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Design and synthesis of novel arylisoxazole-chromenone carboxamides: Investigation of biological activities associated with Alzheimer's disease

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This paper is dedicated to the memory of our unique teacher in Chemistry and Medicinal Chemistry, Professor Abbas Shafiee (1937-2016).

Abstract: A novel series of hybrid arylisoxazole-chromenone carboxamides were designed, synthesized, and evaluated for their cholinesterase (ChE) inhibitory activity based on the modified Ellman's method. Among synthesized compounds, 5-(3-nitrophenyl)-*N*-(4-((2-oxo-2*H*-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11h**) depicted the most acetylcholinesterase (AChE) inhibitory activity (IC_{50} = 1.23 μM) and 5-(3-chlorophenyl)-*N*-(4-((2-oxo-2*H*-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11e**) was found to be the most potent butyrylcholinesterase (BChE) inhibitor (IC_{50} = 9.71 μM). Compound **11h** was further investigated for its BACE1 inhibitory activity as well as neuroprotectivity and metal chelating ability as important factors involved in onset and progress of Alzheimer's disease. It could inhibit BACE1 by 48.46% at 50 μM. Also, it showed 6.4% protection at 25 μM and satisfactory chelating ability toward Zn^{2+}, Fe^{2+}, and Cu^{2+} ions. Also, docking studies of compounds **11h** and **11e** confirmed desired interactions with those amino acid residues of the AChE and BChE, respectively.

Key words: Alzheimer's disease, BACE1, Cholinesterase, Chromenone, Coumarin, Docking, Isoxazole, Metal chelating, Neuroprotectivity

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder, known as the most common cause of dementia in elderly people leading to the various difficulties such as dependence, disability, and mortality in patients.[1] It is estimated that 44 million people currently live with dementia worldwide and is predicted to increase to more than triple by 2050.[2] In this regard, AD has been recognized as a global public health priority by the World Health Organization due to the versatile and controversial health and economic issues. AD is characterized by decline in memory, language, problem solving and other cognitive skills that decrease the ability to perform daily

activities due to destruction of neurons in the brain.[3] At the molecular level, AD is associated with reduced synaptic levels of acetylcholine (ACh), abnormal deposits of β-amyloid peptide (Aβ), and the presence of neurofibrillary tangles in the special areas of the brain such as the hippocampus and cortex (cholinergic hypothesis) which are related to the memory and cognition.[4] Loss of pre-cholinergic cells and decreased access to synaptic acetylcholine can lead to cognitive impairment in the patients with AD.[5] AD is the first disease in which a broad erosion of the frontal cholinergic system has been observed and cholinergic neurons may be lost up to 75% in the basal forehead and septum area.[6-10] Therefore, one of the therapeutic approaches is to increase the level of acetylcholine (ACh) in the brain by blocking the cholinesterase (ChE) enzymes responsible for the ACh hydrolysis. Furthermore, several lines of recent evidence have suggested that when the level of AChE gradually decreases the level of BChE significantly increases in the hippocampus and temporal cortex of the brain of patients with AD.[11] Accordingly, the concurrent inhibition of both AChE and BChE may improve AD signs and symptoms.[12] Due to the fact that AD is a multi-factorial disease, design and synthesis of compounds affecting multiple pathological pathways are highly in demand. A wide range of heterocyclic compounds possessing ChEI activity have been documented in the literature.[13] Coumarine derivatives have been investigated for their effectiveness on various pathological paths in AD. For example, various hydroxycoumarin derivatives were synthesized and tested as AChE inhibitors and among them compound **A** (Fig. 1) was found to be active toward AChE (IC_{50} = 4.5 μM).[14] Another study showed that compounds possessing coumarin-amide moiety showed metal chelating abilities toward Cu^{2+} and Fe^{2+} ions as well as neuroprotective properties on pc12 cell-line. In this respect, compound **B** depicted 84.9% cell viability at the concentration of 50 μM (Fig. 1).[15] Also, we recently reported that compound **C** (Fig. 1) demonstrated important selective BChEI activity (IC_{50} = 0.32 μM) comparing with

AChEI activity (IC_{50} = 32.38 μM). Moreover, it could inhibit the BACE1 by 22%.[16] According to our previous study, new derivatives of arylisoxazole-1,2,3-triazole showed moderate to good anti-AChE activity. It was found that compound **D** (Fig.1) was the most potent AChE inhibitor (IC_{50} = 0.90 μM), however, it inhibited BChE with IC_{50} value of 31.20 μM.[17] In addition, synthesis and anti-ChE activity of arylisoxazole-*N*-phenylpiperazine hybrids were investigated and compound **E** (Fig. 1) depicted good anti-AChE activity (IC_{50} = 21.85 μM).[18] Also, hybridization of arylisoxazole-indole moieties (compound **F**) led to moderate ChEI activity and compound **F** (Fig. 1) showed inhibitory activity toward AChE with IC_{50} value of 29.52 μM.[19] In continuation of research on ChEI activity of arylisoxazole hybrids arylisoxazole-*N*-benzylpyridinium derivatives were synthesized and among them compound **G** (Fig.1) was the most potent and selective BChE inhibitor (IC_{50} = 0.32 μM); however it was less active toward AChE (IC_{50} = 16.82 μM).[20] It should be noted that the presence of the amide moiety played an important role in inducing appropriate ChEI activity which has been considered in several studies (**B-G**). Herein, in continuation of our studies on developing anti-ChE compounds (**C-G**) new arylisoxazole-chromenone hybrids were designed, synthesized, and evaluated for biological tests involved in onset and progress of AD (Fig. 1).

Results and discussion

Chemistry

Synthesis of required starting materials and target compounds **11a-m** has been schematically described in Schemes 1-3. Required precursors **3** were prepared by the reaction of diethyl oxalate (**1**) and acetophenone derivatives **2** in the presence of sodium in dry ethanol. Then, reaction of the later compounds and hydroxylamine hydrochloride (**4**) in ethanol under reflux conditions for 3 h

gave related ethyl 5-arylisoxazole-3-carboxylate derivatives **5** which tolerated hydrolysis under basic conditions leading to the formation of the corresponding carboxylic acid derivatives **6** [21] (Scheme 1). On the other hand, 7-(4-aminophenoxy)-2*H*-chromen-2-one (**10**)[22] was prepared by the reaction of 7-hydroxy-coumarin (**7**) and 1-fluoro-4-nitrobenzene (**8**) in DMF at 60-80 °C following with the reduction of nitro group in the presence of Zn/NH_4Cl in the mixture of H_2O/MeOH at room temperature (Scheme 2). Finally, the reaction of compounds **6** and **10** in the presence of hydroxybenzotriazole (HOBT) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) in dry acetonitrile at room temperature led to the formation of arylisoxazole- chromonenone carboxamide hybrids **11a-m** (Scheme 3).

Anticholinesterase activity

All synthesized compounds **11a-m** were tested *in vitro* through the modified Ellman's method comparing with donepezil as the reference drug. For each compound, 50% inhibitory concentration (IC_{50}) was determined and reported in Table 1. It is clear that electronic and steric properties of substituents on the aryl ring connected to the isoxazole moiety as well as their position played significant roles in the inhibitory activity.

In the case of AChEI activity, compound **11h** possessing 3-nitroaryl group was found as the most potent compound (IC_{50} = 1.23 μM). Interestingly, changing the position of nitro from 3- to 4- (compound **11i**) led to the deletion of activity (IC_{50} > 100 μM). It seems that the efficacy of strong electron-withdrawing group (NO_2) directly depended on the position of that group on the aryl ring. Our data related to the AChEI activity also demonstrated that the presence of strong electron-donating group (OMe) on the aryl ring completely removed anti-ChE activity in such a manner that compounds **11j-l** possessing OMe group either at 3- or 4- position did not show activity (IC_{50} > 100

μM). It should be noted that the presence of moderate electron-donating group (Me) led to the relatively weak inhibitory activity (IC_{50} = 71.55 μM). However, the absence of substituents on the aryl ring (compound **11a**) afforded moderate activity (IC_{50} = 44.94 μM). Another point comes back to the derivatives **11b-g** containing halogens. It was found that the presence of halogens (F, Cl, and Br) led to good AChE activity; however, the activity was affected by their positions. Among halogenated compounds **11b-g**, compound **11e** having 3-chloroaryl moiety showed the best inhibitory activity (IC_{50} = 6.49 μM). Changing the position of Cl to 2- or 4- did not afford better activity and IC_{50} values for compound **11d** and **11f** were calculated as 16.62 and 17.47 μM, approximately 3 times weaker than compound **11e**. The presence of bromine at 4- position of aryl ring (compound **11g**) led to the lower activity (IC_{50} = 20.14 μM) comparing with compound **11f** at the same position. Also, compound **11c** having 4-fluoroaryl group showed similar activity (IC_{50} = 20.02 μM) to compound **11g**. It seems that the efficacy of halogens can be considered as Cl>F~Br for 4-halosubstituted derivatives. It is worth mentioning that compound **11b** having F at 2- position of aryl group (IC_{50} = 13.63 μM) was found to be more active than its analogue, compound **11c**. In addition, it demonstrated relatively higher activity than its counterpart, compound **11d**.

In the case of BChEI activity, the best activity was related to compound **11e** possessing 3-chloroaryl group connected to isoxazole moiety (IC_{50} = 9.71 μM), however, the presence of chlorine at 2- and 4- position (compounds **11d** and **11f**) afforded no BChE activity (IC_{50} > 100 μM). Also, the same result was obtained by compound **11g** having 4-brominated moiety (IC_{50} > 100 μM). Unlike compounds **11f** and **11g**, 4-fluorinated derivative **11c** showed good BChEI activity (IC_{50} = 22.57 μM) but its analogue was not active and the calculated IC_{50} was more than 100 μM. Another point was related to compound **11h** (the most active AChE inhibitor) which was inactive towards BChE and compound **11i** was inversely active and IC_{50} value was calculated as 19.55 μM.

It should be noted that in the case of electron-donating groups (Me and OMe) and lacking substituents (H), no anti-BChE activity was observed. It can be concluded that the presence of electron-donating group (Me and OMe) induced no ChEI activity neither anti-AChE nor anti-BChE activity. The exception comes back to the compound **11m** which depicted weak anti-AChE activity (IC_{50} = 71.55 μM). On the contrary, strong electron-withdrawing group (NO_2) and halogens (F, Cl, and Br) were found to be beneficial for inducing ChE inhibitory activity, however, it should be mentioned that most compounds were inactive toward BChE. Concisely, the presence of NO_2 and Cl groups at 3- position of aryl group connected to the isoxazole moiety played important roles in the ChEI activity. Compound **11h** selectively inhibited AChE (IC_{50} = 1.23 μM) while it showed no activity against BChE and compound **11i** lacking AChEI activity showed relatively good BChE activity (IC_{50} = 19.55 μM).

Comparing our results from anti-AChE activity of arylisoxazole-coumarine hybrids with previous studies on arylisoxazole hybrids (**E**-**G**) showed that compound **11h** (IC_{50} = 1.23 μM) was stronger than arylisoxazole-*N*-phenylpiperazine **E** (IC_{50} = 21.85 μM), arylisoxazole-indole **F** (29.52), and arylisoxazole-*N*-pyridinium **G** (IC_{50} = 16.82) hybrids, but it was not as active as compound **E**, arylisoxazole-1,2,3-triazole hybrid (IC_{50} = 0.90 μM).[18] BChEI activity of newly synthesized compound **11** was comparable to those compounds **D**-**F**, however, they were much less active than arylisoxazole-*N*-pyridinium hybrid **G** (IC_{50} = 0.32) and coumarin-*N*-pyridinium hybrid **C** (IC_{50} = 0.32).[20]

Kinetic Studies

Kinetic studies were performed to investigate the mechanism of inhibition by compounds **11h** and **11e** against AChE and BChE, respectively. Graphical analysis of the reciprocal Lineweaver–Burk plot

related to compound **11h** described a competitive inhibition (Fig. 2) which prevented the substrate from binding to the active site. Also, the *Ki* value was calculated using the secondary plot as *Ki* = 2.28 μM. In the case of compound **11e**, the same study demonstrated mixed type inhibition against BChE confirming that it could simultaneously bind to the CAS and PAS of the enzyme. It should be noted that *Ki* = 9.11 μM was calculated for the corresponding inhibition.

2.4. Metal chelating Activity

Compound **11h** was tested for its metal chelating ability towards Fe^{2+}, Cu^{2+}, and Zn^{2+} ions (Fig. 4) The UV spectrum of methanolic solution (20 μM) of that compound showed two characteristic absorption peaks at 316.3 and 273.6 nm. After interaction of compound **11h** with Zn^{2+} ions for 30 min, increase of absorption at the same wavelengths was observed. Interaction of compound **11h** with Fe^{2+} ions demonstrated three absorption peaks at 318.4, 312.0 and 273.6 nm indicating two red and blue shifts which are associated with the formation of different complexes. Also, similar results were obtained in the case of interaction of compound **11h** with Cu^{2+} ions which showed three absorption peaks at 320.5, 312.0, and 273.6 nm.

The stoichiometry of complex **11h**-Cu^{2+} was also studied (Fig. 5). The concentration of the test compound **11h** was 20 μM and the final concentration of Cu^{2+} ranged from 0-40 μM with 4 μM intervals at 273.6 nm. The plot was obtained by the corresponding absorption against the mole fraction of Cu^{2+} to ligand **11h**. According to the plot, the ratio 1:1 complexation ration of **11h**-Cu^{2+} can be seen at the fracture point of the plot with the mole fraction of 0.6.

BACE1 enzymatic assay

β-Secretase (BACE-1) inhibitors have been emerged as the important approach to the treatment of AD due to the role of BACE1 in the proteolytic cleavage of the amyloid protein precursor (APP).[23]

Accordingly, the BACE-1 inhibitory activity of the most potent AChE inhibitor, compound **11h** was evaluated *via* a fluorescence resonance emission transfer (FRET) method. The used kit consisted of BACE1 enzyme and APP peptide based substrate (Rh-EVNLDAEFK- quencher) comparing with OM99-2 (IC_{50} = 0.014 μM) as the reference drug. It was found that compound **11h** induced moderate inhibitory activity; 48.46% and 31.66% inhibition at the concentrations of 50 and 10 μM, respectively.

Neuroprotective effect against Aβ-Induced Damage measured in PC12 Cells

Neuroprotective effect of compound **11h** against damage induced by Aβ25–35 was investigated in PC12 cells by MTT assay.[24] This compound showed 6.4% protection at the concentration of 25 μM comparing with rutin with 13.4% protection at the same concentration.

Molecular docking study

The observed AChE and BChE inhibitory activity of the target arylisoxazole-chromonenone was confirmed through active site-docking simulation of compounds **11h** and **11 j** into 1EVE and **11e**, into 1P0P active sites (Figs. 6-9). The corresponding protein crystal structures for AChE (1EVE) and BChE (1P0P) were retrieved from the protein data bank and co-crystal ligand donepezil was re-docked using smina in Linux platform.[25] The range of minimized affinity values of the poses of ligands **11h, 11j** and **11e** were −12.82 to −11.73 , −12.50 to −11.10 and −11.1 to −10.4 kcal/mol, respectively.

Binding interactions mode of compounds

The interactions of the best-docked conformation of the most potent AChE inhibitor (**11h**) with the active site residues was presented in Fig. 6. According to the 1EVE simulated inhibition docking evaluation, compound **11h** constructed two hydrogen bonds with the active site. The nitrogen of the isoxazole ring formed a hydrogen bond with Gly123 residue and the oxygen of nitroaryl moiety also formed a hydrogen bond with His440 residue of the 1EVE which confirmed the efficacy of the isoxazole and 3-nitroaryl moieties for inducing the desired bioactivity. Furthermore, on the other side of the molecule, chromenone ring formed pi-anion, pi-cation and pi-pi stacking interactions with Trp272, Asp72 and Tyr334, respectively which fixed compound **11h** in the active site. Hydrophobic interactions well stabilized the compound–1EVE complex into the active site (Fig. 6). This cooperative binding might be beneficial to increase the inhibitory activity of the compound–1EVE complex to describe the promising efficacy of compound **11h** against AChE.[26]

The proposed binding mode of compound **11j** is illustrated in Fig. 7. The oxygen of methoxy group formed a hydrogen bond with Gly118 residue of the enzyme, however, it was not apparently beneficial to induce desired AChE inhibitory activity comparing with compound **11h**. In another docking study (Fig. 8.), both compounds **11j** and **11h** as well as native ligand were superimposed into the active site. It was found that the orientation of 3-nitroaryl moiety of compound **11h** resembled that of the native ligand, donepezil. The proposed binding was thus very similar to that of donepezil. The interactions were dominated in the region of His440 and Trp84 amino acid residues due to pronouncing the existence of hydrogen bonds and pi-pi stacking interaction at the catalytic anionic site. However, 3-methoyaryl moiety of compound **11j** represented perpendicular orientation into the active site which misled proper interactions.

Fig. 9. represented binding interactions of compound **11e** over 1P0P. Targeted compound produced conventional H-bonding interaction with the backbone of Pro285 *via* NH of amide moiety. Furthermore, the aryl group was stabilized at specific pocket by side chain of Ala277 *via* pi-alkyl interaction. Also, formation of hydrophobic interactions through π-anion and π-π stack interactions of chromenone moiety of compound **11e** with Gly115 as well as Glu197 and His438 from different position were observed.[27] The aromatic linker between arylisoxazole and chromenone moieties was completely fixed through π-alkyl and π-π stack interactions with Ala328 and Tyr332 residues in the protein's active site.

Conclusions

In conclusion, different and novel arylisoxazole-chromonenone carboxamides were designed, synthesized, and evaluated for their ChEI activity. Among them, compounds **11h** (IC_{50} = 1.23 μM) and **11e** (IC_{50} = 9.71 μM) respectively possessing 3-nitroaryl and 3-chloroaryl groups connected to the arylisoxazole moiety, were found to be the most potent AChE and BChE inhibitors. Furthermore, evaluation of BACE1 inhibitory activity, neuroprotectivity, and metal chelating ability of compound **11h** demonstrated good results which make the compound worthy to be considered for further biological activities associated with AD.

Experimental

All chemicals and reagents were purchased from Merck and Aldrich. Melting points were determined using Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (potassium bromide disks). NMR spectra were recorded on a Bruker 500 spectrometer and chemical shifts were expressed as δ (ppm) with tetramethylsilane

as internal standard. Mass spectra were determined on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV.

Chemistry

*Synthesis of 2,4-dioxo-4-aryl butanoates **3***

Initially, sodium (1 mol) was added to dry ethanol (to 250 ml) and the solution was stirred in an ice bath while a mixture of diethyl oxalate (**1**) (1 mol) and the acetophenone derivative **2** (1 mol) was added dropwise to the solution. The yellow pasty material was left at room temperature for 15 h and after that, it was stirred at 80 °C in water bath for 90 min. The crude product was treated with diluted sulfuric acid to adjust pH = 2. Then, the oily product was extracted using chloroform and washed using saturated $NaHCO_3$ solution and brine. After drying the organic phase using sodium sulfate, the solvent was removed under vacuum to obtain 2,4-dioxo-4-aryl butanoate **3** which was recrystallized from chloroform and hexane.[21]

*4.1.2. Synthesis of ethyl 5-arylisoxazole-3-carboxylates **5***

The mixture of hydroxylamine hydrochloride (**4**) (0.23 mol) and 2,4-dioxo-4-aryl butanoate derivative **3** (0.08 mol) was heated at reflux in ethanol (220 ml) for 3 h. After the completion of the reaction (checking by TLC), half of the solvent was removed and the mixture was poured onto the ice and the precipitate was extracted by dichloromethane and washed with water, NaOH solution (4%), and brine, respectively. The organic phase was dried and the solvent was removed under vacuum to afford ethyl 5-arylisoxazole-3-carboxylate **5**.[21]

*4.1.3. Synthesis of ethyl 5-arylisoxazole 3-carboxylic acids **6***

Ethyl 5-arylisoxazole-3-carboxylate **5** (1 mmol) was hydrolyzed in the presence of KOH (3 mmol) in methanol (15 ml) under reflux conditions for 3 h. After the completion of the reaction (checking

by TLC), the mixture was poured into the water and ice, and the concentrated HCl was added drop wise to form a white precipitated product. The mixture was stirred at room temperature for 20 min and the precipitate was filtered off resulting in the corresponding acid **6**.

*4.1.4. Synthesis of 7-(4-nitrophenoxy)-2H-chromen-2-one (**9**)*

The mixture of 7-hydroxy-coumarin (**7**) (10 mmol) and potassium carbonate (K_2CO_3, 10 mmol) in DMF (10 ml) was stirred at 60-80 °C for 1 h. Then, 1-fluoro-4-nitrobenzene (**8**) (10 mmol) was added and the reaction mixture was stirred at 60-80 °C for 24 h. After the completion of the reaction (checking by TLC), the mixture was added to ice and water, the precipitated product was filtered off, and washed thoroughly with water; resulting in 7-(4-nitrophenoxy)-2*H*-chromen-2-one (**9**).

*4.1.5. Synthesis of 7-(4-aminophenoxy)-2H-chromen-2-one (**10**)*

The mixture of compound **9** (5 mmol) and ammonium chloride (30 mmol) in the mixture of methanol and water (80 ml, 7:1) was stirred at room temperature for 5 min. Then, zinc powder (100 mmol) was gradually added within 10 min and the reaction was stirred for 40 min. The reaction was checked by TLC and at the end of reaction, the mixture was filtered using sintered glass funnel and celite, and the filtered solution was added onto the ice. The resulting precipitated product was filtered off to give 7-(4-aminophenoxy)-2*H*-chromen-2-one (**10**).

*4.1.6. General procedure for the preparation of chromonen-3-aryl isoxazole-5-carboxamide derivatives **11 a-m***

The mixture of 5-arylisoxazole-3- carboxylic acid derivative **6** (1 mmol), HOBT (1 mmol) and EDCI (1 mmol) was stirred in dry acetonitrile at room temperature for 1 h, then compound **10** (1 mmol) was added to the mixture and the reaction was continued overnight. After the completion of the reaction (checking by TLC), the solvent was evaporated under vacuum and the crude was extracted using chloroform and washed with water and solutions of $NaHCO_3$ 10%, NaCl and citric acid,

respectively. After drying the organic phase over sodium sulfate, the solvent was removed and the solid product was recrystallized from ethyl acetate and *n*-hexane.

*N-(4-((2-Oxo-2H-chromen-7-yl)oxy)phenyl)-5-phenylisoxazole-3-carboxamide (**11a**)*

Yield: 67.1%; M.p. = 215-225 °C; IR (KBr): 3330, 3150, 1732, 1672, 1657, 1541 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.92 (s, 1H, NH), 8.04 (d, *J* = 9.5 Hz, 1H, H4), 7.98 (d, *J* = 6.4 Hz, 2H, H2", H6"), 7.90 (d, J = 8.8 Hz, 1H, H3', H5'), 7.71 (d, J = 8.6 Hz, 1H, H5), 7.58-7.55 (m, 3H, H3", H4", H5"), 7.52 (s, 1H, isoxazole), 7.19 (d, *J* = 8.8 Hz, 1H, H2', H6'), 6.96 (dd, *J* = 8.6, 2.2 Hz, 1H, H6), 6.93 (d, *J* = 2.0 Hz, H8), 6.37 (d, *J* = 9.5 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 170.6, 160.6, 160.0, 159.9, 157.2, 155.1, 151.0, 144.1, 135.0, 131.0, 130.1, 129.4, 126.3, 125.9, 122.4, 120.5, 114.2, 114.0, 113.8, 104.5, 100.2 ppm. MS: *m/z* (%) = 424 $[M]^+$ (100), 306 (95), 279 (79), 251 (58), 172 (20), 105 (53), 89 (41), 77 (32). Anal. calcd. for $C_{25}H_{16}N_2O_5$: C, 70.75; H, 3.80; N, 6.60. Found: C, 70.51; H, 3.62; N, 6.83.

*5-(2-Fluorophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11b**)*

Yield: 53.9%; M.p. = 240-244 °C; IR (KBr): 3315, 3184, 1726, 1666, 1620, 1535, 1495 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.91 (s, 1H, NH), 8.04-8.02 (m, 2H, H4, H6"), 7.90 (d, *J* = 8.4 Hz, 1H, H3', H5'), 7.71 (d, *J* = 8.4 Hz, 1H, H5), 7.64-7.63 (m, 1H, H4"), 7.48 (t, *J* = 9.5 Hz, 1H, H5"), 7.45-7.42 (m, 1H, H3"), 7.30 (s, 1H, isoxazole), 7.19 (d, *J* = 8.4 Hz, 1H, H2', H6'), 6.96 (d, *J* = 8.4 Hz, 1H, H6), 6.92 (s, 1H, H8), 6.36 (d, *J* = 9.4 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 165.0, 160.6, 160.0, 159.8, 158.5 (d, J_{C-F} = 251.2 Hz), 156.9, 155.1, 151.1, 144.1, 135.0, 133.3, 130.1, 128.0, 125.5, 122.5, 120.5, 116.8 (d, J_{C-F} = 20.9 Hz), 114.4 (d, J_{C-F} = 11.8 Hz), 114.2, 114.0, 113.8, 104.6, 103.0 (d, J_{C-F} = 8.8 Hz) ppm. MS: *m/z* (%) = 442 $[M]^+$ (85), 401 (17), 279 (23), 251 (25), 190 (20), 135 (100), 123 (26), 89 (21), 77 (14). Anal. calcd. for $C_{25}H_{15}FN_2O_5$: C, 67.87; H, 3.42; N, 6.33. Found: C, 67.95; H, 3.12; N, 6.60.

*5-(4-Fluorophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11c**)*

Yield: 44.5%; M.p.> 250 °C; IR (KBr): 3369, 3149, 1732, 1673, 1617, 1537, 1507 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.92 (s, 1H, NH), 8.06-8.03 (m, 2H, H4, H5), 7.89 (d, *J* = 8.7, 2H, H3', H5'), 7.73-7.68 (t, *J* = 8.7 Hz, 2H, H2", H6"), 7.51 (s, 1H, isoxazole), 7.43 (t, *J* = 8.7 Hz, 2H, H3", H5"), 7.19 (d, *J* = 8.7 Hz, 2H, H2', H6'), 6.96 (d, *J* = 8.6 Hz, 1H, H6), 6.93 (s, 1H, H8), 6.37 (d, *J* = 9.6 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 170.0, 166.1 (d, J_{C-F} = 250.0 Hz), 160.6, 160.4, 160.0, 157.1, 155.1, 151.0, 144.1, 136.8, 135.0, 131.8 (d, J_{C-F} = 12.5 Hz), 130.1, 128.5 (d, J_{C-F} = 11.2 Hz), 122.4, 120.5, 116.6 (d, J_{C-F} = 22.4 Hz), 114.1 (d, J_{C-F} = 17.2 Hz), 113.8, 104.5, 100.4 (d, J_{C-F} = 10.1 Hz) ppm. MS: *m/z* (%) = 442 $[M]^+$ (10), 401 (26), 191 (13), 135 (100), 121 (13), 92 (17), 77 (17), 55 (21), 41 (25). Anal. calcd. for $C_{25}H_{15}FN_2O_5$: C, 67.87; H, 3.42; N, 6.33. Found: C, 67.54; H, 3.65; N, 6.11.

*5-(2-Chlorophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11d**)*

Yield: 63.6%; M.p. = 210-215 °C; IR (KBr): 3330, 3094, 1732, 1676, 1675, 1544, 1480 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.62 (s, 1H, NH), 7.95 (m, 1H, H4"), 7.75-7.73 (m, 3H, H3", H6", H5), 7.65 (d, *J* = 9.5 Hz, 1H, H4), 7.55 (t, *J* = 4.5 Hz, 1H, H5"), 7.46-7.43 (t, *J* = 4.5 Hz, 2H, H3', H5'), 7.41 (s, 1H, isoxazole), 7.12 (d, *J* = 8.0 Hz, 1H, H2', H6'), 6.91 (d, *J* = 9.0 Hz, 1H, H6), 6.89 (s, 1H, H8), 6.30 (d, *J* = 9.5 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 167,5, 160,6, 160.0, 159.6, 157.0, 155.0, 151.1, 144.1, 135.0, 132.4, 131.1, 131.0, 130.1, 130.0, 128.1, 125.0, 122.5, 120.5, 114.2, 114.0, 113.8, 104.6, 104.1 ppm. MS: *m/z* (%) = 460 $[M+2]^+$ (31), 458 $[M]^+$ (93), 279 (100), 251 (77), 222 (18), 206 (20), 139 (90), 111 (35), 89 (55), 75 (23), 63 (37), 50 (17). Anal. calcd. for $C_{25}H_{15}ClN_2O_5$: C, 65.44; H, 3.29; N, 6.11. Found: C, 65.27; H, 3.48; N, 5.97.

*5-(3-Chlorophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11e**)*

Yield: 44%; M.p.= 242-244°C; IR (KBr): 3340, 3078, 1724, 1671, 1620, 1544, 1508 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.83 (s, 1H, NH), 8.02 (m, 2H, H5", H5), 7.93-7.89 (m, 3H, H6", H3', H5'), 7.70 (d, *J* = 8.6 Hz, 1H, H4"), 7.60-7.59 (m, 3H, H4, H2", Isoxazole), 7.18 (d, *J* = 8.9 Hz, 2H, H2', H6'), 6.95 (dd, *J* = 2.0, 8.5 Hz 1H, H6), 6.91 (d, *J* = 2.2 Hz, 1H, H8), 6.35 (d, *J* = 9.5 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 169.5, 161.0, 160.4, 160.4, 157.4, 155.5, 151.5, 144.5, 135.4, 134.6, 131.7, 131.1, 130.5, 128.6, 126.0, 124.9, 122.9, 120.9, 114.6, 114.5, 114.2, 105.0, 101.8 (d, J_{C-F} = 8.8 Hz) ppm. MS: *m/z* (%) = 460 $[M+2]^+$ (33), 458 $[M]^+$ (100), 279 (27), 252 (39), 206 (21), 139 (37), 111 (20), 89 (38). Anal. calcd. for $C_{25}H_{15}ClN_2O_5$: C, 65.44; H, 3.29; N, 6.11. Found: C, 65.62; H, 3.08; N, 6.36.

*5-(4-Chlorophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11f**)*

Yield: 36.5%; M.p. >250 °C; IR (KBr): 3327, 3093, 1726, 1669, 1611, 1538 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.58 (s, 1H, NH), 7.77 (d, *J* = 8.0 Hz, 2H, H3", H5"), 7.73 (d, *J* = 8.0 Hz, 2H, H2", H6"), 7.65 (d, *J* = 9.5 Hz, 1H, H4), 7.50 (d, *J* = 8.0 Hz, 2H, H3', H5'), 7.42 (d, *J* = 8.5 Hz, 1H, H5), 7.13 (d, *J* = 8.0 Hz, 2H, H2', H6'), 7.05 (s, 1H, isoxazole), 6.92 (d, *J* = 8.5 Hz, 1H, H6), 6.89 (s, 1H, H8), 6.31 (d, *J* = 9.5 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 169.5, 160.8, 160.0, 159.0, 157.2, 155.1, 151.2, 144.2, 135.8, 135.1, 132.7, 130.2, 129.5, 127.7, 122.4, 120.6, 114.2, 114.0, 113.9, 104.5, 101.1 ppm. MS: *m/z* (%) = 460 $[M+2]^+$ (19), 458 $[M]^+$ (57), 279 (100), 251 (54), 139 (52), 111 (24), 89 (40), 75 (14), 63 (20). Anal. calcd. for $C_{25}H_{15}ClN_2O_5$: C, 65.44; H, 3.29; N, 6.11. Found: C, 65.15; H, 3.13; N, 5.80.

*5-(4-Bromophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11g**)*

Yield: 42%; M.p. = 235-240 °C; IR (KBr): 3332, 1728, 1671, 1615, 1535, 1502 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.58 (s, 1H, NH), 7.74-7.65 (m, 6H, H3", H5", H2", H6", H3', H5'), 7.56 (d, *J* = 8.0 Hz, 1H, H5), 7.42 (d, *J* = 8.5 Hz, 1H, H4), 7.12 (d, *J* = 8.5 Hz, 2H, H2', H6'), 7.07 (s,

1H, isoxazole), 6.92 (d, *J* = 8.0 Hz, 1H, H6), 6.89 (s, 1H, H8), 6.30 (d, *J* = 8.5 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ = 169.9, 162.4, 160.3, 159.4, 155.8, 150.5, 149.6, 143.2, 135.2, 131.1, 128.3, 126.0, 123.7, 123.0, 121.7, 120.9, 116.5, 114.4, 113.9, 106.1, 101.5 ppm. MS: *m/z* (%) = 504 $[M+2]^+$ (80), 502 $[M]^+$ (80), 279 (100), 251 (76), 222 (15), 234 (12), 196 (10), 185 (53), 155 (25), 133 (14), 115 (10), 89 (53), 76 (21), 63 (25), 50 (10). Anal. calcd. for $C_{25}H_{15}BrN_2O_5$: C, 59.66; H, 3.00; N, 5.57. Found: C, 59.47; H, 3.24; N, 5.73.

*5-(3-Nitrophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11h**)*

Yield: 55.6%; M.p. = 230-243 °C; IR (KBr): 3327, 3090, 1711, 1662, 1614, 1522, 1554 and 1354 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.70 (s, 1H, NH), 8.58 (S, 1H, H2"), 8.36 (d, *J* = 8.0 Hz, 1H, H6"), 8.15 (d, *J* = 8.0 Hz, 1H, H4"), 7.75-7.34 (m, 3H, H5", H5', H3'), 7.65 (d, *J* = 9.5 Hz, 1H, H4), 7.42 (d, *J* = 8.0 Hz, 1H, H5), 7.22 (s, 1H, isoxazole), 7.13 (d, *J* = 8.0 Hz, ٢H, H2', H6'), 6.92 (d, *J* = 8.0 Hz, 1H, H6), 6.89 (s, 1H, H8), 6.30 (d, *J* = 9.5 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ = 169.2, 161.0, 160.5, 160.0, 157.3, 155.5, 151.5, 148.8, 144.5, 135.4, 132.3, 131.5, 130.5, 127.8, 125.7, 122.9, 121.8, 120.8, 115.3, 114.4, 114.2, 104.9, 103.2 ppm. MS: *m/z* (%) = 469 $[M]^+$ (1), 279 (100), 251 (37), 222 (10), 150 (53), 104 (14), 89 (20), 76 (17), 63 (19), 50 (13). Anal. calcd. for $C_{25}H_{15}N_3O_7$: C, 63.97; H, 3.22; N, 8.95. Found: C, 64.22; H, 3.01; N, 8.73.

*5-(4-Nitrophenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11i**)*

Yield: 32.1%; M.p. >250 °C; IR (KBr): 3729, 3327, 1726, 1675, 1617, 1529, 1578 and 1383 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.59 (s, 1H, NH), 8.40 (d, *J* = 8.0 Hz, 2H, H3", H5"), 8.04 (d, *J* = 8.0 Hz, 2H, H2", H6"), 7.75 (d, *J* = 8.0 Hz, 2H, H3', H5'), 7.66 (d, *J* = 9.0 Hz, 1H, H4), 7.42-7.40 (m, 2H, H5, isoxazole), 7.14 (d, *J* = 8.0 Hz, 2H, H2', H6'), 6.93 (d, *J* = 8.0 Hz, 1H, H6), 6.88 (s, 1H, H8), 6.31 (d, *J* = 9.0 Hz, 1H, H3) ppm. ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ = 169.3, 161.8,

160.4, 160.0, 154.9, 151.2, 150.7, 146.8, 143.4, 132.6, 131.6, 129.1, 125.1, 124.1, 122.9, 120.8, 116.0, 114.0, 113.5, 107.0, 101.7 ppm. MS: *m/z* (%) = 469 $[M]^+$ (100), 279 (73), 251 (59), 217 (17), 150 (27), 104 (10), 89 (25), 63 (10). Anal. calcd. for $C_{25}H_{15}N_3O_7$: C, 63.97; H, 3.22; N, 8.95. Found: C, 63.72; H, 3.54; N, 9.16.

*5-(3-Methoxyphenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11j**)*

Yield: 60%; M.p.= 225-229 °C; IR (KBr): 3348, 3120, 1735, 1681, 1602, 1541, 1492 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ = 8.61 (s, 1H, NH), 7.74 (d, *J* = 8.5 Hz, 2H, H3', H5'), 7.65 (d, *J* = 9.5 Hz, 1H, H4), 7.43-7.41 (m, 3H, H6", H5", H5), 7.36 (s, 1H, isoxazole), 7.13 (s, 1H, H2"), 7.12 (s, 1H, H4"), 7.04 (d, *J* = 8.5 Hz, 2H, H2', H6'), 6.91 (d, *J* = 9.0 Hz, 1H, H6), 6.89 (s, 1H, H8), 6.30 (d, *J* = 9.5 Hz, 1H, H3), 3.90 (s, 3H, OCH_3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 169.0, 165.2, 161.0, 160.3, 157.7, 155.5, 151.6, 148.6, 144.6, 135.3, 131.2, 130.6, 129.8, 123.0, 120.9, 118.5, 117.4, 115.2, 114.6, 114.2, 111.4, 105.0, 100.8, 55.9 ppm. MS: *m/z* (%) = 454 $[M]^+$ (100), 279 (47), 252 (56), 222 (11), 202 (23), 135 (55), 107 (20), 89 (47), 77 (23), 63 (24). Anal. calcd. for $C_{26}H_{18}N_2O_6$: C, 68.72; H, 3.99; N, 6.16. Found: C, 68.53; H, 3.71; N, 6.35.

*5-(4-Methoxyphenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide (**11k**)*

Yield: 44.4%; M.p. >250 °C; IR (KBr): 3411, 2925, 1733, 1672, 1617, 1537, 1507 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.85 (s, 1H, NH), 8.02 (d, 2H, *J* = 10.0 Hz, H4), 7.90-7.87 (m, 4H, H3', H5', H2", H6"), 7.71 (d, *J* = 8.5 Hz, 1H, H5), 7.31 (s, 1H, isoxazole), 7.18 (d, *J* = 6 Hz, 2H, H3", H5"), 7.11 (d, *J* = 7.0 Hz, 2H, H2', H6'), 6.96 (d, *J* = 8.5 Hz, 1H, H6), 6.91 (s, 1H, H8), 6.36 (d, *J* = 10.0 Hz, 1H, H3), 3.83 (s, 3H, OCH_3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 168.4, 162.1, 161.7, 161.6, 158.7, 155.3, 153.2, 151.5, 144.6, 130.5, 128.1, 125.8, 122.9, 120.9, 119.2, 115.3, 114.2, 113.5, 112.8, 109.6, 98.9, 55.9 ppm. MS: *m/z* (%) = 454 $[M]^+$ (100), 279 (25), 252

(27), 135 (50), 89 (23), 77 (10). Anal. calcd. for $C_{26}H_{18}N_2O_6$: C, 68.72; H, 3.99; N, 6.16. Found: C, 68.94; H, 3.67; N, 6.21.

5-(3,4-Dimethoxyphenyl)-N-(4-((2-oxo-2H-chromen-7-yl)oxy)phenyl)isoxazole-3-carboxamide ***(11l)***

Yield: 54.4%; M.p.>250 °C; IR (KBr): 3322, 2931, 1731, 1672, 1617, 1538, 1511 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.86 (s, 1H, NH), 8.02 (d, *J* = 9.6 Hz, 1H, H4), 7.88 (d, *J* = 8.5 Hz, 2H, H3', H5'), 7.70 (d, *J* = 8.6 Hz, 1H, H6"), 7.52 (d, *J* = 8.5 Hz, 1H, H5), 7.48 (s, 1H, isoxazole), 7.40 (s, 1H, H2"), 7.18 (d, *J* = 8.5 Hz, 2H, H2', H6'), 7.11 (d, *J* = 8.6 Hz, 1H, H5"), 6.95 (d, *J* = 8.5 Hz, 1H, H6), 6.90 (s, 1H, H8), 6.35 (d, *J* = 9.6 Hz, 1H, H3), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 171.3, 161.0, 160.2, 157.8, 155.5, 151.5, 151.4, 149.7, 149.6, 144.5, 135.4, 130.5, 123.1, 123.0, 120.9, 119.4, 114.5, 114.2, 112.5, 109.7, 104.9, 103.4, 99.3, 56.3, 56.1 ppm. MS: *m/z* (%) = 484 $[M]^+$ (100), 279 (43), 251 (43), 205 (21), 176 (15), 165 (83), 133 (15), 89 (43), 77 (17), 63 (21). Anal. calcd. for $C_{27}H_{20}N_2O_7$: C, 66.94; H, 4.16; N, 5.78. Found: C, 66.75; H, 3.98; N, 5.54.

*N-(4-((2-Oxo-2H-chromen-7-yl)oxy)phenyl)-5-(p-tolyl)isoxazole-3-carboxamide (**11m**)*

Yield: 55%; M.p. >250 °C; IR (KBr): 3416, 3340, 1729, 1673, 1617, 1536, 1506 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ = 10.90 (s, 1H, NH), 8.01-7.70 (m, 6H, H2", H6", H3', H5', H4, H5), 7.38 (m, 3H, H3", H5", isoxazole), 7.18 (d, *J* = 8.4 Hz, 2H, H2', H6'), 6.92 (m, 2H, H6, H8), 6.36 (d, *J* = 9.5 Hz, 1H, H3), 2.37 (s, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 170.8, 164.9, 160.2, 159.6, 155.4, 151.4, 149.5, 144.4, 130.4, 129.3, 128.5, 127.9, 126.2, 123.0, 122.9, 120.9, 117.9, 114.2, 114.1, 106.0, 99.8, 22.6 ppm. MS: *m/z* (%) = 438 $[M]^+$ (100), 279 (41), 252 (58), 222 (11), 186 (24), 133 (11), 119 (75), 89 (47), 63 (17). Anal. calcd. for $C_{26}H_{18}N_2O_5$: C, 71.23; H, 4.14; N, 6.39. Found: C, 71.52; H, 4.33; N, 6.12.

Biological Activity

Anticholinesterase activity

The *in vitro* anticholinesterase activity of all synthesized compounds **11a-m** was assayed using modified Ellman's method, exactly according to our previous study.[16, 28]

Kinetic Studies

The kinetic study for the inhibition of AChE and BChE by compounds **11h** and **11e** was carried out according to the Ellman's method used for the inhibition assay[28] using four different concentrations of inhibitors. For the kinetic study of AChE, compound **11h** was used at the concentrations of 0, 0.43, 3.19, and 6.39 μM. The Lineweaver–Burk reciprocal plot was constructed by plotting 1/V against 1/[S] at variable concentrations of the substrate acetylthiocholine (187.5, 750, 1500, 3000 μM). The inhibition constant *Ki* was achieved by the plot of slopes versus the corresponding concentrations of the compound **11h**. The same method was performed for the kinetic study of BChE using four different concentrations of compound **11e** (0, 5.45, 21.79, and 43.59 μM) and butyrylthiocholine (187.5, 750, 1500, 3000 μM).

Metal chelating Activity

To study the metal binding ability of compound **11h**, a mixture of methanolic solutions of that compound (1 mL) and the related metal (1 mL) with the same final concentrations (20 μM) in a quartz cuvette was incubated at room temperature for 30 min. Then, the absorption spectra were recorded in the range of 200–600 nm. The stoichiometry of complex **11h**-Cu^{2+} was also studied using the molar ratio method.[29] The concentration of compound **11h** was 20 μM and the final

concentration of Cu^{2+} ranged from 0-40 μM with 4 μM intervals at 273.6 nm. The plot was obtained by the corresponding absorption versus mole fraction of Cu^{2+} to ligand **11h.**

BACE1 enzymatic assay

A FRET-based BACE1 enzyme assay kit was used to evaluate the inhibitory activity of compound **11h** against BACE1. The kit was purchased from Invitrogen (former Pan Vera Corporation, Madison, WI) and evaluation procedure was conducted according to the manufacturer's instructions.[23]

Neuroprotection effect against Aβ-induced damage

The neuroprotective effect of compound **11h** in protecting neuronal PC12 cells against damage induced by $A\beta_{25-35}$ was examined according to our previous report.[24]

Molecular docking study

A docking study was performed using Auto Dock Tools (version 1.5.6) and the pdb structures of 1EVE (AChE) and 1P0I (BChE) were taken from the Brookhaven protein database (http://www.rcsb.org). The 3D structures of the selected compounds were created by MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and by Auto Dock Tools converted to pdbqt coordinates. Moreover, the pdbqt coordinates of the enzymes were prepared using the Auto Dock Tools. Before preparation of the pdbqt form of the enzymes, the water molecules and inhibitors were removed. Then, by using Auto Dock Tools, polar hydrogen atoms were added, Kollman charges were assigned, and the obtained protein structures were used as input files for the AUTOGRID program. In AUTOGRID for each atom type in the inhibitors, maps were calculated with 0.375 Å

spacing between grid points, and the center of the grid box was placed at x=2.023, y=63.295, and z=67.062 for AChE and x=137.985, y=122.725, and z=38.78 for BChE. The dimensions of the active site box were set at 40×40×40 Å for AChE and 55×55×55 Å for BChE. Flexible ligand dockings were accomplished for the selected compounds. Each docked system was carried out by 50 runs of the AUTODOCK search by the Lamarckian genetic algorithm (LGA). The best position of each compound was selected for analyzing the interactions between AChE or BChE and the inhibitor, and the results were visualized using Discovery Studio 4.0 Client.

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Author Contribution Statement

Mina Saeedi contributed to the design of compounds and preparation of manuscript. Arezoo Rastegari and Mohammad Mahdavi contributed to the synthesis and characterization of compounds. Seyedeh Sara Mirfazli performed the docking study. Roshanak Hariri performed the biological tests. Najmeh Edraki and Omidreza Firuzi supervised biological tests. Tahmineh Akbarzadeh supervised all phases of the study.

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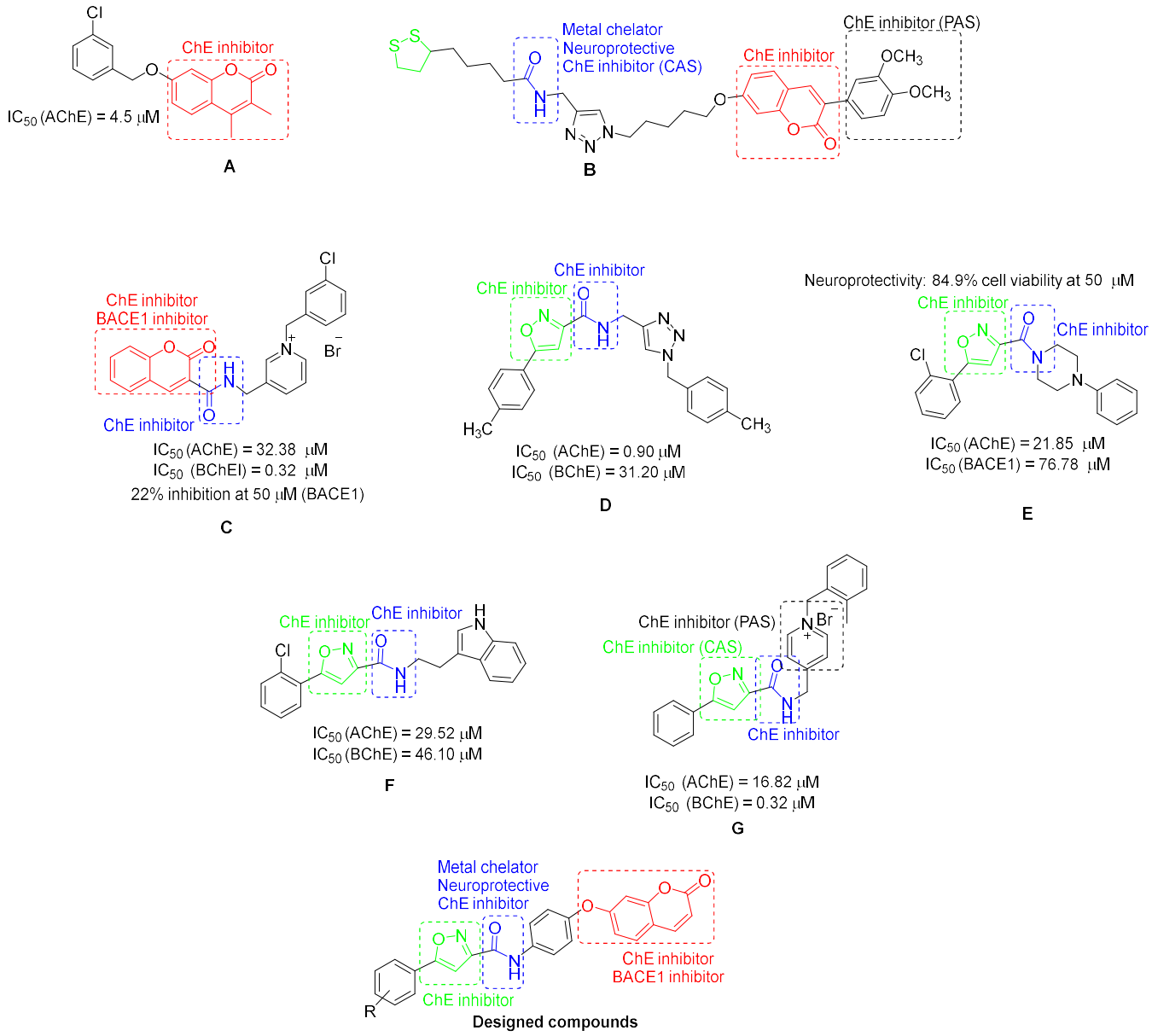

Fig. 1. The structure of ChE inhibitors **A-G** and designed compounds. Compounds **A-G** contain coumarin, arylisoxazole, and amide moieties and designed compounds are new coumarin-arylisoxazole hybrids.

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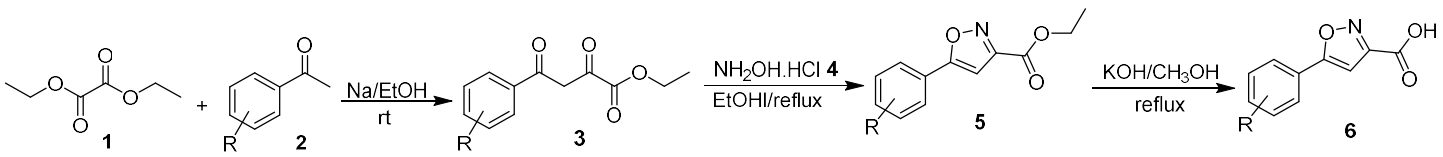

Scheme 1. Synthesis of 5-arylisoxazole 3-carboxylic acids **6**.

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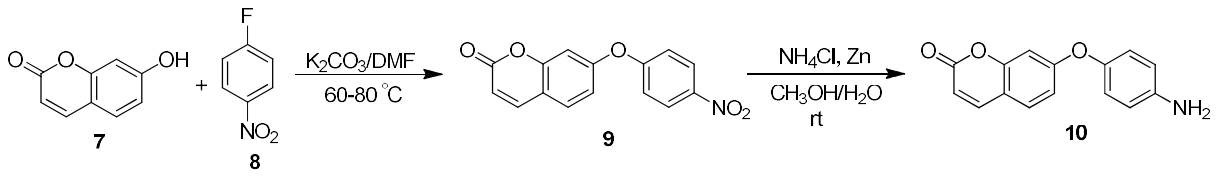

Scheme 2. Synthesis of 7-(4-aminophenoxy)-2*H*-chromen-2-one (**10**).

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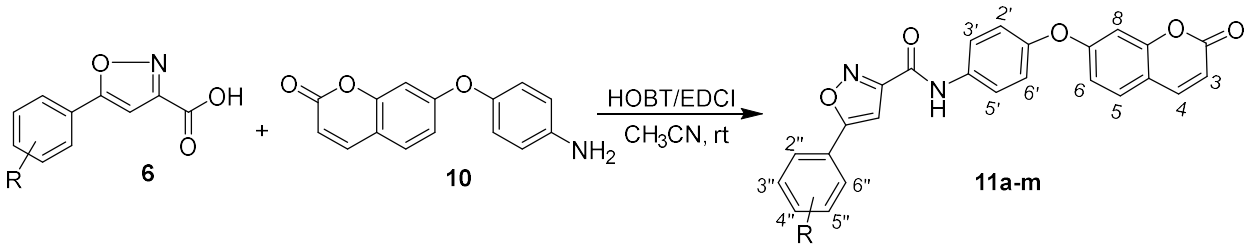

11a: R = H, **11b**: R = 2-F, **11c**: R = 4-F, **11d**: R = 2-Cl, **11e**: 3-Cl, **11f**: R = 4-Cl, **11g**: R = 4-Br, **11h**: R = 3-NO_2, **11i**: R = 4-NO_2, **11j**: R = 3-OCH_3, **11k**: R = 4-OCH_3, **11l**: R = 3,4-diOCH_3, **11m**: R = 4-CH_3

Scheme 3. Synthesis of chromenone-5-arylisoxazole-3-carboxamides **11a-m**.

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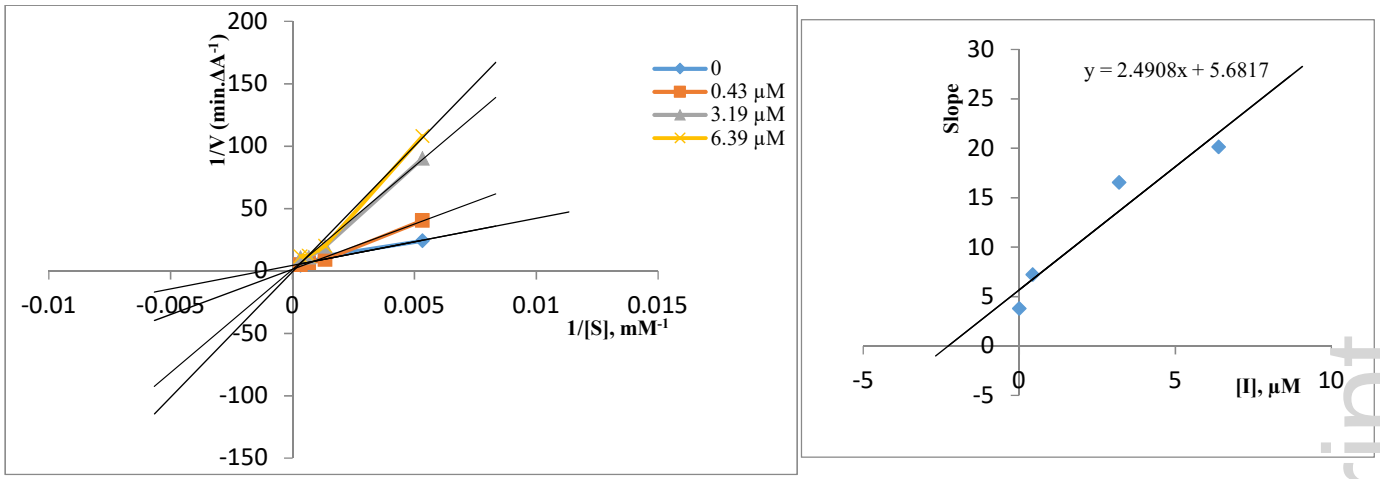

Fig. 2. Kinetic study of inhibitor **11h** (I) against AChE. Lineweaver-Burk plot (left) and double reciprocal Lineweaver-Burk plot (right) are shown.

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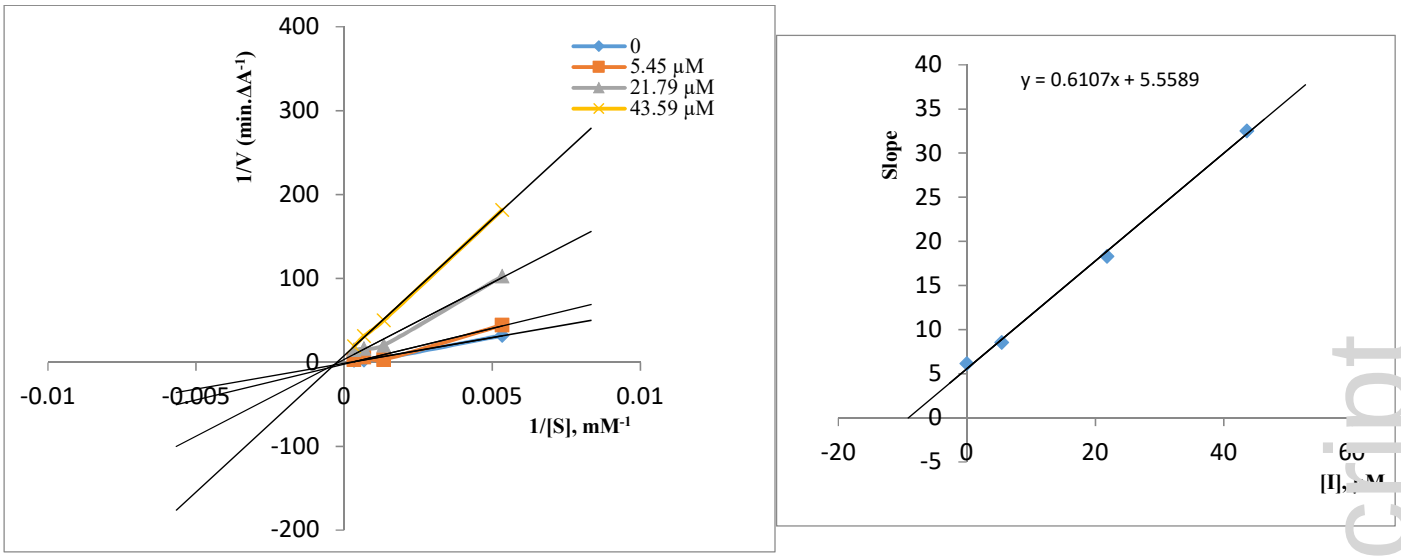

Fig. 3. Kinetic study of inhibitor **11e** (I) against BChE. Lineweaver-Burk plot (left) and double reciprocal Lineweaver-Burk plot (right) are shown.

10.1002/cbdv.201900746

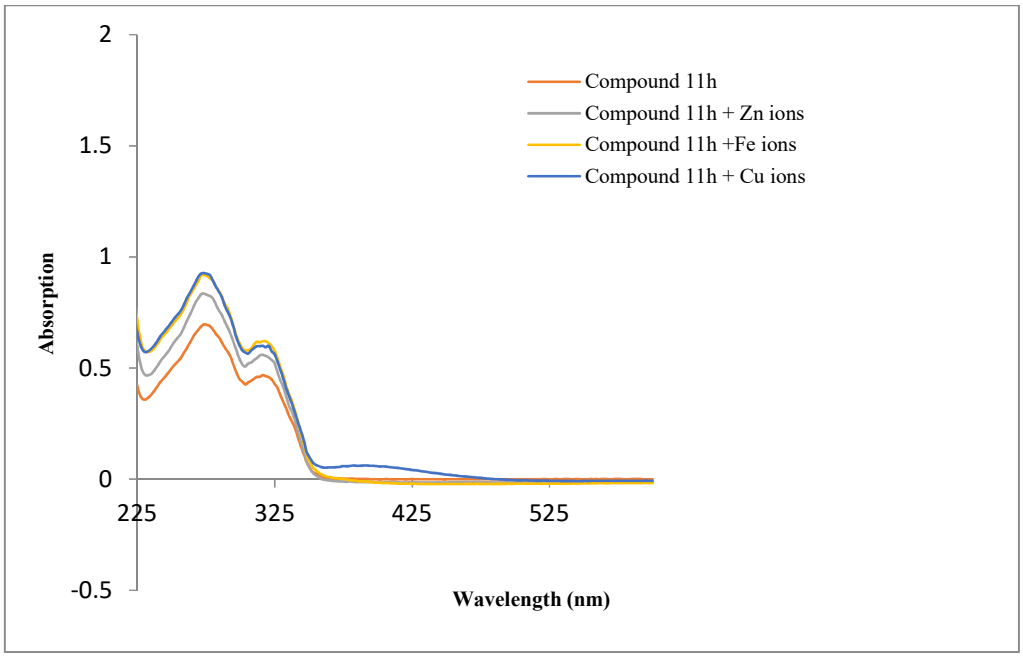

Fig. 4. The absorbance change of compound **11h** alone and in the presence of Zn^{2+}, Fe^{2+}, and Cu^{2+}.

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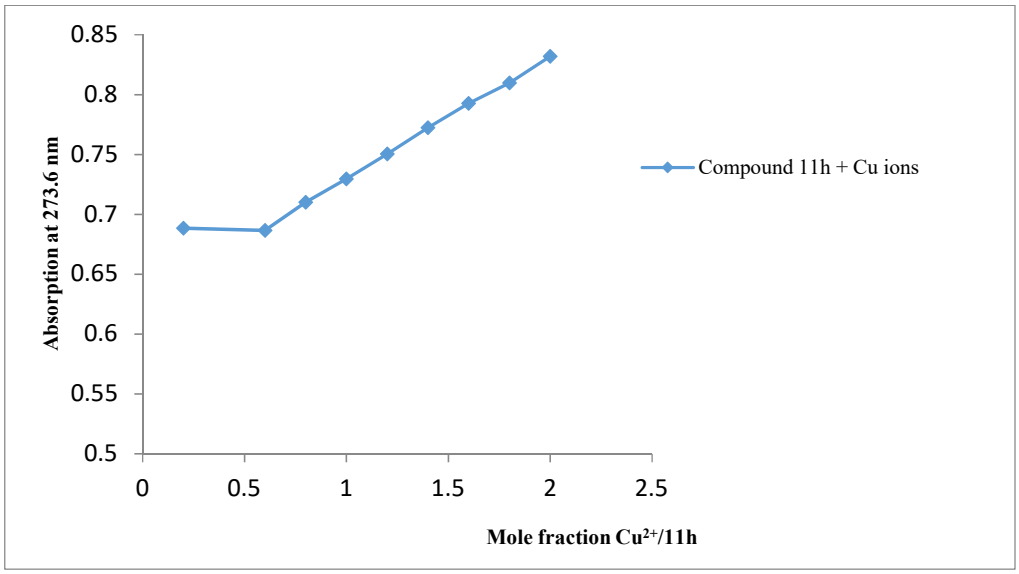

Fig. 5. Absorption at 273 nm (λ_{max}) depending on the mole fraction of Cu^{2+} to compound **11h**.

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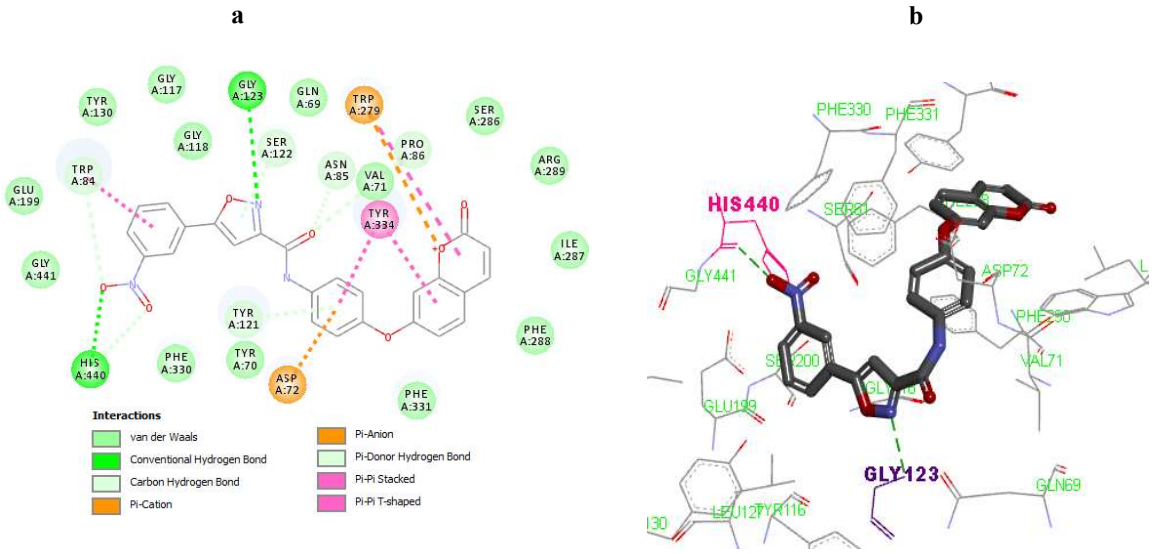

Fig. 6. Two-dimensional orientation of compound **11h** docked into the active site of 1EVE (**a**). Three-dimensional conformation of compound **11h** docked into the active site (**b**).

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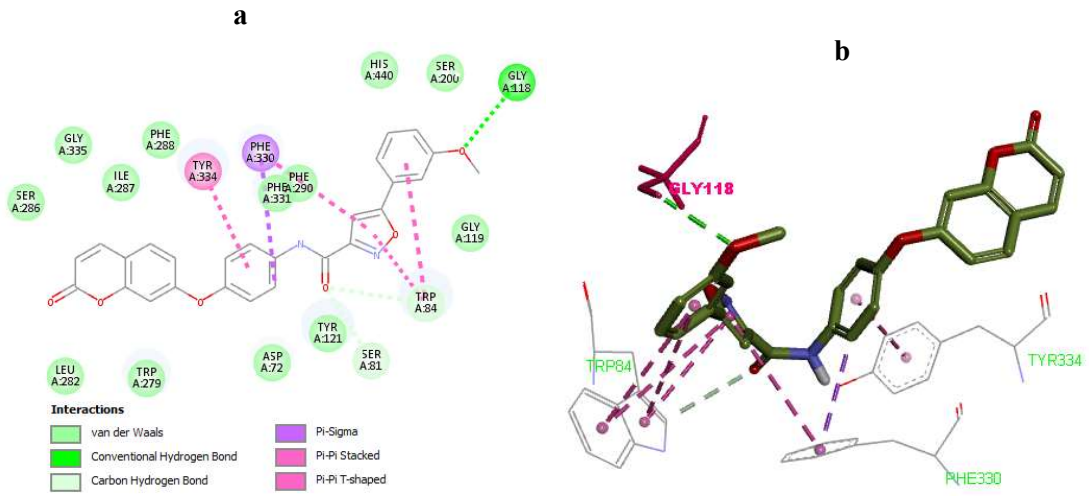

Fig. 7. Two-dimensional orientation of compound **11j** docked into the active site of 1EVE (**a**). Three-dimensional conformation of compound **11j** docked into the active site (**b**).

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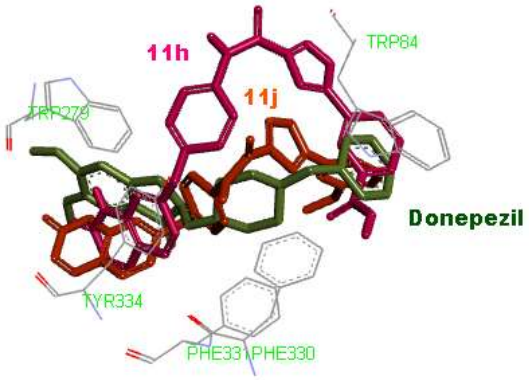

Fig. 8. The lateral representation of re-docked donepezil structure (in green), compounds **11h** (in pink) and **11j** (in orange) 1EVE active site.

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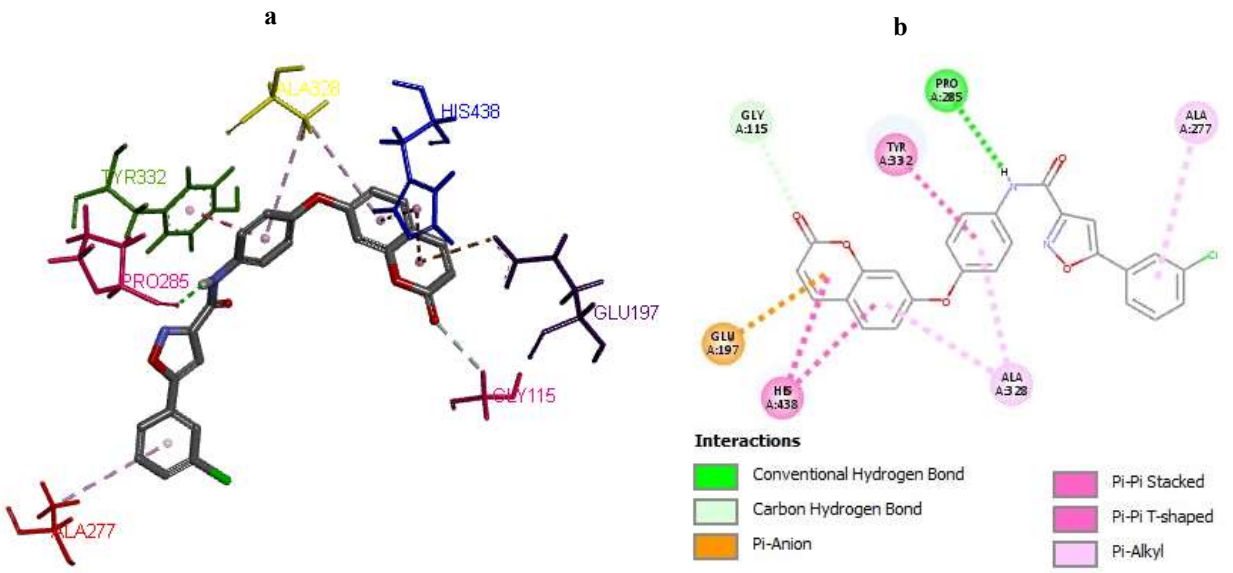

Fig. 9. Two-dimensional orientation of compound **11e** docked into the active site of 1P0P (**a**). Three-dimensional conformation of compound **11e** docked into the active site (**b**).

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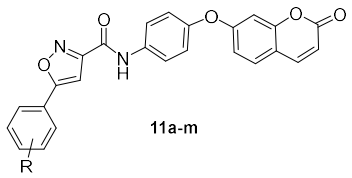
Table 1. Anticholinesterase activity (IC_{50}, μM)[a] of compounds **11a-m**.



11a-m

Entry	Compound **11**	R	AChEI [IC_{50} (μM)]	BChEI [IC_{50} (μM)]
1	**11a**	H	44.94 ± 1.50	>100
2	**11b**	2-F	13.63 ± 0.49	>100
3	**11c**	4-F	20.02 ± 1.54	22.57 ± 0.38
4	**11d**	2-Cl	16.62 ± 0.52	>100
5	**11e**	3-Cl	6.49 ± 0.14	9.71 ± 0.16
6	**11f**	4-Cl	17.47 ± 0.65	>100
7	**11g**	4-Br	20.14 ± 0.63	>100
8	**11h**	3-NO_2	1.23 ± 0.05	>100
9	**11i**	4-NO_2	>100	19.55 ± 0.15
10	**11j**	3-OCH_3	>100	>100
11	**11k**	4-OCH_3	>100	>100
12	**11l**	3,4-diOCH_3	>100	>100
13	**11m**	4-CH_3	71.55 ± 0.95	>100
-	Donepezil	-	0.079±0.002	5.19±0.38

[a] Data represented in terms of mean±SD.