MOLECULAR DOCKING AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF PSORALEN DERIVATIVES

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ABSTRACT

Alzheimer Disease (AD) is a deadly neurodegenerative disease which cause irreversible memory loss and progressive cognitive dysfunction, together with impaired language skill and personality changes. Even though the exact cause of AD is not fully understood, some factors such as low levels of neurotransmitter acetylcholine (ACh) is believed to play a vital role in the progress of AD. Hence, the most promising method for the treatment of AD is to increase the levels of ACh in the brain by inhibiting the acetylcholinesterase (AChE) enzyme. However, due to the complex nature of AD, standard drugs with AChE inhibitors such as galanthamine, donepezil, rivastigmine and tacrine can only alleviate the symptoms but cannot cure neurodegeneration. Thus, it is significant to develop multifunctional drugs which are Multi-Target Directed Ligands (MTDLs) as the best approach for the treatment of AD. Based on previous studies, coumarin derivatives possess a wide range of biological activities such as a potent AChE inhibitor. Thus, the objectives of this study are to carry *in silico* evaluation of the extracted AChE protein and perform molecular docking of psoralen derivatives which is also known as furocoumarin, with AChE protein. Acetylcholinesterase inhibitory activity of psoralen derivatives was also conducted. Results from molecular docking shows potential of compound **(21)** as AChE inhibitors due to its highest binding energy value. It was further supported by the result from acetylcholinesterase inhibitor activity, whereby compound **(21)** has 91.69% inhibition which is comparable to galantamine (94.12%).

ABSTRAK

Penyakit Alzheimer (AD) adalah penyakit neurodegeneratif yang menyebabkan pesakit mengalami kehilangan ingatan yang sukar dipulihkan serta menyebabkan kegagalan dalam kemampuan berkomunikasi dan perubahan personality. Walaupun punca sebenar AD masih belum dijumpai, beberapa faktor seperti tahap pemancar-neuro asetilkolin (ACh) yang rendah dipercayai memainkan peranan penting dalam perkembangan AD. Oleh itu, kaedah yang paling berpotensi untuk rawatan AD adalah meningkatkan tahap ACh di otak dengan merencat enzim asetilkolinesterase (AChE). Walau bagaimanapun, disebabkan sifat AD yang kompleks, ubat-ubatan yang sedia ada dengan perencat AChE seperti galantamine, donepezil, rivastigmine dan tacrine hanya dapat mengurangkan gejala tetapi tidak dapat menyembuhkan neurodegenerasi. Oleh itu, adalah sangat penting untuk menghasilkan ubat-ubatan pelbagai fungsi yang merupakan ‘Multi-Target Directed Ligands (MTDLs)’ sebagai pendekatan terbaik untuk rawatan AD. Berdasarkan kajian sebelum ini, terbitan kumarin mempunyai pelbagai aktiviti biologi, salah satu nya adalah perencat AChE. Oleh itu, objektif kajian ini adalah untuk melakukan penilaian *in silico* terhadap protein AChE yang diekstrak dan proses mengedok molekul antara terbitan psoralen yang juga dikenali sebagai furocoumarin, dengan protein AChE telah dijalankan. Ujian perencatan asetilkolinesterase bagi terbitan psoralen juga telah dilakukan. Hasil dari molekul dok menunjukkan potensi sebatian **(21)** sebagai perencat AChE kerana ia mempunyai nilai tenaga pengikatannya yang tertinggi. Ini disokong lagi oleh hasil dari aktiviti perencat asetilkolinesterase, di mana sebatian **(21)** mempunyai perencatan sebanyak 91.69% yang setanding dengan galantamine (94.12%).

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LIST OF ABBREVIATIONS

AD	-	Alzheimer Disease
ACh	-	Acetylcholine
AChE	-	Acetylcholinesterase
BuChE	-	Butyrylcholinesterase
IC_{50}	-	Half-maximal inhibition concentration

LIST OF SYMBOLS

Å - angstrom

nM - nanomolar

µL - microlitre

π - pi

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Alzheimer’s disease (AD), the most common form of dementia (Fernandez-Martos *et al.* (2017), is a deadly neurodegenerative disease that commonly attack elderly people around the world. Nowadays, dementia is estimated to affect more than 46 million people worldwide and the World Health Organization estimates that the number may reach approximately 131 million by 2050, with the number doubling every 20 years (Zhang *et al.*, 2018). In persons with Alzheimer’s disease (AD), stigma and how others treat them, such as acting in ways that discriminate, patronize, or isolate them can affect how they perceive themselves, such as feeling they are less worthy or are incompetent (Stites *et al.*, 2018).

Most patients with AD experience neuropsychiatric symptoms (NPSs) as they are progressing with their illness. These symptoms include depression, anxiety, apathy, agitation, disinhibition, motor disturbances, delusions, and hallucinations, whereby most are identified to have multiple symptoms, with around half experiencing four or more (Connors *et al.,* 2018). Apart from that, they might have difficulties with memory, language, problem-solving and other cognitive skills that make a person loses the ability to carry out everyday activities. According to Alzheimer’s Association Report on ‘2018 Alzheimer Disease’s Facts and Figures’, neurons in other parts of the brain of AD patients are eventually damaged, including those that responsible in enabling a person to perform basic bodily functions such as walking and swallowing. The profound irreversible memory loss and progressive cognitive dysfunction, together with impaired language skill and personality changes, make AD a terrible disease for patients and their families, as well as making it a potential social and economic crisis of 21st century (Zhang *et al.*, 2018). People in the final stages of the disease are bedridden and require full-time care. Alzheimer's disease is ultimately fatal.

This disease has drawn attention among the researchers to find new potent drugs to treat AD more efficiently. Although the etiology of AD is not fully understood, some factors such as low levels of neurotransmitter acetylcholine (ACh), the aggregation of b-amyloid peptide, dyshomeostasis of biometals, hyperphosphorylation of τ-protein, and oxidative stress, are believed to play important roles in the pathogenesis of AD (Yang *et al.*, 2017). Accordingly, the most promising approach for the treatment of AD is to increase the levels of ACh in the brain by inhibiting the acetylcholinesterase (AChE) enzyme, which is mainly responsible for its hydrolysis and termination of action. AChE inhibitors such as galantamine, donepezil, rivastigmine and tacrine are the main stay drugs for the clinical management of AD (Anand *et al.*, 2012). However, due to the complex nature of AD, these drugs only work in alleviating the symptoms but cannot cure neurodegeneration and stop brain damage (Xie *et al.*, 2016). Thus, multifunctional drugs which are Multi-Target Directed Ligands (MTDLs) have been chosen as the best approach for the treatment of AD.

Coumarins are the leading scaffold in design novel drugs because the compounds are proven to possess a wide range of biological activities as reported by previous studies. Coumarins are an important class of natural compounds, used as additives in both foods and cosmetics (Ali *et al.*, 2016). Coumarin has been reported to have antibacterial (Hu *et al.*, 2018), anti-oxidant (Nenad Vukovic *et al.*, 2010), anti-inflammatory (Witaicenis *et al.*, 2014), and anticoagulant (Monti *et al.*, 2007), antituberculosis (Keri *et al.*, 2015), anticancer (Lv *et al.*, 2017) and anti-AD activities (Lan *et al.*, 2017). Coumarin's structure consists offused benzene and a pyrone ring that serves as the structural nucleus. Studies have shown that naturally occurring as well as the chemically synthesized coumarin analogs exhibit potent AChE inhibitory activity (Anand *et al.*, 2012).

Recent studies have shown the use of computational methods as one of effective, less time-consuming and cost-saving method in drug design (Prada-Gracia *et al.*, 2016). It consists of a powerful toolbox for discovery and optimization of drug candidate molecules. Basically, there are two methods for computational drug design, which are structure based and ligand based, depending on the available information on the target (Katsila *et al.*, 2016). The software and techniques to perform the computational methods are selected based on the purpose of study. Among them, molecular docking and molecular dynamic are the most common approaches in the development of drugs (Tautermann *et al.*, 2015).

Docking is a technique which involves predicting the best orientation of ligand in the active site of the receptor when bound to each other to form a stable complex (Gupta Meenakshi *et al.*, 2018). Due to its ability to determine the binding-conformation of small molecule ligand to the suitable target binding site, this method was frequently used in structure-based drug design (Leach *et al.*, 2006). Correspondingly, the number of molecules can be virtually screened for biological activity in the early stage of drug development. There are two types of docking, Rigid docking and Flexible docking (Forli *et al.*, 2016). In rigid docking, protein and ligand are fixed so that there is no change in the bond angles or lengths. Even though this type of docking is extremely fast, it lacks its practical use since it neglects the conformational degrees of freedom of ligands. On the other hand, flexible docking requires much more time, but it is more preferably and widely used since it allows conformational shifts.

Thus, synthesizing potential novel anti-Alzheimer agents based on coumarin compounds will be beneficial to human and pharmacological studies. The role of specific position in the structure will be evaluated with respect to biological activity.

1.2 Problem Statement

Multifactorial nature of AD causes limited therapeutic success when treated with acetylcholinesterase (AChE) inhibitors. Thus, the development of novel multifunctional drugs which are Multi-Target Directed Ligands (MTDLs) is a suitable approach strategy for the treatment of AD. Coumarins are chosen as a leading scaffold in designing novel drugs because the compound has been proven to possess high potential for AChE inhibitor as reported by previous studies.

Since data on the structure of AChE is available, it is possible to use computational methods to study new drug (ligands) via molecular docking. In this way, a deeper understanding on the drug and targeted enzyme interactions can be obtained. For AD patients with developed resistance to most of the available drugs, this study focused on the possible use of novel psoralen derivatives as AChE inhibitors.

A series of psoralen derivative have been synthesized using the point of diversity at amide portion as inactivating agent. It is expected that one of the compounds would have an excellent potency in the *in-silico* investigation. Data from the docking results would be able to show which of the synthesized compounds has more potential as AChE inhibitor, since the results evaluation are based on binding free energy (BE). Biological evaluation for the most potent compounds will be carry out to test for its cholinesterase inhibitory activity.

1.3 Research Objective

The objectives of this research are:

i. To carry *in silico* evaluation for the extracted AChE protein 3D structure and to confirm its binding site.
ii. To perform molecular docking of the psoralen derivatives with AChE protein.
iii. To conduct biological evaluation for cholinesterase inhibitory activity of psoralen derivatives.

1.4 Scope of Study

The scopes of this study are to carry *in silico* techniques such as molecular docking to find out more details on catalytic binding mode of the AChE protein with the psoralen derivatives. The 3D structure of the AChE protein (code: 1EVE) used in this study was extracted from Protein Data Bank server (PDB). The psoralen derivatives were taken from previous researcher in the same laboratory (Faten, 2017). The compound was chosen due to the presence of chlorine and methoxy group, which has been proven to enhance AChE inhibition by previous researches. Evaluation programs such as PROCHECK, ERRAT and Verify3D were used to assess the quality of the AChE protein 3D structure.

AChE protein-ligand complex structure conformations were modelled computationally by molecular docking calculations. Discovery Studio (DS) was also used to analyze and visualize the 2D diagram to confirm the exact ligand –AChE protein binding site. The compounds with highest potential as AChE inhibitor will also be tested for cholinesterase inhibitory activity.

1.5 Significance of Study

The significance of this research is to produce a potent coumarin derivatives, specifically for the treatment of Alzheimer due to the drawbacks of current commercialized drugs. Previous studies has found the potential of coumarin as acetylcholinesterase (AChE) inhibitor. Thus, producing potential novel anti-Alzheimer drugs based on coumarin compounds would be beneficial to human and pharmacological studies. The presence of functional groups such as chlorine, methoxy and amide has been found to enhance the inhibiting function. The use of in-silico method will help to narrow down choices into only a few potent AChE inhibitors, making the process less time-consuming.

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