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SCREENING OF ANGIOTENSIN-CONVERTING ENZYME-2 AND FURIN INHIBITORS AS DRUG LEADS AGAINST SARS-COV2

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ABSTRACT

A database of angiotensin-converting enzyme-2 (ACE2) and furin inhibitors were selected as ligands to screen them as drug leads against SARS CoV2. The methodology employed for screening the inhibitors entailed the use of ADMET-SAR server, which facilitated the assessment of drug likeliness, absorption, distribution, metabolism, excretion, and toxicity analysis. Molecular docking analysis was done by using AutoDock Vina software, and it revealed that three potential inhibitors *viz*., captopril, nicotianamine, and perindopril, exhibited strong binding affinity within the active site of ACE2 protein. Captopril exhibited highest binding affinity of -5.5 kcal mol^{-1} to ACE2 protein with low dock score of -26.074. 4-hydroxycoumarin showed strongest binding with furin active site residues with a binding score of -6.8 kcal mol^{-1} followed by scopoletin and barlerin whose binding scores were slightly lower than 4-hydroxycoumarin. All the 3 ligands showed strong hydrogen binding with conserved Trp-531. The inhibitor 4-hydroxycoumarin exhibited binding with furin involving the amino acid residues Glu-271, Gln-488, Asn-310, Pro-266, Ala-532, and Trp-531. The study revealed that ACE2 and furin inhibitors can serve as lead molecules for optimization and drug development against SARS CoV2. The study has significance in the exploration and development of effective drugs against coronavirus.

Keywords: ACE2 inhibitors, captopril, furin inhibitors, 4-hydroxycoumarin, SARS-CoV2

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infections continue to pose a serious threat to human health worldwide (El-Zowalaty *et al*., 2020). The World Health Organization (WHO) on March 11, 2020 declared novel coronavirus (COVID-19) outbreak as global pandemic (Maza *et al*., 2020). More than 767,984,989 cases of novel coronavirus, including at least 6,943,390 deaths have been reported worldwide to the WHO as on June 7, 2023. SARS-CoV-2 virus is a member of Coronaviridae family. The most common symptoms of infection were fever (88.7%), cough (67.8%) and diarrhoea (3.8%) (Guan *et al*., 2020). Despite strenuous efforts the researchers are still unable to design specific antiviral drugs against this disease (Wang *et al*., 2020).

Angiotensin-converting enzyme-2 (ACE-2) is one of the important receptors on cell membrane of host cells which interacts with spike protein (SP) with a furin-cleavage site and results in SARS-CoV-2 invasion (Hasan *et al*., 2020). The inhibition of early SARS-CoV2 - ACE2 gene interactions using appropriate inhibitors has proven to be an effective treatment against coronavirus infection. Since furin is highly expressed in lungs, an enveloped virus that infects the respiratory tract may successfully exploit this convertase to activate its surface glycoprotein (Mbikay *et al*., 1997). Small molecular inhibitors can limit the interaction of ACE-2 and furin with SP so can be exploited as potential therapeutic to combat coronavirus.

Virtual screening approaches have gained immense popularity and have become an integral part of industrial and academic research on drug design and discovery (Shoichet, 2004; Villoutreix *et al*., 2007, Jahn *et al*, 2009). The discovery of innovative leads is an expensive and time-consuming process (Lavecchia *et al*., 2013). In contrast to the conventional methods of drug screening which involves high throughput screening (HTS), recently virtual high throughput screening (vHTS) term has been coined to **accelerate** the drug discovery for time-efficient identification of cost effective novel and selective drug leads (Subramaniam *et al*., 2008). Computer-aided drug discovery has recently has gained significance as new biologically-active compounds can be predicted along with their receptor-bound structures and in several cases the achieved hit rates (ligands discovered per molecules tested) have been significantly greater than with HTS (Andricopulo, *et al*., 2009). The present study was aimed to screen the most potential compounds which could inhibit ACE2 protein and furin protein. A set of 38 ACE2 inhibitors and 28 furin inhibitors already reported (Teralı *et al*., 2020) were virtually screened through structure based drug design approach. The study undertakes virtual ligand screening to find the most effective inhibitors that could prevent the interaction of ACE-2 and furin proteins with spike proteins of SARS-CoV-2 and can be used as potential therapeutic platforms to combat the spreading coronavirus epidemic.

MATERIALS AND METHODS

Retrieval of receptor proteins structures

The structures of ACE2 and furin proteins were retrieved from Protein Data Bank (PDB) with PDB IDs 1r4l and 4z2a, respectively (*https://www.rcsb.org/*). The protein structures were prepared for docking by using PyRx and AutoDockTools-1.5.6 software.

Selection of ligands

An intensive literature search was performed to screen the potential ligands that have been reported as inhibitors against ACE2 and furin proteins. A total of 38 ACE2 inhibitors and 28 furin inhibitors were evaluated in the present study. 3D structures of inhibitors were downloaded from Pubchem in .sdf file format (*https://pubchem.ncbi.nlm.nih.gov/*). Energy minimization of each ligand was done using PyRx prior to docking (*https://pyrx.sourceforge.io/*).

Target and ligand preparation for docking

The target proteins were prepared for docking by removing polar hydrogens and adding Kollman charges using AutoDockTools. Hetatm and the side chains, if present, were deleted using Discovery Studio Visualizer (*https://discover.3ds.com/discovery-studio-visualizer*). The ligands were also prepared for docking using AutoDockTools, the root was detected, and the number of torsions and the aromaticity criterion were set to 6 and 7.5, respectively. The Grid Box dimensions were set as 60 x 60 x 60 and the output file was saved as a.gpf in Cygwin folder. LamarkianGA(4.2) algorithm was selected and the file was saved as a.dpf in Cygwin folder.

Molecular docking

Molecular docking of ACE2 and furin proteins with their selected inhibitors were performed using AutoDock 4.2 with Cygwin terminal commands. The docked complexes were viewed using Discovery Studio Visualizer (BIOVIA Discovery Studio Visualizer 4.5) to view the 10 possible ligand-target complex poses, the best model among those was picked and checked for amino acid residues attached to the H bonds involved in binding. The binding affinities were calculated using PyRx software. The docking scores were predicted using HPEPDOCK (*http://huanglab.phys.hust.edu.cn/hpepdock/*).

Ligand receptor interaction analysis

For a clear view of receptor ligand interaction of the best docked complexes, 2D plots of receptor

ligand interactions were analysed using Discovery Studio Visualizer that generates 2D representation of the number of hydrogen bonds and amino acids residues involved in bonding between the target and ligand in the active site of the target protein. The electrostatic interactions, hydrogen bonding, Van der Waals forces and hydrophobic interactions which contribute to the affinity of inhibitors within the active site of the proteins were also observed. 3D images of docked protein-ligand complexes were visualized using Discovery Studio Visualizer.

ADME toxicity

The ligands used in this study were initially tested for their drug likeliness on the basis of thresholds set by "Lipinski's Rule of Five". Screened inhibitors were further checked for their absorption, decomposition, metabolism, excretion and toxicity profile virtually with admetSAR server. In addition to this, AMES toxicity and carcinogenic likelihood of inhibitors was also evaluated (*http://lmmd.ecust.edu.cn/admetsar2*).

RESULTS AND DISCUSSION

Screening of potential inhibitors and molecular docking

In present study, the screening was conducted to identify potential inhibitors using molecular docking. Out of the 38 ACE2 inhibitors and 28 furin inhibitors, 10 ACE2 inhibitors and 8 furin inhibitors were shortlisted based on ranking criteria, considering higher binding affinities and lower docking scores (Table 1). Notably, three inhibitors, namely captopril, nicotianamine, and perindopril, exhibited strong binding affinity within the active site of the ACE2 protein. Among them, captopril demonstrated the highest binding affinity of -5.5 kcal mol^{-1} and a dock score of -26.074. Captopril formed hydrogen bonds with specific amino acid residues, including Val-209, Leu-95, Pro-565, Lys-562, Glu-208, and Asp-206 of ACE2, with additional close contact residues identified (Fig. 1, A,B). Nicotianamine and perindopril also displayed favourable binding scores of -6.3 and -6.7 kcal mol^{-1}, respectively.

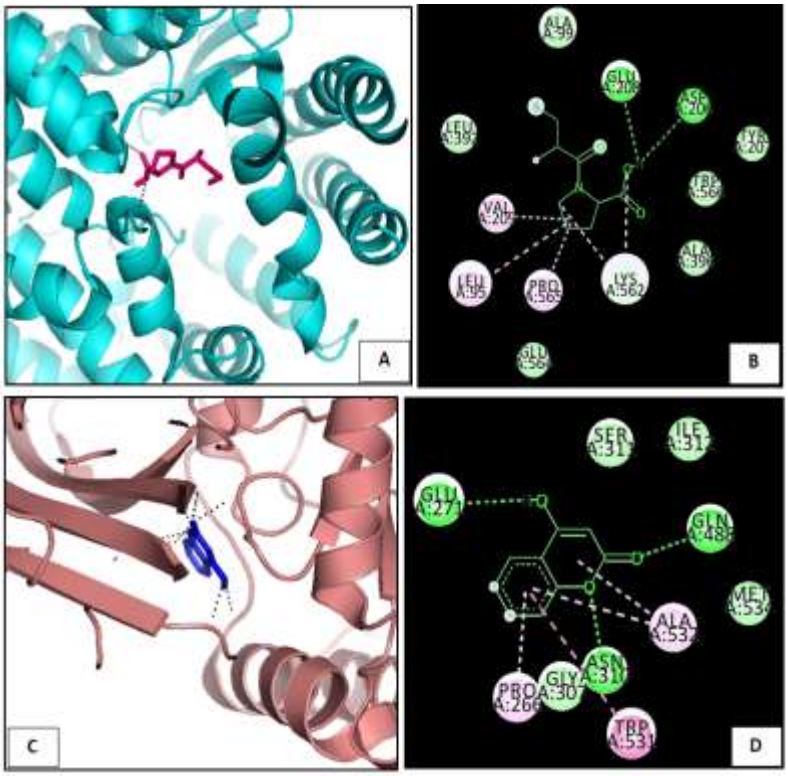

Fig. 1: A) 3D representation of inhibitor captopril docked with ACE2 protein; B) The interactions of inhibitor captopril with the active site of ACE2 receptor showing conventional hydrogen bond (green dotted lines), alkyl interactions (pink dotted lines) and closer contact amino acid residues (green circles only); C) 3D representation of inhibitor 4-hydroxycoumarin docked with furin protein D) interactions of inhibitor 4-hydroxycoumarin with the active site of furin receptor showing conventional hydrogen bonds (green dotted lines), alkyl interactions (pink dotted lines) and closer contact amino acid residues (green circles only)

Table 1: Summary of top ranked inhibitors screened against ACE2 and furin with their respective binding affinities, dock scores, interacting and closer contact residues

Inhibitors	Binding affinity (kcal mol^{-1})	Docking score	Residues interacting with ligand through H-bonding	Closer contact residues
A} ACE2 inhibitors				
Captopril	-5.5	-26.074	Val-209, Leu-95, Pro-565, Lys-562, Glu-208, Asp-206	Leu-392, Ala-99, Tyr-207, Trp-566, Ala-396, Glu-564
Perindopril	-6.7	-93.677	Asp-382, Ala-348,Asp-350,Trp-349	His-401, His-378, Leu-351, Ser-44,Ser-47,Ser-43,Phe-40,Thr-34, Asn-394Arg-514,Tyr-510
Enalaprilat	-7.2	-48.052	Tyr-510, Asn-394, His-401, Ala-348	His-505,Phe-504, His-349, Tyr-515, Arg-514,Asp-350, Asp-382, Tyr-389,Trp-349,Glu-402
Trandolapril	-7.2	-93.677	Ala-562, Lys-99	Leu-392, Leu-391, Gln-102, Tyr-196, Gly-205, Asp-206, Tyr-202, Asn-394
Libenzapril	-7.2	-130.334	Gly-205, Gly-211, Glu-208, Val-205, Pro-565, Asp-206, Lys-562, Leu-95	Gln-98, Asn-210, Val-212,Ala-396, Asn-39,Trp-566, Glu-564, Leu-392
Nicotianamine	-6.3	-26.014	Glu-346, Pro-37, Ala-348, Asp-382, Arg-514, Tyr-515	His-374, Trp-349, His-378,Thr-34, His-345, His-505, Glu-402,Asn-394, Tyr-385, His-401, Asp-350
Perindoprilat	-7.6	-26.074	Asp-382, Ala-348, Arg-514, Tyr-510	His-378, Glu-402, Trp-349, Glu-379, Thr-34, Phe-504, His-345,Pro-346, Tyr-515, Phe-40, Asp-350, His-401, Ile-379
MLN-4760	-7.9	-36.191	Phe-274, Asn-149, His-345, Glu-145	Thr-445, Thr-276, Phe-504, Pro-346, Tyr-12, Trp-271, His-505, Lys-363, Cys-344, Arg-273, Met-360, Asp-368, Thr-371, Asp-367, Arg-518
Zabicipril	-7.4	-89.270	Glu-208, Lys-562, Leu-392, Ala-99	Tyr-202, Tyr-196, Gly-205, Asp-206, Gln-98, Gln-96, Leu-391, Leu-95, Asn-210, Val-209, Pro-565, Trp-566, Ala-396
Enalaprilic acid	-7.7	-48.052	Pro-346, Asp-368, Thr-371, Lys-363, Glu-145, Trp-271	Cys-361, Asp-269, Phe-274, Arg-273, His-345, Leu-144, Asn-149, Cys-344, Asp-36
B) Furin inhibitors				
Andro-grapholide	-7.6	-31.298	Ser-311, Trp-531, Ala-532, Asn-310	Asn-529, Gly-307, Pro-266,Ile-312, Gln-488, Glu-271, Arg-490
Chrysin	-7.6	-26.474	Ala-532, Asn-310, Gln-488, Trp-531	Gly-307, Met-534, Ser-311, Ile-312, Tyr-313, Glu-271, Pro-266, Gly-269
4-Hydroxy-coumarin	-6.8	-24.033	Glu-271, Gln-488, Asn-310, Pro-266, Ala-532, Trp-531	Tyr-313, Ser-311, Ile-312, Met-534, Gly-265
Scopoletin	-6.6	-22.289	Gln-488, Asn-310, Pro-266, Ala-532, Trp-531, Ser-311	Glu-271, Tyr-313, Ile-312, Met-534, Gly-307, Gly-265
Barlerin	-7.3	-24.934	Tyr-571, Arg-490, Ala-532, Trp-531	Ser-311, Glu-271, Lys-449, Tyr-313, Gln-488, Ile-312, Asn-529, Asn-310, Gly-307, Pro-266, Gly-265
Baicalein	-7.7	-20.813	Arg-490, Gln-488, Gly-307, Asn-310, Trp-531, Ala-532	Asp-264, Gly-265, Pro-266, Ser-311, Ile-312
Luteolin	-7.8	-22.632	Asp-264, Asn-310, Ala-532, Glu-271	Asp-530, Arg-490, Asn-529, Trp-531, Pro-26, Gly-307, Ser-311, Ile-312, Gln-488
Oroxylin A	-7.8	-23.350	Gln-488, Arg-490, Ala-532, Trp-531, Ser-311	Met-534, Asn-310, Gly-307, Pro-266, Gly-265, Asp-264, Glu-271, Ile-312

Regarding furin inhibitors, 4-hydroxycoumarin exhibited the strongest binding with furin active site residues, with a binding score of -6.8 kcal mol^{-1} (Fig. 1C, D). Scopoletin and barlerin showed slightly lower binding scores compared to 4-hydroxycoumarin. Notably, all three ligands displayed strong hydrogen bonding with the conserved Trp-531 residue. For example, 4-hydroxycoumarin interacted with amino acid residues Glu-271, Gln-488, Asn-310, Pro-266, Ala-532, and Trp-531 in the binding site of furin. Furin inhibitors are being explored as potential antiviral agents for SARS-CoV-2 due to the important role furin plays in the maturation and activation of the spike protein. By blocking the furin-mediated cleavage, these inhibitors aim to hinder viral entry, replication, and the subsequent spread of the virus within the host (Walls *et al*., 2020). Scopoletin formed hydrogen bonds with Gln-488, Asn-310, Pro-266, Ala-532, Trp-531, and Ser-311, exhibited a binding score of -6.6 kcal mol^{-1}. The study provided detailed insights into the receptor-ligand interactions through 2D representation and 3D images of docked protein-ligand complexes using Discovery Studio Visualizer. These visualizations highlighted the hydrogen bonding, Van der Waals forces, and alkyl interactions involved in the binding of potential inhibitors within the active sites of ACE2 and furin proteins.

ADMET results

Drug likeness of almost all the inhibitors for ACE2 and furin proteins obeyed Lipinski's rule of their molecular mass less than 500 Da, high lipophilicity expressed as LogP to be less than 5, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and molar refractivity between 40-130. The ADMETsar analyses the compounds based on the five parameters *viz.*, adsorption, distribution, metabolism, excretion and toxicity. The evaluation based on a number of thresholds showed that the potential inhibitors captopril and 4-hydroxycoumarin passed ADMETsar threshold ability to be effective inhibitors of proteins as a drug (Table 2). The number of coronavirus infection cases established

Table 2: ADMET profiling enlisting adsorption, distribution, metabolism, excretion and toxicity related drug like parameters of ACE2 inhibitors and furin inhibitors

S. No.	Inhibitors	Adsorption		Distribution		Metabolism		Excretion	Toxicity		
		HIA	Caco-2	BBB	GP S/I	CP 450S	P450I	ROCTI	MES	Carcinogen	Acute
A.	ACE2 inhibitors										
1.	Captopril	+	+	+	-/-	-	-	-	-	-	III
2.	Perindopril	+	-	-	+/-	-	-	-	-	-	III
3.	Enalaprilat	+	-	-	+/-	-	-	-	-	-	III
4.	Trandolapril	+	-	-	+/+	-	-	-	-	-	III
5.	Libenzapril	-	-	+	+/-	-	-	-	-	-	III
6.	Nicotianamine	-	-	-	+/-	-	-	-	-	-	III
7.	Perindoprilat	+	-	-	+/-	-	-	-	-	-	III
8.	MLN-4760	+	-	-	+/-	-	-	-	-	-	III
9.	Zabicipril	-	-	-	+/-	-	-	-	-	-	III
10.	Enalaprilic acid	+	-	-	+/-	-	-	-	-	-	III
B.	Furin inhibitors										
1.	Andrographolide	+	-	+	+/-	-	-	-	-	-	III
2.	Chrysin	+	+	+	-/-	-	+	-	-	-	III
3.	4-hydroxycoumarin	+	+	+	-/-	-	-	-	-	-	III
4.	Scopoletin	+	+	+	+/-	-	-	-	-	-	III
5.	Barlerin	-	-	-	+/-	-	-	-	-	-	III
6.	Baicalein	+	-	-	+/-	-	-	-	-	-	II
7.	Luteolin	+	-	-	+/-	-	-	-	-	-	II
8.	Oroxylin A	+	+	-	+/-	-	-	-	-	-	III

*HIA: Human intestinal absorption; Caco-2: Caco-2 Permeability; BBB: Blood-brain barrier; GP S/I: P-glycoprotein substrate/ inhibitor; P450S: CYP450 2C9 substrate; P450I: CYP450 2C9 inhibitor; ROCTI: Renal organic cation transporter inhibitor. + indicates positive; - indicates negative; I- highly toxic; II- moderately toxic; III- slightly toxic

to rise significantly, represent the prodigious acute unmet medical need. In current surge of attempts to discover anti-coronavirus drugs, no FDA approved therapeutic or preventive regime to cure coronavirus infection has yet reached to the market. Through the Solidarity Clinical Trial, WHO is leveraging its ability to drive global collaboration in research to test a range of possible treatment options, ensure access of patients to safe and effective medicines and, ultimately, save the lives (Mortaz *et al*., 2022). Based on the evidences from laboratory animal and clinical studies, WHO's Solidarity clinical trial for COVID-19 includes the treatment options: Remdesivir; Lopinavir/Ritonavir; Lopinavir/ Ritonavir with interferon beta-1a; and chloroquine or hydroxychloroquine (Tabassum *et al*., 2022). There are currently 10 registered trials globally to study their efficacy against COVID-19 (Lythgoe *et al*., 2020).

In present study, the most potential inhibitors of ACE2 proteins with desired ADME-Tox characteristics were virtually screened by exploiting the literature of ACE2 inhibitors. Three potent ACE2 inhibitors i.e. captopril, nicotianamine and perindopril were screened based on their binding affinity and docking score. ACE2 receptors mediate the entry into the cell of three strains of coronavirus: SARS-CoV, NL63 and SARS-CoV-2 (Verdecchia *et al*., 2020). An important salutary function of membrane-bound and soluble ACE2 is the degradation of angiotensin II to angiotensin (Hoffmann *et al*., 2020). Consequently, ACE2 receptors minimize several detrimental effects due to the binding of angiotensin II to AT1 receptors, which include vasoconstriction, enhanced inflammation and thrombosis (Li *et al*., 2003). Huang *et al*. (2003) reported novel peptide inhibitors of angiotensin-converting enzyme 2. The ACE-inhibitors, angiotensin receptor blockers and severe acute respiratory syndrome caused by coronavirus have recently been studied (Verdecchia *et al*., 2020).

The predicted potential furin inhibitors in present study including 4-hydroxycoumarin, scopoletin and barlerin were found to form strong H-bonds to furin proteins with higher binding scores that serve as a crucial step in preventing coronavirus entry and replication, thereby inhibit DV infectivity. This validates that interactions predicted by docking were stable and strong. Hardes *et al*. (2015) proposed novel furin inhibitors with potent anti-infectious activity and confirmed that the inhibition of furin is a promising strategy for a short-term treatment of acute infectious diseases. The SARS-CoV-2 S glycoprotein reportedly harbour a furin cleavage site at the boundary between S1/S2 subunits, which is processed during biogenesis and sets this virus apart from SARS-CoV and SARS-related CoVs.

Though ACE inhibitors primarily target the renin-angiotensin-aldosterone system (RAAS) to regulate blood pressure, there has been interest in investigating their potential effects on SARS-CoV-2 infection. The most potential ACE inhibitor screened in present study, captopril apart for the treatment of arterial hypertension and cardiovascular diseases (Peng *et al*., 2005), has been proved to express immune regulating, antioxidant as well as anti-inflammatory properties (Albuquerque *et al*., 2010). Similarly, 4-hydroxy-coumarin, predicted as the most potential furin inhibitor in current study, reportedly display important pharmacological effects, including analgesic, anti-arthritis, anti-inflammatory, anti-pyretic, anti-bacterial, anti-viral, and anti-cancer properties (Luchini *et al.*, 2008). 4-hydroxycoumarin and its derivatives have effectively been used as anticoagulants for treating disorders causing excessive or undesirable clotting, such as thrombophlebitis, pulmonary embolism, and certain cardiac conditions (Shapiro and Sherwin, 1943). While our findings hold promise, it is important to note that further research including *in vitro* and *in vivo* studies, as well as clinical trials, is necessary to fully evaluate the efficacy, safety, and potential of ACE2 and furin inhibitors as therapeutic agents against SARS-CoV2. Besides, the development of specific inhibitors with optimal pharmacokinetic and pharmacodynamic properties would be crucial for effective intervention.

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