

Molecular Docking Analysis of Human Somatic and Testicular Angiotensin Converting Enzyme Complexed with a Novel Compound Gly-Val-Arg

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Abstract Hypertension is one of the most common chronic diseases affecting millions of people worldwide. The structure-based drug design targeting domains of the ACE is important in the treatment of hypertension. The domain specificity inhibition of somatic ACE and testicular ACE by tripeptide GVR by binding to the specified active site of ACE has not been previously described. From this study, it was shown that tripeptide GVR was significantly bound to the active site of the C-domain of somatic ACE as compared to the N-domain. Although tripeptide GVR was mimicking captopril, a strong inhibition of the C-domain's active site and a weak inhibition of the N-domain by GVR probably led to less significant side effects to the patients as compared to the strong non-domain specific inhibitor, captopril. Besides, the ability of tripeptide GVR to strongly inhibit testicular ACE was also shown. From this study, it has been shown that tripeptide GVR was able to bind on both somatic ACE and testicular ACE. It was also shown that the existence of arginine amino acid at the C-terminal of a peptide sequence was essential to inhibit ACE significantly.

Keywords C- and N-Domains, Enzyme Inhibition, Hypertension, Molecular Docking

1. Introduction

Hypertension or high blood pressure is one of the most common chronic diseases affecting the human population. Approximately 1.13 billion adults have high blood pressure in 2015 [1]. Hypertension, if left untreated or remain uncontrolled, can lead to serious health conditions like heart attack, cerebrovascular accident, kidney failure and premature death [2]. Hypertension is linked to 7.6 million deaths yearly across the globe, making it the primary risk factor for cardiovascular diseases [3]. In many countries, nearly 30% of the adult population is affected by hypertension and over 50% of individuals are oblivious about their health status [4]. The Renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure where it is widely acknowledged by its

involvement in the regulation of arterial blood pressure. In this pathway, the presence of an angiotensin-I-converting enzyme (ACE) plays an essential role in the regulation of blood pressure. ACE converts inactive angiotensin-I to a potent vasoconstrictor, angiotensin-II which constricts the blood vessels and elevates the blood pressure. Other than cleaving angiotensin-I to angiotensin-II in RAS, ACE also degrades bradykinin, a potent vasodilator in the kallikrein-kinin system to an inactive form. In humans, there are two main types of the ACE present; somatic ACE (sACE) which comprises of two domains (N-domain and C-domain) and testicular ACE (tACE) which comprises only one domain [5-6]. Suppression of ACE is vital as a treatment to reduce elevated blood pressure [7]. Many commercially available drugs like captopril and enalapril have been used to inhibit ACE. Despite the effectiveness in treating hypertensive patients, these drugs are known to cause some serious side effects including dry cough and organ-related problems [8]. Therefore, the search for a new antihypertensive drug with no or less significant side effects is necessary. Lately, much attention has been focused on the isolation and characterisation of the ACE inhibitory peptides from food sources as an alternative to the currently existing commercial drugs. To the best of our knowledge, none of these food isolated peptides were included for the domain-specific inhibition of ACE. Commercially available ACE inhibitors are non-domain specific inhibitors [9]. Consumption of such ACE inhibitors leads to the accumulation of bradykinin which is broadly accepted as a causative agent for the side effects of dry cough and angioedema [10]. On the other hand, C-domain selective ACE inhibitors would not only be capable of reducing elevated blood pressure but would also prevent or reduce the adverse effects by not interrupting the N-domain activity [11]. It has been shown that a novel tripeptide glycine-valine-arginine (GVR), derived from *Pleurotus pulmonarius* or commonly known as grey oyster mushroom was able to inhibit ACE *in vitro* and reduce the elevated systolic blood pressure *in vivo* using spontaneously hypertensive rats (SHRs) [12-13]. This tripeptide was resistant towards enzymatic digestion of trypsin, pepsin and chymotrypsin (A). Moreover, during the long-term study, it has also been shown that tripeptide GVR did not produce any toxic effect on the liver and kidney tissues studied. Furthermore, it induced a vasorelaxation effect on the constricted endothelium intact aortas but not on endothelium denuded aortas. Analysis of the metabolomic and proteomic profiles have shown that the reduction of elevated systolic blood pressure by tripeptide GVR probably affected the RAS [13]. The structure-based drug design targeting domains of the ACE is important in the treatment of hypertension. Interestingly, through a Lineweaver-Burk plot analysis, it has been shown earlier that this peptide competitively inhibited ACE [12]. The current study was hence designed to validate the Lineweaver-Burk plot analysis by predicting

and identifying the binding affinity and interactions of tripeptide GVR towards the active site of sACE domains (C- and N-domains) and tACE. It was hypothesised that strong inhibition of the C-domain's active site and weak or no inhibition of N-domain by tripeptide GVR will produce fewer side effects to the patients as compared to the captopril due to uninterrupted degradation of bradykinin.

2. Materials and Methods

Molecular docking analysis was carried out with the AutoDock 4.2 [14] and AutoDockTools 1.5.6 software [15]. The three-dimensional (3D) structures of tACE (PDB ID: 1UZE), C-domain of sACE (PDB ID: 4APH) and N-domain of sACE (PDB ID: 2C6F) were downloaded from the Protein Data Bank (PDB) and were used as the receptors in this study. Prior to the molecular docking procedure, the tACE and sACE C-domain protein structures were included for a structure loop remodelling whereby the missing residues on the structures were addressed by utilising MODELLER 9.19 [16]. The evaluation of the modelled structures was then conducted using PROCHECK [17] to obtain the Ramachandran plot profiles. Since there were no missing residues recorded, this step was not carried out on the sACE N-domain structure. Before the docking simulation, all the hetero atoms together with crystallised water molecules were extracted out while the zinc atom was retained for each of the proteins. The atomic coordinates of the proteins were then kept as another folder and introduced as input to the AutoDock Tools. In this stage, polar hydrogens, Kollman charges as well as solvation parameters were later included for docking preparations. The ligand GVR was then made with ACD/ChemSketch Freeware (Advanced Chemistry Development Inc. Ontario, Canada) and geometry-optimised with MMFF94 force field [18] with the Avogadro software [19]. The GVR was readied for a molecular docking study by combining the non-polar hydrogen atoms and defining their rotatable bonds. The active site was determined from ExPASy Prosite whereby the residues VAHHEMGHIQ for sACE and the residues TAHHEMGHIQ for tACE were identified as the active regions on the protein. Grid maps with the dimension of $50 \times 50 \times 50$ with 0.375 Angstroms (Å) grid spacing were used and centred at the active regions on each of the proteins to outline the binding site for protein structures. The Lamarckian genetic algorithm with local search was employed as the search gateway, with an entirety of 100 runs. In every run, a total of 150 individuals with 27,000 generations as well as 250,000 energy evaluations were used. Operator weights for crossover, mutations and elitism were established at 0.8, 0.02, and 1.0, individually. In addition to the mentioned parameters, each of the molecular docking simulations was performed with an extended AutoDock4Zn force field to take into account the

metalloprotein properties of ACE [20].

3. Results and Discussions

The capability of tripeptide GVR to form a complex with sACE domains and tACE was carried out in this experiment. Further visualisation of the formed complex was conducted using UCSF Chimera [21]. The structure remodelling was carried out first to replace the missing residues reported in the sACE and tACE protein structures (figure 1). The quality of the remodelled structures was later determined by the Ramachandran plot (figure 2). Briefly, based on the Ramachandran plot statistics, the most favoured regions comprised about 94.7% and 93.5% for the whole structure of the modelled sACE C-domain and tACE respectively. Plus, there were no residues in the disallowed regions for both of the modelled structures. The other 28 residues of modelled sACE C-domain and 34 residues of modelled tACE were within the allowed region. Hence, the quality of the remodelled structure of the sACE C-domain and tACE is considered as good which can be employed in the molecular docking study since there were more than 90% of residues in the most favoured regions.

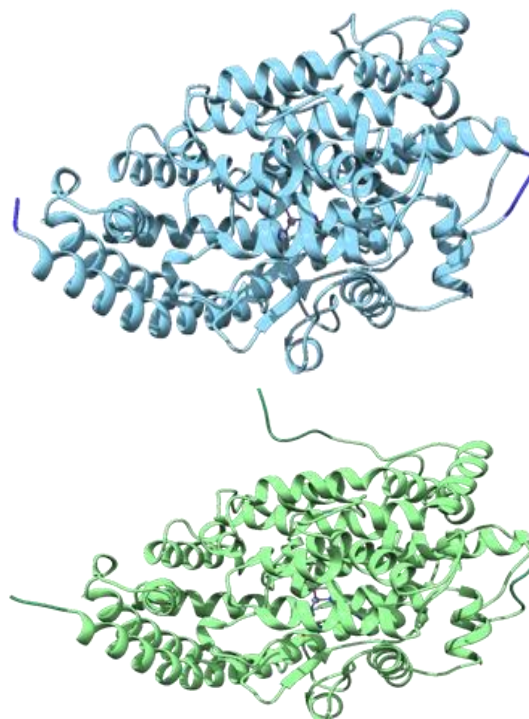
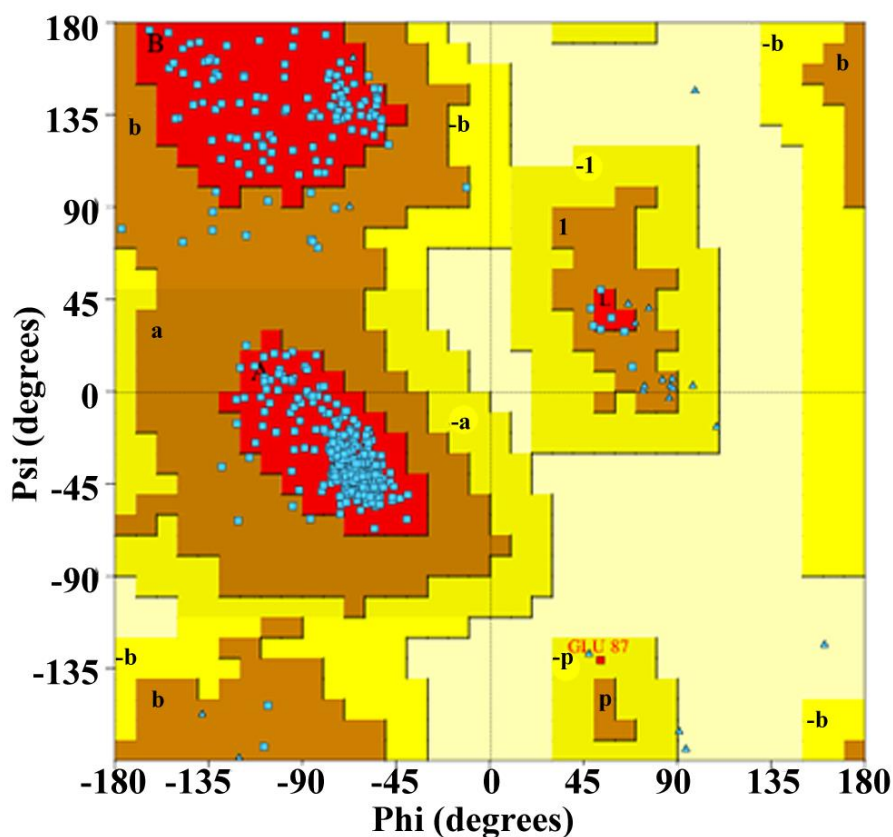


Figure 1. The remodelled protein structures of the sACE C-domain (blue) and tACE (green).



(A)

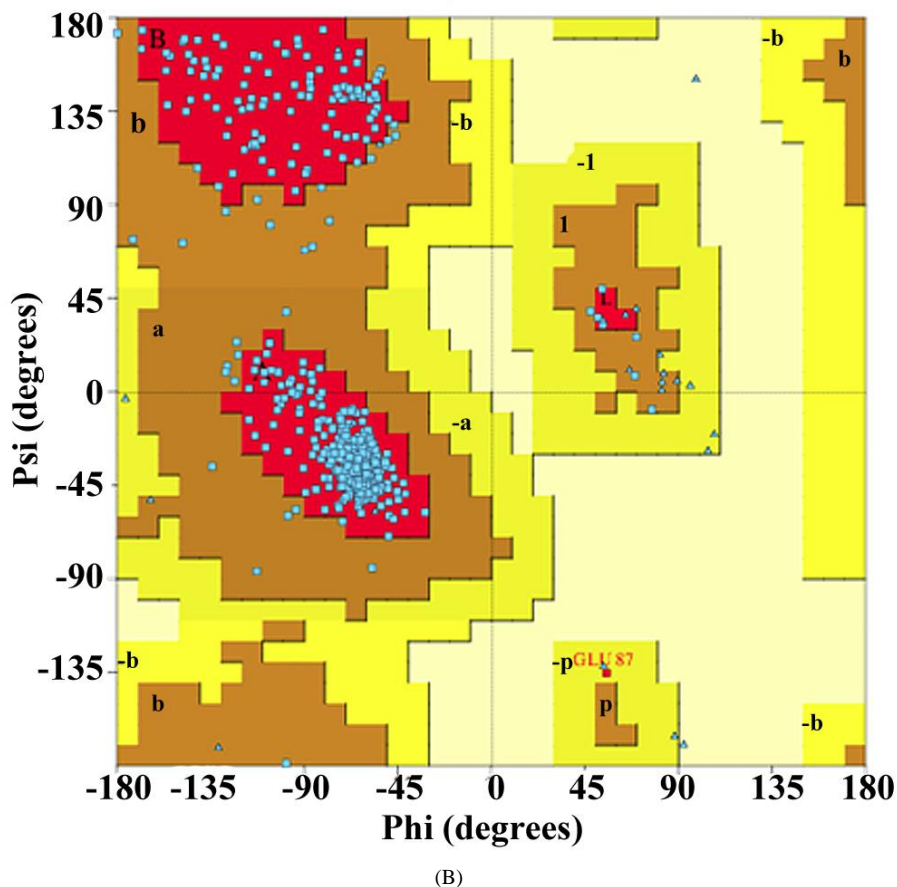


Figure 2. The Ramachandran plot of the remodelled structure of sACE C-domain (Fig 2A) and tACE (Fig 2B)

Table 1 represents the 10 lowermost binding energies from 100 runs of molecular docking simulations. The GVR-ACE complex with the lowermost binding energy was later employed for the binding mode study of the complex (N- and C-domains) and GVR-tACE complex.

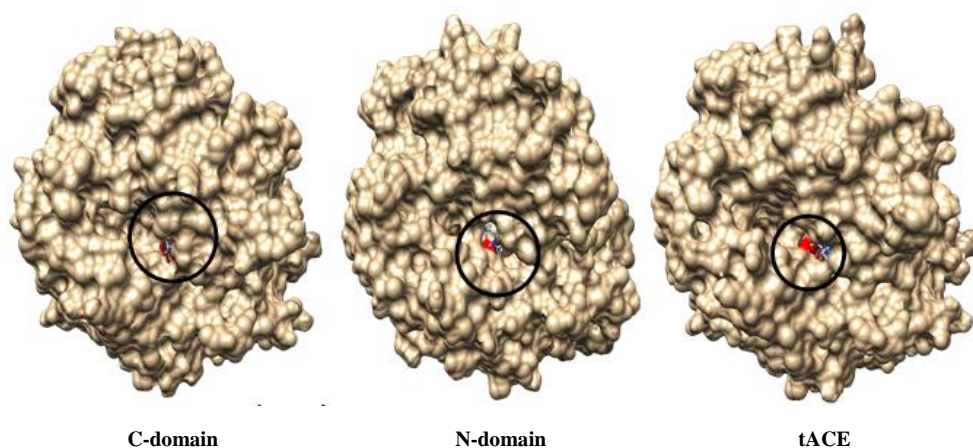
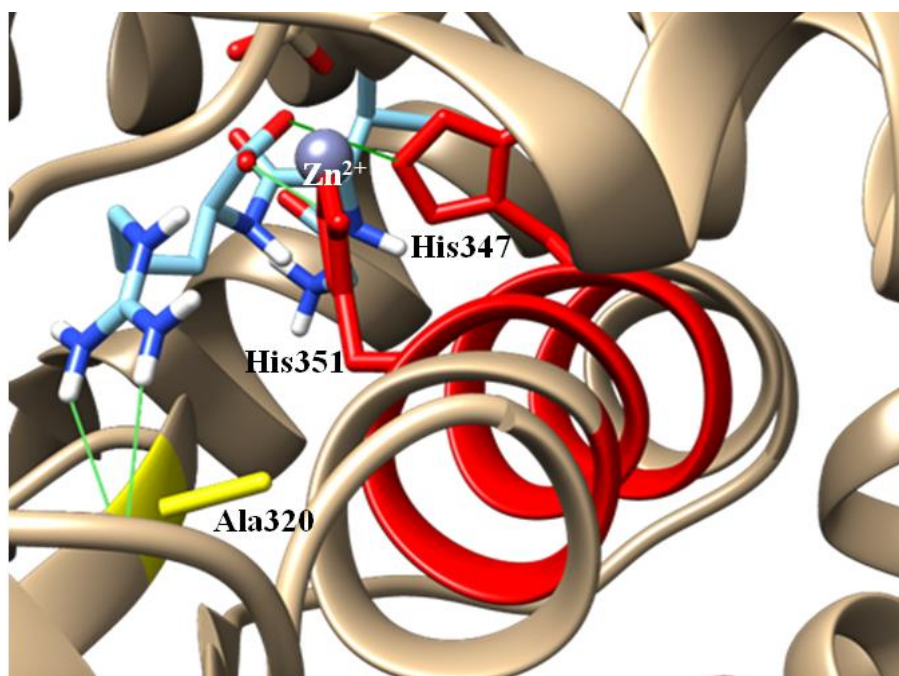
The bulkiness of sACE and tACE proteins in the presence of ligand GVR are revealed in Figure 3 with the binding site occupied by the peptide. From Figures 4, 5 and 6, the images represent the binding sites which are zoomed in to expose the forecasted interactions between the peptide and the protein. For C-domain, N-domain of sACE and tACE, there were 3, 4 and 4 interacting residues with 4, 2 and 5 hydrogen bonds interaction that stabilised the structure, respectively. The forecasted hydrogen bonding interactions and the gap (\AA) of the formed bonds are simplified in Table 2.

Table 1. Top ten lowermost binding energies identified from 100 runs of molecular docking simulations for sACE and tACE

Conformation	sACE		tACE (kcal/mol)
	C-domain (kcal/mol)	N-domain (kcal/mol)	
1	-9.17	-9.11	-10.52
2	-7.44	-8.59	-10.27
3	-7.41	-8.49	-10.01
4	-7.19	-8.42	-9.57
5	-6.48	-8.21	-9.46
6	-4.31	-8.02	-9.26
7	-4.06	-7.65	-9.03
8	-3.94	-7.65	-8.91
9	-3.91	-7.15	-8.74
10	-3.65	-6.86	-8.59

Table 2. The forecasted hydrogen bonding and the gap formed between interacting residues of sACE domains and tACE with GVR

Complex	Protein	Ligand	Distance (Å)
GVR- C domain	Ala320:O	Arg3:HH1	1.73
	Ala320:O	Arg3:HH2	2.11
	His 351:NE2	Arg3: OXT	2.65
	His 347:NE2	Arg3:O	2.73
GVR-N domain	His316:NE2	Arg3:H	2.53
	Tyr501:HH	Arg3:OXT	1.64
GVR-tACE	Ala320:O	Arg3:HH2	2.14
	Ala320:O	Arg3:HH1	1.91
	His351:NE2	Arg3:O	2.77
	His347:NE2	Arg3:OXT	3.10
	Tyr487:HH1	Arg3:OXT	1.85

**Figure 3.** The molecular surface of sACE domains and tACE. The binding pocket of sACE domains and tACE with the ligand are recognised to be located at the middle part of the protein (circled black). The red colour within the binding site of the ACE protein was identified as the active region.**Figure 4.** The predicted orientation of GVR at the catalytic site (coloured in red) of the C-domain of sACE.

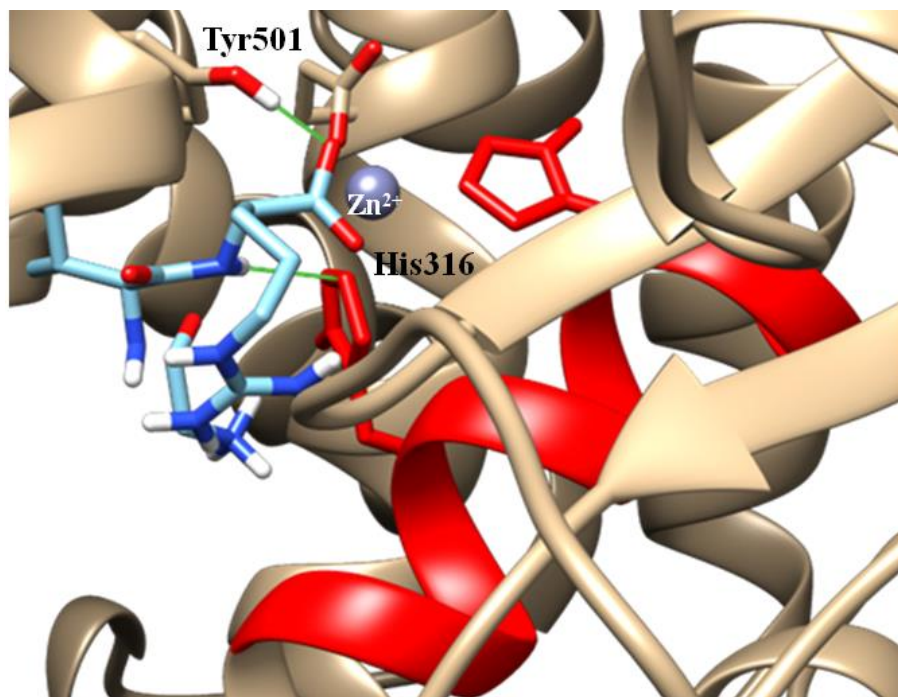


Figure 5. The predicted orientation of GVR at the catalytic site (coloured in red) of the N-domain of sACE.

The comparison between N- and C- domains of sACE revealed that not much difference in their sequences was found [6]. Despite that, these two domains differed in both substrate and inhibitor specificity. It is known that C-domain is mainly involved in the regulation of blood pressure. On top of that, it has been revealed that inhibition of one of these two domains *in vivo* halted the cleavage of angiotensin I. The inhibition of the C-domain probably is important and sufficient to treat certain cardiorenal diseases since this site is known as a dominant site for the production of angiotensin II [6]. In this study, tripeptide GVR was predicted to bind at the C-domain active site of sACE with the lowest binding energy of -9.17 kcal/mol. It is noted from Figure 4 that tripeptide GVR was able to interact with the residues from the active site. Two active site residues on sACE C-domain known as His347 and His351 (known as His383 and His387 from the original structure) were found interacting with the arginine residue of the tripeptide. Previously, these two residues were reported by Masuyer *et al.* [11], as residues from the active site acting on the zinc ion. Although tripeptide GVR did not directly act on the zinc ion, the arginine residue of the tripeptide GVR was found to form hydrogen bonds interaction with the residues (His347 and His351) on the sACE C-domain active region. These interactions may eventually lead to the inhibition of the sACE C-domain. This is supported by Abdelhedi *et al.* [22] who mentioned that arachin ACE-inhibitory peptides did not directly interact with the Zn^{2+} atom. However, the peptides' interaction to His383, His387 produced strong inhibitory activity. Another residue on the sACE C-domain, Ala320 (known as Ala356 from the original structure) was also found to form an interaction with the ligand. It was

observed that two hydrogen bonds were formed in between the ligand and Ala320. Previous studies have also shown that tripeptide IEW, IEY, IKY, IKP, QKEPMIGV and KYIPIQ were found to form an interaction with Ala320 [23-24]. The ACE protein had three main active site pockets known as S1, S2 and S1' [22] whereby Ala318 (Ala354 for the original structure) was a residue within the S1 pocket. Since Ala320 was closely located to Ala318, thus it is predicted that the formation of the two hydrogen bonds between ligand and Ala320 may also contribute to the inhibition of sACE. The formation of strong hydrogen bonds between the ligand and His347, His351 plus with the interaction of the ligand with Ala320 could conclude the ability of tripeptide GVR to bind to the C-domain of sACE. Furthermore, the short distance (1.73 Å to 2.73 Å) of hydrogen bonds between the ligand and the C-domain of sACE interacting residues could contribute to the stability of the ligand-sACE complex. Other than binding to the C-domain of sACE, GVR was also predicted to bind at the active site of the N-domain with the lowest binding energy of -9.11 kcal/mol. Although inhibition of the C-domain alone was considered sufficient to reduce blood pressure and produce vasorelaxation, N-domain was also shown to possess the same efficiency as the C-domain in cleaving active bradykinin to the inactive form [6,25]. Nevertheless, weaker binding interactions of the N-domain were produced by GVR as compared to C-domain interactions. Although the lowest binding energies (Table 1) was comparable between these two domains, the formation of the hydrogen bonds between the ACE residues and ligand varies. Four hydrogen bonds were formed in between the ACE residues and the tripeptide ligand in the C-domain while only two were formed for N-domain. It is speculated

that a strong C-domain inhibition could be achieved since there were two active site residues that formed interactions with the tripeptide. On the other hand, only one active site residue (His316) was found to interact with the tripeptide. With only two hydrogen bonds formed, it is further predicted that the stability of the GVR-ACE complex (N-domain) is considerably low. Based on these analyses, even though the tripeptide GVR was able to bind to the active site of the N-domain, a weak inhibition is predicted. Although inhibition of both domains will further reduce the elevated blood pressure, it will also result in the accumulation of bradykinin which can lead to dry cough. In this study, unlike captopril, a weaker inhibition of the N-domain was found. Therefore, it is concluded that tripeptide GVR was able to inhibit C-domain significantly than N-domain. Moreover, it is further hypothesised that GVR would produce a less significant adverse effect as compared to the captopril.

This study has also shown a strong binding capability produced by GVR on tACE (figure 6) with the lowest binding energy of -10.52 kcal/mol. Five hydrogen bonds were formed in between the hydrogen interacting residues. The ability of the peptide to interact with the two active site residues (His351 and His347) and a residue from the S1 position of the active site, Tyr487 (known as Tyr523 in original structure) may lead to a strong inhibition of ACE. Further analysis showed that there were two hydrogen bonds that formed closely to the S1 position of the ACE active site in between Ala320 and the arginine of GVR which would further strengthen the ACE inhibition. Good stability of the GVR-ACE complex is predicted with the presence of five hydrogen bonds while short distances in between the ACE residues and the ligand can further strengthen the complex. The ability of tripeptide GVR to block testicular ACE raises a question regarding the potentiality of GVR to cause infertility in males. Tripeptide GVR is an ACE inhibitor. Two well-known ACE inhibitors known as captopril and lisinopril which also produce testicular ACE inhibition were previously studied and reported. Approximately 14 research studies related to

captopril's involvement in the male reproduction system was reviewed [28]. The author stated that captopril does not seem to affect the quality of the sperm significantly. Furthermore, the ACE activity in the epididymal fluid had no effect after long term administration of captopril. This is because captopril could not easily penetrate the blood-epididymis barrier. In contrast, based on three additional published reports, although captopril did not significantly affect the quality of sperm, the authors did not deny that captopril may affect the fusion rate of spermatozoa-egg. The authors further suggested that clinical trials need to be conducted to confirm this statement. In a separate report, lisinopril was found to improve sperm count and motility. When the dose of lisinopril was reduced, the sperm count and motility were reduced as well. The effect was dose-dependent. The authors also added that infertile males with low quality sperm cells could benefit from the low dose of ACE inhibition. A low dose of ACE inhibition could be achieved with a low dose intake of lisinopril and the arterial blood pressure will not significantly change [29]. Based on the available reports, tripeptide GVR may not affect the quality of sperms significantly but the fusion rate of sperm-egg needs to be studied further. Based on this study, it can be proven that the long-term reduction of the elevated systolic blood pressure *in vivo* after the administration of tripeptide GVR [13] was probably due to the inhibition of the C-domain of sACE by GVR. Furthermore, the importance of the arginine at the C-terminal of the peptide for ACE inhibition has been mentioned earlier [26] and is also proven from this study by the formed interactions. Thus, considering arginine at the C-terminal is important while designing a peptide for ACE inhibition. In addition, it is also shown that although both domains of sACE are alike, they differ in terms of substrate specificity which could be due to the variation in the single amino acid at the N-terminal on the active site. Furthermore, these findings probably explained the reason behind the differences in the IC₅₀ values of both ACE inhibitors [27,12].

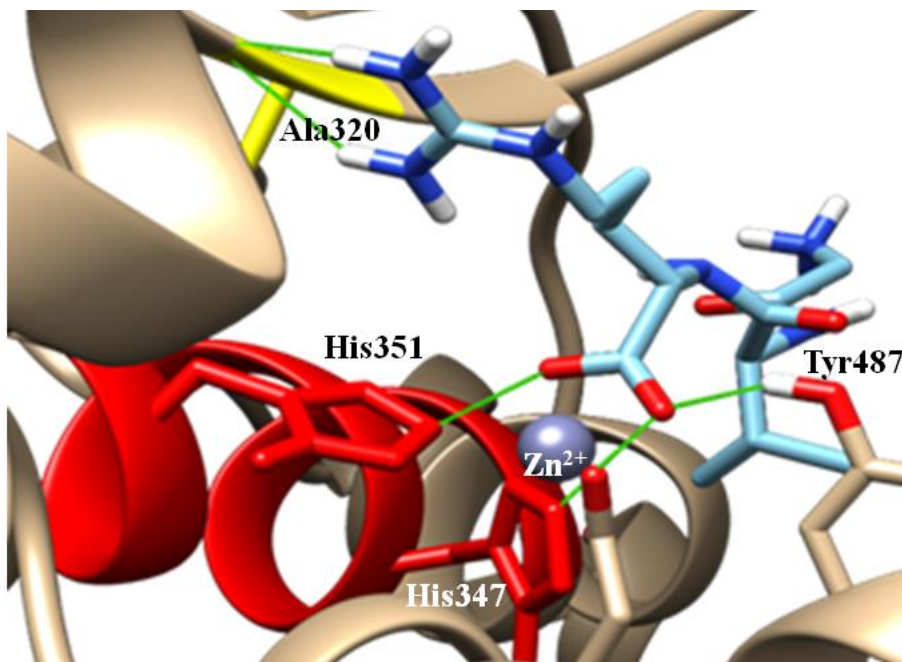


Figure 6. The predicted orientation of GVR at the catalytic site (in red) of tACE

4. Conclusion

In conclusion, this study further strengthens the capability of the bioactive tripeptide, GVR derived from *P. pulmonarius* to significantly reduce hypertension in SHR. The reduction of hypertension *in vivo* was probably due to the inhibition of the C-domain of sACE despite it being known as an ACE inhibitor *in vitro*. Although GVR is not considered as a specific domain inhibitor, it has shown the preference to bind at C-domain rather than N-domain. Hence, the inhibition of the C-domain will lead to the reduction of the blood pressure and improvement in the relaxation of the blood vessels minus the adverse effects and thus, would improve the quality of patients' lives.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

- [1] NCD Risk Factor Collaboration (NCD-RisC). "Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants," *Lancet*, vol. 389, pp. 37-55, 2017.
- [2] Naing C., Yeoh PN., Wai VN., Win NN., Kuan LP., Aung K., "Hypertension in Malaysia: an analysis of trends from the National Surveys 1996 to 2011," *Medicine (Baltimore)*, vol. 95, pp. e2417, 2016.
- [3] Chow CK., Teo KK., Rangarajan S., Islam S., Gupta R., Avezum A., Bahonar A., Chifamba J., Dagenais G., Diaz R., Kazmi K., Lanan F., Wei L., Lopez-Jaramillo P., Fanghong L., Ismail NH., Puoane T., Rosengren A., Szuba A., Temizha, A., Wielgosz A., Yusuf R., Yusufali A., McKee M., Liu L., Mony P., Yusuf S., PURE (Prospective Urban Rural Epidemiology) Study investigators, "Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries," *JAMA*, vol. 310, pp. 959-968, 2013.
- [4] Balti R., Nedjar-Arroume N., Bougatef A., Guillochon D., Nasri M., "Three novel angiotensin-I-converting enzyme (ACE) inhibitory peptides from cuttlefish (*Sepia officinalis*) using digestive proteases," *Food Research International*, vol 43, pp. 1136, 2010.
- [5] Michaud A., Acharya KR., Masuyer G., Quenech'du N., Gribouval O., Moriniere V., Gubler MC., Corvol P., "Absence of cell surface expression of human ACE leads to perinatal death," *Human Molecular Genetics*, vol. 23, pp. 1479, 2014.

- [6] Natesh R., Schwager SLU., Evans HR., Sturrock ED., Acharya KR, "Structural details on the binding of antihypertensive drugs captopril and enalaprilat to human testicular angiotensin-I-converting enzyme," *Biochemistry*, vol. 43, pp. 8718, 2004.
- [7] Manoharan S., Shuib AS., Abdullah N, "Structural characteristics and antihypertensive effects of angiotensin-I-converting enzyme inhibitory peptides in the renin-angiotensin and kallikrein kinin systems," *African Journal of Traditional, Complementary and Alternative Medicine*, vol. 14, pp. 383, 2017.
- [8] Marczak ED., Usui H., Fujita H., Yang Y., Yokoo M., Lipkowski AW., Yoshikawa M, "New antihypertensive peptides isolated from rapeseed," *Peptides*, vol. 24, pp.791, 2003.
- [9] Sharp S., Poglitsch M., Zilla P., Davies NH., Sturrock ED, "Pharmacodynamic effects of C-domain-specific ACE inhibitors on the renin-angiotensin system in myocardial infarcted rats," *Journal of Renin Angiotensin and Aldosterone System*, vol. 16, pp. 1149, 2015.
- [10] Anthony CS., Masuyer G., Sturrock ED., Acharya KR, "Structure based drug design of angiotensin-I-converting enzyme inhibitors," *Current Medicinal Chemistry*, vol. 19, pp. 845, 2012.
- [11] Masuyer G., Schwager SLU., Sturrock ED., Isaac RE., Acharya KR, "Molecular recognition and regulation of human angiotensin-I-converting enzyme (ACE) activity by natural inhibitory peptides," *Scientific Reports*, vol. 2, pp. 717, 2012.
- [12] Manoharan S., Shuib AS., Abdullah N., Mohamad SB., Aminudin N, "Characterisation of novel angiotensin-I-converting enzyme inhibitory tripeptide, Gly-Val-Arg derived from mycelium of *Pleurotus pulmonarius*," *Process Biochemistry*, vol. 62, pp. 215, 2017.
- [13] Manoharan S., Shuib AS., Abdullah N., Ashrafzadeh A., Kabir N, "Gly-Val-Arg, an angiotensin-I-converting enzyme inhibitory tripeptide ameliorates hypertension on spontaneously hypertensive rats," *Process Biochemistry*, vol. 69, pp. 224, 2018.
- [14] Goodsell DS., Morris GM., Olson AJ, "Automated docking of flexible ligands: applications of AutoDock," *Journal of Molecular Recognition*, vol. 9, pp. 1, 1999.
- [15] Sanner MH, "Python: a programming language for software integration and development," *Journal of Molecular Graphics and Modelling*, vol. 17, pp. 57, 1999.
- [16] Sali A., Blundell TL, "Comparative protein modelling by satisfaction of spatial restraints," *Journal of Molecular Biology*, vol. 234, pp. 779, 1993.
- [17] Laskowski RA., MacArthur MW., Moss DS., Thornton JM, "PROCHECK: a program to check the stereochemical quality of protein structures," *Journal of Applied Crystallography*, vol. 26, pp. 283, 1993.
- [18] Halgren TA, "Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94," *Journal of Computational Chemistry*, vol. 17, pp. 490, 1996.
- [19] Hanwell MD., Curtis DE., Lonie DC., Vandermeersch T., Zurek E., Hutchison GR, "Avogadro: an advanced semantic chemical editor, visualization, and analysis platform," *Journal of Cheminformatics*, vol. 4, pp. 17, 2012.
- [20] Santos-Martins D., Forli DS., Ramos MJ., Olson AJ, "AutoDock4Zn: An improved AutoDock force field for small-molecule docking to zinc metalloproteins," *Journal of Chemical Information and Modeling*, vol. 54, pp. 2371, 2014.
- [21] Pettersen EF., Goddard TD., Huang CC., Couch GS., Greenblatt DM., Meng EC, "UCSF Chimera-a visualization system for exploratory research and analysis," *Journal of Computational Chemistry*, vol. 25, pp. 1605, 2004.
- [22] Abdelhedi O., Nasri R., Mora L., Jridi M., Toldr áF., Nasri M, "*In silico* analysis and molecular docking study of angiotensin-I-converting enzyme inhibitory peptides from smooth-hound viscera protein hydrolysates fractionated by ultrafiltration," *Food Chemistry*, vol. 239, pp. 453, 2018.
- [23] Jimsheena VK., Gowda LR, "Arachin derived peptides as selective angiotensin-I-converting enzyme (ACE) inhibitors: Structure-activity relationship," *Peptides*, vol. 31, pp. 1165, 2010.
- [24] Lin K., Zhang LW., Han X., Cheng DY, "Novel angiotensin-I-converting enzyme inhibitory peptides from protease hydrolysates of Qula casein: quantitative structure-activity relationship modelling and molecular docking study," *Journal of Functional Foods*, vol. 32, pp. 266, 2017.
- [25] van Esch JHM., Tom B., Dive V., Batenburg WW., Georgiadis D., Yiotakis A., van Gool JM., de Bruijn RJ., de Vries R., Danser AH, "Selective angiotensin-converting enzyme C-domain inhibition is sufficient to prevent angiotensin I-induced vasoconstriction," *Hypertension*, vol. 45, pp. 120, 2005.
- [26] Norris R., FitzGerald RJ, "Antihypertensive peptides from food proteins. In: *Bioactive food peptides in health and disease*," (Ed. by Hernandez-Ledesma & CC Hsieh; Intech, New York), pp. 45, 2013.
- [27] Ibadallah BX., Abdullah N., Shuib AS, "Identification of angiotensin-converting enzyme inhibitory proteins from mycelium of *Pleurotus pulmonarius* (Oyster Mushroom)," *Planta Medica*, vol. 81, pp. 123, 2015.
- [28] Banihani SA, "Effect of captopril on semen quality," *Andrologia*, vol. 49, pp. e12641, 2017.
- [29] Okeahialam BN., Amadi K., Ameh AS, "Effect of lisinopril, an angiotensin converting enzyme (ACE) inhibitor on spermatogenesis in rats," *Archives of Andrology*, vol. 52, pp. 209, 2006.