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Synthesis and molecular docking studies of some 4-phthalimidobenzenesulfonamide derivatives as acetylcholinesterase and butyrylcholinesterase inhibitors

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ABSTRACT

A series of 4-phthalimidobenzenesulfonamide derivatives were designed, synthesized and evaluated for the inhibitory activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Structures of the title compounds were confirmed by spectral and elemental analyses. The cholinesterase (ChE) inhibitory activity studies were carried out using Ellman's colorimetric method. The biological activity results revealed that all of the title compounds (except for compound **8**) displayed high selectivity against AChE. Among the tested compounds, compound **7** was found to be the most potent against AChE (IC_{50}= 1.35 ± 0.08 μM), while compound **3** exhibited the highest inhibition against BuChE (IC_{50}= 13.41 ± 0.62 μM). Molecular docking studies of the most active compound **7** in AChE showed that this compound can interact with both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE.

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Introduction

Alzheimer's disease (AD), characterized by memory loss and other cognitive impairments, is currently one of the most difficult progressive neurodegenerative disorders to treat[1–4]. In the past decades, various pathogenesis hypothesis of AD have been proposed, such as cholinergic hypothesis, amyloid cascade hypothesis, oxidative stress hypothesis and tau protein hypothesis. Among them, cholinergic hypothesis was a widely accepted theory, which suggests that the low level of acetylcholine in specific regions of the brain is the major cause leading to learning and memory dysfunctions. Based on the cholinergic hypothesis, one possible approach to treat AD is to restore the level of acetylcholine by using reversible inhibitors to inhibit cholinesterases that include acetylcholinesterase (AChE) and butyrlcholinesterase (BuChE)[3–10]. Currently, four AChE inhibitors have been approved by European and US agency: tacrine, donepezil, galantamine and rivastigmine (Figure 1). These agents are important for the palliative treatment of AD, but their clinically efficacy is limited, mainly due to their poor selectivity, bioavailability and adverse side effects on peripheral nervous system and liver. Thus, the search for new ChE inhibitors is still of great interest[3,4,11–19].

The crystal structure of AChE in complex with inhibitors revealed that there are two binding sites, a peripheral anionic site (PAS) and catalytic active site (CAS). According to the structure characteristics of AChE, several new compounds were synthesized as anti-AD agents[3,11,12,14,20–22].

Phthalimide derivatives are important compounds due to their various bioactivities such as anticancer, anti-inflammatory, anticonvulsant and AChE inhibitory activity. Literature survey revealed that phthalimide structure had been proved to interact with the active site of AChE and several novel AChE inhibitors were designed based on this pharmacophore[23–29]. Meanwhile, sulfonamide derivatives are another important class of pharmacophores in medicinal chemistry effective in a number of different therapeutic areas. They act as antibacterials, diuretics, carbonic anhydrase inhibitors, anticonvulsants, anti-inflammatory, anticancers, antihypertensives and AChE inhibitors[30–34].

In this study, we designed a series of 4-phthalimidobenzenesulfonamide derivatives as potent cholinesterase inhibitors, in which two pharmacophores (phthalimide and sulfonamide) were combined. Accordingly, the synthesis, AChE and BuChE inhibitory activities and molecular docking studies of designed compounds are reported.

Materials and methods

Chemistry

All chemicals, reagents and solvents were high-grade commercial products and used without further purification. Reactions were checked by thin-layer chromatography (TLC) on precoated silica gel aluminum plates (Kieselgel 60, F254, E. Merck, Germany); spots were visualized by UV at 254 nm. Melting points were determined using a Stuart SMP30 (Staffordshire, ST15 OSA, United Kingdom) melting point apparatus and are not corrected. IR spectra of the compounds were recorded on a Perkin Elmer 100 Fourier transform FT-IR (ATR) spectrophotometer (Perkin Elmer Inc., MA). ^{1}H NMR spectra were recorded on a Varian AS 400 Mercury Plus NMR spectrometer (Varian, Palo Alto, CA) at 400 MHz using DMSO-d_6 and Aceton-d_6 as solvent. Chemical shifts were given in ppm (δ) with TMS as an internal standard. *J* values were given in Hertz. Abbreviations for ^{1}H NMR data quoted are as follows: s (singlet); d, (doublet); t, (triplet); q, (quartet); m, (multiplet); bs, (broad

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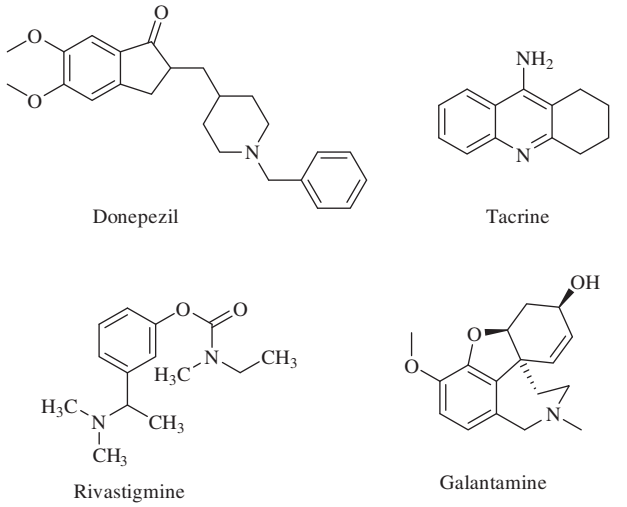


Figure 1. Chemical structures of FDA approved AChE inhibitors.

singlet). Mass spectra (APCI-MS) were measured on a Thermo MSQ Plus LC/MS (Thermoscientific Inc., San Jose, CA). Elemental analyses (C, H, N and S) were performed by Leco TruSpec Micro (Leco, St. Joseph, MI). The analytical results for the elements were within ±0.4% of the theoretical values.

General procedure for the synthesis of N-*phenylphthalimide (1a)*

Phthalic anhydride (3.38 mmol) and aniline (4.06 mmol) were heated at 160 °C in the sand bath, until completely melted. After cooling, the residue was crystallized from water[35]. Yield is 45%. Mp 204–205 °C.

General procedure for the synthesis of 4-phthalimidobenzenesulfonyl chloride (1b)

To a solution of chlorosulfonic acid (1.35 mmol) and phosphorus pentachloride (0.67 mmol) that had been stirred for 10 min, compound 1a (0.67 mmol) was added in small portions. After the mixture was stirred and heated at 50 °C for 30 min, it was poured into ice water and extracted with chloroform. The organic phase was separated, dried over anhydrous sodium sulfate and evaporated at reduced pressure to furnish compound **1b**[36]. Yield is 86%. Mp 256 °C.

General procedure for the synthesis of final compounds (1–11)

4-Phthalimidobenzenesulfonyl chloride (1.1 mmol) and appropriate amines (2.2 mmol) were refluxed in acetone (25 mL). After completion of reaction (monitored by TLC), reaction mixture was poured into ice water, then obtained precipitate was filtered and recrystallized from ethanol[37].

4-(1,3-Dioxoisoindolin-2-yl)-N-(o-tolyl)benzenesulfonamide (1) Yield 66%. mp 235 °C; 391–393 °C[38]. ^{1}H NMR (DMSO-d_6): δ 9.68 (1H, bs, NH), 7.99–7.92 (2H, *m*, phthalimide-H), 7.92–7.90 (2H, *m*, phthalimide-H), 7.80 (2H, d, *J* = 8.8 Hz, benzene-H), 7.65 (2H, d, *J* = 8.8 Hz, benzene-H), 7.14–7.11 (1H, *m*, anilide-H), 7.10 (2H, *t*, *J* = 4.4 Hz, anilide-H), 7.01–6.98 (1H, *m*, anilide-H), 2.04 (3H, *s*, CH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3485, 3312, 3085, 2797, 1785, 1717, 1592, 1501, 1327, 1161. Anal. calcd. for $C_{21}H_{16}N_2O_4S$: C, 64.27; H, 4.11; N, 7.14; S, 8.17. Found C, 64.26; H, 4.28; N, 7.56; S, 8.04. MS (APCI) *m/z* (%): 393 ($M+H^+$, 100).

4-(1,3-Dioxoisoindolin-2-yl)-N-(2-methoxyphenyl) benzenesulfonamide (2) Yield 49%. mp 205 °C. ^{1}H NMR (DMSO-d_6): δ 9.57 (1H, bs, NH), 7.98–7.94 (2H, *m*, phthalimide-H), 7.92–7.89 (2H, *m*, phthalimide-H), 7.80 (2H, d, *J* = 9.2 Hz, benzene-H), 7.61 (2H, d, *J* = 7.6 Hz, benzene-H), 7.24 (1H, d, *J* = 6.8 Hz, anilide-H), 7.13 (1H, *t*, *J* = 7.8 Hz, anilide-H), 6.90 (2H, d, *J* = 7.6 Hz, anilide-H), 3.48 (3H, *s*, OCH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3465, 3298, 3100, 3011, 2963, 2837, 1786, 1707, 1337, 1166. Anal. calcd. for $C_{21}H_6N_2O_5S$: C, 61.76; H, 3.95; N, 6.86; S, 7.85. Found C, 61.51; H, 4.15; N, 7.21; S, 7.86. MS (APCI) *m/z* (%): 288 (100), 409 ($M+H^+$, 75).

4-(1,3-Dioxoisoindolin-2-yl)-N-(2-isopropylphenyl) benzenesulfonamide (3) Yield 66%. mp 186 °C. ^{1}H NMR (DMSO-d_6): δ 9.76 (1H, bs, NH), 7.98–7.93 (2H, *m*, phthalimide-H), 7.92–7.89 (2H, *m*, phthalimide-H), 7.79 (2H, d, *J* = 7.2 Hz, benzene-H), 7.65 (2H, d, *J* = 6.4 Hz, benzene-H), 7.26 (1H, d, *J* = 8.4 Hz, anilide-H), 7.19 (1H, *t*, *J* = 7.8 Hz, anilide-H), 7.07 (1H, *t*, *J* = 7.2 Hz, anilide-H), 6.94 (1H, d, *J* = 8.0 Hz, anilide-H), 3.15–3.10 (1H, *m*, CH), 0.92 (6H, d, *J* = 6.8 Hz, 2xCH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3474, 3249, 3201, 2978, 2966, 2929, 2868, 2821, 2749, 1787, 1721, 1335, 1162. Anal. calcd. for $C_{23}H_{20}N_2O_4S$: C, 65.70; H, 4.79; N, 6.66; S, 7.63. Found C,65.53; H, 4.99; N, 7.07; S,7.84. MS (APCI) *m/z* (%): 421 ($M+H^+$, 100).

4-(1,3-Dioxoisoindolin-2-yl)-N-(p-tolyl)benzenesulfonamide (4) Yield 52%. mp 212 °C; 224–226 °C[38]. ^{1}H NMR (DMSO-d_6): δ 10.22 (1H, bs, NH), 7.98–7.94 (2H, *m*, phthalimide-H), 7.92–7.89 (2H, *m*, phthalimide-H), 7.86 (2H, d, *J* = 8.0 Hz, benzene-H), 7.62 (2H, d, *J* = 8.4 Hz, benzene-H), 7.03 (2H, d, *J* = 8.8 Hz, anilide-H), 6.99 (2H, d, *J* = 8.4 Hz, anilide-H), 2.17 (3H, *s*, CH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3282, 1786, 1713, 1594, 1509, 1340, 1161. Anal. calc. for $C_{21}H_{16}N_2O_4S$. 0.3 C_2H_6O: C, 63.86; H, 4.42; N, 6.98; S, 7.89. Found C, 64.17; H, 4.64; N, 7.28; S, 7.72. MS (APCI) *m/z* (%): 379 (100), 393 ($M+H^+$, 11).

4-(1,3-Dioxoisoindolin-2-yl)-N-(4-methoxyphenyl) benzenesulfonamide (5) Yield 48%. mp 161 °C; 168–170 °C[38]. ^{1}H NMR (DMSO-d_6): δ 10.04 (1H, bs, NH), 7.97–7.94 (2H, *m*, phthalimide-H), 7.92–7.89 (2H, *m*, phthalimide-H), 7.82 (2H, d, *J* = 8.4 Hz, benzene-H), 7.63 (2H, d, *J* = 9.2 Hz, benzene-H), 7.01 (2H, d, *J* = 8.8 Hz, anilide-H), 6.81 (2H, d, *J* = 8.8 Hz, anilide-H), 3.65 (3H, *s*, OCH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3243, 1790, 1713, 1592, 1508, 1334, 1172. Anal. calcd. for $C_{21}H_{16}N_2O_5S$. 0.01 C_2H_6O: C, 61.74; H, 3.96; N, 6.85; S, 7.84. Found C, 62.15; H, 4.39; N, 7.10; S, 7.70. MS (APCI) *m/z* (%): 288 (100), 409 ($M+H^+$, 38).

4-(1,3-Dioxoisoindolin-2-yl)-N-(4-chlorophenyl)benzenesulfonamide (6) Yield 59%. mp 212 °C; 212–214 °C[38]. ^{1}H NMR (DMSO-d_6): δ 10.56 (1H, bs, NH), 7.98–7.94 (2H, *m*, phthalimide-H), 7.91–7.89 (2H, *m*, phthalimide), 7.89 (2H, d, *J* = 8.4 Hz, benzene-H), 7.66 (2H, d, *J* = 8.8 Hz, anilide-H), 7.30 (2H, d, *J* = 8.4 Hz, benzene-H), 7.13 (2H, d, *J* = 8.6 Hz, anilide-H) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3252, 1784, 1714, 1592, 1334, 1164, 712. Anal. calcd. for $C_{20}H_{13}ClN_2O_4S$. 0.9 C_3H_6O: C, 58.57; H, 3.95; N, 6.02; S, 6.88. Found C, 58.17; H, 3.55; N, 5.56; S, 6.88. MS (APCI) *m/z* (%): 222 (100), 413 ($M+H^+$, 13), 415 ($M+H+2^+$, 4).

4-(1,3-Dioxoisoindolin-2-yl)-N,N-diethylbenzenesulfonamide (7) Yield 68%. mp 181 °C; 178–180 °C[38]. ^{1}H NMR (DMSO-d_6): δ 7.99–7.97 (2H, *m*, phthalimide-H), 7.92–7.90 (2H, *m*, phthalimide-H), 7.94 (2H, d, *J* = 8.4 Hz, benzene-H), 7.69 (2H, d, *J* = 8.4 Hz,

benzene-H), 3.19 (4H, *q*, *J* = 7.2 Hz, 2xCH_2), 1.06 (6H, *t*, *J* = 7.0 Hz, 2xCH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 2976, 1782, 1711, 1593, 1349, 1290, 1180. Anal. calcd. for $C_{18}H_{18}N_2O_4S$: C, 60.32; H, 5.06; N, 7.82; S, 8.95. Found C, 60.40; H, 5.19; N, 7.53; S, 8.76. MS (APCI) *m/z* (%): 359 ($M+H^+$, 100).

2-(4-(Pyrrolidin-1-ylsulfonyl)phenyl)isoindoline-1,3-dione (8) Yield 45%. mp 175 °C. ^{1}H NMR (Acetone-d_6): δ 8.02–8.00 (2H, *m*, phthalimide-H), 7.98 (2H, d, *J* = 8.4 Hz, benzene-H), 7.96–7.95 (2H, *m*, phthalimide-H), 7.82 (2H, d, *J* = 8.4 Hz, benzene-H), 3.30–3.26 (4H, *m*, pyrolidine-H), 1.81–1.77 (4H, *m*, pyrolidine-H) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 2979, 2879, 1792, 1716, 1592, 1341, 1251, 1161. Anal. calcd. for $C_{18}H_{16}N_2O_4S$. H_2O: C, 57.74; H, 4.85; N, 7.48; S, 8.56. Found C, 58.11; H, 4.60; N, 7.08; S, 8.91. MS (APCI) *m/z* (%): 357 ($M+H^+$, 100).

2-(4-(Piperidin-1-ylsulfonyl)phenyl)isoindoline-1,3-dione (9) Yield 85%. mp 176 °C; 165–167 °C[38]. ^{1}H NMR (Acetone-d_6): δ 8.01–7.95 (4H, *m*, phthalimide-H), 7.93 (2H, d, *J* = 8.4 Hz, benzene-H), 7.83 (2H, d, *J* = 8.4 Hz, benzene-H), 3.06–3.03 (4H, *m*, piperidine-H), 1.68–1.62 (4H, *m*, piperidine-H), 1.50–1.47 (2H, *m*, piperidine-H) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 3281, 2973, 2933, 1781, 1712, 1672, 1575, 1354, 1237, 1177. Anal. calcd. for $C_{19}H_{18}N_2O_4S$. 0.25H_2O: C, 60.87; H, 4.97; N, 7.47; S, 8.55. Found C, 60.53; H, 4.68; N, 7.37; S, 8.60. MS (APCI) *m/z* (%): 371 ($M+H^+$, 100).

2-(4-((2-Methylpiperidin-1-yl)sulfonyl)phenyl)isoindoline-1,3-dione (10) Yield 18%. mp 182 °C; 182–184 °C[39]. ^{1}H NMR (DMSO-d_6): δ 7.99–7.97 (2H, *m*, phthalimide-H), 7.94 (2H, d, *J* = 8.8 Hz, benzene-H), 7.92–7.90 (2H, *m*, phthalimide-H), 7.70 (2H, d, *J* = 8.0 Hz, benzene-H), 4.18–4.12 (1H, *m*, piperidine-H), 3.64–3.61 (1H, *m*, piperidine-H), 3.03–2.96 (1H, *m*, piperidine-H), 1.56–1.43 (5H, *m*, piperidine-H), 1.26–1.17 (1H, *m*, piperidine-H), 1.02 (3H, d, *J* = 6.4 Hz, CH_3) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 2933, 2872, 1788, 1719, 1592, 1335, 1263, 1163. Anal. calcd. for $C_{20}H_{20}N_2O_4S$. H_2O: C, 59.69; H, 5.51; N, 6.96; S, 7.97. Found C, 59.27; H, 5.16; N, 6.88; S, 8.31. MS (APCI) *m/z* (%): 385 ($M+H^+$, 100).

2-(4-(Morpholinosulfonyl)phenyl)isoindoline-1,3-dione (11) Yield 11%. mp 222 °C; 212 °C[40]. ^{1}H NMR (DMSO-d_6): δ 8.01–7.98 (2H, *m*, phthalimide-H), 7.95–7.92 (2H, *m*, phthalimide-H), 7.90 (2H, d, *J* = 8.0 Hz, benzene-H), 7.77 (2H, d, *J* = 8.0 Hz, benzene-H), 3.63 (4H, *t*, *J* = 4.4 Hz, morpholine-H), 2.91 (4H, *t*, *J* = 4.6 Hz, morpholine-H) ppm. IR (υ_{maks} cm^{-1}) (FT/ATR): 2952, 2895, 2866, 2828, 1776, 1715, 1682, 1590, 1349, 1260, 1162. Anal. calcd. for $C_{18}H_{16}N_2O_5S$. 0.3 C_2H_6O: C, 57.84; H, 4.65; N, 7.25; S, 8.30. Found C, 57.40; H, 4.73; N, 7.33; S,7.91. MS (APCI) *m/z* (%): 373 ($M+H^+$, 100).

Biological activity

AChE (E.C.3.1.1.7., Type VI-S, from electric eel) and BuChE (E.C.3.1.1.8., from equine serum) were purchased from Sigma-Aldrich (Steinheim, Germany). 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) acetylthiocholine iodide (AChI) and butyrylthiocholine iodide (BChI) used as substrates were obtained from Fluka. Buffer compounds (potassium dihydrogen phosphate, potassium hydroxide) and sodium hydrogen carbonate were purchased from Merck (Darmstadt, Germany). Spectrophotometric measurements were performed on a Shimadzu 160-A UV-Vis spectrophotometer.

Acetylcholinesterase/butyrylcholinesterase activity assay

The inhibitory effects of the synthesized compounds on AChE and BuChE were evaluated using a slightly modified colorimetric method of Ellman et al., with galantamine as the reference compound[41–45]. Prior to use, all solutions were adjusted to 20 °C. Enzyme solution (100 μL) and inhibitor solution (100 μL) were added into a cuvette containing the phosphate buffer (3.0 mL, 0.1 M; pH 8.0). After 5-min incubation, required aliquots of the DTNB solution (100 μL) and of the AChI/BChI (20 μL) were added. After rapid and immediate mixing, the absorption was measured at 412 nm by UV spectroscopy. As a reference, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 200 μL water, 100 μL DTNB and 20 μL substrate. The enzyme activity was determined in the presence of at least five different concentrations of an inhibitor. Each concentration was assayed in triplicate. The samples were investigated immediately after preparation. The AChE/BuChE inhibitory activities of the title compounds are summarized in Table 1.

Table 1. AChE and BuChE inhibitory activities and log P values of the title compounds.

Compound No	IC_{50}±SEM (μM)*		log P†
	AChE	BuChE	
1	6.84 ± 0.17	74.36 ± 2.34	3.60
2	6.79 ± 0.41	44.24 ± 13.38	3.30
3	8.19 ± 0.13	13.41 ± 0.62	4.41
4	8.61 ± 0.18	85.01 ± 13.67	3.60
5	8.52 ± 0.18	87.16 ± 13.18	3.30
6	8.21 ± 0.14	78.15 ± 5.39	3.94
7	1.35 ± 0.08	>100	2.52
8	>100	>100	2.27
9	10.39 ± 0.61	>100	2.66
10	8.05 ± 0.54	75.18 ± 23.82	3.05
11	7.95 ± 0.22	56.67 ± 3.26	1.51

*Data are means ± standard error of the main of triplicate independent experiments.
†Log P values calculated using MOE 2011.10.

Molecular docking study

The crystal structures of donepezil in complex with AChE (PDB code 1EVE resolved at 2.5 Å) were taken from the Protein Data Bank. Heteroatoms and water molecules in the PDB file were removed and hydrogen atoms were added to the protein by using MOE 2014.09.1[46]. Prior to the docking calculations, an energy minimization using the AMBER99 force field was performed on the enzyme. Compound **7** was built and protonated using the protonate 3D protocol and energy minimized using the MMFF94 force field via MOE 2014.09.1. Docking of the ligand was carried out using the GOLD 5.2.1 program with default settings[47,48]. A sphere of 22 Å around the carbonyl group of Glu199 was defined as the binding site for the ligand docking and 250 confirmations was allowed. The Chemscore and Goldscore standard precision (sp) were calculated and analyzed. The putative binding mode was carried out through visual inspection (Figure 2).

Results and discussion

Chemistry

As shown in Scheme 1, the synthesis of the title compounds was realized in three steps according to the procedure in the literature[35–37]. Initially, phthalic anhydride and aniline were reacted to yield *N*-phenylphthalimide. Then, *N*-phenylphthalimide was treated with chlorosulfonic acid to give the 4-phthalimidobenzenesulfonyl chloride. Finally, 4-phthalimidobenzenesulfonyl chloride was

promptly converted to final sulfonamide derivatives by SN2 nucleophilic reaction with appropriate amine.

The synthesis of the compounds **1, 4–7, 9–11** were reported previously[36,38–40,49,50]. Compound **8** is listed compound with registry number CASRN 898471–20-0, whereas corresponding scientific data are not available. The AChE and BuChE inhibitory activities of the all compounds have not been described in the literature, and were reported for the first time in this study.

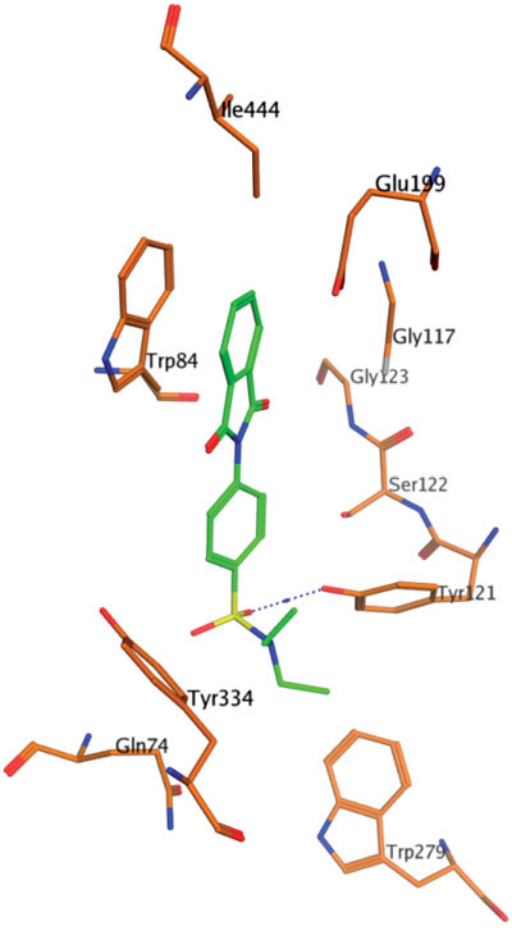


Figure 2. Proposed binding mode for compound 7 inside AChE (1EVE pdb code). The active compound is showed as green stick in AChE. The most involved residues are named and represented as brown sticks for AChE. Hydrogen bond interactions are represented as blue dashed lines.

The structures of the title compounds were confirmed by spectral and elemental analyses.

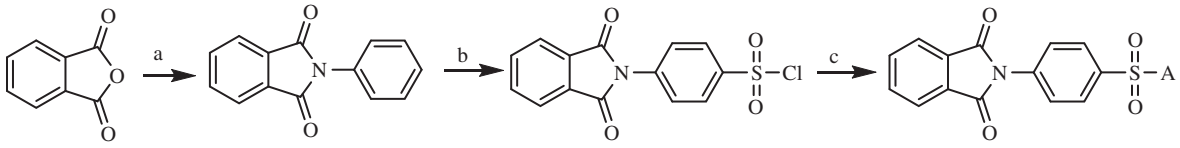
With regard to IR data, diagnostic vibrational bands were provided by sulfonamide and phthalimide moieties of the final compounds. SO_2-stretching bands of sulfonamide chromophore were observed between1327–1354 and 1161–1180 cm^{-1}, in addition, NH-stretching bands for compounds **1–6** were detected in the range of 3243–3485 cm^{-1}. Two characteristic absorption bands were appeared at around 1782–1792 and 1707–1721 cm^{-1} in spectra indicating the presence of phthalimide carbonyl groups[51]. ^{1}H NMR spectra of the compounds were consistent with expected resonance signals in terms of chemical shifts and integrations. In ^{1}H NMR, the NH proton of secondary sulfonamide group was seen as a broad singlet between at δ 9.57–10.56 ppm (for compounds **1–6**). The protons of phthalimide ring were observed as multiplets in the range of δ 8.02–7.89 ppm. Aromatic protons of phenyl ring linked to phthalimide structure were detected at δ 7.61–7.98 ppm as two doublets with 2H integration according to the AA'BB' pattern. On the other hand, resonance signals of all the aromatic and aliphatic protons were observed in the expected regions with expected multiplicities confirming the proposed structure[52].

The structures of the title compounds were further verified by APCI spectra where the *m/z* values of molecular ion peaks were in complete agreement with the calculated molecular weight for each compound.

The purity levels of the compounds were determined by elemental analyzes (C, H, N, S) and results were within 0.4% of the calculated values.

Biological activity

Inhibitory activities of the synthesized compounds against AChE and BuChE were evaluated by modified Ellman method, using galantamine as the reference compound[41–45]. The AChE inhibitory activity was determined by using electric eel acetylcholinesterase and the BuChE inhibitory activity was tested by using equine serum butyrylcholinesterase. The IC_{50} values for AChE and BuChE inhibitions are summarized in Table 1.



A= 2-methylaniline (**1**)
2-methoxyaniline(**2**)
2-isopropylaniline(**3**)
4-methylaniline(**4**)
4-methoxyaniline(**5**)
4-chloroaniline(**6**)
diethylamine(**7**)
pyrolidine(**8**)
piperidine(**9**)
2-methylpiperidine(**10**)
morpholine(**11**)

a) Aniline, 30 min. **b**) $ClSO_3H$, PCl_5, 30 min. **c**) Amines, acetone, 2h

Scheme 1. Synthesis of the final compounds **1–11.**

According to the biological activity results, target compounds exhibited higher inhibitory activity against AChE than BuChE. In addition, all compounds displayed high selectivity against AChE. Among the tested compounds, compounds **1**, **2** and **3** bearing the substituent at *ortho* position of *N*-phenyl ring on sulfonamide group showed slightly better AChE inhibitory activity compared to the *para*-substituted derivatives. Compound **7** with diethyl substituent on nitrogen atom of the sulfonamide is the most active compound with IC_{50} value of 1.35 μM against AChE. The conversion of sulfonanilide structure of the compounds **1–6** and cyclic sulfonamide structure of the compounds **8–11** to *N*,*N*-dietil sulfonamide produced the most active compound **7** against AChE. This finding let us to consider that the characterization of amide nitrogen is important for AChE inhibitory activity.

Regarding BuChE activity results, generally, it is found that the tested compounds have moderate to weak inhibition potency. Compound **3** bearing isopropyl substituent at *ortho* position of *N*-phenyl ring on sulfonamide exhibited the highest inhibitory activity in the series. However, compound **7,** the most active compound against AChE, did not exhibit any inhibition against BuChE. This situation possibly results from the differences of the amino acids in the active site of the both enzymes.

Based on these activity results, 4-phthalimidobenzenesulfonamide derivatives could be described as selective AChE inhibitors.

As a potential compound for treatment of AD, log *p* was thought as an important physicochemical parameter to evaluate or predict the ability to cross blood–brain barrier (BBB). It was reported that log *p* with the optimum central nervous system (CNS) penetration was around 2 ± 0.7[53]. The lipophilicities of the synthesized compounds were calculated using MOE 2011.10 (Molecular Operating Environment Chemical Computing Group). As shown in Table 1, log *p* values of synthesized compounds ranged from 1.51–4.41, which indicated that all the compounds are sufficiently lipophilic to pass the BBB.

Docking studies

To explore the possible binding mode of the phthalimide derivatives with AChE (PDB code 1EVE), docking studies were performed using Gold 5.2.1. for the most active compound **7** in the series. As shown in Figure 2, compound **7** exhibited two binding modes with AChE. In the bottom of the gorge, the phthalimide moiety interact with Trp84 via the π–π stacking interaction and the oxygen atom of sulfonamide group create a hydrogen bond with hydroxyl group of Tyr121. Summing up, it can be proposed that compound **7** can interact both with CAS and PAS of AChE.

Conclusion

In this study, eleven 4-phthalimidobenzenesulfonamide derivatives were synthesized and evaluated for their *in vitro* AChE and BuChE inhibition. Among the series, compound **7** and **3** showed the highest activity against AChE and BuChE, respectively. Generally, all the tested compounds displayed selectivity for AChE. Molecular modeling study of the most active compound **7** against AChE demonstrated binding interactions with both PAS and CAS of the enzyme. According to the calculated log *p* values, all the compounds might pass to the BBB. These sulfonamide derivatives could be considered as new lead compounds to develop more potent AChE and BuChE inhibitors.

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Disclosure statement

The authors declare no conflicts of interests.

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