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Synthesis and evaluation of acetylcholinesterase inhibitory effects of 2-(2-(4-benzoylpiperazin-1-yl)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione derivatives with potential anti-Alzheimer activity

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia in elderly people. The disease is characterized as age-dependent chronic and neurodegenerative disorder. Cognitive decline is supposed to be related to the degeneration of cholinergic neurons in the affected brain structures. Design and development of novel acetylcholinesterase inhibitors is one of the interesting areas in medicinal chemistry. In the current project, a new series of naphthalimide-based anti-acetylcholinesterase were synthesized and their enzyme inhibitory potency was assessed using Ellman's protocol. Fortunately, the most of tested derivatives demonstrated favorable acetylcholinesterase activity. Amongst tested derivatives, compound ***4k*** *(3-OCH_3) was the most active compounds in these series (IC_{50} = 0.26 µM).*

Keywords*:* Synthesis, Naphthalimide, Acetylcholinesterase, Alzheimer

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in elderly people. The disease is characterized as age-dependent chronic and neurodegenerative disorder. Some clinical presentations such as cognitive decline and behavioral changes are featured that lastly led to the reduction of intellectual as well as mental activities [1-4]. AD patients demonstrated impairment in mental abilities and performances that consequently causing them to be unable to perform normal daily functions[5-8]. AD is responsible for about 50-60% of the total cases of dementia in patient higher 65 years old[9].Unfortunately, the true etiology and pathology of the disease is still unknown and therefore, the development of efficacious anti-alzheimer agents is one of the encouraging areas in current medicinal chemistry researches [5].

The exact ethiology of AD is still unclear. But, three pathological reasons have been recognized for the origin of disease namely amyloid-βplaques, neurofibrillary tangles (NFTs) and synaptic loss [1].Neocortex and hippocampus as sections with superior mental activities are the most regions of the brain that afflicted by AD. This includes the extracellular deposits of β-amyloid [9].Cognitive decline is supposed to be related to the degeneration of cholinergic neurons in the affected brain structures. According to the cholinergic hypothesis, some parts of the cognitive decline

in AD patients are originated from the deficiency in acetylcholine (ACh) and consequently cholinergic neurotransmission [10]. One of the effective strategies for combating AD in the recent years is the administration of acetylcholinesterase(AChE) inhibitors. Inhibitory agents of the acetylchlinesterase enzyme enhance the duration of action of acetylcholine in synaptic cleft. These medications have beneficial effects on cognitive, functional, and behavioral symptoms of AD. Therefore, an increase and improvement in cognition and memory of patients is observed. Tacrine, rivastigmine, galantamine and donepezil are common and well-known AChE inhibitors in the market[10,11]. Donepezil as dimethoxyindanone derivatives is a potent, long acting, selective and reversible acetylcholinesterase inhibitor and has been prescribed worldwide for the treatment of AD**(Figure 1)**[12-15].

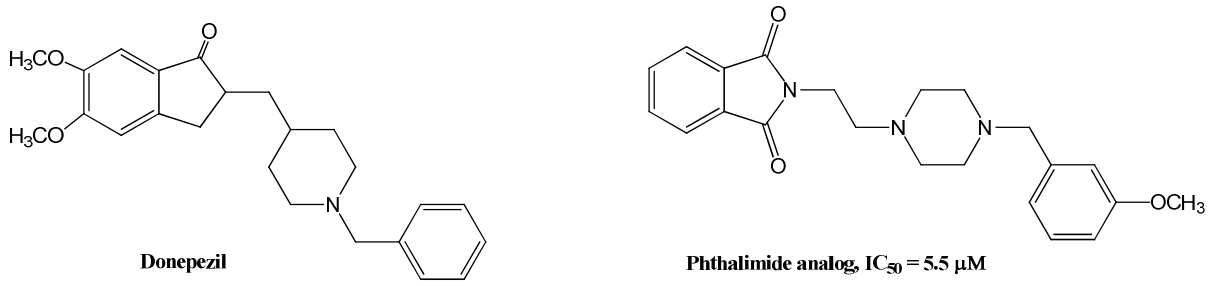


Figure 1.Structure of donepezil as acetylcholinesterase inhibitor and a previously reported phthalimide-based inhibitor

According to our recent reports about the efficacy of phthalimide derivatives in inhibition of AChE, (**Figure 1**) [5, 14], phthalimide-based compounds have same pharmacophoric sections to donepezil. In the current project, we encouraged on the design and synthesis of new anticholinesterase agents with naphthalimide-based structure to achieve more active analogs. Naphthalimide residue has an aromatic phenyl ring more than phthalimide residue that enhances their lipophilic property and subsequently improves the blood brain barrier penetration. In recent years, some biological activity of naphthalimide derivatives have been confirmed revealed [16, 17].Hence, in continuation of our previous investigations, we embarked on the design and synthesis of new acetylcholinesterase inhibitors.

MATERIALS AND METHODS

Chemistry

Merck and Sigma-Aldrich companies were selected as valid commercial suppliers for preparation of chemical substances such as solvents, starting materials and reagents. coated Silica gel-coated aluminum TLC sheets were utilized for thin layer chromatography. Purification of the intermediate and final compounds was carried out using silica gel (70-230 mesh). ^{1}HNMR spectra acquisition was done by nuclear magnetic resonance (NMR) Bruker 500 MHz instrument. All intended compounds were dissolved in deutrated solvents such as dimethylsulfoxide chloroform ($CDCl_3$). Chemical shifts for each proton were presented as δ (ppm) proportionally to tetramethylsilane (TMS) as internal standard. Potassium bromide (KBr) disk was prepared for infrared (IR) spectra in Shimadzu 470 spectrophotometer. Mass spectroscopy was performed using a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV and mass of each fragment were provided with its frequency percentage. Melting points for final compounds was also obtained using melting point analyzer apparatus electrothermal 9001A model in open capillary tubes.

*Synthesis of 2-(2-(Piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione***(3)**

In a flat bottom flask, 3 g (15.2 mmol) of naphthalene-1,8-dicarboxylic anhydride, 1.97 g (15.2 mmol) 2-(piperazin-1-yl)ethanamine and 2.10 ml (15.2 mmol) triethylamine were refluxed in toluene for 24 h. Thin layer chromatography (TLC) was utilized to determine the reaction end. Toluene was evaporated under reduced pressure and the obtained residue was washed by diethyl ether (Et_2O) and *n*-hexane to afford a creamy solid. The obtained powder was applied for the next step without any extra purification [16, 17].

^{1}HNMR (DMSO-d_6, 250 MHz) δ (ppm): 0.80 (brs, 4H, N-CH_2-C$\underline{H}_2$-N), 1.35 (brs, 4H, N-C$\underline{H}_2$-CH_2-N), 2.50 (t, 2H, naphthalimide-CH_2-C$\underline{H}_2$-piperazine), 4.15 (t, 2H, naphthalimide-C$\underline{H}_2$-CH_2-piperazine), 7.84 (t, 2H, $H_{5,8}$-naphthalimide), 8.45 (t, 2H, $H_{4,6,7,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3059 (C-H, stretch, aromatic), 2881, 2951 (stretch, C-H, aliphatic), 1693 (stretch, C=O). MS (*m/z*, %): 309 (M^+, Weak), 267 (20) 224 (20), 180 (20), 99 (100), 91 (90).

General procedure for synthesis of compounds **(4a-4l)**

In a flat bottom flask, 0.2 g (0.65 mmol) of compound **3**wastreated with equimolar quantities of dicyclohexylcarbodiimide (DCC) and hydroxybenzotirazole (HOBt) in 20 ml tetrahydrofuran (THF) an ice bath. Besides, an appropriate benzoic acid derivative was also added to the reaction medium for amidation. The reaction

mixture was stirred for 1 h. Then, stirring was continued overnight. Thin layer chromatography (TLC) was utilized to determine the reaction end. After completion, the THF was evaporated and ethyl acetate/water was added to the residue. Organic layer was washed two times by sodium bicarbonate 5% and brine. The aqueous layer was discarded and organic layer was dried over anhydrous sodium sulfate. Sodium sulfate was filtered off and ethyl acetate was evaporated under reduced pressure using rotary evaporator apparatus. Diethyl ether and *n*-hexane were also utilized for washing of the afforded powder to remove some impurities. Furthermore, column chromatography (Ethyl acetate/petroleum ether; 70/30) was implemented to purify the final products[17].

2-(2-(4-(2-Fluorobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4a)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 3.04 (brs, 4H, piperazine), 3.52 (t, 2H, -CH_2-C$\underline{H}_2$-piperazine), 3.90 (brs, 4H, piperazine), 4.28 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.13 (t, 1H, *J* = 9 Hz, H_4-2-fluorophenyl), 7.25 (t, 1H, *J* = 9 Hz, H_5-2-fluorophenyl), 7.40-7.7.46 (m, 2H, $H_{3,6}$-2-fluorophenyl), 7.79 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.26 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.62 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3035 (C-H, stretch, aromatic), 2924 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1624 (C=C, stretch, aromatic), 1438 (C=C, stretch, aromatic), 1238 (C-N, stretch).

2-(2-(4-(3-Fluorobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4b)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 3.04 (brs, 4H, piperazine), 3.51 (t, 2H, -CH_2-C$\underline{H}_2$-piperazine), 3.85 (brs, 4H, piperazine), 4.16 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.16 (m, 3H, 3-fluorophenyl), 7.43 (m, 1H, H_6-3-fluorophenyl), 7.81 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.27 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.64 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3059 (C-H, stretch, aromatic), 2924 (C-H, asymmetric stretch, aliphatic), 2854 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1627 (C=C, stretch, aromatic), 1442 (C=C, stretch, aromatic), 1234 (C-N, stretch).

2-(2-(4-(4-Fluorobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4c)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.69 (brs, 4H, piperazine), 2.85 (t, 2H, -C$\underline{H}_2$-piperazine), 3.49 (brs, 4H, piperazine), 4.41 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.12 (t, 2H, *J* = 8.5 Hz, $H_{2,6}$-4-fluorophenyl), 7.43 (dd, 2H, *J* = 13.5, 5 Hz, $H_{3,5}$-4-fluorophenyl), 7.79 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.24 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.62 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide).IR (KBr, cm^{-1}) ῡ: 3059 (C-H, stretch, aromatic), 2927 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1701 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1624 (C=C, stretch, aromatic), 1438 (C=C, stretch, aromatic), 1238 (C-N, stretch).MS (*m/z*, %): 431 (M^+, 10), 362 (10), 292 (10), 267 (20), 221 (100), 180 (15), 123 (70), 95 (15).

2-(2-(4-(2-Chlorobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4d)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.77 (brs, 4H, piperazine), 2.85 (t, 2H, -C$\underline{H}_2$-piperazine), 3.52 (brs, 4H, piperazine), 4.42 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.34-7.45 (m, 2H, 2-chlorophenyl), 7.57 (dd, 1H, *J* = 7.5, 2.5 Hz, 2-chlorophenyl), 7.73 (dd, 1H, *J* = 7.5, 2.5 Hz, 2-chlorophenyl), 7.80 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.26 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.62 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3329 3035 (C-H, stretch, aromatic), 2927 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1624 (C=C, stretch, aromatic), 1465 (C=C, stretch, aromatic), 1238 (C-N, stretch).

2-(2-(4-(3-Chlorobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4e)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.75 (brs, 4H, piperazine), 2.84 (t, 2H, -C$\underline{H}_2$-piperazine), 3.52 (brs, 4H, piperazine), 4.42 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.19 (d, 1H, *J* = 7.5 Hz, H_6-3-chlorophenyl), 7.29 (t, H, *J* = 7.5 Hz, H_5-3-chlorophenyl), 7.34 (s, 1H, H_2-3-chlorophenyl), 7.36 (d, 1H, *J* = 7.5 Hz, H_4-3-chlorophenyl), 7.69 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.16 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.52 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3035 (C-H, stretch, aromatic), 2924 (C-H, asymmetric stretch, aliphatic), 2854 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1627 (C=C, stretch, aromatic), 1438 (C=C, stretch, aromatic), 1238 (C-N, stretch).

2-(2-(4-(4-Chlorobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4f)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.57 (brs, 4H, piperazine), 2.84 (t, 2H, -C$\underline{H}_2$-piperazine), 3.49 (brs, 4H, piperazine), 4.46 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.36 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-chlorophenyl), 7.77 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 7.85 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-chlorophenyl), 8.22 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.59 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3035 (C-H, stretch, aromatic), 2927 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1693 (C=O, stretch, imide), 1658 (C=O, stretch, amide), 1627 (C=C, stretch, aromatic), 1434 (C=C, stretch, aromatic), 1242 (C-N, stretch). MS (*m/z*, %): 449 (M^++2, 5), 447 (10), 292 (12), 267 (30), 237 (100), 226 (30), 210 (10), 180 (30), 139 (90), 99 (30), 56 (60).

2-(2-(4-(2-Nitrobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4g)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.62 (brs, 4H, piperazine), 2.85 (t, 2H, -C$\underline{H}_2$-piperazine), 3.49 (brs, 2H, piperazine), 4.27 (brs, 2H, piperazine), 4.40 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.57 (m, 2H, 2-nitrophenyl), 7.74 (m, 1H, 2-nitrophenyl), 7.87 (d, 1H, 2-nitrophenyl), 7.79 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.24 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.62 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3062 (C-H, stretch, aromatic), 2924 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1624 (C=C, stretch, aromatic), 1531 (NO_2, asymmetric stretch), 1442 (C=C, stretch, aromatic), 1342 (NO_2, symmetric stretch), 1238 (C-N, stretch).

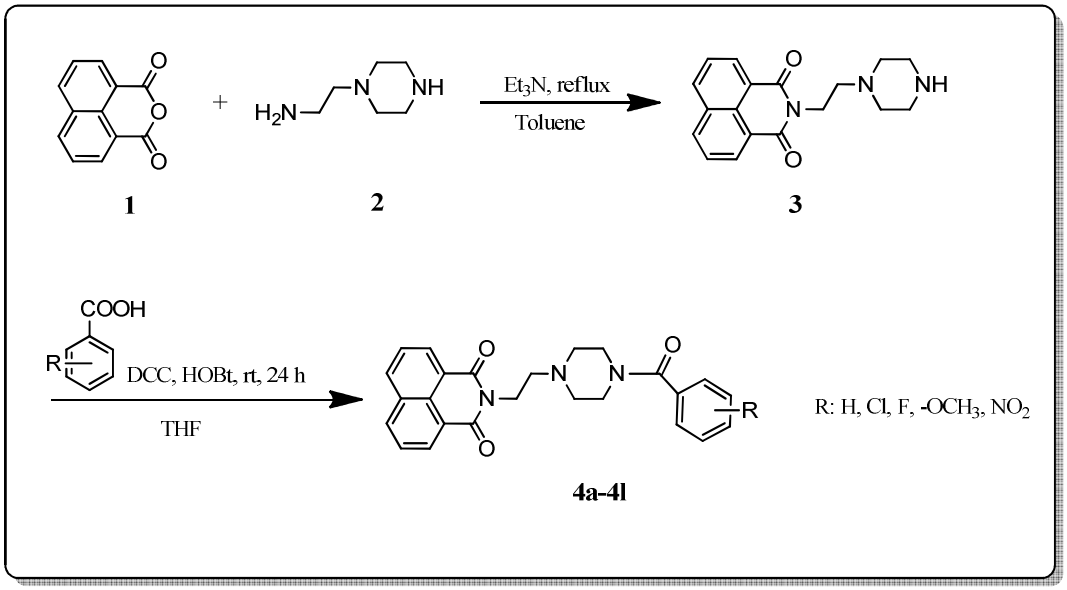
2-(2-(4-(3-Nitrobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4h)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.59 (brs, 2H, piperazine), 2.69 (brs, 2H, piperazine), 2.78 (t, 2H, -C$\underline{H}_2$-piperazine), 3.37 (brs, 2H, piperazine), 3.77 (brs, 2H, piperazine), 4.36 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.61 (t, 1H, *J* = 7.5 Hz, H_5-3-nitrophenyl), 7.73 (d, 1H, *J* = 7.5 Hz, H_6-2-nitrophneyl), 7.77 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.23 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.26 (s, 1H, H_2-3-nitrophenyl), 8.27 (d, 1H, *J* = 7.5 Hz, H_6-2-nitrophneyl), 8.60 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3062 (C-H, stretch, aromatic), 2924 (C-H, asymmetric stretch, aliphatic), 2854 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1627 (C=C, stretch, aromatic), 1531 (NO_2, asymmetric stretch), 1438 (C=C, stretch, aromatic), 1346 (NO_2, symmetric stretch), 1238 (C-N, stretch).

2-(2-(4-(4-Nitrobenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4i)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.57 (brs, 2H, piperazine), 2.70 (brs, 2H, piperazine), 2.78 (t, 2H, -C$\underline{H}_2$-piperazine), 3.37 (brs, 2H, piperazine), 3.77 (brs, 2H, piperazine), 4.36 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 7.55 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-nitrophenyl), 7.77 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.23 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.27 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-nitrophenyl), 8.60 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3062 (C-H, stretch, aromatic), 2927 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1627 (C=C, stretch, aromatic), 1523 (NO_2, asymmetric stretch), 1465 (C=C, stretch, aromatic), 1346 (NO_2, symmetric stretch), 1238 (C-N, stretch). MS (*m/z*, %): 458 (M^+, Weak), 248 (30), 224 (20), 180 (20), 150 (20), 127 (25), 104 (25), 77 (25), 56 (35), 45 (100).

2-(2-(4-(2-Methoxybenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4j)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.51 (brs, 2H, piperazine), 2.65 (brs, 2H, piperazine), 2.79 (t, 2H, -C$\underline{H}_2$-piperazine), 3.26 (brs, 2H, piperazine), 3.52 (brs, 2H, piperazine), 3.86 (s, 3H, -OCH_3), 4.39 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 6.93 (d, 1H, *J* = 8.5 Hz, H_3-2-methoxyphenyl), 7.01 (t, 1H, *J* = 7 Hz, H_5-2-methoxyphenyl), 7.25 (d, 1H, *J* = 8.5 Hz, H_6-2-methoxyphenyl), 7.37 (t, 1H, *J* = 7 Hz, H_4-2-methoxyphenyl), 7.80 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.26 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.63 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3059 (C-H, stretch, aromatic), 2927 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1701 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1624 (C=C, stretch, aromatic), 1465 (C=C, stretch, aromatic), 1242 (C-N, stretch).

2-(2-(4-(3-Methoxybenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4k)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.57 (brs, 2H, piperazine), 2.68 (brs, 2H, piperazine), 2.77 (t, 2H, -C$\underline{H}_2$-piperazine), 3.40 (brs, 2H, piperazine), 3.76 (brs, 2H, piperazine), 3.82 (s, 3H, -OCH_3), 4.36 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 6.93 (m, 3H, 3-methoxyphenyl), 7.29 (t, 1H, *J* = 7.5 Hz, H_5-3-methoxyphenyl), 7.76 (t, 2H, *J* = 7.5 Hz, $H_{5,8}$-naphthalimide), 8.21 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.59 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3059 (C-H, stretch, aromatic), 2927 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1701 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1624 (C=C, stretch, aromatic), 1465 (C=C, stretch, aromatic), 1242 (C-N, stretch).

2-(2-(4-(4-Methoxybenzoyl)piperazin-1-yl)ethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione ***(4l)***
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 2.95 (brs, 4H, piperazine), 2.77 (t, 2H, -C$\underline{H}_2$-piperazine), 3.49 (brs, 4H, piperazine), 3.89 (s, 3H, -OCH_3), 4.71 (t, 2H, -C$\underline{H}_2$-CH_2-piperazine), 6.98 (d, 2H, *J* = 8 Hz, $H_{3,5}$-4-methoxyphenyl), 7.46 (d, 2H, *J* = 8 Hz, $H_{2,6}$-4-methoxyphenyl), 7.81 (t, 1H, *J* = 8Hz, $H_{5,8}$-naphthalimide), 8.28 (d, 2H, *J* = 8.5 Hz, $H_{6,7}$-naphthalimide), 8.64 (d, 2H, *J* = 7.5 Hz, $H_{4,9}$-naphthalimide). IR (KBr, cm^{-1}) ῡ: 3325 (C-H, stretch, aromatic), 2924 (C-H, asymmetric stretch, aliphatic), 2850 (C-H, symmetric stretch, aliphatic), 1697 (C=O, stretch, imide), 1654 (C=O, stretch, amide), 1620 (C=C, stretch, aromatic), 1465 (C=C, stretch, aromatic), 1249 (C-N, stretch). MS (*m/z*, %): 443 (M^+, Weak), 224 (25), 198 (15), 154 (25), 135 (25), 99 (25), 70 (20), 56 (100), 41 (60).



Scheme 1.Synthetic pathway of compounds 4a-4l

Ellman's test

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 (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), potassium hydroxide (KOH), sodium hydrogen carbonate ($NaHCO_3$), and acetylthiocholine iodide were purchased from Fluka (Buchs, Switzerland). Spectrophotometric measurements were run on a Cecil BioAquarius CE 7250 Double Beam Spectrophotometer.

Compounds **4a-4l** were dissolved in a mixture of 20 ml distilled water and 5 ml methanol and then diluted in 0.1 M KH_2PO_4/K_2HPO_4 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 ^{0}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 IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration vs percent of inhibition) [18, 19].

RESULTS AND DISCUSSION

Chemistry

All intended final derivatives **4a-4l** were synthesized according to **scheme 1** through preparation of the intermediate compound **3**.Compound **3** was synthesized via a Gabriel like synthetic procedure of phthalimides. Naphthalene-1,8-dicarboxylic anhydride was reacted with 2-(piperazin-1-yl)ethanamine under reflux condition in toluene. The intended product was afforded with 84% yield and 105 ^{o}C was recorded as its melting point. Consequently, final products **4a-4l**were synthesized using DCC as carbodiimide coupling agents as well as hydroxybenzotriazole as additive agent. Compounds **4a-4l** obtained with a moderate yields 40-75 % (**Table 1**). Purification was carried out with *n*-hexane and diethyl ether. Furthermore, column chromatography (EtOAC/Petroleum ether) was applied to achieve purred compounds. Melting points were also determined using open capillary tubes and a range of 115-195 ^{o}C was recorded.

Table 1.Properties of synthesized compounds

Compounds	R	MW (g/mol)	mp (°C)	Chemical Formula	Yield (%)
3	-	309	105	$C_{18}H_{19}N_3O_2$	84
4a	2-F	431.46	170	$C_{25}H_{22}FN_3O_3$	66
4b	3-F	431.46	174	$C_{25}H_{22}FN_3O_3$	59
4c	4-F	431.46	191	$C_{25}H_{22}FN_3O_3$	48
4d	2-Cl	447.91	184	$C_{25}H_{22}ClN_3O_3$	40
4e	3-Cl	447.91	160	$C_{25}H_{22}ClN_3O_3$	71
4f	4-Cl	447.91	160	$C_{25}H_{22}ClN_3O_3$	41
4g	2-NO_2	458.47	175	$C_{25}H_{22}N_4O_5$	49
4h	3-NO_2	458.47	175	$C_{25}H_{22}N_4O_5$	75
4i	4-NO_2	458.47	190	$C_{25}H_{22}N_4O_5$	52
4j	2-OCH_3	443.49	195	$C_{26}H_{25}N_3O_4$	53
4k	3-OCH_3	443.49	115	$C_{26}H_{25}N_3O_4$	67
4l	4-OCH_3	443.49	180	$C_{26}H_{25}N_3O_4$	46

Enzyme inhibitory assay

A new series of naphthalimide**s** bearing piperazinyl moiety were synthesized and related anti-acetylcholinesterase potency were investigated using Ellman's method. All tested derivatives were compared to donepezil as standard acetylcholinesterase inhibitor (**Table 2**).Various substituents such as fluorine, chlorine, nitro and methoxy were examined on the phenyl residue to investigate the role of electronic effects as well as other physicochemical parameters. Generally, compounds that containing methoxy with electron donating property possessed superior enzyme inhibitory activity compared to other tested compounds with electron withdrawing moieties. The methoxy substituent at *meta* position (IC_{50} = 0.26 µM) of the phenyl residue demonstrated more inhibitory effect than *ortho*(IC_{50} = 91.6 µM) and *para* (IC_{50} = 0.39 µM). Fluorine atom as electron withdrawing moiety with a small size as same as hydrogen rendered its enhancing positive impact on the *ortho* position (IC_{50} = 1.9 µM) more clearly than other positions. Replacement of the fluorine with chlorine atom caused an increase in enzyme inhibitory property at position *ortho* (IC_{50} = 1.1 µM). It is likely that lipophilic property of the chlorine atom at position *ortho* is a beneficial parameter for potency. Furthermore, moving the chlorine to the position *meta* also exerted an acceptable potency. The lowest anti-acetylcholinesterase activity was observed while chlorine substituted at *para*. Using nitro moiety as another electron withdrawing group also caused an interesting enhancement especially at positions *ortho* and *meta*. Totally, it could be concluded that substitution of the electron withdrawing moieties at position *ortho* and *meta* is so beneficial for potency. At position *para*, only positioning of the fluorine substituent potentiated the activity whereas, chlorine and nitro bearing compounds did not show this trend. As known, nitro and chlorine have more volume than fluorine. However, it is probable that steric effect that produced by nitro and chlorine moieties may be a limiting and detrimental factor for enzyme inhibition. The corresponding section of the active site of the acetylcholinesterse does not have enough space for acceptance of the large moieties. Overall, compounds **4h**, **4k** and**4l** displayed higher enzyme inhibitory effect than donepezil.

Comparison of the obtained results in this research with our previous reports confirmed that *ortho* positioning of the chlorine is a suitable selection for the moiety that is substituted on the phenyl ring(5). In the other words, chlorine atom potentiated the anti-cholinesterase activity in phthalimides as well as naphthalimides. According to the previous results methoxy at position *meta* caused also remarkable activity in comparison with other congeners in these series. In the present study, replacement of the former phthalimide residue with naphthalimide residue enhanced the enzyme inhibitory capability. It is likely that more hydrophobicity as well as more π-interactions may be responsible for potent receptor interactions of the corresponding ligands.

Table 2.Results (IC_{50}, µM) of anti-acetylcholinesterase activity of compounds 4a-4l

Compound	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	Donepezil
R	**2-F**	**3-F**	**4-F**	**2-Cl**	**3-Cl**	**4-Cl**	**2-NO_2**	**3-NO_2**	**4-NO_2**	**2-OCH_3**	**3-OCH_3**	**4-OCH_3**	-
IC_{50}	1.9	8	2.1	1.1	6.3	163	4.3	0.35	294	91.6	0.26	0.39	0.41

CONCLUSION

A new series of chemical entities based on naphthalimide structure were synthesized and their acetylcholinesterase activities were assessed. The intended derivatives exerted significant anti-cholinesterase potency and could be suggested as potential lead compounds. Some potent derivatives such as compound **4k** (3-OCH_3) are proposed to be investigated for further *in vitro* as well as *in vivo* experimental assays.

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