Arch. Pharm. Chem. Life Sci. 2017, *350*, e1700076

ARCH PHARM
Archiv der Pharmazie

Full Paper

Acetamide Derivatives of Chromen-2-ones as Potent Cholinesterase Inhibitors

Suchita Prasad[1], Bipul Kumar[2], Shiv Kumar[1], Karam Chand[1,3], Shashank S. Kamble[2], Hemant K. Gautam[2], and Sunil K. Sharma[1]*

[1] Department of Chemistry, University of Delhi, Delhi, India
[2] CSIR-Institute of Genomics and Integrative Biology, Sukhdev Vihar, Delhi, India
[3] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Alzheimer's disease (AD), a neurodegenerative disorder, is a serious medical issue worldwide with drastic social consequences. Inhibition of cholinesterase is one of the rational and effective approaches to retard the symptoms of AD and, hence, consistent efforts are being made to develop efficient anti-cholinesterase agents. In pursuit of this, a series of 19 acetamide derivatives of chromen-2-ones were synthesized and evaluated for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory potential. All the synthesized compounds exhibited significant anti-AChE and anti-BChE activity, with IC_{50} values in the range of 0.24–10.19 μM and 0.64–30.08 μM, respectively, using donepezil hydrochloride as the standard. Out of 19 compounds screened, 3 compounds, *viz*. **22**, **40**, and **43**, caused 50% inhibition of AChE at 0.24, 0.25, and 0.25 μM, respectively. A kinetic study revealed them to be mixed-type inhibitors, binding with both the CAS and PAS sites of AChE. The above-selected compounds were found to be effective inhibitors of AChE-induced and self-mediated $A\beta_{1-42}$ aggregation. ADMET predictions demonstrated that these compounds may possess suitable blood–brain barrier (BBB) permeability. Hemolytic assay results revealed that these compounds did not lyse human RBCs up to a thousand times of their IC_{50} value. MTT assays performed for the shortlisted compounds showed them to be negligibly toxic after 24 h of treatment with the SH-SY5Y neuroblastoma cells. These results provide insights for further optimization of the scaffolds for designing the next generation of compounds as lead cholinesterase inhibitors.

Keywords: Cholinesterase inhibition / Chromen-2-ones / Docking study / Hemolytic assay / MTT assay

Received: March 1, 2017; Revised: June 6, 2017; Accepted: June 13, 2017

DOI 10.1002/ardp.201700076

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

Alzheimer's disease (AD), which is delineated by overshadowed cognitive characteristics such as intelligence, memory, language, and speech, is the most prevalent form of dementia. It accounts for more than 80% of dementia cases worldwide in elderly people [1]. Currently, 38 million people are affected by AD worldwide and it is projected that by 2050, 115 million people will be under the ravages of this neurodegenerative syndrome [2]. With the advent of medical

Correspondence: Prof. Sunil K. Sharma, Department of Chemistry, University of Delhi, Delhi 110007, India.
E-mail: sksharma@chemistry.du.ac.in
Fax: +91-11-27666646

*Additional correspondence: Dr. Hemant K. Gautam,
E-mail: hemant@igib.res.in

ARCH PHARM
Archiv der Pharmazie

and social issues associated with the rapid growth of AD in both developed and developing countries, a stimulated research in this field is required in order to trigger the synthesis of therapeutic agents with higher efficacy.

The presence of numerous amyloid β-peptide (Aβ) plaques, neurofibrilliary tangles (NFT), and atrophy of the basal forebrain cholinergic neurons leading to declined acetylcholine (ACh) levels in the hippocampus and cortex are the hallmarks of cognitive impairment associated with AD [3]. Acetylcholinesterase (AChE) belongs to the family of hydrolases that augments the hydrolysis of neurotransmitter ACh. In addition to this, it has been found to accelerate the formation of β-amyloid fibril through attachment of non-amyloidogenic form of Aβ protein to the peripheral site of AChE resulting in peptide deposition in a non-catalytic manner [4–6]. A deep and narrow gorge mainly composed of Ser-His-Glu catalytic site located at the bottom and the peripheral anionic binding site (PAS) located at the entrance of the gorge, as two distinct binding sites constitutes the active site of AChE [7]. Hence, escalation of synaptic levels of ACh concomitant with the prevention of Aβ aggregation and AChE activity, fueled by compounds capable of binding to both active and peripheral sites of AChE, holds substantial promise to mitigate the neurological deficits associated with AD [8, 9]. Butyrylcholinesterase (BChE), another important enzyme co-localizing with the senile plaques and NFTs, has also been reported to play a physiological role in hydrolyzing ACh and optimizing cholinergic neurotransmission in the healthy brain [10]. Recent studies have shown that AChE activity decreases progressively in specific brain regions of AD patients, while BChE activity remains the same or is increased [11]. Thus, dual inhibition of AChE and BChE represents an important strategy in the treatment of AD [12–14].

Remarkable development has been done to identify AChE inhibitors which ameliorate the symptoms of AD and within the most successful AChE inhibitors introduced as drugs on the market, galantamine (**1**), rivastigmine (**2**), donepezil (**3**), and tacrine (**4**) acquired prominent position (Fig. 1) [15]. However, the adverse effects such as angina, vasogenic cerebral edema, hepatotoxicity, skin cancer and the potential for retinal pathology associated with different AChE inhibitors have limited their clinical efficacy [16, 17].

Coumarins, naturally occurring phytochemicals with fragrance found in many plant species and characterized by the presence of fused benzene and α-pyrone ring as a structural nucleus, have received a large amount of attention in the literature, as a consequence of their exciting and wide array of biological properties and their role as pharmacophores of considerable importance [18]. The inhibitory efficacy of naturally occurring as well as the chemically synthesized coumarin analogs toward AChE through π–π stacking interactions with PAS of AChE have been well documented in literature [19–21]. Moreover, analogs developed from the functionalization of the aromatic moiety of coumarins have been identified as inhibitors of AChE-induced Aβ aggregation [22]. Studies on different experimental models of amnesia have revealed anti-amnestic and the memory restorative properties associated with coumarin derivatives [23]. These have also been identified as protecting agents to neurons against Aβ-induced oxidative stress and free radicals [24]. Ensaculin (**5**) and AP2238 (**6**) containing coumarin motif have been introduced as anti-Alzheimer's agents [25]. Literature reports reveal that coumarin carboxamides (**7**/**8**) and *N*-benzylpyridinium salt (**9**) exhibit AChE inhibitory potency in micromolar and nanomolar range (Fig. 2) [25, 26]. Furthermore, extracts of *Angelica gigas*, *Mesua elegans*, *Scopolia carniolica*, *Citrus hystrix*, and *Psoraleae fructus* possess anti-AChE activity which has been attributed to the presence of coumarins [27]. Brühlmann and co-workers [3] have shown that the replacement of coumarin (benzopyran-2-one) with chromone (benzopyran-4-one) template leads to the reduction in AChE inhibitory activity suggesting the crucial role of the coumarin ring itself in AChE inhibition. Thus, coumarins represent an elite class of compounds that warrant further investigation as AChE inhibitors.

In accord with the literature reports and in pursuit of dual binding site AChE inhibitors with higher therapeutic index, herein a series of hybrids connecting the coumarin skeleton to the acyclic analog (*N*,*N*-dialkylaminoethylamines) of piperazine characterizing ensaculin (**5**) were designed. To elucidate the structure–activity relationship (SAR), *N*,*N*-dialkylaminopropylamines were linked with coumarin through acetamide spacer. In addition, *N*-(1-benzylpiperidin-4-yl) unit taken from donepezil (**3**) was tethered with the coumarin core (Fig. 3).

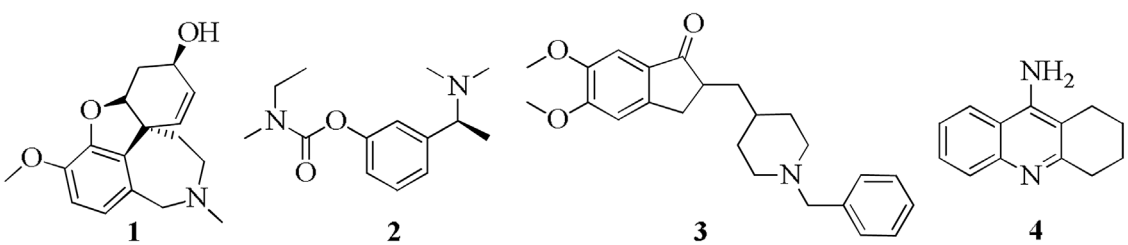


Figure 1. Acetylcholinesterase inhibitors as FDA approved Alzheimer's disease therapeutics.

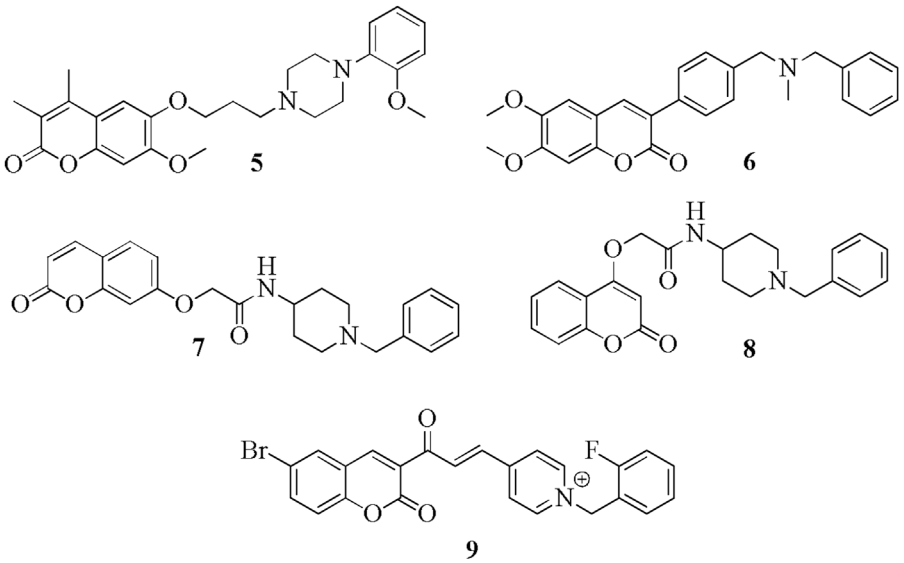


Figure 2. AChE inhibitors along with coumarin containing motifs.

All of these compounds were evaluated for their AChE and BChE inhibitory potency and the shortlisted active compounds subjected to kinetic, AChE-induced and self-mediated anti-Aβ aggregation, docking, hemolytic and cytotoxicity studies.

Results and discussion

Chemistry

A class of 19 (coumarinyloxy)acetamide derivatives (**21–35** and **40–43**) were synthesized by the reaction of (coumarinyloxy)-acetates (**17–20**) and (coumarinyloxy)acetic acids (**36–39**) with various *N*,*N*-dialkylaminoalkylamines and 1-benzylpiperidin-4-amine, respectively, using the strategy as outlined in Scheme 1.

7-Hydroxy-4-methylcoumarins (**13–15**) were synthesized from resorcinol (**10**) and ethyl acetoacetate as well as alkylated ethyl acetoacetate via Pechmann condensation using sulfuric acid as a catalyst by following the method reported earlier from our group [28]. However, for the synthesis of 6-hydroxy-4-methyl-2*H*-chromen-2-one (**16**), 4-methoxyphenol (**11**) was used as a starting material which was subjected to Pechmann reaction conditions followed by demethylation using HBr-acetic acid. The chromen-2-ones (**13–16**) were then *O*-alkylated using ethyl bromoacetate to get the desired (coumarinyloxy)acetamide precursors (**17–20**), which on further reaction with *N*,*N*-dialkylaminoal-kylamines and using *n*-butanol as solvent afforded the desired products (**21–35**) in almost quantitative yields (Scheme 1).

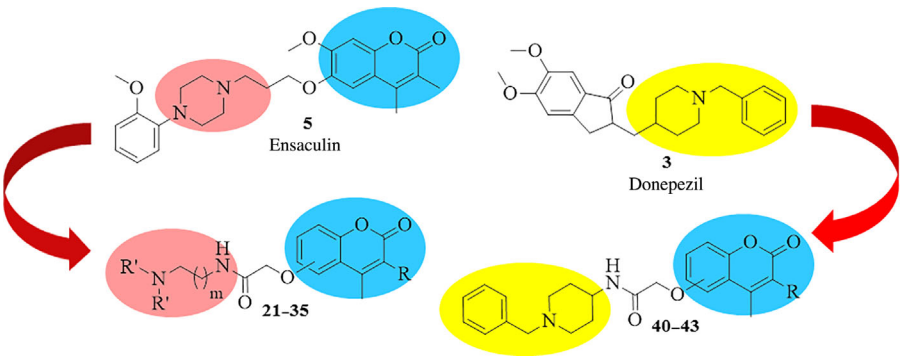
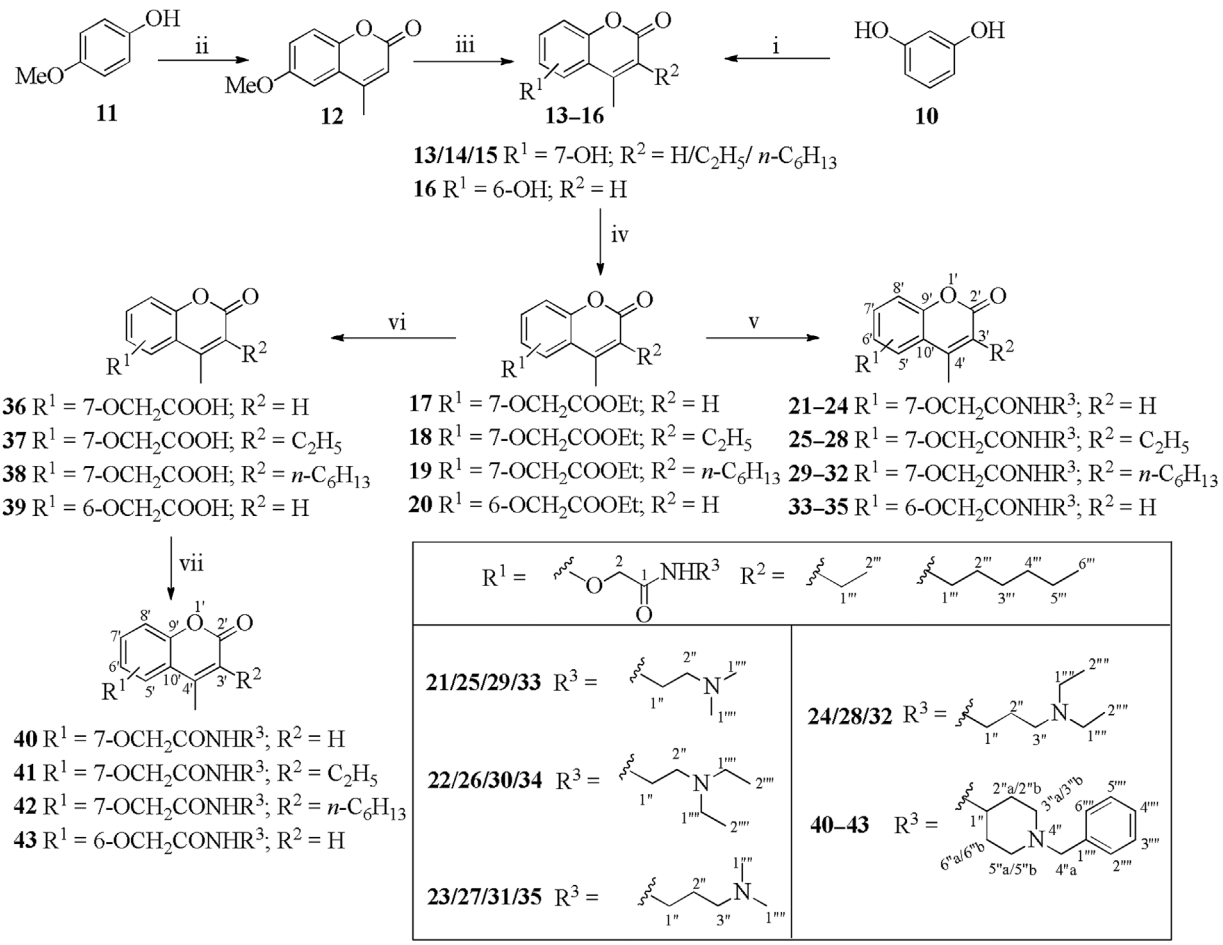


Figure 3. Designed AChE inhibitors.

ARCH PHARM
Archiv der Pharmazie



Scheme 1. Synthesis of (coumarinyloxy)acetamide derivatives. Reagents and conditions: (i) ethyl 2-alkylacetoacetates, conc. H_2SO_4, RT, 12 h; (ii) ethyl acetoacetate, H_2SO_4-C_2H_5OH (7:3), RT, 24 h; (iii) HBr-AcOH (7:3), 110°C, 24 h; (iv) ethyl bromoacetate, K_2CO_3, DMF, RT, 12 h; (v) R^3NH_2, *n*-butanol, RT, 48 h; (vi) NaOH (5%), ethanol, reflux, 2 h; (vii) EDC, HBT, 1-benzylpiperidin-4-amine, CH_3CN, RT, 24 h.

However, on treatment of **17–20** with 1-benzylpiperidin-4-amine, very little conversion was observed. Hence, in order to synthesize (coumarinyloxy)acetamides containing *N*-(1-benzylpiperidin-4-yl) unit, various esters (**17–20**) were hydrolyzed using aqueous sodium hydroxide solution to obtain the corresponding acids (**36–39**). (Coumarinyloxy)acetic acids (**36–39**) were then coupled with 1-benzylpiperidin-4-amine in the presence of *N*-(3-dimethylaminopropyl)-*N*-ethyl-carbodiimide hydrochloride (EDC) and hydroxybenzotriazole (HOBT) to yield the corresponding amides (**40–43**) in 73–82% yield (Scheme 1).

Of all the synthesized derivatives, compounds **21**, **22**, **25**, **26**, **30**, **34**, and **41–43** are reported for the first time, while compounds **24**, **27–29**, **31–33**, and **35** have been reported earlier by our group [28]. Though, compounds **23** and **40** are commercially available, however, no literature reports are available for them. All the synthesized compounds have been fully characterized from their physical and spectral data and the data compared with the literature values for known compounds. The purity of compounds **22**, **40**, and **43** was found to be greater than 95% using HPLC (high performance liquid chromatography) technique.

Biology

Cholinesterase inhibitory activity

The *in vitro* acetylcholinesterase and butyrylcholinesterase inhibitory potency of all the synthesized (coumarinyloxy)-acetamide derivatives (**21–35** and **40–43**) was determined and evaluated using *Electrophorus electricus* acetylcholinesterase (*ee*lAChE) and butyrylcholinesterase from equine serum employing the method described by Nunes et al. [29] with some modifications. Different concentrations (30–0.1 μg/mL) of the acetamide derivatives were used for AChE and BChE inhibition study and to determine IC_{50} values with donepezil

hydrochloride as a reference drug. The acetylcholinesterase and butyrylcholinesterase inhibition activity is summarized in Table 1.

From the activity results, it was observed that all the synthesized compounds exhibited potent AChE inhibition with IC_{50} values in the micromolar range. Out of 19 compounds screened, 3 compounds i.e., **22**, **40**, and **43** possessed promising anti-acetylcholinesterase activity with IC_{50} value of 0.24, 0.25, and 0.25 μM, respectively. SAR study suggested that the nature of the acetamide pendant group along with its position on the C-3 unsubstituted coumarin ring is crucial for the compound to exhibit potent AChE inhibition. On comparing the results, it was observed that the incorporation of *N*-(1-benzylpiperidin-4-yl) and *N*-(2-(diethylamino)-ethyl) functionality in the molecule enhanced the enzyme inhibition in a significant manner as observed for compounds **22**, **26**, **40**, and **43** (Table 1). However, comparison of the IC_{50} values of compounds **22**, **26**, **30**, and **40–42** led us to conclude that *n*-alkyl substitution at the C-3 position of the coumarin moiety adversely affected AChE inhibition, with pronounced decrease in the activity on increasing the alkyl chain length. The same trend was observed for compounds **21**; **25**; **29**, **23**; **27**; **31** and **24**; **28**; **32**. Furthermore, a comparison of IC_{50} values of compounds **21**, **22**, **25**, **26**, **29**, and **30** with **23**, **24**, **27**, **28**, **31**, and **32**, respectively, revealed that approximately, two to sevenfold decrease in AChE inhibition was observed on increasing the number of carbons from two to three in the diamine used for amide bond formation. The evaluation of IC_{50} values of compounds **21**; **22**, **23**; **24**, **25**; **26**, **27**; **28**, **29**; **30** and **31**; **32** gave a vivid picture that the replacement of *N*-(ω-(dimethylamino)alkyl) by *N*-(ω-(diethylamino)alkyl) group significantly decreased the IC_{50} value and consequently escalated the AChE inhibitory potential. Moreover, a comparison of the inhibitory activity of compounds **21–23** with **33–35**, respectively, demonstrated that on changing the position of the acetamide group from C-7 to C-6 on the coumarin skeleton, the AChE inhibitory potential decreased with the exception in case of compounds **40** and **43** which does not exhibit any change in inhibitory potential.

The IC_{50} values of compounds **21–35** and **40–43** against BChE were in the range of 0.64–30.08 μM and significantly higher than that against AChE. Furthermore, there was a remarkable difference in the trend of AChE and BChE inhibition e.g., the presence of lipophilic moiety was found to enhance in particular the BChE inhibition activity, i.e., acetamide derivatives **30**, **32**, and **42** that exhibit higher BChE inhibition activity bears a hexyl chain on the coumarin nucleus. On the other hand, the most active compounds **20**, **40**, and **43** for AChE inhibition lack the hexyl moiety. The results displayed that compound **42** exhibiting the highest IC_{50} value against AChE was the most potent anti-BChE agent. Notably, the three most potent anti-AChE compounds i.e., **22**, **40**, and **43** were found to exhibit selective activity against AChE, with the highest selectivity observed for *N*-(2-(diethylamino)ethyl)-2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetamide (**22**). Among the tested compounds, the SAR

Table 1. AChE and BChE inhibitory activity of compounds 21–35 and 40–43 along with donepezil as standard.

Compound	IC_{50} (μM)[a]		Selectivity for AChE[b]
	AChE inhibition	BChE inhibition	
21	3.61 ± 0.25	16.43 ± 0.68	4.55
22	**0.24 ± 0.01**	30.08 ± 0.83	125.33
23	6.28 ± 0.23	3.93 ± 0.25	0.63
24	1.73 ± 0.16	7.22 ± 0.23	4.17
25	3.91 ± 0.19	1.88 ± 0.15	0.48
26	0.45 ± 0.005	6.94 ± 0.34	15.42
27	6.62 ± 0.31	1.80 ± 0.16	0.27
28	1.87 ± 0.09	3.34 ± 0.12	1.79
29	4.12 ± 0.16	1.61 ± 0.06	0.39
30	3.87 ± 0.09	**0.75 ± 0.05**	0.19
31	6.71 ± 0.30	1.55 ± 0.20	0.23
32	5.81 ± 0.57	**0.72 ± 0.02**	0.12
33	4.98 ± 0.01	16.43 ± 0.36	3.3
34	4.14 ± 0.15	1.88 ± 0.15	0.45
35	8.82 ± 0.83	3.93 ± 0.27	0.45
40	**0.25 ± 0.009**	3.08 ± 0.19	12.32
41	2.30 ± 0.14	1.44 ± 0.09	0.63
42	10.19 ± 0.32	**0.64 ± 0.008**	0.06
43	**0.25 ± 0.025**	1.54 ± 0.11	6.16
Donepezil	0.05 ± 0.003	4.87 ± 0.24	97.4

Bold values indicate active compounds.
[a]The concentration at which the enzyme activity is inhibited by 50%. All the experiments were carried out in duplicate.
[b]Selectivity for AChE = IC_{50} (BChE)/IC_{50} (AChE).

study revealed that the acetamide derivatives incorporated with *n*-hexyl (**29**–**32** and **42**) and ethyl chain (**25**, **27**, and **41** with the exception in case of presence of *N*-(ω-(diethylamino)-alkyl) group) at the C-3 position of coumarin skeleton were found to exhibit selective activity against BChE. Moreover, C-3 unsubstituted derivatives functionalized with *N*-(3-(dimethyl-amino)propyl) group (**23** and **35**) possessed superior activity against BChE than that against AChE. On comparison of the results obtained for compounds **29**; **25**; **21**, **30**; **26**; **22**, **31**; **27**; **23**, **32**; **28**; **24** and **42**; **41**; **40**, it was inferred that the hydrophobic substituents affected BChE inhibition i.e., on decreasing the alkyl chain length from six to two to zero, the BChE inhibitory potential decreased. Furthermore, it was observed that on increasing the number of carbon atoms of *N*-(ω-(dialkylamino)alkyl) group from two ((*N*-(ω-(dialkyla-mino)ethyl)) to three ((*N*-(ω-(dialkylamino)propyl)), BChE inhibition by the compounds increased as was exemplified by the compounds **21**; **23**, **22**; **24**, **25**; **27**, **26**; **28**, **29**; **31**, **30**; **32** and **33**; **35**. The change in the position of the acetamide functionality from C-6 to C-7 resulted in an increase in the IC_{50} value against BChE in case of compounds **34**; **22** and **43**; **40**, with no change observed for compounds **33**; **21** and **35**; **23**.

Kinetic study of AChE inhibition

To gain an insight into the mechanism of AChE inhibition by this family of compounds, the two most potent compounds **22** and **43** were subjected to an enzyme kinetic study. Line-weaver–Burk double reciprocal plots were created by plotting the reciprocal of the reaction rates versus the reciprocal of substrate (acetylthiocholine) concentrations at different inhibitor concentrations (**22**: 0.3, 0.6, and 1.2 μM; **43**: 0.25, 0.5, and 1 μM). Graphical analysis of the plots (Fig. 4a and b) revealed that with an increase in inhibitor concentration, both slopes and intercepts increased, implying decreased V_{max} (maximum reaction velocity) and higher K_m (inhibition constant), respectively.

This characteristic pattern elucidated a mixed-type inhibition and therefore unveiled that compounds **22** and **43** might be possibly interacting with both the catalytic active site as well as the peripheral anionic site of AChE, which were consistent with our design strategy. Further, the estimation of inhibition constant, K_i for these compounds was achieved using secondary plot obtained by plotting the slopes against the concentrations of the inhibitor (K_i: **22** and **43**: 0.12 μM) (Fig. 4b and d).

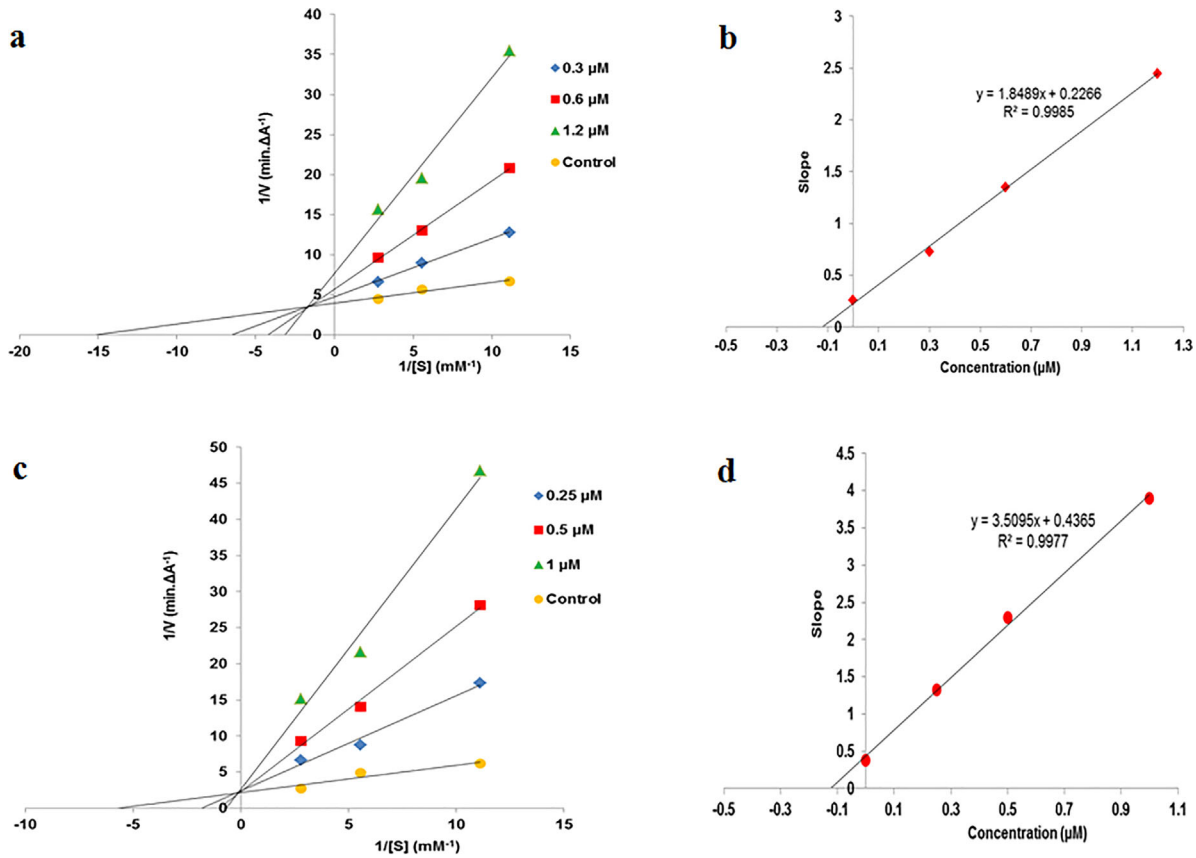


Figure 4. Lineweaver–Burk plots of compounds (a) **22** and (c) **43**. Secondary plot for calculating inhibition constant of compounds (b) **22** and (d) **43**.

Table 2. **AChE-induced and self-mediated anti-Aβ_{1-42} aggregation potential of compounds 22, 40, and 43.**

Compound	Inhibition of AChE-induced Aβ_{1-42} aggregation (%)[a)]	Inhibition of self-mediated Aβ_{1-42} aggregation (%)[a)]
22	66.67 ± 1.23	72.73 ± 1.36
40	40.00 ± 0.8	69.69 ± 0.90
43	40.00 ± 1.0	67.68 ± 1.04
Donepezil	20.82 ± 1.09	40.12 ± 0.94
Curcumin	24.90 ± 1.45	58.05 ± 1.72

[a)] All the experiments were carried out in duplicate.

Inhibition of AChE-induced and self-mediated Aβ_{1-42} aggregation

AD pathogenesis has been associated with the generation of Aβ from amyloid precursor protein due to their propensity to form fibrillar aggregates which are neurotoxic in nature. The aggregation of Aβ peptide fragments is also fostered by AChE through its non-cholinergic function. The peripheral anionic site of AChE primarily mediates this action by co-localizing with the Aβ peptide forming stable AChE-Aβ complexes and thereby promoting Aβ fibrillogenesis. Thus, the compounds having the ability to bind with PAS can inhibit AChE-induced Aβ aggregation. These results prompted us to investigate the AChE-induced and self-mediated anti-Aβ_{1-42} aggregation potential of potent AChE inhibitors **22**, **40**, and **43** using thioflavin T-based fluorometric assay. The results revealed that the selected compounds **22**, **40**, and **43** were effective inhibitors of AChE-induced and self-mediated Aβ_{1-42} aggregation, with inhibition values ranging from 40.00 to 66.67% and 67.68 to 72.73% at 100 μM inhibitor concentration, respectively (Table 2).

In particular, compound **22** was found to be the most potent inhibitor of both AChE-induced (66.67%) and self-mediated (72.73%) Aβ_{1-42} aggregation.

ADMET prediction

Subsequently, the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of most potent compounds **22**, **40**, and **43** was predicted using admetSAR web-based application such as BBB (blood–brain barrier) penetration, HIA (human intestinal absorption), Caco-2 cell permeability, and Ames test [30]. LC_{50} values (lethal concentration 50%) were predicted using http://lazar.in-silico.de/predict. *c*Log*P* (calculated partition coefficient) and acid dissociation constant (pK_a) were determined using ChemBioDraw Ultra 12.0, together with TPSA (topological polar surface area) using http://www.molinspiration.com/cgi-bin/properties. Table 3 summarizes the predicted ADMET data along with *c*Log*P*, pK_a, and TPSA of compounds **22**, **40**, and **43**.

On the basis of the predicted values for BBB penetration, it could be inferred that compounds **22**, **40**, and **43** might be able to penetrate into the CNS (central nervous system) and therefore, could be contemplated as CNS active compounds [31]. Furthermore, the calculated LC_{50} values and Ames test data revealed that the selected compounds did not exhibit either acute toxicity or mutagenic effect.

Further, designing of CNS drugs with better brain permeation, properties such as molecular weight, water solubility, lipophilicity, and membrane permeability should be taken care of. Literature reports reveal that for drugs to diffuse through the BBB, molecular weight lower than 450 Da, pK_a value between 7.5 and 10.5, *c*Log*P* value of 3.4, and TPSA of less than 76 Å^2 have been suggested [32, 33]. As shown in Table 3, the *c*Log*P*, pK_a, and TPSA values for the selected compounds are in the range of other known CNS drugs. In addition, the molecular weight of compounds **22**, **40**, and **43** are 332, 406, and 406 Da, respectively, which is well below the

Table 3. **Predicted ADMET properties, *c*Log*P*, pK_a, and TPSA of compounds 22, 40, and 43 along with the standard used.**

Compound	BBB penetration		HIA[a)] (%)	Caco-2 permeability[b)]	Ames toxicity[c)]	Acute toxicity LC_{50}[d)] (μM)	*c*Log*P*	pK_a	TPSA (Å^2)
	%	CNS activity							
22	83	+	90	+	−	507.0	2.71	8.95	71.78
40	91	+	96	+	−	105.0	2.85	8.78	71.78
43	90	+	95	+	−	94.6	2.85	8.78	71.78
Donepezil	99	+	99	+	−	62.1	4.59	9.28	38.78

[a)] Human intestinal absorption. [b)] The value of more than 50 was considered as +. [c)] Mutagenic potential of compounds. [d)] EPA v4b Fathead Minnow Acute Toxicity.

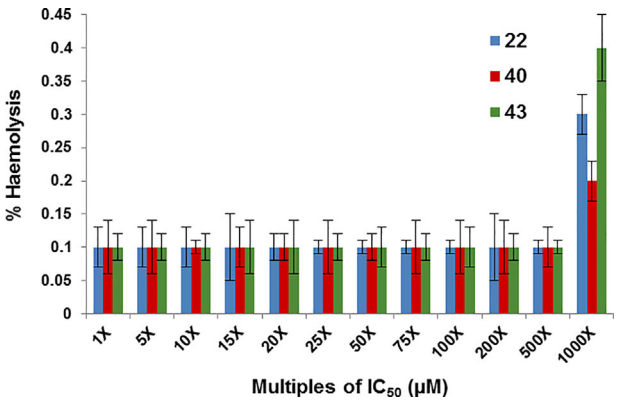


Figure 5. Hemolytic assay of compounds **22**, **40**, and **43** against human erythrocytes.

cut-off for BBB-penetrable molecules [32, 33]. Finally, they contain a small number of hydrogen bond donors and acceptors and rotatable bonds, another commonality among CNS drugs. Together, these properties suggest that the potent compounds **22**, **40**, and **43** identified from AChE inhibition assay might have suitable BBB permeability.

Hemolytic study

In order to shed light on toxicity, the potent compounds **22**, **40**, and **43** were further investigated for the viability of human erythrocytes by hemolytic assay [34–36] at various multiples of their IC_{50} values with 1× being 0.25 μM. From the activity results, it was inferred that these compounds were non-hemolytic with minimal toxicity of 0.4% even up to concentration of 1000×, i.e., 250 μM (Fig. 5).

Cytotoxicity study

In order to develop efficient therapeutics, the cytotoxicity of the compounds is one of the major areas of concern that needs to be addressed. Therefore, in order to examine the *in vitro* cytotoxicity of the active anti-AChE compounds **22**, **40**, and **43**, SH-SY5Y neuroblastoma cell line was treated with varying concentrations of test compounds and the viability was assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)-based colorimetric assay after 24 and 48 h [37].

Figure 6 unveils the cytotoxicity of compounds **22**, **40**, and **43** and from the results it could be observed that the compounds did not show significant effect on cell viability at the tested concentrations after 24 h (Fig. 6a). Furthermore, compounds **22** and **40** caused negligible cell death after 48 h at all the concentrations tested (Fig. 6b). However, the cell viability reduced when the cells were incubated with 25 and 50 μM concentrations of compound **43** after 48 h.

Molecular docking study

It is well known that the AChE enzyme contains a deep pocket, at the bottom of which is located the catalytic site, known as the "catalytic triad" of AChE formed by three amino acids, Ser200, His440, and Glu327 (sequence numbering of *Torpedo californica* AChE, *Tc*AChE) [38]. Apart from this, it possesses two more active sites, catalytic anionic site (CAS) and peripheral anionic site (PAS) [38]. Three important amino acids, Phe330, Trp84, and Glu199, form CAS which is situated at the lower part of the gorge, while PAS which is located at the entrance of the gorge is formed by Trp279, Asp72, and Tyr70 [38]. It is evidenced from previous studies that in this deep pocket of the AChE enzyme, acetylcholine (substrate) can slip inside with water molecule and get hydrolyzed into acetic acid and choline, which after hydrolysis no longer

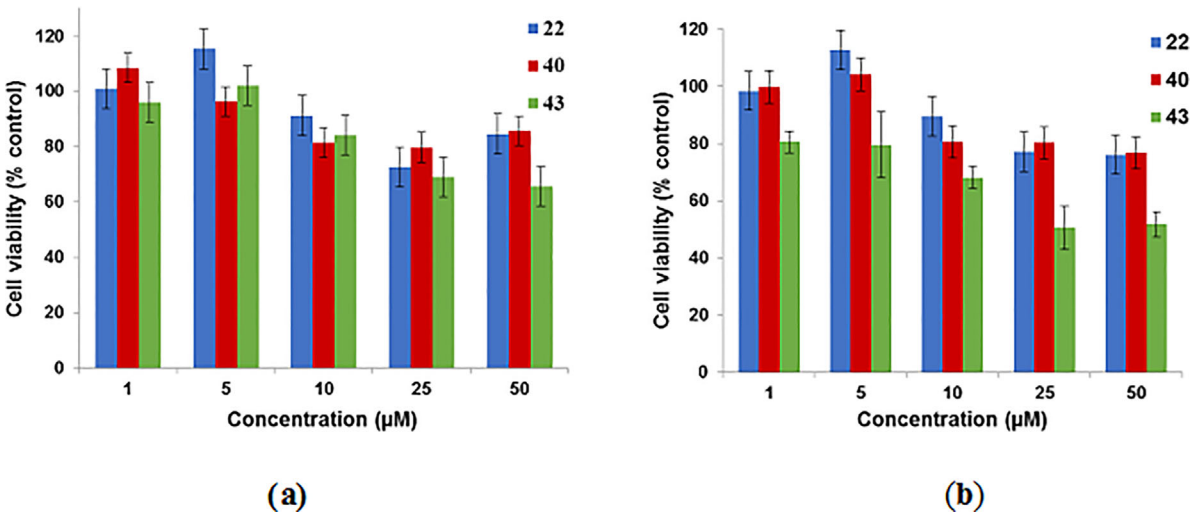


Figure 6. Cytotoxicity evaluation of compounds **22**, **40**, and **43** on viability of SH-SY5Y cell line by MTT assay after (a) 24 h and (b) 48 h.

remains a neurotransmitter and contributes toward AD progression. The compounds which have the capability to interact at both CAS and PAS sites of the enzyme can fill up this deep pocket and have proved to be good AChE inhibitors. Therefore, the strategy followed herein for the design of new potential AChE inhibitors was the coupling of two main moieties – chromen-2-one and benzylpiperidine or dialkylaminoalkane unit through an acetamide spacer in such a way that one moiety can interact with CAS site while at the same time other moiety can interact with the PAS site to produce maximum AChE inhibition. The length of the acetamide spacer between these selected moieties of designed inhibitors was decided by the docking study using program GOLD, v. 5.1 [39]. The crystal structure of *Tc*AChE complexed with an inhibitor (donepezil) was taken from RCSB Protein Data Bank (PDB, entry 1ODC) [40]. The docking calculations were performed using the ASP scoring function, since this function has previously proved to give the best docking predictions for AChE inhibitors [29, 41]. For each compound, one conformation was selected to give a good compromise between the best consensus score and that with the closest alignment to the original ligand.

In order to get insight into the binding mode of the inhibitor, molecular docking study of the best AChE inhibitors (compounds **22**, **40**, and **43**) obtained through *in vitro* screening is presented in Fig. 7. The docking studies revealed favorable interactions for the new inhibitors (Fig. 7), with many similarities in their binding conformations to that of original ligand (donepezil) and it was observed that these compounds were settling inside the pocket of AChE in a similar fashion to that of donepezil drug. Figure 7 unraveled that the 1-benzylpiperidine ring and *N*,*N*-diethylaminoethyl chain of compounds **40**, **43**, and **22**, respectively, get inserted in the pockets of the enzyme, binding to the catalytic active site (CAS) by π–π stacking with the aromatic rings of Trp84 and Phe330 of protein and overlapping almost perfectly with

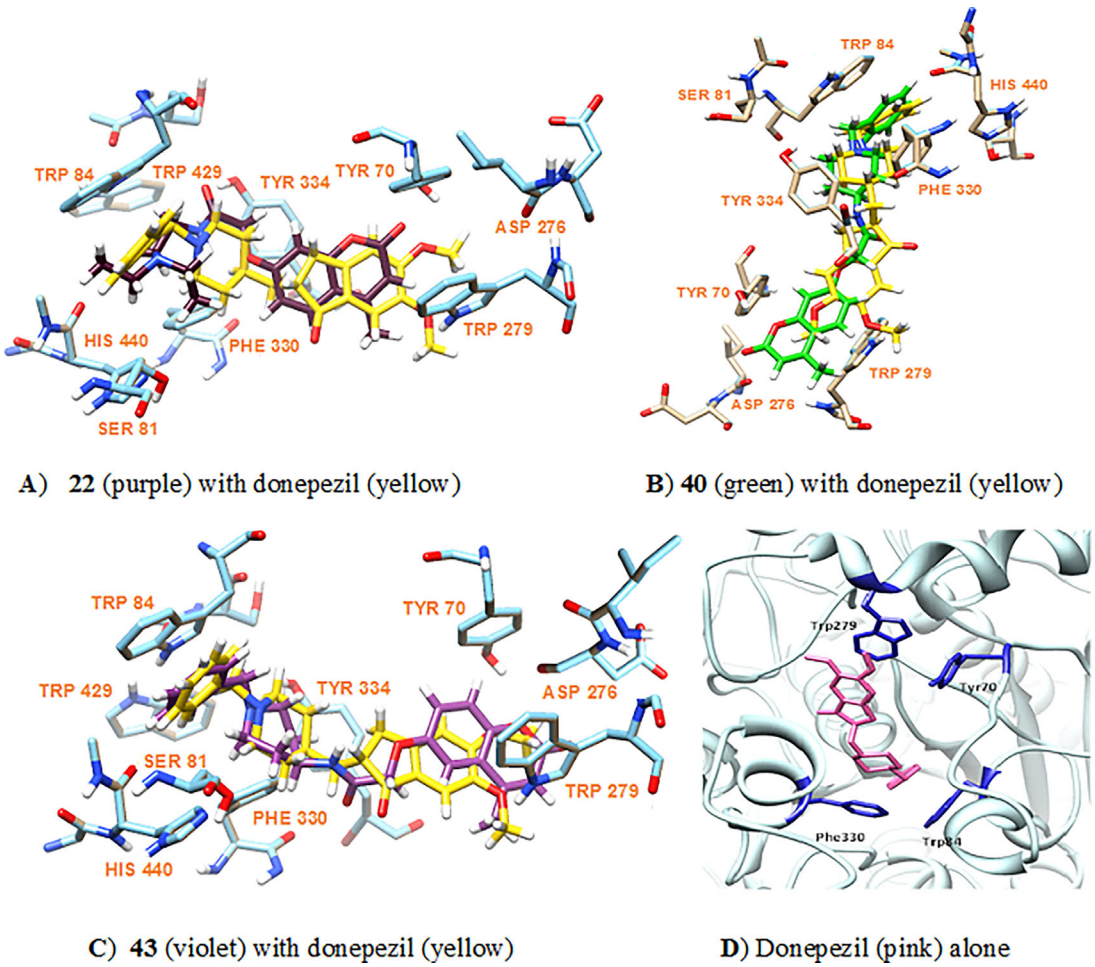


Figure 7. 3D representation of compounds **22**, **40**, **43** and donepezil docked in the active site of *Tc*AChE.

the 1-benzylpiperidine ring of the donepezil. Also, the chromenone ring of the designed inhibitor was placed at the entrance of the gorge, being able to fit in the void created by the protein rings Trp279 and Tyr70 of peripheral active site (PAS), thus allowing to maintain strong interactions with the enzyme. The literature reports reveal that π–cation interaction of Phe330 with the ligand at the bottom of the active site is responsible for ligand recognition and trafficking [26], similarly the benzylpiperidine part of compounds **40** and **43** also exhibited such interaction with the Phe330 residue. Overall, the docking into AChE of the designed compounds suggests their ability to interact with both the CAS and PAS sites, therefore being able to act as dual-binding site AChE inhibitors, which is undoubtedly encouraging to pursuit their synthesis and evaluation as potential multifunctional anti-AD drugs.

Conclusions

In summary, a total of 19 (coumarinyloxy)acetamides were synthesized and fully characterized by ^{1}H NMR, ^{13}C NMR, UV, FT-IR, and high resolution mass spectrometry (HRMS). All of the compounds synthesized were evaluated for their cholinesterase inhibitory potential using *Electrophorus electricus* acetylcholinesterase (*ee*AChE) and butyrylcholinesterase from equine serum. The anti-cholinesterase activity results demonstrated that all the synthesized compounds exhibited significant AChE and BChE inhibition with IC_{50} values in the micromolar range. Moreover, out of 19 compounds screened, 3 compounds i.e., **22**, **40**, and **43** led to 50% inhibition of AChE at a concentration of 0.24, 0.25, and 0.25 μM. From the SAR study, it could be concluded that the nature as well as the position of the acetamide pendant group is crucial for the compound to be a potent anti-AChE candidate. Moreover, it was worthy to note that the presence of lipophilic unit could dramatically escalate the BChE inhibition activity. Kinetic study revealed them to be a mixed-type inhibitor, binding with both CAS and PAS sites of AChE. The results revealed that the selected compounds **22**, **40**, and **43** were effective inhibitors of AChE-induced and self-mediated $A\beta_{1-42}$ aggregation. Prediction of ADMET properties suggests that these active anti-AChE compounds might have suitable BBB permeability. Further, the hemolysis results provided a vivid picture that the human RBCs were negligibly lysed by the shortlisted compounds even up to thousand times of their IC_{50} values. Investigation of the cytotoxicity profile of compounds **22**, **40**, and **43** revealed that the compounds were negligibly toxic after 24 and 48 h of treatment with the cells at all the tested concentrations with reduced cell viability on incubation of cells with 25 and 50 μM concentrations of compound **43** after 48 h. The docking study of the potent compounds **22**, **40**, and **43** unraveled that these compounds were settling inside the pocket of AChE in a similar fashion to that of donepezil drug. These results can be used for the design and synthesis of newer pharmacophores with improved anti-cholinesterase activity.

Experimental

Chemistry

General

The organic solvents were dried and distilled prior to their use. Reactions were monitored by pre-coated TLC plates (Merck silica gel $60F_{254}$); the spots were visualized by UV light and ninhydrin stain. Silica gel (100–200 mesh) was used for column chromatography. All of the chemicals and reagents were procured from Spectrochem Pvt. Ltd., India and Sigma–Aldrich Chemicals Pvt. Ltd., USA. Melting points were measured on a Buchi M-560 equipment and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Jeol ECX-400P (400 MHz, 100.5 MHz) NMR spectrometer using tetramethylsilane (TMS) as internal standard. The chemical shift values are on a δ scale and the coupling constant values (*J*) are in Hertz. The UV data were recorded on Cary 300 UV-Vis spectrophotometer, Agilent Technologies. The HRMS data were recorded on Waters (Micromass) LCT 1, Q-TOF LCMS-Agilent Technology-6530 and HPLC/MS – Agilent 6210 (Agilent Technologies). The purity of active anti-AChE compounds was determined by HPLC on Agilent 1200 series instrument using Eclipse plus C18 column. Methanol was used as the mobile phase with a flow rate of 0.5 mL/min and injection volume was 10 μL for all samples.

The NMR and HRMS spectra as well as the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*General procedure for the synthesis of (coumarinyloxy)-acetamide derivatives (**21–35**)*

To a stirred solution of (coumarinyloxy)acetate (**17–20**) (1 g) in *n*-butanol (20 mL) was added *N*,*N*-dialkylaminoalkylamine (4 equivalents) and the reaction was stirred at room temperature for 48 h. The progress of reaction was monitored by TLC and on completion of the reaction, the solvent was removed *in vacuo*. The solid so obtained was washed with hexane (3 × 20 mL) and crystallized from ethanol to give (coumarinyloxy)acetamide (**21–35**) as white/off-white solid.

*N-(2-(Dimethylamino)ethyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**21**)*

The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)-oxy)acetate (**17**) (1 g, 3.81 mmol) with N^1,N^1-dimethylethane-1,2-diamine (1.34 g, 15.24 mmol) gave the title compound **21** as a white solid (0.95 g, 82%) by following the general procedure; m.p.: 140.5–141.3°C; R_f: 0.40 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 318 nm; IR ($CHCl_3$) ν_{max}: 2924.78, 2855.90, 1721.34 (C=O), 1675.40 (NH*CO*-), 1617.32, 1544.77, 1390.78, 1276.98, 1152.58, 1075.82, 849.02 cm^{-1}; ^{1}H NMR (400 MHz,

$CDCl_3$): δ 2.21 (s, 6H, H-1‴′), 2.39 (s, 3H, C-4′ CH_3), 2.43 (t, 2H, $J = 6.56$ Hz, H-2″), 3.38–3.43 (m, 2H, H-1″), 4.53 (s, 2H, H-2), 6.16 (s, 1H, H-3′), 6.84 (d, 1H, $J = 2.20$ Hz, H-8′), 6.89 (dd, 1H, $J = 2.96$ and 8.80 Hz, H-6′), 7.02 (*brs*, 1H, NH), 7.53 (d, 1H, $J = 8.80$ Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.82 (C-4′ CH_3), 36.66 (C-1″), 45.34 (C-1‴′), 57.85 (C-2″), 67.71 (C-2), 102.78 (C-8′), 111.96 (C-6′), 112.91 (C-3′), 114.84 (C-10′), 126.06 (C-5′), 152.38 (C-4′), 155.21 and 160.14 (C-7′ and C-9′), 161.02 and 167.21 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{16}H_{20}N_2O_4$: 305.1423. Found: 305.1477.

*N-(2-(Diethylamino)ethyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**22**)*

The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)-oxy)acetate (**17**) (1 g, 3.81 mmol) with N^1,N^1-diethylethane-1,2-diamine (1.77 g, 15.24 mmol) gave the title compound **22** as a white solid (1.08 g, 85%) by following the general procedure; m.p.: 119.4–119.7°C; R_f: 0.40 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 317 nm; IR ($CHCl_3$) ν_{max}: 2964.50, 2924.32, 2854.71, 1725.63 (C=O), 1665.86 (NH*CO*-), 1618.50, 1544.06, 1389.39, 1281.29, 1151.97, 1041.24, 848.30 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (t, 6H, $J = 6.60$ Hz, H-2‴′), 2.39 (s, 3H, C-4′ CH_3), 2.49 (q, 4H, $J = 6.56$ Hz, H-1‴′) 2.55 (t, 2H, $J = 5.88$ Hz, H-2″), 3.32–3.39 (m, 2H, H-1″), 4.54 (s, 2H, H-2), 6.16 (d, 1H, $J = 1.48$ Hz, H-3′), 6.84 (d, 1H, $J = 2.96$ Hz, H-8′), 6.87 (dd, 1H, $J = 2.92$ and 8.80 Hz, H-6′), 7.20 (*brs*, 1H, NH), 7.53 (d, 1H, $J = 8.80$ Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 12.26 (C-2‴′), 18.83 (C-4′ CH_3), 36.73 (C-1″), 46.94 (C-1‴′), 51.32 (C-2″), 67.67 (C-2), 102.74 (C-8′), 111.71 (C-6′), 112.90 (C-3′), 114.84 (C-10′), 126.13 (C-5′), 152.38 (C-4′), 155.25 and 160.17 (C-7′ and C-9′), 161.03 and 167.00 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{18}H_{24}N_2O_4$: 333.1736. Found: 333.1793.

*N-(3-(Dimethylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**23**)*

The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)-oxy)acetate (**17**) (1 g, 3.81 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1.55 g, 15.24 mmol) gave the title compound **23** as a white solid (1.06 g, 87%) by following the general procedure; m.p.: 132.8–133.3°C; R_f: 0.40 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 318 nm; IR ($CHCl_3$) ν_{max}: 2924.78, 2855.96, 1723.88 (C=O), 1670.61 (NH*CO*-), 1616.78, 1544.99, 1389.62, 1272.86, 1152.15, 1075.74, 848.87 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.66–1.74 (m, 2H, H-2″), 2.20 (s, 6H, H-1‴′), 2.39–2.44 (m, 5H, C-4′ CH_3 and H-3″), 3.42–3.48 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.17 (d, 1H, $J = 1.12$ Hz, H-3′), 6.83 (d, 1H, $J = 2.20$ Hz, H-8′), 6.87 (dd, 1H, $J = 2.20$ and 8.80 Hz, H-6′), 7.54 (d, 1H, $J = 8.80$ Hz, H-5′), 8.34 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.84 (C-4′ CH_3), 25.55 (C-2″), 39.56 (C-1″), 45.46 (C-1‴′), 58.97 (C-3″), 67.69 (C-2), 102.70 (C-8′), 111.61 (C-6′), 112.87 (C-3′), 114.77 (C-10′), 126.14 (C-5′), 152.42 (C-4′), 155.25 and 160.37 (C-7′ and C-9′), 161.08 and 167.16 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{17}H_{22}N_2O_4$: 319.1580. Found: 319.1666.

*N-(3-(Diethylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**24**) [28]*

The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)-oxy)acetate (**17**) (1 g, 3.81 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.98 g, 15.24 mmol) gave the title compound **24** as a white solid (1.08 g, 82%) by following the general procedure; m.p.: 106.2–107.7°C; R_f: 0.40 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 292 and 318 nm; IR (KBr) ν_{max}: 3255.61 (N-H str), 2956.53, 1730.14 (C=O), 1679.02 (NH*CO*-), 1617.18, 1527.43, 1276.70, 1158.08, 1073.82, 846.35, 735.40, 589.81 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (t, 6H, $J = 7.32$ Hz, H-2‴′), 1.65–1.73 (m, 2H, H-2″), 2.40 (d, 3H, $J = 1.48$ Hz, C-4′ CH_3), 2.48–2.56 (m, 6H, H-1‴′ and H-3″), 3.43–3.49 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.16 (d, 1H, $J = 1.48$ Hz, H-3′), 6.83 (d, 1H, $J = 2.20$ Hz, H-8′), 6.87 (dd, 1H, $J = 2.92$ and 8.80 Hz, H-6′), 7.54 (d, 1H, $J = 8.76$ Hz, H-5′), 8.47 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.34 (C-2‴′), 18.64 (C-4′ CH_3), 25.11 (C-2″), 39.66 (C-1″), 46.92 (C-1‴′), 52.21 (C-3″), 67.58 (C-2), 102.58 (C-8′), 111.44 (C-6′), 112.66 (C-3′), 114.57 (C-10′), 125.94 (C-5′), 152.23 (C-4′), 155.04 and 160.16 (C-7′ and C-9′), 160.93 and 166.89 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{19}H_{26}N_2O_4$: 369.1790. Found: 369.1789.

*N-(2-(Dimethylamino)ethyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**25**)*

The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**18**) (1 g, 3.44 mmol) with N^1,N^1-dimethylethane-1,2-diamine (1.21 g, 13.76 mmol) gave the title compound **25** as a white solid (0.92 g, 80%) by following the general procedure; m.p.: 153.8–154.0°C; R_f: 0.41 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 290 and 318 nm; IR (KBr) ν_{max}: 3373.03 (N-H str), 2961.77, 1709.96 (C=O), 1660.68 (NH*CO*-), 1621.17, 1554.47, 1160.21, 1096.14, 870.22, 778.80, 598.65 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, 3H, $J = 7.68$ Hz, H-2‴′), 2.23 (s, 6H, H-1‴′), 2.37 (s, 3H, C-4′ CH_3), 2.45 (t, 2H, $J = 5.88$ Hz, H-2″), 2.66 (q, 2H, $J = 7.32$ Hz, H-1‴′), 3.39–3.44 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.82 (d, 1H, $J = 2.16$ Hz, H-8′), 6.89 (dd, 1H, $J = 2.92$ and 8.80 Hz, H-6′), 7.07 (*brs*, 1H, NH), 7.52 (d, 1H, $J = 9.52$ Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.22 (C-2‴′), 14.71 (C-4′ CH_3), 21.00 (C-1‴′), 36.61 (C-1″), 45.33 (C-1‴′), 57.89 (C-2″), 67.67 (C-2), 102.40 (C-8′), 111.75 (C-6′), 115.58 (C-10′), 125.87 (C-3′), 125.92 (C-5′), 145.60 (C-4′), 153.59 and 159.08 (C-7′ and C-9′), 161.79 and 167.44 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{18}H_{24}N_2O_4$: 333.1814. Found: 333.1798.

*N-(2-(Diethylamino)ethyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**26**)*

The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**18**) (1 g, 3.44 mmol) with N^1,N^1-diethylethane-1,2-diamine (1.59 g, 13.76 mmol) gave the title compound **26** as a white solid (1.01 g, 81%) by following the general procedure; m.p.: 131.7–132.0°C; R_f: 0.41 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 291 and 318 nm; IR (KBr) ν_{max}: 3373.01 (N-H str), 2965.50, 1713.40 (C=O), 1658.38 (NH*CO*-), 1618.54, 1544.31, 1160.26, 1096.03, 873.26, 778.52, 597.49 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98 (t, 6H, J = 7.32 Hz, H-2‴′), 1.13 (t, 3H, J = 6.96 Hz, H-2‴), 2.38 (s, 3H, C-4′ CH_3), 2.51 (q, 4H, J = 7.36 Hz, H-1‴′), 2.57 (t, 2H, J = 5.88 Hz, H-2″), 2.65 (q, 2H, J = 7.32 Hz, H-1‴), 3.34–3.40 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.81 (d, 1H, J = 2.92 Hz, H-8′), 6.85 (dd, 1H, J = 2.92 and 8.80 Hz, H-6′), 7.26 (*brs*, 1H, NH), 7.52 (d, 1H, J = 8.80 Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 12.12 (C-2‴′), 13.22 (C-2‴), 14.71 (C-4′ CH_3), 21.00 (C-1‴), 36.66 (C-1″), 46.98 (C-1‴′), 51.38 (C-2″), 67.61 (C-2), 102.34 (C-8′), 111.52 (C-6′), 115.56 (C-10′), 125.90 (C-3′), 125.92 (C-5′), 145.59 (C-4′), 153.62 and 159.10 (C-7′ and C-9′), 161.80 and 167.24 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{20}H_{28}N_2O_4$: 361.2127. Found: 361.2131.

*N-(3-(Dimethylamino)propyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**27**) [28]*

The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**18**) (1 g, 3.44 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1.40 g, 13.76 mmol) gave the title compound **27** as a white solid (0.95 g, 80%) by following the general procedure; m.p.: 136.6–137.5°C; R_f: 0.42 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 290 and 317 nm; IR (KBr) ν_{max}: 3375.80 (N-H str), 2966.80, 1713.47 (C=O), 1666.42 (NH*CO*-), 1618.60, 1549.02, 1159.70, 1095.12, 871.43, 777.79, 599.98 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12 (t, 3H, J = 7.32 Hz, H-2‴), 1.65–1.71 (m, 2H, H-2″), 2.18 (s, 6H, H-1‴′), 2.38 (s, 3H, C-4′ CH_3), 2.40 (t, 2H, J = 5.84 Hz, H-3″), 2.65 (q, 2H, J = 7.32 Hz, H-1‴), 3.41–3.47 (m, 2H, H-1″), 4.50 (s, 2H, H-2), 6.79 (d, 1H, J = 2.92 Hz, H-8′), 6.83 (dd, 1H, J = 2.92 and 8.80 Hz, H-6′), 7.53 (d, 1H, J = 8.80 Hz, H-5′), 8.31 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.24 (C-2‴), 14.72 (C-4′ CH_3), 21.01 (C-1‴), 25.59 (C-2″), 39.55 (C-1″), 45.48 (C-1‴′), 58.99 (C-3″), 67.66 (C-2), 102.36 (C-8′), 111.34 (C-6′), 115.50 (C-10′), 125.89 (C-3′), 125.95 (C-5′), 145.61 (C-4′), 153.64 and 159.29 (C-7′ and C-9′), 161.82 and 167.31 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{19}H_{26}N_2O_4$: 369.1790. Found: 369.1789.

*N-(3-(Diethylamino)propyl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**28**) [28]*

The reaction of ethyl 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**18**) (1 g, 3.44 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.79 g, 13.76 mmol) gave the title compound **28** as a white solid (1.04 g, 81%) by following the general procedure; m.p.: 122.1–122.9°C; R_f: 0.42 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 291 and 318 nm; IR (KBr) ν_{max}: 3375.70 (N-H str), 2964.89, 1712.57 (C=O), 1661.90 (NH*CO*-), 1618.23, 1545.43, 1161.61, 1096.47, 872.25, 778.04, 596.54 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (t, 6H, J = 7.32 Hz, H-2‴′), 1.13 (t, 3H, J = 7.32 Hz, H-2‴), 1.65–1.72 (m, 2H, H-2″), 2.38 (s, 3H, C-4′ CH_3), 2.48–2.55 (m, 6H, H-1‴′ and H-3″), 2.66 (q, 2H, J = 7.32 Hz, H-1‴), 3.43–3.47 (m, 2H, H-1″), 4.50 (s, 2H, H-2), 6.80 (d, 1H, J = 2.20 Hz, H-8′), 6.84 (dd, 1H, J = 2.20 and 8.80 Hz, H-6′), 7.53 (d, 1H, J = 8.80 Hz, H-5′), 8.44 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.61 (C-2‴′), 13.26 (C-2‴), 14.73 (C-4′ CH_3), 21.02 (C-1‴), 25.38 (C-2″), 39.88 (C-1″), 47.13 (C-1‴′), 52.45 (C-3″), 67.77 (C-2), 102.47 (C-8′), 111.35 (C-6′), 115.52 (C-10′), 125.91 (C-3′), 125.95 (C-5′), 145.62 (C-4′), 153.64 and 159.29 (C-7′ and C-9′), 161.87 and 167.23 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{21}H_{30}N_2O_4$: 397.2103. Found: 397.2093.

*N-(2-(Dimethylamino)ethyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**29**) [28]*

The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**19**) (1 g, 2.88 mmol) with N^1,N^1-dimethylethane-1,2-diamine (1.01 g, 11.52 mmol) gave the title compound **29** as a white solid (0.89 g, 79%) by following the general procedure; m.p.: 133.4–135.0°C; R_f: 0.48 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 319 nm; IR (KBr) ν_{max}: 3372.39 (N-H str), 2923.11, 1709.01 (C=O), 1661.57 (NH*CO*-), 1619.11, 1552.59, 1430.03, 1287.14, 1160.80, 1089.68, 869.87, 781.53, 602.82, 533.51 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.32 Hz, H-6‴), 1.28–1.31 (m, 4H, H-4‴ and H-5‴), 1.35–1.46 (m, 2H, H-3‴), 1.47–1.54 (m, 2H, H-2‴), 2.22 (s, 6H, H-1‴′), 2.37 (s, 3H, C-4′ CH_3), 2.43 (t, 2H, J = 5.86 Hz, H-2″), 2.62 (t, 2H, J = 8.05 Hz, H-1‴), 3.39–3.43 (m, 2H, H-1″), 4.53 (s, 2H, H-2), 6.83 (d, 1H, J = 2.20 Hz, H-8′), 6.87 (dd, 1H, J = 2.20 and 8.75 Hz, H-6′), 7.04 (*brs*, 1H, NH), 7.52 (d, 1H, J = 8.75 Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.01 (C-6‴), 14.78 (C-4′ CH_3), 22.55 (C-5‴), 27.52, 28.68, 29.30, and 31.63 (C-1‴–C-4‴), 36.46 (C-1″), 45.14 (C-1‴′), 57.69 (C-2″), 67.49 (C-2), 102.21 (C-8′), 111. 49 (C-6′), 115.39 (C-10′), 124.63 (C-3′), 125.66 (C-5′), 145.54 (C-4′), 153.39 and 158.85 (C-7′ and C-9′), 161.70 and 167.21 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{22}H_{32}N_2O_4$: 411.2260. Found: 411.2260.

*N-(2-(Diethylamino)ethyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**30**)*

The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**19**) (1 g, 2.88 mmol) with N^1,N^1-diethylethane-1,2-diamine (1.34 g, 11.52 mmol) gave the title compound **30** as a white solid (1.07 g, 89%) by following the general procedure; m.p.: 130–131°C; R_f: 0.48 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 319 nm; IR (KBr) ν_{max}: 3367 (N-H str.), 2959, 1706 (C=O), 1673 (NH*CO*-) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.8 Hz, H-6‴), 0.95 (t, 6H, J = 7.3 Hz, H-2‴′), 1.26–1.29 (m, 4H, H-2‴ and H-3‴), 1.32–1.39 (m, 2H, H-4‴), 1.45–1.52 (m, 2H, H-5‴), 2.36 (s, 3H, C-4′ CH_3), 2.48 (q, 4H, J = 7.3 Hz, H-1‴′), 2.54 (t, 2H, J = 6.4 Hz, H-1‴), 2.60 (t, 2H, J = 7.3 Hz, H-2″), 3.32–3.37 (m, 2H, H-1″), 4.51 (s, 2H, H-2), 6.80 (d, 1H, J = 2.2 Hz, H-8′), 6.84 (dd, 1H, J = 2.29 and 8.7 Hz, H-6′), 7.22 (*brs*, 1H, NH), 7.51 (d, 1H, J = 8.7 Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 12.03 (C-2‴′), 14.02 (C-6‴), 14.79 (C-4′ CH_3), 22.56 (C-5‴), 27.52, 28.69, 29.30, and 31.63 (C-1‴–C-4‴), 36.49 (C-1″), 46.72 (C-1‴′), 51.10 (C-2″), 67.39 (C-2), 102.14 (C-8′), 111.22 (C-6′), 115.35 (C-10′), 124.57 (C-3′), 125.72 (C-5′), 145.55 (C-4′), 153.41 and 158.85 (C-7′ and C-9′), 161.71 and 166.97 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{24}H_{36}N_2O_4$: 417.2748. Found: 417.2740.

*N-(3-(Dimethylamino)propyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**31**) [28]*
The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**19**) (1 g, 2.88 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1.17 g, 11.52 mmol) gave the title compound **31** as a white solid (0.93 g, 80%) by following the general procedure; m.p.: 136.0–137.1°C; R_f: 0.47 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 318 nm; IR (KBr) ν_{max}: 3373.96 (N-H str), 2923.56, 1709.94 (C=O), 1662.74 (NH*CO*-), 1618.85, 1550.06, 1430.12, 1285.53, 1162.34, 1090.23, 871.91, 780.88, 601.50 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.96 Hz, H-6‴), 1.29–1.31 (m, 4H, H-4‴ and H-5‴), 1.36–1.41 (m, 2H, H-3‴), 1.47–1.54 (m, 2H, H-2‴), 1.66–1.70 (m, 2H, H-2″), 2.18 (s, 6H, H-1⁗), 2.38 (s, 3H, C-4′ CH_3), 2.39 (t, 2H, J = 5.86 Hz, H-3″), 2.62 (t, 2H, J = 8.05 Hz, H-1‴), 3.42–3.47 (m, 2H, H-1″), 4.51 (s, 2H, H-2), 6.80 (d, 1H, J = 2.20 Hz, H-8′), 6.83 (dd, 1H, J = 2.20 and 8.79 Hz, H-6′), 7.53 (d, 1H, J = 8.79 Hz, H-5′), 8.33 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.02 (C-6‴), 14.79 (C-4′ CH_3), 22.56 (C-5‴), 25.43 (C-2″), 27.53, 28.70, 29.31, and 31.64 (C-1‴–C-4‴), 39.43 (C-1″), 45.34 (C-1⁗), 58.86 (C-3″), 67.46 (C-2), 102.15 (C-8′), 111.06 (C-6′), 115.30 (C-10′), 124.57 (C-3′), 125.73 (C-5′), 145.56 (C-4′), 153.43 and 159.06 (C-7′ and C-9′), 161.74 and 167.08 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{23}H_{34}N_2O_4$: 425.2416. Found: 425.2402.

*N-(3-(Diethylamino)propyl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**32**) [28]*
The reaction of ethyl 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (**19**) (1 g, 2.88 mmol) with N^1,N^1-diethylpropane-1,3-diamine (1.50 g, 11.52 mmol) gave the title compound **32** as a white solid (1 g, 81%) by following the general procedure; m.p.: 115.6–117.2°C; R_f: 0.48 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 319 nm; IR (KBr) ν_{max}: 3370.84 (N-H str), 2922.59, 1710.76 (C=O), 1662.03 (NH*CO*-), 1618.44, 1549.66, 1426.54, 1286.31, 1160.00, 1088.95, 781.66, 599.06 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 6.87 Hz, H-6‴), 0.98 (t, 6H, J = 7.33 Hz, H-2⁗), 1.27–1.31 (m, 4H, H-4‴ and H-5‴), 1.33–1.40 (m, 2H, H-3‴), 1.45–1.53 (m, 2H, H-2‴), 1.63–1.69 (m, 2H, H-2″), 2.36 (s, 3H, C-4′ CH_3), 2.47 (q, 4H, J = 7.33 Hz, H-1⁗), 2.51 (t, 2H, J = 5.95 Hz, H-3″), 2.61 (t, 2H, J = 7.33 Hz, H-1‴), 3.42–3.46 (m, 2H, H-1″), 4.49 (s, 2H, H-2), 6.79 (d, 1H, J = 2.29 Hz, H-8′), 6.68 (dd, 1H, J = 2.29 and 8.70 Hz, H-6′), 7.52 (d, 1H, J = 8.70 Hz, H-5′), 8.47 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.52 (C-2⁗), 14.04 (C-6‴), 14.80 (C-4′ CH_3), 22.57 (C-5‴), 25.19 (C-2″), 27.53, 28.71, 29.31, and 31.65 (C-1‴ C-4‴), 39.82 (C-1″), 46.95 (C-1⁗), 52.32 (C-3″), 67.55 (C-2), 102.26 (C-8′), 111.04 (C-6′), 115.30 (C-10′), 124.56 (C-3′), 125.73 (C-5′), 145.59 (C-4′), 153.41 and 159.03 (C-7′ and C-9′), 161.79 and 166.96 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{25}H_{38}N_2O_4$: 453.2729. Found: 453.2729.

*N-(2-(Dimethylamino)ethyl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide (**33**) [28]*
The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)-oxy)-acetate (**20**) (1 g, 3.81 mmol) with N^1,N^1-dimethylethane-1,2-diamine (1.37 g, 15.24 mmol) gave the title compound **33** as a white solid (0.87 g, 75%) by following the general procedure; m.p.: 116.5–117.4°C; R_f: 0.41 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 272 nm; IR (KBr) ν_{max}: 3483.31 (N-H str), 3266.03, 2831.81, 1711.00 (C=O), 1652.01 (NH*CO*-), 1573.45, 1552.15, 1431.37, 1246.74, 1168.79, 1060.91, 930.85, 837.95, 607.85, 548.75 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 6H, H-1⁗), 2.39 (s, 3H, C-4′ CH_3), 2.43 (t, 2H, J = 5.95 Hz, H-2″), 3.38–3.43 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.29 (s, 1H, H-3′), 7.04 (d, 1H, J = 2.75 Hz, H-5′), 7.08 (*brs*, 1H, NH), 7.14 (dd, 1H, J = 2.75 and 9.16 Hz, H-7′), 7.27 (d, 1H, J = 9.16 Hz, H-8′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.64 (C-4′ CH_3), 36.45 (C-1″), 45.15 (C-1⁗), 57.78 (C-2″), 68.02 (C-2), 108.77 (C-5′), 115.78 (C-3′), 118.27 (C-7′), 119.46 (C-8′), 120.55 (C-10′), 148.54, 151.69, and 153.61 (C-4′, C-6′, and C-9′), 160.63 and 167.62 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{16}H_{20}N_2O_4$: 327.1321. Found: 327.1331.

*N-(2-(Diethylamino)ethyl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide (**34**)*
The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)-oxy)acetate (**20**) (1 g, 3.81 mmol) with N^1,N^1-diethylethane-1,2-diamine (1.77 g, 15.24 mmol) gave the title compound **34** as a light brown solid (1.13 g, 60%) by following the general procedure; m.p.: 118–119°C; R_f: 0.45 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 273 nm; IR (KBr) ν_{max}: 3299 (N-H str), 2975, 1716, (C=O), 1650 (NH*CO*-) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, 6H, J = 7.3 Hz, H-2⁗), 2.39 (d, 3H, J = 0.9 Hz, C-4′ CH_3), 2.48 (q, 4H, J = 7.1 Hz, H-1⁗), 2.54 (t, 2H, J = 5.9 Hz, H-2″), 3.33–3.37 (m, 2H, H-1″), 4.52 (s, 2H, H-2), 6.29 (d, 1H, J = 0.9 Hz, H-3′), 7.02 (d, 1H, J = 2.7 Hz, H-5′), 7.11 (dd, 1H, J = 2.7 and 9.1 Hz, H-7′), 7.24 (*brs*, 1H, NH), 7.27 (d, 1H, J = 9.1 Hz, H-8′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 11.91 (C-2⁗), 18.60 (C-4′ CH_3), 36.55 (C-1″), 46.73 (C-1⁗), 51.24 (C-2″), 67.91 (C-2), 108.48 (C-5′), 115.74 (C-3′), 118.31 (C-7′), 119.32 (C-8′), 120.54 (C-10′), 148.50, 151.64, and 153.64 (C-4′, C-6′, and C-9′), 160.61 and 167.42 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{18}H_{24}N_2O_4$: 333.1809. Found: 333.1805.

*N-(3-(Dimethylamino)propyl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide (**35**) [28]*
The reaction of ethyl 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)-oxy)acetate (**20**) (1 g, 3.81 mmol) with N^1,N^1-dimethylpropane-1,3-diamine (1.55 g, 15.24 mmol) gave the title compound **35** as a white solid (0.89 g, 73%) by following the general procedure; m.p.: 119.7–118.8°C; R_f: 0.46 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 272 nm; IR (KBr) ν_{max}: 3467.99 (N-H str), 2815.93, 1715.96 (C=O), 1648.52 (NH*CO*-), 1574.59, 1432.04, 1245.18, 1173.60, 1058.26, 929.85, 838.82, 610.28 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.63–1.69 (m, 2H, H-2″), 2.15 (s, 6H, H-1⁗), 2.23 (t, 2H, J = 6.40 Hz, H-3″), 2.39 (d, 3H, J = 0.92 Hz, C-4′ CH_3), 3.39–3.44 (m, 2H, H-1″), 4.49 (s, 2H, H-2), 6.29 (d, 1H, J = 0.92 Hz, H-3′), 7.00 (d, 1H, J = 2.75 Hz, H-5′), 7.08 (dd, 1H, J = 2.75 and 9.16 Hz, H-7′), 7.27 (d, 1H, J = 9.16 Hz, H-8′), 8.15 (*brs*, 1H, NH) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.63 (C-4′ CH_3), 25.66 (C-2″), 39.13 (C-1″), 45.33 (C-1⁗), 58.55

(C-3″), 67.86 (C-2), 108.28 (C-5′), 115.77 (C-3′), 118.31 (C-7′), 119.21 (C-8′), 120.55 (C-10′), 148.43, 151.62, and 153.76 (C-4′, C-6′, and C-9′), 160.61 and 167.47 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+Na]^+$ calculated for $C_{17}H_{22}N_2O_4$: 341.1477. Found: 341.1490.

*General procedure for the synthesis of (coumarinyloxy)-acetamide derivatives (**40–43**)*

(Coumarinyloxy)acetic acid (**36**–**39**) (1 g/0.7 g) was taken in a round bottom flask. Dry acetonitrile (20 mL/15 mL) was added and stirred, followed by addition of EDC (1 equivalent) and HBT (1 equivalent). The reaction mixture was stirred at room temperature for 30 min and 1-benzylpiperidin-4-amine (1 equivalent) was added to the mixture. The solution was stirred at room temperature for 24 h. On completion of the reaction, the reaction mixture was diluted with water, extracted with chloroform (3 × 50 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product obtained was purified by column chromatography (MeOH/$CHCl_3$ 1:24) to give (coumarinyloxy)acetamide (**40**–**43**) as a white solid.

*N-(1-Benzylpiperidin-4-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**40**)*

The reaction of 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetic acid (**36**) (0.7 g, 2.99 mmol) with 1-benzylpiperidin-4-amine (0.57 g, 2.99 mmol) gave the title compound **40** as a white solid (0.99 g, 82%) by following the general procedure; m.p.: 172.5–173.3°C; R_f: 0.35 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 319 nm; IR ($CHCl_3$) ν_{max}: 3448.37 (N-H str), 2923.25, 2852.90, 2368.20, 1718.89 (C=O), 1654.21 (NH*CO*-), 1617.62, 1542.35, 1388.80, 1150.58, 1075.73, 849.68, 746.59 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48–1.59 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 1.90–1.97 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 2.10–2.18 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 2.41 (d, 3H, J = 1.12 Hz, C-4′ CH_3), 2.79–2.86 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 3.50 (s, 2H, H-4″a), 3.87–3.97 (m, 1H, H-1″), 4.52 (s, 2H, H-2), 6.18 (d, 1H, J = 1.52 Hz, H-3′), 6.36 (d, 1H, J = 7.64 Hz, NH), 6.85 (d, 1H, J = 2.28 Hz, H-8′), 6.89 (dd, 1H, J = 2.28 and 8.40 Hz, H-6′), 7.25–7.31 (m, 5H, H-2‴–H-6‴), 7.55 (d, 1H, J = 8.40 Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.85 (C-4′ CH_3), 32.24 (C-2″ and C-6″), 46.55 (C-1″), 52.29 (C-3″ and C-5″), 63.13 (C-4″a), 67.63 (C-2), 102.70 (C-8′), 111.89 (C-6′), 113.06 (C-3′), 115.00 (C-10′), 126.18 (C-5′), 127.27 (C-4‴), 128.41 (C-3‴ and C-5‴), 129.24 (C-2‴ and C-6‴), 138.37 (C-1‴), 152.36 (C-4′), 155.25 and 159.93 (C-7′ and C-9′), 161.01 and 166.45 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{24}H_{26}N_2O_4$: 407.1893. Found: 407.1934.

*N-(1-Benzylpiperidin-4-yl)-2-((3-ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**41**)*

The reaction of 2-((3-ethyl-4-methyl-2-oxo-2*H*-chromen-7-yl)-oxy)acetic acid (**37**) (1 g, 3.82 mmol) with 1-benzylpiperidin-4-amine (0.73 g, 3.82 mmol) gave the title compound **41** as a white solid (1.32 g, 80%) by following the general procedure; m.p.: 148.0–148.5°C; R_f: 0.35 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 319 nm; IR ($CHCl_3$) ν_{max}: 2925.30, 2855.23, 1706.47 (C=O), 1666.42 (NH*CO*-), 1614.65, 1539.97, 1456.29, 1169.54, 1090.39, 839.81 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (t, 3H, J = 6.88 Hz, H-2‴), 1.46–1.58 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 1.89–1.97 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 2.09–2.18 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 2.39 (s, 3H, C-4′ CH_3), 2.67 (q, 2H, J = 7.64 Hz, H-1‴), 2.77–2.85 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 3.48 (s, 2H, H-4″a), 3.86–3.97 (m, 1H, H-1″), 4.50 (s, 2H, H-2), 6.35 (d, 1H, J = 8.4 Hz, NH), 6.81 (d, 1H, J = 2.32 Hz, H-8′), 6.86 (dd, 1H, J = 2.28 and 8.40 Hz, H-6′), 7.24–7.30 (m, 5H, H-2‴–H-6‴), 7.53 (d, 1H, J = 8.40 Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.24 (C-2‴), 14.73 (C-4′ *C*H_3), 21.05 (C-1‴), 32.28 (C-2″ and C-6″), 46.52 (C-1″), 52.30 (C-3″ and C-5″), 63.15 (C-4″a), 67.64 (C-2), 102.37 (C-8′), 111.66 (C-6′), 115.76 (C-10′), 125.98 (C-5′), 126.15 (C-3′), 127.24 (C-4‴), 128.40 (C-3‴ and C-5‴), 129.23 (C-2‴ and C-6‴), 138.48 (C-1‴), 145.51 (C-4′), 153.67 and 158.88 (C-7′ and C-9′), 161.76 and 166.62 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{26}H_{30}N_2O_4$: 435.2206. Found: 435.2276.

*N-(1-Benzylpiperidin-4-yl)-2-((3-hexyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**42**)*

The reaction of 2-((3-hexyl-4-methyl-2-oxo-2*H*-chromen-7-yl)-oxy)acetic acid (**38**) (0.7 g, 2.20 mmol) with 1-benzylpiperidin-4-amine (0.42 g, 2.20 mmol) gave the title compound **42** as a white solid (0.81 g, 75%) by following the general procedure; m.p.: 148.9–149.4°C; R_f: 0.35 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}: 319 nm; IR ($CHCl_3$) ν_{max}: 3443.01 (N-H str), 2925.17, 2856.07, 1707.43 (C=O), 1654.27 (NH*CO*-), 1617.76, 1610.47, 1541.64, 1387.05, 1170.97, 1088.11, 743.16 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.84 Hz, H-6‴), 1.26–1.58 (m, 10H, H-2‴–H-5‴, H-2″a/H-2″b and H-6″a/H-6″b), 1.89–1.97 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 2.09–2.19 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 2.38 (s, 3H, C-4′ CH_3), 2.63 (t, 2H, J = 7.64 Hz, H-1‴), 2.78–2.85 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 3.49 (s, 2H, H-4″a), 3.86–3.97 (m, 1H, H-1″), 4.50 (s, 2H, H-2), 6.35 (d, 1H, J = 8.4 Hz, NH), 6.81 (d, 1H, J = 2.28 Hz, H-8′), 6.86 (dd, 1H, J = 2.28 and 9.16 Hz, H-6′), 7.28–7.32 (m, 5H, H-2‴–H-6‴), 7.53 (d, 1H, J = 9.16 Hz, H-5′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.07 (C-6‴), 14.84 (C-4′ CH_3), 22.60 (C-5‴), 27.58, 28.73, 29.35, and 31.67 (C-1‴–C-4‴), 32.05 (C-2″ and C-6″), 46.11 (C-1″), 52.09 (C-3″ and C-5″), 62.94 (C-4″a), 67.40 (C-2), 102.11 (C-8′), 111.44 (C-6′), 115.55 (C-10′), 124.81 (C-5′), 125.79 (C-3′), 127.06 (C-4‴), 128.21 (C-3‴ and C-5‴), 129.04 (C-2‴ and C-6‴), 138.09 (C-1‴), 145.52 (C-4′), 153.44 and 158.61 (C-7′ and C-9′), 161.71 and 166.42 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{30}H_{38}N_2O_4$: 491.2832. Found: 491.2876.

*N-(1-Benzylpiperidin-4-yl)-2-((4-methyl-2-oxo-2H-chromen-6-yl)oxy)acetamide (**43**)*

The reaction of 2-((4-methyl-2-oxo-2*H*-chromen-6-yl)oxy)acetic acid (**39**) (0.7 g, 2.99 mmol) with 1-benzylpiperidin-4-amine (0.57 g, 2.99 mmol) gave the title compound **43** as a white solid (0.89 g, 73%) by following the general procedure; m.p.: 163.8–164.5°C; R_f: 0.35 (MeOH/$CHCl_3$ 1:49); UV (MeOH) λ_{max}:

319 nm; IR ($CHCl_3$) ν_{max}: 3439.80 (N-H str), 2925.44, 2361.91, 2343.68, 1718.43 (C=O), 1670.28 (NH*CO*-), 1572.45, 1431.31, 1170.58, 1059.63, 746.26 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.48–1.60 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 1.89–1.98 (m, 2H, H-2″a/H-2″b and H-6″a/H-6″b), 2.11–2.19 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 2.41 (s, 3H, C-4′ CH_3), 2.79–2.88 (m, 2H, H-3″a/H-3″b and H-5″a/H-5″b), 3.50 (s, 2H, H-4″a), 3.88–3.98 (m, 1H, H-1″), 4.51 (s, 2H, H-2), 6.33 (s, 1H, H-3′), 6.40 (d, 1H, $J = 7.64$ Hz, NH), 7.04 (d, 1H, $J = 3.08$ Hz, H-5′), 7.13 (dd, 1H, $J = 3.04$ and 9.16 Hz, H-7′), 7.28–7.33 (m, 6H, H-2‴′–H-6‴′ and H-8′) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 18.89 (C-4′ CH_3), 32.29 (C-2″ and C-6″), 46.53 (C-1″), 52.31 (C-3″ and C-5″), 63.15 (C-4″a), 68.17 (C-2), 108.98 (C-5′), 116.14 (C-3′), 118.62 (C-7′), 119.43 (C-8′), 120.85 (C-10′), 127.29 (C-4‴′), 128.42 (C-3‴′ and C-5‴′), 129.24 (C-2‴′ and C-6‴′), 138.28 (C-1‴′), 148.78, 151.75, 153.62 (C-4′, C-6′, and C-9′), 160.65 and 166.96 (C-2′ and C-1) ppm; HRMS: *m/z* $[M+H]^+$ calculated for $C_{24}H_{26}N_2O_4$: 407.1893. Found: 407.1945.

Biology

Materials

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type VI-S, lyophilized powder, from electric eel, 500 UN), butyrylcholinesterase (BChE, E.C. 3.1.1.8, lyophilized powder, from equine serum, 1000 UN), butyrylthiocholine iodide, donepezil hydrochloride, Triton X-100, DMSO, and methanol were procured from Sigma–Aldrich Chemicals Pvt. Ltd., USA. 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine idodide, Tris buffer (pH 8.0) were procured from HiMedia, Mumbai, India. Dulbecco's Modified Eagle's Media, glutamine, fetal bovine serum (FBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Thermo Fisher Scientific, USA. SH-SY5Y neuroblastoma cell line and 24-well tissue culture plates were obtained from ATCC (ATCC CRL2266), USA and Corning Inc., USA, respectively.

Cholinesterase inhibitory activity

The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory assay was performed for (coumarinyloxy)-acetamides to evaluate the inhibition activity percentage using the protocol as described by Nunes et al. [29] with some modifications. In brief, different concentrations (30–0.1 μg/mL) of the acetamide derivatives taken from the stock solution of the compounds prepared in methanol were tested to determine the IC_{50} values with donepezil hydrochloride as a reference drug. The Ellman's reagent, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB, 3 mM) and the stock solution of the substrate (acetylthiocholine iodide (AChI), 16 mM) were prepared in Tris buffer (20 mM, pH 8) and distilled water, respectively. AChE (500 UN) from electric eel was diluted to 2 UN/mL using Tris buffer (20 mM, pH 8) which was used for the inhibitory activity with the final concentration of AChE being 0.05 UN/mL in the assay. A total of 93.5 μL of Tris buffer (50 mM, pH 8), 119 μL of DTNB, 6.25 μL of AChE solution, range of different concentrations of the inhibitor prepared from the stock solution and the amount of methanol necessary to have 231.25 μL of the sample were mixed and left to incubate for 15 min. Subsequently, 18.75 μL of AChI was added and the initial rate of the enzymatic reaction was followed by reading the solution absorbance at 412 nm during the first 5 min of the reaction using Tecan Infinite 200 Pro. A control reaction was performed having no inhibitor, which was considered as 100% activity. The percentage of inhibition (%*I*) was calculated as follows:

$$\%I = v_I/v_0 \times 100$$

where v_I is the initial rate in the presence of the inhibitor and v_0 is the initial rate of the control reaction. The inhibition curves were obtained by plotting the percentage of enzymatic inhibition with inhibitor concentration. All the experiments were performed in duplicate and the temperature of all the solutions was set to 25°C prior to use. The described method was also used for BChE inhibition assay using butyrylthiocholine iodide as a substrate.

Kinetic study of AChE inhibition

The Lineweaver–Burk plot (plot of 1/velocity vs. 1/[substrate]) was used to determine the type of AChE inhibition by the compounds. The two most active compounds **22** and **43** were subjected to kinetic study in order to obtain the mechanism of AChE inhibition. Double reciprocal plots of 1/*V* versus 1/[S] were constructed at different concentrations of the substrate acetylthiocholine (0.09–0.36 mM) by using Ellman's method. Three concentrations of the compounds were selected for this study (**22**: 0.3, 0.6, and 1.2 μM; **43**: 0.25, 0.5, and 1.0 μM). The rate of enzyme activity was measured in the presence and absence of inhibitor for proposed concentrations of the substrate. After the addition of acetylthiocholine, progress curves were monitored at 412 nm for 5 min. Using the slopes of progress curves, double reciprocal plots (1/*V* vs. 1/[S]) were constructed to obtain the type of inhibition. Secondary plots obtained using the slopes of the reciprocal plots and the concentrations of inhibitors gave the inhibition constant K_i. Data analysis was performed with Microsoft Excel 2010.

Inhibition of AChE-induced and self-mediated $A\beta_{1-42}$ peptide aggregation assay

In order to determine AChE-induced $A\beta_{1-42}$ peptide aggregation [42], aliquots of $A\beta_{1-42}$ peptide (dissolved in DMSO and diluted in 0.215 M sodium phosphate buffer, pH 8.0, final concentration: 50 μM) and AChE (dissolved in 0.1 M sodium phosphate buffer, pH 8.0, final concentration: 0.02 U) from electric eel, in the presence and absence of the test inhibitors (final concentration: 100 μM) were incubated for 48 h at room temperature. For self-mediated $A\beta_{1-42}$ peptide aggregation, aliquot of $A\beta_{1-42}$ peptide (dissolved in DMSO and diluted in 50 mM phosphate buffer saline, pH 7.4, final concentration: 20 μM) was incubated in the presence and absence of test compounds (final concentration: 100 μM) for 48 h at 37°C. Following co-incubation for 48 h, 180 μL of 5 μM thioflavin T in 50 mM glycine-NaOH buffer (pH 8.0) was added. Subsequently, fluorescence intensities were measured after 5 min

with excitation and emission wavelength at 450 and 485 nm, respectively. The percent inhibition of AChE-induced and self-mediated $A\beta_{1-42}$ peptide aggregation was calculated using the expression: 100 – ($IF_i/IF_o \times 100$), where IF_i and IF_o are the fluorescence intensities in the presence and absence of the test inhibitors, respectively, minus the fluorescence intensities due to the respective blanks. All the experiments were carried out in duplicate.

Hemolytic study

In order to have an insight of the *in vitro* human red blood cells (hRBCs) toxicity, potent compounds **22**, **40**, and **43** were subjected to hemolytic assay as described by Ciornei et al. [34], Dobrovolskaia et al. [35], and Kumar et al. [36] with some minor modifications. Briefly, fresh human erythrocytes washed and centrifuged with phosphate buffer saline (PBS) thrice were suspended in PBS (pH 7.4) to make 4% (v/v) solution. A total of 100 μL of hRBC suspension, added to each well of 96-well flat bottom tissue culture plate along with different concentrations of the compounds **22**, **40**, and **43** [1× (0.25 μM), 5× (1.25 μM), 10× (2.5 μM), 15× (3.75 μM), 20× (5 μM), 25× (6.25 μM), 50× (12.5 μM), 75× (18.75 μM), 100× (25 μM), 200× (50 μM), 500× (125 μM), and 1000× (250 μM)] with the final volume made to 200 μL using 1× PBS, were incubated for 1 h at 37°C. This was followed by centrifugation at 1000 rpm for 10 min at 4°C. Aliquots (100 μL) of supernatant were transferred to fresh 96-well culture plate and OD_{540} was recorded using ELISA plate reader (BioTek Instruments, Inc.). The absorbance of hRBC suspension treated with Triton X-100 was taken as 100% hemolysis and used as positive control. On the other hand, cell suspension treated with PBS was used as a negative control. Percentage of hemolysis was calculated using the following formula:

$$\%\text{Hemolysis} = \left(\frac{(OD_{540nm}\text{ of Sample} - OD_{540nm}\text{ of PBS})}{(OD_{540nm}\text{ of 0.1\% Triton X-100} - OD_{540nm}\text{ of PBS})}\right) \times 100$$

Cytotoxicity study

In vitro cell cytotoxicity assay of potent anti-AChE compounds **22**, **40**, and **43** was performed using SH-SY5Y neuroblastoma cell line by MTT colorimetric assay as described by Riss et al. [37]. Cells were maintained in antibiotic-free Dulbecco's Modified Eagle's Medium supplemented with glucose (1 g/L), glutamine (4 mM), and 10% FBS at 37°C in a humidified atmosphere of 5% (v/v) CO_2. At 60–70% confluency, cells were passaged and seeded in 24-well plates at the seeding density of 5×10^4 per well. The different concentrations of the compounds (1–50 μM) were added and cells were incubated at 37°C in an atmosphere of 5% (v/v) CO_2 for 24 and 48 h. Finally, 10 μL of MTT solution (5 mg/mL) was added to each well and incubated for 3 h at 37°C in 5% CO_2. Formation of formazan crystals was confirmed microscopically and 100 μL of DMSO was added in each well to solubilize these crystals and the absorbance (A) was measured at 570 nm using a plate reader (Tecan Infinite 200 Pro). The untreated cells with ~100% cell viability were taken as control. The experiment was performed in triplicate. The relative cell viability percentage as compared to the control was calculated using the formula [43]:

$$A_{Sample}/A_{Control} \times 100$$

Molecular docking study

The docking calculations were performed following a procedure identical to that previously reported [24]. The X-ray crystallographic structure of acetylcholinesterase *Torpedo californica* – AChE complexed with an inhibitor was taken from RCSB Protein Data Bank (PDB entry 1ODC), in order to be used as receptor in the docking simulations. The original complex structure was treated using Maestro v. 9.3 [44], by removing the original ligand, solvent and co-crystalization molecules and adding hydrogen atoms. This program was also used to design the ligand structures, which were submitted to random conformational search (RCS) of 1000 cycles and 2500 optimization steps with the program Ghemical v 2.0 [45]. The ligands were docked into the AChE structure, using GOLD v. 5.1. [39], with the default parameters of Gold (except "allow early termination" option) and the ASP scoring function. The zone of interest was defined as the residues within 10 Å from the original position of the ligand in the crystal structure.

We gratefully acknowledge the financial assistance from University of Delhi and CSIR-TOUCH (BSC0302) project.

The authors have declared no conflicts of interest.

References

[1] A. Kumar, A. Singh, Ekavali, *Pharmacol. Rep*. **2015**, *67*, 195–203.
[2] H. Akrami, B. F. Mirjalili, M. Khoobi, A. Moradi, H. Nadri, S. Emami, A. Foroumadi, M. Vosooghi, A. Shafiee, *Arch. Pharm. Chem. Life Sci*. **2015**, *348*, 1–9.
[3] C. Brühlmann, F. Ooms, P. Carrupt, B. Testa, M. Catto, F. Leonetti, C. Altomare, A. Carotti, *J. Med. Chem*. **2001**, *44*, 3195–3198.
[4] J. Hardy, D. J. Selkoe, *Science* **2002**, *297*, 353–356.
[5] G. V. De Ferrari, M. A. Canales, I. Shin, L. M. Weiner, I. Silman, N. C. Inestrosa, *Biochemistry* **2001**, *40*, 10447–10457.
[6] M. Bartolini, C. Bertucci, V. Cavrini, V. Andrisano, *Biochem. Pharmacol*. **2003**, *65*, 407–416.
[7] N. Mishra, D. Sasmal, *Bioorg. Med. Chem. Lett*. **2013**, *23*, 702–705.
[8] L. Piazzi, A. Rampa, A. Bisi, S. Gobbi, F. Belluti, A. Cavalli, M. Bartolini, V. Andrisano, P. Valenti, M. Recanatini, *J. Med. Chem*. **2003**, *46*, 2279–2282.

[9] M. Alipour, M. Khoobi, A. Foroumadi, H. Nadri, A. Moradi, A. Sakhteman, M. Ghandi, A. Shafiee, *Bioorg. Med. Chem*. **2012**, *20*, 7214–7222.
[10] M. A. Kamal, P. Klein, Q. Yu, D. Tweedie, Y. Li, H. W. Holloway, N. H. Greig, *J. Alzheimers Dis*. **2006**, *10*, 43–51.
[11] M. Decker, B. Kraus, J. Heilmann, *Bioorg. Med. Chem*. **2008**, *16*, 4252–4261.
[12] E. Giacobini, *Pharmacol. Res*. **2004**, *50*, 433–440.
[13] M. A. Kamal, P. Klein, W. Luo, Y. Li, H. W. Holloway, D. Tweedie, N. H. Greig, *Neurochem. Res*. **2008**, *33*, 745–753.
[14] R. M. Lane, S. G. Potkin, A. Enz, *Int. J. Neuropsychopharmacol*. **2005**, *9*, 1–24.
[15] S. O. Nama, D. H. Park, Y. H. Lee, J. H. Ryu, Y. S. Lee, *Bioorg. Med. Chem*. **2014**, *22*, 1262–1267.
[16] K. Mullane, M. Williams, *Biochem. Pharmacol*. **2013**, *85*, 289–305.
[17] M. Shidore, J. Machhi, K. Shingala, P. Murumkar, M. K. Sharma, N. Agrawal, A. Tripathi, Z. Parikh, P. Pillai, M. R. Yadav, *J. Med. Chem*. **2016**, *59*, 5823–5846.
[18] C. Kontogiorgis, A. Detsi, D. Hadjipavlou-Litina, *Expert Opin. Ther. Pat*. **2012**, *22*, 437–454.
[19] A. Fallareroa, P. Oinonenb, S. Gupta, P. Bloma, A. Galkine, C. G. Mohand, P. M. Vuorela, *Pharmacol. Res*. **2008**, *58*, 215–221.
[20] M. Alipour, M. Khoobi, H. Nadri, A. Sakhteman, A. Moradi, M. Ghandi, A. Foroumadi, A. Shafiee. *Arch. Pharm. Chem. Life Sci*. **2013**, *346*, 577–587.
[21] N. Changwong, C. Sabphon, K. Ingkaninan, P. Sawasdee, *Phytother. Res*. **2012**, *26*, 392–396.
[22] L. Piazzi, A. Cavalli, F. Colizzi, F. Belluti, M. Bartolini, F. Mancini, M. Recanatini, V. Andrisano, A. Rampa, *Bioorg. Med. Chem. Lett*. **2008**, *18*, 423–426.
[23] C. R. Wu, C. L. Chang, P. Y. Hsieh, L. W. Lin, H. Ching, *Planta Med*. **2007**, *73*, 275–278.
[24] C. A. Kontogiorgis, Y. Xu, D. Hadjipavlou-Litina, Y. Luo, *Free Radic. Res*. **2007**, *41*, 1168–1180.
[25] M. Alipour, M. Khoobi, A. Moradi, H. Nadri, F. H. Moghadam, S. Emami, Z. Hasanpour, A. Foroumadi, A. Shafiee, *Eur. J. Med. Chem*. **2014**, *82*, 536–544.
[26] S. F. Razavi, M. Khoobi, H. Nadri, A. Sakhteman, A. Moradi, S. Emami, A. Foroumadi, A. Shafiee, *Eur. J. Med. Chem*. **2013**, *64*, 252–259.
[27] P. Anand, B. Singh, N. Singh, *Bioorg. Med. Chem*. **2012**, *20*, 1175–1180.
[28] S. Prasad, S. Kumar, B. Kumar, A. K. Singh, H. K. Gautam, S. K. Sharma, *Med. Chem. Res*. **2015**, *24*, 2297–2313.
[29] A. Nunes, S. M. Marques, C. Quintanova, D. F. Silva, S. M. Cardoso, S. Chaves, M. A. Santos, *Dalton Trans*. **2013**, *42*, 6058–6073.
[30] F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P. W. Lee, Y. Tang, *J. Chem. Inf. Model*. **2012**, *52*, 3099–3105.
[31] H. Akrami, B. F. Mirjalili, M. Khoobi, H. Nadri, A. Moradi, A. Sakhteman, S. Emami, A. Foroumadi, A. Shafiee, *Eur. J. Med. Chem*. **2014**, *84*, 375–381.
[32] H. Pajouhesh, G. R. Lenz, *NeuroRX* **2005**, *2*, 541–553.
[33] A. K. Ghose, T. Herbertz, R. L. Hudkins, B. D. Dorsey, J. P. Mallamo, *ACS Chem. Neurosci*. **2012**, *3*, 50–68.
[34] C. D. Ciornei, T. Sigurdardóttir, A. Schmidtchen, M. Bodelsson, *Antimicrob. Agents Chemother*. **2005**, *49*, 2845–2850.
[35] M. A. Dobrovolskaia, J. D. Clogston, B. W. Neun, J. B. Hall, A. K. Patri, S. E. McNeil, *Nano Lett*. **2008**, *8*, 2180–2187.
[36] B. Kumar, A. Mathur, R. Pathak, K. Sardana, H. K. Gautam, P. Kumar, *J. Bioact. Compat. Polym*. **2016**, *31*, 467–480.
[37] T. L. Riss, R. A. Moravec, A. L. Niles, H. A. Benink, T. J. Worzella, L. Minor, Cell viability assays, in *Assay Guidance Manual* (Eds.: G. S. Sittampalam, N. P. Coussens, H. Nelson, M. Arkin, D. Auld, C. Austin, B. Bejcek, M. Glicksman, J. Inglese, P. W. Iversen, Z. Li, J. McGee, O. McManus, L. Minor, A. Napper, J. M. Peltier, T. Riss, O. J. Trask, Jr., J. Weidner, Eli Lilly and Company and the National Center for Advancing Translational Sciences, Bethesda, Maryland, USA **2013**, pp. 1–13.
[38] H. Dvir, I. Silman, M. Harel, T. L. Rosenberry, J. L. Sussman, *Chem. Biol. Interact*. **2010**, *187*, 10–22.
[39] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol*. **1997**, *267*, 727–748.
[40] Protein Data Base (PDB). http://www.pdb.org/pdb/home/home.do (accessed July **2016**).
[41] J. Sebestik, S. M. Marques, P. L. Fale, S. Santos, D. M. Arduino, S. M. Cardoso, C. R. Oliveira, M. L. Serralheiro, M. A. Santos, *J. Enzyme Inhib. Med. Chem*. **2011**, *26*, 485–497.
[42] J. Kumar, P. Meena, A. Singh, E. Jameel, M. Maqbool, M. Mobashir, A. Shandilya, M. Tiwari, N. Hoda, B. Jayaram, *Eur. J. Med. Chem*. **2016**, *119*, 260–277.
[43] S. Yadav, M. Mahato, R. Pathak, D. Jha, B. Kumar, S. R. Deka, H. K. Gautam, A. K. Sharma, *J. Mater. Chem. B* **2014**, *2*, 4848–4861.
[44] Maestro, Version 9.3, Schrödinger Inc., Portland, OR, **2012**.
[45] T. Hassinen, M. Peräkylä, *J. Comput. Chem*. **2001**, *22*, 1229–1242.