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Synthesis and Acetylcholinesterase Inhibitory Assessment of 3-(2-(4-benzoylpiperazin-1-yl) ethylimino) indolin-2-one Derivatives with Potential Anti-Alzheimer Effects

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Alzheimer's disease (AD) is a neurodegenerative disorder in geriatric people that characterized by reduction in memory and learning ability. Attenuation of cholinergic system is the most likely hypothesis. Therefore, potentiation of the cholinergic system via inhibition of acetylcholinesterase enzyme could improve and alleviate the symptoms of AD. A new series of isatin-based derivatives (**4a-4h**) were designed and synthesized according to the pharmacophore of donepezil and consequently related anti-cholinesterase effect was evaluated by Ellman's test. Obtained results were compared with donepezil as a standard drug. All of target derivatives (**4a-4h**) synthesized with moderate yields. In Ellman's test as an enzymatic assay for acetylcholinesterase, the most of them displayed better inhibitory potency in comparison with donepezil. In summary, isatin-based derivatives could be potential anti-Alzheimer agents. But, more experimental investigations are needed to prove this statement in the future.

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Introduction

Alzheimer's disease (AD) one of the most common forms of dementia in elderly persons is a chronic neurodegenerative disorder that is featured by a progressive loss in memory, learning and cognitive functions, language, emotion and personality. Nowadays, it is estimated that about 36 million people suffer from AD worldwide and expecting to reach 66 million by 2030 [1-3]. Approximately, it estimates the universal cost of dementia to be 604 billion dollars in 2010 and the burden is expected to increase as the population ages [4]. Decline in presynaptic cholinergic neurons in some areas of the brain is responsible for memory and learning. This neurodegenerative disease is also associated with the presence of amyloid β-peptide (Aβ) deposits and neurofibrillary tangles in the brain [2]. Potentiation of cholinergic neurotransmission by enhancement of acetylcholine (ACh) levels would be an effective route to overwhelm the occurrence, symptoms and progression of AD. Hence, the inhibition of acetylcholinesterase (AChE) which is responsible for the termination of action and metabolic cleavage of ACh has been considered as one of the most promising strategies for combating AD [2, 5-8]. Acetylcholinesterase (AChE) as a key enzyme in cholinergic pathway of autonomic system presented in the termination of nerve signals through the hydrolysis of acetylcholine. It is a target of drug development to combat the neuromuscular disorders such as myasthenia gravis, glaucoma and Alzheimer's disease [9-12]. Tacrine, rivastigmine, galantamine and donepezil are the currently in use anticholinesterase in clinic for treatment of AD. None of the mentioned drugs are so effective in the advanced steps of the disease and also present some unwanted side effects. However, discovery of novel anticholinesterase is a deep need in medicine and medicinal chemistry [13, 14].

Isatin or 2, 3-dioxindole is a bright orange-coloured powder with a long history and a broad range of biological and pharmacological functions. It seems that isatin may have physiological effects in some tissues like brain because of its presence in remarkable levels. It can be both anxiogenic and sedative. Brain monoamine levels may increase by isatin. It is an MAO inhibitor, especially of MAO_B. Isatin is an endogenous indole derivative vastly present in both human and other mammalian tissues and fluids. Perhaps, it occurs as a result of the tryptophan metabolic pathway. Recent researches and reports states that many isatin derivatives exhibit a broad range of biological activities such as anticancer, antidepressant, anticonvulsant, antifungal, anti-HIV and anti-inflammatory, etc. [15-19].

Former studies revealed that ACE enzyme has two binding sites: catalytic anionic site (CAS) and peripheral anionic site (PAS). It was proposed that PAS has important role in the deposition and aggregation of Aβ in the brain. Compounds bearing benzyl piperidine, benzylamino, phenylpiperazine, and anilino moieties are strong inhibitors of catalytic domain of the enzyme. On the other hands, the presence of aromatic rings in ACE inhibitors facilitates the binding profile to the PAS section of the enzyme. According to the previous reports, some aromatic rings such as indoline have this capability [2]. In the present study, we designed a new series of donepezil-like derivatives with isatin substructure **(Fig. 1)** and assessed their anticholinesterase activity *in vitro*. In fact, we obeyed the pharmacophoric necessities of the donepezil structure to design target compounds.

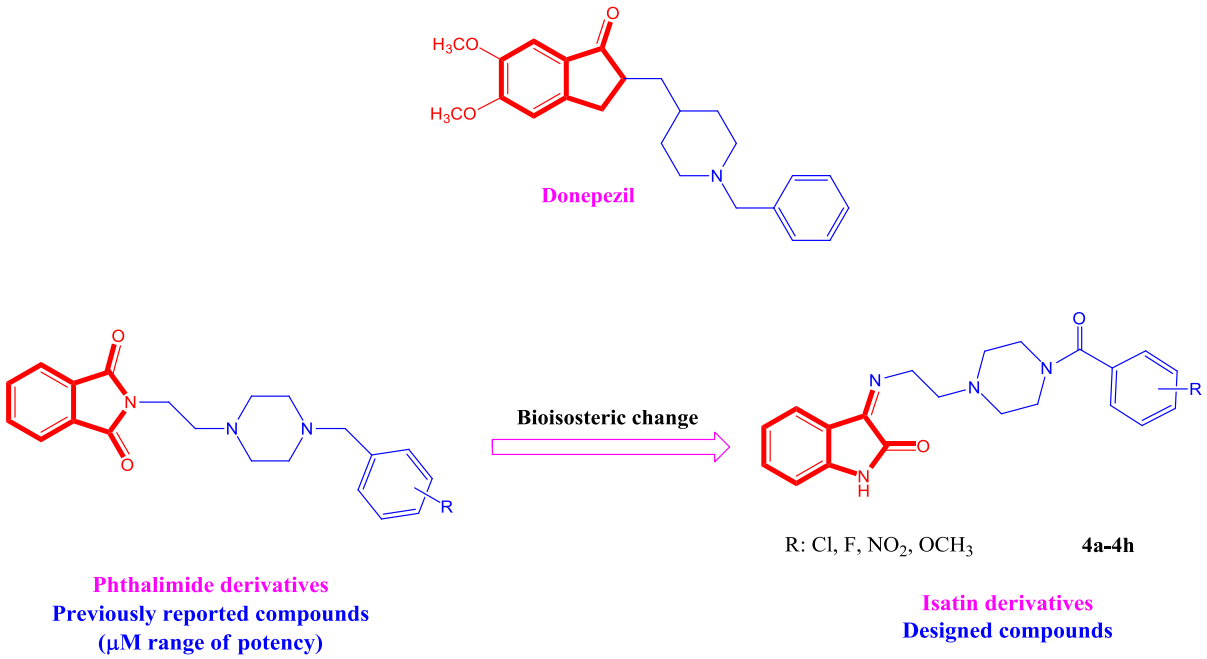


Fig. 1. Design of target compounds **4a-4h** according to the structure of donepezil and phthalimde derivatives.

Material and methods

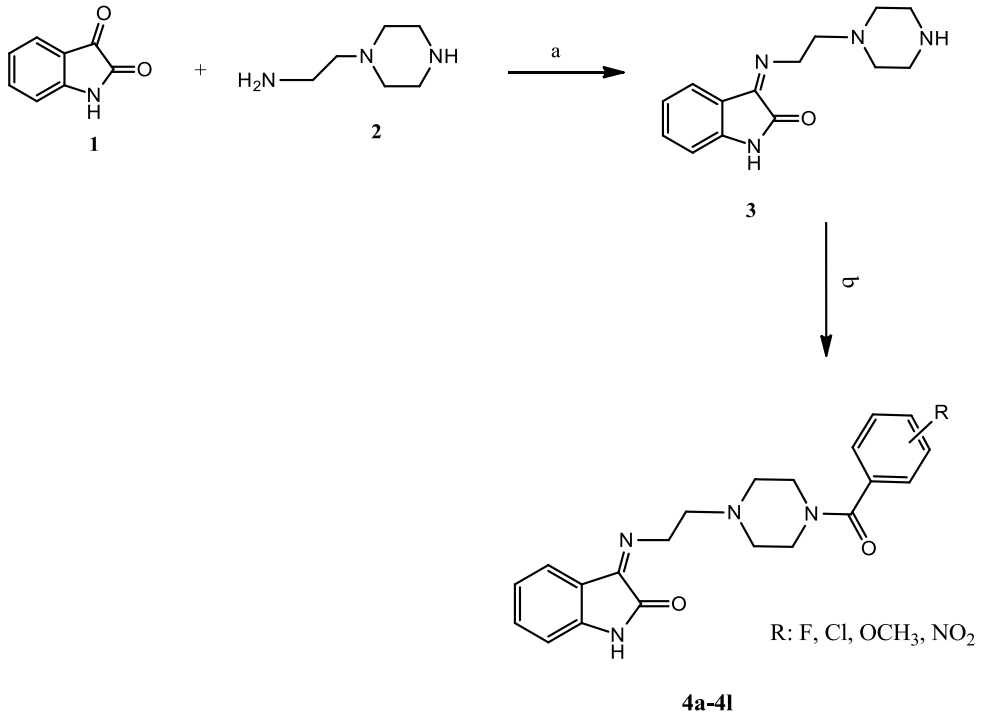
Chemistry

Merck and Sigma-Aldrich companies were selected as valid commercial suppliers for preparation of chemical substances such as solvents, starting materials and reagents. 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 (DMSO-d_6) and 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 (Z)-3-((2-(Piperazin-1-yl)ethyl)imino)indolin-2-one (3)

In a flat bottom flask 3 g (20 mmol) of isatin was stirred with 2.6 ml (20 mmol) *N*-(2-aminoethyl)piperazine in the presence of glacial acetic acid (1 ml) (**Scheme 1**). The reaction mixture was stirred at room temperature for 96 h. Then, thin layer chromatography (TLC) was applied for determining the reaction end. The brick colored precipitate was filtered and washed by diethyl ether (Et_2O) and *n*-hexane. Column chromatography (EtOAC/Petroleum ether; 70/30) was performed for extra purification [20].
^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.95 (m, 4H, Piperazine), 2.33 (m, 4H, Piperazine), 2.79 (t, 2H, -CH_2-C$\underline{H_2}$-Piperazine), 3.12 (t, 2H, -C$\underline{H_2}$-CH_2-Piperazine), 7.32 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.41 (t, 1H, *J* = 10 Hz, H_6-Isatin), 7.57 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.83 (d, 1H, *J* = 10 Hz, H_4-Isatin), 7.98 (brs, NH, Piperazine). IR (KBr, cm^{-1}) ῡ: 3429, 3059,

2939, 2821, 1716, 1635, 1620, 1469, 1440, 1402, 1327, 1186, 1008, 754. MS (*m/z*, %): 258 (M^+, 30), 217, (40), 162 (100), 146 (30), 132 (60), 120 (30), 104 (45), 85 (60), 56 (75).



1 2 3 4a-4l

Scheme 1. Synthetic pathway for target compounds. Reagents and conditions: a) Glacial acetic acid, rt, 96 h. b) Benzoic acid derivatives, EDC, HOBt, Acetonitrile.

General procedure for synthesis of final derivatives (4a-4l)

A mixture of equimolar quantities of appropriate benzoic acid derivative (0.775 mmol), 148 mg (0.775 mmol) of *N*-ethyl-*N*-dimethylaminopropyl carbodimide (EDC) and 105 mg (0.775 mmol) of hydroxybenzotriazole (HOBt) were stirred in acetonitrile (20 ml) for 30 min. Then, 200 mg (0.775 mmol) of compound **3** was added to the reaction medium and stirring was continued for 24 h. Thin layer chromatography (TLC) was utilized for reaction monitoring as well as reaction end determination. Finally, acetonitrile was evaporated under reduced pressure and the obtained residue was extracted by water/ethylacetate (50/50). Aqueous layer was discarded and organic layer was washed three times by sodium bicarbonate 5% and brine. Organic phase was dried over anhydrous sodium sulfate and then filtered and evaporated. The obtained powders were treated by *n*-hexane and diethyl ether (Et_2O). Further purification was done using column chromatography (EtOAC/Petroleum ether; 70/30) [21-26].

(*Z*)-3-((2-(4-(2-Chlorobenzoyl) piperazin-1-yl) ethyl)imino)indolin-2-one (4a)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.08 (m, 4H, Piperazine), 1.33 (m, 4H, Piperazine), 1.68 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 1.93 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 7.34 (m, 4H, Aromatic), 7.80 (m, 2H, Aromatic), 7.95 (d, (m, 2H, Aromatic). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1724 (Stretch, C=O, isatin), 1627 (Stretch, C=O, amide). MS (*m/z*, %): 395 (M^++2, 5), 397 (M^+, 2), 141 (25), 120 (20), 139 (100), 111 (80), 90 (15), 75 (50), 63 (25), 52 (30), 50 (35), 41 (50).

(*Z*)-3-((2-(4-(3-Chlorobenzoyl) piperazin-1-yl) ethyl)imino)indolin-2-one (4b)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.10 (m, 4H, Piperazine), 1.33 (m, 4H, Piperazine), 1.69 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 1.92 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 7.29-7.41 (m, 2H, Isatin), 7.54-7.62 (m, 2H, Isatin), 7.75 (t, 1H, *J* = 10 Hz, H_5-3-Chlorophenyl), 7.86 (d, 1H, *J* = 10 Hz, H_6-3-Chlorophenyl), 8.11 (s, 1H, H_2-3-Chlorophenyl), 8.23 (d, 1H, *J* = 10 Hz, H_4-3-Chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1724 (Stretch, C=O, isatin), 1627 (Stretch, C=O, amide). MS (*m/z*, %): 395 (M^++2, 12), 397 (M^+, 5), 141 (15), 120 (30), 139 (100), 111 (30), 90 (25), 75 (55), 63 (15), 52 (30), 50 (65), 41 (50).

(*Z*)-3-((2-(4-(4-Chlorobenzoyl) piperazin-1-yl) ethyl)imino)indolin-2-one (4c)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.12 (m, 4H, Piperazine), 1.28 (m, 4H, Piperazine), 1.69 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 1.92 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 7.29-7.37 9 (m, 2H, Isatin), 7.40 (d, 1H, *J* = 10 Hz, $H_{2,6}$-4-Chlorophenyl), 7.65 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.77 (d, 1H, *J* = 10 Hz, H_4-Isatin), 7.99 (d, 1H, *J* = 10 Hz, $H_{3,5}$-4-Chlorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1685 (Stretch, C=O, isatin), 1627 (Stretch, C=O, amide). MS (*m/z*, %): 395 (M^++2, 15), 397 (M^+, 5), 141 (20), 120 (40), 139 (100), 111 (55), 90 (25), 75 (35), 63 (25), 52 (40), 50 (15), 41 (20).

(*Z*)-3-((2-(4-(2-Methoxybenzoyl) piperazin-1-yl) ethyl)imino)indolin-2-one (4d)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.11 (m, 4H, Piperazine), 1.29 (m, 4H, Piperazine), 1.68 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 1.90 (t, 2H, -C$\underline{H}_2$-CH_2-Piperazine), 4.07 (s, 3H, -OCH_3), 7.06 (d, 1H, *J* = 10 Hz, H_3-2-Methoxyphenyl), 7.14 (t, 1H, *J* = 10 Hz, H_5-2-Methoxyphenyl), 7.32 (t, 1H, *J* = 10 Hz, H_4-2-Methoxyphenyl), 7.38 (t, 1H, *J* = 10 Hz, H_5-isatin), 7.57 (t, 1H, *J* = 10 Hz, H_6-isatin), 7.64 (t, 1H, *J* = 10 Hz, H_6-2-Methoxyphenyl), 7.84 (d, 1H, *J* = 10 Hz, H_7-isatin), 8.17 (d, 1H, *J* = 10 Hz, H_4-isatin). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1724 (Stretch, C=O, isatin), 1627 (Stretch, C=O, amide). MS (*m/z*, %): 392 (M^+, 15), 354 (20), 303 (20), 286 (25), 254 (35), 233 (20), 210 (35), 146 (55), 135 (100), 107 (20), 92 (50), 77 (45).

(*Z*)-3-((2-(4-(3-Methoxybenzoyl) piperazin-1-yl) ethyl) imino)indolin-2-one (4e)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.11 (m, 4H, Piperazine), 1.33 (m, 4H, Piperazine), 1.70 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 1.93 (t, 2H, -C$\underline{H}_2$-CH_2-Piperazine), 3.81 (s, 3H, -OCH_3), 6.90-7.04 (m, 2H, 3-Methoxyphenyl), 7.29-7.38 (m, 4H, 3-Methoxyphenyl, Isatin), 7.62 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.84 (d, 1H, *J* = 10 Hz, H_4-Isatin). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1724 (Stretch, C=O, isatin), 1624 (Stretch, C=O, amide). MS (*m/z*, %): 392 (M^+, 5), 354 (15), 286 (25), 254 (25), 233 (40), 210 (15), 146 (35), 135 (100), 107 (30), 92 (35), 77 (65).

(*Z*)-3-((2-(4-(4-Methoxybenzoyl) piperazin-1-yl)ethyl)imino)indolin-2-one (4f)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.01 (m, 4H, piperazine), 1.22 (m, 4H, piperazine), 1.58 (t, 2H, -CH_2-piperazine), 1.68 (t, 2H, -CH_2-piperazine), 3.90 (s, 3H, -OCH_3), 6.96 (d, 2H, *J* = 10 Hz, $H_{3,5}$-4-Methoxyphenyl), 7.35 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.46 (t, 1H, *J* = 10 Hz, H_6-Isatin), 7.63 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.85 (d, 2H, *J* = 10 Hz, $H_{3,5}$-4-Methoxyphenyl), 7.87 (d, 1H, *J* = 10 Hz, H_4-Isatin). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1728 (Stretch, C=O, isatin), 1624 (Stretch, C=O, amide). MS (*m/z*, %): 392 (M^+, Weak), 390 (5), 354 (12), 303 (12), 286 (15), 254 (15), 233 (20), 210 (25), 146 (25), 135 (100), 107 (40), 92 (80), 77 (80).

(*Z*)-3-((2-(4-(4-Fluorobenzoyl) piperazin-1-yl) ethyl)imino)indolin-2-one (4g)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.13 (m, 4H, Piperazine), 1.32 (m, 4H, Piperazine), 1.69 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 1.91 (t, 2H, -CH_2-C$\underline{H}_2$-Piperazine), 7.31 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.36 (t, 1H, *J* = 10 Hz, H_6-Isatin), 7.43 (dd, 2H, *J* = 15, 5.5 Hz, $H_{2,6}$-4-Fluorophenyl), 7.62 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.80 (d, 1H, *J* = 10 Hz, H_4-Isatin), 8.08 (dd, 2H, *J* = 15, 5.5 Hz, $H_{3,5}$-4-Fluorophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1724 (Stretch, C=O, isatin), 1627 (Stretch, C=O, amide). MS (*m/z*, %): 380 (M^+, 2), 312 (10), 281 (5), 239 (35), 217 (12), 193 (15), 163 (20), 143 (60), 104 (60), 67 (100), 41 (100).

(*Z*)-3-((2-(4-(4-nitrobenzoyl) piperazin-1-yl) ethyl)imino)indolin-2-one (4h)

^{1}HNMR ($CDCl_3$, 500 MHz) δ (ppm): 1.03 (m, 4H, Piperazine), 1.69 (m, 4H, Piperazine), 1.56 (t, 2H, -CH_2-$\underline{CH}_2$-Piperazine), 1.67 (t, 2H, -$\underline{CH}_2$-CH_2-Piperazine), 6.87 (d, 1H, *J* = 10 Hz, H_7-Isatin), 7.03 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.45 (d, 1H, *J* = 10 Hz, H_4-Isatin), 7.53 (t, 1H, *J* = 10 Hz, H_5-Isatin), 7.67 (d, 2H, *J* = 10 Hz, $H_{3,5}$-4-Nitrophenyl), 8.26 (d, 2H, *J* = 10 Hz, $H_{2,6}$-4-Nitrophenyl). IR (KBr, cm^{-1}) ῡ: 3325 (Stretch, NH), 2927 (Stretch, CH aliphatic), 2850 (Stretch, CH aliphatic), 1728 (Stretch, C=O, isatin), 1627 (Stretch, C=O, amide), 1523 (Stretch, Asymmetric, NO_2), 1350 (Stretch, Symmetric, NO_2). MS (*m/z*, %): 407 (M^+, Weak), 406 (5), 384 (15), 331 (15), 263 (90), 234 (50), 205 (20), 150 (100), 120 (75).

Ellman's test

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-4h** 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-4e** were added to the assay solution and preincubated at 25 ^oC 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) [13, 14].

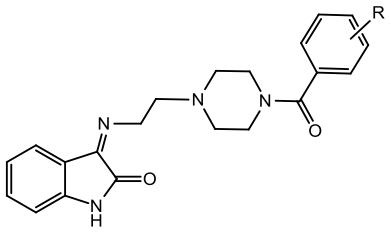
Results

Chemistry

Various isatin-based derivatives **(4a-4h)** were synthesized with accordance to the **scheme 1 (Table 1)**. Isatin was treated with equimolar quantities of *N*-(2-aminoethyl)piperazine under stirring condition in the presence of catalytic amount of glacial acetic acid. Compound **3** was reacted with various benzoic acid derivatives using EDC as coupling agent. HOBt was also added to the reaction medium as an additive agent to prevent the formation of *N*-acylurea as side product. All target compounds were obtained with an average to moderate yield. Amongst them, compound **4h** with *para* fluorine moiety showed the highest yield (82.9 %). Compound **4a** with *ortho* positioning of the chlorine substituent demonstrated the lowest yield of synthesis. Generally, *ortho* substituted derivatives exhibited higher yield compared to *meta* as well as *para*. Melting points for all synthesized derivatives were

measured and provided in **Table 1**.Compound **4b** (3-Cl) exerted the highest melting point (222 ^{o}C) in this series and compounds **4g** (4-NO_2) and **4h** (4-F) rendered the lowest melting points (160 ^{o}C for both of them). All prepared compounds were characterized by spectroscopic methods such as IR, MS and ^{1}HNMR.In the most of synthesized derivatives a weak peak was seen for molecular ion in MS spectra. A M^{+}+2 peak was observed for chlorinated derivatives (**4a-4c**).The main peak in IR spectra namely, carbonyl groups were reported. ^{1}HNMR spectra were acquired in deutrated DMSO-d_6 or chloroform.

Table 1. Physicochemical properties of synthesized compounds.



Compound	R	Yield (%)	mp (^{o}C)	Molecular Formula	MW (g/mol)
4a	2-Cl	44.7	202	$C_{21}H_{21}ClN_4O_2$	396.87
4b	3-Cl	48.7	222	$C_{21}H_{21}ClN_4O_2$	396.87
4c	4-Cl	71.5	179	$C_{21}H_{21}ClN_4O_2$	396.87
4d	2-OCH_3	48.4	180	$C_{22}H_{24}N_4O_3$	392.45
4e	3-OCH_3	51.7	193	$C_{22}H_{24}N_4O_3$	392.45
4f	4-OCH_3	66.2	178	$C_{22}H_{24}N_4O_3$	392.45
4g	4-NO_2	49.3	160	$C_{21}H_{21}N_5O_4$	407.42
4h	4-F	82.9	160	$C_{21}H_{21}FN_4O_2$	380.42

Enzymatic assay

Inhibitory capacity of all target compounds was investigated against acetylcholinesterase and inhibitory potency was compared to donepezil as a standard drug (**Table 2**). Fortunately, most of them displayed superior activity than donepezil. Only, compound **4g** (IC_{50} = 34.7 nM) with *p*-nitro moiety showed a lower activity than donepezil (IC_{50} = 9 nM). Compound **4f** (4-OCH_3) exhibited the highest activity in comparison with other congeners in this series. Compound **4e** with *meta* positioning of methoxy group was twelve times weaker than compound **4f**. Movement of the methoxy to the *ortho* position also caused to decline in activity. Replacement of the *p*-methoxy (IC_{50} = 0.01 nM) with *p*-chlorine (IC_{50} = 0.10 nM) atom led to the enhancement of the activity. Displacement of the chlorine to the *meta* as well as *ortho* position afforded derivatives with inferior potency. Substitution of fluorine atom at position *para* of the phenyl residue also caused stronger inhibitory effect than donepezil.

Table 2. Enzymatic results (IC_{50}, nM) of compounds **4a-4h**.

Compound	4a	4b	4c	4d	4e	4f	4g	4h	Donepezil
R	2-Cl	3-Cl	4-Cl	2-OCH_3	3-OCH_3	4-OCH_3	4-NO_2	4-F	-
IC_{50} (nM)	1.6	7.5	0.10	0.58	0.12	0.01	34.7	0.24	9

Discussion

Chemistry

Depicted protocol in **scheme 1** was applied for synthesis of **4a-4h**. In first step glacial acetic acid was utilized for catalyzing the reaction. In fact, protonation of the ketone type carbonyl of the isatin residue facilitate the nucleophilic attack of the primary amine of the *N*-(2-aminoethyl) piperazine. The reaction was carried out under mild condition of stirring. Refluxing condition led to the low yield. Stirring for a long time (96 h) afforded compound **3** with 73% yield. Compounds **4a-4h** were synthesized via an amidic reaction using EDC as direct coupling agent. Namely, the step of acyl halide formation was surpassed. HOBt was also utilized in the coupling step as an additive reagent. This reagent prevents the formation of side products such as *N*-acylurea as well as facilitates the coupling process by EDC. All final derivatives **4a-4h** were obtained with moderate yields. Derivatives bearing substituent at *para* position (4-Cl, 4-OCH_3, 4-F) of the phenyl residue showed higher yield compared to others, except for *para* positioning of the nitro moiety. It is probable that *para* positioning of the corresponding substituent causes less steric hindrance in the coupling reaction. Interestingly, the lowest yields were observed while substitution was implemented at *ortho* position of the phenyl residue. A median yield was also afforded for *meta* substituted derivatives. It means that reduction in steric effect causes a better reaction and eventually higher yielding. Related melting points for each compound were measured using melting point analyzer. The lowest melting points were displayed by *para* substituted derivatives. Whereas, *meta* substituted derivatives showed the highest melting points.

Enzymatic assay

Ellman's protocol was performed for enzymatic assay to explore the inhibitory potency of the final derivatives **4a-4h**. Obtained results were compared to donepezil. Generally, methoxylated derivatives (**4d-4f**) rendered a higher inhibitory activity towards AChE. It is likely electron donating property of the methoxy moiety is a beneficial parameter for enhancing the enzyme inhibitory activity. *Para* position of the phenyl residue was a better position for improvement of the potency. Electron donating activity is more effective at positions *ortho* as well as *para*. But, *m*-methoxy derivative (compound **4e**) demonstrated superior activity than *o*-methoxy. It could be hypothesized that a hydrogen bonding interaction may responsible for high potency of the *p*-methoxy and movement to the *meta* position attenuate the inhibitory activity. Steric effect may be another determining factor for methoxy group. Namely, methoxy group at position *ortho* may interfere with correct positioning of the molecule in the active site of enzyme and also interrupt the proper interaction. Replacement of the methoxy substituent with electron withdrawing moieties such as Cl, F and nitro led to the moderate decrease in activity. Nitro moiety reduced the enzyme inhibitory activity more significantly in comparison with other electron withdrawing groups. Fluorine substitution was also an efficacious change for enhancing the anticholinesterase activity remarkably.

Overall, isatin-based anticholinesterase that studied in the current project exerted higher efficacy compared to previous reported phthalimide derivatives by our research group(13, 14). In fact, isatin substructure causes a better and more favorable interaction with active site of the enzyme.

Conclusion

A new isatin-based and donepezil-like anticholinesterase were designed, synthesized and related biological activity was also investigated using Ellman's test. The most of tested compounds demonstrated superior activity than donepezil as reference anticholinesterase. Further biological and experimental tests are necessary to confirm the potent compounds as potential anti-Alzheimer agents.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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