



Research article

Synthesis and evaluation of the acetylcholinesterase inhibitory effects of some new pentanamide derivatives bearing a Naphthalen-2-yl Scaffold

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ABSTRACT

Acetylcholinesterase (AChE) is an important enzyme in the synaptic cleft of the cholinergic nervous system, many studies have shown that a deficiency in this enzyme can lead to cognitive decline in Alzheimer's disease. Therefore, it is a crucial target in the development of new drugs for the treatment of this disease. Five potential new compounds (**IVa**, **IVb**, **IVc**, **IVd**, **IVe**) were synthesized through a sequence of five reactions: [3+2] cycloaddition, N-alkylation, ester hydrolysis, acidification, and amidation, with the starting material being 2-naphthalene carbonitrile. Thin layer chromatography (TLC) and melting point determination are two methods used to assess the purity of the synthesized compounds. The structures of the compounds were confirmed through three spectroscopic methods, including high-resolution mass spectrometry (HRMS), proton nuclear magnetic resonance (¹H-NMR) spectroscopy, and carbon nuclear magnetic resonance (¹³C-NMR) spectroscopy. After that, the five synthesized compounds were evaluated for the biological activity based on their ability to inhibit the AChE enzyme and their drug-like properties. Among the five synthesized compounds, three demonstrated the ability to inhibit AChE with IC₅₀ values ranging from 236.80 ± 8.17 μM to 354.88 ± 8.17 μM. All five compounds met the criteria of Lipinski's Rule of Five, and one compound was found to be within the range capable of crossing the blood-brain barrier according to Veber's rules. This research suggested the potential structural frameworks in synthesizing AChE inhibitor derivatives for treating Alzheimer's disease.

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INTRODUCTION

Alzheimer's disease (AD) is a common condition that leads to memory loss and a progressive decline in cognitive functions. It was first reported at a psychiatric conference in Germany in 1906 by Alois Alzheimer, after he was introduced to a 51-year-old female patient named Auguste Deter [1-3]. The cholinergic hypothesis suggests that a deficiency in the crucial neurotransmitter acetylcholine (ACh) contributes to cognitive decline in AD, with one of the causes of this deficiency being the hydrolysis of ACh by acetylcholinesterase (AChE) [4, 5]. Consequently, AChE has become a potential therapeutic target, prompting research and the development of new drugs aimed at inhibiting this enzyme for the treatment of AD [6].

The structure of acetylcholinesterase (AChE) consists of three important regions: the catalytic active site (CAS), the peripheral

site (PAS), and the narrow channel connecting the CAS and PAS [7]. Compounds capable of effectively inhibiting AChE need to possess components that bind to either the CAS or PAS, or have the ability to bind to both regions, with an appropriate linker that interacts with the narrow channel region [8].

Naphthalene is a bicyclic aromatic system containing 10 carbon atoms and 8 hydrogen atoms. Naphthalene-based derivatives exhibit a wide range of potential biological activities, including anticancer, antimicrobial, anti-inflammatory, antiviral, neuroprotective, and antipsychotic effects [9]. Moreover, some studies have shown that naphthalene-based derivatives exhibit strong activity against certain molecular targets in the treatment of Alzheimer's disease, with the inhibition of acetylcholinesterase (AChE) enzyme gaining significant attention [10, 11]. In 2020, Fareeha Anwar and her colleagues synthesized

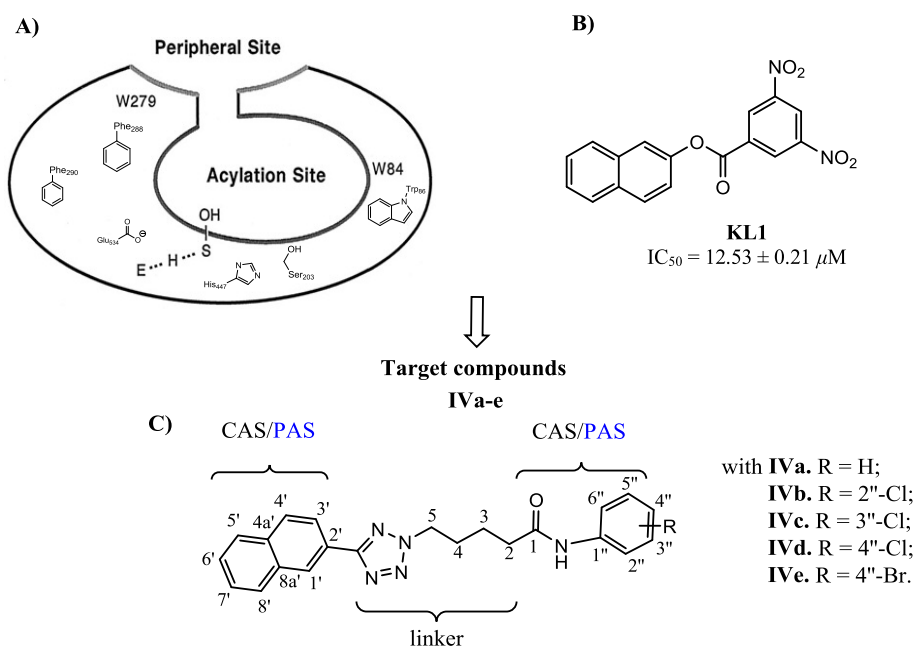


Figure 1. A) Illustration of the structure of AchE including: active center region (CAS), border barrier region (PAS) and channel lumen region. B) Structures of naphthalene derivatives. C) Structures of target compounds.

compounds **KL1**, which exhibited IC_{50} values of $12.53 \pm 0.21 \mu\text{M}$ against AChE (**Figure 1B**) [10]. In addition, derivatives containing an amide group demonstrate several potential effects, such as anticancer, Parkinson's disease treatment, protection against acute lung injury, and ulcerative colitis, among others [12]. Furthermore, numerous studies indicate that compounds with an amide group exhibit strong inhibition of AChE, making these compounds a focus of research for AD treatment [3].

In this study, based on the known structure of AChE, previously published AChE inhibitors, and the potential of the naphthalene scaffold and amide group, we synthesized and evaluated the acetylcholinesterase inhibitory activity and drug-like properties of five novel compounds with the structure 5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)-N-phenylpentanamide (**Figure 1C**).

MATERIALS AND METHODS

Chemistry

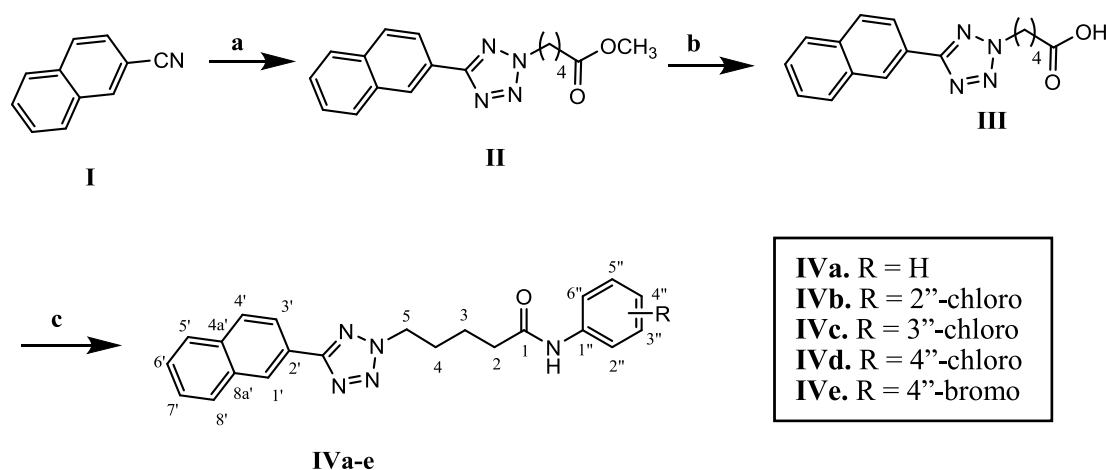
Reagents were purchased from domestic or foreign suppliers (Merck, Sigma-Aldrich) are

used directly without purification. Solvents were purchased from Merck (Germany), Sigma-Aldrich, Fisher (USA), South Korea and China suppliers. The progression of reactions was monitored by thin layer chromatography (TLC) using Merck Kieselgel 60F₂₅₄ thin plates and visualized under UV light at 254 nm.

The potential compounds **IVa**, **IVb**, **IVc**, **IVd**, **IVe** bearing the 5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)-N-phenylpentanamide scaffold were synthesized from 2-naphthalene carbonitrile (**I**) according to **Scheme 1** below.

Scheme 1. Synthesis of naphthalene derivatives (**IVa-e**). Reagents and conditions: (a) i) NaN_3 , ZnCl_2 , DMF, 110°C , 8h; ii) methyl-5-chloropentanoate, 60°C , 2h; (b) i) NaOH , MeOH, room temperature, 1.5h; ii) HCl đặc, pH = 1-2; (c) aniline derivatives, HOBt, EDC.HCl, Et_3N , DCM, DMF, room temperature, 60°C , 8h.

2-pyridin carbonitrile (1.0 eq, 10 mmol) was dissolved in *N,N*-dimethylformamide (DMF), and then NaN_3 (1.5 eq, 15 mmol) and ZnCl_2 (0.1 eq, 1 mmol) was added. The mixture was stirred at 110°C for 8 hours. The



Scheme 1: Synthesis scheme of five **IVa-e** derivatives

reaction progress was monitored by TLC. Upon completion of the reaction, the temperature was lowered to room temperature, and methyl-5-chloropentanoate (1.5 eq, 15 mmol) was added. The reaction was heated and maintained at 60°C for 2 hours. The mixture was cooled, and a saturated solution of Na₂CO₃ (25 mL) was added to precipitate Zn²⁺. The mixture was extracted with ethyl acetate (EA) (30 mL x 3 times), the organic layer phase was collected, dried with anhydrous Na₂SO₄, and organic solvent was removed under reduced pressure. The resulting mixture was purified by silica gel column chromatography with mobile phase of EA:n-hexane (30% EA) to yield compound **II**. Compound **II** was used as the starting material for subsequent reactions.

Compound **III** was synthesized from ester **II** in two stages: first, hydrolysis of ester **II** was carried out using NaOH in methanol solvent, and then the resulting mixture was acidified with concentrated HCl to yield compound **III**. The intermediate **II** was dissolved in MeOH followed hydrolysis with NaOH (2 eq). The reaction mixture was stirred for about 1.5h at room temperature. Then concentrated HCl was added to the mixture until the pH reached 1-2. The mixture was then evaporated to remove methanol, obtaining a mixture of compound **III** and NaCl salt. Next, a solvent mixture of methanol: dichloromethane (MeOH:DCM = 4:1) was added to the solid mixture, and the NaCl salt was filtered off, obtaining the filtrate containing acid **III**.

Next, compound **III** was dissolved in a minimal amount of DMF, then hydroxybenzotriol (HOBt, 1.2 eq), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl, 1.2 eq), aniline derivatives were added into the reaction vessel

(1.2 eq). A sufficient amount of DCM solvent was added. The reaction was stirred for 8h at 60°C. When the reaction completed, the solvent was removed by evaporating. Then 10% NaHCO₃ was added to the mixture, extracted the mixture with EA to collect the EA layer. The EA layer was dried with anhydrous Na₂SO₄ then evaporated under reduce pressure. The mixture was purified by silica gel column chromatography with mobile phase EA:n-hexane (10% EA), and the final product **IVa-e** was dried in a freeze dryer.

Melting points of synthesized compounds were measured using a Stuart Scientific melting point apparatus SMP3 (made in the UK). Chemical shifts are reported in parts per million (ppm) relative to TMS. Mass spectra were obtained using an Agilent 6530 Accurate-Mass QTOF LC/MS (United States) with electron spray ionization (ESI).

AChE inhibitory activity

SPL 96-well plate (Korea), tip 1000, 200 microliter from AHL (Germany), micropipette 1000, 200 microliter Nichiryo (Japan); AChE enzyme (Sigma-Aldrich, USA), acetylthiocholine iodide (ATCI, Sigma-Aldrich, USA), 5,5-dithiobis(2-nitrobenzoic acid (DTNB, TCI, Japan), galantamine hydrobromide (TCI, Japan) were used in the experiment to evaluate the ability to inhibit AChE of the synthesized compounds by the spectrophotometric method of Ellman [13] with slight modification [14]. AChE inhibitory effect was evaluated on a Varioskan Lux 96-well plate reader. 20 μL of different concentrations of test solutions in phosphate buffer pH 8.0, 20 μL 19.2 mM DTNB solution, 20 μL 19.2 mM ATCI solution and 20 μL of 0.25 IU/ml AChE solution, was added with phosphate buffer pH 8.0 until the total volume was 200 μL. Incubate the mixture at 25°C for 30 minutes, then measure the absorbance of the mixture at 412 nm

wavelength. Each substance was tested for its ability to inhibit AChE at five concentrations of 256 μM , 128 μM , 64 μM , 32 μM , 16 μM , the test was repeated three times independently. The percentage (%) of AChE inhibition of the substances at the tested concentrations is calculated according to the formula:

$$\% \text{ inhibition} = \left(1 - \frac{\text{control} - \text{test sample}}{\text{control} - \text{blank}}\right) \times 100\%$$

In which: Blank: ATCI, DNTB in pH 8.0 buffer solution

Control: ATCI, DTNB and enzyme in pH 8.0 buffer

Test sample: test substance (or galantamine hydrobromide control), ATCI, DTNB and enzyme in pH 8.0 buffer solution

The analyses were performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and the values are expressed as mean \pm SD. The AChE inhibitory activity of each sample was expressed in terms of the IC_{50} value (μM required to inhibit the hydrolysis of the substrate, ACTI by 50%), as calculated using TableCurve 2Dv4 software.

Molecular docking simulations

Five synthesized compounds were docked into AChE by AutoDock Vina to characterize the binding pose and binding affinity of ligands to AChE. In particular, the structure of human AChE was downloaded from the Protein Data Bank (PDB) with an ID of 4EY6 [14]. AutoDock Tools was employed to topologies both the receptor and ligands. The docking grid center was selected as the native ligand center of mass. The grid size was selected as $30 \times 30 \times 30 \text{ \AA}$, the center coordinates of the grid box were set to $X = -12.053$, $Y = -42.677$, $Z = 28.902$. The largest energy difference between docking modes was $7.0 \text{ kcal mol}^{-1}$. Molecular docking simulations were performed using AutoDock Vina (version

1.2.5) with the exhaustiveness parameter reaching 20. The docking poses of free binding energy conformations were visualized using BIOVIA Discovery Studio 2025 Client.

Physicochemical properties studies

Some physicochemical properties and drug-likeness predictions of three-synthesized compounds were predicted by using the SwissADME platform [15] and the BOILED-Egg permeation method [16]. A potential AChE inhibitor should observe the criteria of Lipinski's rule of five [17] and Veber's rule [18].

RESULTS AND DISCUSSIONS

RESULTS

The designed compounds **IVa-e** were synthesized following **Scheme 1**. Then their purity was confirmed by measuring the melting point and using TLC; their structures were determined by HR-MS and NMR spectra. The detailed information of their spectra is given below.

5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)-N-phenylpentanamide (IVa). Off-white solid, yield: 50 %. Mp: 210.7-211.9°C, $R_f = 0.45$ (EA:n-hexane = 1:1). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 8.68 (s, 1H, H1'), 8.21 (dd, $J = 9.5 \text{ Hz}$, 1H, H3'), 7.95 (d, $J = 6.8 \text{ Hz}$, 2H, H4', H8'), 7.88 (d, $J = 6.5 \text{ Hz}$, 1H, H5'), 7.55 (m, 2H, H6', H7'), 7.50 (d, $J = 8.0 \text{ Hz}$, 2H, H2'', H6''), 7.30 (t, $J = 7.0 \text{ Hz}$, 2H, H3'', H5''), 7.10 (t, $J = 7.3 \text{ Hz}$, 1H, H4''), 4.74 (t, $J = 5.3 \text{ Hz}$, 2H, H5), 2.44 (t, $J = 7.3 \text{ Hz}$, 2H, H2), 2.21-1.85 (m, 4H, H3, H4). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ 170.1 (C=O), 165.3 (N=C-N), 137.7 (C1''), 134.2 (C4a'), 133.2 (C8a'), 129.0 (C4'), 128.8 (C8'), 127.9 (C3'', C5''), 127.1 (C2', C5'), 126.7 (C6', C7'), 124.7 (C1'), 124.4 (C3'), 123.9 (C4''), 119.8 (C2'', C6''), 52.8 (C5), 36.5 (C2), 28.7 (C4), 22.3 (C3). HR-MS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{22}\text{N}_5\text{O}^+$ ($[\text{M}+\text{H}]^+$) = 372.1819, found HR-MS m/z for $[\text{M}+\text{H}]^+$ = 372.1807.

***N*-(2-chlorophenyl)-5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)pentanamide (IVb).** Off-white solid, yield: 53 %. Mp: 212.1-212.9°C, $R_f = 0.42$ (EA:n-hexane = 1:1). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 8.71 (s, 1H, H1'), 8.38 (d, $J = 8.5$ Hz, 1H, H3''), 8.24 (dd, $J = 8.5$ Hz, 1H, H3'), 7.98 (d, $J = 8.5$ Hz, 2H, H4', H8'), 7.91 (d, $J = 7.0$ Hz, 1H, H5'), 7.63 (s, 1H, CO-NH), 7.56 (m, 2H, H6', H7'), 7.38 (dd, $J = 8.0$ Hz, 1H, H6''), 7.30 (d, $J = 7.8$ Hz, 1H, H5''), 7.06 (d, $J = 7.8$ Hz, 1H, H4''), 4.78 (t, $J = 7.3$ Hz, 2H, H5), 2.55 (t, $J = 7.3$ Hz, 2H, H2), 2.26 (quint, $J = 7.4$ Hz, 2H, H4), 1.91 (quint, $J = 7.5$ Hz, 2H, H3). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ 170.2 (C=O), 165.4 (N=C-N), 134.4 (C1''), 134.2 (C4a'), 133.2 (C8a'), 129.0 (C4'), 128.8 (C8', C3''), 127.9 (C5''), 127.8 (C2''), 127.1 (C2', C5'), 126.7 (C6', C7'), 124.7 (C4'', C1'), 123.9 (C3'), 121.7 (C6''), 52.8 (C5), 36.7 (C2), 28.7 (C4), 22.2 (C3). HR-MS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{20}\text{ClN}_5\text{ONa}^+$ ($[\text{M}+\text{Na}]^+$) = 428.1249/430.1219, found HR-MS m/z for $[\text{M}+\text{Na}]^+$ = 428.1069/430.0992.

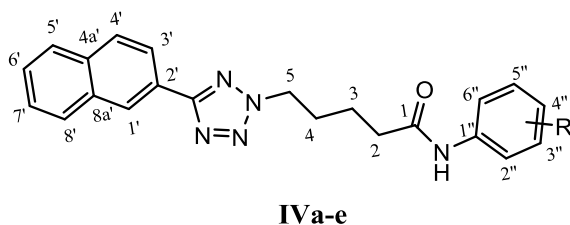
***N*-(3-chlorophenyl)-5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)pentanamide (IVc).** Off-white solid, yield: 45 %. Mp: 211.8-213.3°C, $R_f = 0.47$ (EA:n-hexane = 1:1). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 8.70 (s, 1H, H1'), 8.23 (dd, $J = 8.5$ Hz, 1H, H3'), 7.98 (dd, $J = 8.5$ Hz, 2H, H4', H8'), 7.91 (d, $J = 7.5$ Hz, 1H, H5'), 7.67 (s, 1H, CO-NH), 7.58 (m, 4H, H6', H7'), 7.35 (d, $J = 8.0$ Hz, 1H, H6''), 7.23 (m, 2H, H2'', H5''), 7.09 (d, $J = 7.5$ Hz, 1H, H4''), 4.78 (t, $J = 6.8$ Hz, 2H, H5), 2.46 (t, $J = 7.3$ Hz, 2H, H2), 2.24 (quint, $J = 7.3$ Hz, 2H, H4), 1.88 (quint, $J = 7.5$ Hz, 2H, H3). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ 170.2 (C=O), 165.4 (N=C-N), 138.8 (C1''), 134.7 (C3''), 134.3 (C4a'), 133.2 (C8a'), 130.0 (C2''), 128.8 (C4'), 128.70 (C8'), 127.9 (C5''), 127.2 (C2', C5'), 126.7 (C6', C7'), 124.5 (C1', C4''), 123.9 (C3'),

119.9 (C6''), 52.8 (C5), 36.4 (C2), 28.6 (C4), 22.1 (C3). HR-MS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{21}\text{ClN}_5\text{O}^+$ ($[\text{M}+\text{H}]^+$) = 406.1429/408.1400, found HR-MS m/z for $[\text{M}+\text{H}]^+$ = 406.1417/408.1404.

***N*-(4-chlorophenyl)-5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)pentanamide (IVd).** Off-white solid, yield: 59 %. Mp: 214.7-215.9°C, $R_f = 0.40$ (EA:n-hexane = 1:1). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 8.59 (s, 1H, H1'), 8.12 (dd, $J = 8.5$ Hz, 1H, H3'), 7.87 (d, $J = 8.5$ Hz, 2H, H4', H8'), 7.81 (d, $J = 7.0$ Hz, 1H, H5'), 7.48 (m, 2H, H6', H7'), 7.37 (d, $J = 9.0$ Hz, 2H, H2'', H6''), 7.16 (d, $J = 8.0$ Hz, 2H, H3'', H5''), 4.67 (t, $J = 7.0$ Hz, 2H, H5), 2.36 (t, $J = 7.3$ Hz, 2H, H2), 2.14 (quint, $J = 7.3$ Hz, 2H, H4), 1.77 (quint, $J = 7.5$ Hz, 2H, H3). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ 170.2 (C=O), 165.4 (N=C-N), 136.3 (C1''), 134.3 (C4a'), 133.2 (C8a'), 129.0 (C4'), 128.8 (C8'), 128.7 (C4''), 127.9 (C3'', C5''), 127.2 (C2', C5'), 126.7 (C6', C7'), 124.6 (C1'), 123.8 (C3'), 121.0 (C2'', C6''), 52.8 (C5), 36.4 (C2), 28.6 (C4), 22.2 (C3). HR-MS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{21}\text{ClN}_5\text{O}^+$ ($[\text{M}+\text{H}]^+$) = 406.1429/408.1400, found HR-MS m/z for $[\text{M}+\text{H}]^+$ = 406.1417/408.1404.

***N*-(4-bromophenyl)-5-(5-(naphthalene-2-yl)-2H-tetrazole-2-yl)pentanamide (IVe).** Off-white solid, yield: 55 %. Mp: 219.1-220.5°C, $R_f = 0.38$ (EA:n-hexane = 1:1). $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 8.67 (s, 1H, H1'), 8.20 (dd, $J = 8.5$ Hz, 1H, H3'), 7.95 (d, $J = 8.5$ Hz, 2H, H4', H8'), 7.89 (d, $J = 7.0$ Hz, 1H, H5'), 7.55 (m, 2H, H6', H7'), 7.40 (m, 4H, H2'', H3'', H5'', H6''), 7.13 (s, 1H, CO-NH), 4.75 (t, $J = 7.0$ Hz, 2H, H5), 2.43 (t, $J = 7.3$ Hz, 2H, H2), 2.22 (quint, $J = 7.3$ Hz, 2H, H4), 1.85 (quint, $J = 7.4$ Hz, 2H, H3). $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ 170.1 (C=O), 165.4 (N=C-N), 136.8 (C1''), 134.3 (C4a'), 133.2 (C8a'), 132.0 (C4'), 128.8 (C8'), 128.7 (C4''),

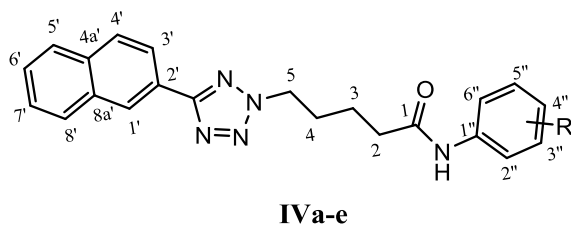
Table 1. IC₅₀ value of compounds **IVa**, **IVb**, **IVc**, **IVd** and **IVe**



IVa. R = H
IVb. R = 2''-Cl
IVc. R = 3''-Cl
IVd. R = 4''-Cl
IVe. R = 4''-Br

Compounds	IC ₅₀ (μM)
IVa	354.88 ± 8.17
IVb	> 1000
IVc	236.80 ± 20.09
IVd	243.83 ± 14.77
IVe	> 1000
Negative control	0
Galantamine hydrobromide	2.51 ± 0.20 μM

Table 2. The binding energy of compounds **IVa-e**



IVa. R = H
IVb. R = 2''-Cl
IVc. R = 3''-Cl
IVd. R = 4''-Cl
IVe. R = 4''-Br

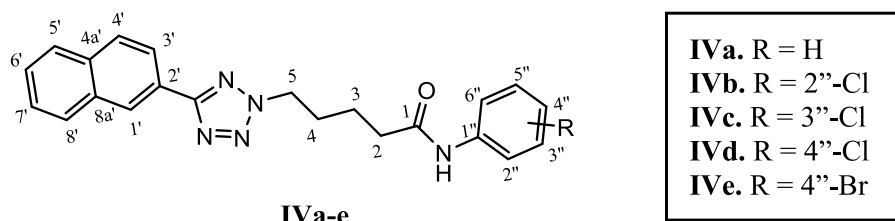
Compounds	Binding energy (kcal.mol ⁻¹)
IVa	-8.7
IVb	-8.0
IVc	-10.0
IVd	-8.1
IVe	-7.8
Galantamine	-10.3

127.9 (C3'', C5''), 127.2 (C2', C5'), 126.72 (C6'), 126.67 (C7'), 124.7 (C1'), 123.9 (C3'), 121.3 (C2'', C6''), 52.8 (C5), 36.4 (C2), 28.6 (C4), 22.2 (C3). HR-MS (ESI) m/z calculated for C₂₂H₂₁BrN₅O⁺ ([M+H]⁺) = 406.1429/408.1400, found HR-MS m/z for [M+H]⁺ = 406.1417/408.1404.

After that, five compounds **IVa**, **IVb**, **IVc**, **IVd** and **IVe** were evaluated by IC₅₀ and compared with galantamine hydrobromide as the positive control. Results expressed as IC₅₀ value are presented in **Table 1**.

We conduct further research on the interaction of the 5 synthesized compounds

Table 3. Calculated physicochemical properties data for compounds **IVa-e**



Compounds	Molecular weight (Da)	MlogP	HBA*	HBD*	TPSA* (Å ²)	NORT*	WlogP
IVa	371.44	3.06	6	1	72.70	8	4.11
IVb	405.88	3.54	6	1	72.70	8	4.76
IVc	405.88	3.54	6	1	72.70	8	4.76
IVd	405.88	3.54	6	1	72.70	8	4.76
IVe	450.33	3.65	6	1	72.70	8	4.87
RO5**	≤ 500	≤ 5	≤ 10	≤ 5			
Veber's rule			Total H-bond ≤ 12		≤ 140	≤ 10	

*HBA: Hydrogen Bonding Acceptor; HBD: Hydrogen Bonding Donnor; TPSA: Topological Polar Surface Area (Å²); NORT: Number of Rotatable Bond. **RO5: Lipinski's rule of five

with AChE by performing molecular docking simulations using AutoDock Vina. Galantamine is predicted to bind with AChE at the binding site with energy of -10.3 kcal/mol (**Table 2**).

In addition, the calculation of the physicochemical properties of synthesized compounds **IVa**, **IVb**, **IVc**, **IVd** and **IVe** are shown in **Table 3**.

DISCUSSION

The above spectral analysis confirmed the structures of the synthesized derivatives as initially designated. The ¹H-NMR spectra of all compounds exhibited peaks between 1.77 and 8.71 ppm. The protons of the methylene and ethylene groups were clearly visible in the ¹H-NMR spectra. However, the amine proton (NH) was weak and difficult to detect due to

its exchange with the solvent. The ¹H-NMR spectrum of all compounds showed a single peak with a chemical shift of about 8.59 to 8.71 ppm (in CDCl₃) corresponding to the proton of C1'. Moreover, the peak of H3' shows splitting due to interaction with H4' and its proximity to the tetrazole ring. The ¹³C-NMR data showed all expected carbon peaks, ranging from 22.1 to 170.2 ppm. The mass spectra displayed the ion molecular peak ([M+H]⁺ or [M+Na]⁺), which corresponded to the expected molecular weight of the target compounds. For the four compounds **IVb-d** with a chloro group, and compound **IVe** with a bromo group, these four compounds have two isotopes. Therefore, we need to determine two m/z values of these compounds and confirm them with the obtained mass spectrum. The m/z isotopic values of

compounds **IVb-e** are also displayed as corresponding molecular ion peaks in the mass spectrum. The spectral structure confirmation results showed agreement with the expected structures.

In **Table 1**, There are three compounds with IC_{50} values from $236.80 \pm 8.17 \mu\text{M}$ to $354.88 \pm 8.17 \mu\text{M}$. Compound **IVc** showing the best activity among them, having the smallest IC_{50} . Two compounds **IVb** and **IVe** exhibit poor AChE inhibitory activity with $IC_{50} > 1000 \mu\text{M}$. Compounds **IVc** and **IVd** exhibit better activity than **IVa**, while compounds **IVb** and **IVe** show lower activity compared to **IVa**. Therefore, there is no clear pattern regarding the introduction of different halogen substituents at different positions on the phenyl ring in relation to AChE inhibitory activity.

The results of molecular docking simulations (**Table 2**) show that in the structure of the synthesized compounds, the naphthalene ring is attached to the CAS region of AChE, through Trp86. Trp86 is a critical amino acid residue in the structure of AChE, as it serves as the initial binding site for the acetylcholine molecule, thereby initiating the catalytic hydrolysis reaction, thus this results indicate that these compounds possess potential as AChE inhibitors. Our molecular docking simulations also revealed binding energies for the five compounds ranging from -10.0 to -7.8 kcal/mol (**Table 2**), showing a strong affinity for AChE. The correlation between the IC_{50} values and the binding energy of the compounds with AChE is not yet clear, and further studies are needed to establish the structure-activity relationship of the compounds with a naphthalene scaffold.

To assess the drug-likeness and oral bioavailability of the synthesized compounds, we applied Lipinski's rule and Veber's rule. All five compounds satisfied both of these rules.

Moreover, the BOILED-Egg diagram of the compounds presented in supporting information shows that **IVa** is in the region with the potential for blood-brain barrier (BBB) penetration, thus compound **IVa** demonstrates strong potential as a promising AChE inhibitor for drug development. These findings highlight the potential of naphthalene and amide derivatives as AChE inhibitors for Alzheimer's patients.

CONCLUSION

In this study, we synthesized five compound bearing the naphthalene-2-yl scaffold **IVa-e** starting from 2-naphthalene carbonitrile. Three reaction used was [3+2] cycloaddition to form tetrazole ring, followed by an *N*-alkylation reaction with methyl-5-chloropentanoate reagent. Next is the ester hydrolysis reaction to form a salt, followed by acidifying the salt to obtain the acid carboxylic. Finally reaction was amide coupling, which used acid carboxylic and aniline derivatives to form final compounds **IVa-e**. HRMS and NMR spectroscopy were used to confirm that the structures of the synthesized compounds were as expected. **IVa**, **IVc** and **IVd** have the ability to inhibit AChE, with compound **IVc** showing the best inhibitory activity. Additionally, all five compounds demonstrated drug-like properties and good oral bioavailability according to Lipinski's and Veber's rules. Compound **IVa** is predicted to be able to cross the blood-brain barrier according to the permeability method in the BOILED-EGG model, thus compound **IVa** demonstrates strong potential as a promising AChE inhibitor for drug development.

ADDITIONAL INFORMATION

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CONFLICTS OF INTEREST

None.

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