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SPECIAL ISSUE

Discovery of Potent Dual Binding Site Acetylcholinesterase Inhibitors via Homo- and Heterodimerization of Coumarin-Based Moieties

Leonardo Pisani,*[a] Marco Catto,[a] Annalisa De Palma,[b] Roberta Farina,[a] Saverio Cellamare,[a] and Cosimo D. Altomare[a]

Acetylcholinesterase (AChE) inhibitors still comprise the majority of the marketed drugs for Alzheimer's disease (AD). The structural arrangement of the enzyme, which features a narrow gorge that separates the catalytic and peripheral anionic subsites (CAS and PAS, respectively), inspired the development of bivalent ligands that are able to bind and block the catalytic activity of the CAS as well as the role of the PAS in beta amyloid (Aβ) fibrillogenesis. With the aim of discovering novel AChE dual binders with improved drug-likeness, homo- and heterodimers containing 2*H*-chromen-2-one building blocks were developed. By exploring diverse linkages of neutral and protonatable amino moieties through aliphatic spacers of different length, a nanomolar bivalent AChE inhibitor was identified (3-[2-({4-[(dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)ethoxy]-6,7-dimethoxy-2*H*-chromen-2-one (**6d**), IC_{50} = 59 nM) from originally weakly active fragments. To assess the potential against AD, the disease-related biological properties of **6d** were investigated. It performed mixed-type AChE enzyme kinetics (inhibition constant K_i = 68 nM) and inhibited Aβ self-aggregation. Moreover, it displayed an outstanding ability to protect SH-SY5Y cells from $Aβ_{1-42}$ damage.

Introduction

Alzheimer's disease (AD) is a life-threatening disease that accounts for 60–80% of cases of dementia and that is the sixth-leading cause of death in the United States.[1] In most cases, the definitive diagnosis comes at a late stage of the disease because clinical symptoms are difficult to recognize and AD-related biomarkers are not widely accepted.[2] The histopathologic hallmarks are neurofibrillary tangles and amyloid plaques. Brain lesions appear in cholinergic regions that serve memory, language, and learning skills, and thus impair daily activities and cognitive functions.[3] On this basis, current standard therapies are based on acetylcholinesterase (AChE) inhibitors (that is, rivastigmine, galantamine, and donepezil) aimed at counteracting cognitive decline through the restoration of adequate neurotransmitter levels (acetylcholine, ACh).[4] Apart from this, the most recently marketed drug (that is, memantine) is able to decrease glutamate-mediated excitotoxicity with a different mechanism, namely *N*-methyl-D-aspartate (NMDA) receptor antagonism.[5] Both AChE inhibition and NMDA-receptor blocking provide merely palliative relief of symptoms without hampering the progression of the disease. Hence, the development of effective therapies is urgently needed.[6] Much is known about the histopathology of AD, although the mechanism has not been fully elucidated yet because of its complex multifactorial nature.[7] Among others,[8] the cholinergic hypothesis still remains valuable.[9] AChE is a serine hydrolase that is responsible for catabolic deacetylation of ACh.[10,11] X-Ray studies of the enzyme in complex with both reversible[12] and irreversible inhibitors revealed the presence of two anionic binding regions, termed the catalytic anionic subsite (CAS) and peripheral anionic subsite (PAS).[13] The former is closer to the catalytic triad and binds the substrate and other positively charged ligands (for example, edrophonium).[14] An aromatic gorge separates the CAS from the PAS, where the binding of gorge-spanning bisquaternary ligands occurs through π–cation interactions.[15,16] Increasing lines of evidence support a PAS chaperone-like activity during amyloid deposition.[17] The three-dimensional structural arrangement encourages the design of dual binding site (DBS) inhibitors,[18] namely compounds that are able to occupy the two anionic sites and block catalytic and fibrillogenic activity; thus, we envisage a renewed interest in the oldest AD-validated target. Structural analysis revealed key similarities between CAS and PAS, which share the presence of tryptophan residues (Trp86 and Trp286 in CAS and PAS of human AChE, respectively) that are able to establish π–π and π–cation interactions.[19,20]

[a] *Dr. L. Pisani, Prof. M. Catto, Dr. R. Farina, Prof. S. Cellamare, Prof. C. D. Altomare*
Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", via E. Orabona 4, 70125 Bari (Italy)
E-mail: leonardo.pisani@uniba.it

[b] *Prof. A. De Palma*
Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari "Aldo Moro", via E. Orabona 4, 70125 Bari (Italy)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/cmdc.201700282.

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Our research group has long been involved in the design and synthesis of AChE inhibitors[21] with a particular focus on bivalent ligands[22] and we have proved the ability of the 2*H*-chromen-2-one core to fit the enzymatic site,[23,24] as also demonstrated by others.[25] The homo- and heterodimerization approach has been extensively applied to the design of DBS bifunctional inhibitors by connecting two moieties through a spacer of appropriate length and flexibility. In most cases, the repeating monomer or at least one of the two fragments is derived from a smaller active compound (for example, tacrine)[26] or a more complex known inhibitor after molecular simplification.[27] Depending on the physicochemical features, the bridge connecting the two heads may produce additional binding interactions in the mid-gorge region.[28]

The tethering approach might improve binding affinities and lead to more active compounds than the original units. In fact, the linker can decrease the desolvation penalty if simpler and more hydrophilic monomers are joined in more lipophilic compounds and promotes the "chelate effect,"[29] which results in a thermodynamic stability gain for the protein–ligand complex. If two fragments that are able to interact simultaneously with different binding sites are present in the same molecule, the binding of one head drives the binding of the distal tail by increasing the binding probability for the second fragment or through the "proximity and orientation" effect,[30] which decreases the entropic penalty upon binding a larger bifunctional ligand with respect to two simpler monomers.

In previous reports, we identified an edrophonium–coumarin conjugate endowed with outstanding potency in the picomolar range[23] and studied the isosteric replacement of its heterocyclic core.[31] In this work, we aimed to improve the drug-like properties by means of removing toxicophore alerts and metabolic reactive groups, namely the aniline and the phenolic groups present in the edrophonium fragment. Thus, structural modifications were planned by scaffold hopping replacement of the polar and neutral 5-(dimethylamino)benzene-1,3-diol with a protonatable 4-((dimethylamino)methyl)-7-hydroxy-2*H*-chromen-2-one building block (Figure 1). On this basis, the design of new achiral bivalent ligands containing at least one basic monomer was envisaged through a dimerization strategy of connecting different hydroxycoumarin moieties with a flexible polymethylene linker anchored to the most favorable 3-, 6-, and 7- positions, as anticipated earlier.[23]

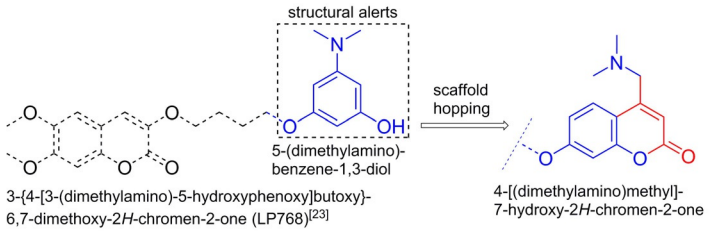
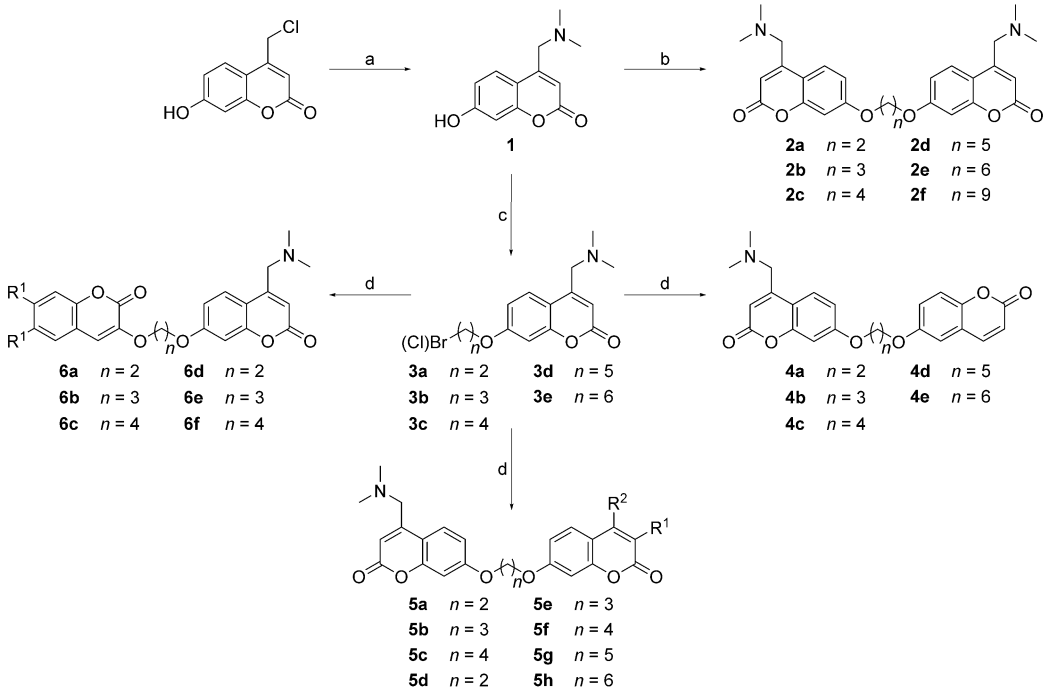


Figure 1. Scaffold hopping modifications to remove metabolic and toxic liabilities from the previous hit compound (previously described in ref. [23] as compound **13**).

Results and Discussion

Chemistry

The simple and straightforward synthesis of the desired compounds **2a–f**, **4a–e**, **5a–h** and **6a–f** was accomplished as illus-



Scheme 1. *Reagents and conditions*: a) 2.0 N *N*,*N*-dimethylamine in THF, dry THF, room temperature, 6 H, b) suitable dibromoalkane, Cs_2CO_3, KI, dry acetonitrile, 160 °C, microwave (MW), 30 min; c) 1-bromo-2-chloroethane or suitable dibromoalkane, Cs_2CO_3, KI, dry acetone, 130 °C, MW, 30 min; d) suitable hydroxycoumarin, Cs_2CO_3, KI, dry acetone, 130 °C, MW, 30 min.

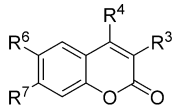
trated in Scheme 1. The initial monomer **M5** (**1**) was obtained by treating previously described 4-(chloromethyl)-7-hydroxy-2*H*-chromen-2-one[32] with a commercially available solution of *N*,*N*-dimethylamine in THF. The reaction of suitable linear dibromoalkanes with excess **M5** (**1**) under microwave irradiation afforded derivatives **2a**–**f**. Alkylation of **M5** (**1**) with an excess dibromo(chloro)alkane, in the presence of Cs_2CO_3 as the base and KI as the catalyst, yielded the monobromo(chloro) intermediates **3a**–**e**, which underwent nucleophilic substitution with suitable hydroxycoumarins under the standard microwave-assisted protocol to furnish the heterodimers **4a**–**e**, **5a**–**h**, and **6a**–**f**.

Biological assays

Monomeric hydroxycoumarins **M1**–**6** and dimers **2a**–**f**, **4a**–**e**, **5a**–**h**, and **6a**–**f** were screened in vitro as inhibitors of electric eel acetylcholinesterase (*ee*AChE) and butyrylcholinesterase from horse serum (*hs*BChE) by using the well-known Ellman's spectrophotometric method.[33] Data are reported in Tables 1 and 2 and are expressed as the half-maximal inhibitory concentration (IC_{50}) or as a percentage of inhibition at 10 μM concentration for the least active compounds.

It can be inferred from the data in Table 1 that all coumarin-based monomers were moderately active as AChE inhibitors,

Table 1. Cholinesterase inhibition data for the initial monomeric hydroxycoumarins **M1**–**6**.



Compd	R^3	R^4	R^6	R^7	Inhibition [%][a] AChE[b]	BChE[c]
M1	H	H	H	OH	43	13
M2	H	H	OH	H	38	32
M3	OH	H	H	H	49	21
M4	Cl	Me	H	OH	50	40
M5	H	$CH_2N(CH_3)_2$	H	OH	50	48
M6	OH	H	OMe	OMe	47	15

[a] Determined at 10 μM; values are means of three independent experiments; SEM < 10%. [b] AChE from electric eel. [c] BChE from horse serum.

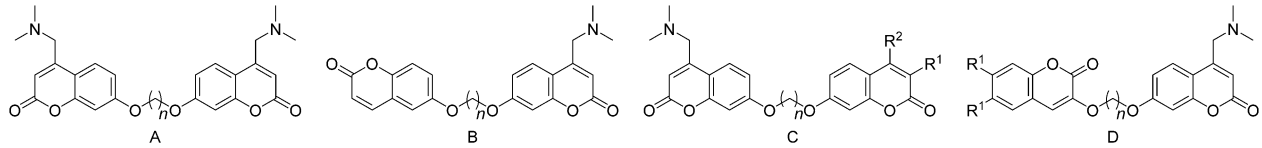
with IC_{50} values ≥ 10 μM, and displayed poor selectivity against BChE, with the exception of basic compound **M5** (**1**), which proved to be equipotent on both targets (50 and 48% inhibition toward AChE and BChE, respectively).

Interestingly, a remarkable increase in AChE inhibitory potencies (Table 2) was shown by the bivalent compounds, the binding of which to the target protein is thermodynamically supported by the so-called "chelate effect." All building blocks employed (that is, the basic coumarin moiety, as well as the planar and neutral fragments) might establish cation–π or π–π stacking interactions with the aromatic residues characterizing the PAS and CAS pockets in AChE. This structural framework is missing in the other cholinesterase (BChE), which might account for the observed selectivity, with a few exceptions (**4d** and **4e**).[34,35]

In symmetric ligands **2a**–**f**, the spacer was increased from 2 to 9 methylene groups in order to study its influence on the binding affinities. The shorter linker produced the most potent homodimeric AChE inhibitor, **2a**, which was endowed with a sub-micromolar affinity (IC_{50} = 0.87 μM) and a good selectivity over BChE. Further spacer homologation in ligands **2b**–**d** dramatically decreased the AChE inhibition until the chain was five carbon atoms long. Surprisingly, with addition of an additional methylene unit, an active compound was obtained (**2e**, IC_{50} = 1.98 μM). No clear structure–affinity relationships can be derived from this, because the introduction of a longer chain in derivative **2f** resulted in a weakly active compound again.

In the heterodimeric series, the influence of the spacer length was different depending on the attachment point of the neutral coumarin fragment. Irrespective of the coumarin substitution pattern, at least one compound endowed with a sub-micromolar activity toward AChE was found in each class, depending on the spacer length. Among the compounds of general structure B, bearing a 6-substituted coumarin moiety, the AChE inhibitory potency showed a nonlinear evolution because it increased as the polymethylene chain was lengthened from 2 to 4 units (**4a** < **4b** < **4c**) and then dropped down as the length moved from 4 to 6 units (**4c** > **4d** > **4e**). The same trend was observed if the tether anchoring position was shifted from the coumarin 6- to 7-position (general structure C, **5a** < **5b** and **5b** > **5c**). In the presence of lipophilic substituents at the 3- (Cl) and 4- (Me) positions in one building block, a linear inverse correlation between spacer length and AChE inhibition can be derived, because the IC_{50} values progressively increased from 0.92 μM with the shorter spacer (**5d**, n = 2) to more than 10 μM with the longest chain (**5h**, n = 6). By reversing the branching side and attaching the polymethylene bridge at the coumarin 3-position, which moves the lactone groups closer, the most active compounds were disclosed (**6a** and **6d**), both bearing an ethylene linker. Compounds of structure D (**6a**–**f**) displayed very high inhibitory potencies, with IC_{50} values from the low micromolar to the nanomolar range (0.059 μM < IC_{50} < 1.54 μM). In this case, the length of the spacer also markedly influenced AChE activity, and the lowest activity was associated with the longest spacer (n = 2 > n = 3 > n = 4; **6a** > **6b** > **6c** and **6d** > **6e** > **6f**, respectively). The introduction of a dimethoxy functionality in the neutral moiety strongly improved the AChE binding affinity and the most potent inhibitor of the whole series (**6d**) can be found in this subset. The potency drop relative to that of the previous hit compound can be adequately counteracted by increased drug-like features (Table 3), thanks to the removal of the potential toxicophore features that characterize the lead compound. In addition, the presence of a protonatable amino group in compound **6d** shifted the lipophilicity (and distribution coefficient) to a value closer to that of central nervous system drugs and improved aqueous solubility to a lesser extent (Table 3).

Table 2. Cholinesterase inhibition data for dimers **2a–f**, **4a–e**, **5a–h**, and **6a–f**.



Compd	*n*	General structure	R^1	R^2	IC_{50} [μM] (or inhibition [%] at 10 μM)[a] AChE[b]	BChE[c]
2a	2	A			0.870 ± 0.056	(11 ± 1)
2b	3	A			0.990 ± 0.024	(7 ± 1)
2c	4	A			(28 ± 2)	(13 ± 1)
2d	5	A			(25 ± 1)	(< 5)
2e	6	A			1.98 ± 0.361	(21 ± 2)
2f	9	A			(31 ± 2)	(< 5)
4a	2	B			6.59 ± 0.885	(19 ± 2)
4b	3	B			2.50 ± 0.410	(23 ± 3)
4c	4	B			0.730 ± 0.083	(19 ± 2)
4d	5	B			(49 ± 1)	(47 ± 1)
4e	6	B			(38 ± 2)	(48 ± 2)
5a	2	C	H	H	1.36 ± 0.040	(17 ± 3)
5b	3	C	H	H	0.900 ± 0.180	(37 ± 2)
5c	4	C	H	H	1.32 ± 0.140	(46 ± 3)
5d	2	C	Cl	Me	0.920 ± 0.069	(22 ± 2)
5e	3	C	Cl	Me	7.10 ± 0.567	(21 ± 1)
5f	4	C	Cl	Me	9.90 ± 0.744	(28 ± 2)
5g	5	C	Cl	Me	(23 ± 3)	(21 ± 1)
5h	6	C	Cl	Me	(22 ± 2)	(21 ± 2)
6a	2	D	H	H	0.380 ± 0.020	(20 ± 1)
6b	3	D	H	H	0.730 ± 0.043	(48 ± 2)
6c	4	D	H	H	1.54 ± 0.400	(48 ± 3)
6d	2	D	OMe	OMe	0.059 ± 0.010	(15 ± 1)
6e	3	D	OMe	OMe	0.850 ± 0.070	(27 ± 1)
6f	4	D	OMe	OMe	1.40 ± 0.100	(37 ± 2)
donepezil					0.021 ± 0.002	2.31 ± 0.120

[a] Values are means of three independent experiments; SEM < 10%. [b] AChE from electric eel. [c] BChE from horse serum.

Table 3. Comparison of druglike and biological properties of **6d** and LP768.[23]

Parameter	**6d**	LP768[a]
AChE inhibition (IC_{50}) [nM]	59	0.236
Amyloid aggregation inhibition [%][b]	55	< 5
Neuroprotection from $A\beta_{1-42}$ at 24/48 h [%][c]	62/46	not tested
clog*P*[d]	2.52	3.36
clog$D_{7.4}$[d]	1.65	3.36
Aqueous solubility at pH 7.4 [μM][e]	23	10

[a] Edrophonium–coumarin conjugate reported in ref. [23] as compound **13**. [b] Percentage of $A\beta_{1-40}$ (30 μM) self-aggregation inhibition by compounds **6d** and LP768 (100 μM). [c] Measured as the difference between the percentage of viable cells (relative to the control) co-incubated with amyloid in the presence and absence of **6d**. [d] Calculated from ChemAxon. [e] Calculated from ACDLabs 6.00.

Heterodimer **6d** showed an outstanding inhibitory potency (IC_{50} = 59 nM) and was therefore submitted to more detailed investigations aimed at determining the AD-related biological activities.

Biochemical investigations of 6d

To demonstrate the dual binding features of **6d** to occupy simultaneously the CAS and PAS in AChE, a kinetic investigation of the inhibition was carried out. Figure 2 shows the Lineweaver–Burk plot at different inhibitor concentrations, which indicates a mixed-type or non-competitive mechanism (inhibition constant K_i = (68 ± 6) nM), which likely indicates at least a partial occupation of the PAS.

In addition, the well-established thioflavin T fluorescence (ThT) assay[36] demonstrated that compound **6d** behaved as an anti-amyloid aggregation agent and showed good inhibition of $A\beta_{1-40}$ deposition [(55 ± 4)% at 100 μM; Table 3]. Thus, the potential of **6d** to interfere with the amyloid cascade might arise from interaction with Aβ peptide oligomers, as well as from occupancy of the PAS and the consequent blockade of its chaperone-like activity during the fibrillization process.

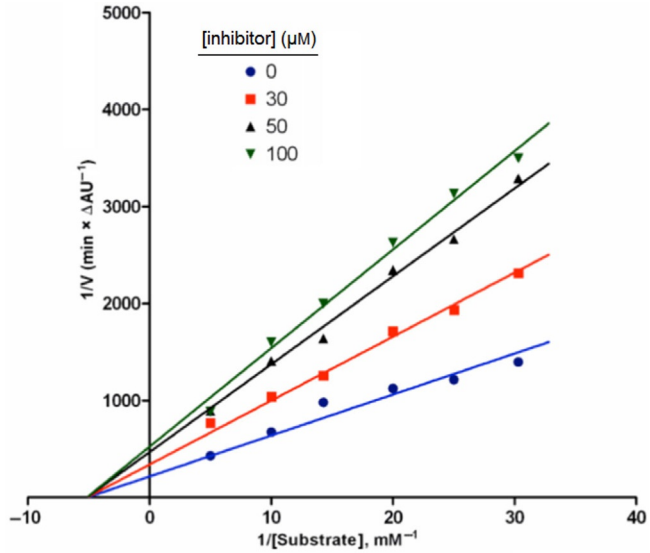


Figure 2. Lineweaver–Burk diagram of AChE inhibition kinetics for compound **6d**: reciprocals of enzyme activity (*ee*AChE) versus reciprocals of substrate (*S*-acetylthiocholine) concentration in the presence of various inhibitor concentrations, as indicated.

The cytoprotective effect of **6d** from $A\beta_{1-42}$ damage in SH-SY5Y human neuroblastoma cell lines was determined through a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As shown in Figure 3, at low concentration, compound **6d** was able to neutralize amyloid-induced neuronal death. It markedly increased cell viability if co-incubated for 24 and 48 h in the presence of $A\beta_{1-42}$ (5 μM); untreated cells were used as a control.

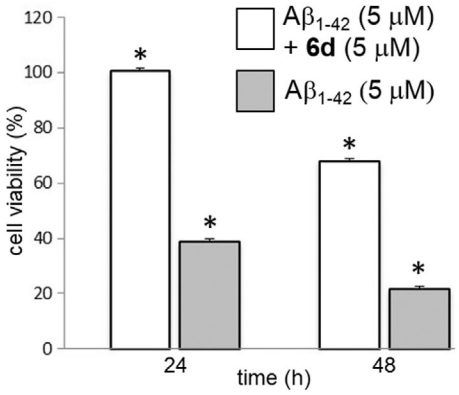


Figure 3. Cytoprotective effects of **6d** (5 μM) on SH-SY5Y human neuroblastoma cells. Viability was measured by the MTT assay after 24 and 48 h of incubation at 37 °C with $A\beta_{1-42}$ (5 μM) in the absence (gray bars) and presence (white bars) of **6d**. The percentage of MTT reduction is relative to control cells in medium. Values are expressed as the mean ± SEM of six replicates, which is significantly different from the respective control as estimated by the Student's *t* test (*$p < 0.01$).

Conclusions

The discovery of disease-modifying therapies for AD is a challenging research topic owing to the multifactorial nature and elusive understanding of the causative mechanisms. Even if great efforts in academic and industrial settings added to knowledge of the disease and several druggable targets gained consensus,[37] AChE inhibition would still remain a valuable option. Aimed at improving the druglike features of DBS AChE inhibitors previously identified, a scaffold hopping approach was envisaged in order to replace structural alerts. Herein, we studied the effect of linker length and ramification pattern on dimeric inhibitors built from coumarin-based moieties. From the initial weakly active and novel monomeric ligands, sub-micromolar bivalent AChE inhibitors with high selectivity over BChE were discovered. In particular, heterodimer **6d** demonstrated a promising multifunctional profile because it is a nanomolar AChE inhibitor ($IC_{50} = 59$ nM) endowed with mixed-mode kinetics, which supports PAS binding. Moreover, it acts as a cytoprotective agent that is capable of decreasing amyloid aggregation and cellular damage induced by $A\beta_{1-42}$ in SH-SY5Y lines.

Experimental Section

Chemistry

Materials and methods: Starting materials, reagents, and analytical grade solvents were purchased from Sigma–Aldrich (Europe). Intermediates **M1**–**4** were obtained from commercial sources. Hydroxycoumarin **M6** was prepared by following literature reports.[38] The purity of all intermediates, checked by ^{1}H NMR spectroscopy and HPLC, was always better than 95%. All newly prepared and tested compounds showed HPLC purity higher than 98%, as determined by high-performance, reverse-phase liquid chromatography (RPLC). RPLC analyses were performed on an Analytic Agilent 1260 Infinity multidetector system equipped with an automatic sampler and a 1200 series UV-diode array detector by using a Kinetex 2.6 μm C18 column (150 mm × 2.1 mm in diameter). UV detection was performed at 230, 254, 280 and 320 nm. Each tested compound was analyzed by isocratic elution with two different mobile-phase systems: in system 1, compounds were eluted with a 70:30 methanol/ammonium formate buffer (20 mM, pH 5.0) mixture at a flow rate of 0.2 or 0.3 mL min^{-1}, and in system 2, compounds were eluted with a 65:35 acetonitrile/ammonium formate buffer (20 mM, pH 5.0) mixture at a flow rate of 0.2 or 0.3 mL min^{-1}. Flash chromatography separations were performed on a Biotage SP1 purification system by using flash cartridges prepacked with KP-Sil 32–63 μm 60 Å silica. Microwave reactions were performed in a Milestone MicroSynth apparatus, by setting the temperature and hold times with the maximum irradiation power fixed at 500 W and with heating ramp times fixed at 2 min. All reactions were routinely checked by TLC on Merck Kieselgel 60 F254 aluminum plates with UV light visualization. NMR spectra were recorded on a Varian Mercury 300 instrument (at 300 MHz) or on an Agilent Technologies 500 apparatus (at 500 MHz) at ambient temperature in the specified deuterated solvent. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. The coupling constants *J* are given in Hertz (Hz). The following abbreviations were used: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quadruplet), qn (quintuplet), m (multiplet), and brs (broad signal). Signals that are the result of OH and NH protons were located by deuterium exchange with D_2O. Elemental analyses of the final compounds that were tested as inhibitors were performed on a EuroEA 3000 analyzer. The measured values for C, H,

and N agreed to within ±0.40% of the theoretical values. Melting points were determined by the capillary method on a Stuart Scientific SMP3 electrothermal apparatus and are uncorrected.

4-[(Dimethylamino)methyl]-7-hydroxy-2*H*-chromen-2-one (M5; 1): 4-(Chloromethyl)-7-hydroxy-2*H*-chromen-2-one[32] (80 mmol, 16.8 g) was dissolved in dry THF (150 mL), before commercially available 2.0 N solution of *N*,*N*-dimethylamine in THF (100 mL, 200 mmol) was added. The mixture was kept at room temperature for 6 h with magnetic stirring, and then the inorganic residue was filtered off. The solution was concentrated under rotary evaporation and purified by flash chromatography (gradient eluent: ethyl acetate in *n*-hexane 30%→100%) to yield the desired product as a yellow solid (80%, 14.0 g). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 10.49 (s, 1H, dis. with D$_2$O; OH), 7.73 (d, J = 8.8 Hz, 1H, H5), 6.76 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.68 (d, J = 2.5 Hz, 1H, H8), 6.17 (s, 1H, H3), 3.50 (s, 2H, CH$_2$N), 2.20 ppm (s, 6H, N(CH$_3$)$_2$).

General procedure A for the synthesis of homodimers 2a–f: A Pyrex microwave process vial was charged with a magnetic stirring bar. Intermediate **M5** (2.0 mmol, 439 mg) was suspended in acetonitrile (5 mL), before cesium carbonate (2.0 mmol, 652 mg), the appropriate dibromoalkane (0.80 mmol), and a catalytic amount of KI were added. The vessel was placed in the microwave apparatus and heated at 160 °C for 30 min. The mixture was cooled to room temperature, and the inorganic residue was filtered off after thorough washing with CH$_2$Cl$_2$. The solvent was evaporated under rotary evaporation, and the resulting crude solid was purified by flash chromatography with different eluent gradients as indicated below to obtain the desired homodimers **2a–f**.

7,7′-[Ethane-1,2-diylbis(oxy)]bis{4-[(dimethylamino)methyl]-2*H*-chromen-2-one} (2a): Prepared according to general procedure A from **M5** (2.0 mmol, 439 mg) and 1,2-dibromoethane (0.80 mmol, 0.069 mL). Isolation procedure: flash column chromatography (gradient eluent: methanol in ethyl acetate 0%→5%). Yellow solid (44%, 164 mg); mp: 167–169 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.86 (d, J = 8.8 Hz, 2H, H5), 7.05 (d, J = 2.5 Hz, 2H, H8), 6.99 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 2H, H6), 6.27 (s, 2H, H3), 4.45 (s, 4H, CH$_2$O), 3.54 (s, 4H, CH$_2$N), 2.21 ppm (s, 12H, N(CH$_3$)$_2$); elemental analysis: calcd for C$_{26}$H$_{28}$N$_2$O$_6$: C 67.23, H 6.08, N 6.03; found: C 67.56, H 6.11, N 5.90.

7,7′-[Propane-1,3-diylbis(oxy)]bis{4-[(dimethylamino)methyl]-2*H*-chromen-2-one} (2b): Prepared according to general procedure A from **M5** (2.0 mmol, 439 mg) and 1,3-dibromopropane (0.80 mmol, 0.081 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 50%→100%). White solid (63%, 241 mg); mp: 178–180 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.83 (d, J = 8.8 Hz, 2H, H5), 6.98 (m, 2H, H8), 6.94 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 2H, H6), 6.25 (s, 2H, H3), 4.24 (t, J = 6.3 Hz, 4H, OC*H*$_2$CH$_2$C*H*$_2$O), 3.53 (s, 4H, CH$_2$N), 2.21 (s, 12H, N(CH$_3$)$_2$), 2.19–2.27 ppm (m, 2H, OCH$_2$C*H*$_2$CH$_2$O); elemental analysis: calcd for C$_{27}$H$_{30}$N$_2$O$_6$: C 67.77, H 6.32, N 5.85; found: C 68.02, H 6.36, N 5.80.

7,7′-[Butane-1,4-diylbis(oxy)]bis{4-[(dimethylamino)methyl]-2*H*-chromen-2-one} (2c): Prepared according to general procedure A from **M5** (2.0 mmol, 439 mg) and 1,4-dibromobutane (0.80 mmol, 0.096 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 50%→100%). White solid (70%, 276 mg); mp: 142–144 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.83 (d, J = 8.8 Hz, 2H, H5), 6.97 (d, J = 2.5 Hz, 2H, H8), 6.93 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 2H, H6), 6.25 (s, 2H, H3), 4.14 (br s, 4H, OC*H*$_2$CH$_2$CH$_2$C*H*$_2$O), 3.53 (s, 4H, CH$_2$N), 2.21 (s, 12H, N(CH$_3$)$_2$), 1.89 ppm (s, 4H, OCH$_2$C*H*$_2$C*H*$_2$CH$_2$O); elemental analysis: calcd for C$_{28}$H$_{32}$N$_2$O$_6$: C 68.28, H 6.55, N 5.69; found: C 68.50, H 6.42, N 5.65.

7,7′-[Pentane-1,5-diylbis(oxy)]bis{4-[(dimethylamino)methyl]-2*H*-chromen-2-one} (2d): Prepared according to general procedure A from **M5** (2.0 mmol, 439 mg) and 1,5-dibromopentane (0.80 mmol, 0.109 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 50%→90%). Off-white solid (51%, 207 mg); mp: 146–148 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.82 (d, J = 8.8 Hz, 2H, H5), 6.95 (d, J = 2.5 Hz, 2H, H8), 6.91 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 2H, H6), 6.24 (s, 2H, H3), 4.09 (t, J = 6.3 Hz, 4H, OC*H*$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$O), 3.53 (s, 4H, CH$_2$N), 2.21 (s, 12H, N(CH$_3$)$_2$), 1.75–1.82 (m, 4H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$O), 1.54–1.62 ppm (m, 2H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$O); elemental analysis: calcd for C$_{29}$H$_{34}$N$_2$O$_6$: C 68.76, H 6.76, N 5.53; found: C 68.81, H 6.74, N 5.46.

7,7′-[Hexane-1,6-diylbis(oxy)]bis{4-[(dimethylamino)methyl]-2*H*-chromen-2-one} (2e): Prepared according to general procedure A from **M5** (2.0 mmol, 439 mg) and 1,6-dibromohexane (0.80 mmol, 0.123 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 30%→100%). White solid (91%, 379 mg); mp: 125–127 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.82 (d, J = 8.8 Hz, 2H, H5), 6.94 (d, J = 2.2 Hz, 2H, H8), 6.91 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H, H6), 6.24 (s, 2H, H3), 4.04–4.09 (m, 4H, OC*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$O), 3.53 (s, 4H, CH$_2$N), 2.21 (s, 12H, N(CH$_3$)$_2$), 1.72–1.79 (m, 4H, OCH$_2$C*H*$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$O), 1.44–1.50 ppm (m, 4H, OCH$_2$CH$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$O); elemental analysis: calcd for C$_{30}$H$_{36}$N$_2$O$_6$: C 69.21, H 6.97, N 5.38; found: C 69.41, H 7.04, N 5.29.

7,7′-[Nonane-1,9-diylbis(oxy)]bis{4-[(dimethylamino)methyl]-2*H*-chromen-2-one} (2f): Prepared according to general procedure A from **M5** (2.0 mmol, 439 mg) and 1,9-dibromononane (0.80 mmol, 0.163 mL). Isolation procedure: crystallization from hot ethanol. Yellow solid (68%, 306 mg); mp: 121–123 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 7.81 (d, J = 8.8 Hz, 2H, H5), 6.90–6.94 (m, 4H, H6, H8), 6.24 (s, 2H, H3), 4.05 (t, J = 5.4 Hz, 4H, OCH$_2$), 3.53 (s, 4H, CH$_2$N), 2.21 (s, 12H, N(CH$_3$)$_2$), 1.67–1.74 (m, 4H, OCH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$O), 1.38–1.43 (m, 4H, OCH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$C*H*$_2$CH$_2$CH$_2$O), 1.29–1.33 (m, 6H, OCH$_2$CH$_2$CH$_2$C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$CH$_2$CH$_2$O); elemental analysis: calcd for C$_{33}$H$_{42}$N$_2$O$_6$: C 70.44, H 7.52, N 4.98; found: C 70.69, H 7.46, N 4.91.

General procedure B for the synthesis of intermediates 3a–e: In a Pyrex microwave vessel, intermediate **M5** (8.0 mmol, 1.75 g) was suspended in dry acetone (25 mL), and then cesium carbonate (10.0 mmol, 3.26 g), 1-bromo-2-chloroethane (32.0 mmol, for **3a**) or the appropriate dibromoalkane (32.0 mmol, for **3b–e**), and a catalytic amount of KI were added under magnetic stirring. After being heated at 130 °C for 30 min under microwave irradiation, the mixture was cooled to room temperature and diluted with CH$_2$Cl$_2$. The inorganic residue was filtered off, and the resulting solution was concentrated to dryness under reduced pressure. The crude oil was purified by flash chromatography with different gradients of ethyl acetate in *n*-hexane as indicated below to furnish intermediates **3a–e**.

7-(2-Chloroethoxy)-4-[(dimethylamino)methyl]-2*H*-chromen-2-one (3a): Prepared according to general procedure B from **M5** (8.0 mmol, 1.75 g) and 1-bromo-2-chloroethane (32.0 mmol, 2.66 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 0%→60%). Yellow solid (80%, 1803 mg); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.85 (d, J = 8.8 Hz, 1H, H5), 7.01 (d, J = 2.5 Hz, 1H, H8), 6.97 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.27 (s, 1H, H3), 4.35 (t, J = 4.9 Hz, 2H, OCH$_2$), 3.97 (t, J = 4.9 Hz, 2H, CH$_2$Cl), 3.54 (s, 2H, CH$_2$N), 2.21 ppm (s, 6H, N(CH$_3$)$_2$).

7-(3-Bromopropoxy)-4-[(dimethylamino)methyl]-2*H*-chromen-2-one (3b): Prepared according to general procedure B from **M5** (8.0 mmol, 1.75 g) and 1,3-dibromopropane (32.0 mmol, 3.25 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 0%→50%). Yellow solid (69%, 1878 mg); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 8.17–8.26 (m, 1H, H5), 7.11–7.15 (m, 1H, H8), 7.00–7.10 (m, 1H, H6), 6.71 (s, 1H, H3), 4.71–4.81 (m, 2H, CH_2O), 4.22 (s, 2H, CH_2N), 3.55–3.78 (m, 2H, CH_2Br), 3.14 (s, 6H, $N(CH_3)_2$), 2.21–2.36 ppm (m, 2H, $OCH_2CH_2CH_2Br$).

7-(4-Bromobutoxy)-4-[(dimethylamino)methyl]-2*H*-chromen-2-one (3c): Prepared according to general procedure B from **M5** (8.0 mmol, 1.75 g) and 1,4-dibromobutane (32.0 mmol, 3.82 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 0%→60%). White solid (75%, 2125 mg); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.81 (d, J = 8.8 Hz, 1H, H5), 6.88 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1H, H6), 6.81 (d, J = 2.0 Hz, 1H, H8), 6.44 (s, 1H, H3), 4.06 (t, J = 5.9 Hz, 2H, CH_2O), 3.82 (s, 2H, CH_2N), 3.49 (t, J = 6.4 Hz, 2H, CH_2Br), 2.54 (s, 6H, $N(CH_3)_2$), 2.04–2.10 (m, 2H, $OCH_2CH_2CH_2CH_2Br$), 1.96–2.01 ppm (m, 2H, $OCH_2CH_2CH_2CH_2Br$).

7-[(5-Bromopentyl)oxy]-4-[(dimethylamino)methyl]-2*H*-chromen-2-one (3d): Prepared according to general procedure B from **M5** (8.0 mmol, 1.75 g) and 1,5-dibromopentane (32.0 mmol, 4.36 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 0%→50%). Yellow solid (81%, 2386 mg); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.83 (d, J = 8.8 Hz, 1H, H5), 7.03 (d, J = 2.0 Hz, 1H, H8), 6.84 (dd, J_1 = 8.8 Hz, J_2 = 2.0, 1H, H6), 6.34 (s, 1H, H3), 4.04 (t, J = 6.6 Hz, 2H, OCH_2), 3.55 (t, J = 6.6 Hz, 2H, CH_2Br), 3.44 (s, 2H, CH_2N), 2.36 (s, 6H, $N(CH_3)_2$), 1.85 (qn, J = 6.6 Hz, 2H, OCH_2CH_2), 1.74 (qn, J = 6.6 Hz, 2H, CH_2CH_2Br), 1.46–1.59 ppm (m, 2H, $OCH_2CH_2CH_2CH_2CH_2Br$).

7-[(6-Bromohexyl)oxy]-4-[(dimethylamino)methyl]-2*H*-chromen-2-one (3e): Prepared according to general procedure B from **M5** (8.0 mmol, 1.75 g) and 1,6-dibromohexane (32.0 mmol, 4.92 mL). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 0%→50%). Yellow solid (84%, 2569 mg); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.79 (d, J = 8.8 Hz, 1H, H5), 6.97 (d, J = 2.5 Hz, 1H, H8), 6.87 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.30 (s, 1H, H3), 4.03 (t, J = 6.7 Hz, 2H, CH_2O), 3.58 (s, 2H, CH_2N), 3.51 (t, J = 6.7 Hz, 2H, CH_2Br), 2.39 (s, 6H, $N(CH_3)_2$), 1.79–1.90 (m, 2H, OCH_2CH_2), 1.68–1.77 (m, 2H, CH_2CH_2Br), 1.41–1.47 ppm (m, 4H, $OCH_2CH_2CH_2CH_2CH_2CH_2Br$).

General procedure C for the synthesis of heterodimers (4a–e, 5a–h, and 6a–f): The appropriate chloro or bromo intermediate **3a–e** (0.50 mmol) was dissolved in dry acetone (5 mL), and then the suitable hydroxycoumarin **M1–4 and M6** (0.60 mmol), Cs_2CO_3 (0.60 mmol, 195 mg), and a catalytic amount of KI were added in a Pyrex microwave process vessel. After being heated at 130 °C for 30 under microwave irradiation, the mixture was cooled to room temperature and the resulting precipitate was filtered off after washing with CH_2Cl_2. The solvent was removed under rotary evaporation, and the resultant crude residue was diluted with CH_2Cl_2 or EtOAc (50 mL) and washed with NaOH 2 N (3×10 mL). The organic layer was dried over Na_2SO_4, and the solvent was removed under vacuum. The resultant crude solid was purified as indicated below to obtain the desired compounds **4a–e**, **5a–h**, and **6a–f**.

4-[(Dimethylamino)methyl]-7-{2-[(2-oxo-2*H*-chromen-6-yl)oxy]-ethoxy}-2*H*-chromen-2-one (4a): Prepared according to general procedure C from **3a** (0.50 mmol, 141 mg) and 6-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 30%→90%). Yellow solid (48%, 98 mg); mp: 128–130 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.99 (d, J = 9.6 Hz, 1H, H4′), 7.85 (d, J = 8.8 Hz, 1H, H5), 7.33–7.36 (m, 2H, H8′, H5′), 7.23–7.27 (m, 1H, H7′), 7.05 (d, J = 2.5 Hz, 1H, H8), 6.98 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.49 (d, J = 9.6 Hz, 1H, H3′), 6.27 (s, 1H, H3), 4.44–4.45 (m, 2H, OCH_2CH_2O), 4.37–4.39 (m, 2H, OCH_2CH_2O), 3.54 (s, 2H, CH_2N), 2.21 ppm (s, 6H, $N(CH_3)_2$); elemental analysis: calcd for $C_{23}H_{21}NO_6$: C 67.80, H 5.20, N 3.44; found: C 67.91, H 5.10, N 3.39.

4-[(Dimethylamino)methyl]-7-{3-[(2-oxo-2*H*-chromen-6-yl)oxy]-propoxy}-2*H*-chromen-2-one (4b): Prepared according to general procedure C from **3b** (0.50 mmol, 170 mg) and 6-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 40%→100%). White solid (73%, 154 mg); mp: 141–143 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.98 (d, J = 9.6 Hz, 1H, H4′), 7.83 (d, J = 8.8 Hz, 1H, H5), 7.31–7.34 (m, 2H, H5′, H8′), 7.21 (dd, J_1 = 8.8 Hz, J_2 = 3.0 Hz, 1H, H7′), 6.99 (d, J = 2.5 Hz, 1H, H8), 6.95 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.47 (d, J = 9.6 Hz, 1H, H3′), 6.25 (s, 1H, H3), 4.22–4.26 (m, 2H, $OCH_2CH_2CH_2O$), 4.15–4.19 (m, 2H, $OCH_2CH_2CH_2O$), 3.52 (s, 2H, CH_2N), 2.20 (s, 6H, $N(CH_3)_2$), 2.13–2.25 ppm (m, 2H, $OCH_2CH_2CH_2O$); elemental analysis: calcd for $C_{24}H_{23}NO_6$: C 68.40, H 5.50, N 3.32; found: C 68.53, H 5.42, N 3.26.

4-[(Dimethylamino)methyl]-7-{4-[(2-oxo-2*H*-chromen-6-yl)oxy]butoxy}-2*H*-chromen-2-one hydrochloride (4c): Prepared according to general procedure C from **3c** (0.50 mmol, 177 mg) and 6-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 40%→90%). The hydrochloride salt was obtained by treating the oily free base with 1.25 N HCl in ethanol. White solid (90%, 212 mg); mp: 165–167 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.11 (s, 1H, dis. with D_2O; NH^+), 7.98 (d, J = 9.6 Hz, 1H, H4′), 7.88 (d, J = 8.8 Hz, 1H, H5), 7.32 (d, J = 9.1 Hz, 1H, H8′), 7.28 (d, J = 3.0 Hz, 1H, H5′), 7.19 (dd, J_1 = 9.1 Hz, J_2 = 3.0 Hz, 1H, H7′), 7.00–7.06 (m, 2H, H6, H8), 6.59 (s, 1H, H3), 6.48 (d, J = 9.6 Hz, 1H, H3′), 4.52–4.55 (m, 2H, $OCH_2CH_2CH_2CH_2O$), 4.14–4.18 (m, 2H, $OCH_2CH_2CH_2CH_2O$), 4.08 (s, 2H, CH_2N), 2.84 (s, 6H, $N(CH_3)_2$), 1.86–1.93 ppm (m, 4H, $OCH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{25}H_{26}ClNO_6$: C 63.63, H 5.55, N 2.97; found: C 63.88, H 5.40, N 2.94.

4-[(Dimethylamino)methyl]-7-({5-[(2-oxo-2*H*-chromen-6-yl)oxy]-pentyl}oxy)-2*H*-chromen-2-one (4d): Prepared according to general procedure C from **3d** (0.50 mmol, 184 mg) and 6-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 40%→90%). White solid (89%, 200 mg); mp: 129–131 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.97 (d, J = 9.6 Hz, 1H, H4′), 7.82 (d, J = 8.8 Hz, 1H, H5), 7.31 (d, J = 9.1 Hz, 1H, H8′), 7.27 (d, J = 2.8 Hz, 1H, H5′), 7.17 (dd, J_1 = 9.1 Hz, J_2 = 2.5 Hz, 1H, H7′), 6.96 (d, J = 2.5 Hz, 1H, H8), 6.91 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.46 (d, J = 9.6 Hz, 1H, H3′), 6.24 (s, 1H, H3), 4.08 (t, J = 6.3 Hz, 2H, $OCH_2CH_2CH_2CH_2CH_2O$), 4.02 (t, J = 6.6 Hz, 2H, $OCH_2CH_2CH_2CH_2CH_2O$), 3.52 (s, 2H, CH_2N), 2.20 (s, 6H, $N(CH_3)_2$), 1.75–1.82 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2O$), 1.51–1.61 ppm (m, 2H, $OCH_2CH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{26}H_{27}NO_6$: C 69.47, H 6.05, N 3.12; found: C 69.55, H 5.98, N 3.06.

4-[(Dimethylamino)methyl]-7-({6-[(2-oxo-2*H*-chromen-6-yl)oxy]-hexyl}oxy)-2*H*-chromen-2-one (4e): Prepared according to general procedure C from **3e** (0.50 mmol, 191 mg) and 6-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 40%→100%). White solid (48%, 111 mg); mp: 96–98 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.98 (d, J = 9.6 Hz, 1H, H4′), 7.81 (d, J = 8.8 Hz, 1H,

H5), 7.30 (d, J = 9.1 Hz, 1H, H8′), 7.26 (d, J = 2.8 Hz, 1H, H5′), 7.17 (dd, J_1 = 9.1 Hz, J_2 = 2.8 Hz, 1H, H7′), 6.95 (d, J = 2.5 Hz, 1H, H8), 6.90 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.46 (d, J = 9.6 Hz, 1H, H3′), 6.24 (s, 1H, H3), 4.06 (t, J = 6.3 Hz, 2H, $OCH_2CH_2CH_2CH_2CH_2O$), 4.00 (t, J = 6.3 Hz, 2H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 3.52 (s, 2H, CH_2N), 2.20 (s, 6H, $N(CH_3)_2$), 1.72–1.75 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2O$), 1.47–1.50 ppm (m, 4H, $OCH_2CH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{27}H_{29}NO_6$: C 69.96, H 6.31, N 3.02; found: C 70.10, H 6.26, N 2.97.

4-[(Dimethylamino)methyl]-7-{2-[(2-oxo-2*H*-chromen-7-yl)oxy]-ethoxy}-2*H*-chromen-2-one (5a): Prepared according to general procedure C from **3a** (0.50 mmol, 141 mg) and 7-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 60%→100%). White solid (41%, 84 mg); mp: 82–83 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.98 (d, J = 9.8 Hz, 1H, H4′), 7.85 (d, J = 8.8 Hz, 1H, H5), 7.63 (d, J = 8.3 Hz, 1H, H5′), 7.05 (d, J = 2.5 Hz, 1H, H8/H8′), 7.04 (d, J = 2.5 Hz, 1H, H8/H8′), 6.97–7.00 (m, 2H, H6, H6′), 6.30 (s, 1H, H3), 6.27 (d, J = 9.8 Hz, 1H, H3′), 4.46 (br s, 4H, OCH_2CH_2O), 3.54 (s, 2H, CH_2N), 2.21 ppm (s, 6H, $N(CH_3)_2$); elemental analysis: calcd for $C_{23}H_{21}NO_6$: C 67.81, H 5.20, N 3.44; found: C 67.99, H 5.11, N 3.27.

4-[(Dimethylamino)methyl]-7-{3-[(2-oxo-2*H*-chromen-7-yl)oxy]-propoxy}-2*H*-chromen-2-one (5b): Prepared according to general procedure C from **3b** (0.50 mmol, 170 mg) and 7-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: crystallization from hot ethanol. White solid (61%, 129 mg); mp: 170–172 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.96 (d, J = 9.4 Hz, 1H, H4′), 7.82 (d, J = 8.8 Hz, 1H, H5), 7.60 (d, J = 8.2 Hz, 1H, H5′), 6.99 (d, J = 2.3 Hz, 1H, H8′), 6.97 (d, J = 2.5 Hz, 1H, H8), 6.96 (dd, J_1 = 8.2 Hz, J_2 = 2.3 Hz, 1H, H6′), 6.93 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.28 (s, 1H, H3), 6.25 (d, J = 9.4 Hz, 1H, H3′), 4.23 (t, J = 5.9 Hz, 4H, $OCH_2CH_2CH_2O$), 3.53 (s, 2H, CH_2N), 2.23–2.27 (m, 2H, $OCH_2CH_2CH_2O$), 2.20 ppm (s, 6H, $N(CH_3)_2$); elemental analysis: calcd for $C_{24}H_{23}NO_6$: C 68.40, H 5.50, N 3.32; found: C 68.55, H 5.51, N 3.28.

4-[(Dimethylamino)methyl]-7-{4-[(2-oxo-2*H*-chromen-7-yl)oxy]butoxy}-2*H*-chromen-2-one (5c): Prepared according to general procedure C from **3c** (0.50 mmol, 177 mg) and 7-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: crystallization from hot ethanol. Yellow solid (69%, 150 mg); mp: 141–142 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.97 (d, J = 9.8 Hz, 1H, H4′), 7.82 (d, J = 8.8 Hz, 1H, H5), 7.60 (d, J = 8.3 Hz, 1H, H5′), 6.97 (d, J = 2.9 Hz, 1H, H8′), 6.96 (d, J = 2.5 Hz, 1H, H8), 6.94 (dd, J_1 = 8.3 Hz, J_2 = 2.9 Hz, 1H, H6′), 6.92 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.27 (s, 1H, H3), 6.25 (d, J = 9.8 Hz, 1H, H3′), 4.14 (br s, 4H, $OCH_2CH_2CH_2CH_2O$), 3.53 (s, 2H, CH_2N), 2.21 (s, 6H, $N(CH_3)_2$), 1.89–1.90 ppm (m, 4H, $OCH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{25}H_{25}NO_6$: C 68.95, H 5.79, N 3.22; found: C 69.10, H 5.62, N 3.06.

3-Chloro-7-[2-({4-[(dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)ethoxy]-4-methyl-2*H*-chromen-2-one (5d): Prepared according to general procedure C from **3a** (0.50 mmol, 141 mg) and 3-chloro-7-hydroxy-4-methylcoumarin (0.60 mmol, 126 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 30%→80%). White solid (55%, 125 mg); mp: 160–162 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.85 (d, J = 8.8 Hz, 1H, H5′/H5), 7.78 (d, J = 8.8 Hz, 1H, H5′/H5), 7.12 (d, J = 2.5 Hz, 1H, H8), 7.04–7.08 (m, 2H, H8′, H6), 6.98 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6′), 6.27 (s, 1H, H3′), 4.38–4.55 (m, 4H, OCH_2CH_2O), 3.54 (s, 2H, CH_2N), 2.53 (s, 3H, CH_3), 2.21 ppm (s, 6H, $N(CH_3)_2$); elemental analysis: calcd for $C_{24}H_{22}ClNO_6$: C 63.23, H 4.86, N 3.07; found: C 63.30, H 4.85, N 3.02.

3-Chloro-7-[3-({4-[(dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)propoxy]-4-methyl-2*H*-chromen-2-one (5e): Prepared according to general procedure C from **3b** (0.50 mmol, 170 mg) and 3-chloro-7-hydroxy-4-methylcoumarin (0.60 mmol, 126 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 30%→80%). White solid (54%, 127 mg); mp: 170–172 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.83 (d, J = 8.8 Hz, 1H, H5′/H5), 7.76 (d, J = 8.8 Hz, 1H, H5′/H5), 7.06 (d, J = 2.5 Hz, 1H, H8), 7.02 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.98 (d, J = 2.5 Hz, 1H, H8′), 6.94 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6′), 6.25 (s, 1H, H3′), 4.22–4.28 (m, 4H, $OCH_2CH_2CH_2O$), 3.52 (s, 2H, CH_2N), 2.52 (s, 3H, CH_3), 2.18–2.26 (m, 2H, $OCH_2CH_2CH_2O$), 2.20 ppm (s, 6H, $N(CH_3)_2$); elemental analysis: calcd for $C_{25}H_{24}ClNO_6$: C 63.90, H 5.15, N 2.98; found: C 63.83, H 5.21, N 3.00.

3-Chloro-7-[4-({4-[(dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)butoxy]-4-methyl-2*H*-chromen-2-one (5f): Prepared according to general procedure C from **3c** (0.50 mmol, 177 mg) and 3-chloro-7-hydroxy-4-methylcoumarin (0.60 mmol, 126 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 20%→80%). White solid (60%, 145 mg); mp: 150–152 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.82 (d, J = 8.8 Hz, 1H, H5′/H5), 7.75 (d, J = 8.8 Hz, 1H, H5′/H5), 7.03 (d, J = 2.5 Hz, 1H, H8), 7.00 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.95 (d, J = 2.5 Hz, 1H, H8′), 6.92 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6′), 6.24 (s, 1H, H3′), 4.14–4.16 (m, 4H, $OCH_2CH_2CH_2CH_2O$), 3.53 (s, 2H, CH_2N), 2.52 (s, 3H, CH_3), 2.20 (s, 6H, $N(CH_3)_2$), 1.86–1.93 ppm (m, 4H, $OCH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{26}H_{26}ClNO_6$: C 64.53, H 5.41, N 2.89; found: C 64.60, H 5.40, N 2.87.

3-Chloro-7-{[5-({4-[(dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)pentyl]oxy}-4-methyl-2*H*-chromen-2-one (5g): Prepared according to general procedure C from **3d** (0.50 mmol, 184 mg) and 3-chloro-7-hydroxy-4-methylcoumarin (0.60 mmol, 126 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 40%→90%). White solid (82%, 204 mg); mp: 102–104 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.81 (d, J = 8.8 Hz, 1H, H5′/H5), 7.74 (d, J = 8.8 Hz, 1H, H5′/H5), 6.97–7.05 (m, 2H, H8, H6/H8′), 6.89–6.96 (m, 2H, H6′, H6/H8′), 6.24 (s, 1H, H3′), 4.06–4.13 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2O$), 3.52 (s, 2H, CH_2N), 2.52 (s, 3H, CH_3), 2.20 (s, 6H, $N(CH_3)_2$), 1.76–1.84 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2O$), 1.55–1.64 ppm (m, 2H, $OCH_2CH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{27}H_{28}ClNO_6$: C 65.12, H 5.67, N 2.81; found: C 65.10, H 5.60, N 2.76.

3-Chloro-7-{[6-({4-[(dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)hexyl]oxy}-4-methyl-2*H*-chromen-2-one (5h): Prepared according to general procedure C from **3e** (0.50 mmol, 191 mg) and 3-chloro-7-hydroxy-4-methylcoumarin (0.60 mmol, 126 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in *n*-hexane 40%→100%). White solid (76%, 195 mg); mp: 89–91 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.81 (d, J = 8.5 Hz, 1H, H5′/H5), 7.74 (d, J = 8.5 Hz, 1H H5′/H5), 6.96–7.01 (m, 2H, H8, H6/H8′), 6.88–6.94 (m, 2H, H6′, H6/H8′), 6.24 (s, 1H, H3′), 4.04–4.10 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 3.99 (s, 2H, CH_2N), 2.51 (s, 3H, CH_3), 2.20 (s, 6H, $N(CH_3)_2$), 1.60–1.79 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$), 1.44–1.51 ppm (m, 4H, $OCH_2CH_2CH_2CH_2CH_2CH_2O$); elemental analysis: calcd for $C_{28}H_{30}ClNO_6$: C 65.69, H 5.91, N 2.74; found: C 65.80, H 5.83, N 2.65.

4-[(Dimethylamino)methyl]-7-{2-[(2-oxo-2*H*-chromen-3-yl)oxy]-ethoxy}-2*H*-chromen-2-one (6a): Prepared according to general procedure C from **3a** (0.50 mmol, 141 mg) and 3-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in n-hexane 60%→100%).

White solid (50%, 102 mg); mp: 96–98 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.86 (d, J = 8.8 Hz, 1H, H5), 7.59 (d, J = 7.8 Hz, 1H, H5′), 7.41–7.44 (m, 2H, H4′, H6′/H7′), 7.30–7.36 (m, 2H, H6′/H7′, H8′), 7.06 (d, J = 2.5 Hz, 1H, H8), 6.99 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.27 (s, 1H, H3), 4.41–4.48 (brs, 4H, OCH_2CH_2O), 3.55 (s, 2H, CH_2N), 2.21 ppm (s, 6H, N(CH_3)$_2$); elemental analysis: calcd for $C_{23}H_{21}NO_6$: C 67.81, H 5.20, N 3.44; found: C 67.92, H 5.21, N 3.38.

4-[(Dimethylamino)methyl]-7-{3-[(2-oxo-2*H*-chromen-3-yl)oxy]-propoxy}-2*H*-chromen-2-one (6b): Prepared according to general procedure C from **3b** (0.50 mmol, 170 mg) and 3-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: crystallization from hot ethanol. White solid (56%, 118 mg); mp: 151–153 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.82 (d, J = 8.8 Hz, 1H, H5), 7.57 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 1H, H5′), 7.41–7.44 (m, 1H, H6′/H7′), 7.39 (s, 1H, H4′), 7.26–7.38 (m, 2H, H6′/H7′, H8′), 6.99 (d, J = 2.9 Hz, 1H, H8), 6.95 (dd, J_1 = 8.8 Hz, J_2 = 2.9 Hz, 1H, H6), 6.25 (s, 1H, H3), 4.23 (t, J = 5.9 Hz, 2H, OCH$_2$CH$_2$$CH_2$O), 4.18 (t, J = 5.9 Hz, 2H, OCH_2CH$_2$CH$_2$O), 3.53 (s, 2H, CH_2N), 2.22–2.26 (m, 2H, OCH$_2$$CH_2CH_2$O), 2.20 ppm (s, 6H, N($CH_3$)$_2$); elemental analysis: calcd for $C_{24}H_{23}NO_6$: C 68.40, H 5.50, N 3.32; found: C 68.51, H 5.36, N 3.29.

4-[(Dimethylamino)methyl]-7-{4-[(2-oxo-2*H*-chromen-3-yl)oxy]butoxy}-2*H*-chromen-2-one (6c): Prepared according to general procedure C from **3c** (0.50 mmol, 177 mg) and 3-hydroxycoumarin (0.60 mmol, 97 mg). Isolation procedure: crystallization from hot ethanol. Yellow solid (97%, 211 mg); mp: 121–123 °C; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 7.88 (d, J = 8.8 Hz, 1H, H5), 7.56 (dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 1H, H5′), 7.41–7.45 (m, 1H, H6′/H7′), 7.28–7.31 (m, 2H, H6′/H7′, H8′), 7.24 (s, 1H, H4′), 6.92 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6), 6.89 (d, J = 2.5 Hz, 1H, H8), 6.25 (s, 1H, H3), 4.25 (t, J = 5.9 Hz, 2H, OCH$_2$CH$_2$CH$_2$$CH_2$O), 4.21 (t, J = 5.9 Hz, 2H, OCH_2CH$_2$CH$_2$CH$_2$O), 3.58 (s, 2H, CH_2N), 2.30 (s, 6H, N(CH_3)$_2$), 2.04–2.07 ppm (m, 4H, OCH$_2$$CH_2$$CH_2CH_2$O); elemental analysis: calcd for $C_{25}H_{25}NO_6$: C 68.95, H 5.79, N 3.22; found: C 69.04, H 5.70, N 3.06.

3-[2-({4-[(Dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)-ethoxy]-6,7-dimethoxy-2*H*-chromen-2-one (6d): Prepared according to general procedure C from **3a** (0.50 mmol, 141 mg) and 3-hydroxy-6,7-dimethoxycoumarin (0.60 mmol, 133 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in n-hexane 70%→100%). Brown solid (47%, 110 mg); mp: 176–177 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.79 (d, J = 8.8 Hz, 1H, H5′), 7.32 (s, 1H, H4), 7.06 (s, 1H, H5), 6.97–6.99 (m, 2H, H8, H6′/H8′), 6.93 (m, 1H, H6′/H8′), 6.21 (s, 1H, H3′), 4.29–4.40 (brs, 4H, OCH$_2$CH$_2$O), 3.75 (s, 3H, OCH$_3$), 3.72 (s, 3H, OCH$_3$), 3.49 (s, 2H, CH_2N), 2.15 ppm (s, 6H, N(CH_3)$_2$); elemental analysis: calcd for $C_{25}H_{25}NO_8$: C 64.23, H 5.39, N 3.00; found: C 64.51, H 5.40, N 2.93.

3-[3-({4-[(Dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)-propoxy]-6,7-dimethoxy-2*H*-chromen-2-one (6e): Prepared according to general procedure C from **3b** (0.50 mmol, 170 mg) and 3-hydroxy-6,7-dimethoxycoumarin (0.60 mmol, 133 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in n-hexane 70%→100%). Off-white solid (81%, 195 mg); mp: 125–127 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.89 (d, J = 8.8 Hz, 1H, H5′), 7.23 (s, 1H, H4), 7.07 (s, 1H, H5), 6.94 (s, 1H, H8), 6.92 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6′), 6.90 (d, J = 2.5 Hz, 1H, H8′), 6.25 (s, 1H, H3′), 4.35 (t, J = 5.9 Hz, 2H, OCH$_2$CH$_2$$CH_2$O), 4.27 (t, J = 5.9 Hz, 2H, OCH_2CH$_2$CH$_2$O), 3.89 (s, 3H, OCH$_3$), 3.83 (s, 3H, OCH$_3$), 3.58 (s, 2H, CH_2N), 2.33–2.35 (m, 2H, OCH$_2$$CH_2CH_2$O), 2.29 ppm (s, 6H, N($CH_3$)$_2$); elemental analysis: calcd for $C_{26}H_{27}NO_8$: C 64.86, H 5.65, N 2.91; found: C 65.16, H 5.50, N 2.68.

3-[4-({4-[(Dimethylamino)methyl]-2-oxo-2*H*-chromen-7-yl}oxy)-butoxy]-6,7-dimethoxy-2*H*-chromen-2-one (6f): Prepared according to general procedure C from **3c** (0.50 mmol, 177 mg) and 3-hydroxy-6,7-dimethoxycoumarin (0.60 mmol, 133 mg). Isolation procedure: flash column chromatography (gradient eluent: ethyl acetate in n-hexane 70%→100%). Off-white solid (78%, 193 mg); mp: 162–164 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 7.89 (d, J = 8.8 Hz, 1H, H5′), 7.18 (s, 1H, H4), 7.07 (s, 1H, H5), 6.93 (s, 1H, H8), 6.92 (dd, J_1 = 8.8 Hz, J_2 = 2.5 Hz, 1H, H6′), 6.88 (d, J = 2.5 Hz, 1H, H8′), 6.25 (s, 1H, H3′), 4.24 (t, J = 5.9 Hz, 2H, OCH$_2$CH$_2$CH$_2$$CH_2$O), 4.15 (t, J = 5.9 Hz, 2H, OCH_2CH$_2$CH$_2$CH$_2$O), 3.90 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 3.59 (s, 2H, CH_2N), 2.29 (s, 6H, N(CH_3)$_2$), 2.04–2.06 ppm (m, 4H, OCH$_2$$CH_2$$CH_2CH_2$O); elemental analysis: calcd for $C_{27}H_{29}NO_8$: C 65.45, H 5.90, N 2.83; found: C 65.69, H 5.78, N 2.71.

Biological assays

Cholinesterase inhibition assays: A modified protocol of Ellman's spectrophotometric assay[33] was adapted to a 96-well plate procedure. Inhibition assays of AChE from electric eel (*ee*AChE, 463 U mL^{-}; Sigma–Aldrich) and BChE from horse serum (*hs*BChE, 13 U mL^{-}; Sigma–Aldrich) were performed in 0.1 M phosphate buffer at pH 8.0. Acetyl- and butyrylthiocholine iodide (Sigma–Aldrich) were used, respectively, as substrates, and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB; Sigma–Aldrich) was used as the chromophoric reagent. Incubations were carried out in clear flat-bottomed 96-well plates (Greiner Bio-One, Kremsmünster, Austria) in duplicate for each concentration. Seven solutions (ranging from 30 to 0.03 μM as the final concentrations) of inhibitors were prepared by diluting a stock 1000 μM DMSO solution with the working buffer. Incubation samples contained 20 μL of the enzyme solution (0.9 U mL^{-1} in work buffer), 20 μL of a 3.3 mM solution of DTNB in 0.1 M phosphate buffer (pH 7.0), 20 μL of a solution of the inhibitor, and 120 μL of the working buffer. After incubation of the mixture for 20 min at 25 °C, thiocholine substrate (20 μL of 5 mM aqueous solution) was added, and AChE-catalyzed hydrolysis was followed by measuring the increase in absorbance at 412 nm for 5 min at 25 °C with the Tecan Infinite M100 Pro multiplate reader. Inhibition values were calculated with GraphPad Prism software as the mean of three independent experiments. Kinetic studies of the *ee*AChE inhibition mechanism were performed with the same test conditions, by using six concentrations of substrate (from 0.033 to 0.2 mM) and four concentrations of inhibitor (0, 30, 50, and 100 μM). Apparent inhibition constants and kinetic parameters were calculated within the "Enzyme kinetics" module of the Prism software.

ThT assay of $A\beta_{1-40}$ aggregation: Inhibition of $A\beta_{1-40}$ self-aggregation was studied by means of a spectrofluorimetric test based on ThT fluorescence, which was slightly modified from a previously reported protocol.[36] $A\beta_{1-40}$ (EZBiolab, Carmel, IN, USA) was pretreated as described[39] and dissolved to a concentration of 300 μM in 10 mM phosphate-buffered saline (PBS, pH 7.4). Stock solution of inhibitor **6d** was 1000 μM in DMSO. Incubations were carried out in black round-bottomed 96-well plates (Greiner Bio-One, Kremsmünster, Austria) in triplicate. Incubation samples were set by adding 5 μL each of $A\beta_{1-40}$ stock, inhibitor solution, and 20% solution of 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma–Aldrich) in PBS and then adding PBS to a final volume of 50 μL. Samples devoid of inhibitor were prepared as a control for free self-aggregation. After 2 h of incubation at 25 °C, 25 μM ThT solution in phosphate buffer at pH 6.0 (150 μL) was added and the fluorescence was read with an Infinite M100 Pro multiplate reader (Tecan, Cernusco S.N., Italy).

$A\beta_{1-42}$-induced neurotoxicity: By following already reported protocols, SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich) supplemented with 10% (*v/v*) inactivated fetal bovine serum (Sigma–Aldrich), 2 mM L-glutamine (Sigma–Aldrich), 100 μg mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (Sigma–Aldrich) at 37 °C in 5% CO_2. For assays, the cells were grown to 70% confluence and seeded for experiments in 96-well plates at a density of 10000 cells/well in 125 μL of cell culture medium. Experiments were performed 24 h after cells were seeded. SH-SY5Y cell viability was determined by using a conventional MTT reduction assay.[40] This method is based on the ability of viable cells to metabolize MTT (Sigma–Aldrich), a water-soluble salt (yellow color), by cellular oxidoreductase into a water-insoluble blue formazan product. Therefore, the amount of produced formazan is proportional to the quantity of viable cells. Briefly, viable cells (10^4/well) in 96-well cell culture plates were supplemented with a DMEM solution of $A\beta_{1-42}$, alone or in a mixture with **6d** (5 μM final concentration of both) and incubated at 37 °C in 5% CO_2. At the end of incubation time (24 and 48 h), the culture medium was replaced by DMEM supplemented with a solution of MTT in PBS (50 μg mL^{-1} final concentration). After 2 h of incubation at 37 °C in 5% CO_2, this solution was removed and DMSO (200 μL) was added to each well to dissolve the formazan product. Absorbance values were measured at 570 nm by using a Victor^3 V multilabel plate counter (Perkin–Elmer), with DMSO medium as the blank solution. The experiments, repeated twice, were carried out in six replicates, and the results were given as the mean ± SEM.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Alzheimer's disease · coumarins · dimerization · enzyme catalysis · inhibitors

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