

Design of a new inhibitory tripeptide to affect the ACE enzyme in Covid-19 patients by bioinformatics software

Mohammad Darvish Khadem¹, Saeed Pirmoradi¹, *Ali Shahriari¹

1- Department of Basic Sciences, Division of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz 61357-831351, Iran

a.shahriari@scu.ac.ir

Abstract

Background: Many natural peptide compounds have inhibitory effects on the angiotensin converting enzyme (ACE), effective in the treatment of cardiovascular disease and reducing mortality in patients with Covid-19.

Aim of the study: This study aims to use one of these peptides as a template to achieve a new enzyme inhibitor with the help of bioinformatics servers.

Materials and Methods: A new inhibitory compound for the ACE enzyme was introduced by modeling a series of peptide compounds with the help of the ZINC15 server. Then, toxicity prediction of the compound was investigated by SWISS-ADME. Molecular docking with ACE were evaluated using MVD and Auto Dock-Vina and their interactions were mapped using Discovery Studio and Ligplot.

Results: This introduced compound has proper binding energy and due to appropriate interactions with amino acids of the enzyme active site, non-toxicity in the nervous system, cardiovascular, liver, and skin sensitivity and following Lipinski's rules probably acts as an Effective inhibitory enzyme ligand.

Conclusions: This new compound could be used as a new and safer option with low side effects and appropriate efficacy for controlling blood pressure in the treatment of patients with cardiovascular diseases, as well as decreasing mortality in patients with Covid-19 with hypertension.

Keywords: Angiotensin-converting enzyme, Cardiovascular diseases, Covid-19, Lipinski's rules, Molecular docking, Natural tripeptides.

طراحی لیگاند مهاري جديد بر پايه برخي تريپتيدهاي طبيعي جهت مهار آنزيم تبديل کننده آنزوتانسین در بیماران مبتلا به کووید ۱۹ توسط نرم افزارهای بیوانفورماتیک

محمد درویش خادم^۱، سعید پیرمرادی^۱ علی شهریاری^{۱*}

^۱ گروه علوم پایه، بخش بیوشیمی و بیولوژی مولکولی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران.

a.shahriari@scu.ac.ir

چکیده

زمینه و هدف: ترکیبات پپتیدی با منشأ طبیعی وجود دارند که اثراتی در مهار آنزیم مبدل آنژیوتانسین (ACE) نشان می‌دهند که می‌توانند در درمان بیماری‌های قلبی-عروقی و کاهش مرگ‌ومیر در بیماران مبتلا به کووید ۱۹ مؤثر باشند.

هدف این مطالعه: الگوبرداری از یکی از این پپتیدها با کمک نرم‌افزارهای بیوانفورماتیک جهت دستیابی به یک ترکیب جدید مهاري برای آنزیم ACE می‌باشد.

مواد و روش‌ها: با الگوبرداری از یک سری ترکیب پپتیدی و به کمک سرور ZINC15 یک ترکیب جدید مهاري برای آنزیم ACE معرفی شد. سپس پیش‌بینی سمیت ترکیب موردنظر توسط Swiss-ADME و Pkcsim انجام شد. داکینگ مولکولی آنزیم ACE توسط نرم‌افزارهای Autodock و MVD انجام شد و برهمکنش‌های لیگاند و گیرنده توسط Discovery Studio و Ligplot رسم گردید.

یافته‌ها: ترکیب جدید مهاري، با انرژی اتصال مناسب و ایجاد برهمکنش‌های هیدروژنی و هیدروفوبی مطلوب با جایگاه فعال آنزیم موردنظر و همچنین فقدان سمیت کبدی، عصبی، قلبی-عروقی و حساسیت پوستی و با پیروی از قانون لیپینسکی احتمالاً به‌عنوان یک مهارکننده مؤثر ACE می‌باشد.

نتیجه‌گیری: ترکیب سنتزی جدید احتمالاً با عوارض جانبی کم و مناسب به‌عنوان گزینه جدید و ایمن‌تر جهت کنترل فشار خون در درمان بیماران قلبی-عروقی و کاهش مرگ‌ومیر بیماران کووید ۱۹ دارای فشار خون بالا استفاده شود.

کلید واژگان: آنزیم تبديل کننده آنزوتانسین، بیماری های قلبی عروقی، کووید ۱۹، قوانین لیپینسکی، داکینگ مولکولی، تريپتيدهاي طبيعي.

Introduction

Biologically active peptides are compounds with different arrangements of natural amino acids that have different biological functions with multifunctional properties including antioxidant activities, immune activators, hormone modulators, antibacterials, anti-thrombosis, anti-viral and anti-hypertensive(1). In addition, these peptides are potential candidates for the production of functional peptide drugs and functional food additives due to their high food safety and bioavailability (2). The World Health Organization estimates that cardiovascular disease kills more than 17.5 million people a year. One of the important characteristics of cardiovascular diseases is high blood pressure, the control of which is the main goal for the prevention and treatment of cardiovascular diseases (3). New findings show that a significant number of patients with severe infection or death from Covid-19 have high blood pressure or cardiovascular diseases, which is often treated with ACE inhibitors(4-5). Antihypertensive drugs such as captopril and enalapril are restricted due to side effects such as cough, skin rash, and headache(6). Therefore, due to the importance of cardiovascular problems, some new and safer options such as active food-derived peptides are needed. Recently, significant progress has been made in identifying and screening food components that have a positive effect on cardiovascular health(7), and angiotensin-converting enzyme (ACE) inhibitory peptides derived from a variety of dietary sources, including animal and plant proteins such as soy, wheat, and corn (8), have been shown to have high antihypertensive activity (9). Previously, the hypotensive activity of peptides was determined in vitro by measuring the inhibitory activity of ACE, a dipeptidyl carboxypeptidase located on cell membranes that can disrupt the balance between the two hormonal systems regulating blood pressure and fluid balance and make a lot of damage (10-8). ACE is a glycoprotein responsible for high blood pressure, which is a zinc-dependent metalloprotease enzyme (zinc). Angiotensin I converting enzyme plays an important role in regulating blood pressure by catalyzing the conversion of inactivated angiotensin I to angiotensin II, a potential vasoconstrictor, and inactivating bradykinin, a potential vasodilator. Therefore, the best mechanism for decreasing blood pressure is the effect of ACE inhibitors (11). In addition, food-induced antihypertensive peptides do not cause any toxicity or side effects in patients with hypertension (12). Isolation and purification of antihypertensive peptides from dietary proteins provides a new pathway for the production of natural antihypertensive drugs, based on which new drugs can be designed. According to statistics from the Biopep site, 359 peptides with ACE inhibitory activity have been identified so far. On the other hand, the type of amino acids at the C and N terminals have a very important effect on ACE inhibitors. Hydrophobic amino acids, especially those with aliphatic side chains such as Lys and Val, are characteristic of the N-peptide terminal, and cyclic or aromatic amino acids such as Tyr, Pro, and Trp at the C-terminal are termed ACE inhibitors. Many dietary protein-derived peptides contain proline at their C-terminals. This rule applies mainly to short peptides. The presence of proline at the C-terminus of the peptide, together with the presence of aliphatic amino acids with branched side chains at the N-terminus, affects ACE inhibitory activity. Observations on the effect of physicochemical properties of C and N-terminal amino acids on peptide bioactivity were consistent with previous research (13-14) and studies have reported that highly active peptides should consist of a large hydrophobic aromatic amino acid with a polar functional group at the C terminal, which led to the identification of some structural features for ACE inhibitory tripeptides (14). In the case of tripeptides, the first residue is usually aromatic, the second residue is a positively charged amino acid, and the third amino acid (C terminal) is hydrophobic (13). The present study aims to design a new inhibitor for ACE enzyme based on isoleucine-glutamine-proline tripeptide and their physicochemical and pharmacological properties are investigated using bioinformatics software.

Material and method

Preparation of ACE enzyme files and ligand design

First, the PDB structure (three-dimensional structure) of ACE enzyme combination with access code (1O86) was obtained from the RCSB database. Then, a tripeptide consisting of amino acids with aromatic and positively charged and hydrophobic groups, designed with Pymol software, was inserted into the ZINC15 database and used as a leader template to design new inhibitory ligands to inhibit the desired ACE enzyme (Fig. 1).

ADME prediction (absorption, distribution, metabolism and excretion)

Pharmacodynamic analysis of a proposed molecule that can be used as a drug is important. SWISS-ADME is a website (<https://www.swissadme.ch>) that allows the user to draw the relevant drug ligand or molecule, or by including SMILES data, parameters such as lipophilicity (iLOGP, XLOGP3, SLOGP, MLOGP, SL, LogP0 / w), water solubility LogS (ESOL, Ali, SILICOS-IT), drug similarity rules (Lipinski, Ghose Veber, Egan and Muegge) and medicinal chemistry (PAINS, Brenk, Leadlikeness, Synthetic accessibility) Put analysis (16). SMILES ligand data prepared are available via PubChem (17-18).

Toxicity prediction

Toxicological prediction of small molecules due to prediction the tolerance of those molecules before consumption in human models is essential. pkCSM is an online database in which the information of the studied compound is analyzed by plotting the composition of the compound or by entering the SMILES formula into it. This website can provide details of toxicological effects in the areas of AMES toxicity, maximum human tolerance dose, hERG-I inhibitor, hERG-II inhibitor, LD50, LOAEL, hepatotoxicity, skin toxicity, T. pyriformis toxicity and Minnow toxicity. In this study, SMILES related to inhibitory ligand were analyzed using a website (19).

Docking process

The PBD file of the ACE inhibitor protein ligands was designed and the ACE receptor entered the MOLEGRO VIRTUAL DOCKER (MVD) software as well as the CB-DOCK server. Before the docking process, the necessary preparations were made to correct the protein errors. For this purpose, first, in the molecular preparation stage, a state is selected in which the bonds, the orientation of the hydrogen bonds, the charges as well as the flexible bonds are identified in the ligand, and then the errors of its residues are identified, eliminated and optimized. Next, we need to find the holes in the protein structure and determine which parts of the protein are most likely to interact between the ligand and the protein. After performing these preparations and selecting the ligands, the SCORING mode is set to MolDock scoring (GRID) mode and the connection location is selected based on the holes. Then, select the algorithm based on Mol Dock SE, by choosing the number of runs, the docking process begins. From the designed ligands, the ligand with the highest negative energy in both docking software was selected and used to investigate the interaction with the ACE receptor. In addition, the amino acids involved in this interaction were identified and images of these interactions were obtained using Discovery Studio software.

Result

Results of ACE enzyme files and ligand design

At this stage, based on a tripeptide designed for the ACE protein that was selected as the template (Fig. 1), a virtual search was performed in the ZINC15 database to find new ligands. Then, the SDF or MOL2 file of the obtained inhibitory ligands was prepared based on this pattern (Fig. 1) from this database (15).

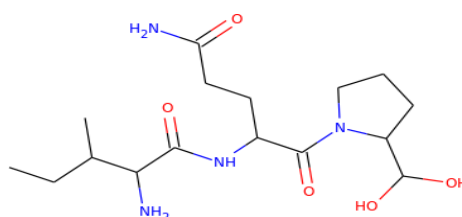


Fig. 1. Inhibitory ligand selected from several ligands based on the tripeptide proteins.

Results of drug similarity prediction

At this stage, with the help of Molsoft server and from the Molecular Properties and Drug-likeness section, the pharmacological similarity of the inhibitory ligand compound was investigated, and it was found that this compound is in the range of compounds with medicinal properties (Fig. 2).

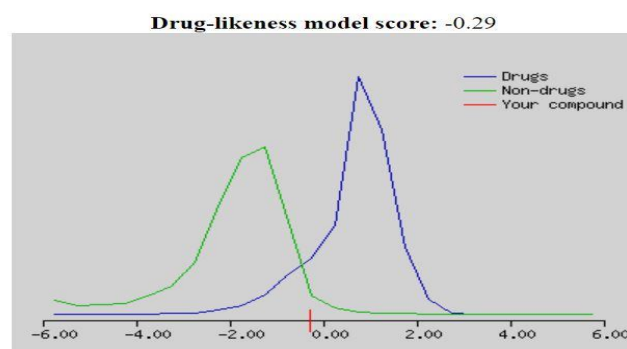


Fig. 2. Diagram related to the drug similarity of the designed ligand (the red line indicates that the ligand is in the range of drug compounds)

Toxicity prediction results

The results of the predicted toxicity in the PKCSM server showed that the examined ligand was free of AMES toxicity; Because the maximum tolerated dose of this compound in humans is about (075 mg/kg/day), it also does not inhibit hERG-I and hERG-II. Although some drugs are usually found to have some hepatotoxicity, bioinformatics studies have shown that the compound is non-hepatotoxic and does not cause skin allergies. During the study process, the molecular and genetic toxicity of the inhibitory ligand was determined using Lazar Toxicity Prediction software. The results of the prediction showed that the ligand composition lacked mutagenic and carcinogenic properties. Evaluation of hepatic, renal, neurological and cardiovascular toxicity was performed through way2drug server that the inhibitory ligand composition was free of cardiovascular toxicity and neurotoxicity. The combination of the inhibitory ligand with 9 rotatable bonds and 6 acceptor groups and 5 hydrogen donor groups has favorable conditions based on Lipinski multiple criteria for drugs and follows Lipinski's rules.

ADME Prediction Results (Absorption, Distribution, Metabolism and Excretion)

After checking the prediction of inhibitory ligand composition by ADME using PkcsM and SWISSADME databases, the following results were obtained (Table 1). The physiological characteristics of this compound are 25 heavy atoms, 6 hydrogen bond recipients, 5 hydrogen bond donors. The values related to the lipophilicity of the molecule are equal to (-0.86) P0/W. Its calculated solubility in water was 2.095, based on which is categorized in highly soluble compounds. Predicted pharmacokinetic data indicate gastrointestinal (GI) absorption. Studies have also demonstrated that this inhibitory compound is not inhibited by cytochromes CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. This combination fully follows Lipinski's rules and also has an achievable score of 0.55 based on drug rating rules such as Ghose, Veber, Egan, Muegge. The compound in question has a molecular weight of 352/228 (less than 500) (Table 1) and has the total polar surface area (TPSA)

Docking properties	binding energy (MVD)	binding energy (Auto dock vina)	Molecular weight	Log P	Log S	hydrogen bond donor	hydrogen bond acceptor	Rotatable bond
Ligand	-88.883	-7	358.22	-4.06	-0.64	5	6	9

of 3.89.

Receptor stability

The stability of ACE enzyme was evaluated by IUPred2 server and the diagram related to this evaluation showed that the protein has good stability, especially in the regions of inhibitory ligand binding (Fig. 3).

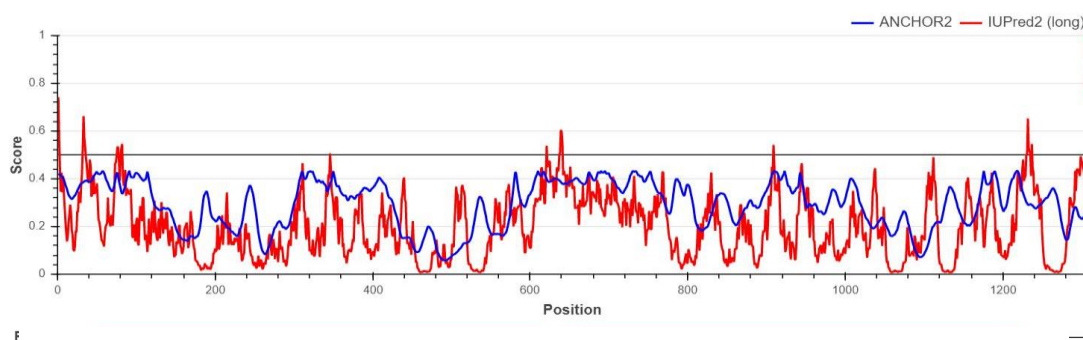


Fig. 3. Receptor stability evaluation by IUPred2 software

Docking results

To perform the docking process, the main ligand of the ACE protein was removed. Then, the ligand designed with the protein was docked with MVD software, and data on binding energy changes, ligand interactions including hydrogen bonds, and ester interactions obtained by MVD and Discovery Studio software were displayed. The docking process was also performed with

the CB-DOCK server, which is based on Autodock Vienna software. The results of these studies indicated that the studied derivatives can inhibit or change the function of the enzyme by binding to a part of the ACE protein. The binding energy results of the inhibitory ligand with ACE of host cells by (-88-883 KJ/mol) MVD and (-7 KJ/mol) CB-DOCK indicate high binding power between inhibitory ligand obtained with ACE protein (Table 2). The results of molecular docking also displayed that the non-polar parts of the designed inhibitory ligand composition mainly had hydrogen interaction with parts of the ACE protein containing the amino acids His353, His513 and Tyr523. Moreover, ester interactions have been established with the amino acids Ser355, Ala354, Phe512, His513, His383, Tyr523, His353, Ala354, and electrostatic interactions with the amino acids Glu411, Asp415, His383 (Fig. 4).

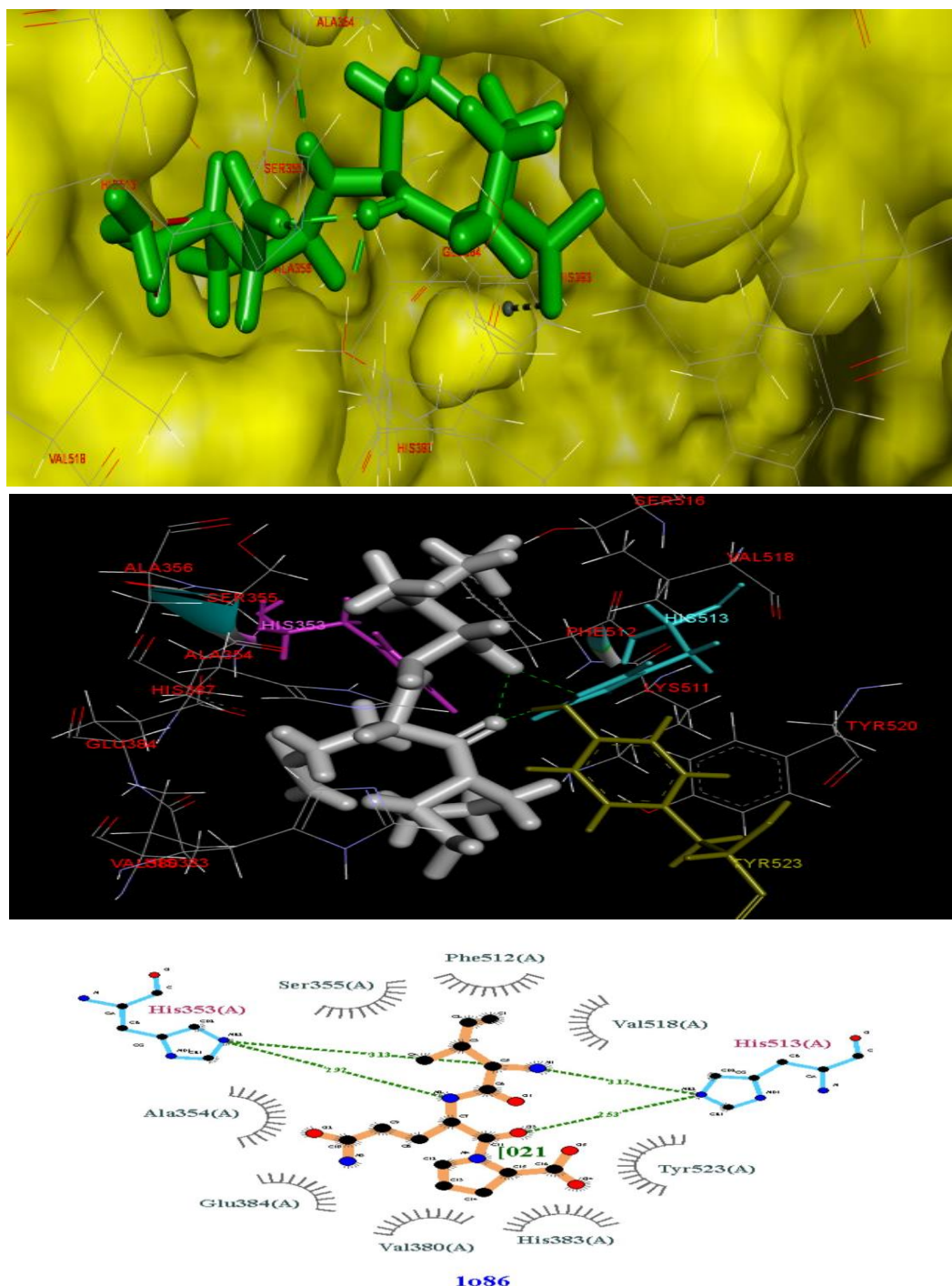


Figure. 4. Interaction of inhibitory ligand with ACE receptor by various bioinformatics software

Discussion and conclusion

The results of this study showed that the designed compound in this study could probably play a role in improving the symptoms of cardiovascular disease by reducing the normal structural function of this enzyme or inhibiting it, as well as reducing mortality in patients with Covid-19.

Standard processes for finding active compounds against a biological target are possible by testing thousands or millions of small molecules (ligands) (20). Although these methods are very useful, they require a lot of laboratory materials and also take much time. Because of this, molecular bonding simulation is now a computer program that can replace the old methods mentioned. Computer methods that dock small molecules into the structure of a protein target molecule and rate the degree to which the ligand pairs with the known active site have been nominated to introduce and optimize selected compounds (21). With these series of computer programs, the correct ligand and receptor conformer is determined. In recent years, many inhibitory compounds have been obtained from plant and animal sources that have played a role in the process of preventing many diseases with minimal side effects (22-23). In the present study, using information from various articles and the role of the presence of specific amino acids in the composition of inhibitory peptides to inhibit the ACE enzyme, selected several key amino acids to design an inhibitory tripeptide as a leader combination for virtual search in databases such as ZINC15 and others. By designing the desired tripeptide through Pimol software, a virtual search was performed to find new ligands, and through this, some ligands were obtained for the docking process with the ACE enzyme. Since the ACE enzyme converts a dipeptide from the carboxylic terminal of angiotensin I to angiotensin II, which is a major factor in the induction of blood pressure (24), the carboxy terminal amino acids of inhibitory peptides play a key role in this (25-26). The presence of aromatic amino acids at the carboxylic end of inhibitory peptides play an important role in their inhibitory process (27). Also, according to other studies, the presence of some branched aliphatic amino acids at the amino-terminal of these peptides contributes to their inhibitory process (28). Based on this evidence, it can be argued that the type and position of amino acids in the structure of inhibitory peptides can play a key role in designing an ideal inhibitory peptide. For this purpose, by selecting three amino acids from this category of amino acids, we designed an inhibitory tripeptide. After obtaining a more favorable ligand from docking results, we studied its biochemical and biophysical properties with the help of various softwares and this compound has no liver toxicity as well as skin allergies. The results of predicting the risk of genetic and cytotoxicity of the designed compound using Lazar Toxicity Prediction software showed that the ligand lacks mutagenic and carcinogenic properties. Renal, neurological and cardiovascular toxicity was also assessed through the way2drug server, which has no inhibitory ligand combination without cardiovascular and neurotoxicity.

Predictability of drug uptake and similarity based on molecular weight (M.W) and number of donor atoms (HBD) and acceptor (HBA) of hydrogen bond are examined (29). According to this rule, compounds with HBD of less than 5, HBA of less than 10, and a molecular mass of less than 500 have quasi-pharmacological properties (Table 1), and Verdonk reported in 2003 that the number of rotational bonds (RF) of pharmaceutical compounds should be less than 3 (30). This inhibitory ligand compound with 9 rotatable bonds, 6 acceptor groups, 5 hydrogen donor groups and a molecular weight of 358.22, has favorable conditions based on Lipinski's multiple criteria for drugs and follows Lipinski's rules. Hydrophobicity of compounds based on Logp is shown to be the coefficient of fractionation of octanol into water, which according to Lipinski's rules should be less than 5 (31). The amount of Logs of compounds with high solubility is higher than zero and the amount of less than -10 indicates the insolubility of the substance and the range of Logs of about 95% of drugs is between 6- to 0.5 (31-33). Its solubility in water has been calculated in ESOL (-0.37) and Ali (-0.30) and SILICOS-IT (-0.26) states that this categorizes into soluble compounds (16).

In this study, it was shown that none of the cytochromes CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 have the ability to inhibit the designed ligand. The results of molecular docking showed that between the non-polar parts of the designed inhibitory ligand composition, mainly with parts of the ACE protein consisting of amino acids, His513 Tyr523, His353, hydrogen interaction took place. They also make ester interactions with the amino acids Ser355, Ala354, Phe512, His513, His383, Tyr523, His353, Ala354, and electrostatic interactions with the amino acids Glu411, Asp415, His383 (34, 21). Based on the results of energy level and docking score obtained by MVD (binding energy -88.883) and cb-dock server (vina score equal to -7) obtained from the present study, it can be concluded that the inhibitory ligand composition interacts with Important amino acids located at the active site of ACE protein that is effective in the pathogenesis of cardiovascular disease and also, it has been reported that ACE inhibitors are associated with a reduced risk of mortality in Covid-19 patients, suggesting that ACE may be involved in increasing mortality in Covid-19 patients (35).

Taken together, due to the importance of high mortality because of cardiovascular disease and Covid-19 patients with hypertension, as well as the limitation of the use of some ACE enzyme inhibitors such as enalapril and captopril due to side effects, the use of active food-derived peptide compounds that have an inhibitory effect on the ACE enzyme, can be used as a new and safer option to control blood pressure and reduce mortality from cardiovascular disease as well as Covid-19. For this purpose, by modeling an ACE-affecting peptide and with the help of bioinformatics software and servers, a new inhibitory compound for the ACE enzyme was designed that has the ability to create optimal inhibitory interactions with the desired

enzyme. The results of bioinformatics studies also indicated that the composition of the ligand is free of hepatotoxic, neurological, cardiovascular and skin allergies and has no mutagenic or carcinogenic properties. Further studies in vitro or in vivo should be performed to evaluate the efficacy and certainty of these compounds.

Reference

- [1] Wang X, Chen H, Fu X, Li S, Wei J. A novel antioxidant and ACE inhibitory peptide from rice bran protein: Biochemical characterization and molecular docking study. *Lwt*. 2017;75:93–9.
- [2] Sarmadi BH, Ismail A. Antioxidative peptides from food proteins: a review. *Peptides*. 2010;31(10):1949–56.
- [3] Celermajer DS, Chow CK, Marijon E, Anstey NM, Woo KS. Cardiovascular disease in the developing world: prevalences, patterns, and the potential of early disease detection. *J Am Coll Cardiol*. 2012;60(14):1207–16.
- [4] Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *lancet Respir Med*. 2020;8(4):e21.
- [5] Pironti G, Andersson DC, Lund LH. Mechanistic and therapeutic implications of extracellular vesicles as a potential link between Covid-19 and cardiovascular disease manifestations. *Frontiers in Cell and Developmental Biology*. 2021 Feb 11;9:197.
- [6] Yu Z, Chen Y, Zhao W, Li J, Liu J, Chen F. Identification and molecular docking study of novel angiotensin - converting enzyme inhibitory peptides from *Salmo salar* using in silico methods. *J Sci Food Agric*. 2018;98(10):3907 – 14.
- [7] Liu C, Fang L, Min W, Liu J, Li H. Exploration of the molecular interactions between angiotensin-I-converting enzyme (ACE) and the inhibitory peptides derived from hazelnut (*Corylus heterophylla* Fisch.). *Food Chem*. 2018;245:471–80.
- [8] Clayton D, Hanchapola I, Thomas WG, Widdop R, Smith AI, Perlmutter P, et al. Structural determinants for binding to angiotensin converting enzyme 2 (ACE2) and angiotensin receptors. *Front Pharmacol*. 2015;6:5.
- [9] Gebre AK, Altaye BM, Atey TM, Tuem KB, Berhe DF. Targeting renin–angiotensin system against Alzheimer’s disease. *Front Pharmacol*. 2018;9:440.
- [10] Martínez-Maqueda D, Miralles B, Recio I, Hernández-Ledesma B. Antihypertensive peptides from food proteins: a review. *Food Funct*. 2012;3(4):350–61.
- [11] Zheng Y, Li Y, Zhang Y, Ruan X, Zhang R. Purification, characterization, synthesis, in vitro ACE inhibition and in vivo antihypertensive activity of bioactive peptides derived from oil palm kernel glutelin-2 hydrolysates. *J Funct Foods*. 2017;28:48–58.
- [12] Hernández-Ledesma B, del Mar Contreras M, Recio I. Antihypertensive peptides: Production, bioavailability and incorporation into foods. *Adv Colloid Interface Sci*. 2011;165(1):23–35.
- [13] Iwaniak A, Minkiewicz P, Darewicz M. Food - originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. *Compr Rev Food Sci Food Saf*. 2014;13(2):114 – 34.
- [14] Wu J, Ding X. Characterization of inhibition and stability of soy-protein-derived angiotensin I-converting enzyme inhibitory peptides. *Food Res Int*. 2002;35(4):367–75.
- [15] Huang S-Y, Zou X. Advances and challenges in protein-ligand docking. *Int J Mol Sci*. 2010;11(8):3016–34.
- [16] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7(1):1–13.
- [17] Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. *Nat Biotechnol*. 2007;25(2):197–206.
- [18] Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res*. 2014;42(W1):W32–8.
- [19] Pires DE V, Blundell TL, Ascher DB. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem*. 2015;58(9):4066–72.
- [20] Yu Y, Hu J, Miyaguchi Y, Bai X, Du Y, Lin B. Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides derived from porcine hemoglobin. *Peptides*. 2006;27(11):2950–6.
- [21] Radifar M, Yuniarti N, Istyastono EP. PyPLIF: Python-based protein-ligand interaction fingerprinting. *Bioinformatics*. 2013;9(6):325.
- [22] Hayashi O, KATO T, OKUWAKI Y. Enhancement of antibody production in mice by dietary *Spirulina platensis*. *J Nutr Sci Vitaminol (Tokyo)*. 1994;40(5):431–41.
- [23] Premkumar K, Abraham SK, Santhiya ST, Ramesh A. Protective effect of *Spirulina fusiformis* on chemical-induced genotoxicity in mice. *Fitoterapia*. 2004;75(1):24–31.
- [24] Timmermans PB, Benfield P, Chiu AT, Herblin WF, Wong PC, Smith RD. Angiotensin II receptors and functional correlates. *Am J Hypertens*. 1992;5(12_Pt_2):221S-235S.

- [2]. Otte J, Shalaby SMA, Zakora M, Nielsen MS. Fractionation and identification of ACE-inhibitory peptides from α -lactalbumin and β -casein produced by thermolysin-catalysed hydrolysis. *Int Dairy J*. 2007;17(12):1460–72.
- [26] Suetsuna K. Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from *Allium sativum* L (garlic). *J Nutr Biochem*. 1998;9(7):415–9.
- [27] HONG SON, CHEUNG HSON, WANG FLAI, MA O, EF S. Binding of peptide substrates and inhibitors of angiotensin-converting enzyme: importance of the COOH-terminal dipeptide sequence. 1980;
- [28] Maeno M, Yamamoto N, Takano T. Identification of an antihypertensive peptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. *J Dairy Sci*. 1996;79(8):1316–21.
- [29] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 1997;23(1–3):3–25.
- [30] Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved protein–ligand docking using GOLD. *Proteins Struct Funct Bioinforma*. 2003;52(4):609–23.
- [31] Peterson L. COVID-19 and flavonoids: In silico molecular dynamics docking to the active catalytic site of SARS-CoV and SARS-CoV-2 main protease. Available SSRN 3599426. 2020;
- [32] Jorgensen WL, Duffy EM. Prediction of drug solubility from Monte Carlo simulations. *Bioorg Med Chem Lett*. 2000;10(11):1155–8.
- [33] Jorgensen WL, Duffy EM. Prediction of drug solubility from structure. *Adv Drug Deliv Rev*. 2002;54(3):355–66.
- [34] Ahmad I. Isolation, elucidation, and molecular docking studies of active compounds from *Phyllanthus niruri* with angiotensin-converting enzyme inhibition. 2018;
- [35] Caldeira D, Alves M, e Melo RG, António PS, Cunha N, Nunes-Ferreira A, et al. Angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers and the risk of COVID-19 infection or severe disease: systematic review and meta-analysis. *IJC Hear Vasc*. 2020;31:100627.