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Original Article

Synthesis, docking, pharmacokinetic prediction, and acetylcholinesterase inhibitory evaluation of *N*-(2-(piperidine-1-yl)ethyl)benzamide derivatives as potential anti-Alzheimer agents

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Abstract

Background and purpose: Alzheimer's disease is the most common form of dementia and the sixth most common cause of death in the US according to the Alzheimer's Association. As regards, to date, no effective treatments are available because of the multifactorial nature of the disease, therefore, a large body of recent research has been allocated to the design and development of multi-target-directed ligands that can become effective drug candidates.
Experimental approach: A novel series of benzamide derivatives (5a-5l) containing piperidine core were synthesized in the current work. After identification of the chemical structures of the members of this series using ^{1}H NMR, IR, and MS spectra, their anti-acetylcholinesterase activity was assessed by the Ellman′s test. Docking studies were also performed to investigate the binding mode and determine the interacting amino acids with the corresponding ligands. Finally, the pharmacokinetic (ADME parameters) of the most potent derivative (5d) was predicted and compared with donepezil.
Findings/Results: Compound 5d possessing the fluorine atom substitution at position ortho was the most active compound in these series (IC_{50} = 13 ± 2.1 nM). This compound demonstrated superior activity than the reference drug donepezil (IC_{50} = 0.6 ± 0.05 µM). Molecular docking showed a significant hydrogen bonding of the carbonyl group of compounds 5d with tyrosine 121 into the active site of acetylcholinesterase. Fortunately, this compound showed better promising ADME properties than donepezil.
Conclusion and implication: The benzamide derivatives introduced in this paper could be proposed as potential anti-acetylcholinesterase.

Keywords: Acetylcholinesterase; Alzheimer's disease; Piperidine; Synthesis.

INTRODUCTION

Alzheimer's disease (AD), a chronic and gradual neurodegenerative disorder (1), distinguished by memory decline, language impairment, and loss the level of intellectual ability, is one of the most frequent diseases in the aged population and the prime reason for death in developed countries (2-5). AD as a neurodegenerative illness is a multifaceted disorder and is associated with various molecular events.

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Misfolding and aggregation of proteins inside the central nervous system (CNS), oxidative stress, mitochondrial abnormalities, and neuroinflammatory causes play an important role in the pathophysiology of AD (6). One of the principal causes of cognitive decline is the impairment in the neurotransmission activity of the cholinergic pathways. Acetylcholine (ACh) as a neurotransmitter conducts the related signals in the neuronal pathways. The action of the ACh terminates *via* the hydrolysis reaction by acetylcholinesterase (AChE). The mentioned enzyme has a critical role in cholinergic neurotransmission. Accumulation of the amyloid beta plaques and neurofibrillary tangles in neural cells and junction of the synapses especially in the hippocampus area is the main hallmark of the disease. This phenomenon impairs memory function and cognition (7). The disturbance in the pathways related to the other neurotransmitters like *N*-methyl-*D*-aspartate (NMDA) may also affect AD. Antagonistic activity on the NMDA receptor by administration of the memantine drug attenuates the disease progression (8,9). Other studies confirm the role of monoamine oxidase (MAO) in the pathogenesis of AD. MAO-A inhibitors are applied for the treatment of depression and anxiety, whereas MAO-B inhibitors are beneficial for AD and Parkinson's disease. Extreme levels of MAO-B gene expression would have resulted in the beginning of some biochemical events concerning neuronal dysfunction. Generated free radicals due to MAO activity have an essential impact on the pathogenesis of AD. MAO-B inhibitors protect the neuronal cells from oxidative stress (10,11).

The advantageous effects of acetylcholinesterase inhibitors on cognitive, functional, and behavioral signs of the disease are considered one of the pharmacological therapy of AD. Currently, rivastigmine, tacrine, galantamine, and donepezil (Fig. 1), fashionable medications in the market for the pharmacological therapy of AD as AChE inhibitors, have been prescribed (4,12). These inhibitors can merely delay the progression of AD *via* enhancement of the half-life of the acetylcholine, but not fully stop the disease. According to the acquired knowledge from X-ray crystallography, it has been identified that two dissimilar parts are noticeable in the active position of AChE (13). The first one is placed at the bottom of the active site and named the catalytic anionic site (CAS) and the second one is the peripheral anionic site (PAS) and is located at the entrance (14). The molecule of donepezil as an impressive AChE inhibitor can bind to both sections mentioned as the enzyme's active site. The indanone ring is bonded to the PAS, whereas the benzyl piperidine portion is bonded to the CAS in the protonated state (15-17). Replacement of the piperidine ring with piperazine congener or removal of the nitrogen atom of the piperidine ring reduces the enzyme activity. Furthermore, *N*-acylation of the nitrogen atom of the piperidine moiety decreases the potency of AChE (18-21). Although the therapeutic potential of these inhibitors in the treatment of AD is well-recognized, their application in the treatment of AD is significantly constrained by their associated side effects, which present a considerable challenge in clinical settings. (10-14). In light of the abovementioned suppositions and the escalating demand from patients for more effective therapeutic options, the design and development of novel AChE inhibitors with more potent activity and lower side effects compared with the above four inhibitors remain a formidable challenge for pharmacologists and chemists (15,22). In recent decades, many natural products or their derivatives were discovered as a new potential remedy for the treatment of AD (23-25).

According to the works of the former literature (Fig. 2), benzamide derivatives have the potential activity to inhibit the AChE enzyme (15,26,27). These compounds promote learning and memory and could be beneficial in the treatment of neurodegenerative disorders, cognitive impairment, depression, and AD (28,29). Therefore, the current study synthesized some novel benzamide derivatives containing piperidine core to inhibit AChE enzyme *in vitro*.

In designed compounds (Fig. 3), the benzamide group mimics the aromatic character of the indanone ring in donepezil, and the piperidine ring still appears as the piperidine ring of donepezil. Furthermore, a molecular docking study was also supported to explore the likely binding mode of the most potent derivative (30). This technique was applied to find the probable interactions and to compare the binding state with donepezil.

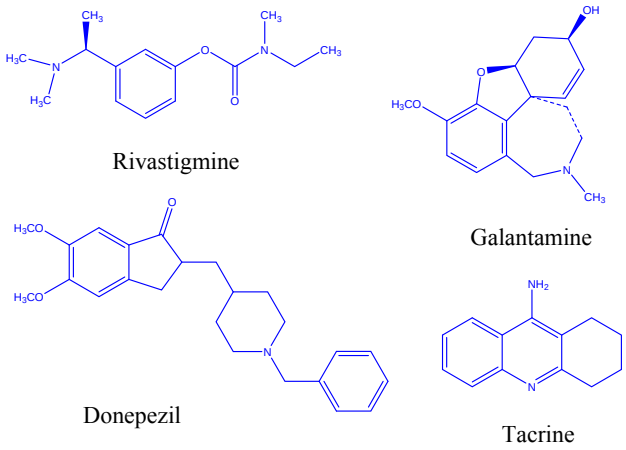


Fig. 1. Structures illustration of FDA (Food and Drug Administration)-licensed acetylcholinesterase inhibitors for the symptomatic treatment of Alzheimer's disease.

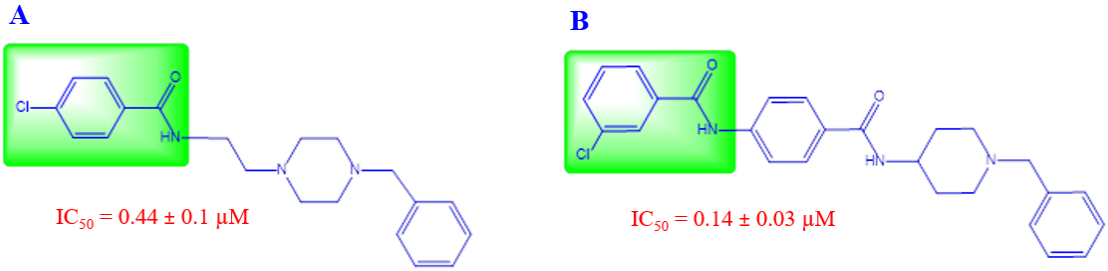


Fig. 2. (A and B) Benzamide derivatives were reported previously as potent acetylcholinesterase inhibitors.

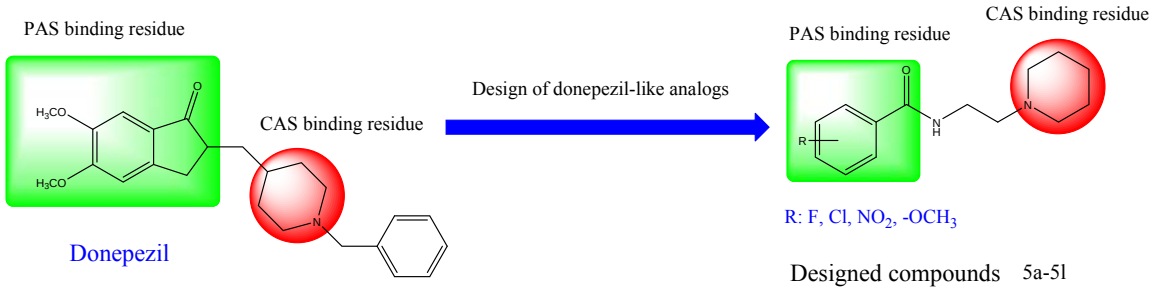


Fig. 3. Design of target compounds 5a-5l. The intended derivatives were designed according to the requisite parts of the donepezil pharmacophore. PAS as well as CAS binding groups were considered in the structures of the final compounds 5a-5l. PAS, Peripheral anionic site; CAS, catalytic anionic site;

MATERIALS AND METHODS

The commercial solvents and reagents were purchased from popular commercial provisions such as Merck and Sigma-Aldrich and used without extra purification. All tests were performed on glassware that was thoroughly cleaned and dried. A rotary vacuum evaporator was used to remove solvents from the product under reduced pressure. Proton nuclear magnetic resonance (^{1}H NMR, 250 MHz) spectra were recorded on a Bruker (Germany) spectrophotometer in deuterated chloroform solvent using tetramethylsilane as an internal

standard. The chemical bonding data of the synthesized compounds were extracted by a recorded infrared (IR) spectrum on a Shimadzu 470 spectrophotometer (Japan) in the range of 400-4000 cm^{-1}. The compounds were monitored on the Merck silica gel GF-254 plates under UV at 254 nm.

Chemistry

Synthesis of 2-(2-(piperidine-1-yl)ethyl) isoindoline-1,3-dione (3)

The synthesis of 2-(2-(piperidine-1-yl)ethyl)isoindoline-1,3-dione (3) started from 1-(2-chloroethyl) piperidine hydrochloride (2) (1 g, 6.80 mmol), in the presence of triethylamine, acetonitrile was responsible for dissolving the reagents. Triethylamine was applied to release compound (2) from the related HCl salt. The reaction mixture was left to be refluxed overnight. After scanning the reaction *via* thin-layer chromatography (TLC) and achieving the endpoint, the solvent was removed under reduced pressure. The residual was diluted with H_2O and extracted with ethyl acetate, detached from the solvent, the isolated brown oil was dissolved in CH_3CN, and potassium phthalimide (1.26 g, 6.80 mmol) was added to the suspension. The mixture reaction refluxed overnight. After completion of the reaction, the solvent was removed using a Rotavap (Heidolph, Germany). The obtained product was diluted with H_2O and extracted with ethyl acetate; the solvent was removed under diminished pressure. Eventually, by cooling, compound 3 was obtained as a white crystalline precipitant (31).

Synthesis of 2-(piperidine-1-yl)ethan-1-amine (4)

Precursor 3 (1 g, 7.81 mmol) and methylamine (0.6 mL, 78.1 mmol) in absolute ethanol were heated under reflux overnight. After observing the TLC evidence, the solvent removal process and product extraction by water and ethyl acetate were performed, the organic solvent was separated under the vacuum, and the yellow liquid of the yield was afforded (31).

Synthesis of N-(2-(piperidine-1-yl) ethyl) benzamide derivatives (5a-5l)

This method was selected as a template to prepare all derivatives (5a-5l). In a 50 mL round-bottom flask, appropriate benzoic acid derivatives (1.56 mmol), compound 4 (0.2 g, 1.56 mmol), hydroxybenzotriazole (HOBt; 0.211 g, 1.56 mmol), and dicyclo-hexylcarbodiimide (DCC; 0.321 g, 1.56 mmol) were dissolved in tetrahydrofuran (THF). The mixture was stirred for 1 h at 0 °C. This process was continued at room temperature for 24 h. After the termination of the process, the solvent was removed by rotary evaporator, and recrystallization with diethyl ether was carried out. Finally, the pure yellow crystal has emerged (32).

Molecular docking

To study the *in silico* molecular interaction of the target compounds, ArgusLab 4.0 software was utilized and a docking procedure was implemented (33,34). The structure of compound 5d was constructed in the Arguslab workspace and afterward was minimized energetically using Austin Model 1 (AM1) as a semi-empirical method. AChE enzyme in complex with donepezil (PDB code: 1EVE) was downloaded from the Brookhaven protein data bank as a PDB file (35). The donepezil as the co-crystallized ligand was docked into the active site of the AChE to validate the software internally. The obtained root mean squared deviation (RMSD) deduced between the docking of the co-crystallized molecule and the newly defined donepezil molecule was 0.064 Å. The calculated binding free energy for this docking process was -12.74 kcal/mol. The structure of the AChE enzyme was optimized geometrically using the universal force field as a molecular mechanic method. The docking process was done for ligand 5d in the workspace of ArgusLab 4 software. Initially, the related groups for the corresponding ligand and AChE were defined. The binding location of donepezil was characterized as a binding site for finding the best pose and conformation for exploring ligands. The binding mode and related interactions of ligand 5d were explored in Molegro molecular viewer software (Fig. 4) (36).

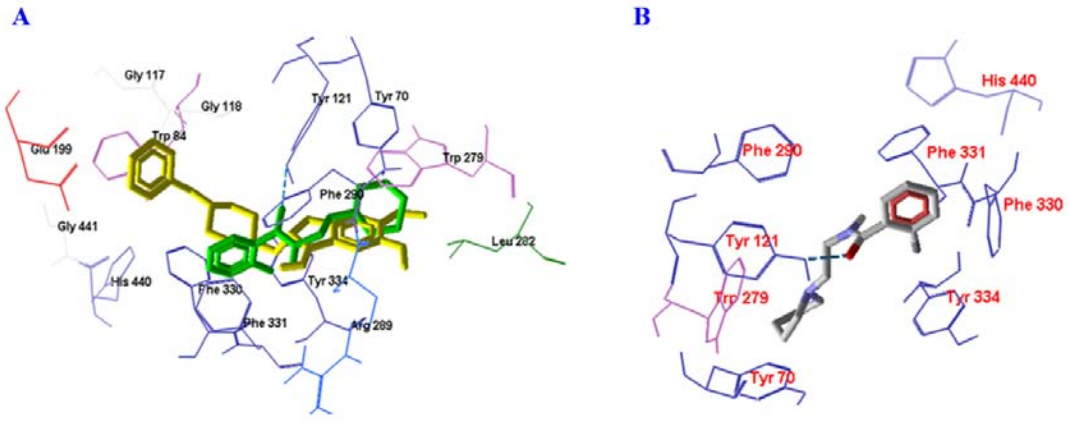
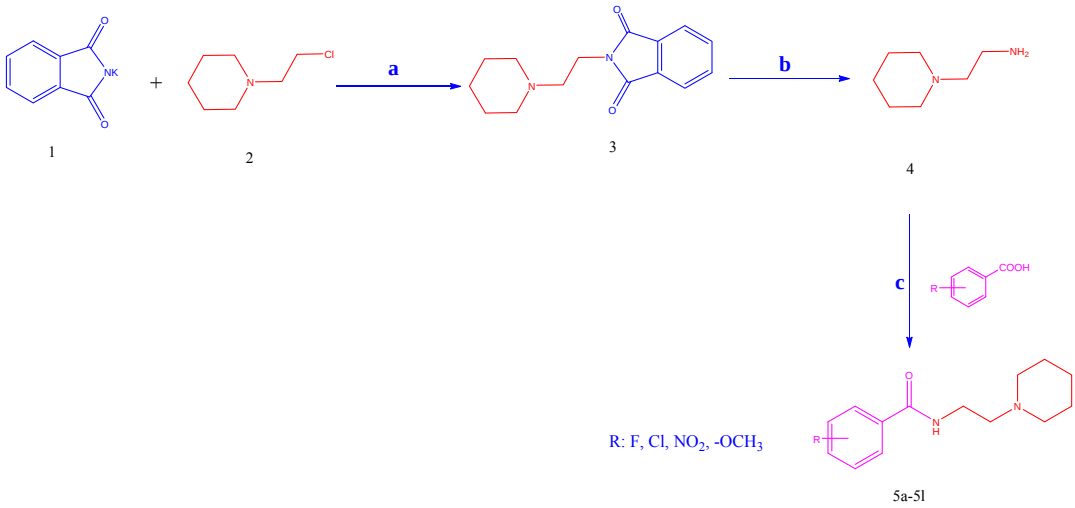


Fig. 4. (A) Superimposition of donepezil (yellow) and compound 5d (green) into the active site of AChE. Essential interacting amino acids are observable in this area; (B) interaction of compound 5d into the active site of AChE. Hydrogenic interaction with Tyr121 has occurred. AChE, Acetylcholinesterase.



Scheme 1. Total illustration for the synthesis of final compounds 5a-5l. a, CH3CN, reflux overnight; b, CH_3NH_2, reflux, EtOH; c, dicyclohexylcarbodiimide, hydroxybenzotriazole, tetrahydrofuran, room temperature.

Ellman's test

Ellman's test is used to evaluate the possible inhibitory effects of the compounds on AChE (37). The procedure was done based on the previous study, with slight modifications (38). Briefly, the agents were solubilized in ethanol and prepared in 8 different concentrations (0, 10^{-3}, 10^{-4}, 10^{-5}, 10-6, 10^{-7}, 10^{-8} and 10^{-9} µM). Ellman's test is based on the acetylthiocholine conversion to acetate and thiocholine. The thio compound then reacts with 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB) to form 5-thio-2-nitrobenzoate, a chromophore with maximum absorption at 412 nM. The test was done in a 96-well microplate. Enzyme inhibition is measured when different drug concentrations are used with Ellman's reagents. The results are then normalized based on the protein content of each well which is determined by the method of Bradford (39). The IC_{50} values were calculated and used to compare the potency of the agents.

Drug-likeness prediction

Pharmacokinetic properties and ADME (absorption, distribution, metabolism, elimination) parameters related to the drug-likeness prediction of the most active compound, namely compound 5d (ortho fluorine) and donepezil, were calculated using http://swissadme.ch website (40).

RESULTS

Chemistry

All target compounds 5a-5l were prepared according to Scheme 1. Compound 2 was purchased as HCl salts and liberation from the hydrochloride salt was done using triethylamine as a basic substance. After the freedom of the related amine as a liquid material, potassium phthalimide as a potent nucleophile was utilized to achieve compound 3 with an average yield of 58%. Phthalimide residue was applied to perform a Gabriel synthetic procedure for primary amine formation (compound 4). However, the phthalimide residue was cleaved from compound 3 using methylamine base under reflux conditions. At last, compound 4 was afforded as presented in Table 1 with a 30% yield. Final benzamide derivatives 5a-5l were synthesized using DCC as a coupling agent and HOBt as an additive agent in THF. Most of these derivatives were obtained with high yields and the corresponding physicochemical features such as melting points were also measured. Furthermore, spectroscopic data (NMR, IR, MS) were also collected to confirm the synthesized substances.

Characterization data for the synthesized compounds

2-(2-(Piperidine-1-yl)ethyl)isoindoline-1,3-dione (3)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.39 (m, 2H, H_4-piperidine), 1.51 (m, 4H, $H_{3,5}$-piperidine), 2.46 (m, 4H, $H_{2,6}$-piperidine), 2.59 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.82 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.70 (m, 2H, $H_{5,6}$-phthalimide), 7.83 (m, 2H, $H_{4,7}$-phthalimide). IR (KBr, cm^{-1}) ῡ: 3086 (stretch, C-H, aromatic), 2939, 2850 (stretch, C-H, aliphatic), 1712 (stretch, C=O phthalimide). MS (*m/z*, %): 258 (M^+, 10), 160 (30), 147 (20), 98 (100).

2-(Piperidine-1-yl)ethan-1-amine (4)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.24 (s, 2H, NH_2), 1.39 (m, 2H, H_4-piperidine), 1.51 (m, 4H, $H_{3,5}$-piperidine), 2.45 (m, 4H, $H_{2,6}$-piperidine), 2.58 (t, 2H, -C$\underline{H}_2$-CH_2-NH_2), 3.81 (t, 2H, -CH_2-C$\underline{H}_2$-NH_2). IR (KBr, cm^{-1}) ῡ: 3411, 3240 (stretch, NH_2), 3082 (stretch, C-H, aromatic), 2939, 2850 (stretch, C-H, aliphatic), 1712 (stretch, C=O phthalimide). MS (*m/z*, %): 128 (M^+, 5), 104 (80), 98 (100).

2-Chloro-N -(2- (piperidine-1-yl) ethyl) benzamide (5a)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.11 (m, 2H, H_4-piperidine), 1.25 (m, 4H, $H_{3,5}$-piperidine), 1.66 (m, 4H, $H_{2,6}$-piperidine), 1.91 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.39 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.63 (t, 1H, J = 7.5 Hz, H_4-2-chlorophenyl), 7.86 (t, 1H, J = 7.5 Hz, H_5-2-chlorophenyl), 8.02 (d, 1H, J = 7.5 Hz, H_6-2-chlorophenyl), 8.52 (d, 1H, J = 7.5 Hz, H_3-2-chlorophenyl), 9.72 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3066 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1675 (stretch, C=O). MS (*m/z*, %): 266 (M^+, weak), 160 (55), 139 (100), 111 (95), 98 (30), 75 (85).

Table 1. Properties of compounds 5a-5l and related obtained yields of the final compounds.

Compound	R	Chemical formula	MW (g/mol)	mp (°C)	Yield (%)
3	-	$C_{15}H_{18}N_2O_2$	258.32	78	58
4	-	$C_7H_{16}N_2$	128.22	186*	30
5a	2-Cl	$C_{14}H_{19}ClN_2O$	266.77	91	94
5b	3-Cl	$C_{14}H_{19}ClN_2O$	266.77	106	42
5c	4-Cl	$C_{14}H_{19}ClN_2O$	266.77	100	63
5d	2-F	$C_{15}H_{22}FN_2O$	265.35	82	69
5e	3-F	$C_{15}H_{22}FN_2O$	265.35	86	86
5f	4-F	$C_{15}H_{22}FN_2O$	265.35	86	53
5g	2-NO_2	$C_{14}H_{19}N_3O_3$	277.32	120	45
5h	3-NO_2	$C_{14}H_{19}N_3O_3$	277.32	102	70
5i	4-NO_2	$C_{14}H_{19}N_3O_3$	277.32	125	98
5j	2-OCH_3	$C_{15}H_{22}N_2O_2$	262.35	78	67
5k	3-OCH_3	$C_{15}H_{22}N_2O_2$	262.35	89	31
5l	4-OCH_3	$C_{15}H_{22}N_2O_2$	262.35	145	61

*, The value is related to the boiling point; MW, molecular weight; mp, melting point.

3-Chloro-N-(2- (piperidine-1-yl) ethyl) benzamide (5b)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.12 (m, 2H, H_4-piperidine), 1.30 (m, 4H, $H_{3,5}$-piperidine), 1.62 (m, 4H, $H_{2,6}$-piperidine), 1.89 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.40 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.47 (d, 1H, *J* = 7.5 Hz, H_6-3-chlorophenyl), 7.57 (t, 1H, *J* = 7.5 Hz, H_5-3-chlorophenyl), 7.76 (d, 1H, *J* = 7.5 Hz, H_4-3-chlorophenyl), 8.27 (s, 1H, H_2-3-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3070 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1693 (stretch, C=O). MS (*m/z*, %): 266 (M^+, 10), 160 (35), 139 (100), 111 (75), 98 (40), 75 (65).

4-Chloro-N- (2- (piperidine-1-yl)ethyl) benzamide (5c)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.14 (m, 2H, H_4-piperidine), 1.30 (m, 4H, $H_{3,5}$-piperidine), 1.68 (m, 4H, $H_{2,6}$-piperidine), 1.90 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.42 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.58 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl), 8.20 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3089 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1624 (stretch, C=O). MS (*m/z*, %): 266 (M^+, 15), 160 (65), 139 (100), 111 (45), 98 (60), 75 (85).

2-Fluoro-N- (2- (piperidine-1-yl) ethyl) benzamide (5d)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.14 (m, 2H, H_4-piperidine), 1.34 (m, 4H, $H_{3,5}$-piperidine), 1.62 (m, 4H, $H_{2,6}$-piperidine), 1.91 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.39 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.09-7.23 (m, 1H, H_4-2-fluorophenyl), 7.40-7.61 (m, 1H, H_5-2-fluorophenyl), 7.68-7.86 (m, 1H, H_6-2-fluorophenyl), 8.03 (t, 1H, *J* = 7.5, H_3-2-fluorophenyl), 10.83 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3089 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1616 (stretch, C=O). MS (*m/z*, %): 250 (M^+, weak), 140 (95), 123 (100), 95 (50).

3-Fluoro-N-(2- (piperidine-1-yl) ethyl) benzamide (5e)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.22 (m, 2H, H_4-piperidine), 1.62 (m, 4H, $H_{3,5}$-piperidine), 1.66 (m, 4H, $H_{2,6}$-piperidine), 1.90 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.39 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.30 (t, 1H, *J* = 7.5 Hz, H_5-3-fluorophenyl), 7.43 (t, 1H, *J* = 7.5 Hz, H_6-4-fluorophenyl), 7.76 (m, 1H, H_4-4-fluorophenyl), 7.89 (m, 1H, H_2-4-fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3066 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1693 (stretch, C=O). MS (*m/z*, %): 250 (M^+, weak), 140 (60), 123 (100), 95 (65).

4-Fluoro-N-(2- (piperidine-1-yl) ethyl) benzamide (5f)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.22 (m, 2H, H_4-piperidine), 1.62 (m, 4H, $H_{3,5}$-piperidine), 1.66 (m, 4H, $H_{2,6}$-piperidine), 1.90 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.39 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.46-7.57 (m, 2H, $H_{2,6}$-4-fluorophenyl), 8.32 (m, 2H, $H_{3,5}$-4-fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3074 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1681 (stretch, C=O). MS (*m/z*, %): 250 (M^+, weak), 140 (85), 123 (100), 95 (40).

2-Nitro-N-(2-(piperidine-1-yl)ethyl)benzamide (5g)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.14 (m, 2H, H_4-piperidine), 1.29 (m, 4H, $H_{3,5}$-piperidine), 1.66 (m, 4H, $H_{2,6}$-piperidine), 1.92 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.40 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.70 (t, 1H, *J* = 7.5 Hz, H_5-2-nitrophenyl), 7.86 (t, 1H, *J* = 7.5 Hz, H_4-2-nitrophenyl), 8.33 (d, 1H, *J* = 7.5 Hz, H_6-2-nitrophenyl), 8.56 (d, 1H, *J* = 7.5 Hz, H_3-2-nitrophenyl). IR (KBr, cm^{-1}) ῡ: 3332 (stretch, NH), 3078 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1620 (stretch, C=O). MS (*m/z*, %): 277 (M^+, weak), 150 (100), 134 (90), 104 (35), 76 (95), 63 (40), 51 (80).

3-Nitro-N-(2-(piperidine-1-yl)ethyl)benzamide (5h)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.22 (m, 2H, H_4-piperidine), 1.36 (m, 4H, $H_{3,5}$-piperidine), 1.63 (m, 4H, $H_{2,6}$-piperidine), 1.92 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.39 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.69 (t, 1H, *J* = 7.5 Hz, H_5-3-nitrophenyl), 8.43 (m, 2H, H_4, H_6, 3-nitrophenyl), 8.94 (s, 1H, H_2-3-nitrophenyl), 10.25 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3089 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1624 (stretch, C=O). MS (*m/z*, %): 277 (M^+, 10), 150 (100), 134 (70), 104 (45), 76 (85), 63 (60), 51 (45).

4-Nitro-N-(2-(piperidine-1-yl)ethyl)benzamide (5i)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.13 (m, 2H, H_4-piperidine), 1.36 (m, 4H, $H_{3,5}$-piperidine), 1.67 (m, 4H, $H_{2,6}$-piperidine), 1.89 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.40 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 7.32 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-nitrophenyl), 7.83 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-nitrophenyl), 10.52 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 3113 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1693 (stretch, C=O). MS (*m/z*, %): 277 (M^+, 12), 150 (100), 134 (70), 104 (55), 76 (85), 63 (20), 51 (70).

2-Methoxy-N-(2- (piperidine-1-yl) ethyl) benzamide (5j)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.10 (m, 2H, H_4-piperidine), 1.25 (m, 4H, $H_{3,5}$-piperidine), 1.63 (m, 4H, $H_{2,6}$-piperidine), 1.86 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.44 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 3.97 (s, 3H, -OCH_3), 7.12 (t, 1H, *J* = 7.5 Hz, H_5-2-methoxyphenyl), 7.42 (d, 1H, *J* = 7.5 Hz, H_3-2-methoxyphenyl), 7.68 (t, 1H, *J* = 7.5 Hz, H_4-2-methoxyphenyl), 8.06 (d, 1H, *J* = 7.5 Hz, H_6-2-methoxyphenyl), 8.85 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3055 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1712 (stretch, C=O). MS (*m/z*, %): 262 (M^+, weak), 152 (75), 135 (100), 123 (50), 105 (95), 92 (45).

3-Methoxy-N-(2- (piperidine-1-yl) ethyl) benzamide (5k)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.12 (m, 2H, H_4-piperidine), 1.27 (m, 4H, $H_{3,5}$-piperidine), 1.67 (m, 4H, $H_{2,6}$-piperidine), 1.90 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.44 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 3.97 (s, 3H, -OCH_3), 6.85 (s, 1H, H_2-3-methoxyphenyl), 7.23 (d, 1H, *J* = 7.5 Hz, H4-3-methoxyphenyl), 7.69 (d, 1H, *J* = 7.5 Hz, H_4-3-methoxyphenyl), 7.77 (t, 1H, *J* = 7.5 Hz, H_5-3-methoxyphenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3062 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1693 (stretch, C=O). MS (*m/z*, %): 262 (M^+, 10), 152 (65), 135 (100), 123 (70), 105 (65), 92 (85).

4-Methoxy-N-(2- (piperidine-1-yl) ethyl) benzamide (5l)

^{1}HNMR ($CDCl_3$, 250 MHz) δ (ppm): 1.11 (m, 2H, H_4-piperidine), 1.36 (m, 4H, $H_{3,5}$-piperidine), 1.66 (m, 4H, $H_{2,6}$-piperidine), 1.90 (m, 2H, -N-CH_2-C$\underline{H}_2$-piperidine), 3.47 (m, 2H, -N-C$\underline{H}_2$-CH_2-piperidine), 3.94 (s, 3H, -OCH_3), 7.06 (d, 2H, $H_{3,5}$-4-methoxyphenyl), 8.24 (d, 2H, $H_{2,6}$-4-methoxyphenyl). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 3062 (stretch, C-H, aromatic), 2927, 2850 (stretch, C-H, aliphatic), 1624 (stretch, C=O). MS (*m/z*, %): 262 (M^+, 15), 152 (45), 135 (100), 123 (65), 105 (85), 92 (90).

AChE inhibition

The synthesized compounds 5a-5l were tested against AChE and the obtained IC_{50} values were compared to donepezil as a reference drug. Various moieties namely Cl, F, NO_2, and OCH_3 were examined on the diverse positions of the phenyl residue in terms of their electronic, polarity, and hydrophilicity/lipophilicity impacts. Compound 5d with the fluorine atom substitution at position ortho was the most active compound in these series (IC_{50} = 13 ± 2.1 nM). Compound 5d demonstrated superior activity than donepezil (IC_{50} = 0.6 ± 0.05 µM). Interestingly, compound 5a with ortho positioning of the chlorine atom also rendered remarkable inhibitory activity toward AChE (IC_{50} = 0.09 ± 0.002 µM) and this inhibitory effect was higher than donepezil. Movement of the chlorine moiety to the meta position (compound 5b) also led to a potent derivative (IC_{50} = 0.63 ± 0.0002 µM) with comparable potency to donepezil. The rest of the synthesized derivatives did not display potency greater than donepezil. According to the results obtained electron-withdrawing substituents that

possess lipophilic properties such as Cl and F indicated an increase in the potency at position ortho. However, this effect is not produced by nitro moiety as an electron-withdrawing substituent at position ortho because of the hydrophilic property of this moiety. Investigation of the methoxylated derivatives as compounds bearing electron-donating moiety exhibited no favorable inhibitory effect against AChE. Changing the position of the lipophilic electron-withdrawing moieties (Cl and F) from ortho to para decreased the inhibitory effect of the enzyme. In contrast, as observed in Table 2, electron-withdrawing groups, the nitro group for instance, exhibited greater polarity and hydrophilic properties and yielded an enhanced outcome at the position para which was the opposite of Cl and F impact.

Molecular docking

Molecular docking showed a significant hydrogen bonding of the carbonyl group of compound 5d with Tyr121 into the AChE active site (Fig. 4). The hydroxyl group of the respective amino acid was responsible for the mentioned hydrogenic interaction. Other important amino acids in the active site of AChE such as Phe330, Phe331, and Trp279 were also observable in the binding area of compound 5d. Bonding interactions of donepezil inside the active site of AChE prepared using Ligandscout 2 software and the regarded image have been demonstrated in Fig. 5. Hydrophobic (Phe330, Trp84, Ile444), electrostatic (Tyr334, Phe330), and π-interaction (Trp84) were the main interactions that have been seen in this *in silico* investigation (Table 3).

Table 2. Results of acetylcholinesterase inhibitory assessment (IC50, µM) of compounds 5a-5l.

Compound	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**Donepezil**
R	2-Cl	3-Cl	4-Cl	2-F	3-F	4-F	-
IC_{50} (µM)	0.09 ± 0.002	0.63 ± 0.0002	46 ± 2.4	13 ± 2.1*	1.6 ± 0.06	3.1 ± 0.47	0.6 ± 0.05
Compound	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**	
R	2-NO_2	3-NO_2	4-NO_2	2-OCH_3	3-OCH_3	4-OCH_3	
IC_{50} (µM)	19 ± 4	2 ± 0.34	2.4 ± 0.2	4.7 ± 1.5	5.2 ± 0.5	5.6 ± 0.3	

*, nM

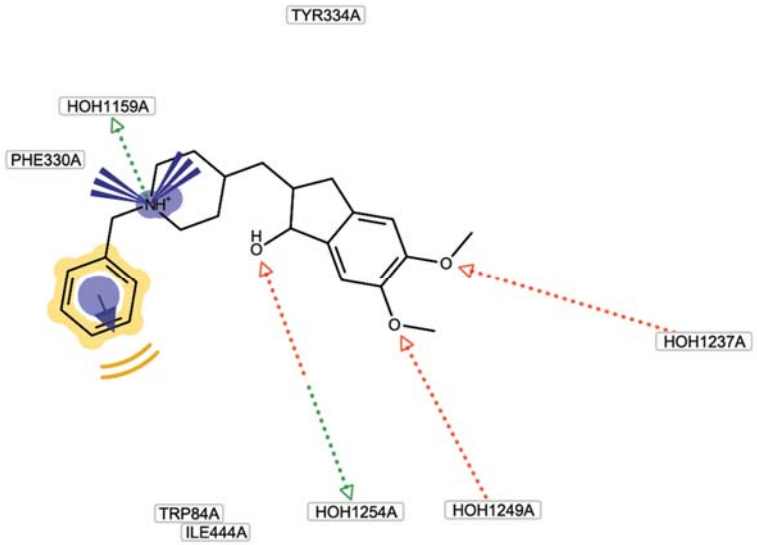


Fig. 5. Bonding interactions of donepezil inside the active site of acetylcholinesterase.

Table 3. Binding free energies and the related interacting amino acids for each ligand.

Compound	R	Binding free energy (kcal/mol)	Interaction
5a	2-Cl	-9.83	Hydrogenic (Tyr121), hydrophobic (Phe331, Phe288, Phe330, Phe290).
5b	3-Cl	-9.92	Hydrophobic (Phe331, Phe330, Tyr334).
5c	4-Cl	-10.17	Hydrophobic (Phe331, Phe330, Tyr334).
5d	2-F	-9.76	Hydrogenic (Tyr121), hydrophobic (Phe331, Phe330, Tyr334).
5e	3-F	-9.63	Hydrogenic (Tyr121), hydrophobic (Tyr70, Tyr334), electrostatic (Trp279).
5f	4-F	-9.53	Hydrogenic (Tyr121), hydrophobic (Phe331, Phe330, Tyr334), electrostatic (Trp279).
5g	2-NO_2	-9.45	Hydrogenic (Tyr121, 2 cases), hydrophobic (Phe331, Phe330, Tyr334), electrostatic (Asp72, Tyr334, Trp279).
5h	3-NO_2	-8.92	Hydrogenic (Tyr334), hydrophobic (Phe331, Phe330), electrostatic (Asp72, Tyr334, Trp279).
5i	4-NO_2	-9.14	Hydrogenic (Tyr334, Tyr121), electrostatic (Asp72).
5j	2-OCH_3	-9.26	Hydrogenic (Tyr121, 2 cases), hydrophobic (Phe331, Phe330).
5k	3-OCH_3	-8.96	Hydrophobic (Phe331, Phe330, Tyr334), electrostatic (Trp279), π-interaction (Phe331).
5l	4-OCH_3	-9.10	Hydrogenic (Tyr121), hydrophobic (Phe331, Phe330, Trp84), electrostatic (Trp279), π-interaction (Phe331).
Donepezil	-	-12.74	Hydrophobic (Phe330, Trp84, Ile444), electrostatic (Tyr334, Phe330), π-interaction (Trp84).

Table 4. Properties of compound 5d and donepezil.

Compound	MW	Rotatable bond	H-bond acceptor	H-bond donor	TPSA	GI absorption	BBB permeation	P-gp substrate	Bio-availability	CYP450 interaction
5d	250.31	5	3	1	32.34	High	Yes	No	0.55	No
Donepezil	379.49	6	4	0	38.77	High	Yes	Yes	0.55	Yes (2D6, 3A4)

MW, Molecular weight; GI, gastrointestinal; BBB, blood-brain barrier; TPSA, topological polar surface area.

The interactions for other compounds were obtained by Ligandscout 2 which are reported in Table 3 provided as a supplementary file (https: //github.com/Alireza1878/RPS-supplementary). The value of the binding free energy for donepezil inhibition of the AChE was -12.74 kcal/mol. This value was higher than the binding free energy for other tested compounds. Binding free energy for compound 5d as the most potent inhibitor in the enzymatic test was also one of the highest obtained values compared to the other ligands. According to Table 3, the main interactions in the enzyme active site were the hydrophobic, hydrogen bonding, π-interaction, and electrostatic interplays. Phe330, Phe331, and Tyr334 were the critical amino acids for hydrophobic interactions. Hydrogen bonding was carried out *via* the Tyr121 in most cases and the Tyr334 in two cases. Compounds 5k and 5l as methoxylated derivatives participated in π-interaction by Phe331.

Drug-likeness prediction

The corresponding results obtained from the http://swissadme.ch website are listed in Table 4. The achieved data exhibited that the properties of compound 5d conform to the drug-likeness factors such as Lipinski, Ghose, Egan, Veber, and Muegge rules. Fortunately, compound 5d did not show any violation of the mentioned rules. Higher gastrointestinal absorption and blood-brain barrier penetration of this compound was the same as donepezil. In contrast to donepezil, the predicted data exhibited no affinity for the P-gp pump. Besides, compound 5d did not rendered any interaction with CYP450 isoenzymes (CYP2C9, CYP2C19, CYP2D6, CYP1A2, CYP3A4), whereas donepezil displayed interaction with CYP2D6 and CYP3A4 isoenzymes. Overall, better pharmacokinetic features were anticipated for compound 5d compared to donepezil.

DISCUSSION

Synthesis of compound 3 as a phthalimide derivative was implemented according to the Gabriel protocol (Scheme 1). 1-(2-Chloroethyl) piperidine hydrochloride (compound 2) was released from the hydrochloride salt and consequently *via* the reaction with potassium phthalimide in toluene, the intended phthalimide derivative (compound 3) was afforded with an acceptable yield (Table 1). The best condition for reaction performance was the reflux process in the presence of triethylamine as a proton acceptor. In the next step, methylamine was applied in absolute ethanol to cleave the phthalimide residue and achieve the corresponding primary amine (compound 4). This reaction was also carried out under refluxing conditions. At last, target compounds 5a-5l were prepared through amidation protocol using DCC and HOBt at room temperature. Most of the final compounds were obtained with an average yield.

Synthesis of compounds 5c (4-Cl), 5h (3-NO_2), and 5i (4-NO_2) have been reported in the previous papers (41,42). A different method was applied for the synthesis of the intended compounds. Fortunately, higher yield was afforded for compounds 5c (63% *vs* 30%) and 5i (98% *vs* 86%) compared to the previously reported procedures. Different melting points for these compounds have been reported previously. Likely, differences in the preparation methods and consequently different probable impurities and purification methods may affect the results of melting point analysis.

Comparison of the obtained results of the benzamide derivatives of the current project with other chemical pharmacophores such as phthalimide analogs showed that replacement of the phthalimide residue with benzamide congener and concomitant replacement of the piperazine ring with piperidine as its bioisosteric group can significantly enhance the enzyme inhibitory activity (30,43). Probably, more flexibility of the amidic functional group compared to rigid phthalimide analogs potentiates the capability of these compounds for more effective interaction with the regarded receptor. In addition, the benzamide derivatives introduced in this research demonstrated higher enzyme inhibitory potency than the previously stated compounds (15). It could be proposed that the hydrogenic interaction that connected the amidic bond to the Tyr121 of the enzyme may be a determinant factor for the potency enhancement. Former synthesized benzamide analogs that bear piperazine heterocycle displayed inferior activity than currently investigated compounds. It could be proposed that the higher lipophilicity of the piperidine heterocycle in the current derivatives is responsible for the remarkable potency.

Recently some benzamide derivatives as AChE inhibitors were synthesized that possessed the inhibitory effects in micromolar concentrations (44). The mentioned compounds did not contain the amine side chain compared to the compounds present in the current project. This evidence confirmed the pivotal role of the amine side chain that appeared in the target derivatives 5a-5l as a piperidine heterocycle. Besides, the molecular docking study also showed that the amine functional group presented in donepezil participated in an electrostatic interaction with Phe330 (18) (Fig. 5). This important interaction also occurred for most of the tested compounds between the nitrogen of the piperidine heterocycle and Trp279 (Table 3).

Exploration of the previous literature showed that compound 5c (4-Cl) positively treats depression *in vivo* (41). Structural similarity of the synthesized compounds is obvious with the antidepressant drug moclobemide. Therefore, it could be proposed that these compounds may be effective as MAO inhibitors in the treatment of CNS disorders such as depression and Alzheimer's disease. Some of the nitrated benzamide derivatives like 5h (3-NO_2) and 5i (4-NO_2) were also synthesized as intermediates to prepare anticancer agents (42).

According to the literature and Table 3, the obtained results for the *in-silico* interactions of donepezil were hydrophobic (Phe330, Trp84, Ile444), electrostatic (Tyr334, Phe330), and π-interaction (Trp84) (18). The afforded results for target compounds 5a-5l showed that the interacting amino acids with donepezil are also observable for the interaction of the docked ligands 5a-5l. In addition, the amidic bond

caused most of the investigated compounds to communicate with the enzyme active site *via* a hydrogenic interaction by Tyr121. The phenyl ring and aliphatic part of the synthesized compounds potentiated the hydrophobic interplays with the receptor, whereas the nitrogen atom of the piperidinyl moiety fortified the electrostatic interaction. Nitrated derivatives also participated in an electrostatic interaction through the nitro group. Methoxylated compounds contribute to π-interaction with Phe331. This type of interaction has not been reported for donepezil.

CONCLUSION

The benzamide derivatives introduced in this paper could be proposed as potential anti-acetylcholinesterase. Compound 5d with ortho fluorine atom as a potent inhibitor (IC_{50} = 13 ± 2.1 nM) is a susceptible drug candidate for *in vivo* investigation. A strong hydrogen bonding was rendered for this compound in molecular docking investigation and a similar in silico binding mode and superimposition were observed. Furthermore, better pharmacokinetic features were predicted for this compound to support the drug-likeness possibility.

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Conflict of interest statements

The authors declared no conflict of interest in this study.

Authors' contributions

All authors contributed to the design of the manuscript. A. Mohammadi-Farani contributed to conceptualization; data curation; formal analysis; and project administration. F. Moradi contributed to the investigation; methodology; resources; validation; visualization; and writing the original draft. A. Hosseini contributed to the investigation, review, and editing of the article. A. Aliabadi contributed to the conceptualization; supervision; writing, review, and editing of the article. The finalized article was read and approved by all authors.

REFERENCES

1. Bagheri M, Joghataei MT, Mohseni S, Roghani M. Genistein ameliorates learning and memory deficits in amyloid β (1-40) rat model of Alzheimer's disease. Neurobiol Learn Mem. 2011;1;95(3):270-276. DOI: 10.1016/j.nlm.2010.12.001.
2. Patel V, Flisher AJ, Hetrick S, McGorry P. Mental health of young people: a global public-health challenge. Lancet 2007;14;369(9569):1302-1313. DOI: 10.1016/S0140-6736(07)60368-7.
3. Batty GD, Deary IJ, Gottfredson LS. Premorbid (early life) IQ and later mortality risk: systematic review. Ann Epidemiol. 2007;17(4):278-288. DOI: 10.1016/j.annepidem.2006.07.010.
4. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, *et al*. Global burden of disease attributable to mental and substance use disorders: findings from the global burden of disease study 2010. Lancet. 2013;382(9904):1575-1586. DOI: 10.1016/S0140-6736(13)61611-6.
5. Gallagher M, Pelleymounter MA. Spatial learning deficits in old rats: a model for memory decline in the aged. Neurobiol Aging. 1988;9(5-6):549-556. DOI: 10.1016/s0197-4580(88)80112-x.
6. Shi DH, Huang W, Li C, Wang LT, Wang SF. Synthesis, biological evaluation and molecular modeling of aloe-emodin derivatives as new acetylcholinesterase inhibitors. Bioorg Med Chem. 2013;21(5):1064-1073. DOI: 10.1016/j.bmc.2013.01.015.
7. Wang CH, Wang LS, Zhu N. Cholinesterase inhibitors and non-steroidal anti-inflammatory drugs as Alzheimer's disease therapies: an updated umbrella review of systematic reviews and meta-analyses. Eur Rev Med Pharmacol Sci. 2016;20(22):4801-4817. PMID: 27906438.
8. Bent-Ennakhil N, Coste F, Xie L, Aigbogun MS, Wang Y, Kariburyo F, *et al*. A real-world analysis of treatment patterns for cholinesterase inhibitors and memantine among newly-diagnosed Alzheimer's disease patients. Neurol Ther. 2017;6(1):131-144. DOI: 10.1007/s40120-017-0067-7.
9. Sun G, Wang J, Guo X, Lei M, Zhang Y, Wang X, *et al*. Design, synthesis and biological evaluation of LX2343 derivatives as neuroprotective agents for the treatment of Alzheimer's disease. Eur J Med Chem. 2018;145:622-633. DOI: 10.1016/j.ejmech.2017.12.080.
10. Y.X. Xu, H. Wang, X.K. Li, S.N. Dong, W.W. Liu, Q. Gong, et al. Discovery of novel propargylamine-modified 4-aminoalkyl imidazole substituted pyrimidinylthiourea derivatives as multifunctional agents for the treatment of Alzheimer's disease, Eur J Med Chem. 143 (2018) 33-47. DOI: 10.1016/j.ejmech.2017.08.025.

11. Joubert J, Foka GB, Repsold BP, Oliver DW, Kapp E, Malan SF. Synthesis and evaluation of 7-substituted coumarin derivatives as multimodal monoamine oxidase-B and cholinesterase inhibitors for the treatment of Alzheimer's disease. Eur J Med Chem. 2017;125:853-864.
DOI: 10.1016/j.ejmech.2016.09.041.
12. Regier DA, Farmer ME, Rae DS, Myers JK, Kramer MR, Robins LN, *et al*. One-month prevalence of mental disorders in the United States and sociodemographic characteristics: the epidemiologic catchment area study. Acta Psychiatr Scand. 1993;88(1):35-47.
DOI: 10.1111/j.1600-0447.1993.tb03411.x.
13. Chiriano G. Computer-and synthesis-based approaches towards the discovery of novel BACE-1 inhibitors as potential anti-Alzheimer's drugs. 2011. pp.18-20.
14. Wong DM, Greenblatt HM, Dvir H, Carlier PR, Han YF, Pang YP, *et al*. Acetylcholinesterase complexed with bivalent ligands related to huperzine a: experimental evidence for species-dependent protein-ligand complementarity. J Am Chem Soc. 2003;125(2):363-373.
DOI: 10.1021/ja021111w.
15. Aliabadi A, Mohammadi-Farani A, Bistouni JR. Synthesis and acetylcholinesterase inhibitory assessment of benzamide derivatives incorporated piperazine moiety as potential anti-Alzheimer agents. J Pharm Sci Res. 2017;9(9):1598-1603.
16. Pourshojaei Y, Abiri A, Eskandari K, Haghighijoo Z, Edraki N, Asadipour A. Phenoxyethyl piperidine/morpholine derivatives as PAS and CAS inhibitors of cholinesterases: insights for future drug design. Sci. Rep. 2019;27;9(1):19855,1-19.
DOI: 10.1038/s41598-019-56463-2.
17. Korabecny J, Spilovska K, Mezeiova E, Benek O, Juza R, Kaping D, *et al*. A systematic review on donepezil-based derivatives as potential cholinesterase inhibitors for Alzheimer's disease. Curr. Med. Chem. 2019;26(30):5625-5648.
DOI: 10.2174/0929867325666180517094023.
18. Sugimoto H, Yamanishi Y, Iimura Y, Kawakami Y. Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors. Curr Med Chem. 2000;7(3):303-339.
DOI: 10.2174/0929867003375191.
19. Mehrabi F, Pourshojaei Y, Moradi A, Sharifzadeh M, Khosravani L, Sabourian R, *et al*. Design, synthesis, molecular modeling and anticholinesterase activity of benzylidene-benzofuran-3-ones containing cyclic amine side chain. Future Med Chem. 2017;9(7):659-671.
DOI: 10.4155/fmc-2016-0237.
20. Shamsimeymandi R, Pourshojaei Y, Eskandari K, Mohammadi-Khanaposhtani M, Abiri A, Khodadadi A, *et al.* Design, synthesis, biological evaluation, and molecular dynamics of novel cholinesterase inhibitors as anti-Alzheimer's agents. Arch Pharm. 2019;352(7):1800352,1-12.
DOI: 10.1002/ardp.201800352.
21. Abolhasani F, Pourshojaei Y, Mohammadi F, Esmaeilpour K, Asadipour A, Ilaghi M, *et al*. Exploring the potential of a novel phenoxyethyl piperidine derivative with cholinesterase inhibitory properties as a treatment for dementia: Insights from STZ animal model of dementia. Neurosci Lett. 2023;27;810:137332.
DOI: 10.1016/j.neulet.2023.137332.
22. McHardy SF, Wang HY, McCowen SV, Valdez MC. Recent advances in acetylcholinesterase Inhibitors and Reactivators: an update on the patent literature (2012-2015). Expert Opin Ther Pat. 2017;27(4):455-476.
DOI: 10.1080/13543776.2017.1272571.
23. Cooper EL, Ma MJ. Alzheimer disease: clues from traditional and complementary medicine. J Tradit Complement Med. 2017;7(4):380-385.
DOI: 10.1016/j.jtcme.2016.12.003.
24. Houghton PJ, Howes MJ. Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease. Neurosignals. 2005;14(1-2):6-22.
DOI: 10.1159/000085382.
25. Bonda DJ, Wang X, Perry G, Nunomura A, Tabaton M, Zhu X, *et al*. Oxidative stress in Alzheimer disease: a possibility for prevention. Neuropharmacology. 2010;59(4-5):290-294.
DOI: 10.1016/j.neuropharm.2010.04.005.
26. Mohammadi-Farani A, Nazari S, Mohammadi M, Navid SJ, Hosseini A, Aliabadi A. Novel acetylcholinesterase inhibitors: synthesis, docking and inhibitory activity evaluation of 4-benzamido-N-(1-benzylpiperidin-4-yl) benzamide derivatives. Results Chem. 2024;7:101273,1-8.
DOI: 10.1016/j.rechem.2023.101273.
27. Mohammadi-Farani A, Takesh M, Mohammadi M, Hosseini A, Aliabadi A, Aliabadi A. Synthesis, docking and acetylcholinesterase inhibitory evaluation of 1, 3, 4-thiadiazole derivatives as potential anti-alzheimer agents. Pharm Sci. 2024;30(3):1-10.
DOI: 10.34172/PS.2024.7.
28. Yanez M, Vina D. Dual inhibitors of monoamine oxidase and cholinesterase for the treatment of Alzheimer disease. Curr Top Med Chem. 2013;13(14):1692-1706.
DOI: 10.2174/15680266113139990120.
29. Rochais C, Lecoutey C, Gaven F, Giannoni P, Hamidouche K, Hedou D, *at al*. Novel multitarget-directed ligands (MTDLs) with acetylcholinesterase (AChE) inhibitory and serotonergic subtype 4 receptor (5-HT4R) agonist activities as potential agents against Alzheimer's disease: the design of donecopride. J Med Chem. 2015;58(7):3172-3187.
DOI: 10.1021/acs.jmedchem.5b00115.
30. Mohammadi-Farani A, Ahmadi A, Nadri H, Aliabadi A. Synthesis, docking and acetylcholinesterase inhibitory assessment of 2-(2-(4-Benzylpiperazin-1-yl) ethyl) isoindoline-1, 3-dione derivatives with potential anti-Alzheimer effects. Daru. 2013;21(1):47,1-9.
DOI: 10.1186/2008-2231-21-47.

31. Aliabadi A, Shamsa F, Ostad SN, Emami S, Shafiee A, Davoodi J, *et al*. Synthesis and biological evaluation of 2-phenylthiazole-4-carboxamide derivatives as anticancer agents. Eur J Med Chem. 2010;45(11):5384-5389. DOI: 10.1016/j.ejmech.2010.08.063.
32. Rahmani Khajouei M, Mohammadi-Farani A, Moradi A, Aliabadi A. Synthesis and evaluation of anticonvulsant activity of (Z)-4-(2-oxoindolin-3-ylideneamino)-N-phenylbenzamide derivatives in mice. Res Pharm Sci. 2018;13(3):262-272. DOI: 10.4103/1735-5362.228956.
33. Thompson MA 2004. ArgusLab 4.0. 1. Planaria Software LLC, Seattle, WA. Available at http://www.arguslab.com.
34. Fernandez D, Avilés FX, Vendrell J. A new type of five-membered heterocyclic inhibitors of basic metallocarboxypeptidases. Eur J Med Chem. 2009;44(8):3266-3271. DOI: 10.1016/j.ejmech.2009.03.034.
35. Burley SK, Berman HM, Bhikadiya C, Bi C, Chen L, Di Costanzo L, *et al*. RCSB protein data bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic Acids Res. 2019;47(D1):D464-D474. DOI: 10.1093/nar/gky1004.
36. Pripp AH. Docking and virtual screening of ACE inhibitory dipeptides. Eur Food Res Technol. 2007;225:589-592. DOI: 10.1007/s00217-006-0450-6.
37. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95. DOI: 10.1016/0006-2952(61)90145-9.
38. ahmani-Khajouei M, Mohammadi-Farani A, Ghorbani H, Aliabadi A. Synthesis and acetylcholinesterase inhibitory assessment of 3-(2-(4-benzoylpiperazin-1-yl) ethylimino) indolin-2-one derivatives with potential anti-alzheimer effects. J Rep Pharm Sci. 2015;4(2):162-171. DOI: 10.4103/2322-1232.222583.
39. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254. DOI: 10.1006/abio.1976.9999.
40. Daina A, Michielin O, Zoete VJ. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717,1-13. DOI: 10.1038/srep42717.
41. Ghanbarpour A, Hadizadeh F, Piri F, Rashidi-Ranjbar P. Synthesis, conformational analysis and antidepressant activity of moclobemide new analogues. Pharm Acta Helv. 1997;72(2): 119-122. DOI: 10.1016/s0031-6865(97)00004-6.
42. Kapuriya N, Kakadiya R, Dong H, Kumar A, Lee P, Zhang X, *et al*. Design, synthesis, and biological evaluation of novel water-soluble N-mustards as potential anticancer agents. Bioorg Med Chem. 2011;19(1):471-485. DOI: 10.1016/j.bmc.2010.11.005.
43. Hassanzadeh M, Hassanzadeh F, Khodarahmi GA, Rostami M, Azimi F, Nadri H, *et al*. Design, synthesis, and bio-evaluation of new isoindoline-1,3-dione derivatives as possible inhibitors of acetylcholinesterase. Res Pharm Sci. 2021;16(5): 482-492. DOI: 10.4103/1735-5362.323915.
44. Drozdowska D, Maliszewski D, Wróbel A, Ratkiewicz A, Sienkiewicz M. New benzamides as multi-targeted compounds: a study on synthesis, AChE and BACE1 inhibitory activity and molecular docking. Int J Mol Sci. 2023;24(19):14901,1-18. DOI: 10.3390/ijms241914901.