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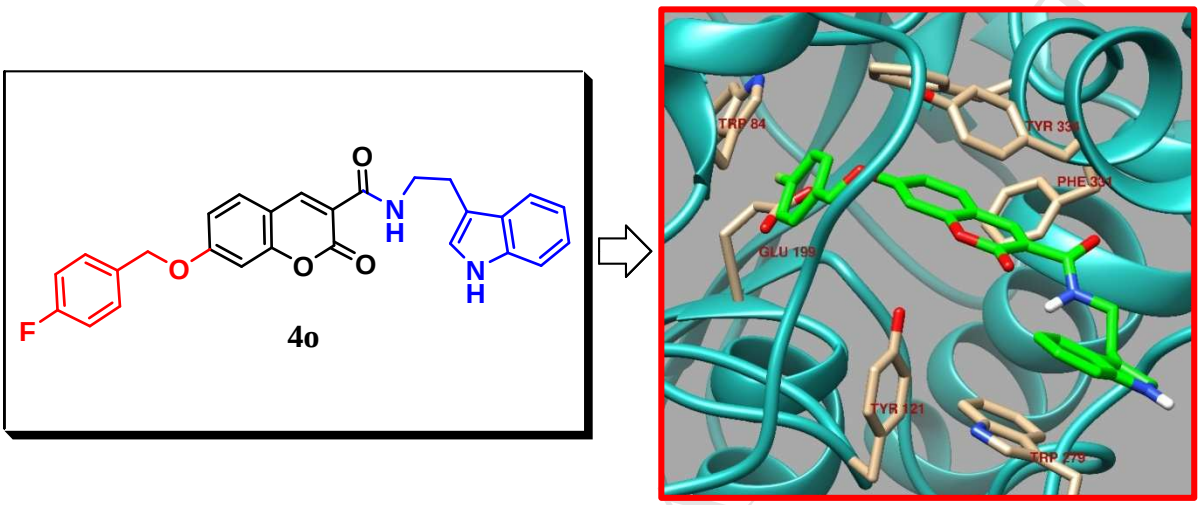

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Synthesis and anticholinesterase activity of coumarin-3-carboxamides bearing tryptamine moiety

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Abstract:

A number of *N*-(2-(1*H*-indol-3-yl)ethyl)-2-oxo-2*H*-chromene-3-carboxamides were synthesized and tested against AChE and BuChE. The in vitro assessment of the synthesized compounds **4a-o** revealed that most of them had significant activity toward AChE. The SAR study demonstrated that the introduction of benzyloxy moiety on the 7-position of coumarin scaffold can improve the anti-AChE activity. The best result was obtained with 7-(4-fluorobenzyl)oxy moiety in the case of compound **4o**, displaying IC_{50} value of 0.16 µM. Based on the docking study of AChE, the prototype compound **4o** was laid across the active site and occupied both peripheral anionic site (PAS) and catalytic anionic site (CAS).

Keywords: Acetylcholinesterase; butyrylcholinesterase; Alzheimer's disease; coumarin; indole.

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1. Introduction

Alzheimer's disease (AD) is an age-related, progressive and multi-factorial neurodegenerative disorder in the elderly population. The neuropathological changes in the brain of patients with AD lead to decline in language skills, progressive cognitive impairment, and dementia [1,2]. This disease which depends on a combination of genetic and environmental factors, reaches epidemic proportions, with a great social, and economic burden worldwide [3,4].

Although the etiopathogenesis of AD is complicated and unknown, several hallmarks including acetylcholine (ACh) deficiency and aggregation of beta-amyloid peptide (Aβ) are found to be involved in the onset and progression of this disease. In particular, the presynaptic decrease of ACh which may be due to damage of cholinergic neurons in some special parts of the brain results in a generalized cognitive decline [5-7]. Based on the cholinergic hypothesis, the increase of ACh via inhibiting acetylcholinesterase (AChE) in the brain is an effective approach to retard the AD's symptoms [8,9]. The AChE is the main enzyme responsible for hydrolysis of ACh in the central nervous system (CNS). Accordingly, most studies have focused on AChE inhibition in the treatment of AD [10,11]. Therefore, AChE inhibitors such as donepezil, rivastigmine, galantamine and tacrine have been demonstrated to have great potential in the treatment of AD patients. All of these drugs with the exception of tacrine are still in clinical use. Tacrine was withdrawn from the market due to the hepatotoxicity [12,13]. Primarily, AChE inhibitors have been considered only as symptomatic therapy for AD, however recent studies have suggested that AChE inhibitors can act as disease-modifying agents by inhibition of the amyloid cascade, modulation of various proteins' activity, regulation of cerebral blood flow and neuroprotection [14-16].

Coumarin moiety is an important aromatic ring with a broad spectrum of biological activity [17]. Among the various classes of compounds studied for design of new AChE inhibitors, coumarin scaffold has received great attention, due to its ability for binding to peripheral anionic site (PAS) of AChE [18]. Recently, we have reported different series of coumarin-based AChE inhibitors [19-23]. On the other hand, some authors have described the usefulness of indole amine framework in the design of new AChE inhibitors or multi-target agents for AD therapy. For example, Luo et al. synthesized a series of indole amine-based benzylpyridinium bromides (**I**, Fig. 1) as multi-functional anti-AD agents with cholinesterase inhibitory, antioxidant, and neuroprotective activities [24]. In a study by Cheng and co-workers, some (−)-meptazinol–indole amine hybrids (**II**) were synthesized as potential multi-

target-directed ligands against AD with dual inhibitory potency against cholinesterases and effective inhibition of Aβ self-aggregation [25]. In order to find new lead candidate for AD therapy, Peng et al. designed *p*-hydroxybenzamide derivatives **III** bearing indole amine scaffold and used it for step-by-step optimization of the framework to obtaining potential multi-site AChE inhibitors [26]. In this context and in continuation of our recent works [27-29], it seems very interesting to develop some coumarin carboxamide derivatives bearing indole amine (tryptamine) scaffold in order to find new AChE inhibitors. Thus, we report here the synthesis and cholinesterases inhibitory activity of *N*-(2-(1*H*-indol-3-yl)ethyl)-2-oxo-2*H*-chromene-3-carboxamides **4a-o** (Fig. 1).

2. Chemistry

The synthetic routes to intermediates **3a-o** and final compounds **4a-o** were depicted in Schemes 1 and 2, respectively. The cyclization reaction of salicylaldehyde derivatives **1a-d** with diethyl malonate in the presence of piperidine in ethanol afforded ethyl coumarin-3-carboxylate **2a-d**. The 7-hydroxy derivative **2b** was reacted with alkyl halides or benzyl halides by using potassium carbonate as a base in DMF to give *O*-alkyl or *O*-benzyl derivatives **2e-o**. The ethyl esters **2a-o** was hydrolyzed in the presence of NaOH to give corresponding coumarin-3-carboxylic acids **3a-o** (Scheme 1). In the final step, the acids **3a-o** were converted to related acid chlorides by using thionyl chloride. Subsequently, the obtained acid chlorides were reacted with tryptamine in the presence of potassium carbonate in dry toluene to afford final compounds **4a-o** (Scheme 2). The final step was also evaluated under microwave irradiations in different solvents, as well as solvent-free condition, to decrease the reaction time. The best result was obtained in acetonitrile as solvent. Interestingly, the time of the reaction was decreased from about 15 hours under conventional condition to around five minutes under microwave irradiation (Supplementary material, Table S1).

3. Results and discussion

3.1. In vitro anticholinesterase activity

The in vitro anticholinesterase activity of designed compounds **4a-o** was determined against AChE and BuChE. The obtained IC_{50} values (in µM) were presented in Table 1. The IC_{50}s against AChE revealed that most of compounds possess significant activity in the range of

0.16 to 43.8 µM. Among them, the (4-fluorobenzyl)oxy derivative **4o** with IC_{50} of 0.16 µM showed the highest activity against AChE.

In the structure-activity point of view, the simplest compound **4a** with no substitution on the core structure exhibited good activity against AChE. Introduction of 6-bromo substituent on the coumarin ring resulted in inactive compound **4b**. In contrast, the 8-methoxy substitution could improve the activity as observed in compound **4c**. While the insertion of 7-hydroxy group dramatically eliminated the anti-cholinesterase activity, but *O*-alkylation or *O*-benzylation of 7-hydroxy could retained the activity. 7-hydroxy analog **4d** was inactive (IC_{50} > 100 µM) however *O*-alkyl and *O*-benzyl derivatives (compounds **4e-h**) showed mild to good activity against AChE. In particular, the *O*-benzyl derivative **4h** with IC_{50} value of 1.5 µM was about four times more potent than parent compound **4a**. In the 7-alkoxy derivatives **4e-g**, the best activity was observed with 7-methoxycoumarin derivative **4e**. The activities of 7-ethoxy and 7-propoxy analogs (**4f** and **4g**) were less than those of unsubstituted counterpart **4a** and 7-methoxy derivate **4e**. Thus, the elongation of *O*-alkyl group diminished the activity.

The comparison of 7-benzyloxy **4h** and unsubstituted compound **4a** demonstrated that the insertion of 7-benzyloxy group could improve the anti-cholinesterase activity. A survey on the IC_{50} values of *O*-benzyl derivatives **4h-o** revealed that the introduction of one or two chlorine atoms significantly decreased the anti-AChE activity. However, the fluoro substituent was tolerated on the benzyl moiety as observed in compounds **4m-o**. The 2-fluoro- and 3-fluorobenzyl derivatives (**4m** and **4n**) with IC_{50} values of 2.5 µM showed same activity against AChE. The displacement of fluorine atom from *ortho* or *meta* positions to *para* position of benzyl moiety greatly improved the activity against AChE. The most potent compound **4o** bearing a (4-fluorobenzyl)oxy residue was 15-fold more potent than compounds **4m** and **4n**. Moreover, the activity of 4-fluorobenzyl derivative **4o** was 9 times superior to that of benzyl analog **4h**.

The obtained data of test compounds **4a-o** against BuChE demonstrated that the most of designed compounds had mild or no activity against this enzyme. The *O*-benzyl derivative **4h** with IC_{50} of 16.2 µM was the most potent compound against BuChE. It was 3-fold more potent than parent compound **4a**. Furthermore, the 8-methoxy analog **4c** showed suitable anti-BuChE activity (IC_{50}= 20.1 µM). The chloro-substituted benzyloxycoumarins **4i-l** were inactive against BuChE, while the fluorobenzyl congeners **4m-o** could properly inhibit this

enzyme (IC_{50}s < 30 µM). In general, the inhibitory activity of all compounds against AChE was higher over BuChE.

3.2. Kinetic study of AChE inhibition

To assess the kinetic mode of AChE inhibition displayed by target compounds, the most active compound **4o** was subjected to kinetic studies. For this purpose, the rate of enzyme activity was measured at four different concentrations of inhibitor **4o** (0, 0.069, 0.206 and 0.618 µM) in the presence of different concentrations of substrate (ATCh). For each inhibitor concentration, the initial velocity was measured at different substrate concentrations (S) and the reciprocal of the initial velocity (1/v) was plotted versus the reciprocal of substrate concentration (1/[s]). As depicted in Fig. 2, the obtained double reciprocal (Lineweavere-Burk) plot showed a mixed-type inhibition pattern for compound **4o**. The *K*i value was also calculated using the secondary plot (*K*i = 0.49 µM, Fig. 2). All experiments were performed as same as cholinesterase inhibition test described in experimental section.

3.3. Docking simulation

As the target compounds showed moderate to high selective inhibitory activity against AChE, the molecular modeling studies were performed to obtain the proposed binding mode of the most active compound **4o** in the active site of AChE. AutoDock Vina (ver. 1.1.2) was used for all docking experiments. Docking validation was based on the co-crystallized ligand (E2020) from crystal structure (1EVE) and it was shown that docking runs were able to reproduce original arrangement of the ligand with low RMSD value below 1 Å. As depicted in Fig. 3, the target compound was laid across the active site and occupied both PAS and CAS (catalytic anionic site) of enzyme active site where the 4-fluorobenzyl moiety oriented toward the bottom of active site. A closer examination of this binding mode was done to find out the interactions of this compound with enzyme residues. As shown in Fig. 3, the 4-fluorophenyl ring formed a face-to-face π-π stacking with Trp84. Another π-π stacking was observed between coumarin ring and Tyr334. The indole ring of this compound also interacted with PAS through a π-π interaction with Trp279.

4. Conclusion

We have synthesized some coumarin carboxamide derivatives bearing indole amine scaffold (tryptamine) as new cholinesterase inhibitors. The in vitro assessment of synthesized

compounds **4a-o** against AChE/BuChE revealed that most of compounds had significant activity toward AChE. The modification of substituent on the coumarin ring could modulate the activity of designed compounds against cholinesterases. The best result was obtained with 7-(4-fluorobenzyl)oxy moiety against AChE in compound **4o**, displaying IC_{50} value of 0.16 µM. The SAR study demonstrated that the introduction of *O*-benzyl moiety on the 7-position of coumarin scaffold can improve the anti-AChE activity. The docking simulation study showed the dual binding mode of the promising compound **4o**. As a potent inhibitor of AChE with dual binding to both PAS and CAS, this prototype compound could be a good candidate for further studies and modifications.

5. Experimental

All commercially available reagents were purchased from Merck AG or Aldrich and used without further purification. TLC was conducted on silica gel 250 micron. Melting points were measured on the Buchi Melting point B-540. FT-IR spectra were run on a Nicolet FT-IR Magna 550 spectrograph (KBr discs). ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR instrument. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million and Hertz, respectively. The final step of synthesis was alternatively carried out under microwave irradiation using a microwave oven (MicroSYNTH, Microwave Labstation).

*5.1. General procedure for the synthesis of compounds **2a-d***

A mixture of salicylaldehyde derivatives **1a-d** (5 mmol), diethyl malonate (6 mmol) and piperidine (0.2 ml) was refluxed in ethanol (10 ml) for 14-16 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled, filtrated and washed with cold ethanol to obtain white solid. The solid was recrystallised from ethanol to give pure compounds **2a-d**.

*5.2. General procedure for the synthesis of compounds **2e-o***

Ethyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**2b**, 5 mmol) and potassium carbonate (6 mmol) were mixed with DMF (10 ml). The mixture was stirred at room temperature for several minutes and then appropriate alkyl halide or benzyl halide derivative (7 mmol) was

added dropwise to the mixture. The reaction mixture was stirred at room temperature for 14-16 h. After completion of the reaction (monitored by TLC), the mixture was diluted with water. The precipitate was filtered, washed with water, and used without further purification.

*5.3. General procedure for the synthesis of compounds **3a-o***

NaOH solution (2N, 25 ml) was added to the ester derivatives **2a-o**, and the mixture was stirred at room temperature for 5-7 h. After cooling, hydrochloric acid (2N) was added to the yellow solution until a white precipitate formed. The precipitated solid was isolated by filtration, washed with water and dried to give compounds **3a-o**.

*5.4. General procedure for the synthesis of compounds **4a-o***

Compounds **3a-o** (1 mmol) were added to thionyl chloride (5 ml) and the mixture was refluxed for 5-6 h. After completion of the reaction, thionyl chloride was removed with simple distillation to give appropriate coumarin-3-carboxylic acid chloride. The crude product was used directly without further purification. In the next step, the corresponding acid chloride, tryptamine (1 mmol) and potassium carbonate (2 mmol) were suspended in dry toluene (15 ml). The mixture was refluxed for 14-16 h under argon atmosphere. After completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure and the residue was washed with water and dried to give compounds **4a-o**. Alternatively the same reaction could carry out under microwave irradiations at 85 ºC for 5 min.

*5.4.1. N-(2-(1H-Indol-3-yl)ethyl)-2-oxo-2H-chromene-3-carboxamide (**4a**).* Pale brown solid; yield 98%; mp 184-188 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, Acetone-d_6) δ: 10.09 (br, 1H, NH), 8.93 (s, 1H, H_4 coumarin), 8.88 (br, 1H, NH), 7.98 (d, *J* = 7.8 Hz, 1H, H_5 coumarin), 7.79 (t, *J* = 7.8 Hz, 1H, H_6 coumarin), 7.71 (d, *J* = 7.8 Hz, 1H, H_8 coumarin), 7.48 (t, *J* = 7.8 Hz, 1H, H_7 coumarin), 7.47 (d, *J* = 8.0 Hz, 1H, H_7 indole), 7.41 (d, *J* = 8.0 Hz, 1H, H_4 indole), 7.28 (s, 1H, H_2 indole), 7.12 (t, *J* = 8.0 Hz, 1H, H_5 indole), 7.04 (t, *J* = 8.0 Hz, 1H, H_6 indole), 3.78 (t, *J* = 7.2 Hz, 2H, NH-C$\underline{H}_2$), 3.11 (t, *J* = 7.2 Hz, 2H, indole-C$\underline{H}_2$). ^{13}C NMR (75 MHz, Acetone-d_6) δ: 160.9, 160.8, 154.4, 147.6, 136.9, 133.8, 130.1, 127.6, 125.1, 122.6, 121.2, 119.0, 118.8, 118.5, 118.4, 116.1, 112.3, 111.3, 40.2, 25.2. Anal. Calcd for $C_{20}H_{16}N_2O_3$ (332.35): C, 72.28; H, 4.85; N, 8.43. Found: C, 72.01; H, 4.57; N, 8.16.

*5.4.2. 6-Bromo-N-(2-(1H-indol-3-yl)ethyl)-2-oxo-2H-chromene-3-carboxamide (**4b**).* Pale brown solid; yield 82%; mp 221-224 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.85 (brs, 2H, NH and H_4 coumarin), 8.06 (s, 1H, NH), 7.84 (t, *J* = 7.8 Hz, 1H, H_7 coumarin), 7.76 (d, *J* = 7.8 Hz, 1H, H_5 coumarin), 7.69 (d, *J* = 7.8 Hz, 1H, H_8 coumarin), 7.41 (d, *J* = 8.1 Hz, 1H, H_7 indole), 7.13-7.36 (m, 4H, $H_{2,\ 4,\ 5,\ 6}$ indole), 3.83 (m, 2H, NH-C$\underline{H}_2$), 3.14 (t, *J* = 6.9 Hz, 2H, indole-C$\underline{H}_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 161.1, 160.36, 153.3, 146.6, 136.7, 136.6, 132.5, 127.5, 123.3, 121.4, 120.8, 120.56, 118.8, 118.8, 118.7, 117.1, 111.8, 40.8, 25.4. Anal. Calcd for $C_{20}H_{15}BrN_2O_3$ (411.25): C, 58.41; H, 19.43; N, 6.81. Found: C, 58.63; H, 19.21; N, 6.48.

*5.4.3. N-(2-(1H-Indol-3-yl)ethyl)-8-methoxy-2-oxo-2H-chromene-3-carboxamide (**4c**).* Pale brown solid; yield 89%; mp 176-179 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, Acetone-d_6) δ: 10.07 (brs, 1H, NH), 8.89 (brs, 2H, H_4 coumarin and NH), 7.72 (d, *J* = 7.8 Hz, 1H, H_5 coumarin), 7.50 (t, *J* = 7.8 Hz, 1H, H_6 coumarin), 7.36-7.46 (m, 3H, H_7 coumarin and $H_{4,7}$ indole), 7.28 (s, 1H, H_2 indole), 7.12 (t, *J* = 7.5 Hz, 1H, H_5 indole), 7.04 (t, *J* = 7.5 Hz, 1H, H_6 indole), 4.01 (s, 3H, CH_3), 3.78 (m, *J* = 7.2 Hz, 2H, NH-C$\underline{H}_2$), 3.11 (t, *J* = 7.2 Hz, 2H, indole-C$\underline{H}_2$).^{13}C NMR (75 MHz, Acetone-d_6) δ: 161.15, 160.66, 147.96, 146.96, 143.91, 136.78, 127.55, 125.13, 122.56, 121.23, 121.06, 119.39, 119.01, 118.53, 118.45, 115.81, 112.10, 111.29, 55.84, 40.28, 25.17. Anal.Calcd for $C_{21}H_{18}N_2O_4$ (362.38): C, 69.60; H, 5.01; N, 7.73. Found: C, 69.46; H, 4.89; N, 7.50.

*5.4.4. N-(2-(1H-Indol-3-yl)ethyl)-7-hydroxy-2-oxo-2H-chromene-3-carboxamide (**4d**).* Pale brown solid; yield 91%; mp 264-266 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.85 (brs, 1H, NH), 8.81 (s, 1H, H_4 coumarin), 8.77 (t, *J* = 5.7 Hz, 1H, NH), 7.82 (d, *J* = 7.8 Hz, 1H, H_5 coumarin), 7.62 (d, *J* = 7.8 Hz, 1H, H_6 coumarin), 7.35 (d, *J* = 8.1 Hz, 1H, H_4 indole), 7.21 (s, 1H, H_8 coumarin), 7.08 (t, *J* = 8.1 Hz, 1H, H_5 indole), 6.99 (t, *J* = 8.1 Hz, 1H, H_6 indole), 6.88 (d, *J* = 8.7 Hz, 1H, H_7 indole), 6.80 (s, 1H, H_2 indole), 3.45 (m, 2H, NH-C$\underline{H}_2$), 2.96 (t, *J* = 7.2 Hz, 2H, C$\underline{H}_2$ indole). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.25, 161.95, 161.49, 156.76, 148.45, 136.77, 132.43, 127.61, 123.27, 121.44, 118.84, 118.71, 114.87, 114.03, 111.98, 111.84, 111.54, 102.26, 25.53, 19.03. Anal. Calcd for $C_{20}H_{16}N_2O_4$ (348.35): C, 68.96; H, 4.63; N, 8.04. Found: C, 66.81; H, 4.90; N, 8.25.

*5.4.5. N-(2-(1H-Indol-3-yl)ethyl)-7-methoxy-2-oxo-2H-chromene-3-carboxamide (**4e**).* Pale brown solid; yield 85%; mp 191-193 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR

(300 MHz, $CDCl_3$) δ: 8.86 (brs, 1H, NH), 8.84 (s, 1H, H_4 coumarin), 8.07 (br, 1H, NH), 7.67 (d, *J* = 7.5 Hz, 1H, H_5 coumarin), 7.56 (d, *J* = 8.7 Hz, 1H, H_7 indole), 7.38 (d, *J* = 8.1 Hz, 1H, H_4 indole), 7.08-7.24 (m, 3H, $H_{6, 8}$ coumarin and H_5 indole), 6.93 (m, 1H, H_6 indole), 6.84 (s, 1H, H_2 indole), 3.91 (s, 3H, OCH_3), 3.79 (q, *J* = 7.5 Hz, 2H, NH-C$\underline{H}_2$), 3.10 (t, *J* = 7.5 Hz, 2H, indole-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.7, 161.9, 161.7, 156.63, 148.1, 136.4, 130.9, 127.3, 122.1, 119.4, 118.8, 115.0, 113.9, 113.1, 112.4, 118.1, 100.3, 56.0, 40.1, 25.3. Anal. Calcd for $C_{21}H_{18}N_2O_4$ (362.38): C, 69.60; H, 5.01; N, 7.73. Found: C, 69.43; H, 4.84; N, 7.55.

*5.4.6. N-(2-(1H-Indol-3-yl)ethyl)-7-ethoxy-2-oxo-2H-chromene-3-carboxamide (**4f**).* Pale brown solid; yield 88%; mp 201-203 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, Acetone-d_6) δ: 10.86 (brs, 1H, NH), 8.86 (s, 1H, H_4 coumarin), 8.78 (brs, 1H, NH), 7.91 (d, *J* = 8.7 Hz, 1H, H_5 coumarin), 7.62 (d, *J* = 7.5 Hz, 1H, H_4 indole), 7.35 (d, *J* = 7.5 Hz, 1H, H_7 indole), 7.21 (s, 1H, H_2 indole), 6.94-7.15 (m, 4H, $H_{6, 8}$ coumarin and $H_{5, 6}$ indole), 4.19 (q, *J* = 7.2 Hz, 2H, OCH_2), 3.61 (q, *J* = 6.9 Hz, 2H, NH-C$\underline{H}_2$), 2.97 (t, *J* = 6.9 Hz, 2H, indole-CH_2), 1.38 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.5, 161.8, 161.3, 156.9, 148.5, 136.5, 136.9, 132.1, 127.4, 123.8, 123.1, 121.9, 118.2, 118.6, 115.9, 114.8, 112.4, 111.1, 101.4, 64.0, 25.3, 14.7. Anal. Calcd for $C_{22}H_{20}N_2O_4$ (376.41): C, 70.20; H, 5.36; N, 7.44. Found: C, 68.98; H, 5.06; N, 7.15.

*5.4.7. N-(2-(1H-Indol-3-yl)ethyl)-2-oxo-7-propoxy-2H-chromene-3-carboxamide (**4g**).* Pale brown solid; yield 90%; mp 174-176 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.51 (brs, 1H, NH), 9.31 (s, 1H, Ar-H_4), 9.28 (brs, 1H, NH), 8.31 (d, *J* = 8.7 Hz, 1H, H_5 coumarin), 8.16 (d, *J* = 7.5 Hz, 1H, H_4 indole), 7.86 (d, *J* = 7.8 Hz, 1H, H_7 indole), 7.72 (s, 1H, H_2 indole), 7.37-7.63 (m, 4H, $H_{6, 8}$ coumarin and $H_{5, 6}$ indole), 4.61 (t, *J* = 6.6 Hz, 2H, OCH_2), 4.21 (q, *J* = 7.2 Hz, 2H, NH-C$\underline{H}_2$), 3.55 (t, *J* = 7.2 Hz, 2H, indole-CH_2), 2.31 (sex, *J* = 7.2 Hz, 2H, CH_3-C$\underline{H}_2$-CH_2), 1.53 (t, *J* = 7.2 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 164.2, 161.8, 161.3, 156.6, 148.2, 136.7, 132.0, 127.6, 123.2, 121.4, 118.8, 118.7, 115.1, 114.3, 112.5, 111.9, 111.8, 101.1, 70.5, 25.5, 22.2, 10.7. Anal. Calcd for $C_{23}H_{22}N_2O_4$ (390.43): C, 70.75; H, 5.68; N, 7.17. Found: C, 70.53; H, 5.39; N, 6.97.

*5.4.8. N-(2-(1H-Indol-3-yl)ethyl)-7-(benzyloxy)-2-oxo-2H-chromene-3-carboxamide (**4h**).* Pale brown solid; yield 91%; mp 195-197 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.80-8.90 (brs, 1H, NH), 8.86 (s, 1H, H_4 indole), 8.12 (brs, 1H, NH), 7.70 (d, *J* = 7.5 Hz, 1H, H_5 indole), 7.60 (d, *J* = 8.7 Hz, 1H, H_6 coumarin), 7.34-7.53

(m, 6H, H_2 indole and 5H benzyl), 7.09-7.27 (m, 3H, $H_{4,7,5}$ indole), 7.03-6.89 (m, 2H, H_6 indole and H_8 coumarin), 5.19 (s, 2H, CH_2-O), 3.82 (q, *J* = 6.9 Hz, 2H, NH-C<u>H</u>$_2$), 3.13 (t, *J* = 6.9 Hz, 2H, indole-C<u>H</u>$_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.8, 161.7, 161.2, 156.4, 148.1, 136.7, 136.4, 132.0, 129.9, 127.6, 123.2, 121.4, 118.8, 118.7, 115.4, 114.6, 112.7, 111.9, 111.8, 101.6, 70.6, 25.5. Anal. Calcd for $C_{27}H_{22}N_2O_4$ (438.47): C, 73.96; H, 5.06; N, 6.39. Found: C, 73.74; H, 4.88; N, 6.11.

*5.4.9. N-(2-(1H-Indol-3-yl)ethyl)-7-((2-chlorobenzyl)oxy)-2-oxo-2H-chromene-3-carboxamide (**4i**).* Pale brown solid; yield 85%; mp 189-192 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.87 (brs, 1H, NH), 8.86 (s, 1H, H_4 coumarin), 8.79 (brs, 1H, NH), 7.94 (d, *J* = 7.8 Hz, 1H, H_5 coumarin), 7.72 (d, *J* = 7.8 Hz, 1H, H_6 coumarin), 7.63 (m, 2H, $H_{3,5}$ benzyl), 7.46 (t, *J* = 7.5 Hz, 1H, H_4 benzyl), 7.33-7.38 (m, 2H, $H_{4,7}$ indole), 7.23 (m, 2H, H_2 indole and H_8 coumarin), 7.04-7.17 (m, 2H, $H_{5,\ 6}$ indole), 6.99 (t, *J* = 7.5 Hz, 1H, H_6 benzyl), 5.28 (s, 2H, CH_2-O), 3.63 (d, *J* = 5.7 Hz, 2H, NH-C<u>H</u>$_2$), 2.97 (t, *J* = 6.3 Hz, 2H, indole-C<u>H</u>$_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.6, 161.8, 161.2, 156.4, 148.1, 136.7, 136.6, 135.2, 133.2, 132.1, 131.3, 131.1, 128.5, 127.5, 123.8, 123.2, 123.1, 127.4, 118.8, 118.7, 115.5, 114.4, 112.9, 111.9, 101.5, 70.5, 25.4. Anal. Calcd for $C_{27}H_{21}ClN_2O_4$ (472.92): C, 68.57; H, 4.48; N, 5.92. Found: C, 68.38; H, 4.69; N, 5.70.

*5.4.10. N-(2-(1H-Indol-3-yl)ethyl)-7-(2,3-dichlorobenzyloxy)-2-oxo-2H-chromene-3-carboxamide (**4j**).* Pale brown solid; yield 82%; mp 219-222 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.87 (brs, 1H, NH), 8.86 (s, 1H, H_4 coumarin), 8.78 (brs, 1H, NH), 7.94 (d, *J* = 8.7 Hz, 1H, H_5 coumarin), 7.79 (d, *J* = 7.8 Hz, 1H, H_4 benzyl), 7.70 (d, *J* = 7.8 Hz, 1H, H_5 benzyl), 7.62 (d, *J* = 7.8 Hz, 1H, H_6 benzyl), 7.50 (d, *J* = 7.8 Hz, 1H, H_7 indole), 7.35 (d, *J* = 7.8 Hz, 1H, H_4 indole), 6.87-7.28 (m, 5H, $H_{6,\ 8}$ coumarin and $H_{5,\ 6,\ 2}$ indole), 5.29 (s, *J* = 7.8 Hz, 2H, OCH_2), 3.63 (q, *J* = 6.5 Hz, 2H, NH-C<u>H</u>$_2$), 2.97 (t, *J* = 6.5 Hz, 2H, indole-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.4, 161.7, 161.2, 156.4, 148.1, 136.6, 136.4, 132.5, 132.1, 131.4, 131.1, 129.4, 128.9, 127.5, 123.1, 121.4, 118.8, 118.7, 115.6, 114.4, 113.0, 111.9, 111.8, 101.6, 68.6, 25.4. Anal. Calcd for $C_{27}H_{20}Cl_2N_2O_4$ (507.36): C, 63.92; H, 3.97; N, 5.52. Found: C, 63.75; H, 3.79; N, 5.23.

*5.4.11. N-(2-(1H-Indol-3-yl)ethyl)-7-(4-chlorobenzyloxy)-2-oxo-2H-chromene-3-carboxamide (**4k**).* Pale brown solid; yield 82%; mp 203-207 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83-8.94 (brs, 1H, NH), 8.87 (s, 1H, H_4

coumarin), 8.07 (brs, 1H, NH), 7.70 (d, *J* = 8.0 Hz, 1H, H_5 coumarin), 7.62 (d, *J* = 8.0 Hz, 1H, H_6 coumarin), 7.35-7.46 (m, 5H, $H_{2,3,6,5}$ benzyl and H_7 indole), 7.12-7.28 (m, 3H, $H_{4,5}$ indole and H_8 coumarin), 7.01 (dd, *J* = 8.7 Hz, *J* = 2.1 Hz, 1H, H_6 indole), 6.91 (d, *J* = 2.4 Hz, 1H, H_2 indole), 5.15 (s, 2H, CH_2-O), 3.82 (q, *J* = 6.8 Hz, 2H, NH-C$\underline{H}_2$), 3.13 (t, *J* = 6.8 Hz, 2H, indole-C$\underline{H}_2$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.5, 161.7, 161.2, 156.4, 148.1, 136.7, 135.5, 133.3, 132.0, 130.3, 129.0, 127.6, 123.2, 121.7, 118.8, 118.7, 115.5, 114.5, 112.8, 111.9, 111.8, 101.6, 69.7, 25.5. Anal. Calcd for $C_{27}H_{21}ClN_2O_4$ (472.92): C, 68.57; H, 4.48; N, 5.92. Found: C, 68.69; H, 4.67; N, 5.73.

*5.4.12. N-(2-(1H-Indol-3-yl)ethyl)-7-(3,4-dichlorobenzyloxy)-2-oxo-2H-chromene-3-carboxamide (**4l**).* Pale brown solid; yield 92%; mp 236-239 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.87 (brs, 1H, NH), 8.88 (s, 1H, H_4 coumarin), 8.79 (t, 1H, *J* = 8.7 Hz, NH), 7.96 (d, *J* = 8.7 Hz, 1H, H_5 coumarin), 6.90-7.75 (m, 10H, $H_{6,8}$ coumarin, $H_{2,4,5,6,7}$ indole and $H_{2,5,6}$ benzyl), 5.37 (s, 2H, OCH_2), 3.64 (t, *J* = 6.9 Hz, 2H, NH-C$\underline{H}_2$), 2.97 (t, *J* = 6.9 Hz, 2H, indole-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 163.4, 161.7, 161.2, 156.4, 148.1, 136.6, 136.4, 132.5, 132.1, 131.4, 131.1, 129.4, 128.9, 127.5, 123.1, 121.4, 118.8, 118.7, 115.6, 114.4, 113.0, 111.9, 111.8, 101.6, 68.6, 25.4. Anal. Calcd for $C_{27}H_{20}Cl_2N_2O_4$ (507.36): C, 63.92; H, 3.97; N, 5.52. Found: C, 63.78; H, 4.25; N, 5.73.

*5.4.13. N-(2-(1H-Indol-3-yl)ethyl)-7-(2-fluorobenzyloxy)-2-oxo-2H-chromene-3-carboxamide (**4m**).* Pale brown solid; yield 89%; mp 173-176 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.86 (brs, 1H, NH), 8.86 (s, 1H, H_4 coumarin), 8.80 (t, *J* = 6.0 Hz, 1H, NH), 7.94 (d, *J* = 8.7 Hz, 1H, H_5 coumarin), 7.56-7.67 (m, 2H, $H_{4,6}$ benzyl), 7.42-7.53 (m, 1H, H_5 benzyl), 7.20-7.40 (m, 4H, H_8 coumarin and $H_{4,7,5}$ indole), 7.03-7.17 (m, 3H, H_6 coumarin and $H_{2,6}$ indole), 6.92-7.03 (m, 1H, H_3 benzyl), 5.31 (s, 2H, OCH_2), 3.28 (q, *J* = 7.2 Hz, 2H, NH-C$\underline{H}_2$), 2.68 (t, *J* = 7.2 Hz, 2H, indole-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.5, 161.8, 161.7, 156.4, 148.0, 136.4, 131.0, 130.5, 130.4, 1129.8, 129.8, 127.3, 124.51, 124.4, 122.6, 122.1, 122.1, 119.4, 118.8, 115.8, 115.5, 115.2, 114.3, 113.1, 112.8, 111.1, 101.3, 40.1, 25.3. Anal. Calcd for $C_{27}H_{21}FN_2O_4$ (456.47): C, 71.04; H, 4.64; N, 6.14. Found: C, 70.82; H, 4.31; N, 5.89.

*5.4.14. N-(2-(1H-Indol-3-yl)ethyl)-7-((3-fluorobenzyl)oxy)-2-oxo-2H-chromene-3-carboxamide (**4n**).* Pale brown solid; yield 87%; mp 173-176 ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (s, 1H, H_4 coumarin), 8.75-8.93 (brs, 1H, NH), 8.08 (brs, 1H, NH), 7.66 (d, *J* = 7.8 Hz, 1H, H_5 coumarin), 7.59 (d, *J* = 8.7 Hz, 1H, H_7

indole), 7.32-7.45 (m, 2H, $H_{4,5}$ benzyl), 6.94-7.24 (m, 7H, H_8 coumarin, $H_{2,4,5,6}$ indole and $H_{2,6}$ benzyl), 6.8 (d, *J* = 7.8 Hz, 1H, H_6 coumarin), 5.15 (s, 2H, OCH_2), 3.79 (q, *J* = 6.9 Hz, 2H, NH-C$\underline{H}_2$), 3.10 (t, *J* = 7.2 Hz, 2H, indole-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.5, 161.8, 161.7, 156.4, 148.0, 136.4, 131.0, 130.5, 130.4, 129.8, 129.8, 127.3, 124.5, 124.4, 122.6, 122.1, 122.1, 119.4, 118.8, 115.8, 115.5, 115.2, 114.3, 113.1, 112.8, 111.1, 101.3, 40.1, 25.3. Anal. Calcd for $C_{27}H_{21}FN_2O_4$ (456.47): C, 71.04; H, 4.64; N, 6.14. Found: C, 71.14; H, 4.42; N, 5.85.

*5.4.15. N-(2-(1H-Indol-3-yl)ethyl)-7-((4-fluorobenzyl)oxy)-2-oxo-2H-chromene-3-carboxamide (**4o**).* Pale brown solid; yield 90%; mp 193-196ºC; IR (KBr, cm^{-1}) ν_{max}: 3500, 1710, 1680; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (s, 1H, H_4 coumarin), 8.78-8.92 (brs, 1H, NH), 8.10 (brs, 1H, NH), 7.66 (d, *J* = 8.2 Hz, 1H, H_5 coumarin), 7.56 (d, *J* = 8.2 Hz, 1H, H_6 coumarin), 7.31-7.47 (m, 3H, $H_{2,6}$ benzyl and H_7 indole), 7.04-7.24 (m, 5H, $H_{3,5}$ benzyl, $H_{4,5}$ indole and H_8 coumarin), 6.98 (d, *J* = 8.6 Hz, 1H, H_6 indole), 6.88 (s, 1H, H_2 indole), 5.10 (s, 2H, OCH_2), 3.79 (q, *J* = 7.0 Hz, 2H, NH-C$\underline{H}_2$), 3.10 (t, *J* = 7.0 Hz, 2H, indole-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.5, 161.8, 161.7, 156.4, 148.0, 136.4, 131.0, 130.5, 130.4, 129.8, 129.8, 127.3, 124.5, 124.4, 122.6, 122.1, 122.1, 119.4, 118.8, 115.8, 115.5, 115.2, 114.3, 113.1, 112.8, 111.1, 101.3, 40.1, 25.3. Anal. Calcd for $C_{27}H_{21}FN_2O_4$ (456.47): C, 71.04; H, 4.64; N, 6.14. Found: C, 71.31; H, 4.37; N, 6.05.

5.5. *Cholinesterases inhibition assay*

The inhibitory potency of target compounds on AChE/BuChE was determined using Ellman's method [30]. The compounds **4a-o** were dissolved in 1 ml DMSO and 9 ml ethanol and then four different concentrations of each compound were tested to obtain the range of 20% to 80% enzyme inhibition for AChE and BuChE. The reaction mixture included 2 ml phosphate buffer (0.1 M, pH= 8.0), 65 µl of DTNB 0.1 M, 35 µl of enzyme [2 U/mL of AChE (E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel*) or BuChE (E.C. 3.1.1.8, from equine serum)] and 35 µl of inhibitor solution. The changing of the absorbance was measured at 412 nm for 2 min (30s intervals) after addition of 10 µl of substrate (acetylthiocholine iodide or butyrylthiocholine iodide, 0.15 M) to the reaction mixture. The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration vs. percent of inhibition). All experiments were performed in 24 well plates on asynergy HTX microplate reader in quadruplicates.

5.6. Molecular modeling study

All docking simulations were performed using Autodock Vina (ver. 1.1.2) [31]. The high resolution crystal structure of acetylcholinesterase complexed with E2020 (code ID: 1EVE, resolution: 2.5 Å) was retrieved from protein data bank (www.rcsb.org). Then, the co-crystallized ligand and water molecules were removed and the protein was converted to pdbqt format using Autodock Tools (1.5.6) [32]. The 2D structures of ligands were prepared using MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to 3D format by Openbabel (ver. 2.3.1) [33]. Finally, pdbqt format of ligands was prepared using Autodock Tools python script, *prepare_ligand4.py*. After preparation of ligands and protein, the docking studies were performed using the following docking parameters: center_x = 2.023; center_y = 63.295; center_z = 67.062; size_x = 20; size_y = 20; size_z = 20; exhaustiveness = 80; num_modes = 15. The other parameters were left as default. At the end, the best docking solutions were selected for further analysis of enzyme-inhibitor interactions. The graphics were depicted using Chimera 1.6 software [34] and PoseView (http://poseview.zbh.uni-hamburg.de/poseview/wizard, 2016.).

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Captions:

Figure 1. structures of previously reported anti-AChE compounds (**I-III**) bearing indole amine moiety and newly designed compounds **4a-o**.

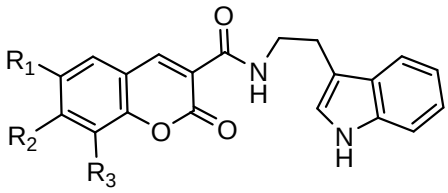
Figure 2. Left: Lineweavere-Burk plot for the inhibition of AChE by compound **4o** at different concentrations of substrate (ATCh); Right: Secondary plot for calculation of steady-state inhibition constant (*K*i) of compound **4o**.

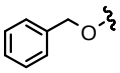
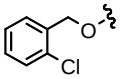
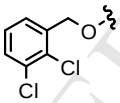
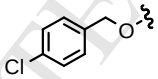
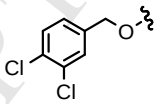
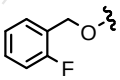
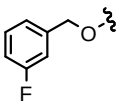
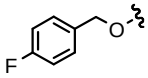
Figure 3. 2D (left) and 3D (right) representation of interactions of compound **4o** in the active site of AChE.

Scheme 1. Synthesis of intermediates **3a-o**. Reagents and conditions: (a) diethyl malonate, piperidine, EtOH, reflux ; (b) NaOH (2N) and then HCl (2N); (c) R-X, K_2CO_3, DMF.

Scheme 2. Synthesis of final compounds **4a-o**. Reagents and conditions: (a) $SOCl_2$; (b) Tryptamine, K_2CO_3, dry toluene, reflux.

Table 1. The IC_{50} values of the target compounds **4a-o** against AChE and BuChE.



Compound	R_1	R_2	R_3	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
4a	H	H	H	5.9	50.5
4b	Br	H	H	>100	>100
4c	H	H	CH_3O	2.8	20.1
4d	H	HO	H	>100	>100
4e	H	CH_3O	H	3.5	34.6
4f	H	CH_3CH_2O	H	43.8	>100
4g	H	$CH_3CH_2CH_2O$	H	18.7	>100
4h	H		H	1.5	16.2
4i	H		H	22.5	>100
4j	H		H	>100	>100
4k	H		H	12.4	>100
4l	H		H	31.5	>100
4m	H		H	2.5	27.5
4n	H		H	2.5	27.8
4o	H		H	0. 16	29.7
Donepezil				0.016	7.4

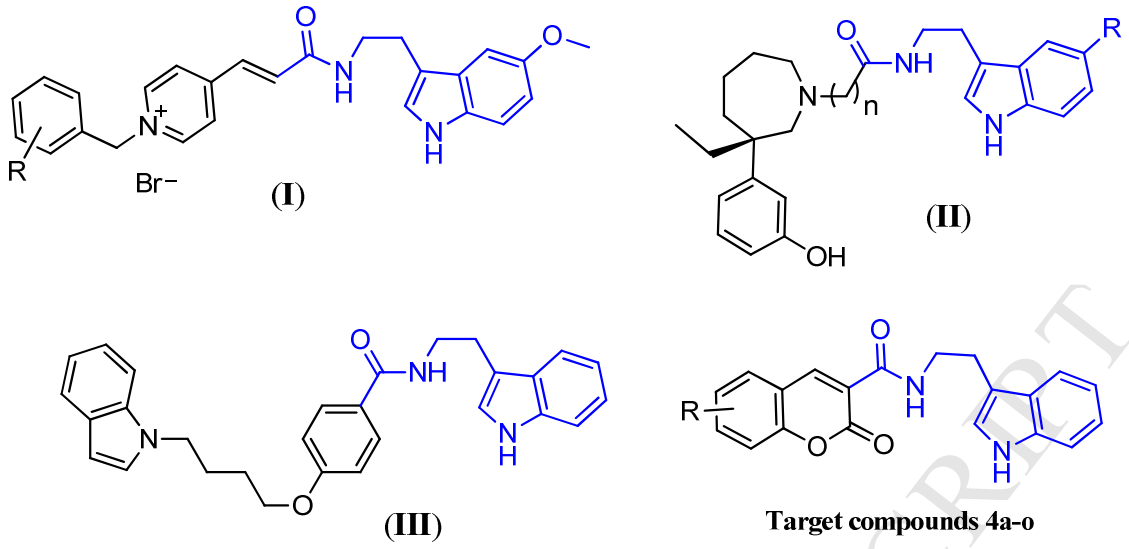
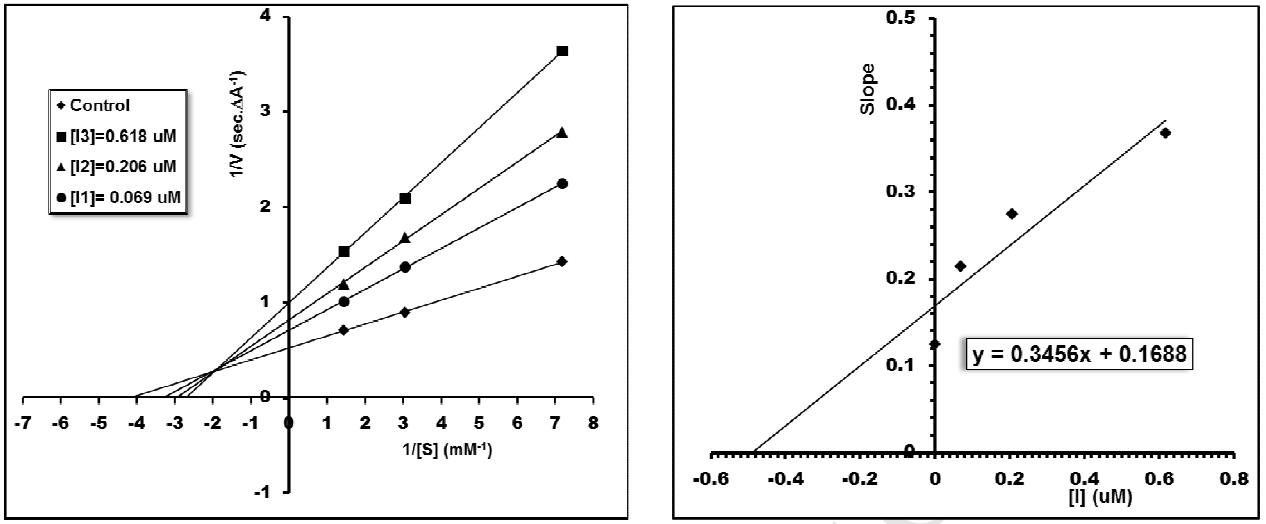


Figure 1. Structures of previously reported anti-AChE compounds (**I-III**) bearing indole amine moiety and newly designed compounds **4a-o**.



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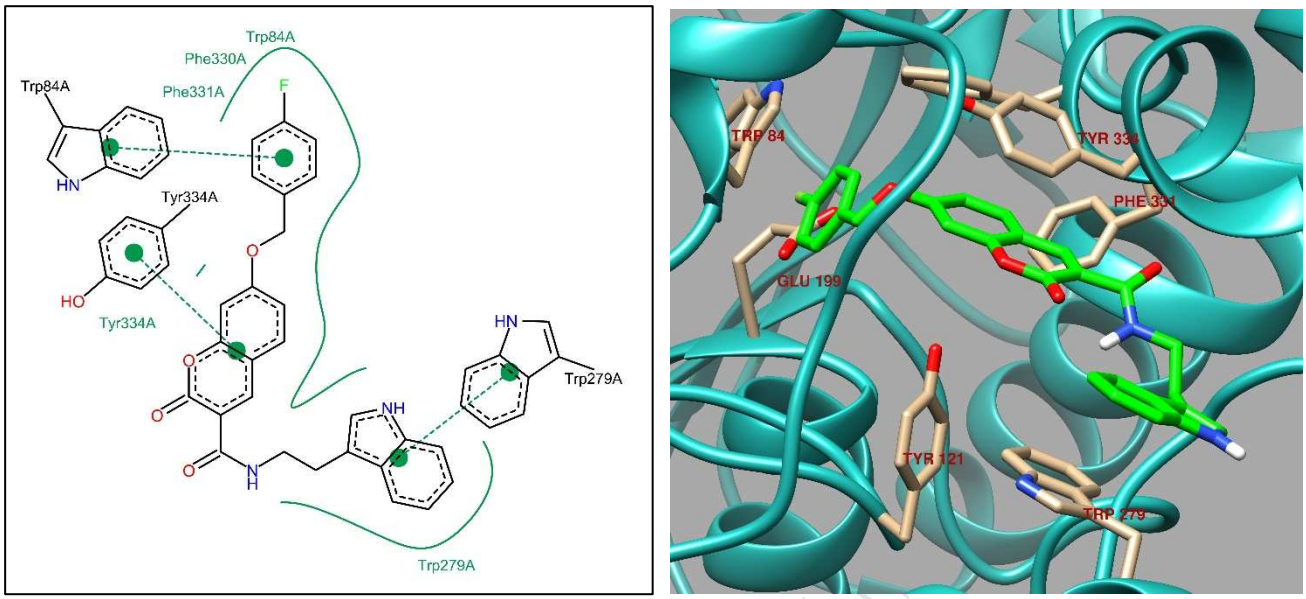
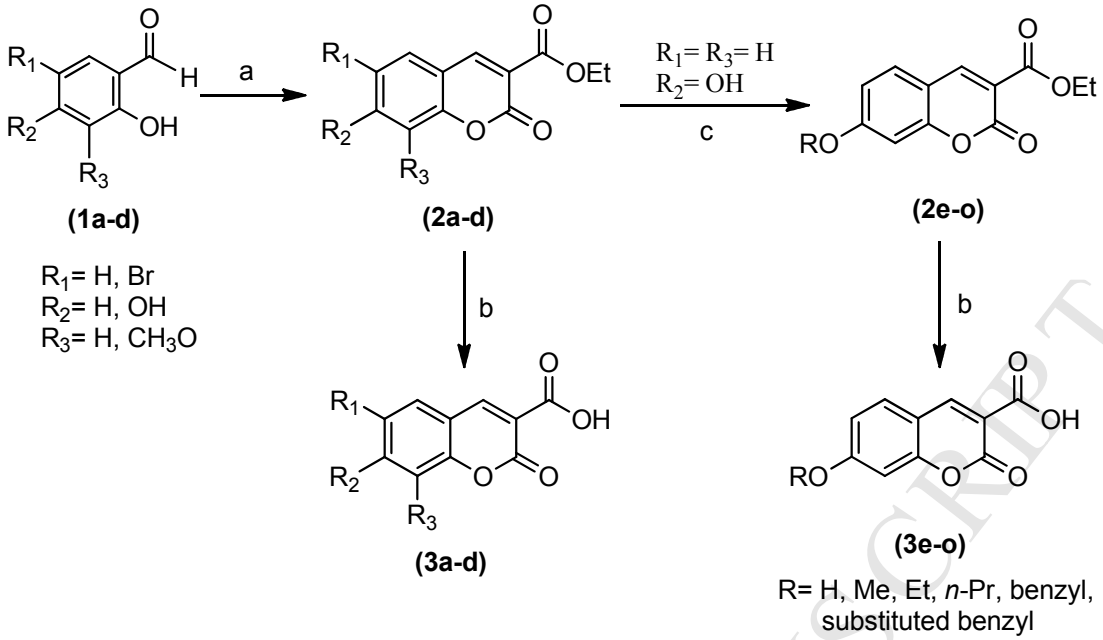
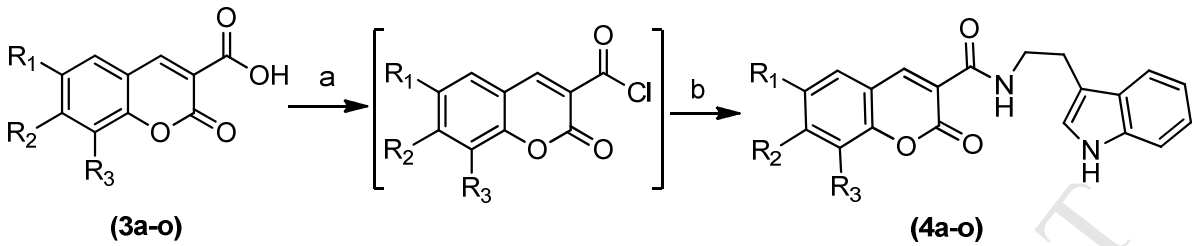


Figure 3. 2D (left) and 3D (right) representation of interactions of compound **4o** in the active site of AChE.



Scheme 1. Synthesis of intermediates **3a**-**p**. Reagents and conditions: (a) diethyl malonate, piperidine, EtOH, reflux ; (b) NaOH (2N) and then HCl (2N); (c) R-X, K_2CO_3, DMF.



Scheme 2. Synthesis of final compounds **4a-o**. Reagents and conditions: (a) $SOCl_2$; (b) Tryptamine, K_2CO_3, dry toluene, reflux.

Research Highlights

- Some coumarin-3-carboxamides of tryptamine were synthesized as anticholinesterases
- All compounds were tested against AChE and BuChE.
- Most of the compounds had significant activity toward AChE.
- The introduction of 7-benzyloxy moiety on coumarin moiety improved the anti-AChE activity.
- The 7-(4-fluorobenzyl)oxy analog **4o** was the most potent compound (IC_{50}= 0.16 µM).