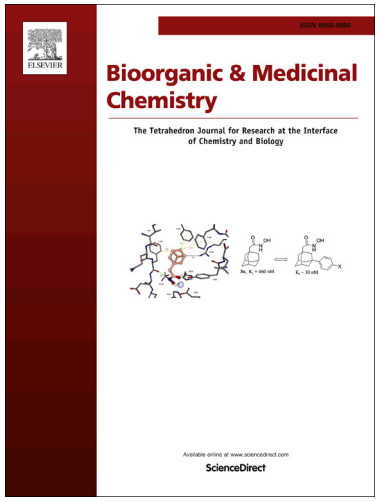

Design, synthesis and evaluation of chromone-2-carboxamido-alkylbenzylamines as multifunctional agents for the treatment of Alzheimer's disease

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Design, synthesis and evaluation of chromone-2-carboxamido-alkylbenzylamines as multifunctional agents for the treatment of Alzheimer's disease

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Abstract

A series of chromone-2-carboxamido-alkylbenzylamines were designed, synthesized and evaluated as multifunctional agents for the treatment of Alzheimer's disease (AD). The results showed that most of these compounds exhibited good multifunctional activities. Among them, compound **49** displayed excellent inhibitory potency toward acetylcholinesterase (AChE), moderate anti-oxidative activity, selective biometal chelating, and possessed good inhibitory effects on self-induced and Cu^{2+}-induced Aβ aggregation. Both kinetic analysis of AChE inhibition and molecular modeling study indicated that **49** was a mixed-type inhibitor, binding simultaneously to the catalytic active site and peripheral anionic site of AChE. These results suggested that **49** might be a potential multifunctional agent for AD treatment.

Keywords: Alzheimer's disease, Chromone-2-carboxamido-alkylbenzylamines, Acetylcholinesterase inhibitors, Anti-oxidative activity, Biometal chelators, Aβ aggregation inhibitors.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects daily living through memory loss and cognitive impairment.[1] Current therapeutic options for the treatment of AD, including cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and *N*-methyl-*D*-aspartate (NMDA) receptor antagonist (memantine), have resulted in a modest improvement in memory and cognitive function, but they do not prevent progressive neurodegeneration.[2,3] Although the etiology of AD remain elusive, multiple factors, such as low levels of acetylcholine (ACh), oxidative stress, accumulation of misfolded amyloid-β (Aβ) and dyshomeostasis of biometals have been considered to play definitive roles in the pathophysiology of AD, and several hypotheses based on these factors have been proposed to explain the mechanism of AD pathogenesis.[4,5] According to the ''cholinergic hypothesis'', AChE acts primarily as a regulatory enzyme at cholinergic synapses, while BuChE functions as an enzyme closely related to AChE and serves as a co-regulator of cholinergic neurotransmission by hydrolysing ACh.[6,7] AChE and BuChE inhibition have been documented as critical targets for the effective management of AD by an increase the availability of ACh in the brain regions.[8] However, BuChE is mainly localized in the peripheral tissues including plasma and very small amount is present in the brain region. Many side effects (e.g. gastrointestinal events, nausea, vomiting, diarrhoea, dizziness) mainly associated with their peripheral AChE inhibitory activity.[9] Therefore, the potential advantage of selective inhibition of AChE over BChE may include lesser degree of associated side effects due to peripheral inhibition of cholinesterase enzyme.[8] So it may be a significative approach to develop specific AChE inhibitors for the treatment of AD, with expectation for their fewer side effects.

Based on "amyloid hypothesis", the production and accumulation of oligomeric aggregates of Aβ in the brain are a central event in the pathogenesis of AD.[10] Aβ_{40} and Aβ_{42} are the main isoforms of Aβ peptides. *In vivo* studies show that Aβ_{42} is a major constituent of amyloid plaques and promulgate that Aβ_{42} aggregation plays a critical role in the initiation of plaque formation and AD pathogenesis.

In vitro, Aβ_{42} displays lower solubility and more toxic and has the tendency to form fibrillar aggregates at lower concentrations than Aβ_{40}.[11] Therefore, the prevention of Aβ_{42} aggregation attracts much current attention. Moreover, excess of metal ions such as Cu^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+} have been found in Aβ plaques of AD brains, and Cu^{2+} and Zn^{2+} have been proved to promote Aβ aggregation as described previously.[12] Interestingly, the abnormally high levels of redox-active metal ions such as Cu^{2+}, Fe^{2+} or impaired regulation of redox-active metals which can induce the formation of cytotoxic reactive oxygen species and neuronal damage.[13] Thus, biometals chelators might be a potential therapeutic strategy for the treatment of AD. In addition, Oxidative stress plays an important role in neuronal degeneration. Oxidative damage marked by lipid peroxidation, nitration, reactive carbonyls, and nucleic acid oxidation is increased in vulnerable neurons in AD.[10] Therefore, the successful protection of neuronal cells from oxidative damage could potentially prevent AD.

Obviously, there are many attractive targets for the development of anti-AD drugs, the compounds possess two or more complementary biological activities have drawn considerable attention for their advancement in the treatment of AD. In this regard, the multitarget-directed ligands strategy (MTDLs) has been performed by many research groups.[14]

< Figure 1. here >

Chromone (**I**), a privileged scaffold in medicinal chemistry, is the core fragment of several flavonoid derivatives such as flavones and isoflavones (**Figure 1**). The derivatives of chromone have attracted much attention in recent years because of their diverse pharmacological properties such as anti-inflammatory effect, metal chelating ability, and neuroprotective effect.[15-17] Recent study has indicated that a new family of tacrine-chromone hybrids showed interesting bioactivities for the potential treatment of AD, such as inhibition of human AChE, BuChE, and β-secretase 1 (BACE-1), as well as radical scavenger activity.[18] However, whether this kind of tacrine-chromone hybrids poccesses the effects on Aβ aggregation is unknown. Recently, our group has reported the synthesis of genistein-*O*-alkylbenzylamines and discovered one 7,4′-*O*-modified genistein derivative (**II**) as a

potential multifunctional agent for the treatment of AD.[19] However, this compound has low inhibitory effects on self-induced Aβ_{1-42} aggregation.

Herein, chromone-2-carboxamide moiety was selected to combine with different length alkylbenzylamine fragments to design a series of novel chromone-2-carboxamido-alkylbenzylamine derivatives (**Figure 1**), to test whether these novel molecules might possess more potency in various multifunctional activities and improve the shortcomings. In this paper, a series of chromone-2-carboxamido-alkylbenzylamine derivatives were designed, synthesized and evaluated biological activities based on the multitarget-directed ligands strategy (MTDLs). Their biological activities including AChE and BuChE inhibition, the kinetics of enzyme inhibition, antioxidant activities, biometal chelation, and effects on Aβ aggregation were also evaluated.

2. Results and discussion

2.1. Chemistry

The key intermediates **11a-d**, **12a-h** and **13a-d** were prepared according to the route outlined in **Scheme 1**. The secondary amines **5a-g** were synthesized by reductive amination of methylamine, ethylamine or propargylamine with the corresponding benzaldehydes **1-4** in the presence of $NaBH_4$,[20] while compound **5h** was obtained from a different route. Compound **7** was prepared from salicylaldehyde **6** through reductive amination by using ethanol amine and $NaBH_4$ and then protection by using di-*tert*-butyl dicarbonate (Boc_2O) and Et_3N in one-pot reaction. Then **5h** was obtained through Mitsunobu Reaction and deprotection by the treatment with trifluoroacetic acid. The primary amine intermediates **11-13** were prepared by conventional method[21] through Gabriel syntheses starting from the commercially available ω-bromoalkylphthalimides and corresponding secondary amines **5a-h**.

< Scheme 1. here >

The general procedure for the synthesis of the chromone-2-carboxamido-alkylbenzylamines **24-50**

was depicted in **Scheme 2**. The chromone-2-carboxylic acid analogs **20-23** were prepared from phloroglucinol **14** and 2,6-dihydroxyacetophenone **19**.[22] First, phloroglucinol **14** was acylated to compound **15** by Fries rearrangement using $BF_3 \cdot Et_2O$.[23] Then treatment of the latter with Me_2SO_4 and K_2CO_3 in acetone gave compound **16**. In the reported method for the synthesis of chromone-2-carboxylic acid involved three steps including condensation, saponification and demethylation generally.[24] But the workup was inconvenient. So we adopted a new one-pot strategy by condensation of **16** with ethyl oxalate in the presence of EtONa in absolute EtOH and then treatment with 40% HBr for 12 h or 48 h afforded compound **20** and **21** respectively. Similar procedure was adopted in the preparation of compound **22** and the key intermediate compound **17** was synthesized by reacting compound **15** with dimethylamine hydrochloride in the presence of $NaHCO_3$ in H_2O under argon. More conveniently, for the synthesis of 7-H substituted chromone moiety, it is easy to obtain the ester **23** from compound **19** directly by using excessive amounts of EtONa in EtOH. Eventually, the chromone-2-carboxamido-alkylbenzylamines **24-50** were eventually obtained by either condensation reaction or amination reaction with the appropriate primary amine **11a-d**, **12a-h** and **13a-d**. All synthesized compounds were purified using recrystallization or chromatography, and the analytical and spectroscopic data confirmed their structures, as detailed in the experimental section.

< Scheme 2. here >

2.2. Pharmacology

2.2.1. Inhibition studies on AChE and BuChE

Inhibitory activities toward AChE and BuChE of the synthesized compounds **24-50** and intermediates **20**-**23** were evaluated according to the modified Ellman's method. Their activities were initially assayed on AChE from rat cortex homogenate (*Rat*AChE) and BuChE from rat serum (*Rat*BuChE). Moreover, the compounds were evaluated using electric eel AChE (*Ee*AChE).

Donepezil was used as reference compound. The IC_{50} values and selectivity index for the inhibition of AChE and BuChE were summarized in **Table 1**.

< Table 1. here >

All the target compounds showed significant AChE inhibitiory activities with IC_{50} values ranging from submicromolar to micromolar, and demonstrated weak BuChE inhibitory activities, indicating that these derivatives were selective inhibitors for AChE. In view of BuChE was mainly localized in the peripheral tissues including plasma and very small amount was present in the brain region.[25] Thus, higher selectivity inhibitors toward AChEover BuChE, resulted in lesser degree of associated side effects, might be beneficial for the treatment of AD. Therefore, it was identical to our design strategy. Compound **49** exhibited the best inhibitory potency for AChE with IC_{50} value of 0.07 μM. According to the above results, the properties of the substitutions at chromone nucleus 7 position had significant effects on AChE inhibitory activities. The trend for AChE inhibitiory activities was 7-H > 7-OCH_3 > 7-$N(CH_3)_2$ > 7-OH. The screening data exhibited that the structure of terminal groups NR_1R_2 of side chain effected the inhibitory activities, generally, the potency to inhibit AChE were in the order *N*-ethyl-2-methoxy-benzenemethanamine ≈ 2-(dimethylamino)-*N*-ethyl-benzenem ethanamine > *N*-benzylethanamine > *N*-(2-methoxybenzy)methanamine > 4-(dimethylamino)-*N*-ethylbenzylethanamine > *N*-benzylmethanamine > *N*-(2-methoxybenzyl)prop-2-yn-1-amine > 2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepine. The compounds (**25**, **27**, **29**, **31**, **37**, **39**) possessing a *N*-ethyl group showed better AChE inhibitory activity than compounds (**24**, **26**, **28**, **30**, **36**, **38**) with a *N*-methyl group, in most cases, and the inhibitory activity dropped dramatically when the *N*-ethyl group was replaced by *N*-propargyl (**34**). With the same scaffold and terminal groups NR_1R_2, the inhibitory activity increased with the length of methylene linkers increased, except compound **49**, where the optimal chain length was four. Finally, compound **49** showed the most potent inhibition for *Rat*AChE with an IC_{50} value of 0.07 ± 0.002 μM, and 735.7-fold selectivity for AChE over BuChE. Moreover, all the target compounds were determined by additional testing using *Ee*AChE, it is

notable that all the tested compounds exhibited a higher inhibitory activity towards *Rat*AChE than *Ee*AChE, except compounds **24**, **28**, **29**, **36**, **37** and **38** (**Table 1**). In addition, the choromone scaffolds (**20-23**) showed low inhibitory activity and none selectivity toward AChE and BuChE, indicating that the introduction of the *O*-alkylamines increased the ChEs inhibitory capacity and improved the selectivity for AChE over BuChE, which is identical to our design strategy. Overall, the results suggested that *N*-(2-methoxybenzyl)ethylamine and *N*-(2-dimethylaminobenzyl)ethylamine might be the optimal substitution patterns for AChE inhibition. Almost all the compounds exhibited very weak activity against BuChE (IC_{50} > 20 μM). This result showed that these target compounds were potent AChE inhibitors with high selectivity toward AChE. This selectivity profile may be a limitation, but this may also be beneficial to diminish peripheral cholinergic side effects and provide lower toxicity. A typical case in point is tacrine, which had severe side effects because of its poor selectivity.[26] Based on the above assay results, we next selected the most potent AChE inhibitor, **49**, for kinetic analysis to investigate the type of inhibition.

2.2.2 Kinetic study for the inhibition of AChE

A kinetic study was carried out on compound **49** using *Ee*AChE to gain further insight into the mechanism of action on AChE of this family of compounds. The Lineweaver-Burk plots (**Figure 2.**) showed both increased slopes (decreased V_{max}) and intercepts (higher K_m) at increasing concentration of the inhibitor, which indicated a mixed-type inhibition. It revealed that compound **49** might be able to bind to the catalytic active site (CAS) as well as peripheral anionic site (PAS) of AChE.

< Figure 2. here >

2.2.3 Molecular modeling study

In order to obtain more information about the binding interactions between compound **49** and *Tc*AChE (PDB code: *1EVE*), docking studies were carried out using the AUTODOCK 4.2 package[11] as shown in **Figure 3**.

< Figure 3. here >

Results showed that compound **49** exhibited multiple binding modes with AChE. In the **49**–*Tc*AChE complex, compound **49** occupied the entire enzymatic CAS, mid-gorge and PAS. The chromone moiety occupied the PAS of AChE and showed π-π stacking with Phe288, Phe290, Phe331 and Tyr334. Notably, a strong hydrogen bond was observed between the 4-carbonyl group at the chromone moiety and Tyr121. Besides, the benzene ring of **49** displayed significant π-stacking interaction in choline binding site represented by Trp84. The long chain of methylene folded in a conformation in the gorge that allowed it to interact with Phe330 via the hydrophobic interaction. The docking study showed that compound **49** exhibited a mixed type of inhibition, consistented with our kinetic analysis result.

2.2.4 Antioxidant activity

The antioxidant activities of compounds **20-50** were evaluated by following the well-established ORAC-FL method.[27,28] Peroxyl radicals were thermally generated from 2,2-azobis-(amidinopropane) dihydrochloride and reacted with fluorescein to form nonfluorescent products at 535 nm. The antioxidant capacity of compounds **20-50** was determined by their competition with fluorescein in the radical capture, using a fluorescence microplate reader. Their ability to scavenge radicals was provided as a Trolox (a vitamin E analog) equivalent, with their relative potency at 5 μM compared with Trolox (**Table 1**). All the target compounds demonstrated moderate to good antioxidant activity. Compounds **33**, **40**, **41 and 42** showed the most potent antioxidant activity of this family with ORAC-FL values of 2.20, 2.10, 2.30 and 2.25 equivalents, respectively. The *N*-(2-dimethyl aminobenzyl)ethylamine moiety seemed to be a potent substitution pattern for the radical scavenging ability. It was noticeable that the free 7-OH of chromone derivatives was found to be crucial to the radical scavenging ability when the ORAC-FL values were compared between that of the 7-H derivatives (**45-50**) and that of the 7-OCH_3 derivatives (**24-39**).

2.2.5 Metal-chelating properties

The chelating ability of compound **49** toward biologically relevant metal ions such as Cu^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+} was studied by UV–vis spectrometry. Electronic spectral of compound **49** in methanol significantly changed in the presence of Cu^{2+} ions while remained unchanged after adding Fe^{2+}, Zn^{2+} and Al^{3+} ions (**Figure 4.**). Upon the addition of $CuCl_2$, the curve had a red shift (the peak at 340 nm shift to 420 nm) suggesting the formation of complex **49**-Cu^{2+}. However, after adding $FeSO_4$, $ZnCl_2$, and $AlCl_3$ no significant shift was observed in the UV spectrum.

< Figure 4. here >

In order to determine the stoichiometry of the complex **49**-Cu^{2+}, the molar ration method was employed by preparing the solution of compound **49** with ascending amounts of $CuCl_2$. The UV spectrum was used to obtain the absorbance of the **49** complex and different concentrations of $CuCl_2$ at 420 nm. The results showed that absorbance linearly increased initially and then plateaued (**Figure 5.**). The two straight lines intersected at a mole fraction of 0.98, revealing a 1:1 stoichiometry for complex **49**-Cu^{2+}.

< Figure 5. here >

2.2.6 Effects on the A*β* Aggregation

The inhibition of self and Cu^{2+}-induced A$\beta_{1\text{-}42}$ aggregation by our synthetic derivatives was determined by using thioflavin T fluorescence method[26,29,30], with curcumin as reference compound.[31] The data for their effects on A$\beta_{1\text{-}42}$ peptide aggregation at concentration of 25 μM was summarized in **Table 2**. The marketed AD drug, donepezil, did not show any significant inhibitory activity under the same experimental conditions. In the ThT assay screening for A$\beta_{1\text{-}42}$ peptide self-aggregation inhibition, the most potent compounds, similarly to AChE inhibition, were found to be **31**, **39**, **49** and **50** featuring a *N*-(2-methoxybenzyl)ethylamine group on the terminal side of carbon chain with respective inhibition ratio of 51.0%, 58.2%, 59.2% and 63.0%, which are higher than that of curcumin (43.1%). Interestingly, these four compounds also exhibited the high inhibitory activity for AChE. For example, compound **49**, as the most potent AChE inhibitor, showed the

second highest inhibitory potency of self-induced A$\beta_{1\text{-}42}$ aggregation with inhibitory percentage of 59.2%. Other target compounds showed moderate to good potencies ranging from 21.1% to 50.6%. The results indicated that substitution on the 7 position of chromone moiety played a pivotal role in inhibition of self-induced A$\beta_{1\text{-}42}$ aggregation. The trend for A$\beta_{1\text{-}42}$ inhibition was 7-H > 7-OCH_3 > 7-$N(CH_3)_2$ > 7-OH (e.g. **49 > 31 > 43 > 42**). Structure-activity relationship analysis showed that the chain length turned out to play an important role, since the inhibitory potency rose as the length of chain increased (e.g. **50 > 49 > 48**).

< Table 2. here >

Regarding the inhibition of Cu^{2+}-induced A$\beta_{1\text{-}42}$ aggregation, all compounds showed moderate potencies ranging from 18.1% to 55.6%, which were lower than that of curcumin. The compounds having potent inhibition of self-induced A$\beta_{1\text{-}42}$ aggregation generally exhibited relatively good inhibition of Cu^{2+}-induced A$\beta_{1\text{-}42}$ aggregation. For example, compound **50** showed the highest inhibitory potency of self-induced A$\beta_{1\text{-}42}$ aggregation (63.0%) and was also the most potent inhibitor of Cu^{2+}-induced A$\beta_{1\text{-}42}$ aggregation (55.6%) among these compounds. Comparing to 7,4′-*O*-modified genistein derivative (**II**), the highlight of this paper reported chromone-2-carboxamido-alkylbenzylamines involves in the improvements of self-induced A$\beta_{1\text{-}42}$ aggregation inhibition. The most potent compounds **31**, **39**, **49** and **50** showed inhibition ratio with 51.0%, 58.2%, 59.2% and 63.0% respectively, which are much potent than 7,4′-*O*-modified genistein derivative (**II**) (35.0%).[19] What is more, according to 'Lipinski's rule of five', chromone-2-carboxamido-alkylbenzylamines derivatives are more likely to be drug candidates than 7,4′-*O*-modified genistein derivative (**II**) in terms of their molecular weight.

Interestingly, the result of Aβ anti-aggregation activity is consistent with the outcome of inhibition against AChE. These chromone derivatives, as was revealed in the kinetic study, were dual binding site AChE inhibitors. This kind of inhibitors are often endowed with Aβ anti-aggregating properties,[32] which arise either from blockade of the AChE peripheral anionic site (PAS)[33-35] or from

a direct interaction with Aβ (blockade of spontaneous Aβ aggregation), in the latter case likely due to the presence of aromatic planar moieties in the inhibitors. To be specific, comparing with 7,4′-*O*-modified genistein derivative (**II**), compounds **49** and **50** are structurally simpler since the genistein 3-phenyl is removed and the linker is attached at the 2-position of chromone scaffold. Without the existence of the bulky 3-phenyl on the chromone scaffold, 4-carbonyl group may be more likely to form a hydrogen bond with the amino acid residues of Aβ and it is reported that this kind of interaction between inhibitors and Aβ can finally increase the inhibitory activity towards the Aβ aggregation.[36]

3. Conclusion

We have designed, synthesized and evaluated 27 new chromone-2-carboxamido-alkylbenzylamines as multifunctional agents against AD. Most of the compounds exhibited potent inhibitory activity towards AChE and displayed high selectivity for AChE over BuChE. Compounds **49** showed the most potent inhibition toward AChE with IC_{50} value of 0.07 ± 0.01 μM. The kinetic analysis suggested that **49** showed mixed-type inhibition, and could bind to both CAS and PAS of AChE, which was consistent with the molecular modeling study. Importantly, compound **50** and **49** showed excellent self-induced Aβ aggregation inhibitory activity (63.0% and 59.2% repectively) and good Cu^{2+}-induced Aβ aggregation inhibitory activity (55.6% and 48.3% respectively). Moreover, compound **49** also possessed the prospective property of acting as a metal chelator. Taken together, compound **49** was a promising multifunctional agent for AD treatment.

4. Experimental section

4.1 Chemistry

Reagents were either purchased from common commercial sources. All solvents were purified and dried by standard procedures. All the reactions were monitored by thin-layer chromatography (TLC)

on silica gel GF254 plates from Qingdao Haiyang Chemical Co. Ltd. (China), visualized in an iodine chamber or with an UV lamp (254 nm). Column chromatography was performed using silica gel (230-400 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. (China). Melting points were recorded on YRT-3 melting-point apparatus (China) and are uncorrected. NMR spectra were recorded at room temperature in $CDCl_3$ or DMSO-d_6 solutions using a Varian INOVA 400 NMR spectrometer or Varian INOVA 600 NMR spectrometer. Chemical shifts are reported in parts per millions (ppm) relative to tetramethylsilane (TMS). Mass spectra were recorded on Agilent-6210 TOF LC-MS Spectrometer.

4.1.1 General procedure for the synthesis of secondary amines (5a-g)

Compounds **5a-g** were prepared as previously described.[20]

4.1.2 2,3,4,5-Tetrahydrobenzo[f][1,4]oxazepine (5h)

4.1.2.1 2-(((2-Hydroxyethyl)amino)methyl)phenol (7)

To a solution of salicylaldehyde (4.3 mL, 41.20 mmol) in MeOH was added ethanolamine (2.48 mL, 41.20 mmol). The mixture was stirred at room temperature for 6 h, and then $NaBH_4$ (780 mg, 20.60 mmol) was added in portions within 2 h. The mixture is stirred at room temperature for 3.5 h, then Et_3N (6.89 mL, 49.44 mmol) and Boc_2O (9.89 g, 45.32 mmol) was added. The mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. H_2O (50 mL) was added to the residue. The mixture was acidified with 3 N HCl to pH = 5 and white solid was formed and collected by filtration. After being washed by water, compound **7** was obtained and used without further purification; 94% yield; white solid; mp 108-109 °C.

4.1.2.2 2,3,4,5-Tetrahydrobenzo[f][1,4]oxazepine (5h)

To a solution of Ph_3P (4.63 g, 22.44 mmol) in THF (15 mL) at -10 °C was added a solution of DIAD (4.4 mL, 22.44 mmol) in THF (10 mL) dropwise. Then a solution of compound **7** (3 g, 11.22 mmol) in THF (20 mL) was added dropwise to the mixture. The reaction was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure. Dichloromethane (40 mL) and TFA (15

mL) were added to the residue and the mixture was stirred at room temperature for 5 h, then the solvent was evaporated under reduced pressure. H_2O was added and the mixture was acidified with 3 N HCI to pH = 2. The aqueous phase was washed with dichloromethane (30 mL × 3). The aqueous phase was then treated with sodium hydroxide until pH 12 was established. The mixture was then extracted with dichloromethane (30 mL × 3). The combined organic phases were washed with saturated aqueous NaCl (100 mL), dried over sodium sulfate, and filtered. The solvent was evaporated to dryness under reduced pressure. Compound **5h** was obtained and used without further purification; 92.0% yield; light yellow oil.

4.1.3 General procedure for the synthesis of compounds 8-13

Compounds **8-13** were prepared as previously described.[21]

4.1.4 1-(2,4,6-Trihydroxyphenyl)ethanone (15)

Phloroglucinol **14** (10 g, 79.4 mmol) and acetic anhydride (18 mL, 190.0 mmol) were dissolved in ethyl acetate (40 mL), and $BF_3 \cdot Et_2O$ (12 mL, 97.2 mmol) was added dropwise. The reaction mixture was heated at 50 °C for 10 h. Then, H_2O (150 mL) was added and the reaction mixture was extracted with ethyl acetate. After evaporation of the solvent the raw material was recrystallised from H_2O to give compound **15**; 64.9% yield; yellow crystal; mp 220-221 °C (lit.[37] 221 °C).

4.1.5 1-(2-Hydroxy-4,6-dimethoxyphenyl)ethanone (16)

To a mixture of 1-(2,4,6-trihydroxyphenyl)ethanone **15** (4.5 g, 24.2mmol) and anhydrous K_2CO_3 (8.29 g, 55.6 mmol) in acetone (90 mL), Me_2SO_4 (6.84 mL, 72.5 mmol) was added dropwise with stirring. The resulting mixture was refluxed for 5 h and then filtered and concentrated under reduced pressure. The residue was purified on a silica gel chromatography using CH_2Cl_2 as eluent, obtaining compound **16**; 84.2% yield; white solid; mp 78-79 °C (lit.[38] 79-80 °C).

4.1.6 1-(4-(Dimethylamino)-2,6-dihydroxyphenyl)ethanone (17)

To a mixture of dimethylamine hydrochloride (1.8 g, mmol) and $NaHCO_3$ (1.6 g, 19.05 mmol) in H_2O (4 mL), 1-(2,4,6-trihydroxyphenyl)ethanone (**15**) (200 mg, 1.07 mmol) was added. The reaction

mixture was warmed to 70 °C and stirred for 10 h under an argon atmosphere. H_2O (10 mL) was added and the reaction mixture was extracted with ethyl acetate (5 mL × 3), the combined organic phases were washed successively with saturated aqueous Na_2CO_3 (5 mL × 3) and saturated aqueous NaCl (10 mL), dried over sodium sulfate, and filtered. The solvent was evaporated to dryness under reduced pressure. The residue was purified on a silica gel chromatography using CH_2Cl_2 as eluent, obtaining compound **17**; 67.83% yield; white solid; mp 192-193 °C. ^{1}H NMR (400MHz, $CDCl_3$) δ 5.65 (s, 2H), 3.06 (s, 6H), 2.63 (s, 3H).

4.1.7 1-(4-(Dimethylamino)-2-hydroxy-6-methoxyphenyl)ethanone (18)

To a mixture of 1-(4-(dimethylamino)-2,6-dihydroxyphenyl)ethanone **17** (810 mg, 4.15 mmol) and anhydrous K_2CO_3 (745 mg, 5.40 mmol) in acetone (16 mL), Me_2SO_4 (0.47 mL, 4.98 mmol) was added dropwise with stirring. The resulting mixture was refluxed for 5 h and then filtered and concentrated under reduced pressure. The residue was purified on a silica gel chromatography using CH_2Cl_2 as eluent, obtaining compound **18**, 97.1% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ 14.17 (s, 1H), 5.77-5.76 (d, J = 2.4 Hz, 1H), 5.62-5.61 (d, J = 2.4 Hz, 1H), 3.87 (s, 3H), 3.04 (s, 6H), 2.56 (s, 3H).

4.1.8 General procedure for the synthesis of compounds 20-22

A solution of compound **16** or **18** (6.1 mmol) in ethyl oxalate (25.7 mmol) was added to a freshly prepared solution of EtONa in EtOH (prepared by addition of 872 mg of sodium to 25 mL of absolute EtOH). The mixture was refluxed for 10 h. After which, the reaction mixture was cooled to room temperature, ethanol was evaporated and 40% aqueous HBr (18 mL) was added. The mixture was refluxed for 12 h or 48 h. After being cooled to room temperature, the mixture was treated with 10% sodium hydroxide solution until pH 12 was established. The mixture was filtered and the filtrate was acidified with 12 N HCI to pH < 2. The resulting solid was collected and was recrystallized from acetic acid to give the corresponding compounds **20-22**.

4.1.8.1 5-Hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (20)

It was synthesized from 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone (**16**) according to the general procedure (treatment with 40% aqueous HBr for 12 h); 57% yield, brown solid; mp > 240 °C. ^{1}H NMR (400MHz, DMSO-d_6) δ 12.41 (s, 1H), 6.86 (s, 1H), 6.71 (s, 1H), 6.44 (s, 1H), 3.87 (s, 3H).

4.1.8.2 5,7-Dihydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (21)

It was synthesized from 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone (**16**) according to the general procedure (treatment with 40% aqueous HBr for 48 h); 61% yield; brown solid, mp > 240 °C (Lit.[39] 305–308 °C).

4.1.8.3 7-(Dimethylamino)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (22)

It was synthesized from 1-(4-(dimethylamino)-2-hydroxy-6-methoxyphenyl)ethanone (**18**) according to the general procedure (treatment with 40% aqueous HBr for 12h); 65% yield; brown solid; mp > 240 °C. ^{1}H NMR (400MHz, DMSO-d_6) δ 12.78 (s, 1H), 6.54 (s, 1H), 6.17 (s, 1H), 6.05 (s, 1H), 3.00 (s, 6H), 1.88 (s, 1H).

4.1.9 Ethyl 5-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (23)

A solution of 2,6-dihydroxyacetophenone (**19**) (1.4 g, 9.2 mmol) in ethyl oxalate (5 mL, 36.8 mmol) was added to a freshly prepared solution of EtONa in EtOH (prepared by addition of 1.06 g of sodium to 60 mL of absolute EtOH). The mixture was refluxed for 10 h and the mixture is cooled to room temperature, and 4 ml of 12 N HCl is added dropwise. The mixture is heated at 90 °C for 30 minutes and cooled, and the solvent is removed under reduced pressure. H_2O (40 mL) was added to the residue and the mixture was extracted with EtOAc (20 mL × 3). The combined organic phases were washed with saturated aqueous NaCl (40 mL), dried over Na_2SO_4, and filtered. The solvent was evaporated to dryness under reduced pressure. The residue was purified on a silica gel chromatography using mixtures of petroleum ether/acetone (15:1) as eluent, obtaining compound **27**; 38% yield, yellow solid; mp 143-146 °C. (Lit.[40] 148 °C)

4.1.10 General procedure for the synthesis of compounds 24-44

To a mixture of the corresponding carboxylic acid derivatives **20-22** (0.21 mmol), EDCI (0.32 mmol) and HOBt (0.21 mmol) in THF were added the appropriate intermediates **11a-d**, **12a-h**, and **13a-d** (0.25 mmol). The reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated under reduced pressure. H_2O (10 mL) was added to the residue and the mixture was extracted with dichloromethane (8 mL × 3). The combined organic phases were washed successively with saturated aqueous $NaHCO_3$ (5 mL × 2) and saturated aqueous NaCl (10 mL), dried over sodium sulfate, and filtered. The solvent was evaporated to dryness under reduced pressure. The residue was purified on a silica gel chromatography using petroleum ether/acetone (12:1) as eluent to obtain the corresponding compounds **24-44**.

4.1.10.1 *N*-(3-(Benzyl(methyl)amino)propyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (24)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-benzyl-*N*-methylpropane-1,3-diamine (**11a**) according to the general procedure and gave compound **24** as a light yellow oil; 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.51 (s, 1H), 9.11 (brs, 1H), 7.31-7.18 (m, 5H), 7.02 (s, 1H), 0.94 (d, *J* = 2 Hz, 1H), 5.95 (s, 1H), 3.74 (s, 3H), 3.59-3.54 (m, 4H), 2.66-2.63 (t, *J* = 5.6 Hz, 2H), 2.36 (s, 3H), 1.84 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.34, 165.77, 162.12, 158.37, 156.56, 155.59, 137.71, 129.08, 128.38, 127.34, 110.30, 106.02, 98.24, 92.73, 62.94, 56.77, 55.60, 41.81, 40.46, 24.60. HRMS-ESI $[M+H]^+$ Calcd for $C_{22}H_{24}N_2O_5$: 397.1685, found 397.1688.

4.1.10.2 *N*-(3-(Benzyl(ethyl)amino)propyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (25)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-benzyl-*N*-ethylpropane-1,3-diamine (**11b**) according to the general procedure and gave compound **25** as a light yellow oil; 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.50 (s, 1H), 9.10 (brs, 1H), 7.32-7.30 (m, 2H), 7.21-7.15 (m, 3H), 7.01 (s, 1H), 6.36-6.35 (d, *J* = 2 Hz, 2H), 6.00 (s, 1H), 3.74 (s,

3H), 3.63 (s, 2H), 3.56-3.52, (q, *J* = 5.6 Hz, 2H), 2.70-2.65 (m, 4H), 1.84 (brs, 2H), 1.18-1.15 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.36, 165.79, 162.16, 158.44, 156.61, 155.60, 137.82, 129.20, 128.35, 127.29, 110.42, 106.11, 98.36, 92.70, 58.49, 55.61, 52.40, 47.24, 40.54, 24.41, 11.11. HRMS-ESI $[M+H]^+$ Calcd for $C_{23}H_{26}N_2O_5$ 411.1842, found 411.1851.

4.1.10.3 5-Hydroxy-7-methoxy-*N*-(3-((2-methoxybenzyl)(methyl)amino)propyl)-4-oxo-4*H*-chromene-2-carboxamide (26)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-(2-methoxybenzyl)-*N*-methylpropane-1,3-diamine (**11c**) according to the general procedure and gave compound **26** as a light yellow oil; 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.49 (s, 1H), 9.72 (s, 1H), 7.23-7.21 (d, *J* = 6.8 Hz, 1H), 7.15-7.11 (t, *J* = 7.2 Hz, 1H), 6.98 (s, 1H), 6.86-6.83 (t, *J* = 7.2 Hz, 1H), 6.67-6.65 (d, *J* = 8 Hz, 1H), 6.32-6.31 (d, *J* = 2 Hz, 1H), 5.74 (s, 1H), 3.72 (s, 3H), 3.61-3.55 (m, 5H), 2.73-2.72 (t, *J* = 4.8 Hz, 2H), 2.36 (s, 3H), 1.86 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.42, 165.74, 162.00, 158.40, 157.90, 156.61, 156.01, 131.43, 129.04, 125.36, 120.11, 110.36, 110.07, 105.98, 98.24, 92.70, 57.68, 57.28, 55.59, 54.84, 41.71, 41.09, 24.18. HRMS-ESI $[M+H]^+$ Calcd for $C_{23}H_{26}N_2O_6$ 427.1791, found 427.1796.

4.1.10.4 *N*-(3-(Ethyl(2-methoxybenzyl)amino)propyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (27)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-ethyl-*N*-(2-methoxybenzyl)propane-1,3-diamine (**11d**) according to the general procedure and gave compound **27** as a light yellow oil; 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.49 (s, 1H), 9.72 (brs, 1H), 7.26 (s, 1H), 7.13-7.10 (t, *J* = 7.2 Hz, 1H), 7.00 (s, 1H), 6.85-6.81 (t, *J* = 7.6 Hz, 1H), 6.66-6.64 (d, *J* = 7.6 Hz), 6.33 (s, 1H), 5.64 (br, 1H), 3.71 (s, 3H), 3.63-3.56 (m,7H), 2.74-2.69 (m, 4H), 1.91 (brs, 2H), 1.14 (brs, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.40, 165.71, 161.95, 158.38, 157.89, 156.57, 155.94, 131.52, 128.94, 125.42, 120.08, 110.32, 110.09, 106.00, 98.33, 92.56, 55.54,

54.80, 53.10, 47.33, 41.24, 23.81, 10.77. HRMS-ESI $[M+H]^+$ Calcd for $C_{24}H_{28}N_2O_6$ 441.1947, found 441.1943.

4.1.10.5 *N*-(4-(Benzyl(methyl)amino)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (28)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-benzyl-*N*-methylbutane-1,4-diamine (**12a**) according to the general procedure and gave compound **28** as a light yellow oil; 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (s, 1H), 7.89 (brs, 1H), 7.38-7.27 (m, 5H), 7.00 (s, 1H), 6.36-6.33 (m, 2H), 3.79 (s, 1H), 3.62 (s, 2H), 3.50-3.49 (m, 2H), 2.55-2.52 (t, *J* = 6 Hz, 2H), 2.27 (s, 3H), 1.74-1.72 (t, *J* = 2.8 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.24, 165.96, 162.03, 158.72, 156.73, 155.39, 136.88, 129.26, 128.40, 127.53, 110.49, 106.08, 98.57, 92.74, 61.63, 56.22, 55.76, 41.80, 39.51, 26.51, 24.18. HRMS-ESI $[M+H]^+$ Calcd for $C_{24}H_{28}N_2O_5$ 425.1998, found 425.1990.

4.1.10.6 *N*-(4-(Benzyl(ethyl)amino)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (29)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-benzyl-*N*-ethylbutane-1,4-diamine (**12b**) according to the general procedure and gave compound **29** as a light yellow oil; 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H), 7.44 (brs, 1H), 7.37 -7.23 (m, 5H), 7.01 (s, 1H), 6.39-6.37 (m, 2H), 3.83 (s, 1H), 3.64 (s, 2H), 3.48-3.43 (q, *J* = 6 Hz, 2H), 2.62-2.52 (m, 4H), 1.71-1.62 (m, 4H), 1.10-1.06 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.33, 166.08, 162.30, 158.71, 156.80, 155.28, 129.10, 128.33, 137.23, 110.76, 106.25, 98.64, 92.86, 57.76, 55.87, 52.28, 47.21, 39.69, 26.92, 24.34, 10.95. HRMS-ESI $[M+H]^+$ Calcd for $C_{25}H_{30}N_2O_5$ 439.2155, found 439.2148.

4.1.10.7 5-Hydroxy-7-methoxy-*N*-(4-((2-methoxybenzyl)(methyl)amino)butyl)-4-oxo-4*H*-chromene-2-carboxamide (30)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-(2-methoxybenzyl)-*N*-methylbutane-1,4-diamine (**12c**) according to the general procedure and gave compound **30** as a light yellow oil; 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.40 (br, 1H), 8.38 (brs, 1H), 7.31-7.29 (d, *J* = 7.2 Hz, 1H), 7.273-7.234(m, 1H), 6.96 (s, 1H), 6.94-6.90 (t, *J* = 7.6 Hz, 1H), 6.87-6.85 (d, *J* = 8.4 Hz, 1H), 6.32-6.31 (d, *J* = 2 Hz, 1H), 6.18-6.17 (d, *J* = 2 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.62 (s, 2H), 3.49-3.46 (m, 2H), 2.55-2.52 (m, 2H), 2.25 (s, 3H), 1.77-1.71 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.28, 165.91, 162.05, 158.70, 157.77, 156.74, 155.68, 131.27, 128.87, 125.04, 120.24, 110.41, 110.35, 106.09, 98.42, 92.76, 56.74, 55.74, 55.49, 55.05, 42.26, 39.43, 26.46, 24.27. HRMS-ESI $[M+H]^+$ Calcd for $C_{25}H_{30}N_2O_6$ 455.2104, found 455.2109.

4.1.10.8 *N*-(4-(Ethyl(2-methoxybenzyl)amino)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (31)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-ethyl-*N*-(2-methoxybenzyl)butane-1,4-diamine (**12d**) according to the general procedure and gave compound **31** as a light yellow oil; 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (s, 1H), 8.04 (brs, 1H), 7.43-7.41 (d, *J* = 7.2 Hz, 1H), 7.28-7.24 (m, 1H), 6.99 (s, 1H), 6.95-6.91 (t, *J* = 7.6 Hz, 1H), 6.87-6.85 (d, *J* = 8.4 Hz, 1H), 6.47 (brs, 1H), 6.37-6.36 (d, *J* = 1.6 Hz, 1H), 3.83-3.80 (m, 8H), 3.50-3.47 (m, 2H), 2.75-2.66 (m, 4H), 1.77-1.71 (m, 4H), 1.74-1.14 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.41, 166.06, 162.10, 158.87, 157.81, 156.92, 155.49, 131.30, 129.20, 120.52, 110.60, 110.48, 106.27, 98.70, 92.93, 55.89, 55.27, 52.23, 51.22, 47.47, 39.28, 26.60, 23.74, 10.27. HRMS-ESI $[M+H]^+$ Calcd for $C_{26}H_{32}N_2O_6$ 469.2260, found 469.2271.

4.1.10.9 *N*-(4-((2-(Dimethylamino)benzyl)(ethyl)amino)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (32)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-(2-(dimethylamino)benzyl)-*N*-ethylbutane-1,4-diamine (**12e**) according to the general procedure and gave compound **32** as a light yellow oil; 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.38 (s, 1H),

8.79 (brs, 1H), 7.85-7.83 (d, *J* = 7.6 Hz, 1H), 7.41-7.37 (t, *J* = 6.8 Hz, 1H), 7.28-7.24 (m, 1H), 7.20-7.16 (t, *J* = 7.6 Hz, 1H), 7.03 (brs, 1H), 7.00 (s, 1H), 6.35-6.35 (d, *J* = 2 Hz, 1H), 4.35 (s, 2H), 3.89 (s, 3H), 3.56-3.51 (q, *J* = 5.6 Hz, 2H), 3.11-3.02 (m, 4H), 2.66 (s, 6H), 2.06-2.01 (m, 2H), 1.77-1.74 (m, 2H), 1.37-1.33 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.50, 166.12, 161.59, 159.24, 157.21, 155.41, 153.81, 131.83, 130.81, 125.05, 124.44, 120.80, 110.19, 106.25, 98.94, 93.34, 55.96, 51.41, 50.68, 46.53, 45.54, 37.81, 25.60, 21.17, 8.11. HRMS-ESI $[M+H]^+$ Calcd for $C_{27}H_{35}N_3O_5$ 482.2577, found 482.2580.

4.1.10.10 *N*-(4-((4-(Dimethylamino)benzyl)(ethyl)amino)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (33)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-(4-(dimethylamino)benzyl)-*N*-ethylbutane-1,4-diamine (**12f**) according to the general procedure and gave compound **33** as a light yellow oil; 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.43 (s, 1H), 8.31 (brs, 1H), 7.27-7.25 (m, 2H), 6.99 (s, 1H), 6.69-6.64 (m, 3H), 6.35-6.35 (d, *J* = 2 Hz, 1H), 3.85 (s, 3H), 3.81 (s, 2H), 3.52-3.48 (q, *J* = 4 Hz, 2H), 2.93 (s, 6H), 2.83-2.72 (m, 4H), 1.87-7.72 (m, 4H), 1.26-1.23 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.35, 165.97, 161.79, 158.95, 156.93, 155.41, 150.34, 130.94, 119.75, 112.10, 110.32, 106.15, 98.67, 93.05, 56.36, 55.84, 51.24, 46.45, 40.19, 38.85, 29.13, 26.26, 22.79, 9.70. HRMS-ESI $[M+H]^+$ Calcd for $C_{27}H_{35}N_3O_5$ 482.2577, found 482.2584.

4.1.10.11 *N*-(4-(Ethyl(2-(prop-2-yn-1-yl)benzyl)amino)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (34)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-ethyl-*N*-(2-(prop-2-yn-1-yl)benzyl)butane-1,4-diamine (**12g**) according to the general procedure and gave compound **34** as a light yellow oil; 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.48 (s, 1H), 7.72 (brs, 1H), 7.36-7.34 (d, *J* = 6.8 Hz, 1H), 7.27-7.23 (m, 1H), 6.99 (s, 1H), 6.94-6.92 (t, *J* = 7.2 Hz, 1H), 6.87-6.85 (d, *J* = 8 Hz, 1H), 6.35 (s, 1H), 6.13 (s, 1H), 3.80 (s, 3H), 3.69 (s, 2H), 3.51-3.49

(m, 2H), 3.36 (s, 2H), 2.70-2.37 (t, *J* = 6 Hz, 2H), 2.24 (brs, 1H), 1.79-1.69 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.30, 166.00, 162.28, 158.64, 157.88, 156.71, 155.50, 131.02, 128.77, 125.70, 120.32, 110.63, 110.48, 106.16, 98.43, 92.74, 78.35, 73.49, 55.82, 55.20, 53.00, 51.54, 42.17, 39.71, 26.70, 24.68. HRMS-ESI $[M+H]^+$ Calcd for $C_{27}H_{30}N_2O_6$ 479.2104, found 479.2112.

4.1.10.12 *N*-(4-(2,3-Dihydrobenzo[f][1,4]oxazepin-4(5*H*)-yl)butyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (35)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and 4-(2,3-dihydrobenzo[f][1,4]oxazepin-4(5*H*)-yl)butan-1-amine (**12h**) according to the general procedure and gave compound **35** as a light yellow oil; 58% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H), 7.27 (brs, 1H), 7.20-7.12 (m, 2H), 7.01-6.97 (m, 3H), 6.44-6.38 (m, 2H), 4.08-4.06 (t, *J* = 4 Hz, 2H), 3.87 (s, 5H), 3.13-3.47 (q, *J* = 6 Hz, 2H), 3.13-3.11 (t, *J* = 4 Hz, 2H), 2.56-2.52 (t, *J* = 6.8 Hz, 2H), 1.73-1.62 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.27, 166.11, 162.38, 159.72, 158.69, 156.74, 155.20, 130.92, 130.55, 128.74, 123.46, 120.76, 110.77, 106.21, 98.59, 92.90, 69.48, 57.99, 57.97, 55.89, 52.89, 39.75, 26.94, 24.67. HRMS-ESI $[M+H]^+$ Calcd for $C_{25}H_{28}N_2O_6$ 453.1947, found 453.1940.

4.1.10.13 *N*-(6-(Benzyl(methyl)amino)hexyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (36)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-benzyl-*N*-methylhexane-1,6-diamine (**13a**) according to the general procedure and gave compound **36** as a light yellow oil; 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.46 (s, 1H), 7.36-7.27 (m, 4H), 7.03 (brs, 2H), 6.49-6.49 (d, *J* = 1.6 Hz, 1H), 6.39-6.38 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 3.59 (s, 2H), 3.49-3.44 (q, *J* = 6.8 Hz, 2H), 2.48-2.44 (t, *J* = 7.2 Hz, 2H), 2.27 (s, 3H), 1.66-1.60 (m, 4H), 1.39 (brs, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.23, 166.01, 162.11, 158.52, 156.67, 155.14, 137.09, 129.28, 128.24, 127.33, 110.50, 106.05, 98.53, 92.86, 61.76, 56.71, 55.81, 41.63, 39.78, 29.08, 26.65, 26.49. HRMS-ESI $[M+H]^+$ Calcd for $C_{25}H_{30}N_2O_5$ 439.2155, found 439.2162.

4.1.10.14 *N*-(6-(Benzyl(ethyl)amino)hexyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (37)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-benzyl-*N*-ethylhexane-1,6-diamine (**13b**) according to the general procedure and gave compound **37** as a light yellow oil; 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (s, 1H), 7.37-7.23 (m, 5H), 7.01 (brs 2H), 6.47-6.46 (d, *J* = 2.4 Hz, 1H), 6.38-6.37 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.63 (s, 2H), 3.47-3.42(q, *J* = 6.8 Hz 2H), 2.61-2.55 (q, *J* = 7.2 Hz, 2H), 2.51-2.47 (t, *J* = 7.2 Hz, 2H), 1.66-1.62 (m, 2H), 1.56-1.53 (m, 2H), 1.37-1.35 (m, 4H), 1.10-1.06 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.21, 166.00, 162.11, 158.48, 156.64, 155.14, 138.15, 129.01, 128.12, 127.01, 110.47, 106.03, 98.52, 92.83, 57.60, 55.79, 52.56, 47.02, 39.81, 29.10, 26.73, 26.22, 11.08. HRMS-ESI $[M+H]^+$ Calcd for $C_{26}H_{32}N_2O_5$ 453.2311, found 453.2303.

4.1.10.15 5-Hydroxy-7-methoxy-N-(6-((2-methoxybenzyl)(methyl)amino)hexyl)-4-oxo-4*H*-chromene-2-carboxamide (38)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-(2-methoxybenzyl)-*N*-methylhexane-1,6-diamine (**13c**) according to the general procedure and gave compound **38** as a light yellow oil; 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.40 (br, 1H), 7.36-7.34 (d, *J* = 7.6 Hz, 1H), 7.27-7.21 (m, 2H), 6.99 (s, 1H), 6.94-6.92 (t, *J* = 7.2 Hz, 1H), 6.88-6.86 (d, *J* = 8.4 Hz, 1H), 6.49-6.48 (d, *J* = 2 Hz, 1H), 6.35-6.34 (d, *J* = 2.0 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.62 (s, 2H), 3.48-3.43 (q, *J* = 6.4 Hz, 2H), 2.51-2.47 (t, *J* = 7.6 Hz, 2H), 2.28 (s, 3H), 1.68-1.60 (m, 4H), 1.39 (brs, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.23, 165.98, 162.10, 158.50, 157.68, 155.13, 130.93, 128.50, 125.14, 120.18, 110.49, 110.30, 106.05, 98.53, 92.83, 57.11, 55.80, 55.27, 54.98, 41.86, 39.78, 29.09, 26.73, 26.53. HRMS-ESI $[M+H]^+$ Calcd for $C_{26}H_{32}N_2O_6$ 469.2260, found 469.2255.

4.1.10.16 *N*-(6-(Ethyl(2-methoxybenzyl)amino)hexyl)-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (39)

It was synthesized from 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (**20**) and *N*-ethyl-*N*-(2-methoxybenzyl)hexane-1,6-diamine (**13d**) according to the general procedure and gave compound **39** as a light yellow oil; 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.44 (s, 1H), 7.66-7.61 (m, 2H), 7.39-7.35 (m, 1H), 7.03 (s, 1H), 7.00-6.96 (dt, *J* = 7.2 Hz, 0.8 Hz, 1H), 6.94-6.91 (d, *J* = 8.4 Hz, 1H) 6.70-6.69 (d, *J* = 2 Hz, 1H), 6.38-6.37 (d, *J* = 2.4 Hz, 1H), 4.16 (s, 2H), 3.88-3.87 (m 6H), 3.49-3.44 (q, *J* = 6.8 Hz, 2H), 3.03-2.97 (q, *J* = 7.2 Hz, 2H), 2.29-2.85 (m, 2H), 1.85 (br, 2H), 1.71-1.67 (m, 2H), 1.43-1.41 (t, *J* = 3.6 Hz, 4H), 1.38-1.34 (t, *J* = 7.2 Hz, 3H). HRMS-ESI $[M+H]^+$ Calcd for $C_{27}H_{34}N_2O_6$ 483.2417, found 483.2415.

4.1.10.17 *N*-(3-(ethyl(2-methoxybenzyl)amino)propyl)-5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (40)

It was synthesized from 5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**21**) and *N*-ethyl-*N*-(2-methoxybenzyl)propane-1,3-diamine (**11d**) according to the general procedure and gave compound **40** as a light yellow oil; 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.31 (br, 1H), 9.27 (brs, 1H), 7.29-7.20 (m, 2H), 6.92-6.83 (m, 2H), 6.78-6.76 (d, *J* = 8 Hz, 1H), 6.18 (s, 1H), 5.98 (s, 1H), 5.77 (br, 1H), 3.86 (s, 2H), 3.72 (s, 3H), 3.49 (s, 2H), 2.86-2.78 (m, 4H), 1.96 (brs, 2H), 1.16-1.13 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.52, 168.12, 161.73, 159.60, 157.76, 157.15, 154.59, 131.75, 130.13, 121.61, 120.56, 110.55, 109.80, 104.27, 100.74, 95.26, 55.18, 51.15, 47.44, 38.58, 39.15, 24.49, 9.66. HRMS-ESI $[M+H]^+$ Calcd for $C_{23}H_{26}N_2O_6$ 427.1791, found 427.1802.

4.1.10.18 *N*-(4-(Ethyl(2-methoxybenzyl)amino)butyl)-5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (41)

It was synthesized from 5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**21**) and *N*-ethyl-*N*-(2-methoxybenzyl)butane-1,4-diamine (**12d**) according to the general procedure and gave compound **41** as a light yellow oil; 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.23 (br, 1H), 8.52 (brs, 1H), 7.41-7.39 (d, *J* = 6.8 Hz, 1H), 7.31-7.27 (m, 1H), 6.90-6.83 (m, 2H), 6.77 (s, 1H), 6.48 (s,

1H), 6.13 (1s, 1H), 5.93 (br, 1H), 4.10 (s, 2H), 3.79 (s, 3H), 3.40 (brs, 2H), 2.98-2.96 (m, 4H), 1.86 (brs, 2H), 1.62 (brs, 2H), 1.29-1.25 (t, J = 6.4 Hz, 3H). HRMS-ESI $[M+H]^+$ Calcd for $C_{24}H_{28}N_2O_6$ 441.1947, found 441.1948.

4.1.10.19 *N*-(6-(Ethyl(2-methoxybenzyl)amino)hexyl)-5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (42)

It was synthesized from 5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**21**) and *N*-ethyl-*N*-(2-methoxybenzyl)hexane-1,6-diamine (**13d**) according to the general procedure and gave compound **42** as a light yellow oil; 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (br, 1H), 7.75 (brs, 1H), 7.37-7.36 (d, J = 6.8 Hz, 1H), 7.30-7.26 (m, 1H), 6.93-6.90 (t, J = 7.2 Hz, 1H), 6.86-6.83 (m, 2H), 6.32 (s, 1H), 6.07 (s, 1H), 4.36 (br, 1H), 3.89 (s, 2H), 3.79 (s, 3H), 3.33-3.31 (m, 2H), 2.82-2.77 (m, 2H), 2.69-2.64 (m, 2H), 1.59-1.47 (m, 4H), 1.26-1.17 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.51, 169.42, 161.70, 159.30, 157.84, 157.37, 154.81, 131.62, 130.04, 120.69, 110.64, 109.79, 104.00, 101.14, 95.50, 55.40, 52.50, 50.85, 47.18, 39.76, 28.94, 26.64, 26.45, 24.87, 9.90. HRMS-ESI $[M+H]^+$ Calcd for $C_{26}H_{32}N_2O_6$ 469.2260, found 469.2263.

4.1.10.20 7-(Dimethylamino)-*N*-(4-(ethyl(2-methoxybenzyl)amino)butyl)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (43)

It was synthesized from 7-(dimethylamino)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**22**) and *N*-ethyl-*N*-(2-methoxybenzyl)butane-1,4-diamine (**12d**) according to the general procedure and gave compound **43** as a light yellow oil; 51% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.42 (s, 1H), 7.82 (brs, 1H),7.44-7.43 (d, J = 7.2 Hz, 1H), 7.27-7.24 (m, 1H), 6.93-6.91 (t, J = 7.8 Hz, 1H), 6.89 (s, 1H), 6.86-6.85 (d, J = 7.8 Hz, 1H), 6.18 (s, 1H), 6.05-6.04 (d, J = 1.8 Hz, 1H), 3.83 (s, 2H), 3.81 (s, 3H), 3.01 (s, 6H), 2.76-2.72 (m, 2H), 2.69-2.67 (m, 2H), 1.77-1.67 (m, 4H), 1.18-1.16 (t, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.95, 161.16, 159.15, 157.70, 156.97, 155.37, 154.40, 131.23, 129.16, 123.41, 120.41, 110.36, 110.11, 102.61, 94.84, 89.34, 55.19, 52.04, 50.86, 47.28, 39.89, 39.10, 26.55, 23.33, 10.19. HRMS-ESI $[M+H]^+$ Calcd for $C_{26}H_{33}N_3O_5$ 468.2420, found 468.2426.

4.1.10.21 7-(Dimethylamino)-*N*-(4-((2-(dimethylamino)benzyl)(ethyl)amino)butyl)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (44)

It was synthesized from 7-(dimethylamino)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**22**) and *N*-(2-(dimethylamino)benzyl)-*N*-ethylbutane-1,4-diamine (**12e**) according to the general procedure and gave compound **44** as a light yellow oil; 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.43 (s, 1H), 7.65-7.63 (d, *J* = 7.6 Hz, 1H), 7.52 (s, 1H), 7.27-7.22 (m, 1H), 7.13-7.11 (d, *J* = 7.6 Hz, 1H), 7.09-7.06 (t, *J* = 7.2 Hz, 1H), 6.91 (s, 1H), 6.18 (s, 1H), 6.07-6.08 (d, *J* = 2Hz, 1H), 3.85 (s, 2H), 3.46-3.44 (m, 2H), 3.04 (s, 6H), 2.70-2.66 (m, 10H), 1.69-1.68 (m, 4H), 1.14-1.11 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.01, 161.31, 159.17, 157.03, 155.46, 154.36, 153.17, 131.20, 130.43, 128.11, 123.46, 119.30, 110.30, 102.72, 94.98, 89.31, 52.43, 51.92, 47.33, 45.20, 39.98, 39.34, 26.73, 23.70, 10.55. HRMS-ESI $[M+H]^+$ Calcd for $C_{27}H_{36}N_4O_4$ 481.2737, found 481.2738.

4.1.11 General procedure for the synthesis of compounds 45-50

To a solution of ethyl 5-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (**23**) (30 mg, 0.13 mmol) in EtOH (3 mL), were added the appropriate intermediates **11a-d**, **12a-h**, and **13a-d** (0.17 mmol). The mixture was refluxed for 10 h. The solvent was evaporated under reduced pressure. H_2O (10 mL) was added to the residue and the mixture was extracted with dichloromethane (8 mL × 3). The combined organic phases were washed with saturated aqueous NaCl (30 mL), dried over Na_2SO_4, and filtered. The solvent was evaporated to dryness under reduced pressure. The residue was purified on a silica gel chromatography using petroleum ether/acetone (15:1) as eluent to obtain the corresponding compounds **45-50**.

4.1.11.1 *N*-(3-(Benzyl(methyl)amino)propyl)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (45)

It was synthesized from *N*-benzyl-*N*-methylpropane-1,3-diamine (**11a**) according to the general procedure and gave compound **45** as a light yellow oil; 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (s, 1H), 9.25 (s, 1H), 7.46-7.23 (m, 6H), 7.07 (s, 1H), 6.81-6.79 (d, *J* = 8 Hz, 1H), 6.49-6.46 (d,

J = 8.4 Hz, 1H), 3.72 (s, 3H), 3.62-3.58 (q, *J* = 5.2 Hz, 2H), 2.77-2.74 (t, *J* = 5.6 Hz, 2H), 2.43 (s, 3H), 1.95-1.91 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.79, 160.70, 158.56, 155.99, 155.34, 136.04, 129.39, 128.66, 128.02, 111.94, 111.26, 110.46, 107.12, 62.67, 56.33, 41.20, 39.93, 24.43. HRMS-ESI $[M+H]^+$ Calcd for $C_{21}H_{22}N_2O_4$ 367.1580, found 367.1584.

4.1.11.2 5-Hydroxy-*N*-(3-((2-methoxybenzyl)(methyl)amino)propyl)-4-oxo-4*H*-chromene-2-carboxamide (46)

It was synthesized from *N*-(2-methoxybenzyl)-*N*-methylpropane-1,3-diamine (**11c**) according to the general procedure and gave compound **46** as a light yellow oil; 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (s, 1H), 9.82 (s, 1H), 7.35-7.30 (t, *J* = 8.4 Hz, 1H), 7.26-7.25 (m, 1H), 7.18-7.14 (m, 1H), 7.05 (s, 1H), 6.90-6.86 (t, *J* = 7.2 Hz, 1H), 6.77-6.74 (d, *J* = 8.4 Hz, 1H), 6.69-6.67 (d, *J* = 8 Hz, 1H), 6.02-6.00 (d, *J* = 8 Hz, 1H), 3.64-3.57 (m, 7H), 2.76-2.73 (t, *J* = 5.6 Hz, 2H), 2.35 (s, 3H), 1.89-1.88 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.76, 160.48, 158.28, 158.02, 156.37, 155.18, 135.84, 131.64, 129.22, 125.31, 120.04, 111.65, 111.06, 110.43, 110.12, 107.04, 57.84, 57.31, 54.84, 41.58, 41.11, 29.14, 24.20. HRMS-ESI $[M+H]^+$ Calcd for $C_{22}H_{24}N_2O_5$ 397.1685, found 397.1688.

4.1.11.3 5-Hydroxy-*N*-(4-((2-methoxybenzyl)(methyl)amino)butyl)-4-oxo-4*H*-chromene-2-carboxamide (47)

It was synthesized from *N*-(2-methoxybenzyl)-*N*-methylbutane-1,4-diamine (**12c**) according to the general procedure and gave compound **47** as a light yellow oil; 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.28 (brs, 1H), 8.74(brs, 1H), 7.47-7.43 (t, *J* = 8.4 Hz, 1H), 7.37-7.28 (m, 2H), 7.04 (s, 1H), 6.98-6.94 (t, *J* = 7.6 Hz, 1H), 6.90-6.88 (d, *J* = 8 Hz, 1H), 6.79-6.77 (d, 8.4 Hz, 1H), 6.61-6.59 (d, 8.8 Hz, 1H), 3.81 (s, 3H), 3.74 (s, 2H), 3.52-3.51 (m, 2H), 2.66-2.65 (m, 2H), 2.32 (s, 3H), 1.81-1.80 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.70, 160.54, 158.72, 157.89, 156.20, 155.36, 135.97, 131.92, 131.92, 129.56, 123.28, 120.38, 111.75, 111.19, 110.53, 110.34, 107.29, 56.27, 55.12, 54.97, 41.62, 39.04, 26.00, 23.62. HRMS-ESI $[M+H]^+$ Calcd for $C_{23}H_{26}N_2O_5$ 411.1842, found 411.1836.

4.1.11.4 *N*-(3-(Ethyl(2-methoxybenzyl)amino)propyl)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (48)

It was synthesized from *N*-benzyl-*N*-methylpropane-1,3-diamine (**11a**) according to the general procedure and gave compound **48** as a light yellow oil; 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (s, 1H), 9.25 (s, 1H), 7.46-7.23 (m, 6H), 7.07 (s, 1H), 6.81-6.79 (d, *J* = 8 Hz, 1H), 6.49-6.46 (d, *J* = 8.4 Hz, 1H), 3.72 (s, 3H), 3.62-3.58 (q, *J* = 5.2 Hz, 2H), 2.77-2.74 (t, *J* = 5.6 Hz, 2H), 2.43 (s, 3H), 1.95-1.91 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.79, 160.70, 158.56, 155.99, 155.34, 136.04, 129.39, 128.66, 128.02, 111.94, 111.26, 110.46, 107.12, 62.67, 56.33, 41.20, 39.93, 24.43. HRMS-ESI $[M+H]^+$ Calcd for $C_{23}H_{26}N_2O_5$ 411.1842, found 411.1840.

4.1.11.5 *N*-(4-(Ethyl(2-methoxybenzyl)amino)butyl)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (49)

It was synthesized from *N*-ethyl-*N*-(2-methoxybenzyl)butane-1,4-diamine (**12d**) according to the general procedure and gave compound **49** as a light yellow oil; 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.28 (brs, 1H), 8.26 (s, 1H), 7.52-7.48 (t, *J* = 8.4 Hz, 1H), 7.41-7.39 (d, *J* = 7.6 Hz, 1H), 7.28-7.23 (m, 1H), 7.05 (s, 1H), 6.95-6.91 (t, *J* = 7.2 Hz, 1H), 6.86-6.84 (d, *J* = 8 Hz, 1H), 6.81-6.79 (d, *J* = 8 Hz, 1H), 6.77-6.75 (d, *J* = 8.4 Hz, 1H), 3.81 (s, 3H), 3.75 (s, 2H), 3.49-3.48 (m, 2H), 2.70-2.63 (m, 4H), 1.75 (brs, 4H), 1.13-1.09 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.71, 160.66, 158.71, 157.78, 156.07, 155.39, 136.03, 131.26, 128.94, 120.37, 111.89, 111.26, 110.53, 110.40, 107.23, 55.17, 52.21, 51.18, 47.37, 39.41, 26.55, 23.95, 10.21. HRMS-ESI $[M+H]^+$ Calcd for $C_{24}H_{28}N_2O_5$ 425.1998, found 425.1992.

4.1.11.6 *N*-(6-(ethyl(2-methoxybenzyl)amino)hexyl)-5-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (50)

It was synthesized from *N*-ethyl-*N*-(2-methoxybenzyl)hexane-1,6-diamine (**13d**) according to the general procedure and gave compound **50** as a light yellow oil; 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (s, 1H), 7.61-7.57 (t, 1H), 7.42-7.40 (d, *J* = 7.2 Hz, 1H), 7.23-7.19 (t, *J* = 8 Hz, 1H),

7.11 (s, 1H), 6.96-6.84 (m, 5H), 3.82 (s, 3H), 3.60 (s, 2H), 3.49-3.44 (q, J = 6.8 Hz, 2H), 2.57-2.45 (m, 4H), 1.67-1.63 (m, 2H), 1.56-1.51 (m, 2H), 1.38-1.37 (m, 4H), 1.07-1.05 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.62, 160.79, 158.49, 157.62, 155.65, 155.34, 136.14, 130.53, 128.24, 120.32, 112.11, 111.24, 110.62, 110.22, 107.09, 55.27, 52.85, 50.99, 47.46, 39.84, 29.08, 26.79, 26.51, 26.19, 11.12. HRMS-ESI $[M+H]^+$ Calcd for $C_{26}H_{32}N_2O_5$ 453.2311, found 453.2315.

4.2 Biological evaluation

4.2.1 Inhibition Experiments of AChE and BuChE

AChE and BuChE activities were measured by Ellman's method with slight modification.[19] Rat cortex homogenate and rat serum were used as resource of *Rat*AChE and BuChE respectively and *Ee*AChE derived from *Electrophorus electricus (Sigma-Aldrich Co.)*. The brain homogenate was preincubated for 5 min with tetraisopropyl pyrophosphoramide (*iso*-OMPA, selective inhibitor of BuChE, 4.0 mmol/L) (Sigma-Aldrich Co.) before use. For rat AChE or BuChE inhibition assays, a reaction mixture (100 μL) containing acetylthiocholine iodide (1mmol/L, 30 μL) (J&K Scientific) or butyrylthiocholine iodide (1 mmol/L, 30 μL) (TCI Shanghai Development), phosphate-buffered solution (0.1 mmol/L, pH=7.4, 40 μL), 5% homogenate or 25% serum (10 μL) and different concentrations of test compounds (20 μL) was incubated at 37°C for 15 min. Then 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, 0.2%, 30 μL) (J&K Scientific) was added and changes in absorbance were detected at 405 nm in a Varioskan Flash Multimode Reader (Thermo Scientific). For Electrophorus electricus AChE inhibition assay, *Ee*AChE (0.05 U/mL, final concentration) was used and the assay was carried out in a phosphate buffer (0.01 mmol/L, pH = 8.0). Changes in absorbance were detected at 412 nm.[41] The other procedure was the same as above. Thus, IC_{50} values were calculated as the concentration of compound that produces 50% AChE or BuChE activity inhibition. Donepezil was applied as positive drug. All samples were assayed in triplicate.

4.2.2 Kinetic Characterization of AChE Inhibition

Kinetic characterization of AChE inhibition was performed based on a reported method using purified AChE from *Electrophorus electricus* (*Ee*AChE).[42] The assay solution (100 μL) consists of 0.1 M phosphate buffer (pH 8.0), with the addition of 30 μL of 0.2% DTNB, 10 μL of 0.5 units/mL *Ee*AChE, and 20 μL of substrate (ATCh). Three different concentrations of inhibitors were added to the assay solution and pre-incubated for 15 min at 37°C with the *Ee*AChE followed by the addition of substrate in different concentrations. Kinetic characterization of the hydrolysis of ATCh catalyzed by *Ee*AChE was done spectrometrically at 412 nm. The parallel control experiments were performed without inhibitor in the assay. The plots were assessed by a weighted least square analysis that assumed the variance of v to be a constant percentage of v for the entire data set. Slopes of these reciprocal plots were then plotted against the concentration of **49** in a weighted analysis, and K_i was determined as the intercept on the negative *x*-axis.

4.2.3 Molecular Docking

The crystal structure of AChE complexed with donepezil (code ID: 1EVE) was obtained from the Protein Data Bank after eliminating the original inhibitors and water molecules. The 3D Structure of **49** was built and performed geometry optimization by molecular mechanics. After addition of Gasteiger charges, removal of hydrogen atoms, addition of their atomic charges to skeleton atoms, and the assignment of proper atomic types, the further preparation of the inhibitor was accomplished. Autotors was then used to define the rotatable bonds in the ligands. Docking studies were performed using the AUTODOCK 4.2 program. By using Autodock Tools (ADT; version 1.5.6), polar hydrogen atoms were added to amino acid residues, and Gasteiger charges were assigned to all atoms of the enzyme. The resulting enzyme structure was used as an input for the AUTOGRID program. AUTOGRID performed a pre-calculated atomic affinity grid maps for each atom type in the ligand, plus an electrostatics map and a separate desolvation map presented in the substrate molecule. All maps were calculated with 0.375 Å spacing between grid points. The center of the grid box was placed at the center of donepezil with coordinates $x = 2.023$, $y = 63.295$, $z = 67.062$. The dimensions

of the active site box were set at 50 × 50 × 50 Å. Flexible ligand docking was performed for the compounds. Each docked system was performed by 100 runs of the AUTODOCK search by the Lamarckian genetic algorithm (LGA). Other than the referred parameters above, the other parameters were accepted as default. A cluster analysis was performed on the docking results using a root mean square (RMS) tolerance of 1.0 and the lowest energy conformation of the highest populated cluster was selected for analysis. Graphic manipulations and visualizations were done by Autodock Tools or Discovery Studio 2.1 software.

4.2.4 Antioxidant Activity Assay

The antioxidant activity was determined by the oxygen radical absorbance capacity fluorescein (ORAC-FL) method.[43] 2,2′-Azobis(amidinopropane) dihydrochloride (AAPH) was purchased from Accela ChemBio Co., Ltd. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and fluorescein (FL) were purchased from TCI (Shanghai) Development. All the assays were conducted with 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. Antioxidant (20 μL) and fluorescein (120 μL, 150 nM final concentration) were placed in the wells of a black 96-well plate. The mixture was pre-incubated for 15 min at 37 °C, and then AAPH solution (60 μL, 12 mM final concentration) was added rapidly using an autosampler. The plate was immediately placed in a Varioskan Flash Multimode Reader (Thermo Scientific) and the fluorescence recorded every minute for 90 min with excitation at 485 nm and emission at 535 nm. The plate was automatically shaken prior to each reading. Trolox was used as standard (1-8 μM, final concentration). A blank (FL + AAPH) using phosphate buffer instead of antioxidant and trolox calibration were carried out in each assay. The samples were measured at different concentration (1-10 μM). All the reaction mixture was prepared in duplicate, and at least three independent assays were performed for each sample. Antioxidant curves (fluorescence versus time) were normalized to the curve of the blank in the same assay, and then the area under the fluorescence decay curve (AUC) was calculated. The net AUC of a sample was obtained by subtracting the AUC of the blank. ORAC-FL values were expressed as

Trolox equivalents by using the standard curve calculated for each sample, where the ORAC-FL value of Trolox was taken as 1, indicating the antioxidant potency of the tested compounds.

4.2.5 Metal Binding Studies

The metal binding studies were carried out in a Shimadzu UV-2450 spectrophotometer. To investigate the metal binding ability of compound, the UV absorption of the tested compound **49** in the absence or presence of $CuCl_2$, $FeSO_4$, $ZnCl_2$, and $AlCl_3$, was recorded with wavelength ranging from 200 to 500 nm after incubating for 30 min at room temperature.[19] The final concentrations of tested compound and metals were 37.5 μM and the final volume of reaction mixture was 1 mL. 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 due to complex formation. The molar ration method was performed to determine the stoichiometry of the complex compound-metal by titrating the methanol solution of tested compound with ascending of $CuCl_2$. The final concentration of tested compound was 37.5 μM, and the final concentration of Cu^{2+} ranged from 0.375 μM to 93.75 μM. The UV spectra were recorded and treated by numerical subtraction of $CuCl_2$ and tested compound at corresponding concentrations, plotted versus the mole fraction of tested compound.

4.2.6 Inhibition of self and Cu^{2+}-induced Aβ_{1-42} Aggregation

In order to investigate the self-induced Aβ_{1-42} aggregation, a Thioflavin T-based flurometric assay was performed.[44] Thioflavin T (Basic Yellow 1) was purchased from TCI (Shanghai) Development. 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were purchased from Energy Chemical. β-Amyloid$_{1-42}$ (Aβ_{1-42}), supplied as trifluoroacetate salt, was purchased from ChinaPeptides Co., Ltd. Briefly, Aβ_{1-42} was dissolved in HFIP (1 mg/mL) and incubated for 24 h at room temperature, and solvent was evaporated. Then the HFIP pretreated Aβ_{1-42} was resolubilized in dry DMSO to a final stock concentration of 200 μM and was kept frozen at -80 °C until use. Solutions of test compounds were prepared in DMSO in 2.5 mM for storage and diluted with phosphate buffer solution (pH 7.4) before use. For the self-induced assay, Aβ_{1-42} (20 μL, 25 μM, final concentration) was incubated with 20 μL

of test compounds at different concentrations ranging from 10-50 μM in 50 mM phosphate buffer solution (pH 7.4) at 37 °C for 24 h. To minimize evaporation effect the wells were sealed by a transparent heat-resistant plastic film. After incubation, 160 μL of 5 μM thioflavin T in 50 mM glycine-NaOH buffer (pH 8.5) was added. Each assay was run in triplicate. Fluorescence was measured on a Varioskan Flash Multimode Reader (Thermo Scientific) with excitation and emission wavelengths at 446 nm and 490 nm, respectively. The fluorescence intensities were compared and the percent inhibition due to the presence of the inhibitor was calculated by the following formula: 100 - ($IF_i/IF_c \times 100$) where IF_i and IF_c were the fluorescence intensities obtained for Aβ_{1-42} in the presence and in the absence of inhibitors, respectively.

For the inhibition of Cu^{2+}-induced Aβ_{1-42} aggregation assay,[45] solutions of Cu^{2+} were prepared from standards to concentration of 75 μM using the HEPES buffer (20 mM, pH 6.6, 150 mM NaCl) and the Aβ_{1-42} stock solution was diluted in HEPES buffer (20 mM, pH 6.6, 150 mM NaCl). The mixture of the peptide (20 μL, 25 μM, final concentration) and Cu^{2+} (20 μL, 25 μM, final concentration), with or without the tested compound at different concentrations (20 μL, 10-35 μM, final concentration) was incubated at 37 °C for 24 h. After incubation, 190 μL of 5 μM thioflavin T in 50 mM glycine-NaOH buffer (pH 8.5) was added. Each assay was run in triplicate. The detection method was the same as that of self-induced Aβ_{1-42} experiment.

Acknowledgments

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Supplementary Material

The ^{1}H NMR and ^{13}C NMR spectra of the target compounds are available as supplementary material.

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Table, Scheme, and Figures Legends

Table 1. AChE and BuChE inhibitory activities, and oxygen radical absorbance capacity (ORAC, trolox equivalent) of intermediates **20**-**23**, compounds **24-50** and donepezil.

Table 2. Inhibition of self-induced and Cu^{2+}-induced A$\beta_{1\text{-}42}$ aggregation by compounds **24-50** and reference compounds.

Table 1.

Compd.	R	n	NR_1R_2	$IC_{50} \pm SD^a$ (μM)			Selectivity Index[e]	ORAC[f]
				*Rat*AChE[b]	*Rat*BuChE[c]	*Ee*AChE[d]		
20	OCH_3	--	--	>200	>200	>200	---	0.87
21	OH	--	--	>200	>200	>200	---	2.01
22	$N(CH_3)_2$	--	--	>200	>200	>200	---	0.95
23	H	--	--	>200	>200	>200	---	0.78
24	OCH_3	3	**a**	15.26±0.03	>200	3.12±0.05	>13.1	0.46
25	OCH_3	3	**b**	7.94±0.32	83.55±1.27	11.2±0.45	10.5	0.66
26	OCH_3	3	**c**	4.01±0.21	170.59±3.23	9.8±0.29	42.5	0.93
27	OCH_3	3	**d**	0.88±0.02	197.21±5.28	1.85±0.01	224.1	0.54
28	OCH_3	4	**a**	7.50±0.04	134.51±4,26	5.86±0.05	17.9	0.79
29	OCH_3	4	**b**	1.81 ± 0.12	128.50±2.31	1.45±0.03	71.0	0.65
30	OCH_3	4	**c**	2.90±0.05	82.10±1.44	3.50±0.21	28.3	0.55
31	OCH_3	4	**d**	0.33±0.01	86.50±3.13	1.16±0.04	262.1	0.62
32	OCH_3	4	**e**	0.31±0.01	74.12±1.23	0.92±0.05	239.1	0.97
33	OCH_3	4	**f**	7.45±0.23	57.51±0.98	9.05±0.12	7.7	2.20
34	OCH_3	4	**g**	13.6±0.40	57.31±1.01	73±1.04	4.2	0.56
35	OCH_3	4	**h**	>200	>200	>200	---	0.68
36	OCH_3	6	**a**	3.03±0.08	50.05±0.54	0.25±0.01	16.5	1.00
37	OCH_3	6	**b**	1.70±0.04	53.51±1.45	1.38±0.45	31.5	0.65
38	OCH_3	6	**c**	1.59±0.02	43.27±1.34	0.58±0.02	27.2	1.30
39	OCH_3	6	**d**	0.23±0.01	49.85±2.52	0.56±0.01	216.7	0.76
40	OH	3	**d**	6.85±0.39	>200	49.75±1.97	>29.20	2.10
41	OH	4	**d**	1.11±0.07	141.1±2.31	36.2±0.11	127.1	2.30
42	OH	6	**d**	0.87±0.01	>200	1.40±0.02	>200	2.25
43	$N(CH_3)_2$	4	**d**	0.91 ± 0.04	109.08±2.20	4.02±0.06	119.9	1.05
44	$N(CH_3)_2$	4	**e**	1.39 ± 0.08	148.88±1.98	3.53±0.07	107.1	1.34
45	H	3	**a**	19.55±0.41	120.07±1.34	24.35±0.76	6.1	0.85
46	H	3	**c**	9.51±0.14	102.19±1.44	n.a.	10.7	1.64
47	H	4	**c**	1.37±0.02	55.00±0.89	3.29±0.46	40.1	0.81
48	H	3	**d**	0.41±0.01	93.43 ± 1.47	13.90±0.18	227.9	1.13
49	H	4	**d**	0.07±0.01	51.50±1.87	0.55±0.03	735.7	0.83
50	H	6	**d**	0.19±0.02	23.25±1.67	0.53±0.12	122.4	0.87
Donepezil				0.015±0.002	20.70±0.36	0.11±0.003	1380.0	

[a] IC_{50} values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of 3 independent experiments, each performed in triplicate (SD = standard deviation).

[b] From 5% rat cortex homogenate.

[c] BuChE from rat serum.

[d] From *Electrophorus electricus.*

[e] selectivity index = IC_{50} (*Rat*BuChE)/IC_{50} (*Rat*AChE).

[f] The mean ± SD of the 3 independent experiments. Data are expressed as µM of Trolox equivalent/µM of tested compound.

Table 2.

Comp.	% inhibition of Aβ aggregation[a]	
	self-induced[b, d]	Cu^{2+}-induce[c, d]
24	23.0±1.7	24.3±1.3
25	21.1±1.3	31.5±2.1
26	43.6±2.3	28.7±1.8
27	47.9±1.1	27.2±1.1
28	24.4±2.1	35.3±3.0
29	39.0±1.4	34.1±2.2
30	47.5±1.2	36.3±1.4
31	51.0±1.1	38.9±1.7
32	25.6±1.4	20.0±2.2
33	37.1±1.3	28.8±1.3
34	22.4±1.2	21.5±1.7
35	40.9±2.6	35.0±2.1
36	33.5±1.6	37.1±2.4
37	28.6±1.5	42.6±2.3
38	43.9±2.8	48.1±1.2
39	58.2±1.5	52.0±1.7
40	35.1±2.7	18.1±2.1
41	37.8±1.1	32.1±1.7
42	39.4±1.7	28.7±1.9
43	41.1±1.0	36.9±1.3
44	40.8±1.8	30.7±1.6
45	43.9±2.8	25.8±1.7
46	50.6±1.7	23.8±2.1
47	49.3±2.6	24.1±1.2
48	48.7 ± 2.2	35.2 ± 2.9
49	59.2 ± 1.6	48.3 ± 1.7
50	63.0 ± 1.3	55.6 ± 1.3
Curcumin	43.1 ± 1.1	58.0 ± 2.3
Donepezil	< 5.0	< 5.0

[a] For Inhibition of Aβ aggregation, the thioflavin-T fluorescence method was used.

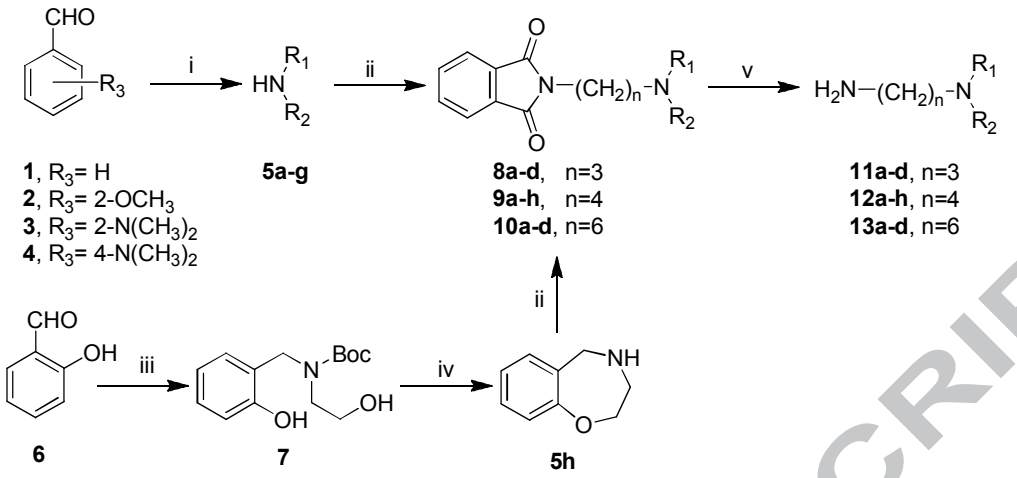
[b] Inhibition of self-induced $A\beta_{1-42}$ aggregation (25 μM) by tested inhibitors at 25 μM.

[c] Inhibition of Cu^{2+}-induced $A\beta_{1-42}$ aggregation. The concentration of tested compounds and Cu^{2+} was 25 μM.

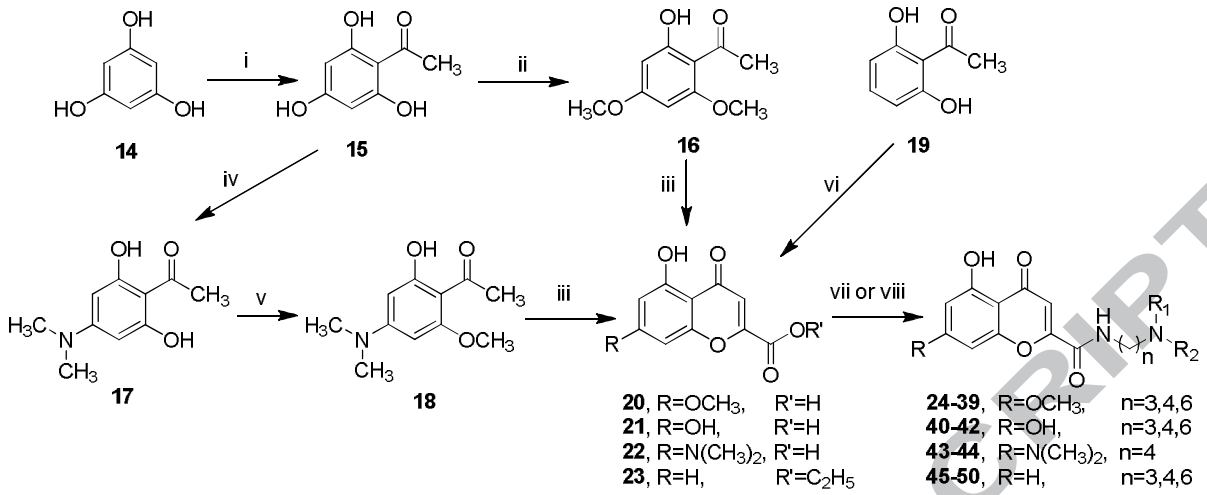
[d] Data are presented as the mean ± SD of three independent experiments.

Scheme 1. Synthesis of compounds **11-13**. *Regents and conditions:* (i) $MeNH_2$, $EtNH_2$, or propargylamine, $NaBH_4$, CH_3OH, at r.t., for 5-6 h; (ii) R_1R_2NH (**5a-h**), ω-bromoalkylphthalimides, K_2CO_3, CH_3CN, reflux for 12-15 h; (iii) $HO(CH_2)_2NH_2$, $NaBH_4$, CH_3OH, at r.t., for 5-6 h and then Et_3N, Boc_2O, at r.t., for 3 h; (iv) Ph_3P, DIAD, dry THF at r.t., for 12 h then TFA, CH_2Cl_2 at r.t., for 5 h; (v) $N_2H_4 \cdot H_2O$, EtOH, reflux for 5 h.

Scheme 2. Synthesis of chromone derivatives **24-50**. *Regents and conditions:* (i) Ac_2O, $BF_3 \cdot Et_2O$, AcOEt, 50 °C for 10 h; (ii) Me_2SO_4, K_2CO_3, acetone, reflux for 5 h; (iii) ethyl oxalate, EtONa, EtOH, reflux for 10h, then 40% aqueous HBr, relux for 12 h (for **20** and **22**) or 48 h (for **21**); (iv) $N(CH_3)_2 \cdot HCl$, $NaHCO_3$, H_2O, 70 °C for 10 h; (v) Me_2SO_4, K_2CO_3, acetone, reflux for 5 h; (vi) ethyl oxalate, EtONa, EtOH, reflux for 10h, then 12N HCl, 90 °C for 30 minutes; (vii)(for **24-44**) $R_1R_2N(CH_2)_nNH_2$, EDCI, HOBt, THF, at r.t., for 24 h. (viii)(for **45-50**) $R_1R_2N(CH_2)_nNH_2$, EtOH, reflux for 10 h.



Scheme 1.



Scheme 2.

Figure 1. Structure of Chromones (**I**), 7,4′-*O*-modified genistein derivative (**II**) and Chromone-2-carboxamido-alkylbenzylamines (**24-50**).

Figure 2. Kinetic study on the mechanism of *Ee*AChE inhibition by compound **49**. Merged Lineweaver-Burk reciprocal plots of AChE initial velocity with increasing substrate concentration (0.1-0.4 mM) in the absence or presence of **49**. Lines were derived from a weighted least-squares analysis of data points.

Figure 3. Hypothetical binding mode of compound **49** (colored by atom type) interacting with residues in the binding site of *Tc*AChE. The protein residues that participate in the main interactions with the inhibitor are labeled.

Figure 4. The UV spectrum of compound **49** (37.5 μM in methanol) alone or in the presence of $CuCl_2$, $FeSO_4$, $ZnCl_2$ or $AlCl_3$ (37.5 μM, in methanol).

Figure 5. Determination of the stoichiometry of complex-Cu^{2+} by using the molar ratio method through titrating the methanol solution of compound **49** with ascending amounts of $CuCl_2$. The final concentration of tested compound was 37.5 μM, and the final concentration of Cu^{2+} ranged from 0.375 μM to 93.75 μM.

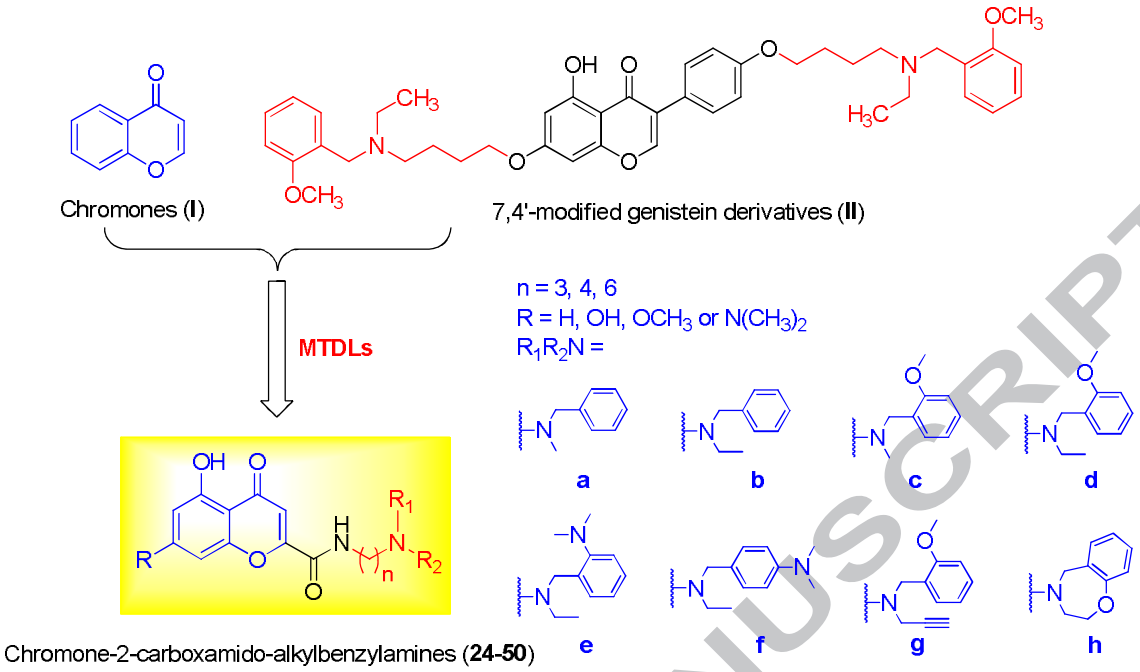


Figure 1.

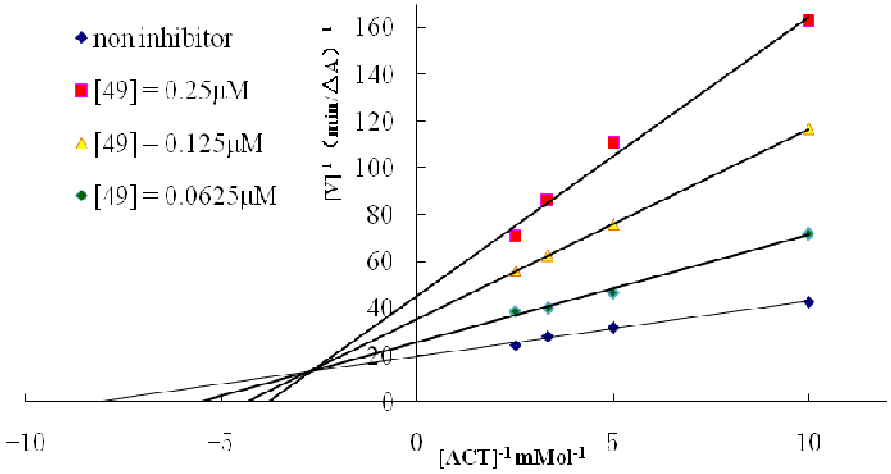


Figure 2.

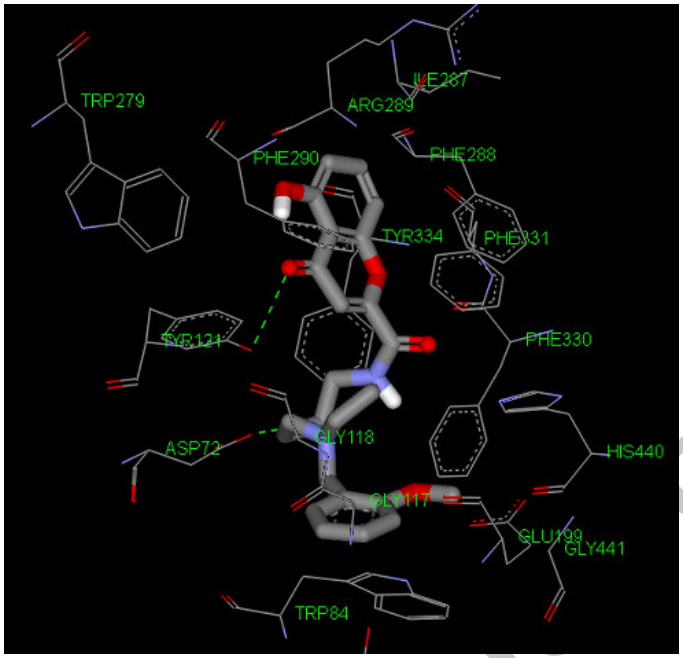


Figure 3.

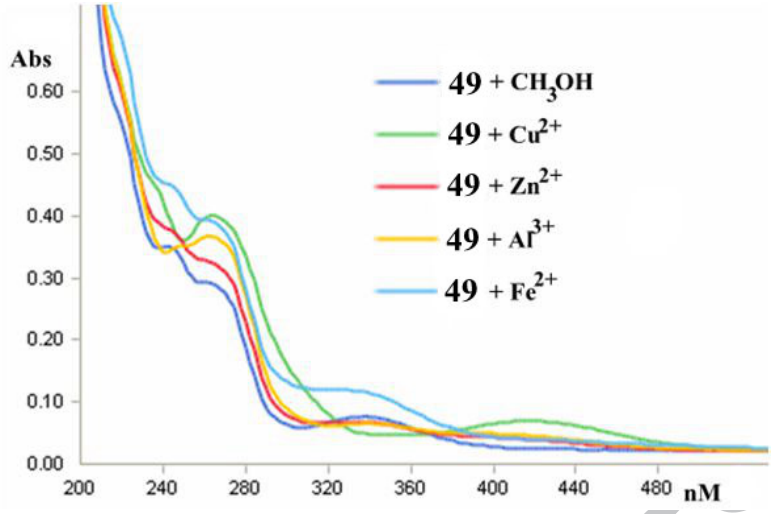


Figure 4.

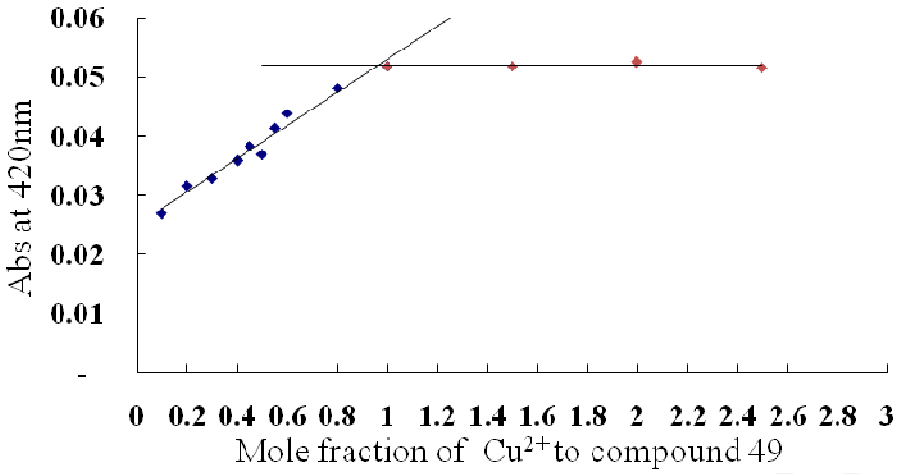
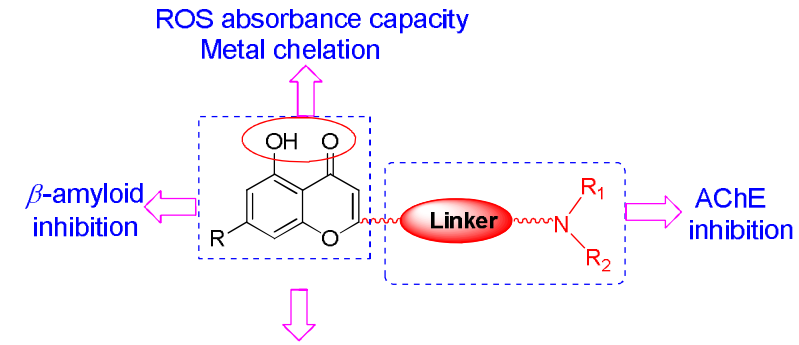
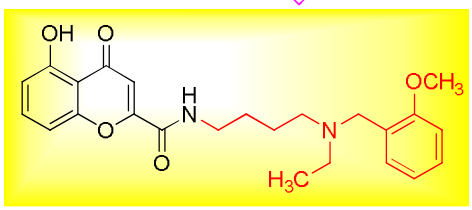


Figure 5.

Graphic Abstract





Compound **49**

Rat AChE IC_{50} : 0.07 μM
Ee AChE IC_{50} : 0.55 μM
ORAC-FL value : 0.83 *eq.* (Trolox equiv)
Self-induced $A\beta_{1\text{-}42}$ aggregation :
59.2% inhibition at 25 μM
Cu^{2+}-induced $A\beta_{1\text{-}42}$ aggregation :
48.3% inhibition at 25 μM