ORIGINAL ARTICLE

In Silico Identification of Potential Losartan Analogs as Inhibitors of ACE 2 through Virtual Screening and Toxicity Studies for the Treatment of Cardiovascular Diseases

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ABSTRACT

The angiotensin-converting enzyme 2 (ACE2) is known to be an important drug target for cardiovascular disease (CVD) control. In an attempt to identify ACE2 inhibitors, recently solved high-resolution crystal structures of the apo and inhibitor-bound forms of ACE2 have provided the basis for a docking strategy. *In silico* ADME/Tox prediction of Losartan analogs satisfied the Lipinski Rule of Five, Ghose, Veber, Egan, and other ADME/Tox parameters. Screened Losartan analogs were docked with binding pocket of ACE2 using molecular docking program, which resulted low binding affinity. The findings of this work affirm the importance of these compounds as potential candidate drugs for CVD and hypertension therapy. In addition, the work also facilitated the study of *in vivo* and *in vitro* evaluation for the proposed compounds intended to confirm the computational findings.

Key words: ADME/Tox, Molecular docking, angiotensin-converting enzyme 2, Losartan, Renin-angiotensin system

INTRODUCTION

Heart attacks and strokes are usually acute events that are often caused by a blockage that prevents blood from being given to the heart or brain. The cause of heart attacks and strokes is typically a combination of risk factors, such as tobacco usage, unhealthy diet and obesity, physical inactivity and harmful use of alcohol, hypertension, diabetes, and hyperlipidemia. In India and internationally, cardiovascular disease (CVD) and stroke create enormous health and economic burdens. In Asia, which has more than 60 percent of the world's population and many of its countries are developing economies, strokes are a particularly serious issue. In Asia, mortality from strokes is higher than in Western Europe, the Americas or Australasia, with the exception of such countries such as Japan.[1] High blood pressure and hypertension are the most important risk factors for CVD, which is the leading cause of mortality.[2,3] The 2013 European Society of Hypertension/European Society of Cardiology Guidelines on Hypertension have recently set a universal target of <140/90 mm Hg for all patients, with the exception of the elderly population category (target, <150/90 mm Hg for those aged 80 years or older).[4] The pharmacological control of the renin-angiotensin system is the widely recognized first-line approach to the treatment of hypertension and CVD (RAS).[5] The renin-angiotensin system (RAS) is a signaling pathway that acts as a homeostatic vascular function regulator.[6] Several studies have investigated the effect of RAS on the development of CVD and chronic kidney disease. However, by regulating regional blood flow and monitoring trophic responses to a variety of stimuli, the RAS also plays a significant local role. A single catalytic metallopeptidase unit contains an angiotensin-converting enzyme 2 (ACE2) enzyme extracellular domain that catalyzes the synthesis of angiotensin 1–7 vasodilator peptides and is therefore responsible for counterbalancing the powerful vasoconstrictor effects of angiotensin II.[7,8] This counterbalancing property of ACE2 is proposed to be significant in the development of novel pharmacotherapy against hypertension and related CVD.[9,10]

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MATERIALS AND METHODS

Virtual Screening

Ligand-based virtual screening of Losartan against GPCR Ligands (ChEMBL) using Swiss similarity web tool.[11]

ADME/Tox Screening

In silico physicochemical, pharmacokinetic, and toxicological properties of Losartan analogs were performed by Swiss ADME[12] web tool.

Preparation of ACE2 Structure

Inhibitor Bound Human Angiotensin-Converting Enzyme-Related Carboxypeptidase (ACE2) (PDB ID: 1R4L) was retrieved from the Protein Data Bank (https://www.rcsb.org/). To make the ACE2 structure ready for molecular docking processes, all the ligands were removed and polar hydrogen added.

Molecular Docking

Screened Losartan analogs were docked with ACE2 using MTiAutoDock (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::MTiAutoDock). The most negative binding affinity score of compound chosen as candidate compound will be analyzed furthermore.

RESULTS AND DISCUSSION

In Silico ADME/Tox Screening of Losartan Analogs

About half of the drug candidates struggled during development due to ADME/Tox deficiencies.

A series of *in silico* ADME/Tox screens were introduced with the goal of discarding compounds during the discovery process to avoid this production failure. The ADME predictions for the passive human gastrointestinal (GI) absorption and blood-brain barrier (BBB) permeation of Candesartan analogs are both based on the BOILED-Egg model, as shown in Table 1. Human intestinal absorption properties are a determinant of the development of drugs and must be administered orally. The BBB plays a significant role in drug pharmacology.[13] Carcinogenicity is a cancer-causing risk in the body. Losartan analogs were accessed by five different rule-based filters such as Lipinski[14] filter implemented rule-of-five, Ghose *et al*.,[15]

Table 1: The prediction of pharmacokinetics and drug likeliness of Losartan analogs and binding energy with ACE2

Sl. No.	GPCRLigands compound Id	GI	BBB	LogKp (Cm/s)	Lipinski	Ghose	Veber	Egan	Muegge	Binding Energy (kcal/mol)
1.	CHEMBL82814	High	No	–6.14	Yes	Yes	Yes	Yes	Yes	–8.21
2.	CHEMBL86292	High	No	–5.92	Yes	Yes	Yes	Yes	Yes	–6.41
3.	CHEMBL87755	High	No	–6.26	Yes	Yes	Yes	Yes	Yes	–8.90
4.	CHEMBL419672	High	No	–5.98	Yes	Yes	Yes	Yes	Yes	–5.53
5.	CHEMBL87343	High	No	–5.49	Yes	Yes	Yes	Yes	Yes	–8.84
6.	CHEMBL323179	High	No	–5.89	Yes	Yes	Yes	Yes	Yes	–8.83
7.	CHEMBL86207	High	No	–5.80	Yes	Yes	Yes	Yes	Yes	–8.96
8.	CHEMBL85692	High	No	–6.01	Yes	Yes	Yes	Yes	Yes	–8.63
9.	CHEMBL82445	High	No	–5.04	Yes	No	Yes	Yes	No	–9.46
10.	CHEMBL313130	High	No	–5.91	Yes	Yes	Yes	Yes	Yes	–7.09
11.	CHEMBL1204443	High	No	–5.94	Yes	Yes	Yes	Yes	Yes	**–9.85**
12.	CHEMBL313715	High	No	–6.53	Yes	No	Yes	Yes	Yes	–8.74
13.	CHEMBL164536	High	No	–5.72	Yes	Yes	Yes	Yes	Yes	–8.83
14.	CHEMBL420778	High	No	–5.72	Yes	Yes	Yes	Yes	Yes	–7.68
15.	CHEMBL312950	High	No	–5.36	Yes	Yes	Yes	Yes	No	–9.09
16.	CHEMBL41421	High	No	–6.48	Yes	Yes	Yes	Yes	Yes	–8.05
17.	CHEMBL163760	Low	No	–6.41	Yes	Yes	Yes	No	Yes	–8.11
18.	CHEMBL25726	High	Yes	–6.30	Yes	Yes	Yes	Yes	Yes	**–9.71**
19.	CHEMBL24849	High	Yes	–6.11	Yes	Yes	Yes	Yes	Yes	–6.79
20.	CHEMBL299105	High	No	–6.41	Yes	No	No	Yes	Yes	–7.52
21.	CHEMBL85028	High	No	–5.19	Yes	No	No	Yes	No	–7.33
22.	CHEMBL278614	High	Yes	–5.76	Yes	Yes	Yes	Yes	Yes	–6.54
23.	CHEMBL322910	Low	No	–5.71	Yes	No	Yes	Yes	No	–8.39
24.	CHEMBL417009	High	No	–5.46	Yes	Yes	No	Yes	No	–7.42
25.	CHEMBL1205016	High	No	–6.42	Yes	Yes	Yes	Yes	Yes	–8.30
26.	CHEMBL105724	High	No	–5.89	Yes	Yes	Yes	Yes	Yes	–8.68
27.	CHEMBL84111	High	No	–5.79	Yes	Yes	Yes	Yes	Yes	–6.53
28.	CHEMBL308493	High	No	–5.31	Yes	Yes	Yes	Yes	No	–7.53
29.	CHEMBL86067	Low	No	–5.69	Yes	No	No	No	No	–8.18
30.	CHEMBL280868	High	No	–6.31	Yes	No	Yes	Yes	Yes	–8.27
31.	CHEMBL48955	High	No	–5.94	Yes	Yes	Yes	Yes	Yes	–6.75
32.	CHEMBL328552	High	No	–5.76	Yes	Yes	Yes	Yes	Yes	–7.56
33.	CHEMBL313534	High	No	–6.08	Yes	Yes	Yes	Yes	Yes	–6.92
34.	CHEMBL89403	High	No	–6.36	Yes	Yes	Yes	Yes	Yes	–8.39
35.	CHEMBL300526	High	No	–5.34	Yes	No	Yes	Yes	No	–6.51
36.	CHEMBL314149	High	No	–6.06	Yes	Yes	Yes	Yes	Yes	–7.86

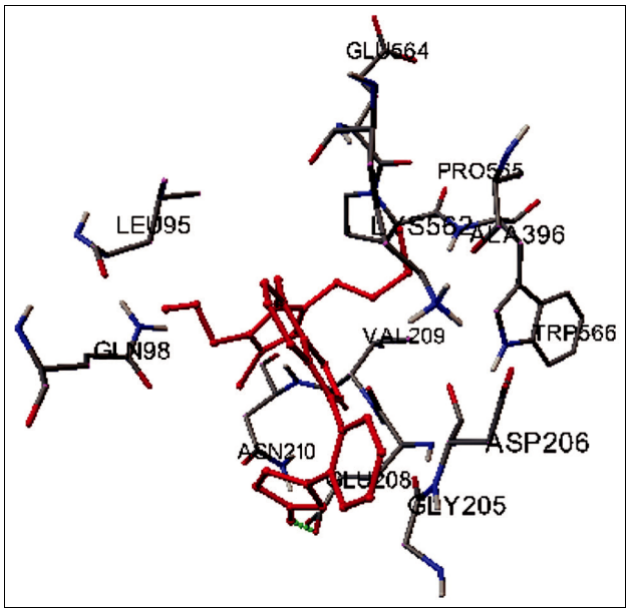

Figure 1: Docking pose of Losartan analog CHEMBL1204443 in binding site of angiotensin-converting enzyme 2. One H-bond was formed between amino acid GLU208 of protein with compound, respectively. Hydrogen bonds are represented with spherical line

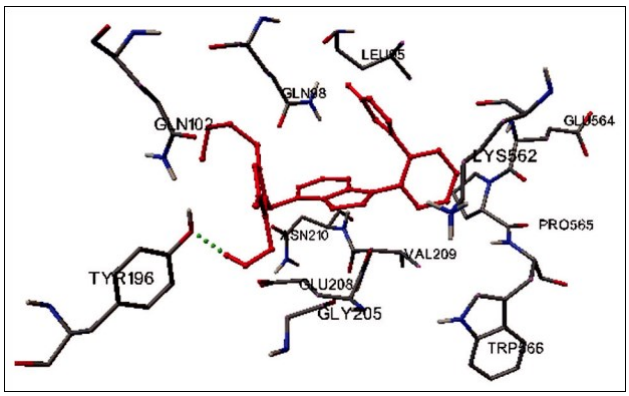

Figure 2: Docking pose of Losartan analog CHEMBL35726 in binding site of angiotensin-converting enzyme 2. One H-bond was formed between amino acid TYR196 of protein with compound, respectively. Hydrogen bonds are represented with spherical line

Veber *et al.*,[16] Egan *et al.*,[17] and Muegge *et al.*[18] methods, respectively, and shown in Table 1. The result presented in Table 1 shows that all of the investigated compounds present high GI absorption and good skin permeation. At the end of the entire ADMET evaluation screening, five compounds, CHEMBL69221, CHEMBL302815, CHEMBL80220, CHEMBL305936, and CHEMBL311188, failed the drug likeliness test.

Analysis of Molecular Docking

Forty-one ADME/Tox parameters qualify Losartan were docked in binding site of ACE2 enzyme using MTiAutoDock server. Binding energy of Losartan analogs is shown in Table 1. On analysis of binding energy of analogs, it was found that two analogs, CHEMBL1204443 and CHEMBL35726, had low binding affinity –10.81 kcal/mol, –9.45 kcal/mol, and –9.34 kcal/mol with ACE2, respectively. Predicted docked complexes were analyzed through Python Molecular Viewer[19] for their interaction study, as shown in Figures 1 and 2. Losartan analogs were represented in sticks and balls model. Losartan analog CHEMBL1204443 interacted with residues GLU564, PRO565, LEU95, LYS562, ALA396, VAL209, GLN98, ASN210, GLU208, GLY205, ASP206, and TRP566. Losartan analog CHEMBL35726 interacted with residues GLU564, PRO565, LYS562, VAL209, TRP566, GLN98, VAL209, ASN210, GLU208, GLY205, TYR196, LEU95, and GLN102. Interacting residues were represented in lines in Figures 1 and 2.

CONCLUSION

For the treatment of CVD, current research uses computer-based virtual screening to identify ACE2 inhibitors. Two Losartan analogs, CHEMBL1204443 and CHEMBL35726, were identified as potential inhibitors for further experimental research, from several chemical structures and a sequence of logical refining steps, including similarity search, ADME properties, and molecular docking.

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