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In-silico molecular docking of Angiotensin-Converting Enzyme 2 receptor with selected inhibitors as potential anti SARS-CoV 2 agent

J.B. Minari*[1], K. O. Adekoya[1], C.I. Ayolabi[2], S.A. Adesida[2], V.O. Osunkalu[3], O.O. Adewumi[4], E.E Agho[1]. F.D. Adebiyi[1] and B.O Sholaja[1]

[1]Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, PMB 1, UNILAG Post Office, Akoka, Yaba, Lagos, Nigeria.
[2]Department of Microbiology, Faculty of Science, University of Lagos, PMB 1, UNILAG Post Office, Akoka, Yaba, Lagos, Nigeria.
[3]Department of Haematology and Blood Transfusion, College of Medicine, University of Lagos, PMB 12003, Lagos, Nigeria.
[4]Distance Learning Institute, University of Lagos, PMB 1, UNILAG Post Office, Akoka, Yaba, Lagos, Nigeria.

*Author for Correspondence. E-mail: jminari@unilag.edu.ng, Tel: +2348032488513

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ABSTRACT: The functional receptor for SARS-CoV 2, the newly discovered pathogen responsible for COVID-19, has been identified as angiotensin-converting enzyme 2 (ACE 2). This invariably suggests that blocking the ACE 2 active site could prevent the virus from gaining entrance into the cell to establish infection. The present study delineated the property of ACE 2 receptor and investigated the five (5) possible compounds that can inhibit its activity using molecular docking tools. The study revealed that the ACE 2 receptor has a molecular weight of 92491.05 Daltons, isoelectric pH 5.36, an aliphatic index of 80.55, instability index was predicted to be 40.10, and GRAVY value of −0.376. InterPro showed that ACE 2 has 19 amino acids used for substrate interaction. The motif and domain analysis done using different computational tools revealed that angiotensin-converting enzyme 2 is mainly found in the outer part of the cell membrane. Multiple Em Motif Elicitation predicted that the sequences of ACE 2 have CQAAKHEGGHIKCDI in position 369–383, HDEDYCD at 195-201, MWGGFW at 270-275, VCNPDNW 132-138, KWRWMK 458-463, RSEVGKALR 169 -177, VGAKNMNVRP, 244-253. The study revealed that trifluoperazine hydrochloride (TFH), a phenothiazine derivative compound, showed a minimum value of binding free energy as −7.90 kcal/mol while the inhibition constant was 1.62 µM. This suggests that this ligand (TFH) had the strongest interaction with ACE 2 when compared with other inhibitors screened. Quinine and thioridazine hydrochloride (TOH) another phenothiazine also exhibited a strong binding affinity for ACE 2, with the binding free energy of −6.54 kcal/mol and −6.33 kcal/mol and inhibition constants (Ki) of 16.20 µM and 22.7 µM respectively. Pentamidine isethionate and allicin both interacted with the active site of ACE 2 receptors and inhibited with binding free energy and inhibition constants (Ki) of −3.94 kcal/mol and 1.29 mM; −3.65 kcal/mol and 2.12 mM respectively. Swiss Institute of Bioinformatics, Switzerland (ADME) analysis established that allicin satisfies the Lipinski's rule with the Molecular

Weight of 162.27(g/mol) (< 500), the lipophilicity of 1.18 (MLog *P*) (< 5), hydrogen bond donors of 0 (< 5) and hydrogen bond acceptors of 1 (<10). This study suggests that trifluoperazine hydrochloride, thioridazine hydrochloride, quinine, and allicin could be possible ACE 2 receptor blockers that can be harnessed to prevent SARS-CoV 2 access into the cell of the host and allicin as a probable drug for COVID-19 patients.

Keywords: ACE 2, SARS-CoV 2, Receptor, Inhibitor, Docking

Introduction

The recent outbreak of COVID-19 which spread into several countries of the world has led to loss of life and has impacted on the global economy negatively. This newly recognized coronavirus (SARS CoV 2) which is a single-stranded and positive-sense RNA virus is the causative organism for COVID-19 [1]. Several authors have provided insight into the mechanisms of the transmission and impacts on human subjects. For example, Tobolowsky *et al.* reported the impact of the outbreak in homeless service sites in Washington [2] while Ghinai *et al* gave scientific perception on community transmission [3]. The characteristics of hospitalized COVID-19 patients and infection rates [4], transmission among health workers during exposures to hospitalized patients [5] and geographical differences in COVID -19 cases [6] have also been documented. Previously, coronavirus (CoVs) outbreaks were believed to cause mild infections with patients recovering with or without specific treatment. Nearly 50 years ago, α-coronaviruses (HCoV-NL63 and HCoV-229E) and β-coronaviruses (HCoV-HKU1 and HCoV-OC43) were isolated [7]. In the previous SARS-CoV outbreak, the HCoV-NL63 and HCoV-HKU1 were recently identified [8]. Each year, the viruses which are endemic in human populations, cause 15–30% of respiratory tract infections. They cause severe and greater disease incidence of lower respiratory tract infection in individuals with underlying illnesses, in neonates and the elderly. The strain HCoV-NL63 has been documented to be linked with acute laryngotracheitis [9].

One fascinating feature of these strains of viruses is their variability in tolerance to genetic diversities. The isolates HCoV-229E acquired from the different parts of the world showed negligible sequence divergence [10] while HCoV-OC43 isolates from the same place but at different years revealed substantial genetic diversity [11]. This probably is the reason HCoV-229E could not cross the species barrier to infect mice. Meanwhile HCoV-OC43 and BCoV (bovine coronavirus) can infect mice and other ruminant species. Mouse Hepatitis Vitus (MHV) which causes demyelinating disease has provided the basis to suggest that human Coronaviruses might play a major role in multiple sclerosis (MS) developemnt. Conversely, to date, no available indication has shown that human Corona Viruses are significantly involved in MS.

An earlier investigation of the S protein receptor binding motif indicated that SARS-CoV and SARS-CoV-2 have conserved amino acid residues crucial for receptor binding. This suggests that both strains can enter the host cell through the same receptor [12]. It has also been reported that SARS-CoV uses Angiotensin-Converting Enzyme 2 (ACE-2) receptor for entry into the host cell [13]. Previous documentation by Zhou *et al.* suggests that SARS-CoV-2 utilizes ACE-2 from Chinese horseshoe bars, humans, civet cats, and pigs to gain entry [14]. Similar findings for human and bat of angiotensin-converting enzyme 2 was reported by Hoffmann *et al* [15].

ACE2 is a glycoprotein of type 1 integral membrane [16] which is mainly expressed in tissues such as the endothelium, heart, the lungs, and in the kidney [17, 16]. Different groups have described that ACE 2 shares homology with an angiotensin-converting enzyme (ACE) in its catalytic domain which gives various functions in the renin-angiotensin system [16, 17]. This has previously been observed to serve as a receptor for the SARS-CoV [13]. Angiotensin-converting enzyme 2 can convert angiotensin I to angiotensin (1–9) and angiotensin II to angiotensin (1–7) [18, 19]. The enzyme does not only act on peptides belonging to the renin-angiotensin system but also on other peptides from other systems such as apelin 13, neurotensin 1–13, dynorphin 1–13 and some of the kinin metabolites, but not on bradykinin [18].

ACE 2 through the activities of the renin-angiotensin system is involved in cardiovascular and renal homeostasis through the regulation of blood pressure, body fluid homeostasis, and other cardiovascular functions, counter-regulating Ang II-induced effects [20]. The Ang 1–7 may act on Ang II being cardio and renoprotective as a negative feedback hormone [21]. Tissues such as heart, kidney and testis effect a local renin-angiotensin system that acts independently of the circulating one [22, 23].

At present, there are no specific antiviral treatments available for CoVs infections, with significant progress only made in the development of vaccines. Therefore, a necessity arises for the quest into finding the possible therapeutic agent for the virus. To achieve this, a study into the enzymes (ACE2) which is involved in the entrance of the CoVs into the cell of the host was carried out.

This study, therefore, focused on investigating the inhibitory potential of some possible inhibitors of the ACE2 receptor sites which could prevent the virus from gaining entry into the host cell. Therefore, we aim to identify compounds that inhibit ACE 2 activity using several virtual screening approaches. The recent discovery of angiotensin-converting enzyme 2 (ACE 2) and its significance in the regulation of renin-angiotensin system activity is relevant as a potentially valuable target for anticoronavirus therapy.

Five inhibitors namely trifluoperazine hydrochloride (TFH), thioridazine hydrochloride (TOH), pentamidine isethionate (PAI), Quinine (QUI) and allicin (ACN) was considered in this investigation for the inhibition ACE 2. Trifluoperazine Hydrochloride (TFH) is an important phenothiazine derivative which has a structural formula $C_{21}H_{24}F_3N_3S.2HCl$, and a molecular weight of 407.496g/mole. It mainly acts on dopamine receptors. The primary indication for trifluoperazine is in the treatment of schizophrenia and also possess antiemetic, sedative, antipruritic, antidyskinetic, analgesic, and antihistaminic properties [24]. Another ligand of interest is Pentamidine isethionate which proved effective during the early 1940s, for the treatment and prophylaxis of human African trypanosomiasis (HAT) or sleeping sickness and visceral leishmaniasis in India [25]. Currently, pentamidine has been reportedly used for prophylaxis and treatment of *Pneumocystis jirovecii* pneumonia and also in the management of first-stage human African trypanosomiasis as well as several forms of American cutaneous leishmaniasis. Quinine, which was the earliest known drug used for the treatment of malaria caused by plasmodium falciparum was also considered. As with other quinolone antimalarial drugs, the action of quinine has not been fully resolved. Although several hypotheses have been given on the mechanism of action of quinine, the most broadly accepted is the one based on the well-studied and closely related quinolone drug, chloroquine. This model suggests the inhibition of hemozoin biocrystalization that leads to the accumulation of cytotoxic heam toxin and the subsequent death of the parasites [26].

Allicin which is otherwise known as allyl 2-propenethiosulfinate or diallyl thiosulfinate or S-allyl cysteine sulfoxide is found in white garlic (*Allium sativum* L.). It is a sulfur-containing volatile compound that is effective towards several gram-positive and gram-negative bacteria. This compound has also demonstrated promising potential in the treatment of cardiovascular diseases (CVD), through its antioxidant properties [27].

The results obtained from this study provides a possible range of chemotherapeutic agents that can be leveraged as potential antiviral treatments available for COVID -19. To achieve this, Investigation into the binding affinities of the selected inhibitors on angiotensin-converting enzyme 2 receptor sites was studied using the molecular docking approach. The molecular docking is a tool commonly used in structure-based drug design. It is complimentary to wet laboratory experiments in studying the structures and functions of biomolecules. Also, drug-likeness and compatibility with gastrointestinal and brain absorption of allicin were investigated to assess the appropriateness as possible therapeutic agents and orally active drug for the treatment of COVID -19. This study, therefore, investigated the computational modeling of the interactions of ACE 2 receptor with TFH, TOH, PAI, QUI and ACN inhibitors as possible prevention of coronavirus infectivity on the host cell.

Materials and Methods

The Prediction of Physiochemical Properties of Human ACE 2

The human ACE 2 physiochemical properties for this study were determined using Protparam (www.ncbi.nlm.gov/pmc/articles). This bioinformatics tool which works based on the Edelhoch method determines the weight value of instability with reference to 400 diverse dipeptides (DIWV) and the extinction coefficients, charge, Theoretical pI, Aliphatic Index, Molecular weight, Chemical formula, the number of amino acids, instability index (II), and GRAVY (Gaverage of Hydropathy) Value.

Membrane Topology of ACE 2

Signal p4.1, OCTOPUS, PolyPhobius and SPOCTOPUS were used to predict the ACE 2 membrane topology.

Prediction of Domain and Motif

ACE 2 domain of humans considered in this study were obtained from the conserved domain database National Center Biotechnology Information (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?). Multiple Em Motif Elicitation software was used in this study to predict the sequence of the enzyme.

Receptor, Active Site and Ligands Prediction

To predict the possible binding of the ligand to the ACE 2 structure, its 3D-structure was retrieved from RCSB-PDB (https://www.rcsb.org/pdb/software/wsreport.do) while the human angiotensin-converting enzyme 2 active sites were obtained from InterPro (https://www.ebi.ac.uk/interpro/). PubChem software that is usually used to select compounds based on their chemical formula and physiochemical properties were used to retrieve the structures of the ligand molecule as obtained in this study.

Preparation of ligands (inhibitors)

In preparation of the ligand for docking, the Gasteiger charge was assigned and then non-polar hydrogens were merged since the ligands used in this study are not peptides just as every other ligand. The rigid roots of each ligand used were also defined automatically rather than picking them manually.

Docking

Larmarckian genetic algorithm was preferred for the study of the best conformers. A maximum of 10 conformers was used for the ligands investigated. The parameters were set using the software ADT (Autodock Tool Kit) on the computer which is associated with AutoDock 4.2. AutoDock 4.2 is the software considered for performing automated docking of the ligands used to the ACE 2 receptor used in this study. The components of the program involved in the study are AutoGrid and AutoDock. AutoGrid was used to pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field while the AutoDock was used to perform the work of the docking. The ligand moves randomly in any one of six degrees of freedom, namely 3 translation and rotation degrees [28, 29], and it also calculates the energy of the new ligand state. If the energy of the new state is lower than that of the old state, the new one is automatically recognized as the subsequent step in docking as the procedure continues [30, 31].

Absorption, Distribution, Metabolism, and Excretion (ADME) Analysis

Swiss Institute of Bioinformatics, Switzerland (Swiss ADME) was used to predict the Drug-likeness of our allicin [32,33]. The absorption of the Allicin in the brain and gastrointestinal tract was predicted using the Brain or IntestinaL EstimateD permeation predictive model (BOILED-Egg) which has a threshold (WLOGP $\leq$ 5.88 and TPSA $\leq$ 131.6). This reveals a 2D graphical representation in which the yolk area signifies the molecules that can passively be absorbed by the blood-brain barrier (BBB) while the molecules found in the white region are revealed to be able to cross into the gastrointestinal (GI) tract.

Results and Discussion

Human ACE 2, with 805 amino acids, was established to show a molecular weight of 92,491.05 Da and isoelectric pH 5.36. A stable protein with an aliphatic index of 80.55 and an instability index of 40.10 was observed in this study. The GRAVY value of ACE 2 is −0.376 as suggested by the result obtained from this study. This suggests that the protein is a hydrophilic peptide (Table 1).

The 3D ribbon structure of human angiotensin-converting enzyme 2 was obtained from the PDB software resource online and visualized with Discovery Studio Visualizer (Figure 1). ACE 2 active site was predicted to possess 19 amino acids the enzyme uses to bind with ligands using InterPro (Table 2).

The different computational tools such as OCTOPUS, SPOCTOPUS, Signal p4.1, and PolyPhobius used in this study predicted that human angiotensin-converting enzyme 2 is located mainly outside of the cell membrane (extracellular) (Figures 2-4). Multiple Em Motif Elicitation revealed that ACE 2 had the sequences CQAAKHEGGHIKCDI in location 369–383, HDEDYCD at 195-201, MWGGFW at 270-275, VCNPDNW 132-138, KWRWMK 458-463, RSEVGKALR 169 -177, VGAKNMNVRP, 244-253.

Figure 1: 3D Structure of Angiotensin-Converting Enzyme 2 as retrieved from RCSB-PDB https://www.rcsb.org/pdb/software/wsreport.do

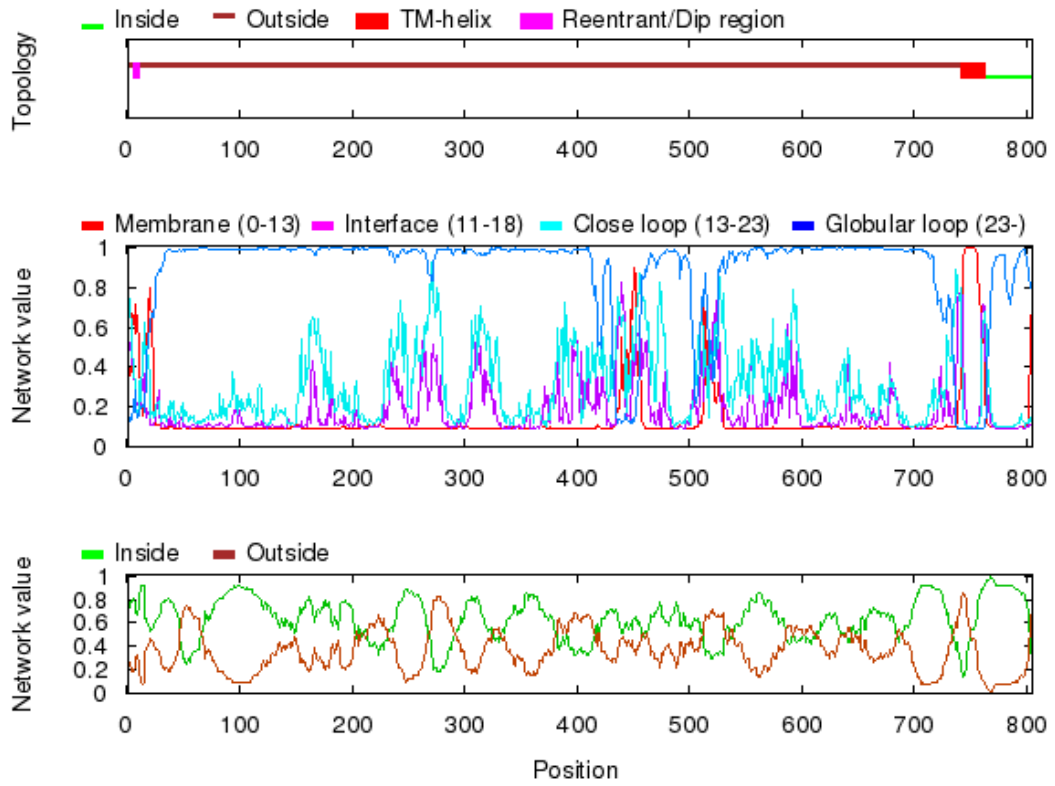

Figure 2: Membrane Topology Prediction of ACE 2 Using Octopus and Spoctopus

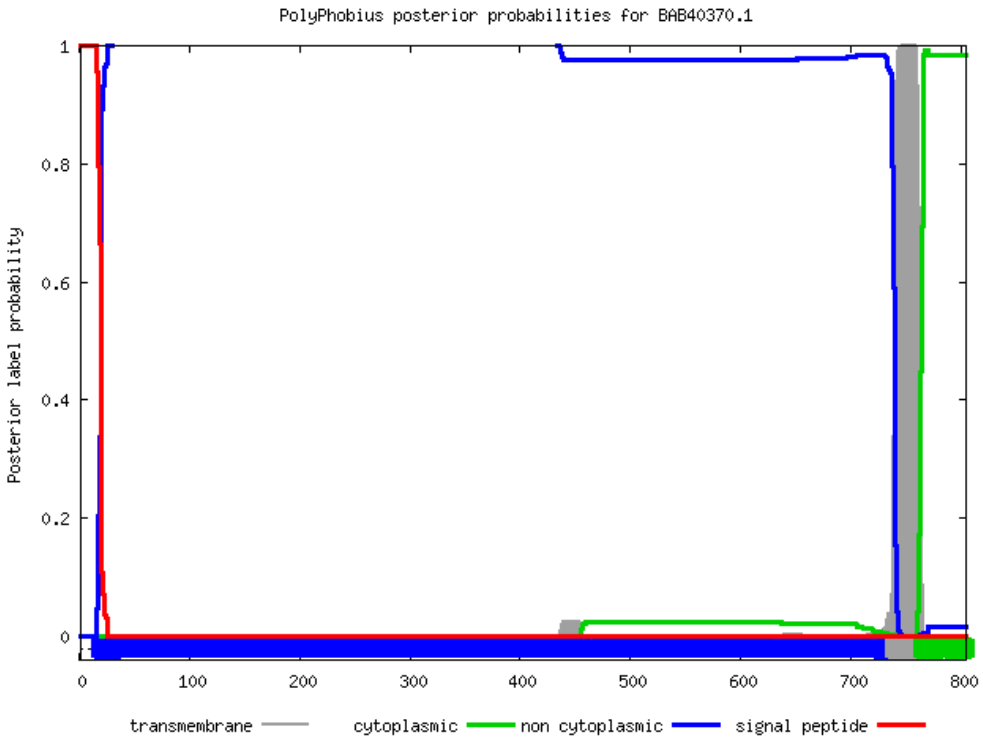

Figure 3: Membrane Topology Prediction of ACE 2 Using PolyPhobius

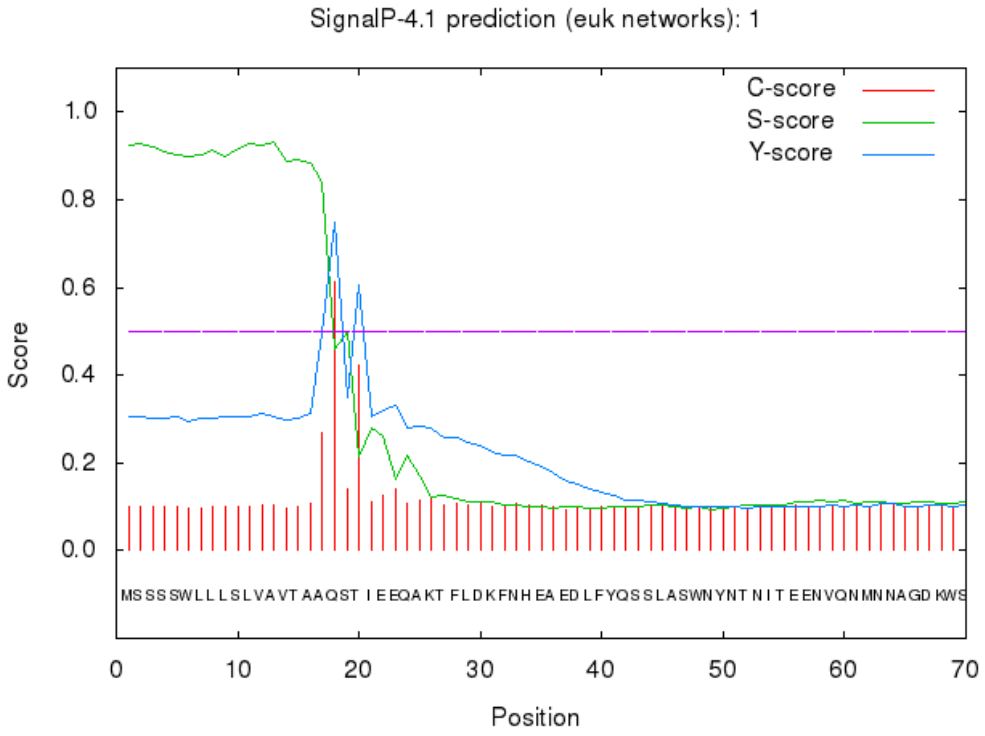

Figure 4: Membrane Topology Prediction of ACE 2 using SignalP 4.1

Table 1: Human Angiotensin-Converting Enzyme 2 Physiochemical Properties

Serial Number	Property	Value
1.	Chemical Formula	$C_{4170}H_{6358}N_{1094}O_{1222}S_{35}$
2.	Total number of atoms	12879
3.	Aliphatic index	80.55
4.	Charge	Negative
5.	Extinction coefficient *	176170 Abs 0.1% (=1 g/l) 1.905, assuming all pairs of Cys residues form cystines
6.	Instability index	40.10
7.	Molecular weight	92491.05 Dalton
8.	Grand average of hydropathicity (GRAVY)	–0.376
9.	Number of amino acids	805
10.	Theoretical pI	5.36

* Extinction coefficients are in units of M-1 cm-1, at 280 nm measured in water.

Table 2: Human Angiotensin-Converting Enzyme 2 Active Site Amino Acids and Their Location

Site Location	Amino Acids
382	Aspartic Acid
347	Threonine
345	Histidine
505	Histidine
406	Glutamic Acid
378	Histidine
375	Glutamic Acid
449	Threonine
515	Tyrosine
510	Tyrosine
504	Phenylalanine
346	Proline
503	Leucine
401	Histidine
348	Alanine
402	Glutamic Acid
368	Aspartic Acid
374	Histidine
512	Phenylalanine

Table 3 shows the estimated binding energy and inhibition constant values obtained from the docking ACE 2 receptor with the selected inhibitors. The binding energy represents the energy released when a ligand associates with a target while the inhibitor constant (Ki) indicates the level of potency of an inhibitor. This represents the concentration required to produce half-maximum inhibition. The result obtained from this study revealed that the tested inhibitors were found to interact with the pocket of the target receptor.

Table 3. Docking Ranked by Binding Energies and Inhibition Constants

	Inhibitors	Binding Free Energy (kcal=mol)	Inhibition Constant (Ki)
1	TOH	-6.33	22.74 µM
2	QUI	-6.54	16.20 µM
3	TFH	-7.90	1.62 µM
4	ACN	-3.65	2.12 mM
5	PAI	-3.94	1.29 mM

KEY:
TFH, Trifluoperazine Hydrochloride; TOH, Thioridazine hydrochloride; PAI, Pentamidine isethionate; QUI, Quinine; ACN, Allicin.

The results showed estimates of the binding free energy of the ligand from a given orientation and inhibition constants. Inhibitors with the lowest estimated binding free energy and inhibition constant tend

to establish a strong interaction with ACE 2 receptors on specific active sites. This study showed trifluoperazine hydrochloride (TFH) which is an important phenothiazine derivative with a minimum value of binding free energy −7.90 kcal/mol and inhibition constant of 1.62 µM (Table 3) is a potent inhibitor of ACE 2. The ligand (TFH) had the strongest interaction with ACE 2 receptor when compared with other inhibitors screened in this study. Quinine which is an antimalarial drug and thioridazine hydrochloride (TOH) another phenothiazine neuroleptic drug used for the treatment of schizophrenia and other psychiatric disorders also exhibited a strong binding affinity for ACE 2, with binding free energy and inhibition constants of −6.54 kcal/mol and 16.20 µM; −6.33 kcal/mol and 22.74 µM respectively (Table 3) even though trifluoperazine hydrochloride showed a better affinity for the receptor. This suggests that these ligands can serve as potential blockers for SARS-CoV 2 entry into the cell through ACE 2 receptor the major gateway into the cell.

It should also be noted that apart from being the passage for SARS-CoV 2 into the host cell, the receptor has been identified to play a major role in the conversion angiotensin II to angiotensin- (1–7) and this makes it to attenuate its effects on vasoconstriction, sodium retention, and fibrosis. Although angiotensin II has been reported to be the primary substrate of ACE2, it's involvement in the cleaveage of angiotensin I to angiotensin (1–9) and in the hydrolysis of other peptides have been well documented [18]. Studies on humans' tissue samples obtained from 15 different organs have revealed that ACE 2 is expressed in most organs as well as on the main target cells for SARS-CoV-2 which also is known as the site of dominant injury (the lung alveolar epithelial cells) [34]. Also to buttress the role of ACE 2 in the infectivity of the SARS-CoV 2 on host cells, and an *In Vitro* experiment demonstrated that expression of ACE 2 positively correlated with the SARS-CoV infection [35]. Genetic variants in ACE2 have also been suggested to influence the interaction of the receptor with the viral spike protein [36].

The findings from this study, suggest that trifluoperazine hydrochloride, quinine and thioridazine hydrochloride due to the high affinity for ACE 2 receptor could potentially prevent the entry of the SARS-CoV 2 into the cell. Pentamidine isethionate which is mainly used for prophylaxis and treatment of *Pneumocystis jirovecii* pneumonia (PCP), and Allicin also interacted with the active site of ACE 2 receptor and inhibited with binding free energy and inhibition constants of −3.94 kcal/mol and 1.29 mM; −3.65 kcal/mol and 2.12 mM respectively (Table 3). These ligands are effective inhibitors for ACE 2 receptors but not necessarily as effective as trifluoperazine hydrochloride, quinine, and thioridazine hydrochloride.

Although, allicin has a low binding affinity for ACE 2 when compared with other inhibitors considered, it is the only natural compound investigated. We observed that the compound showed some levels of effective inhibition of ACE 2. However, the antagonistic interaction of allicin with ACE 2 may not guarantee its suitability as a therapeutic agent. It is a herbal compound found in garlic that has earlier been reported to inhibit the activity of ACE and satisfy all Lipinski's rules with minimal absorption in the brain [37].

However, the antagonistic interaction of allicin with an ACE 2 does not guarantee the appropriateness as a remedy. It is, therefore, necessary to use ADME analysis of inhibitors which helps provide approval of inhibitor for drug development [38]. Absorption, Distribution, Metabolism, and Excretion (ADME) Analysis is majorly based on Lipinski's rule of five also known as Pfizer's rule of five [39]. Lipinski's rule of five or Rule of five (Ro5) was published in 1997 by Christopher A. Lipinski [37]. This rule is used to determine the drug-likeness and to establish whether or not a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans [37]. The analysis most times uses the information provided on the lipophilicity, molecular weight (MW), number of hydrogen bond donors and hydrogen bond acceptors to predict the bioavailability of the ligand studied, the level of permeability across the intestine and its solubility in water. To guarantee this, the molecular weight of the ligand studied must be less than 500 g/mol, the lipophilicity and number of hydrogen bond donors must both be less than 5 while hydrogen bond acceptors must be less than 10. These parameters provide information on the molecular properties necessary to determine the drug's pharmacokinetics in the body of humans and the absorption, distribution, metabolism, and excretion (ADME). Peradventure the ligand did not meet up to the standard of Lipinski's rule of five, this will then suggest that there is a high probability that the ligand could cause ingestion problems after use [40]. The

results of ADME predictions with the BOILED-egg method, Drug-likeness using Lipinski parameters and Pharmacokinetics are shown in Figure 5 and Table 4.

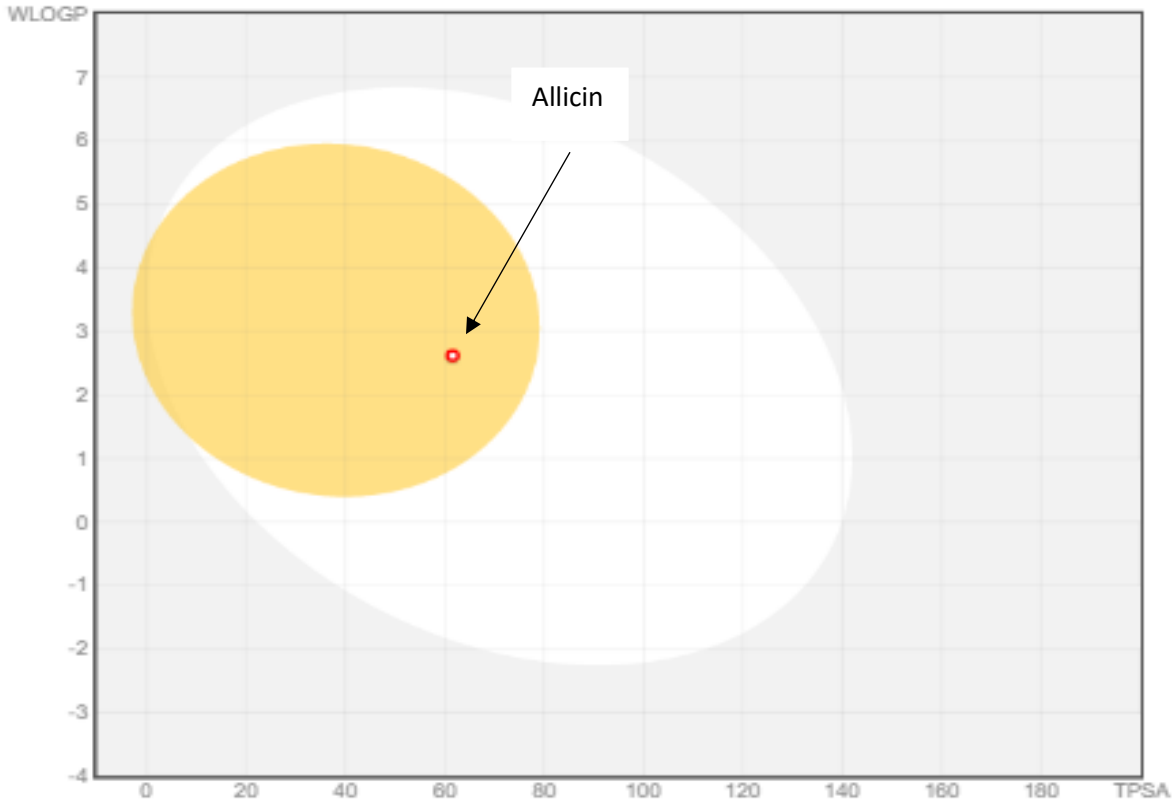

Figure 5: Analysis of Allicin using BIOLED-EGG method of ADME

Table 4: Some Pharmacokinetic Parameters of Allicin from ADME

Gastro-Intestinal absorption	High
Blood-Brain Barrier permeant	Yes
Log K_p (skin permeation)	6.36 cm/s

This investigation revealed that allicin satisfies the Lipinski's rule Molecular Weight 162.27(g/mol) (< 500), lipophilicity of 1.18 (MLog *P*),(< 5) number of hydrogen bond donors is 0 (< 5) and the number of hydrogen bond acceptors is 1 (<10). Swiss ADME BOILED-egg analysis confirmed that allicin can be absorbed by the brain within the acceptable range, thus it promises to be a potential remedy for COVID-19 patients which can be formulated to be taken orally and its antithrombotic properties as elucidated in previous studies [27] could be exploited given current findings where disseminated intravasculopathy (DIC) and microvascular thrombosis have been identified in autopsies of COVID-19 subjects [41,42]. However, more *In Vitro* and *In Vivo* studies still need to be done to scientifically ascertain the potency of allicin and other ligands studied as potential drugs for COVID-19 treatment.

Conflict of Interest

There is no conflict of interest on this research article

Funding
None

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