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Novel coumarin derivatives bearing *N*-benzyl pyridinium moiety: Potent and dual binding site acetylcholinesterase inhibitors

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ABSTRACT

A novel series of coumarin derivatives linked to benzyl pyridinium group were synthesized and biologically evaluated as inhibitors of both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The enzyme inhibitory activity of synthesized compounds was measured using colorimetric Ellman's method. It was revealed that compounds **3e**, **3h**, **3l**, **3r** and **3s** have shown higher activity compared with donepezil hydrochloride as standard drug. Most of the compounds in these series had nanomolar range IC_{50} in which compound **3r** (IC_{50} = 0.11 nM) was the most active compound against acetylcholinesterase enzyme.

1. Introduction

Alzheimer's disease (AD), a progressive and degenerative disorder of the brain, is supposed to be the most common cause of dementia among the aged people. Approximately, 24 million people worldwide suffer from this disease. It was demonstrated that AD is mainly caused by the loss of cholinergic activity in some parts of the brain such as hippocampus which are related to learning and cognition.[1,2] Thus, increasing the synaptic level of acetylcholine (ACh) by means of acetylcholinesterase (AChE) inhibition could be helpful to alleviate AD's symptoms.[3–7]

In addition, some evidence suggests that butyrylcholinesterase (BuChE) levels are unchanged or even rise in advanced AD, while the activity of the acetylcholinesterase is decreased in certain area of the brain.[8–10] As a matter of fact, the inhibition of BuChE can raise ACh levels.[11,12] Consequently, dual inhibition of AChE/BuChE may be beneficial to improve AD symptoms without remarkable side effects.[13]

Besides, it was revealed that AChE augments the neurotoxic effect of amyloid beta through accelerating the formation of beta amyloid deposits in the brain. Therefore, the role of amyloid beta in beginning and progression of AD is a matter of debate.[14,15] It is well known that the enzyme interacts with beta amyloid through peripheral anionic site (PAS) and promotes the formation of fibrils.[16] According to these findings, designing the compounds able to interact with both active site and PAS of AChE would be a new therapeutic approach for effective management of AD's symptoms.[17,18] Coumarins, a groups of naturally occurring substances in many plants exhibit a wide range of biological activities such as anti-inflammatory,[19] anti-tumor,[20] hepatoprotective,[21] anti-HIV-1,[22] antiviral,[23] antifungal,[24] antimicrobial,[25] anti-oxidant,[26] and antidepressant effects.[27] Moreover, the potent anticholinesterase activity of many synthetic coumarins was revealed similar to that observed in the natural compounds.[28–31]

Many of these efforts were conducted by connecting the coumarin scaffold, via different spacers, to the structures with high affinity towards the catalytic site of the enzyme.[32]

Moreover, benzylpyridinium salts (Fig. 1) have been represented an excellent acetylcholinesterase inhibitory in several reports.[33] In our previous work we have reported synthesis and anticholinesterase activity of new benzofuranone derivatives bearing benzyl pyridinium moiety (Fig. 1, **X**=**O**).[33a] Observing a dramatic decrease in anticholinesterase activity of the compounds lacking the benzyl pyridinium moiety, proved that this part is necessarily required for superior activity which was in accord with the previously reported data by Iimura.[33b]

Based on these findings and in pursuit of our previous study on coumarin scaffold,[34] we have presented a novel series of coumarin derivatives that were attached to benzyl pyridinium group through alpha beta unsaturated carbonyl linker (Fig. 2). An outstanding feature of this linker is the conformational restriction caused by the

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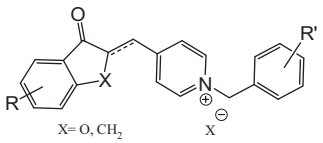


Figure 1. Some potent anticholinesterase structures bearing benzyl pyridinium moiety.

conjugated double bond, which makes it possible to study the substituent modifications regardless of any conformational alterations in the linker.

2. Results and discussion

2.1. Chemistry

Compounds **3** were synthesized via the route outlined in Scheme 1. In the first step, 3-acetylcomarin derivatives **6** were synthesized using a commercial 2-hydroxybenzaldehydes **4** and ethyl acetoacetate **5** in the presence of catalytic amount of piperidine according to the previous reported procedure.[30] In the next step 3-acetylcomarins were transformed into the key intermediate **7** through condensation with pyridine-4-carbaldehyde under microwave irradiation with good to excellent yields (64–77%). For preparation of the latter compounds several conditions were screened (*p*-TsOH in toluene, piperidine in ethanol and piperidine in *n*-butanol both under thermal and microwave conditions) but only in the presence of *n*-butanol as solvent and piperidine as catalyst under microwave irradiation the best result was obtained. The aldol products **7** were only in *E*-configuration as thermodynamically favored structures. The coupling constant of 15–16 Hz between the olefinic protons clearly showed that the compound **7** possess the *E*-configuration. The target compounds **3** were easily prepared through the addition of proper benzyl bromide or chloride derivatives to compounds **7** in dry acetonitrile under reflux condition. On cooling, the precipitates was filtered and washed with diethyl ether.

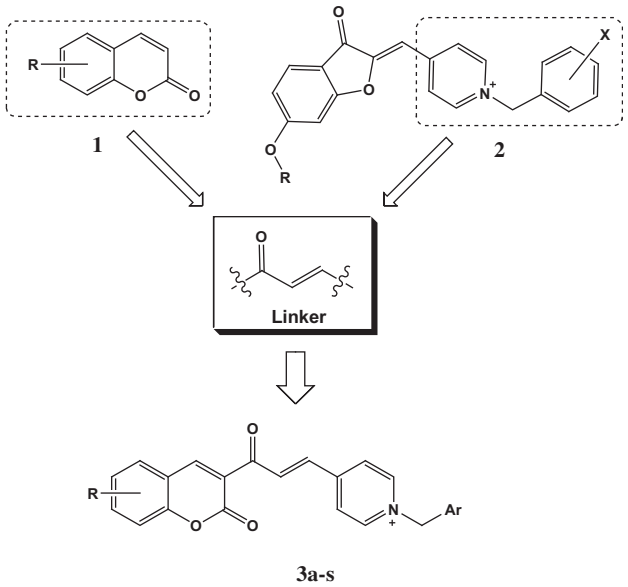


Figure 2. Design strategy of the target compounds.

2.2. Acetylcholinesterase and butyrylcholinesterase inhibition and preliminary SAR studies

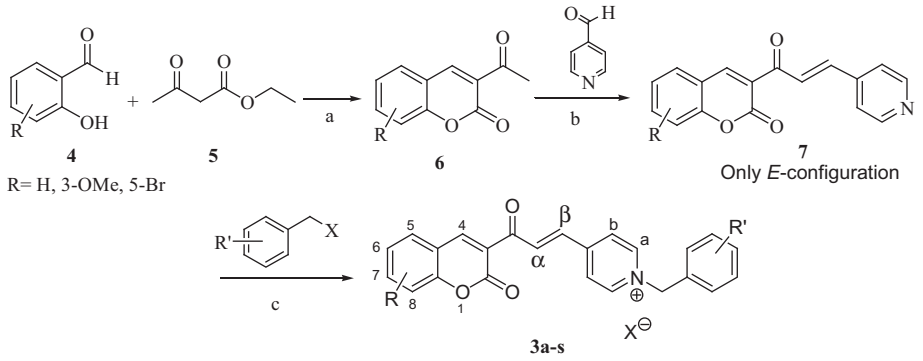
The target compounds were assayed for their inhibitory activity against AChE from *Electrophorus electricus* (*eel*) and BuChE from equine serum using the previously reported method and Donepezil hydrochloride was taken as the positive control.[33a] According to the enzyme inhibition data, as depicted in Table 1, most of the target compounds showed potent AChE inhibition activity with IC_{50} values within pico and micro molar ranges. Among the synthesized compounds, **3r** (IC_{50} = 0.11 nM) showed superior activity in comparison to the reference drug, Donepezil hydrochloride (IC_{50} = 14 nM). By contrast, compound **3h** gave the most potent inhibition for BuChE (IC_{50} = 125 nM). Compound **3e** exhibited the most selectivity for AChE. It was also revealed that anticholinesterase activity of the target compounds depends largely to the steric and electronic features of the substituents. For example, a fourfold reduction in the activity was observed by the movement of fluoro from position 2 in compound **3r** to position 4 in compound **3s** (IC_{50} = 0.46 nM). This fact supported by the docking studies. According to computational and experimental studies, it is suggested that combination of steric and electronic features can determine the activity of this series of compounds. Based on docking studies, presence of fluoro at *orto* position can disrupt the π–π stacking interactions via rotation of the phenyl ring as in compound **3d**. Movement of fluoro to position *meta* as in compound **3e** has therefore resulted in better activity due to proper stacking of the phenyl ring with Trp 84. On the other hand the activity would slightly diminish when the flouro shifts to the *para* position. It is assumed that the steric hindrance of substituents with amino acids at the bottom of AChE gorge may be responsible for this effect. Furthermore the activity of target compounds is very sensitive to the size of the substituent at *para* position. Thus compounds such as **3p** and **3q** having bulky groups at *para* position showed weaker activity. It is worth noting that in case of **3a** and **3b,** removing 6-bromo substiuent in coumarin ring, led to a large decrease in the activity of the target compounds (IC_{50} = 540; 760 nM, respectively). To study the effects of more hydrophilic substituents on coumarin ring, some compounds bearing methoxy group on 8 position of coumarin ring were synthesized (**3c**–**q**). As it could be observed from Table 1, the size of the substituent in position 2, regardless of its electronic properties, is important in order to show considerable activity. Larger substituents have therefore exhibited better activities in comparison to those with the smaller size. The anticholinesterase activity was in the order: CH_3 > Cl > F according to the Van der Waals radii 200, 180 and 135 pm, respectively (IC_{50} = **3l**: 0.16, **3h**: 0.46, **3d**: 26 nM). In addition, the data showed that compounds with more electron-withdrawing substituent on position 3 of benzyl moiety exhibited higher activity in the order of **3e** (F, 0.47 nM) > **3o** (CN, 76 nM) > **3i** (Cl, 86 nM) > **3m** (CH_3, 800 nM). Finally, insertion of second substituent at any position of benzyl moiety dramatically decreased the activity of the resulted compound (**3g**, **3j**, **3k**).

2.3. Antioxidant activity assay

Two different methods; DPPH (1,1-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays were used to evaluate target compounds for their antioxidant efficacy. The antioxidant assay data were summarized in Table 2. According to the data none of the tested compounds showed significant antioxidant ability in both methods compared with reference compounds.

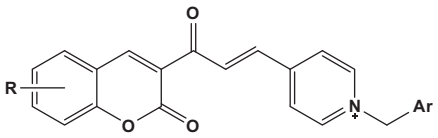
2.4. Molecular docking studies

In an attempt to study the binding mode for the interaction of the target compounds within gorge of AChE from *Torpedo*



Scheme 1. Reagents and conditions: (a) piperidine, 50 °C; (b) piperidine, MW, 30 min; (c) benzyl halide derivatives, CH_3CN, reflux, 2–4 h.

Table 1
AChE and BuChE inhibition data and their selectivity for synthesized compounds **3a–s**



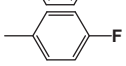
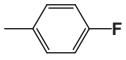
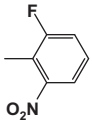
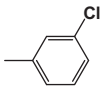
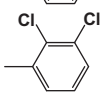
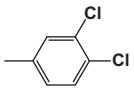
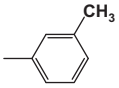
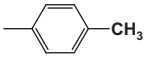
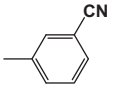
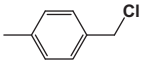
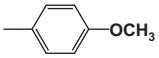
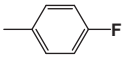
Compound	R	Ar	IC_{50} AChE[a,b] (nM)	IC_{50} BuChE[a,b] (nM)	Selectivity for AChE[d]
3a	H		540	848	1.57
3b	H		760	910	1.2
3c	8-OMe		ND[c]	ND[c]	ND[c]
3d	8-OMe		26	329	12.6
3e	8-OMe		0.47	2100	4468
3f	8-OMe		472	931	1.97
3g	8-OMe		330	685	2.1
3h	8-OMe		0.46	125	272
3i	8-OMe		86	560	6.5
3j	8-OMe		120	6800	57
3k	8-OMe		440	5790	13.6

Table 1 (*continued*)

Compound	R	Ar	IC_{50} AChE[a,b] (nM)	IC_{50} BuChE[a,b] (nM)	Selectivity for AChE[d]
3l	8-OMe		0.16	342	2137
3m	8-OMe		800	11,200	14
3n	8-OMe		3650	27,000	7.4
3o	8-OMe		76	1000	13.1
3p	8-OMe		1600	15,100	9.4
3q	8-OMe		1470	971	0.7
3r	6-Br		0.11	489	4445
3s	6-Br		0.46	494	1074
Donepezil			14	5380	384

[a] The concentration of inhibitor required to produce 50% enzyme inhibition.
[b] Data are means of triplicate independent experiments.
[c] Data was not determined.
[d] Selectivity for AChE = IC_{50} (BuChE)/IC_{50} (AChE).

Table 2
The antioxidant activity of target compounds

Compounds	DPPH IC_{50} (μg/ml)	FRAP value
3a	>25	<150
3b	>25	<150
3c	>25	<150
3d	>25	<150
3e	>25	<150
3f	>25	<150
3g	>25	<150
3h	>25	<150
3i	>25	<150
3j	>25	<150
3k	>25	<150
3l	>25	<150
3m	>25	<150
3n	>25	<150
3o	>25	<150
3p	>25	<150
3q	>25	<150
3r	>25	<150
3s	>25	<150
Ascorbic acid	3.6	1890
BHT[a]	—	430

[a] Butylated hydroxytoluene.

californica (*Tc*AChE) active site, molecular docking was conducted using autodock vina.[36] Among the synthesized series of compounds, **3r**, the most potent compound, was subjected to docking studies. Superposition of the best pose of **3r** and the co-crystallized Donepezil in the active site revealed that its binding mode at the gorge of *Tc*AChE resembled very much to that of Donepezil (Fig. 3).

Subsequently, compound **3r** was analyzed in order to investigate the possible interactions with amino acid residues on the active site of the enzyme. As depicted in Figures 4 and 5, π-cation interaction of the positively charged nitrogen with Tyr334, was playing an important role in the penetration of the ligand into active site as well as in the inhibition process. This finding was in agreement with the previously reported data.[37] Additionally, the three π–π stacking interactions between the phenyl group of benzyl moiety and coumarin with the indole ring of catalytic anionic site (CAS) Trp84 and Trp279 (PAS), respectively, and pyridine ring with phenyl ring of Phe330 seems to stabilize the orientation of the molecule in the gorge of *Tc*AChE. The latter feature allowed for one hydrogen bonding interaction between the oxygen of coumarin carbonyl group with nitrogen of Phe288. Therefore, ligand **3r** is a dual PAS and CAS binding inhibitor of AChE that is in good agreement with our rational design.

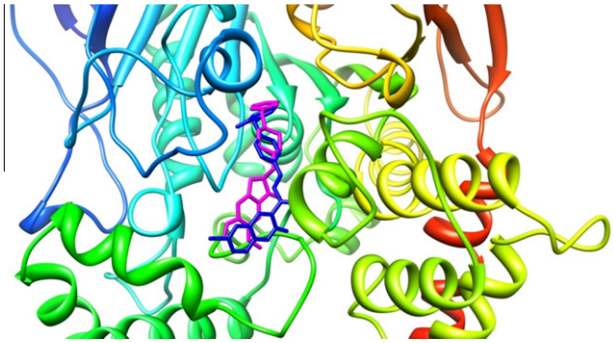


Figure 3. Superimposition of **3r** (blue) and donepezil (magenta) in the gorge of *Tc*AChE.

3. Conclusions

A new series of coumarin derivatives linked to benzyl pyridinium group has been synthesized and evaluated for AChE and BuChE inhibitory activity. Acetylcholinesterase activity of these compounds was sensitive to substitiuent steric and electronic properties at position 2 and 3 of the benzyl moiety, respectively. Among them compound **3r** (IC_{50} = 0.11 nM) was identified as the

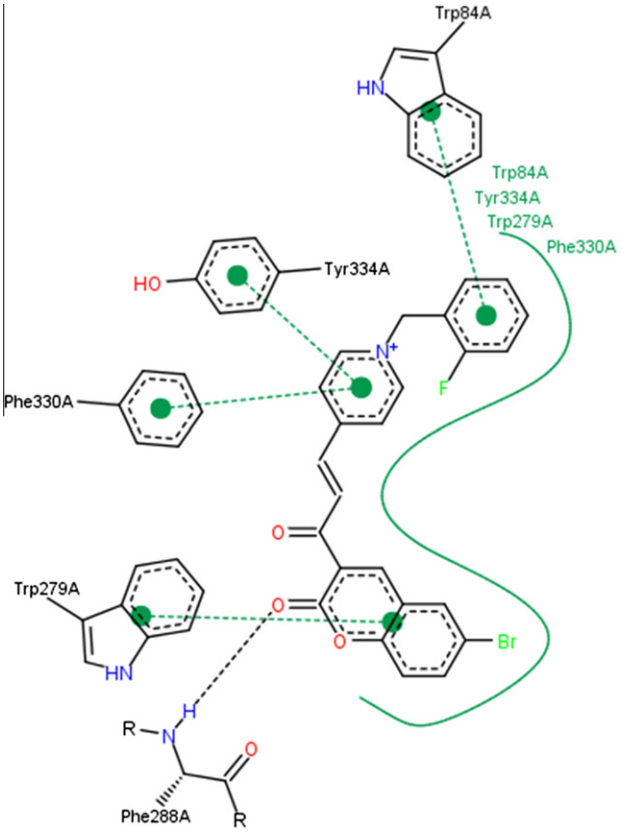


Figure 4. 2D representation of binding mode of **3r** and amino acid residues in the gorge of *Tc*AChE created by poseview.

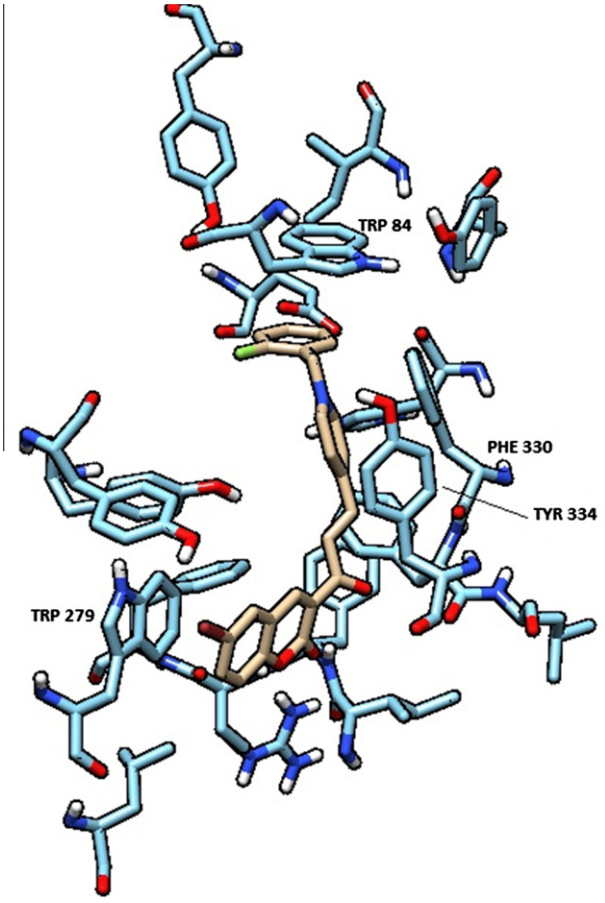


Figure 5. Docking of **3r** in the active site of of *Tc*AChE. Hydrogen atoms are not shown for clarity.

most potent AChE inhibitor. Modeling studies showed similar modes for the interaction of the compound and donepezil a well known potent inhibitor of the enzyme. According to computational and experimental studies, it is suggested that combination of steric and electronic features can determine the activity of this series of compounds. Based on docking studies, presence of fluoro at *orto* position can disrupt the π–π stacking interactions via rotation of the phenyl ring as in compound **3d**. This finding is largely dependent to electronic features and size of the substituents. Consequently, it can be concluded that, regardless of the electronic features, substitution at *para* position is not normally favored (i.e. **3p**,**3q**,**3n**, **3k** etc).

4. Experimental section

4.1. General

All commercially available reagents were purchased from Merck AG, Aldrich or Acros Organics and used without further purification. Column chromatography was carried out on silica gel (70–230 mesh). TLC was conducted on silica gel 250 micron, F_{254} plates. For the synthesis of compounds **7** the experiments were performed using a microwave oven (ETHOS 1600, Milestone) with a power of 600 W specially designed for an organic synthesis and modified with a condenser and mechanical stirrer. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrographs (KBr disks). Mass spectra of the products were obtained with an HP (Agilent technologies) 5937 Mass Selective Detector. ^{1}H NMR spectra were recorded on a Bruker 400 or 500 MHz NMR instruments. The atoms numbering of the target compounds used for ^{1}H NMR data are depicted in Scheme 1. The chemical shifts (δ) and coupling constants (J) are expressed in parts per million and hertz, respectively. Elemental analyses were carried out by a CHN-Rapid Heraeus elemental analyzer. The results of elemental analyses (C, H, N) were within ±0.4% of the calculated values.

4.2. Representative procedure for the preparation of 3-acetyl coumarin derivatives 6

To a rapid stirring cold mixture of 2-hydroxy benzaldehyde derivatives **4** (0.2 mol) and ethyl acetoacetate **5** (0.2 mol), piperidine (2 ml) was added. The mixture was allowed to warm to 50 °C. Progress of the reaction was monitored by TLC. After completion of the reaction, the solid was filtered off and subsequently washed with ethanol and recrystallized from water/ethanol (30:70).[35]

4.3. Representative procedure for the preparation of (*E*)-3-(3-(pyridin-4-yl)acryloyl)-2*H*-chromen-2-one drivatives (7)

To a mixture of 3-acetyl coumarin drivatives **6** (0.12 mmol) and pyridine-4-carbaldehyde (0.12 mmol) in 3 ml *n*-butanol were added catalytic amount of piperidine (2 drops). The mixture was irradiated with microwaves at 150 °C for 30 min. After completion of the reactions, mixture was cooled and the precipitated solid was filtered off and washed with ethanol. The organic phase was evaporated and the residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (7:3) as the mobile phase to give the pure product.

4.3.1. (*E*)-3-(3-(Pyridin-4-yl)acryloyl)-2*H*-chromen-2-one (7a)

Yield 77%, yellow solid, mp 110–112 °C, IR υ_{max}/cm^{-1} (KBr): 1720, 1668 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 7.45 (t, 1H, H_6 coumarin, J = 8.0 Hz), 7.51 (d, 1H, H_8 coumarin, J = 8.0 Hz), 7.69–7.73 (m, 3H, H_b-pyridine and H_α vinylic), 7.77 (t, 1H, H_7 coumarin, J = 8.0 Hz), 7.86 (d, 1H, H_β vinylic, J = 16.4 Hz), 7.96 (d, 1H, H_5 coumarin, J = 8.0 Hz), 8.67 (d, 2H, H_a-pyridine, J = 4.6 Hz), 8.73 (s, 1H, H_4 coumarin). Anal. Calcd for $C_{17}H_{11}NO_3$: C, 73.64; H, 4.00; N, 5.05. Found: C, 73.28; H, 4.22; N, 5.17.

4.3.2. (*E*)-8-Methoxy-3-(3-(pyridin-4-yl)acryloyl)-2*H*-chromen-2-one (7b)

Yield 72%, yellow solid, mp 103–105 °C, IR υ_{max}/cm^{-1} (KBr): 1719, 1664 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.73 (s, 3H, OMe), 7.21–7.35 (m, 3H, $H_{5,6,7}$ coumarin) 7.82 (d, 1H, H_α vinylic, J = 16.3 Hz), 7.88 (d, 2H, H_b-pyridine, J = 4.6 Hz), 8.12 (d, 1H, H_β vinylic, J = 16.3 Hz), 8.74 (s, 1H, H_4 coumarin), 8.82 (d, 2H, H_a-pyridine, J = 4.6 Hz). Anal. Calcd for $C_{18}H_{13}NO_4$: C, 70.35; H, 4.26; N, 4.56. Found: C, 70.18; H, 4.41; N, 4.33.

4.3.3. (*E*)-6-Bromo-3-(3-(pyridin-4-yl)acryloyl)-2*H*-chromen-2-one 7c)

Yield 64%, yellow solid, mp 114–116 °C, IR υ_{max}/cm^{-1} (KBr): 1710, 1656 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 7.14 (d, 1H, H_8 coumarin, J = 8.7 Hz), 7.29 (d, 1H, H_7 coumarin, J = 8.7 Hz), 7.67 (d, 1H, H_α vinylic, J = 15.5 Hz), 7.88 (s, 1H, H_5 coumarin), 8.00 (m, 3H, H_b-pyridine and H_β vinylic), 8.71 (s, 1H, H_4 coumarin), 8.83 (d, 2H, H_a-pyridine, J = 4.6 Hz). Anal. Calcd for $C_{17}H_{10}BrNO_3$: C, 57.33; H, 2.83; N, 3.93. Found: C, 57.61; H, 2.52; N, 3.70.

4.4. General procedure for synthesis of pyridinium halide derivatives 3a–s

Dry acetonitrile (7 ml) was added to (*E*)-3-(3-(pyridin-4-yl)acryloyl)-2*H*-chromen-2-one drivatives **7** (1 equiv), and the mixture was dissolved by heating under reflux. Then appropriate benzyl halides (1.2 equiv) and a catalytic amount of KI were added. After heating under reflux for 2–4 h, it was left for cooling to room temperature. The precipitated solids were separated by filtration, washed with diethyl ether and dried. The resulting products were further purified if needed by flash chromatography employing chloroform/methanol (99:1) as the mobile phase to afford compounds **3a–s**.

4.4.1. (*E*)-1-(2-Fluorobenzyl)-4-(3-oxo-3-(2-oxo-2*H*-chromen-3-yl)prop-1-enyl)pyridinium chloride (3a)

Following Section 4.4, from compound **7a** (R = H, 1 mmol, 0.277 g) and 2-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 3 h, product **3a** was obtained, yield 72%, yellow solid, mp 246–248 °C, IR υ_{max}/cm^{-1} (KBr): 1721, 1666 (C=O), ^1H NMR (DMSO-d_6, 500 MHz), 5.95 (s, 2H, $-CH_2N^+$), 7.32–7.36 (m, 2H, $H_{4,6}$ phenyl), 7.47 (t, 1H, H_6 coumarin, J = 7.3 Hz), 7.50–7.54 (m, 2H, H_8 coumarin and H_3 phenyl), 7.62 (t, 1H, H_5 phenyl, J = 7.3 Hz), 7.80 (t, 1H, H_7 coumarin, J = 7.3 Hz), 7.83 (d, 1H, H_α vinylic, J = 16.0 Hz), 7.99 (d, 1H, H_5 coumarin, J = 7.3 Hz), 8.13 (d, 1H, H_β vinylic, J = 16.0 Hz), 8.46 (d, 2H, H_b-pyridine, J = 6.0 Hz), 8.82 (s, 1H, H_4 coumarin), 9.18 (d, 2H, H_a-pyridine, J = 6.0 Hz). EI-MS m/z (%) 386 (M^+, 11), 385 (M^+-1, 39), 363 (26), 277 (78), 248 (47), 220 (32), 173 (89), 109 (100), 83 (67). Anal. Calcd for $C_{24}H_{17}ClFNO_3$: C, 68.33; H, 4.06; N, 3.32. Found: C, 68.54; H, 3.82; N, 3.61.

4.4.2. (*E*)-1-(4-Fluorobenzyl)-4-(3-oxo-3-(2-oxo-2*H*-chromen-3-yl)prop-1-enyl)pyridinium chloride (3b)

Following Section 4.4, from compound **7a** (R = H, 1 mmol, 0.277 g) and 4-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 2 h, product **3b** was obtained, yield 90%, yellow solid, mp 213–215 °C, IR υ_{max}/cm^{-1} (KBr): 1724, 1675 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 5.90 (s, 2H, $-CH_2N^+$), 7.28–7.32 (m, 2H, $H_{2,6}$ phenyl), 7.44–7.53 (m, 3H, $H_{6,7,8}$ coumarin), 7.69–7.73 (m, 2H, $H_{3,5}$ phenyl), 7.81 (d, 1H, H_α vinylic, J = 16.4 Hz), 8.01 (d, 1H, H_5 coumarin, J = 7.2 Hz), 8.15 (d, 1H, H_β vinylic, J = 16.4 Hz), 8.49 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.86 (s, 1H, H_4 coumarin), 9.32 (d, 2H, H_a-pyridine, J = 6.4 Hz). Anal. Calcd for $C_{24}H_{17}ClFNO_3$: C, 68.33; H, 4.06; N, 3.32; Found: C, 68.24; H, 4.32; N, 3.65.

4.4.3. (*E*)-1-Benzyl-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (3c)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and benzyl chloride (1.2 mmol, 0.252 g), for 2 h, product **3c** was obtained, yield 75%, yellow solid, mp 255–257 °C, IR υ_{max}/cm^{-1} (KBr): 1720, 1679 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.95 (s, 3H, OMe), 5.88 (s, 2H, $-CH_2N^+$), 7.37–7.58 (m, 8H, $H_{5,6,7}$ coumarin and 5H phenyl), 7.82 (d, 1H, H_α vinylic, J = 15.6 Hz), 8.14 (d, 1H, H_β vinylic, J = 15.6 Hz), 8.48 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.82 (s, 1H, H_4 coumarin), 9.31 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 398 (M^+, 15), 397 (M^+-1, 52), 307 (74), 278 (26), 251 (28), 203 (33), 91 (100), 65 (31). Anal. Calcd for $C_{25}H_{20}ClNO_4$: C, 69.20; H, 4.65; N, 3.23. Found: C, 68.94; H, 4.73; N, 3.49.

4.4.4. (*E*)-1-(2-Fluorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl) pyridinium bromide (3d)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 2-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 3 h, product **3d** was obtained, yield 75%, yellow solid, mp 215–217 °C, IR υ_{max}/cm^{-1} (KBr): 1723, 1664 (C=O), ^1H NMR (DMSO-d_6, 500 MHz), 3.94 (s, 3H, OMe), 5.97 (s, 2H, $-CH_2N^+$), 7.32–7.39 (m, 3H, H_7 coumarin and $H_{4,6}$ phenyl), 7.46–7.53 (m, 3H, $H_{5,6}$ coumarin and H_5 phenyl), 7.63–7.65 (m, 1H, H_3 phenyl), 7.83 (d, 1H, H_α vinylic, J = 13.9 Hz), 8.14 (d, 1H, H_β vinylic, J = 13.9 Hz), 8.47 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.82 (s, 1H, H_4 coumarin), 9.21 (d, 2H, H_a-pyridine, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz) 56.2, 57.3, 115.8, 116.0, 116.8, 118.7, 121.2, 121.6, 124.5, 125.0, 125.2, 126.4, 131.4, 132.0, 134.6, 136.2, 143.9, 145.4, 146.3, 148.5, 150.8, 157.9, 161.4, 186.9. EI-MS m/z (%) 416 (M^+, 11), 415 (M^+-1, 50), 307 (83), 278 (35), 203 (65), 109 (100). Anal. Calcd for $C_{25}H_{19}BrFNO_4$: C, 60.50; H, 3.86; N, 2.82. Found: C, 60.37; H, 3.75; N, 2.94.

4.4.5. (*E*)-1-(3-Fluorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (3e)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 3-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 4 h, product **3e** was obtained, yield 80%, yellow solid, mp 222–224 °C, IR υ_{max}/cm^{-1} (KBr): 1726, 1661 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.99 (s, 3H, OMe), 5.83 (s, 2H, $-CH_2N^+$), 7.28 (t, 1H, H_5 phenyl, J = 7.3 Hz), 7.38–7.55 (m, 6H, $H_{5,6,7}$ coumarin and H phenyl$_{2,4,6}$), 7.82 (d, 1H, H_α vinylic, J = 16.4 Hz), 8.12 (d, 1H, H_β vinylic, J = 16.4 Hz), 8.46 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.78 (s, 1H, H_4 coumarin), 9.24 (d, 2H, H_a-pyridine, J = 6.4 Hz). Anal. Calcd for $C_{25}H_{19}ClFNO_4$: C, 66.45; H, 4.24; N, 3.10. Found: C, 66.71; H, 4.58; N, 2.86. EI-MS m/z (%) 416 (M^+, 3), 415 (M^+-1, 8), 308 (81), 307 (63), 203 (88), 109 (100), 83 (86).

4.4.6. (*E*)-1-(4-Fluorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (7f)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 4-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 2 h, product **3f** was obtained, yield 85%, yellow solid, mp 262–264 °C, IR υ_{max}/cm^{-1} (KBr): 1726, 1675 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.91 (s, 3H, OMe), 5.77 (s, 2H, $-CH_2N^+$), 7.20–7.24 (m, 2H, H_α vinylic and H_7 coumarin), 7.35–7.38 (m, 2H, $H_{2,6}$ phenyl),

7.44–7.47 (m, 2H, $H_{5,6}$ coumarin), 7.52–7.56 (m, 3H, H_β vinylic and $H_{3,5}$ phenyl), 8.12 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.57 (s, 1H, H_4 coumarin), 9.12 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 416 (M^+, 3), 415 (M^+-1, 9), 307 (91), 278 (30), 251 (31), 203 (35), 109 (100). Anal. Calcd for $C_{25}H_{19}ClFNO_4$: C, 66.45; H, 4.24; N, 3.10. Found: C, 66.68; H, 4.18; N, 3.35.

4.4.7. (*E*)-1-(2-Fluoro-6-nitrobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (3g)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 2-fluoro-6-nitrobenzyl chloride (1.2 mmol, 0.227 g), for 2 h, product **3g** was obtained, yield 70%, yellow solid, mp 223–225 °C, IR υ_{max}/cm^{-1} (KBr): 1728, 1679 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.96 (s, 3H, OMe), 6.20 (s, 2H, $-CH_2N^+$), 7.41 (t, 1H, H_6 coumarin, J = 7.8 Hz), 7.49 (d, 1H, H_7 coumarin, J = 7.8 Hz), 7.53 (d, 1H, H_5 coumarin, J = 7.8 Hz), 7.83–7.91 (m, 3H, H_α vinylic and $H_{4,5}$ phenyl), 8.11–8.15 (m, 2H, H_3 phenyl and H_β vinylic), 8.43 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.79 (s, 1H, H_4 coumarin), 9.12 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 461 (M^+, 2), 307 (90), 278 (62), 251 (24), 203 (55), 154 (67), 132 (100), 107 (69). Anal. Calcd for $C_{25}H_{18}ClFN_2O_6$: C, 60.43; H, 3.65; N, 5.64. Found: C, 60.26; H, 3.93; N, 5.77.

4.4.8. (*E*)-1-(2-Chlorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl) pyridinium chloride (3h)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 2-chlorobenzyl chloride (1.2 mmol, 0.193 g), for 2 h, product **3h** was obtained, yield 73%, yellow solid, mp 222–224 °C, IR υ_{max}/cm^{-1} (KBr): 1725, 1678 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.95 (s, 3H, OMe), 6.00 (s, 2H, $-CH_2N^+$), 7.37–7.60 (m, 7H, $H_{5,6,7}$ coumarin and 4H phenyl), 7.84 (d, 1H, H_α vinylic, J = 16.4 Hz), 8.15 (d, 1H, H_β vinylic, J = 16.4 Hz), 8.48 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.82 (s, 1H, H_4 coumarin), 9.18 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 434 (M^++2, 1), 432 (M^+, 3), 396 (41), 307 (67), 278 (22), 251 (21), 203 (22), 125 (100), 91 (96). Anal. Calcd for $C_{25}H_{19}Cl_2NO_4$: C, 64.11; H, 4.09; N, 2.99. Found: C, 64.36; H, 4.24; N, 3.21.

4.4.9. (*E*)-1-(3-Chlorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (3i)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and (3-chlorobenzyl chloride (1.2 mmol, 0.193 g), for 3 h, product **3i** was obtained, yield 80%, yellow solid, mp 213–215 °C, IR υ_{max}/cm^{-1} (KBr): 1726, 1679 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.95 (s, 3H, OMe), 5.85 (s, 2H, $-CH_2N^+$), 7.38–7.61 (m, 6H, $H_{5,6,7}$ coumarin and $H_{4,5,6}$ phenyl), 7.72 (s, 1H, H_2 phenyl), 7.82 (d, 1H, H_α vinylic, J = 16.4 Hz), 8.12 (d, 1H, H_β vinylic, J = 16.4 Hz), 8.46 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.79 (s, 1H, H_4 coumarin), 9.25 (d, 2H, H_a-pyridine, J = 6.4 Hz). Anal. Calcd for $C_{25}H_{19}Cl_2NO_4$: C, 64.11; H, 4.09; N, 2.99. Found: C, 64.33; H, 4.20; N, 2.78.

4.4.10. (*E*)-1-(2,3-Dichlorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl) pyridinium chloride (3j)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 2,3-dichlorobenzyl chloride (1.2 mmol, 0.234 g), for 4 h, product **3j** was obtained, yield 75%, brown solid, mp 240–242 °C, IR υ_{max}/cm^{-1} (KBr): 1725, 1664 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.96 (s, 3H, OMe), 6.15 (s, 2H, $-CH_2N^+$), 7.41 (d, 1H, H_7 coumarin, J = 9.0 Hz), 7.46–7.54 (m, 4H, $H_{5,6}$ coumarin and $H_{5,6}$ phenyl), 7.77 (d, 1H, H_4 phenyl, J = 7.2 Hz), 7.85 (d, 1H, H_α vinylic, J = 15.2 Hz), 8.16 (d, 1H, H_β vinylic, J = 15.2 Hz), 8.50 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.83 (s, 1H, H_4 coumarin), 9.18 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 471 (M^++4, 1), 469 (M^++2, 6), 467 (M^+, 9), 396 (15), 307 (47), 203 (31), 159 (100), 142 (33), 125 (27). Anal. Calcd for $C_{25}H_{18}Cl_3NO_4$: C, 59.72; H, 3.61; N, 2.79. Found: C, 59.94; H, 3.39; N, 2.55.

4.4.11. (*E*)-1-(3,4-Dichlorobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (3k)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 3,4-dichlorobenzyl chloride (1.2 mmol, 0.234 g), for 3 h, product **3k** was obtained, yield 79%, brown solid, mp 238–240 °C, IR υ_{max}/cm^{-1} (KBr): 1727, 1663 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.99 (s, 3H, OMe), 5.85 (s, 2H, $-CH_2N^+$), 7.45 (d, 1H, H_7 coumarin, J = 8.4 Hz), 7.52–7.64 (m, 3H, $H_{5,6}$ phenyl and H_6 coumarin), 7.79 (d, 1H, H_5 coumarin, J = 8.4 Hz), 7.86 (d, 1H, H_α vinylic, J = 15.2 Hz), 8.01 (s, 1H, H_2 phenyl), 8.17 (d, 1H, H_β vinylic, J = 15.2 Hz), 8.50 (d, 2H, H_b-pyridine, J = 6.2 Hz), 8.84 (s, 1H, H_4 coumarin), 9.29 (d, 2H, H_a-pyridine, J = 6.2 Hz). EI-MS m/z (%) 470 (M^++4, 1), 468 (M^++2, 6), 466 (M^+, 9), 307 (40), 278 (15), 251 (14), 203 (30), 163 (32), 161(94), 159 (100). Anal. Calcd for $C_{25}H_{18}Cl_3NO_4$: C, 59.72; H, 3.61; N, 2.79. Found: C, 59.45; H, 3.76; N, 2.51.

4.4.12. (*E*)-4-(3-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)-1-(2-methylbenzyl)pyridinium chloride (3l)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 2-methylbenzyl chloride (1.2 mmol, 0.168 g), for 2 h, product **3l** was obtained, yield 90%, yellow solid, mp 241–243 °C, IR υ_{max}/cm^{-1} (KBr): 1727, 1664 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 2.32 (s, 3H, CH_3), 3.95 (s, 3H, OMe), 5.92 (s, 2H, $-CH_2N^+$), 7.18 (d, 1H, H_3 phenyl, J = 7.0 Hz), 7.27–7.41 (m, 4H, $H_{6,7}$ coumarin and $H_{4,5}$ phenyl), 7.48 (d, 1H, H_6 phenyl, J = 7.0 Hz), 7.53 (d, 1H, H_5 coumarin, J = 7.2 Hz), 7.85 (d, 1H, H_α vinylic, J = 15.6 Hz), 8.15 (d, 1H, H_β vinylic, J = 15.6 Hz), 8.47 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.82 (s, 1H, H_4 coumarin), 9.11 (d, 2H, H_a-pyridine, J = 6.4 Hz). Anal. Calcd for $C_{26}H_{22}ClNO_4$: C, 69.72; H, 4.95; N, 3.13. Found: C, 69.51; H, 5.18; N, 3.32.

4.4.13. (*E*)-4-(3-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)-1-(3-methylbenzyl)pyridinium chloride (3m)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 3-methylbenzyl chloride (1.2 mmol, 0.168 g), for 3 h, product **3m** was obtained, yield 92%, yellow solid, mp 223–226 °C, IR υ_{max}/cm^{-1} (KBr): 1719, 1674 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 2.31 (s, 3H, CH_3), 3.95 (s, 3H, OMe), 5.90 (s, 2H, $-CH_2N^+$), 7.24 (s, 1H, H_2 phenyl), 7.33–7.53 (m, 6H, $H_{5,6,7}$ coumarin, $H_{4,5,6}$ phenyl), 7.82 (d, 1H, H_α vinylic, J = 15.6 Hz), 8.12 (d, 1H, H_β vinylic, J = 15.6 Hz), 8.45 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.81 (s, 1H, H_4 coumarin), 9.25 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 412 (M^+, 2), 307 (23), 278 (13), 251 (10), 203 (8), 127 (13), 105 (100), 77 (24). Anal. Calcd for $C_{26}H_{22}ClNO_4$: C, 69.72; H, 4.95; N, 3.13. Found: C, 69.55; H, 4.71; N, 3.45.

4.4.14. (*E*)-4-(3-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)-1-(4-methylbenzyl)pyridinium chloride (3n)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 4-methylbenzyl chloride (1.2 mmol, 0.168 g), for 2 h, product **3n** was obtained, yield 95%, yellow solid, mp 250–252 °C, IR υ_{max}/cm^{-1} (KBr): 1715, 1678 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 2.31 (s, 3H, CH_3), 3.95 (s, 3H, OMe), 5.78 (s, 2H, $-CH_2N^+$), 7.26 (d, 2H, $H_{2,6}$ phenyl, J = 8.0 Hz), 7.37.7.52 (m, 5H, $H_{5,6,7}$ coumarin and $H_{3,5}$ phenyl), 7.82 (d, 1H, $H_\alpha$vinylic, J = 16.2 Hz), 8.10 (d, 1H, H_β vinylic, J = 16.2 Hz), 8.43 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.77 (s, 1H, H_4 coumarin), 9.20 (d, 2H, H_a-pyridine, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 20.6, 56.2, 95.3, 116.8, 118.7, 121.6, 124.5, 125.0, 126.4, 128.7, 129.7, 131.1, 134.4, 136.2, 138.9, 142.0, 144.9, 146.3, 148.4, 150.4, 158.0, 186.9. Anal. Calcd for $C_{26}H_{22}ClNO_4$: C, 69.72; H, 4.95; N, 3.13. Found: C, 69.61; H, 4.77; N, 3.31.

4.4.15. (*E*)-1-(3-Cyanobenzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium bromide (3o)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 3-cyanobenzyl chloride (1.2 mmol, 0.181 g), for 3 h, product **3o** was obtained, Yield 83%, Yellow solid, mp 262–264 °C; IR υ_{max}/cm^{-1} (KBr): 1718, 1677 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.95 (s, 3H, OMe), 5.93 (s, 2H, $-CH_2N^+$), 7.39 (t, 1H, H_5 phenyl, J = 6.6 Hz), 7.47 (d, 1H, H_7 coumarin, J = 7.5 Hz), 7.52 (d, 1H, H_5 coumarin, J = 7.5 Hz), 7.67 (t, 1H, H_6 coumarin, J = 7.5 Hz), 7.82 (d, 1H, H_α vinylic, J = 16.8 Hz), 7.89–7.93 (m, 2H, $H_{4,6}$ phenyl), 8.10 (s, 1H, H_2 phenyl), 8.13 (d, 1H, H_β vinylic, J = 16.8 Hz), 8.47 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.79 (s, 1H, H_4 coumarin), 9.27 (d, 2H, H_a-pyridine, J = 6.4 Hz). EI-MS m/z (%) 423 (M^+, 6), 422 (M^+-1, 19), 307 (100), 278 (47), 251 (46), 203 (46), 116 (65). Anal. Calcd for $C_{26}H_{19}BrN_2O_4$: C, 62.04; H, 3.80; N, 5.57. Found: C, 62.31; H, 3.69; N, 5.78.

4.4.16. (*E*)-1-(4-(Chloromethyl)benzyl)-4-(3-(8-methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)pyridinium chloride (3p)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 1,4-bis(chloromethyl)benzene (1.2 mmol, 0.578 g), for 4 h, product **3p** was obtained, yield 72%, brown solid, mp 222–224 °C, IR υ_{max}/cm^{-1} (KBr): 1716, 1681 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.95 (s, 3H, OMe), 4.77 (s, 2H, $-CH_2Cl$), 5.87 (s, 2H, $-CH_2N^+$), 7.38–7.55 (m, 7H, $H_{5,6,7}$ coumarin and 4H phenyl), 7.81 (d, 1H, H_α vinylic, J = 16.4 Hz), 8.12 (d, 1H, H_β vinylic, J = 16.4 Hz), 8.45 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.78 (s, 1H, H_4 coumarin), 9.23 (d, 2H, H_a-pyridine, J = 6.4 Hz). Anal. Calcd for $C_{26}H_{21}Cl_2NO_4$: C, 64.74; H, 4.39; N, 2.90. Found: C, 64.43; H, 4.48; N, 2.57.

4.4.17. (*E*)-4-(3-(8-Methoxy-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)-1-(4-methoxybenzyl)pyridinium chloride (3q)

Following Section 4.4, from compound **7b** (R = 8-OMe, 1 mmol, 0.307 g) and 4-methoxybenzyl chloride (1.2 mmol, 0.187 g), for 2 h, product **3q** was obtained, yield 80%, brown solid, mp 193–195 °C, IR υ_{max}/cm^{-1} (KBr): 1727, 1677 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 3.76 (s, 3H, OMe), 3.95 (s, 3H, OMe coumarin), 5.89 (s, 2H, $-CH_2N^+$), 7.00 (d, 2H, $H_{3,5}$ phenyl, J = 8.4 Hz), 7.40 (t, 1H, H_6 coumarin, J = 8.0 Hz), 7.48 (d, 1H, H_7 coumarin, J = 8.0 Hz), 7.53 (d, 1H, H_5 coumarin, J = 8.0 Hz), 7.56 (d, 2H, $H_{2,6}$ phenyl, J = 8.4 Hz), 7.81 (d, 1H, H_α vinylic, J = 16.4 Hz), 8.12 (d, 1H, H_β vinylic, J = 16.4 Hz), 8.45 (d, 2H, H_b-pyridine, J = 5.8 Hz), 8.81 (s, 1H, H_4 coumarin), 9.26 (d, 2H, H_a-pyridine, J = 5.8 Hz). EI-MS m/z (%) 428 (M^+, 9), 427 (M^+-1, 31), 307 (47), 203 (54), 121 (100), 106 (65), 77 (65). Anal. Calcd for $C_{26}H_{22}ClNO_5$: C, 67.31; H, 4.78; N, 3.02. Found: C, 67.01; H, 5.12; N, 3.34.

4.4.18. (*E*)-4-(3-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)-1-(2-fluorobenzyl)pyridinium chloride (3r)

Following Section 4.4, from compound **7c** (R = 6-Br, 1 mmol, 0.356 g) and 2-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 4 h, product **3r** was obtained, yield 78%, brown solid, mp 218–220 °C, IR υ_{max}/cm^{-1} (KBr): 1752, 1664 (C=O), ^1H NMR (DMSO-d_6, 400 MHz), 5.98 (s, 2H, $-CH_2N^+$), 7.30–7.35 (m, 2H, $H_{4,6}$ phenyl), 7.49–7.54 (m, 2H, H_8 coumarin and H_3 phenyl), 7.64 (t, 1H, H_5 phenyl, J = 7.4 Hz), 7.84 (d, 1H, H_α vinylic, J = 15.6 Hz), 7.94 (dd, 1H, H_7 coumarin, J = 2.4, 9.2 Hz), 8.11 (d, 1H, H_β vinylic, J = 15.6 Hz), 8.25 (d, 1H, H_5 coumarin, J = 2.4 Hz), 8.48 (d, 2H, H_b-pyridine, J = 6.4 Hz), 8.80 (s, 1H, H_4 coumarin), 9.22 (d, 2H, H_a-pyridine, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 57.6, 115.9, 116.1, 116.6, 118.7, 120.2, 125.3, 125.5, 126.5, 131.5, 132.2, 132.6, 134.5, 136.6, 136.9, 144.6, 145.5, 146.9, 150.8, 153.7, 157.9, 159.5, 161.5, 186.9. EI-MS m/z (%) 466 (M^++2, 9), 464 (M^+, 11), 357 (15), 248 (12), 109 (100), 83 (18). Anal. Calcd for $C_{24}H_{16}BrClFNO_3$: C, 57.57; H, 3.22; N, 2.80. Found: C, 57.44; H, 3.51; N, 2.97.

4.4.19. (*E*)-4-(3-(6-Bromo-2-oxo-2*H*-chromen-3-yl)-3-oxoprop-1-enyl)-1-(4-fluorobenzyl)pyridinium chloride (3s)

Following Section 4.4, from compound **7c** (R = 6-Br, 1 mmol, 0.356 g) and 4-fluorobenzyl chloride (1.2 mmol, 0.173 g), for 4 h, product **3s** was obtained, yield 86%, yellow solid, mp 252–254 °C, IR υ_{max}/cm^{-1} (KBr): 1745, 1663 (C=O), ^1H NMR (DMSO-d_6, 400 MHz) 5.86 (s, 2H, $-CH_2N^+$), 7.28–7.33 (m, 2H, $H_{2,6}$ phenyl), 7.51 (d, 1H, H_8 coumarin, J =8.8 Hz), 7.66–7.70 (m, 2H, $H_{3,5}$ phenyl), 7.83 (d, 1H, H_α vinylic, J = 15.6 Hz), 7.94 (dd, 1H, H_7 coumarin, J = 2.4, 8.8 Hz), 8.10 (d, 1H, H_β vinylic, J = 15.6 Hz), 8.25 (d, 1H, H_5 coumarin, J = 2.4 Hz), 8.47 (d, 2H, H_b-pyridine, J = 6.8 Hz), 8.78 (s, 1H, H_4 coumarin), 9.28 (d, 2H, H_a-pyridine, J = 6.8 Hz). EI-MS m/z (%) 466 (M^++2, 27), 464 (M^+, 30), 357 (61), 356 (51), 355 (51), 248 (40), 109 (100), 83 (45). Anal. Calcd for $C_{24}H_{16}BrClFNO_3$ C, 57.57; H, 3.22; N, 2.80. Found: C, 57.39; H, 3.42; N, 2.47.

4.5. Molecular docking studies

For docking purpose, the pdb structure of 3I6Z was retrieved from the Brookhaven protein database[38] as a complex bound with inhibitor N-sacharinohexyl-galantamine. Then, the water molecules and co-crystallized ligand were removed from the protein. The structure of target compound was constructed by Marvine-Sketch which was converted to 3D structure using Openbabel version (2.3.1). Finally the pdbqt formats (The format is used by docking software) of the protein and ligand were prepared using Autodock Tools (ver. 1.5.4)[39] using default parameters. Docking was carried out using Autodock vina (ver. 1.1.1) with default parameters and exhaustiveness was set at 100. The docking site was defined by establishing a box at geometrical center of the native ligand present in the above mentioned PDB structure with the dimensions of 36, 34, 56, that covering all binding sites occurred in the active site of the enzyme. The grid spacing of 0.375 Å was used and the coordinates x, y, z for the center of grid box were 1.75, 63.1, 67.11, respectively. Finally the lowest energy conformations were selected for analyzing the interactions between the AChE and inhibitor. The results were shown using PosViewWeb 1.97.0[40] and Chimera 1.6.[41]

4.6. AChE and BuChE inhibition assay

Acetylcholinesterase (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel,* 1000 unit), butylcholinesterase (BuChE, E.C. 3.1.1.8, from equine serum), butylthiocholine iodode (BTC), was purchased from Sigma–Aldrich. 5,5′-Dithiobis-(2-nitrobenzoic acid), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydrogen carbonate, and acetylthiocholine iodide were obtained from Fluka. The stock solutions of tested compounds were prepared in a mixture of 1 ml DMSO and 9 ml methanol followed by dilution in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to obtain final assay concentrations. The previously reported method was applied to evaluate enzyme inhibition activity of tested compounds. All solution temperatures were adjusted to 25 °C prior to use. Five different concentrations of each compound were tested in triplicate to obtain 20% to 80% inhibition of AChE and BuChE activity. The assay medium contained 3 ml of 0.1 M phosphate buffer pH 8.0, 100 μl of 0.01 M 5,5′-dithio-bis(2-nitrobenzoic acid), 100 μl of 2.5 unit/ml enzyme solution (AChE, E.C. 3.1.1.7, Type V-S, lyophilized powder, from *electric eel*) (Sigma Chemical). 100 μl of each tested compounds, were added to the assay medium and incubated at 25 °C for 15 min followed by adding 20 μl of substrate (acetylthiocholine iodide). After that the rate of absorbance change was measured at 412 nm for 6 minutes. The blank reading solution was used to justify non-enzymatic hydrolysis of substrate during the assay. The blank solution contained 3 ml buffer, 200 μl water, 100 μl DTNB

and 20 μl substrate. As a reference, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The rate of the substrate enzymatic hydrolysis was calculated, and inhibition percent of the test compounds was determined. The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration Vs percent of inhibition). Spectrophotometric measurements were performed on a UV-2100 Rayleigh Double Beam Spectrophotometer. The same method was used for BuChE inhibition assay.

4.7. Antioxidant activity

4.7.1. FRAP assay

The modified method was taken to evaluate the antioxidant activity of the synthesized compounds using the FRAP assay.[42] 300 mM acetate buffer (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6 and 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM hydrochloric acid and 20 mM ferric chloride hexahydrate solution were mixed. The temperature of the solution was raised to 37 °C before the assay. After that 300 μl of the compound stock solution was added thereto in the dark and the change of absorbance was monitored at the 593 nm. The concentration of the reduced ferrous ion (Fe^{2+}) in the solution as colored ferrous tripyridyltriazine complex was measured. Results are expressed in μM ferrous/g dry mass according to the plotted standard curve of ferrous sulfate. Ascorbic acid and BHT were used as references.

4.7.2. DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

To assess the scavenging ability of the compounds the reported DPPH method was applied.[43] The target compounds were tested within the range of 0–25 μg/ml in methanol. To 2.5 ml of tested compound in 5 different concentrations, 1 ml of 0.3 mM DPPH ethanol solution was added. In the meantime 1 ml of methanol was added and the solution was allowed to react for 30 min in the dark at room temperature. The change of the absorbance was read at 518 nm. The reading blank consisted of 2.5 ml of tested compound and 1 ml of methanol meanwhile the mixture of 1 ml DPPH and 2.5 ml of methanol was used as negative control. The percent of the antioxidant activity was calculated as follow:% Inhibition=$[(A_B-A_A)/A_B] \times 100$. Where: A_B: absorption of blank sample, A_A: absorption of tested samples. The IC_{50} value was calculated and compared with ascorbic acid as reference.

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References and notes

1. Terry, A. V., Jr.; Buccafusco, J. J. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 821.
2. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
3. Shvaloff, A.; Neuman, E.; Guez, D. *Psychopharmacol. Bull.* **1996**, *32*, 343.
4. Wilcock, G. K. *Neurodegeneration* **1996**, *5*, 505.
5. Perry, E. K. *Br. Med. Bull.* **1986**, *42*, 63.
6. Bartus, R. T.; Dean, R. L., III; Beer, B.; Lippa, A. S. *Science* **1982**, *217*, 408.
7. Bullock, R.; Dengiz, A. *Int. J. Clin. Pract.* **2005**, *59*, 817.
8. Darvesh, S.; Hopkins, D. A.; Geula, C. *Nat. Rev. Neurosci.* **2003**, *4*, 131.
9. Eskander, M. F.; Nagykery, N. G.; Leung, E. Y.; Khelghati, B.; Geula, C. *Brain Res.* **2005**, *1060*, 144.
10. Decker, M.; Krauth, F.; Lehmann, J. *Bioorg. Med. Chem.* **1966**, *2006*, 14.
11. Greig, N. H.; Utsuki, T.; Ingram, D. K.; Wang, Y.; Pepeu, G.; Scali, C.; Yu, Q. S.; Mamczarz, J.; Holloway, H. W.; Giordano, T.; Chen, D.; Furukawa, K.; Sambamurti, K.; Brossi, A.; Lahiri, D. K. *Proc. Natl. Acad. Sci. U.S.A* **2005**, *102*, 17213.
12. Marcelo, F.; Silva, F. V.; Goulart, M.; Justino, J.; Sinay, P.; Bleriot, Y.; Rauter, A. P. *Bioorg. Med. Chem.* **2009**, *17*, 5106.
13. Lane, R. M.; Potkin, S. G.; Enz, A. *Int. J. Neuropsychopharmacol.* **2006**, *9*, 101.
14. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
15. Selkoe, D. J. *Annu. Rev. Neurosci.* **1994**, *17*, 489.
16. Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, J. *Neuron* **1996**, *16*, 881.
17. Castro, A.; Martinez, A. *Mini. Rev. Med. Chem.* **2001**, *1*, 267.
18. Giacobini, E. *Neurochem. Res.* **2003**, *28*, 515.
19. Lee, S. J.; Lee, U. S.; Kim, W. J.; Moon, S. K. *Mol. Med. Report* **2011**, *4*, 337.
20. Huang, X. Y.; Shan, Z. J.; Zhai, H. L.; Su, L.; Zhang, X. Y. *Chem. Biol. Drug Des.* **2011**, *78*, 651.
21. Bahadir, O.; Citoglu, G. S.; Ozbek, H.; Dall'Acqua, S.; Hosek, J.; Smejkal, K. *Fitoterapia* **2011**, *82*, 454.
22. Huang, L.; Yuan, X.; Yu, D.; Lee, K. H.; Chen, C. H. *Virology* **2005**, *332*, 623.
23. Hwu, J. R.; Singha, R.; Hong, S. C.; Chang, Y. H.; Das, A. R.; Vliegen, I.; De, C. E.; Neyts, J. *Antiviral Res.* **2008**, *77*, 157.
24. Kumar, R.; Saha, A.; Saha, D. *Fitoterapia* **2012**, *83*, 230.
25. Arshad, A.; Osman, H.; Bagley, M. C.; Lam, C. K.; Mohamad, S.; Zahariluddin, A. S. *Eur. J. Med. Chem.* **2011**, *46*, 3788.
26. Roussaki, M.; Kontogiorgis, C. A.; Hadjipavlou-Litina, D.; Hamilakis, S.; Detsi, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3889.
27. Sashidhara, K. V.; Kumar, A.; Chatterjee, M.; Rao, K. B.; Singh, S.; Verma, A. K.; Palit, G. *Bioorg. Med. Chem. Lett.* **1937**, *2011*, 21.
28. Zhou, X.; Wang, X. B.; Wang, T.; Kong, L. Y. *Bioorg. Med. Chem.* **2008**, *16*, 8011.
29. Fallarero, A.; Oinonen, P.; Gupta, S.; Blom, P.; Galkin, A.; Mohan, C. G.; Vuorela, P. M. *Pharmacol. Res.* **2008**, *58*, 215.
30. Shen, Q.; Peng, Q.; Shao, J.; Liu, X.; Huang, Z.; Pu, X.; Ma, L.; Li, Y. M.; Chan, A. S.; Gu, L. *Eur. J. Med. Chem.* **2005**, *40*, 1307.
31. Soto-Ortega, D. D.; Murphy, B. P.; Gonzalez-Velasquez, F. J.; Wilson, K. A.; Xie, F.; Wang, Q.; Moss, M. A. *Bioorg. Med. Chem.* **2011**, *19*, 2596.
32. Anand, P.; Singh, B.; Singh, N. *Bioorg. Med. Chem.* **2012**, *20*, 1175.
33. (a) Nadri, H.; Pirali-Hamedani, M.; Shekarchi, M.; Abdollahi, M.; Sheibani, V.; Amanlou, M.; Shafiee, A.; Foroumadi, A. *Bioorg. Med. Chem.* **2010**, *18*, 6360; (b) Iimura, Y.; Kosasa, T. **2004**, US 6,706,741 B2.
34. (a) Khoobi, M.; Foroumadi, A.; Emami, S.; Safavi, M.; Dehghan, Gh.; Alizadeh, B. H.; Ramazani, A.; Ardestani, S. K.; Shafiee, A. *Chem. Biol. Drug Des.* **2011**, *78*, 580–586; (b) Khoobi, M.; Emami, S.; Dehghan, Gh.; Foroumadi, A.; Ramazani, A.; Shafiee, A. *Arch. Pharm. Chem. Life Sci.* **2011**, *344*, 588–594; (c) Khoobi, M.; Ramazani, A.; Foroumadi, A.; Hamadi, H.; Hojjati, Z.; Shafiee, A. *J. Iran. Chem. Soc.* **2011**, *8*, 1036–1042; (d) Khoobi, M.; Ramazani, A.; Foroumadi, A.; Emami, S.; Jafarpour, F.; Mahyari, A.; Ślepokura, K.; Lis, T.; Shafiee, A. *Helvetica Chimica Acta* **2012**, *95*, 660–671; (e) Khoobi, M.; Alipour, M.; Zarei, S.; Jafarpour, F.; Shafiee, A. *Chem. Commun.* **2012**, *48*, 2985–2987.
35. Khode, S.; Maddi, V.; Aragade, P.; Palkar, M.; Ronad, P. K.; Mamledesai, S.; Thippeswamy, A. H.; Satyanarayana, D. *Eur. J. Med. Chem.* **2009**, *44*, 1682.
36. Trott, O.; Olson, A. J. *J. Comput. Chem.* **2010**, *31*, 455.
37. Branduardi, D.; Gervasio, F. L.; Cavalli, A.; Recanatini, M.; Parrinello, M. *J. Am. Chem. Soc.* **2005**, *127*, 9147.
38. http://www.rcsb.org.
39. http://autodock.scripps.edu/resources/adt.
40. PoseViewWeb 1.97.0, http://poseview.zbh.uni-hamburg.de/poseview/wizard. 2012.
41. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.
42. Benzie, I. F. F.; Strain, J. J. *Meth. Enzymol.* **1999**, *299*, 15.
43. Choi, W. C.; Kim, S. C.; Hwang, S. S.; Choi, B. K.; Ahn, H. J.; Lee, M. Y.; Park, S. H.; Kim, S. K. *Plant Sci.* **2002**, *163*, 1161.