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Synthesis, biological evaluation, and molecular docking of N'-(Aryl/alkylsulfonyl)-1-(phenylsulfonyl) piperidine-4-carbohydrazide derivatives

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Abstract: A series of new N'-[(alkyl/aryl)sulfonyl]-1-(phenylsulfonyl)piperidine-4-carbohydrazide derivatives were synthesized. Starting from ethyl piperidine-4-carboxylate **(a)**, first ethyl 1-(phenylsulfonyl)piperidine-4-carboxylate **(1)**, second 1-(phenylsulfonyl)piperidine-4-carbohydrazide **(2)**, and finally N'-[(alkyl/aryl)sulfonyl]-1-(phenylsulfonyl)piperidine-4-carbohydrazides **(4a–n)** were synthesized by reacting **2** with alkyl/aryl sulfonyl chlorides **(3a–n)**. The structures of the synthesized compounds were characterized by IR, ^{1}H-NMR, and EI-MS spectra and all were screened in vitro for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibition activities. Molecular docking was accomplished for these compounds to examine their binding interactions with AChE and BChE human proteins. The strategy we applied for this purpose was a direct receptor-based approach. The binding modes of the inhibitors under study were determined using an automated docking program (AutoDock) and were compared with antienzymatic IC_{50} values. Both studies confirmed the potential of compounds as excellent inhibitors for AChE and BChE.

Key words: Ethyl piperidine-4-carboxylate, benzenesulfonyl chloride, piperidine-4-carbohydrazide, enzyme inhibition, molecular docking, spectral analysis, AChE/BChE inhibitors

1. Introduction

Animal-source cholinesterases are prevalent enzymes present in body fluids and cholinergic as well as noncholinergic tissues.[1–3] Cholinesterase inhibitors are important for treatment of various diseases like organophosphate poisoning,[4] myasthenia gravis, glaucoma, and promisingly Alzheimer's disease (AD).[5,6]

AD is a neurodegenerative disorder that is attributed to a deficit in acetylcholine (ACho)[7] among other neurotransmitters, which affects the elderly population. The most prevalent strategy to treat AD is based on the cholinergic hypothesis and cholinesterase inhibitors, which enhance the level of ACho at cholinergic synapses.[8,9] Acetylcholinesterase (AChE; C. 3.1.1.7) and butyrylcholinesterase (BChE; E.C. 3.1.1.8) represent 2 main classes of cholinesterases on the basis of their specificity, behavior to substrate, and vulnerability to inhibitors. AChE and BChE are specific in their tissue distribution. AChE is profuse in the brain, erythrocyte membrane, and

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muscle, whereas BChE is distributed in the liver, intestine, heart, kidney, and lungs.[10,11] AChE catalytic activity involves acylation or deacylation of serine moiety present in the active center, which consists of 2 subsites, i.e. esteratic and anionic.[12] It has been demonstrated that AChE activity involves allosteric regulation of the ligand binding to an anionic site.[13] BChE favorably acts on butyrylcholine (BCho) as a substrate but also hydrolyzes ACho.[14,15]

At present, tacrine, donepezil, galantamine, and rivastigmine are the most frequently prescribed anti-cholinesterase drugs.[16] However, they have adverse effect profiles and some toxicity. Therefore, there is a great need for cholinesterase inhibitor drug candidates with high potency and reduced toxicity. Recently, a number of active compounds have been identified with potential therapeutic activity against cholinergic disorders.[17]

The present work is a continuation of our successful efforts for the synthesis of potentially bioactive compounds bearing the piperidine nucleus.[18] The reported derivatives were found to be potent against AChE and BChE. Molecular docking studies were carried out to rationalize these activities at molecular level and to identify the binding patterns and affinities of ligands in the binding pockets of AChE and BChE. Binding models of compounds **4g**, **4m**, **4i**, and **4n** are shown in Figure 1 (a–d), which were the most potent compounds of this series.

2. Experimental

2.1. General

Melting points of the synthesized compounds were recorded on a Griffin and George melting point apparatus by open capillary tube and were uncorrected. Purity was checked by thin layer chromatography (TLC) on precoated silica gel G-25-UV$_{254}$ plates with different polarity solvent systems using ethyl acetate and n-hexane giving a single spot. Identification of spots was carried out at 254 nm with a UV lamp, and by ceric sulfate reagent. The IR spectra were recorded by KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker spectrometer operating at 300 MHz. Chemical shifts are given in ppm taking TMS as reference. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system (Scheme).

2.2. Preparation of ethyl 1-(phenylsulfonyl)piperidine-4-carboxylate in aqueous media (1)

Ethyl piperidine-4-carboxylate (**a**; 20.0 mL, 10.0 mmol) was suspended in 50 mL of water and the pH was maintained at 9.0 during the whole reaction by adding basic aqueous solution of Na_2CO_3 (5%) at 0–5 °C. Then benzenesulfonyl chloride (**b**; 29.0 mL, 10.0 mmol) was slowly added over 10–15 min. After complete addition of **b**, the temperature was allowed to rise slowly to 30 °C. The reaction mixture was stirred and monitored with TLC. After the reaction was finished, conc. HCl (2.0 mL, 11 M) was added slowly to adjust the pH to 2.0. The precipitate was filtered, washed with distilled water, and dried to afford the off-white solid compound **1**.

Ethyl 1-(phenylsulfonyl)piperidine-4-carboxylate (1): IR (KBr, cm^{-1}): v_{max}: 3015 (Ar-CH), 2925 (C-H), 1760 (C=O), 1531 (C=C), 1329 (S=O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.78 (dd, J = 7.8, 1.8 Hz, 2H, H-2', H-6'), 7.68–7.65 (m, 1H, H-4'), 7.62 (dd, J = 7.5, 1.8 Hz, 2H, H-3', H-5'), 4.11 (q, J = 7.2 Hz, 2H, O-CH_2), 3.64 (t, J = 3.6 Hz, 2H, H_{eq}-2, H_{eq}-6), 3.59 (t, J = 3.6 Hz, 2H, H_{ax}-2, H_{ax}-6), 2.13–2.10 (m, 1H, H-4), 1.75–1.72 (m, 4H, H-3, H-5), 1.20 (t, J = 6.9 Hz, CH_3); EIMS (m/z): 297 (7%) $[M]^+$, 224 (10%), 156 (90%), 141 (14%), 77 (32%), 82 (100%).

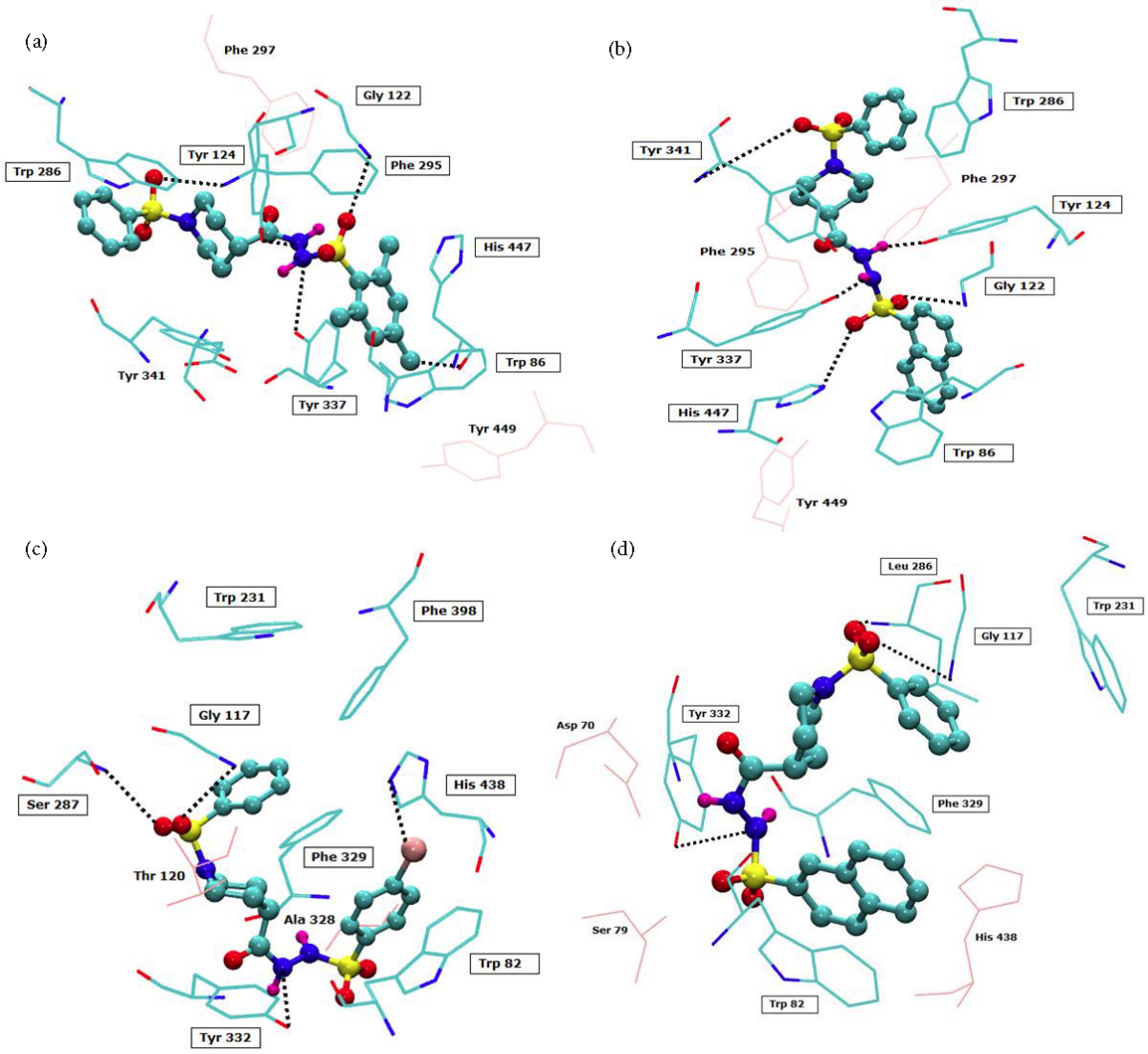
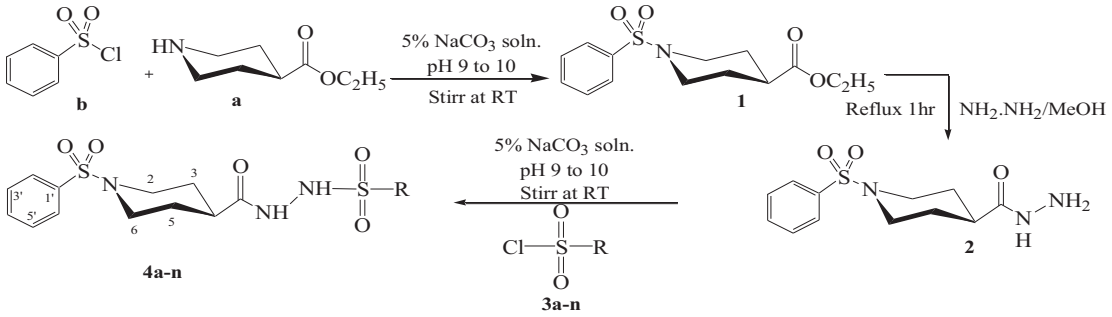


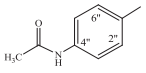
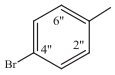
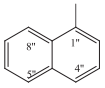
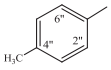
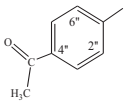
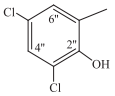
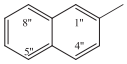
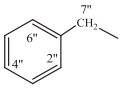
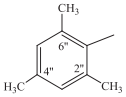
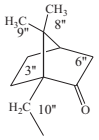
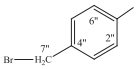
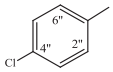
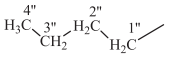
Figure 1. Binding models of compounds **4g** (a), **4m** (b), **4i** (c), and **4n** (d). **(a)**: Compound **4g** is nicely bound to AChE through Tyr124 and Tyr337, Gly122, Phe295, His447 and 3 $\pi - \pi$ interactions with Trp86, Trp286, and Tyr341. **(b):** Compound **4m** is well bound to AChE through Gly122, Tyr341, His447, Tyr124, and Tyr337, Trp86, Trp286, and Phe297 and 3 $\pi - \pi$ interactions. **(c):** Compound **4i** is nicely bound to BChE with Tyr332, Gly117, and Ser207 and 4 $\pi - \pi$ interactions with Trp82, Trp231, Phe329, and Phe398 **(d):** Compound **4n** is adequately bound to BChE with Tyr332, Gly117, and Leu286. There are 4 $\pi - \pi$ interactions with Trp82, Trp231, Phe329, and His438.

2.3. Preparation of 1-(phenylsulfonyl)piperidine-4-carbohydrazide (2)

Ethyl 1-(phenylsulfonyl)piperidine-4-carboxylate (**1**; 0.03 mol, 10 g) was dissolved in 50 mL of methanol as solvent in a 250-mL RB flask and the mixture was cooled to 0–5 °C. Then 30.0 mL of hydrazine hydrate (80%) was added dropwise to the reaction mixture and the solution was stirred for 1 h at 0–5 °C. Reaction completion was monitored by TLC until a single spot was obtained. After the reaction was finished, excess solvent was removed from the reaction mixture by distillation and white crystalline product **2** was formed, filtered off, and washed with n-hexane.[19,20]



b a 1 2 4a-n 3a-n

Compd.	R	Compd.	R	Compd.	R	Compd.	R
4a		**4e**		**4i**		**4m**	
4b		**4f**		**4j**		**4n**	
4c		**4g**		**4k**			
4d		**4h**		**4l**			

Scheme. Outline for the synthesis of N'-(1-(phenylsulfonyl)piperidine-4-carbonyl)sulfonohydrazide derivatives.

(2): IR (KBr, cm^{-1}): v_{max}: 3310 (N-H), 3018 (Ar-CH), 2926 (C-H), 1630 (C=O), 1529 (C=C), 1325 (S=O); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.85 (s, 1H, NH-CO), 7.78 (dd, J = 7.8, 1.8 Hz, 2H, H-2', H-6'), 7.66–7.63 (m, 3H, H-3' to H-5'), 3.79 (t, J = 3.6 Hz, 2H, H_{eq}-2, H_{eq}-6), 3.75 (t, J = 3.6 Hz, 2H, H_{ax}-2, H_{ax}-6), 2.13-2.11 (m, 1H, H-4), 1.9 (s, 2H, NH_2) 1.79–1.75 (m, 4H, H-3, H-5); EIMS (m/z): 283 (13%) $[M]^+$, 252 (10%), 224 (12%), 156 (12%), 84 (95%), 77 (100%).

2.4. General procedure for the synthesis of N'-[(alkyl/aryl)sulfonyl]-1-(phenylsulfonyl)piperidine-4-carbohydrazides derivatives in aqueous media (4a–n)

1-(Phenylsulfonyl) piperidine-4-carbohydrazide (**2**; 0.2 g, 0.007 mol) was suspended in 10.0 mL of water and the pH was maintained at 9.0 by adding basic aqueous solution of a Na_2CO_3 at 0–5 °C. Then the alkyl/alkylaryl/aryl sulfonyl chlorides (**3a**–**n**; 0.007 mol) were slowly added to the reaction mixture. After the addition was finished, the temperature was allowed to rise slowly to room temperature. The reaction mixture was kept on stirring for 2–3 h and was monitored via TLC until a single spot was obtained. Then conc. HCl (around 0.5 mL) was slowly added to adjust the pH to 2.0. The formed precipitate was filtered and washed with distilled water to afford the title compounds **4a**–**n** on drying. Recrystallization was performed using methanol.

***N'*, 1-bis (phenylsulfonyl)piperidine-4-carbohydrazide (4a):** IR (KBr, cm^{-1}): v_{max}: 3449 (N-H), 3011 (Ar-CH), 2924 (C-H), 1639 (C=O), 1530 (C=C), 1328 (S=O), 828 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$,

δ/ppm): 8.02 (s, 1H, NH-CO), 7.85 (d, J = 7.2 Hz, 2H, H-2" & H-6"), 7.73 (dd, J = 6.8, 1.6 Hz, 2H, H-2' & H-6'), 7.66–7.61 (m, 2H, H-4' & H-4"), 7.61–7.59 (m, 2H, H-3' & H-5'), 7.51 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.34 (dt, J = 11.6, 2.8 Hz, 2H, H_{ax}-2 & H_{ax}-6), 2.04–2.00 (m, 1H, H-4), 1.65–1.45 (m, 4H, H-3 & H-5); EIMS (m/z): 423 (1.2%) $[M]^+$, 281 (28%), 252 (46%), 224 (55%), 170 (14%), 156 (71%), 77 (100%), 82 (56%).

***N'*-[(4-methylphenylsulfonyl)]-1-(phenylsulfonyl)piperidine-4-carbohydrazide (4b):** IR (KBr, cm^{-1}): v_{max}: 3443 (N-H), 3015 (Ar-CH), 2910 (C-H), 1640 (C=O), 1531 (C=C), 1329 (S=O), 838 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.05 (s, 1H, NH-CO), 7.75 (d, J = 7.6 Hz, 2H, H-2" & H-6"), 7.73–7.67 (m, 2H, H-2' & H-6'), 7.66–7.64 (m, 1H, H-4'), 7.61–7.59 (m, 2H, H-3' & H-5'), 7.30 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 3.66–3.63 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.41 (s, 3H, -CH_3), 2.32 (dt, J = 11.6, 2.4 Hz, 2H, H_{ax}-2 & H_{ax}-6), 2.02–1.62 (m, 1H, H-4), 1.49–1.47 (m, 4H, H-3 & H-5); EIMS (m/z): 437 (0.7%) $[M]^+$, 280 (27%), 252 (47%), 224 (50%), 170 (11%), 156 (100%), 91 (72%), 77 (50%), 84 (44%).

***N'*-(benzylsulfonyl)-1-(phenylsulfonyl)piperidine-4-carbohydrazide (4c):** IR (KBr, cm^{-1}): v_{max}: 3440 (N-H), 3013 (Ar-CH), 2924 (C-H), 1638 (C=O), 1530 (C=C), 1327 (S=O), 841 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.07 (s, 1H, NH-CO), 7.79 (d, J = 6.8 Hz, 2H, H-2' & H-6'), 7.64 (d, J = 7.2 Hz, 1H, H-4'), 7.60 (d, J = 7.6 Hz, 2H, H-3' & H-5'), 7.43–7.33 (m, 5H, H-2" to H-6"), 4.30 (s, 2H, H-7"), 3.77–3.74 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.43–2.38 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.21–2.18 (m, 1H, H-4), 1.85–1.77 (m, 4H, H-3 & H-5); EIMS (m/z): 437 (0.8%) $[M]^+$, 280 (25%), 252 (49%), 224 (51%), 141 (65%), 91 (100), 84 (40%), 42 (43%).

***N'*-{[4-(bromomethyl)phenyl]sulfonyl} -1-(phenylsulfonyl)piperidin-4-carbohydrazide (4d):** IR (KBr, cm^{-1}): v_{max}: 3439 (N-H), 3017 (Ar-CH), 2921 (C-H), 1635 (C=O), 1533 (C=C), 1322 (S=O), 842 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.05 (s, 1H, NH-CO), 7.77 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.76 (d, J = 7.2 Hz, 2H, H-2' & H-6'), 7.64–7.61 (m, 2H, H-3' & H-5'), 7.59 (m, 1H, H-4'), 7.45 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 4.57 (s, 2H, C4"-CH_2), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.47–2.41 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.29–2.24 (m, 1H, H-4), 1.97–1.69 (m, 4H, H-3 & H-5); EIMS (m/z): 516 (0.7%) $[M]^+$, 280 (2%), 279 (8%), 167 (33%), 149 (100%), 71 (46%), 57 (65%), 43 (50%).

***N'*-{[4-(acetamido)phenyl]sulfonyl} -1-(phenylsulfonyl)piperidin-4-carbohydrazide (4e):** IR (KBr, cm^{-1}): v_{max}: 3431 (N-H), 3019 (Ar-CH), 2920 (C-H), 1631 (C=O), 1529 (C=C), 1319 (S=O), 835 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.09 (s, 1H, NH-CO), 7.86 (d, J = 7.6 Hz, 2H, H-2" & H-6"), 7.77–7.75 (m, 2H, H-2' & H-6'), 7.70–7.69 (m, 1H, H-4'), 7.69–7.64 (m, 2H, H-3' & H-5'), 7.58 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 7.33 (s, 1H, NH-$COCH_3$), 3.65–3.62 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.32 (dt, J = 11.6, 2.4 Hz, 2H, H_{ax}-2 & H_{ax}-6), 2.15 (s, 3H, -CH_3), 2.03–1.61 (m, 1H, H-4), 1.53–1.47 (m, 4H, H-3 & H-5); EIMS (m/z): 480 (0.5%) $[M]^+$, 282 (17%), 252 (44%), 224 (62%), 134 (78%), 77(65%), 55 (33%), 42 (47%).

***N'*-[(4-(acetylphenyl)sulfonyl]-1-(phenylsulfonyl)piperidin-4-carbohydrazide (4f):** IR (KBr, cm^{-1}): v_{max}: 3432 (N-H), 3011 (Ar-CH), 2897 (C-H), 1705 (R-C=O-R) 1624 (C=O), 1522 (C=C), 1317 (S=O), 833 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.11 (s, 1H, NH-CO), 7.77 (d, J = 7.2 Hz, 2H, H-2" & H-6"), 7.75 (d, J = 7.2 Hz, 2H, H-2' & H-6'), 7.64 (m, 1H, H-4'), 7.64–7.61 (m, 2H, H-3' & H-5'), 7.59 (d, J = 7.6 Hz, 2H, H-3" & H-5"), 3.62–3.57 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.54–2.47 (m, 2H, H_{ax}-2 & H_{ax}-6), 1.96–1.69 (m, 1H, H-4), 1.57–1.28 (m, 4H, H-3 & H-5); EIMS (m/z): 465 (1.5%) $[M]^+$, 282 (15%), 252 (49%), 224 (99%), 112 (22%), 84 (31%), 77 (68%), 42 (55%).

***N′*-[(2,4,6-trimethylphenyl)sulfonyl]-1-(phenylsulfonyl)piperidin-4-carbohydrazide (4g):** IR (KBr, cm^{-1}): v_{max}: 3437 (N-H), 3013 (Ar-CH), 2923 (C-H), 1620 (C=O), 1521 (C=C), 1319 (S=O), 855 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.09 (s, 1H, NH-CO), 7.76–7.72 (m, 2H, H-2' & H-6'), 7.65–7.61 (m, 1H, H-4'), 7.60–7.58 (m, 2H, H-3' & H-5'), 6.96 (s, 2H, H-3" & H-5"), 3.69–3.60 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.62 (s, 6H, CH_3-2", CH_3-6"), 2.28 (s, 3H, CH_3-4"), 2.32-2.22 (m, 2H, H_{ax}-2 & H_{ax}-6), 1.99–1.94 (m, 1H, H-4), 1.56–1.46 (m, 4H, H-3 & H-5); EIMS (m/z): 466 (2%) $[M]^+$, 281 (28%), 252 (77%), 224 (100%), 119 (14%), 84 (25%), 55 (70%), 42 (63%).

***N′*-[(4-chlorophenyl)sulfonyl]-1-(phenylsulfonyl)piperidin-4-carbohydrazide (4h):** IR (KBr, cm^{-1}): v_{max}: 3429 (N-H), 3025 (Ar-CH), 2921 (C-H), 1637 (C=O), 1524 (C=C), 1318 (S=O), 847 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.07 (s, 1H, NH-CO), 7.82 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.73 (dd, J = 8.4, 1.6 Hz, 2H, H-2' & H-6'), 7.66–7.64 (m, 1H, H-4'), 7.61–7.59 (m, 2H, H-3' & H-5'), 7.51 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 3.65–3.62 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.34 (dt, J = 11.6, 2.8 Hz, 2H, H_{ax}-2 & H_{ax}-6), 2.02–1.60 (m, 1H, H-4), 1.52–1.45 (m, 4H, H-3 & H-5); EIMS (m/z): 458 (0.8%) $[M]^+$, 282 (68%), 252 (86%), 224 (100%), 111 (15%), 77 (67%), 55 (58%), 43 (73%).

***N′*-[(4-bromophenyl)sulfonyl]-1-(phenylsulfonyl)piperidin-4-carbohydrazide (4i):** IR (KBr, cm^{-1}): v_{max}: 3427 (N-H), 3023 (Ar-CH), 2925 (C-H), 1640 (C=O), 1529 (C=C), 1316 (S=O), 849 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.11 (s, 1H, NH-CO), 7.85 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.77 (d, J = 6.8 Hz, 2H, H-2' & H-6'), 7.65–7.64 (m, 1H, H-4'), 7.59 (d, J = 6.8 Hz, 2H, H-3' & H-5'), 7.50 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.52–2.47 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.38–2.35 (m, 1H, H-4), 2.34–1.96 (m, 4H, H-3 & H-5); EIMS (m/z): 502 (1.7%) $[M]^+$, 282 (67%), 252 (83%), 224 (78%), 156 (100%), 77 (54%), 54 (41%), 43 (57%).

***N′*-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl)-1-(phenylsulfonyl) piperidin-4-carbohydrazide (4j):** IR (KBr, cm^{-1}): v_{max}: 3440 (N-H), 3320 (O-H), 3017 (Ar-CH), 2921 (C-H), 1632 (C=O), 1526 (C=C), 1320 (S=O), 855 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.11 (s, 1H, NH-CO), 7.73 (d, J = 7.2 Hz, 2H, H-2' & H-6'), 7.64 (d, J = 2.4 Hz, 1H, H-6"), 7.63 (d, J = 2.4 Hz, 1H, H-4"), 7.64–7.61 (m, 1H, H-4'), 7.59 (d, J = 6.8 Hz, 2H, H-3' & H-5'), 3.60–3.57 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.40–2.35 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.10–1.65 (m, 1H, H-4), 1.52–1.50 (m, 4H, H-3 & H-5); EIMS (m/z): 508 (1.2%) $[M]^+$, 282 (23%), 252 (47%), 224 (82%), 161 (52%), 150 (100%), 77 (63%), 55 (48%).

***N′*-{[(1R,4R)-7,7-dimethyl-2-oxobicyclo[2.2.1]hept-1-yl)methyl] sulfonyl} -1-(phenylsulfonyl) piperidine-4-carbohydrazide (4k):** IR (KBr, cm^{-1}): v_{max}: 3448 (N-H), 3014 (Ar-CH), 2924 (C-H), 1715 (R-C=O-R), 1637 (C=O), 1530 (C=C), 1328 (S=O), 828 (N-S), 860 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 8.12 (s, 1H, NH-CO), 7.77–7.76 (m, 2H, H-2' & H-6'), 7.66–7.64 (m, 1H, H-4'), 7.61–7.60 (m, 2H, H-3' & H-5'), 3.71 (brs, 2H, H-10"), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.52–2.47 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.37–2.31 (m, 2H, H-3"), 2.30–2.28 (m, 1H, H-4), 2.19–2.16 (m, 4H, H-3 & H-5), 1.97–1.94 (m, 2H, H-6"), 1.86-1.81 (m, 2H, H-5"), 1.83–1.69 (m, 1H, H-4"), 1.06 (s, 3H, H-9"), 0.74 (s, 3H, H-8"); EIMS (m/z): 497 (0.8%) $[M]^+$, 252 (49%), 224 (68%), 215 (42%), 175 (51%), 151 (100%), 84 (35%), 77 (27%).

***N′*-(butylsulfonyl)-1-(phenylsulfonyl)piperidine-4-carbohydrazide (4l):** IR (KBr, cm^{-1}): v_{max}: 3445 (N-H), 3011 (Ar-CH), 2920 (C-H), 1638 (C=O), 1525 (C=C), 1317 (S=O), 878 (N-S); ^{1}H- NMR (300 MHz, $CDCl_3$, δ/ppm): 8.10 (s, 1H, NH-CO), 7.77 (d, J = 6.8 Hz, 2H, H-2' & H-6'), 7.66–7.64 (m, 1H, H-4'),

7.59 (d, J = 6.8 Hz, 2H, H-3' & H-5'), 3.74 (brs, 2H, H-1"), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.52–2.47 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.38–2.35 (m, 1H, H-4), 2.34–1.96 (m, 4H, H-3 & H-5), 1.89–1.77 (m, 2H, H-3"), 1.76–1.69 (m, 2H, H-2"), 1.28 (brs, 3H, H-4"); EIMS (m/z): 403 (2.2%) $[M]^+$, 282 (66%), 252 (49%), 224 (80%), 121 (32%), 84 (21%), 77 (39%), 54 (29%).

***N'*-(naphthalene-1-ylsulfonyl)-1-(phenylsulfonyl)piperidine-4-carbohydrazide (4m):** IR (KBr, cm^{-1}): v_{max}: 3447 (N-H), 3016 (Ar-CH), 2923 (C-H), 1637 (C=O), 1529 (C=C), 1324 (S=O), 831 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$ δ/ppm): 8.71 (s, 1H, NH-CO), 8.53–8.02 (m, 7H, H-2" to H-8"), 7.98 (d, J = 6.8 Hz, 2H, H-2' & H-6'), 7.85–7.80 (m, 1H, H-4'), 7.75 (d, J = 6.8 Hz, 2H, H-3' & H-5'), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.52–2.47 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.38-2.35 (m, 1H, H-4), 2.34–1.96 (m, 4H, H-3 & H-5); EIMS (m/z): 473 (2.5%) $[M]^+$, 252 (52%), 224 (44%), 127 (89%), 119 (39%), 101 (100%), 84 (21%), 77 (15%).

***N'*-(naphthalene-2-ylsulfonyl)-1-(phenylsulfonyl)piperidine-4-carboohydrazide (4n):** IR (KBr, cm^{-1}): v_{max}: 3443 (N-H), 3018 (Ar-CH), 2921 (C-H), 1635 (C=O), 1525 (C=C), 1327 (S=O), 834 (N-S); ^{1}H-NMR (300 MHz, $CDCl_3$ δ/ppm): 8.70 (s, 1H, NH-CO), 8.68 (s, 1H, H-1"), 8.51–8.00 (m, 6H, H-3" to H-8"), 7.96 (d, J = 6.8 Hz, 2H, H-2' & H-6'), 7.82–7.78 (m, 1H, H-4'), 7.73 (d, J = 6.8 Hz, 2H, H-3' & H-5'), 3.61–3.58 (m, 2H, H_{eq}-2 & H_{eq}-6), 2.52–2.47 (m, 2H, H_{ax}-2 & H_{ax}-6), 2.38–2.35 (m, 1H, H-4), 2.34–1.96 (m, 4H, H-3 & H-5); EIMS (m/z): 473 (2%) $[M]^+$, 252 (51%), 224 (42%), 127 (88%), 119 (38%), 101 (100%), 84 (21%), 77 (17%).

2.5. Cholinesterase assays

The AChE and BChE inhibition activities were determined according to the spectrophotometric method of Ellman[21] with small modifications. A volume of 100 μL comprising 60 μL of Na_2HPO_4 buffer with concentration of 50 mM (pH 7.7), 10 μL of test compound (0.5 mM $well^{-1}$), and 10 μL (0.005 unit $well^{-1}$ for AChE and 0.5 unit $well^{-1}$ for BChE) of enzyme was developed. This homogeneous mixture was pre-read at 405 nm followed by pre-incubation for 10 min at 37 °C. The reaction started by the addition of 10 μL of 0.5 mM $well^{-1}$ substrate (acetylthiocholine iodide for AChE and butyrylthiocholine chloride for BChE) and the addition of 10 μL of DTNB (0.5 mM $well^{-1}$). After 15 min of incubation at 37 °C, absorbance was measured at 405 nm using a 96-well plate reader (Synergy HT, Biotek, USA). All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM $well^{-1}$) was used as a positive control. The percent inhibition was calculated by the following equation: Inhibition (%) = (Abs of Control – Abs of Test Comp)/Abs of Control $\times$ 100,

where control is the activity without inhibitor and test is the activity in the presence of test compound. IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc., Amherst, NH, USA). The IC_{50} values were the average of 3 independent experiments.

All the measurements were executed in triplicate and statistical analysis was performed using Microsoft Excel 2010. Results are presented as mean ± SEM.

2.6. Molecular docking

The AChE crystal structure (PDB accession code, *1B41*)[22] was retrieved from the Protein Databank (PDB). The missing residues in the crystal structure were constructed by using the program UCSF Chimera 1.6.16.[23] The pdb file (PDB accession code, *2WID*) was retrieved from the protein databank and its missing residues

were constructed by aligning it to the other pdb file (PDB accession code, *1P0P*). All water molecules were removed from the retrieved crystal structures using the program Visual Molecular Dynamics, VMD 1.9.[24] Both AChE and BChE were allowed to dock to experimentally synthesize 16 active compounds including parent compounds. The 3D structures of all compounds were constructed in pdb format and subsequently optimized at semiempirical RM1 level of theory using by the programs Gabedit[25] and MOPAC 2012,[26] respectively. The docking study of all compounds was accomplished by the software AutoDock Vina,[27] using the built-in Lamarckian genetic algorithm method. A total of 20 runs were performed for each docking and rests of parameters were set to default values.

The search space was restricted to a grid box size of 46 × 46 × 46 in the x, y, and z dimensions, respectively, centered on the binding site of protein with x, y, and z coordinates of 120.491, 106.059, and –136.443 Å, respectively. All the docking runs were performed on Intel Core i5-2410M CPU @ 2.30 GHz of Sony origin, with 6.0 GB DDR RAM. AutoDock Vina was compiled and run under the Windows 7 Professional 64-bit operating system.

3. Result and discussion

3.1. Chemistry

In the present work, *N′*-(aryl/alkylsulfonyl)(1-(phenylsulfonyl)piperidine-4-carbohydrazide derivatives were prepared in a 3-step synthesis. Then they were screened against AChE and BChE enzymes. The parent compound, ethyl-1-(phenylsulfonyl)piperidine-4-carboxylate **(1)** was prepared by the reaction of ethyl piperidine-4-carboxylate (**a**) and benzenesulfonyl chloride (**b**) at dynamic pH control in aqueous media.[28–32] The reaction was processed in basic media at pH 9 to neutralize the hydrochloric acid developed by sulfonyl chloride during the reaction. The produced acid suppresses the nucleophilic character of the amine by capturing the lone pair of nitrogen, thus rendering the corresponding salt and influencing also the rate of reaction. At the end, the reaction medium was acidified to remove the unreacted amine in the form of salt and also to convert the salt form of sulfonamide into acidic form. The excess of acid should be restrained because it lessens yield due to another salt formation of sulfonamide. Further, the compound **(1)** was converted to 1-(phenylsulfonyl)piperidine-4-carbohydrazide **(2)** by refluxing with hydrated hydrazine (80%) in methanol as solvent for 3–4 h. The product was collected by filtration after the evaporation of half of the solvent. The third and last step comprises the synthesis of all derivatives **4a–n** by coupling **2** with the different alkyl/aryl sulfonyl chlorides **(3a–n)** in the aqueous media under low basic pH. The final products were filtered off after acidifying the reaction mixture. The structures of the synthesized compounds **1**, **2,** and **4a–n** were elucidated by spectral data as described in the experimental section. The physical data of the synthesized compounds are provided in Table 1.

Compound **2** was synthesized as a white crystalline solid with melting point of 119 °C and 80% yield. The molecular formula $C_{12}H_{19}N_3O_3S$ was established by molecular ion peak at m/z 283 in EI-MS and by counting the number of protons in its ^{1}H-NMR spectrum. The infrared spectrum showed absorption bands at 3310 cm^{-1}, 3018 cm^{-1}, 2926 cm^{-1}, 1630 cm^{-1}, 1529 cm^{-1}, and 1325 cm^{-1}, which were assigned to N-H (stretching), C-H (aromatic stretching), C-H (aliphatic stretching), C=O (stretching), C=C (aromatic stretching), and S=O (stretching of sulfonyl group), respectively. The EI-MS gave characteristic peaks at m/z224 and 156, which were attributed to the loss of (phenylsulfonyl)piperidine and benzene sulfonamide groups, respectively. In the aromatic region of the ^{1}H-NMR spectrum, signals appearing at δ 7.78 (dd, J = 7.8, 1.8 Hz, 2H, H-2', H-6') and 7.66–7.63 (m, 3H, H-3' to H-5') were assigned to the benzenesulfonyl ring. In the aliphatic region of the

^{1}H-NMR spectrum, signals appearing at δ 3.79 (t, J = 3.6 Hz, 2H, H_{eq}-2', H_{eq}-6'), 3.75 (t, J = 3.6 Hz, 2H, H_{ax}-2', H_{ax}-6'), 2.12–1.99 (m, 1H, H-4'), and 1.79–1.75 (m, 4H, H-3' & H-5') for δ 3.79 (t, J = 3.6 Hz, 2H, H_{eq}-2, H_{eq}-6'), 3.75 (t, J = 3.6 Hz, 2H, H_{ax}-2' H_{ax}-6'), 2.12–1.99 (m, 1H, H-4), and 1.79–1.75 (m, 4H, H-3 & H-5) indicated the presence of a piperidine nucleus in the molecule. One signal emerging at δ 7.85 as a singlet was specified for the proton of nitrogen directly attached to the carbonyl group. On the basis of these data, the structure of **2** was assigned as 1-(phenylsulfonyl)piperidine-4-carbohydrazide. Similarly, the structures of other compounds were characterized by ^{1}H-NMR, IR, and mass spectral data as described in the experimental section.

Table 1. Physical data of synthesized compounds.

Compound	Appearance	Melting point (°C)	Molecular formula	%Yield
1	Creamy white powder	60–62	$C_{14}H_{19}NO_4S$	90
2	White crystals	119	$C_{12}H_{19}N_3O_3S$	80
4a	Creamy white powder	150–152	$C_{18}H_{21}N_3O_5S_2$	88
4b	White powder	176–178	$C_{19}H_{23}N_3O_5S_2$	75
4c	White fibrous solid	168–169	$C_{19}H_{23}N_3O_5S_2$	78
4d	White powder	140–142	$C_{19}H_{22}BrN_3O_5S_2$	77
4e	White powder	115–117	$C_{20}H_{24}N_4O_6S_2$	78
4f	Off white powder	110–112	$C_{20}H_{23}N_3O_6S_2$	73
4g	Creamy white powder	72–74	$C_{21}H_{27}N_3O_5S_2$	72
4h	White powder	165–166	$C_{18}H_{20}ClN_3O_5S_2$	78
4i	Creamy white powder	149–150	$C_{18}H_{20}BrN_3O_5S_2$	80
4j	White powder	210–212	$C_{18}H_{19}Cl_2N_3O_5S_2$	83
4k	Lemon yellow liquid	–	$C_{22}H_{31}N_3O_6S_2$	76
4l	Mustard sticky liquid	–	$C_{16}H_{25}N_3O_5S_2$	79
4m	Light yellow powder	59–60	$C_{22}H_{23}N_3O_5S_2$	82
4n	Off white powder	126–127	$C_{22}H_{23}N_3O_5S_2$	76

*Melting points of the synthesized compounds were recorded on a Griffin and George melting point apparatus by open capillary tube; Molecular formulas were confirmed by EI-MS calculations

3.2. Biological assay and docking analysis

In order to elucidate the probable mechanism by which the title compounds could induce anticholinesterase activity and to rationalize the ligand–protein interaction at molecular level for establishing structure activity relationships, molecular docking of the potent inhibitors from a series of compounds was accomplished into the receptor site of the crystal structures of AChE and BChE. The highly potent compounds from the series were selected on the basis of IC_{50}, which was determined experimentally. The enzyme inhibition data are provided for all the synthesized compounds in Table 2. Compounds **4g** and **4m** were found to be the most active against AChE and compounds **4i** and **4n** against BChE among the series of the compounds. Their candidacy of being highly potent was also supported by docking score as these compounds had good binding energy with the respective proteins. Compounds **4g** and **4m** had docking scores of –10.00 kcal/mol and –11.60 kcal/mol, respectively, against AChE as compared to the reference inhibitor, eserine, i.e. –7.10 kcal/mol. Compounds **4i** and **4n** had docking scores of –9.80 kcal/mol and –11.50 kcal/mol, respectively, against BChE as compared to the reference inhibitor, eserine, i.e. –7.50 kcal/mol. The binding energies of the highlighted compounds are given in Table 3.

Table 2. Enzymatic activity profile of the synthesized compounds.

Sample code	AChE			BChE		
	Conc./well (mM)	Inhibition (%)	IC_{50} μM	Conc./well (mM)	Inhibition (%)	IC_{50} μM
1	0.5	41.00 ± 0.15	-	0.5	22.48 ± 0.16	-
2	0.5	43.33 ± 0.15	-	0.5	26.68 ± 0.16	-
4a	0.5	48.96 ± 0.61	-	0.5	41.21 ± 0.54	-
4b	0.5	78.86 ± 0.94	104.21 ± 0.11	0.5	51.54 ± 0.11	> 400
4c	0.5	62.31 ± 0.69	212.31 ± 0.11	0.5	32.46 ± 0.15	-
4d	0.5	57.61 ± 0.44	256.81 ± 0.14	0.5	81.85 ± 0.22	68.71 ± 0.51
4e	0.5	60.46 ± 0.31	230.14 ± 0.91	0.5	47.54 ± 0.55	-
4f	0.5	73.71 ± 0.11	121.41 ± 0.04	0.5	28.41 ± 0.17	-
4g	0.5	91.57 ± 0.78	58.91 ± 0.14	0.5	86.92 ± 0.62	63.21 ± 0.91
4h	0.5	68.02 ± 0.14	141.51 ± 0.17	0.5	52.46 ± 0.77	> 400
4i	0.5	87.51 ± 0.22	72.41 ± 0.71	0.5	92.31 ± 0.21	48.91 ± 0.11
4j	0.5	38.12 ± 0.18	-	0.5	43.96 ± 0.69	-
4k	0.5	76.01 ± 0.14	116.31 ± 0.12	0.5	66.62 ± 0.89	202.31 ± 0.14
4l	0.5	11.72 ± 0.47	-	0.5	27.64 ± 0.18	-
4m	0.5	90.80 ± 0.55	64.11 ± 0.04	0.5	72.46 ± 0.47	103.21 ± 0.11
4n	0.5	87.73 ± 0.87	78.51 ± 0.91	0.5	93.85 ± 0.64	44.52 ± 0.12
Positive control	0.25	Eserine 91.29 ± 1.17	0.04 ± 0.0001	0.25	Eserine 82.82 ± 1.09	0.85 ± 0.0001

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc., Amherst, NH, USA).
AChE = Acetylcholinesterase. BChE = Butyrylcholinesterase.

Table 3. Results obtained from the molecular docking of most active compounds from series.

Enzyme	Compd.	B.E. (kcal/mol)	H-Bonding		π–H interaction	π–π interaction	Cation–π interaction	Electrostatic interactions	VDW
			Interacting residues	Distance (Å)					
AChE	4g	–10.00	Tyr124 Tyr337 Phe295 Gly122 His447	2.839 2.360 3.704 3.148 3.017	Tyr341	Trp286 Trp86	-	-	Tyr449
	4m	–11.60	Tyr124 Tyr337 *Tyr341 Gly122 *His447	3.209 3.305 5.760 3.148 4.551	Phe297	Trp286 Trp86	-	-	Tyr449 Phe295
BChE	4i	–9.80	Tyr332 Gly117 Ser287	2.893 3.724 4.077	Phe398	Trp231 Trp82 Phe329	-	His438	-
	4n	–11.50	Tyr332 Gly117 Leu286	3.037 3.692 4.220	-	Trp231 Trp82 Phe329	His438	-	-

* The binding energies >4.5 Å show weak H-bonding AChE = Acetyl cholinesterase BChE = Butyrylcholinesterase
B.E = Binding energy VDW = Van der Waal interaction

Compounds **4g** and **4m** were the most active against AChE with IC_{50} values of 58.91 ± 0.14 μM and 64.11 ± 0.04 μM, respectively, with respect to the reference standard eserine, having an IC_{50} value of 0.04 ± 0.0001 μM. The binding models of compounds **4g** and **4m** in the active site of AChE are depicted in Figure 1 (a and b, respectively). Compound **4g** was nicely bound to AChE through H-bonds involving amino and amido groups projecting toward the hydroxyl oxygen of Tyr124 (2.59 Å) and Tyr337 (3.84 Å). The oxygen of both sulfonyl groups formed H-bonds with the backbone nitrogen atom of Gly122 (3.08 Å) and Phe295 (3.70 Å). Protons of methyl group of mesitylene were also involved in H-bonding with carboxylic oxygen of His447 (3.02 Å). There are 2 $\pi - \pi$ interactions among Trp86, Trp286, and the inhibitor (Figure 1a). Compound **4m** was well bound to the AChE with its oxygen atoms of the sulfonyl group projecting toward the amino group of Gly122 (3.15 Å) and His447 (4.55 Å), the protons of amino and amido groups bound to oxygen atoms of hydroxyl groups of Tyr124 (3.21 Å) and Tyr337 (3.31 Å). Trp86 and Trp286 formed 2 $\pi - \pi$ interactions with the inhibitor (Figure 1b). The binding of ligands with common residues Tyr124, Tyr337, Gly122, and His447 is vital for their activities as AChE inhibitors. All these residues were found in the binding model of both **4g** and **4m** compounds, making them highly potent for AChE (Table 3).

Compounds **4i** and **4n** were the most potent inhibitors of BChE with IC_{50} values of 48.91 ± 0.11 μM and 44.52 ± 0.12 μM, respectively, with eserine, the reference standard having IC_{50} value of 0.85 ± 0.0001 μM. The binding models of compounds **4i** and **4n** in the active site of BChE are depicted in Figure 1(c and d, respectively). Compound **4i** is bound to the BChE in an adequate manner through its amino group projecting toward the hydroxyl oxygen of Tyr 332 (2.89 Å) and oxygen atoms of the sulfonyl group projected toward the amino group of Gly 117 (3.72 Å) and Ser 287 (4.08 Å). The bromine at the para position of the benzene ring is bound to the amino group of His438 through weak electrostatic interactions. Three $\pi - \pi$ interactions were formed among Trp82, Trp231, Phe329, and Phe398 and the inhibitor (Figure 1c). Compound **4n** is nicely bound to BChE through its amino group with hydroxyl oxygen of Tyr332 (3.04 Å) and the oxygen atoms of the sulfonyl group are bound to the amino group of Gly117 (3.69 Å) and Leu286 (4.22 Å). There are 3 $\pi - \pi$ interactions between Trp82, Trp231, Phe329, and the inhibitor (Figure 1d). The binding of ligands with common residues Tyr332, Gly117, Trp82, Trp231, and Phe329 is vital for their activities as BChE inhibitors. All these residues were found in the binding model of both **4i** and **4n** compounds, making them rather potent for AChE (Table 3).

4. Conclusion

The synthesis of this series of compounds bearing a heterocyclic moiety of piperidine and 2 different functionalities involving sulfonamide and amide linkages was proved to be highly active due to remarkable inhibitory action for AChE and BChE enzymes. In this study, insights into the interaction of cholinesterase with synthesized inhibitory compounds were elucidated through the use of molecular docking. AutoDock was used to model the interaction between cholinesterase (AChE and BChE) and its inhibitors. The results revealed that Tyr124, Tyr337, Gly122, and His447 and Tyr332, Gly117, Trp82, Trp231, and Phe329 residues were important for the interaction of inhibitors with both AChE and BChE. The knowledge accomplished with this study has important implications for the design of new AChE and BuChE inhibitors. To further enhance the anticholinesterase efficiency of piperidine bearing sulfonamides, further structural modifications of existing scaffolds or development of new structures is a promising venue to explore.

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