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Synthesis and biological activity of some benzochromenoquinolinones: Tacrine analogues as potent anti-Alzheimer's agents

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

Abstract

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Alzheimer's disease (AD) is a well-known neurodegenerative disorder affecting millions of old people worldwide and the corresponding epidemiological data emphasize the importance of the disease. As AD is a multifactorial illness, various single-target directed drugs that have reached clinical trials have failed. Therefore, various factors associated with outset of AD have been considered in targeted drug discovery. In this work, various benzochromenoquinolinones were synthesized and evaluated for their cholinesterase and BACE1 inhibitory activities as well as neuroprotective and metal-chelating properties. Among the synthesized compounds, 14-amino-13-(3-nitrophenyl)-3,4-dihydro-1*H*-benzo[6,7]chromeno[2,3-*b*]quinoline-7,12(2*H*,13*H*)-dione (**6m**) depicted the best inhibitory activity toward acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with IC_{50}s of 0.86 and 6.03 μM, respectively. Also, it could inhibit β-secretase 1 (BACE1) with IC_{50} = 19.60 μM and showed metal chelating ability toward Cu^{2+}, Fe^{2+} and Zn^{2+}. In addition, docking study demonstrated desirable interactions of compound **6m** with amino acid residues characterizing AChE, BChE, and BACE1.

Keywords: Alzheimer's, BACE1, Benzochromenoquinolinones, Cholinesterase, Docking study, Neuroprotectivity, Tacrine

Introduction

Alzheimer's disease (AD) has recently emerged as a controversial neurodegenerative diseases among elderly people.[1] Cognitive impairment and memory loss along with aggressive behavior and severe changes in the personality are the remarkable problems faced by patients with AD.[2] However, there is no definite cure to stop the progression of the disease and the current drugs only provide symptomatic treatment of AD which is achieved by the acetylcholinesterase inhibitors (AChEIs).[3] Now, it has been proved that different cellular processes such as reduction

of acetylcholine (ACh) in the brain,[4] tau protein aggregation,[5] extracellular plaque deposits of the β-amyloid peptide (Aβ),[6] and metal homeostasis dysfunction[7] are involved in the creation and progression of AD. Multi aspects of the disease have made it difficult and sometimes impossible to be cured and hence, recent research has focused on this fact to develop efficient multi-target compounds against AD.[8,9]

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two enzymes which contribute to the hydrolysis of ACh into acetic acid and choline to allow the corresponding pieces to be reused and renovated into new neurotransmitters for the next message.[10] Cholinesterase inhibitors (ChEIs) are compounds which inhibit those enzymes from breaking down ACh in order to increase its level and duration of action.[11] Currently, there are three FDA approved drugs including rivastigmine, galantamine, and donepezil which are ChEIs.[12] It should be noted that tacrine was the first accepted ChEI by FDA for the treatment of AD, however, it was removed from the market due to its hepatotoxicity and lots of efforts were dedicated to develop synthesis of tacrine analogs.[13-15] As the most effective approach to the symptomatic treatment of AD is still inhibition of AChE and BChE, tacrine has been considered as the main scaffold and various tacrine derivatives[16-20] especially multifunctional agents inhibiting β-amyloid aggregation, β-secretase, monoamino oxidases, and tau protein phosphorylation have been designed and synthesized. They also are capable of binding to biometals and scavenging reactive oxygen species (ROS) (Fig. 1).[21,22] Recently, analogues of anticholinesterase drugs such as donepezil and tacrine have been investigated against depression.[23,24] It was revealed that they could improve cognition in depressed people which may delay conversion to diagnosis of AD.

In order to meet our goal in developing novel anticholinesterase agents[25-29] which may improve cognitive impairment, herein, we synthesized some benzochromenoquinolinone

derivatives and evaluated for their biological activities with the aim of developing anti-Alzheimer's agents.

Results and discussion

Design of Compounds

The rationale behind the design of compounds comes back to the hybridization of scaffolds which have been endorsed for desired biological activities (Fig. 2). Tacrine moiety was considered as the main core for inducing ChEI activity. It has been revealed that the replacement of benzene fused ring by pyran moiety has led to potent pyranotacrines as ChE inhibitors.[30] As indicated by Marco et al., 4*H*-pyrano[2,3-*b*]quinolone derivative (**A**)[31] showed AChEI activity with IC_{50} of 0.87 μM. Also, our previous study revealed that aminopyrazolopyranoquinolines (**B** and **C**) have depicted potent AChE (IC_{50} = 2.77 μM) and BChE (IC_{50} = 0.06 μM) inhibitory activity.[18] Moreover, Shafiee et al. reported different tacrine-based analogues and among them compound **D** was found to be effective ChEI (AChEI, IC_{50} = 0.052 μM and BChEI, IC_{50} = 0.006 μM).[32] In a study reported by Han et al.,[33] BACE1 inhibitory activity of bis(7)-tacrine was confirmed. Also, 1,4-naphthoquinones such as 2-hydroxy-1,4-naphthoquinone (**E**) have shown BACE1 inhibitory activity (IC_{50} = 5.96 μM).[34] It seems that hybridization of the corresponding scaffolds leads to obtain multi-target activity of synthesized compounds against AD.

Chemistry

Synthetic route for the preparation of benzochromenoquinolinone derivatives **6a-p** is demonstrated in Scheme 1. Initially, required 2-amino-4aryl-5,10-dioxo-5,10-dihydro-4*H*-benzo[*g*]chromene-3-carbonitriles **4** were prepared easily according to the reaction reported in the

literature[35] by the reaction of 2-hydroxynaphthalene-1,4-dione **1**, aromatic aldehydes **2**, malononitrile **3** in the presence of piperidine in refluxing ethanol. Finally, all derivatives **6** were synthesized through the reaction of compound **4** and cyclohexanone **5** in the presence of $AlCl_3$ in dry 1,2-dichloroethan (DCE) under reflux conditions.

Pharmacology

AChE and BChE inhibitory activity

All synthesized novel benzochromenoquinolinone derivatives **6a-p** were evaluated for their cholinesterase inhibitory activity based on the modified Ellman's method[36] comparing with tacrine and rivastigmine as the reference drugs (Table 1). As can be seen in Table 1, the best AChEI activity was obtained by 14-amino-13-(3-nitrophenyl)-2,3,4,13-tetrahydro-1*H*-benzo[6,7]chromeno[2,3-*b*]quinoline-7,12-dione (**6m**) with IC_{50} = 0.86 μM. It should be noted that 14-amino-13-(4-fluorophenyl)-2,3,4,13-tetrahydro-1*H*-benzo[6,7]chromeno[2,3-*b*]quinoline-7,12-dione (**6c**) was also found as a strong anti-AChE agent with IC_{50} = 0.89 μM. It was revealed that the presence of nitro and fluorine groups at 3- and 4- position of aryl group connected to benzochromenoquinolinone moiety led to high AChEI activity. The significant point is related to analogues of compounds **6m** and **6c**; compounds **6l**/**6n** (IC_{50}s = 4.22 μM /inactive) and **6b** (IC_{50} = >100 μM), respectively, proving remarkable reduction of AChEI activity. It was concluded that the anti-AChE activity was directly depended on the electronic property and position of substituent on the aryl ring. Changing substituents to chlorine afforded interesting results. The presence of chlorine at 3- position of aryl group (**6e**) resulted in good AChEI activity (IC_{50} = 1.447 μM). Changing the position of Cl led to the reduction of activity in such a manner that compounds **6d** and **6f** demonstrated lower activity with IC_{50}s = 5.312 and 14.395 μM, respectively. Also,

increasing the number of chlorine afforded good and moderate anti-AChE activity as compounds **6g** and **6h** showed IC_{50}s = 7.57 and 27.52 μM, respectively. It was indicated that the presence of Cl at 3- position of aryl group induced better activity as observed for the compound **6e** but increasing the number of Cl atom not only did not increase inhibitory activity but also decreased the anti-AChE activity. Another instructive point came back to the presence of bromine on the aryl group in compounds **6i-k**. The best activity was obtained by compound **6j** possessing 3-bromophenyl with IC_{50} = 1.80 μM, whereas compounds **6i** and **6k** showed lower activity with IC_{50}s = 2.77 and 13.79 μM, respectively. Comparing the AChEI activity of halogenated derivative (**6b-k**) revealed that the size of halogens played no significant role and their position on the aryl moiety was found to be much more important factor in such a manner that the order of AChEI activity of 2-halosubstituted derivatives was 2-Br>2-Cl>2-F. Conversely, the order of activity was observed as 4-F>4-Br>4-Cl for 4-halosubstituted derivatives. The absence of a substituent on the aryl moiety also led to good activity in compound **6a** (IC_{50} = 1.71 μM) and the presence of methyl group afforded good to moderated inhibitory activity in compounds **6o** and **6p** (IC_{50}s = 3.01 and 13.95 μM, respectively).

As shown in Table 1, the anti-BChE activity of compounds **6a**, **6b**, **6f-i**, **6k**, **6l**, **6n-p** was not significant, however, the best BChEI activity was obtained by compound **6m** (IC_{50} = 6.03 μM) which was also the most active AChEI. Compounds **6c**, **6d**, **6e**, and **6j** showed good to moderate activity with IC_{50}s = 13.85, 29.02, 27.31, and 15.63 μM, respectively. It seems that the presence of an electron-withdrawing group at 3- position of aryl moiety induced strengthening effect on BChEI activity as observed in the case of AChEI activity.

Kinetic studies of AChE and BChE inhibition

To study the inhibition mode of AChE and BChE induced by the most potent compound **6m**, a kinetic study was conducted as the same as ChE inhibition assay.[15] As it is clear in Fig. 3 and 4, graphical analysis of the Lineweaver-Burk reciprocal plots showed a mixed type of inhibition confirming the fact that compound **6m** binds to the both peripheral anionic site (PAS) and the catalytic anionic site (CAS) of both AChE and BChE. The inhibition constant for AChEI and BChEI were calculated as K_i = 1.22 and 7.89 μM, respectively.

Beta secretase (BACE1) inhibitory activity

β-Secretase (BACE1) plays a remarkable role in the synthesis of neurotoxic β-amyloid (Aβ) peptides in the brain and it remains to be a versatile and challenging target in AD drug discovery development and clinical trials are under underway.[37] In this regard, the most active anti-AChE compound **6m** was evaluated for its BACE1 inhibitory activity which showed IC_{50} = 19.60± 0.9 μM comparing with OM99-2 (IC_{50} = 0.014±0.003 μM).

Neuroprotection effect against Aβ-induced damage

Neuroprotective effect of compound **6m** against damage induced by $A\beta_{25-35}$ was investigated in PC12 cells by MTT assay. This compound showed no significant activity up to the concentration of 25 μM.

Metal chelating

Role of oxidative stress in the pathogenesis of AD has been widely supported in the literature since the level of lipid peroxidation, DNA and protein oxidation products increases in the brain of patients with AD. Unregulated reaction of molecular oxygen with the redox active

metals such as Fe, Cu, and Zn leads to the generation of ROS.[38] However, the role of metal-ion homeostasis especially in the central nervous system needs to be considered in design of anti-AD agents. Herein, the most active ChE inhibitor **6m** was studied for its chelating ability toward Cu^{2+}, Fe^{2+} and Zn^{2+} in methanol using UV-vis spectrophotometer. As can be seen in Fig. 5, UV absorption of compound **6m** depicted a blue shift with addition of Cu^{2+}, Zn^{2+}, and Fe^{2+} confirming the interaction of **6m** and those metal ions. It worth mentioning that compound **6m** depicted more strong interaction with Fe^{2+} as the higher change was observed in the wavelength of absorbance of treated solution of compound **6m** and ferrous ions.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Now, it is clear that oxidative damage is present within the brains of patients with AD.[39] In this respect, compound **6m** was candidate for the evaluation of anti-oxidant activity, DPPH radical scavenging activity in comparison to hydroxyanisole (BHA) as a standard antioxidant. However, it showed no remarkable antioxidant activitiy (IC_{50}>500 μM).

Docking studies

*AChE and BChE inhibitory activity of compound **6m***

Molecular docking study was performed using smina in Linux platform. Compound **6m** was subjected to docking into the 3D structure of AChE (1EVE) and BChE (1P0P) by smina. The range of minimized affinity values of the poses of compound **6m** is −17.35 and −15.35 kcal/mol, respectively. The interactions of the best-docked conformation of compound **6m** with the active site residues of 1EVE is depicted in Fig. 6. Significantly, it formed a hydrogen bond with Tyr334. Comparative analysis of the interactions of compound **6m** with the binding site residues of BChE

is depicted in Fig. 7. As shown in Fig. 7, ligand has formed four hydrogen bonds with Trp82, Trp430, Tyr440 and His438 through nitrogen and oxygen of the ligand which enhanced binding of ligand to the active site.

*BACE1 inhibitory activity of compound **6m***

Docking results related to BACE1 inhibitory activity indicated that compound **6m** is located in the binding site of BACE1 as the same as SC7 (crystal ligand). BACE1 inhibition values of the compound **6m** ligand is −10.33 kcal/mol. Interactions of compound **6m** with the active site residues of 2QP8 is figured out in Fig. 8. Hydrogen bond between Ser71 and nitro group is clearly shown which fixed ligand binding to the active site. Other interactions are depicted in 2D diagram presented in Fig. 8.

*Binding mode of compound **6m***

1EVE: It is well accommodated in the peripheral anionic site (PAS) and the catalytic site (CAS) of AChE.[40] As shown in Fig. 6, there is a hydrogen bond between nitro group and Tyr334 in the PAS. There is a remarkable sandwich π-π hydrophobic interactions between southern site of selected ligand (tetrahydroquinoline) and two amino acids residue (Trp84 and Phe330) from the anionic site at the gorge opening. Another hydrophobic interaction was provided similarly between quinoline moiety and Phe330. Van der Waals and pi-alkyl interactions were presented with Trp84 and Phe330 residues.

1P0P: Similarly, compound **6m** oriented rigidly in the active site of 1P0P1. Nitro and amino groups formed four hydrogen bonds with Tyr440, Trp430, Trp82 and His438 in the catalytic triad and anionic site. Several pi-alkyl interactions were provided through the interactions of

quinoline as well as aryl moieties (of naphthoquinone) and Ala328. On the other side, quinoline moiety and aryl ring connected to benzochromenoquinolinone moiety facilitated numerous π-π hydrophobic interactions with Trp82 and His438 residues.

2QP8: Fig. 8 represents the binding mode of compound **6m** with 2QP8. Hydrogen bonding with Ser71 and hydrophobic interactions with Tyr132, Thr292, Gly291 and Ilu171 around quinoline moiety and aryl ring as well as similar interaction with Gln134 in other side led to well orientation of compound **6m** in the active site. Also, Van der Waals interaction with amino acid Gly74 and pi-alkyl interaction between aryl ring and Leu91 residue well fixed the compound in the BACE1 active site.

Conclusion

In conclusion, a wide range of benzochromenoquinolinone derivatives were synthesized and evaluated for their biological activity responsible for the creation of AD. They included cholinesterase and BACE1 inhibitory activities as well as neuroprotective and metal-chelating properties. Among the synthesized compounds, compound **6m** depicted the best ChE and BACE1 inhibitory activity. Also, it demonstrated metal chelating ability toward Cu^{2+}, Fe^{2+} and Zn^{2+}. Desired biological activities and low hepatotoxicity[19] made these compounds as the versatile anti-AD agents.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-500, using TMS as an internal standard. IR

spectra were obtained on a Nicolet Magna FTIR 550 spectrophotometer (KBr disks). Elemental analysis was performed on an Elementar Analysensysteme GmbH Vario EL III CHNS mode.

*General procedure for the synthesis of 2-amino-4arryl-5,10-dioxo-5,10-dihydro-4H-benzo[g]chromene-3-carbonitriles **4***

A mixture of hydroxynaphthalene-1,4-dione **1** (1 mmol), aromatic aldehydes **2** (1 mmol), and malononitrile **3** (1.1 mmol) in the presence of piperidine (1 mmol) was heated in EtOH (10 mL) for 5 h under reflux conditions. After completion of reaction (checked by TLC), the solvent was evaporated, the crude was extracted by chloroform and used for further reaction without purification.

*General procedure for the synthesis of benzochromenoquinolinone derivatives **6a-p***

A mixture of compound **4** (1 mmol) and cyclohexanone (**5**) (1 mmol) was added to the suspension of $AlCl_3$ in dry DCE (2 mmol in 30 mL) and heated at reflux for 12 h. After completion of reaction (checked by TLC), DCE was evaporated, the crude was extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and the solvent was evaporated under vacuum. All compounds were recrystallized from petroleum and ethyl acetate to give pure products **6**.

*14-Amino-13-phenyl-2,3,4,13-tetrahydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12-dione **(6a)***

Yield: 75%, mp> 250 °C, IR (KBr): 3435, 3250, 3055, 2850, 1650, 1452 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.03 (d, J = 7.0 Hz, 1H, H8), 7.92 (d, J = 7.0 Hz, 1H, H11), 7.82-7.81 (m , 2H, H9, H10), 7.44 (d, J = 7.5 Hz, 2H, H2', H6'), 7.22 (t, J = 7.5 Hz, 2H, H3', H5'), 7.12 (t, J = 7.5 Hz, 1H, H4'), 5.82 (s, 2H, NH_2), 5.43 (s, 1H, H13), 2.61-2.62 (m, 2H, H4), 2.35 (d, J = 16.5 Hz, 1H, H1),

2.19 (d, *J* = 16.5 Hz, 1H, H1), 1.71-1.69 (m, 4H, H2, H3). ^{13}C NMR (125MHz, DMSO-*d*$_6$): 182.6, 177.9, 153.4, 152.8, 151.5, 150.4, 142.8, 134.4, 133.8, 131.2, 130.6, 128.4, 128.2, 126.7, 126.0, 125.9, 122.9, 113.8, 97.7, 33.8, 32.0, 23.0, 22.2, 21.9. Anal. Calcd for $C_{26}H_{20}N_2O_3$: C, 76.45; H, 4.94; N, 6.86. Found: C, 76.21; H, 4.73; N, 6.64.

*14-Amino-13-(2-fluorophenyl)-2,3,4,13-tetrahydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12-dione (**6b**)*

Yield: 70%, mp > 250 °C. IR (KBr): 3420, 3252, 3055, 2850, 1645, 1443 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): 8.04 (d, *J* = 6.5 Hz, 1H, H8), 7.90 (d, *J* = 6.5 Hz, 1H, H11), 7.84-7.80 (m , 2H, H9, H10), 7.72 (d, *J* = 8.0 Hz, 1H, H4'), 7.21 (dd, *J* = 13.0, 8.0 Hz, 1H, H3'), 7.12-7.09 (m, 1H, H6'), 7.07-7.03 (m, 1H, H5'), 5.60 (s, 2H, NH_2), 5.50 (s, 1H, H13), 2.59-2.57 (m, 2H, H4), 2.35 (d, *J* = 15.5 Hz, 1H, H1), 2.18 (d, *J* = 15.5 Hz, 1H, H1), 1.70-1.69 (m, 4H, H2, H3). ^{13}CNMR (125 MHz, DMSO-*d*$_6$): 182.5, 177.9, 160.4 (d, J_{C-F} = 245 Hz), 153.2, 152.9, 151.4, 151.0, 142.0, 134.5, 133.9, 131.9, 131.1, 130.5, 129.2, 128.6 (d, J_{C-F} = 12.5 Hz), 126.0 (d, J_{C-F} = 21.5 Hz), 124.2, 120.5, 115.6 (d, J_{C-F} = 21.5 Hz), 114.0, 96.2, 31.9, 30.0, 23.1, 22.9, 22.1. Anal. Calcd for $C_{26}H_{19}FN_2O_3$: C, 73.23; H, 4.49; N, 6.57. Found: C, 73.53; H, 4.29; N, 6.27.

*14-Amino-13-(4-fluorophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6c**)*

Yield: 74%, mp > 250 °C. IR (KBr): 3431, 3251, 3050, 2850, 1650, 1455 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): 8.03 (d, *J* = 7.0 Hz, 1H, H8), 7.92 (d, *J* = 7.0 Hz, 1H, H11), 7.82-7.81 (m , 2H, H9, H10), 7.45-7.44 (m, 2H, H2', H6'), 7.03 (t, *J* = 7.0 Hz, 2H, H3', H5'), 5.84 (s, 2H, NH_2), 5.44 (s, 1H, H13), 2.58-2.57 (m, 2H, H4), 2.35 (d, *J* = 16.0 Hz, 1H, H1), 2.18 (d, *J* = 16.0 Hz, 1H, H1), 1.70-1.69 (m, 4H, H2, H3). ^{13}CNMR (125 MHz, DMSO-*d*$_6$): 182.7, 177.9, 160.9 (d, J_{C-F} = 242.5 Hz), 153.4, 152.9, 151.5, 150.5, 143.0, 139.0, 134.4, 133.9, 131.2, 130.5 (d, J_{C-F} = 20.0 Hz), 126.1,

125.9, 122.7, 114.8 (d, J_{C-F} = 22.5 Hz), 113.9, 97.5, 33.1, 32.1, 23.0, 22.1, 21.9. $C_{26}H_{19}FN_2O_3$: C, 73.23; H, 4.49; N, 6.57. Found: C, 73.12; H, 4.29; N, 6.25.

*14-Amino-13-(2-chlorophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6d**)*

Yield: 75%, mp > 250 °C. IR (KBr): 3430, 3252, 3050, 2851, 1645, 1450 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.05 (d, J = 7.0 Hz, 1H, H8), 7.87-7.81 (m, 3H, H9, H10, H11), 7.49 (d, J = 7.0 Hz, 1H, H3'), 7.28 (d, J = 7.0 Hz, 1H, H6'), 7.24-7.17 (m, 2H, H5', H4'), 5.51(s, 1H, H13), 5.44 (s, 2H, NH_2), 2.57-2.56 (m, 2H, H4), 2.31 (d, J = 16.0 Hz, 1H, H1), 2.17 (d, J = 16.0 Hz, 1H, H1), 1.68-1.67 (m, 4H, H2 , H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.5, 177.8, 153.2, 153.0, 151.5, 150.8, 139.3, 134.5, 133.9, 132.4, 132.1, 131.0, 130.5, 129.6, 128.9, 127.7, 126.1, 125.9, 120.9, 114.0, 96.8, 33.1, 31.8, 22.9, 22.1, 21.8 . $C_{26}H_{19}ClN_2O_3$: C, 70.51; H, 4.32; N, 6.33. Found: C, 70.21; H, 4.52; N, 6.52.

*14-Amino-13-(3-chlorophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6e**)*

Yield: 75%, mp > 250 °C. IR (KBr): 3425, 3251, 3050, 2850, 1651, 1438 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.05 (d, J = 6.5 Hz, 1H, H8), 7.88-7.82 (m, 3H, H9, H10, H11), 7.49 (s, 1H, H2') 7.28 (d, J = 7.5 Hz, 1H, H4'), 7.24-7.17 (m, 2H, H5', H6'), 5.52 (s, 1H, H13), 5.45 (s, 2H, NH_2), 2.58-2.57 (m, 2H, H4), 2.32 (d, J = 15.5 Hz, 1H, H1), 2.18 (d, J = 15.5 Hz, 1H, H1), 1.70-1.68 (m, 4H, H_2 , H_3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.5, 177.8, 153.2, 153.0, 151.5, 150.8, 139.3, 134.6, 134.0 132.4, 132.1, 131.1, 130.5, 129.6, 128.9, 127.7, 126.0, 125.9, 120.9, 114.0, 96.8, 33.1, 31.8, 22.9, 22.1, 21.8. $C_{26}H_{19}ClN_2O_3$: C, 70.51; H, 4.32; N, 6.33. Found: C, 70.21; H, 4.55; N, 6.63.

14-Amino-13-(4-chlorophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione ***(6f)***

Yield: 75%, mp > 250 °C. IR (KBr): 3450, 3261, 3055, 2856, 1651, 1428 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.05 (d, J = 7.0 Hz, 1H, H8), 7.94 (dd, J = 7.0, 1.5 Hz, 1H, H11), 7.88-7.83 (m, 2H, H9, H10), 7.46 (d, J = 8.0 Hz, 2H, H3', H5') 7.28 (d, J = 8.0 Hz, 2H, H2', H6'), 5.87 (s, 2H, NH_2), 5.46 (s, 1H, H13), 2.60-2.58 (m, 2H, H4), 2.38-2.35 (d, J = 17.5 Hz, 1H, H1), 2.21-2.18 (d, J = 17.5 Hz, 1H, H1), 1.70-1.68 (m, 2H, H2, H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.6, 177.8, 153.3, 152.9, 151.5, 150.6, 141.7, 134.5, 133.5, 131.4, 131.2, 130.6, 130.5, 128.6, 128.0, 125.9, 122.4, 113.9, 97.5, 33.2, 31.9, 23.0, 22.2, 21.9. $C_{26}H_{19}ClN_2O_3$: C, 70.51; H, 4.32; N, 6.33. Found: C, 70.31; H, 4.52; N, 6.13.

*14-Amino-13-(2,3-dichlorophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (****6g****)*

Yield: 70%, mp > 250 °C. IR (KBr): 3432, 3250, 3050, 2852, 1650, 1440 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.07 (d, J = 7.0 Hz, 1H, H8), 7.91 (d, J = 7.0 Hz, 1H, H11), 7.86-7.85 (m, 2H, H9, H10), 7.55 (d, J = 7.5 Hz, 1H, H4') 7.49 (d, J = 7.5 Hz, 1H, H6'), 7.28 (t, J = 7.5 Hz, 1H, H5'), 5.67(s, 1H, H13), 5.47 (s, 2H, NH_2), 2.60-2.58 (m, 2H, H4), 2.35 (d, J = 17.0 Hz, 1H, H1), 2.20 (d, J = 17.0 Hz, 1H, H1), 1.71-1.69 (m, 4H, H2, H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.5, 177.9, 153.5, 153.2, 151.7, 150.8, 142.1, 139.5, 134.6, 132.4, 132.0, 131.1, 130.6, 129.8, 128.5, 126.0, 125.9, 120.3, 116.8, 114.3, 96.6, 33.3, 31.9, 22.9, 22.1, 21.8. $C_{26}H_{18}Cl_2N_2O_3$: C, 65.42; H, 3.80; N, 5.87. Found: C, 65.62; H, 4.1; N, 5.67.

*14-Amino-13-(2,4-dichlorophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (****6h****)*

Yield: 70%, mp > 250 °C. IR (KBr): 3425, 3245, 3056, 2852, 1650, 1450 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.06 (d, J = 6.5 Hz, 1H, H8), 7.90 (d, J = 6.5 Hz, 1H, H11), 7.84-7.83 (m, 2H, H9, H10), 7.62 (d, J = 8.5 Hz, 1H, H5'), 7.51(s, 1H, H3'), 7.34 (d, J = 8.5 Hz, 1H, H6'), 5.56 (s, 1H, H13), 5.46 (s, 2H, NH_2), 2.59-2.58 (m, 2H, H4), 2.34 (d, J = 16.0 Hz, 1H, H1), 2.19 (d, J = 16.0 Hz, 1H, H1), 1.70-1.69 (m, 4H, H2, H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.5, 177.7, 153.3, 153.0, 151.5, 151.1, 138.3, 134.5, 133.9, 133.1, 132.5, 131.0, 130.5, 128.9, 127.8, 127.6, 126.2, 126.0, 120.2, 114.2, 96.3, 33.0, 31.9, 22.9, 22.1, 21.8. $C_{26}H_{18}Cl_2N_2O_3$: C, 65.42; H, 3.80; N, 5.87. Found: C, 65.72; H, 3.65; N, 5.57.

14-Amino-13-(2-bromophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6i**)

Yield: 77%, mp > 250 °C. IR (KBr): 3430, 3250, 3045, 2856, 1645, 1450 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): 8.15 (d, J = 7.0 Hz, 1H, H8), 8.01(d, J = 7.0 Hz, 1H, H11), 7.70-7.69 (m, 2H, H9, H10), 7.53 (d, J = 7.0 Hz, 1H, H3'), 7.33 (d, J = 7.0 Hz, 1H, H6'), 7.18-7.04 (m, 2H, H4', H5'), 5.59 (s, 2H, H13), 4.62 (s, 2H, NH_2), 2.80-2.78 (m, 2H, H4), 2.35-2.24 (m, 2H, H1), 1.82-1.79 (m, 4H, H2, H3). ^{13}C NMR (125 MHz, $CDCl_3$): 183.1, 177.7, 154.6, 153.2, 151.1, 150.9, 145.2, 141.6, 134.2, 133.6, 132.8, 131.5, 130.8, 129.1, 128.7, 126.6, 126.3, 123.2, 122.6, 114.3, 98.3, 35.6, 32.1, 23.1, 22.4, 22.2. $C_{26}H_{19}BrN_2O_3$**:** C, 64.08; H, 3.93; N, 5.75. Found: C, 64.38; H, 4.13; N, 5.42.

14-Amino-13-(3-bromophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6j**)

Yield: 77%, mp > 250 °C. IR (KBr): 3430, 3245, 3055, 2850, 1642, 1450 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): 8.11 (d, J = 7.0 Hz, 1H, H8), 8.00 (d, J = 7.0 Hz, 1H, H11), 7.70-7.68 (m, 2H, H9, H10), 7.62 (s, 1H, H2'), 7.22 (d, J = 7.5 Hz, 1H, H4'), 7.07-7.01 (m, 2H, H5', H6'), 5.61 (s, 2H,

H13), 4.60 (s, 2H, NH_2), 2.81-2.79 (m, 2H, H4), 2.33-2.28 (m, 2H, H1), 1.81-1.79 (m, 4H, H2, H3). ^{13}C NMR (125 MHz, $CDCl_3$): 183.2, 178.0, 154.3, 153.0, 151.2, 150.4, 145.0, 141.2, 134.0, 133.4, 132.2, 131.3, 130.4, 129.0, 128.6, 126.4, 126.0, 123.7, 122.1, 114.4, 98.5, 35.4, 32.1, 23.2, 22.4, 22.1. $C_{26}H_{19}BrN_2O_3$**:** C, 64.08; H, 3.93; N, 5.75. Found: C, 64.30; H, 4.21; N, 5.40.

*14-Amino-13-(4-bromophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6k**)*

Yield: 78%, mp > 250 °C. IR (KBr): 3430, 3245, 3052, 2850, 1650, 1450 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 8.13 (d, *J* = 6.5 Hz, 1H, H8), 7.99 (d, *J* = 6.5 Hz, 1H, H11), 7.69-7.68 (m, 2H, H9, H10), 7.39-7.33 (m, 4H, H2', H3', H5', H6'), 5.24 (s, 1H, H13), 4.28 (s, 2H, NH_2), 2.84-2.82 (m, 2H, H4), 2.38-2.30 (m, 2H, H1), 1.85-1.83 (m, 2H, H2, H3). ^{13}C NMR (125 MHz, DMSO-d_6): 183.2, 177.8, 155.0, 153.5, 150.9, 150.3, 140.6, 134.2, 133.7, 132.1, 131.6, 130.8, 130.6, 126.6, 126.3, 122.1, 121.7, 114.8, 98.0, 35.4, 32.3, 23.1, 22.4, 22.2. $C_{26}H_{19}BrN_2O_3$: C, 64.08; H, 3.93; N, 5.75. Found : C, 63.88; H, 3.68; N, 5.39.

14-Amino-13-(2-nitrophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6l**)

Yield: 72%, mp> 250 °C, IR (KBr): 3430, 3250, 3055, 2852, 1650, 1535, 1450, 1375 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.01 (d, *J* = 6.5 Hz , 1H, H8), 7.89 (d, *J* = 6.5 Hz , 1H, H11), 7.83-7.80 (m, 3H, H9, H10, H3'), 7.58 (t, *J* = 7.0 Hz , 1H, H5'), 7.43 (t, *J* = 7.0 Hz , 1H, H4'), 7.28 (d, *J* = 7.0 Hz, 1H, H6'), 5.92 (s, 2H, NH_2), 5.79 (s, 1H, H13), 2.63-2.61 (m, 2H, H4), 2.36 (d, *J* = 16.5 Hz, 1H, H1), 2.21 (d, *J* = 16.5 Hz, 1H, H1), 1.73-1.71 (m, 4H, H2 , H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.8, 177.6, 153.7, 153.4, 151.7, 150.0, 149.1, 136.5, 134.5, 134.2, 131.6, 130.7, 130.6, 128.6, 126.1, 125.9, 125.7, 124.1, 121.2, 114.3, 95.9, 31.9, 30.1, 22.9, 22.1, 21.8. $C_{26}H_{19}N_3O_5$: C, 68.87; H, 4.22; N, 9.27. Found : C, 69.17; H, 4.52; N, 8.94.

*14-Amino-13-(3-nitrophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6m**)*

Yield: 70%, mp> 250 °C, IR (KBr): 3432, 3250, 3052, 2850, 1650, 1550, 1450, 1375 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.49 (s, 1H, H2'), 8.04 (d, *J* = 7.5 Hz, 1H, H8), 7.99 (d, *J* = 7.5 Hz, 1H, H4'), 7.92 (d, *J* = 7.5 Hz, 1H, H11), 7.80-7.79 (m, 3H, H9, H10, H6'), 7.51 (t, *J* = 7.5 Hz, 1H, H5'), 5.99 (s, 2H, NH2), 5.64 (s, 1H, H13), 2.61-2.59 (m, 2H, H4), 2.35 (d, *J* = 16.5 Hz, 1H, H1), 2.10 (d, *J* = 16.5 Hz, 1H, H1), 1.71-1.69 (m, 4H, H2 , H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.6, 177.8, 153.3, 151.6, 150.9, 147.4, 144.8, 135.1, 134.4, 133.9, 131.1, 130.7, 129.7, 126.1, 125.8, 123.5, 122.1, 121.8, 121.6, 114.0, 95.8, 33.6, 32.1, 23.1, 22.1, 21.9.

$C_{26}H_{19}N_3O_5$: C, 68.87; H, 4.22; N, 9.27. Found: C, 68.52; H, 3.92; N, 8.94.

*14-Amino-13-(4-nitrophenyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6n**)*

Yield: 75%, mp> 250 °C, IR (KBr): 3430, 3250, 3050, 2855, 1652, 1550, 1455, 1370 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.10 (d, *J* = 8.0 Hz, 2H, H3', H5'), 8.05 (d, *J* = 7.5 Hz, 1H, H8), 7.92 (d, *J* = 7.5 Hz, 1H, H11), 7.85-7.82 (m, 2H, H9 , H10), 7.74 (d, *J* = 8.0 Hz, 2H, H2', H6'), 5.94 (s, 2H, NH2), 5.64 (s, 1H, H13), 2.61-2.60 (m, 2H, H4), 2.36 (d, *J* = 17.5 Hz, 1H, H1), 2.16 (d, *J* = 17.5 Hz, 1H, H1), 1.72-1.70 (m, 4H, H2 , H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.6, 177.8, 153.3, 151.7, 150.9, 150.2, 146.3, 134.5, 133.9, 131.1, 130.7, 129.9, 126.1, 125.9, 123.3, 123.2, 121.6, 114.1, 98.7, 33.9, 32.1, 23.0, 22.1, 21.9 . $C_{26}H_{19}N_3O_5$: C, 68.87; H, 4.22; N, 9.27. Found: C, 68.57; H, 4.45; N, 9.39.

*14-Amino-13-(o-tolyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6o**)*

Yield: 77%, mp> 250 °C, IR (KBr): 3435, 3250, 3050, 2850, 1650, 1452 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.05 (d, *J* = 7.5 Hz, 1H, H8), 7.90 (d, *J* = 7.5 Hz, 1H, H11), 7.83-7.82 (m, 2H, H9, H10), 7.21-7.05 (m, 4H, H3', H4', H5', H6'), 5.39 (s, 1H, H13), 5.24 (s, 2H, NH_2), 2.64-2.60 (m, 5H, H4, CH_3), 2.32 (d, *J* = 16.5 Hz, 1H, H1), 2.20 (d, *J* = 16.5 Hz, 1H, H1), 1.71-1.69 (m, 4H, H2, H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.9, 177.9, 153.2, 153.0, 151.5, 150.4, 140.9, 135.5, 134.5, 133.9, 131.1, 130.6, 130.5, 130.2, 126.9, 126.5, 126.1, 125.8, 122.6, 114.3, 98.6, 32.3, 31.9, 22.9, 22.1, 21.9, 19.2. $C_{27}H_{22}N_2O_3$: C, 76.76; H, 5.25; N, 6.63. Found: C, 76.46; H, 5.55; N, 6.43.

*14-Amino-13-(m-tolyl)-3,4-dihydro-1H-benzo[6,7]chromeno[2,3-b]quinoline-7,12(2H,13H)-dione (**6p**)*

Yield: 72%, mp> 250 °C, IR (KBr): 3432, 3255, 3050, 2852, 1650, 1450 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.11 (d, *J* = 7.0 Hz, 1H, H8), 7.98 (d, *J* = 7.0 Hz, 1H, H 11), 7.67-7.66 (m, 2H, H9, H10), 7.28-7.00 (m, 4H, H2', H4', H5', H6'), 5.87 (s, 2H, NH_2), 5.36 (s, 1H, H13), 2.60-2.58 (m, 5H, H4, CH_3), 2.24-2.16 (m, 2H, H_1), 1.70-1.68 (m, 4H, H2, H3). ^{13}C NMR (125 MHz, DMSO-d_6): 182.9, 177.9, 153.2, 153.0, 151.5, 150.4, 140.9, 135.5, 134.5, 133.9, 131.1, 130.6, 130.5, 130.2, 126.9, 126.5, 126.1, 125.8, 122.6, 114.3, 98.6, 32.3, 31.9, 22.9, 22.1, 21.9, 19.2. $C_{27}H_{22}N_2O_3$: C, 76.76; H, 5.25; N, 6.63. Found: C, 76.96; H, 5.15; N, 6.75.

AChE and BChE inhibition assay

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from electric eel), butylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI) and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate,

potassium hydroxide, and sodium hydrogen carbonate were purchased from Fluka. The assay was performed according to our previous reports based on modified Ellman's method.[15]

Kinetic studies of AChE and BChE inhibition

For estimates of the inhibition model and inhibition constant Ki, reciprocal plots of 1/V versus 1/[S] were obtained using different concentrations of the substrate. For this purpose, experiments were performed similar to enzyme inhibition assay.[15] The rate of enzymatic reaction was obtained with different concentrations of inhibitor and in the absence of inhibitor. For each experiment, reaction was started by adding substrate and progress curves were recorded at 405 nm within 2 min. Next, double reciprocal plots (1/v *vs*. 1/[s]) were made using the slopes of progress curves to obtain the type of inhibition. Slopes of these reciprocal plots were then plotted against the concentration of compound in a weighted analysis, and Ki was determined as the intercept on the negative x-axis. All rate measurements were performed in triplicate and data analysis was performed with Microsoft Excel 2003.

BACE1 inhibitory activity

The BACE1 enzyme inhibition assay was achieved by a FRET (Forster resonance energy transfer) kit, from Invitrogen (former Pan Vera corporation, Madison, WI) as described by the manufacturer instructions comparing with OM99-2 as the reference inhibitor.[41]

Protection of neuronal PC12 cells against Aβ-induced damage

The ability of compound **6m** in protecting neuronal PC12 cells against damage induced by $A\beta_{25-35}$ was examined by the MTT assay as previously described in the literature.[41] PC12 (rat

pheochromocytoma) cells were a generous gift from Professor Lloyd A. Greene (Department of Pathology and Cell Biology, Columbia University, New York, NY).

Metal chelating

All solutions used in metal-chelating study were prepared in methanol and Fe^{2+}, Cu^{2+}, and Zn^{2+} solutions were prepared from $FeSO_4$, $CuCl_2$ or and $ZnCl_2$ respectively. To study the metal binding ability, a mixture of compound **6m** (1 mL) and metal solution (1 mL) with the same concentration (20 μM) in a 1 cm quartz cuvette was incubated at room temperature for 30 min. Then, the absorption spectra were recorded with wavelength ranging from 200–500 nm.[42]

DPPH radical scavenging activity

Antioxidant activity of compound **6m** was tested using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to our previous report.[15]

Molecular docking study

Molecular docking study was performed to explore the possible binding mode of the most active compound **6m** with AChE (PDB code 1EVE), BChE (PDB code 1P0P) and BACE1 (PDB code 2QP8) using smina in Linux platform. Smina is a fork of AutoDock Vina that provides enhanced support for scoring function.[43] The scoring function of smina does a better job sampling low root mean squared deviation (RMSD) poses when compared to the default Auto Dock Vina scoring function. The bounding box for docking is specified automatically with the autobox ligand option. It generates a box with an 8 Å buffer around the reference ligand in the active site. For each final pose, the affinity was reported as the negative value, the higher value indicated a more

favorable binding pose. In order to confirm the validity of the docking protocol, RMSD value was measured between the crystal ligand (donepezil) pose and the generated nine poses of redocked donepezil pose (2 Å) in 1EVE and 1P0P. The minimized affinity values for generated poses of redocked crystal ligand pose were −10.92 kcal/mol. In addition, all RMSD values are less than 2 Å for 2QP8 and the minimized affinity values for generated poses of redocked crystal ligand pose were −9.29 kcal/mol.

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Author Contribution Statement

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

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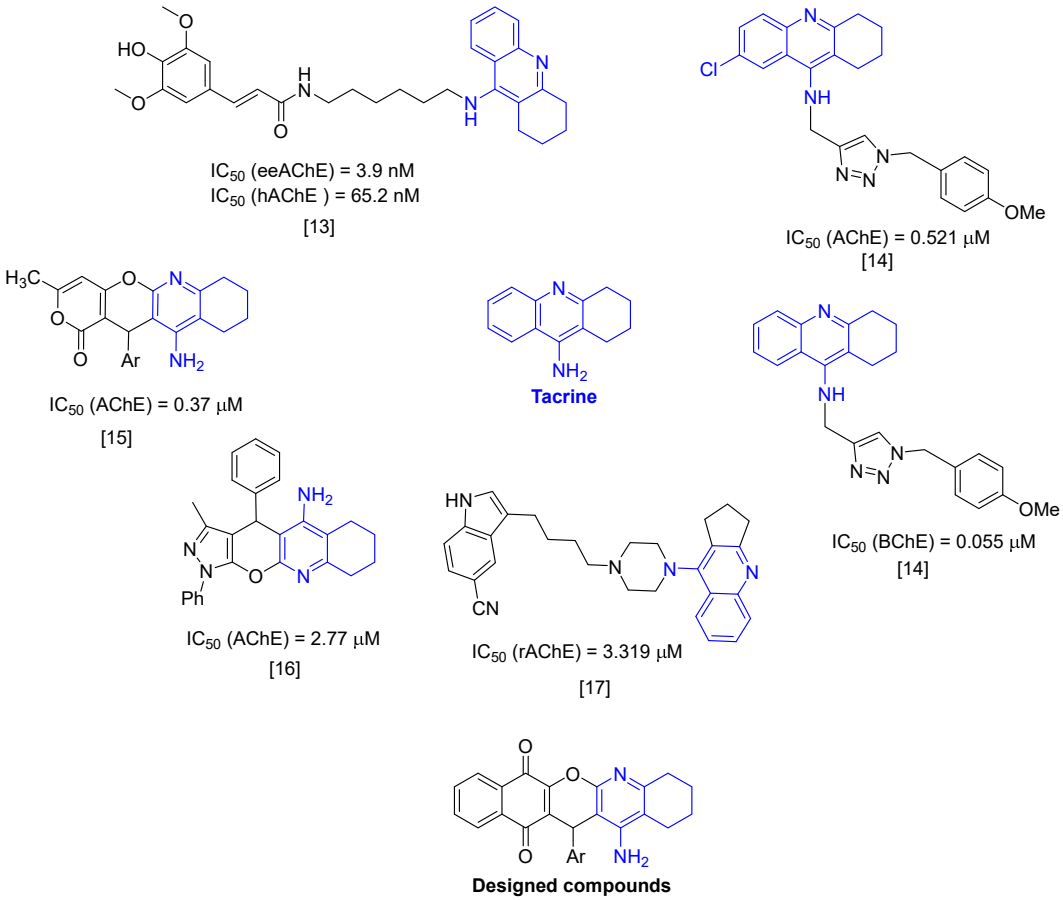

Fig. 1. Different tacrine analogues possessing anti-ChE activity.

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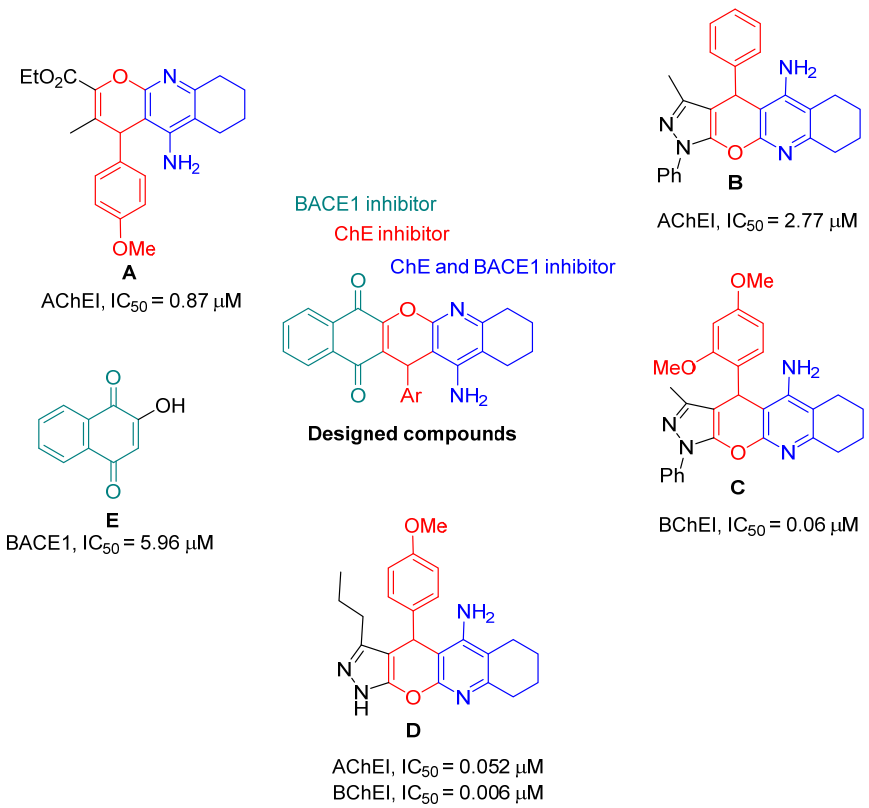

Fig. 2. Design of benzochromenoquinolinones.

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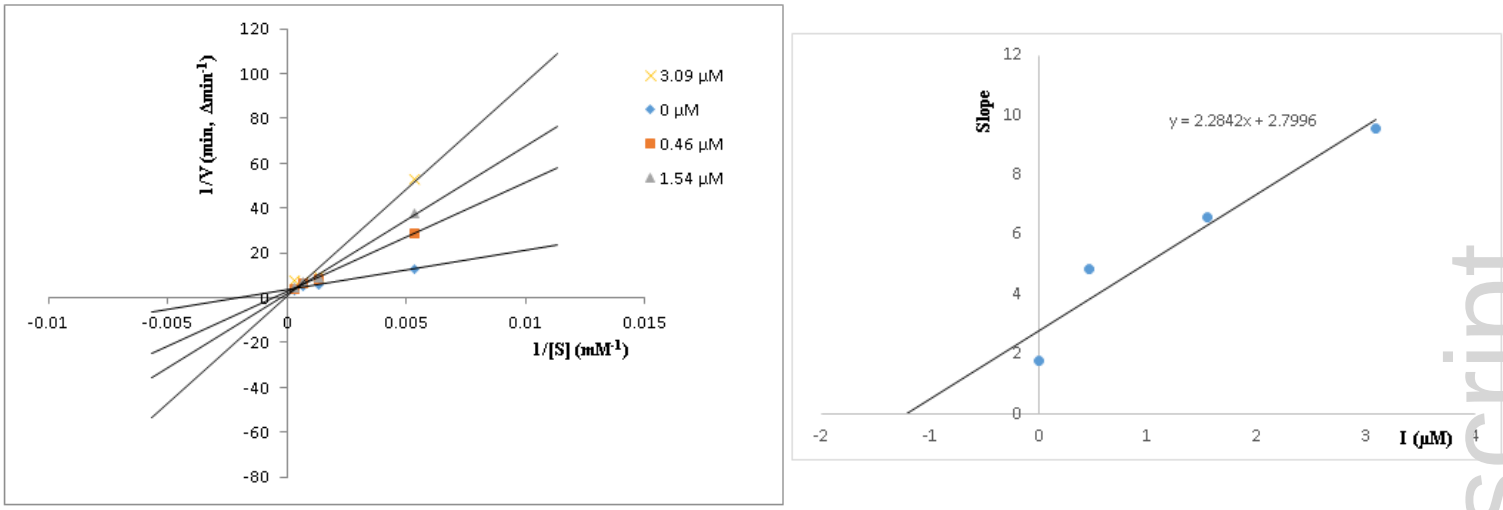

Fig. 3. Left: Lineweaver-Burk plot for the inhibition of AChE by compound **6m** at different concentrations of acetylthiocholine (ATCh). Right: Steady-state inhibition constant (Ki) of compound **6m**.

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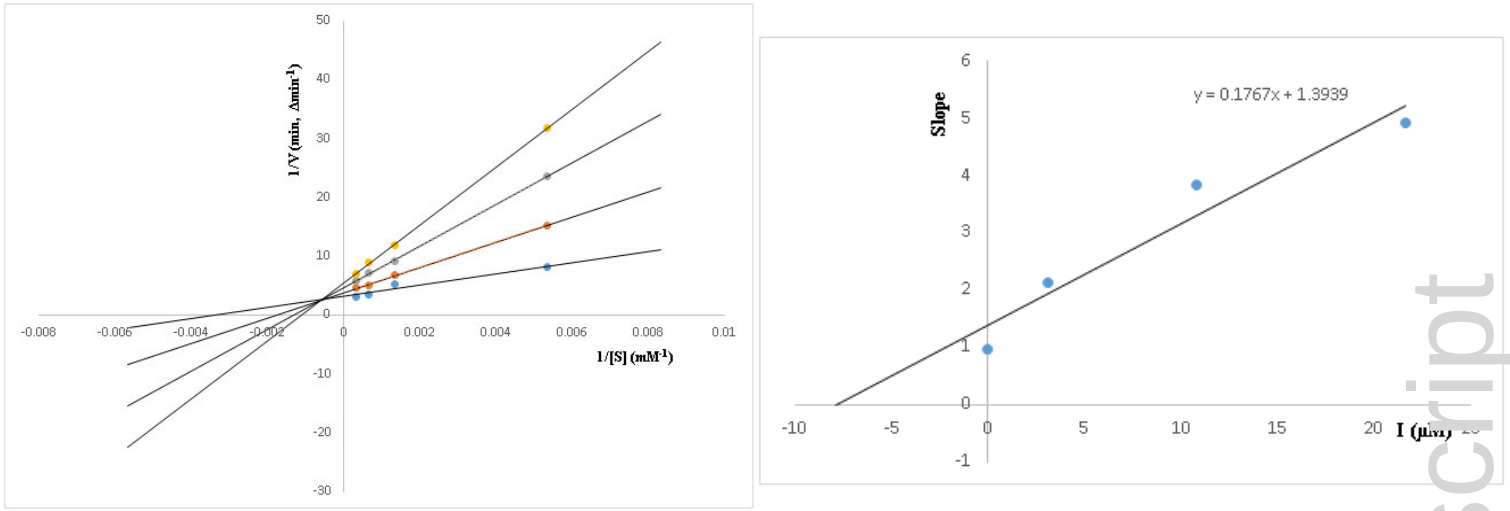

Fig. 4. Left: Lineweaver-Burk plot for the inhibition of BChE by compound **6m** at different concentrations of butyrylthiocholine (BTCh). Right: Steady-state inhibition constant (Ki) of compound **6m**.

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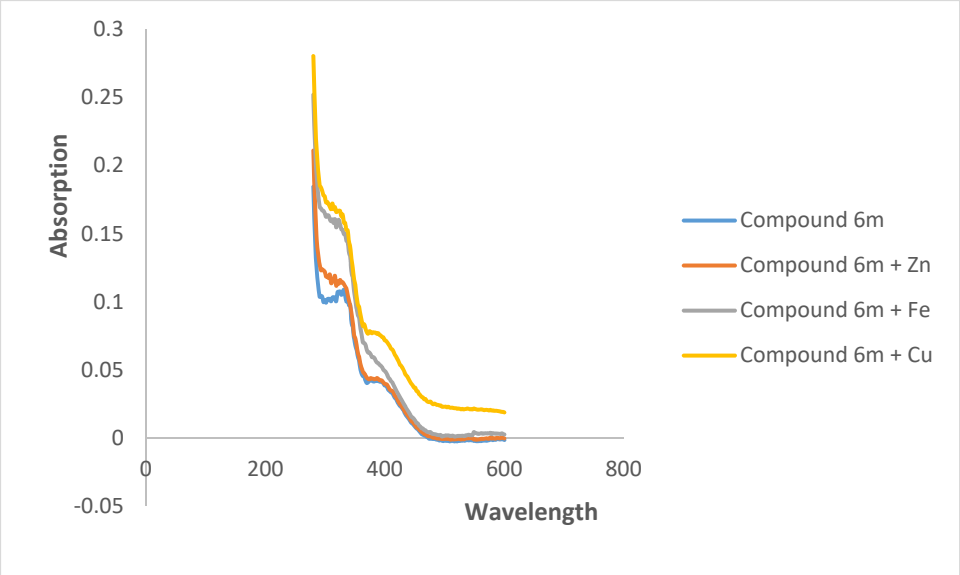

Fig. 5. The UV spectrum of compound **6m** (20 μM) alone or in the presence of Fe^{2+}, Cu^{2+}, and Zn^{2+} (20 μM); all solutions were prepared in methanol.

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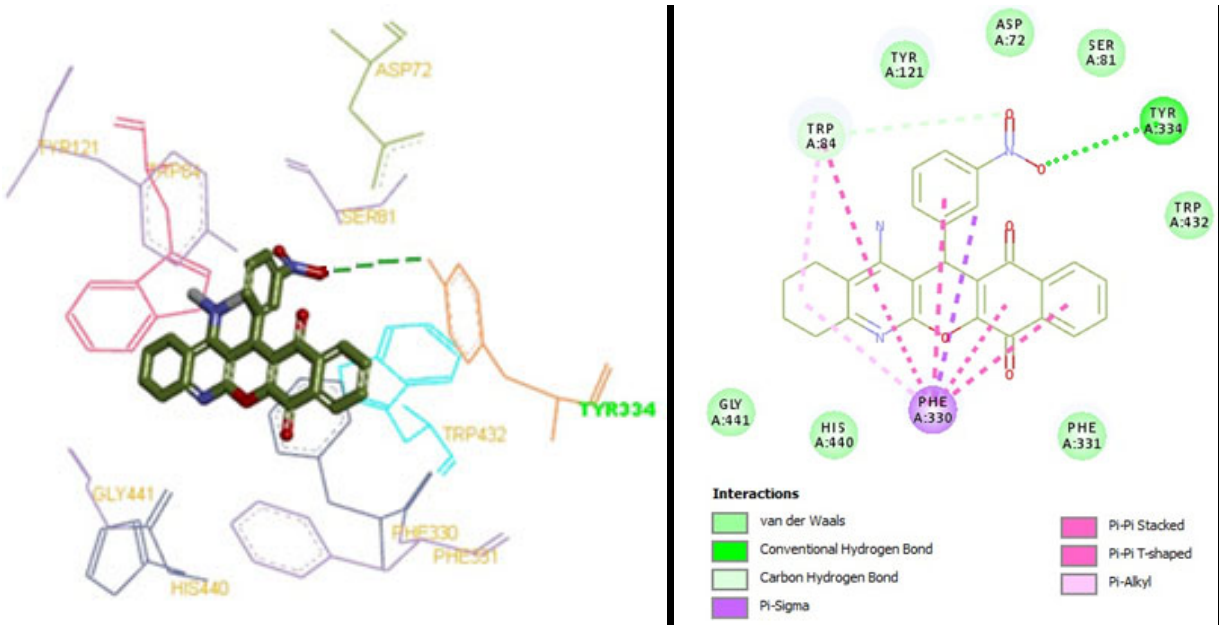

Fig. 6. 3D analysis of the interactions between AChE (1EVE) and compound **6m** 2D presentation of different interactions of compound **6m** and 1EVE.

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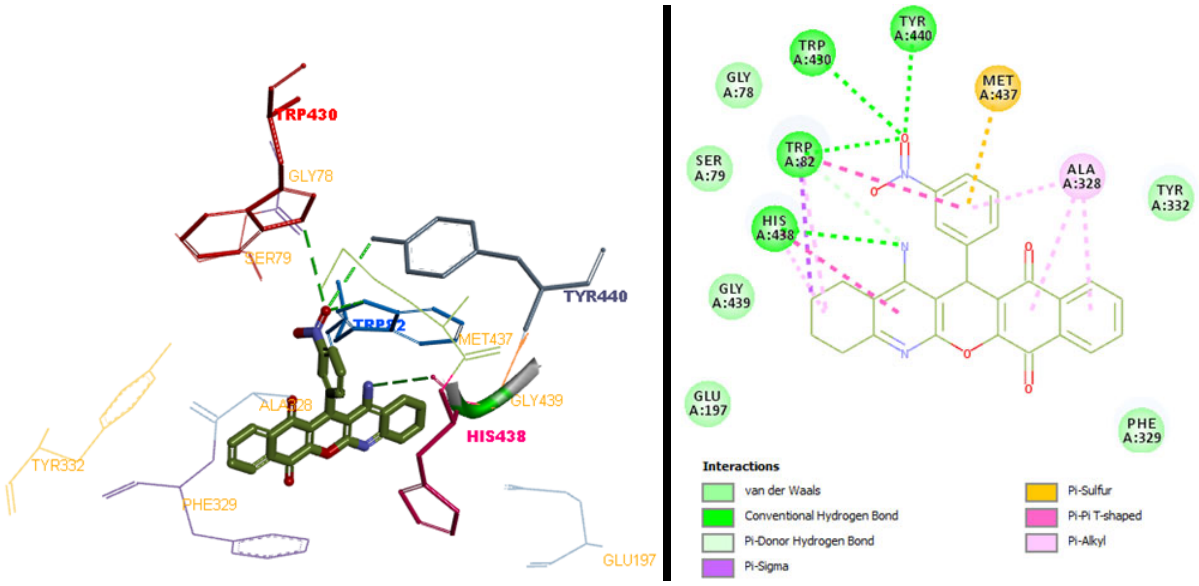

Fig. 7. Binding mode of compound **6m** within the active site of BChE (1P0P) b) schematic presentation of selected ligand with 1P0P.

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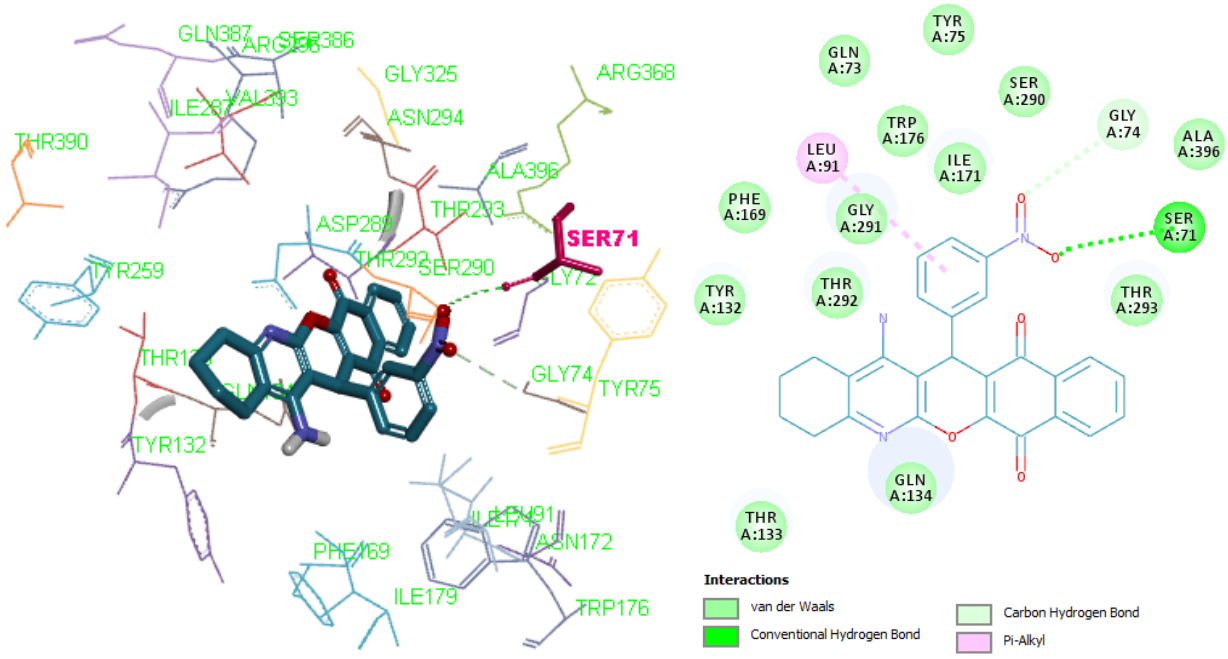

Fig. 8. 3D and 2D representation of BACE1 (2QP8) active site. Hydrogen bonds are shown by dashed lines, amino acids are shown by sticks.

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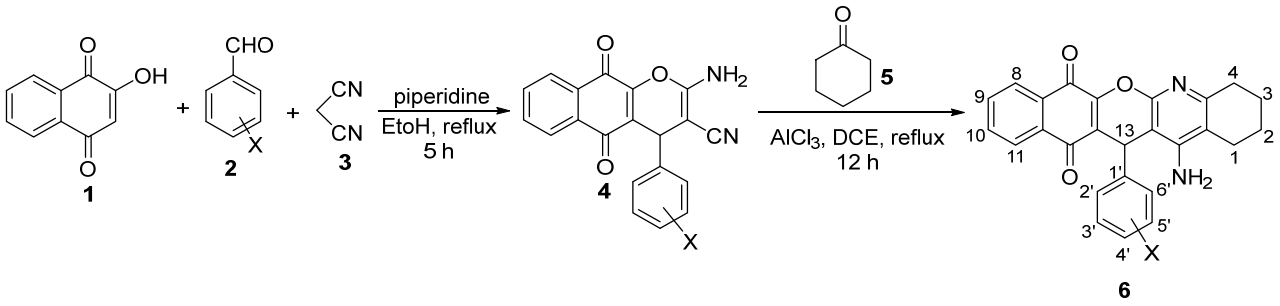

Scheme 1. Synthesis of benzo[6,7]chromeno[2,3-*b*]quinoline-7,12-dione **6**.

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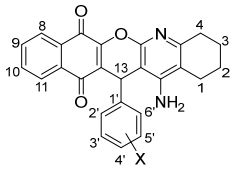
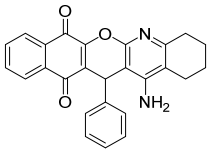
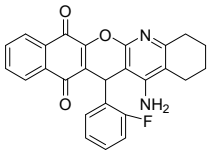
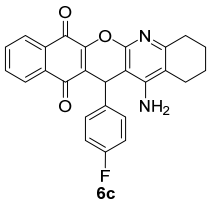
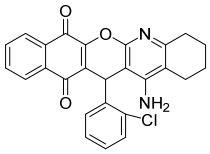
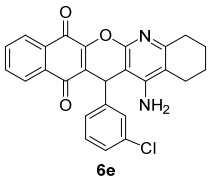
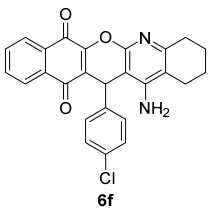
Table 1. Inhibitory activity of compounds **6** against AChE and BChE.



6

Entry	Compound **6**	AChEI IC_{50} (μM)[a]	BChEI IC_{50} (μM)[a]
1	 **6a**	1.71± 0.14	>100
2	 **6b**	>100	>100
3	 **6c**	0.89±0.02	13.85 ± 0.13
4	 **6d**	5.31 ±0.34	29.02± 0.56
5	 **6e**	1.45 ± 0.08	27.31 ± 1.19
6	 **6f**	14.39 ± 2.14	>100

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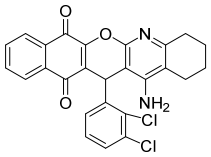
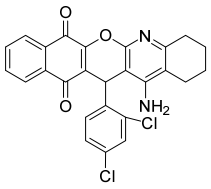
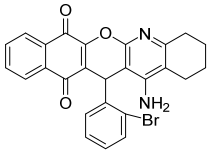
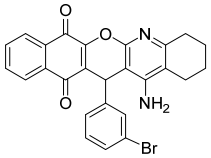
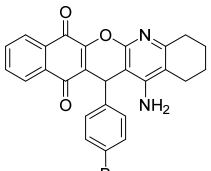
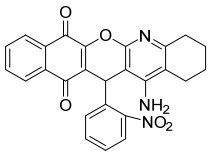
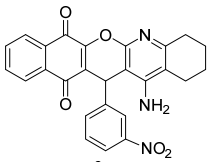
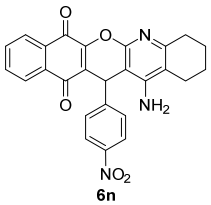
7	 **6g**	7.57 ± 0.25	>100
8	 **6h**	27.52± 1.95	>100
9	 **6i**	2.77± 0.13	>100
10	 **6j**	1.80 ±0.11	15.63 ± 0.41
11	 **6k**	13.79 ±0.46	>100
12	 **6l**	4.22 ± 0.15	>100
13	 **6m**	0.86 ±0.04	6.03 ± 0.34
14	 **6n**	>100	>100

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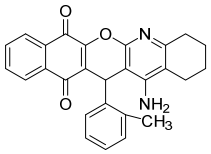
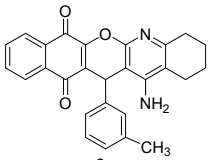
15	 **6o**	3.01 ±0.02	>100
16	 **6p**	13.95 ± 0.74	>100
17	Rivastigmine	11.07±0.01	7.72±0.02
18	Tacrine	0.05±0.01	0.01 ±0.00

[a] Inhibitor concentration (mean ± SD of three experiments) required for 50% inactivation of AChE.