STRUCTURAL REQUIREMENTS OF ANGIOTENSIN RECEPTOR: PREFERRED MODIFICATIONS FOR ANTAGONIST DESIGN

Tatyana Dzimbova[1,2], Atanas Chapkanov[1]

[1]South-West University "Neofit Rilski"
66 Ivan Mihailov St., Blagoevgrad 2700, Bulgaria
[2] Institute of Molecular Biology "Roumen Tsanev"
21 Acad. Georgi Bonchev St., Sofia 1311, Bulgaria
E-mail: tania_dzimbova@abv.bg

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ABSTRACT

Blood pressure and fluid balance are regulated hormonally by renin-angiotensin system (RAS). Influence on this system could be achieved by different compounds that act as angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin receptor blockers and renin inhibitors. The purpose of the present study is to predict the structures of the potent ACE inhibitors on the base of His-Leu peptide structural element of angiotensine I using computational methods. Different modifications were made in the structure of this dipeptide and the energy of binding with the enzyme were calculated. The docking results were analyzed and it was found that along with the important amino acid residue of the receptor molecule Arg167, the Tyr residues (35, 87, 88 and 92) as well as Cys180 are extremely important for the strong binding to the receptor and, accordingly, the manifestation of antagonistic action by the analogues. To block the receptor, the ligand molecule must have an intact terminal carboxyl group and an imidazole nucleus to participate in appropriate interactions. The inclusion of functional groups in the side chains of the amino acid residues of the dipeptide create an additional site for binding to the receptor. With the help of docking, the ligand molecule can be optimized, and this process is fast, saving the synthesis of many compounds and their biological testing. And finally, the most potent analogues will be synthesized and biologically tested.

Keywords: GOLD 5.2, molecular modeling, structure-activity relationship.

INTRODUCTION

Cardiovascular disease remains one of the main causes of death throughout the world despite impressive advances in diagnosis and therapeutics during the past few decades [1]. Hypertension is the most common modifiable risk factor in cardiovascular disease, as myocardial infarction, stroke, heart failure, and renal disease can be greatly reduced by lowering blood pressure [2]. The hypertension, cardiac hypertrophy, heart failure, ischemic heart disease, and nephropathy are the result of the over-stimulation of RAS. Various angiotensin peptides were generated in the RAS but the most important is angiotensin II (AngII). It acts as vasoconstrictors. In humans, AngII binds to two subtypes of angiotensin G protein-coupled receptors (GPCRs), angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R) [4]. Almost all physiological and pathophysiological effects of AngII are mediated by AT1R [5].

The purpose of the present study is to predict the structures of the potent ACE inhibitors on the base of His-Leu peptide structural element of angiotensine I using computational methods.

EXPERIMENTAL

Various software was used in the present study: Avogargo [6]. GOLD 5.2 (Genetic Optimization for Ligand Docking) [7], and Molegro Molecular Viewer

[8]. Six ligands, investigated for their binding to AR were selected for docking studies. The binding site for AT1R (PDB id: 4zud), we assumed like in all G-protein couple receptor, was on the third transmembrane helix (TM). For the docking we use Arg^{167} residue from the TM and the space within 10Å radius of them. GoldScore scoring function of GOLD was used. The conformations of the ligands with best scoring functions were selected and the total energies of the complexes with AT1R were used for analysis.

RESULTS AND DISCUSSION

In all G-protein coupled receptors, residues located on TM3 are important for ligand recognition. In AT1R, their binding site is defined as a 10 Å space around Arg167. The results of this docking have already been published and are presented in Table 1 [9].

The total energies of ligand-receptor complexes can be taken to represent the affinity of the respective ligand for the receptor, as it reflects the binding strength between them. Their values show that the formed complexes are stable enough, and three of them could act as antagonists, blocking the binding site of the receptor for a long time. The mode of binding also largely determines the action of the ligands. His-sNle3 forms a large number of hydrogen bonds as well as interacts electrostatically and hydrophobically with AT1R (Fig. 1), making it potentially the most active antagonist in the series of compounds tested.

In all three analogues there is one functional group introduced – sulfo group. This group makes it

Table 1. The total energies of the ligand-receptor complexes with AT1R [9].

Ligand	Total energies of complexes with AT1R
His-Leu	-34.27
His-sIle	-25.08
His-sLeu	-49.05
His-sNle	-82.91
His-sNle2	-55.45
His-sNle3	-85.72

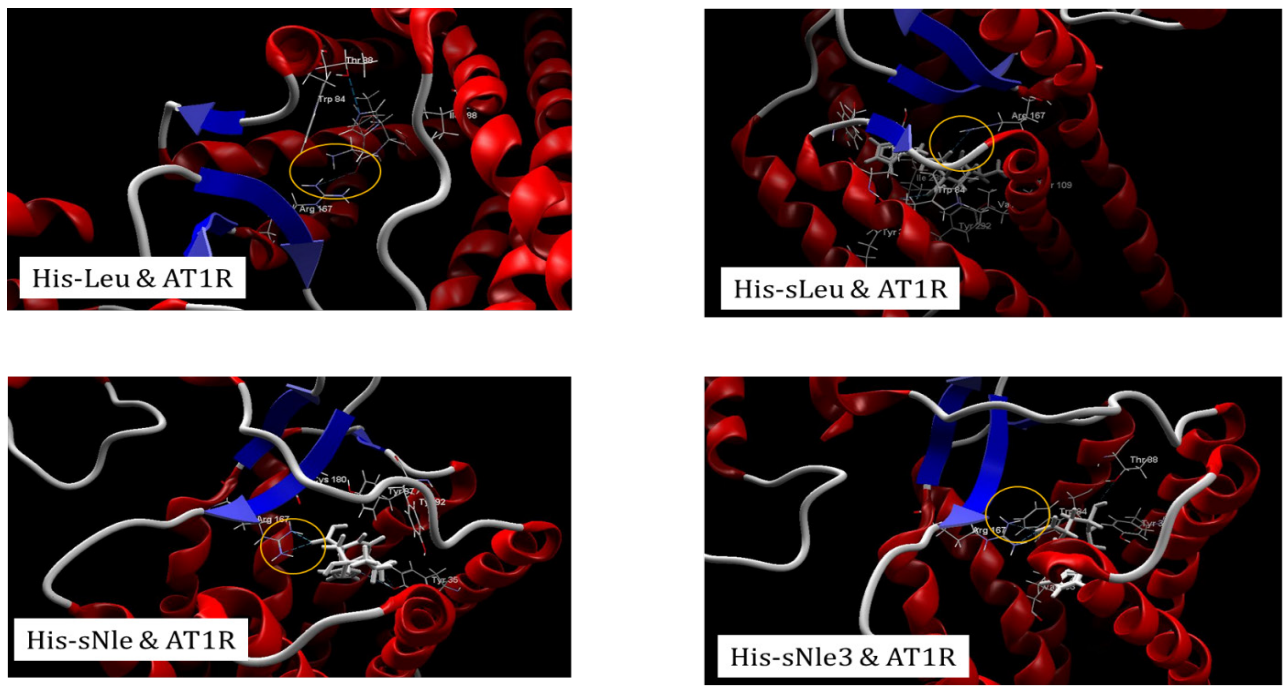

Fig. 1. Binding mode of His-Leu, His-sLeu, His-sNle and His-sNle3 with AT1R.

Table 2. Interactions in the binding site of receptor.

Ligands	Interacting amino acid residue of the receptor
His-Leu	Trp^{84}, Tyr^{88}, Arg^{167}, Ile^{288}
His-sIle	Ser^{105}, Ser^{109}, Ala^{159}, Arg^{167}, Ile^{288}, Tyr^{292}
His-sLeu	Trp^{84}, Tyr^{87}, Tyr^{92}, Val108, Ser^{109}, Arg^{167}, Ile^{288}, Tyr^{292}
His-sNle	Tyr^{35}. Tyr^{87}, Tyr^{92}, Arg^{167}, Cys^{180}
His-sNle2	Tyr^{35}, Tyr^{88}, Tyr^{92}, Arg^{167}
His-slLe3	Tyr^{35}, Tyr^{87}, Tyr^{92}, Arg^{167}, Cys^{180}

possible for the ligand to interact with the receptor, the interactions contributing to stronger binding and correspondingly occupying the binding site of the receptor for a longer time, interacting with Arg167. Additional interaction with Tyr35 is beneficial for the stabilization of the complex and for the effect of the most potent ligands.

The interaction between His-sNle and His-sNle3 is the strongest, and this interaction releases the most energy (-6.81 J mol^{-1}), followed by the energy released during the interaction of His-Leu (-4.3 J mol^{-1}). In all three cases, the guanidino group of Arg^{167} interacts with the free COOH group of the second amino acid residue of the dipeptide. This shows that it is extremely important for binding to the receptor to keep this group unchanged, and even if modifications are undertaken, they retain the character and are capable of binding with a guanidino group.

The inclusion of a sulfo group in the side chain creates an additional site for binding to the receptor and this is evident from the results in Table 2, which presents the amino acid residues with which the ligands interact. Their number increases with His-Leu analogues. This indicates that the introduction of a functional group into the ligands contributes to an increase in their ability to bind to the receptor.

Interaction with tyrosine residues in the receptor, and in particular Tyr^{35}, Tyr^{87}, Tyr^{88}, Tyr^{92}, makes the binding stronger. Such an interaction is absent for His-sIle and its binding energy is the highest in the series of ligands studied. The tyrosine residues interact with the imidazole nucleus of histidine and therefore it is necessary to keep it unchanged in the molecule of the analogues.

Another important interaction that contributes to the stabilization of the ligand-receptor complex ex with Cys180. The ligands with the lowest energy of the formed complexes, His-sNle and His-sNle3 interact with it through their free COOH group.

His-sLeu, His-sNle and His-sNle3 have the potetial to block RAS by inhibiting both ACE, as we showed in our previous study [9] and AT1R.

CONCLUSIONS

As a result of the presented study, we showed that the most important residue in the receptor molecule is Arg^{167} as all synthetic analogues bind to this residue. As many electrostatic interactions or/and H-bonds the ligand form with the receptor as lower the energy of the receptor-ligand complex is and, respectively, as strong the binding to the receptor is (i.e. the energy of the complex is lower) the inhibitory action of the ligand will be higher as it will stay bounded to the receptor longer.

This research once again showed that docking could be used for rapid, cheap and accurate drug design as many compounds could be tested virtually and finally the most promising candidates to be tested *in vitro* and *in vivo*.

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