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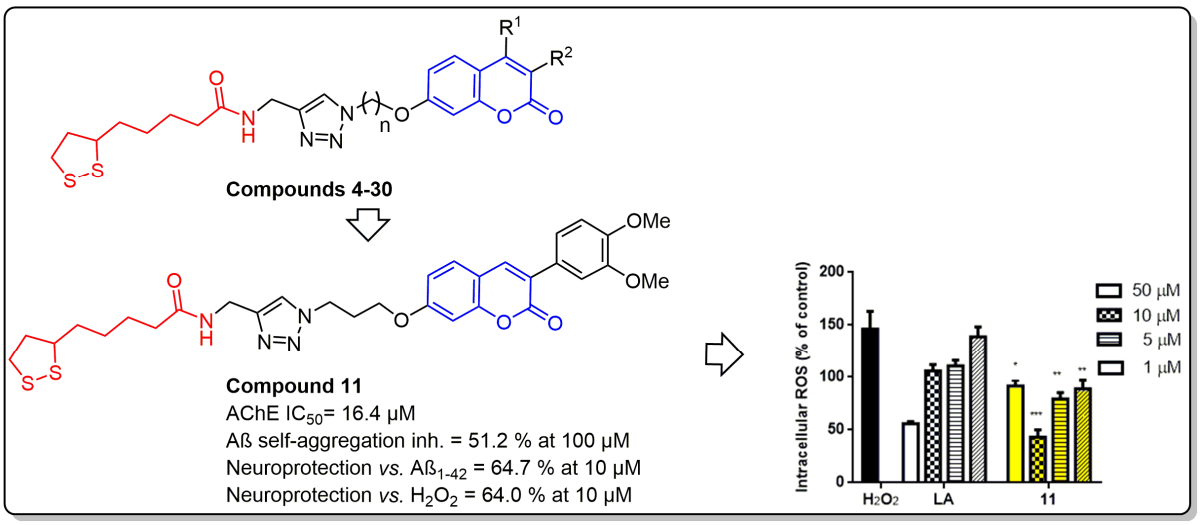

Design, synthesis and evaluation of novel multi-target-directed ligands for treatment of Alzheimer's disease based on coumarin and lipoic acid scaffolds

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Abstract: A novel series of coumarin-lipoic acid conjugates were synthesized via cycloaddition click reaction to find out new multi-target-directed ligands (MTDLs) for treatment of Alzheimer's disease (AD). All of synthesized compounds were screened for neuroprotective and anti-cholinesterase activities. Based on primary screening, two compounds (**5** and **11**) were subjected to further biological evaluations. In particular, compound **11** which was the most potent AChE inhibitor showed good inhibitory effect on Aβ-aggregation and intracellular ROS (reactive oxygen species) formation, as well as the ability of selective bio-metal chelation and neuroprotection against H_2O_2- and $A\beta_{1-42}$-induced cytotoxicity. In the light of these results, the applied hybridization approach introduced new promising lead compound with desired multifunctional properties, being useful in the treatment of Alzheimer's disease.

***Keywords*:** Alzheimer's disease; Coumarin; Lipoic acid; Neuroprotective agents; Antioxidant; MTDLs

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system [1]. In response to increasing distribution of AD in individuals under age 65, several treatment strategies have been explored to increase elderly population functionally and life expectancy [2]. The etiology of AD is not fully understood, but diverse factors including low levels of acetylcholine, β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress, inflammation, and dyshomeostasis of bio-metals have been correlated with AD [3]. The cholinergic hypothesis was the first theory based on the fact that neuronal degeneration is caused by the loss of cholinergic neurotransmitters in the brains of AD patients [4,5]. This theory has led to the development of the acetylcholinesterase (AChE) inhibitors which are still the first used symptomatic treatments for AD by elevating cholinergic neurotransmission. The level of ACh can be also decreased by butyrylcholinesterase (BuChE) as a co-regulator of the ACh degradation, so inhibition of both enzymes is helpful therapeutic approach for AD [6].

The Aβ deposition is another hypothesis, suggesting prevention of the self-assembled Aβ peptide formation is an important parameter to slow down the pathological complications of the AD [7]. Several studies suggested that blocking of peripheral anionic binding site (PAS) of AChE prevents the initiation of Aβ-aggregation [8].

Besides the pathological signs of the disease, AD patient brains show evidence of oxidative damages [9]. Supported by the oxidative stress hypothesis, reactive oxygen species (ROS) play a key role in onset and progression of AD [10]. ROS induce Aβ overproduction by elevating β-secretase expression and promotes the amyloidogenic pathway as one of the initial events in the AD [11,12]. ROS are originated by mitochondrial abnormalities and Aβ peptides aggregation particularly in the presence of biometal ions (Cu(II), Fe(II), Zn(II), etc.) [13]. The abnormal high level of metal ions which has been found at the injured locations of the AD brains, is associated with Aβ aggregates and promotes Aβ neurotoxicity and oxidative stress through the generation of free radicals [14]. In this context, antioxidant agents can be nominated as helpful therapeutics. Moreover, modulation of biometals dyshomeostasis by chelation in redox-inactive forms is also promised as an additional therapeutic strategy to inhibit metal-catalyzed oxidation of biomolecules in AD treatment [15]. Therefore, development of multitarget-directed ligands (MTDLs) with two or more complementary biological activities may display significant advantages against complex diseases like AD [16,17].

Coumarins are pharmacologically active natural compounds having antioxidant and ROS scavenging activities [18-20]. AP2469 (Fig. 1) is one of the famous coumarin-based MTDL having AChE and beta-secretase inhibitory activities, as well as anti-Aβ aggregating, antioxidant, and neuroprotective properties which has been generated from lead AP2238 [21]. Furthermore, ensaculin (Fig. 1) is another example of coumarin drugs with potential use in the treatment of AD [8].

On the other hand, Lipoic acid (LA, Fig. 1) is a naturally occurring antioxidant featured by its disulfide bond [22,23]. Since LA as a mitochondria-targeted antioxidant is able to trap free radicals and chelat with bio-metal ions, it has been called as universal antioxidant [24-27]. LA has also shown a neuroprotective effect against Aβ-induced cytotoxicity [28] and can stabilize cognitive functions in patients with AD [29]. In addition, Lipocrine as a conjugate of Tacrin and LA has been also introduced as potent AChE inhibitor with the ability to inhibit AChE-induced Aβ-aggregation as well as neuroprotection activity against ROS (Fig. 1) [30]. Moreover, coumarin-3-carboxamides bearing LA have shown acceptable anti-inflammatory and antioxidant activity [31].

In this work, on the basis of important findings about role of oxidative stress as a crucial and early event in mediating the AD pathogenesis and in continues of our previous studies for developing new anti-Alzheimer agents [32], we designed new MTDLs for treatment of AD based on the LA and coumarin scaffolds. To reach this goal, LA and the 7-hydroxycoumarin scaffolds were connected via click chemistry and formation of 1,2,3-triazole ring [33-35] (Fig. 1). We report here, synthesis of coumarin-lipoic acid conjugates **4-30**, and evaluation of their antioxidant potential for neuroprotection as well as their ability to inhibit cholinesterases, lipoxygenase (LOX), and Aβ aggregation.

2. Results and discussion

2.1. Chemistry

As illustrated in Scheme 1, alkyne-azide click chemistry was employed for the preparation of target compounds **4-30**. The key intermediates 7-hydroxycoumarins **1b-k** were prepared based on well-known methods. Briefly, the reaction of resorcinol with ethyl acetoacetate in the

presence of H_2SO_4 gave 7-hydroxy-4-methyl-coumarin (**1b**) [36]. The acetyl and ethyl ester analogs (**1c** and **1d**) were prepared form the reaction of 2,4-dihydroxybenzaldehyde and ethyl acetoacetate or diethyl malonate, respectively. The acid analog **1e** was prepared via hydrolyzation of compound **1d** in an aqueous solution of sodium hydroxide (5%) at room temperature [37]. Also, 2,4-dihydroxybenzaldehyde underwent to cyclization with appropriate phenylacetic acid derivatives in the presence of anhydrous CH_3COONa in refluxing Ac_2O to afford 7-hydroxy-3-phenyl-coumarin derivatives **1f-k** [38]. The coumarin derivatives **1a-k** were reacted with an excess amount of different dibromoalkanes to achieve compounds **2** [39]. On the other hand, the *N*-propargylamide of LA (compound **3**) was synthesized via EDCI–DMAP mediated amide bond formation between LA and propargyl amine [40]. Finally, target compounds **4-30** were synthesized by one-pot three-component reaction of bromoalkyl derivatives **2**, propargylated compound **3** and sodium azide [41].

2.2. Biological screenings

Primarily, all target compounds **4-30** were screened for their neuroprotective activity against oxidative stress in PC12 cells, anti-cholinesterase activity and inhibitory activity against 15-LOX as possible targets for treatment of AD.

2.2.1. Neuroprotective activity against H_2O_2-induced cell death

Neurotoxicity and oxidative damage generated by H_2O_2 are key parameters in the progression of neurodegenerative diseases [42]. Thus, the neuroprotective potential of compounds **4-30** (at the concentrations of 0.1, 1, 5, 10, 20 and 50 µM) in PC12 cells against the H_2O_2-induced cell death was tested using MTT assay. As seen in Table 1, except for compounds **9**, **16**, **24**, and **26** which did not show significant effect on cell viability, all other tested compounds significantly increased PC12 cell viability in the presence of H_2O_2 in a concentration-dependent manner. Most of the compounds significantly increased cell viability even at the low concentration of 0.1 µM ($p < 0.001$).

The higher protection was observed with compounds **5**, **8**, **12**, **13**, **15**, **20,** **22** and **23** as compared in the different concentrations. The neuroprotective effect of these compounds was

higher than that of reference drug quercetin. As seen in Table 1, structurally the target compounds have a 3, 4 or 5 atom linker (n= 3-5). The more promising compounds were amongst the propylene (n=3) or butylene (n=4) series. Furthermore, compounds **5** and **23** contain 4-methylcoumarin moiety, and compounds **13** and **20** derived from 3-(4-chlorophenyl)coumarin showed the best activity among other coumarin derivatives. Notably, the unsubstituted phenyl derivatives **9**, **16** and **26** had no significant effect on the H_2O_2-induced cell death. However, introduction of halo- or methoxy- substituents resulted in effective compounds (compare compounds **10-14** with **9**, compound **17-21** with **16**, and compounds **27-30** with **26**).

2.2.2. Anti-cholinesterase activity

It is well-known that the coumarin-containing compounds can act as PAS binder and inhibit AChE enzyme [32]. Accordingly, all final compound **4-30** were screened for their inhibitory activity against AChE and BuChE in comparison to donepezil as reference drug. The obtained IC_{50} values were listed in Table 2. Although most of compounds exhibited weak or no activity on AChE, but 3,4-dimethoxyphenyl-coumarin derivative **11** with IC_{50} values of 16.4 µM showed acceptable activity against this enzyme. The latter compound contains a propylene liker (n= 3). Elongation of linker (n= 4 and 5) led to homologs **18** and **28** with no activity on cholinesterases. On the other hand, the 3,4-dichlorophenyl-coumarin derivatives **21** and **30** with 4C or 5C linker showed suitable inhibitory activity against BuChE (IC_{50} values of 10.3 and 7.8 µM, respectively). The 3,4-dichlorophenyl-coumarin analog **14** with propylene spacer (n= 3) showed marginal activity against BuChE (IC_{50}= 73.5 µM). Therefore, it can be concluded that in 3,4-dichlorophenyl-coumarin derivatives, the elongation of linker increased the anti-BuChE potency.

To investigate the AChE inhibition mechanism, the most active compound **11** was selected for kinetic studies. Reciprocal Lineweaver-Burk plots at different concentrations of the substrate indicated a mixed-type inhibition against AChE (Fig. 2A) which means compound **11** might be able to bind to the PAS as well as CAS (catalytic active site). Re-plotting of slope versus concentration of compound **11** (Fig. 2B) showed an estimate of non-competitive inhibition with K_i = 6.91 µM.

2.2.2.1. Docking study with AChE

The binding mode of most active compound **11** with acetylcholinesterase (1EVE) was defined by docking study using the MOE software. As shown in Figure 3, compound **11** located in the entire enzymatic gorge. The dimethoxyphenyl moiety of compound **11** formed π-anion interaction with Asp71, and also made π-π stacking with phenyl ring of Tyr333, near the top of the gorge (the PAS). In this situation, the pyrone part of coumarin made two π-π interactions with Tyr333 and Tyr69, and the oxygen of the pyrone ring formed a hydrogen bond with Try69. Noteworthy, in the middle of the gorge (the CAS), the oxygen of amidic group was involved in a hydrogen bond with Tyr120 along with a van der Waals interaction with Phe330, indicating the role of amidic group on ligand activity. Moreover, the triazole ring formed a π-alkyl interaction with Ile286 near the bottom of the gorge. The 1,2-dithiolane ring makes a π-sulfur interaction with the imidazole ring of His439. All these facts provide an explanation for micromolar inhibitory activity of compound **11** against AChE.

2.2.3. 15-LOX inhibitory activity

Previous studies revealed that lipid hydroperoxides as key mediators of mitochondrial dysfunction alter tissue inflammatory responses and contribute to the pathogenesis of neurodegenerative disorders like AD [43]. Lipoxygenase (LOX) enzymes are the biggest contributor to the synthesis of lipid hydroperoxides through oxidation of poly-unsaturated fatty acids such as linoleic and arachidonic acid to hydroperoxy acids [44]. Moreover, higher levels of LOX were found in post-mortem brain samples from Alzheimer's patients compared to controls [45,46]. Due to the relevant of the LOX enzyme in AD, all target compounds **4-30** were also tested for their inhibitory activity against LOX enzyme. A perusal of the inhibition values showed that all compounds were not active against 15-LOX (IC_{50} values more than 100 μM). It was presumed that the large scaffold of the compound banned the molecule to accommodate in the active site.

2.3. Complementary evaluations of the selected compounds

The initial screening of the target compounds **4-30** against oxidative stress-induced cell death in PC12 cells as well as their activity against cholinesterases revealed that the most of the compounds had significant neuroprotective activity while a limited number of them found to be

potent AChE and/or BuChE inhibitor. Based on the results, compound **5** with significant neuroprotective activity and marginal anti-AChE potency as well as compound **11** possessing more potent anti-AChE activity and moderate neuroprotective property, were selected for further biological evaluations to check the possible usefulness of compounds for Alzheimer's disease.

2.3.1. Inhibitory effects on the Aβ peptide aggregation

In the light of Aβ anti-aggregating properties of coumarin and LA derivatives [47,48], the potential of compounds **5** and **11** to inhibit Aβ-aggregation was evaluated using thioflavin T (ThT) assay. The obtained results indicated that compounds **5** and **11** displayed good inhibitory activity (26.6 and 51.2% inhibition, respectively) in comparison to the reference compounds donepezil (22%) and rifampicin (27.5%, Table 3). Compound **11** was about 2-fold more effective than compound **5** and reference drugs in the inhibition of Aβ aggregation. The more inhibitory activity of compound **11** than compound **5** suggests the great effect of dimethoxyphenyl-coumarin scaffold on the interaction with Aβ. The potential of compounds **5** and **11** to inhibit Aβ-aggregation induced by AChE were also evaluated (Table 3). Compound **5** showed weak inhibition activity toward AChE-induced Aβ aggregation (2.9% inhibition), while the inhibitory activity of compound **11** was about 2-fold higher than that of donepezil (47.4% compared to 26.1%). This result was in agreement with the kinetic and docking studies confirming potential binding of compound **11** with PAS residues of AChE.

2.3.2. Neuroprotective activity against H_2O_2- or $A\beta_{1-42}$-induced cytotoxicity in SH-SY5Y cells

The viability of human neuroblastoma SH-SY5Y cells against H_2O_2 in the presence of compounds **5** and **11** were also examined. As depicted in Fig. 4B, these compounds can more effectively (55.6 and 64%, respectively) protect SH-SY5Y cells against H_2O_2-induced cytotoxicity compared to donepezil as a reference drug (33%). As described in section 2.2.1, these compounds could significantly protect neuronal PC12 cells (neuroblastic and eosinophilic cells) against the H_2O_2-induced cell death. In PC12 cells, oxidative stress and subsequently cell death were induced by adding 150 µM of H_2O_2; while H_2O_2 was used at the higher concentration of 250 µM in SH-SY5Y cells. Accordingly, we could not compare the obtained

results between PC12 and SH-SY5Y cells. However, it should be noted that in PC12 model compound **5** was more effective than compound **11**. In contrast, the obtained results against SH-SY5Y cells indicated that compound **11** is better than **5**.

It has been reported that Aβ senile plaques have toxic effects on neuronal cells through stimulating the formation of free radicals [49]. Therefore, the neuroprotective effects of representative compounds **5** and **11** towards $A\beta_{1-42}$-induced cytotoxicity in human neuroblastoma SH-SY5Y cells were evaluated. According to the obtained results (Fig. 4A), compounds **5** and **11** had significant protective capability against $A\beta_{1-42}$-induced cytotoxicity with the percentage of cell viability of 72.9 and 64.7 % receptivity, higher than reference drug donepezil (39.9%). The neuroprotective behavior of compound **5** and **11** suggests that pre-treatment of neurons with these compounds before exposure to Aβ or H_2O_2 can significantly reduce oxidative stress and increase cell survival in human SH-SY5Y neuroblastoma cells.

2.3.3. Effect on the intracellular ROS formation

To determine the potential use of compounds **5** and **11** for the treatment of AD as a new antioxidant MTDLs, their antioxidant activity against intracellular ROS generation in PC12 cells after treatment with H_2O_2 were investigated by dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. We used a range of concentrations of the tested compounds that did not affect the cell viability (1, 5, 10 and 50 µM). The results showed a significant decrease of ROS production in a concentration-independent manner for both tested compounds (Fig. 5, for details, see supporting information, Table S1). In particular, compound **11** in all concentrations significantly decreased the intracellular ROS generation in treated cells compared with untreated control cells. Moreover, compound **5** and LA showed the same results at the highest concentration of 50 µM. The results confirmed the successful role of the compounds in the reduction of endogenous ROS which are formed as a natural byproduct of the normal metabolism of oxygen in cells [50].

2.3.4. Total Antioxidant Power

The ferric reducing antioxidant power (FRAP) of the representative compounds **5** and **11** was assessed in comparison with lipoic acid, ascorbic acid, and quercetin. The results indicated that compounds **5** and **11** have excellent reducing power (84.7 and 87.5 mM Fe^{+2}, respectively).

Compound **11** showed slightly more ability to reducing ferric ion than quercetin and lipoic acid (Table 4).

2.3.5. Metal-chelating property

Binding to biometal ion is one of the mechanisms by which antioxidants control autoxidation reactions and could be a potential therapeutic strategy for the treatment of AD [51]. The chelation activity of the selected compound **11** for biometals such as Mg^{+2}, Ca^{+2}, Al^{+3}, Cu^{+2}, Fe^{+2}, and Zn^{+2} which have been found in Aβ plaques, was studied by UV-vis spectrometry. An increase in maximum absorption intensity at 322 nm and also a shoulder at 356 nm was observed after addition of $CuCl_2$ or $FeSO_4$ (Fig. 6), indicating the formation of complex **11**-metal (II). However, no significant change was observed when Mg^{+2}, Ca^{+2}, Al^{+3}, and Zn^{+2} were added. The chelating property of compound **11** could be attributed to the presence of amidic group and triazole scaffold or the sulfurs at the LA structure [52]. The stoichiometry of the **11**-Cu (II) complex was also determined by titration of the methanolic solution of compound **11** with ascending amounts of $CuCl_2$ (The molar ratio method). As shown in Figure 6, initially, the absorbance increased at 365 nm and then became stable. The two straight lines crossed at a mole fraction of 0.5, indicating a 2:1 stoichiometry for the complex. It has been approved that Cu^{+2} promote the formation of senile plaques and reactive oxygen species because of its superior affinity to complex with $A\beta_{1-42}$ [53]. Therefore, the selective chelation of Cu^{+2} ions by compound **11** suggest the possible capability of the compound to stop formation of amyloid plaques and decrease oxidative stress.

3. Conclusion

We described synthesis and biological characterization of 27 new triazole-containing coumarin-lipoic acid conjugates as novel multifunctional antioxidants for the development of potential anti-Alzheimer agents. The in vitro assays showed that most of the compounds had significant neuroprotective activity against H_2O_2-induced oxidative stress in PC12 cells. Investigation on anti-cholinesterase activity of compounds revealed that some of them such as compounds **11** and **30** have remarkable activity against AChE and BuChE, respectively. Based on primary screening, compound **5** as a highly effective neuroprotective agent and compound **11**

with superior activity against AChE were selected for further studies. Based on the obtained results, compound **11** was about 2-fold more effective than reference drug donepezil in terms of the inhibitory activity on self-induced and AChE-induced $A\beta_{1-42}$ aggregation. Moreover, cell-based assay indicated that compounds **11** displays significant protection against $A\beta_{1-42}$-induced cytotoxicity, being superior to donepezil. The additional ability of compound **11** in the diminution of intracellular ROS generation, reduction of ferric ion and selective metal chelation makes compound **11** as a promising multifunctional antioxidant for further studies to the development of new anti-Alzheimer agents.

4. Experimental

4.1. Chemistry

7-hydroxy coumarin (**1a**), lipoic acid (LA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 4-dimethylaminopyridine (DMAP) and all other starting materials, reagents and solvents were purchased from commercial sources and used without further purification. Melting points were measured on a Kofler hot stage apparatus. The IR spectra (KBr disks) were taken by Nicolet FT-IR Magna 550 spectrometer. ^{1}H and ^{13}C NMR spectra were measured using Brucker 500 MHz instrument. The chemical shifts (δ) and coupling constants (*J*) are presented in parts per million (ppm) and Hertz (Hz), respectively. The results of elemental analyses (C, H, and N) were within ±0.4% of the calculated values and determined with CHN-Rapid Heraeus Elemental Analyzer.

*4.1.1. General procedure for the preparation of 7-Hydroxy-4-methyl-coumarin (**1b**)*

Concentrated H_2SO_4 (0.5 mL) was added dropwise to an ice-cold solution of resorcinol (2.0 g, 18.2 mmol) in dioxane. Ethyl acetoacetate (2.8 g, 21.8 mmol) was then added, and the mixture was heated to 60 °C for 4 h. The mixture was next poured into ice and water, and the precipitate was filtered and dried. The product was finally recrystallized from methanol [36].

*4.1.2. General procedure for the preparation of 3-substituted coumarins **1c-e***

Ethyl acetoacetate (0.128 g, 1 mmol) or diethyl malonate (0.176 g, 1 mmol) was added to a solution of 2,4-dihydroxybenzaldehyde (0.122 g, 1 mmol) in absolute ethanol. Two drops of piperidine were then added and the solution was heated under reflux for 3 h. After completion of the reaction, the hot solution was diluted with water and stored overnight in a refrigerator. The crystalline product filtered off, washed with a cold solution of ethanol/water (1:1.5) and dried in the air. The acid analog **1e** was prepared via hydrolyzation of compound **1d** in an aqueous solution of sodium hydroxide (5%) at room temperature for 12 h. After completion of the reaction, the solution was acidified (pH=4) with HCl (10%) and the precipitated product was washed with water and dried on air [37].

*4.1.3. General procedure for the preparation of 3-phenyl-coumarin derivatives **1f-k***

Anhydrous CH_3COONa (92 mg, 1.4 mmol), appropriate phenylacetic acid derivative (1 mmol), and 2,4-dihydroxybenzaldehyde (1 mmol) was refluxed in Ac_2O (1.2 mL) for 2 h. The reaction mixture was cooled, neutralized with 10% aqueous $NaHCO_3$, and filtered. The solid product was washed with water and ethyl acetate and dried [38].

*4.1.4. General procedure for the preparation of bromoalkoxy intermediate **2***

A mixture of substituted 7-hydroxycoumarin (5 mmol), appropriate dibromoalkane (50 mmol) and anhydrous K_2CO_3 (1.4 g, 10 mmol) was refluxed in acetone (15 mL) for 4 h. After cooling, the reaction mixture was filtered and the organic phase was evaporated under reduced pressure. The obtained residue was triturated with *n*-hexane to give the product **2** as white solid [39].

*4.1.5. General procedure for the preparation of 1,2-dithiolane-3-pentanoic acid-N-propargylamide (**3**)*

Lipoic acid (LA, 1 mmol), EDCI (1.2 mmol), and DMAP (1.2 mmol) were dissolved in anhydrous CH_2Cl_2 (10 mL). The reaction mixture was cooled to 0 °C and propargylamine (1.2 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise. Then the mixture was stirred at room

temperature for 12 h. After completion of the reaction (monitored by TLC), the mixture was filtered and the organic phase was evaporated. The obtained crude product was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (1:2) as the eluent, to give yellow solid [40].

*4.1.6. General procedure for the preparation of compounds **4-30***

A mixture of the intermediate **2** (1 mmol), NaN_3 (1 mmol) and NEt_3 (1 mmol) in *t*-BuOH/H_2O (1:1) were heated at 70 °C for 30 min. 1,2-Dithiolane-3-pentanoic acid-*N*-propargylamide (**3**, 1 mmol), sodium ascorbate (0.2 mmol), and $CuSO_4$ (0.2 mmol) were then added to the latter mixture. After completion of the reaction (12-48 h, monitored by TLC), the mixture was diluted with cold water and the precipitate was filtered off and washed with water. The resulting crude product was purified by flash chromatography on silica gel eluting with ethyl acetate/petroleum ether (1:1) to give compounds **4-30** [41].

*4.1.6.1. 5-(1,2-dithiolan-3-yl)-N-((1-(3-((2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**4**).* Yeild 99%; Off white solid; mp 126-128 ºC; IR (KBr, cm^{-1}) ν_{max}: 3284 (NH), 2926 (C-H), 1732 and 1627 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.29 (t, 1H, *J*=5.5 Hz, NH), 7.98 (d, 1H, *J*=9.5 Hz, H_4), 7.94 (s, 1H, triazole), 7.62 (d, 1H, *J*=8.5 Hz, H_5), 6.97 (d, 1H, *J*=2.0 Hz, H_8), 6.92 (dd, 1H, *J*=8.5 Hz, *J*=2.0 Hz, H_6), 6.30 (d, 1H, *J*=9.5 Hz, H_3), 4.51 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.08 (t, 2H, *J*=6.0 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.18-3.09 (m, 2H, CH_2S), 2.38 (sextet, 1H, *J*=6.7 Hz, S-CH_2-C<u>H</u>$_2$), 2.28 (t, 2H, *J*=6.0 Hz, aliphatic), 2.08 (t, 2H, *J*=7.5 Hz, $COCH_2$), 1.84 (sextet, 1H, *J*=7.0 Hz, S-CH_2-C<u>H</u>$_2$), 1.58-1.65 (m, 1H, aliphatic), 1.54-1.49 (m, 3H, aliphatic), 1.33-1.30 (m, 2H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.8, 167.4, 161.4, 160.2, 155.3, 145.0, 144.3, 129.5, 122.9, 112.6, 112.4, 101.2, 65.3, 56.1, 46.3, 38.0, 34.9, 34.0, 29.2, 28.3, 24.9. Anal. Calcd for $C_{23}H_{28}N_4O_4S_2$: C, 56.54; H, 5.78; N, 11.47. Found: C, 56.70; H, 5.86; N, 11.71.

*4.1.6.2. 5-(1,2-dithiolan-3-yl)-N-((1-(3-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**5**).* Yeild 95%; Off white solid; mp 83-85 ºC; IR (KBr, cm^{-1}) ν_{max}: 3295 (NH), 2930 (C-H), 1721 and 1616 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.29 (bs, 1H, N<u>H</u>-CO), 7.93 (s, 1H, triazole), 7.67 (d, 1H, *J*=8.5 Hz, H_5), 6.96-6.93 (m, 2H, $H_{6,8}$), 6.21 (s, 1H, H_3), 4.51 (t, 2H, *J*=7.0 Hz, CH_2-N-), 4.26 (d, 2H, *J*=5.5 Hz, CH_2-NH), 4.08 (t,

2H, *J*=6.0 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.5 Hz, CH-S), 3.16-3.11 (m, 2H, CH_2-S), 2.39-2.36 (m, 4H, CH_3 and 1H, S-CH_2-C<u>H</u>$_2$), 2.28 (quintet, 2H, aliphatic), 2.08 (m, 2H, $COCH_2$), 1.84 (sextet, 1H, *J*=7.0 Hz, S-CH_2-C<u>H</u>$_2$), 1.61-1.69 (m, 1H, aliphatic), 1.53-1.48 (m, 3H, aliphatic), 1.26-1.35 (m, 2H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 172.1, 161.4, 160.1, 154.7, 153.4, 445.1, 126.4, 122.8, 113.2, 112.4, 111.2, 101.2, 65.3, 56.1, 46.3, 34.9, 34.0, 29.2, 28.2, 24.9, 18.1. Anal. Calcd for $C_{24}H_{30}N_4O_4S_2$: C, 57.35; H, 6.02; N, 11.15. Found: C, 57.72; H, 6.36; N, 11.29.

*4.1.6.3. N-((1-(3-((3-acetyl-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**6**).* Yield 90%; Off white solid; mp 115-117 ºC; IR (KBr, cm^{-1}) ν_{max}: 3489 (NH), 2927 (C-H), 1715 and 1627 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.63 (s, 1H, H_4), 8.25 (bs, 1H, NH), 7.93 (s, 1H, triazole), 7.87 (d, 1H, *J*=8.5 Hz, H_5), 7.03 (s, 1H, H_8), 6.98 (d, 1H, *J*=8.5 Hz, H_6), 4.51 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=5.5 Hz, CH_2-NH), 4.13 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.16-3.09 (m, 2H, CH_2-S), 2.56 (s, 3H, $COCH_3$), 2.39 (sextet, 1H, *J*=6.7 Hz, S-CH_2-C<u>H</u>$_2$), 2.30 (t, 2H, *J*=6.0 Hz, aliphatic), 2.08 (t, 2H, *J*=7.5 Hz, $COCH_2$), 1.85 (sextet, 1H, *J*=7.0 Hz, S-CH_2-C<u>H</u>$_2$), 1.64-1.62 (m, 1H, aliphatic), 1.51 (bs, 3H, aliphatic), 1.31-1.33 (m, 2H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 194.6, 171.7, 163.7, 158.7, 156.9, 147.4, 145.0, 132.1, 122.7, 120.42, 113.6, 111.8, 100.6, 65.6, 56.0, 46.2, 39.8, 34.9, 34.0, 33.9, 29.8, 29.1, 28.1, 24.8. Anal. Calcd for $C_{25}H_{30}N_4O_5S_2$: C, 56.59; H, 5.70; N, 10.56. Found: C, 56.81; H, 5.99; N, 10.85.

*4.1.6.4. ethyl 7-(3-(4-((5-(1,2-dithiolan-3-yl)pentanamido)methyl)-1H-1,2,3-triazol-1-yl)propoxy)-2-oxo-2H-chromene-3-carboxylate (**7**).* Yield 85%; Off white solid; mp 121-123 ºC; IR (KBr, cm^{-1}) ν_{max}: 3317 (NH), 2925 (C-H), 1737, 1641 and 1606 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.70 (s, 1H, H_4), 8. 22 (t, 1H, *J*=5.5 Hz, NH), 7.91 (s, 1H, triazole), 7.82 (d, 1H, *J*=8.5 Hz, H_5), 6.99 (s, 1H, H_8), 6.97 (d, 1H, *J*=8.5 Hz, H_6), 4.51 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (m, 4H, CH_2-NH and O-C<u>H</u>$_2$-CH_3), 4.13 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.19-3.10 (m, 2H, CH_2-S), 2.39 (sextet, 1H, *J*=6.7 Hz, S-CH_2-C<u>H</u>$_2$), 2.30 (t, 2H, *J*=6.0 Hz, aliphatic), 2.08 (t, 2H, *J*=7.5 Hz, $COCH_2$), 1.83 (sextet, 1H, *J*=7.0 Hz, S-CH_2-C<u>H</u>$_2$), 1.52-1.49 (m, 4H aliphatic), 1.33-1.29 (m, 2H aliphatic and 3H CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 172.4, 164.1, 156.9, 145.7, 132.0, 113.6, 111.4, 101.7, 89.4, 65.6, 60.8, 56.0, 46.2, 37.9, 34.9, 33.9, 29.1, 18.1, 24.8, 14.0. Anal. Calcd for $C_{26}H_{32}N_4O_6S_2$: C, 55.70; H, 5.75; N, 9.99. Found: C, 55.99; H, 5.66; N, 9.81.

*4.1.6.5. 7-(3-(4-((5-(1,2-dithiolan-3-yl)pentanamido)methyl)-1H-1,2,3-triazol-1-yl)propoxy)-2-oxo-2H-chromene-3-carboxylic acid (**8**).* Yield 80%; Off white solid; mp 140-143 ºC; IR (KBr, cm^{-1}) ν_{max}: 3289 (NH, OH), 2932 (C-H), 1749, 1677 and 1612 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 13 (s, 1H, OH), 8.72 (s, 1H, H_4), 8.26 (s, 1H, NH), 7.93 (s, 1H, triazole), 7.82 (d, 1H, *J*=8.5 Hz, H_5), 7.01 (s, 1H, H_8), 6.97 (d, 1H, *J*=8.5 Hz, H_6), 4.51 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=5.5 Hz, CH_2-NH), 4.12 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.17-3.09 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.7 Hz, S-CH_2-C$\underline{H}_2$), 2.30 (t, 2H, *J*=6.0 Hz, $COCH_2$), 2.08 (t, 2H, *J*=7.5 Hz, aliphatic), 1.83 (sextet, 1H, *J*=7.0 Hz, S-CH_2-C$\underline{H}_2$), 1.55-165 (m, 1H, aliphatic), 1.54-1.49 (m, 3H, aliphatic), 1.33-1.30 (m, 2H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 163.9, 163.6, 157.1, 156.7, 148.9, 145.0, 131.5, 122.8, 113.8, 113.4, 111.6, 100.7, 65.6, 56.0, 46.2, 38.0, 34.9, 34.0, 29.1, 28.2, 24.8. Anal. Calcd for $C_{24}H_{28}N_4O_6S_2$: C, 54.12; H, 5.30; N, 10.52. Found: C, 54.08; H, 5.15; N, 10.31.

*4.1.6.6. 5-(1,2-dithiolan-3-yl)-N-((1-(3-((2-oxo-3-phenyl-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**9**).* Yield 75%; Off white solid; mp 125-127 ºC; IR (KBr, cm^{-1}) ν_{max}: 3295 (NH), 2946 (C-H), 1719 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.28 (t, 1H, *J*=5.5 Hz, NH), 8.21 (s, 1H, H_4) 7.94 (s, 1H, triazole), 7.70 (m, 3H, phenyl), 7.44 (m, 2H, phenyl), 7.39 (d, 1H, *J*=7.0 Hz, H_5), 7.02 (s, 1H, H_8), 6.96 (d, 1H, *J*=9.0 Hz, H_6), 4.52 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.10 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.57 (m, 1H, CH-S), 3.18-3.07 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C$\underline{H}_2$), 2.30 (m, 2H, CH_2, aliphatic chain), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.82 (sextet, 1H, S-CH_2-C$\underline{H}_2$), 1.65-1.60 (m, 1H, CO-CH_2-C$\underline{H}_2$), 1.55-1.49 (m, 3H, aliphatic chain), 1.33-1.30 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.8, 161.4, 159.9, 140.7, 134.8, 129.7, 128.2, 128.0, 123.2, 122.9, 113.1, 65.3, 56.0, 46.3, 34.7, 34.0, 29.2, 28.2, 24.9. Anal. Calcd for $C_{29}H_{32}N_4O_4S_2$: C, 61.68; H, 5.71; N, 9.92. Found: C, 61.80; H, 5.89; N, 10.18.

*4.1.6.7. 5-(1,2-dithiolan-3-yl)-N-((1-(3-((3-(4-methoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**10**).* Yield 75%; Off white solid; mp 143-145 ºC; IR (KBr, cm^{-1}) ν_{max}: 3291 (NH), 2933 (C-H), 1721 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.26 (t, 1H, *J*=5.5 Hz, NH), 8.13 (s, 1H, H_4) 7.93 (s, 1H, triazole), 7.66 (m, 3H, phenyl and H_5), 7.04-6.99 (m, 3H, phenyl and H_8), 6.94 (d, 1H, *J*=9.0 Hz, H_6), 4.52 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=5.0 Hz, CH_2-NH), 4.09 (t, 2H, *J*=5.5 Hz, CH_2-O), 3.80 (s, 3H, OMe), 3.56 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.18-3.08 (m, 2H, CH_2-S), 2.37 (sextet, 1H,

J=6.0 Hz, S-CH_2-C<u>H</u>), 2.30 (q, 2H, CH_2, aliphatic chain), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.83 (sextet, 1H, S-CH_2-C<u>H</u>), 1.65-1.55 (m, 1H, CO-CH_2-C<u>H_2</u>), 1.54-1.48 (m, 3H, aliphatic chain), 1.33-1.30 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.0, 160.0, 159.26, 154.4, 145.0, 139.3, 139.2, 130.3, 129.5, 128.6, 127.0, 122.9, 113.2, 112.9, 100.6, 65.3, 56.0, 55.2, 46.3, 38.0, 34.9, 34.0, 29.2, 28.2, 24.9. Anal. Calcd for $C_{30}H_{34}N_4O_5S_2$: C, 60.59; H, 5.76; N, 9.42. Found: C, 60.46; H, 5.37; N, 9.23.

*4.1.6.8. N-((1-(3-((3-(3,4-dimethoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**11**)*. Yield 80%; Off white solid; mp 136-138 ºC; IR (KBr, cm^{-1}) ν_{max}: 3295 (NH), 2931 (C-H), 1719 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.27 (t, 1H, *J*=5.5 Hz, NH), 8.17 (s, 1H, H_4), 7.93 (s, 1H, triazole), 7.66 (d, 1H, *J*=9.0 Hz, H_5), 7.32 (m, 2H, phenyl), 7.02-7.00 (m, 1H, phenyl and 1H_8), 6.95 (d, 1H, *J*=9.0 Hz, H_6), 4.52 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.26 (d, 2H, *J*=5.0 Hz, CH_2-NH), 4.09 (t, 2H, *J*=5.5 Hz, CH_2-O), 3.80 (s, 6H, 2MeO), 3.56 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.22-3.08 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.29 (q, 2H, CH_2, aliphatic chain), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.75-165 (m, 1H, S-CH_2-C<u>H</u>), 1.65-160 (m, aliphatic), 1.53-1.50 (m, 3H, aliphatic chain), 1.33-1.29 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.9, 166.1, 161.1, 160.0, 154.4, 149.0, 145.1, 142.0, 127.3, 122.9, 113.3, 112.9, 112.2, 111.4, 100.7, 65.3, 56.1, 55.5, 46.3, 38.0, 35.0, 34.0, 29.3, 28.2, 24.9. Anal. Calcd for $C_{31}H_{36}N_4O_6S_2$: C, 59.60; H, 5.81; N, 8.97. Found: C, 59.64; H, 5.83; N, 9.07.

*4.1.6.9. N-((1-(3-((3-(4-bromophenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**12**)*. Yield 87%; Off white solid; mp 150-152 ºC; IR (KBr, cm^{-1}) ν_{max}: 3282 (NH), 2928 (C-H), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.26 (m, 1H, NH and 1H, H_4), 7.93 (s, 1H, triazole) 7.70-7.64 (m, 5H, phenyl and H_5), 7.01(s, 1H, H_8), 6.96 (d, 1H, *J*=9.0 Hz, H_6), 4.52 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=5.0 Hz, CH_2-NH), 4.01 (t, 2H, *J*=5.5 Hz, CH_2-O), 3.56 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.20-3.08 (m, 2H, CH_2-S), 2.37 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.30 (q, 2H, CH_2, aliphatic chain), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.85-1.80 (m, 1H, S-CH_2-C<u>H</u>), 1.67-1.60 (m, 1H, aliphatic), 1.53-1.49 (m, 3H, aliphatic chain), 1.34-1.30 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 176.1, 171.8, 161.9, 159.7, 156.8, 154.8, 145.1, 140.8, 133.9, 131.1, 130.3, 121.4, 113.0, 100.7, 65.4, 56.0, 46.3, 38.0, 34.9, 34.0, 29.2, 28.2, 24.9. Anal. Calcd for $C_{29}H_{31}BrN_4O_4S_2$: C, 54.12; H, 4.86; N, 8.71. Found: C, 54.32; H, 5.02; N, 8.33.

*4.1.6.10. N-((1-(3-((3-(4-chlorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**13**)*. Yield 92%; Off white solid; mp 163-165 ºC; IR (KBr, cm^{-1}) ν_{max}: 3320 (NH), 2932 (C-H), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.27-8.25 (m, 1H, NH and 1H, H_4), 7.94 (s, 1H, triazole), 7.75 (d, 2H, *J*=7.5 Hz, phenyl), 7.68 (d, 1H, *J*=8.5 Hz, H_5), 7.51 (d, 2H, *J*=8.0 Hz, phenyl), 7.01(s, 1H, H_8), 6.96 (d, 1H, *J*=9.0 Hz, H_6), 4.52 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=5.0 Hz, CH_2-NH), 4.01 (t, 2H, *J*=5.5 Hz, CH_2-O), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.16-3.09 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.31-2.29 (m, 2H, CH_2, aliphatic chain), 2.08 (bs, 2H, CH_2-CO), 1.85-1.81 (m, 1H, S-CH_2-C<u>H</u>), 1.64-1.61 (m, 1H, CO-CH_2-C<u>H_2</u>), 1.52-1.51 (m, 3H, aliphatic chain), 1.33-1.31 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.5, 159.7, 154.7, 154.0, 141.0, 133.6, 132.8, 130.0, 128.3, 128.0, 122.7, 121.9, 113.0, 65.4, 56.0, 46.3, 38.0, 34.9, 34.0, 29.2, 28.2, 24.8. Anal. Calcd for $C_{29}H_{31}ClN_4O_4S_2$: C, 58.13; H, 5.22; N, 9.35. Found: C, 58.35; H, 5.46; N, 9.47.

*4.1.6.11. N-((1-(3-((3-(3,4-dichlorophenyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**14**)*. Yield 97%; Off white solid; mp 150-152 ºC; IR (KBr, cm^{-1}) ν_{max}: 3280 (NH), 2927 (C-H), 1716 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.34 (s, 1H, H_4), 8.26 (bs, 1H, NH), 8.00 (s, 1H, H_5), 7.93 (s, 1H, triazole), 7.73-7.68 (m, 3H, phenyl), 7.01 (s, 1H, H_8), 6.96 (d, 1H, *J*=8.5 Hz, H_6), 4.52 (bs, 2H, CH_2-N), 4.28 (bs, 2H, CH_2-NH), 4.10 (bs, 2H, CH_2-O), 3.56 (bs, 1H, CH-S), 3.15-3.10 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.30 (bs, 2H, aliphatic), 2.08 (s, 2H, CH_2CO), 1.85-1.80 (m, 1H, S-CH_2-C<u>H</u>), 1.63-1.57 (m, 1H, aliphatic), 1.52-1.50 (m, 3H, aliphatic chain), 1.33-1.31 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.7, 159.5, 154.9, 145.0, 141.8, 135.4, 130.6, 130.2, 129.9, 129.8, 128.3, 122.9, 122.7, 120.4, 112.9, 100.8, 65.4, 56.0, 46.3, 38.0, 34.9, 34.0, 29.2, 28.2, 24.8. Anal. Calcd for $C_{29}H_{30}Cl_2N_4O_4S_2$: C, 54.97; H, 4.77; N, 8.84. Found: C, 54.76; H, 4.43; N, 8.55.

*4.1.6.12. 5-(1,2-dithiolan-3-yl)-N-((1-(4-((2-oxo-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**15**)*. Yield 96%; Off white solid; mp 128-130 ºC; IR (KBr, cm^{-1}) ν_{max}: 3285 (NH), 2926 (C-H), 1733, 1628 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.24 (t, 1H, *J*=5.5 Hz, NH), 7.97 (d, 1H, *J*=9.5 Hz, H_4), 7.90 (s, 1H, triazole), 7.61 (d, 1H, *J*=8.5 Hz, H_5), 6.96 (d, 1H, *J*=2.0 Hz, H_8), 6.92 (dd, 1H, *J*=8.5 Hz, *J*=2.0 Hz, H_6), 6.27 (d, 1H, *J*=9.5 Hz, H_3), 4.40 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.09 (t, 2H, *J*=6.0 Hz, CH_2-O),

3.58 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.19-3.07 (m, 2H, CH_2-S), 2.39 (sextet, 1H, *J*=6.7 Hz, C<u>H</u>-CH_2-S), 2.09 (t, 2H, *J*=6.0 Hz, C<u>H</u>$_2$-CH_2-N), 1.96 (quintet, 2H, *J*=7.0 Hz, $COCH_2$), 1.83 (sextet, 1H, *J*=7.0 Hz, C<u>H</u>-CH_2-S), 1.71-169 (m, 3H, aliphatic), 1.55-1.50 (m, 3H, aliphatic), 1.34-1.32 (m, 2H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 151.4, 144.2, 129.3, 122.6, 112.6, 112.3, 101.1, 67.5, 56.0, 45.9, 38.0, 34.9, 34.0, 28.1, 26.4, 25.3, 24.8. Anal. Calcd for $C_{24}H_{30}N_4O_4S_2$: C, 57.35; H, 6.02; N, 11.15. Found: C, 57.52; H, 6.29; N, 11.23.

*4.1.6.13. 5-(1,2-dithiolan-3-yl)-N-((1-(4-((2-oxo-3-phenyl-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**16**)*. Yield 76%; Off white solid; mp 134-136 ºC; IR (KBr, cm^{-1}) ν_{max}: 3292 (NH), 2924 (C-H), 1722 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.26 (t, 1H, NH), 8.20 (s, 1H, H_4), 7.91 (s, 1H, triazole), 7.71-7.68 (m, 3H, phenyl), 7.45-7.39 (m, 2H, phenyl and 1H, H_5), 7.02 (s, 1H, H_8), 6.97 (d, 1H, *J*=8.0 Hz, H_6), 4.41 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.12 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.19-3.10 (m, 2H, CH_2-S), 2.37 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>-CH_2-S), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.97 (bs, 2H, C<u>H</u>$_2$-CH_2-N), 1.84-1.83 (m, 1H, C<u>H</u>-CH_2-S), 1.72-1.64 (m, 2H, O-CH_2-C<u>H</u>$_2$ and 1H, C<u>H</u>-CH_2-CO), 1.55-1.49 (m, 3H, aliphatic chain), 1.35-1.32 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.9, 162.3, 159.8, 145.0, 141.0, 134.7, 128.4, 128.2, 127.9, 123.2, 122.9, 112.9, 67.9, 56.1, 48.8, 37.9, 34.9, 33.9, 28.1, 26.4, 25.4, 24.8. Anal. Calcd for $C_{30}H_{34}N_4O_4S_2$: C, 62.26; H, 5.92; N, 9.68. Found: C, 62.09; H, 6.19; N, 9.84.

*4.1.6.14. 5-(1,2-dithiolan-3-yl)-N-((1-(4-((3-(4-methoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**17**)*. Yield 75%; Off white solid; mp 145-146 ºC; IR (KBr, cm^{-1}) ν_{max}: 3292 (NH), 2931 (C-H), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.26 (t, 1H, *J*=5.5 Hz, NH), 8.13 (s, 1H, H_4), 7.91 (s, 1H, triazole), 7.67 (m, 3H, phenyl and H_5), 7.00 (m, 3H, phenyl and H_8), 6.94 (d, 1H, *J*=8.5 Hz, H_6), 4.41 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.10 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.80 (s, 3H, OMe), 3.58 (m, 1H, CH-S), 3.19-3.09 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.08 (s, 2H, CH_2-CO), 1.98-1.94 (m, 2H, C<u>H</u>$_2$-CH_2-N), 1.83 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 1.73-1.70 (m, 2H, O-CH_2-C<u>H</u>$_2$), 1.65-1.60 (m, 1H, C<u>H</u>-CH_2-CO), 1.54-1.49 (m, 3H, aliphatic chain), 1.34-1.31(m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.8, 161.2, 160.0, 159.2, 154.4, 144.9, 139.4, 139.3, 129.5, 127.0, 122.7, 114.2, 113.6, 113.1, 100.7, 67.5, 56.0,

55.1, 48.8, 34.9, 34.0, 28.2, 26.4, 25.4, 24.9. Anal. Calcd for $C_{31}H_{36}N_4O_5S_2$: C, 61.16; H, 5.96; N, 9.20. Found: C, 61.31; H, 6.15; N, 9.54.

*4.1.6.15. N-((1-(4-((3-(3,4-dimethoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**18**)*. Yield 74%; Off white solid; mp 134-135 ºC; IR (KBr, cm^{-1}) ν_{max}: 3298 (NH), 2934 (C-H), 1716 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.28 (t, 1H, NH), 8.17 (s, 1H, H_4), 7.91 (s, 1H, triazole), 7.66 (d, 1H, *J*=9.0 Hz, H_5), 7.31 (m, 2H, phenyl), 7.02-7.00 (m, 2H, phenyl and H_8), 6.95 (d, 1H, *J*=9.0 Hz, H_6), 4.41 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.10 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.80 (bs, 6H, 2OMe), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.16-3.10 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>-CH_2-S), 2.09 (s, 2H, CH_2-CO), 1.98-1.93 (m, 2H, C<u>H$_2$</u>-CH_2-N), 1.83 (sextet, *J*=6.5 Hz, 1H, C<u>H</u>-CH_2-S), 1.72-1.70 (m, 2H, O-CH_2-C<u>H$_2$</u>), 1.66-1.62 (m, 1H, C<u>H</u>-CH_2-CO), 1.52-1.49 (m, 3H, aliphatic chain), 1.33-1.30 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.9, 161.3, 160.1, 154.5, 149.0, 148.3, 145.0, 127.3, 122.8, 121.1, 113.1, 112.9, 112.1, 111.4, 100.7, 67.6, 56.1, 55.6, 48.9, 38.1, 35.0, 34.1, 28.3, 26.5, 25.5, 24.9. Anal. Calcd for $C_{32}H_{38}N_4O_6S_2$: C, 60.17; H, 6.00; N, 8.77. Found: C, 59.98; H, 5.88; N, 8.39.

*4.1.6.16. N-((1-(4-((3-(4-bromophenyl)-2-oxo-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**19**)*. Yield 83%; Off white solid; mp 158-160 ºC; IR (KBr, cm^{-1}) ν_{max}: 3291 (NH), 2923 (C-H), 1719 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.24 (m, 1H, NH and H_4), 7.91 (s, 1H, triazole), 7.69-7.63 (m, 5H, phenyl and H_5), 7.02 (s, 1H, H_8), 6.97 (d, 1H, *J*=9.0 Hz, H_6), 4.41 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.11 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.16-3.09 (m, 2H, CH_2-S), 2.39 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.08 (s, 2H, CH_2-CO), 1.95 (m, 2H, C<u>H$_2$</u>-CH_2-N), 1.82 (sextet, 1H, *J*=6.5 Hz, S-CH_2-C<u>H</u>), 1.73-1.70 (m, 2H, O-CH_2-C<u>H$_2$</u>), 1.65-160 (m, 1H, C<u>H</u>-CH_2-CO), 1.54-1.48 (m, 3H, aliphatic chain), 1.34-1.31 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.8, 161.7, 159.9, 154.7, 145.1, 134.0, 131.1, 130.2, 122.9, 121.4, 113.0, 112.9, 100.7, 67.6, 56.0, 48.8, 38.0, 34.9, 34.0, 28.2, 26.4, 25.4, 24.8. Anal. Calcd for $C_{30}H_{33}BrN_4O_4S_2$: C, 54.79; H, 5.06; N, 8.52. Found: C, 54.80; H, 5.12; N, 8.59.

*4.1.6.17. N-((1-(4-((3-(4-chlorophenyl)-2-oxo-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**20**)*. Yield 97%; Off white solid; mp 170-172

ºC; IR (KBr, cm^{-1}) ν_{max}: 3320 (NH), 2932 (C-H), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.27 (t, 1H, *J*=5.0 Hz NH), 8.24 (s, 1H, H_4), 7.91 (s, 1H, triazole), 7.74 (d, 2H, *J*=8.0 Hz, phenyl), 7.67 (d, 1H, *J*=8.5 Hz, H_5), 7.50 (d, 2H, *J*=8.0 Hz, phenyl), 7.02 (s, 1H, H_8), 6.96 (d, 1H, *J*=8.5 Hz, H_6), 4.40 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.26 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.11 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.17-3.10 (m, 2H, CH_2-S), 2.37 (sextet, 1H, *J*=6.0 Hz, S-CH_2-C<u>H</u>), 2.09 (t, 2H, *J*=7.5 Hz, CH_2-CO), 1.98-1.95 (m, 2H, C<u>H$_2$</u>-CH_2-N), 1.83 (sextet, 1H, S-CH_2-C<u>H</u>), 1.73-1.70 (m, 2H, O-CH_2-C<u>H$_2$</u>), 1.67-1.62 (m, 1H, C<u>H</u>-CH_2-CO), 1.54-1.51 (m, 3H, aliphatic chain), 1.33-1.30 (m, 2H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.9, 161.8, 159.8, 154.8, 145.0, 141.2, 133.7, 132.8, 130.0, 128.1, 122.6, 121.8, 113.0, 104.4, 100.9, 67.7, 56.1, 48.8, 38.0, 34.9, 34.0, 28.2, 26.5, 25.4. Anal. Calcd for $C_{30}H_{33}ClN_4O_4S_2$: C, 58.76; H, 5.42; N, 9.14. Found: C, 58.82; H, 5.50; N, 9.31.

*4.1.6.18. N-((1-(4-((3-(3,4-dichlorophenyl)-2-oxo-2H-chromen-7-yl)oxy)butyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**21**)*. Yield 95%; Off white solid; mp 113-115 ºC; IR (KBr, cm^{-1}) ν_{max}: 3290 (NH), 2931 (C-H), 1718 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.35 (s, 1H, H_4), 8.26 (bs, 1H, NH), 8.01 (s, 1H, H_5), 7.89 (s, 1H, triazole), 7.75-7.68 (m, 3H, phenyl), 7.04-6.97 (m, 2H, $H_{8,6}$), 4.35 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.26 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.07 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.62-3.57 (m, 1H, CH-S), 3.15-3.10 (m, 2H, CH_2-S), 2.39-2.35 (m, 1H, S-CH_2-C<u>H</u>), 2.08 (bs, 2H, CH_2-CO), 1.98-1.97 (m, 2H, C<u>H$_2$</u>-CH_2-N), 1.87-1.76 (m, 1H, S-CH_2-C<u>H</u> and 2H, O-CH_2-C<u>H$_2$</u>), 1.67-1.63 (m, 1H, CO-CH_2-C<u>H</u>), 1.51-1.32 (m, 5H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 170.2, 161.9, 155.2, 146.8, 141.1, 135.3, 131.3, 130.0, 128.4, 122.1, 119.2, 112.8, 100.5, 67.6, 56.0, 48.9, 38.3, 35.0, 34.0, 29.2, 28.2, 27.6, 22.5. Anal. Calcd for $C_{30}H_{32}Cl_2N_4O_4S_2$: C, 55.64; H, 4.98; N, 8.65. Found: C, 55.98; H, 5.23; N, 8.91.

*4.1.6.19. 5-(1,2-dithiolan-3-yl)-N-((1-(5-((2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**22**)*. Yield 96%; Off white solid; mp 93-94 ºC; IR (KBr, cm^{-1}) ν_{max}: 3295 (NH), 2929 (C-H), 1731, 1621 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.23 (t, 1H, *J*=5.5 Hz, NH), 7.97 (d, 1H, J=9.0 Hz, H_4), 7.87 (s, 1H, triazole), 7.60 (d, 1H, *J*=8.5 Hz, H_5), 6.96 (s, 1H, H_8), 6.92 (dd, 1H, *J*=8.5 Hz, *J*=2.0 Hz, H_6), 6.27 (d, 1H, *J*=9.0 Hz, H_3), 4.34 (t, 2H, *J*=6.5 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.05 (t, 2H, *J*=6.0 Hz, CH_2-O), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.19-3.07 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.7 Hz, C<u>H</u>CH_2S), 2.09 (t, 2H, *J*=6.0 Hz, COC<u>H$_2$</u>), 1.88-1.73 (m, 5H, aliphatic), 1.66-1.62 (m, 1H,

C<u>H</u>CH_2-CO), 1.53-1.31 (m, 7H aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.7, 161.7, 160.1, 155.3, 144.8, 144.1, 129.3, 122.4, 112.5, 112.2, 112.1, 101.0, 67.9, 56.0, 49.0, 37.9, 34.9, 34.0, 33.9, 29.3, 28.1, 27.6, 24.8, 22.3. Anal. Calcd for $C_{25}H_{32}N_4O_4S_2$: C, 58.12; H, 6.24; N, 10.84. Found: C, 57.9; H, 6.19; N, 11.10.

*4.1.6.20. 5-(1,2-dithiolan-3-yl)-N-((1-(5-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**23**).* Yield 98%; Off white solid; mp 66-67 ºC; IR (KBr, cm^{-1}) ν_{max}: 3295 (NH), 2862 (C-H), 1721, 1616 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.29 (t, 1H, *J*=5.0 Hz, NH), 7.89 (s, 1H, triazole), 7.67 (d, 1H, *J*=8.5 Hz, H_5), 6.95-6.93 (m, 2H, $H_{6,8}$), 6.20 (s, 1H, H_3), 4.35 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.26 (d, 2H, *J*=5.5 Hz, CH_2-NH), 4.04 (t, 2H, *J*=6.0 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.5 Hz, CH-S), 3.15-3.11 (m, 2H, CH_2-S), 2.49-2.39 (m, 3H, CH_3 and 1H C<u>H</u>-CH_2S), 2.09 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.86-1.76 (m, 5H, aliphatic), 1.65-1.60 (m, 1H, aliphatic), 1.51-1.31 (m, 7H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.7, 154.7, 153.4, 144.9, 131.6, 126.4, 122.6, 113.0, 112.4, 111.0, 101.1, 68.0, 56.1, 49.1, 38.0, 34.9, 34.10, 29.4, 28.3, 27.8, 24.9, 22.4, 18.1. Anal. Calcd for $C_{26}H_{34}N_4O_4S_2$: C, 58.84; H, 6.46; N, 10.56. Found: C, 59.09; H, 6.68; N, 10.83.

*4.1.6.21. N-((1-(5-((3-acetyl-2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**24**).* Yield 72%; Off white solid; mp 120-122 ºC; IR (KBr, cm^{-1}) ν_{max}: 3489 (NH), 2927 (C-H), 1715, 1627 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.62 (s, 1H, H_4), 8.22 (s, 1H, NH), 7.87 (s, 1H, triazole), 7.85 (d, 1H, *J*=8.5 Hz, H_5), 7.03 (s, 1H, H_8), 6.99 (d, 1H, *J*=8.5 Hz, H_6), 4.34 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.26 (d, 2H, *J*=5.5 Hz, CH_2-NH), 4.11 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.19-3.07 (m, 2H, CH_2-S), 2.55 (s, 3H, $COCH_3$), 2.39-2.37 (m, 1H, C<u>H</u>CH_2S), 2.09 (t, 2H, *J*=7.5 Hz, CH_2CO), 1.87-1.76 (m, 5H, aliphatic), 1.65-1.62 (m, 1H, aliphatic), 1.54-1.50 (m, 3H, aliphatic), 1.40-1.31 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 194.6, 171.7, 151.7, 147.5, 144.8, 132.1, 122.4, 113.7, 100.6, 68.3, 56.0, 49.0, 37.9, 34.9, 34.0, 33.9, 29.9, 29.3, 28.1, 27.6, 24.8, 22.3. Anal. Calcd for $C_{27}H_{34}N_4O_5S_2$: C, 58.04; H, 6.13; N, 10.3. Found: C, 58.19; H, 5.93; N, 9.98.

*4.1.6.22. ethyl 7-((5-(4-((5-(1,2-dithiolan-3-yl)pentanamido)methyl)-1H-1,2,3-triazol-1-yl)pentyl)oxy)-2-oxo-2H-chromene-3-carboxylate (**25**).* Yield 83%; Off white solid; mp 108-110 ºC; IR (KBr, cm^{-1}) ν_{max}: 3308 (NH), 2851 (C-H), 1736 and 1603 (C=O). ^{1}H NMR

(DMSO-d_6, 500 MHz) δ: 8.70 (s, 1H, H_4), 8.22 (t, 1H, *J*=5.5 Hz, NH), 7.87 (s, 1H, triazole), 7.82 (d, 1H, *J*=8.5 Hz, H_5), 7.00 (s, 1H, H_8), 6.97 (dd, 1H, *J*=2.0 Hz, *J*=8.5 Hz, H_6), 4.34 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.28-4.25 (m, 2H, CH_2-NH and 2H, O-C<u>H</u>$_2$-CH_3), 4.10 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.58 (quintet, 1H, *J*=6 Hz, CH-S), 3.19-3.07 (m, 2H, CH_2-S), 2.39 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>CH_2S), 2.09 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.88-1.76 (m, 5H, aliphatic), 1.67-1.61 (m, 1H, C<u>H</u>CH_2CO), 1.53-1.50 (m, 3H, aliphatic), 1.39-1.28 (t, 4H, aliphatic and 3H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz) *δ*: 171.7, 164.0, 162.8, 156.1, 148.9, 144.8, 131.5, 122.4, 113.5, 111.2, 100.5, 68.3, 60.7, 56.0, 49.0, 37.9, 34.9, 34.0, 33.9, 29.2, 28.1, 27.6, 24.8, 22.3, 13.9. Anal. Calcd for $C_{28}H_{36}N_4O_6S_2$: C, 57.12; H, 6.16; N, 9.52. Found: C, 56.95; H, 5.89; N, 9.25.

*4.1.6.23. 5-(1,2-dithiolan-3-yl)-N-((1-(5-((2-oxo-3-phenyl-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**26**).* Yield 77%; Off white solid; mp 128-130 ºC; IR (KBr, cm^{-1}) ν_{max}: 3330 (NH), 2930 (C-H), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.26 (t, 1H, *J*=5.5 Hz, NH), 8.20 (s, 1H, H_4), 7.89 (s, 1H, triazole), 7.69 (m, 3H, phenyl), 7.43 (m, 2H, phenyl), 7.39 (t, 1H, *J*=7.0 Hz, H_5), 7.01 (s, 1H, H_8), 6.95 (d, 1H, *J*=8.0 Hz, H_6), 4.36 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.08 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.56-3.52 (m, 1H, CH-S), 3.17-3.10 (m, 2H, CH_2-S), 2.39 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>-CH_2-S), 2.09 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.89-1.76 (m, 2H, N-CH_2-C<u>H</u>$_2$, 2H, O-CH_2-C<u>H</u>$_2$ and 1H, C<u>H</u>-CH_2-S), 1.65-1.62 (m, 1H, C<u>H</u>CH_2CO), 1.54-1.50 (m, 3H, aliphatic), 1.41-1.30 (m, 4H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.7, 159.9, 144.9, 140.8, 134.9, 129.6, 128. 2, 123.0, 112.9, 68.0, 56.0, 49.0, 34.9, 34.0, 29.4, 28.2, 27.7, 24.9, 22.4. Anal. Calcd for $C_{31}H_{36}N_4O_4S_2$: C, 62.81; H, 6.12; N, 9.45. Found: C, 63.01; H, 6.30; N, 9.21.

*4.1.6.24. 5-(1,2-dithiolan-3-yl)-N-((1-(5-((3-(4-methoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)pentanamide (**27**).* Yield 78%; Off white solid; mp 120-122 ºC; IR (KBr, cm^{-1}) ν_{max}: 3291 (NH), 2934 (C-H), 1722 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.26 (t, 1H, *J*=5.5Hz, NH), 8.12 (s, 1H, H_4), 7.89 (s, 1H, triazole), 7.66 (m, 3H, phenyl and H_5), 7.01-6.98 (m, 3H, phenyl and H_8), 6.93 (d, 1H, *J*=8.0 Hz, H_6), 4.35 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.06 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.80 (s, 3H, OMe), 3.57 (m, 1H, CH-S), 3.16-3.10 (m, 2H, CH_2-S), 2.37 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>-CH_2-S), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.89-176 (m, 2H, N-CH_2-C<u>H</u>$_2$ and 2H, O-CH_2-C<u>H</u>$_2$ and 1H, C<u>H</u>-CH_2-S), 1.66-1.62 (m, 1H, C<u>H</u>CH_2CO), 1.54-1.48 (m, 3H, aliphatic), 1.41-1.30 (m,

4H, aliphatic chain). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.3, 160.0, 149.2, 154.5, 144.9, 139.4, 129.5, 127.1, 122.7, 122.5, 113.6, 113.5, 113.0, 112.6, 100.6, 68.0, 56.0, 55.1, 49.0, 38.0, 34.9, 34.0, 29.4, 28.2, 27.7, 24.9, 22.4. Anal. Calcd for $C_{32}H_{38}N_4O_5S_2$: C, 61.71; H, 6.15; N, 9.00. Found: C, 61.80; H, 6.31; N, 9.25.

*4.1.6.25. N-((1-(5-((3-(3,4-dimethoxyphenyl)-2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**28**)*. Yield 915; Off white solid; mp 114-117 ºC; IR (KBr, cm^{-1}) ν_{max}: 3297 (NH), 2935 (C-H), 1717 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.25 (t, 1H, NH), 8.16 (s, 1H, H_4), 7.88 (s, 1H, triazole ring), 7.65 (d, 1H, *J*=9.0 Hz, H_5), 7.31 (m, 2H, phenyl), 7.02-6.99 (m, 2H, phenyl and H_8), 6.94 (d, 1H, *J*=9.0 Hz, H_6), 4.35 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.06 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.80 (d, 6H, *J*=5.0 Hz, 2OMe), 3.57 (m, 1H, CH-S), 3.16-3.10 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>-CH_2-S), 2.09 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.88-1.75 (m, 2H, N-CH_2-C<u>H</u>$_2$ and 2H and O-CH_2-C<u>H</u>$_2$, 1H, C<u>H</u>-CH_2-S), 1.65-1.62 (1H, C<u>H</u>CH_2CO), 1.52-1.50 (m, 3H, aliphatic chain), 1.41-1.30 (4H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.4, 160.0, 154.4, 148.9. 148.2, 144.9, 129.3, 127.3, 122.7, 121.0, 113.0, 112.1, 111.4, 100.6, 68.0, 56.0, 55.5, 49.0, 38.0, 34.9, 34.0, 34.0, 29.4, 28.2, 27.7. Anal. Calcd for $C_{33}H_{40}N_4O_6S_2$: C, 60.72; H, 6.18; N, 8.58. Found: C, 60.54; H, 6.34; N, 8.62.

*4.1.6.26. N-((1-(5-((3-(4-bromophenyl)-2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**29**)*. Yield 82%; Off white solid; mp 136-139 ºC; IR (KBr, cm^{-1}) ν_{max}: 3290 (NH), 2930 (C-H), 1720 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.26-8.24 (m, 1H, NH and H_4), 7.89 (s, 1H, triazole), 7.68-7.63 (m, 5H, phenyl and H_5), 7.01 (s, 1H, H_8), 6.95 (d, 1H, *J*=8.0 Hz, H_6), 4.32 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.26 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.07 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.57 (m, 1H, CH-S), 3.20-3.09 (m, 2H, CH_2-S), 2.40-2.36 (m, 1H, C<u>H</u>-CH_2-S), 2.09 (m, 2H, *J*= 7.0 Hz, CH_2-CO), 1.87-1.77 (m, 2H, N-CH_2-C<u>H</u>$_2$ and 2H, O-CH_2-C<u>H</u>$_2$ and 1H, C<u>H</u>-CH_2-S), 1.63-1.61 (m, 1H, C<u>H</u>CH_2CO), 1.51-1.31 (m, 7H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 161.9, 159.6, 154.9, 145.1, 141.0, 134.1, 131.1, 130.3, 122.7, 122.0, 121.4, 113.9, 113.0, 112.8, 100.6, 68.1, 56.1, 49.1, 38.0, 34.9, 34.1, 34.0, 29.4, 28.2, 27.7, 24.9, 22.4. Anal. Calcd for $C_{31}H_{35}BrN_4O_4S_2$: C, 55.44; H, 5.25; N, 8.34. Found: C, 55.49; H, 5.30; N, 8.42.

*4.1.6.27. N-((1-(5-((3-(3,4-dichlorophenyl)-2-oxo-2H-chromen-7-yl)oxy)pentyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(1,2-dithiolan-3-yl)pentanamide (**30**)*. Yield 100%; Off white solid; mp 132-134 ºC; IR (KBr, cm^{-1}) ν_{max}: 3285 (NH), 2924 (C-H), 1717 (C=O). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.34 (s, 1H, H_4), 8.27 (t, 1H, *J*=5.5 Hz, NH), 8.00 (s, 1H, H_5), 7.89 (s, 1H, triazole), 7.74-7.66 (m, 3H, phenyl), 7.01 (s, 1H, H_8), 6.96 (d, 1H, *J*=8.0 Hz, H_6), 4.35 (t, 2H, *J*=7.0 Hz, CH_2-N), 4.27 (d, 2H, *J*=6.0 Hz, CH_2-NH), 4.08 (t, 2H, *J*=6.5 Hz, CH_2-O), 3.57 (quintet, 1H, *J*=6.0 Hz, CH-S), 3.17-3.10 (m, 2H, CH_2-S), 2.38 (sextet, 1H, *J*=6.0 Hz, C<u>H</u>-CH_2-S), 2.08 (t, 2H, *J*=7.0 Hz, CH_2-CO), 1.89-1.75 (m, 2H, N-CH_2-C<u>H</u>$_2$ and 2H, O-CH_2-C<u>H</u>$_2$ and 1H, C<u>H</u>-CH_2-S), 1.65-1.61 (m, 1H, C<u>H</u>CH_2CO), 1.52-1.48 (m, 3H, aliphatic), 1.39-1.30 (m, 4H, aliphatic). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 171.8, 162.1, 159.5, 155.0, 144.9, 141.9, 135.4, 130.9, 130.6, 129.9, 129.8, 128.3, 122.6, 120.3, 113.3, 112.7, 100.7, 68.1, 56.1, 49.1, 38.0, 34.9, 34.0, 29.4, 28.2, 27.7, 24.9, 22.4. Anal. Calcd for $C_{31}H_{34}Cl_2N_4O_4S_2$: C, 56.27; H, 5.18; N, 8.47. Found: C, 56.30; H, 5.24; N, 8.71.

4.2. Biological assays

4.2.1. Neuroprotection assay against H_2O_2-induced cell death in PC12 cells

Cell viability of rat differentiated PC12 cells was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay according to the previously reported method [54]. PC12 cells (purchased from the Iranian Biological Resource Center, IBRC, Tehran, Iran) were seeded in 96-well plates (10,000 cells/well) and incubated at 37 ºC in a humidified atmosphere containing 5% CO_2. After 24 h, cells were treated with different concentrations (0.1, 1, 5, 10, 20, and 50 µM) of each tested compounds and incubated for 3 h followed by exposing cells with H_2O_2 (150 µM) for another 2 h. MTT solution (20 µL, 5 mg/mL) was consequently added after the removal of the previous medium and incubated in a CO_2 incubator for 4 h. Afterward, the medium was removed and the produced formazan crystals were solubilized using DMSO (100 µL). Finally, the related absorbance was recorded at 570 nm using a Synergy 2 multi-mode plate reader (Biotek, Winooski, VT, USA). Results were expressed as the percentage of untreated control cells (PC12 cells in the absence of tested compound and H_2O_2). All above-mentioned experiments were repeated for three times and mean±SEM of the obtained results were reported.

4.2.2. In vitro AChE/BuChE inhibition assay

The inhibitory activities of the compounds **4-30** towards AChE (from *Electrophorus electricus* (electric *eel*AChE), Sigma-Aldrich) and horse serum BuChE (*eq*BuChE, Sigma-Aldrich) were measured by Ellman's method [55]. In 24-well plates, a mixture of phosphate buffer (0.1 M, pH 8.0, 2 mL), 5,5´-dithiobis-2-nitrobenzoic acid (DTNB, 60 µL), acetylcholinesterase or butyrylcholinesterase (20 µL, 5 IU/mL) and different concentration of the compound solution (30 µL) was pre-incubated for 5 min followed by the addition of the substrate (acetylthiocholine iodide or butyrylthiocholine iodide, 20 µL). Changes in absorbance were measured at 412 nm by using a multi-mode reader (BioTek Synergy HT). To evaluate the IC_{50} (concentration of compound produced 50% of enzyme activity inhibition), log concentration versus percentage of inhibition curve was plotted and analyzed. All experiments were performed in triplicate and donepezil was used as a positive control.

4.2.3. Determination of the inhibitory potency on self-induced and AChE-induced $A\beta_{1-42}$ aggregation

In order to investigate the self-induced and AChE-induced $A\beta_{1-42}$ aggregation, a thioflavin T (ThT)-based fluorescence assay was performed [56]. For prefibrillation, amyloid-β protein 1-42 (Sigma A9810) 50 µM was dissolved in Phosphate Buffer Saline pH 7.4 (PBS, HyClone Thermo Scientific) containing 1% ammonium hydroxide, and incubated for 72 h at 37 °C. For the assay $A\beta_{1-42}$ (10 µL) ± human recombinant AChE (0.01 u/mL, Sigma C1682) were added to 0.05 M KP buffer pH 7.4 and incubated at 37 °C for 48 h in the absence and presence of compounds (100 µM). Then, 50 µL of thioflavin T (ThT, 200 µM, in 50 mM glycine-NaOH buffer, pH 8.5) was added to the mixture. Fluorescence was measured by Microplate Reader (SpectraMax) at λ_{ex}=448 and λ_{em}=490 nm. Rifampicin (100 µM, Sigma R-3501) and Donepezil (100 µM, Sigma D-6821) were also tested as reference drugs. The percent of inhibition of self or AChE-induced aggregation for each compound was determined by the following formula: [(IFi/IFo) × 100] where IFi and IFo are the fluorescence intensities obtained for Aβ ± AChE in the presence and in the absence of inhibitors.

4.2.4. 15-LOX inhibition assay

Soybean lipoxygenase (type 1-B lyophilized powder ≥50000 units/mg solid, Sigma) inhibition assay was performed as reported previously [57]. The tested compounds dissolved in DMSO and diluted in phosphate buffer (0.1 M, pH=8) then incubated at room temperature with linoleic acid in ethanol (134 mM) and enzyme solution (final concentration: 167 U/mL). The conversion of linoleic acid to 13-hydroperoxylinoleic acid at 234 nm was recorded. The experiment was done on Unico double beam spectrophotometer.

4.2.5. Neuroprotection assay against H_2O_2 or $A\beta_{1-42}$-induced cytotoxicity in SH-SY5Y cells

Cell viability was determined using MTT assay [54]. Human neuroblastoma SH-SY5Y cells (purchased from the American Type Culture Collection, ATCC, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 1% *L*-glutamine, and 1% antibiotic mix at 37 °C in a humidified atmosphere containing 5% CO_2. SH-SY5Y cells were seeded into 96-well plates (5×10^3 cells/well) and treated with compounds at a concentration of 10 µM or 50 µM for 3 h, prior to the addition of 250 µM H_2O_2 or 10 µM $A\beta_{1-42}$. After 24 hours of incubation, 10 µL of the MTT reagent (5 mg/mL) was added to each well. 100 µL of DMSO was used to solve formazan crystals. Absorbance values were measured at 570 nm using a microplate reader (Biotek Power Wave XS). Cell viability was reported as the percentage of untreated control cells as below:

Viability=100× [OD of treated wells – OD of blank wells/ OD of control wells – OD of blank wells]

4.2.6. Determination of intracellular H_2O_2-induced ROS production in PC12 cells

Intracellular ROS formation was determined by using the ROS-sensitive dye, 2´,7´-dichloro-fluorescein diacetate (DCFH-DA), as a probe [58]. Briefly, PC12 cells were seeded in 96-well plates at 1×10^4 cells/well and cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin and incubated at 37 ºC in humidified 5% CO_2 for 24 h. PC12 cells were then incubated in the presence or absence of

various concentrations of the tested samples (1, 5, 10 and 50 µM, which were dissolved in ≤ 0.5% DMSO and diluted with fresh medium) for 6 h, next washed with PBS and incubated with H_2O_2 (150 µM) for additional 18 h. Treated cells were then washed with PBS and incubated with a concentration of 10 µM DCFH-DA for 30 min at 37 ºC in the dark. At the end of incubation, cells were washed two times with PBS. The fluorescence of the dichlorofluorescein (DCF) was measured (λ_{ex}= 485 nm, λ_{em}= 528 nm) by ELISA fluorimeter (BioTek). The results were expressed as a percentage of intracellular ROS compared to untreated control cells.

4.2.7. FRAP assay

To evaluate the antioxidant power of the potent compounds, the method of Benzie and Strain was employed with slight modifications [59]. FRAP solution was prepared with a fresh mixture of 300 mM acetate buffer (pH 3.6), 10 mM hydrochloric (40 mM) solution of TPTZ (2,4,6-tripyridyl-*s*-triazine) and 20 mM ferric chloride. 240 µL FRAP solution was added to 10 µL of the tested compound solution (10 µM) and incubated for 15 min. The reduced ferric ion formed a colored ferrous-TPTZ complex at low pH. The absorbance was measured at 593 nm using a microplate reader (BioTek Synergy HT). FRAP values were reported in mmol of ferrous according to the plotted standard curve of ferrous sulfate.

4.2.8. Metal binding studies

The metal binding studies were carried out in a dual-beam GBC Cintra 101 spectrophotometer with 1-cm quartz cells. To investigate the metal binding ability of compound **11**, the UV absorption was recorded in the absence and presence of $CuCl_2$, $FeSO_4$, $ZnCl_2$, $AlCl_3$, $MgCl_2$ and $CaCl_2$ with a wavelength ranging from 200 to 500 nm after incubating for 30 min at room temperature. Final volume of the reaction mixture was 1 mL, and final concentrations of the tested compound and metals were 20 µM. Numerical subtraction of the spectra of the metal alone and the compound alone from the spectra of the mixture gave the difference UV-vis spectra indicating the complex formation. The molar ratio method was performed to determine the stoichiometry of the complex. The volume of the ligand solution was kept constant (2.5 mL, 40 µM) and titrated by $CuCl_2$ (0.01 mL, 1000 µM) [60].

4.3. Docking simulations

Docking studies were performed using the Molecular Operating Environment (MOE) software (Chemical Computing Group, Montreal, Canada). The crystal structure of acetylcholinesterase complexed with donepezil (1EVE) was retrieved from the Protein Data Bank (PDB). The preparation and optimization of the receptor were done by Autodock Tools (4.2) [61]. The ligand 3D structure and minimization prepared by Openbabel (2.3.1) [62]. The dimensions of active site box were set as 15×15×15 Å, the center of grid box was placed at x = 2.023, y = 63.295, z = 67.062. The 3D molecular visualization of the strongest cluster was done by Discovery Studio 2.0 software [63].

4.4. Statistical analysis

Data are reported as mean ±SEM of at least 3 independent experiments. Statistical analysis was performed using one-way ANOVA with Dunnett post hoc test and *p*-values less than 0.05 were considered statistically significant. Analyses were performed using PRISM 6 software on a Windows platform.

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Legends:

Fig. 1. Design of the target compounds **4-30** based on the structures of well-known drugs useful in the treatment of Alzheimer's disease.

Fig. 2. A) Lineweaver-Burk plot for the inhibition of AChE by compound **11** at different concentrations of substrate (ATCh), B) Secondary plot for calculation of steady-state inhibition constant (K_i) of compound **11**.

Fig. 3. Illustration of binding mode of compound **11** in the AChE active site.

Fig. 4. A) Protective effects of compounds **5** and **11** on cell injury induced by $A\beta_{1-42}$ in SH-SY5Y cells. B) Protective effects of compounds **5** and **11** on cell injury induced by H_2O_2 in SH-SY5Y cells. Values are the mean ± SEM of three independent experiments ($^{*}P < 0.05$, $^{***}P < 0.001$) *vs.* $A\beta_{1-42}$ or H_2O_2 group.

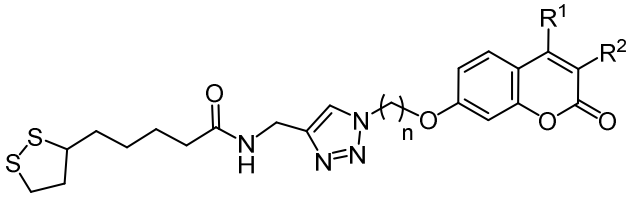
Fig. 5. Effects of the compound **5**, **11** and Lipoic acid on intracellular ROS formation in neuronal PC12 cells. Values are the mean ± SEM of three independent experiments ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) *vs.* H_2O_2 group (See supporting information for details, Table S1).

Fig. 6. A) The UV spectrum of compound **11** (final concentration, 20 µM in methanol) alone or in the presence of $CuCl_2$, $ZnCl_2$, $AlCl_3$, $CaCl_2$, $MgCl_2$ and $FeSO_4$ (final concentration, 20 µM in methanol). B) Determination of the stoichiometry of complex Cu^{+2}-**11** using molar ratio method through titrating the methanol solution of compound **11** (40 µM) with ascending amounts of $CuCl_2$ (1000 µM in methanol, titration step 0.01 mL).

Scheme 1. Synthesis of compounds **4-30**. Reagents and conditions: (a) ethyl acetoacetate, concentrated H_2SO_4, dioxane; (b) ethyl acetoacetate or diethyl malonate, piperidine, absolute ethanol; (c) anhydrous CH_3COONa, appropriate phenylacetic acid derivative, Ac_2O; (d) aqueous solution of sodium hydroxide (5%); (e) $Br(CH_2)_nBr$ (n = 3-5), anhydrous K_2CO_3, acetone, reflux, 4h; (f) propargylamine, EDCI, DMAP, CH_2Cl_2, rt, 12 h; (g) NaN_3, $CuSO_4$, sodium ascorbate, *t*-Butanol/H_2O, 70 °C, 24-48 h.

Table 1

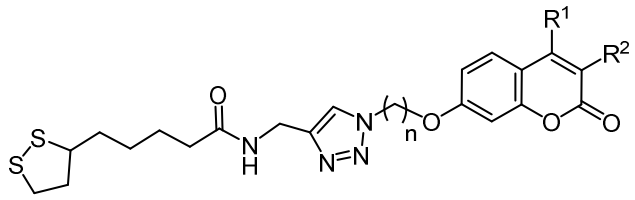
The protective effect of compounds **4-30** against H_2O_2 (150 µM) induced injury in PC12 cell line at different concentrations in comparison to Quercetin.[a]



Code	n	R^1	R^2	PC12 cells viability (% of control)						
				H_2O_2	0.1 µM	1 µM	5 µM	10 µM	20 µM	50 µM
4	3	H	H	27.1±1.2	37.8±1.9**	37.2±0.8**	39.2±0.9**	40.0±1.3**	42.2±1.3**	43.5±1.4**
5	3	Me	H	32.4±1.7	44.8±1.3**	54.5±2.2**	64.6±0.7**	69.4±1.8**	74.3±1.6**	75.4±1.1**
6	3	H	COMe	31.2±2.8	34.6±1.3**	35.5±0.4**	37.5±1.9**	38.0±0.8**	39.8±0.8**	40.7±2.2**
7	3	H	COOEt	29.5±1.4	28.0±1.2^{ns}	28.3±1.6^{ns}	32.4±1.3*	36.1±1.6**	42.1±0.8**	49.2±0.9**
8	3	H	COOH	34.0±0.4	43.4±2.0**	52.1±1.7**	57.0±2.3**	59.9±2.8**	66.8±1.1**	73.5±0.9**
9	3	H	Ph	36.0±1.1	33.9±1.7^{ns}	31.9±0.5^{ns}	29.8±0.4^{ns}	29.1±1.4^{ns}	27.2±0.7^{ns}	23.1±1.1^{ns}
10	3	H	4-MeO-Ph	36.7±2.2	42.0±2.4**	47.4±0.5**	52.8±2.7**	57.6±2.9**	62.0±1.8**	65.7±3.1**
11	3	H	3,4-$(MeO)_2$-Ph	34.3±1.8	36.4±1.6^{ns}	39.3±1.8**	40.6±0.8**	43.5±2.4**	45.6±0.8**	49.5±2**
12	3	H	4-Br-Ph	33.4±2.6	39.5±2.5**	42.8±2.2**	57.3±3.6**	63.3±1.6**	69.1±1**	74.7±3.6**
13	3	H	4-Cl-Ph	27.7±2.3	40.9±3.8**	49.3±0.8**	60.6±4.7**	67.8±1.8**	75.6±2.1**	79.4±3.8**
14	3	H	3,4-Cl_2-Ph	33.0±1.4	38.1±1.4**	42.4±2.6**	44.7±2.8**	49.6±1.1**	51.2±0.7**	54.8±1.2**
15	4	H	H	25.2±1.4	40.5±0.6**	45.9±1.6**	52.8±0.3**	65.1±2.0**	69.8±1.4**	75.8±3.2**
16	4	H	Ph	41.8±2.3	38.6±0.6^{ns}	34.8±0.5^{ns}	32.8±3.7^{ns}	30.5±2.8^{ns}	26.0±2.7^{ns}	20.5±2.3^{ns}
17	4	H	4-MeO-Ph	30.2±1.5	32.1±1.5^{ns}	36.8±1.3**	44.9±2.3**	48.6±2.4**	52.3±1.6**	55.3±2.2**
18	4	H	3,4-$(MeO)_2$-Ph	32.2±2.4	40.7±1.5**	53.0±1.7**	57.2±2.2**	64.0±2.7**	68.4±2.5**	71.9±1.9**
19	4	H	4-Br-Ph	31.2±2.6	34.2±1.7**	37.4±1.1**	40.5±0.4**	42.6±1.3**	45.0±0.9**	47.6±1.7**
20	4	H	4-Cl-Ph	33.6±1.8	38.8±0.7**	46.2±0.8**	52.0±3.1**	61.8±1.0**	68.4±2.7**	74.9±3.8**
21	4	H	3,4-Cl_2-Ph	34.2±1.1	39.6±1.3**	49.3±1.5**	53.2±2.2**	58.9±1.1**	62.9±1.3**	69.5±2.6**
22	5	H	H	30.3±1.8	46.3±1.8**	57.2±1.9**	64.5±3.0**	71.3±2.1**	78.5±3.9**	84.9±4.1**
23	5	Me	H	30.9±2.3	45.6±1.7**	56.5±0.5**	61.0±2.8**	66.3±2.3**	70.3±2.0**	73.7±0.2**
24	5	H	COMe	33.9±2.7	28.9±1.7^{ns}	27.4±1.5^{ns}	22.2±2.1^{ns}	20.8±2.6^{ns}	20.7±1.6^{ns}	18.2±0.8^{ns}
25	5	H	COOEt	27.9±0.7	34.4±0.4**	35.1±0.6**	36.4±1.7**	38.3±1.2**	39.5±1.0**	40.7±1.3**
26	5	H	Ph	36.8±1.6	35.5±1.4^{ns}	30.7±0.4^{ns}	28.0±0.3^{ns}	26.3±0.7^{ns}	20.7±0.5^{ns}	17.7±1.1^{ns}
27	5	H	4-MeO-Ph	32.1±1.7	34.5±1.2^{ns}	37.1±1.9**	41.0±2.1**	45.5±0.6**	49.0±1.4**	52.8±2.6**
28	5	H	3,4-$(MeO)_2$-Ph	31.0±3.3	38.5±0.9**	42.2±2.2**	46.9±2.8**	50.5±2.2**	51.9±2.5**	55.0±2.9**
29	5	H	4-Br-Ph	36.4±3.0	41.8±2.1**	43.7±2.7**	45.7±2.9**	48.4±2.3**	52.3±1.3**	53.6±2.2**
30	5	H	3,4-Cl_2-Ph	29.5±2.1	39.7±2.2**	50.6±3.1**	54.8±1.6**	59.4±1.7**	65.5±2.0**	66.2±2.4**
Quercetin		-	-	28.6±0.9	36.2±2.3**	40.7±1.4**	47.3±0.8**	53.7±1.2**	56.9±1.6**	58.8±2.1**

[a] Cell viability was determined using MTT assay protocol. Data are expressed as the mean ± SEM of three independent replicates. The significant ($^{**}p < 0.001$, $^{*}p < 0.01$) and not significant (ns) values with respect to H_2O_2 group.

Table 2
Inhibitory activity of the synthesized compound **4-30** against AChE and BuChE.



Compound	n	R^1	R^2	AChE[a] IC_{50} (μM)	BuChE[a] IC_{50} (μM)
4	3	H	H	66.7±1.2	>100
5	3	Me	H	55.0±2.0	>100
6	3	H	COMe	54.6±1.5	>100
7	3	H	COOEt	55.0±1.7	>100
8	3	H	COOH	>100	>100
9	3	H	Ph	>100	>100
10	3	H	4-MeO-Ph	40.2±0.7	>100
11	3	H	3,4-$(MeO)_2$-Ph	16.4±1.3	49.37±4.2
12	3	H	4-Br-Ph	46.5±3.1	>100
13	3	H	4-Cl-Ph	>100	>100
14	3	H	3,4-Cl_2-Ph	>100	73.5±1.1
15	4	H	H	65.5±1.4	>100
16	4	H	Ph	>100	>100
17	4	H	4-MeO-Ph	>100	>100
18	4	H	3,4-$(MeO)_2$-Ph	>100	>100
19	4	H	4-Br-Ph	>100	>100
20	4	H	4-Cl-Ph	>100	52.0±1.5
21	4	H	3,4-Cl_2-Ph	>100	10.3±0.2
22	5	H	H	70.6±2.4	>100
23	5	Me	H	>100	>100
24	5	H	COMe	>100	>100
25	5	H	COOEt	59.7±3.3	>100
26	5	H	Ph	>100	>100
27	5	H	4-MeO-Ph	>100	>100
28	5	H	3,4-$(MeO)_2$-Ph	>100	>100
29	5	H	4-Br-Ph	>100	>100
30	5	H	3,4-Cl_2-Ph	>100	7.8±3.6
Donepezil	-	-	-	0.02±1.9	3.4±1.4

[a] Inhibitor concentration (mean ± SEM of three experiments) required for 50% inhibition.

Table 3
Inhibition of self and AChE-induced Aβ aggregation by compounds **5** and **11**.

Compound	Inhibition of Aβ aggregation (%)	
	Self-induced[a]	AChE-induced[b]
5	26.6 ± .5	2.9 ± 2.6
11	51.2 ± 3.1	47.4 ± 1.9
Rifampicin	27.5 ± 4.3	12.2 ± 3.0
Donepezil	22.0 ± 5.4	26.1 ± 2.5

[a] Inhibition of self-induced Aβ(1-42) aggregation (10 μM) produced by the tested compounds at 100 μM concentration. Values are expressed as means ± SEM of three experiments.

[b] Co-aggregation inhibition of Aβ(1-42) and AChE (0.01 u/ml) by the tested compounds at 100 μM concentration was detected by ThT assay. Values are expressed as means ± SEM of three experiments.

Table 4

Antioxidant activity of compounds **5** and **11** determined by FRAP assay.

Compound	**FRAP (mM)**[a]
5	84.7 ± 0.7
11	87.5 ± 0.4
Lipoic acid	84.2 ± 0.8
Ascorbic acid	81.2 ± 0.5
Quercetin	84.7 ± 1.2

[a] The data are expressed as Mean ± SEM of three experiments.

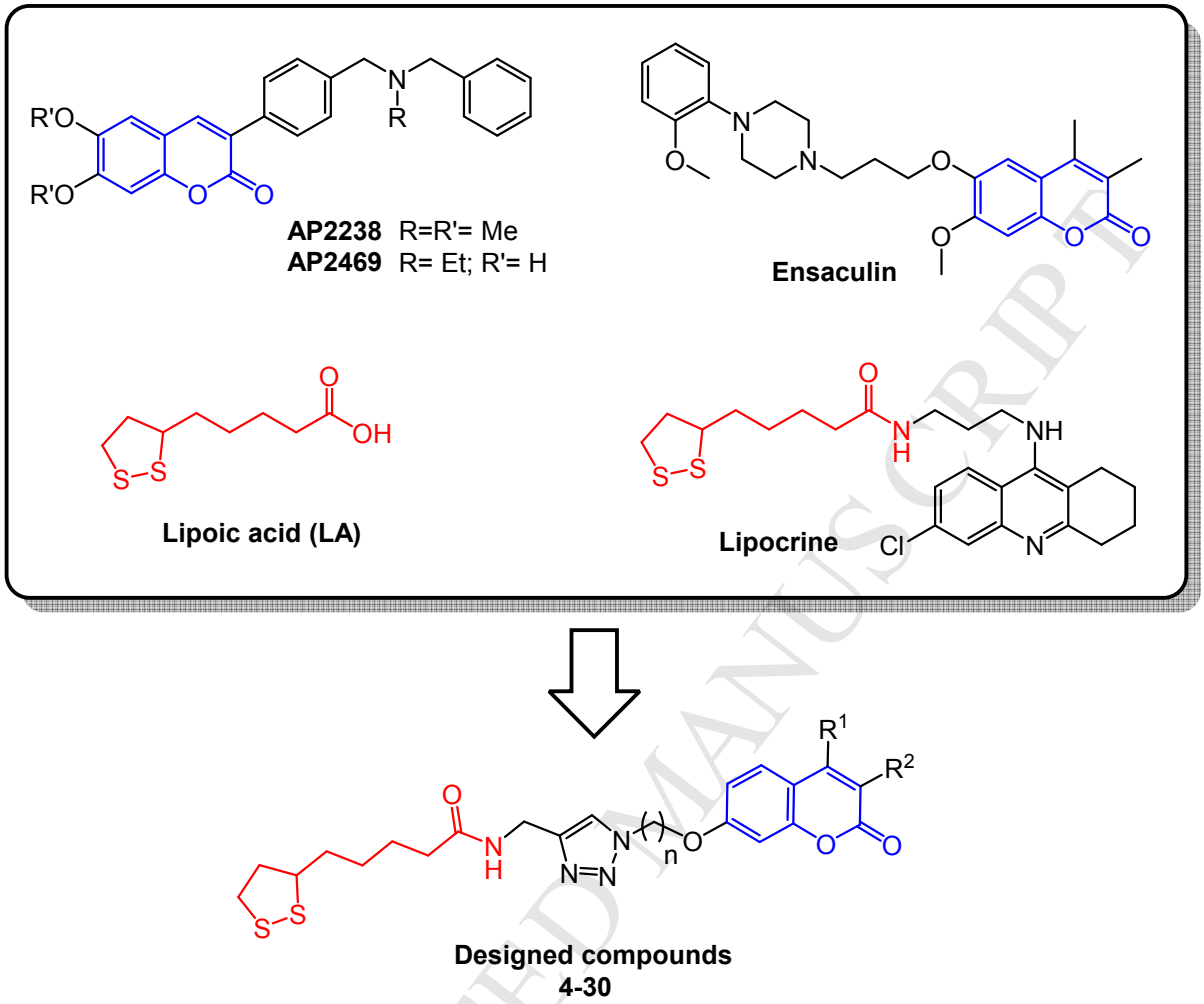


Fig. 1. Design of the target compounds **4-30** based on the structures of well-known drugs useful in the treatment of Alzheimer's disease.

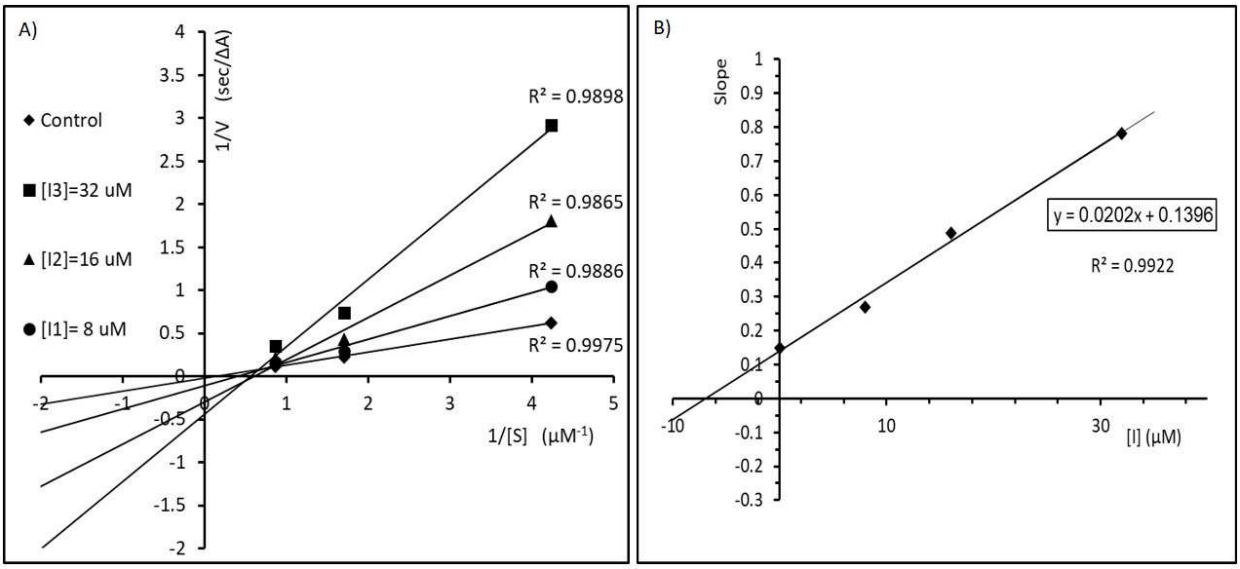


Fig. 2. A) Lineweaver-Burk plot for the inhibition of AChE by compound **11** at different concentrations of substrate (ATCh), B) Secondary plot for calculation of steady-state inhibition constant (K_i) of compound **11**.

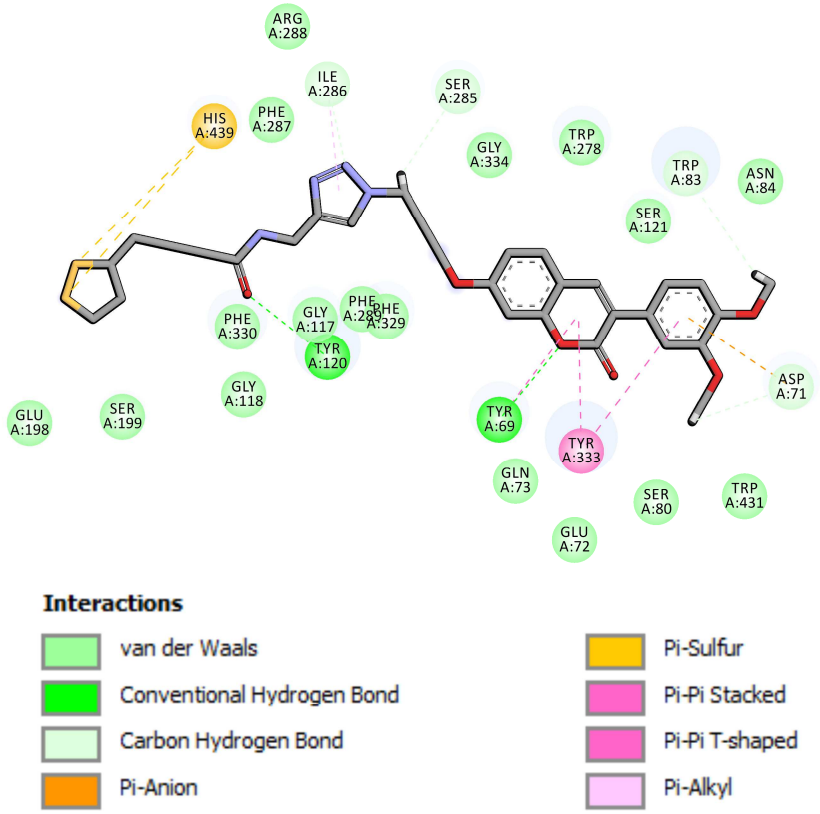


Fig. 3. Illustration of binding mode of compound **11** in the AChE active site.

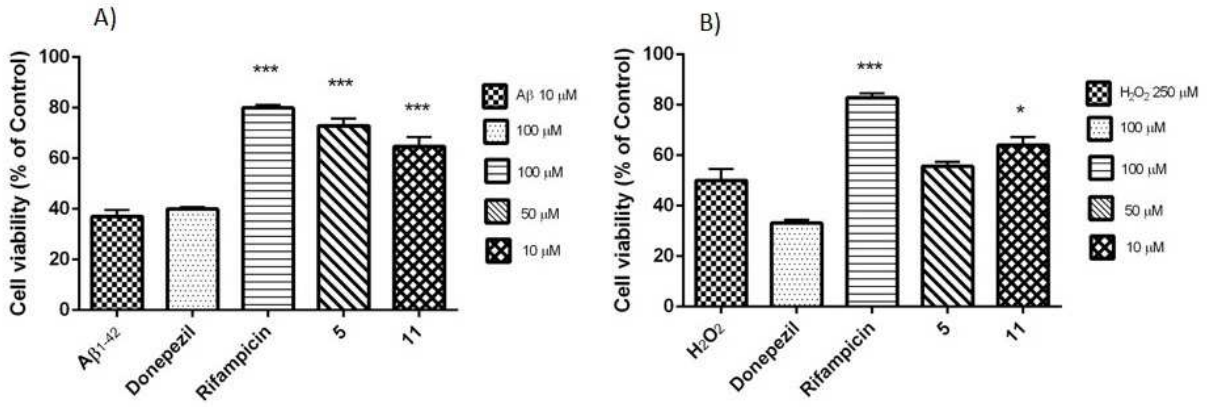


Fig. 4. A) Protective effects of compounds **5** and **11** on cell injury induced by $A\beta_{1-42}$ in SH-SY5Y cells. B) Protective effects of compounds **5** and **11** on cell injury induced by H_2O_2 in SH-SY5Y cells. Values are the mean ± SEM of three independent experiments ($^{*}P < 0.05$, $^{***}P < 0.001$) *vs.* $A\beta_{1-42}$ or H_2O_2 group.

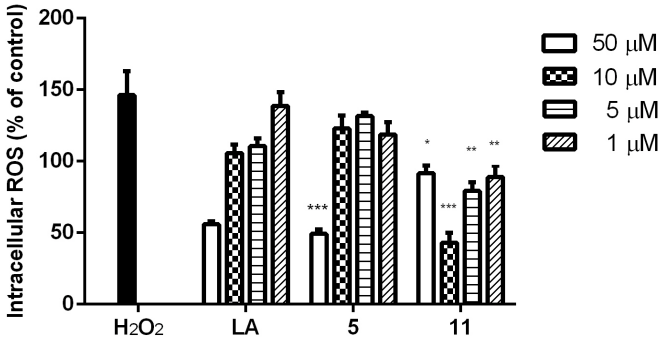


Fig. 5. Effects of the compound **5**, **11** and Lipoic acid on intracellular ROS formation in neuronal PC12 cells. Values are the mean ± SEM of three independent experiments ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) *vs.* H_2O_2 group (See supporting information for details, Table S1).

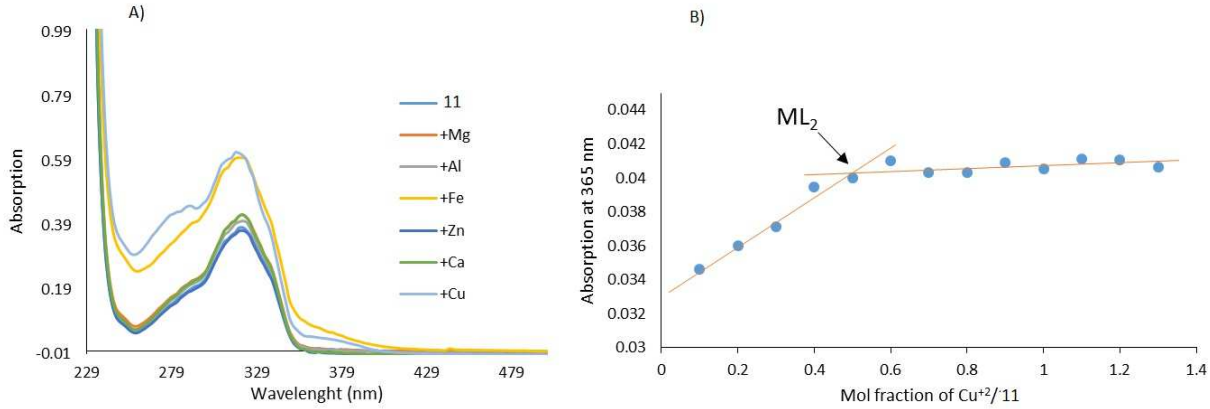
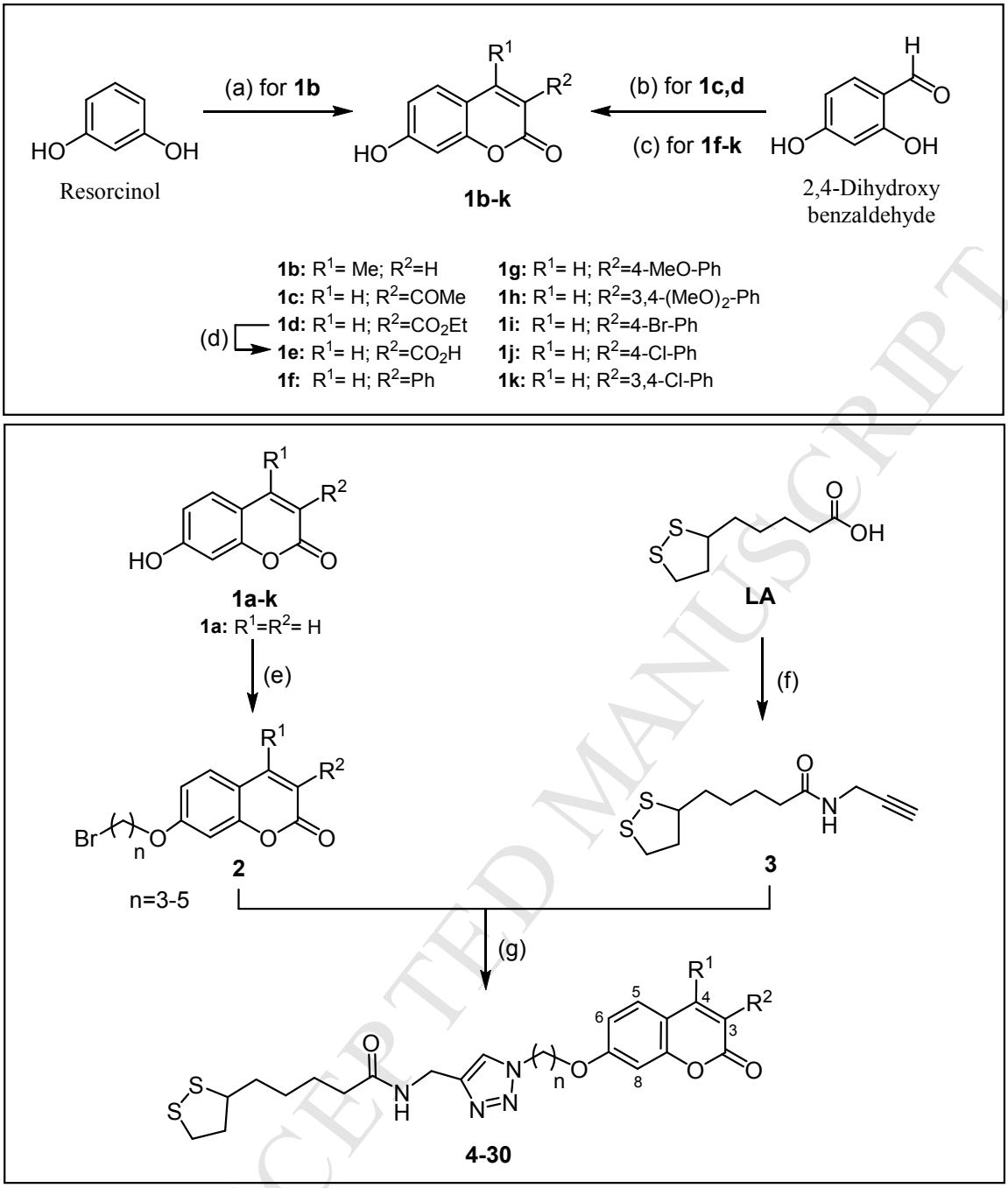


Fig. 6. A) The UV spectrum of compound **11** (final concentration, 20 µM in methanol) alone or in the presence of $CuCl_2$, $ZnCl_2$, $AlCl_3$, $CaCl_2$, $MgCl_2$ and $FeSO_4$ (final concentration, 20 µM in methanol). B) Determination of the stoichiometry of complex Cu^{+2}-**11** using molar ratio method through titrating the methanol solution of compound **11** (40 µM) with ascending amounts of $CuCl_2$ (1000 µM in methanol, titration step 0.01 mL).



Scheme 1. Synthesis of compounds **4-30**. Reagents and conditions: (a) ethyl acetoacetate, concentrated H_2SO_4, dioxane; (b) ethyl acetoacetate or diethyl malonate, piperidine, absolute ethanol; (c) anhydrous CH_3COONa, appropriate phenylacetic acid derivative, Ac_2O; (d) aqueous solution of sodium hydroxide (5%); (e) $Br(CH_2)_nBr$ (n = 3-5), anhydrous K_2CO_3, acetone, reflux, 4h; (f) propargylamine, EDCI, DMAP, CH_2Cl_2, rt, 12 h; (g) NaN_3, $CuSO_4$, sodium ascorbate, *t*-Butanol/H_2O, 70 °C, 24-48 h.

Highlights

- 27 new coumarin-lipoic acid conjugates were synthesized as anti-Alzheimer agents.
- Most compounds could significantly protect PC12 cells against H_2O_2-induced death.
- The 3-(dimethoxyphenyl)coumarin analog **11** was the most potent compound against AChE.
- Compound **11** could significantly inhibit Aβ-induced neurotoxicity in SH-SY5Y cells.
- Compound **11** showed anti-Aβ aggregation, antioxidant and metal chelating activities.