Investigation of Acetylcholinesterase Inhibitory Effects of Some New Hydrazides and Molecular Modeling Studies

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Abstract

In this study, the ability to suppress enzyme activity was explored for three substances, namely 5, 6, and 9 compounds. One possible method for controlling Alzheimer's disease and its related health concerns is to reduce the action of acetylcholinesterase (AChE). All three chemicals, 5, 6, and 9, showed a decrease in AChE (acetylcholinesterase) enzyme activity, according to the data. Visual depictions (Figures 1, 3, and 5) make the inhibition by concentration dependency very evident. Based on these results, these chemicals may be useful in treating Alzheimer's disease and other conditions associated with acetylcholinesterase (AChE) enzyme activity. You can see how each chemical interacts with the AChE (acetylcholinesterase) enzyme down to the amino acid level in the two-dimensional interaction maps (Figures 2, 4, and 6). Importantly, amino acids such as ARG 296 and SER 293 were shown to interact via hydrogen bonding, highlighting their function in the inhibitory mechanism. Furthermore, the red separation connections in the interaction maps indicate that the water molecules have left the enzyme's active site, which causes a change in the enzyme's structure and the end of catalytic events. This work lends credence to the idea that chemicals 5, 6, and 9 compounds might be useful as therapeutics for diseases like Alzheimer's that are characterized by increased activity of the acetylcholinesterase enzyme. The safety and effectiveness of these chemicals as possible ant Alzheimer medicines might be further evaluated with more study, such as in vivo investigations and clinical trials

Keywords: *AChE (acetylcholinesterase), Inhibition, Alzheimer*

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder associated with memory impairment and cognitive deficit. It is characterized by low levels of acetylcholine in the brain of AD patients. According to the cholinergic hypothesis, the inhibition of acetylcholinesterase (AChE), an enzyme that catalyzes acetylcholine hydrolysis, increases the levels of acetylcholine in the brain, thus improving cholinergic functions in AD patients. Furthermore, although the general consensus concludes that AChE inhibitors (AChEi) can alleviate AD symptoms, they neither delay nor reverse the disease progress. Most of the drugs currently available for the treatment of AD are AChEi: tacrine (1), donezepil (2), rivastigmine (3) and galanthamine (4), all of which have limited effectiveness and some kind of side effect [1]. Tacrine (1) and donepezil (2), both from synthetic origin, were the first drugs approved for the treatment of cognitive loss in AD patients by US-FDA in 1993 and 1996, respectively. Rivastigmine (3) was approved in 2000 (US-FDA) and was designed from the lead compound physostigmine, a natural AChEi alkaloid. Galanthamine (4), a natural alkaloid first obtained from Galanthus spp. was approved by US-FDA in 2001. Huperzine A (5), an alkaloid found in Huperzia spp., is an AChEi commercialized as a dietary supplement for memory support and it is used to treat AD symptoms in China. This alkaloid has been thoroughly studied with promising results yielded particularly from the evaluation of cognitive performance of animals as well as from studies on its efficacy, tolerance and safety. Taking into account that inhibitors 3, 4 and 5 are related to natural products and that AChEi are an important therapeutic strategy for the treatment of AD, many research groups have focused their studies on naturally-occurring compounds from plants as potential sources of either new or more effective AChEi. These studies led to the discovery of an important number of secondary metabolites as well as plant extracts, both of which are characterized by their ability to inhibit AChE. On the other hand, the fact that a significantly relevant number of

research papers has been recorded in this field during the last decades can be clearly attributed to the development of colorimetric methods which allow a rapid and facile screening of a large number of samples. Ellman's method is the most widely used for the detection of AChEi, even in complex mixtures, and for the quantification of anti-AChE inhibitory activity [2-3]. Several reviews on the newly discovered AChEi obtained from plants, fungus and marine organisms have also been published over the last years [4-5]. The majority of these AChEi belong to the

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alkaloid group, including indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. On the other hand, several non-alkaloidal and potent AChEi have been obtained from natural sources, including terpenoids, flavonoids and other phenolic compounds. Interestingly, although literature demonstrates to be rich in the study on AChEi obtained from plants, this issue keeps on being the center of attention for research as confirmed by the increasing number of studies published every year. Therefore, the purpose of this review is to provide a comprehensive summary of the, on plant-derived compounds, plant

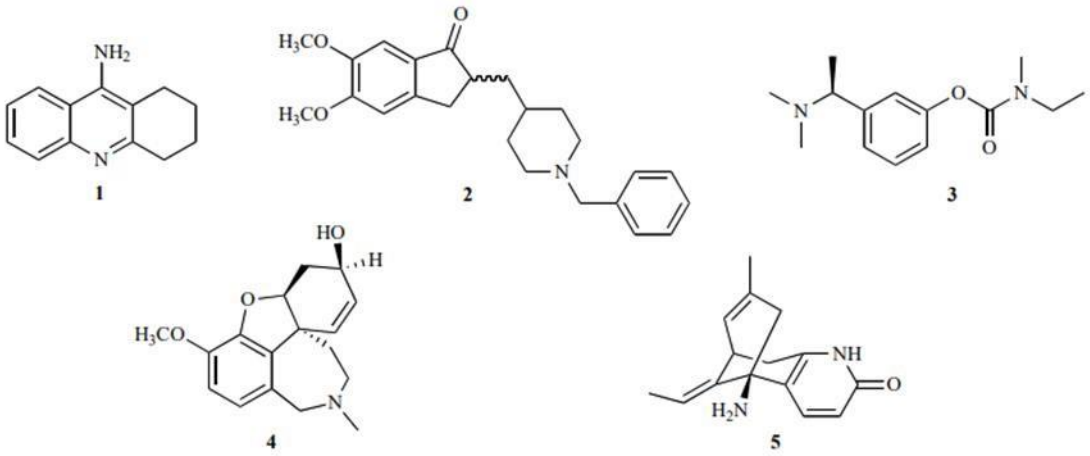


Figure 1 The compounds of some medications currently available to treat Alzheimer's disease are AChEi extracts and essential oils which have been reported to inhibit AChE. Readers interested not only in previous findings but also in synthetic/semisynthetic AChEi or natural AChEi of fungal, marine or microbial origin are recommended to see the above-mentioned reviews [i.e. 4-5]. For the sake of brevity and in order to focus our attention on the most relevant findings, only those research papers reporting quantified results (IC50 and/or percentage of inhibition at a given concentration) were included. Extracts or essential oils with IC50 > 0.5 mg/ml were considered weakly active and were therefore not taken into account in the present review. With a few exceptions, only molecules with IC50 < 50 M have been considered. Furthermore, unless otherwise stated, those results on AChE inhibition included in the present review refer to in vitro assays carried out with AChE from electric eel.

2. Materials and Methods

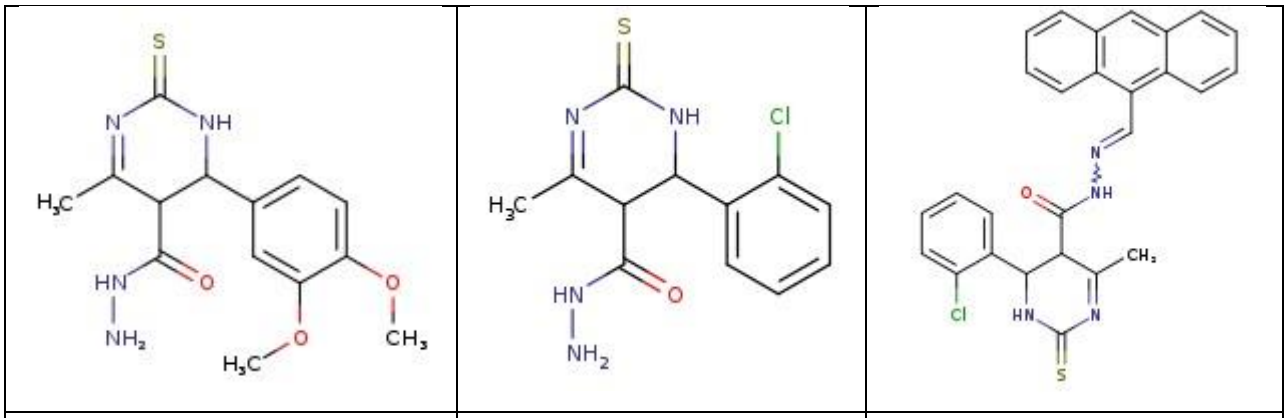
One popular method for determining whether a chemical or substance inhibits AChE activity in vitro is the AChE (acetylcholinesterase) enzyme inhibition test. Neuroscience, toxicology, and drug development are just a few of the areas that stand to benefit from inhibiting acetylcholinesterase (AChE), the enzyme that breaks down the neurotransmitter acetylcholine. The drug or chemical that has to be evaluated for AChE inhibition is first dissolved in 1 milligramme per millilitre of dimethyl sulfoxide (DMSO). Various concentrations for the test are achieved by diluting the stock solution. The enzyme supply is made ready by using appropriate procedures to isolate or purify AChE. In most cases, the enzyme is kept frozen and then thawed just before the test. In order to facilitate the AChE enzyme's optimum functioning, a buffer system is set up. The presence of Tris-HCl in the buffer ensures that the enzymes remain stable and active. Common substrates for AChE activity measurements include acetylthiocholine (ATCh). In order to get the substrate to the right concentration, it is dissolved in the

assay buffer. The drug to be evaluated, together with the buffer and AChE enzyme, is combined to form the reaction mixture. Finding the sweet spot for enzyme and substrate concentrations is essential. In a positive control, the AChE enzyme activity is completely inhibited, while in a negative control, the enzyme activity is at its highest without inhibition. The inclusion of these controls in the test ensures the validity of the data. To enable the enzyme-substrate reaction to take place, the reaction mixture is incubated at a certain temperature, usually 37°C, for a specified duration. Optimal incubation times could vary from experiment to experiment. The activity of the AChE enzyme is evaluated after the incubation time using an appropriate detection technique. Thiocholine is a typical end product that may be detected by watching its production; when it combines with a chromogenic or fluorogenic reagent, it produces a signal that is either coloured or fluorescent.
A spectrophotometer is used to measure the signal intensity. For each concentration of the tested drug or chemical, the percentage of AChE inhibition is determined using the acquired signal values. By plotting the enzyme's activity against dosage, one may get the inhibitory concentration (IC50), which is the concentration needed to block 50% of the AChE enzyme's activity.

Molecular Docking

In order to predict the docking of molecules in the active sites of enzymes, docking studies were performed using the Molegro Virtual Docker software [6]. The Experimentally-determined X-ray crystal structures of AChE were retrieved from the RCSB Protein Data Bank (RCSB PDB) website with 4EY7 PDB ID [7]. The 2D structures of the synthesized molecules were drawn in Chemdraw and transferred to the MarvinSketch program for checking as structure and obtaining the 3D SDF structures. Molegro Virtual Docker performed regularization and optimization for enzymes and new molecules in docking studies. A grid box with the coordinates of the crystal ligands in the center was created at the active site of the enzymes. Ten trials were performed at the active sites of the targets for each molecule. The high-scoring poses were selected and were visualized and analyzed using Discovery Studio Visualizer.

3. Results and Discussion



5	6	9

Figure 2 Structures of the tested compounds

Table 1 displays the symbols used in this study as well as the outcomes for these samples.

Table 1 Shows the signals used and the IC_{50} of the sample

Compounds	IC_{50} values µM
5	46.210
6	165.035
9	99.021

Given that compound 5 has been shown to inhibit the activity of the acetylcholinesterase (AChE) enzyme, it has the potential to be used in the treatment of pathological conditions such as Alzheimer's disease, which are associated with AChE. Figure 3 provides a detailed explanation of the activity and visually shows the relationship between the percentage of enzyme activity and the concentration of compound 5. Enzyme AChE (acetylcholinesterase) activity drops with increasing 5 values, as shown in the graph. A two-dimensional interaction map depicting the unique interactions between 5 and the amino acids of the enzyme may be seen in Figure 4. Amino acids ARG 296 and SER 293 played crucial roles in the binding process via their contributions to hydrogen bond interactions. The binding is stabilised and the inhibitory effects are amplified via these interactions. Also, when you see red separation contacts, it means that water molecules have left the enzyme's active site. The end result is a change in the enzyme's structure and a cessation of reaction catalysis. Thus, compound 5 blocks the action of the acetylcholinesterase (AChE) enzyme.

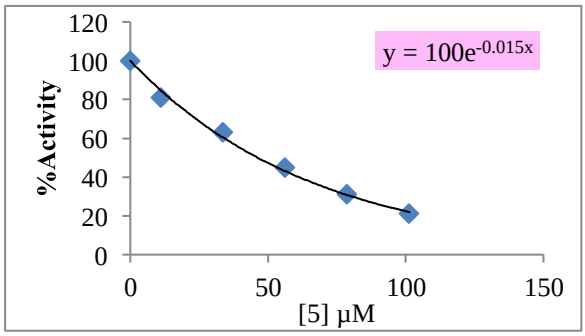


Figure 3 Reduce AChE (acetylcholinesterase) enzyme activity from the compound 5

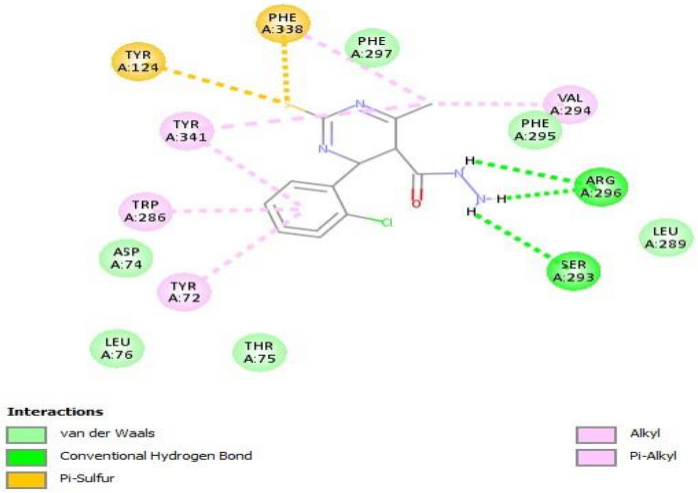


Figure 4 The 5 compound's two-dimensional structures with AChE (acetylcholinesterase) enzyme

Given that 6 has been shown to inhibit the activity of the acetylcholinesterase (AChE) enzyme, it has the potential to be used in the treatment of pathological conditions such as Alzheimer's disease, which are associated with AChE. Figure 5 provides a detailed explanation of the activity and visually shows the relationship between the percentage of enzyme activity and the concentration of compound 6. Enzyme AChE (acetylcholinesterase) activity drops with increasing 6 values, as shown in the graph. A two-dimensional interaction map depicting the unique interactions between 6 and the amino acids of the enzyme may be seen in Figure 6. Amino acids SER 203, GLY121 and ASP 74 played crucial roles in the binding process via their contributions to hydrogen bond interactions. The binding is stabilised and the inhibitory effects are amplified via these interactions. Also, when you see red separation contacts, it means that water molecules have left the

enzyme's active site. The end result is a change in the enzyme's structure and a cessation of reaction catalysis. Thus, 6 blocks the action of the acetylcholinesterase (AChE) enzyme.

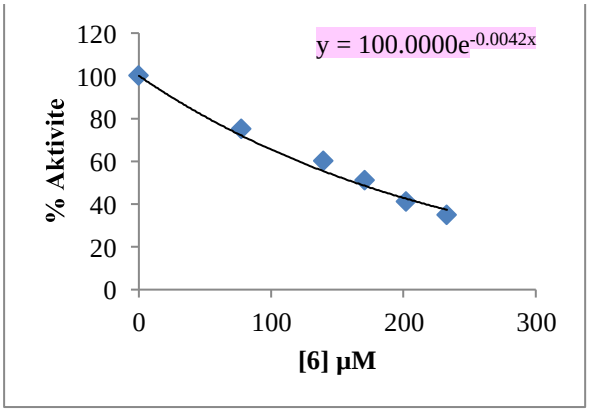


Figure 5 Reduce AChE (acetylcholinesterase enzyme activity from the compound 6

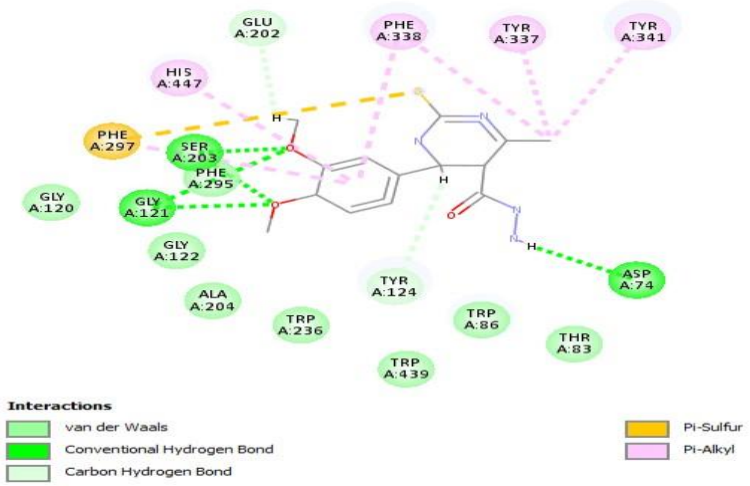


Figure 6 The 6 compound's two-dimensional structures with AChE (acetylcholinesterase) enzyme

Given that compound 9 has been shown to inhibit the activity of the acetylcholinesterase (AChE) enzyme, it has the potential to be used in the treatment of pathological conditions such as Alzheimer's disease, which are associated with AChE. Figure 7 provides a detailed explanation of the activity and visually shows the relationship between the percentage of enzyme activity and the concentration of compound 9. Enzyme AChE (acetylcholinesterase) activity drops with increasing 6 values, as shown in the graph. A two-dimensional interaction map depicting the unique interactions between 9 and the amino acids of the enzyme may be seen in Figure 8. Amino acids GLY 122, HIS 447 and SER 203 played crucial roles in the binding process via their contributions to hydrogen bond interactions. The binding is stabilised and the inhibitory effects are amplified via these interactions. Also, when you see red separation contacts, it means that water molecules have left the enzyme's active site. The end result is a change in the enzyme's structure and a cessation of reaction catalysis. Thus, 9 blocks the action of the acetylcholinesterase (AChE) enzyme.

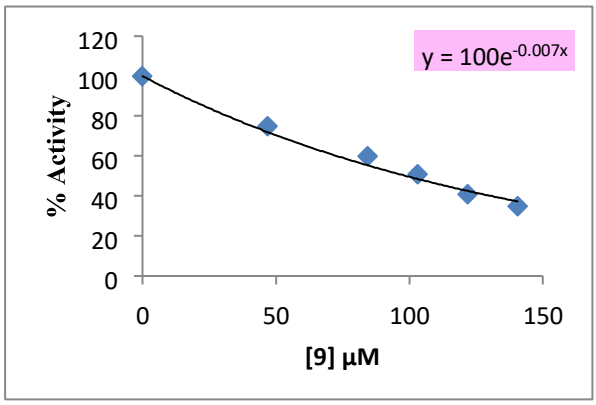


Figure 7 Reduce AChE (acetylcholinesterase) enzyme activity from the compound 9

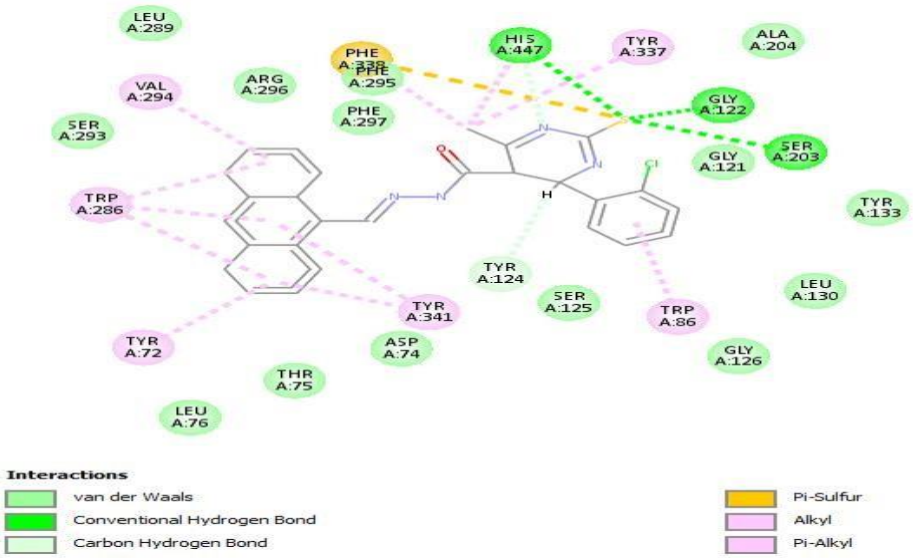


Figure 8 The 9 compound's two-dimensional structures with AChE (acetylcholinesterase) enzyme

4. Conclusion

This study examined the potential of three substances—5, 6, and 9—to inhibit the activity of AChE (acetylcholinesterase). Obesity and other conditions linked to elevated acetylcholinesterase (AChE) activity may now have a new treatment option. The acetylcholinesterase (AChE) activity was concentrationdependently reduced by the three medications. The 2D interaction maps highlighted the inhibitory mechanism by revealing interactions with specific amino acids via hydrogen bonds. The red separation contacts indicate that the enzyme's catalytic activity was impaired because water molecules were displaced from its active site. The findings indicate that compounds 5, 6, and 9 have potential as a therapy for Alzheimer's disease and other conditions associated with elevated AChE activity. Additional research, including clinical trials and in vivo studies, is necessary to validate these results and assess the efficacy and safety of these compounds as antiAlzheimer's medications. Research like this is a huge step forward in the hunt for novel therapeutic techniques, and it might mean improved health and a better quality of life for people with AChE (acetylcholinesterase)related illnesses in the future.

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