Molecular Docking and Anti-acetylcholinesterase Activity of (N-(1-benzyl-1H-1,2,3-triazol-4yl)methyl)-4-hydroxy-3-methoxycinnamamide

โมเลกุลาร์ด็อกกิ้งและฤทธิ์การยับยั้งอะซิทิลโคลีนเอสเทอเรสของสาร (N-(1-benzyl-1H-1,2,3-triazol-4yl)methyl)-4-hydroxy-3-methoxycinnamamide

Sivanan Dongyai (ศิวนันท์ ดวงใหญ่)* Dr.Worawan Kitphathi (ดร.วรวรรณ กิจผาติ)**
Dr.Sumarn Saraya (ดร.สุมาลย์ สาระยา)*** Dr. Kittisak Sripha (ดร.กิตติศักดิ์ ศรีภา)****
Dr. Jaturong Pratuangdejkul (ดร. จตุรงค์ ประเทืองเดชกุล)*****

ABSTRACT

Ferulic acid, a phenolic compound is found in a variety of fruits and vegetables. Ferulic acid has antioxidant activity, anti-inflammation and inhibition of β-amyloid fibrils formation. Thus, derivative of ferulic acid has gained much interest in drug discovery for treatment of neurodegenerative diseases. Recently, triazolyl feruloyl amide derivatives were synthesized in our laboratory. It was found that (N-(1-benzyl-1H-1,2,3-triazol-4yl)methyl)-4-hydroxy-3-methoxycinnamamide (compound 4a) produce a good inhibitory activity for $A\beta_{1-42}$ aggregation. In this study, the inhibitory activity of compound 4a for electric eel acetylcholinesterase (*Ee*AChE) and equine serum butyrylcholiesterase (eqBuChE) were evaluated and donepezil was used as a reference. Compound 4a was selective inhibitor for *Ee*AChE as compared to eqBuChE. However, compound 4a shows less potent (IC_{50} 255.85 μM), as compared to donepezil (IC_{50} 0.01 μM) for inhibition of *Ee*AChE. From results of molecular docking, compound 4a interacted with dual binding sites: catalytic anionic site (CAS) residues Trp86 and His447; and peripheral anionic site (PAS) residue Trp286 in the binding cavity of *Ee*AChE. The nature of binding between compound 4a and *Ee*AChE were mainly π–π and π–cation interactions. Moreover, compound 4a also bound into the hydrophobic pocket of *Ee*AChE. Our study found that compound 4a was worthy for further investigation and development for treatment of Alzheimer's disease.

บทคัดย่อ

กรดเฟอรูลิคเป็นสารฟีโนลิคที่พบในผลไม้และผักหลากหลายชนิด กรดเฟอรูลิคมีฤทธิ์ต้านออกซิเดชัน ต้านการอักเสบและยับยั้งการสร้างเบต้าอะไมลอยด์ไฟบริล ดั้งนั้นอนุพันธ์ของกรดฟูเลอริคจึงได้รับความสนใจมากในการค้นพบยาสำหรับรักษาโรคที่เกิดจากความเสื่อมของระบบประสาท เมื่อเร็วๆ นี้อนุพันธ์ไทรอะโซลิลเฟอรูโลอิลเอไมด์ได้ถูกสังเคราะห์ในห้องปฏิบัติการของเรา พบว่า (N-(1-benzyl-1H-1,2,3-triazol-4yl)methyl)-4-hydroxy-3-methoxycinnamamide (สาร 4a) แสดงฤทธิ์ยับยั้งการเกาะกลุ่มของ $A\beta_{1-42}$ ที่ดี ในการศึกษานี้ ฤทธิ์ยับยั้งของสาร 4a ต่อเอนไซม์อะซิทิลโคลีนเอสเทอเรสจากปลาไหลไฟฟ้า (*Ee*AChE) และบิวทีริลโคลีนเอสเทอเรสจากเซรั่มม้า (eqBuChE) ได้ถูกประเมินและโดเนเพซิลถูกใช้เป็นสารอ้างอิง สาร 4a เป็นสารยับยั้งที่จำเพาะต่อ *Ee*AChE เมื่อเทียบกับ eqBuChE อย่างไรก็ตามสาร 4a มีความแรง (IC_{50} 255.85 μM) น้อยกว่าเมื่อเทียบกับโดเนเพซิล (IC_{50} 0.01 μM) ในการยับยั้ง *Ee*AChE จากผลของโมเลกุลาร์ด็อกกิ้ง สาร 4a เกิดอันตรกิริยากับกรดอะมิโนในโพรงการจับของ *Ee*AChE ในสองตำแหน่ง คือ Trp86 และ His447 ใน catalytic anionic site (CAS) และ Trp286 ใน peripheral anionic site (PAS) การจับกันระหว่างสาร 4a และ *Ee*AChE เป็นอันตรกิริยาชนิด π–π และ π–cation นอกจากนี้สาร 4a ยังจับกับโพรง hydrophobic ของ *Ee*AChE การศึกษานี้พบว่าสาร 4a มีความน่าสนใจที่จะนำมาศึกษาและพัฒนาต่อไปเพื่อการรักษาโรคอัลไซเมอร์

Key Words: Triazolyl feruloyl amide, Anti-cholinesterase, Molecular docking
คำสำคัญ: ไทรอะโซลิลเฟอรูโลอิลเอไมด์ ต้านเอนไซม์โคลีนเอสเทอเรส โมเลกุลาร์ด็อกกิ้ง

*Student, Master of Science Program in Biopharmaceutical Science (International Program), Department of Microbiology, Faculty of Pharmacy, Mahidol University
** Lecturer, Department of Physiology, Faculty of Pharmacy, Mahidol University
***Associate Professor, Department of Microbiology, Faculty of Pharmacy, Mahidol University
**** Associate Professor, Department of Pharmaceutical chemistry, Faculty of Pharmacy, Mahidol University
***** Lecturer, Department of Microbiology, Faculty of Pharmacy, Mahidol University

Introduction

Alzheimer's disease (AD), the most common form of dementia in the elderly population has become the health problem in the developed countries. Currently, AD is increasing in people over 65 years and affects over 36 million people worldwide. The number of the affected population is expected to triple by 2050 if no efficient treatment is discovered. It has become one of the most costly diseases which bring heavy social and financial burden to both society and families (Cavalli et al., 2008). The current major therapeutic approach to treating AD is directed to the inhibition of acetylcholinesterase (AChE), based on the hypothesis that this disease results from a defect in the cholinergic system (Nunomura et al., 2006). Thus, AChE inhibitors are the most widely developed compounds for the symptomatic treatment of this disease. Examples of AChE inhibitor are rivastigmine, heptyl-physostigmine, tacrine, metrifonate, bis-tacrine, galantamine and donepezil (Rakonczay., 2003). However, these drugs have proved usefully for treatment of mild to moderate severe cases only, and do not reverse or heal the disease (Terry et al., 2003). Therefore, many researchers have focused on the modification of natural substance in order to find more potent active compounds.

The ferulic acid is one of the dominating natural phenolic acids and occurs, often together with caffeic acid, in the secondary metabolite spectrum of important economic and medicinal plants such as wheat (*Triticum aestivum*) or eucalyptus (*Eucalyptus globulus*). Many secondary metabolites that contain ferulic acid substructures or ferulic acid esters showed very potent anti-oxidative activity (Heilmann et al., 2000). An *in vivo* study in mice showed that long-term administration of ferulic acid induced resistance to $A\beta_{1-42}$ toxicity in the brain (Yan et al., 2001). The modified ferulic acid also exhibits the action on acetylcholinesterase (AChE) inhibitory activity and antioxidant activity (Wonglerdsiri et al., 2012). In this context, ferulic acid derivative seems to be of a special interest for development as a drug for treatment of AD.

The aim of this study is to evaluate the acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity of (N-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-4-hydroxy-3-methoxycinnamamide compound 4a, see Figure 1). In the assay, the inhibitor concentration that causes 50% inhibitory activity (IC_{50}) values of compound 4a for inhibition of *Electrophorus electricus* acetylcholinesterase (*Ee*AChE) and equine serum butyrylcholinesterase (eqBuChE) were calculated to define the selectivity of compound 4a. Finally, the putative binding mode of compound 4a in the binding site of *Ee*AChE and eqBuChE were predicted and analyzed using docking and molecular modeling tools.

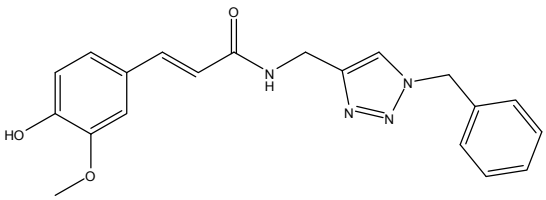

Figure 1 Structure of (N-(1-benzyl-1H-1,2,3-triazol-4yl) methyl)-4-hydroxy-3-methoxycinnamamide (compound 4a).

Objective of study

The aim of this study was to evaluate anti-acetylcholinesterase and butyrylcholiesterase activities and molecular docking study of (N-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-4-hydroxy-3-methoxycinnamamide (compound 4a).

Methodology

1. Cholinesterase inhibitory assay

1.1. Chemicals

Electrophorus electricus acetylcholinesterase from (*Ee*AChE), equine serum butyrylcholinesterase (eqBuChE), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), and 5,5-dithiobis[2-nitrobenzoic acid] (DTNB) were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Fluka. Donepezil was obtained from T.O. Chemicals (1979) Ltd. The synthesized(N-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-4-hydroxy-3-methoxycinnamamide (compound 4a) was kindly provided by Dr. Kittisak Sripha and his working group (Wonglerdsiri et al., 2012). 50 mM Tris–HCl pH 8.0 was used as a buffer throughout the experiment unless otherwise stated. The enzyme stock solution was kept at -80°C. The further enzyme-dilution was dissolved in 0.1% BSA in buffer. DTNB was dissolved in the buffer containing 0.1 M NaCl and 0.02 M $MgCl_2$. ATCI was dissolved in deionized water

1.2. Cholinesterase assay

The assay of compound 4a for *Ee*AChE and eqBuChE inhibitory activity was performed according to the methods developed by (Ellman et al., 1961 and Ingkaninan et al., 2000) Donepezil was used as a positive control. Briefiy, 25 µl of 15 mM ATCI or 1.5 mM BTCI, 125 µl of 3 mM DTNB, 50 µl of Tris-buffer, and 25 µl of sample solution were added to the wells followed by 25 µl of 0.22 U/ml *Ee*AChE or 0.5 U/ml eqBuChE. The microplate was read at 405 nm every 5 min until 2 hours by a microplate reader (Infinite 200 PRO, Switzerland). Then the results were analyzed at 20 min. The percentage inhibition of compound 4a for *Ee*AChE and eqBuChE were calculated by comparing the rates for the samples to the blank (0.1% BSA in 50 mM Tris–HCl pH 8.0). Each experiment was done in triplicate. The percentage inhibition was calculated using the following formula:

$$\text{Percentage Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100\%$$

Finally, the IC_{50} value was determined with the software package Prism (Graph Pad Inc, San Diego, USA) using 10-12 different concentrations of the inhibitors with Equation: log concentration (inhibitor) vs. normalized response (variable slope).

2. Molecular modeling study

2.1 Preparation of compound 4a and donepezil structures

Structures of compound 4a and donepezil were constructed and submitted to energy minimization *via* molecular mechanic method using Discovery Studio® 2.5 with the CHARMm forcefield and Momany-Rone partial atomic charges. The energy-minimizations were performed using Steepest descent and followed by Adopted-based Newton-Raphson Algorithm (ABNR). The structures were considered as fully optimized structure when the energy changes between iterations of calculation steps were less than 0.001 kcal/mol.

2.2 Molecular docking of Compounds 4a and donepezil into *Ee*AChE

Compound 4a and donepezil were docked into *Electrophorus electricus* AChE (*Ee*AChE). The coordinates of *Ee*AChE (PDB entry 1C2B) were obtained from the Protein Data Bank (PDB). For docking studies, initial protein was prepared by removing all water molecules, heteroatoms, any co-

crystallized solvent, and the ligand. CHARMm forcefield was applied using the simulation tool in Discovery Studio® 2.5. The energy minimizations were then performed using the Steepest descent and followed by Adopted-based Newton-Raphson (ABNR) algorithm with 40 kcal/mol of backbone harmonic restraint until the energy changes between iterations of calculation steps were less than 0.001 kcal/mol. Docking calculations were performed with the Flexible docking (Koska et al., 2008) program implemented in Discovery Studio® 2.5. The binding site sphere was defined according to amino acid residues for flexible docking (Tyr72, Asp74, Trp86, Gly121, Gly122, Glu202, Ser203, Ala204, Trp236, Trp286, Phe295, Phe297, Glu334, Tyr337, Phe338, and His447) with 20 Å radius. The default parameters were used. The results were clustered by analysis of conformations tool. The representative docking pose of each cluster was selected using the best CDOCKER interaction energy score. Then chosen complex pose were submitted for *in situ* ligand minimization protocol prior to calculate the binding energy. The binding interaction of donepezil and compound 4a were finally analyzed.

Results

In vitro inhibition of EeAChE and eqBuChE by donepezil and compound 4a

To determine the potential application of target compound 4a for treatment of AD, the AChE inhibitory activity was examined by the method of Ellman et al. (Ellman et al., 1961) on AChE from electric eel, using commercial donepezil as the reference standard. BuChE inhibitory activity on equine serum BuChE was also determined using the same method. The IC_{50} values of compound 4a and donepezil for *Ee*AChE and eqBuChE inhibition were depicted in Figure 2a and 2b, respectively. Donepezil was found to be more selective and more potent inhibitor of *Ee*AChE (IC_{50} value of 0.01 μM) than eqBuChE (IC_{50} value of 2.86 μM) (Table 1). Compound 4a was found to inhibit *Ee*AChE with the IC_{50} value of 255.85 μM and exhibited very poor inhibitory activity of eqBuChE with IC_{50} value of 8,830.80 μM (Table 1).

Molecular modeling studies

Molecular modeling studies were performed to gain an insight into the interaction of donepezil and compound 4a with amino acid residues in the binding site of *Ee*AChE. The program Flexible Docking in Discovery studio® 2.5 software was used to simulate protein flexibility and dock ligand with an induced fit receptor optimization (Koska et al., 2008). In this study, donepezil and compound 4a were docked into a single catalytic subunit of *Ee*AChE (PDB entry 1C2B).

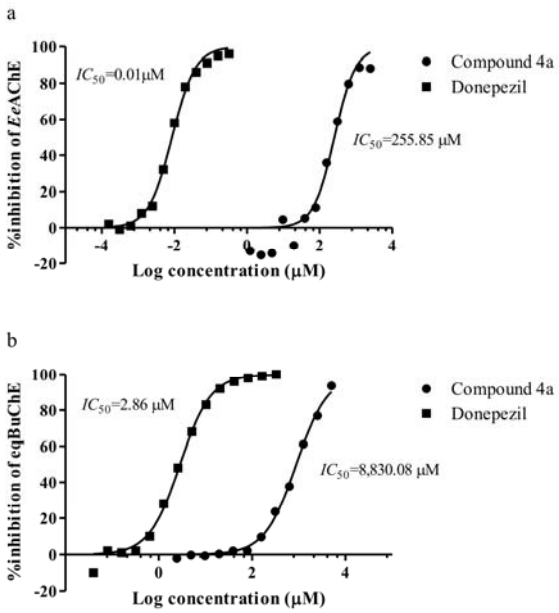

Figure 2 Inhibition of *Ee*AChE (a) and eqBuChE (b) by compounds 4a and donepezil.

To account for flexibility during docking, flexible torsion in the donepezil and compound 4a were assigned, and the torsional angles were allowed

to rotate freely. The account for protein flexibility is widely accepted for docking algorithm. This allows small movements in the side chain or backbone of the protein that can increase or decrease the occupied volume of active site, or to modify the hydrogen-bonding pattern between ligand and protein. In AChE studies, the flexibility of protein structure in docking of ligand protocol has been commonly used (Samadi et al., 2011 and Bartolini et al., 2011 and Martins et al., 2011 and Samadi et al., 2012). Our studies, the flexible residues include; Tyr72, Asp74, Trp286 in the peripheral anionic site (PAS); Trp86, Glu202 and Tyr337 in catalytic anionic site (CAS); Ser203, Glu334 and His447 in catalytic triad; Gly121, Gly122 and Ala204 in oxyanion hole and Trp236, Phe338, Phe295 and Phe297 in acyl binding site of *Ee*AChE.

The docking results showed that total of 23 and 31 poses of donepezil and compound 4a were obtained, respectively. The docking pose with the highest CDOCKER interaction energy score of -54.90 and -49.96 kcal/mol for donepezil and compound 4a were selected, respectively (see Table 2). After *in situ* ligand minimization, the binding energies of donepezil and compound 4a with *Ee*AChE were calculated and their corresponding energetic terms are summarized in Table 2. The binding energy of donepezil was greater (-151.48 kcal/mol) than that of compound 4a (-66.89 kcal/mol) toward *Ee*AChE.

To determine the binding modes of donepezil and compound 4a in the binding site of *Ee*AChE, the conformation, orientation and interactions of ligands and amino acid residues were analyzed. Figure **3** shows the overlay image of donepezil and compound 4a docked into the active site gorge of *Ee*AChE. Interestingly, both donepezil and compound 4a were found to occupy the narrow and deep gorge of *Ee*AChE active site, which comprised of catalytic anionic site (CAS) at the base of gorge and the peripheral anionic site (PAS) at the entrance of gorge. The result indicated that both donepezil and compound 4a represented a dual-site binding, interacts with key CAS residues Trp86 and His447 as well as key PAS residue Trp286 of *Ee*AChE active site.

The binding interactions of compound 4a in the *Ee*AChE active site were further analyzed using the Analyze Docking Results module in Discovery studio® 2.5 to visualize the interactions navigating through ligands. The 3D image and 2D diagram representing for interactions of compound 4a in the active site gorge of *Ee*AChE were shown in Figure 4 and Figure 5, respectively. The main interactions between compound 4a and *Ee*AChE are: π–π interaction between aromatic ring of cinnamamide moiety and PAS residue Trp286; π–π and π–cation interactions between benzyl ring connecting 1H-1,2,3-triazole moiety and CAS residues Trp86 and His447, respectively. As depicted in Figure 4, the aromatic ring of cinnamamide moiety appears almost in the parallel plane to the indole nucleus of Trp286 with strongly packed force as indicated by the distance of 3.49 Å and 5.08 Å. While phenyl ring connecting 1H-1,2,3-triazole moiety of compound 4a shows strong π–π interaction almost in the parallel plane to the indole nucleus of Trp86 with the distance of 4.25 Å and 3.82 Å. Furthermore, compound 4a accommodates within a hydrophobic pocket formed by Phe295, Phe297, and Phe338 of acyl pocket residues; Tyr337 of CAS residue; and Tyr341 of bottle neck residue (see Figure **5**).

Table 1 *In vitro* inhibition and selectivity of Donepezil and Compound 4a for cholinesterase.

Inhibitor	IC_{50} (μM)		Selectivity [a]
	*Ee*AChE	eqBuChE	
Compound 4a	255.85	8,830.80	34.52
Donepezil	0.01	2.86	331.94

[a] Selectivity for *Ee*AChE is defined as IC_{50}(eqBuChE)/IC_{50}(*Ee*AChE).

Table 2 Ligand- *Ee*AChE complex energy terms[a,b,c]

Ligand	CDOCKER interaction energy of the best docking pose	$E_{binding}$	E_{ligand}	E_{enzyme}	$E_{liand\text{-}enzyme}$
Compound 4a	-49.96	-66.89	9.53	-26774	-26831.84
Donepezil	-54.90	-151.48	66.74	-26796	-26880.30

[a] Energy terms were calculated using CHARMm forcefield in Discovery studio® 2.5 program from Accelrys Inc. (San Diego, CA).

[b] $E_{binding}$ = energy of binding, E_{ligand} = energy of ligand, E_{enzyme} = energy of enzyme, $E_{liand\text{-}enzyme}$ = energy of ligand-enzyme complex.

[c] Expressed in kcal/mol.

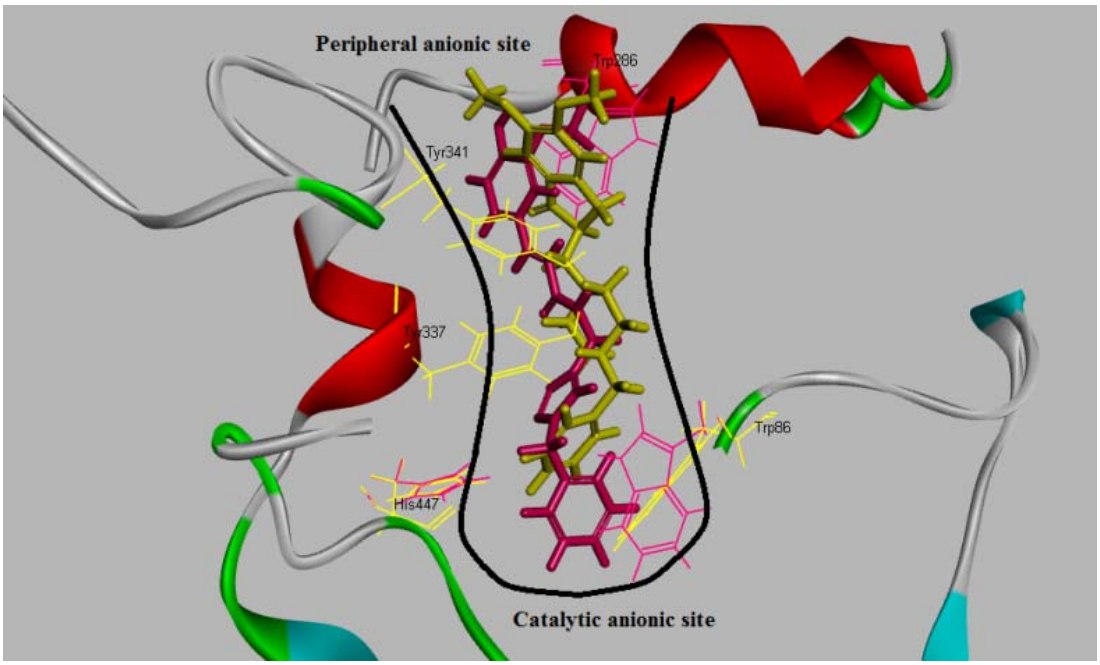

Figure 3 Overlay image of compound 4a and donepezil in the *Ee*AChE active site. The pink colored represented for compound 4a and its interacting residues, whereas yellow colored represented for donepezil and its interacting residues

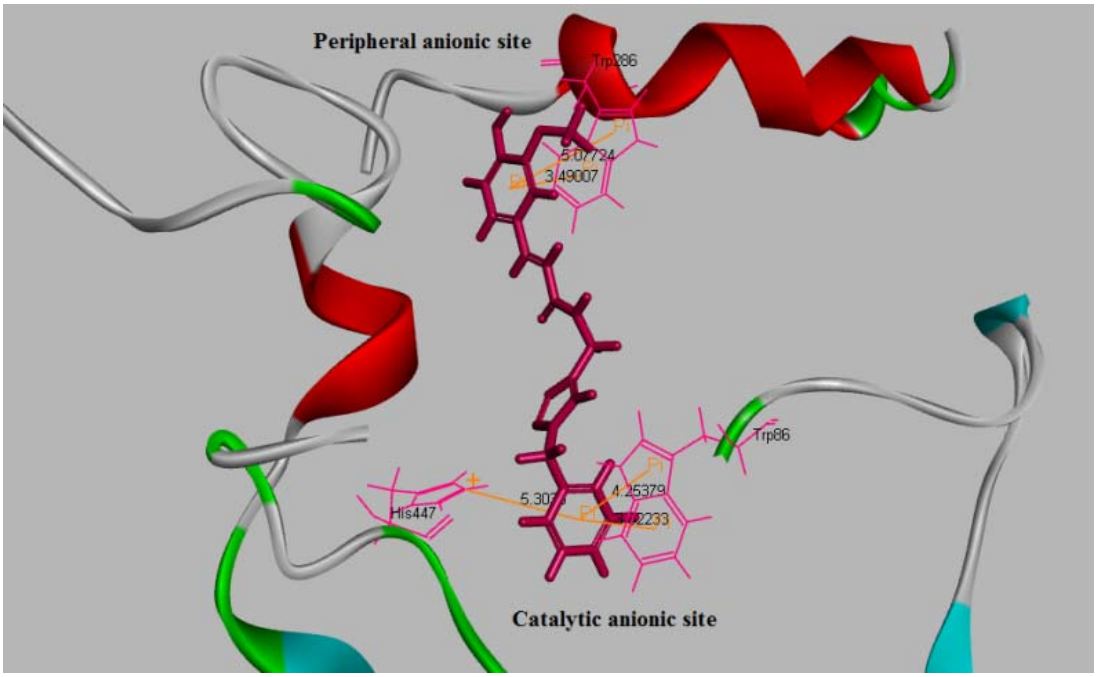

Figure 4 Compound 4a docked into the active site gorge of *Ee*AChE (PDB: 1C2B). π–π interaction, π–cation interaction and distances of ligand from key residues in Å are shown.

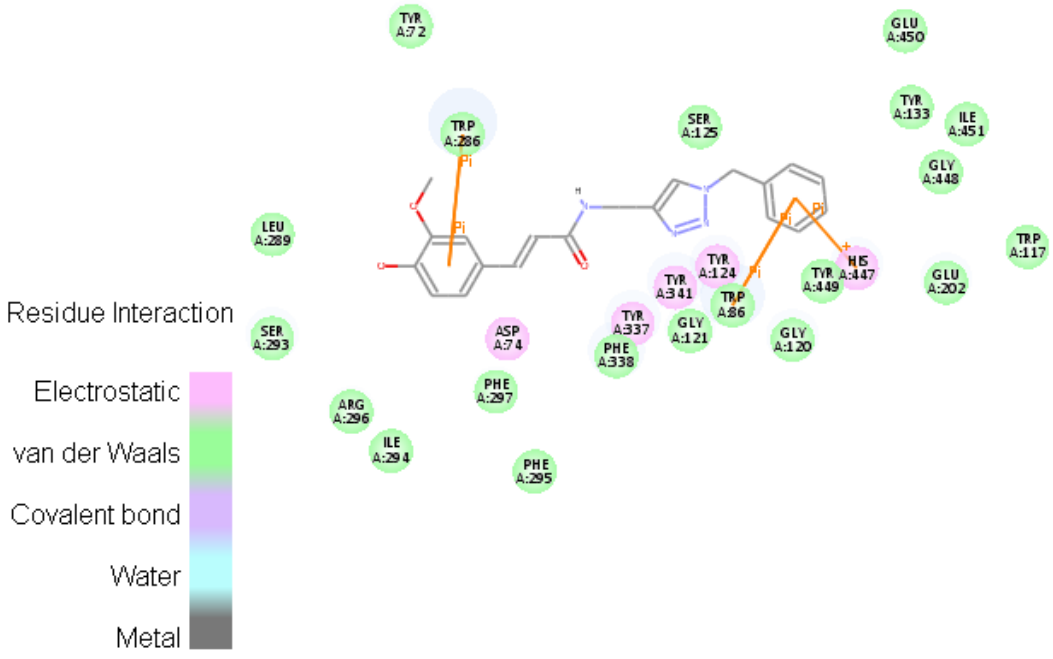

Figure 5 2D diagram interaction of compound 4a in *Ee*AChE binding site.

Discussion and Conclusions

Previous study of a triazolyl feruloyl amide derivative,(N-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-4-hydroxy-3-methoxycinnamamide (compound 4a) shows potential activity for treatment of Alzheimer's disease through the inhibition of $A\beta_{1-42}$ aggregation (Wonglerdsiri et al., 2012). In this study, the inhibition and selectivity of compound 4a against *Ee*AChE and eqBuChE were evaluated. Donepezil was used as positive control. Our results indicated that compound 4a was selective inhibitor for *Ee*AChE (35-fold selectivity) compared with eqBuChE. Compound 4a showns less potent for inhibition of *Ee*AChE (IC_{50} 255.85 μM) as compared to donepezil (IC_{50} 0.01 μM). However, compound 4a has gained attention for our group because it can produce both inhibitory activities of $A\beta_{1-42}$ aggregation and acetylcholinesterase. Thus, compound 4a has potential to develop as compound with dual actions for treatment of Alzheimer's disease. To attain this goal, understanding the binding mode of compound 4a in the binding site of AChE was need. The interaction of compound 4a in the *Ee*AChE active site could be predicted by molecular modeling technique.

In this study, compound 4a and donepezil were docked into the active site gorge of *Ee*AChE by defining the flexibility of key residues in various binding regions, for instance acyl pocket catalytic triad, CAS, PAS and bottle neck. Our result indicated that compound 4a bounds into active site gorge with both catalytic as well as peripheric site residues, which was similar to the binding pattern of donepezil (Figure 3). Based on the docking studies, the lower potency of compound 4a in comparison to donepezil (as show in IC_{50} value) could be attributed to the reasons of: (a) CDOCKER interaction energy and binding energy of compound 4a were less stable (Table 2); (b) there were no hydrogen bonding interactions; and (c) lower extent of hydrophobic fitting and π–π interactions.

It was observed that π—π interactions played an important role in stabilizing both donepezil and compound 4a complex. Compound 4a interacts with Trp286 (PAS) forming a face-to-face π—π interaction with the aromatic ring of cinnamamide moiety. The hydrophobic interaction between alkene chain of cinnamamide moiety and rich aromatic residues (Phe295, Phe297, Tyr337, Phe338 and Tyr341) along the gorge could direct the phenyl ring of triazole moiety to penetrate into the catalytic anionic site region in the choline-binding site and forming π—π stacking interaction between Trp86. From docking results of compound 4a and donepezil, it was suggested that increasing structural hydrophobicity of compound, lining along the hydrophobic residues of acetylcholinesterase gorge may increase its binding interaction, hence the inhibitory activity of compound may be improved.

In conclusion, a triazolyl feruloyl amide derivative,(N-(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-4-hydroxy-3-methoxycinnamamide (compound 4a) produced an inhibitory activity for acetylcholesterase by interacting with catalytic anionic site (CAS) and peripheral anionic site (PAS) through π–π and π–cation interactions. In addition to pivotal role of anti-AChE activity, compound 4a was previously reported as inhibitor for amyloid-β aggregation (Wonglerdsiri et al., 2012). Thus, compound 4a can be used as a starting point to discover feruloyl amide-based compounds, with dual actions of anti-acetylcholinesterase and anti-amyloid-β aggregation activities for treatment of Alzheimer's disease

Acknowledgements

This study was supported by the National Research University (NRU) grant No. NRU-PY550106, Faculty of Pharmacy, Mahidol University, Thailand.

References

Bartolini M, Pistolozzi M, Andrisano V, Egea J, Lopez MG, Iriepa I, et al. Chemical and pharmacological studies on enantiomerically pure p-methoxytacripyrines, promising multi-target-directed ligands for the treatment of Alzheimer's disease. ChemMedChem 2011; 6(11): 1990-7.

Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, et al. Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem 2008; 51(3): 347-72.

Ellman GL, Courtney DK, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88–90.

Heilmann J, Quadri-Spinelli T, Rali T, Sticher O. Bioactive coumarin derivatives from the fern Cyclosorus interruptus. Planta Med 2000; 66(8): 728-33.

Ingkaninan K, de Best CM, van der Heijden R, Hofte AJ, Karabatak B, Irth H, et al. High performance liquid chromatography with on-line coupled UV-mass spectrophotometric-biochemical detection for identification of acetyl cholinesterase inhibitors from natural products. J Chromatogr A 2000; 872(1-2): 61–73.

Koska J, Spassov VZ, Maynard AJ, Yan L, Austin N, Flook PK, et al. Fully automated molecular mechanics based induced fit protein-ligand docking method. J Chem Inf Model. 2008; 48(10): 1965-73.

Martins C, Carreiras MC, Leon R, de los Rios C, Bartolini M, Andrisano V, et al. Synthesis and biological assessment of diversely substituted furo[2,3-b]quinolin-4-amine and pyrrolo[2,3-b]quinolin-4-amine derivatives, as novel tacrine analogues. Eur J Med Chem 2011; 46(12): 6119-30.

Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA. Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 2006; 65(7): 631-41.

Rakonczay Z. Potencies and selectivities of inhibitors of acetylcholinesterase and its molecular forms in normal and alzheimer's disease brain. Acta Biol Hung 2003; 54(2): 183–9.

Samadi A, Chioua M, Bolea I, de los Ríos C, Iriepa I, Moraleda I, et al. Synthesis, biological assessment and molecular modeling of new multipotent MAO and cholinesterase inhibitors as potential drugs for the treatment of Alzheimers disease. Eur J Med Chem 2011; 46(9): 4665-8.

Samadi A, de los Ríos C, Bolea I, Chioua M, Iriepa I, Moraleda I, et al. Multipotent MAO and cholinesterase inhibitors for the treatment of Alzheimer's disease: Synthesis, pharmacological analysis and molecular modeling of heterocyclic substituted alkyl and cycloalkyl propargyl amine. Eur J Med Chem 2012; 52: 251-62.

Terry AV Jr, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer'sdisease-related cognitive deficits: recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 2003; 306(3): 821-7.

Wonglerdsiri W, Chatket A. Discovery of new anti Aβ aggregation and acetyl- and butyrylcholinesterase inhibitors developed from ferulic and vanillic acid derivatives. [A special project submitted in partial fulfilment of the requirements for the bachelor degree of science in pharmacy]. Bangkok: Faculty of Pharmacy, Mahidol University; 2012. [in thai].

Yan JJ, Cho J, Kim HS, Kim KL, Jung JS, Huh SO, et al. Protection against β-amyloid peptide toxicity *in vivo* with long term administration of ferulic acid. Br J Pharmacol 2001; 133(1): 89-96.