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Synthesis, Acetylcholinesterase and Molecular Modelling Analysis of (*3E*,*5E*)-3,5-Diarylidene-1-Phenylethylpiperidine-4-One Derivatives as a Therapeutic Agent for Alzheimer's Disease

(Sintesis, Asetilkolinesterase dan Analisis Pemodelan Molekul Terbitan (*3E,5E*)-3,5-Diarilidena-1-Feniletilpiperidina-4-One sebagai Agen Terapeutik untuk Penyakit Alzheimer)

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ABSTRACT

New diarylidiene-1-phenylethylpiperidine-4-one derivatives **2a-h** were successfully synthesised with yields ranging from 57% to 96% and characterised with various spectroscopic methods. The compounds were evaluated for their *in vitro* acetylcholinesterase inhibition activity with galantamine as positive control. Among the synthesised compounds, compounds **2g** and **2h** were found to be most potent against acetylcholinesterase enzyme with half-maximal inhibitory concentration (IC_{50}) of 46.3 ± 0.53 µM and IC_{50} = 29.7 ± 0.41 µM, respectively. The binding energy and interaction of compounds **2g** and **2h** with acetylcholinesterase protein were further studied through molecular docking studies. Compound **2h** (-12.1 ± 0.0 kcal mol^{-1}) showed a strong binding affinity than compound **2g** (-10.6 ± 0.0 kcal mol^{-1}) and both compounds exhibit several binding interactions with amino acids in acetylcholinesterase protein. The molecular docking studies of these compounds supports the *in vitro* acetylcholinesterase inhibition activity, suggesting that diarylidene phenylethyl-1-piperidone scaffold might be a promising drug candidate for a new acetylcholinesterase inhibitor drug development.

Keywords: Acetylcholinesterase inhibitor; Alzheimer's disease; diarylidene; molecular docking; piperidone

ABSTRAK

Sebatian terbitan baharu diarilidena-1-fenetilpiperidina-4-one **2a–h** telah berjaya disintesis dengan peratusan hasil antara 57% hingga 96% dan dicirikan menggunakan pelbagai kaedah spektroskopi. Penilaian terhadap aktiviti perencatan enzim asetilkolinesterase secara *in vitro* telah dijalankan dengan galantamina sebagai kawalan positif. Dalam kalangan sebatian yang disintesis, sebatian **2g** dan **2h** didapati menunjukkan keupayaan paling kuat dalam perencatan enzim asetilkolinesterase dengan nilai kepekatan perencatan (IC_{50}) masing-masing 46.3 ± 0.53 µM dan 29.7 ± 0.41 µM. Tenaga pengikatan dan interaksi antara sebatian **2g** dan **2h** dengan protein asetilkolinesterase telah dikaji dengan lebih lanjut melalui analisis pengedokan molekul. Sebatian **2h** (-12.1 ± 0.0 kcal/mol) menunjukkan tenaga pengikatan yang lebih tinggi berbanding sebatian **2g** (-10.6 ± 0.0 kcal/mol) dan kedua-dua sebatian membentuk beberapa interaksi dengan asid amino dalam protein asetilkolinesterase. Kajian pemadanan dok molekul ini menyokong keputusan aktiviti perencatan secara *in vitro*, sekali gus mencadangkan bahawa sebatian diarilidena piperidona berpotensi sebagai calon drug untuk pembangunan perencat asetilkolinesterase yang baharu.

Kata kunci: Diarilidene; pengedokan molekul; penyakit Alzheimer; perencat asetilkolinesterase; piperidona

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that begins with the gradual loss of neurons in the brain causing deterioration of brain function with symptoms such as memory loss, declining movement and cognitive capabilities (Porsteinsson et al. 2021). Currently, almost 50 million people around the world are suffering from AD and it is estimated to affect 152.8 million people in 2050, along with growing population and aging (Schwarzinger & Dufouil 2022). In Malaysia, the economic burden of AD is estimated USD 1.7-1.9 billion in 2022 (Ong et al. 2025).

Current treatment only slows down the progression and relieves the symptoms of AD due to the complex and multifactorial nature of AD (Gong et al. 2022). This has led to several hypotheses being proposed by modern research supported by experimental evidence. Among the proposed hypothesis that widely accepted are cholinergic hypothesis (Chen et al. 2022), tau and neuroinflammation (Chen & Yu 2023), β-amyloid deposition (Wu et al. 2022), heavy metal toxicity (Zhang et al. 2021), metabolic health disorder (Vinuesa et al. 2021), and oxidative stress (Bai et al. 2022).

The cholinergic hypothesis has been the most extensively studied and impactful. It has led to three out of four drugs that we have today (Aysegul et al. 2025). According to cholinergic hypothesis, the decline of cognitive skill and memory loss in AD are caused by the degradation of acetylcholine (ACh) level at the synaptic cleft, which lead to the loss of cholinergic transmission at the pre-synaptic cleft neuron in the brain (Vecchio et al. 2021). However, this degradation of ACh can be reduced or maintained by inhibition of acetylcholinesterase (AChE) enzyme which regulates the concentration of ACh in the synaptic cleft (David et al. 2021).

Substances or compounds that can inhibit AChE enzyme are known as cholinesterase inhibitors. The use of cholinesterase inhibitors reduces the symptoms of AD by inhibiting AChE enzyme which will improve the memory and cognitive function of an AD patient. Currently, cholinesterase inhibitors drugs such as donepezil, galantamine and rivastigmine have been approved by United States Food and Drug Administration (USFDA) to treat mild to severe AD patients (Ju & Tam 2022).

While the current USFDA approved inhibitors gives good symptomatic relief to the patients, they have no influence to slow down the progression of AD (Moreira et al. 2022). They also give adverse side effects such as nausea, vomiting, and diarrhea especially during the dose titration process (Xi et al. 2021). These have inspired researcher to discover more cholinesterase inhibitor candidates and other critical information related to AD in last few decades, with major interest towards synthesising new compounds that can provide less adverse side effects to the patients.

With this ambition, recently, our group have synthesised (3*E*,5*E*)-3,5-diarylidene-1-phenethyl piperidine-4-one derivatives *via* Claisen-Smith condensation reaction that have promising AChE inhibition activity (Kia et al. 2013; Mohamed Yusoff et al. 2022). According to our previous study, we have known that the phenyl moiety attached to the piperidin-4-one core was interacted with acyl binding pocket and PAS of the AChE enzyme through molecular docking study. With aim to develop a potent AChE inhibitor, now in the current study, a series of (3*E*,5*E*)-3,5-diarylidene-1-phenethyl piperidine-4-one derivatives bearing different electron donating group and electron withdrawing group substituent have been synthesise, characterise and investigate for their *in-vitro* acetylcholinesterase activity.

MATERIALS AND METHODS

All chemicals and materials were purchased from Sigma Aldrich Co. and Merck Chemical Co. The purification of synthesised compounds *via* column chromatography was performed using silica gel (0.040-0.063 mm). Thin-layer chromatography (TLC) was performed using silica-coated aluminium sheets (silica gel 60 F_{254}) and visualised by using vanillin-sulphuric acid solution. The melting points were determined using a Stuart SMP30 apparatus. The Fourier-Transform Infrared (FT-IR) spectra were recorded using Shimadzu FTIR equipped with ATR. Nuclear magnetic resonance (NMR) spectra were obtained using 500 MHz Bruker Advance NMR (500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR) spectrometer system and the data was analysed using MNova Software Version 14.2.1 (Mestrelab Research, Santiago de Compostela, Spain). The chemical shifts were internally calibrated using the residual $CDCl_3$ peak (^{1}H: 7.26 ppm, ^{13}C:77.0 ppm) or the tetramethyl silane (TMS) signal at 0.00 ppm for both ^{1}H and ^{13}C-NMR.

*Synthesis of 1-phenethylpiperidine-4-one (**1**)*

The synthesis of 1-phenethylpiperidine-4-one was carried out with the reaction between 4-piperidone monohydrate hydrochloride (10 mmol, 1 equiv.), sodium carbonate (24 mmol, 2.4 equiv.) and 2-bromoethyl benzene (12 mmol, 1.2 equiv.) in 80 mL acetonitrile. The suspension was stirred and reflux at 65 °C. After 12 h, the reaction mixture was cooled to ambient temperature and extracted with dichloromethane. The organic layer was washed with water and dried over anhydrous sodium sulphate and concentrated by using rotary evaporator. The resulting yellow oil was subjected to silica column chromatography (hexane/ethyl acetate 50%) to give the desired compound **1** (Zhuang et al. 2022).

General synthesis of (3E,5E)-3,5-diarylidene-1-phenethylpiperidine-4-one derivatives

The synthesis of (3E,5E)-3,5-diarylidene-1-phenethylpiperidine-4-one derivatives (**2a-h**) is shown in Scheme 1. A mixture of 1-phenethylpiperidine-4-one (**1**) (1 mmol, 1 equiv.), appropriate aldehyde **2** (2 mmol, 2 equiv.) was dissolve in ethanol (20 mL), 10 mL of 30% sodium hydroxide solution added dropwise in 15 min period and stirred with magnetic stirrer for 2-6 h at ambient temperature. After completion of the reaction as evidence from TLC, the mixture was poured into crushed ice. The precipitated solid was filtered and washed with cold ethanol to afford the final products **2a-h** (Mohamed Yusoff et al. 2022). The details about the reactions and spectroscopic data are shown in supporting information.

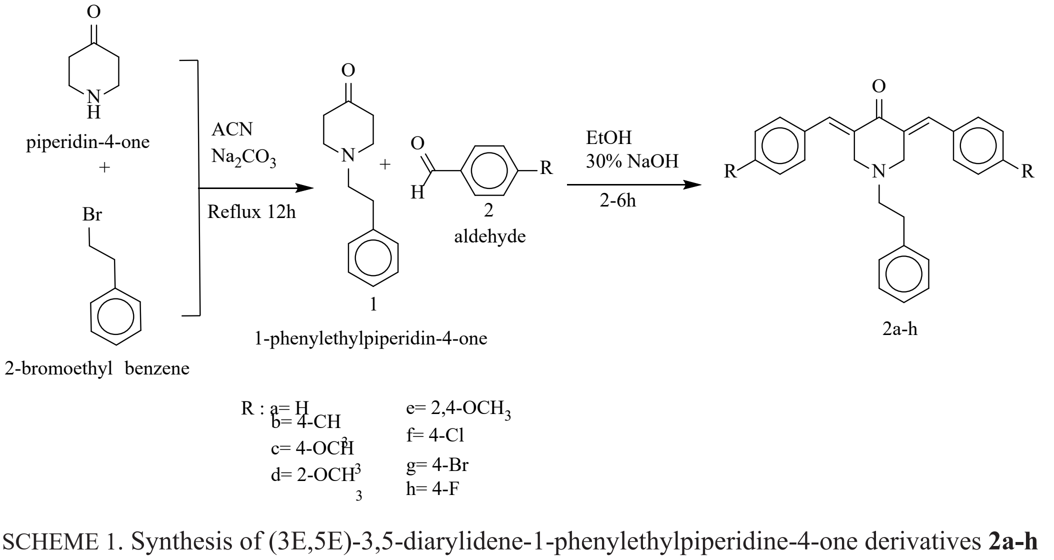
In vitro Acetylcholinesterase Inhibition Assay

The acetylcholinesterase (AChE) enzyme activity was evaluated by using Ellman's colourimetric method describe previously (Idris et al. 2025). 2.5 mg (0.5 unit/mL) of AChE enzyme dissolved in 1 mL of 0.05 M sodium phosphate buffer (pH 7.5). A series concentration of compounds (500-1 µM) has been prepared in dimethyl sulphoxide (DMSO) and the final concentration of the solution was mark up by using 0.05 M sodium phosphate solution (pH 7.5). The compounds were initially dissolved in DMSO and serially diluted with 0.05 M sodium phosphate buffer (pH 7.5) to obtain a concentration range of 500 to 1 µM. 179 µL of phosphate buffer, 10 µL of AChE and 1 µL of different concentrations of compounds were pre-incubated at 37 °C for 15 min in 96-well plates. Then, 5 µL of 14 mM ATCI and 10 mM DTNB were added into the wells to give the final volume of 200 µL. The absorbance was recorded for the wells over 30 min at 412 nm. Galantamine was used as a positive control. The inhibition percentage was calculated using the following formula, and the inhibitory concentration at 50% (IC_{50}) for each sample was determined using GraphPad Prism version 8.0. (GraphPad Software Inc., San Diego, CA, USA).

$$\text{Percentage inhibition (\%)} = \frac{\text{Abs of control (DMSO)} - \text{Abs of sample}}{\text{Abs of control (DMSO)}} \times 100$$

Molecular docking study

The structure of the synthesised compounds identified as ligands were drawn using ChemDraw Professional 22.00 software (Perkin Elmer Informatics, Massachusetts, USA). The ligands were then transformed into three-dimensional (3D) structures using the Chem3D tools in the ChemDraw software. The energy was minimised, and the structure was optimised using MM2 force field. The structure was then saved in the protein databank (.pdb) format. Protein (PDB ID: 4EY6) were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb) and prepared for docking. The DockPrep tools in UCSF Chimera software (Regents of University of California, USA) were used for the protein preparation. During this process, various adjustment has been made to the ligand and protein such as polar hydrogen atom were added, merging non-polar hydrogen atoms, setting solvation parameters followed by the inclusion of Gasteiger charges and the prepared structures were saved in (.pdbqt) format. This adjustment was targeted to improve the affinity of the protein binding sites (Idris et al. 2025). The grid size along the X, Y, Z axes were set to 18.522 Å, 23.934 Å, and 11.629 Å, respectively, with default lattice spacing of 0.375 Å. The lattice centre for acetylcholinesterase binding sites set to 8.817 Å, -60.624 Å and -23.964 Å along the X, Y, and Z axes, respectively (Silva et al. 2023). The docking experiment was performed



SCHEME 1. Synthesis of (3E,5E)-3,5-diarylidene-1-phenylethylpiperidine-4-one derivatives **2a-h**

with AutoDock Vina 1.5.7 in three independent molecular docking run. The docking method was validated by performing docking experiment between the protein and galantamine which serve as a guide. After completing the molecular docking simulation, 10 ligand-protein models were generated and evaluated based on binding affinity. Finally, the ligand-receptor model that exhibit the most favourable binding energy and best interaction was selected for further visualization and analysis of their active interaction in 2D and 3D conformations by using BIOVIA Discovery Studio Visualiser 2021 (Dassault Systems, California, USA).

RESULTS AND DISCUSSION

Synthesis of (3E,5E)-3,5-diarylidene-1-phenylethylpiperidine-4-one derivatives

The (3*E*,5*E*)-3,5-diarylidene-1-phenylethylpiperidine-4-one derivatives (**2a-h**) were synthesised *via* a 2-step reaction with several substituents as illustrated in Scheme 1. All the reported compounds (**2a-h**) have been synthesised in satisfactory to good yields between 57% and 96%) and were characterised by spectroscopic method including ^{1}H and ^{13}C NMR, FT-IR and HRMS analysis (for supporting information, kindly contact the corresponding author).

Compound **2g** was selected as a representative example for further discussion of spectral data. This compound exhibits peaks at 3022, 1673, 1602, and 1205 cm^{-1} corresponding to the peaks of aromatic C-H stretching, C=O stretching for ketone, C=C stretching for alkene group, and C-N stretching for tertiary amine group. In the ^{1}H-NMR spectrum of compound **2g**, 23 total protons have been integrated within the range δ_H 2.72-7.73. The formation of alkene group (C=C) H-10 was confirmed by the appearance of the most downfield singlet peak at δ_H 7.73. Doublet at δ_H 7.55 corresponds to aromatic proton H-12, multiplet at δ_H 7.20-7.25 corresponds to aromatic proton H-4 and H-13. In the upfield region, a singlet peak at δ_H 3.83 corresponds to H-7. The multiplet at δ_H 2.81-2.84 and δ_H 2.72-2.75 corresponds to H-1 and H-2, respectively. In the ^{13}C-NMR, a total of 27 carbon was observed between δ_C 34.3-187.1. The signals for quaternary and aromatic carbons are expected to be within δ_C 123.7-187.1 range. A carbon signal at δ_C 135.6 and δ_C 133.7 correspond to carbon C-10 and C-8 of the alkene group, respectively. The structure of the compounds was further confirmed by DEPT-135, HSQC, and HMBC NMR spectroscopy. The stereoselectivity of the synthesise compound **2a-h** were also confirmed with our previously reported literature (Mohamed Yusoff et al. 2022).

Acetylcholinesterase inhibitory activity

The acetylcholinesterase inhibitory activity of the (3*E*,5*E*)-3,5-diarylidene-1-phenylethylpiperidine-4-one derivatives (**2a-f**) were determined by Ellman's method using acetylcholinesterase enzyme from *Electrophorus electricus* (electric eel). The effect of the substituent on the phenyl ring of the compounds was evaluated in term of their half-maximal inhibitory concentration IC$_{50}$ values. Table 1 shows the IC$_{50}$ (μM) values of the synthesised compounds against AChE enzyme. Compounds with electron donating group (methyl and methoxy group at ortho and para position) (**2a-e**) showed an IC$_{50}$ of more than 100 μM. This suggest that electron donating group either at ortho or para position did not show inhibitory potential towards AChE enzymes. This might be due to electron donating group may increase the repulsive interactions between the aromatic ring and the active site of the enzyme protein (Guevara-Salazar et al. 2007). On the other hand, between all the electron-withdrawing group (halogenated) compounds (**2f**-**h**), the best inhibition potential with satisfactory IC$_{50}$ values was obtained by bromine substituted compound **2g** (IC$_{50}$=46.3 ± 0.53 μM) and fluorine substituted compound **2h** (IC$_{50}$=29.7 ± 0.41 μM). This indicates that halogenated compounds **2g-h** can inhibit the cholinesterase enzyme, although their inhibitory potency is weaker than that of the positive control, galantamine (4.4 ± 0.13 μM). The switching from bromine atom in **2g** to fluorine atom in **2h** at the same para position led to lower IC$_{50}$ values suggest that fluorine atom is more favourable towards the inhibition of AChE activity. This may be due to the smaller size fluorine atom compared to bromine atom. Nonetheless, these findings collectively suggest that the compounds with an electron-withdrawing group (**2g-h**) in the phenyl ring exhibit lower IC$_{50}$ values than compounds with an electron-donating group (**2a-e**). Based on this result, we carry out molecular docking study to further explore binding interactions between compounds (**2g** and **2h**) and the active site of the AChE enzyme.

Molecular docking study

Based on the results obtained from *in vitro* assay, molecular docking studies were performed to understand the binding interactions and binding energy between the ligands (compounds) and the active site of the enzyme. Compound **2g** and **2h** have been selected for molecular docking analysis. The analysis was done by using crystal structures of human acetylcholinesterase in complex with galantamine (PDB ID: 4EY6) as reference complexes for the docking process (Silva et al. 2023). The docking result in Table 2 showed excellent binding affinity of compound **2g** (-10.6 ± 0.0 kcal mol^{-1}) and compound **2h** (-12.1 ± 0.0 kcal mol^{-1}) compared to galantamine (-9.6 ± 0.0 kcal mol^{-1}) with amino acid in AChE protein. This is due to the binding energy that are lower or equal to -8.0 kcal mol^{-1} is considered as active compound in drug design (Ivanova & Karelson 2022).

Binding interaction in 2D and 3D mode of compounds **2h** and **2g** with AChE protein residues are shown in Figures 1 and 2 with details of their binding interactions in the active site in Table 3. The binding interactions of compound **2g** showed hydrophobic interactions between phenyl group and amino acids residues TRP613. Polar interaction is formed between halogen group and amino acid residue SER818. Multiple π-π interactions between bromo atom and amino acid residues LEU657 and TYR660 also indicates that the ligand (compound **2g**) and active site of the protein is effectively stabilised by both interactions.

On the other hand, compound **2h** exhibited a conventional hydrogen bond between fluoro atom and amino acid TYR660. A carbon-hydrogen bond interaction between phenyl group and amino acid GLY653 and VAL819 may provide additional stabilisation to the ligand (compound **2g**) and amino acid. Furthermore, there are π-π stacking and π-alkyl hydrophobic interactions between the phenyl group and amino acids residues TRP613 and HIS972. From this analysis, it could be that the presence of phenylethyl piperidone moiety are compulsory, while the presence of halogen group such as bromo and fluoro substituent also play an important role in order to obtained high binding energy with AChE protein.

Structure-activity relationship studies (SARs)

In vitro acetylcholinesterase inhibitory activity of synthesised compounds **2a-h** was performed with galantamine as control. The IC_{50} values of the compounds against AChE inhibition activity was shown in Table 1. The presence of substituent on the benzylidene moiety are carefully selected to represent different electronic environment to the molecule. Thus, electron withdrawing group such as chlorine, bromine and fluorine and electron donating group such as methoxy and methyl group was selected. Compounds with electron donating group (**2a-e**) does not give any inhibition on AChE enzyme (IC_{50}=>100 μM). However, by replacing the electron donating group with electron withdrawing group (halogens), we observe that compound **2g** (IC_{50}=46.3 ± 0.53 μM) bearing bromine atom and **2h** (IC_{50}=29.7 ± 0.41 μM) bearing fluorine atom showed lower IC_{50} values compared to non-substituent compound **2a** (IC_{50}=>100 μM). Compound **2h** bears fluorine atom at para position on the phenyl moiety appears to increase the inhibition on AChE activity by 1.6-fold compared to compound **2g** bearing bromine atom. In addition, derivatives bearing halogens group (**2f**, **2g**, **2h**), the order of the inhibitory potency was observed as: fluorine>bromine>chlorine. This result can be explained by electron density of the compounds. It has been reported that electron withdrawing group decreases the electron density on the benzylidene moiety which will strengthen van der Waals forces and improves electrostatic interaction between the compounds and the amino acid of AChE protein (Guevara- Salazar et al. 2007). Consistent with molecular docking study of **2g** and **2h**, the polar interaction form between bromine and fluorine atoms of compounds **2g** and **2h**, respectively, are formed with the amino acid residue SER818 in the active site of AChE enzymes. The presence of hydrogen bond between fluorine atom and amino acid TYR660, explains the better inhibition of **2h** compared to **2g**. Figure 3 summarizes the structure-activity relationship (SAR) studies of these diarylidene phenethylpiperidine-4-one derivatives.

TABLE 1. In vitro acetylcholinesterase inhibitory potential of compounds **2a-h**

Compound	AChE, IC_{50} ± SD (μM)[a]
2a	>100
2b	>100
2c	>100
2d	>100
2e	>100
2f	>100
2g	46.3 ± 0.53
2h	29.7 ± 0.41
Galantamine	4.4 ± 0.13

[a]Data are means ± standard deviation of triplicate independent experiments

TABLE 2. Binding energy of compounds **2g**, **2h** and galantamine with acetylcholinesterase protein

Protein	Compound	Binding energy (kcal mol^{-1})
Acetylcholinesterase (PDB ID: 4EY6)	**2g**	-10.6 ± 0.0
	2h	-12.1 ± 0.0
	Galantamine	-9.6 ± 0.0

Results are expressed as mean ± standard deviation (SD) for n=3 experiments

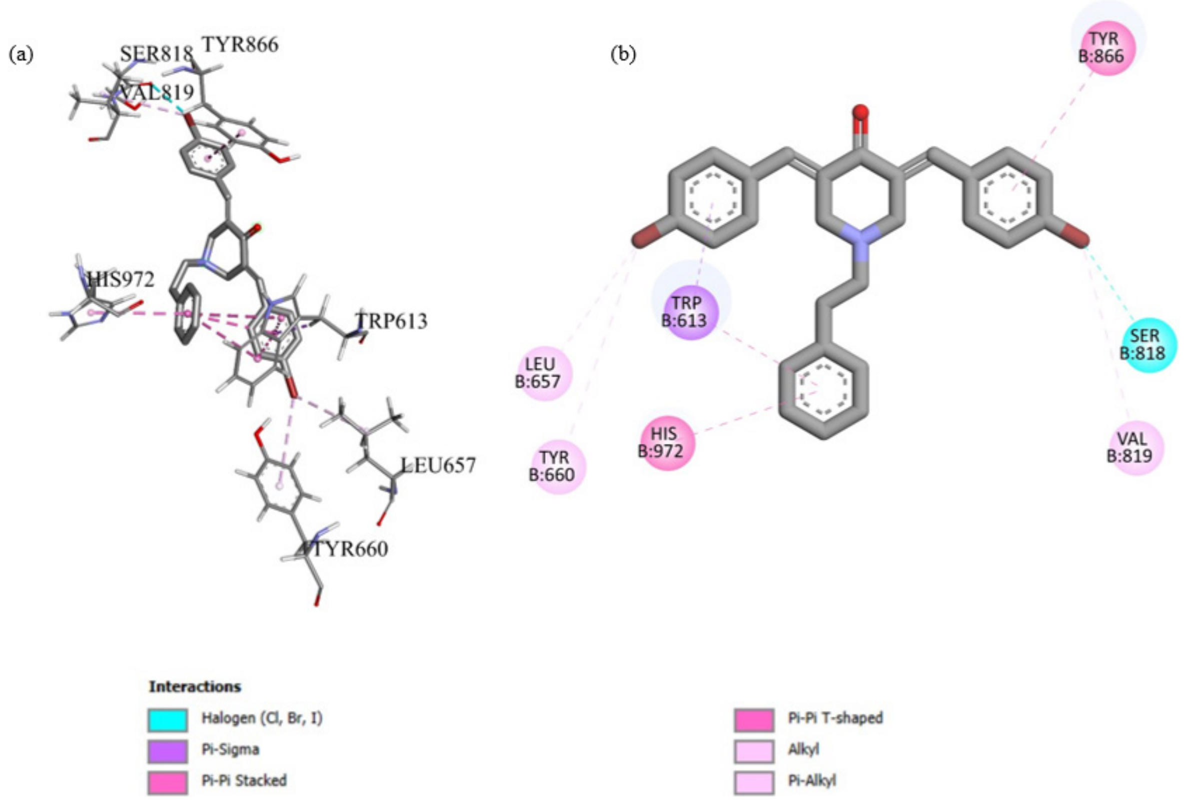


FIGURE 1. Binding interaction of compound **2g** and 4EY6 protein in (a) 3D form (b) 2D form

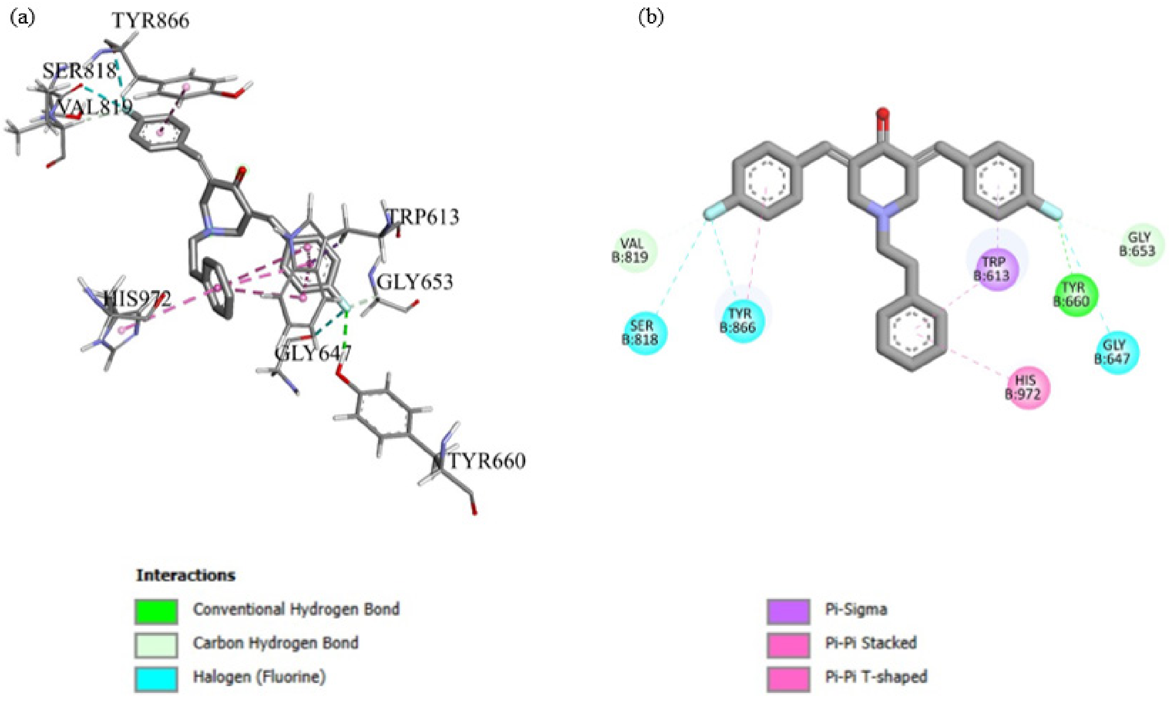


FIGURE 2. Binding interaction of compound **2h** and 4EY6 protein in (a) 3D form (b) 2D form

TABLE 3. Key binding interactions of compounds **2g** and **2h** with 4EY6 protein

Protein	Compound	Protein residue	Interacting unit of compound	Types of interaction
Acetylcholinesterase (PDB ID: 4EY6)	**2g**	SER818	4-bromo phenyl	Halogen
		TRP613	phenyl	π - sigma
		HIS972	phenyl	π – π stacked
		TYR866	phenyl	π – π T-shaped
		LEU657	4-bromo	alkyl
		TYR660	4-bromo	π - alkyl
	2h	TYR660	4-fluoro	H-bond (2.66 Å)
		GLY653	4-fluoro	Carbon H-bond
		VAL819	4-fluoro	Carbon H-bond
		GLY647	4-fluoro	Halogen
		TRP613	Phenyl	π - sigma
		HIS972	Phenyl	π – π stacked

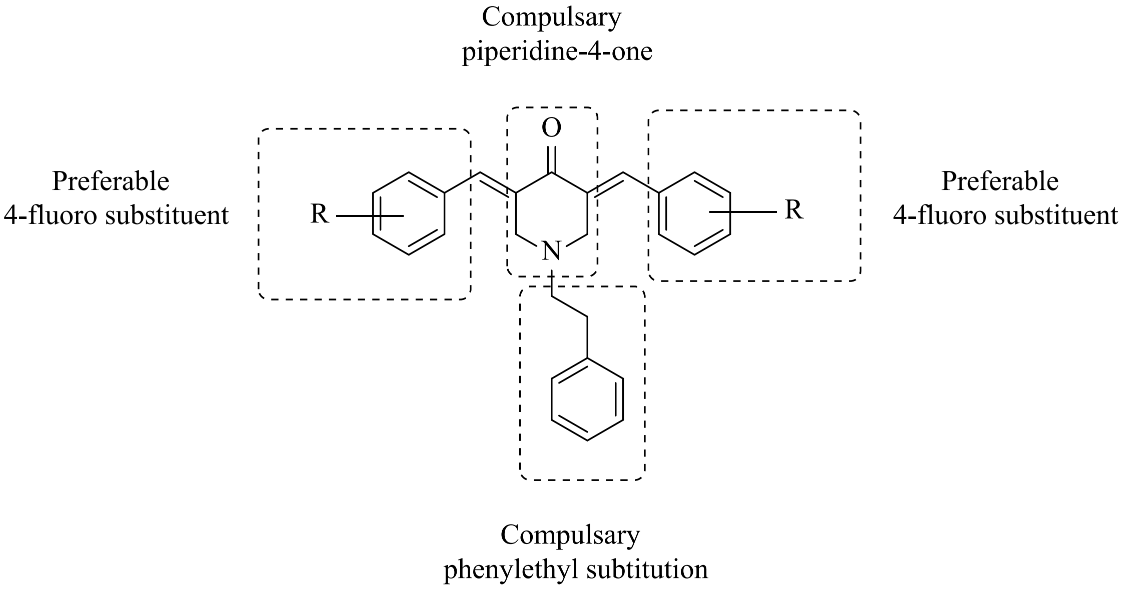


FIGURE 3. SAR study of diarylidene-1-phenethyl piperidine-4-one compounds

CONCLUSION

In summary, a series of diarylidene-1-phenethylpiperidine-4-one derivatives were successfully synthesised in moderate to high yield (57% to 96%) and characterised by NMR, FTIR, and HRMS techniques. The target compounds (**2a-h**) were explored for their *in vitro* acetylcholinesterase inhibitory activity. Two of the synthesised compounds, **2g** and **2h** were moderately potent with $IC_{50} = 46.3 \pm 0.53$ and 29.7 ± 0.41, respectively, against AChE enzyme. This suggests that the introduction of an electron-withdrawing group (halogen) on the phenyl ring improves the AChE inhibitory activity compared to an electron-donating group. The *in vitro* acetylcholinesterase inhibition activity result is also in line with molecular docking studies for these two potent compounds. Compound **2h** showed lower binding energy (-12.1 ± 0.0 kcal mol^{-1}) compared to **2g** (-10.6 ± 0.0 kcal mol^{-1}). Only compound **2h** formed a hydrogen bond with the amino acid TRY660 of acetylcholinesterase protein. It can be stated that this hydrogen bond may play an important role in increasing acetylcholinesterase inhibition activity. Therefore, compound **2h** bearing fluoro atom in the benzylidene moiety might be a promising scaffold for the development of new potent AChE inhibitor drug.

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REFERENCES

Aysegul, K.I., Sıcak, Y., Uysal, D.B., Tugba, T.T., Ozturk, M. & Oruc, E.E.E. 2025. Chiral thioureas containing naphthalene moiety as selective butyrylcholinesterase inhibitors: Design, synthesis, cholinesterase inhibition activity and molecular docking studies. *Journal of Molecular Structure* 1319: 139333.

Bai, R., Guo, J., Ye, X.Y., Xie, Y. & Xie, T. 2022. Oxidative stress: The core pathogenesis and mechanism of Alzheimer's disease. *Ageing Research Reviews* 77: 101619.

Chen, Y. & Yu, Y. 2023. Tau and neuroinflammation in Alzheimer's disease: Interplay mechanisms and clinical translation. *Journal of Neuroinflammation* 20(1): 165.

Chen, Z.R., Huang, J.B., Yang, S.L. & Hong, F.F. 2022. Role of cholinergic signaling in Alzheimer's disease. *Molecules* 27(6): 1816.

David, B., Pascal, S., Philipp, S., Jorg, P. & Gohlke, H. 2021. Discovery of new acetylcholinesterase inhibitors for Alzheimer's disease: Virtual screening and *in vitro* characterisation. *Journal of Enzyme Inhibition and Medicinal Chemistry* 36(1): 491-496.

Gong, C.X., Dai, C.L. Liu, F. & Iqbal, K. 2022. Multi-targets: An unconventional drug development strategy for Alzheimer's disease. *Frontiers in Aging Neuroscience* 14: 837649.

Guevara-Salazar, J.A., Espinoza-Fonseca, M., Beltrán, H.I., Correa-Basurto, J., Zavala, D.Q. & Trujillo-Ferrara, J.G. 2007. The electronic influence on the active site-directed inhibition of acetylcholinesterase by *N*-aryl-substituted succinimides. *Journal of the Mexican Chemical Society* 51(4): 222-227.

Idris, M., Parumasivam, T., Awang, K., Zahari, A., Litaudon, M., Apel, C., Supratman, U., Che Omar, M.T. & Azmi, M.N. 2025. Bioassay-guided isolation of acetylcholinesterase and butyrylcholinesterase inhibitors from *Horsfieldia tomentosa* fruits (Myristicaceae). *Phytochemistry Letters* 65: 133-140.

Ivanova, L. & Karelson, M. 2022. The impact of software used and the type of target protein on molecular docking accuracy. *Molecules* 27(24): 9041.

Ju, Y. & Tam, K.Y. 2022. Pathological mechanisms and therapeutic strategies for Alzheimer's disease. *Neural Regeneration Research* 17(3): 543-549.

Kia, Y., Osman, H., Kumar, R.S., Murugaiyah, V., Basiri, A., Perumal, S., Wahab, H.A. & Bing, C.S. 2013. Synthesis and discovery of novel piperidone-grafted mono- and bis-spirooxindole-hexahydropyrrolizines as potent cholinesterase inhibitors. *Bioorganic & Medicinal Chemistry* 21(7): 1696-1707.

Mohamed Yusoff, N., Osman, H., Katemba, V., Abd Ghani, M.S., Supratman, U., Che Omar, M.T., Murugaiyah, V., Xiang, R., Six, Y. & Azmi, M.N. 2022. Design, synthesis and cholinesterase inhibitory activity of new dispiro pyrrolidine derivatives. *Tetrahedron* 128: 133115.

Moreira, N.C.d.S., Lima, J.E.B.d.F., Marchiori, M.F., Carvalho, I. & Sakamoto-Hojo, E.T. 2022. Neuroprotective effects of cholinesterase inhibitors: Current scenario in therapies for Alzheimer's disease and future perspectives. *Journal of Alzheimer's Disease Reports* 6(1): 177-193.

Ong, S.C., Tay, L.X., Ong, H.M., Tiong, I.K., Ch'ng, A.S.H. & Parumasivam, T. 2025. Annual societal cost of Alzheimer's disease in Malaysia: A micro-costing approach. *BMC Geriatrics* 25(1): 154.

Porsteinsson, A.P., Isaacson, R.S., Knox, S., Sabbagh, M.N. & Rubino, I. 2021. Diagnosis of early Alzheimer's disease: Clinical practice in 2021. *The Journal of Prevention of Alzheimer's Disease* 8(3): 371-386.

Schwarzinger, M. & Dufouil, C. 2022. Forecasting the prevalence of dementia. *The Lancet Public Health* 7(2): 94-95.

Silva, L.B., Ferreira, E.F.B., Maryam, Espejo-Román, J.M., Costa, G.V., Cruz, J.V., Kimani, N.M., Costa, J.S., Bittencourt, J.A.H.M., Cruz, J.N., Campos, J.M. & Santos, C.B.R. 2023. Galantamine based novel acetylcholinesterase enzyme inhibitors: A molecular modeling design approach. *Molecules* 28(3): 1035.

Vecchio, I., Sorrentino, L., Paoletti, A., Marra, R. & Arbitrio, M. 2021. The state of the art on acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. *Journal of Central Nervous System Disease* 13: 11795735211029113.

Vinuesa, A., Pomilio, C., Gregosa, A., Bentivegna, M., Presa, J., Bellotto, M., Saravia, F. & Beauquis, J. 2021. Inflammation and insulin resistance as risk factors and potential therapeutic targets for Alzheimer's disease. *Frontiers in Neuroscience* 15: 653651.

Wu, T., Lin, D., Cheng, Y., Jiang, S., Riaz, M.W., Fu, N., Mou, C., Ye, M. & Zheng, Y. 2022. Amyloid cascade hypothesis for the treatment of Alzheimer's disease: Progress and challenges. *Journal of Aging and Diseases* 13(6): 1745-1758.

Xi, R., Liwei, J., Meijie, Q., Hassan, S.S.U. & Zongchao, L. 2021. Enhancing therapeutic efficacy of donepezil by combined therapy: A comprehensive review. *Current Pharmaceutical Design* 27(3): 332-344.

Zhang, N., Yu, X., Xie, J. & Xu, H. 2021. New insights into the role of ferritin in iron homeostasis and neurodegenerative diseases. *Molecular Neurobiology* 58(6): 2812-2823.

Zhuang, T., Xiong, J., Ren, X., Liang, L., Qi, Z., Zhang, S., Du, W., Chen, Y., Liu, X. & Zhang, G. 2022. Benzylaminofentanyl derivates: Discovery of bifunctional opioid and receptor ligands as novel analgesics with reduced adverse effects. *European Journal of Medicinal Chemistry* 241: 114649.

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