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AN IN SILICO STUDY ON THE MOLECULAR INTERACTIONS OF ANACYCLIN WITH SARS CoV-2 ACE-2 BINDING RECEPTOR

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***Abstract*:** Coronavirus disease 2019 (COVID-19) is an infectious disease, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). Angiotensin-converting enzyme 2 (ACE2) is not only an enzyme but also a functional receptor on cell surfaces through which SARS-CoV-2 enters the host cells and is highly expressed in the heart, kidneys, and lungs and shed into the plasma. It was reported that natural compounds obtained from plants have the ability to suppress the effect of SARS CoV-2. Therefore, the current study was carried on to investigate the inhibitory action of anacyclin against SARS-CoV2 entry into the cell using molecular docking approach to inhibit spike receptor binding domain bound to angiotensin- converting enzyme 2 (ACE2). The structure of SARS- CoV-2 spike receptor binding domain (RBD) and anacyclin were retrieved from PDB and IMPPAT(Indian Medicinal Plants, Phytochemistry And Therapeutics database). Molinspiration was used to check the drugability of anacyclin. AutoDock 4.2 software was used to dock the SARS- CoV-2 spike-RBD with anacyclin. Anacyclin satisfied the Lipinski rule and can be used as a drug. The binding energy of anacyclin to the Spike-RBD to ACE2 was -6.26 kcal/mol. In conclusion, *in-silico* studies of anacyclin from *Anacyclus pyrethrum* with SARS- CoV-2 ACE2 receptors showed that anacyclin may interfere with the attachment of spike with the human cell membrane and further it can be used as a potential drug candidate for COVID-19.

Index terms: **SARS-CoV-2, Anacyclin, ACE2 receptor, Drug**

I. INTRODUCTION

Coronaviruses are involved in human and other vertebrate diseases. They are members of the subfamily Coronavirinae in the family Coronaviridae and the order Nidovirales. The recent emergence of a novel coronavirus with an outbreak of unusual viral pneumonia in Wuhan, China, and then pandemic outbreak is 2019-CoV or COVID-19 (Mousavizadeh and Ghasemi., 2021). SARS-CoV-2 enters host cells via angiotensin-converting enzyme 2 (ACE2), which is highly expressed in the heart, kidneys, and lungs and shed into the plasma. ACE2 regulates the renin-angiotensin-aldosterone system (RAAS). SARS-CoV-2 disrupts the ACE/ACE2 balance and activates the RAAS, ultimately leading to COVID-19 progression, particularly in patients with comorbidities such as hypertension, diabetes, and cardiovascular disease. As a result, ACE2 expression may have contradictory effects, aiding SARS-CoV-2 pathogenicity while limiting viral infection (Beyerstedt *et al*, 2021).

Through a new scheme called Arokkiam, the state of Tamil Nadu proposed the use of Siddha formulations in the management of asymptomatic and mild COVID-19, with Kabasura Kudineer being one such polyherbal Siddha formulation. In the Siddha system of medicine, Kabasura Kudineer is indicated for use in fever (Aiyasuram) and respiratory diseases (Aiyanoigal). Kabasura Kudineer is made up of 15 herbal ingredients, five of which have been shown to have antiviral activity. Cucurbitacin B, cardiofoliolide, apigenin, and pyrethrin were found to be effective in preventing SARS-CoV-2 binding and replication in the herbal decoction. Standardization of kabasura kudineer has resulted in the documentation of organoleptic properties, physicochemical values, thin layer chromatographic profiles(TLC), and high performance TLC fingerprints. Furthermore, toxicological studies have shown that kabasura kudineer is safe and it has antipyretic, anti-inflammatory, and antibacterial properties (Natrajan *et al*, 2021).

Anacyclus pyrethrum, also known as 'Akarkara' in India, is a small hairy herbaceous perennial in the Asteraceae family. The plant is native to Arabia and but they are found in India along the Himalaya, Jammu and Kashmir, Bengal, and in the northern parts. In Ayurveda and Unani medicine, Akarkara described an aphrodisiac drug with multiple uses. Pyrethrum root, also known as pellitory root, has medicinal properties such as mollusidal and anti-inflammatory activity, analgesic, anti-rheumatic, carminative, antiviral, anti-catarrh, improve digestion, emmenagogue, febrifuge, vermifuge, and nervine activity. (Panday *et al*, 2018)

Great efforts are being made to evaluate the similar 'drug-like' properties of molecules in the early stages of the discovery-research process in order to advance the discovery and development of new drugs. There are several approaches to solve this problem, but the simplest and most widely used approach was developed by Chris Lipinski and his colleagues at Pfizer and is known as the Lipinski Rules or the Rule of Five (ROF). An ideal drug molecule would comply with the physicochemical property guidelines of Lipinski's Rule of Five (Chen *et al*, 2013). To obtain parameters such as MiLogP, TPSA, and drug likeness, molinspiration software was used. Molecular docking has proven to be an extremely effective tool for discovering new drugs that target proteins. Protein-ligand docking is of particular interest due to its application in the pharmaceutical industry (Azam and Abbasi 2013). For the docking purpose of the study current version of Autodock was used. In current work, our aim was to examine the possibility of an existing relationship between the experimental bioactivities of the inhibitors under study and the docking scores. In order to get accurate results, all the docking experiments were performed with the default parameters.

COVID-19 has been flooding the world over the past two years, claiming the lives of lakhs of individuals. To deal with a difficult circumstance like this pandemic, it may be possible to provide efficient therapeutic approaches based on the region's established traditional knowledge. Traditional herbal remedies are a typical complex mixture of diverse phytochemicals, and their pharmacology and synergetic activity may provide relief from symptoms when used alone or in combination with modern antiviral medications. Despite continuous efforts at drug development and vaccine development, no proven therapies exist. As a result, this research may shed light on the role of anacyclin as a potential drug of SARS-COV-2 binding in the ACE-2 receptor, which could be useful in the quest for viable treatments.

II. RESEARCH METHODOLOGY

The pdb structure of the target protein was obtained from the protein data bank website and anacyclin's three-dimensional structure was obtained in pdb format from IMPPAT. Molinspiration analysis was performed using canonical smiles. Using Lipinski's rule of five, the bioactivity score of the ligand molecule predicted from molinspiration which was used to determine whether it can act as an orally active drug or not. Autodock4 was used to dock the protein and ligand. In autodock 4.2, the target protein's structure was uploaded. The target protein was created by the addition and deletion of molecules in chains and saving it in pdbqt format. In autodock 4.2, the ligand molecule was uploaded and prepared for grid box formation.

The three-dimensional gridbox was 214 x 222 x 264 with a spacing of 0.500 on the geometric centre of the protein. Autogrid was used to create glg files and map files. The dlg files were created using autodock. The best poses with the lowest binding energy were chosen from the RMSD table in the dlg file after docking to visualize the ligand protein interaction. The interactions between the molecule is studied using Biovia Discovery Studio.

III. RESULTS AND DISCUSSION

The present study was carried out to understand the drug likeness property of Anacyclin and its binding affinity with SARS CoV2 ACE2 receptors and also to analyse the druglikeness property of anacyclin using the parameters of molinspiration analysis.

Analysis of Lipinski's rule of 5

The Molinspiration data of compounds were analyzed in this study using the basic concept of Lipinski's rule of five. Anacyclin's molecular descriptor value and bioactive properties were investigated (Table 2 and 3) and it obeys the rule of five with all ideal values.

Table 1: Drug likeness properties of Anacyclin

LIPINSKI'S RULE OF FIVE	Acceptable values	Anacyclin
Number of hydrogen bond donor	<5	2
Number of hydrogen bond acceptor	<10	1
Molecular weight	<500	271.40
LogP lipophilicity	<5	4.99
Number of rotatable bonds22	<10	7

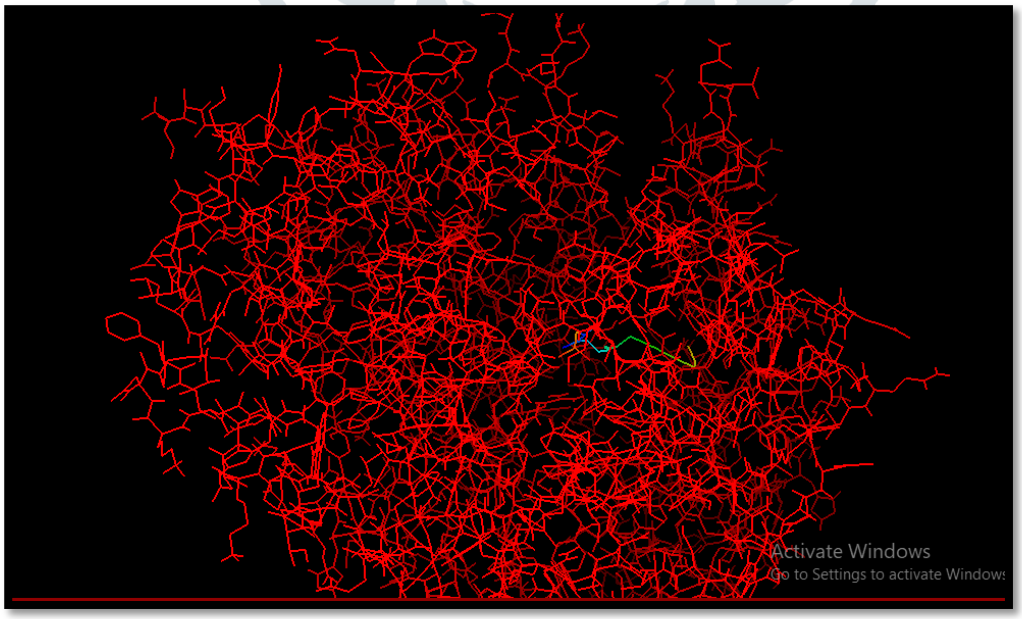
Table 2: Molecular descriptor and drug likeness property of Anacyclin

PROPERTIES	Anacyclin	Acceptable values
Polar surface area	29.10Å	140 Å
Molecular volume	293.66	**0.0=active** **Between -0.5 to 0.0= moderately active** **0.5= inactive (Dar *et al*, 2015)**
GPCR ligand	0.28	
Ion channel modulator	0.36	
Kinase inhibitor	-0.17	
Nuclear receptor ligand	0.23	
Protease inhibitor	0.20	
Enzyme inhibitor	0.32	

Molecular Docking Studies

Molecular docking is a one of the important tool in the drug design, which is carried out in this study to evaluate the binding efficiency of the anacylin against target ACE2 binding receptor[7DMU] using autodock 4.2 software. It has visualized the hydrogen interaction between anacyclin and SARS CoV2 ACE2 binding 2 receptor using AutoDock 4.2 was visualized shown in the figure 1 and 2. This study revealed the molecular interaction of anacyclin with ASP350, GLU375, HIS345, HIS375, HIS505, PRO346, TRP349, THR347, TYR510 and TYR515 amino acids of protein 7dmu (shown in figure 1). The least binding energy was found to be -6.26 kcal/mol. The binding energy histogram of anacyclin with SARS CoV2 ACE2 receptor is shown in figure 2.

(a)



(b)

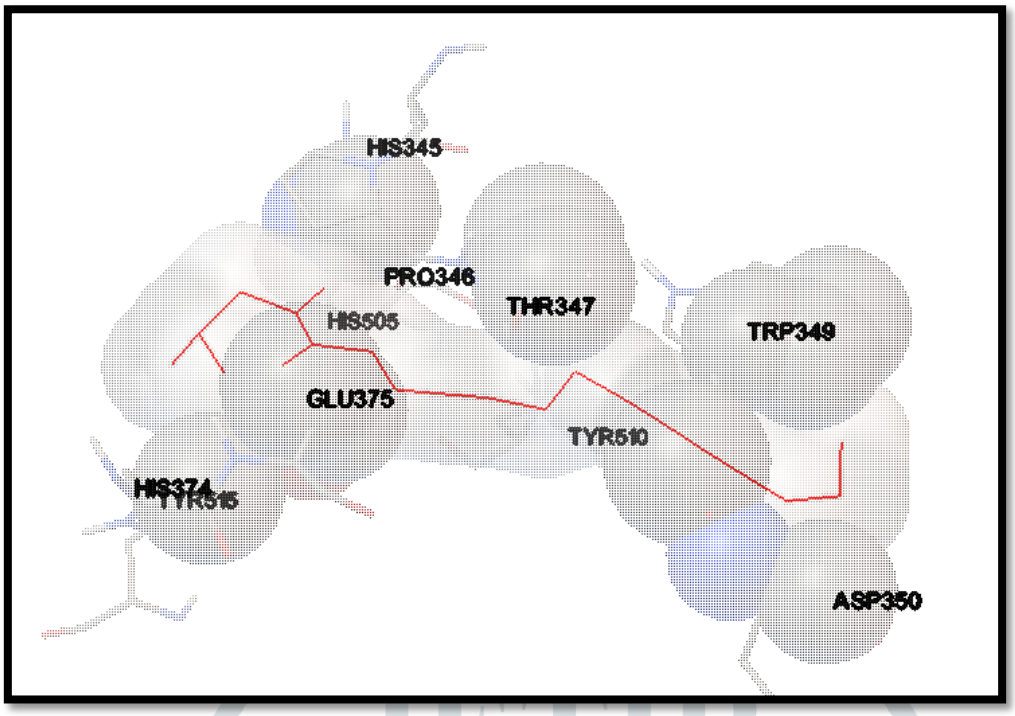


Figure 1 (a & b): Autodock view of Anacyclin with ACE2 receptor

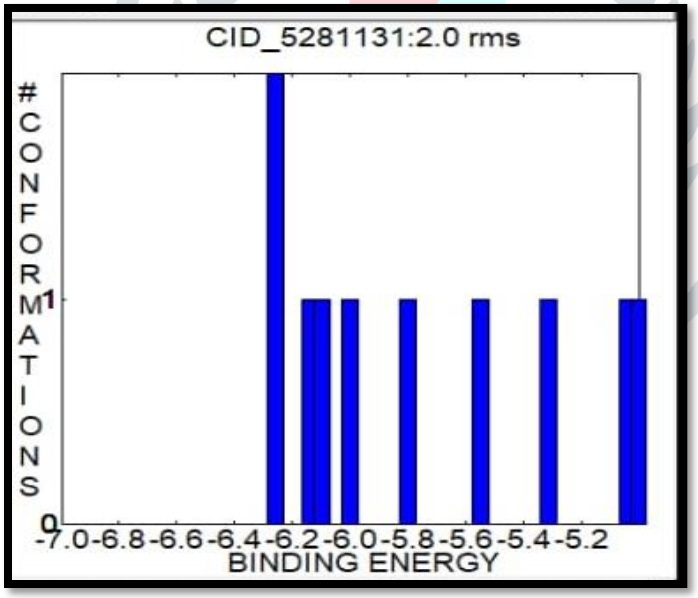


Figure 2: Binding energy histogram of Anacyclin with ACE2 receptor

Angiotensin converting enzyme 2 (ACE2) is a transmembrane protein which is considered as a spike protein binding receptor for novel corona virus. Since there is no medication available for the treatment of COVID-19, it is important and essential to design a new drug. *In-silico* analysis plays a major role because of its rapidness and cost effectiveness. Natural compounds are safe and are easily available for the treatment of COVID affected patients. In the present study, Anacyclin have been selected for molecular docking against spike protein of SARS-CoV2 with its human receptor ACE2 molecule.

Natural products are regarded as the most important resource for drug research and development, particularly in the treatment of infectious diseases (Alrasheid *et al*,2021). The primary goal of the drug is to bind with the biological target. Lipinski's rule of five is used to assess the druglikeness of a chemical compound that has properties that make it a likely or potential drug in humans (Lipinski *et al*,2001). To be potentially used as an oral drug, the biologically active molecule must meet five conditions. Poor absorption or permeation are most likely if:

- ➢ Molar mass >500,
- ➢ Number of H-bond acceptors >10,

➢ Number of H-bond donors > 5,
➢ log P > 5 (or M log P > 4.15)
➢ Bioactivity score 0-4

which means that a potential drug has no more than one violation of the exposed criteria (Lipinski *et al*,1997).

However, Lipinski points out that such molecules should not be completely dismissed; it is well known that many drugs do not undergo ROF.

Certain molecular parameters, such as log P (partition coefficient), number of rotatable bonds, number of hydrogen bond donors, number of hydrogen bond acceptors, and molecular weight, are used to predict the oral activity of the drug compound. According to the rule, most metal complexes with good membrane permeability have log P value as 5, 10 hydrogen bond acceptors, 5 hydrogen bond donors, and 10 rotatable bonds (Dar *et al*, 2016). An orally active drug should have no more than one violation of the given criteria. Anacyclin was found to be in good agreement with the given criteria in the current study and can be said to have good oral bioavailability (Table 2). Anacyclin has a topological polar surface area of 29.10, which is less than 140, making it a suitable drug for transport.

Anacyclin's bioactivity scores were calculated for various parameters such as binding to GPCR ligand and nuclear receptor ligand, ion channel modulation, kinase inhibition, protease inhibition, and enzyme activity inhibition. All of the parameters were calculated using the online software Molinspiration (www.molinspiration.com), which predicted that anacyclin had moderate biological activity.Table 3 shows the bioactivity score. It is known that if a drug candidate's bioactivity score is greater than 0.0, the complex is active; if it is between 5.0 and 0.0, the complex is moderately active; and if it is less than 5.0, the complex is inactive. As shown in Table3, the bioactivity scores of anacyclin were greater than 0.0, indicating that they possess the necessary properties to act as potential drug (Dar *et al*, 2016).

The interaction of SARS-CoV2 spike protein fragment with ACE2 receptor protein was analysed by molecular docking. The docked model with the lowest binding energy and highest binding affinity indicated the most stable ligand-target protein binding.The affinity of SARS-CoV2 for anacyclin was calculated to be -6.26 kcal/mol. Anacyclin interacted with the SARS-CoV2 spike ACE-2 receptor *via* the amino acids His374, Tyr515, His505, Glu375, Pro346, HIS345, Thr347, Tyr510, Trp349, and Asp350. The 2D structure interaction of the molecules are retrieved and studied using the Biovia Discovery Studio (Figure 3).

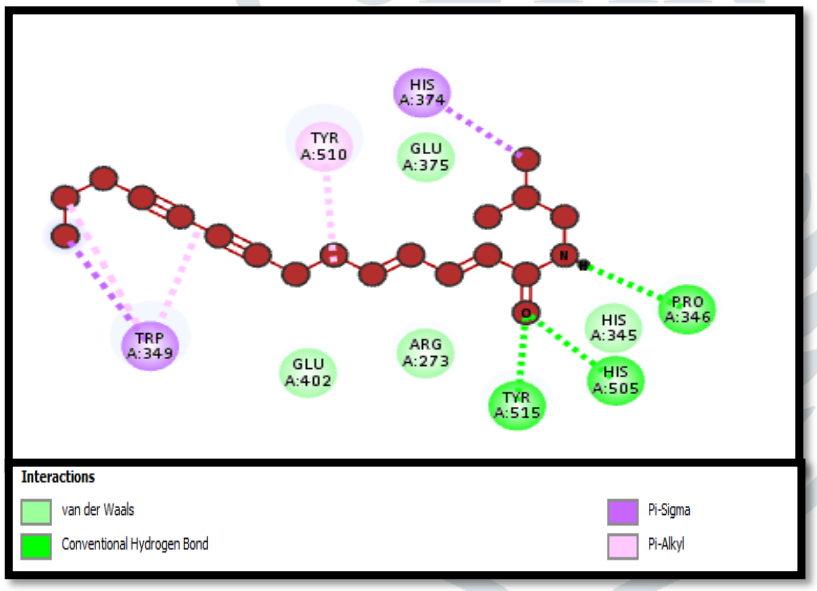


Figure 3: 2D interaction view of ACE2 receptor with anacyclin using Biovia discovery studio

Dhanasekaran *et al* (2020) conducted a study in which 28 lead molecules from well-documented medicinal herbs were subjected to molecular docking analysis targeting the ACE2 receptor and their potential for impeding the host-viral interface were evaluated. According to the findings, nearly 11 bioactive lead molecules out of 28 had a potential binding affinity of about 100 percent with the target amino acid residue (31 Lys and 353 Lys).

Oliveira and colleagues used 60 drugs to perform molecular docking with the ACE2 receptor in complex with the S-glycoprotein SARS-CoV-2 (PDB ID: 6M0J). According to the docking results, paritaprevir and ivermectin have the highest binding affinity to the ACE2 receptor. Paritaprevir had the highest binding affinity with the ACE2 receptor, owing to two factors: the hydrogen bonding interaction with Asn394 residue and the hydrophobic interaction of aminophenanthrene with Trp349 residue. In-silico results suggested that both candidates could be used to treat COVID-19. Remdesivir and azithromycin are among the promising drugs in the second group. The repurposed medications hydroxychloroquine and chloroquine were not effective as monotherapies against SARS-CoV-2 infection when compared to other medications (Micael and Kelson, 2020).

Abdelli and colleagues used natural compounds (-terpinene, p-cymene, limonene, thymol, and isothymol) obtained from the anti-viral plant *Ammoides verticillata* to conduct virtual screening of drugs capable of blocking the action of ACE2. They tested the active principles of *Ammoides verticillatea* (-terpinene, p-cymene, limonene, thymol, and isothymol) against the enzyme ACE2 and performed a molecular docking analysis against the ACE2 enzyme containing -d-mannose co-crystallized ligand using regular parameter settings in the MOE-2013 software package (PDB ID: 6vw1). The docking result analysis revealed that docked isothymol outperformed the co-crystallized ligand -d-mannose in terms of docking score (Abdelli *et al*, 2021).

According to the literature, there is no evidence for SARS-CoV2 ACE2 with anacyclin,.The current study found that anacyclin meets all of the criteria for a drug. Anacyclin had the lowest binding energy with the SARS-CoV ACE2 binding receptor, indicating that it could be used as a potential drug for the treatment of COVID-19 disease. The results of this study concluded that anacyclin

with active binding sites has a high affinity.The anacyclin can be used as a lead molecule in the development of a COVID -19 drug candidate.

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