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Coumarin derivatives bearing benzoheterocycle moiety: synthesis, cholinesterase inhibitory, and docking simulation study

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ABSTRACT

Objective(s): To investigate the efficiency of a novel series of coumarin derivatives bearing benzoheterocycle moiety as novel cholinesterase inhibitors.

Materials and Methods: Different 7-hydroxycoumarin derivatives were synthesized via Pechmann or Knoevenagel condensation and conjugated to different benzoheterocycle (8-hydroxyquinoline, 2-mercaptobenzoxazole or 2-mercaptobenzimidazole) using dibromoalkanes **3a-m**. Final compounds were evaluated against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) by Ellman's method. Kinetic study of AChE inhibition and ligand-protein docking simulation were also carried out for the most potent compound **3b**.

Results: Some of the compounds revealed potent and selective activity against AChE. Compound **3b** containing the quinoline group showed the best activity with an IC_{50} value of 8.80 μM against AChE. Kinetic study of AChE inhibition revealed the mixed-type inhibition of the enzyme by compound **3b**. Ligand-protein docking simulation also showed that the flexibility of the hydrophobic five carbons linker allows the quinoline ring to form π-π interaction with Trp279 in the PAS.

Conclusion: We suggest these synthesized compounds could become potential leads for AChE inhibition and prevention of AD symptoms.

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to loss of memory and cognitive ability. It was estimated that more than 18 million people suffer from AD worldwide and the number of patients will be increased to 70 million by 2050 (1). It has been reported that reduction of the acetylcholine level in the brain, accumulation of beta-amyloid (βA) plaques, neurofibrillary tangles, and oxidative stress are the most common causes of AD (2). The detailed mechanism of AD is still ambiguous and there is a progressive attention to discover and explain the pathogenesis of AD (3, 4). The most common approach for the treatment of AD is application of cholinesterase inhibitors (ChEIs) increasing the synaptic levels of ACh in the brain (5–7). Galantamine, donepezil, and rivastigmine are the mainstay of AD patient management (8, 9). The AChE has a nearly 20 Aº deep narrow gorge with two binding sites of the catalytic active site (CAS) at the bottom of the gorge and peripheral anionic site (PAS) near the entry of the gorge. PAS as Aβ binding domain leads formation of the stable and toxic AChE-βA complex (10-11). It has been revealed that the activity of BuchE is more than AChE in patients suffering AD and plays an important role in regulation of ACh level in the neurosynaptic area (12).

Coumarins are natural plant species with diverse biological activities including antiinflammatory (13), anti-tumor (14), anti-oxidant (15), and anti-diabetic (16). Several studies demonstrated that coumarins can bind to the PAS of AChE by their aromatic ring preventing the formation of the Aβ-AChE adducts (16-20). Coumarin derivatives have been also considered as neuroprotective agents against oxidative stress and free radical generation (21).

On the other hand, benzoheterocyclic frame-works such as benzoxazole **(A)** (19) and 8-hydroxy-

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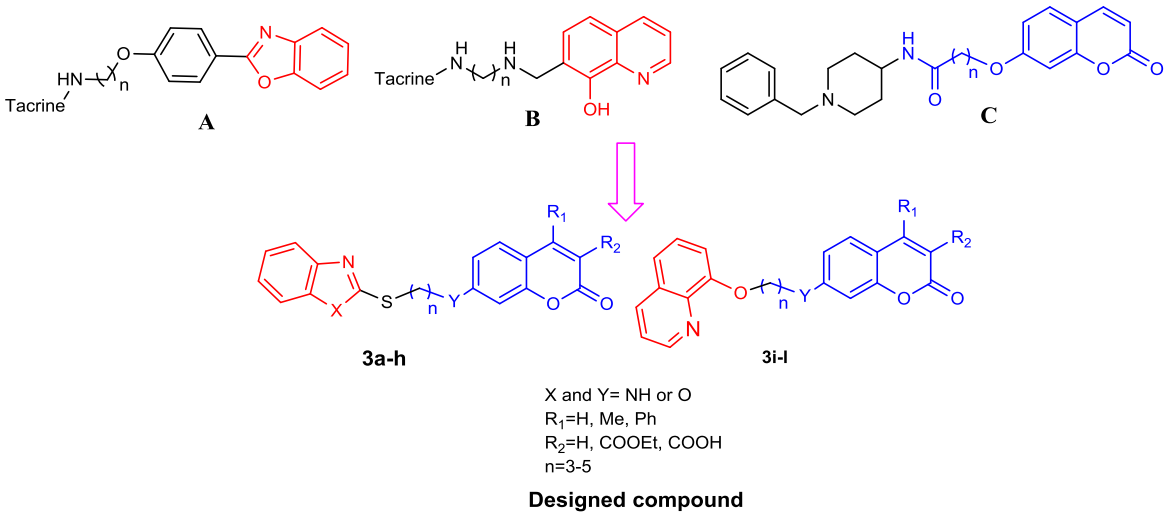


Figure 1. Some previously reported anti-AChE compounds bearing benzoheterocycle moiety (**A** and **B**), 7-hydroxycoumarin derivatives as AChE inhibitors reported in our previous study (**C**) and new designed AChE inhibitors **3a-l**

quinoline **(B)** (20) analogs have been described as multifunctional anti-AD agents with the ability to target cholinesterase and prevent deposition of amyloid aggregation as well as promote the clearance of Aβ by neutralizing deposits (Figure 1).

Considering the above results and in continuation of our previous works on coumarin derivatives (Figure 1, **C**) (21-25), herein we report preparation, evaluation, and molecular modeling study of a series of novel coumarin hybrids (**3a-l**) bearing different benzoheterocycles as ChE inhibitors (Figure 1).

Materials and Methods

Chemistry

All commercially available chemical reagents were used without further purification. The progress of the reactions was monitored by TLC on silica gel 250 micron, F254 plates. Melting points were measured on a Kofler hot stage. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrograph (KBr discs). ^{1}H NMR spectra were recorded on Brucker 500 MHz. The chemical shifts (δ) and coupling constants (*J*) are expressed in parts per million and Hertz, respectively. The atoms numbering of the target compounds used for ^{1}H NMR is shown in Scheme 1. Elemental analyses were carried out by a CHN- Rapid Heraeus elemental analyzer and the results were within ±0.5% of the calculated values.

Preparation of compound **3a-l**

A mixture of benzoheterocycle (0.5 mmol), anhydrous K_2CO_3 (0.5 mmol), and the corresponding coumarin derivate **2a-l** (0.5 mmol) in dry *N*, *N*-dimethylformamide (8 ml) was stirred at 70 °C for 8 hr. After completion of the reaction (monitored by TLC), the mixture was cooled to the room temperature and ice water was then added to the mixture and stirred for 30 min. The obtained solid was finally filtered and dried to obtain **3a-l**.

Docking study

Ligand-protein docking was conducted using Autodock Vina (1.1.2) (26) and the binding poses were retrieved. For this purpose, the 3D structure of the AChE was obtained from Protein Data Bank (PDB) at http://www.rcsb.org/pdb/home/home.do. The crystal structure of AChE (PDB ID: 1eve) in complex with donepezil was chosen. None-protein atoms were removed in the protein preparation process. The appropriate pdbqt format of the receptor was then prepared using Autodock Tools (1.5.6) (27). To prepare ligands, the 2D chemical structure of ligands was sketched using MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to 3D format by Openbabel (ver 2.3.1) (28). Finally, the required pdbqt format of ligands was prepared using an Autodock Tools python script, *prepare_ligand4. py*. The grid box with the size of 15×15×15 Å was determined and the center of the box was fixed on the center of co-crystalized ligand. After docking, the best pose was selected for further analysis. The graphics are depicted using Chimera 1.10 software (29).

Pharmacology

The capacity of the target compounds against AChE and BuChE was determined by Ellman's method (30). The stock solution of the tested compounds was dissolved in DMSO 1% and diluted in 3 ml phosphate buffer (0.1 mol/l, pH 8.0), 100 µl of 5,5′-dithio-bis(2-nitrobenzoic acid), 100 µl of 2.5 IU/ml of acetylcholinesterase or butyrylcholinesterase. Assays were measured at 412 nm for 6 min at 25 °C by using a UV Unico Double Beam spectrophotometer. The IC_{50}

values were determined graphically from log concentration vs. % of inhibition curves. All experiments were performed in three different experiments.

Results

Chemistry

All synthesized derivatives were characterized by spectroscopic methods such as ^{1}H NMR, IR, and CHN.

7-(4-(Quinolin-8-yloxy)butoxy)-2*H*-chromen-2-one (3a).

White solid; Yield (55%) mp 131-133 ºC; IR (KBr, cm^{-1}) ῡ: 3050 (N-H), 2951(C-H), 1717 (C=O), 1615 (C=N), 1552 (C=C). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.86 (d, 1H, *J*= 4.0Hz, H_2quinoline), 8.29 (d, 1H, *J*= 8.5 Hz, H_4quinoline), 7.97 (d, 1H, *J*= 10.0 Hz, H_4coumarin), 7.60 (d, 1H, *J* = 8.5 Hz, H_5coumarin), 7.53-7.48 (m, 3H, $H_{5,6,7}$quinoline), 7.19-7.20 (m, 1H, H_3quinoline), 7.01 (s, 1H, H_8coumarin), 6.96 (d, 1H, *J* = 8.5 Hz, H_6coumarin), 6.27 (d, 1H, *J* = 10.0 Hz, H_3coumarin), 4.25 (s, 4H, CH_2-O), 2.02 (s, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 161.8, 160.1, 155.3, 154.4, 148.8, 144.2, 139.7, 135.6, 129.3, 128.9, 126.7, 121.6, 119.4, 112.6, 112.2, 112.1, 109.3, 101.1, 68.1, 67.9, 25.5, 25.2. Anal. Calcd for: $C_{22}H_{19}NO_4$ (361.39): C, 73.12; H, 5.30; N, 3.88. Found: C, 73.36; H, 5.66; N, 3.44.

7-((5-(Quinolin-8-yloxy)pentyl)oxy)-2*H*-chromen-2-one (3b)

White solid; Yield (94%) mp 107-109 ºC; IR (KBr, cm^{-1}) ῡ: 2947 (C-H), 1726 (C=O), 1616 (C=N). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.85 (dd, 1H, *J*= 2.0 Hz, *J*= 4.0 Hz, H_2quinoline), 8.29 (dd, 1H, *J*= 2.0 Hz, *J* = 4.0 Hz, H_4quinoline), 7.96 (d, 1H, *J*= 9.5 Hz, H_4coumarin), 7.60 (d, 1H, *J*= 8.5 Hz, H_5coumarin), 7.50-7.53 (m, 1H, H_5quinoline), 7.49-7.46 (m, 2H, $H_{6,7}$quinoline), 7.18-7.20 (m, 1H, H_3quinoline), 6.97 (s, 1H, H_8coumarin), 6.93 (d, 1H, *J*= 8.5 Hz, H_6coumarin), 6.26 (d, 1H, *J* = 9.5 Hz, H_3coumarin), 4.2 (t, 2H, *J* = 6.5 Hz, CH_2-O), 4.13 (t, 2H, *J* = 6.5 Hz, CH_2-O), 1.94 (quint, 2H, *J* = 6.5 Hz, CH_2), 1.87 (quint, 2H, *J* = 6.5 Hz,CH_2), 1.68-1.71 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 161.8, 160.1, 155.3, 154.5, 148.7, 144.2, 139.7, 135.6, 129.3, 128.9, 126.7, 121.6, 119.3, 112.6, 112.2, 112.1, 109.3, 101.1, 68.2, 68.1, 28.3, 28.1, 22.2. Anal. Calcd for: $C_{21}H_{17}NO_4$ (375.42): C, 72.61; H, 4.93; N, 4.03. Found: C, 72.31; H, 4.33; N, 4.28.

4-Methyl-7-(3-(quinolin-8-yloxy)propoxy)-2H-chromen-2-one (3c)

Red solid; Yield (72%); mp 112-114 ºC; IR (KBr, cm^{-1}) ῡ: 3407 (N-H), 2949 (C-H), 1715 (C=O), 1616 (C=N). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.86 (d, 1H, *J* = 2.5 Hz, H_2quinoline), 8.29 (d, 1H, *J* = 8.0 Hz, H_4quinoline), 7.65 (d, 1H, *J*=8.5 Hz, H_5coumarin), 7.53 (m, 1H, H_5quinoline), 7.50-7.47 (m, 2H, $H_{6,7}$coumarin), 7.23 (t, 1H, *J* = 2.5Hz, H_3quinoline), 7.00 (s,1H, H_8), 6.98 (d, 1H, *J* = 2.5Hz, H_6quinoline), 6.18 (s, 1H, H_3coumarin), 4.35-4.37 (m, 4H, CH_2-O), 2.37 (s, 3H, CH_3), 2.34-2.36 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ:161.5, 159.9, 154.6, 154.2, 153.1, 148.8, 139.7, 135.6, 128.9, 126.6, 126.3, 121.6, 119.6, 113.0, 112.2, 111.0, 109.6, 101.2, 65.1, 64.9, 28.4, 17.9. Anal. Calcd for: $C_{22}H_{19}NO_4$ (361.39): C, 73.12; H, 5.30; N, 3.88. Found: C, 73.36; H, 5.56; N, 3.96.

4-Methyl-7-((5-(quinolin-8-yloxy)pentyl)oxy)-2H-chromen-2-one (3d)

White solid; Yield (50%); mp 100-102 ºC; IR (KBr, cm^{-1}) ῡ: 2943-2871 (C-H), 1723 (C=O), 1612 (C=N), 1107 (C-O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.85 (d, 1H, *J*=3.0 Hz, H_2 quinoline), 8.29 (dd, 1H, *J* = 8.0 and *J*=2.0 Hz, H_4 quinoline), 7.65 (d, 1H, *J*=9.0 Hz, H_5 coumarin), 7.50-7.53 (m, 1H, H_5 quinoline), 7.49-7.47 (m, 2H, $H_{6,7}$ quinoline), 7.18-7.20 (m, 1H, H_3 quinoline), 6.96 (s, 1H, H_8 coumarin), 6.94 (d, 1H, *J*=2.5 Hz, H_6 coumarin), 6.18 (s, 1H, H_3 coumarin), 4.20 (t, 2H, *J*=6.5 Hz, CH_2-O), 4.13 (t, 2H, *J*=6.5 Hz, CH_2-O), 2.38 (s, 3H, CH_3), 1.94 (quint, 2H, *J*=7.0 Hz,CH_2), 1.87 (quint, 2H, *J*=8.0 Hz, CH_2), 1.69 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 161.6, 160.0, 154.6, 154.4, 153.2, 148.7, 139.7, 135.6, 128.9, 126.6, 126.2, 121.6, 119.3, 112.9, 112.3, 110.9, 109.3, 101.1, 68.1, 28.3, 28.1, 22.2, 17.9. Anal. Calcd for: $C_{24}H_{23}NO_4$ (375.42): C, 74.02; H, 5.95; N, 3.60. Found: C, 74.15; H, 5.68; N, 3.85.

Ethyl 2-oxo-7-(3-(quinolin-8-yloxy)propoxy)-2*H*-chromene-3-carboxylate(3e).

Red solid; Yield (95%); mp 63-65 ºC; IR (KBr, cm^{-1}) ῡ: 2963-2878 (C-H), 1747 (C=O), 1610 (C=N), 1552 (C=C). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.87 (bs, 1H, H_2quinoline), 8.68 (s,1H, H_4coumarin), 8.29 (d, 1H, *J* = 7.0 Hz, H_4quinoline), 7.81(d, 1H, *J* = 8.0 Hz, H_5coumarin), 7.51-7.50 (m, 3H, $H_{5,6,7}$quinoline), 7.23 (bs, 1H, H_3quinoline), 7.07-7.03 (m, 2H, $H_{6,8}$coumarin), 4.40-4.27 (m, 2H, CH_2CH_3 and 4H, CH_2-O), 2.36 (s, 2H, CH_2), 1.30 (t, 3H, *J* = 7.0 Hz CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ:163.9, 162.7, 156.8, 156.1, 154.2, 148.9, 139.7, 135.6, 131.5, 128.9, 126.6, 121.7, 119.7, 113.4, 113.3, 111.3, 109.6, 100.7, 65.5, 64.8, 60.8, 28.4, 14.0. Anal.Calcd for: $C_{24}H_{21}NO_6$ (419.43): C, 68.73; H, 5.05; N, 3.34. Found: C, 68.42; H, 5.28; N, 3.25.

Ethyl 2-oxo-7-((5-(quinolin-8-yloxy)pentyl)oxy)-2*H*-chromene-3-carboxylate (3f).

Red solid; Yield (94%); mp 66-68 ºC; IR (KBr cm^{-1}) ῡ: 2941-2877 (C-H), 1764 (C=O), 1609 (C=N). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.84 (s, 1H, H_2quinoline), 8.68 (s,1H, H_4coumarin), 8.27 (d, 1H, *J* = 7.0 Hz, H_4quinoline), 7.80 (d, 1H, *J* = 8.0 Hz, H_5coumarin), 7.50-7.47 (m, 3H, $H_{5,7,6}$ quinoline), 7.18 (bs,1H, H_3quinoline), 6.99 (m, 2H; $H_{6,8}$coumarin),

4.28-4.18 (m, 2H, $C\underline{H}_2CH_3$ and 4H, CH_2-O), 1.93-1.88 (m, 4H, CH_2), 1.68 (s, 2H, CH_2), 1.30 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 164.1, 162.7, 156.8, 156.1, 154.5, 148.9, 148.7, 139.7, 135.5, 131.5, 128.9, 126.6, 121.6, 119.3, 113.4, 113.1, 111.2, 109.3, 100.6, 68.5, 68.1, 60.7, 28.3, 28.0, 22.2, 14.0. Anal.Calcd for: $C_{26}H_{25}NO_6$ (477.41): C, 69.79; H, 5.63; N, 3.13. Found: C, 69.58; H, 5.95; N, 4.31.

2-Oxo-7-(3-(quinolin-8-yloxy)propoxy)-2*H*-chromene-3-carboxylic acid (3g)

Light brown solid; Yield (95%) mp 63 ºC; IR (KBr, cm^{-1}) ῡ: 3438 (O-H), 2930 (C-H), 1760 (C=O), 1623 (C=N). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 9.14 (bs, 1H, H_2quinoline), 9.01 (d, 1H, *J* = 7.0 Hz H_4quinoline), 8.68 (s, 1H, H_4 coumarin), 8.01 (bs, 1H, H_5 coumarin), 7.80-7.78 (m, 3H, $H_{5,6,7}$quinoline), 7.59 (d, 1H, *J* = 7.0Hz, H_3quinoline), 7.04-7.00 (m, 2H, $H_{6,8}$coumarin), 4.50-4.47 (m, 4H, CH_2-O), 2.39 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 125 MHz) δ:164.1, 163.8, 157.1, 156.7, 149.9, 148.9, 145.9, 144.1, 131.5, 129.4, 122.7, 120.0, 113.8, 113.5, 112.7, 111.6, 100.8, 65.8, 65.5, 28.1, 14.0. Anal.Calcd for: $C_{20}H_{15}NO_6S$ (397.06): C, 60.45; H, 3.80; N, 3.52. Found: C, 60.71; H, 3.63; N, 3.77.

4-Phenyl-7-((3-(quinolin-8-yloxy)propyl)amino)-2*H*-chromen-2-one (3h)

Cream solid; Yield (48%) mp 74-76 ºC; IR (KBr, cm^{-1}) ῡ: 3339 (NH), 2925-2871(C-H), 1713 (C=O), 1106 (C-O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.91 (bs, 1H, H_2quinoline), 8.30 (d, 1H, *J* = 8.0Hz, H_4quinoline), 7.53-7.47 (m, 6H, 5Hphenyl and 1H, H_5coumarin), 7.21-7.00 (m,3H, $H_{5,6,3}$quinoline), 6.66 (d, 1H, *J* = 9.0 Hz, H_6coumarin), 6.58 (s, 1H, H_8coumarin), 5.90 (s,1H, H_3coumarin), 4.31 (bs, 2H, CH_2-O), 3.41 (bs, 2H, CH_2-NH), 2.16 (bs, 2H, CH_2). ^{13}CNMR (DMSO-d_6, 125 MHz) δ: 160.4, 156.4, 155.6, 154.2, 152.5, 149.0, 139.7, 135.7, 135.6, 129.2, 128.9, 128.6, 128.1, 127.3, 126.7, 121.7, 119.5, 110.5, 109.4, 107.2, 107.0, 96.6, 66.5, 39.8, 28.0. Anal. Calcd for: $C_{27}H_{22}N_2O_3$ (422.48): C, 76.76; H, 5.25; N, 6.66. Found: C, 76.30; H, 4.92; N, 6.33.

7-((5-((1H-Benzo[d]imidazol-2-yl)thio) pentyl) oxy)-2H-chromen-2-one (3i)

White solid; Yield (84%) mp 107-109 °C; IR (KBr, cm^{-1}) ῡ: 3173 (N-H), 2929-2880 (C-H), 1700 (C=O), 1618 (C=N). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.04 (d, 1H, *J* = 9.7 Hz, 1H, H_4coumarin), 7.61(d, 1H, *J* = 9.7 Hz, H_7 benzimidazole), 7.50 (bs, 1H, H_4 benzimidazole), 7.31 (d, 1H, *J* = 8.5 Hz, H_5coumarin), 7.17 (m, 2H, $H_{5,6}$ benzimidazole), 6.77 (d, 1H, *J* = 8.5 Hz, H_6coumarin), 6.73 (s, 1H, H_8cumarin), 6.23 (d, 1H, *J* = 9.7 Hz, H_3coumarin), 3.93 (t, 2H, *J* = 6.5 Hz, CH_2-O), 3.35 (t, 2H, *J* = 6.5 Hz, CH_2-S), 1.83 (quint, 2H, *J* = 7.4 Hz, CH_2), 1.77 (quint, 2H, *J* = 7.4 Hz, CH_2), 1.58 (quint, 2H, *J*=7.4 Hz, CH_2). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 162.2, 161.4, 155.7, 150.4, 143.5, 128.7, 122.1, 114.0, 112.9, 112.8, 112.3, 101.3, 68.2, 32.2, 29.2, 28.3, 24.9. Anal. Calcd for: $C_{21}H_{21}N_3O_2S$ (380.46): C, 66.47; H, 5.58; N, 11.07. Found: C, 66.71; H, 5.22; N, 10.89.

7-((5-(Benzo[d]oxazol-2-ylthio)pentyl)oxy)-2*H*-chromen-2-one (3j)

White solid; Yield (89%) mp 54-56 ºC; IR (KBr, cm^{-1}) ῡ: 2931-2974 (C-H), 1711(C=O), 1623 (C=N). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.58-7.62 (m, 2H, H_4coumarin and H_7benzoxazole), 7.42 (d, 1H, *J* = 7.4 Hz, H_4benzoxazole), 7.34 (d, 1H, *J* = 8.5 Hz, H_5coumarin), 7.29-7.21 (m, 2H, $H_{5,6}$ benzoxazole), 6.81 (d, 1H, *J* = 8.5Hz, H_6coumarin), 6.79 (s, 1H, H_8coumarin), 6.23 (d, 1H, *J* = 9.7 Hz, H_3coumarin), 4.03 (t, 2H, *J* = 6.5 Hz, CH_2-O), 3.35 (t, 2H, *J*=6.5 Hz, CH_2-S), 1.94 (quint, 2H, *J* = 7.5 Hz, CH_2), 1.88 (quint, 2H, *J* = 7.5 Hz, CH_2), 1.69 (quint, 2H, *J* = 7.5 Hz, CH_2). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 176.8, 162.2, 155.9, 143.35, 128.6, 124.2, 123.8, 118.3, 112.9, 112.4, 109.8, 101.3, 68.1, 32.0, 28.9, 28.4, 25.0. Anal. Calcd for: $C_{21}H_{19}NO_4S$ (381.44): C, 66.12; H, 5.02; N, 3.67. Found: C, 66.32; H, 5.36; N, 3.54.

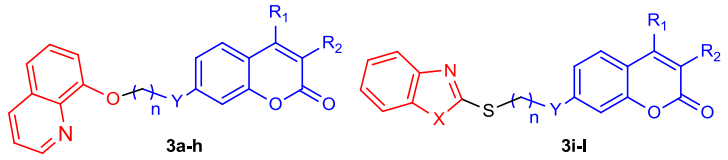
7-(3-(Benzo[d]oxazol-2-ylthio)propoxy)-4-methyl-2*H*-chromen-2-one (3k)

White solid; Yield (50%) mp 107-109 ºC; IR (KBr, cm^{-1}) ῡ: 2952 (C-H), 1731 (C=O), 1615 (C=N). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.57 (d, 1H, *J* = 7.5 Hz, H_7benzoxazole), 7.47 (d, 1H, *J* = 7.5 Hz, H_4benzoxazole), 7.41 (d, 1H, *J* = 7.5 Hz, H_5coumarin), 7.28-7.22 (m, 2H, $H_{5,6}$ benzoxazole), 6.85 (d, 1H, *J* = 7.5 Hz, H_6coumarin), 6.81(s, 1H, H_8coumarin), 6.13 (s, 1H, H_3coumarin), 4.20 (t, 2H, *J* = 6.0 Hz, CH_2-O), 3.51 (t, 2H, *J* = 7.0 Hz, CH_2-S), 2.39 (bs, 5H, 2H, CH_2 and 3H, CH_3). ^{13}C NMR($CDCl_3$, 125 MHz) δ: 164.3, 161.6, 161.1, 155.2, 152.3, 151.8, 141.8, 125.5, 124.2, 123.9, 118.4, 113.7, 112.4, 112.0, 109.8, 101.5, 66.2, 28.8, 28.7, 18.6. Anal. Calcd for: $C_{20}H_{17}NO_4S$ (367.42): C, 65.38; H, 4.66; N, 3.81. Found C, 65.59; H, 4.03; N, 3.98.

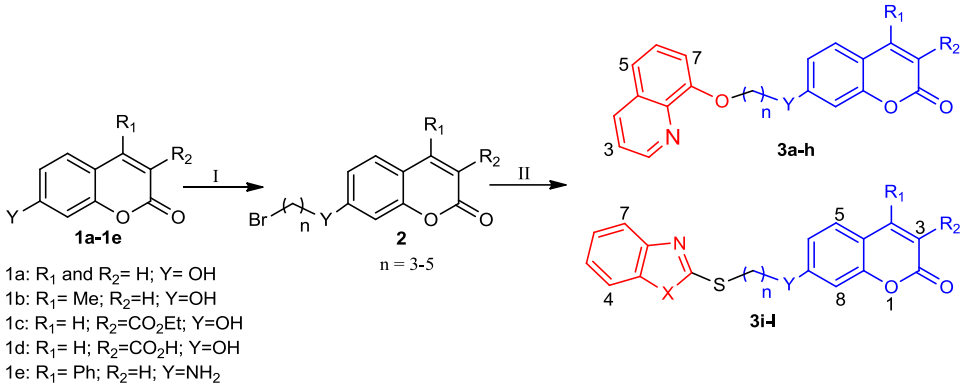
7-((3-(Benzo[d]oxazol-2-ylthio)propyl)amino)-4-phenyl-2H-chromen-2-one (3l)

Cream solid; Yield (54%) mp 135-137 °C; IR (KBr, cm^{-1}) ῡ: 3329 (NH), 2930 (C-H), 1704 (C=O). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.60 (d, 1H, *J*= 7.0 Hz, H_7benzoxazole), 7.48-7.42 (m, 3H, benzoxazol and 1H, H_5coumarin), 7.29-7.22 (m, 5H, phenyl), 6.56 (s, 1H, H_3coumarin), 6.50 (d, 1H, *J* = 8.0 Hz, H_6coumarin), 6.05 (s, 1H, H_8coumarin), 5.16 (s, 1H, NH), 3.39-3.43 (m, 2H, CH_2-NH and 2H, CH_2-S), 2.19-2.21 (m, 2H, CH_2). ^{13}CNMR ($CDCl_3$, 125 MHz) δ: 161.8, 156.7, 156.1, 151.9, 151.4, 141.6, 136.1, 129.2, 128.6, 128.3, 128.0, 124.4, 124.0, 118.2, 110.2, 109.9, 109.4, 109.1, 98.2, 41.4, 29.2, 28.8. Anal. Calcd for: $C_{25}H_{20}N_2O_3S$ (422.48): C, 70.07; H, 4.70; N, 6.54. Found: C, 70.31; H, 4.62; N, 6.27.

Table 1. Inhibitory activity of the target compounds **3a-m** against AChE and BuChE



Compounds	R_1	R_2	n	X	Y	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
3a	H	H	4	-	O	48.00% at 35 μM	28.30
3b	H	H	5	-	O	8.80	26.50
3c	Me	H	3	-	O	11.29	32.40
3d	Me	H	5	-	O	13.96	34.60% at 35 μM
3e	H	CO_2Et	3	-	O	16.19	46.00% at 35 μM
3f	H	CO_2Et	5	-	O	8.94	29.16% at 35 μM
3g	H	CO_2H	3	-	O	15.00% at 35 μM	2.00% at 35 μM
3h	Ph	H	3	-	NH	30.20	37.00% at 35 μM
3i	H	H	5	NH	O	11.72	38.00% at 35 μM
3j	H	H	5	O	O	25.37	14.00% at 35 μM
3k	Me	H	3	O	O	44.00% at 35 μM	11.00% at 35 μM
3l	Ph	H	3	O	NH	13.00	26.00% at 35 μM
Donepezil						0.016	5.41



Scheme 1. Synthesis of target compounds **3a-l**. Reagent and condition: (I) $Br(CH_2)_nBr$ (n= 3-5), K_2CO_3, acetone, reflux; (II) DMF, K_2CO_3, appropriate benzoheterocycle, 24 hr, 70 °C

Enzymatic assay

All synthesized compounds **3a-l** were evaluated against AChE and BuChE in comparison with commercial donepezil as standard drug. The activities were summarized in (Table 1) as IC_{50} values. Also, Kinetic study was used to determine the mechanism of enzyme inhibition by compound **3b**. The relative velocity of the enzyme was determined on three increasing concentrations of acetylthio-choline (ATChI) (Figures 2, 3).

Docking

The most active compound **3b** was optimized and docked into the active site of the enzyme using Autodock Vina program to investigate binding mode of the ligand to AChE (Figure 4).

Discussion

Chemistry

Target compounds **3a-l** were easily synthesized via the procedure outlined in (scheme 1). The coumarin derivatives **1a-e** were initially prepared via Pechmann or Knoevenagel condensation according to the previously reported procedure (31-33). 7-Hydroxycoumarin **1a** was commercially purchased. Compound **1b** was synthesized via Pechmann condensation between resorcinol and ethyl acetoacetate in the presence of sulfuric acid as a catalyst (31). Compound **1c** was prepared through Knoevenagel condensation between diethyl malonate and 2,4-dihydroxybenzaldehyde catalyzed by a few drops of piperidine which was then hydrolyzed in an aqueous solution of sodium hydroxide to prepare acid analog **1d** (32). Compound **1e** was also prepared via reaction of ethyl (3-hydroxyphenyl) carbamate with phenyl acetoacetate (33). Different dibromoalkanes (n=3-5) were then employed as cross-linkers to bridge between coumarin ring and different benzoheterocycles. This substitution nucleophilic reaction was performed in the presence of acetone as solvent and K_2CO_3 as the base with an excess amount of appropriate dibromoalkanes

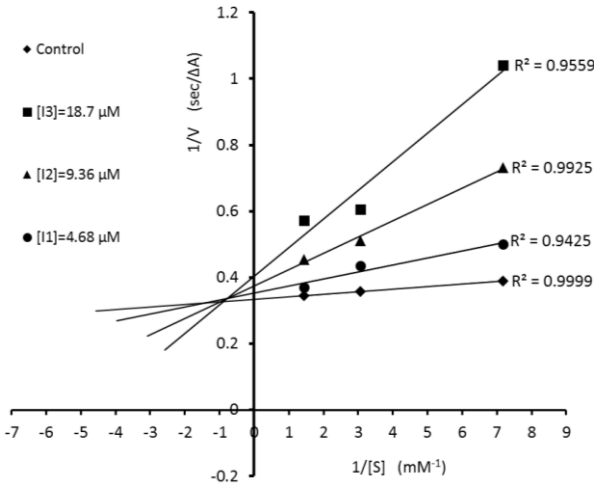


Figure 2. Lineweaver-Burk plot for the inhibition of AChE by **3b**

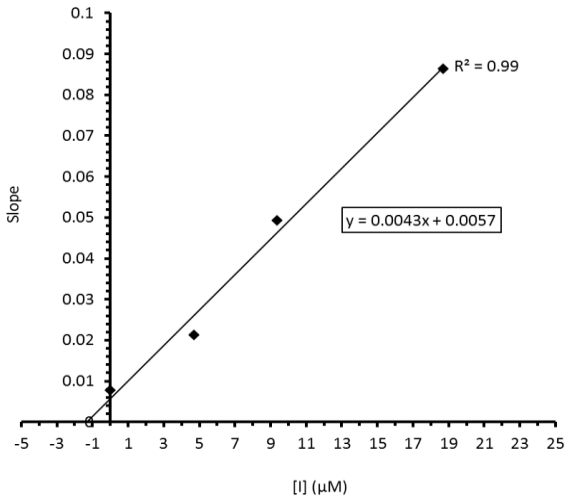


Figure 3. Lineweaver-Burk secondary plot for Ki calculation

(10 equivalents). Compound **2** was finally reacted with different benzoheterocycles in DMF as solvent and K_2CO_3 as the base at 70ºC to prepare target compounds **3a-l**.

Ligand-protein docking simulation

As depicted in Figure 4, the coumarin ring of the compound is placed in the mid gorge of AChE active site in parallel with phenyl ring of Phe330 and makes a π-π stacking with this residue. The quinoline head is oriented toward the PAS. The flexibility of the linker allows the quinoline ring to form another π-π interaction with Trp279 in the PAS. The mentioned interactions are responsible for the affinity between the ligand and the enzyme. The aliphatic linker also participates in the binding of the ligand to the enzyme through hydrophobic interactions with side chains of amino acids in the active site of AChE.

Anti-cholinesterase activity

As indicated in Table 1, target compounds showed a diverse range of activity and different parameters affect their inhibitory. Compounds **3d** and **3k** containing 8-hydroxyquinoline linked to simple and ethyl 3-coumarincarboxylate, respectively, through a 5-carbon spacer, exhibited the most potent inhibitory activity against AChE (IC_{50}=8.80 and 8.94 µM). The results revealed that the AChE inhibitory was closely dependent on the length of the alkylene chain and 5-carbon bond spacer is the proper length for derivatives without any substituent at 4-position of the coumarin ring. The comparison of compounds **3e** and **3g** showed that conversion of ester groups at 3-position of coumarin ring to the corresponding acid profoundly diminished the AChE inhibitory (IC_{50}=16.19 µM vs. 15.00% inhibition at 35 µM). The results also revealed that the presence of substituent at 4-position of coumarin ring could affect the inhibitory activity. Compound **3b** showed superior activity compared with compound **3d** (IC_{50}=8.80 vs. 13.96 µM). The comparison of unsubstituted coumarins with five carbons linker (n= 5) in terms of anti-AChE activity showed that the order of activity by considering the type of benzoheterocycle moiety was as follow: quinoline> benzimidazole > benzoxazole (**3b**, **3i**, and **3j,** respectively). But, the presence of bulky phenyl group at 4-position of coumarin ring changed the results and the anti-AChE activity of compound **3l** was higher than compound **3h** (IC_{50}= 13.00 vs. 30.20 µM).

All of the target compounds showed significantly less activity against BuChE than those of AChE except for compound **3a**. Compounds **3a-c** were the most potent compounds for inhibition of BuChE with an IC_{50} value of less than 3250 µM. But, all other compounds show no proper activity against BuChE. Compound **3b** containing unsubstituted coumarin attached to the 8-hydroxyquinoline via five carbons linker was the most superior compound for both enzymes.

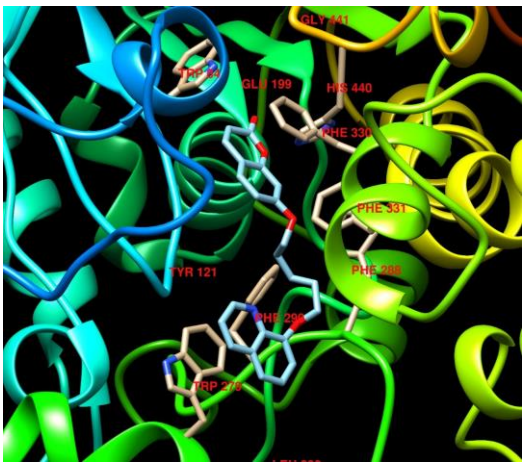


Figure 4. Schematic interaction of compound **3b** with the active site of AChE

Kinetic study of AChE inhibition

In order to depict the Lineweaver-Bruke plot, the enzyme velocity was measured in the presence of inhibitor **3b** at following concentrations: 0.575, 0.115, and 0.23 μM. The Lineweaver-Burke plot was then schemed using the reciprocal of velocity (1/v) and substrate concentration (S) (Figure 2). A mixed type inhibition of AChE was established by compound **3b**. The Lineweaver-Burk secondary plot (Figure 3) was also applied to determine the K_i value for **3b** (1.32 μM).

Conclusion

In summary, we designed and synthesized a novel series of coumarin derivatives bearing quinoline, benzoxazole, or benzoimidazole to evaluate their anti-AChE/BuChE activity. Compound **3b** with quinoline pendent group displayed the highest AChE and BuChE inhibitory activity (IC_{50}= 8.80 and 26.50 μM, respectively). The docking study of the most potent compound **3b** revealed that the target ligand can interact with the PAS of AChE preventing the formation of stable and toxic AChE-Aβ complex. These results make the prototype compound **3b** a promising cholinesterase inhibitor for further developments.

Acknowledgment

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