

Short Communication:**Structure-Based Design and Molecular Dynamics Simulations of Pentapeptide AEYTR as a Potential Acetylcholinesterase Inhibitor**Vivitri Dewi Prasasty¹ and Enade Perdana Istyastono^{2,*}¹Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta 12930, Indonesia²Faculty of Pharmacy, Sanata Dharma University, Paingan, Maguwoharjo, Depok, Sleman, Yogyakarta 55282, Indonesia*** Corresponding author:**

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Abstract: Structure-based virtual screening protocol to identify potent acetylcholinesterase inhibitors was retrospectively validated. The protocol could be employed to examine the potential of designed compounds as novel acetylcholinesterase inhibitors. In a research project designing short peptides as acetylcholinesterase inhibitors, peptide AEYTR emerged as one of the potential inhibitors. This article presents the design of AEYTR assisted by the validated protocol and guided by literature reviews followed by molecular dynamics studies to examine the interactions of the pentapeptide in the binding pocket of the acetylcholinesterase enzyme. The molecular dynamics simulations were performed using YASARA Structure in Google Cloud Platform. The peptide AEYTR was identified *in silico* as a potent acetylcholinesterase inhibitor with the average free energy of binding (ΔG) of -19.138 kcal/mol.

Keywords: acetylcholinesterase; pentapeptide; molecular dynamics; YASARA Structure; Google Cloud Platform

■ INTRODUCTION

The approval of potent acetylcholinesterase inhibitors (AChEIs) to treat Alzheimer's Disease (AD) has shown benefits both medically and economically [2], since the approval of tacrine in 1993 [1]. For example, the cost saving of the medication use of AChEI donepezil (AriceptTM) could reach \$2408 in 1 year during 1997–1999 [2]. Recent studies reported that the continuing use of donepezil for treating AD was more cost-effective than discontinuation [3]. The studies also reported that the use of donepezil alone is not less cost-effective compared to the use of donepezil-memantine combined [3]. On the other hand, it is predicted that the number of people with dementia will increase in the coming years [4]. Targeting acetylcholinesterase (AChE) to develop novel drugs to treat AD has therefore, become of considerable interest [1,5] despite the expiration of the AriceptTM patent in November 2010.

Current advances in computational medicinal chemistry can significantly increase the efficiency of drug

discovery [6]. Integration of available and accessible computational tools is highly encouraged to avoid pitfalls in computer-aided drug discovery [6-11]. One of the suggested integration is the “focused integration”, which employs filtering molecules to compile a focused library that contains only compatible and favorable molecules to be screened subsequently [10-12]. Molecular interaction fingerprints play an important role in this integration [10,12-16]. Combined with other docking scores, molecular interaction fingerprints could produce an ensemble-based scoring function to increase the prediction ability of the developed method [6], e.g., the use of ensemble Protein-Ligand Interaction Fingerprints (ensPLIF) to increase the prediction ability of structure-based virtual screening (SBVS) protocols targeting estrogen receptor alpha [17] and AChE [18]. The structure-based approach to discover new AChEIs was developed to publicly provide a computer-aided protocol to predict the activity of designed compounds as potent AChEIs [18]. The *F*-measure value of the SBVS

protocol to identify potent AChEIs is 0.413 [18], which is higher than the reference SBVS protocol (F -measure = 0.226) [19].

In this article, a structure-based *de novo* design of a pentapeptide as a potential AChEI guided by literature [20] and assisted by a validated SBVS protocol [18] is presented. Following the design, molecular dynamics (MD) simulations of the selected peptide in the binding pocket of AChE using YASARA Structure [21] have been performed to obtain more insights into the enzyme-inhibitor interactions. Due to current issues of energy efficiency in green computing [22], we were tempted to perform the MD simulations in Google Cloud Platform (<https://cloud.google.com/>). The usage of this platform has not been reported yet in running MD simulation before. The design led to the identification of pentapeptide AEYTR as a potential AChEI, which was confirmed by the production run of the MD simulations resulting in the average free energy of binding (ΔG) of -19.138 kcal/mol.

■ COMPUTATIONAL DETAILS

Materials

All 400 SMILES formats of tetrapeptides starting with AE as the two first amino acids were provided by Prasasty et al. [20]. Module *molconvert* (Molecule File Converter) version 17.13.0 from ChemAxon Ltd. (<https://chemaxon.com/>) was used to build the SMILES format of the designed pentapeptides from their one-letter-code amino acid sequences. The retrospectively validated SBVS protocol developed by Riswanto et al. [18] installed in a gold package of virtual private server provided by Rumahweb Indonesia (<https://www.rumahweb.com/vps-indonesia/>) was used to perform *in silico* tests on the designed peptides. Molecular dynamics simulations were performed by employing YASARA Structure [21] version 19.5.5 in a virtual machine with 8 virtual central processing units (CPUs), 30 GB memory, 20 GB persistent disk and Ubuntu 16.04 LTS as the operating system provided by Google Cloud Platform (<https://cloud.google.com/>). A working station with Intel® Pentium® Silver N5000 as the CPU and 4 GB memory and Windows 10 Home as the operating system were used as

the computer client to communicate and to control the virtual servers.

Procedure

Design and in silico tests of peptides

The SMILES formats of all 400 tetrapeptides starting with AE as the first two amino acids were objected to previously published SBVS protocol to identify potent AChEIs [18,20]. Based on the results of the virtual screening and guided by literature reviews [20], some pentapeptides were designed. The list of one-letter-code amino acid sequences of the designed compounds was then converted to their SMILES forms by employing module *molconverter* and virtually screened by employing the SBVS protocol [18].

Molecular dynamics simulations

The pentapeptide identified as a potential AChEIs was subjected to MD simulations by using YASARA Structure. The best pose of the hit in the virtual screening was selected and later prepared in a scene mode in YASARA Structure using the default mode. The scene mode was then subjected to MD simulations using the default settings of YASARA Structure macro for MD run (http://www.yasara.org/md_run.mcr) [21], except for the length of MD and the intervals of taking snapshots. In this research, the length of the MD run was 10 ns, and the snapshots were taken at every 10 ps intervals [23]. The production runs were analyzed in the last 5 ns of the simulations, and the equilibrium states were considered reached if the average deviation of the root-mean-squared distances (RMSD) value of the backbone atoms was less than 1 Å [23]. The free energy of binding (ΔG) of the value of each snapshot of the enzyme-ligand binding in the last 5 ns of the MD simulations was also calculated by employing VINA [24] local search in YASARA structure after the enzyme and peptides atoms were fixed [21].

■ RESULTS AND DISCUSSION

This research aimed to design a pentapeptide as a potential AChEI, where the retrospectively validated SBVS protocol [18] was employed, and literature reviews were performed [20]. Molecular dynamics simulations

of the designed pentapeptide in the binding pocket of AChE were also performed to gain insights for further drug discovery processes. To explore the applicability of Google Cloud Platform as a cloud computing service, the MD simulations were performed in a virtual machine in the platform.

The virtual screening of all 400 tetrapeptides starting with amino acids AE resulted in 4 peptides identified as potent AChEIs. The identified tetrapeptides were AEKY, AERW, AEYQ, and AEYT (Fig. 1). The starting AE amino acids were selected since the most potent peptide as AChEI extracted from human was AEFHRWSSYMVHWK [20]. Additional K amino acid at the beginning of AEFHRWSSYMVHWK was reported detrimental for the activity [20]. The literature review reported that the shortest peptides as AChEIs were pentapeptides and the

5th amino acid in the most potent peptide from human as AChEI and in the most potent AChI colivelin, SALLRSIPAPAGASRLLLLTGEIDL was R [20]. Following the findings resulted from the *in silico* tests and equipped by the information about the 5th amino acid, pentapeptides AEKYR, AERWR, AEYQR, and AEYTR were constructed and virtually tested [18].

The pentapeptide AEYTR (Fig. 1) was the only pentapeptide identified as the potent AChEI in the *in silico* tests. The peptide showed ensPLIF #302 value ≥ 0.878 (0.952), ensPLIF #365 value < 0.678 (0.460), and ensPLIF #208 value ≥ 0.164 (0.312) which were the 1st key to be identified as potent AChEI [18]. These ensPLIFs #302, #365, and #208 represent hydrophobic interaction to F³³¹, hydrophobic interaction to G⁴⁴¹, and hydrogen bond to S²⁰⁰, respectively, in the binding pocket

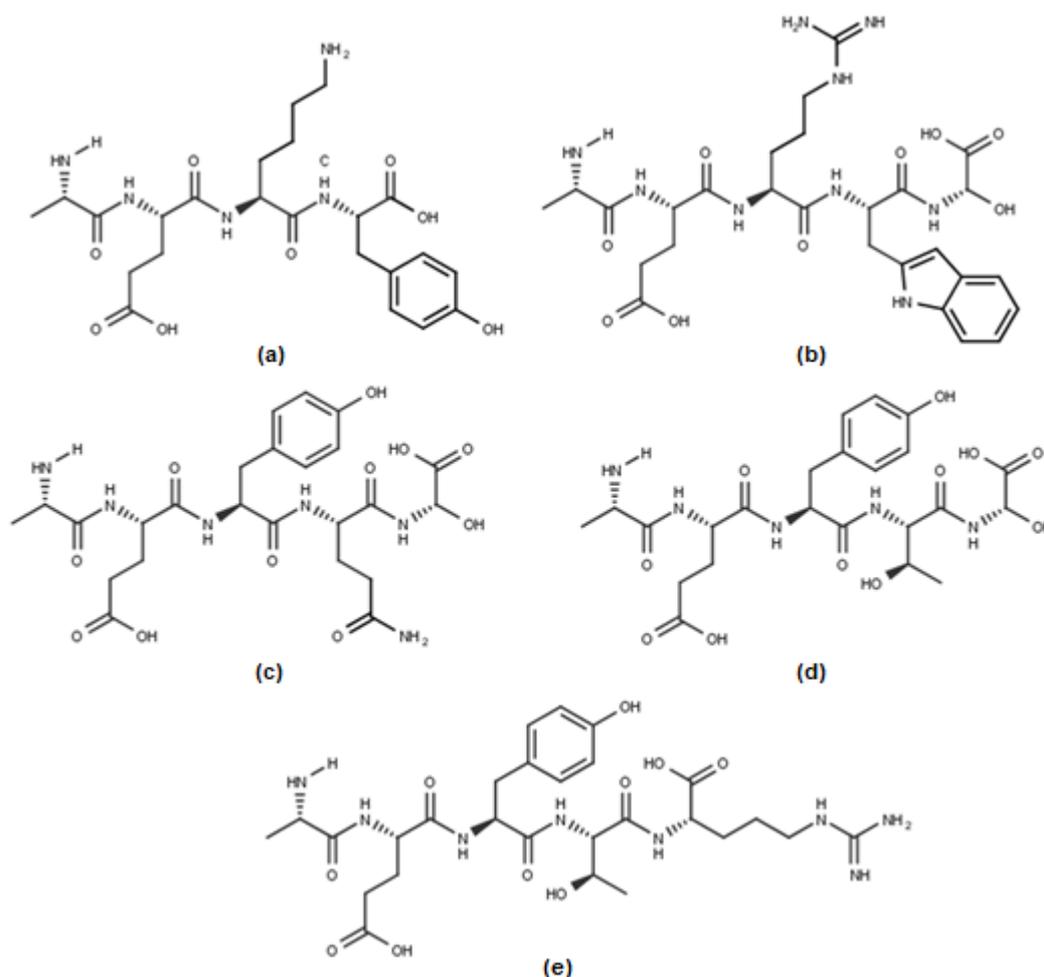


Fig 1. The two-dimensional structures of the AChE inhibitors identified in the virtual screening campaigns: AEKY (a), AERW (b), AEYQ (c), AEYT (d), and AEYTR (e)

of AChE (Fig. 2(a)) [18,25]. In the point of view of the endogenous substrate acetylcholine, the AE amino acids could act as the bioisostere of the acetyl moiety, while the R amino acid could serve as the bioisostere of choline. The additional YT amino acids in the sequence numbers 3 and 4 increase the probabilities to have a hydrophobic interaction to F³³¹ and a hydrogen bond to S²⁰⁰ in the binding pocket of AChE.

Molecular dynamics simulations by employing the best pose of AEYTR in AChE resulted from the previous SBVS campaigns as the initial point were then performed to examine the stability of the enzyme-inhibitor binding [23]. The average deviation value of the RMSD of the backbone atoms of the enzyme during the production run

was 0.204 Å with a standard deviation value of 0.089 Å (Fig. 3(a)). It indicated that the equilibrium had been reached before 5 ns in the MD simulations. These results are in line with those suggested by Liu et al. based on their examination on 10 ns MD simulations of 120 protein-ligand complexes [23]. Therefore, the stability of the AEYTR binding to AChE could be analyzed, and the ΔG could be calculated.

The RMSD values of the AEYTR in the production run of the MD simulations ranged from 2.068 Å to 2.539 Å. The average deviation value of the RMSD was 0.223 Å with a standard deviation value of 0.070 Å (Fig. 3(a)). This small shift reduced the chance to have a hydrogen bond to S²⁰⁰, but the chance of the hydrophobic interaction

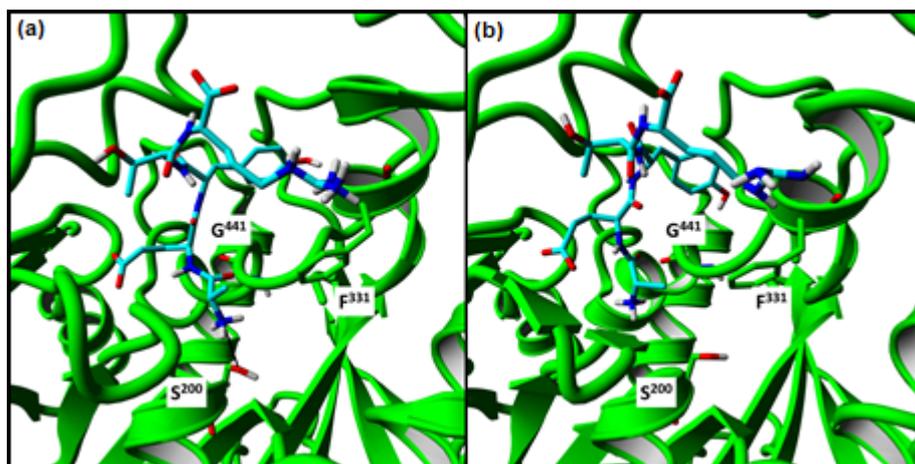


Fig 2. The pentapeptide AEYTR (depicted as sticks with carbon atoms colored with cyan) in the binding pocket of AChE (depicted as cartoon with carbon atoms colored green) resulted from the SVBS campaigns and used as the initial point of the MD simulations (a) and the pose of the MD simulations at 10 ns (b). The residues S²⁰⁰, F³³¹, and G⁴⁴¹ are shown since these residues are the important residues in the identification of AEYTR as a potent AChEI. Nitrogen, oxygen, and hydrogen atoms are colored with blue, red and white respectively. The black line represents the hydrogen bond. For the sake of clarity, non-polar hydrogens and residues 275 to 300 are not shown

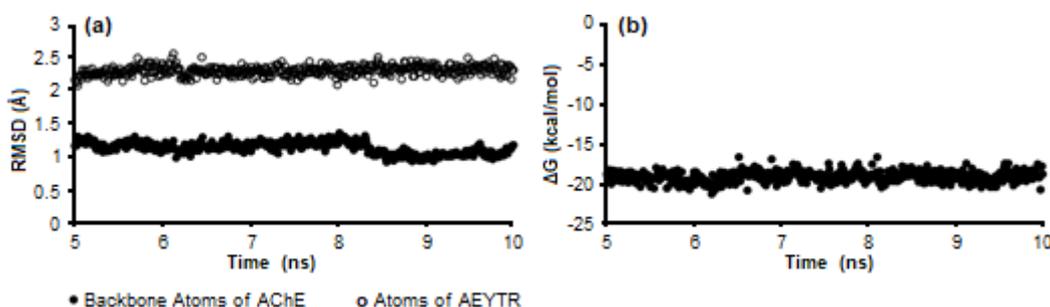


Fig 3. The RMSD values of the backbone atoms of AChE and the atoms of pentapeptide AEYTR during production run (a); The free energy of binding (ΔG) values of AChE-AEYTR during production run (b)

to F³³¹, as the most important interaction in this enzyme-inhibitor case, and the hydrophobic interaction to G⁴⁴¹ [18], remained high (Fig. 2(b)). Although the poses of AEYTR was not stable during equilibrium processes indicated by RMSD values of more than 2.0 Å, the poses remained stable in the production run of the MD simulations [23]. The ΔG calculations were, therefore, valid for the poses in the production run [21,23]. The calculation of ΔG was still considered underdeveloped in YASARA Structure [21]. Fortunately, the ΔG calculation could be performed by employing Autodock VINA [24] in YASARA Structure. The docking simulations employed the macro *dock_runlocal.mcr* with VINA as the docking software in YASARA Structure [21]. The enzyme AChE and the peptide AEYTR from each snapshot of the MD's production run were converted to pdb files and were subsequently fixed to avoid the pose searching phase in the docking simulations. These resulted in the average ΔG value of -19.138 kcal/mol with a standard deviation value of 0.701 kcal/mol (Fig. 3(b)). These values were equal to inhibition constant (K_i) values in subpicomolar [21] indicating that the pentapeptide AEYTR was a potent AChEI. *In vitro* tests will be performed as the ultimate confirmation of the *in silico* results [18,26] and discussed elsewhere.

The MD simulations took 1,575 min in Google Cloud Platform. The platform for the simulations cost circa 203.55 Indonesian Rupiah (IDR) or 0.015 US Dollar (USD)/min. Thus, the computational cost of the MD simulations was about 320,584.14 IDR or 22.91 USD in total. It was considered efficient, especially for research groups that do not have access or have limited access to high performance computing facilities. Besides green computing issues [22], this affordable price in using cloud computing platform could serve as a pivotal strategy in computer-aided drug discovery and development in the near future.

■ CONCLUSION

The pentapeptide AEYTR was identified as a potential hit to be developed as AChEI by employing the retrospectively validated SBVS protocol. The subsequent MD simulations using YASARA Structure in Google Cloud Platform showed that the AChE was stable during

the production run. Vice versa, the AEYTR poses in the binding pocket of AChE were also stable in the production run. Moreover, the ΔG values of the AChE-AEYTR indicated that the pentapeptide could serve as a potent AChEI. However, this finding should be confirmed by *in vitro* tests.

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