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RESEARCH ARTICLE

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# Synthesis and anticholinesterase activity of new substituted benzo[*d*]oxazole-based derivatives

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A series of novel benzo[*d*]oxazole derivatives (**6a–n**) have been synthesized and biologically evaluated as potential inhibitors of acetylcholinesterases (AChE) and butyrylcholinesterase (BChE). The chemical structures of all final compounds were confirmed by spectroscopic methods. In vitro studies showed that most of the synthesized compounds are potent acetylcholinesterase and butyrylcholinesterase inhibitors. Among them, compounds **6a** and **6j** strongly inhibited AChE and BChE activities with $IC_{50}$ values of 1.03–1.35 and 6.6–8.1 μM, respectively. Docking studies also provided the binding modes of action and identified hydrophobic pi forces as the main interaction.

**KEYWORDS**
acetylcholinesterase, Alzheimer's disease, benzo[*d*]oxazol, docking study

## 1 | INTRODUCTION

Alzheimer's disease (AD) is one of the critical health problems in the elderly population of the world. AD is an age-related and complex neurodegenerative disorder of the brain characterized by dementia, cognitive impairment, and memory loss.[1,2] Based on World Alzheimer Report in 2010,[3] the estimated number of people who are afflicted by this disease is more than 30 million.

The pathogenesis of Alzheimer's disease is not completely clear; the typical pathological marks are amyloid-β (Aβ) deposits, neurofibrillary tangles (NFT), oxidative stress, and decreased levels of acetylcholine (ACh) in the brain.[4] Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with 65% homology in their structure are responsible enzymes for the metabolic hydrolysis of ACh at the cholinergic synapses. In the same condition, meaning temperature, and pH, ACh is more effectively hydrolyzed by AChE compared to BChE.[5] The increased levels of ACh are observed as a result of an effective inhibitory activity against these enzymes which led to attenuated levels of behavioral and cognitive symptoms in AD patients.[6] Most of the FDA-approved drugs are based on the inhibition of AChE. Cholinesterase inhibitor drugs (ChEI) involving donepezil, rivastigmine, and

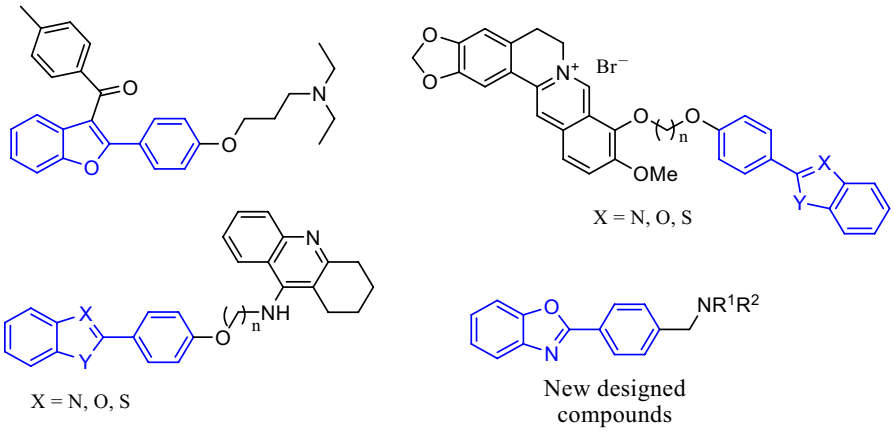



**FIGURE 1** Phenyl-benzoheterocyclic moieties as anticholinesterase agents [Colour figure can be viewed at wileyonlinelibrary.com]

galantamine have been proposed as the most important therapeutic option for the treatment of Alzheimer's disease.[7] The main focuses of these agents are their simultaneous action as disease-modifying agents and symptomatic therapy.[8] However, toxicity[9] and severe side-effects in clinical studies diminished their effectiveness.[10] So, BChE should be alternatively pursued as a remarkable target due to its hydrolyzing role in advanced stages of the disease and fewer side-effects of selective BChEIs.[11] The design of new ligands targeting different molecular abnormalities has directed many research projects to discover effective and novel ChEIs agents[12–14] with improved pharmacokinetics properties.

Oxazoles are important scaffold in heterocyclic compounds, which are extensively found in diverse pharmacologically active substances and in naturally occurring compounds. Furthermore, benzo[*d*]oxazoles play an important role as a key building block in β-adrenergic receptor antagonists,[15] anti-tumor,[16] antihyperglycemic,[17] antibacterial,[18] anti-HIV,[19] and anti-inflammatory agents.[20] In this case, oxazole derivatives have raised considerable attention of medicinal chemists; hence, a large number of investigations on their synthesis and biological activities have been reported during recent years.[21–25]

The presence of heterocyclic rings in AD drugs promoted medicinal chemists to examine different skeletons in novel hybrid molecules. In addition to being in newly discovered anticholinesterase agents, benzofuran presented a venerable pharmacophore in molecules with inhibitory activity against the formation of Aβ fibril. Furthermore, hydrophobic and π–π interactions from aromatic residue on the entrance and inside of AChE gorge add much interests to find novel benzofuran–phenyl analogues.[26–29] In this regard, we rationally combined benzoxazole and phenyl ring system, bearing cyclic and acyclic nitrogen-containing moieties which are logically essential for effective enzyme–compound interactions (Figure 1). Following our interests in designing novel cholinesterase inhibitors,[30] we synthesized a novel series of *N*-((4-(benzo[*d*]oxazol-2-yl)phenyl)methyl)-amine derivatives as potential acetylcholinesterase and butyrylcholinesterase inhibitors. Further, molecular docking studies interpreted the inhibitory activity of the enzyme.

## 2 | METHODS AND MATERIALS

### 2.1 | General chemistry

Melting points are uncorrected and were determined with a Kofler hot-stage apparatus (Reichert, Vienna, Austria). $^{1}$H and $^{13}$C NMR spectra were recorded using Bruker 500 spectrometers. Chemical shifts (δ) are reported in ppm relative to TMS as internal standard. Coupling constant (*J*) values are presented in Hz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet). The IR spectra were obtained on a Nicolet Magna FT-IR 550 spectrophotometer (potassium bromide disks). Mass spectra were determined on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis for C, H, and N was carried out with an Elementar Analysensysteme GmbH. All reagents and solvents used in this study were commercially available (Merck) and were used without further purification.

### 2.2 | General procedure for the synthesis of 2-*p*-tolylbenzo[*d*]oxazole (3)

A mixture of 2-aminophenol (**1**, 5 mmol) and 4-methylbenzoyl chloride (**2**, 5 mmol) in a mixed solvent system (toluene 20 ml and triethyl amine 0.5 ml) was irradiated at 110°C for two cycles of 30 min. The precipitate was removed by filtration. The filtrate was concentrated, and the residue was recrystallized from ethanol to give compound **3** (90%). $^{1}$H NMR (500 MHz, DMSO-$d_6$): δ = 2.41 (s, 3H, $CH_3$), 7.40–7.41 (m, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.75–7.79 (m, 2H), 8.09 (d, *J* = 8.0 Hz, 2H).

### 2.3 | General procedure for the synthesis of 2-(4-(bromomethyl)phenyl)benzo[*d*]oxazole (4)

A solution of compound **3** (10 mmol) and NBS (10 mmol) in toluene (30 ml) was refluxed under nitrogen for 24 h. The

solvent was evaporated and solid was recrystallized from ethanol to give compound **4** as a cream solid. (85%). $^1$H NMR (500 MHz, DMSO-$d_6$): δ = 4.80 (s, 2H), 7.40–7.46 (m, 2H), 7.69 (d, $J$ = 8.0 Hz, 2H), 7.78–7.86 (m, 2H), 8.19 (d, $J$ = 8.0 Hz, 2H).

## 2.4 | General procedure for the synthesis of *N*-((4-(benzo[*d*]oxazol-2-yl)phenyl)methyl)-amine (6a–n)

Treatment of 2-(4-(bromomethyl)phenyl)benzo[*d*]oxazole **4** (1 mmol) with potassium carbonate (1.5 mmol) and amines **5a–n** (1 mmol) in DMF (10 ml) afforded corresponding compounds **6a–n** in good yields.

### 2.4.1 | 2-(4-((Piperidin-1-yl)methyl)phenyl) benzo[*d*]oxazole (6a)

Pale yellow solid; yield: 0.17 g (60%); mp. 100–102°C. IR ($\nu_{max}$, cm$^{-1}$): 3,041, 2,930, 1,450, 851, 742. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 1.40 (s, 2H), 1.51 (s, 4H), 2.36 (s, 4H), 3.52 (s, 2H, $CH_2$), 7.38 (t, $J$ = 7.0 Hz, 2H), 7.52 (d, $J$ = 7.5 Hz, 2H), 7.77 (t, $J$ = 8.0 Hz, 2H), 8.15 (d, $J$ = 7.5 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 23.8, 25.5, 53.9, 62.3, 110.7, 119.6, 124.7, 125.3, 127.1, 129.5, 140.6, 143.2, 144.2, 156.7, 161.9. Anal. Calcd. For $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58%. Found: C, 77.98; H, 6.78; N, 9.65%.

### 2.4.2 | 2-(4-((4-Benzylpiperidin-1-yl)methyl) phenyl)benzo[*d*]oxazole (6b)

Pale yellow solid; yield: 0.25 g (65%); mp. 120–121°C. IR ($\nu_{max}$, cm$^{-1}$): 3,025, 2,912, 1,450, 835, 745. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 1.22–1.26 (m, 1H), 1.50–1.55 (m, 4H), 1.92 (t, $J$ = 11.0 Hz, 4H), 2.79 (d, $J$ = 11.0 Hz, 2H), 3.52 (s, 2H, $CH_2$), 7.15 (t, $J$ = 7.5 Hz, 3H), 7.25 (t, $J$ = 7.1 Hz, 2H), 7.38–7.43 (m, 2H), 7.51 (d, $J$ = 8 Hz, 2H), 7.75–7.79 (m, 2H), 8.13 (d, $J$ = 8.1 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 31.6, 37.1, 42.2, 53.2, 61.8, 110.7, 119.6, 124.8, 125.2, 125.5, 127.0, 127.8, 127.9, 128.8, 129.2, 140.2, 141.4, 143.2, 150.3, 162.1. Anal. Calcd. For $C_{26}H_{26}N_2O$: C, 81.64; H, 6.85; N, 7.32%. Found: C, 81.55; H, 6.76; N, 7.44%.

### 2.4.3 | 2-(4-(Morpholinomethyl)phenyl) benzo[*d*]oxazole (6c)

Pale yellow solid; yield: 0.19 g (65%); mp. 106–108°C. IR ($\nu_{max}$, cm$^{-1}$): 3,033, 2,953, 1,451, 822, 750. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.40 (s, 4H), 3.56 (s, 2H, $CH_2$), 3.60 (s, 4H), 7.39–7.44 (m, 2H), 7.54 (d, $J$ = 7.5 Hz, 2H), 7.76–7.81 (m, 2H), 8.15 (d, $J$ = 7.5 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 53.1, 61.9, 66.1, 110.7, 119.6, 124.7, 126.1, 127.1, 129.5, 130.1, 141.4, 142.2, 150.1, 162.2. Anal. Calcd. For $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52%. Found: C, 73.52; H, 6.25; N, 9.61%.

### 2.4.4 | 2-(4-(Thiomorpholinomethyl)phenyl) benzo[*d*]oxazole (6d)

Pale yellow solid; yield: 0.18 g (60%); mp. 110–112°C. IR ($\nu_{max}$, cm$^{-1}$): 3,029, 2,907, 1,450, 803, 745. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.64 (s, 4H), 2.66 (s, 4H), 3.60 (s, 2H, $CH_2$), 7.39–7.44 (m, 2H), 7.53 (d, $J$ = 8.0 Hz, 2H), 7.76–7.80 (m, 2H), 8.15 (d, $J$ = 8.0 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 27.1, 54.4, 62.0, 110.7, 119.6, 124.7, 125.0, 127.1, 129.4, 130.1, 141.4, 142.5, 150.1, 162.2. Anal. Calcd. For $C_{18}H_{18}N_2OS$: C, 69.65; H, 5.84; N, 9.02%. Found: C, 69.70; H, 5.77; N, 9.12%.

### 2.4.5 | 2-(4-((Piperazin-1-yl)methyl)phenyl) benzo[*d*]oxazole (6e)

Pale yellow solid; yield: 0.20 g (70%); mp. 273–275°C. IR ($\nu_{max}$, cm$^{-1}$): 3,401, 2,930, 1,450, 812, 742. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.47 (s, 4H), 3.15 (s, 4H), 3.67 (s, 2H, $CH_2$), 7.33 (t, $J$ = 7.2 Hz, 2H), 7.40 (d, $J$ = 8.2 Hz, 2H), 7.55–7.81 (m, 2H), 8.41 (d, $J$ = 8.2 Hz, 2H), 10.13 (s, 1H, NH). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 23.1, 53.4, 59.1, 110.7, 119.6, 124.7, 125.2, 127.1, 127.8, 129.0, 130.1, 143.8, 150.1, 161.5. Anal. Calcd. For $C_{18}H_{19}N_3O$: C, 73.69; H, 6.53; N, 14.32%. Found: C, 73.78; H, 6.46; N, 14.42%.

### 2.4.6 | 2-(4-((4-Methylpiperazin-1-yl)methyl) phenyl)benzo[*d*]oxazole (6f)

Pale yellow solid; yield: 0.21 g (67%); mp. 140–142°C. IR ($\nu_{max}$, cm$^{-1}$): 3,025, 2,912, 1,450, 821, 745. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.16 (s, 3H, $CH_3$), 2.34 (s, 4H), 2.41 (s, 4H), 3.55 (s, 2H, $CH_2$), 7.40 (t, $J$ = 7.5 Hz, 2H), 7.41 (d, $J$ = 7.3 Hz, 2H), 7.76–7.79 (m, 2H), 8.13 (d, $J$ = 7.3 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 45.6, 52.5, 54.6, 61.5, 110.7, 120.1, 124.7, 125.3, 126.1, 127.8, 129.4, 130.1, 142.8, 150.1, 162.2. Anal. Calcd. For $C_{19}H_{21}N_3O$: C, 74.24; H, 6.89; N, 13.67%. Found: C, 74.38; H, 7.00; N, 13.55%.

### 2.4.7 | 2-(4-((4-Ethylpiperazin-1-yl)methyl) phenyl)benzo[*d*]oxazole (6g)

Pale yellow solid; yield: 0.23 g (71%); mp. 168–170°C. IR ($\nu_{max}$, cm$^{-1}$): 3,061, 2,904, 1,452, 810, 739. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 0.97 (t, $J$ = 11.0 Hz, 3H), 2.30 (q, $J$ = 12.1 Hz, 2H), 2.36–2.40 (m, 8H), 3.49 (s, 2H, $CH_2$), 7.25 (t, $J$ = 7.5 Hz, 1H), 7.28 (t, $J$ = 8.1 Hz, 1H), 7.32 (d, $J$ = 8.0 Hz, 2H), 7.63 (d, $J$ = 7.7 Hz, 1H), 7.67 (d, $J$ = 7.7 Hz, 1H), 7.86 (d, $J$ = 8.0 Hz, 2H). $^{13}$C NMR (125 MHz,

DMSO-$d_6$): δ = 12.4, 51.5, 52.3, 52.6, 61.6, 111.0, 119.6, 120.1, 124.7, 125.0, 126.1, 127.8, 129.4, 142.8, 152.1, 160.9. Anal. Calcd. For $C_{20}H_{23}N_3O$: C, 74.74; H, 7.21; N, 13.07%. Found: C, 74.65; H, 7.19; N, 12.92%.

### 2.4.8 | 2-(4-((4-Phenylpiperazine-1-yl)methyl) phenyl)benzo[*d*]oxazole (6h)

Pale yellow solid; yield: 0.27 g (72%); mp. 100–103°C. IR ($\nu_{max}$, $cm^{-1}$): 3,064, 2,930, 1,450, 855, 742. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.56 (s, 4H), 3.15 (s, 4H), 3.63 (s, 2H, $CH_2$), 6.76 (t, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 2H), 7.19 (t, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 6 Hz, 2H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.71–7.81 (m, 2H), 8.18 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 48.1, 52.5, 61.5, 110.7, 115.3, 118.6, 119.6, 124.7, 125.1, 125.3, 127.1, 128.7, 129.5, 141.5, 142.5, 150.1, 150.9, 162.2. Anal. Calcd. For $C_{24}H_{23}N_3O$: C, 78.02; H, 6.27; N, 11.37%. Found: C, 78.08; H, 6.18; N, 11.45%.

### 2.4.9 | 2-(4-((4-Benzylpiperazin-1-yl)methyl) phenyl)benzo[*d*]oxazole (6i)

Pale yellow solid; yield: 0.27 g (70%); mp. 160–163°C. IR ($\nu_{max}$, $cm^{-1}$): 3,438, 2,931, 1,448, 801. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.41 (s, 8H), 3.46 (s, 2H, $CH_2$), 3.56 (s, 2H, $CH_2$), 6.76 (t, *J* = 7.5 Hz, 1H), 6.91 (t, *J* = 8.1 Hz, 2H), 7.19 (t, *J* = 7.5 Hz, 2H), 7.36–7.44 (m, 2H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.77–7.79 (m, 2H), 8.17 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 52.5, 61.5, 110.7, 115.3, 118.6, 119.6, 124.7, 125.1, 125.3, 127.1, 128.7, 129.5, 141.5, 142.5, 150.1, 150.9, 162.2. Anal. Calcd. For $C_{25}H_{25}N_3O$: C, 78.30; H, 6.57; N, 10.96%. Found: C, 78.19; H, 6.42; N, 11.10%.

### 2.4.10 | 2-(4-((Pyrrolidin-1-yl)methyl) phenyl)benzo[*d*]oxazole (6j)

Pale yellow solid; yield: 0.18 g (65%); mp. 110–112°C. IR ($\nu_{max}$, $cm^{-1}$): 3,031, 2,950, 1,450, 829, 749. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 1.70–1.72 (m, 4H), 2.43–2.45 (m, 4H), 3.62 (s, 2H, $CH_2$), 7.25 (t, *J* = 7.5 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.65 (d, *J* = 7.2 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 23.1, 53.4, 59.1, 110.7, 119.6, 124.8, 125.2, 127.1, 127.8, 129.0, 130.1, 143.8, 150.1, 162.3. Anal. Calcd. For $C_{18}H_{18}N_2O$: C, 77.67; H, 6.52; N, 10.06%. Found: C, 77.79; H, 6.45; N, 10.16%.

### 2.4.11 | (4-(Benzo[*d*]oxazol-2-yl)phenyl)-*N*-benzylmethylamine (6k)

Pale yellow solid; yield: 0.24 g (76%); mp. 120–123°C. IR ($\nu_{max}$, $cm^{-1}$): 3,405, 3,030, 2,968, 1,450, 833, 743. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 3.72 (s, 2H, $CH_2$), 3.79 (s, 2H, $CH_2$), 7.22 (t, *J* = 7.2 Hz, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.1 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.76–7.80 (m, 2H), 8.15 (d, *J* = 7.5 Hz, 2H), 8.60 (s, 1H, NH). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 51.8, 52.2, 110.7, 119.6, 124.7, 125.2, 126.4, 127.0, 127.5, 127.8, 128.0, 128.5, 140.6, 141.5, 145.3, 150.1, 162.3. Anal. Calcd. For $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91%. Found: C, 80.12; H, 5.63; N, 8.98%.

### 2.4.12 | (4-(Benzo[*d*]oxazol-2-yl)phenyl)-*N*-benzyl-*N*-methylmethanamine (6l)

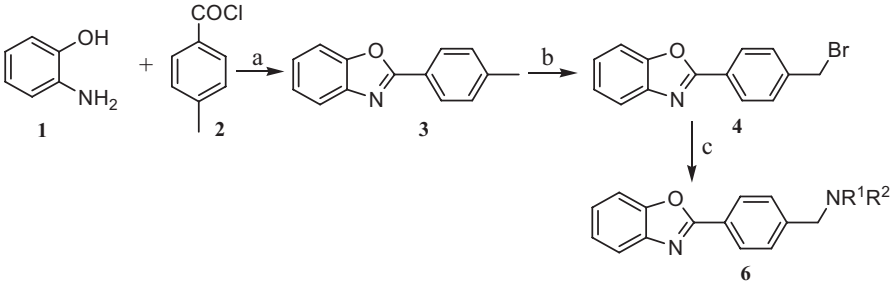
Pale yellow solid; yield: 0.22 g (68%); mp. 106–107°C. IR ($\nu_{max}$, $cm^{-1}$): 3,022, 2,983, 1,465, 819, 744. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 2.12 (s, 3H, $CH_3$), 3.54 (s, 2H, $CH_2$), 3.60 (s, 2H, $CH_2$), 7.26 (t, *J* = 6.5 Hz, 2H), 7.33–7.36 (m, 4H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.77 (d, *J* = 7.4 Hz, 1H), 7.79 (d, *J* = 7.4 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 41.6, 60.5, 61.1, 110.7, 119.6, 124.7, 125.3, 126.8, 127.1, 128.1, 128.5, 129.2, 138.8, 141.5, 143.5, 150.1, 154.2, 162.2. Anal. Calcd. For $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53%. Found: C, 80.56; H, 6.25; N, 8.52%.

### 2.4.13 | *N*-((4-(benzo[*d*]oxazol-2-yl)phenyl) methyl)-*N*-benzylethanamine (6m)

Pale yellow solid; yield: 0.26 g (76%); mp. 120–122°C. IR ($\nu_{max}$, $cm^{-1}$): 3,028, 2,976, 1,449, 879, 744. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 1.04 (t, *J* = 7.0 Hz, 3H, $CH_3$), 2.48 (q, *J* = 6.3 Hz, 2H, $CH_2$), 3.58 (s, 2H, $CH_2$), 3.64 (s, 2H, $CH_2$), 7.23 (t, *J* = 7.1 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.36 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.1 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.78 (d, *J* = 7.3 Hz, 1H), 8.15 (d, *J* = 7.5 Hz, 2H). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 11.5, 46.5, 56.7, 57.1, 110.7, 119.6, 124.7, 124.9, 125.2, 126.7, 127.1, 128.1, 128.4, 129.1, 139.3, 141.5, 144.2, 150.1, 162.2. Anal. Calcd. For $C_{23}H_{22}N_2O$: C, 80.67; H, 6.48; N, 8.18%. Found: C, 80.78; H, 6.46; N, 8.07%.

### 2.4.14 | *N*-((4-(benzo[*d*]oxazol-2-yl)phenyl) methyl)-1-benzylpiperidin-4-amine (6n)

Pale yellow solid; yield: 0.29 g (74%); mp. 114–115°C. IR ($\nu_{max}$, $cm^{-1}$): 3,421, 3,028, 2,937, 1,451, 834, 744. $^1$H NMR (500 MHz, DMSO-$d_6$) δ = 1.31–1.33 (m, 4H), 1.94 (t, *J* = 10.2 Hz, 4H), 2.74 (t, *J* = 10.2 Hz, 1H), 3.42 (s, 2H, $CH_2$), 3.84 (s, 2H, $CH_2$), 7.22 (t, *J* = 7.3 Hz, 1H), 7.26–7.28 (m, 3H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.38–7.43 (m, 2H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.75–7.78 (m, 2H), 8.13 (d, *J* = 8.5 Hz, 2H), 8.50 (s, 1H, NH). $^{13}$C NMR (125 MHz, DMSO-$d_6$): δ = 32.0, 49.4, 51.3, 53.7, 62.1, 110.7, 119.6,




**SCHEME 1** Synthesis of compounds **6a–n**. Reagents and conditions: (a) Toluene, $NEt_3$, MW, 110°C, 1 h; (b) NBS, $CCl_4$, reflux, 24 h; (c) $NHR^1R^2$ (**5a–n**), $K_2CO_3$, DMF, r.t. [Colour figure can be viewed at wileyonlinelibrary.com]

124.7, 125.2, 126.6 (2C), 127.0, 127.4, 127.9, 128.5, 128.6 (2C), 138.6, 145.9, 162.2. Anal. Calcd. $C_{26}H_{27}N_3O$: C, 78.56; H, 6.85; N, 10.57%. Found: C, 78.58; H, 6.89; N, 10.48%.

## 2.5 | Cholinesterases inhibition assay

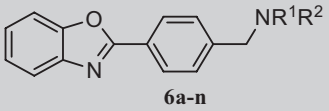
Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type VeS, lyophilized powder, from electric eel, 1,000 unit), butyrylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), and butyrylthiocholine iodide (BTCh) were provided from Sigma-Aldrich. 5,50-Dithiobis-(2-nitrobenzoic acid) (DTNB), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide (ATCh) were purchased from Fluka. The stock solutions of the target compounds were prepared in a mixture of DMSO (1 ml) and ethanol (9 ml) and diluted with 0.1 $MKH_2PO_4/K_2HPO_4$ buffer (pH ¼ 8.0) to obtain final concentrations. 20 ml of substrate (acetylthiocholine iodide 0.075 M) was added to the test solution to obtain final concentration of 466 mM. All experiments were performed based on the previously described method.[31] Spectrophotometric measurements were performed on a UV Unico double-beam spectrophotometer. The same method was also taken for BuChE inhibition assay.

# 3 | RESULTS AND DISCUSSION

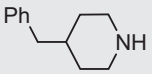
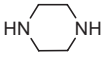
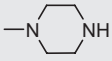
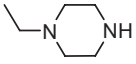
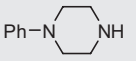
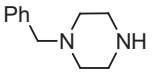
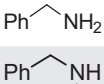
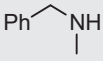
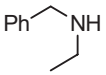
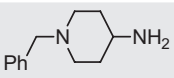
## 3.1 | Chemistry

The synthesis of target compounds **6a–n** was accomplished using the pathway, illustrated in Scheme 1. First, the condensation reaction between 2-aminophenol **1** and 4-methyl benzoyl chloride **2** in toluene under microwave irradiation at 110°C under microwave irradiation resulted in 2-*p*-tolylbenzo[*d*]oxazole **3**, which was brominated in $CCl_4$ using NBS to afford the corresponding 2-(4-(bromomethyl) phenyl)benzo[*d*]oxazole **4** in high yield. Further reaction with proper amines **5a–n** (Table 1) in the presence of $K_2CO_3$ produced *N*-((4-(benzo[*d*]oxazol-2-yl)phenyl)methyl)-amine derivatives (**6a–n**). The structures of all the newly synthesized compounds were characterized by IR, $^1H$ NMR, and $^{13}C$ NMR spectroscopy. The spectra of target compounds are provided in Appendix S1 file.

**TABLE 1** In vitro inhibitory activity of compounds **6a–n** against AChE and BuChE




**6a-n**

| Compound | $NHR^1R^2$ | AChE $IC_{50}$ (μM)[a] | BuChE $IC_{50}$ (μM) |
|---|---|---|---|
| **6a** |  | 1.03 ± 0.2 | 6.6 ± 0.9 |
| **6b** |  | >100 | >100 |
| **6c** |  | 20.10 ± 2.7 | >100 |
| **6d** |  | 2.55 ± 0.5 | 16.9 ± 2.6 |
| **6e** |  | 3.45 ± 0.7 | 21.5 ± 2.1 |
| **6f** |  | 62.23 ± 3.5 | >100 |
| **6g** |  | 38.17 ± 3.1 | >100 |
| **6h** |  | >100 | >100 |
| **6i** |  | >100 | >100 |
| **6j** |  | 1.35 ± 0.2 | 8.1 ± 0.6 |
| **6k** |  | 25.44 ± 1.8 | >100 |
| **6l** |  | 30.76 ± 2.1 | >100 |
| **6m** |  | 56.36 ± 3.4 | >100 |
| **6n** |  | 7.76 ± 0.7 | 50.1 ± 4.2 |
| Donepezil | — | 0.018 ± 0.002 | 12.0 ± 1.4 |

[a]Data are expressed as mean ± SD (three independent experiments).

## 3.2 | Pharmacology

### 3.2.1 | Cholinesterase inhibitory activity

The inhibitory potency of compounds **6a–n** toward AChE and BChE were determined in terms of $IC_{50}$ values, using modified Ellman's colorimetric method,[32] demonstrated in Table 1. All the synthesized compounds were active against AChE except **6b**, **6h**, and **6i**. Less number of compounds showed remarkable anti-BChE activities, but more promising results were obtained in comparison with donepezil. Five- and six-membered heterocycles containing nitrogen were more favorable than cycles with more than one heteroatom. In this regard, piperidine and pyrolidine substituted benzoxazoles, meaning **6a** and **6j** exhibited excellent activities against the AChE and BChE with $IC_{50}$ values of 1.03, 1.35, and 6.6, 8.1 μM, respectively. Utilizing acyclic amines led to totally inactive compounds against BChE.

### 3.2.2 | Docking study

The ligand–protein docking was performed by AUTODOCK VINA (1.1.2)[33] to predict the binding mode of the ligands in the active site of AChE. The 3D co-ordinate of the AChE (PDB ID: 1eve) in complex with donepezil was retrieved from Protein Data Bank (PDB) at http://www.rcsb.org/pdb/home/home.do. For protein preparation, all of the non-protein atoms were removed and then minimized using OPLS3 force field (RMSD = 0.3 Å). The grid box with the size of 25 × 25 × 25 was determined, and the center of the box was fixed on co-crystal ligand. After docking, the best pose was selected for further analysis.

The binding model of titled compound against AChE was represented in Figure 2. All compounds were docked in the active site and then overlaid. As the orientation of the compounds were similar, therefore, compound **6a**, the most active compound, was selected for further analysis. The hydrophobic pi forces are the main feature of interactions. The ligand was anchored in the active site through a π–cation interaction between positively charged nitrogen of piperidine ring and Trp83 and Phe329. Moreover, π-alkyl forces may occur in contact with Trp83. The phenyl ring in the middle of the molecule made a T-shaped π–π interaction with Tyr333. According to the docking data, the benzoxazole moiety was oriented to the rim of the active site and made a hydrogen bond with Phe287.

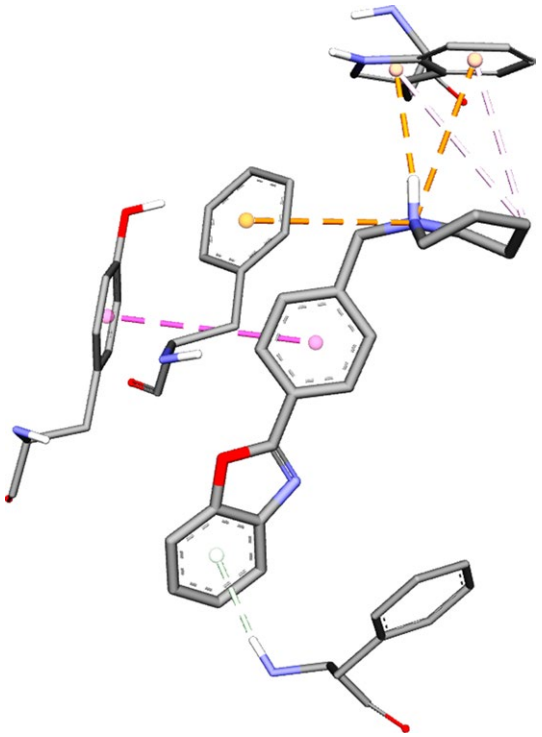


**FIGURE 2** The interaction of compound **6a** with AChE active site [Colour figure can be viewed at wileyonlinelibrary.com]

## 4 | CONCLUSION

In this study, a range of new benzoxazole derivatives have been synthesized and evaluated for their anticholinesterase activities. Our preliminary data revealed that most of the synthesized compounds had inhibitory activity against AChE and BChE. Particularly, piperidine and pyrolidine derivatives (**6a**, **6j**) were the most potent compound against AChE and BChE. Keeping in mind that dual AChE/BChE inhibitory activity is necessary to improve the signs of AD, our promising results would be considered as a new lead in this research field.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

[1] (a) L. Huang, H. Miao, Y. Sun, F. Meng, X. Li, *Eur. J. Med. Chem.* **2014**, *87*, 429. (b) H. F. Kung, C.-W. Lee, Z.-P. Zhuang, M.-P. Kung, C. Hou, K. Pleossl, *J. Am. Chem. Soc.* **2001**, *123*, 12740.

[2] (a) W. Thies, L. Bleiler, *Alzheimer's Dement* **2013**, *9*, 208. (b) H. Feorstl, A. Kurz, *Eur. Arch. Psychiatry Clin. Neurosci.* **1999**, *249*, 288.

[3] M. Prinz, S. Parlar, G. Bayraktar, V. Alptuzun, E. Erciyas, A. Fallarero, D. Karlsson, P. Vuorela, M. Burek, C. Forster, E. Turunc, G. Armagan, A. Yalcin, C. Schiller, K. Leuner, M. Krug, C. A. Sotriffer, U. Holzgrabe, *Eur. J. Pharm. Sci.* **2013**, *49*, 603.

[4] E. Giacobini, *Neurochem. Res.* **2003**, *28*, 515.
[5] P. J. Houghton, Y. Ren, M.-J. Howes, *Nat. Prod. Rep.* **2006**, *23*, 181.
[6] J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, *Science* **1991**, *253*, 872.
[7] L. L. Shen, G. X. Liu, Y. Tang, *Acta Pharmacol. Sin.* **2007**, *28*, 2053.
[8] C. Mount, C. Downton, *Nat. Med.* **2006**, *12*, 780.
[9] J. Marco-Contelles, M. D. C. Carreiras, C. Rodriguez, M. Villarroya, A. G. Garcia, *Chem. Rev.* **2006**, *106*, 116.
[10] R. Leon, A. G. Garcia, J. Marco-Contelles, *Med. Res. Rev.* **2013**, *33*, 139.
[11] (a) E. Giacobini, *Drugs Aging* **2001**, *18*, 891. (b) N. H. Greig, T. Utsuki, Q.-S. Yu, X. Zhu, H. W. Holloway, T. A. Perry, B. Lee, D. K. Ingram, D. K. Lahiri, *Curr. Med. Res. Opin.* **2001**, *17*, 159. (c) E. Giacobini, Butyrylcholinesterase-Its Function and Inhibitors, Martin Dunitz, London **2003**.
[12] A. Lleo, S. M. Greenberg, J. H. Growdon, *Annu. Rev. Med.* **2006**, *57*, 513.
[13] H. Y. Zhang, *FEBS Lett.* **2005**, *579*, 5260.
[14] K. S. Diasa, C. Viegas Jr, *Curr. Neuropharmacol.* **2014**, *12*, 239.
[15] P. Conti, C. Dallanoce, M. D. A. Amici, C. D. Micheli, K. N. Klotz, *Bioorg. Med. Chem.* **1998**, *6*, 401.
[16] S. Aiello, G. Wells, E. L. Stone, H. Kadri, R. Bazzi, D. R. Bell, M. F. Stevens, C. S. Matthews, T. D. Bradshaw, A. D. Westwell, *J. Med. Chem.* **2008**, *51*, 5135.
[17] R. Jeon, S. Y. Park, *Arch. Pharm. Res.* **2004**, *27*, 1099.
[18] Y. K. Kang, K. J. Shin, K. H. Yoo, K. J. Seo, C. Y. Hong, C. S. Lee, S. Y. Park, D. J. Kim, S. W. Park, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 95.
[19] S. Srivastava, L. K. Bajpai, S. Batra, A. P. Bhaduri, J. P. Maikhuri, G. Gupta, J. D. Dhar, *Bioorg. Med. Chem.* **1999**, *7*, 2607.
[20] H. Gulcan, S. Unlu, E. Banoglu, Sahin. M. Fethi, *Turk. J. Chem.* **2003**, *27*, 467.
[21] H. Razavi, E. T. Powers, H. E. Purkey, S. L. Adamski-Werner, K. P. Chiang, M. T. Dendle, J. W. Kelly, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1075.
[22] P. C. Lv, H. Q. Li, J. Y. Xue, L. Shi, H. L. Zhu, *Eur. J. Med. Chem.* **2009**, *44*, 908.
[23] P. Cao, X. F. Huang, H. Ding, H. M. Ge, H. Q. Li, H. L. Zhu, *Chem. Biodivers.* **2007**, *4*, 881.
[24] J. M. Contreras, I. Parrot, W. Sippl, Y. M. Rival, C. G. Wermuth, *J. Med. Chem.* **2001**, *44*, 2707.
[25] D. Shao, C. Zou, C. Luo, X. Tang, Y. Li, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4639.
[26] F. Baharloo, M. H. Moslemin, H. Nadri, A. Asadipour, M. Mahdavi, S. Emami, L. Firoozpour, R. Mohebat, A. Shafiee, A. Foroumadi, *Eur. J. Med. Chem.* **2015**, *93*, 196.
[27] L. Huang, T. Su, W. Shan, Z. Luo, Y. Sun, F. He, X. Li, *Bioorg. Med. Chem.* **2012**, *20*, 3038.
[28] D. R. Howlett, A. E. Perry, F. Godfrey, J. E. Swatton, K. H. Jennings, C. Spitzfaden, H. Wadsworth, S. J. Wood, R. E. Markwell, *Biochem. J.* **1999**, *340*, 283.
[29] A. S. Alpan, S. Parlar, L. Carlino, A. H. Tarikogullari, V. Alptuzun, H. S. Gunes, *Bioorg. Med. Chem.* **2013**, *21*, 4928.
[30] (a) S. Arab, S. E. Sadat-Ebrahimi, M. Mohammadi-Khanaposhtani, A. Moradi, H. Nadri, M. Mahdavi, S. Moghimi, M. Asadi, L. Firoozpour, M. Pirali-Hamedani, A. Shafiee, A. Foroumadi, *Arch. Pharm. Chem. Life Sci.* **2015**, *348*, 643. (b) M. Mohammadi-Khanaposhtani, M. Saeedi, N. Shamsaei Zafarghandi, M. Mahdavi, R. Sabourian, E. Karimpour Razkenari, H. Alinezhad, M. Khanavi, A. Foroumadi, A. Shafiee, T. Akbarzadeh, *Eur. J. Med. Chem.* **2015**, *92*, 799. (c) Y. Pourshojaei, A. Gouranourimi, S. Hekmat, A. Asadipour, S. Rahmani-Nezhad, A. Moradi, H. Nadri, Moghadam. F. Homayouni, S. Emami, A. Foroumadi, A. Shafiee, *Eur. J. Med. Chem.* **2015**, *97*, 181.
[31] H. Nadri, H. Pirali-Hamedani, M. Shekarchi, M. Abdollahi, V. Sheibani, M. Amanlou, A. Shafiee, A. Foroumadi, *Bioorg. Med. Chem.* **2010**, *18*, 6360.
[32] G. L. Ellman, K. D. Courtney, V. Andres Jr, R. M. Feather-Stone, *Biochem. Pharmacol.* **1961**, *7*, 88.
[33] O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455.

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