# FLAVONES AND FLAVANONES AS ACHETYLCHOLINESTERASE INHIBITORS: THE STRUCTURE-ACTIVITY RELATIONSHIP AND MOLECULAR DOCKING STUDIES

**Hoang-Phuc Nguyen[1, 2], Thi-Kim-Chi Huynh[1, 3], Thi-Kim-Dung Hoang[1, 3, *]**

[1]*Institute of Chemical Technology – VAST, 1 Mac Dinh Chi Str., Dist.1,*
*Ho Chi Minh city, Viet Nam*
[2]*Ton Duc Thang University, 19 Nguyen Huu Tho, Dist. 7, Ho Chi Minh city, Viet Nam*

[3]*Graduate University of Science and Technology – VAST, 18 Hoang Quoc Viet Str.,*
*Cau Giay Dist., Ha Noi, Viet Nam*

[*]Emails: *hoangthikimdung@gmail.com, htkdung@vast.ict.vn*

**Abstract.** For decades now, the discovery and development of Alzheimer's (AD) drugs have been an important goal. Many studies showed that, acetylcholinesterase (AChE) plays an important role in the treatment of AD. Flavonoid compounds are known to have a variety of biological effects, including inhibitory AChE activity. In this study, we report the correlation between the structure and activity of flavone and the flavone derivatives that semi-synthesized and synthesized from the flower buds of the *Styphnolobium japonicum* (Leguminosae family) and the citrus peel, with effects against AChE. Results showed that the new functional groups of compounds **Q2** and **Q4** increased AChE inhibition 3 times better than quercetin. The molecular docking study was investigated in order to illuminate the experimental results and find binding modes of these prospective derivatives.

*Keywords:* flavones, flavanones, achetylcholinesterase, structure-activity relationship, molecular docking.

*Classification numbers:* 1.2.1, 1.2.4.

## 1. INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease that a patient suffering from gradually loses memory, hardly remembers the recent events. Moreover, the sequential symptoms include disorientation (more easily getting lost), language impairment, loss of motivation, not taking self-care of and behavioral issues [1 - 3]. The patients with AD were declined the person's condition and lost the bodily functions leading to death. Despite the difference in the speed of progression, the typical longevity is three to nine years, from diagnosis [4 - 5].

One of the crucial hypotheses to illuminate the cause of disease involves in the drastic decrease of acetylcholine (ACh) activity in cerebral cortex and hippocampus [6 - 8], so recent

publications proposed that decreasing acetylcholinesterase (AChE) activity would be a promising treatment for AD patients [9 - 11]. Besides, the crystallographic structure of human AChE contains two binding sites as a long gorge with length of approximately 20 Å, namely catalytic active site (CAS) and peripheral anionic site (PAS). The CAS is at bottom of gorge and responsible for the hydrolysis of AChE, including Ser200, Glu327 and His440 residues. The PAS is at bottom of deep narrow gorge and near the entrance of gorge, including Tyr70, Tyr121, and Trp279 [12 - 13]. Consequently, the ligands that simultaneously link with CAS and PAS have been advocated to design as the potent AChE inhibitors [14].

Flavonoids or their subclasses as flavones and flavanones are part of a family of naturally occurring polyphenolic compounds characterized by a common benzo-γ-pyrone structure. They have been extensively attracted because of their antioxidant properties and favorable health sciences [15 - 17]. They possess a wide range of biological activities such as antimicrobial, antifungal, antioxidant, neuroprotective and anticancer activities [18 - 19]. Furthermore, they not only expressed the low toxicity but also considered as potential anti AD agents [20 - 22]. Therefore, the demand of screening of flavonoids chemical structures increased significantly in order to develop the new drugs for AD treatment. As the initially advantageous condition, we possessed the large number of flavones and flavanones that semi-synthesized and synesized from flower buds of *Styphnolobium japonicum* (Leguminosae) and citrus peels, moreover, the flavone and flavanone derivatives were obtained by different reagents [23 - 24]. In this study, we evaluated the *in vitro* AChE inhibition assay of twenty-seven flavones and flavanones and established the structure-activity relationship (SAR) for tested compounds to emphasize the characters of the lead compound of anti AChE activity. Finally, the molecular docking study was carried out to gain insight into the binding mode of the most potential compounds.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Twenty seven flavones and flavanones (H1-5, L1-5, D1-4, Q1-13) used in this work were synthesized, purified, and characterized for the structures which have been published in the previous reports [23 - 24].

### 2.2. *In vitro* AChE inhibition assay

Acetylcholinesterase, acetylthiocholine iodide (ATCI), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. The AChE inhibitory activity was performed by Ellman's method [25]. The tested compounds were dissolved in a minimum volume of DMSO (10 % of concentration) and diluted using the buffer solution (0.1 M Tris-HCl, pH = 7 and 3 mM NaHCO$_3$) to obtain a final concentration range. Enzyme solution was diluted in the buffer solution (0.1 M Tris-HCl, pH = 8) to give 0.415 units/mL. ATCI 5 mM was obtained by diluting 0.29 g ATCI in 100 mL distilled water and stored in low temperature. DTNB was dissolved in Tris-HCl pH 7 0.1 M and NaHCO$_3$ to collect the solution of 3.3 mM concentration. In 96-well plates, the assays were performed in the same condition for tested compounds, blank sample and Donepezil (as the reference drug). Firstly, the buffer pH 8, tested compound, DTNB and AChE solutions were mixed in a well of 96-plate and incubated for 15 mins at 25 °C. Subsequently, the solution of ATCI was added; shaken and incubated again in 2 mins. The activity was determined in absorbance at 415 nm using an Elisa Multiskan Ascent MicroplateReader system. All samples were measured in triplicate. Percentage of inhibitory of samples was calculated as follows:

$$\% \text{ Inhibition} = \frac{\left[\left(A_{tr1} - A_{tr0}\right) - \left(A_{m1} - A_{m0}\right)\right]}{A_{tr1} - A_{tr0}} \times 100\%$$

where: $A_{tr0}$ is the absorbance value of blank sample; $A_{tr1}$ is the absorbance value of blank sample plus enzyme; $A_{m0}$ is the absorbance value of tested compound, and $A_{m1}$ is the absorbance value of tested compound plus enzyme. The quantity of each assay components in different samples were illustrated in the Table 1.

*Table 1.* The quantity of assay chemical that presented in the well plate of each sample.

| Components | Volumes in well plate (µL) | | | |
|---|---|---|---|---|
| | $A_{tr0}$ | $A_{tr1}$ | $A_{m0}$ | $A_{m1}$ |
| Buffer pH 8 | 240 | 180 | 168 | 126 |
| Tested compound | - | - | 72 | 54 |
| DTNB | 30 | 30 | 30 | 30 |
| AChE | - | 60 | - | 60 |
| ATCI | 30 | 30 | 30 | 30 |

(-): absent

The $IC_{50}$ values (µM) were calculated for tested samples, which had % Inhibition > 50 % in the initial screening, using the non-linear regression analysis.

## 2.3. Molecular docking study

Molecular docking study was performed by software packages BiosolveIT LeadIT 2.1.8, ChemDraw 19.1, Molecular Operating Environment (MOE) version 2015.10 and Sybyl-X 1.1.

The X-ray crystallographic structure of 1-benzyl-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl]piperdine (Donepezil) complexed with AChE was obtained from the Protein Data Bank (PDB code: 1EVE) and used as the receptor model with the co-crystal ligand Donepezil. The 3D structure of the crystallographic complex was distributed by BiosolveIT LeadIT 2.1.8. The active site was prescribed by the reference ligand and enclosed within the radius sphere of 6.5 Å from the reference ligand. All of unbound water molecules were erased and the structure of enzyme was checked before re-establishing the active site.

The 2D and 3D chemical structures of **Q2** and **Q4** were built by ChemDraw 19.1 and MOE 2015.10, respectively. The structures of **Q2** and **Q4** were optimized using the energy minimization and molecular dynamic functions in Sybyl-X 1.1. In the process of energy minimization, the method to minimizing was Conj Grad and the structures of **Q2** and **Q4** were optimized until reached to a minimum energy change of 0.001 kcal.mol$^{-1}$. Gasteiger-Huckel charges were carried out to the structure atoms and the maximum number of iterations was set to 10,000 to operate during minimization. Molecular dynamic process was conducted to obtain the conformations which were the minimum global energy. The Simulated Annealing method was used in this process and in this method, the **Q2** and **Q4** molecules were heated at 700 K in 1000 femtoseconds, after that, they were cooled down to 200 K in the same period to access to the stable states which their final conformations were accomplished. This process was operated in ten cycles to find out the different necessary structures. Lastly, the energy minimization process was conducted one more time and the minimum energy of final conformations was figured out.

The docking protocols were validated by the re-docking process which reproduces co-crystallized binding geometry along with the orientation of the associated reference ligands. The co-crystallized reference ligand (Donepezil) was re-docked within the active site of the prepared AChE complex. The successful docking protocol is relative to Root Mean Squared Deviation (RMSD) between the native conformation and the best re-docked one. The docking protocol is considered good in case of the RMSD value less than 2.0 Å [26-27]. The docking of **Q2** and **Q4** that optimized the minimum energy to the prepared enzyme above was conducted using BiosolveIT LeadIT 2.1.8 as follows: the maximum number of solutions per iteration was 1000, the maximum number of solutions per fragmentation was set to 200, the number of poses to keep for interaction analysis was set to 1 (Top 1). The best conformation that possessed the most negative docking score was kept for the further analysis. The Figures 1 and 2 were exported from LeatIT and MOE softwares after docking processes.

## 3. RESULTS AND DISCUSSION

### 3.1. AChE inhibitory activity

The synthesized compounds were evaluated for AChE enzyme inhibitory activity and used Donepezil as reference drug. The results are shown in the Table 2 below as $IC_{50}$ values.

*Table 2.* Inhibition of *in vitro* AChE activity of flavones and flavanones derivatives.

| Cps. | Structure | $IC_{50}$ (µM) | Cps. | Structure | $IC_{50}$ (µM) |
|------|-----------|-----------|------|-----------|-----------|
| H1 |  | > 100 | L1 |  | > 100 |
| H2 |  | > 100 | L2 |  | > 100 |
| H3 |  | > 100 | L3 |  | > 100 |
| H4 |  | > 100 | L4 |  | > 100 |
| H5 |  | > 100 | L5 |  | > 100 |

| | | | | | |
|---|---|---|---|---|---|
| D1 |  | > 100 | Q1 |  | 96.32 ± 0.56 |
| D2 |  | > 100 | Q2 |  | 32.10 ± 0.21 |
| D3 |  | > 100 | Q3 |  | > 100 |
| D4 |  | > 100 | Q4 |  | 38.93 ± 2.86 |
| Q5 |  | > 100 | Q10 |  | > 100 |
| Q6 |  | > 100 | Q11 |  | 87.36 ± 6.67 |
| Q7 |  | > 100 | Q12 |  | > 100 |
| Q8 |  | > 100 | Q13 |  | > 100 |
| Q9 |  | > 100 | Donepezil | | 0.06 ± 0.02 |

The results indicated that most of tested flavanones (**H1-5**) and flavones (**L1-5**, **M1-4** and **Q1-13**) displayed weak to moderate inhibition of AChE. Take a look on the compounds that

owned the flavanone ring, the bioactivity of original hesperetin (**H1**) had been remained at the same level ($IC_{50} > 100$ μM) with and without appearance of new substituents in its structure (**H2-5**). The same fashion in activity of luteolin (**L1**), diosmin (**D1**) and their derivatives (**L2-5** and **D2-4**) that possessed the flavone ring took place. Fortunately, quercetin (**Q1**) and their derivatives (**Q2-13**) expressed the AChE inhibitory activity in the distinctive way, in other words, the activity of **Q2** and **Q4** had outperformed that of **Q1** as the initial compound. In detail, the replacement the hydroxyl functional groups of **Q1** at 3, 7, 3', 4'-positions by butylcarbamate groups and at 3, 5, 7, 3', 4'-positions by benzoate groups to produce **Q2** and **Q4**, respectively, leads to their AChE inhibitory activity increase significantly. As an evidence, $IC_{50}$ values of **Q2** and **Q4** (32.10 and 38.93 μM, respectively) dropped off approximately three-fold compared to that of **Q1** (96.32 μM). The remained substitutions on quercetin skeleton in this study ultimately led to decrease the AChE inhibition activity.

## 3.2. Molecular docking result

The re-docking showed that the RMSD value of Donepezil was detected to be 1.23 Å ($< 2$ Å) that showed the reliability of docking mode. The docking score of Donepezil was -16.7263 kcal/mol. Figures 1 and 2 illustrated the docking results of the most effective compounds **Q2** and **Q4** into the active site of AChE. The docking results suggested that compound **Q2** and **Q4** span along almost the whole length of the CAS and PAS of active site. The compound **Q2** was binding not only CAS but also PAS of AChE and interacted with His440 of CAS and Tyr121 and Trp279 of PAS. It involved in hydrogen bonding interaction between NH function group at 7-position with C=O function group of His440. In the same fashion, there was a hydrogen bond between OH group of Tyr121 and oxygen at 4-position in **Q2** structure.
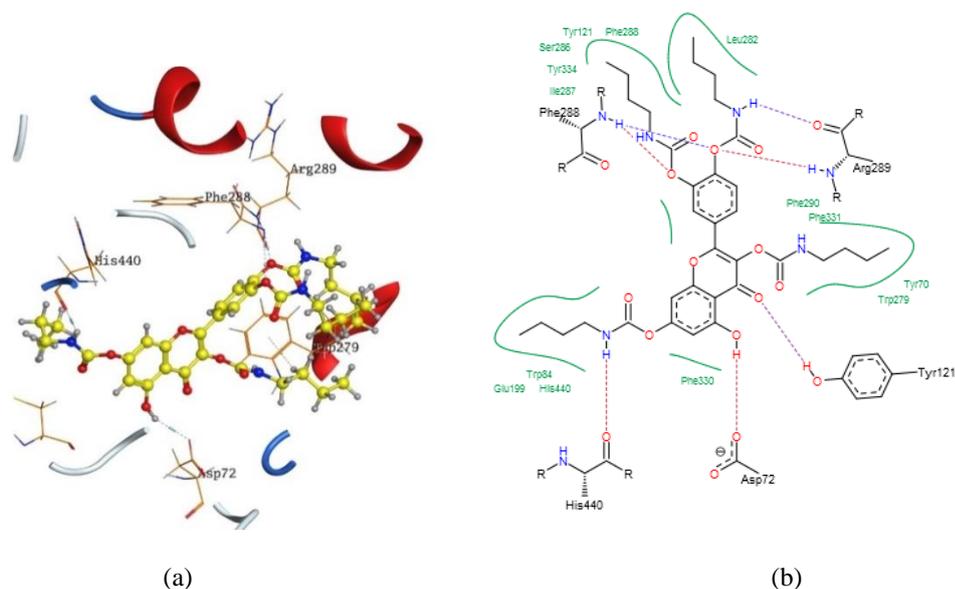


|       (a)       |       (b)       |

*Figure 1.* (a) 3D docking model of compound **Q2** with residues of target AChE. Atom colors: yellow – carbon atoms of **Q2**, dark blue – nitrogen atoms, red – oxygen atoms; (b) 2D schematic diagram of docking model of compound **Q2** with residues of target AChE. The dashed lines illustrate the interactions between the protein and the ligand.

Moreover, **Q2** could bind to PAS of AChE through a H-π interaction of H-3 atoms in the butylcarbamate group at 3-position with Tyr279. Additionally, **Q2** also interacted with the side chains of residues Asp72, Arg289 and Phe288 via hydrogen bonding. On the other hand, **Q4** formed the hydrogen bond interaction between the OH group of Tyr121 and oxygen of carbonyl group of benzoate group at 3-position. According to the analysis of docking results above, the more residues in the active sites compound **Q2** interacted, the better AChE inhibitory activity compound **Q2** was. That is the reason why the AChE inhibition of **Q2** was better than that of **Q4** and the docking scores of **Q2** and **Q4** were -13.47 kcal/mol and -2.85 kcal/mol, respectively.
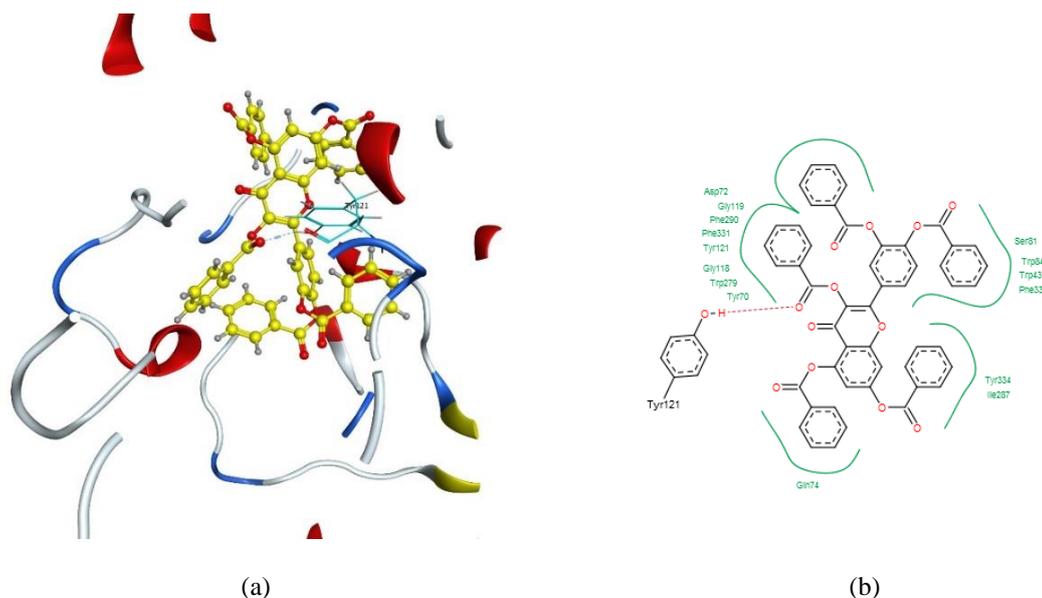


(a)                                         (b)

*Figure 2*. (a) 3D docking model of compound **Q4** with target AChE. Atom colors: yellow – carbon atoms of **Q4**, dark blue – nitrogen atoms, red – oxygen atoms; (b) 2D schematic diagram of docking model of compound **Q4** with target AChE. The dashed lines illustrate the interactions between the protein and the ligand.

## 4. CONCLUSIONS

Twenty seven flavone and flavanone derivatives were evaluated the bioactivity against AChE. The appearance of the new substituents in quercetin derivatives leads to rise the *in vitro* AChE inhibition activity, compared to that of quercetin. The compound **Q2** displayed the AChE inhibitory activity better than the compound **Q4** and the bioactivity of **Q2** and **Q4** had been more advantageous than that of **Q1** as the initial compound in this study. Molecular modeling studies was carried out to confirm and illuminate the binding effect of **Q2** and **Q4** on catalytic active site (CAS) and peripheral anionic site (PAS) of active site of AChE. In the further studies, the complexes of our flavone and flavanone derivatives with metals will be synthesized to discover the new agents that possessed the outstanding biological activity than original ligands.

***CRediT authorship contribution statement.*** HPN and TKCH contributed equally to this work and are co-first authors. HPN: methodology, data curation, formal analysis, software, validation, visualization, writing-original draft, writing-review & editing; TKCH: conceptualization, methodology, resources, data curation, formal analysis, software, supervision, validation, visualization, writing-original draft, writing-

review & editing; TKDH: conceptualization, methodology, resources, data curation, formal analysis, finance, investigation, software, supervision, validation, visualization, writing-original draft, writing-review & editing. All authors have read and agreed to the published version of the manuscript.

*Declaration of competing interest.* The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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