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Research Article

ANTICHOLINESTERASE ACTIVITY OF OCTA PEPTIDES RELATED TO HUMAN HISTATIN 8: *IN-SILICO* DRUG DESIGN AND *IN-VITRO*

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ABSTRACT

Objective: To evaluate the octapeptides related to human histatin 8 by *in-silico* and *in-vitro* studies.

Method: Schrodinger, LLC and Ellman's method.

Results: The compound HH1 and HH2 was found to be potent docking score of −9.494 and −7.401 against acetylcholinesterase (AChE) enzyme. The IC_{50} value of HH1 and HH2 was found to be 0.39±0.28 and 0.78±0.15 µg/mL. However, these compounds are shown to be highly effective as compared with the control AChE inhibitor donepezil (0.065±0.0050 µg/mL).

Conclusion: *In-silico* docking study was conducted for the designed octapeptides related to human histatin 8 against AChE enzyme shows significance binding affinity toward HH1 and HH2 peptides and the AChE inhibitory activity of octapeptides shown to be a highly potent inhibitor as compared with control donepezil.

Keywords: Alzheimer's disease, Octapeptides, *In-silico, In-vitro,* Acetyl cholinesterase.

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INTRODUCTION

Most of the drugs approved for AD treatment are acetylcholinesterase (AChE) inhibitors, which improve the AChE level in the brain by decreasing the hydrolysis of AChE. β amyloid (Aβ) formed by the continuously proteolytic processing of β-amyloid precursor protein by β-secretase and γ-secretase, plays a vital part in the pathogenesis of AD [1]. Recent evidence indicated certain links between Aβ and AChE [2]. Right now the development of single molecule may possess multiple concomitant biological properties and would have more advantages than combination therapy due to deficient of drugs which are monofunctional, defeat only a single target. Furthermore, the risk of drug-drug interactions can be reduced, and the therapeutic treatment has significantly simplified and better patient compliance [3]. Current scenario, peptides are the alternative of heterocyclic compounds in pharmaceutical industry. Short peptides are linear molecules having 2-20 amino acids present in the sequence of the peptide. Right now more than 50 healing peptides are available in the market and some of the peptides are in clinical trials [4] (phase I-III). To overcome the risk factor of peptide delivery, balancing of hydrophobic and hydrophilic characters (amphiphile) [5]. Short and ultra-short peptides were inhibiting aggregation of Aβ and reduce its toxic effects. It is also shown to be effective in AD rodent animal models [6]. Based on the above factors, to evaluate the octapeptides related to human histatin 8 by *in-silico* and *in-vitro* method and correlate both. Synthetic part of the octapeptides related to human histatin 8 was published earlier [7].

MATERIALS AND METHODS

Materials

Chemicals

Acetylthiocholine iodide (ATCI), sodium phosphate buffer and AChE were purchased from SIGMA-ALDRICH. 5, 5-dithio-bis-(2-nitrobenzoic acid) (DTNB) from Thermo Fisher Scientific. Buffers and other chemicals were of analytical grade.

Instruments

Ultraviolet–visible *spectroscopy* spectrophotometer from SHIMADZU and incubator from Thermo Scientific.

Methods

Molecular docking studies with octapeptides

Initially, the octapeptides (HH1, HH2, and HH3) sequences *de novo* peptide structure was predicted using the PEP-FOLD server at mobile server portal [bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/].

Before docking the X-ray crystal structures of acetyl cholinesterase (AChE) with pdb id: 1EVE were retrieved from the RCSB Protein Data Bank [8-10]. Their corresponding protein structures were subsequently prepared using the Protein Preparation Wizard in the Schrödinger software suite [11]. The respective AChE structure is optimized by removing the water molecules, heteroatoms, and cofactors. Hydrogen, missing atoms, bonds, and charges were computed. Further, all the three octapeptides were docked to AChE using guide peptide docking procedure [11].

Further, receptor grid was generated around the AChE enzyme active site by choosing centroid of AChE enzyme complexed ligand (aricept), with the grid box size set to 20 Å radius using receptor grid generation panel implemented in Glide [11].

The ligand structures (Fig. 1) were constructed using the splinter dictionary of Maestro 9.3 (Schrodinger, LLC) using the Optimized Potentials for Liquid Simulations-All Atom (OPLS-AA) force field with the steepest descent followed by curtailed Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-AA force field.

All the octapeptides (HH1, HH2, and HH3) docking calculations were performed using standard precision peptide docking mode. The Glide

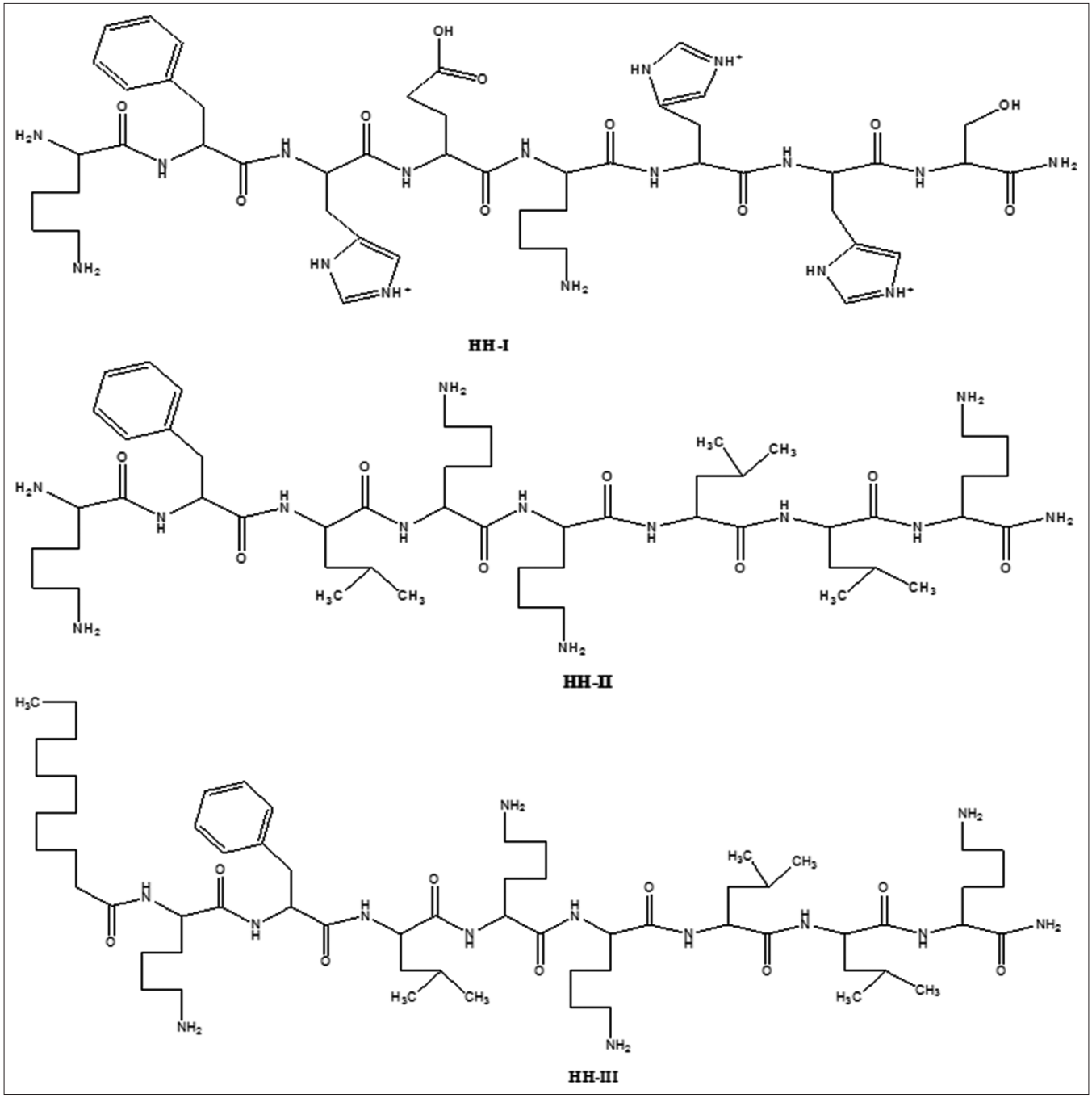

Fig. 1: The ligand structures

docking score was used to determine the best-docked structure from the output. The binding affinity of the AChE/octapeptides complexes was expressed as docking scores. The interactions of these docked complexes were further analyzed using PyMOL [12].

Anti-alzheimer's activity

In-vitro AchE assay

The assay for measuring AChE activity was evaluated based on Ellman's method [13,14]. The principle of this method is based on enzyme hydrolyzes the substrate ATCI into thiocholine and acetic acid. Then, thiocholine is allowed to react with DTNB, and formation of yellow color. The intensity of color formation proportional to the activity of the enzyme.

Briefly, 150 µl of 0.1 M sodium phosphate buffer (pH 8.0), 10 µl of the test compound, and 20 µl of the enzyme solution (0.1 units/mL) were added and incubated for 15 minutes at 25°C. After that followed by mixed with 10 µl of DTNB (10 mM) and 10 µl of ATCI (14 mM) to initiate the reaction, incubate for 10 minutes. The color formation was measured at 410 nm wavelength. The controls contained the solvent to dissolve the compound instead of test compound. The percentage inhibition for each test solution was then calculated using the following equation:

Inhibition (%) = (1- absorbance sample/absorbance control) × 100

RESULTS AND DISCUSSION

Docking with octapeptides on AchE

From docking analysis of all the three octapeptides, it was found that HH1 and HH2 dock to AChE with docking score of −9.494 and −7.401 with the corresponding $IC_{50:}$ 0.39±0.28 and $IC_{50:}$ 0.78±0.15 µg/mL, while HH3 peptide did not dock as the peptide was not stable to bind

to the AChE active site. This result is also in accordance with HH3 $IC_{50:}$ 86.45±0.32 value, showing declined anticholinesterase enzyme inhibition activity. The binding mode of the HH1 peptide to AChE structure shows that the amino terminal end residue Lys1 backbone NH form a hydrogen bond with side chain oxygen of Asn280. While the phe2 of HH1 forms a hydrophobic interaction with side chain of Tyr70. Following it HH1 peptide residue His3 side chain imidazole ring nitrogen forms a hydrogen bond with Gln74 side chain NH, while Glu4 of peptide did not show any key interaction. In the case of Lys6, its side chain aliphatic alkane part forms a hydrophobic interaction with Phe290, Phe331, and Tyr334. Interestingly, face-to-face π-π stacking between Phe330 and with imidazole ring of His6, while the His7 imidazole NH forms a hydrogen bond with Tyr70 backbone oxygen. In addition, the peptide Ser8 backbone forms a hydrogen bond with Tyr130 side chain OH and finally the terminal OH forms a hydrogen bond with one of the side chain oxygen of Glu199 (Fig. 2a).

The binding mode of the HH2 peptide (gray stick) in the AChE active site, where the HH2 peptide residues Lys1 and Lys4 side chain's one of the NH of $NH3^+$ forms a hydrogen bond with Glu73 backbone oxygen and side chain oxygen of Asn280, respectively. While the Leu6 backbone NH forms a hydrogen bond with side chain OH of Tyr70. Meanwhile, the backbone oxygen and NH of Leu7 forms hydrogen bonds with Tyr121 side chain OH. Finally, the carboxyl terminal residue Lys8's side chain forms a hydrogen bond with backbone oxygen is 440 (Fig. 2b).

In-vitro AchE assay

The assay of AChE was based on an improved Ellman's method in a 96 well plate reader using QuantiChrome assay kit (USA). One of the characteristic changes that occur in AD is an increase in AChE activity, the enzyme responsible for acetylcholine hydrolysis, from both cholinergic and non-cholinergic neurons of the brain. The results obtained from the octapeptides related to human histatin 8 against AChE enzyme inhibition activity and the percentage inhibition was evaluated and tabulated in Table 1. IC_{50} value of HH1 and HH2 was found to be 0.39±0.28 and 0.78±0.15 µg/mL. However, these compounds are shown to be highly effective as compared with the control AChE inhibitor donepezil (0.065±0.0050 µg/mL).

Table 1: IC_{50} value of octapeptides related to human histatin 8 and control

S. No	Compounds code	IC_{50} (µg/mL)
	Concentration of compounds (µg/mL)	
1	HH1	0.39±0.28
2	HH2	0.78±0.15
3	HH3	86.45±0.32

Results were expressed as mean±SD (n=3), IC_{50} for donepezil control for AChE inhibition assay was 0.065±0.0050 µg/mL, AChE: Acetylcholinesterase

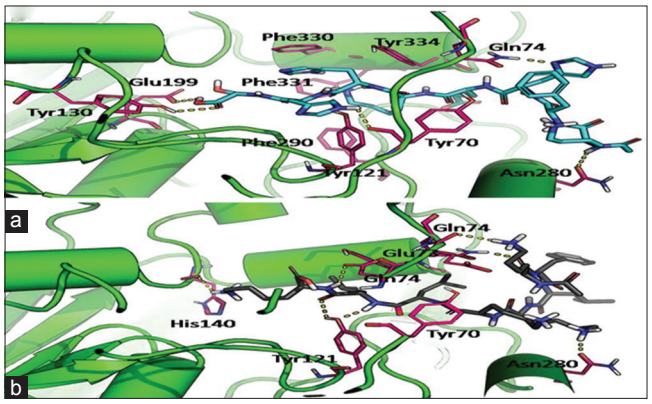

Fig. 2: Shows the binding mode (a) HH1 peptide (blue stick), (b) HH2 peptide (gray stick), in the acetyl cholinesterase active site. The key interacting residues are shown in magenta color lines, and the hydrogen bonds are represented as yellow dashed lines

Statistical analysis

Data were presented as mean±standard deviation. All analyses were performed in triplicates. GraphPad Prism 5 and Microsoft Excel 2007 were used for the statistical and graphical evaluations.

CONCLUSION

In-silico docking study was conducted for the designed octapeptides related to human histatin 8 against AChE enzyme shows significance binding affinity toward HH1 and HH2 peptides. The AChE inhibitory activity of octapeptides related to human histatin 8 was evaluated using Ellman's method, and these compounds are shown to be a highly potent inhibitor as compared with control AChE inhibitor donepezil. *In-silico* results correlates with the *in-vitro* results. The result showed that HH1 and HH2 exhibited more capability to inhibit AChE enzyme.

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