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Design, synthesis and anti-Alzheimer's activity of novel 1,2,3-triazole-chromenone carboxamide derivatives

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

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ABSTRACT

Alzheimer's disease (AD) is a well-known neurodegenerative disorder affecting millions of old people worldwide and the corresponding epidemiological data highlights the significance 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 and development. In this work, a wide range of 1,2,3-triazole-chromenone carboxamides were designed, synthesized, and evaluated for their cholinesterase inhibitory activity. Among them, *N*-(1-benzylpiperidin-4-yl)-7-((1-(3,4-dimethylbenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-oxo-2*H*-chromene-3-carboxamide (**11b**) showed the best acetylcholinesterase inhibitory activity (IC_{50} = 1.80 μM), however, it was inactive toward butyrylcholinesterase. It should be noted that compound **11b** was evaluated for its BACE1 inhibitory activity and calculated IC_{50} = 21.13 μM confirmed desired inhibitory activity. Also, this compound revealed satisfactory neuroprotective effect against H_2O_2-induced cell death in PC12 neurons at 50 μM as well as metal chelating ability toward Fe^{2+}, Cu^{2+}, and Zn^{2+} ions.

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia among older adults characterized by the several complex pathological fluctuations in the brain. It is manifested by the failure of memory and thinking skills leading to disability to perform the simplest tasks. The illness progress is well-known by the advent of aphasia, apraxia, executive disorders, mood disturbances, and psychiatric symptoms to such an extent a huge economic burden in the form of healthcare and nursing home costs has been imposed on the families, communities, and governments [1].

The exact origin of AD is ambiguous and combinatorial reasons such as genetic, lifestyle, and environmental factors are involved in onset and progression of the disease. Formation of toxic amyloid beta (Aβ) protein in the brain [2], tau protein hyperphosphorylation and aggregation [3], biometals dysfunction (ions; copper, iron, and zinc) [4,5], alteration of calcium homeostasis [6], inflammation and oxidative stress due to generation of reactive oxygen species (ROS) [7], and deficits in the cholinergic transmission [8] have been considered as the main causes of AD. Although several single factor theories have been suggested for AD, no one led to definite treatment even more recent pathological hallmarks of AD related to the accumulation of neurofibrillary tangles and amyloid plaques [9,10] have failed in clinical trials. Up to now, only temporary symptomatic relief has obtained from FDA approved anti-cholinesterase (ChE) drugs *via* improving the cholinergic neurotransmitter systems [11].

Acetylcholine (ACh) is a chemical compound synthesized from two precursors; choline and acetyl coenzyme A by enzyme choline *O*-acetyltransferase. It is well-known to be involved in physiological processes and regulation of endothelial cells and immune system cells functions

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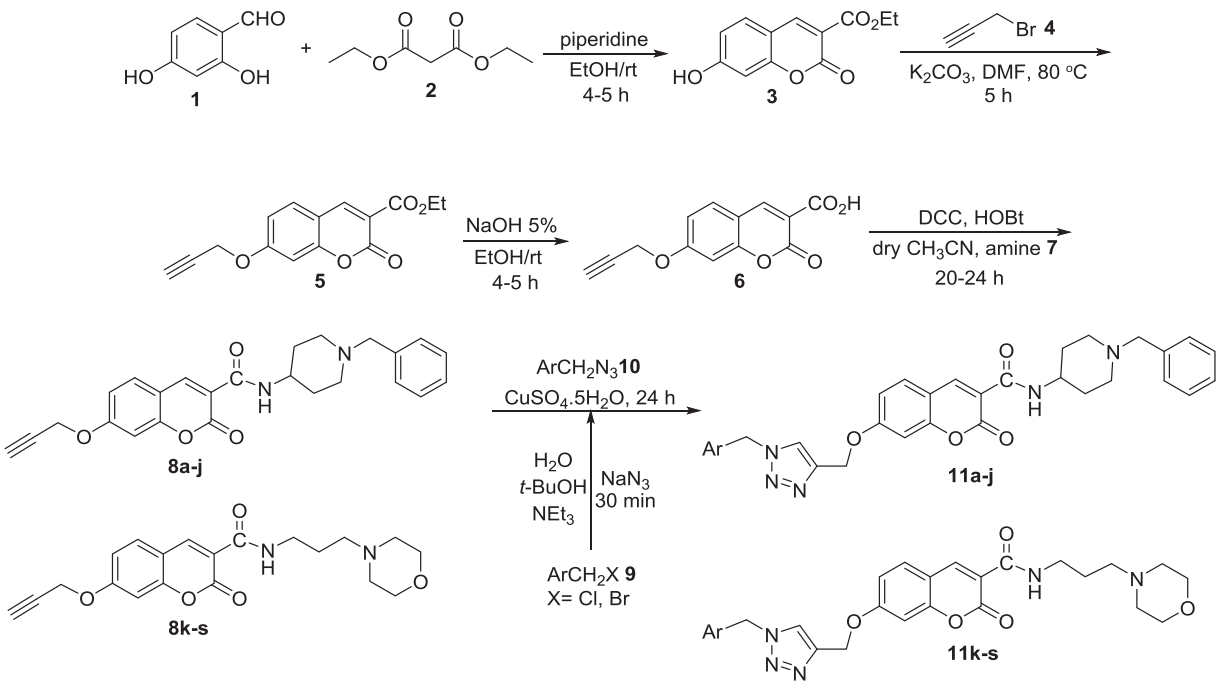


Scheme 1. Synthesis of 1,2,3-triazole-chromenone carboxamides **11**.

[12]. There are two enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which are ubiquitous in the body in large amounts targeting ACh and other choline esters that function as neurotransmitters. AChE has catalytic anionic site (CAS) and peripheral anionic site (PAS) which are selectively accepting ACh [13]. Hence, ChE inhibitors (ChEIs) have been involved in a wide range of AD drug discovery research [14]. The efficacy of anti-ChE agents comes back to the fact that the cholinergic system is extremely affected by the pathological processes in AD to increase accessibility of ACh [15]. It has been also postulated that there are possible interactions between Aβ and cholinergic neurotransmission especially with the PAS of AChE [16]. Although the ChEIs usually are known for their symptomatic effects, they have shown new strategies through neuroprotective effects [17]. Moreover, new version of the cholinergic hypothesis described by McDonald et al. [18] can be extended to the compensatory role of cholinergic system in providing protection for hippocampal neurons.

Herein, in continuation of our efforts to develop anti-AD agents [19–24], we designed and synthesized a novel series of 1,2,3-triazole-chromenone carboxamide derivatives **11** (Scheme 1) which were evaluated for their ChE inhibitory activity. Also, the most potent AChE inhibitor was investigated for BACE1 inhibitory activity as well as neuroprotective and metal-chelating properties.

2. Results and discussion

2.1. Design of compounds *11*

The design of the target compounds **11** is endorsed by the versatile anti-AD activity of coumarin and 1,2,3-triazole scaffolds [25–28] (Fig. 1). In this regard, anti-AChE activity of ensaculine **A** reported by Hoerr and Noeldner [29] demonstrated high anti-AD activity and its hydrochloride salt depicted desirable physiochemical properties. Also, the study reported by Foroumadi et al. [30] has evidently highlighted anti-AChE activity of coumarin-3-carboxamide derivative **B**. Study by Sharpless et al. [27] demonstrated that 1,2,3-triazole-linked tacrine and phenanthridinium derivatives **C** and **D** possessed remarkable AChEI activity by incorporation of 1,2,3-triazole ring. However, introduction of 1,2,3-triazole moiety into the iminochromene-2*H*-carboxamide derivative **E** led to beta secretase (BACE1) inhibitory activity as reported by Edraki et al. [31]. Hybridization of desired scaffolds marked in color in Fig. 1 led to the reasonable synthesis of target compounds **11**.

2.2. Chemistry

Scheme 1 demonstrates schematic synthesis of desired compound **11**. For this purpose, required starting material, ethyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate **3** was obtained by the reaction of 2,4-dihydroxybenzaldehyde **1** and diethyl malonate **2** in the presence of piperidine in EtOH at room temperature. Then, reaction of compound **3** and propargyl bromide **4** in the presence of K_2CO_3 in DMF at 80 °C gave ethyl 2-oxo-7-(prop-2-yn-1-yloxy)-2*H*-chromene-3-carboxylate **5**. Hydrolysis of compound **5** using NaOH (5%) in EtOH at room temperature led to the formation of the corresponding acid, 2-oxo-7-(prop-2-yn-1-yloxy)-2*H*-chromene-3-carboxylic acid **6**. Reaction of compound **6** and appropriated amine **7** in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole hydrate (HOBt) in dry acetonitrile afforded compound **8**. Finally, $CuSO_4{\cdot}5H_2O$-catalyzed click reaction of compound **8** and *in situ* prepared benzyl azide derivatives **10** obtained from the reaction of benzyl chloride/bromide derivative **9** and sodium azide in the presence of trimethylamine in water/*tert*-butyl alcohol afforded final products **11**.

2.3. In vitro anti-AChE and anti-BChE activity

In vitro anti-AChE and anti-BChE activity of synthesized compounds **11** was conducted based on modified Ellman's method [32] (Table 1). The synthesized compounds **11** can be generally divided into two categories based on the different amine moiety connected to amide functional group, benzylpiperidinyl (**11a-j**) and 3-morpholinopropyl groups (**11k-s**).

As can be seen in Table 1, the best AChEI activity was obtained by compound **11b** (IC_{50} = 1.80 μM) in comparison to donepezil as the reference drug (IC_{50} = 0.027 μM). The compound possessed benzylpiperidinyl moiety connected to amide functional group and 3,4-dimethylbenzyl connected to 1,2,3-traizole moiety. Reduction of the number of methyl group as well as changing its position reduced the inhibitory activity of compounds **11c** and **11d** with IC_{50}s = 20.00 and 3.70 μM, respectively. Also, changing 3,4-dimethylbenzyl to 3,4-

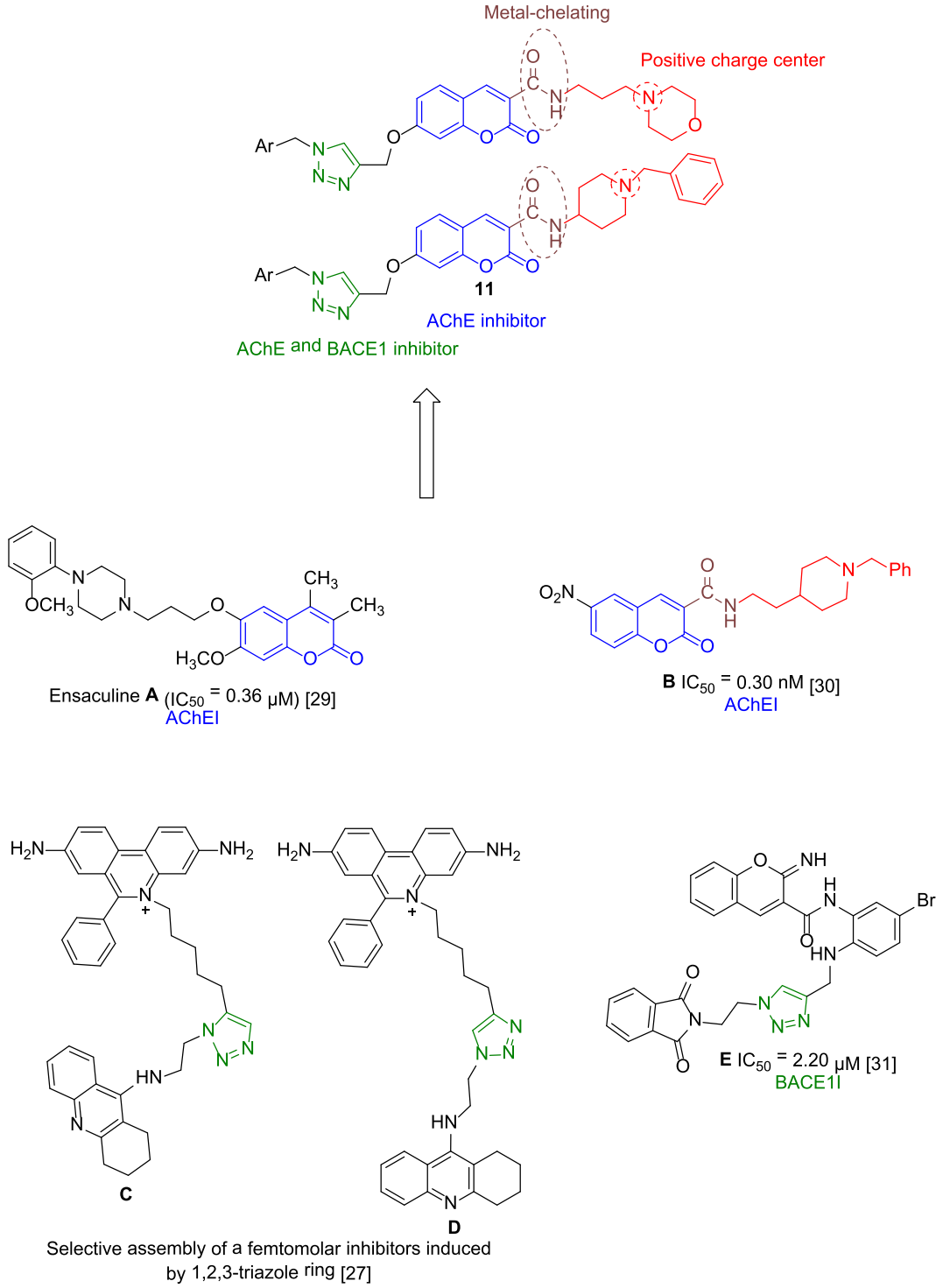


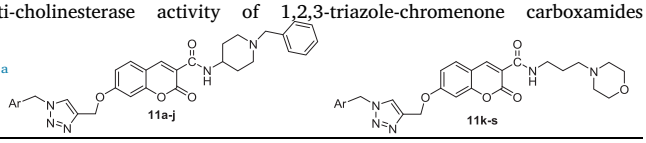
Fig. 1. Design of compounds **11**.

dichlorobenzyl group led to a brief reduction of AChEI activity of compound **11i** with IC_{50} = 2.04 μM, however, reduction of the number of chlorine group and changing position led to the reduction of anti-AChE activity of compounds **11g** and **11h** with IC_{50}s = 3.30 and 18.56 μM, respectively.

Compounds **11f**, **11g**, and **11d** showed similar activity with IC_{50}s = 3.29, 3.30, and 3.70 μM demonstrating that compounds containing 2-substituted benzyl moieties induced similar anti-AChE activity independent of electronic property of substituents in the series of compounds **11a-j**. Compound **11a** having benzyl group with no substituents afforded moderate activity toward AChE (IC_{50} = 4.50 μM). Its analogue **11j** containing fluorine showed lower activity (IC_{50} = 10.10 μM) despite the similarity between atomic size of hydrogen and fluorine. Of course, the lowest active compound in this series was compound **11e** having 4-methoxybenzyl group. Apparently, the presence of strong electron donating substituent deteriorated inhibitory activity toward AChE (IC_{50} = 36.50 μM).

In the series of compounds **11k-s**, compound **11m** having 3-morpholinopropyl and 2-bromobenzyl moieties showed good inhibitory activity toward AChE (IC_{50} = 2.10 μM). However, the activity was not

Table 1
Anti-cholinesterase activity of 1,2,3-triazole-chromenone carboxamides **11**.[a]



Entry	Ar	Product **11**	AChEI IC_{50} (μM)	BChEI IC_{50} (μM)
1	H	**11a**	4.50 ± 0.45	> 100
2	3,4-Me	**11b**	1.80 ± 0.09	> 100
3	4-Me	**11c**	20.00 ± 1.80	> 100
4	2-Me	**11d**	3.70 ± 0.26	> 100
5	4-OMe	**11e**	36.50 ± 2.56	6.18 ± 1.32
6	2-Br	**11f**	3.29 ± 0.20	> 100
7	2-Cl	**11g**	3.30 ± 0.20	> 100
8	2,3-Cl	**11h**	18.56 ± 1.86	> 100
9	3,4-Cl	**11i**	2.04 ± 0.16	> 100
10	4-F	**11j**	10.10 ± 0.91	> 100
11	4-Me	**11k**	43.54 ± 3.48	7.13 ± 1.65
12	2-Me	**11l**	9.83 ± 0.69	62.00 ± 2.48
13	2-Br	**11m**	2.10 ± 0.11	6.92 ± 0.56
14	2,3-Cl	**11n**	5.03 ± 0.35	1.71 ± 0.21
15	3,4-Cl	**11o**	102.68 ± 5.13	32.08 ± 1.85
16	4-Cl	**11p**	29.40 ± 3.20	8.70 ± 1.10
17	3-Cl	**11q**	16.20 ± 1.30	1.85 ± 0.32
18	4-F	**11r**	16.22 ± 0.81	5.40 ± 0.86
19	3-F	**11s**	22.50 ± 2.30	74.30 ± 4.12
20	**donepezil**		0.027 ± 0.002	4.32 ± 0.65

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

significantly lower than two compounds **11b** and **11i**. Replacement of bromine by other halogens led to lower activity in compounds **11n-s**. It was interesting that changing the position of chlorine in compounds **11n** and **11o** afforded different activity. Compound **11n** having 2,3-dichlorobenzyl group showed IC_{50} = 5.03 μM whereas compound **11o** having 3,4-dichlorobenzyl group showed IC_{50} = 102.68 μM. Compounds **11p** and **11q** having 4- and 3-chlorobenzyl group showed IC_{50}s = 29.40 and 16.20 μM, respectively. In the case of fluorine substituted compounds (**11r** and **11s**), changing the position of fluorine did not show a significant difference; however, 4-fluorobenzyl induced better activity. It should be noted that compounds **11k** and **11l** possessing 4- and 2-methylbenzyl moieties, respectively demonstrated similar manner comparing with their counterparts **11c** and **11d** as 2-methyl substituted derivative depicted better activity than 4-methyl substituted derivative.

It seems that benzylpiperidinyl moiety induced better anti-AChE activity than 3-morpholinopropyl which can be associated to similar moiety (benzylpiperidinyl group) ubiquitous in donepezil. Also, the presence of benzylpiperidinyl moiety and 3,4-disubstituted benzyl group connected to 1,2,3-triazole ring is significant for induction of AChEI activity in this series of 1,2,3-triazole-chromenone carboxamides **11**.

In the case of anti-BChE activity, compounds possessing benzylpiperidinyl moiety except **11e** showed no activity. However, compound **11e** showed moderate BChEI activity with IC_{50} = 6.18 μM comparing with donepezil (IC_{50} = 4.32 μM). As can be seen in Table 1, the presence of 3-morpholinopropyl induced much more better inhibitory activity than the first category of compounds **11a-j**. Among anti-BChE compounds **11k-s**, compounds **11n** and **11q** having chlorine on the benzyl group connected to 1,2,3-triazole moiety were found to be the most potent agents with IC_{50} = 1.71 and 1.85 μM, respectively. Changing the position of chlorine to 3,4- and 4- in compounds **11o** and **11p** led to the reduction of activity (IC_{50}s = 8.70 and 32.08 μM, respectively). It is clear that the presence of chlorine at 3-position of benzyl group played a significant role in BChEI activity. Also, introduction of fluorine instead of chlorine led to different results. Generally, fluorinated derivatives **11r** and **11s** depicted lower activity (IC_{50}s = 5.40 and 74.30 μM, respectively) than chlorinated counterparts **11n-q** and inversely 4-F derivative **11r** was more active than 3-F derivative **11s** by 15 times. It should be noted that compounds **11m** possessing bromine showed moderate activity (IC_{50} = 6.92 μM). Finally, our results related to compounds possessing methyl group on the benzyl group connected to 1,2,3-triazole ring showed that compound **11k** having 4-methylbenzyl (IC_{50} = 7.13 μM) was more active than compound **11l** possessing 3-methylbenzyl (IC_{50} = 62.00 μM). It seems that anti-BChE activity is relatively affected by the type of amine connected to the amide moiety as well as position and electronic properties of substituents on the benzyl group connected to 1,2,3-triazole ring.

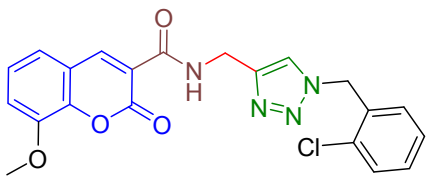
To conclude, comparing our results with those previously reported on ChEI activity of different 1,2,3-triazole-chromenone carboxamides [19] (**F**, Fig. 2) revealed that changing the manner of hybridization along with incorporation of desired positive charge centers led to much better results in this work. Also, compounds **11** had supremacy over 1,2,3-triazole-chromenones **G** [33] which depicted no ChEI activity confirming the fact that carboxamide moiety plays an important role in inducing anti-ChE activity.

2.4. Kinetic study of AChE inhibition

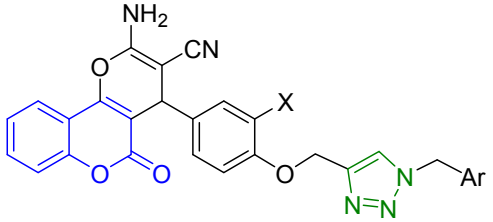
The kinetic study of AChE inhibition was studied for the most active compound **11b** with IC_{50} value of 1.80 μM. For this purpose, the rate of AChE inhibition by different concentrations (0, 1, 2 and 4 μM) of the potent inhibitor **11b** was measured in the presence of acetylthiocholine iodide (ATCh). The cholinesterase inhibition was based on the modified Ellman's method [32]. For each inhibitor concentration, the initial velocity was measured at different substrate concentrations (S) and the reciprocal of the initial velocity (1/V) was plotted respect to the reciprocal of substrate concentration (1/[S]). Accordingly, the Lineweaver-Burk plot for the inhibition of AChE and the secondary plot for calculation of steady-state inhibition constant (*Ki*) for compound **11b** were illustrated in Fig. 3. The Lineweaver-Burk plot showed a mixed-type inhibition pattern for compound **11b** against AChE. The *Ki* value was 1.37 μM as calculated from the secondary plot in Fig. 3.

2.5. β-secretase (BACE1) inhibitory activity of compound *11b*

Cleavage of amyloid precursor protein (APP) by β-secretase (BACE1) results in the formation of soluble *N*-terminal fragment and



F AChEI (IC_{50} = 15.42 μM) [19]



G Weak ChEIs [33]

Fig. 2. Previously reported 1,2,3-triazole-chromenone hybrids as ChEIs **F** and **G**.

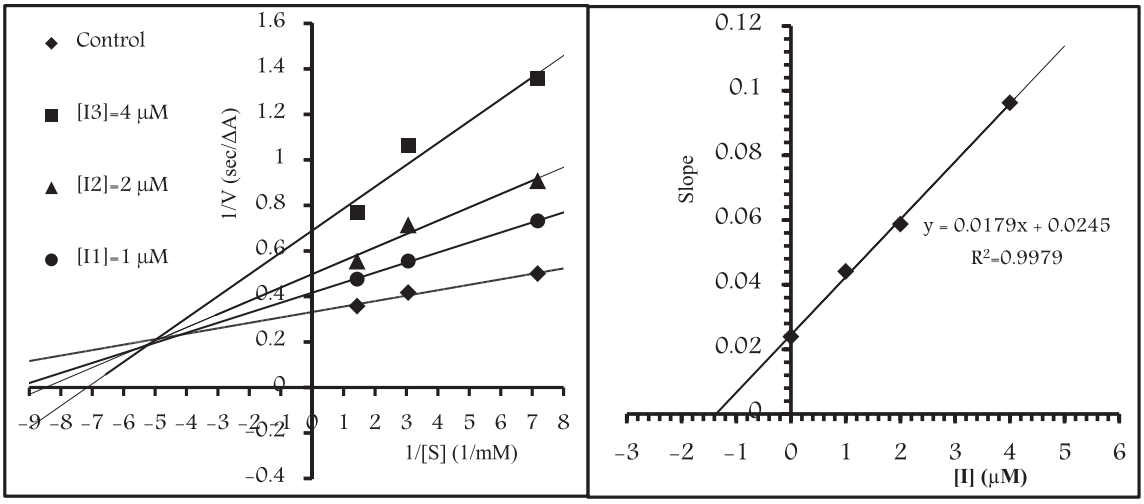


Fig. 3. *Left*: Lineweaver-Burk plot for the inhibition of AChE by compound **11b** at different concentrations of substrate (ATCh), *Right:* Secondary plot for calculation of steady-state inhibition constant (*Ki*) of compound **11b**.

the 99-residue C-terminal fragment of APP (C99). Further cleavage of C99 by another enzyme known as γ-secretase produces Aβ peptides. Since BACE1 clearly plays a direct role in the production of the Aβ peptides, several studies have focused on design and synthesis of selective inhibitors [31]. In this study, the most active anti-AChE compound **11b** was evaluated for its BACE1 inhibitory activity using FRET assay kit comparing with OM99-2 (IC_{50} = 0.014 μM) [19,31]. Compound **11b** showed relatively good inhibitory activity with IC_{50} = 21.13 μM.

2.6. Neuroprotective effect of compound **11b** against H_2O_2-induced cell death in PC12 neurons

Protective effect of compound **11b** was evaluated in comparison to intact (normal, no intervention), quercetin + H_2O_2-treated (positive control) and H_2O_2-treated (negative control) cells (Fig. 4). Compound **11b** showed moderate to good neuroprotectivity at 50 and 100 μM

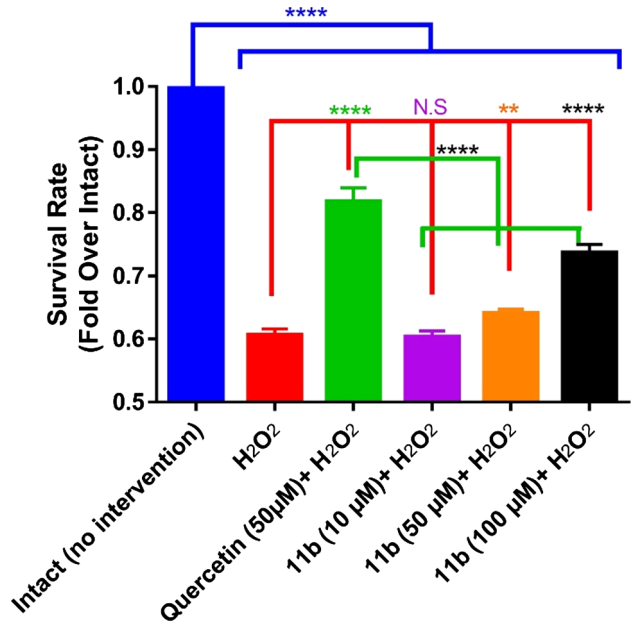


Fig. 4. Neuroprotective effect of compound **11b** on survival of H_2O_2-treated neurons. Data are expressed as mean ± SEM and one-way analysis of variance ANOVA followed by Dunnett's multiple comparisons test was used to determine the level of significance ($^{****}P < 0.0001$, $^{**}P < 0.01$).

($P < 0.01$ & $P < 0.0001$ vs. H_2O_2-treated, respectively). It should be noted that compound **11b** did not show stronger activity than quercetin ($P < 0.0001$, all **11b** treated groups vs. quercetin + H_2O_2-treated). There were significant differences between intact and other H_2O_2-treated groups indicating that apoptosis was successfully occurred in all of the H_2O_2-treated groups ($P < 0.0001$, intact vs. other groups).

2.7. Metal chelating

Role of oxidative stress in the pathogenesis of AD has been widely supported in the literature since levels of lipid peroxidation, DNA and protein oxidation products increase 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 [34]. 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 AChE inhibitor **11b** 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 **11b** depicted a blue shift with addition of Cu^{2+} or Zn^{2+} confirming the interaction of **11b** and those metal ions (Fig. 5, **A**). However, little change was observed by adding Fe^{2+}. The stoichiometry of complex **11b**-Zn^{2+} was determined using the molar ratio method [35] through titrating solutions of compound **11b** by increased amounts of $ZnCl_2$ (Fig. 5, **B**). Accordingly, intersection of two straight lines was obtained at the mole fraction of 1.00 representing a 1:1 stoichiometry for the **11b**-Zn^{2+} complex.

2.8. Ligand-protein docking study

The most promising compound **11b** was selected for docking study with acetylcholinesterase (1EVE) by using the AutoDock vina software. As depicted in Figs. 6 and 7, compound **11b** was positioned in the enzyme active site in such a way to interact with the amino acids at the rim of the cavity, and to act as a barrier preventing the entry of the substrates into the active site. Furthermore, the rest of the molecule passes through the gorge and interacts with the CAS. The coumarin fragment, which has an approximately planar structure, made π-π stacking with the aromatic amino acids of the mid-gorge including tyrosine 69 and tryptophan 278. Moreover, the 1,2,3-triazole linker bound to tryptophan 278 by a π-π interaction. In this situation, the NH of amide bond made a hydrogen bond with tyrosine 120. The pendent substituted benzyl group connected to 1,2,3-triazole was placed in the hydrophobic cavity made by isoleucine 286 and phenylalanine 289. On

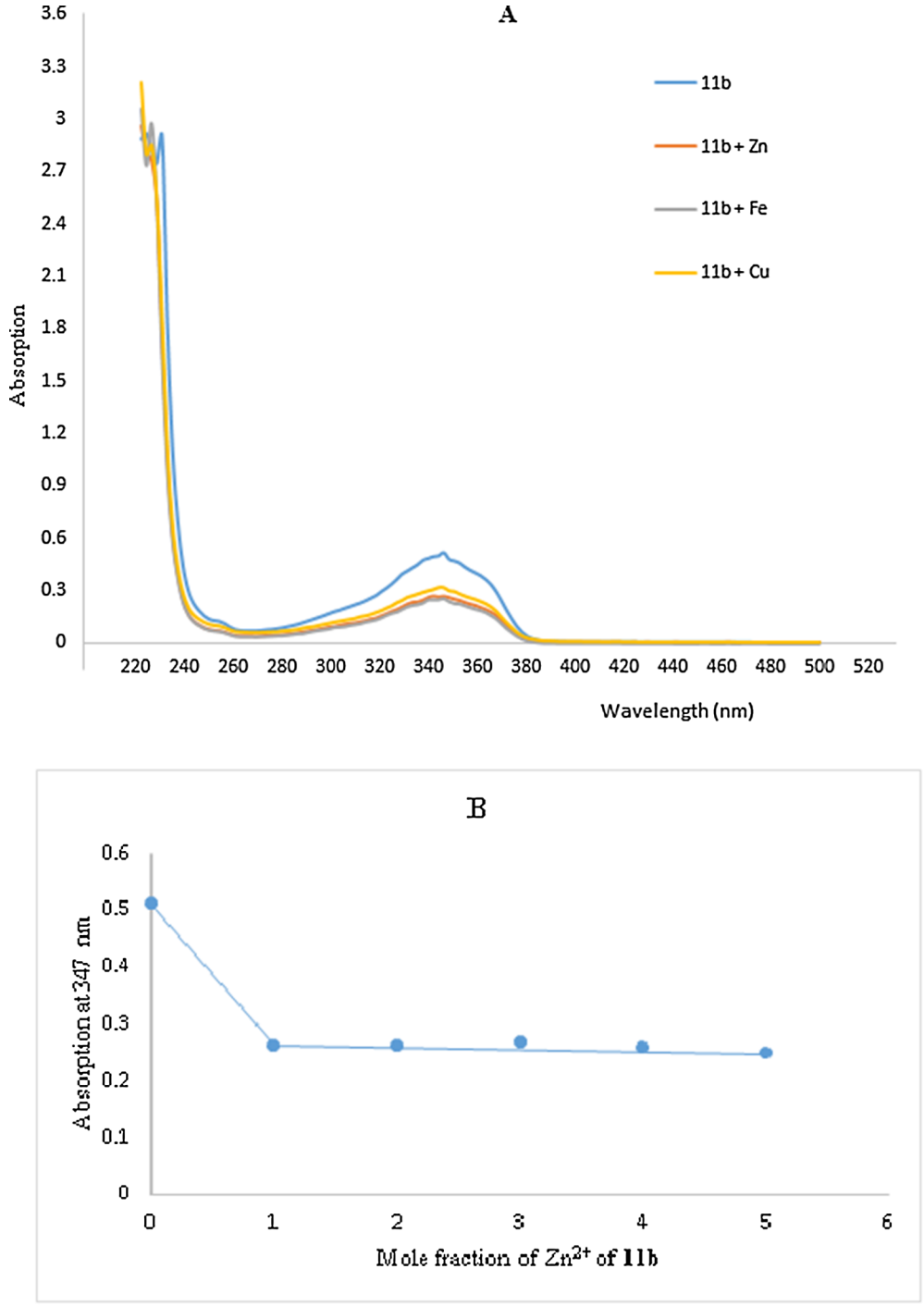


Fig. 5. (A) The UV spectrum of compound **11b** (20 μM) alone or in the presence of Zn^{2+}, Fe^{2+}, and Cu^{2+} (20 μM); all solutions were prepared in methanol. (**B**) Determination of the stoichiometry of complex **11b**-Zn^{2+} by using molar ratio method through titrating methanol solution of compound **11b** with increased amounts of Zn^{2+} solution.

the opposite side of the ligand, benzyl moiety made a π- π interaction with tryptophan 83.

3. Conclusion

In conclusion, a proficient synthetic route was described for the design and synthesis of novel 1,2,3-triazole-chromenone carboxamide derivatives as potent anti-AD agents. Most of synthesized compounds showed good AChEI activity and among them, compound **11b** was found to be the most potent inhibitor with IC_{50} = 1.80 μM. Kinetic study of compound **11b** revealed mixed type inhibition toward the CAS and the PAS of AChE. Furthermore, compound **11n** showed good anti-BChE activity (IC_{50} = 1.71 μM). It should be noted that compound **11b** showed inhibitory activity toward BACE1 with calculate IC_{50} = 21.13 μM and satisfactory metal-chelating property toward Cu^{2+} and Zn^{2+} ions. However, our results demonstrated that it possessed desirable neuroprotectivity which can be considered for further investigations for anti-AD drug developments. Comparing our results with those reported in the literature associated with hybridization of 1,2,3-triazole and chromenone moieties [19,33] confirmed the necessity of the presence of carboxamide moiety as well as the arrangement of 1,2,3-triazole and coumarin scaffolds. However, ChEI activity

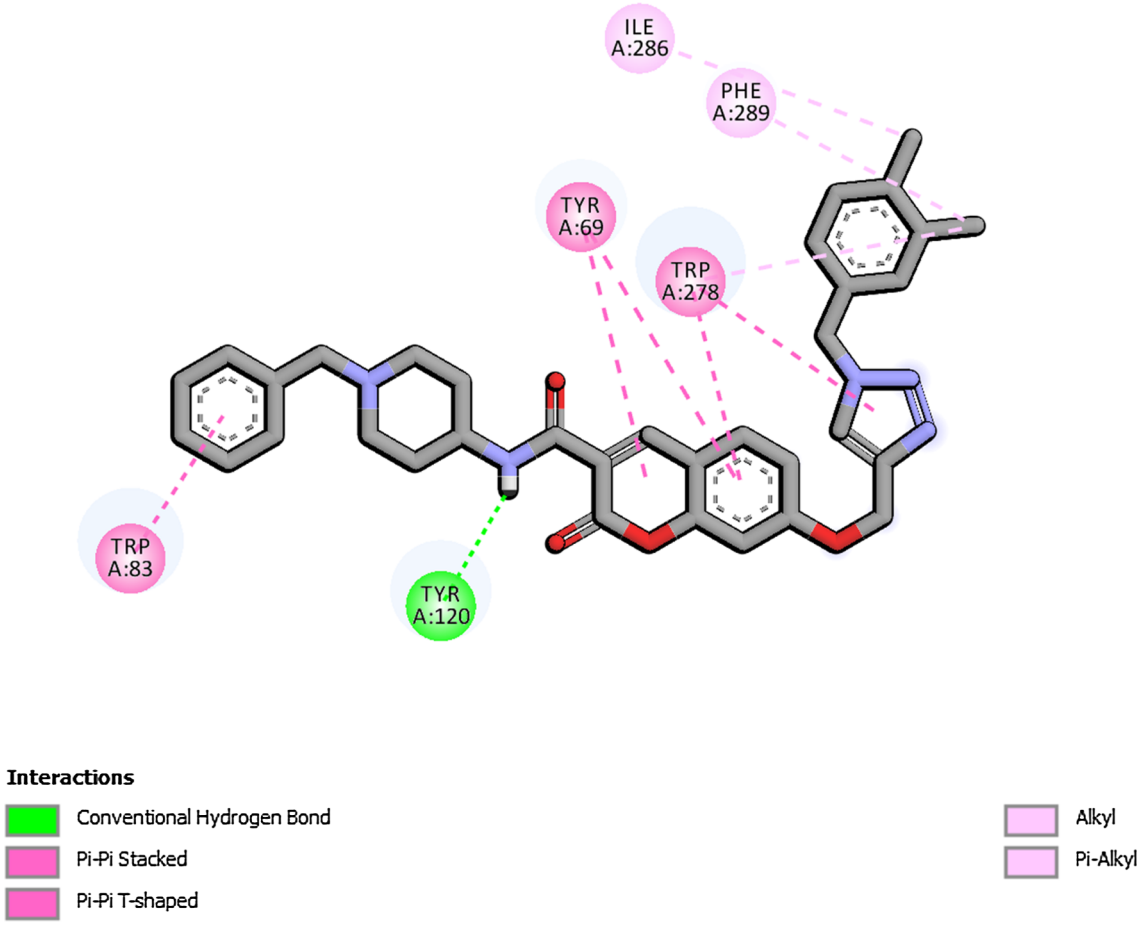


Fig. 6. The proposed orientation of compound **11b** in the active site of AChE.

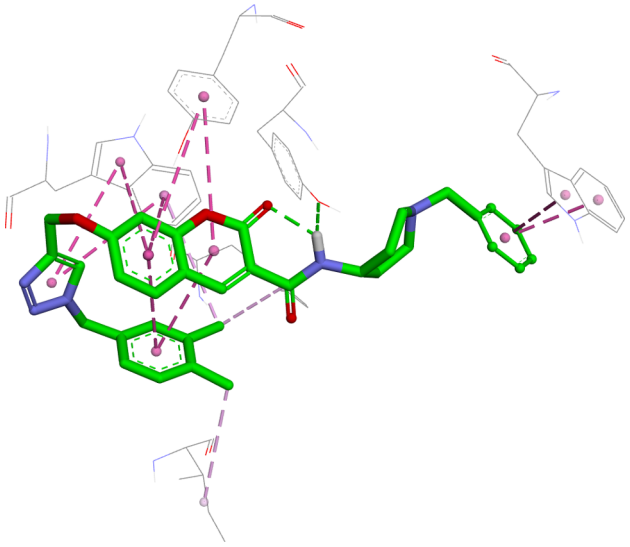


Fig. 7. Illustration of compound **11b** binding mode in the active site of AChE.

was directly affected by the positive charge center and electronic properties of substituents on the benzyl group connected to 1,2,3-triazole ring.

4. 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 Analysensystem GmbH VarioEL CHNS mode. The UV–Vis absorption spectra were recorded on a double beam GBC Cintra 101.

*4.1. General procedure for the synthesis of 1,2,3-triazole-chromenone carboxamides **11***

A mixture of 2,4-dihydroxybenzaldehyde **1** (1 mmol), diethyl malonate **2** (1 mmol), and piperidine (1 mmol) in EtOH (10 mL) was stirred at room temperature for 4–5 h. After completion of the reaction (checked by TLC), the mixture was poured into water to precipitate ethyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate **3**. Next, compound **3** (1 mmol) reacted with propargyl bromide **4** (1 mmol) in the presence of K_2CO_3 (1 mmol) in DMF (5 mL) at 80 °C for 5 h. Then, the mixture was poured into cold water and the precipitates were filtered off and washed with water to obtain ethyl 2-oxo-7-(prop-2-yn-1-yloxy)-2*H*-chromene-3-carboxylate **5**. Compound **5** (1 mmol) was stirred in the presence of NaOH 5% (5 mL) in EtOH (5 mL) at room temperature for 4–5 h. After completion of the reaction (checked by TLC), the mixture was neutralized with HCl 5%, the precipitates were filtered off, washed with water to afford the corresponding acid **6**. A solution of compound **6** (1 mmol), DCC (1.1 mmol), and HOBt (1 mmol) in dry acetonitrile (5 mL) was stirred at room temperature for 30 min. Then, appropriate amine **7** (1 mmol) was added to the mixture and the reaction was continued at room temperature for 20–24 h. After completion of the reaction, the solvent was reduced under vacuum and the residue was dissolved in dichloromethane and washed with citric acid and sodium hydrogen carbonate (10%). The organic phase was dried over

anhydrous Na_2SO_4 and the solvent was evaporated. The obtained compound **8** was completely pure and used for further reaction. Finally, a solution of benzyl chloride/bromide derivative **9** (1.1 mmol), sodium azide (0.06 g, 0.9 mmol), and trimethylamine (0.13 g, 1.3 mmol) in water (4 mL) and *tert*-butyl alcohol (4 mL) was stirred at room temperature for 30 min. Then, compound **8** (0.5 mmol) and $CuSO_4{\cdot}5H_2O$ (7 mol%) were added to the reaction mixture and it was continued for 24 h. Upon completion of the reaction, monitored by TLC, the mixture was diluted with water, extracted with chloroform, and dried over anhydrous Na_2SO_4. After evaporation of solvent, the residue was recrystallized from ethyl acetate and petroleum ether to give pure product **11**.

*4.1.1. 7-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-N-(1-benzylpiperidin-4-yl)-2-oxo-2H-chromene-3-carboxamide (**11a**)*

Yield: 75%, white solids, mp 206–208 °C. IR (KBr): 3337, 2932, 2806, 1710, 1650, 1610, 1560 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.52–1.54 (m, 2H, CH_2), 1.84–1.86 (m, 2H, CH_2), 2.14–2.16 (m, 2H, CH_2), 2.70–2.71 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.30 (s, 2H, CH_2), 5.62 (s, 2H, CH_2), 7.09 (d, J = 9.0 Hz, 1H, H6), 7.27–7.37 (m, 11*H*, Ph, H8), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.35 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.4, 46.5, 51.5, 52.9, 61.9, 62.1, 95.2, 101.1, 112.4, 114.1, 115.0, 125.1, 126.9, 128.0, 128.2, 128.8, 131.6, 133.8, 135.9, 138.5, 142.0, 147.7, 156.0, 160.5, 161.5, 163.0. Anal. calcd. for $C_{32}H_{31}N_5O_4$: C, 69.93; H, 5.69; N, 12.74. Found: C, 70.17; H, 5.82; N, 12.61.

*4.1.2. N-(1-Benzylpiperidin-4-yl)-7-((1-(3,4-dimethylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11b**)*

Yield: 70%, white solids, mp 185–187 °C. IR (KBr): 3330, 2940, 2807, 1707, 1652, 1610, 1530 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.50–1.56 (m, 2H, CH_2), 1.85–1.87 (m, 2H, CH_2), 2.14–2.16 (m, 2H, CH_2), 2.23 (s, 6H, 2 × CH_3), 2.69–2.71 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.30 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.93–6.95 (m, 3H, H8, H2′, H6′), 7.09 (dd, J = 9.0, 2.0 Hz, 1H, H6), 7.24–7.34 (m, 6H, Ph, H4′), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.32 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.81 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 20.8, 31.4, 46.2, 51.5, 52.9, 61.9, 62.1, 95.4, 101.1, 112.4, 114.1, 115.0, 125.1, 125.7, 126.9, 128.1, 128.7, 129.6, 131.6, 135.7, 137.9, 141.9, 147.7, 156.0, 160.6, 161.0, 163.0. Anal. calcd. for $C_{34}H_{35}N_5O_4$: C, 70.69; H, 6.11; N, 12.12. Found: C, 70.53; H, 6.27; N, 12.28.

*4.1.3. N-(1-Benzylpiperidin-4-yl)-7-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11c**)*

Yield: 72%, white solids, mp 170–172 °C. IR (KBr): 3328, 3050, 2939, 2809, 1706, 1654, 1610, 1542 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.50–1.56 (m, 2H, CH_2), 1.85–1.87 (m, 2H, CH_2), 2.15–2.17 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.70–2.72 (m, 2H, CH_2), 3.48 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.29 (s, 2H, CH_2), 5.56 (s, 2H, CH_2), 7.09 (dd, J = 9.0, 2.5 Hz, 1H, H6), 7.17–7.34 (m, 10*H*, Ph, H3′, H4′, H5′, H6′, H8), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.31 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 20.7, 31.4, 47.5, 51.5, 52.7, 61.9, 62.1, 95.1, 101.1, 112.4, 114.1, 115.3, 125.0, 126.8, 128.0, 128.1, 128.7, 129.3, 131.5, 133.0, 137.5, 141.9, 147.7, 156.0, 160.7, 161.3, 163.2. Anal. calcd. for $C_{33}H_{33}N_5O_4$: C, 70.32; H, 5.90; N, 12.43. Found: C, 70.45; H, 6.11; N, 12.56.

*4.1.4. N-(1-Benzylpiperidin-4-yl)-7-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11d**)*

Yield: 70%, white solids, mp 199–201 °C. IR (KBr): 3339, 3050, 2928, 2850, 1710, 1650, 1610, 1560 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.52–1.54 (m, 2H, CH_2), 1.85–1.86 (m, 2H, CH_2), 2.16–2.17 (m, 2H, CH_2), 2.31 (s, 3H, CH_3), 2.71–2.72 (m, 2H, CH_2), 3.48 (s, 2H, CH_2), 3.78–3.80 (m, 1H, CH), 5.30 (s, 2H, CH_2), 5.63 (s, 2H, CH_2), 7.08–7.32 (m, 11*H*, Ph, H3′, H4′, H5′, H6′, H6, H8), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.25 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.81 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 18.6, 31.3, 48.1, 51.0, 51.4, 61.9, 62.0, 95.1, 101.1, 112.3, 114.1, 115.0, 125.2, 126.2, 126.9, 128.1, 128.4, 128.8, 130.4, 131.5, 134.0, 136.3, 138.5, 141.9, 147.7, 156.0, 160.5, 161.0, 163.0. Anal. calcd. for $C_{33}H_{33}N_5O_4$: C, 70.32; H, 5.90; N, 12.43. Found: C, 70.51; H, 5.76; N, 12.28.

*4.1.5. N-(1-Benzylpiperidin-4-yl)-7-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11e**)*

Yield: 71%, white solids, mp 183–185 °C. IR (KBr): 3335, 2942, 2825, 1710, 1650, 1610, 1530 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.52–1.54 (m, 2H, CH_2), 1.84–1.85 (m, 2H, CH_2), 2.15–2.17 (m, 2H, CH_2), 2.69–2.71 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.80–3.82 (m, 1H, CH), 5.29 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.92–7.31 (m, 11*H*, Ph, H2′, H3′, H5′, H6′, H6, H8), 7.89 (d, J = 9.0 Hz, 1H, H5), 8.28 (s, 1H, triazole), 8.57 (d, J = 7.5 Hz, 1H, NH), 8.80 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.3, 43.8, 47.5, 51.3, 52.4, 55.1, 62.0, 95.5, 101.1, 114.1, 115.2, 122.8, 123.9, 124.6, 126.8, 128.0, 128.6, 129.5, 131.4, 137.1, 141.9, 147.5, 155.8, 160.2, 160.8, 161.1, 163.4. Anal. calcd. for $C_{33}H_{33}N_5O_5$: C, 68.38; H, 5.74; N, 12.08. Found: C, 68.22; H, 5.59; N, 12.21.

*4.1.6. N-(1-Benzylpiperidin-4-yl)-7-((1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11f**)*

Yield: 75%, white solids, mp 185–187 °C. IR (KBr): 3342, 2939, 2820, 1708, 1650, 1610, 1559, 1526 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.50–1.56 (m, 2H, CH_2), 1.85–1.87 (m, 2H, CH_2), 2.13–2.15 (m, 2H, CH_2), 2.69–2.71 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.79–3.81 (m, 1H, CH), 5.32 (s, 2H, CH_2), 5.71 (s, 2H, CH_2), 7.10 (dd, J = 8.5, 2.0 Hz, 1H, H6), 7.21 (dd, J = 8.0, 1.5 Hz, 1H, H6′), 7.23–7.32 (m, 7H, Ph, H8, H4′), 7.41 (t, J = 8.0 Hz, 1H, H5′), 7.69 (d, J = 8.0 Hz, 1H, H3′), 7.90 (d, J = 8.5 Hz, 1H, H5), 8.32 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.4, 46.3, 51.5, 53.0, 61.8, 62.1, 95.1, 101.1, 112.4, 114.2, 115.0, 125.6, 126.8, 128.1, 128.3, 128.7, 130.5, 130.6, 131.6, 132.9, 134.7, 138.5, 141.8, 147.7, 156.1, 160.8, 161.0, 163.0. Anal. calcd. for $C_{32}H_{30}BrN_5O_4$: C, 61.15; H, 4.81; N, 11.14. Found: C, 61.28; H, 4.67; N, 11.31.

*4.1.7. N-(1-Benzylpiperidin-4-yl)-7-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11g**)*

Yield: 70%, white solids, mp 201–203 °C. IR (KBr): 3340, 3050, 2941, 2806, 1708, 1649, 1610, 1561, 1524 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.50–1.52 (m, 2H, CH_2), 1.78–1.80 (m, 2H, CH_2), 2.14–2.15 (m, 2H, CH_2), 2.70–2.72 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.31 (s, 2H, CH_2), 5.73 (s, 2H, CH_2), 7.09 (d, J = 9.0 Hz, 1H, H6), 7.25–7.40 (m, 9H, Ph, H4′, H5′, H6′, H8), 7.51 (d, J = 7.5 Hz, 1H, H3′), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.33 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.4, 47.2, 50.7, 51.5, 61.8, 62.1, 95.1, 101.1, 112.4, 114.1, 115.0, 125.5, 126.9, 127.7, 128.1, 128.7, 129.6, 130.3, 130.6, 131.5, 132.7, 133.1, 141.8, 147.7, 156.0, 160.6, 161.0, 163.1. Anal. calcd. for $C_{32}H_{30}ClN_5O_4$: C, 65.81; H, 5.18; N, 11.99. Found: C, 67.64; H, 5.31; N, 12.18.

*4.1.8. N-(1-Benzylpiperidin-4-yl)-7-((1-(2,3-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (**11h**)*

Yield: 70%, white solids, mp 217–2019 °C. IR (KBr): 3334, 2932, 2850, 1708, 1650, 1610, 1560, 1529 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.50–1.52 (m, 2H, CH_2), 1.85–1.87 (m, 2H, CH_2), 2.15–2.17 (m, 2H, CH_2), 2.70–2.72 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.32 (s, 2H, CH_2), 5.79 (s, 2H, CH_2), 7.10 (d, J = 9.0 Hz, 1H, H6), 7.20 (d, J = 8.0 Hz, 1H, H6′), 7.25–7.39 (m, 6H, Ph, H8), 7.40 (t, J = 8.0 Hz, 1H, H5′), 7.67 (d, J = 8.0 Hz, 1H, H4′), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.36 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.3, 47.1, 51.2, 51.4, 61.8,

62.1, 95.1, 101.1, 112.4, 114.1, 115.1, 125.1, 126.9, 128.2, 128.7, 128.8, 129.1, 130.6, 131.6, 132.2, 135.8, 137.1, 141.9, 147.7, 156.0, 160.6, 161.0, 163.0. Anal. calcd. for $C_{32}H_{29}Cl_2N_5O_4$: C, 62.14; H, 4.73; N, 11.32. Found: C, 62.31; H, 4.58; N, 11.50.

4.1.9. N-(1-Benzylpiperidin-4-yl)-7-((1-(3,4-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (11i)

Yield: 72%, white solids, mp 184–186 °C. IR (KBr): 3318, 3063, 2945, 2806, 1706, 1651, 1611, 1548 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.50–1.56 (m, 2H, CH_2), 1.85–1.87 (m, 2H, CH_2), 2.13–2.17 (m, 2H, CH_2), 2.69–2.71 (m, 2H, CH_2), 3.47 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.32 (s, 2H, CH_2), 5.65 (s, 2H, CH_2), 7.10 (dd, J = 9.0, 2.0 Hz, 1H, H6), 7.23–7.32 (m, 6H, Ph, H8), 7.64–7.63 (m, 3H, H2′, H5′, H6′), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.40 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.4, 47.0, 51.4, 51.5, 61.8, 62.1, 95.6, 101.1, 108.4, 112.4, 114.1, 115.0, 125.3, 126.8, 128.1, 128.5, 128.7, 130.2, 131.0, 131.6, 136.8, 138.5, 142.1, 147.7, 156.0, 160.6, 161.0, 163.0. Anal. calcd. for $C_{32}H_{29}Cl_2N_5O_4$: C, 62.14; H, 4.73; N, 11.32. Found: C, 62.26; H, 4.84; N, 11.19.

4.1.10. N-(1-Benzylpiperidin-4-yl)-7-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-oxo-2H-chromene-3-carboxamide (11j)

Yield: 75%, white solids, mp 213–215 °C. IR (KBr): 3333, 2931, 2804, 1712, 1650, 1610, 1560, 1529 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.52–1.54 (m, 2H, CH_2), 1.84–1.87 (m, 2H, CH_2), 2.14–2.15 (m, 2H, CH_2), 2.69–2.70 (m, 2H, CH_2), 3.48 (s, 2H, CH_2), 3.80–3.82 (m, 1H, CH), 5.30 (s, 2H, CH_2), 5.61 (s, 2H, CH_2), 7.09 (dd, J = 9.0, 2.5 Hz, 1H, H6), 7.19–7.42 (m, 10*H*, Ph, H2′, H3′, H5′, H6′, H8), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.35 (s, 1H, triazole), 8.59 (d, J = 7.5 Hz, 1H, NH), 8.82 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 31.4, 47.1, 51.4, 52.1, 61.9, 62.1, 95.2, 101.1, 114.1, 115.6 (d, $J_{C\text{-}F}$ = 22.5 Hz), 125.0, 126.9, 128.1, 128.7, 130.4 (d, $J_{C\text{-}F}$ = 8.7 Hz), 131.6, 131.9, 135.9, 138.5, 142.0, 147.7, 156.2, 160.8, 161.3, 163.0, 163.1 (d, $J_{C\text{-}F}$ = 245.0 Hz). Anal. calcd. for $C_{32}H_{30}FN_5O_4$: C, 67.71; H, 5.33; N, 12.34. Found: C, 67.59; H, 5.18; N, 12.56.

4.1.11. 7-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11k)

Yield: 80%, white solids, mp 172–173 °C. IR (KBr) cm^{-1}: 3328, 2942, 2810, 2767, 1707, 1610, 1545. ^1H NMR (500 MHz, DMSO-d_6): 1.25–1.27 (m, 2H, CH_2), 1.81 (s, 3H, CH_3), 2.47–2.49 (m, 6H, 3 × CH_2), 3.47–3.49 (m, 2H, CH_2), 3.74–3.76 (m, 4H, 2 × CH_2), 5.30 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.84 (d, J = 9.0 Hz, 1H, H_6), 7.04–7.05 (m, 4H, H2′, H3′, H5′, H6′), 7.82 (d, J = 9.0 Hz, 1H, H_5), 8.21 (s, 1H, triazole), 8.59 (m, 1H, NH), 8.69 (s, 1H, H_4). ^{13}C NMR (125 MHz, DMSO-d_6): 20.5, 26.0, 38.3, 49.7, 53.8, 56.6, 61.8, 62.5, 95.3, 101.1, 111.5, 113.6, 124.8, 127.9 129.2, 131.5, 132.8, 137.4, 141.9, 148.9, 156.1, 156.7, 162.7, 163.3. Anal. calcd. for $C_{28}H_{31}N_5O_5$: C, 64.98; H, 6.04; N, 13.53. Found: C, 65.28; H, 5.87; N, 13.74.

4.1.12. 7-((1-(2-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11l)

Yield: 72%, white solids, mp 174–175 °C. IR (KBr): 3435, 2935, 2810, 2767, 1709, 1614, 1565 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 1.29–1.32 (m, 2H, CH_2), 2.30 (s, 3H, CH_3), 2.47–2.49 (m, 4H, 2 × CH_2), 3.21–3.30 (m, 4H, 2 × CH_2), 4.26–4.30 (m, 4H, 2 × CH_2), 5.30 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 7.05 (dd, J = 9.0, 1.0 Hz, 1H, H6), 7.09–7.24 (m, 5H, H8, H3′, H4′, H5′, H6′), 7.82 (d, J = 9.0 Hz, 1H, H5), 8.21(s, 1H, triazole), 8.55(bs, 1H, NH), 8.69(s, 1H, H_4). ^{13}C NMR (125 MHz, DMSO-d_6): 18.5, 25.1, 39.0, 50.1, 51.0, 56.0, 60.8, 61.8, 95.1, 101.1, 111.6, 113.6, 125.0, 126.1, 128.2, 128.6, 130.3, 131.5, 133.8, 136.2, 141.8, 148.9, 156.1, 156.7, 162.7, 163.3. Anal. calcd. for $C_{28}H_{31}N_5O_5$: C, 64.98; H, 6.04; N, 13.53. Found: C, 64.70; H, 6.28; N, 13.29.

4.1.13. 7-((1-(2-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11m)

Yield: 65%, white solids, mp 180–181 °C. IR (KBr): 3340, 2945, 2825, 1705, 1655, 1610, 1561 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.78–1.80 (m, 2H, CH_2), 2.33–2.34 (m, 6H, 3 × CH_2), 3.40–3.42 (m, 2H, CH_2), 3.57–3.58 (m, 4H, CH_2), 5.30 (s, 2H, CH_2), 5.79 (s, 2H, CH_2), 7.03–7.69 (m, 5H, H6, H8, H4′, H5′, H6′), 7.90 (d, J = 8.5 Hz, 1H, H3′), 8.30–8.32 (m, 2H, H5, triazole), 8.76 (bs, 1H, NH), 8.23 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 25.5, 38.0, 53.0, 53.4, 56.0, 61.1, 66.1, 95.4, 103.1, 114.4, 115.1, 125.6, 128.3, 129.4, 130.5, 131.3, 132.9, 134.5, 136.0, 142.0, 147.3, 155.8, 160.2, 161.4, 163.0. Anal. calcd. for $C_{27}H_{28}BrN_5O_5$: C, 55.68; H, 4.85; N, 12.02. Found: C, 55.41; H, 4.68; N, 11.84.

4.1.14. 7-((1-(2,3-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11n)

Yield: 70%, white solids, mp 173–175 °C. IR (KBr): 3340, 2940, 2825, 1707, 1650, 1610, 1560 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.69 (quintet, J = 7.0 Hz, 2H, CH_2), 2.34–2.36 (m, 6H, 3 × CH_2), 3.36 (t, J = 7.0 Hz, 2H, CH_2), 3.57–3.59 (m, 4H, 2 × CH_2), 5.33 (s, 2H, CH_2), 5.79 (s, 2H, CH_2), 7.09 (dd, J = 8.5, 2.0 Hz, 1H, H6), 7.20 (d, J = 8.0 Hz, 1H, H6′), 7.27 (d, J = 2.0 Hz, 1H, H8), 7.41 (t, J = 8.0 Hz, 1H, H5′), 7.67 (d, J = 8.0 Hz, 1H, H4′), 7.90 (d, J = 8.5 Hz, 1H, H5), 8.37 (s, 1H, triazole), 8.77 (t, J = 6.0 Hz, 1H, NH), 8.83 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 25.6, 38.1, 51.2, 53.3, 56.1, 61.8, 66.0, 95.4, 110.1, 112.4, 114.0, 115.1, 125.7, 128.6, 129.1, 130.6, 131.5, 132.2, 135.8, 141.9, 147.6, 155.9, 160.7, 161.2, 162.9. Anal. calcd. for $C_{27}H_{27}Cl_2N_5O_5$: C, 56.65; H, 4.75; N, 12.23. Found: C, 56.80; H, 4.58; N, 12.38.

4.1.15. 7-((1-(3,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11o)

Yield: 70%, white solids, mp 144–146 °C. IR (KBr): 3340, 2919, 2820, 1710, 1650, 1610, 1560 cm^{-1}. ^1H NMR (500 MHz: DMSO-d_6): 1.68 (quintet, J = 6.5 Hz, 2H, CH_2), 2.34–2.36 (m, 6H, 3 × CH_2), 3.36 (t, J = 6.5 Hz, 2H, CH_2), 3.57–3.59 (m, 4H, 2 × CH_2), 5.32 (s, 2H, CH_2), 5.66 (s, 2H, CH_2), 7.10 (d, J = 9.0 Hz, 1H, H6), 7.27 (s, 1H, H8), 7.32 (d, J = 8.5 Hz, 1H, H6′), 7.63–7.66 (m, 2H, H2′, H5′), 7.90 (d, J = 9.0 Hz, 1H, H5), 8.41 (s, 1H, triazole), 8.77 (t, J = 6.0 Hz, 1H, NH), 8.83 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 25.6, 38.0, 51.5, 53.3, 56.1, 61.8, 66.0, 95.4, 101.1, 112.4, 114.0, 115.3, 125.3, 128.5, 130.2, 131.0, 131.3, 131.5, 136.9, 142.2, 147.6, 156.0, 160.5, 161.1, 163.0. Anal. calcd. for $C_{27}H_{27}Cl_2N_5O_5$: C, 56.65; H, 4.75; N, 12.23. Found: C, 56.52; H, 4.51; N, 12.42.

4.1.16. 7-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11p)

Yield: 73%, white solids, mp 182–183 °C. IR (KBr): 3347, 3063, 2926, 2856, 1715, 1613, 1532. ^1H NMR (500 MHz, $CDCl_3$): 1.80–1.81 (m, 2H, CH_2), 2.44–2.46 (m, 6H, 3 × CH_2), 3.51–3.522 (m, 2H, CH_2), 3.72–3.74 (m, 4H, 2 × CH_2), 5.27 (s, 2H, CH_2), 5.52 (s, 2H, CH_2), 6.98–7.01 (m, 2H, H6, H8), 7.22 (d, J = 7.0 Hz, 2H, H2′, H6′), 7.36 (d, J = 7.0 Hz, 2H, H3′, H5′), 7.57 (d, J = 7.5 Hz, 1H, H5), 8.82–8.90 (m, 3H, triazole, H4, NH). ^{13}C NMR (125 MHz, $CDCl_3$): 25.1, 38.2, 53.6, 53.8, 56.6, 62.5, 66.9, 95.5, 101.5, 112.9, 114.1, 115.4, 115.7, 122.8, 129.5, 131.0, 135.1, 143.1, 148.1, 156.4, 161.6, 161.9, 163.1. Anal. calcd. for $C_{27}H_{28}ClN_5O_5$: C, 60.28; H, 5.25; N, 13.02. Found: C, 60.11; H, 5.41; N, 12.87.

4.1.17. 7-((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11q)

Yield: 65%, white solids, mp 195–196 °C. IR (KBr): 3346, 3091, 2941, 2806, 1716, 1610, 1532.

^1H NMR (500 MHz, $CDCl_3$): 1.80–1.82 (m, 2H, CH_2), 2.45–2.46 (m, 6H, 3 × CH_2), 3.52–3.53 (m, 2H, CH_2), 3.72–3.73 (m, 4H, 2 × CH_2), 5.29 (s, 2H, CH_2), 5.53 (s, 2H, CH_2), 6.98–7.01 (m, 2H, H6, H8), 7.17

(d, J = 7.0 Hz, 1H, H6′), 7.31–7.34 (m, 2H, H4′, H5′), 7.58 (d, J = 8.5 Hz, 1H, H5), 7.62 (s, 1H, H2′), 8.70–8.90 (m, 3H, triazole, H4, NH). ^{13}C NMR (125 MHz, $CDCl_3$)/(two isomers probably due to delocalization of lone pair on the nitrogen): 26.0, 38.2, 53.6, 53.8, 56.6, 62.5, 66.9, 100.9, 101.5, 112.9, 113.3, 114.1, 115.3, 123.0, 126.1, 128.0, 128.2, 129.1, 129.5, 130.5, 131.0, 135.1, 136.2, 143.2, 147.9, 148.1, 156.4, 161.2, 162.9. Anal. calcd. for $C_{27}H_{28}ClN_5O_5$: C, 60.28; H, 5.25; N, 13.02. Found: C, 60.44; H, 5.15; N, 13.2.

4.1.18. 7-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11r)

Yield: 75%, white solids, mp 171–173 °C. IR (KBr): 3340, 2918, 2820, 1710, 1655, 1610, 1565 cm^{-1}. ^{1}H NMR (500 MHz: DMSO-d_6): 1.68 (quintet, J = 6.5 Hz, 2H, CH_2), 2.33–2.36 (m, 6H, 3 × CH_2), 3.36 (t, J = 6.5 Hz, 2H, CH_2), 3.57–3.59 (m, 4H, 2 × CH_2), 5.31 (s, 2H, CH_2), 5.62 (s, 2H, CH_2), 7.10 (dd, J = 8.5, 2.5 Hz, 1H, H6), 7.22 (t, J = 8.0 Hz, 2H, H3′, H5′), 7.27 (d, J = 2.5 Hz, 1H, H8), 7.41 (dd, J = 8.0, 5.5 Hz, 2H, H2′, H6′), 7.90 (d, J = 8.5 Hz, 1H, H5), 8.35 (s, 1H, triazole), 8.76 (t, J = 5.5 Hz, 1H, NH), 8.83 (s, 1H, H4). ^{13}C NMR (125 MHz: DMSO-d_6): 25.6, 38.1, 52.1, 53.4, 56.1, 61.9, 66.1, 95.5, 101.1, 112.6, 114.0, 115.6 (d, $J_{C\text{-}F}$ = 21.5 Hz), 125.0, 130.3 (d, $J_{C\text{-}F}$ = 7.5 Hz), 131.5, 133.5, 142.1, 147.7, 156.1, 160.5, 161.2, 162.9. 163.1 (d, $J_{C\text{-}F}$ = 245.0 Hz). Anal. calcd. for $C_{27}H_{28}FN_5O_5$: C, 62.18; H, 5.41; N, 13.43. Found: C, 62.29; H, 5.64; N, 13.59.

4.1.19. 7-((1-(3-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-N-(3-morpholinopropyl)-2-oxo-2H-chromene-3-carboxamide (11s)

Yield: 70%, white solids, mp 201–202 °C. IR (KBr): 3348, 3063, 2926, 2856, 1715, 1611, 1532 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): 1.8–1.83 (m, 2H, CH_2), 2.47–249 (m, 6H, 3 × CH_2), 3.52 (m, 2H, CH_2), 3.35–375 (m, 4H, 2 × CH_2), 5.28 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 6.98–7.59 (m, 7H, H5, H6, H8, H2′, H4′, H5′, H6′), 8.81–8.89 (m, 3H, triazole, H4, NH). ^{13}C NMR (125 MHz, DMSO-d_6): 29.7, 38.2, 53.0, 53.7, 56.6, 62.5, 66.7, 95.7, 101.5, 112.6, 114.0, 114.4 (d, $J_{C\text{-}F}$ = 22.1 Hz), 115.6 (d, $J_{C\text{-}F}$ = 21.5 Hz), 125.2, 130.8 (d, $J_{C\text{-}F}$ = 7.5 Hz), 131.0, 132.0, 133.5, 142.0, 147.5, 156.1, 160.3, 161.2, 162.8. 163.2 (d, $J_{C\text{-}F}$ = 247.0 Hz). Anal. calcd. for $C_{27}H_{28}FN_5O_5$: C, 62.18; H, 5.41; N, 13.43. Found: C, 61.91; H, 5.18; N, 13.21.

4.2. In vitro AChE/BChE inhibition assay

The anti-cholinesterase activity of target compounds **11** was assessed *in vitro* against AChE from Electrophorus electricus (eel AChE) and horse serum butyrylcholinesterase (eq BChE) by using the spectrophotometric Ellman's method [32]. In order to obtain a range of 20–80% enzyme inhibition, five different concentrations of each compound were tested. A mixture of phosphate buffer (0.1 mol/L^{-1}, pH 8.0, 3 mL), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB, 100 µL), acetylcholinesterase or butyrylcholinesterase (100 µL, 2.5 IU/mL) and compound solution (100 µL) was pre-incubated for 10 min. Then, the substrate (acetylthiocholine iodide or butyrylthiocholine iodide) was added. In parallel, a blank containing all components without enzyme was used in order to account the non-enzymatic reaction. Changes in absorbance were measured at 412 nm for 6 min at 25 °C by using an UV Unico Double Beam spectrophotometer. The IC_{50} values were determined graphically from log concentration vs. % of inhibition curves. All experiments were performed in triplicate

4.3. B-Secretase inhibitory activity

β-Secretase inhibitory activity of compound **11b** was performed according to our previous report using a FRET-based assay kit, from Invitrogen (former Pan Vera, Madison, WI) comparing with OM99 was used as a reference inhibitor agent [19,31]. Stock solution of compound **11b** was prepared by solving that in DMSO and diluted with the assay buffer (50 mM sodium acetate; pH 4.5). 10 µL of BACE1 substrate (Rh-EVNLDAEFK-Quencher) was mixed with 10 µL of the tested compound and then 10 µL of enzyme (1 U/mL) was added to initiate the reaction. After 90 min of incubation at room temperature, 10 µL sodium acetate (2.5 M) was used to stop the reaction. The fluorescence was monitored at 545 nm (Ex) and 585 nm (Em). The experiment was achieved in triplicate for each concentration in 96-well polystyrene black plates.

4.4. Neuroprotectivity assay

PC12 cell line was obtained from Pasteur institute and they were cultivated in DMEM supplemented with 10% fetal calf serum, 5% horse, and antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin). All culture media and supplements were purchased from Gibco. To induce neuronal differentiation, PC12 cells were re-suspended using trypsin/EDTA (0.25%) and seeded in 96 well culture plate (3000 cells/well) and cultured for 1 week in differentiation medium (DMEM + 2% horse serum + NGF (100 ng/mL) + penicillin & streptomycin). To evaluate the effect of drugs on survival rate of neurons, the culture medium was changed to NGF free medium and different concentrations of candidate drug (10, 50, and 100 µM) were applied on cells. Quercetin (50 µM) was used as a positive control. Compound **11b** was diluted in DMEM and 10 µL of that solution added to each well. After 3 h, induction of ROS-mediated apoptosis was initiated by adding the H_2O_2 (400 µM) to their medium and after 12 h, MTT assay was performed. For this purpose, 10 µL of MTT solution (5 mg/mL) was added to each well and after 3 h, culture medium was replaced by 100 µL of DMSO. Absorbance was measured at 545 nm for each well using an ELISA reader. Each experiment was performed in four replicates.

4.5. 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 **11b** (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 to 500 nm. The stoichiometry of complex **11b**-Zn^{2+} was also studied using the molar ratio method [35]. The concentration of tested compound **11b** was 20 µM and the final concentration of Zn^{2+} ranged from 0 to 100 µM at 347 nm. The plot was obtained by the corresponding absorption versus mole fraction of Zn^{2+}.

4.6. Docking simulations

The program docking simulations was performed using Autodock Vina [36]. The crystal structure of *Torpedo californica* acetylcholinesterase (1EVE) was obtained from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). To provide receptor and ligand structures for the related studies, the following procedure was performed. First, the co-crystallized ligand and water molecules were removed from the protein. Then, atomic coordinates of the ligands was prepared using Marvinsketch, 2012, ChemAxon (http://www.chemaxon.com), the 3D structures were constructed using Openable [37]. Next, the receptor and optimized structure of the ligands were converted to required pdbqt format using Autodock Tools [38]. The Autodock Vina parameters were set as follow; box size: 15 × 15 × 15 Å, the center of box: x = 2.023, y = 63.295, z = 67.062 (geometrical center of co-crystallized ligand), the exhaustiveness: 100, and the remaining parameters were left unchanged. The calculated geometries were ranked in terms of free energy of binding and the best poses were selected for further analysis. All molecular visualizations were carried out in DS Viewer Pro (Accelrys, Inc., San Diego, CA).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.065.

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