# IN SILICO ANALYSIS FOR CHARACTERIZING THE STRUCTURE AND BINDING PROPERTIES OF ALA-HIS-LYS (AHK) TRIPEPTIDE

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**Abstract:** AHK (Alanine-Histidine-Lysine) tripeptide, known as an antioxidant because of its amino acid properties, has been clinically developed for the treatment of hair loss and skin rash. The copper complex of this tripeptide (AHK-Cu) is an analog with a stronger effect than the Gly-His-Lys (GHK-Cu) tripeptide which is used for hair growth. The effects of AHK-Cu on human hair growth were evaluated by *in vitro* studies (Pyo, 2007) and the results showed that AHK-Cu promotes the growth of human hair follicles. In addition, Vitamin C conjugated AHK has been developed to increase collagen synthesis and promote human dermal fibroblast growth. These results provided important data for the development of peptide-based bone regenerative agents for the treatment of bone-related disorders (Jung, 2018).

The aim of this study is to determine the most stable geometric structure of AHK tripeptide by using theoretical methods with different approaches to determine the binding properties with proteins that can act in the body.

Firstly, the most stable molecular structure of tripeptide was determined with the help of quantum mechanical method, which included electronic interactions in the calculation. Then, the most efficient structure of the tripeptide in the water medium such as the human body was determined using Molecular Dynamic (MD) analysis. In addition; the binding properties of title tripeptide was investigated by molecular docking technique. The discovery and improvement of the structure and activity of cosmetic peptide is an active field of study, particularly in biochemistry and pharmacology.

**Keywords:** AHK, L-Alanine-L-Histidine-L-Lysine, tripeptide-copper complex, DFT, MD, Molecular Docking

## Introduction

Drugs are a very important factor that affect human life and health. Unfortunately, the discovery of molecules result in safe and effective drugs involves a process that has high cost, production, development and testing stages; they must go through certain stages as a result of the experiments performed in the laboratory, and then include clinical research. Designing and developing the most effective drug in a short time and with lower costs attracts the attention of many scientists working in different fields. In the process of designing the most effective drug, *in silico* methods are preferred because it minimizes time and cost. The appropriate drug structures obtained in accordance with the calculations made with *in silico* methods also allow more rational drug designs by reducing the processes of organic synthesis with high budget. The aim of molecular modeling methods that define molecular systems at the atomistic level is to show how atoms and molecules can interact with a three-dimensional image and simulation, and to determine the structure of these interaction mechanisms. These models can also be used to interpret existing observations or to predict new chemical behaviors. In the drug design process, in silico methods have become a valuable and necessary tool for the modeling of molecular structures that have been nominated for drugs, for increasing the effectiveness of drugs, and for the design of new drug

  

molecules with unknown molecular structure. With these methods, it is possible to examine the relationship between chemical structure and function from small systems to large biologic molecules and material groups. The contribution of modern computer-aided drug design to the discovery of drugs is an indisputable fact and is understood to have been used by large pharmaceutical companies in many commercially available drugs. Modern computer-aided drug design has contributed to the discovery and development of many medicines such as Captopril, Dorzolamide, Saquinavir, Zanamivir, Oseltamivir, Aliskiren, Boceprevir, Nolatrexed, Rupintrivir, and NVP-AUY922.

AHK (Alanine-Histidine-Lysine) tripeptide which shows antioxidant effect because of its amino acid properties, has been clinically developed for the treatment of hair loss and skin rash (Rushton, 2002 and Shimura, 2017). It is claimed that copper peptides can increase hair follicle size and create a healthier environment for the growth of scalp hairs. AHK copper complex (AHK-Cu) is an analog of Gly-His-Lys copper complex (GHK-Cu), which has antioxidant and anti-inflammatory effects, is recommended for wound healing, enhancing the effect of immune cells, stimulating collagen synthesis, skin fibroblasts and the growth of blood vessels. The effects of AHK-Cu on human hair growth were evaluated many *in-vitro* and *ex-vivo* studies (Pyo, 2007) and it also promotes the growth of human hair follicles (Patt, 2009). It provides an increase dermal cell multiplication and viability to help to production of collagen (Patt, 2010). It has also stronger effect than GHK-Cu for hair growth. Vitamin C conjugated AHK has been developed to increase collagen synthesis and promote human dermal fibroblast growth. These results provided important data for the development of peptide-based bone regenerative agents and for the treatment of bone-related disorders (Jung, 2018). The aim of this study is to determine the most stable molecular structure of AHK tripeptide, which is a very effective field of cosmetic use, and to identify the binding mechanisms by proteins with which it acts.

## Materials and Methods

### Molecular Dynamic Method

Molecular Dynamic Simulation was performed using the GROMACS software (version 5.1.2) (Van Der Spoel, 2005) to determine the conformational change in the water medium on the optimized geometry, calculated at DFT/B3LYP level of theory with the 6-311++ G (d,p) basis set, of the AHK in the vacuum medium obtained by the Gaussian 09 software program (Frisch, 2009). Initially, the GROMOS96 54a7 force field (van Gunsteren, 1996) was chosen where the topology file would be created to perform molecular dynamic simulation. AHK tripeptide was placed at the center, a distance of 1.0 nm between the outside of the molecule and the edge of the solvent box, and simulated with water medium. The cubic box was filled with 978 moles of SPC (simple point charge) water (Smith, 1993) mediums and two Na+ and three Cl- ions were added in the cubic box to neutralize the system. The steepest descent method was chosen for the energy minimization at 200 ps for water medium. To equilibrate the temperature and pressure of the systems, NVT (50 ps) and NPT (500 ps) ensembles were carried out for 310 K temperature using a V-rescale thermostat (Bussi, 2007) and 1 bar pressure using the isotropic Parrinello-Rahman barostat (Parrinello, 1981). To obtain the trajectory files during 5 ns for analysis the systems behaviors', Molecular dynamics (MD) simulations were performed by applying periodic boundary conditions in all three directions. Leap-frog algorithm was used in equation of motion was united in order to generate time-dependent trajectories. All bond lengths were constrained with the LINCS (linear constraint solver) algorithm (Hess, 1997). The Particle Mesh Ewald (PME) method (Darden, 1993) was used to calculate the long-range electrostatic interaction with a grid width of 0.16 nm and a fourth order cubic interpolation. Verlet cut-off scheme (Verlet, 1967) was used with a 0.8 nm cut-off radius for identified the cut-off distances, the van der Waals and the short-range electrostatic interactions. The atom coordinates, velocities and energies were saved every step and obtained the trajectory files. The resulting of trajectory files were viewed and analyzed with the VMD software (Humphrey, 1996).

### Molecular Docking Method and ADME Analysis

The molecular structure of the AHK tripeptide, which was subjected to molecular dynamics simulation in the water medium for 5 ns at GROMACS program introduced to Schrödinger Maestro program for use as a ligand in the calculation of docking. Schrödinger Lig Prep module was used to prepare ligand to docking analysis. AHK was prepared for docking calculations using the OPLS3 force field (Harder, 2015). A maximum of 24

  

stereoisomers were produced for the ligand after the ionization states at pH 7.0 ± 2.0 were selected. The tripeptide-copper complex, defined as a growth factor, stimulates the proliferation of dermal fibroblasts and increases vascular endothelial growth factor production, while at the same time decreasing the secretion of transformed growth factor-beta1 by dermal fibroblasts. For this reason, Vascular endothelial growth factor receptor 2 (pdb code: 3VO3) (Miyamoto, 2013) was prefered as a receptor and was prepared with Protein preparation wizard tool (Sastry, 2013) in Schrödinger software. The docking analysis of AHK tripeptide onto a vascular endothelial growth factor receptor-2 was performed. The receptor was obtained from the PDB database but due to the lack of residues in the protein structure, the crystal structure was obtained using the SWISS-MODEL server (Bienert, 2016). All waters, metals and ions except protein were deleted from the data file. The polar hydrogens were added to the heavy atoms in the protein. The bond orders were assigned, charges were defined at pH 7.0 and the selected receptor was optimized using PROPKA (Søndergaard, 2011). The heavy atoms in the receptor were converged by preferring 0.3A˚ RMSD and the OPLS3 force field. After the grid was generated using glide grid generation tool, drug candidate molecule was docked to the receptors using Glide SP (standard precision) module of the Maestro version 11.4 (Friesner, 2006; Friesner, 2004; Halgren,2004). Determination of the pharmacokinetic properties of drug candidate molecules is very important for the design and synthesis of drugs with better bioavailability. The drug candidate compounds which easily absorbed orally, easily transported to the target region (skin, stomach, blood brain barrier) in the body and easily removed from the body are determined by ADME profiles which are required by the FDA in the drug approval process (Ntie-Kang,2013). The Qik-Prop module was used to determine the ADME profile of the AHK tripeptide.

## Results and Discussion

### Molecular Dynamics Results

Molecular Dynamic Simulation was performed using the GROMACS software (version 5.1.2) to determine the conformational change in the water medium on the optimized geometry, calculated at DFT/B3LYP level of theory with the 6-311++ G (d,p) basis set, of the AHK in the vacuum medium obtained by the Gaussian 09 software program. The AHK tripeptide was trapped in 978 moles of water molecule and two Na+ and three Cl- ions were added to ensure system neutralization in **Fig.1**. For the energy minimization, the steepest descent method was chosen at 200 ps for water medium in **Fig. 2.** Using the NVT (50 ps) and NPT (500 ps) assemblies, the temperature and pressure of the system were brought to 310 K and 1 bar, respectively, and a 5 ns Molecular dynamic (MD) simulation was performed, thereby examining the conformational change of the peptide in a similar environment to the human body. During the simulation, the RMSD value of AHK tripeptide in water medium was found to be in the range of 0.02 - 0.1 nm in **Fig. 3.** A rmsd of about 0.2 nm or less indicates that the peptide is in its original crystal form. The Rg change graph was also plotted for 5 ns. According to this graph, the constant Rg value to a relatively constant mean tells us that the peptide has stable structure in the water medium in **Fig.4.**

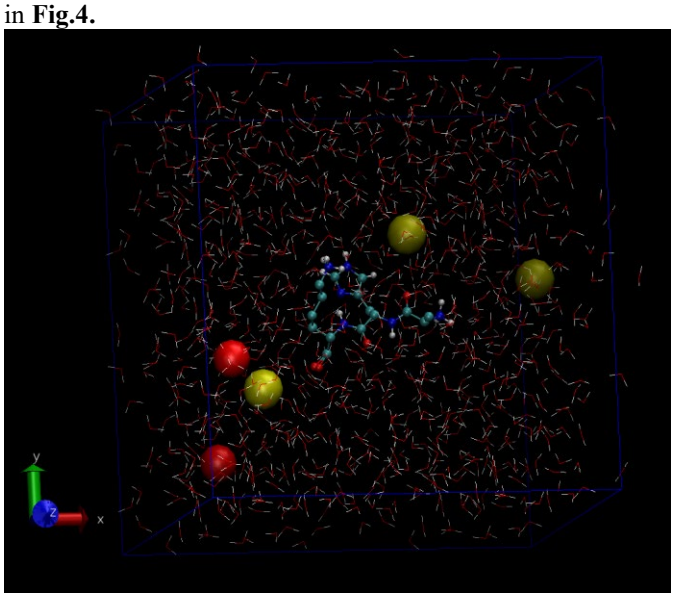

**Figure 1:** AHK tripeptide in a cubic box solvated with 978 SPC water molecules with two $Na^+$ and three $Cl^-$ ions.

  

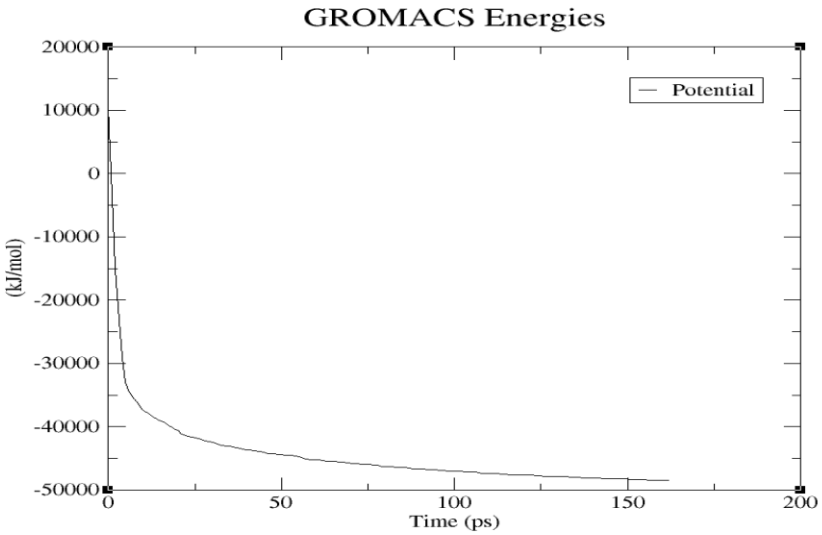


**Figure 2:** The potential energy minimization of the system using the Steepest Descent algorithm for water medium system of AHK tripeptide.

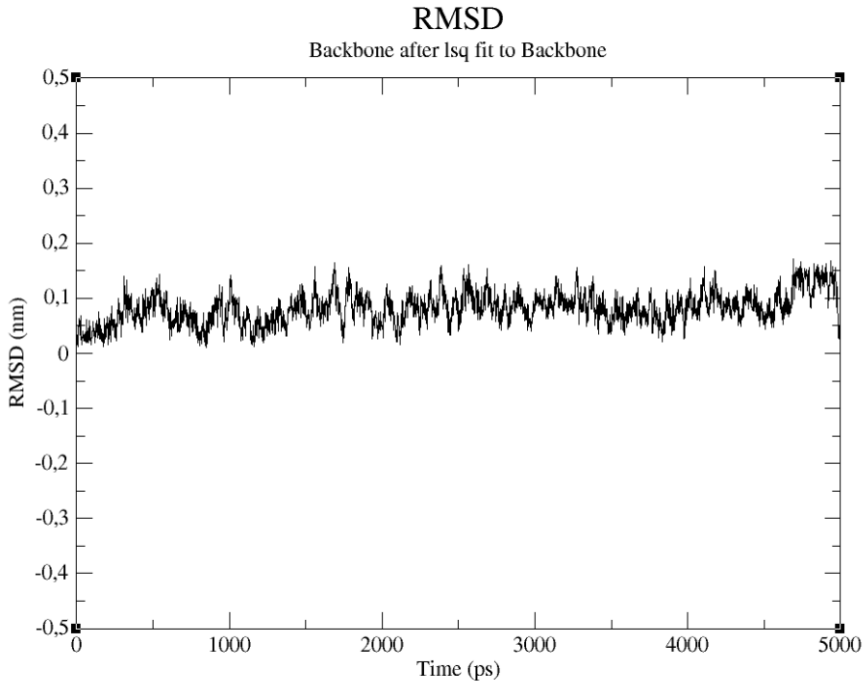


**Figure 3:** The RMSD values of the water medium system of AHK tripeptide.

  

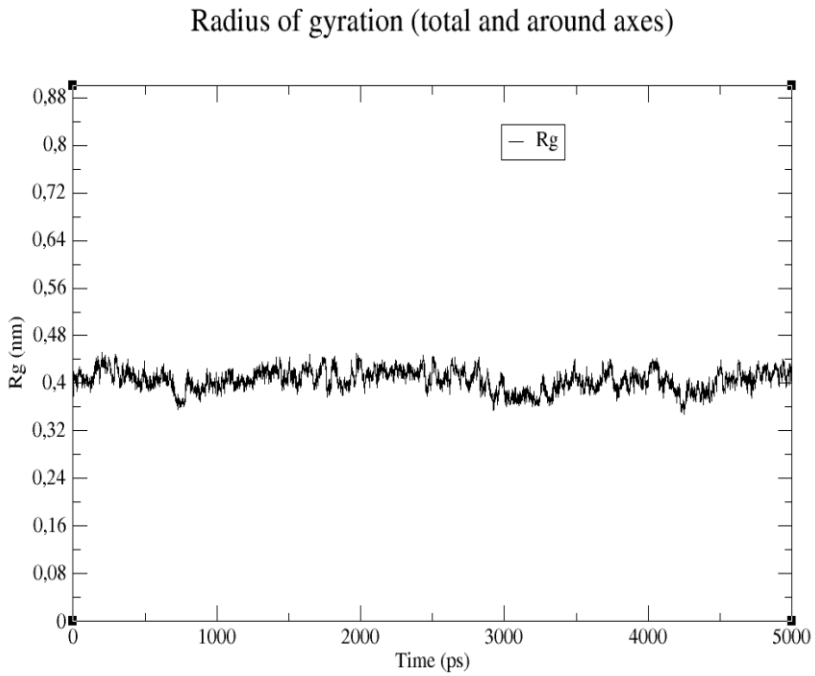


**Figure 4:** The Radius of gyration valuesof the water medium system of AHK tripeptide.

## Molecular Docking and ADME Results

The docking analysis of AHK tripeptide onto a vascular endothelial growth factor receptor 2 (pdb code: 3VO3) was performed and the most possible binding energies were calculated at
-7.769 kcal / mol in **Fig. 5and Fig. 6**. In the active region of the protein in which the AHK tripeptide interacts with, the green, blue, dark blue and orange colored parts represent regions of hydrophobic, polar, positively charged and negatively charged amino acids, respectively in **Fig.7.** The strong hydrogen bonds formed resulted in the formation of stable binding poses between AHK tripeptide and protein. As shown in **Fig.7**. and **Fig.8,** the hydrogen bonds formed with LEU-37 (1,81 Å and 2.39 Å), LYS-117 (1.62 Å), CYS-116 (1.67 Å) and ASN-120 (1.79 Å and 2.18 Å) residues for vascular endothelial growth factor receptor 2. The electrostatic potential of the vascular endothelial growth factor receptor 2 and the docked pose of the AHK was shown in **Fig.9.** The ADME profile, in which the pharmacokinetic properties of AHK was determined by Qikprop tool of the Maestro software in **Table 1.** Pharmacokinetic parameters which are required for predicting the drug-like properties of molecules were defined based on Lipinski 5s rule. According to this rule; the molecular weight should not be greater than 500Mw, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and the octanol / water partition coefficient should not be greater than 5. AHK tripeptide has 354 g/mol molecular weight, 6.5 hydrogen bond donors and 9.5 hydrogen bond acceptors, and the calculated value of octanol / water partition coefficient was-3.615. The rate of skin permeability (SP) is a very important pharmacokinetic property for the transdermal effect of drugs and cosmetics, especially in the fields of medicine and cosmetics. The calculated QP log Kp for skin permeability (Kp in cm/hr) value of AHK tripeptide was -9.743. It is also important to know the ability to cross the blood brain barrier. The calculated brain/blood partition coefficient (QPlogBB) is -2.635 and is within the recommended range of value (-3.0 – 1. 2).

  

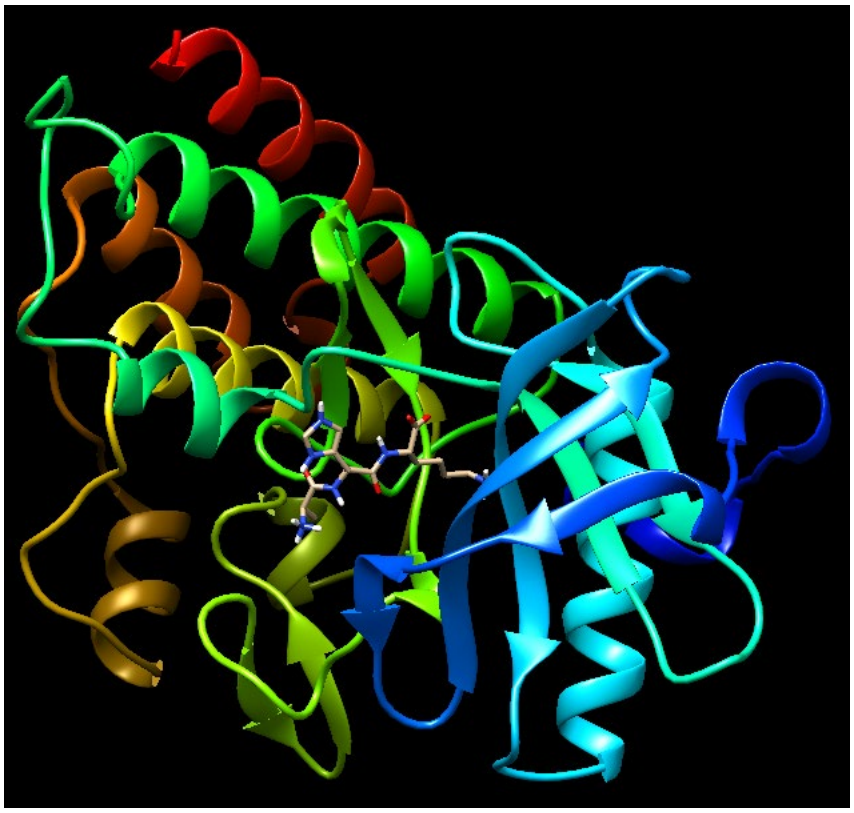

**Figure 5:** The binding poses between the active site of the vascular endothelial growth factor receptor 2 and AHK tripeptide.

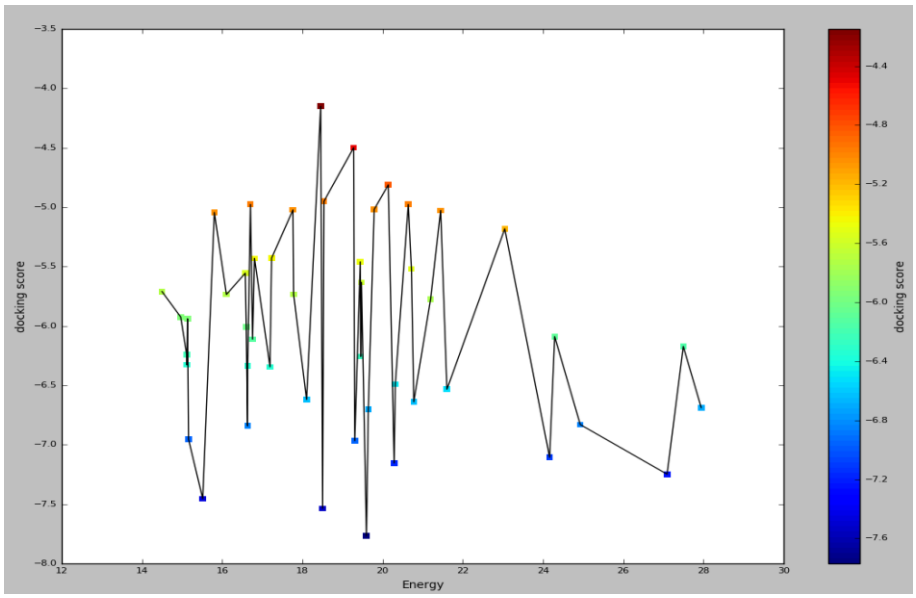


**Figure 6:** The possible docking score values and their energies between the vascular endothelial growth factor receptor 2 and AHK tripeptide.

  

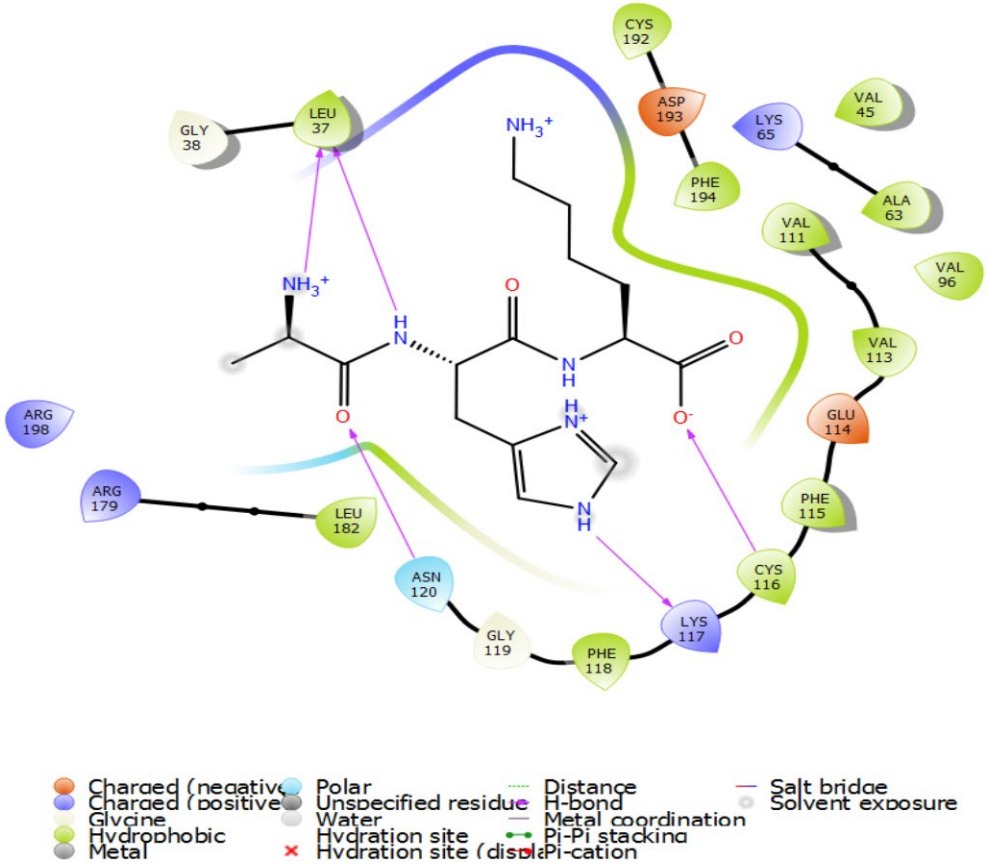


**Figure 7:** 2D ligand interaction of AHK tripeptide in the active side of the vascular endothelial growth factor receptor 2.

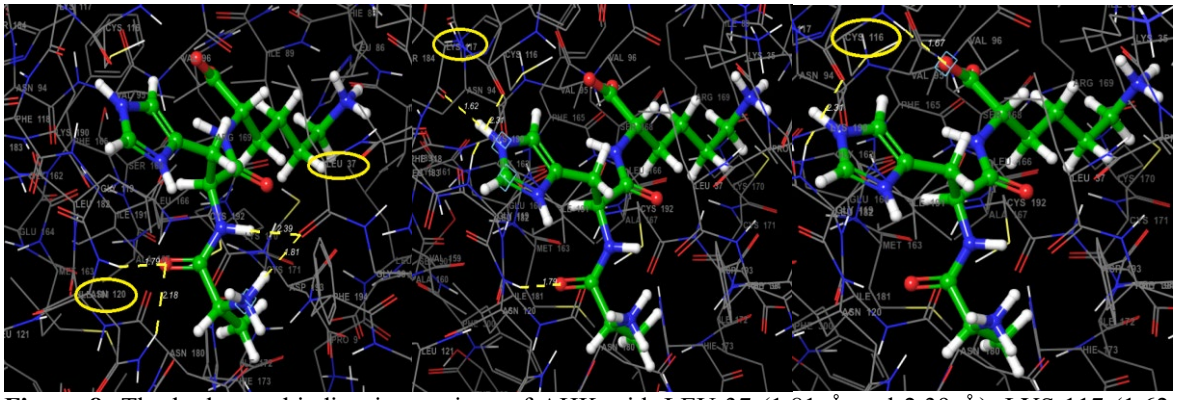

**Figure 8:** The hydrogen binding interactions of AHK with LEU-37 (1,81 Å and 2.39 Å), LYS-117 (1.62 Å), CYS-116 (1.67 Å) and ASN-120 (1.79 Å and 2.18 Å) residues for vascular endothelial growth factor receptor 2

  

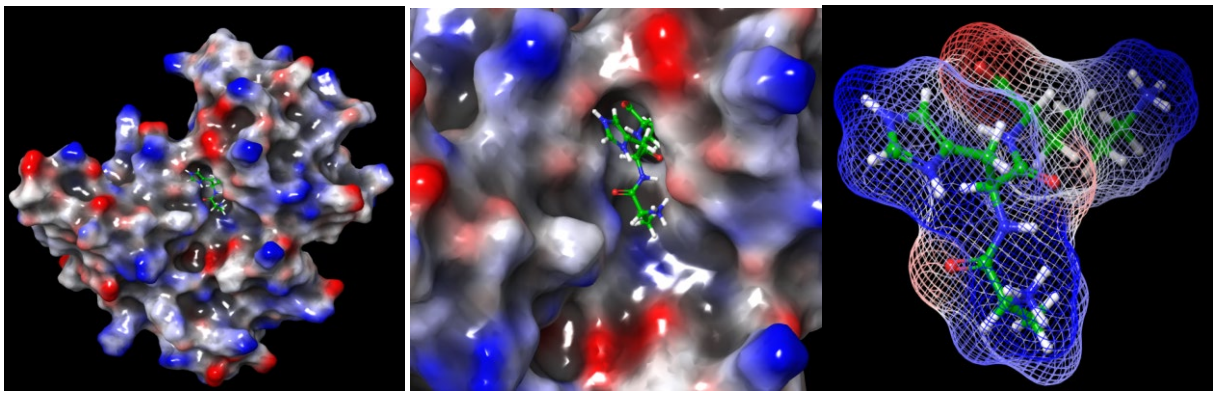

**Figure 9:** The electrostatic potential of the vascular endothelial growth factor receptor 2 and the docked pose of the AHK.

**Table 1:** The calculated ADME properties of AHK tripeptide.

| ***Principal Descriptors:*** | | ***Values*** | ***Recommended Values*** |
|---|---|---|---|
| Solute Molecular Weight | = | 354.408 | (130.0 / 725.0) |
| Solute Dipole Moment (D) | = | 10.434 | (1.0 / 12.5) |
| Solute Total SASA | = | 680.653 | (300.0 /1000.0) |
| Solute Hydrophobic SASA | = | 284.204 | (0.0 / 750.0) |
| Solute Hydrophilic SASA | = | 307.16 | ( 7.0 / 330.0) |
| Solute Carbon Pi SASA | = | 89.289 | ( 0.0 / 450.0) |
| Solute Weakly Polar SASA | = | 0 | ( 0.0 / 175.0) |
| Solute Molecular Volume (A^3) | = | 1191.368 | ( 500.0 /2000.0) |
| Solute vdW Polar SA (PSA) | = | 195.19 | ( 7.0 / 200.0) |
| Solute No. of Rotatable Bonds | = | 13 | ( 0.0 / 15.0) |
| Solute as Donor - Hydrogen Bonds | = | 6.5 | ( 0.0 / 6.0)* |
| Solute as Acceptor - Hydrogen Bonds | = | 9.5 | ( 2.0 / 20.0) |
| Solute Globularity (Sphere = 1) | = | 0.798 | ( 0.75 / 0.95) |
| Solute Ionization Potential (eV) | = | 9.357 | ( 7.9 / 10.5) |
| Solute Electron Affinity (eV) | = | -0.183 | ( -0.9 / 1.7) |
| ***Predictions for Properties:*** | | | |
| QP Polarizability (Angstroms^3) | = | 34.030M | ( 13.0 / 70.0) |
| QP log P for hexadecane/gas | = | 13.579M | ( 4.0 / 18.0) |
| QP log P for octanol/gas | = | 27.160M | ( 8.0 / 35.0) |
| QP log P for water/gas | = | 23.858M | ( 4.0 / 45.0) |
| QP log P for octanol/water | = | -3.615 | ( -2.0 / 6.5)* |
| QP log S for aqueous solubility | = | 0.5 | ( -6.5 / 0.5)* |
| QP log S - conformation independent | = | 0.548 | ( -6.5 / 0.5) |
| QP log K hsa Serum Protein Binding | = | -1.479 | ( -1.5 / 1.5) |
| QP log BB for brain/blood | = | -2.635 | ( -3.0 / 1.2) |
| No. of Primary Metabolites | = | 8 | ( 1.0 / 8.0) |
| Predicted CNS Activity (-- to ++) | = | -- | |
| HERG K+ Channel Blockage: log IC50 | = | -2.114 | (concern below -5) |

  

| | | | |
|---|---|---|---|
| Apparent Caco-2 Permeability (nm/sec) | = | 0 | (<25 poor. >500 great) |
| Apparent MDCK Permeability (nm/sec) | = | 0 | (<25 poor. >500 great) |
| QP log Kp for skin permeability | = | -9.743 | (Kp in cm/hr) |
| Jm. max transdermal transport rate | = | 0 | (micrograms/cm^2-hr) |
| Lipinski Rule of 5 Violations | = | 1 | (maximum is 4) |
| Jorgensen Rule of 3 Violations | = | 2 | (maximum is 3) |
| % Human Oral Absorption in GI (+-20%) | = | 0 | (<25% is poor) |
| Qual. Model for Human Oral Absorption | = | low | (>80% is high) |

## Conclusion

Along with its high antioxidant properties, AHK tripeptide is used in the treatment of hair loss and skin rashes, and it also has an important potential in cosmetics because it helps collagen production and increases dermal cell proliferation and viability. AHK tripeptide which has such important fields of application has been modeled for the first time using *in silico* methods, the most stable geometric structure has been determined, the conformational variations tripeptide in body conditions (water medium) has been examined and also the mechanism of interaction of AHK tripeptide with possible receptor that can effect in the body has been revealed. The discovery and development of peptide structures with a more active and improved mechanism of action is an active field of study, particularly in biochemistry and pharmacology and cosmetic. This study, which reveals the molecular structure, conformational change and mechanism of action of AHK tripeptide, is an original study.

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