properties

Properties	Mitragynine
Molecular weight (mw)	398.50
Consensus log P $_{0/w}$	3.21
TPSA (angstrom)	63.76
Binding energy (kcal/mol)	-6.3
ESOL	-4.29
Ali	-4.43
SILICOS-IT	-5.00
Bioavailability score	0.85
Synthetic accessibility	4.49
GI-absorption	High
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
P-gp substrate	No
BBB permeant	Yes
Skin permeation kinetics (cm/s)	-6.31
PAINS alert	No
AMES test	Negative
hERG I inhibition	Yes
hERG II inhibition	No

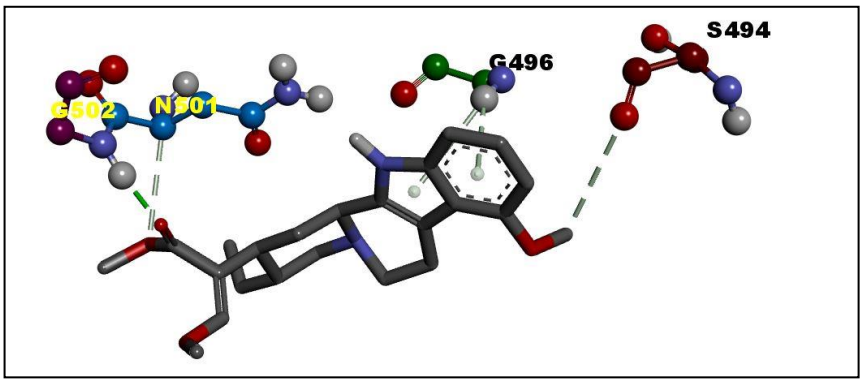

Figure 2. Hydrogen bond interactions between receptor and ligand.

Conclusions

In conclusion, the current *in silico* study offers compelling evidence for mitragynine's efficacy as a SARS-CoV-2 RBD inhibitor. It possesses high druggability based on ADME prediction, following Lipinski's rule of five with suitable pharmacokinetic properties. Molecular docking simulation results revealed that mitragynine interacted with SARS-CoV-RBD with comparable binding energy to other published compounds, and could potentially exhibit the inhibitory effect for SARS-CoV-RBD. The strong interaction between mitragynine and SARS-CoV-RBD was facilitated by the formation of hydrogen bonds. However, more research is required using atomistic molecular dynamics simulations to explore the dynamic interaction of protein-ligand interactions at the atomic level. This research also reveals that mitragynine is a promising drug that should be investigated further *in vitro* and *in vivo* to confirm its potential inhibition. Together with other therapeutic properties of mitragynine for COVID-19 remedy, further works are necessary to establish the efficacy and plausible mechanism for medicinal use of this compound.

Conflicts of Interest

The authors have no conflict of interest in this publication.

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