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Original Article

Design, synthesis, and bio-evaluation of new isoindoline-1,3-dione derivatives as possible inhibitors of acetylcholinesterase

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Abstract

Background and purpose: Alzheimer's disease is considered one of the lead causes of elderly death around the world. A significant decrease in acetylcholine level in the brain is common in most patients with Alzheimer's disease, therefore acetylcholinesterase (AChE) inhibitors such as donepezil and rivastigmine are widely used for patients with limited therapeutic results and major side effects.
Experimental approach: A series of isoindoline-1,3-dione -N-benzyl pyridinium hybrids were designed, synthesized and evaluated as anti-Alzheimer agents with cholinesterase inhibitory activities. The structure of the compounds were confirmed by various methods of analysis such as HNMR, CNMR, and FT-IR. Molecular modeling studies were also performed to identify the possible interactions between neprilysin and synthesized compounds.
Findings/Results: The biological screening results indicated that all synthesized compounds displayed potent inhibitory activity with IC_{50} values ranging from 2.1 to 7.4 µM. Among synthesized compounds, para-fluoro substituted compounds 7a and 7f exhibited the highest inhibitory potency against AChE (IC_{50} = 2.1 µM). Molecular modeling studies indicated that the most potent compounds were able to interact with both catalytic and peripheral active sites of the enzyme. Also, some of the most potent compounds (7a, 7c, and 7f) demonstrated a neuroprotective effect against H_2O_2-induced cell death in PC12 neurons.
Conclusion and implications: The synthesized compounds demonstrated moderate to good AChE inhibitory effect with results higher than rivastigmine.

Keywords: Acetylcholinesterase inhibitors; Alzheimer's disease; Isoindoline-1,3-dione; Molecular docking; N-benzyl pyridinium.

INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disease that contributes to most of the diagnosed cases of dementia (about 80% of all cases). AD was first identified by the decrease in short-term memory, which indicates a defect in the central nervous system. The progression of the disease leads to behavioral changes such as irritability, anxiety, depression, and finally cognitive complaints (1-4). AD has been associated with several factors, including the decline of levels of acetylcholine (ACh), increase activity of monoamine oxidase enzyme, hyper-phosphorylation of tau proteins, amyloid aggregation, and oxidative stress (5). Presently, cholinesterase inhibitors are the main target for the management of AD.

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The current drugs approved by FDA including four acetylcholinesterase inhibitors :tacrine, rivastigmine, galantamine, and donepezil (Fig. 1) (6). These drugs alleviate the symptoms of AD by increasing the levels of the neurotransmitter ACh in the cortex and hippocampus regions of the brain (7). The insufficient efficacy of the currently available treatments, small number of drug candidates in clinical trials, and high failure rate of AD clinical trials suggest there is an urgent need to further research in this area (8,9).

AChE features two distinct binding sites: the catalytic active site (CAS) and the peripheral anionic site (PAS) connected by a gorge (10). In addition, AChE interacts with amyloid-β through PAS which is located at the entrance of the gorge, thereby accelerating its polymerization into oligomers and fibrils and increasing the neurotoxicity of amyloid-β aggregates (11-13). Therefore, the design of new agents that are able to interact with both sites (CAS and PAS) of AChE would be an effective approach for the management of AD's symptoms (11).

Isoindoline-1,3-dione (phthalimide) derivatives are important compounds in medicinal chemistry that possess various biological activities, such as anticancer, anti-inflammatory, monoamine oxidase-B inhibitory potency, α-glucosidase inhibitory,anti-amyloid-β aggregation, antiepileptic and AChE inhibitory activity (14,15). It is proved that phthalimide interacts with the peripheral anionic site of the ACh and therefore it is a noteworthy pharmacophore for the design of a new inhibitor (compound A, Fig. 2) (16). In addition, benzyl pyridinium salts are a privileged scaffold for the design and development of new AChE inhibitors (17,18). Moreover, molecular modeling showed that N-benzyl pyridinium moiety interacted with the CAS of AChE (compound B, Fig. 2) (19).

Starting with these findings, we employed the hybridization strategy to modify the structure of donepezil with the idea of expanding their biological activity on AchE. The design strategy of new compounds is shown in Fig. 2. The N-benzyl pyridinium moiety could inhibit the ChEs through binding to CAS of ChEs, and the phthalimide scaffold might have potential interaction with the PAS of AChE due to the aromatic character of that.

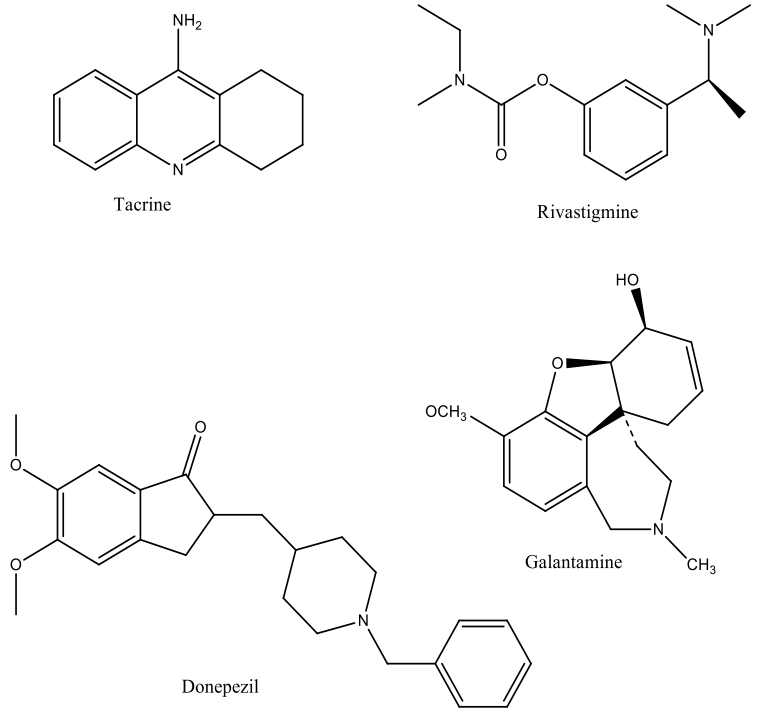


Fig. 1. Chemical structures of marketed anti-Alzheimer's drugs.

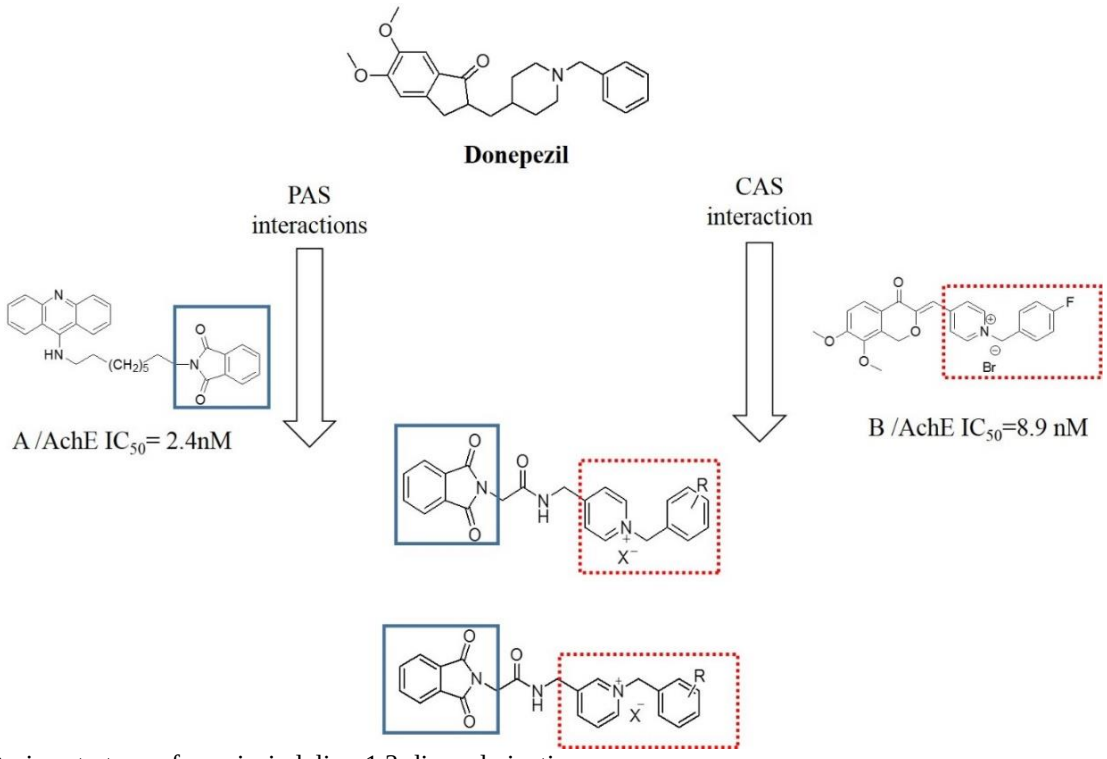


Fig. 2. Design strategy of new isoindoline-1,3-dione derivatives.

MATERIALS AND METHODS

Instrumentation

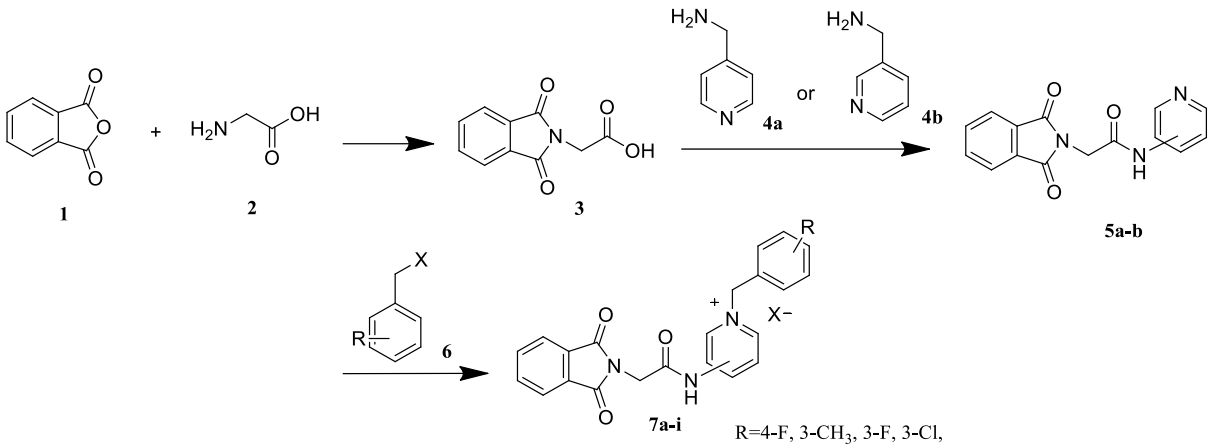
All starting materials, reagents, and solvents were purchased from commercial suppliers like Merck (Germany) and Aldrich (USA) companies. Analytical thin-layer chromatography (TLC) was conducted using Merck silica gel 60 F254 plates (Germany). Proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded by a Bruker 400 MHz spectrometer (Germany) and chemical shifts are expressed as ppm with tetramethylsilane (TMS) as internal standard. Infrared (IR) was recorded using a WQF-510 Fourier-transform IR (FT-IR) spectrophotometer (China). Compounds melting points were determined by electrothermal 9200 melting point apparatus (United Kingdom) and are uncorrected.

Chemistry

The synthesis of target compounds 7a-i was accomplished using the pathways illustrated in Scheme 1. In the first step of the reaction, a mixture of phthalic anhydride 1 (2.5 mmol, 370 mg) and glycine 2 (2.5 mmol, 188 mg) was refluxed at 120 °C for 8 h in glacial acetic acid (20 mL). Progress of the reaction was monitored by TLC. The resulting mixture was cooled to 5 °C at an ice-water bath to produce a white solid precipitate which then was filtered and washed with a cold solution of HCl (1 N) followed by water to produce a white crystalline powder of compound 3 (melting point 195-197 °C, literature.193-196 °C) (20).

2-(1,3-Dioxoisoindolin-2-yl)-N-(pyridin-4-ylmethyl)acetamide 5a and 2-(1,3-dioxoisoindolin-2-yl)-N-(pyridin-3-ylmethyl) acetamide 5b were synthesized *via* an amidation reaction using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBT).

Intermediate 2-(1,3-dioxoisoindolin-2-yl)acetic acid 3 (2 mmol) with 3or4-(aminomethyl)pyridine 4a-b (3 mmol) in the presence of EDC (4 mmol) and HOBT (4 mmol) was stirred in chloroform (15 mL) at room temperature for 15-24 h. After the completion of the reaction, the mixture was poured into water and extracted with methylene chloride (2 × 20 mL). The organic layer was washed two times with a saturated solution of NaCl (50 mL) and once with saturated solution of $NaHCO_3$ (50 mL). Finally, the organic layer was washed with water (25 mL). The organic layer then was concentrated in a rotary evaporator to furnish a white fluffy powder of compound 5a-b.



Scheme 1. Reagents and conditions: (a) K_2CO_3, glacial acetic acid, reflux, 8 h, 97% yield; (b) EDCI, HOBT, chloroform, rt, 15-24 h, 63-70% yield; (c) KI, acetonitrile, 80 C, overnight.

Final compounds 7a-7i were obtained through the addition of proper benzyl halide derivatives 6 to intermediate 5a-b in dry acetonitrile. Finally, in the last step of the synthesis, compound 5a-b (1 mmol) was refluxed with different derivatives of benzyl halide (1.5 mmol) in acetonitrile (15 mL) in 80 °C. The completion of reactions was determined by TLC. Then, the solvent was evaporated from the mixture using a rotary evaporator. Ethyl acetate was added dropwise until crystals were obtained. The resulting crystals were filtered and washed with ethyl acetate.

Cholinesterase inhibition assay

The AChE inhibitory activity was tested using Ellman method (21). Donepezil and rivastigmine have been used as references. AChE (E.C. 3.1.1.7, type V-S, lyophilized powder, from electric eel, 1000 unit) was purchased from Sigma-Aldrich (Steinheim, Germany). 5,5'-Dithiobis-(2-nitrobenzoic acid), dipotassium hydrogen phosphate, potassium dihydrogen phosphate, acetylthiocholine iodide, and potassium hydroxide were purchased from Fluka (Buchs, Switzerland). Synthesized compounds 7a-i were dissolved in dimethyl sulfoxide (DMSO, 5 mL) and then diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to afford a final concentration range. To achieve 20-80% inhibition of AChE activity five different concentrations of target compounds were tested. Each well contained 50 µL potassium phosphate buffer (KH_2PO_4/K_2HPO_4, 0.1 M, pH 8), prepared sample as described above (25 µL), enzyme (25 µL) with a final concentration of 0.22 U/mL in the buffer. They were pre-incubated at 25 °C for 15 min and then substrate (acetylthiocholine iodide, 3 mM in buffer, 125 µL) was added. The change of absorption was measured at 412 nm after 15 min. A control experiment was performed under the same conditions without inhibitor and the blank contained 3 µL buffer, 200 µL water, 100 µL 5,5'-dithiobis-(2-nitrobenzoic acid), and 20 µL substrates. Each concentration was analyzed in triplicate, and IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration *vs* percent of inhibition). Spectrophotometric measurements were performed on a Cecil BioAquarius CE 7250 Double Beam spectrophotometer (22).

Molecular docking studies

Docking studies were performed using the Gold 5.3 program (The Cambridge Crystallographic Data Center, Cambridge, UK). The structures of the most potent compounds (7a and 7f) were built using the ChemDraw program and then were transferred into Discovery Studio 4.1 (AccelrysInc, San Diego, CA, USA). Ligand structures were typed with CHARMm force field and partial charges were calculated by the Momany-Rone option (23). Then, they were minimized with Smart Minimizer which performs 1000 steps of steepest descent with an RMS gradient tolerance of 3 and conjugate gradient minimization (24). The crystal structure of AChE complexed with donepezil (PDB

code: 1EVE) was taken from RCSB Protein Data Bank (PDB, http://www.rcsb.org/pdb/ home/home.do). The protein structures were prepared using the protein preparation protocol in DS. The targets were prepared as follows: the complex was typed with the CHARMm force field, hydrogen atoms were added, water molecules were removed, and the pH of the protein was adjusted to almost neutral, 7.4. ligand-binding site was defined as a sphere with a radius of 10 Å around the native ligand in the crystal structure. Other parameters were set by default protocol settings. GOLD utilizes a genetic algorithm for conformational search and molecular docking. As a result, 10 ligand conformations were obtained and were ranked according to the GOLD fitness function. Finally, the best poses were chosen for binding analysis (25).

Evaluation of the neuroprotective effect of the compounds

The 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to measure the metabolic activity of differentiated PC12 cells as an indicator of cell viability and neuroprotection. The PC12 cell line was purchased from the Pasteur Institute of Iran. Cells were cultivated in Dulbecco′s modified eagle′s medium (DMEM-high glucose, Sigma-Aldrich, D5796) supplemented with 10% fetal bovine serum (Sigma-Aldrich, F7524), 1% non-essential amino acids (Sigma-Aldrich, M7145), and antibiotics (100 unit/mL penicillin, 100 µg/mL streptomycin, Sigma-Aldrich, P4333).

To induce neuronal differentiation, PC12 cells were re-suspended using trypsin/ethylenediaminetetraacetic acid (0.25%) and seeded in 96-well culture plates (3500 cells/well) and cultivated for another one week in the presence of differentiation medium (DMEM + 2% horse serum (Sigma-Aldrich, H1270) + 100 ng/mL beta-nerve growth factor (Sigma-Aldrich, N2513) until neurite appearance. To evaluate the effect of compounds on the survival rate of neurons, the culture medium was changed to serum and nerve growth factor free medium, then, candidate compounds (7a, 7c, and 7f) were added to their culture medium at the final concentrations of 1, 10, 100 µM. Donepezil 10 µM (Sigma-Aldrich D6821) was used as the positive control. Each compound was solved in DMSO (Sigma-Aldrich, C6164) to make 100 mM stock solution from it, and then diluted in DMEM to make 1, 0.1, and 0.01 mM working solutions. DMSO content of working solutions was equally adjusted at 1%. Then, 10 µL of each working solution was added to each well containing 90 µL medium to get 100 µL of culture medium with final concentrations of the compound at 1, 10, 100 µM. The final DMSO concentration which cells were exposed to was 0.1%.

Cells were cultivated in the presence of compounds for 3 h, later ROS-mediated apoptosis was induced by exposing them to H_2O_2 (300 µM) for 12 h. Next, MTT (Sigma-Aldrich, M2128) assay was performed, for this purpose 10 µL of MTT solution (5 mg/mL) was added to each well, and 4-h later culture medium was replaced with 100 µL of DMSO (Sigma-Aldrich, D8418). The optical density (OD) of samples was read at 545 nm and subtracted from the reading at 630 nm using an ELISA reader. Each experiment was performed in three replicates and repeats. Cell survival was calculated using the following equation (26):

$$\text{Cell survival} = \frac{\text{OD of compound} - \text{OD of blank}}{\text{OD of H2O2} - \text{OD of blank}}$$

Statistical analysis

The data were expressed as mean ± SEM. One-way ANOVA followed by the Tukey post hoc test multiple group comparison was used to determine whether there are any statistically significant differences between the groups using GraphPad Prism software (version 6.07). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Chemistry

4- ((2- (1,3-Dioxoisoindolin-2-yl) acetamido) methyl)-1-(4-fluorobenzyl)pyridin-1-ium (7a)

Yield: 63%, mp.140-143 °C, IR V_{max}, 3310(NH), 1780 (C=O), 1728 (C=O), 1661 (C=O), 1611 (C=C) cm^{-1}; HNMR δ: (400 MHz-$CDCl_3$), 9.36 (1H, t, *J* = 6 Hz, N<u>H</u>-CH_2), 9.1 (2H, d, *J* = 8 Hz, CH-N=CH), 8.1 (2H, d, *J* = 8 Hz, <u>CH</u>=CH-N=CH-<u>CH</u>), 7.8 (2H, m, O=C-

CH=CH-CH=CH=C-C=O) 7.7 (2H, m, O=C-CH=CH-CH=CH-C-C=O) 7.6 (2H, m, CH-CH=CF-CH=CH), 7.1 (2H, t, *J* = 10 Hz, CH-CH=CF-CH=CH), 6 (2H, S, N-CH_2-C=O), 4.7 (2H, D, *J* = 6 Hz, N-CH_2), 4.6 (2H, S, N-CH_2-C).

4-((2- (1,3-Dioxoisoindolin-2-yl) acetamido) methyl)-1-(3-methylbenzyl)pyridin-1-ium (7b)

Yield: 49%, mp.144-145 °C, IR V_{max}, 3309 (NH), 2984 (C-H aliphatic), 1778 (C=O) 1730 (C=O), 1659 (C=O); HNMR δ: (400 MHz-$CDCl_3$), 9.5 (1H, t, *J* = 8 Hz, NH-C=O), 9 (2H, d, *J* = 8 Hz, CH-N=CH), 8.1 (2H, d, *J* = 8 Hz, CH=CH-N=CH-CH), 7.8 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.7 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.3-7.2 (4H, m, CH=CH-CH=CH-CH3), 5.9 (2H, S, N-CH_2-C=O), 4.7 (2H, D, *J* = 8 Hz, O=C-NH-CH_2), 4.6 (2H, S, N-CH_2), 2.3 (3H, S, CH_3).

4- ((2- (1,3-Dioxoisoindolin-2-yl)acetamido) methyl)-1-(3-fluorobenzyl)pyridin-1-ium (7c)

Yield: 56%, mp. 121-124 °C, IR V_{max}, 3312 (NH), 3059 (C-H aromatic), 1781 (C=O), 1729 (C=O), 1700 (C=O); HNMR δ: (400 MHz-$CDCl_3$), 9.36 (1H, t, *J* = 8 Hz, O=C-NH-CH_2), 9 (2H, d, *J* = 8 Hz, CH-N=CH), 8 (2H, d, *J* = 8 Hz, CH=CH-N=CH-CH), 7.8 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.7 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.3-7.1 (4H, m, CH=CF-CH=CH-CH), 5.9 (2H, S, N-CH_2-C=O), 4.68 (2H, d, *J* = 8 Hz, O=C-NH-CH_2), 4.6(2H,S,N-CH_2-C).

1-(3-Chlorobenzyl)-4-((2-(1,3-dioxoisoindolin-2-yl)acetamido)methyl)pyridin-1-ium (7d)

Yield: 53%, mp. 194-196 °C, IR V_{max}, 3308 (NH), 3041 (C-H aromatic), 1777 (C=O), 1674 (C=O), 1644 (C=C) cm^{-1}; HNMR δ: (400 MHz-$CDCl_3$), 10 (1H, s, NH-CH_2), 8.7 (1H, t, *J* = 8, CH=CH-CH=N-CH), 8.41 (1H, d, *J* = 8 Hz, CH=CH-CH=N-CH), 8.34 (2H, d, *J* = 8 Hz CH=CH-CH=N-CH) 7.9-7.8 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.7 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.6-7.4 (4H, m, CH=CCl-CH=CH-CH), 5.99 (2H, S, N-CH_2-C=O), 4.82(2H, d, *J* = 8 Hz, NH-CH_2), 4.6(2H, S, N-CH_2-C).

1-Benzyl -3- ((2- (1,3-dioxoisoindolin-2-yl) acetamido)methyl)pyridin-1-ium (7e)

Yield: 76%, mp. 210-211.5 °C, IR V_{max}, 3196 (NH), 2932 (C-H aromatic), 1775 (C=O), 1719 (C=O), 1682 (C=C) cm^{-1}; HNMR δ: (400 MHz-$CDCl_3$), 9.96 (1H,s, NH-CH_2), 8.77(1H, t, *J* = 8 Hz, CH=CH-CH=N), 8.41 (1H, d, *J* = 8 Hz, CH=CH-CH=N), 8.31 (1H, d, *J* = 8 Hz CH=CH-CH=N) 7.85 (3H, m, C-CH-N, O=C-C=CH-CH=CH-CH=C-C=O) 7.75 (2 H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.61 (2 H, m, CH=CH-CH=CH-CH), 7.42 (3 H, m, CH=CH-CH=CH-CH) 5.94 (2H, S, N-CH_2-C=O), 4.8 (2H, d, *J* = 4 Hz, NH-CH_2), 4.6(2H, S, N-CH_2-C).

3- ((2-(1,3-Dioxoisoindolin-2-yl)acetamido) methyl)-1-(4-fluorobenzyl)pyridin-1-ium (7f)

Yield: 38%, mp. 226.5-227 °C, IR V_{max}, 3278 (NH), 3050 (C-H aromatic), 2928 (C-H aliphatic) 1771(C=O), 1716 (C=O), 1676 (C=O) cm^{-1}; HNMR δ: (400 MHz-$CDCl_3$), 10.1 (1H, s, NH-CH_2), 9.32 (1H, t, *J* = 8 Hz, C-CH=CH-CH=N-CH), 8.4 (2H, m, CH=CH-CH=N-CH), 7.8 (3H, m, CH=CH-CH=N-CH, O=C-C=CH-CH=CH-CH=C-C=O) 7.75-7.6 (4H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.2 (2H, t, *J* = 12 Hz CH=CH-CF=CH-CH), 5.9 (2H, S, N-CH_2-C=O), 4.8(2H, d, *J* = 8 Hz, NH-CH_2), 4.7 (2H, S, N-CH_2-C).

3- ((2-(1,3-Dioxoisoindolin-2-yl)acetamido) methyl)-1-(3-methylbenzyl)pyridin-1-ium (7g)

Yield: 67%, mp. 218-220 °C, IR V_{max}, 3198 (NH), 3012 (C-H aromatic), 1770 (C=O), 1714 (C=O), 1675 (C=C) cm^{-1}; HNMR δ (400 MHz-$CDCl_3$), 9.69 (1H, s, O=C-NH-CH_2), 9.41 (1H, t, *J* = 8 Hz, CH=CH-CH=N), 8.76 (1H, d, *J* = 8 Hz,C-CH=CH-CH=N), 8.45 (1H, d, *J* = 8 Hz, C-CH=CH-CH=N) 7.89 (1H, m, C-CH-N) 7.84 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.82 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.4-7.23 (4H, m, CH=CCH_3-CH=CH-CH), 5.94 (2H, S, N-CH_2-C=O), 4.7 (2H, d, *J* = 4 Hz, O=C-NH-CH_2), 4.64 (2H, S, N-CH_2-C), 2.35 (3H, S, CH_3).

3- ((2-(1,3-Dioxoisoindolin-2-yl)acetamido) methyl)-1-(3-fluorobenzyl)pyridin-1-ium (7h)

Yield: 55%, mp. 206-208.5 °C, IR V_{max}, 3254 (NH), 3027 (C-H aromatic), 1773 (C=O), 1717 (C=O), 1629 (C=O), 1592 (C=C) cm^{-1}; HNMR δ: (400 MHz-$CDCl_3$), 10 (1H, s, NH-CH_2), 8.7 (1H, m, C-CH=CH-CH=N-CH), 8.4 (1H, d, *J* = 8 Hz, C-CH=CH-CH=N-CH), 8.28 (1H, d, *J* = 8 Hz CH=CH-CH=N-CH) 7.8 (3H, m, CH=CH-CH-N-CH, O=C-C=CH-CH=CH-

CH=C-C=O) 7.74 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.5-4.2 (4H, m, CH=CF-CH=CH-CH), 5.95 (2H, S, N-CH_2-C=O), 4.8 (2H, d, *J* = 8 Hz, NH-CH_2), 4.65 (2H, S, N-CH_2-C).

1-(3-Chlorobenzyl)-3-((2-(1,3-dioxoisoindolin-2-yl)acetamido)methyl)pyridin-1-ium (7i)

Yield: 62%, mp. 180-182 °C, IR V_{max}, 3259 (NH), 3006 (C-H aromatic), 1775 (C=O), 1718 (C=O), 1680 (C=O) 1620 (C=C) cm^{-1}; HNMR δ: (400 MHz-DMSO), 9.2 (1H, t, *J* = 8 Hz, O=C-NH-CH_2), 9.1 (2H, m, C=CH-CH=CH-N=CH), 8.5 (1H, d, *J* = 8 Hz, CH=CH-CH=CCl-CH), 8.2 (1H, t, *J* = 8 Hz, CH=CH-CH=CCl-CH) 8-7.8 (4H, m, O=C-C=CH-CH=CH-CH=C-C=O) 7.7 (1H, s, C=CH-CCl), 7.5 (1H, d, *J* = 8 Hz,CH=CH-CH=CCl), 7.49 (2H, m, C=CH-CH=CH-N=CH), 5.9 (2H, S, N-CH2-C=O), 4.55 (2H, d, *J* = 8 Hz,NH-CH2), 4.35 (2H, S, N-CH2-C).

2-(1,3-Dioxoisoindolin-2-yl)-N-(pyridin-4-ylmethyl)acetamide (5a)

Yield: 53% HNMR δ: (400 MHz-DMSO), 8.87 (1H, t, *J* = 8 Hz, NH-C=O), 8.51 (2H, d, J = 8 Hz, CH=CH-N=CH-CH), 7.95 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O), 7.85 (2H, m, O=C-C=CH-CH=CH-CH=C-C=O), 7.25 (2H, d, *J* = 8 Hz, CH-N=CH) 4.33(2H, d, *J* = 8 Hz, N-CH_2), 4.3 (2H, S, CH_2-C=O).

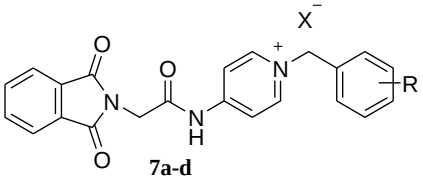
AChE test results

The IC_{50} values are reported in Table 1. All compounds demonstrated good inhibitory activity in the range of IC_{50} = 2.1 ± 0.6-6.7 ± 1.1 μM as compare to standard rivastigmine. Compounds 7a and 7f showed the best anti-AChE activity. It should be noted that the inhibitory activity of target compounds was1.5-5.5 folds higher than rivastigmine.

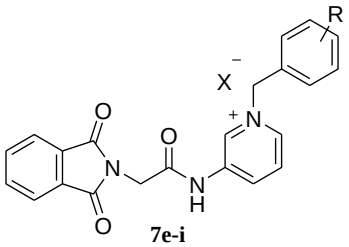
Molecular docking study

Given the hypothesis that the synthesized compounds are AChE inhibitors, molecular docking studies were conducted on selected compounds to rationalize the obtained experimental results and elucidate their potential interaction with the active site of the enzyme. The success rate in retrieving binding modes of known protein-ligand complexes is an important validation for docking programs.

Table 1. Synthesis and acetylcholinesterase inhibitory activity of compounds 7a-7i. Data are expressed as mean ± SEM, n = 3. **P* < 0.01 and ***P* < 0.001 indicate significant differences in comparison with rivastigmine; $^{\#}P$ < 0.001 *vs* donepezil.



7a-d



7e-i

Entry	Compound 7	R	X	IC_{50} (μM)
1	7a	4-F	Br	$2.1 \pm 0.6^{**,\#}$
2	7b	3-Me	Br	$5.4 \pm 0.9^{*,\#}$
3	7c	3-F	Cl	$2.7 \pm 0.2^{**,\#}$
4	7d	3-Cl	Cl	$7.4 \pm 0.1^{*,\#}$
5	7e	H	Cl	$2.6 \pm 0.1^{**,\#}$
6	7f	4-F	Br	$2.1 \pm 0.8^{**,\#}$
7	7g	3-Me	Br	$4.8 \pm 0.5^{**,\#}$
8	7h	3-F	Cl	$2.9 \pm 0.6^{**,\#}$
9	7i	3-Cl	Cl	$6.7 \pm 1.1^{*,\#}$
10		Donepezil		0.018 ± 0.004
11		Rivastigmine		11.07 ± 0.01

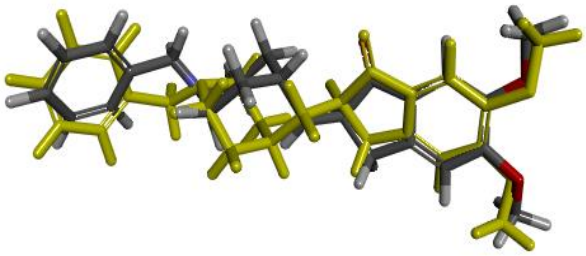


Fig. 3. The best-docked conformation of donepezil (yellow) overlapped with co-crystal ligand (gray).

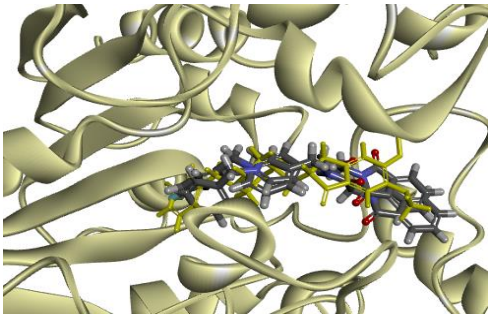


Fig. 4. Superimposition of the most potent compounds 7a and 7f (gray) and donepezil (yellow) in the active site of acetylcholinesterase.

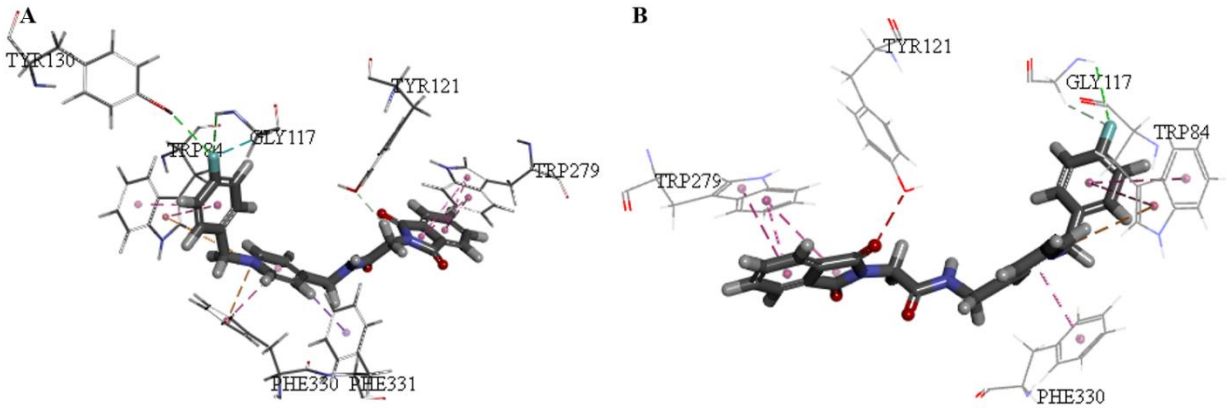


Fig. 5. The most active compounds (A) 7a and (B) 7f in the active site of acetylcholinesterase enzyme. The hydrogen bond, pi-pi stacking, and hydrophobic interactions are shown as green, violet, and black dashed lines, respectively.

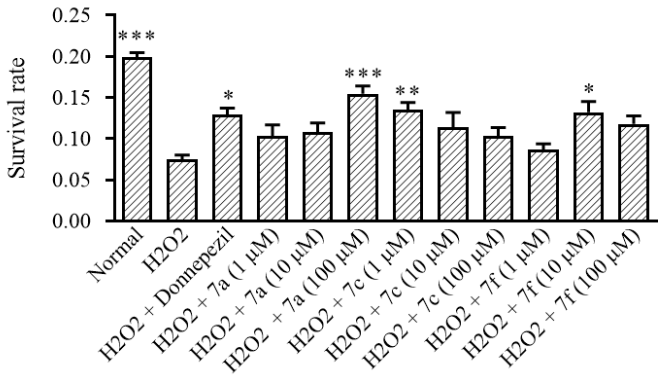


Fig. 6. Neuroprotective activity of compounds 7a, 7c, and 7f based on cell viability of differentiated PC_{12} cells under H_2O_2-mediated oxidative stress. Donepezil (10 µM) was used as the positive control. Data are expressed as mean ± SEM, n = 3. *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$ indicate significant differences compared the positive control group.

The measure that is usually used to determine whether a binding mode prediction is successful is the root mean square deviation (RMSD). Thus, to evaluate whether the docking results were reasonable, the molecular docking protocol was validated by re-docking the donepezil into the binding site of AChE using GOLD. Analysis of the obtained results for the best-docked conformation of donepezil displayed exactly the same binding mode compared to the co-crystallized donepezil, with a good RMSD value of 0.95Å (Fig. 3). Also, the best-docked pose of the most active compounds (7a and 7f) and their interaction with the enzyme active site are shown in Figs.4 and 5, respectively.

MTT assay results

Among the candidate compounds, 7a at 100 μM had the most potent neuroprotective effect compared to the control group (H_2O_2-treated), while donepezil (10 μM), 7c (1 μM), and 7f (10 μM) were also exerted neuroprotective effect as compared to the control group. Comparing the protective efficacy of these compounds with donepezil 10 μM showed no significant differences (Fig. 6).

DISCUSSION

Structure-activity relationship

All compounds (7a-7i) were tested against acetylcholinesterase enzyme and compared with donepezil and rivastigmine as the reference drugs. In order to optimize the inhibitory activity and better understanding of structure-activity relationship (SAR), a number of substituted benzyl halide derivatives were used to synthesize target compounds. The best anti-AChE activity was obtained by compounds 7a and 7f (IC_{50} = 2.1 μM comparing with rivastigmine, IC_{50} = 11.07 μM) possessing 4-fluorobenzyl pyridinium moiety. The shift of the fluoro group from the para to the meta position in 7c and 7h led to a reduction in inhibitory activity (IC_{50s} = 2.7 ± 0.2 and 2.9 ± 0.6 μM, respectively). However, this indicates the importance of the fluoro group in inhibiting the enzym, which is probably due to the ability of the fluoro group to establish an additional hydrogen bond with the catalytic site (CAS-site). In continuing the study of the SAR in halogenated compounds, the switching from fluoro to chloro group in compounds 7d (IC_{50} = 7.4 ± 0.1 μM) and 7i (IC_{50} = 6.7 ± 1.1 μM) led to more than two-fold reduction in the inhibitory activity compared to 7c and 7h. So that chloro-substituted compounds showed the weakest inhibitory activity among the synthesized compounds. For further investigation, the effect of methyl substitution with different electronic properties was studied. The para-methyl substituted compound 7b (IC_{50} = 5.4 ± 0.9 μM) and 7g (IC_{50} = 4.8 ± 0.5 μM) showed improved AChE inhibitory activities compared to chloro-substituted compounds 7d and 7i. A similar trend in SAR was observed by Aliabadi *et al.* in acetylcholinesterase inhibitory evaluation of isoindoline-1,3-dione derivatives (27). Comparison of the inhibitory activity of substituted compounds with the non-substituted compound 7e showed that substitutions on benzyl moiety did not have a significant effect on the inhibitory activity. It was well known that the fluoro and hydrogen are similar in size. Thus, it was reasonable to presume that the size of the substituent on benzyl moiety was important to the activity of the target compounds. A study by Lan *et al.* on coumarin-N-benzyl pyridinium hybrids, substituents with different sizes, and electronic properties on the benzyl group of the N-benzyl pyridinium moiety, also confirm this conclusion (28).

The binding mode of most active compounds 7a and 7f with AChE (1EVE) was investigated by a docking study using the GOLD program. The superimposition structure of donepezil as the reference inhibitor and most potent compounds 7a and 7f in the active site of AChE is presented in Fig. 4. According to the obtained results, these compounds located in the entire enzymatic gorge and indicated a similar binding mode with that of donepezil. In detail, the binding mode of the most active inhibitors 7a and 7f are shown in Fig. 5. Docking studies have shown that these compounds are bound to both the CAS and PAS at the same time thus providing an explanation for its potent inhibitory activity against AChE. The phthalimide moiety was engaged in pi-pi stacking interaction with Trp279 located in the PAS of AChE. Also, the carbonyl group of phthalimide ring formed a hydrogen bond with Tyr121. The pyridinium moiety created a pi-pi stacking with Phe330 while the charged nitrogen of the same moiety also forms cation-pi interactions with Trp84. As shown in Fig. 5, the benzyl moiety was oriented toward Trp84 in the CAS through pi-pi stacking interaction and plays a vital role in ligand recognition in this site of the enzyme. However, it should be noted that the fluorine atom in the para position of the benzyl moiety created two hydrogen bonds with Gly117. Additionally, in the case of compound 7f, the fluorine atom forms another hydrogen bond with the hydroxyl group of Tyr130. Moreover, hydrophobic interactions could be observed between the 7a and aromatic residues of Phe330 in the middle of the active gorge.

Such binding mode may be partially explained by the high potency in inhibiting AChE. Compounds 7c and 7f displayed the best docking scores with values of 72.50 and 72.01, respectively. These compounds showed higher scores than the donepezil (47.91), indicating that the docking method was not able to predict correctly.

CONCLUSION

In conclusion, a novel series of isoindoline-1,3-dione-N-benzyl pyridinium hybrids were synthesized and evaluated for their anti-ChE activity. All compounds were found to demonstrate good inhibitory activity in the range of IC_{50} = 2.1 ± 0.6-6.7±1.1 µM as compare to standard rivastigmine (IC_{50} = 11.07 µM). It should be noted that the inhibitory activity of target compounds was 1.5-5.5 folds higher than rivastigmine. The best anti-AChE activity was obtained by compounds 7a and 7f (IC_{50} = 2.1 µM comparing with rivastigmine) possessing 4-fluorobenzyl pyridinium moiety. It was understood from molecular modeling studies that compounds bound to both the CAS and PAS at the same time, thus providing an explanation for its potent inhibitory activity against AChE. Also, three of the most potent compounds (7a, 7c, and 7f) demonstrated neuroprotective. Generally, our study has presented new potent inhibitors of AChE with therapeutic potential for the treatment of AD.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contribution

All authors contributed equally to this work.

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