2-(2-(4-Benzoylpiperazin-1-yl)ethyl)isoindoline-1,3-dione derivatives: Synthesis, docking and acetylcholinesterase inhibitory evaluation as anti-Alzheimer agents

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Introduction

Alzheimer’s disease (AD), discovered by Dr Alois Alzheimer in 1907, is described as a degenerative disease of the central nervous system (CNS) characterized especially by premature senile mental deterioration. AD patients exhibit marked decline in cognitive ability and severe behavioral abnormalities such as irritability, anxiety, depression, disorientation, and restlessness (1). The disease is characterized by the appearance of senile plaques mainly composed of amyloid β (Aβ), and by the development of neurofibrillary tangles in patients’ brain (2). AD is associated with certain neurological changes such as selective loss of cholinergic neurons in the forebrain, extracellular deposition of amyloid peptide, accumulation of intracellular neurofibrillary tangles, and disordered cognitive functions. It was recently reported that in a significant proportion of AD patients there is also a slowed motor activity and extrapyramidal dysfunction resembling that seen in Parkinson’s disease (PD) (3).

Treatment for AD is a daily challenge for physicians and pharmacists. The cognitive symptoms that characterize Alzheimer’s disease are thought to be related to the degeneration of cholinergic neurons in the cerebral cortex and subcortical structures. A number of preclinical studies have suggested an association between the cholinergic system and cognition. For example, experimental lesions of the basal forebrain cholinergic system and treatment of animals with muscarinic antagonists produce memory deficits (4, 5). There has been substantial progress in the therapeutic approach to AD in the past few years (Figure 1). Among different strategies investigated to...
Recently, some (8-10) reports about the efficacy of phthalimide derivatives in inhibition of AChE have been presented (Figure 2). Hence, in the present study, we focused on the design and synthesis of new anti-acetylcholinesterase agents with phthalimide-based structure. In fact, phthalimide based compounds have similar pharmacophoric portions like indanone ring of the donepezil and are able to act as peripheral binding site inhibitor of AChE (8-14).

Materials and Methods

Chemistry

All chemicals consisting starter materials, reagents and solvents were purchased from commercial vendors such as Merck and Sigma-Aldrich. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F_{254} plates were applied for analytical TLC. Column chromatography was performed on Merck silica gel (70-230 mesh) to purify the intermediate and final compounds. 1H-NMR and 13C NMR spectra were recorded using a Bruker 400 and 250 MHz spectrometers respectively, and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. Intended compounds were dissolved in deuterated chloroform for NMR spectra acquisition. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined electrothermal apparatus. The purity of the reaction was determined. Acetonitrile was applied for analytical TLC. Column chromatography (TLC) using various solvents of different polarities such as Merck and Sigma-Aldrich. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F_{254} plates were applied for analytical TLC. Chromatograms were run on a Finigan TSQ 70 spectrometer (Finigan, USA) at 70 eV. Elemental analyses were carried out on a CHNO rapid elemental analyzer (GmbH-Germany) for C, H, N, and O and the results are within ± 0.4% of the theoretical values.

Figure 2. Structure of a phthalimide based compound as acetylcholinesterase inhibitor

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

In a flat bottom flask, equimolar quantities of phthalic anhydride, N-aminoethylpiperazine and triethylamine (Et$_3$N) were mixed in appropriate amount of toluene solvent. The reaction mixture was refluxed for 24 hr and the termination of the reaction and formation of the desired product was confirmed using TLC. The discolor-ration of the reaction medium and formation of a yellow precipitate was also an indicator of the progress of the reaction. Then, toluene was evaporated under reduced pressure using rotary evaporator apparatus and the obtained yellow viscose and oily residue was washed several times by ethyl acetate (EtOAc) and diethyl ether (Et$_2$O) (9).

General procedure for synthesis of compounds 4a-4j:

In a flat bottom flask, equimolar quantities of appropriate benzoic acid derivative, N-ethyl-N-dimethylaminopropyl carbodiimide (EDC) and HOBT were stirred in a acetonitrile solvent for 30 min. Then, equimolar quantity of compound 3 was added and stirring was continued for 24 hr. The product formation was proved using TLC and therefore the end of the reaction was determined. Acetonitrile was evaporated using rotary evaporator apparatus and the residue was dissolved in ethyl acetate/water (50:50). The mixture was moved to the separatory extraction funnel and the aqueous phase was removed. The organic phase was extracted two times by sodium bicarbonate 5% and brine. Anhydrous sodium sulfate was added for drying and then filtered. The ethyl acetate was evaporated under reduced pressure and the obtained oily residue was treated by methanolic HCl to form the hydrochloride salt of the corresponding base for each compound (9).

Preliminary design

According to the literature, there is three distinct parts in the structure of donepezil that are necessary for anticholinesterase activity (Figure 3) (1, 12). Namely, phenyl ring of the indanone moiety (part A), nitrogen of the piperidine ring (part B) and phenyl...
ring of the benzyl group (part C) are the critical parts for interacting with the corresponding amino acids in the active site of acetyl cholinesterase enzyme. Phenyl ring of the indanone moiety participates in a π-π stacking interaction with Trp 279. Nitrogen of the piperidine ring is important for creating a cation-π interaction with Phe 330. There is another π-π stacking interaction between phenyl ring of the benzyl moiety of the donepezil and indanone ring of the Trp 84. Hence, according to the pharmacophoric necessities that mentioned above for donepezil interaction, a new series of phthalimide based derivatives were designed (Figure 3).

Docking
The study of molecular docking of all ligands was performed using ArgusLab 4.0 software [20]. All intended ligands were built in arguslab workspace and energy minimization was performed using AM1 as semiempirical method for all ligands. The pdb files of acetyl cholinesterase in complex with donepezil (pdb code: 1EVE) was downloaded from brookhaven protein databank (21, 22). The geometry optimization of the protein structure was optimized geometrically by universal force field (UFF) as a molecular mechanic method. The docking process was done for all ligands in the workspace of ArgusLab software after defining the related groups for each ligand as well as protein. The binding site of donepezil was defined as binding site for searching the best pose and conformation for all ligands. Binding mode and related interactions of ligands with acetyl cholinesterase enzyme were explored in Molegro molecular viewer software (23).

Pharmacology
Ellman test was applied to investigate the capability of the synthesized compounds toward the inhibition of the acetylcholinesterase enzyme. Lyophilized powder of acetylcholinesterase from electric eel source (AChE, E.C. 3.1.1.7, Type V-S, 1000 unit) was purchased from Sigma-Aldrich (Steinheim, Germany). 5,5'-Dithiobis-(2- nitrobenzoic acid, DTNB), potassium dihydrogen phosphate (KH₂PO₄), dipotassium hydrogen phosphate (K₂HPO₄), potassium hydroxide (KOH), sodium hydrogen carbonate (NaHCO₃), and acetylthiocholine iodide were purchased from Fluka (Buchs, Switzerland). Spectrophotometric measurements were run on a Cecil BioAquarius CE 7250 Double Beam Spectrophotometer.

Compounds 4a-4j were dissolved in a mixture of 20 ml distilled water and 5 ml methanol and then diluted in 0.1 M KH₂PO₄/K₂HPO₄ buffer (pH 8.0) to yield a final concentration range. According to the literature, the Ellman test was performed for assessment of the anticholinesterase activity of intended compounds in vitro. To achieve 20-80% inhibition of AChE activity five different concentrations of each compound were tested. Compounds 4a-4j were added to the assay solution and preincubated at 25 °C with the enzyme for 15 min followed by adding 0.075 M of acetylthiocholine iodide. After rapid and immediate mixing the change of absorption was measured at 412 nm.

The blank reading contained 3 ml buffer, 200 µl water, 100 µl DTNB and 20 µl substrate. The reaction rates were calculated, and the percent inhibition of test compounds was determined. Each concentration was analyzed in triplicate, and IC₅₀ values were determined graphically from inhibition curves (log inhibitor concentration vs percent of inhibition) (24, 25).

Results

Chemistry
All synthesized derivatives were characterized by spectroscopic methods such as ¹H-NMR, IR and MS. Melting points were also measured and obtained yields were calculated (Table 1).

2-(2-(Piperazin-1-yl) ethyl) isoindoline-1,3-dione (3)
¹H NMR (CDCl₃, 400 MHz) δ (ppm): 2.37 (m, pipеразине), 2.54 (m, pipеразине), 3.22 (t, пхталимид-С₂Н₂-пиперазине), 3.44 (t, пхталимид-С₂Н₂-пиперазине), 4.73 (NH, piperazine), 7.35-7.85 (m, 4H,
Table 1. Properties of compounds 4a-4j

| Compound | (R) | Yield (%) | mp (°C) | MW (g/mol) | Chemical formula |
|----------|-----|-----------|---------|-----------|-----------------|
| 3        | -   | 61        | 105-109 | 259       | C_6H_11N_2O_2   |
| 4a       | 2-Cl | 23        | 229     | 397       | C_4H_8N_2O_2Cl  |
| 4b       | 3-Cl | 18        | 203     | 397       | C_4H_8N_2O_2Cl  |
| 4c       | 4-Cl | 15        | 229     | 397       | C_4H_8N_2O_2Cl  |
| 4d       | 2-F  | 35        | 247     | 381       | C_2H_5F N_2O_3 |
| 4e       | 3-F  | 13        | 243     | 381       | C_2H_5F N_2O_3 |
| 4f       | 4-F  | 52        | 229     | 381       | C_2H_5F N_2O_3 |
| 4g       | 2-CH_3| 14        | 211     | 393       | C_2H_5N_2O_4   |
| 4h       | 3-CH_3| 30        | 127     | 393       | C_2H_5N_2O_4   |
| 4i       | 4-CH_3| 36        | 127     | 393       | C_2H_5N_2O_4   |
| 4j       | 2-NO_2| 49        | 135     | 408       | C_2H_3N_2O_5   |

2-(2-(4-(2-Chlorobenzoyl) piperazin-1-yl) ethyl) isoindoline-1,3-dione (4a)

1H NMR (CDCl_3, 400 MHz) δ (ppm): 2.91 (brs, 4H, Piperazine), 3.41 (brs, 4H, Piperazine), 3.88 (m, 4H, Phthalimide-CH_2-CH_2-Piperazine), 7.43-7.49 (m, 4H, 3-Chlorophenyl), 7.78 (q, 2H, H_5=Phthalimide), 7.91 (q, 2H, H_4=Phthalimide). 13C NMR (DMSO-d_6, 62.5 MHz) δ: 35.8 (Phthalimide-C-C-Piperazine), 54.2 (C_2-Piperazine), 45.9 (C_9-Piperazine), 52.7 (Phthalimide-C-C-Piperazine), 55.3 (C_2-Piperazine), 124.6 (C_5=Phthalimide), 127.1 (C_3-3-Chlorophenyl), 129.7 (C_3-3-Chlorophenyl), 131.8 (C_7-3-Chlorophenyl), 133.1 (C_3=3-Chlorophenyl), 134.9 (C_5=3-Chlorophenyl), 135.2 (C_2=Phthalimide), 136.9 (C_1=3-Chlorophenyl), 166.2 (C=O, Phthalimide), 169.5 (C=O, 3-Chlorobenzoyl). IR (KBr, cm⁻¹): 3066 (C=H, Stretch, Aromatic), 2924 (C-H, Stretch, Asymmetric, Aliphatic), 2854 (C-H, Stretch, Symmetric, Aliphatic), 1712 (C=O, Stretch, Phthalimide), 1631 (C=O, Stretch, Amide), 1429 (C-N, Stretch).

2-(2-(4-(4-Chlorobenzoyl) piperazin-1-yl) ethyl) isoindoline-1,3-dione (4c)

1H NMR (CDCl_3, 400 MHz) δ (ppm): 1.78 (brs, 4H, Piperazine), 2.91 (brs, 4H, Piperazine), 3.42 (m, 2H, Phthalimide-CH_2-CH_2-Piperazine), 4.20 (m, 2H, Phthalimide-CH_2-CH_2-Piperazine), 7.36-7.46 (m, 4H, 2-Chlorophenyl), 7.78 (q, 2H, H_5=Phthalimide), 7.90 (q, 2H, H_4=Phthalimide). 13C NMR (DMSO-d_6, 62.5 MHz) δ: 32.1 (Phthalimide-C-C-Piperazine), 37.8 (C_5=3-Chlorophenyl), 42.8 (C_5=Phthalimide), 50.4 (Phthalimide-C-C-Piperazine), 53.2 (C_2=Phthalimide), 123.3 (C_5=Phthalimide), 126.9 (C_5=2-Chlorophenyl), 127.2 (C_5=4-Chlorophenyl), 127.8 (C_5=2-Chlorophenyl), 129.2 (C_5=2-Chlorophenyl), 129.6 (C_5=2-Chlorophenyl), 131.1 (C_5=Phthalimide), 132.1 (C_5=2-Chlorophenyl), 134.5 (C_2=2-Chlorophenyl), 165.7 (C=O, Phtha...
2-(2-(4-Fluorobenzoyl) piperazin-1-yl) ethyl isoinodine-1,3-dione (4d)

δ (ppm): 2.98 (brs, 4H, Piperazine), 3.46 (brs, 4H, Piperazine), 3.69-4.21 (m, 4H, Phthalimide-CH$_2$-CH$_2$-Piperazine), 7.12-7.55 (m, 4H, 2-Fluorophenyl), 7.77 (q, 2H, H$_5$-Phthalimide), 7.88 (q, 2H, H$_4$-Phthalimide). ¹³C NMR (DMSO-d$_6$, 62.5 MHz) δ: 34.6 (Phthalimide-C$_2$-Piperazine), 42.2 (C$_7$-Piperazine), 41.1 (C$_5$-Piperazine), 50.9 (Phthalimide-C$_7$-Piperazine), 53.6 (C$_2$-Piperazine), 116.2 (d, C$_6$-2-Fluorophenyl), 120.2 (d, C$_6$-2-Fluorophenyl), 123.8 (C$_{3a}-$Phthalimide), 125.6 (C$_5$-2-Fluorophenyl), 127.5 (C$_4$-2-Fluorophenyl), 130.6 (C$_{3a,7a}$-Phthalimide), 132.1 (C$_2$-2-Fluorophenyl), 136.1 (C$_5$-Phthalimide), 161.1 (d, C$_5$-2-Fluorophenyl), 166.2 (d, C$_6$, 2-Fluorobenzoyl), 167.3 (C$_3$, Phthalimide), IR (KBr, cm$^{-1}$): 3132 (C-H, Stretch, Aromatic), 2954 (C-H, Stretch, Asymmetric, Aliphatic), 2927 (C-H, Stretch, Symmetric, Aliphatic), 1712 (C=O, Stretch, Phthalimide), 1650 (C=O, Stretch, Amidé), 1608 (C=C, Stretch, Aromatic), 1470, 1253 (C$_N$, Stretch). MS (m/z %): 381 (M$^+$, 1), 221 (100), 166 (10), 123 (50), 95 (10), 75 (3), 56 (3).

2-(2-(4-Fluorobenzoyl) piperazin-1-yl) ethyl isoinodine-1,3-dione (4e)

δ (ppm): 2.91 (brs, 4H, Piperazine), 3.44 (brs, 4H, Piperazine), 3.63-4.21 (m, 4H, Phthalimide-CH$_2$-CH$_2$-Piperazine), 7.22 (m, 2H, H$_5$-3-Fluorophenyl), 7.45 (m, 1H, H$_4$-3-Fluorophenyl), 7.73 (m, 1H, H$_3$-3-Fluorophenyl), 7.77 (q, 2H, H$_5$-Phthalimide), 7.89 (q, 2H, H$_4$-Phthalimide). ¹³C NMR (DMSO-d$_6$, 62.5 MHz) δ: 37.3 (Phthalimide-C$_5$-Piperazine), 44.6 (C$_7$-Piperazine), 45.5 (C$_7$-Piperazine), 53.1 (Phthalimide-C$_7$-Piperazine), 55.6 (C$_5$-2-Fluorophenyl), 114.5 (d, C$_2$-3-Fluorophenyl), 119.2 (d, C$_2$-3-Fluorophenyl), 122.4 (C$_{4a,7a}$-Phthalimide), 125.2 (C$_{3a}$-3-Fluorophenyl), 130.5 (d, C$_5$-3-Fluorophenyl), 132.6 (C$_{3a,7a}$-3-Fluorophenyl), 133.3 (d, C$_I$-3-Fluorophenyl), 136.4 (C$_5$-Phthalimide), 159.2 (C$_3$-3-Fluorophenyl), 167.2 (C$_3$, Phthalimide), 168.4 (d, C$_1$-2-Fluorophenyl).
Phthalimide. $^{13}$C NMR (DMSO-d$_6$, 62.5 MHz) $\delta$: 35.8 (Phthalimide-C-C-Piperazine), 44.3 (C$_2$-Piperazine), 47.4 (C$_3$-Piperazine), 53.1 (Phthalimide-C-C-Piperazine), 54.5 (C$_2$-Piperazine), 57.1 (C$_6$-Piperazine), 114.2 ($\text{C}_1$-$\text{C}_4$-Methoxyphenyl), 123.6 ($\text{C}_1$-$\text{C}_4$-Phthalimide), 127.8 ($\text{C}_1$-$\text{C}_4$-Methoxyphenyl), 128.2 ($\text{C}_1$-$\text{C}_4$-Methoxyphenyl), 132.1 ($\text{C}_1$-$\text{C}_4$-Phthalimide), 135.9 ($\text{C}_1$-$\text{C}_4$-Phthalimide), 156.9 ($\text{C}_1$-$\text{C}_4$-Methoxyphenyl), 167.1 (C=O, Phthalimide), 169.1 (C=O, 4-Methoxybenzoyl). IR (KBr, cm$^{-1}$) $\tilde{\nu}$: 3080 (C-H, Stretch, Aromatic), 2924 (C-H, Stretch, Asymmetric, Aliphatic), 2858 (C-H, Stretch, Symmetric, Aliphatic), 1712 (C=O, Stretch, Phthalimide), 1647, 1435, 1253 (C-N, Stretch).

**2-(2-(4-(2-Nitrobenzoyl) piperazin-1-yl) ethyl) isoindoline-1,3-dione (4j)**

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ (ppm): 2.96 (brs, 4H, Piperazine), 3.44 (brs, 4H, Piperazine), 3.78-3.94 (m, 4H, Phthalimide-C$_2$-CH$_2$-Piperazine), 6.93 (m, 2H, 2-Nitrophenyl), 7.43 (m, 1H, 2-Nitrophenyl), 7.75 (m, 2H, H$_{5a}$-Phthalimide), 7.86 (m, 2H, H$_{5b}$-Phthalimide), 8.01 (m, 1H, 2-Nitrophenyl). $^{13}$C NMR (DMSO-d$_6$, 62.5 MHz) $\delta$: 37.4 (Phthalimide-C-C-Piperazine), 43.2 (C$_3$-Piperazine), 45.5 (C$_2$-Piperazine), 53.6 (Phthalimide-C-C-Piperazine), 54.8 (C$_2$-Piperazine), 123.7 (C$_4$-Phthalimide), 128.6 (C$_2$-Nitrophenyl), 128.9 (C$_4$-Nitrophenyl), 131.1 (C$_2$-Nitrophenyl), 131.7 (C$_3$-$\text{C}_5$-Phthalimide), 132.2 (C$_2$-2-Nitrophenyl), 133.1 (C$_2$-2-Nitrophenyl), 135.4 (C$_6$-Phthalimide), 140.6 (C$_2$-Nitrophenyl), 166.1 (C=O, Phthalimide), 167.2 (C=O, 2-Nitrobenzoyl). IR (KBr, cm$^{-1}$) $\tilde{\nu}$: 2967 (C-H, Stretch, Asymmetric, Aliphatic), 2927 (C-H, Stretch, Symmetric, Aliphatic), 1712 (C=O, Stretch, Phthalimide), 1650 (C=O, Stretch, Amide), 1608 (C=C, Stretch, Aromatic), 1470 (C=C, Stretch, Aromatic), 1253 (C-N, Stretch).

**Docking study**

All ligands were docked into the active site of acetylcholinesterase and the binding state and interacted amino acids were compared to donepezil (Figure 4, Figure 5).

**Enzymatic assay**

All prepared compounds (4a-4j) were tested against acetylcholinesterase by Ellman’s test and obtained results were listed in Table 2. Observed potencies were compared with donepezil as reference drug.
Table 2. Results of anti-acetylcholinesterase activity of compounds 4a-4j (IC50, µM)

| Compound | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | 4i | 4j | Donepezil |
|----------|----|----|----|----|----|----|----|----|----|----|-----------|
| R        | 2-Cl | 3-Cl | 4-Cl | 2-F | 3-F | 2-F | 2-OCH3 | 3-OCH3 | 4-OCH3 | 2-NO2 | -         |
| IC50 (µM) | 333.4 | 70.2 | >1000 | 3.6 | 7.1* | 50.6 | 100.1 | 1.3 | 20.3* | 140.6 | 0.41      |

* IC50 of compounds 4e and 4i have been presented in nanomolar [nM] range

**Discussion**

**Chemistry**

A new series of isoindoline-1,3-dione (phthalimide) derivatives were synthesized through the treatment of phthalic anhydride and N-aminoethylpiperazine in the presence of triethylamine as proton acceptor. Refluxing condition was applied in toluene solvent to achieve compound 3 with a moderate yield (61%). Then, compound 3 was used for preparation of final product 4a-4j. Final products were synthesized via a carbodiimide coupling process. Namely, appropriate benzoic acid derivative was mixed with N-ethyl-N-dimethyiaminopropyl carbodiimide (EDC) and hydroxybenzotriazole (HOBt) in acetonitrile (CH3CN) solvent and after 30 min equimolar quantities of compound 3 was added. The reaction mixture was stirred for 24 hr. Spectroscopic methods such as H NMR, IR and Mass were utilized for characterization of intermediate compound 3 as well as final compounds 4a-4j. Melting point analyser was used to measure the corresponding melting points using open capillary tube and recorded as centigrade degree. Compound 4d with ortho fluorine substituent demonstrated the highest melting point (247 °C), whereas, compounds 4h and 4i with methoxy moiety rendered the lowest melting points (127 °C).

**Docking**

According to the Figure 3, it is obvious that donepezil has three distinct interactions with acetylcholinesterase enzyme. In the other words, Trp 279, Phe 330 and Trp 84 are the most critical amino acids in the active site of AChE. The binding mode of the tested compounds was investigated by docking method using ArgusLab software. According to the obtained results, there is a similar binding mode and interactions between the docked ligands and donepezil into the active site of acetylcholinesterase (Figure 4, Figure 5). According to the Figure 4, the critical amino acids (Trp 279, Phe 330 and Trp 84) are visible surrounding the docked ligand and also with paying attention to the Figure 5, a similar conformation and orientation like donepezil towards the pivotal amino acids is observable for this ligand in overlaid state.

**Structure activity relationship**

All final compounds 4a-4j were tested against acetylcholinesterase enzyme and the obtained results were recorded as IC50 in Table 2. Fortunately, the synthesized derivatives demonstrated a remarkable inhibitory activity towards acetylcholinesterase.

Various substituents such as Cl, F, methoxy and nitro were introduced on the phenyl ring to explore the impact of electronic effects of the moiety on the potency of these compounds in inhibition of acetylcholinesterase activity. In the other words, electron withdrawing as well as electron donating moiety were examined. According to the Table 2, it is obvious that both the electron withdrawing and electron donating moiety have beneficial effect on the potency of the synthesized derivatives. Compound 4e with meta fluorine moiety was the most active compound in this series (IC50 = 7.1 nM). Generally, electron withdrawing groups like chlorine and fluorine at position meta of the phenyl ring provided a better activity compared to positioning of these moieties at ortho and para. Positioning of the methoxy at para (compound 4i, IC50= 20.3 nM) also rendered a favorable potency but lower than compound 4e. Compounds 4d and 4h were also exhibited an acceptable activity in µM range but lower than donepezil. It means that methoxy as an electron donating moiety at position meta could also enhance the anticholinesterase activity in comparison with ortho position. Ortho positioning of chlorine and nitro moieties did not caused a significant increase in activity. It is probable that steric effect that caused by chlorine and nitro moiety be an interrupting factor for proper interaction of these ligands with receptor at position ortho.

Totally, electron withdrawing atoms enhanced the anticholinesterase activity especially at position 3 of the phenyl ring. Increase in electron withdrawing effects was also beneficial for activity. In the other words, replacement of the chlorine with fluorine atom led to the improvement in activity in all positions of the phenyl ring. Electron donating groups is better to substitute at position 3 and 4 of the phenyl ring.

**Conclusion**

A new series of phthalimide (isoindoline-1,3-dione) derivatives were synthesized and corresponding anti-acetylcholinesterase activity were assessed. Docking study was carried out for exploration of the probable binding mode of the ligands and also for comparison with donepezil. Totally, electron withdrawing groups (Cl, F) at position 3 and electron donating group (methoxy) at position 4 of the phenyl ring enhanced the activity. Compound 4e (m-Fluoro, IC50 = 7.1 nM) and 4i (p-Methoxy, IC50 = 20.3 nM) were the most active compounds in this series and exerted superior potency than donepezil (410 nM). Molecular modeling by docking method also confirmed
the results of enzymatic test. Moreover, a similar binding mode was observed for all ligands in superimposition state with donepezil into the active site of acetylcholinesterase.

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