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New Tacrine−4-Oxo-4*H*-chromene Hybrids as Multifunctional Agents for the Treatment of Alzheimer's Disease, with Cholinergic, Antioxidant, and *β*-Amyloid-Reducing Properties[†]

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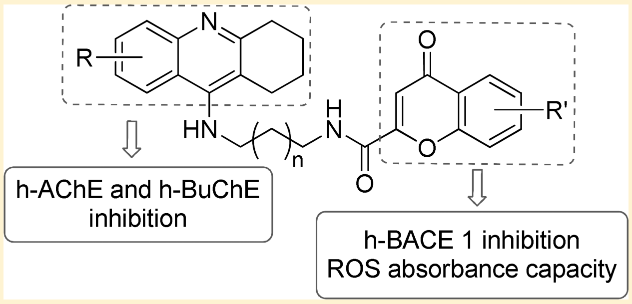
S *Supporting Information*



ABSTRACT: By using fragments endowed with interesting and complementary properties for the treatment of Alzheimer's disease (AD), a new family of tacrine−4-oxo-4*H*-chromene hybrids has been designed, synthesized, and evaluated biologically. The tacrine fragment was selected for its inhibition of cholinesterases, and the flavonoid scaffold derived from 4-oxo-4*H* -chromene was chosen for its radical capture and *β*-secretase 1 (BACE-1) inhibitory activities. At nano- and picomolar concentrations, the new tacrine−4-oxo-4*H*-chromene hybrids inhibit human acetyl- and butyrylcholinesterase (h-AChE and h-BuChE), being more potent than the parent inhibitor, tacrine. They are also potent inhibitors of human BACE-1, better than the parent flavonoid, apigenin. They show interesting antioxidant properties and could be able to penetrate into the CNS according to the in vitro PAMPA-BBB assay. Among the hybrids investigated, 6-hydroxy-4-oxo- *N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4 *H*-chromene-2-carboxamide (**19**) shows potent combined inhibition of human BACE-1 and ChEs, as well as good antioxidant and CNS-permeable properties.

INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative process occurring in the central nervous system (CNS), characterized by deposits of aberrant proteins namely *β*-amyloid (A*β*) and *τ*-protein, oxidative stress, loss of synapses, and death of cells, especially cholinergic neurons.[1] Although several research strategies have been envisaged in recent decades,[2,3] the current therapeutic options for the treatment of AD are limited to three acetylcholinesterase (AChE) inhibitors,[4] namely donepezil, rivastigmine, and galantamine, and one *N*-methyl-D-aspartate receptor antagonist, memantine.[5]

Converging lines of evidence suggest that progressive cerebral deposition of A*β* plays a central role in the pathogenesis and development of AD.[6] Therefore, lowering the concentration of this peptide in the brain appears to be a rational therapeutic approach for treating AD.[7] This goal can be achieved by decreasing A*β* production through inhibition of *β*-secretase (BACE-1) or *γ*-secretase,[8] by interfering with A*β* aggregation by using dual binding sites AChE inhibitors[9] or by promoting A*β* clearance by using selective metal chelators.[10,11] BACE-1, which is involved in the first and rate-limiting step of A*β* formation from its amyloid precursor protein (APP), has generated a great interest, and nowadays several BACE-1 inhibitors are under clinical trials.[12]

The enzyme AChE, besides its important role in the cholinergic transmission, also participates in other functions related to neuronal development, differentiation, and adhesion. Several studies have also indicated that AChE promotes the formation of A*β* fibrils in vitro[13] and A*β* plaques in the cerebral cortex of transgenic mice models of AD.[14] The assumption that this dark side of AChE is mediated by an interaction between A*β* and the peripheral anionic site of the enzyme (PAS)[15] has led to the development of dual binding site inhibitors of both catalytic active site (CAS) and PAS. These compounds are promising disease-modifying AD drug candidates, because they can simultaneously improve cognition and slow down the rate of A*β* degeneration. Recently, this hypothesis has been validated in murine models of AD that showed an improvement in cognition and a reduction of brain amyloid burden when they were treated with dual binding site AChE inhibitors.[16−18]

In healthy brains, AChE hydrolyzes the majority of acetylcholine while butyrylcholinesterase (BuChE) plays a

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secondary role. However, as AD progresses, the activity of AChE decreases, while that of BuChE significantly increases in the hippocampus and temporal cortex.[19] Consequently, in recent years both selective and nonselective BuChE inhibitors have received increasing attention.[20,21] In this sense, recent clinical trials have demonstrated that patients treated with rivastigmine, an inhibitor of both AChE and BuChE, showed minor cortical atrophic changes and attenuated loss of brain volumes.[22−24] These findings are consistent with the hypothesis that inhibition of both enzymes may have neuroprotective and disease-modifying effects.[25]

During aging, the endogenous antioxidant system progressively decays, and an increasing body of evidence supports the involvement of oxidative stress in different pathologies, such as cancer, cardiovascular, and neurodegenerative diseases. In the case of AD, oxidative damage in cellular structures is an event that precedes the appearance of other pathological hallmarks of AD, namely senile plaques and neurofibrillary tangles,[26,27] pointing out the early involvement of oxidative stress in the pathogenesis and progression of this disease.[28,29] Moreover, a recent statistical study involving 23 developed countries suggests that higher consumption of dietary antioxidants such as flavonoids is associated with lower population rates of dementia.[30] Thus, drugs that specifically scavenge oxygen radicals could be useful for either the prevention or the treatment of AD.[31,32]

Tacrine (**1**) is a potent inhibitor of both AChE and BuChE that suffers from therapy-limiting liver toxicity, which can be prevented with free radical scavengers.[33,34] Thus, the development of tacrine derivatives endowed with additional antioxidant properties is an active field in the current AD research.[35] Recent examples of such molecules are lipocrine,[36] tacrine−melatonin hybrids,[37] and tacrine−8-hydroxyquinoline derivatives,[38] that protect cells against oxidative stress, and NO-donor−tacrine hybrids, that showed hepatoprotective properties.[39] Flavonoids (**2**), which are ubiquitously present in fruits and vegetables, have attracted much attention in the last years because they can limit the neurodegeneration associated with a variety of neurological disorders.[40] Flavonoids mediate their effects by several routes, including their capacity to scavenge neurotoxic species, such as free radicals, or their interactions with important neuronal receptors, such as BACE-1.[41−43]

Due to the pathological complexity found in AD, multifunctional molecules with two or more complementary biological activities may represent an important advance for the treatment of this disease.[44,45] Continuing with our research on various heterocyclic families with potential application in the AD field,[46−49] in recent years we reported the synthesis of multifunctional compounds that combine neuroprotective and AChE inhibition,[38,50−53] including a tacrine−melatonin hybrid that is able to reduce amyloid burden and behavioral deficits in a mouse model of AD.[18,37,54]

Currently, our work is focused on the design of new multifunctional molecules endowed with cholinergic, antioxidant, and Aβ-lowering activities, by connecting moieties with such properties. In this work, we planned to use tacrine (**1**) for its inhibition of cholinesterases (ChEs) through the CAS and a flavonoid scaffold derived from 4-oxo-4*H*-chromene (**2**) for its antioxidant and BACE-1 inhibitory activities,[42,55] as well as for its potential interaction with the AChE−PAS due to its aromatic character (Figure 1). Regarding the possible structural modifications on the tacrine fragment, we planned to insert one or two chlorine atoms to study possible effects on ChE inhibition. To reach radical capture capacity, we envisaged introducing one or two phenol groups to the 4-oxo-4*H*-chromene fragment. These hydroxyl groups could be obtained from the corresponding methoxy functionality that, interestingly, could also improve the interaction of these precursors with AChE−PAS, as described for donepezil.[56] According to the well-known structure of AChE, we considered connecting tacrine and 4-oxo-4*H*-chromene fragments by alkylenediamine tethers of different lengths. Such flexible linkers could be lodged in the narrow enzymatic cavity, allowing simultaneous interaction between the heteroaromatic fragments and both the CAS and PAS of AChE.

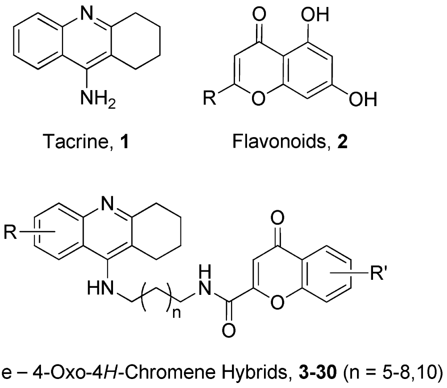


Figure 1. Structures of tacrine (**1**), flavonoids (**2**), and tacrine−4-oxo-4*H*-chromene hybrids (**3**−**30**).

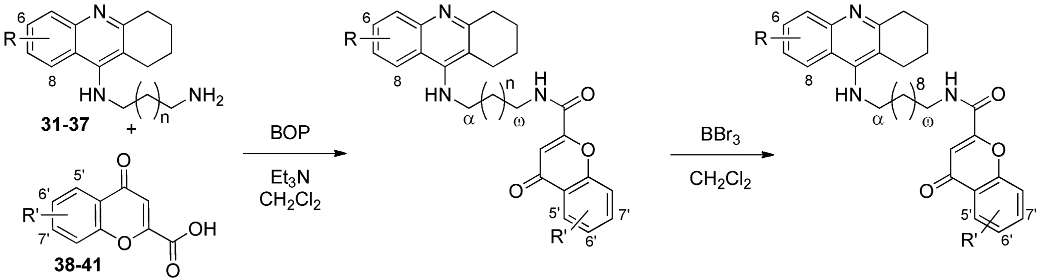
In this paper, we describe the synthesis of new tacrine−4-oxo-4*H*-chromene hybrids (**3**−**30**) and their biological evaluation that includes inhibition of human BACE-1, AChE, and BuChE, oxygen-radical absorbance capacity (ORAC), and in vitro CNS penetration.

RESULTS AND DISCUSSION

Synthesis of Tacrine−4-Oxo-4*H*-chromene Hybrids and Inhibition of Mammalian AChE and BuChE. Scheme 1 depicts the general procedure for the synthesis of tacrine−4-oxo-4*H*-chromene hybrids **3**−**30**. N^1-(1,2,3,4-Tetrahydroacridin-9-yl)alkane-1, *n*-diamines (**31**−**35**), N^1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)decane-1,10-diamine (**36**), and N^1-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)decane-1,10-diamine (**37**) were obtained in good yields, following a described method.[57] 4-Oxo-4*H*-chromene-2-carboxylic acid (**38**) is commercially available, whereas 6-methoxy-,[58] 5,7-dimethoxy-,[59] and 6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid[60] (**38**−**41**) were synthesized according to a described route.[61]

Initially, to determine the optimal length of the aliphatic linker between the two heterocyclic fragments for ChEs inhibition, the synthesis of tacrine−flavonoid hybrids without any substituent in both heterocyclic structures was carried out. In a preliminary experiment, the treatment of N^1-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (**31**) with 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) progressed adequately, but the amide **3** was obtained with an excessive amount of dicyclohexylurea, even after several chromatographic separations. For this reason, we investigated another coupling agent, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP). Thus, the acid **38** was activated with

Scheme 1. Synthesis of Tacrine−4-Oxo-4*H*-chromene Hybrids 3−30



31, R = H, n = 5
32, R = H, n = 6
33, R = H, n = 7
34, R = H, n = 8
35, R = H, n = 10
36, R = 6-Cl, n = 8
37, R = 6,8-diCl, n = 8
38, R' = H
39, R' = 6-OCH_3
40, R' = 5,7-diOCH_3
41, R' = 6,7-diOCH_3

3, R = R' = H, n = 5
4, R = R' = H, n = 6
5, R = R' = H, n = 7
6, R = R' = H, n = 8
7, R = R' = H, n = 10
8, R = 6-Cl, R' = H, n = 8
9, R = 6,8-diCl, R' = H, n = 8
10, R = H, R' = 6-OCH_3, n = 8
11, R = 6-Cl, R' = 6-OCH_3, n = 8
12, R = 6,8-diCl, R' = 6-OCH_3, n = 8
13, R = H, R' = 5,7-diOCH_3, n = 8
14, R = 6-Cl, R' = 5,7-diOCH_3, n = 8
15, R = 6,8-diCl, R' = 5,7-diOCH_3, n = 8
16, R = H, R' = 6,7-diOCH_3, n = 8
17, R = 6-Cl, R' = 6,7-diOCH_3, n = 8
18, R = 6,8-diCl, R' = 6,7-diOCH_3, n = 8

19, R = H, R' = 6-OH
20, R = 6-Cl, R' = 6-OH
21, R = 6,8-diCl, R' = 6-OH
22, R = H, R' = 5-OH,7-OCH_3
23, R = H, R' = 5,7-diOH
24, R = 6-Cl, R' = 5-OH,7-OCH_3
25, R = 6-Cl, R' = 5,7-diOH
26, R = 6,8-diCl, R' = 5-OH,7-OCH_3
27, R = 6,8-diCl, R' = 5,7-diOH
28, R = H, R' = 6,7-diOH
29, R = 6-Cl, R' = 6,7-diOH
30, R = 6,8-diCl, R' = 6,7-diOH

BOP and then coupled with the corresponding N^1-(1,2,3,4-tetrahydroacridin-9-yl)alkane-1,*n*-diamine (**31**−**35**) in the presence of triethylamine in dichloromethane solutions at room temperature. After silica gel column chromatography, the desired tacrine−flavonoid amides **3**−**7** were isolated with a high purity degree (>98%), although in moderate yields (42−67%). These compounds, like all tacrine−4-oxo-4*H*-chromene hybrids described here, were further transformed into their hydrochloride salt by treatment with gaseous hydrochloric acid in dichloromethane solution. In general, free bases were used to obtain spectroscopic (^{1}H NMR, ^{13}C NMR, and MS) data, and hydrochloride salts were employed to determine both purity (HPLC and combustion analysis) and biological activities (AChE, BuChE, and BACE-1 inhibition, antioxidant properties, and CNS penetration).

The tacrine−flavonoid amides **3**−**7** were evaluated as inhibitors of AChE and BuChE following the Ellman method.[62] Proteins of animal origin were initially used, namely AChE from bovine erythrocytes and BuChE from horse serum, due to their lower cost and their high degree of sequence identity to the human enzymes.[63] Precursor compounds **1** and **38** were also evaluated for comparative purposes.

Tacrine−4-oxo-4*H*-chromene derivatives **3**−**7** were found to be potent inhibitors of mammalian cholinesterases with IC_{50} ranging from the submicromolar to the subnanomolar concentration scale. They showed a clear selectivity for BuChE, being about 2 orders of magnitude more potent than that of tacrine toward this enzyme. The most active inhibitor in both ChEs was hybrid **6**, bearing a decane chain between the amine and the amide groups, pointing out that this linker allows optimal interaction between the aromatic fragments of such an inhibitor and the CAS and PAS of enzymes. As expected, the 4-oxo-4*H*-chromene precursor **38** did not inhibit either ChE (data not shown).

Once established that the optimal length of the linker was a chain of ten methylenes, we planned to introduce other substituents to both heterocyclic fragments: (i) one or two chlorine atoms to the 1,2,3,4-tetrahydroacridine group to study possible effects on effectiveness and selectivity in ChE inhibition and (ii) one or two phenol groups to the 4-oxo-4*H*-chromene fragment, looking for radical capture capacity. For the first objective, in addition to 1,2,3,4-tetrahydroacridin-9-yl)decane-1,10-diamine (**34**) employed before, we considered using 6-chloro and 6,8-dichloro analogues (**36** and **37**, respectively), according to our previous results in the tacrine−melatonin series.[37,54] Thus, hybrids **8** and **9** were obtained by coupling amines **36** or **37** with acid **38**, using the above-mentioned conditions.

The second objective was envisaged by demethylation of the corresponding methoxy function.[64] Thus, 4-oxo-4*H*-chromene-2-carboxylic acids **39**−**41**, bearing one or two methoxy groups at different positions (see Scheme 1), were obtained according to a described method.[61] They were activated with BOP and subsequently coupled with the corresponding 9-decylaminotetrahydroacridine (**34**, **36**, or **37**), in the presence of triethylamine in dichloromethane solutions at room temperature to afford the desired tacrine−4-oxo-4*H*-chromene hybrids **10**−**18** in moderate yields (33−68%). Finally, demethylation of the above methoxylated compounds was accomplished with boron tribromide under mild conditions, which is compatible with a large number of chemical functionalities including the amide group.[65] Initially, the treatment of the 6-methoxychromene hybrid **10** with 3 or 5 equiv of BBr_3 in dichloromethane at −78 °C under an inert atmosphere gave a mixture of the desired phenolic product **19** and the starting material, pointing out that the reaction was not completed. When the above-mentioned reaction was repeated using 7 equiv of BBr_3, the 6-hydroxychromene hybrid **19** was isolated in 70% yield and initial product **10** was not detected in the reaction crude product. This result appeared to be in accordance with the suggestion from McOmie et al. for the use of 1 equiv of BBr_3 per ether group for cleavage, plus an additional 1 equiv per nitrogen or oxygen atom included in the molecule.[65] In the same way, the treatment of 6-methoxy compounds **11** and **12**

Table 1. Yield (%) and Inhibition of Mammalian AChE and BuChE by Tacrine−4-Oxo-4*H*-chromene Hybrids 3−30[a]

					IC$_{50}$ ± SD (nM)[c]	
compd	*n*	R	R′	% yield[b]	AChE[d]	BuChE[e]
3	5	H	H	67	100 ± 5	1.75 ± 0.07
4	6	H	H	53	150 ± 7	0.50 ± 0.02
5	7	H	H	42	125 ± 6	0.58 ± 0.02
6	8	H	H	45	75 ± 3	0.18 ± 0.01
7	10	H	H	66	150 ± 6	0.25 ± 0.01
8	8	6-Cl	H	80	5.0 ± 0.3	0.180 ± 0.009
9	8	6,8-diCl	H	70	1000 ± 50	100 ± 5
10	8	H	6-OCH$_3$	59	35 ± 2	0.324 ± 0.003
11	8	6-Cl	6-OCH$_3$	46	5.0 ± 0.1	0.425 ± 0.008
12	8	6,8-diCl	6-OCH$_3$	68	550 ± 6	7.5 ± 0.2
13	8	H	5,7-diOCH$_3$	45	100 ± 1	0.325 ± 0.003
14	8	6-Cl	5,7-diOCH$_3$	49	50 ± 1	1.00 ± 0.01
15	8	6,8-diCl	5,7-diOCH$_3$	68	150 ± 3	0.75 ± 0.03
16	8	H	6,7-diOCH$_3$	64	85 ± 4	0.55 ± 0.01
17	8	6-Cl	6,7-diOCH$_3$	34	6.5 ± 0.1	0.85 ± 0.02
18	8	6,8-diCl	6,7-diOCH$_3$	33	175 ± 3	100 ± 2
19	8	H	6-OH	70	75 ± 3	1.00 ± 0.02
20	8	6-Cl	6-OH	37	80 ± 1	3.50 ± 0.07
21	8	6,8-diCl	6-OH	51	350 ± 17	17.5 ± 0.7
22	8	H	5-OH, 7-OCH$_3$	18	250 ± 5	2.00 ± 0.04
23	8	H	5,7-diOH	42	500 ± 15	0.35 ± 0.01
24	8	6-Cl	5-OH, 7-OCH$_3$	15	38 ± 2	0.175 ± 0.003
25	8	6-Cl	5,7-diOH	43	10.0 ± 0.1	3.00 ± 0.06
26	8	6,8-diCl	5-OH, 7-OCH$_3$	24	80 ± 2	35 ± 1
27	8	6,8-diCl	5,7-diOH	55	75 ± 2	35 ± 1
28	8	H	6,7-diOH	66	300 ± 9	8.0 ± 0.2
29	8	6-Cl	6,7-diOH	80	17.5 ± 0.5	28 ± 1
30	8	6,8-diCl	6,7-diOH	92	90 ± 1	88 ± 2
1	−	−	−	−	40 ± 2	10.0 ± 0.4

[a]Compounds are evaluated as hydrochlorides. [b]Percentage of isolated product (%). [c]Results are presented as the mean of three independent experiments (*n* = 3) ± DS. [d]AChE (EC 3.1.1.7) from bovine erythrocytes. [e]BuChE (EC 3.1.1.8) from horse serum.

with 7 equiv of BBr$_3$ gave the corresponding 6-hydroxychromone derivative **20** and **21** in moderated yields.

Therefore, the demethylation of hybrids containing two methoxy groups was planned by using 8 equiv of BBr$_3$, which afforded 6,7-dihydroxychromones (**28−30**) in good yields (66−92%) from the corresponding 6,7-dimethoxy derivative (**16−18**). When the above conditions were applied to 5,7-dimethoxychromone hybrids (**13−15**), two products were isolated in each case: the 5,7-dihydroxy derivative (**23**, **25**, **27**) in moderate yield (42−55%) and the 5-hydroxy-7-methoxychromene hybrid (**22**, **24**, **26**) in a minor amount (15−24%). These products were separated by silica gel column chromatography, and the position of the methoxy function in compounds **22**, **24**, and **26** was unequivocally established by ^{1}H NMR using the nuclear Overhauser (NOE) effect. The selective irradiation of the methyl group, which appeared as a singlet at ∼3.8 ppm, produced a NOE effect on two aromatic protons located in positions C-6 (at ∼6.5 ppm) and C-8 (at ∼6.8 ppm) of the chromene nucleus, pointing out that the methoxy function was located at the position C-7 in compounds **22**, **24**, and **26**. This result can be explained by the formation of a cyclic intermediate in which the boron atom attached simultaneously to the carbonyl oxygen of the chromone and the ethereal oxygen atom attached to C-5, making this position as the most favorable for the ether cleavage, in a manner similar to that previously proposed for catechol dimethyl ethers.[65]

The newly synthesized hybrids **8−30** were evaluated as inhibitors of mammalian cholinesterases, as previously explained, and the results are summarized in Table 1. As found for compounds **3−7**, hybrids **8−30** also inhibited BuChE more effectively than AChE, with IC$_{50}$ values ranging from submicro- to subnanomolar concentrations. Comparing products possessing the same substituents in the 4-oxo-4*H*-chromene fragment, it was possible to study the influence of changes in the tacrine substructure on the inhibition of ChEs. The presence of a chlorine atom in position 6 improved the AChE inhibition by 1 order of magnitude, maintaining in general the affinity for BuChE. Introduction of a second chlorine atom in position 8, however, decreased the inhibition of both enzymes. Considering now hybrids with the same substituents in the tacrine substructure, the introduction of methoxy groups in the chromone fragment had little influence on the inhibition of both enzymes. On the contrary, the presence of phenolic groups diminished the ability of these compounds to inhibit ChEs by 1 order of magnitude.

In Vitro Evaluation of the Inhibition of Human Cholinesterases, the Oxygen Radical Absorbance Capacity, and the Blood−Brain Barrier Permeation. The most active compounds in mammalian ChEs, derived from tacrine and 6-chlorotacrine, were then evaluated as inhibitors of human ChEs and as free radical scavengers. Their CNS penetration was also evaluated by a PAMPA−BBB assay, and the results are summarized in Table 2.

Table 2. Inhibition of Human Cholinesterases, Oxygen Radical Absorbance Capacity (ORAC, trolox equivalents), and Permeability Results from the PAMPA−BBB Assay (P_e, 10^{-6} cm s^{-1}) by Tacrine− and 6-Chlorotacrine−4-Oxo-4*H*-chromene Hybrids[a]

			IC$_{50}$ (nM)			
compd	R	R′	h-AChE[b]	h-BuChE[c]	ORAC[d]	P_e (10^{-6} cm s^{-1})[e] for PAMPA−BBB assay
6	H	H	17.5 ± 0.9	0.150 ± 0.007	0.10 ± 0.01	9.10 ± 0.04
8	6-Cl	H	0.30 ± 0.01	0.080 ± 0.004	<0.01	7.1 ± 0.2
10	H	6-OCH$_3$	0.775 ± 0.015	0.038 ± 0.001	0.10 ± 0.01	14.9 ± 0.1
11	6-Cl	6-OCH$_3$	0.100 ± 0.002	0.100 ± 0.003	<0.01	7.5 ± 0.4
13	H	5,7-diOCH$_3$	2.3 ± 0.2	0.10 ± 0.01	0.10 ± 0.01	14.7 ± 0.2
14	6-Cl	5,7-diOCH$_3$	0.35 ± 0.02	0.100 ± 0.005	<0.01	10.70 ± 0.03
16	H	6,7-diOCH$_3$	1.8 ± 0.1	15.0 ± 0.5	0.20 ± 0.01	15.0 ± 0.1
17	6-Cl	6,7-diOCH$_3$	0.200 ± 0.008	50 ± 2	0.10 ± 0.01	7.4 ± 0.2
19	H	6-OH	8.0 ± 0.2	1.50 ± 0.04	1.30 ± 0.04	23.1 ± 0.1
20	6-Cl	6-OH	1.00 ± 0.04	1.50 ± 0.02	0.30 ± 0.02	11.50 ± 0.04
22	H	5-OH-7-OCH$_3$	1.00 ± 0.02	1.00 ± 0.01	0.50 ± 0.02	10.6 ± 0.2
23	H	5,7-diOH	2.3 ± 0.1	0.80 ± 0.02	0.40 ± 0.02	13.10 ± 0.02
24	6-Cl	5-OH-7-OCH$_3$	0.035 ± 0.001	5.0 ± 0.2	0.30 ± 0.01	8.7 ± 0.3
25	6-Cl	5,7-diOH	0.065 ± 0.002	2.50 ± 0.05	0.20 ± 0.01	7.8 ± 0.2
28	H	6,7-diOH	6.5 ± 0.2	30 ± 1	0.50 ± 0.03	6.3 ± 0.1
29	6-Cl	6,7-diOH	0.090 ± 0.003	95 ± 4	0.40 ± 0.01	5.3 ± 0.1
1	−	−	350 ± 10	40 ± 2	<0.01	nd
2 (R = 4-OH-Ph)			nd	nd	6.7[f]	nd
melatonin			nd	nd	2.3 ± 0.1	nd

[a] Results are the mean of three independent experiments ($n = 3$) ± SD. [b] AChE (EC 3.1.1.7) from human erythrocytes. [c] BuChE (EC 3.1.1.8) from human serum. [d] Data are expressed as μmol of trolox equivalents/μmol of tested compound. [e] PBS/EtOH (70:30) was used as solvent. [f] Taken from ref 69. nd: not determined.

All tested derivatives showed an IC$_{50}$ against the human AChE (h-AChE) between the nano- and the picomolar range. Hybrid **24**, derived from 6-chlorotacrine and 5-hydroxy-7-methoxy-4-oxo-4*H*-chromene, was the best h-AChE inhibitor of this family, with an IC$_{50}$ of 35 pM that was 10 000-fold better than that of the parent fragment tacrine. Interestingly, they were 4- to 1070-fold more efficient for inhibition of the human enzyme than of the bovine enzyme. This superior affinity of tacrine−flavonoid hybrids toward h-AChE could be due to a better fit between the 4-oxo-4*H*-chromene fragment and the h-AChE−PAS region, because both enzymes show a higher degree of similarity in the CAS than in the PAS region.[63] The presence of substituents in the flavonoid fragment appeared to reinforce these differences, because hybrids bearing a substituted 4-oxo-4*H*-chromene subunit were 1 or 2 orders of magnitude more potent than the nonsubstituted counterparts.

Selected compounds were also potent inhibitors of human BuChE (h-BuChE) with IC$_{50}$ values between the nano- and the picomolar range. Hybrid **10** derived from tacrine and 6-methoxy-4-oxo-4*H*-chromene was the most active of the series (IC$_{50}$ = 38 pM), being 1052-fold more potent than tacrine. To the best of our knowledge, **10** is one of the most potent inhibitors of human BuChE described to date.

Comparing inhibition of h-AChE and h-BuChE, the majority of tested hybrids did not show a clear selectivity, although there were some exceptions. Compound **6**, derived from both nonsubstituted tacrine and 4-oxo-4*H*-chromene, was 117-fold more active toward h-BuChE than toward h-AChE. On the contrary, hybrids **17** (6-chlorotacrine−6,7-dimethoxy-4-oxo-4*H*-chromene), **24** (6-chlorotacrine−5-hydroxy-7-methoxy-4-oxo-4 *H*-chromene), and **29** (6-chlorotacrine−6,7-dihydroxy-4-oxo-4*H*-chromene) showed a clear preference for h-AChE, being 250-, 143-, and 1056-fold more potent inhibiting this enzyme than inhibiting h-BuChE.

The antioxidant activities of the above selected tacrine−flavonoid hybrids were evaluated by following the well-established ORAC-FL method (oxygen radical absorbance capacity by fluorescence)[66,67] that was recently applied by us to other compounds.[37,38,54] Peroxyl radicals were thermally generated from 2,2-azobis(amidinopropane) dihydrochloride and reacted with fluorescein to form nonfluorescent products at 520 nm. The antioxidant capacity of new tacrine−4-oxo-4*H*-chromene hybrids was determined by their competition with fluorescein in the radical capture, using a fluorescence microplate reader. Trolox, a vitamin E analogue, was used as a standard, and the results were expressed as trolox equivalents (μmol of trolox/μmol of tested compound), in a relative scale where ORAC (trolox) = 1. Melatonin was also tested, giving an ORAC value of 2.3 that fully agreed with the value previously described by Sofic et al. (2.0 μmol of trolox/μmol of melatonin),[68] pointing out the reliability of our experiments. Tacrine showed negligible radical-capture ability, whereas hybrids bearing hydroxyl groups exhibited interesting antioxidant capacities, although lower than the naturally related flavone 5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-chromen-4-one, apigenin (ORAC-FL = 6.7).[69] Compound **19**, bearing an unsubstituted tacrine and a 6-hydroxy-4-oxo-4*H*-chromene fragment, was 1.3-fold more potent than the vitamin E analogue and the best antioxidant of this family (Table 2).

Because the first requirement for successful CNS drugs is to reach their therapeutic targets, screening for the blood−brain barrier (BBB) penetration is of great importance. To explore whether the selected tacrine−4-oxo-4*H*-chromene derivatives would be able to penetrate into the brain, we used a parallel artificial membrane permeation assay for blood−brain barrier (PAMPA−BBB). This simple and rapid model, described by Di et al.[70] and successfully applied by us to different compounds,[37,51−54,71−75] has the advantage of predicting passive

Table 3. Inhibition of Human BACE-1 by Selected Tacrine−4-Oxo-4*H*-chromene Hybrids[a]

compd	R	R′	inhibition (%)[b]	IC$_{50}$ (μM)[c]	K_I (μM)[d]
8	6-Cl	H	25.9 ± 0.1	22.40 ± 0.04	6.58 ± 0.01
11	6-Cl	6-OCH$_3$	34.8 ± 0.1	14.40 ± 0.03	4.23 ± 0.08
13	H	5,7-diOCH$_3$	74.9 ± 0.2	2.10 ± 0.04	0.62 ± 0.02
16	H	6,7-diOCH$_3$	72.3 ± 0.1	3.00 ± 0.01	0.88 ± 0.03
19	H	6-OH	53.8 ± 0.1	2.80 ± 0.01	0.82 ± 0.01
22	H	5-OH-7-OCH$_3$	82.9 ± 0.2	2.90 ± 0.01	0.85 ± 0.01
23	H	5,7-diOH	73.9 ± 0.2	3.60 ± 0.01	1.06 ± 0.01
29	6-Cl	6,7-diOH	46.4 ± 0.1	13.60 ± 0.02	3.99 ± 0.03
2 (R = 4-OH-Ph)				38.5[e]	11.31
OM99-2			nd	0.033 ± 0.001	0.0097 ± 0.00002

[a] Human recombinant BACE-1 (EC 3.4.23.46) was used, and the results are the mean of two independent experiments ± SD. [b] Percentage of BACE-1 inhibition of compounds at 10 μM. [c] Eight different concentrations of inhibitors were used, between 0.5 μM and 100 μM. [d] The inhibition constants were calculated from their IC$_{50}$ values, using the equation of Cheng and Prusoff ($K_I = IC_{50}/(1 + [S]/K_M)$)[81] and considering the following parameters: competitive binding mode, [S] = 250 nM and $K_M = 0.104 \pm 0.004$ μM. [e] Taken from ref 42. nd: not determined.

BBB permeation with high success. The in vitro permeabilities (P_e) of the above selected tacrine−flavonoid hybrids and 15 commercial drugs through a lipid extract of porcine brain were determined using a mixture of PBS:EtOH (70:30). Assay validation was made by comparing the experimental permeability with the reported values of these commercial drugs that gave a good lineal correlation, P_e (exptl) = 1.24 P_e (bibl) + 1.98 (R^2 = 0.93) (see Supporting Information). From this equation, and taking into account the limits established by Di et al. for BBB permeation,[70] we found that molecules with a permeability >7.0 × 10^{-6} cm s^{-1} would be able to cross the BBB by passive permeation. The majority of tested tacrine−flavonoid hybrids showed permeability values over the above limit, as the known CNS drugs used in the assay validation, pointing out that these molecules would cross the BBB by passive diffusion (Table 2). Only derivatives **28** and **29**, bearing a 6,7-dihydroxy-4-oxo-4*H*-chromene fragment, may experience some difficulties to reach the CNS.

Inhibition of Human BACE-1. Then, tacrine−4-oxo-4 *H*-chromene hybrids, covering all the different structural features in the flavonoid fragment, were evaluated as inhibitors of the human recombinant BACE-1 protein, which was expressed in a eukaryotic system as a glycoprotein. For measuring the enzyme activity, a FRET-based (fluorescence resonance energy transfer) assay system was applied.[76,77] The well-known competitive inhibitor of BACE-1, OM99-2, was used as a reference compound, displaying in our experiments an IC$_{50}$ value of 33 nM (K_I = 9.68 ± 0.02 nM) that is in accordance with the previously published values (K_I = 1.2−9.8 nM)[78−80] (Table 3).

Initially, compounds were screened at a single concentration (10 μM) displaying interesting BACE-1 inhibitory activities with percentages of inhibition ranging from 26% to 83%, whereas tacrine was inactive (data not shown). Then, the IC$_{50}$ values were calculated from the plot of BACE-1 activity vs the inhibitor concentrations, which were ranging between 0.5 μM and 100 μM (see Supporting Information). The inhibition constants were calculated from IC$_{50}$ values, using the equation of Cheng and Prusoff ($K_I = IC_{50}/(1 + [S]/K_M)$).[81] IC$_{50}$ and K_I results are also gathered in Table 3.

Tacrine−4-oxo-4*H*-chromene derivatives were found to be potent inhibitors of human BACE-1 with IC$_{50}$s from 2 to 22 μM, better than that of apigenin (IC$_{50}$ = 38.5 μM).[42] In general, the presence of substituents in 4-oxo-4*H*-chromene improved inhibition, giving derivatives that were 1 order of magnitude more potent than that of the unsubstituted counterpart. Hybrid **13**, derived from tacrine and 5,7-dimethoxy-4-oxo-4*H*-chromene, was the most active of the series, showing an IC$_{50}$ = 2.1 μM. Because it is described that strong inhibition of BACE-1 could be associated with some toxic effects,[82,83] the fact that these new tacrine−4-oxo-4*H*-chromene hybrids showed nanomolar inhibition of h-ChEs but micromolar inhibition of h-BACE-1 could not be a disadvantage, as recently found for bis(7)-tacrine that displays the same profile toward AChE and BACE-1 as that of the new molecules described here.[84]

■ CONCLUSIONS

From the above results, we can summarize that the new tacrine−4-oxo-4 *H*-chromene hybrids showed interesting in vitro biological activities for the potential treatment of Alzheimer's disease, such as inhibition of human AChE, BuChE, and BACE-1, as well as radical scavenger activity. They were potent inhibitors of both human AChE and BuChE, with IC$_{50}$ values in the nano- and picomolar ranges, being in general more potent than the parent inhibitor tacrine. Hybrid **24** (derived from 6-chlorotacrine and 5-hydroxy-7-methoxy-4-oxo-4 *H*-chromene) was the best h-AChE inhibitor of this family (IC$_{50}$ = 35 pM), whereas compound **10** (derived from tacrine and 6-methoxy-4-oxo-4*H*-chromene) was the most active toward h-BuChE (IC$_{50}$ = 38 pM). In general, these tacrine−flavonoid hybrids did not show a clear selectivity toward human ChEs, although some outstanding exceptions were found. Hybrid **29**, derived from 6-chlorotacrine and 6,7-dihydroxy-4-oxo-4 *H*-chromene, was 1056-fold more potent toward h-AChE than toward h-BuChE. In contrast, compound **6**, bearing unsubstituted heterocycles, showed a clear selectivity for h-BuChE, exhibiting a ratio h-AChE/h-BuChE of 117. In relation to the inhibition of human BACE-1, the best results were obtained with substituted flavonoid fragments, such as hybrid **13** (tacrine−5,7-dimethoxy-4-oxo-4*H*-chromene) that showed an IC$_{50}$ value of 2.1 μM. With regard to antioxidant properties, the radical capture capacity was found to be related to the presence of phenolic groups in the flavonoid fragment, compound **19** (tacrine−6-hydroxy-4-oxo-4*H*-chromene) being 1.3-fold more potent than trolox, a vitamin E analogue. In addition, almost all new compounds were able to penetrate into the CNS according to the well-known PAMPA-BBB assay.

Among the different molecules investigated, hybrid **19** derived from unsubstituted tacrine and 6-hydroxy-4-oxo-4*H*-chromene showed potent combined inhibition of human BACE-1 and ChEs, as well as good antioxidant properties.

Thus, it is expected that this hybrid could augment patient cognition (by increasing levels of acetylcholine), protect neurons from oxidative stress (by capturing free radicals), and reduce the formation of senile plaques (from inhibition of BACE-1). Such biological properties, along with its ability to reach the CNS, highlight hybrid **19** as a very interesting prototype in the search for new disease-modifying drugs useful in the treatment of AD. Work is now scheduled to test hybrid **19** in more complex biological models of AD, involving neuronal cells, brain slices, and APP/Ps1 mice.

■ EXPERIMENTAL SECTION

Chemistry. General Methods. Reagents were purchased from common commercial suppliers and were used without further purification. Solvents were purified and dried by standard procedures. Chromatographic separations were performed either on silica gel (Kielgel 60 Merck of 230−400 mesh) or C18 reversed-phase (Sep-Pak Vac C18 cartridges). Compounds were detected with UV light (λ = 254 nm). HPLC analyses were used to confirm the purity of all compounds (>95%) and were performed on Waters 6000 equipment, at a flow rate of 1.0 mL/min, with a UV detector (λ = 214−274 nm), and using a Delta Pak C_{18} 5 μm, 300 Å column.

Melting points (uncorrected) were determined with a Reichert-Jung Thermovar apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded in CD_3OD or $CDCl_3$ solutions using a Varian XL-300 spectrometer. Chemical shifts are reported in δ scale (ppm) relative to internal Me_4Si. *J* values are given in hertz, and spin multiplicities are expressed as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintuplet), or m (multiplet). Mass spectra (MS) were obtained by electron spray ionization (ESI) in positive mode using a Hewlett-Packard MSD 1100 spectrometer. Elemental analyses were carried out in a Perkin-Elmer 240C equipment in the Centro de Química Orgánica 'Manuel Lora-Tamayo' (CSIC), and the results are within ±0.4% of the theoretical values.

General Procedure for the Synthesis of Tacrine−4-Oxo-4 *H*-chromene Hybrids (3−18). To a mixture of the corresponding 4-oxo-4*H*-chromene-2-carboxylic acid derivative **38−41** (1.0 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 1.3 mmol) in CH_2Cl_2 (20 mL) were added the appropriate 9-alkylaminotetrahydroacridine **31−37** (1.0 mmol) and then triethylamine (2.6 mmol). The reaction mixture was stirred at room temperature overnight and then diluted with CH_2Cl_2 (50 mL). The resulting mixture was consecutively washed with 10% aq citric acid solution (3 × 30 mL), 10% aq $NaHCO_3$ solution (3 × 30 mL), and H_2O (30 mL). The organic phase was dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was purified on a silica gel column using mixtures of EtOAc/CH_3OH/aqueous 30% NH_3 or CH_2Cl_2/CH_3OH as eluent, obtaining the corresponding tacrine−flavonoid compound as a syrup that was identified by ^{1}H NMR, ^{13}C NMR, and MS. Then, the treatment of the previous syrup with HCl (g) in dichloromethane yielded the hydrochloride salt that was collected by filtration as a pure solid, which was used for obtaining combustion analysis results and biological activities.

4-Oxo-*N*-{7-[(1,2,3,4-tetrahydroacridin-9-yl)amino]heptyl}-4*H*-chromene-2-carboxamide (3). Reagents were N^1-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (**31**) (150 mg, 0.48 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (92 mg, 0.48 mg), BOP (277 mg, 0.63 mmol), and Et_3N (170 μL, 1.26 mmol). Purification involved the use of EtOAc/CH_3OH/aqueous 30% NH_3 (from 9:1:0.2 to 6:1:0.2) as eluent. **3**: Pale oil (156 mg, 67%). ESI-MS *m*/*z* 484 $[M + H]^+$. ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, 1H, *J* = 8.1 Hz, *J* = 1.3 Hz, H5′), 7.91 (dd, 1H, *J* = 8.3 Hz, *J* = 1.3 Hz, H8), 7.78 (dd, 1H, *J* = 8.3 Hz, *J* = 1.3 Hz, H5), 7.61 (ddd, 1H, *J* = 8.1 Hz, *J* = 7.1 Hz, *J* = 1.3 Hz, H7′), 7.49 (dd, 1H, *J* = 8.1 Hz, *J* = 1.3 Hz, H8′), 7.45 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H6), 7.35 (ddd, 1H, *J* = 8.1 Hz, *J* = 7.1 Hz, *J* = 1.3 Hz, H6′), 7.27 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H7), 7.10 (t, 1H, *J* = 6.9 Hz, CONH), 7.07 (s, 1H, H3′), 4.40 (broad s, 1H), 3.45 (t, 2H, *J* = 6.9 Hz, $CH_2\alpha$), 3.37 (q, 2H, *J* = 6.9 Hz, $CH_2\omega$), 2.95 (m, 2H, H4), 2.61 (m, 2H, H1), 1.82 (m, 4H, H2, 3), 1.59 (quint, 2H, *J* = 6.9 Hz), 1.52 (quint, 2H, *J* = 6.9 Hz), 1.25 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 178.3 (C4′), 159.2 (CONH), 157.3 (C4a), 155.2 (C2′), 155.0 (C8a′), 151.2 (C9), 146.5 (C10a), 134.4 (C7′), 128.5 (C6), 127.3 (C5), 125.7 (C5′), 125.6 (C6′), 124.0 (C4a′), 123.6 (C7), 123.0 (C8), 119.5 (C8a), 118.2 (C8′), 115.2 (C9a), 111.6 (C3′), 49.1 ($CH_2\alpha$), 39.8 ($CH_2\omega$), 33.2 (C4), 31.4, 29.0, 28.7, 26.5 (2C), 24.4 (C1), 22.7 (C2), 22.4 (C3). **3**·HCl: colorless solid (mp 161−163 °C). Purity: 99% (by HPLC). Anal. ($C_{30}H_{33}N_3O_3$·HCl) C, H, N.

4-Oxo-*N*-{8-[(1,2,3,4-tetrahydroacridin-9-yl)amino]octyl}-4*H*-chromene-2-carboxamide (4). Reagents were N^1-(1,2,3,4-tetrahydroacridin-9-yl)-1,8-octanodiamine (**32**) (200 mg, 0.62 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (117 mg, 0.62 mmol), BOP (353 mg, 0.80 mmol), and Et_3N (212 μL, 1.60 mmol). Purification involved the use of EtOAc/CH_3OH/aqueous 30% NH_3 (from 8:1:0.2 to 6:1:0.2) as eluent. **4**: Pale oil (162 mg, 53%). ESI-MS: *m*/*z* 498 $[M + H]^+$. ^{1}H NMR ($CDCl_3$) δ 8.14 (dd, 1H, *J* = 8.0 Hz, *J* = 1.3 Hz, H5′), 7.93 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, H8), 7.85 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, H5), 7.64 (ddd, 1H, *J* = 8.0 Hz, *J* = 7.1 Hz, *J* = 1.5 Hz, H7′), 7.49 (dd, 1H, *J* = 8.0 Hz, *J* = 1.5 Hz, H8′), 7.47 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.2 Hz, H6), 7.37 (ddd, 1H, *J* = 8.0 Hz, *J* = 7.1 Hz, *J* = 1.5 Hz, H6′), 7.29 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.2 Hz, H7), 7.14 (t, 1H, *J* = 7.1 Hz, CONH), 7.09 (s, 1H, H3′), 4.80 (broad s, 1H), 3.49 (t, 2H, *J* = 7.1 Hz, $CH_2\alpha$), 3.39 (q, 2H, *J* = 7.1 Hz, $CH_2\omega$), 2.99 (m, 2H, H4), 2.65 (m, 2H, H1), 1.84 (m, 4H, H2, 3), 1.62 (quint, 2H, *J* = 7.1 Hz), 1.56 (quint, 2H, *J* = 7.1 Hz), 1.26 (m, 8H). ^{13}C NMR ($CDCl_3$) δ 178.2 (C4′), 159.2 (CONH), 156.6 (C4a), 155.2 (C2′), 155.1 (C8a′), 151.6 (9), 145.5 (C10a), 134.2 (C7′), 128.8 (C6), 126.6 (C5), 125.5 (C5′), 125.4 (C6′), 124.0 (C4a′), 123.6 (C7), 123.1 (C8), 119.1 (C8a), 118.2 (C8′), 114.7 (C9a), 111.5 (C3′), 48.9 ($CH_2\alpha$), 39.8 ($CH_2\omega$), 32.6 (C4), 31.3, 29.0 (2C), 28.8, 26.5 (2C), 24.3 (C1), 22.6 (C2), 22.2 (C3). **4**·HCl: colorless solid (mp 87−90 °C). Purity: 100% (by HPLC). Anal. ($C_{31}H_{35}N_3O_3$·HCl) C, H, N.

4-Oxo-*N*-{9-[(1,2,3,4-tetrahydroacridin-9-yl)amino]nonyl}-4*H*-chromene-2-carboxamide (5). Reagents were N^1-(1,2,3,4-tetrahydroacridin-9-yl)-1,9-nonanodiamine (**33**) (105 mg, 0.31 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (59 mg, 0.31 mmol), BOP (180 mg, 0.40 mmol), and Et_3N (110 μL, 0.81 mmol). Purification involved the use of EtOAc/CH_3OH/aqueous 30% NH_3 (from 10:1:0.2 to 7:1:0.2) as eluent. **5**: Pale oil (67 mg, 42%). ESI-MS: *m*/*z* 512 $[M + H]^+$. ^{1}H NMR ($CDCl_3$) δ 8.11 (dd, 1H, *J* = 8.1 Hz, *J* = 1.3 Hz, H5′), 7.91 (dd, 1H, *J* = 8.2 Hz, *J* = 1.0 Hz, H8), 7.82 (dd, 1H, *J* = 8.2 Hz, *J* = 1.0 Hz, H5), 7.63 (ddd, *J* = 8.1 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H7′), 7.49 (dd, 1H, *J* = 8.1 Hz, *J* = 1.3 Hz, H8′), 7.47 (ddd, 1H, *J* = 8.2 Hz, *J* = 6.9 Hz, *J* = 1.0 Hz, H6), 7.35 (ddd, 1H, *J* = 8.1 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H6′), 7.27 (ddd, 1H, *J* = 8.2 Hz, *J* = 6.9 Hz, *J* = 1.0 Hz, H7), 7.09 (t, 1H, *J* = 7.1 Hz, CONH), 7.08 (s, 1H, H3′), 4.80 (broad s, 1H), 3.49 (t, 2H, *J* = 7.1, $CH_2\alpha$), 3.39 (q, 2H, *J* = 7.1 Hz, $CH_2\omega$), 2.97 (m, 2H, H4), 2.63 (m, 2H, H1), 1.84 (m, 4H, H2, 3), 1.59 (quint, 2H, *J* = 7.1 Hz), 1.56 (quint, 2H, *J* = 7.1 Hz), 1.25 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 178.2 (C4′), 159.2 (CONH), 157.5 (C4a), 155.2 (C2′), 155.0 (C8a′), 151.2 (C9), 146.5 (C10a), 134.3 (C7′), 128.5 (C6), 127.6 (C5), 126.7 (C6′), 125.6 (C5′), 124.1 (C4a′), 123.5 (C7), 122.9 (C8), 119.6 (C8a), 118.1 (C8′), 115.2 (C9a), 111.7 (C3′), 49.2 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 33.3 (C4), 31.5, 29.2, 29.1, 29.0, 28.9, 26.7, 26.6, 24.5 (C1), 22.8 (C2), 22.4 (C3). **5**·HCl: yellow solid (mp 80−82 °C). Purity: 100% (by HPLC). Anal. ($C_{32}H_{37}N_3O_3$·HCl) C, H, N.

4-Oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4*H*-chromene-2-carboxamide (6). Reagents were N^1-(1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**34**) (210 mg, 0.59 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (113 mg, 0.59 mmol), BOP (339 mg, 0.77 mmol), and Et_3N (210 μL, 1.53 mmol Purification involved the use of EtOAc/CH_3OH/aqueous 30% NH_3 (from 10:1:0.2 to 5:1:0.2) as eluent. **6**: Pale oil (140 mg, 45%). ESI-MS: *m*/*z* 526 $[M + H]^+$. ^{1}H NMR ($CDCl_3$) δ 8.19 (dd, 1H, *J* = 8.3 Hz, *J* = 1.3 Hz, H5′), 7.97 (dd, 1H, *J* = 8.3 Hz, *J* = 1.0 Hz, H8), 7.92 (dd, 1H, *J* = 8.3 Hz, *J* = 1.0 Hz, H5), 7.69 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H7′), 7.53 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, H6), 7.49 (dd, 1H, *J* = 8.3 Hz, *J* = 1.3 Hz, H8′), 7.42 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, H7), 7.35 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J*

= 1.3 Hz, H6′), 7.14 (s, 1H, H3′), 7.09 (t, 1H, *J* = 6.8 Hz, CONH), 4.40 (broad s, 1H), 3.55 (t, 2H, *J* = 6.8 Hz, $CH_2\alpha$), 3.45 (c, 2H, *J* = 6.8 Hz, $CH_2\omega$), 3.04 (m, 2H, H4), 2.64 (m, 2H, H1), 1.84 (m, 4H, H2, 3), 1.62 (quint, 2H, *J* = 6.8 Hz), 1.57 (quint, 2H, *J* = 6.8 Hz), 1.30 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 178.3 (C4′), 159.2 (CONH), 156.7 (C4a), 155.2 (C2′), 155.1 (C8a′), 151.9 (C9), 145.7 (C10a), 134.4 (C7′), 128.8 (C6), 126.7 (C5), 125.6 (C5′), 125.5 (C6′), 123.9 (C4a′), 123.6 (C7), 123.2 (C8), 119.2 (C8a), 118.3 (C8′), 114.7 (C9a), 111.5 (C3′), 49.1 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 32.7 (C4), 31.4, 29.1 (2C), 29.0 (2C), 28.9, 26.7 (2C), 24.3 (C1), 22.6 (C2), 22.4 (C3). **6**·HCl: yellow solid (mp 84−86 °C). Purity: 98% (by HPLC). Anal. ($C_{33}H_{39}N_3O_3$·HCl) C, H, N.

4-Oxo-*N*-{12-[(1,2,3,4-tetrahydroacridin-9-yl)amino]dodecyl}-4*H*-chromene-2-carboxamide (7). Reagents were N^1-(1,2,3,4-tetrahydroacridin-9-yl)-1,12-dodecanodiamine (**35**) (260 mg, 0.68 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (130 mg, 0.68 mmol), BOP (392 mg, 0.87 mmol), and Et_3N (250 μL, 1.77 mmol). Purification involved the use of EtOAc/CH_3OH/aqueous 30% NH_3 (from 10:1:0.2 to 5:1:0.2) as eluent. **7**: Pale oil (250 mg, 66%). ESI-MS: *m/z* 554 $[M + H]^+$. ^1H NMR ($CDCl_3$) δ 8.11 (dd, 1H, *J* = 8.3 Hz, *J* = 1.3 Hz, H5′), 8.00 (dd, 1H, *J* = 8.3 Hz, *J* = 1.0 Hz, H8), 7.80 (dd, 1H, *J* = 8.3 Hz, *J* = 1.0 Hz, H5), 7.69 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H7′), 7.54 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, H6), 7.49 (dd, 1H, *J* = 8.3 Hz, *J* = 1.3 Hz, H8′), 7.35 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.3 Hz, H6′), 7.30 (ddd, 1H, *J* = 8.3 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, H7), 7.14 (s, 1H, H3′), 7.09 (t, 1H, *J* = 6.8 Hz, CONH), 4.40 (broad s, 1H), 3.55 (t, 2H, *J* = 6.9 Hz, $CH_2\alpha$), 3.45 (q, 2H, *J* = 6.9 Hz, $CH_2\omega$), 2.96 (m, 2H, H4), 2.64 (m, 2H, H1), 1.84 (m, 4H, H2, 3), 1.67 (quint, 2H, *J* = 6.9 Hz), 1.61 (quint, 2H, *J* = 6.9 Hz), 1.25 (m, 16H). ^{13}C NMR ($CDCl_3$) δ 178.4 (C4′), 159.2 (CONH), 155.3 (C4a), 155.2 (C2′), 155.1 (C8a′), 152.8 (C9), 143.9 (C10a), 134.5 (C7′), 129.9 (C6), 125.7 (C5), 125.5 (C5′), 124.7 (C6′), 124.0 (2C, C7 and C4a′), 123.8 (C8), 118.4 (C8a), 118.3 (C8′), 113.8 (C9a), 111.5 (C3′), 49.1 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 31.6 (C4), 31.2, 29.3 (2C), 29.2 (2C), 29.1, 29.0 (2C), 26.7, 26.6, 24.0 (C1), 22.4 (C2), 21.8 (C3). **7**·HCl: yellow solid (mp 140−142 °C). Purity: 99% (by HPLC). Anal. ($C_{35}H_{43}N_3O_3$·HCl) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4-oxo-4*H*-chromene-2-carboxamide (8).** Reagents were N^1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**36**) (100 mg, 0.26 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (49 mg, 0.26 mmol), BOP (148 mg, 0.33 mmol), and Et_3N (90 μL, 0.67 mmol). Purification involved the use of CH_2Cl_2/CH_3OH (from 10:1 to 5:1), as eluent. **8**: Pale oil (115 mg, 80%). ESI-MS: *m/z* 560 $[M + H]^+$. ^1H NMR ($CDCl_3$) δ 8.17 (dd, 1H, *J* = 8.5 Hz, *J* = 1.0 Hz, H5′), 7.93 (d, 1H, *J* = 8.5 Hz, H8), 7.80 (d, 1H, *J* = 2.2 Hz, H5), 7.70 (ddd, 1H, *J* = 8.5 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, H7′), 7.52 (dd, 1H, *J* = 8.5 Hz, *J* = 1.0 Hz, H8′), 7.41 (ddd, 1H, *J* = 8.5 Hz, *J* = 7.0 Hz, *J* = 1.0 Hz, H6′), 7.26 (dd, 1H, *J* = 8.5 Hz, *J* = 2.2 Hz, H7), 7.12 (s, 1H, H3′), 7.10 (t, 1H, *J* = 7.0 Hz, CONH), 4.40 (broad s, 1H), 3.55 (t, 2H, *J* = 7.0 Hz, $CH_2\alpha$), 3.44 (q, 2H, *J* = 7.0 Hz, $CH_2\omega$), 2.97 (m, 2H, H4), 2.62 (m, 2H, H1), 1.88 (m, 4H, H2,3), 1.68 (quint, 2H, *J* = 7.0 Hz), 1.60 (quint, 2H, *J* = 7.0 Hz), 1.31 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 178.4 (C4′), 159.2 (CONH), 156.4 (C4a), 155.2 (C2′), 152.6 (C9), 155.1 (C8a′), 145.0 (C10a), 135.7 (C6), 134.6 (C7′), 125.8 (C6′), 125.6 (C5′), 125.4 (C8), 124.6 (C7), 124.0 (C4a′), 123.9 (C5), 118.4 (C8′), 116.7 (C8a), 114.1 (C9a), 111.6 (C3′), 49.2 ($CH_2\alpha$), 40.0 ($CH_2\omega$), 31.9 (2C, C4), 29.2 (2C), 29.1, 29.0 (2C), 26.7, 26.6, 23.9 (C1), 22.3 (C2), 21.8 (C3). **8**·HCl: yellow solid (mp 105−107 °C). Purity: 100% (by HPLC). Anal. ($C_{33}H_{38}ClN_3O_3$·HCl·H_2O) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4-oxo-4*H*-chromene-2-carboxamide (9).** Reagents were N^1-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**37**) (100 mg, 0.24 mmol), 4-oxo-4*H*-chromene-2-carboxylic acid (**38**) (45 mg, 0.24 mmol), BOP (136 mg, 0.31 mmol), and Et_3N (88 μL, 0.62 mmol). Purification involved the use of CH_2Cl_2/CH_3OH (from 20:1 to 10:1), as eluent. **9**: Pale oil (97 mg, 70%). ESI-MS: *m/z* 594 $[M + H]^+$. ^1H NMR ($CDCl_3$) δ 8.15 (dd, 1H, *J* = 8.5 Hz, *J* = 1.3 Hz, H5′), 7.74 (d, 1H, *J* = 2.2 Hz, H5), 7.66 (ddd, 1H, *J* = 8.5 Hz, *J* = 7.3 Hz, *J* = 1.3 Hz, H7′), 7.43 (dd, 1H, *J* = 8.5 Hz, *J* = 1.3 Hz, H8′), 7.39 (ddd, 1H, *J* = 8.5 Hz, *J* = 7.3 Hz, *J* = 1.3 Hz, H6′), 7.27 (d, 1H, *J* = 2.2 Hz, H7), 7.13 (t, 1H, *J* = 7.1 Hz, CONH), 7.09 (s, 1H, H3′), 5.80 (broad s, 1H), 3.43 (c, 2H, *J* = 7.1 Hz, $CH_2\omega$), 3.16 (t, 2H, *J* = 7.1 Hz, $CH_2\alpha$), 2.95 (m, 2H, H4), 2.68 (m, 2H, H1), 1.85 (m, 4H, H2, 3), 1.57 (quint, 4H, *J* = 7.1 Hz), 1.33 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 178.1 (C4′), 160.7 (C4a), 159.1 (CONH), 155.1 (C2′), 152.1 (C9 and C8a′), 148.6 (C10a), 134.4 (C7′), 132.5 (C6), 128.4 (C8), 127.5 (C5), 127.3 (C7), 125.9 (C5′), 125.8 (C6′), 124.2 (C4a′), 120.1 (C9a), 117.9 (C8′), 116.9 (C8a), 111.9 (C3′), 49.2 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 33.3 (C4), 30.8, 29.3 (2C), 29.2 (2C), 29.1, 29.0, 26.8 (2C, C1), 22.9 (C2), 22.5 (C3). **9**·HCl: yellow solid (mp 70−72 °C). Purity: 100% (by HPLC). Anal. ($C_{33}H_{37}Cl_2N_3O_3$·HCl) C, H, N.

6-Methoxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4*H*-chromene-2-carboxamide (10). Reagents were N^1-(1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**34**) (74 mg, 0.21 mmol), 6-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid **39** (46 mg, 0.21 mmol), BOP (121 mg, 0.27 mmol), and Et_3N (75 μL, 0.55 mmol). Purification involved the use of EtOAc/CH_3OH/aqueous 30% NH_3 (from 10:1:0.2 to 5:1:0.2), as eluent. **10**: Pale oil (68 mg, 59%). ESI-MS: *m/z* 556 $[M + H]^+$. ^1H NMR ($CDCl_3$) δ 7.97 (d, 1H, *J* = 8.0 Hz, H8), 7.94 (d, 1H, *J* = 8.0 Hz, H5), 7.52 (t, 1H, *J* = 8.0 Hz, H6), 7.51 (d, 1H, *J* = 2.4 Hz, H5′), 7.43 (d, 1H, *J* = 9.0 Hz, H8′), 7.40 (t, 1H, *J* = 5.8 Hz, CONH), 7.28 (t, 1H, *J* = 8.0 Hz, H7), 7.25 (dd, 1H, *J* = 9.0 Hz, *J* = 2.4 Hz, H7′), 7.12 (s, 1H, H3′), 4.20 (broad s, 1H), 3.86 (s, 3H, OCH_3), 3.49 (t, 2H, *J* = 7.1 Hz, $CH_2\alpha$), 3.40 (q, 2H, *J* = 7.1 Hz, $CH_2\omega$), 3.00 (m, 2H, H4), 2.62 (m, 2H, H1), 1.84 (m, 4H, H2, 3), 1.62 (quint, 4H, *J* = 7.1 Hz), 1.35 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 178.0 (C4′), 159.3 (CONH), 157.3 (C4a and C6′), 154.7 (C2′), 151.4 (C9), 149.9 (C10a and C8a′), 128.7 (C6), 127.2 (C5), 124.9 (C4a′), 124.8 (C7′), 123.6 (C7), 123.0 (C8), 119.5 (C8′), 119.4 (C8a), 114.8 (C9a), 110.9 (C3′), 104.8 (C5′), 55.8 (OCH_3), 49.2 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 33.4 (C4), 31.5, 29.3, 29.2, 29.1 (2C), 29.0, 26.8, 26.7, 24.6 (C1), 22.9 (C2), 22.5 (C3). **10**·HCl: yellow solid (mp 113−115 °C). Purity: 100% (by HPLC). Anal. ($C_{34}H_{41}N_3O_4$·HCl·H_2O) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-6-methoxy-4-oxo-4*H*-chromene-2-carboxamide (11).** Reagents were N^1-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**36**) (150 mg, 0.39 mmol), 6-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid **39** (85 mg, 0.39 mmol), BOP (222 mg, 0.50 mmol), and Et_3N (140 μL, 1.0 mmol). Purification involved the use of EtOAc/CH_3OH (from 12:1 to 6:1) as eluent. **11**: Pale oil (105 mg, 46%). ESI-MS: *m/z* 590 $[M + H]^+$. ^1H NMR ($CDCl_3$) δ 7.95 (d, 1H, *J* = 9.0 Hz, H8), 7.80 (d, 1H, *J* = 2.0 Hz, H5), 7.51 (d, 1H, *J* = 3.0 Hz, H5′), 7.46 (d, 1H, *J* = 9.3 Hz, H8′), 7.28 (dd, 1H, *J* = 9.3 Hz, *J* = 3.0 Hz, H7′), 7.25 (dd, 1H, *J* = 9.0 Hz, *J* = 2.0 Hz, H7), 7.10 (s, 1H, H3′), 7.08 (t, 1H, *J* = 5.9 Hz, CONH), 4.52 (broad s, 1H), 3.87 (s, 3H, OCH_3), 3.58 (t, 2H, *J* = 7.0 Hz, $CH_2\alpha$), 3.44 (q, 2H, *J* = 7.0 Hz, $CH_2\omega$), 3.00 (t, 2H, *J* = 5.9 Hz, H4), 2.62 (t, 2H, *J* = 5.9 Hz, H1), 1.88 (m, 4H, H2, 3), 1.65 (m, 4H), 1.30 (m,12H). ^{13}C NMR ($CDCl_3$) δ 178.1 (C4′), 159.3 (CONH), 157.4 (C6′), 157.2 (C4a), 154.8 (C2′), 152.2 (C9), 150.0 (C8a′), 145.8 (C10a), 135.3 (C6), 125.2 (C8), 125.0 (C4a′), 124.9 (C5), 124.6 (C7), 124.5 (C7′), 119.6 (C8′), 117.2 (C8a), 114.6 (C9a), 110.8 (C3′), 104.9 (C5′), 55.9 (OCH_3), 49.3 ($CH_2\alpha$), 40.0 ($CH_2\omega$), 33.4 (C4), 31.3, 29.3, 29.2, 29.1, 29.0 (2C), 26.8, 26.7, 24.1 (C1), 22.5 (C2), 22.0 (C3). **11**·HCl: yellow solid (mp 104−106 °C). Purity: 100% (by HPLC). Anal. ($C_{34}H_{40}ClN_3O_4$·HCl·H_2O) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-6-methoxy-4-oxo-4*H*-chromene-2-carboxamide (12).** Reagents were N^1-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**37**) (100 mg, 0.24 mmol), 6-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid **39** (52 mg, 0.24 mmol), BOP (136 mg, 0.31 mmol), and Et_3N (88 μL, 0.62 mmol). Purification involved the use of EtOAc/CH_3OH (from 15:1 to 7:1) as eluent. **12**: Pale oil (100 mg, 68%). ESI-MS: *m/z* 624 $[M + H]^+$. ^1H NMR ($CDCl_3$) δ 7.74 (d, 1H, *J* = 2.2 Hz, H5), 7.49 (d, 1H, *J* = 3.2 Hz, H5′), 7.37 (d, 1H, *J* = 9.3 Hz, H8′), 7.27 (d, 1H, *J* = 2.2 Hz, H7), 7.24 (dd, 1H, *J* = 9.3 Hz, *J* = 3.2 Hz, H7′), 7.08 (s, 1H, H3′), 6.87 (t, 1H, *J* = 5.8 Hz, CONH), 5.73 (broad s, 1H), 3.84 (s, 3H, OCH_3), 3.40 (t, 2H, *J* = 7.1 Hz, $CH_2\omega$), 3.14 (t, 2H, *J* = 7.1 Hz, $CH_2\alpha$), 2.95 (t, 2H, *J* = 6.6 Hz, H4), 2.66 (t, 2H, *J* = 6.6 Hz, H1), 1.80 (m, 4H, H2, 3), 1.55 (quint, 4H, *J* = 7.1 Hz), 1.30 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 178.0 (C4′), 160.7 (C4a), 159.2 (CONH), 157.4 (C6′), 154.6 (C2′), 152.0 (C9), 149.9 (C8a′), 148.7 (C10a), 132.5 (C6), 128.4 (C8), 127.6 (C7), 127.4 (C5), 125.0 (C4a′), 124.5 (C7′), 120.2 (C9a), 119.3 (C8′), 116.9 (C8a), 111.1 (C3′), 105.0 (C5′), 55.9 (OCH$_3$), 49.2 (CH$_2\alpha$), 39.9 (CH$_2\omega$), 33.4 (C4), 30.8, 29.5, 29.4, 29.3, 29.2, 29.1, 26.9, 26.8, 26.7 (C1), 22.9 (C2), 22.6 (C3). **12**·HCl: yellow solid (mp 83−85 °C). Purity: 100% (by HPLC). Anal. (C$_{34}$H$_{39}$Cl$_2$N$_3$O$_4$·HCl·H$_2$O) C, H, N.

5,7-Dimethoxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4*H*-chromene-2-carboxamide (13). Reagents were *N*^{1}-(1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**34**) (100 mg, 0.28 mmol), 5,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid **40** (71 mg, 0.28 mmol), BOP (163 mg, 0.37 mmol), and Et$_3$N (100 μL, 0.73 mmol). Purification involved the use of EtOAc/CH$_3$OH/aqueous 30% NH$_3$ (from 12:1:0.2 to 7:1:0.2) as eluents. **13**: Pale oil (75 mg, 45%). ESI-MS: *m*/*z* 586 [M + H]$^+$. ^{1}H NMR (CDCl$_3$) δ 7.93 (dd, 2H, *J* = 8.5 Hz, *J* = 1.2 Hz, H5, 8), 7.51 (ddd, 1H, *J* = 8.5 Hz, *J* = 6.8 Hz, *J* = 1.2 Hz, H6), 7.30 (ddd, 1H, *J* = 8.5 Hz, *J* = 6.8 Hz, *J* = 1.2 Hz, H7), 7.18 (t, 1H, *J* = 5.4 Hz, CONH), 6.93 (s, 1H, H3′), 6.48 (d, 1H, *J* = 2.2 Hz, H8′), 6.33 (d, 1H, *J* = 2.2 Hz, H6′), 5.60 (broad s, 1H), 3.89 (s, 3H, OCH$_3$-5′), 3.82 (s, 3H, OCH$_3$-7′), 3.46 (t, 2H, *J* = 7.2 Hz, CH$_2\alpha$), 3.40 (q, 2H, *J* = 6.9 Hz, CH$_2\omega$), 3.01 (m, 2H, H4), 2.67 (m, 2H, H1), 2.01 (m, 4H, H2, 3), 1.62 (m, 4H), 1.29 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 177.0 (C4′), 164.5 (C7′), 161.0 (C5′), 159.3 (C2′), 158.9 (CONH), 158.0 (C4a), 152.5 (C8a′), 150.9 (C9), 147.0 (C10a), 128.5 (C5), 128.3 (C6) 123.5 (C7), 122.9 (C8), 119.9 (C8a), 115,5 (C9a), 113.6 (C3′), 109.6 (C4a′), 96.4 (C6′), 92.7 (C8′), 56.3 (OCH$_3$ 5′), 55.7 (OCH$_3$ 7′), 49.3 (CH$_2\alpha$), 39.8 (CH$_2\omega$), 33.7 (C4), 31.6, 29.4, 29.2 (3C), 29.0 (2C), 26.8, 24.6 (C1), 22.9 (C2), 22.6 (C3). **13**·HCl: yellow solid (mp 140−142 °C). Purity: 100% (by HPLC). Anal. (C$_{35}$H$_{43}$N$_3$O$_5$·HCl·H$_2$O) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-5,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxamide (14).** Reagents were *N*^{1}-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**36**) (150 mg, 0.39 mmol), 5,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid **40** (97 mg, 0.39 mmol), BOP (222 mg, 0.50 mmol), and Et$_3$N (140 μL, 1.0 mmol). Purification involved the use of EtOAc/CH$_3$OH (from 12:1 to 6:1) as eluent. **14**: Pale oil (118 mg, 49%). ESI-MS: *m*/*z* 620 [M + H]$^+$. ^{1}H NMR (CDCl$_3$) δ 7.89 (d, 1H, *J* = 9.0 Hz, H8), 7.87 (d, 1H, *J* = 2.2 Hz, H5), 7.25 (dd, 1H, *J* = 9.0 Hz, *J* = 2.2 Hz, H7), 6.96 (s, 1H, H3′), 6.87 (t, 1H, *J* = 5.8 Hz, CONH), 6.47 (d, 1H, *J* = 2.2 Hz, H8′), 6.37 (d, 1H, *J* = 2.2 Hz, H6′), 5.80 (broad s, 1H), 3.93 (s, 3H, OCH$_3$-5′), 3.87 (s, 3H, OCH$_3$-7′), 3.48 (t, 2H, *J* = 7.0 Hz, CH$_2\alpha$), 3.43 (q, 2H, *J* = 7.0 Hz, CH$_2\omega$), 3.01 (m, 2H, H4), 2.66 (m, 2H, H1), 1.90 (m, 4H, H2, 3), 1.64 (quint, 4H, *J* = 7.3 Hz), 1.30 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 176.9 (C4′), 164.5 (C5′), 161.2 (C7′), 159.4 (C4a), 159.3 (C2′), 158.9 (CONH), 152.4 (C8a′), 150.9 (C9), 148.0 (C10a), 133.9 (C6), 127.4 (C5), 124.6 (C8), 124.2 (C7), 118.3 (C8a), 115.6 (C9a), 113.8 (C3′), 109.8 (C4a′), 96.5 (C6′), 92.8 (C8′), 56.4 (−OCH$_3$-5′), 55.8 (−OCH$_3$-7′), 49.5 (CH$_2\alpha$), 39.9 (CH$_2\omega$), 33.9 (C4), 31.7, 29.5, 29.4, 29.3, 29.2, 29.1, 28.7, 26.8, 24.5 (C1), 22.9 (C2), 22.6 (C3). **14**·HCl: yellow solid (mp 129−130 °C). Purity: 98% (by HPLC). Anal. (C$_{35}$H$_{42}$ClN$_3$O$_5$·HCl·2H$_2$O) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-5,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxamide (15).** Reagents were *N*^{1}-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**37**) (100 mg, 0.24 mmol), 5,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid **40** (59 mg, 0.24 mmol), BOP (136 mg, 0.31 mmol), and Et$_3$N (80 μL, 0.62 mmol). Purification involved the use of EtOAc/CH$_3$OH (from 12:1 to 7:1) as eluents. **15**: Pale oil (106 mg, 68%). ESI-MS: *m*/*z* 656 [M + H]$^+$. ^{1}H NMR (CDCl$_3$) δ 7.77 (d, 1H, *J* = 2.2 Hz, H5), 7.30 (d, 1H, *J* = 2.2 Hz, H7), 6.93 (s, 1H, H3′), 6.90 (t, 1H, *J* = 5.8 Hz, CONH), 6.46 (d, 1H, *J* = 2.2 Hz, H8′), 6.34 (d, 1H, *J* = 2.2 Hz, H6′), 5.78 (broad s, 1H), 3.90 (OCH$_3$ 5′), 3.85 (OCH$_3$ 7′), 3.42 (q, 2H, *J* = 6.8 Hz, CH$_2\omega$), 3.19 (t, 2H, *J* = 6.6 Hz, CH$_2\alpha$), 2.98 (t, 2H, *J* = 6.4 Hz, H4), 2.70 (t, 2H, *J* = 6.4 Hz, H1), 1.85 (m, 4H, H2, 3), 1.60 (m, 4H), 1.30 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 177.4 (C4′), 164.7 (C5′), 160.8 (C7′), 160.6 (C4a), 159.1 (C2′), 158.9 (CONH), 152.2 (C9 and C8a′), 148.6 (C10a), 132.5 (C6), 128.4 (C8), 127.5 (C5), 127.4 (C7), 120.1 (C9a), 116.9 (C8a), 113.4 (C3′), 109.5 (C4a′), 96.7 (C6′), 93.0 (C8′), 56.5 (−OCH$_3$-5′), 55.9 (−OCH$_3$-7′), 49.2 (CH$_2\alpha$), 39.9 (CH$_2\omega$), 33.4 (C4), 30.8, 29.3 (2C), 29.2 (2C), 29.1 (2C), 26.8, 26.9 (C1), 23.0 (C2), 22.5 (C3). **15**·HCl: yellow solid (mp 104−106 °C). Purity: 100% (by HPLC). Anal. (C$_{35}$H$_{41}$Cl$_2$N$_3$O$_5$·HCl·H$_2$O) C, H, N.

6,7-Dimethoxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4*H*-chromene-2-carboxamide (16). Reagents were *N*^{1}-(1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**34**) (218 mg, 0.62 mmol), 6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid **41** (154 mg, 0.62 mmol), BOP (355 mg, 0.80 mmol), and Et$_3$N (222 μL, 1.60 mmol). Purification involved the use of EtOAc/CH$_3$OH/aqueous 30% NH$_3$ (from 10:1:0.2 to 5:1:0.2) as eluents. **16**: Pale oil (230 mg, 64%). ESI-MS: *m*/*z* 586 [M + H]$^+$. ^{1}H NMR (CDCl$_3$) δ 8.06 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, H8), 7.86 (dd, 1H, *J* = 8.3 Hz, *J* = 1.2 Hz, H5), 7.58 (ddd, 1H, *J* = 8.3 Hz, *J* = 6.8 Hz, *J* = 1.2 Hz, H6), 7.46 (s, 1H, H5′), 7.38 (ddd, 1H, *J* = 8.3 Hz, *J* = 6.8 Hz, *J* = 1.2 Hz, H7), 7.36 (t, 1H, *J* = 5.4 Hz, CONH), 7.24 (s, 1H, H8′), 7.09 (s, 1H, H3′), 4.78 (broad s, 1H), 3.95 (s, 3H, OCH$_3$ 6′), 3.93 (s, 3H, OCH$_3$ 7′), 3.63 (t, 2H, *J* = 7.0 Hz, CH$_2\alpha$), 3.44 (q, 2H, *J* = 6.8 Hz, CH$_2\omega$), 3.07 (m, 2H, H4), 2.65 (m, 2H, H1), 1.89 (m, 4H, H2, 3), 1.69 (quint, 2H, *J* = 7.0 Hz), 1.62 (quint, 2H, *J* = 6.8 Hz), 1.30 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 177.3 (C4′), 159.4 (CONH), 155.6 (C4a), 154.9 (C2′), 154.5 (C7′), 152.6 (C8a′), 151.4 (C9), 148.0 (C10a), 144.3 (C6′), 129.7 (C6), 125.4 (C5), 124.1 (C8), 123.5 (C7), 118.5 (C8a), 117.7 (C4a′), 113.9 (C9a), 111.2 (C3′), 104.1 (C5′), 99.9 (C8′), 56.5, 56.3, 49.0 (CH$_2\alpha$), 39.9 (CH$_2\omega$), 31.9 (C4), 31.3, 29.3, 29.2, 29.1, 29.0, 28.9, 26.8, 26.5, 24.2 (C1), 22.5 (C2), 21.9 (C3). **16**·HCl: yellow solid (mp 130−131 °C). Purity: 100% (by HPLC). Anal. (C$_{35}$H$_{43}$N$_3$O$_5$·HCl·H$_2$O) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxamide (17).** Reagents were *N*^{1}-(6-chloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**36**) (150 mg, 0.39 mmol), 6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid **41** (97 mg, 0.39 mmol), BOP (222 mg, 0.50 mmol), and Et$_3$N (140 μL, 1.0 mmol). Purification involved the use of EtOAc/CH$_3$OH (from 12:1 to 7:1) as eluents. **17**: Pale oil (80 mg, 34%). ESI-MS: *m*/*z* 620 [M + H]$^+$. ^{1}H NMR (CDCl$_3$) δ 7.93 (d, 1H, *J* = 9.0 Hz, H8), 7.84 (d, 1H, *J* = 2.2 Hz, H5), 7.50 (s, 1H, H5′), 7.28 (dd, 1H, *J* = 9.0 Hz, *J* = 22 Hz, H7), 7.23 (s, 1H, H8′), 7.09 (s, 1H, H3′), 7.03 (t, 1H, *J* = 5.8 Hz, CONH), 4.31 (broad s, 1H), 3.96 (s, 3H, OCH$_3$ 6′), 3.94 (s, 3H, OCH$_3$ 7′), 3.47 (t, 2H, *J* = 7.1 Hz, CH$_2\alpha$), 3.43 (q, 2H, *J* = 6.3 Hz, CH$_2\omega$), 2.98 (m, 2H, H4), 2.65 (m, 2H, H1), 2.02 (m, 4H, H2, 3), 1.68 (quint, 2H, *J* = 7.1 Hz), 1.60 (quint, 2H, *J* = 6.3 Hz), 1.30 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 177.3 (C4′), 159.4 (CONH), 157.7 (C4a), 155.1 (C2′), 154.4 (C7′), 152.0 (C8a′), 151.4 (C9), 148.1 (C10a), 146.1 (C6′), 135.2 (C6), 125.4 (C5), 125.2 (C8), 124.6 (C7), 117.8 (C8a), 117.3 (C4a′), 114.7 (C9a), 111.4 (C3′), 104.2 (C5′), 99.8 (C8′), 56.6, 56.4, 49.3 (CH$_2\alpha$), 39.9 (CH$_2\omega$), 32.7 (C4), 31.4, 29.3, 29.1 (2C), 29.0 (2C), 26.8, 26.6, 24.2 (C1), 22.6 (C2), 22.1 (C3). **17**·HCl: yellow solid (mp 127−128 °C). Purity: 100% (by HPLC). Anal. (C$_{35}$H$_{42}$ClN$_3$O$_5$·HCl·H$_2$O) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxamide (18).** Reagents were *N*^{1}-(6,8-dichloro-1,2,3,4-tetrahydroacridin-9-yl)-1,10-decanodiamine (**37**) (100 mg, 0.24 mmol), 6,7-dimethoxy-4-oxo-4*H*-chromene-2-carboxylic acid **41** (59 mg, 0.24 mmol), BOP (136 mg, 0.31 mmol), and Et$_3$N (80 μL, 0.62 mmol). Purification involved the use of EtOAc/CH$_3$OH (from 13:1 to 7:1) as eluent. **18**: Pale oil (51 mg, 33%). ESI-MS: *m*/*z* 656 [M + H]$^+$. ^{1}H NMR (CDCl$_3$) δ 7.84 (d, 1H, *J* = 2.2 Hz, H5), 7.48 (s, 1H, H5′), 7.30 (d, 1H, *J* = 2.2 Hz, H7), 7.24 (s, 1H, H8′), 7.03 (s, 1H, H3′), 6.92 (t, 1H, *J* = 5.6 Hz, CONH), 5.80 (broad s, 1H), 3.98 (s, 3H, OCH$_3$ 6′), 3.95 (s, 3H, OCH$_3$ 7′), 3.44 (q, 2H, *J* = 6.9 Hz, CH$_2\omega$), 3.19 (t, 2H, *J* = 7.1 Hz, CH$_2\alpha$), 2.98 (t, 2H, *J* = 6.2 Hz, H4), 2.70 (t, 2H, *J* = 6.2 Hz, H1), 1.85 (m, 4H, H2, 3), 1.59 (quint, 4H, *J* = 7.1 Hz), 1.30 (m, 12H). ^{13}C NMR (CDCl$_3$) δ 177.1 (C4′), 160.7 (C4a), 159.2 (CONH), 154.9 (C2′), 154.3 (C7′), 152.1 (C9 and C8a′), 148.7 (C10a), 148.0 (C6′), 132.5 (C6), 128.4 (C8), 127.4 (C5), 127.3 (C7), 120.1 (C9a), 117.8

(C4a′), 116.9 (C8a), 111.5 (C3′), 104.4 (C5′), 99.5 (C8′), 56.3, 55.7, 49.2 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 33.3 (C4), 30.8, 29.4 (3C), 29.2 (3C), 26.8, 26.9 (C1), 23.0 (C2), 22.5 (C3). **18**·HCl: yellow solid (mp 96−98 °C). Purity: 100% (by HPLC). Anal. ($C_{35}H_{41}Cl_2N_3O_5$·HCl) C, H, N.

General Procedure for the Synthesis of Tacrine−Phenolic-4-oxo-4*H*-chromene Hybrids (19−30) from the Corresponding Methoxylated Derivatives. Under nitrogen atmosphere, to a solution of the corresponding tacrine−methoxylated-4-oxo-4*H*-chromene hybrid (**10**−**18**) (1.0 mmol) in dry CH_2Cl_2 (5 mL) at −78 °C was added a solution of 1.0 M BBr_3 in CH_2Cl_2 (7.0−8.0 mmol), and the reaction was stirred overnight, allowing it to reach room temperature. Then, the mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated $NaHCO_3$ solution (3 × 30 mL) and H_2O (3 × 30 mL). The organic phase was dried over sodium sulfate and filtered, and the solvent was evaporated to dryness under reduced pressure. The residue was purified, employing one of the following methods:

Method A involved flash chromatography on a silica gel column using as eluent mixtures of EtOAC/CH_3OH/aqueous 30% NH_3 of increasing polarity. The corresponding tacrine−flavonoid compound was obtained as a syrup and identified by ^1H NMR, ^{13}C NMR, and MS. Then, the treatment of the previous syrup with HCl (g) in dichloromethane yielded the hydrochloride derivative as a pure solid that was collected by filtration and used for obtaining the combustion analysis and the biological activities.

In method B, the crude oil was treated with HCl aq (10%) and evaporated to dryness. Then, it was purified by reverse phase chromatography employing a C18 Sep-Park Vac 35 cm^3 (10 g) column using a mixture of H_2O/CH_3OH as eluent. In this case, tacrine−flavonoid derivatives were obtained as hydrochlorides that were used for both structural elucidation and biological activities.

6-Hydroxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)-amino]decyl}-4*H*-chromene-2-carboxamide (19). Reagents were **10** (32 mg, 0.06 mmol) and BBr_3 (420 μL, 0.42 mmol). Purification involved method A, EtOAc/CH_3OH/aqueous 30% NH_3 (from 8:1:0.2 to 5:1:0.2). **19**: Pale oil (22 mg, 70%). ESI-MS: *m*/*z* 524 $[M + H]^+$. ^1H NMR (CD_3OD) δ 8.41 (dd, 1H, *J* = 8.2 Hz, *J* = 1.3 Hz, H8), 7.91 (dd, 1H, *J* = 8.2 Hz, *J* = 1.3 Hz, H5), 7.89 (ddd, 1H, *J* = 8.2 Hz, *J* = 6.9 Hz, *J* = 1.3 Hz, H6), 7.76 (d, 1H, *J* = 9.0 Hz, H8′), 7.64 (ddd, 1H, *J* = 8.2 Hz, *J* = 6.9 Hz, *J* = 1.3 Hz, H7), 7.53 (d, 1H, *J* = 3.0 Hz, H5′), 7.46 (dd, 1H, *J* = 9.0 Hz, *J* = 3.0 Hz, H7′), 7.05 (s, 1H, H3′), 5.09 (broad s, 3H, NH, OH and CONH), 3.92 (t, 2H, *J* = 7.3 Hz, $CH_2\alpha$), 3.57 (t, 2H, *J* = 7.1 Hz, $CH_2\omega$), 3.15 (m, 2H, H4), 2.86 (m, 2H, H1), 2.10 (m, 4H, H2,3), 1.91 (quint, 2H, *J* = 7.3 Hz), 1.81 (quint, 2H, *J* = 7.1 Hz), 1.55 (m, 12H). ^{13}C NMR (CD_3OD) δ 180.3 (C4′), 161.3 (CONH), 157.4 (C8a′), 157.1 (C4a), 156.0 (C2′), 154.5 (C9), 150.8 (C6′), 142.9 (C10a), 132.3 (C6), 125.9 (C7′), 125.6 (3C, C7, C8 and C4a′), 123.2 (C5), 121.1 (C8a), 118.6 (C8′), 114.3 (C9a), 110.6 (C3′), 108.7 (C5′), 49.3 ($CH_2\alpha$), 40.9 ($CH_2\omega$), 31.8, 31.2 (C4), 30.4 (4C), 30.1, 27.9, 27.7, 25.3 (C1), 23.4 (C2), 22.6 (C3). **19**·HCl: yellow solid (mp 129−131 °C). Purity: 100% (by HPLC). Anal. ($C_{33}H_{39}N_3O_4$·HCl·H_2O) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-decyl}-6-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (20).** Reagents were **11** (84 mg, 0.14 mmol) and BBr_3 (980 μL, 0.98 mmol). Purification involved method A, EtOAc/CH_3OH/aqueous 30% NH_3 (from 9:1:0.2 to 5:1:0.2). **20**: Pale oil (30 mg, 37%). ESI-MS: *m*/*z* 576 $[M + H]^+$. ^1H NMR (CD_3OD) δ 8.31 (d, 1H, *J* = 9.0 Hz, H8), 7.90 (d, 1H, *J* = 2.2 Hz, H5), 7.57 (d, 1H, *J* = 9.0 Hz, H8′), 7.49 (dd, 1H, *J* = 9.0 Hz, *J* = 2.2 Hz, H7), 7.36 (d, 1H, *J* = 2.5 Hz, H5′), 7.30 (dd, 1H, *J* = 9.0 Hz, *J* = 2.5 Hz, H7′), 6.84 (s, 1H, H3′), 4.94 (broad s, 3H, NH, OH and CONH), 3.84 (t, 2H, *J* = 7.2 Hz, $CH_2\alpha$), 3.59 (t, 2H, *J* = 7.0 Hz, $CH_2\omega$), 3.15 (m, 2H, H4), 2.87 (m, 2H, H1), 2.12 (m, 4H, H2, 3), 1.88 (quint, 2H, *J* = 7.1 Hz), 1.80 (quint, 2H, *J* = 7.1 Hz), 1.50 (m, 12H). ^{13}C NMR (CD_3OD) δ 180.2 (C4′), 161.3 (CONH), 157.6 (C9), 157.1 (2C, C2′ and C8a′), 151.9 (C4a), 150.8 (C6′), 140.4 (C10a), 140.0 (C6), 128.7 (C8), 126.7 (C7), 125.8 (C4a′), 125.4 (C7′), 121.1 (C8′), 119.1 (C5), 115.3 (C8a), 113.2 (C9a), 110.6 (C3′), 108.6 (C5′), 49.5 ($CH_2\alpha$), 40.9 ($CH_2\omega$), 31.2, 30.4 (4C), 30.1, 29.3 (C4), 27.8, 27.6, 24.7 (C1), 22.8 (C2), 21.7 (C3). **20**·HCl: yellow solid (mp 134−136 °C). Purity: 100% (by HPLC). Anal. ($C_{33}H_{39}ClN_3O_4$·HCl) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-decyl}-6-hydroxy-4-oxo-4*H*-chromene-2-carboxamide (21).** Reagents were **12** (50 mg, 0.08 mmol) and BBr_3 (560 μL, 0.56 mmol). Purification involved method A, EtOAc/CH_3OH/aqueous 30% NH_3 (from 8:1:0.2 to 5:1:0.2). **21**: Pale oil (41 mg, 51%). ESI-MS: *m*/*z* 610 $[M + H]^+$. ^1H NMR (CD_3OD) δ 7.74 (d, 1H, *J* = 2.2 Hz, H5), 7.50 (d, 1H, *J* = 2.2 Hz, H5′), 7.33 (d, 1H, *J* = 8.8 Hz, H8′), 7.27 (d, 1H, *J* = 2.2 Hz, H7), 7.24 (broad s, 1H, CONH), 7.22 (dd, 1H, *J* = 8.8 Hz, *J* = 2.2 Hz, H7′), 7.03 (s, 1H, H3′), 5.90 (broad s, 2H, NH and OH), 3.42 (t, 2H, *J* = 7.2 Hz, $CH_2\alpha$), 3.20 (t, 2H, *J* = 7.2 Hz, $CH_2\omega$), 2.97 (t, 2H, *J* = 6.0 Hz, H4), 2.68 (t, 2H, *J* = 6.0 Hz, H1), 1.83 (m, 4H, H2, 3), 1.65 (m, 4H), 1.35 (m, 12H). ^{13}C NMR (CD_3OD) δ 178.7 (C4′), 160.4 (C4a), 159.3 (CONH), 155.5 (C8a′), 154.7 (C2′), 152.6 (C9), 149.1 (C10a), 148.0 (C6′), 132.9 (C6), 128.6 (C8), 127.5 (C5), 126.5 (C7), 124.8 (C4a′), 124.4 (C7′), 119.6 (C9a), 119.3 (C8′), 116.6 (C8a), 110.5 (C3′), 108.5 (C5′), 49.1 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 32.7 (C4), 30.7, 29.3 (2C), 29.2, 29.1, 29.0, 26.9, 26.8 (2C, C1), 22.9 (C2), 22.3 (C3). **21**·HCl: yellow solid (mp 96−98 °C). Purity: 100% (by HPLC). Anal. ($C_{33}H_{37}Cl_2N_3O_4$·HCl·H_2O) C, H, N.

5-Hydroxy-7-methoxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-4*H*-chromene-2-carboxamide (22) and 5,7-Dihydroxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)-amino]decyl}-4*H*-chromene-2-carboxamide (23). Reagents were **13** (100 mg, 0.17 mmol) and BBr_3 (1.4 mL, 1.4 mmol). Purification involved method A, EtOAc/CH_3OH/aqueous 30% NH_3 (from 8:1:0.2 to 5:1:0.2). From the fractions of R_f 0.7 (EtOAc/CH_3OH/aqueous 30% NH_3 (8:1:0.2), compound **22** (18 mg, 18%) was isolated as a pale oil. ESI-MS: *m*/*z* 572 $[M + H]^+$. ^1H NMR (CD_3OD) δ 8.33 (d, 1H, *J* = 8.0 Hz, H8), 7.92 (d, 1H, *J* = 8.0 Hz, H5), 7.81 (td, 1H, *J* = 8.0 Hz, *J* = 1.0 Hz, H6), 7.59 (td, 1H, *J* = 8.0 Hz, *J* = 1.0 Hz, H7), 7.00 (s, 1H, H3′), 6.81 (d, 1H, *J* = 2.4 Hz, H8′), 6.54 (d, 1H, *J* = 2.4 Hz, H6′), 4.98 (broad s, 3H, NH, OH and CONH), 4.06 (s, 3H, OCH_3 7′), 3.82 (t, 2H, *J* = 7.1 Hz, $CH_2\alpha$), 3.57 (t, 2H, *J* = 7.1 Hz, $CH_2\omega$), 3.16 (m, 2H, H4), 2.90 (m, 2H, H1), 2.11 (m, 4H, H2, 3), 1.87 (quint, 2H, *J* = 7.3 Hz), 1.80 (quint, 2H, *J* = 7.3 Hz), 1.49 (m, 12H). ^{13}C NMR (CD_3OD) δ 183.9 (C4′), 167.9 (C7′), 163.3 (C5′), 160.8 (CONH), 158.7 (C8a′), 157.5 (C2′), 156.6 (C4a), 154.8 (C9), 145.7 (C10a), 131.2 (C6), 125.3 (C8), 125.2 (C5), 125.0 (C7), 119.8 (C8a), 115.4 (C9a), 110.9 (C3′), 107.1 (C4a′), 99.7 (C6′), 94.8 (C8′), 56.6 (OCH_3 7′), 49.3 ($CH_2\alpha$), 40.9 ($CH_2\omega$), 32.5 (C4), 31.9, 30.3 (3C), 27.9 (2C), 27.7 (2C), 25.7 (C1), 23.7 (C2), 23.1 (C3). **22**·HCl: yellow solid (mp 113−115 °C). Purity: 100% (by HPLC). Anal. ($C_{34}H_{41}N_3O_5$·HCl·H_2O) C, H, N.

From the fractions of R_f 0.2 (EtOAC/CH_3OH/aqueous 30% NH_3 (8:1:0.2) compound **23** (40 mg, 42%) was isolated as a pale oil. ESI-MS: *m*/*z* 558 $[M + H]^+$. ^1H NMR (CD_3OD) δ 8.55 (d, 1H, *J* = 8.0 Hz, H8), 7.99 (d, 1H, *J* = 8.0 Hz, H5), 7.85 (td, 1H, *J* = 8.0 Hz, *J* = 1.0 Hz, H6), 7.73 (td, 1H, *J* = 8.0 Hz, *J* = 1.0 Hz, H7), 6.92 (s, 1H, H3′), 6.72 (d, 1H, *J* = 2.2 Hz, H8′), 6.36 (d, 1H, *J* = 2.2 Hz, H6′), 5.20 (4H, NH, 2-OH and CONH), 4.10 (t, 2H, *J* = 7.3 Hz, $CH_2\alpha$), 3.57 (t, 2H, *J* = 7.1 Hz, $CH_2\omega$), 3.21 (m, 2H, H4), 2.87 (m, 2H, H1), 2.13 (m, 4H, H2, 3), 2.01 (m, 2H), 1.86 (m, 2H), 1.58 (m, 12H). ^{13}C NMR (CD_3OD) δ 182.3 (C4′), 165.7 (C7′), 161.9 (C5′), 159.6 (CONH), 157.5 (C8a′), 156.5 (C9), 155.9 (C2′), 150.3 (C4a), 138.5 (C10a), 132.8 (C6), 125.3 (C7), 125.1 (C8), 118.9 (C5), 115.7 (C8a), 111.6 (C9a), 109.5 (C3′), 104.9 (C4a′), 99.6 (C6′), 94.7 (C8′), 48.2 ($CH_2\alpha$), 39.8 ($CH_2\omega$), 30.3, 29.1 (2C), 29.0 (2C), 28.9, 28.2 (C4), 26.7, 26.5, 23.8 (C1), 21.8 (C2), 20.7 (C3). **23**·HCl: yellow solid (mp 180−182 °C). Purity: 100% (by HPLC). Anal. ($C_{33}H_{39}N_3O_5$·HCl) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-decyl}-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (24) and *N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (25).** Reagents were **14** (85 mg, 0.14 mmol) and BBr_3 (1.1 mL, 1.1 mmol). Purification involved method A, EtOAc/CH_3OH/aqueous 30% NH_3 (from 8:1:0.2 to 5:1:0.2). From the fractions of R_f 0.8 (EtOAc/CH_3OH/aqueous 30% NH_3 (8:1:0.2), compound **24** (12 mg, 15%) was isolated as a pale oil. ESI-MS: *m*/*z* 606 $[M + H]^+$. ^1H NMR

(CD_3OD) δ 9.22 (broad s, 1H, OH), 7.88 (d, 1H, J = 9.0 Hz, H8), 7.87 (d, 1H, J = 2.2 Hz, H5), 7.24 (dd, 1H, J = 9.0 Hz, J = 2.2 Hz, H7), 7.00 (s, 1H, H3′), 6.39 (d, 1H, J = 2.2 Hz, H8′), 6.34 (d, 1H, J = 2.2 Hz, H6′), 5.20 (broad s, 2H, NH and CONH), 3.83 (s, 3H, OCH_3 7′), 3.49 (t, 2H, J = 7.2 Hz, $CH_2\alpha$), 3.42 (t, 2H, J = 6.2 Hz, $CH_2\omega$), 299 (m, 2H, H4), 2.62 (m, 2H, H1), 1.87 (m, 4H, H2, 3), 1.63 (quint, 4H, J = 7.1 Hz), 1.25 (m, 12H). ^{13}C NMR (CD_3OD) δ 182.3 (C4′), 166.1 (C7′), 162.4 (C5′), 158.7 (CONH), 158.6 (C4a), 156.7 (C8a′), 155.1 (C2′), 151.3 (C9), 147.2 (C10a), 134.4 (C6), 126.6 (C5), 124.7 (C8), 124.3 (C7), 117.8 (C8a), 115.5 (C9a), 110.7 (C3′), 106.2 (C4a′), 98.5 (C6′), 92.9 (C8′), 55.9 (OCH_3 7′), 49.4 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 33.3 (C4), 31.6, 29.7 (2C), 29.3, 29.2, 29.1 (2C), 29.0, 26.8,24.4 (C1), 22.7 (C2), 22.4 (C3). **24**·HCl: yellow solid (mp 133−135 °C). Purity: 99% (by HPLC). Anal. ($C_{34}H_{40}ClN_3O_5$·HCl) C, H, N.

From the fractions of R_f 0.2 (EtOAc/CH_3OH/aqueous 30% NH_3 (8:1:0.2), derivative **25** (35 mg, 43%) was isolated as a yellow solid. ESI-MS: m/z 592 [M + H]$^+$. ^1H NMR (CD_3OD) δ 8.52 (d, 1H, J = 9.3 Hz, H8), 7.92 (d, 1H, J = 2.0 Hz, H5), 7.71 (dd, 1H, J = 9.3 Hz, J = 2.0 Hz, H7), 6.94 (s, 1H, H3′), 6.67 (d, 1H, J = 2.2 Hz, H8′), 6.38 (d, 1H, J = 2.2 Hz, H6′), 5.10 (broad s, 4H, NH, CONH and OH), 4.08 (t, 2H, J = 7.5 Hz, $CH_2\alpha$), 3.58 (t, 2H, J = 7.1 Hz, $CH_2\omega$), 3.17 (m, 2H, H4), 2.91 (m, 2H, H1), 2.15 (m, 4H, H2,3), 2.00 (quint, 2H, J = 6.7 Hz), 1.83 (quint, 2H, J = 6.7 Hz), 1.55 (m, 12H). ^{13}C NMR (CD_3OD) δ 183.6 (C4′), 166.8 (C7′), 163.3 (C5′), 160.9 (CONH), 158.8 (C2′), 157.8 (C9), 157.3 (C8a′), 151.9 (C4a), 140.5 (C10a), 140.1 (C6), 128.7 (C8), 126.7 (C7), 119.2 (C5), 115.4 (C8a), 113.3 (C9a), 110.6 (C3′), 106.2 (C4a′), 100.6 (C6′), 95.6 (C8′), 49.2 ($CH_2\alpha$), 40.9 ($CH_2\omega$), 31.2, 31.1, 30.2, 30.0 (3C), 29.3 (C4), 27.7, 27.6, 24.6 (C1), 22.8 (C2), 21.7 (C3). **25**·HCl: yellow solid (mp 132−134 °C). Purity: 98% (by HPLC). Anal. ($C_{33}H_{38}ClN_3O_5$·HCl) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-5-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxamide (26) and *N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]decyl}-5,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (27).** Reagents were **15** (75 mg, 0.12 mmol) and BBr_3 (0.96 mL, 0.96 mmol). Purification involved method A, EtOAc/CH_3OH/aqueous 30% NH_3 (from 10:1:0.1 to 5:1:0.2). From the fractions of R_f 0.8 (EtOAC/CH_3OH/aqueous 30% NH_3 (8:1:0.1), derivative **26** (18 mg, 24%) was isolated as a pale oil. ESI-MS: m/z 640 [M + H]$^+$. ^1H NMR (CD_3OD) δ 12.43 (broad s, 1H, OH), 7.81 (d, 1H, J = 2.2 Hz, H5), 7.34 (d, 1H, J = 2.2 Hz, H8), 7.02 (s, 1H, H3′), 6.98 (broad s, 1H, CONH), 6.43 (d, 1H, J = 2.2 Hz, H8′), 6.39 (d, 1H, J = 2.2 Hz, H6′), 5.90 (broad s, 1H, NH), 3.86 (s, 3H, OCH_3 7′), 3.45 (c, 2H, J = 6.6 Hz, $CH_2\omega$), 3.23 (t, 2H, J = 7.1 Hz, $CH_2\alpha$), 3.00 (m, 2H, H4), 2.73 (m, 2H, H1), 1.89 (m, 4H, H2, 3), 1.62 (m, 2H), 1.35 (m, 14H). ^{13}C NMR (CD_3OD) δ 182.3 (C4′), 166.0 (C7′), 162.3 (C5′), 158.6 (2C, CONH and C2′), 156.7 (3C, C4,9,8a′), 140.9 (C10a), 140.2 (C6), 128.6 (C8), 127.8 (C7), 119.8 (C5), 115.2 (C8a), 113.7 (C9a), 110.7 (C3′), 106.2 (C4a′), 98.5 (C6′), 93.0 (C8′), 55.9 (−OCH_3-C7′), 49.2 ($CH_2\alpha$), 40.0 ($CH_2\omega$), 30.7, 29.4, 29.3, 29.2, 29.1, 27.8 (C4), 27.0, 26.9, 26.8, 22.8 (C1), 21.3 (C2), 21.0 (C3). **26**·HCl: yellow solid (mp 110−113 °C). Purity: 99% (by HPLC). Anal. ($C_{34}H_{39}Cl_2N_3O_4$·HCl) C, H, N.

From the fractions of R_f 0.3 (EtOAc/CH_3OH/aqueous 30% NH_3 (8:1:0.1), compound **27** (40 mg, 55%) was isolated as a pale oil. ESI-MS: m/z 626 [M + H]$^+$. ^1H NMR (CD_3OD) δ 9.22 (broad s, 2H, OH), 7.88 (d, 1H, J = 2.0 Hz, H5), 7.82 (d, 1H, J = 2.0 Hz, H7), 6.69 (d, 1H, J = 1.7 Hz, H8′), 6.37 (d, 1H, J = 1.7 Hz, H6′), 5.10 (broad s, 2H, NH and CONH), 3.94 (t, 2H, J = 7.2 Hz, $CH_2\alpha$), 3.57 (t, 2H, J = 7.2 Hz, $CH_2\omega$), 3.19 (m, 2H, H4), 3.00 (m, 2H, H1), 2.12 (m, 4H, H2, 3), 1.94 (m, 4H), 1.45 (m, 12H). ^{13}C NMR (CD_3OD) δ 183.5 (C4′), 166.7 (C7′), 163.2 (C5′), 160.7 (CONH), 159.3 (C9), 158.7 (C2′), 157.2 (C8a′), 152.4 (C4a), 141.8 (C10a), 139.0 (C6), 132.6 (C8), 129.2 (C7), 118.2 (C5), 114.4 (C8a), 113.6 (C9a), 110.6 (C3′), 104.7 (C4a′), 100.6 (C6′), 95.6 (C8′), 47.8 ($CH_2\alpha$), 39.9 ($CH_2\omega$), 31.6, 30.3, 30.2, 30.1, 29.9, 29.7, 29.0, 27.8 (2C, C4), 23.4 (C1), 22.9 (C2), 21.7 (C3). **27**·HCl: yellow solid (mp 120−122 °C). Purity: 98% (by HPLC). Anal. ($C_{33}H_{37}Cl_2N_3O_5$·HCl) C, H, N.

6,7-Dihydroxy-4-oxo-*N*-{10-[(1,2,3,4-tetrahydroacridin-9-yl)-amino]decyl}-4*H*-chromene-2-carboxamide (28). Reagents were **16** (80 mg, 0.14 mmol) and BBr_3 (1.1 mL, 1.1 mmol). Purification involved method B, H_2O/CH_3OH (from 90:10 to 50:50). **28**·HCl: yellow solid (50 mg, 66%), mp 140−142 °C. ESI-MS: m/z 558 [M + H]$^+$. ^1H NMR (CD_3OD) δ 12.80 (broad s, 2H, OH), 8.54 (dd, 1H, J = 8.5 Hz, J = 1.3 Hz, H8), 8.02 (ddd, 1H, J = 8.5 Hz, J = 6.8 Hz, J = 1.3 Hz, H6), 7.92 (dd, 1H, J = 8.5 Hz, J = 1.3 Hz, H5), 7.74 (ddd, 1H, J = 8.5 Hz, J = 6.8 Hz, J = 1.3 Hz, H7), 7.51 (s, 1H, H5′), 7.25 (s, 1H, H8′), 7.04 (s, 1H, H3′), 5.10 (broad s, 2H, NH and CONH), 4.10 (t, 2H, J = 7.1 Hz, $CH_2\alpha$), 3.59 (t, 2H, J = 7.1 Hz, $CH_2\omega$), 3.19 (m, 2H, H4), 2.89 (m, 2H, H1), 2.15 (m, 4H, H2, 3), 2.03 (quint, 2H, J = 6.5 Hz), 1.83 (quint, 2H, J = 6.5 Hz), 1.58 (m, 12H). ^{13}C NMR (CD_3OD) δ 178.3 (C4′), 160.2 (CONH), 156.7 (C9), 155.5 (C2′), 154.1 (C8a′), 151.1 (C7′), 150.4 (C4a), 145.7 (C6′), 138.5 (C10a), 132.9 (C6), 125.3 (C7), 125.1 (C8), 118.9 (C5), 116.8 (C4a′), 115.8 (C8a), 111.6 (C9a), 109.5 (C3′), 107.0 (C5′), 102.9 (C8′), 47.9 ($CH_2\alpha$), 39.7 ($CH_2\omega$), 30.2, 29.3, 29.1, 29.0, 28.9 (3C), 28.1 (C4), 26.6, 26.5, 23.6 (C1), 21.7 (C2), 20.6 (C3). Purity: 100% (by HPLC). Anal. ($C_{33}H_{39}N_3O_5$·HCl) C, H, N.

***N*-{10-[(6-Chloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-decyl}-6,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (29).** Reagents were **17** (54 mg, 0.09 mmol) and BBr_3 (720 μL, 0.72 mmol). Purification involved method B, H_2O/CH_3OH (from 70:10 to 50:50). **29**·HCl: yellow solid (41 mg, 80%), mp 139−140 °C. ESI-MS: m/z 592 [M + H]$^+$. ^1H NMR (CD_3OD) δ 8.51 (d, 1H, J = 9.3 Hz, H8), 7.92 (d, 1H, J = 2.1 Hz, H5), 7.70 (dd, 1H, J = 9.3 Hz, J = 2.1 Hz, H7), 7.51 (s, 1H, H5′), 7.24 (s, 1H, H8′), 7.04 (s, 1H, H3′), 5.07 (broad s, 4H, NH, CONH and OH), 4.11 (t, 2H, J = 7.3 Hz, $CH_2\alpha$), 3.58 (t, 2H, J = 7.0 Hz, $CH_2\omega$), 3.17 (m, 2H, H4), 2.84 (m, 2H, H1), 2.14 (m, 4H, H2, 3), 2.00 (quint, 2H, J = 7.2 Hz), 1.72 (quint, 2H, J = 7.2 Hz), 1.55 (m, 12H). ^{13}C NMR (CD_3OD) δ 179.5 (C4′), 161.3 (CONH), 157.6 (C9), 156.5 (C2′), 155.2 (C8a′), 152.8 (C4a), 151.9 (C7′), 146.8 (C6′), 140.4 (C10a), 140.0 (C6), 128.6 (C8), 126.7 (C7), 119.0 (C5), 117.9 (C4a′), 115.3 (C8a), 113.2 (C9a), 110.7 (C3′), 108.2 (C5′), 104.1 (C8′), 48.1 ($CH_2\alpha$), 40.9 ($CH_2\omega$), 31.3, 30.3 (2C), 30.2, 30.1, 30.0, 29.3 (C4), 27.8, 27.6, 24.7 (C1), 22.8 (C2), 21.7 (C3). Purity: 100% (by HPLC). Anal. ($C_{33}H_{38}ClN_3O_5$·HCl) C, H, N.

***N*-{10-[(6,8-Dichloro-1,2,3,4-tetrahydroacridin-9-yl)amino]-decyl}-6,7-dihydroxy-4-oxo-4*H*-chromene-2-carboxamide (30).** Reagents were **18** (40 mg, 0.06 mmol) and BBr_3 (480 μL, 0.48 mmol). Purification involved method B, H_2O/CH_3OH (from 80:20 to 40:60). **30**·HCl: yellow solid (35 mg, 92%), mp 120−123 °C. ESI-MS: m/z 626 [M + H]$^+$. ^1H NMR (CD_3OD) δ 7.90 (d, 1H, J = 2.1 Hz, H5), 7.82 (d, 1H, J = 2.1 Hz, H7), 7.50 (s, 1H, H5′), 7.29 (s, 1H, H8′), 7.01 (s, 1H, H3′), 5.07 (broad s, 4H, NH, CONH and OH), 3.93 (t, 2H, J = 6.9 Hz, $CH_2\alpha$), 3.58 (t, 2H, J = 6.9 Hz, $CH_2\omega$), 3.19 (m, 2H, H4), 3.00 (m, 2H, H1), 2.13 (m, 4H, H2, 3), 1.96 (m, 4H), 1.50 (m, 12H). ^{13}C NMR (CD_3OD) δ 179.6 (C4′), 161.4 (C9), 159.3 (CONH), 156.6 (C2′), 155.2 (C8a′), 152.8 (2C, C4a and C7′), 146.8 (C6′), 141.1 (C10a), 139.0 (C6), 132.6 (C8), 129.2 (C7), 118.2 (C5), 118.0 (C4a′), 114.5 (C8a), 113.6 (C9a), 110.7 (C3′), 108.3 (C5′), 104.2 (C8′), 51.2 ($CH_2\alpha$), 40.9 ($CH_2\omega$), 31.6, 30.2 (2C), 30.1, 29.9, 29.1, 27.8 (C4), 27.5, 26.4 (2C, C1), 22.9 (C2), 21.7 (C3). Purity: 99% (by HPLC). Anal. ($C_{33}H_{37}Cl_2N_3O_5$·HCl) C, H, N.

Biochemical Studies. Cholinesterase Inhibitory Activities. Acetylcholinesterase (AChE) from bovine erythrocytes (0.25−1.0 unit/mg, lyophilized powder), AChE from human erythrocytes (min. 500 units/mg protein in buffered aqueous solution), butyrylcholinesterase (BuChE) from equine serum (10 units/mg protein, lyophilized powder), and BuChE from human serum (3 units/mg protein, lyophilized powder) were purchased from Sigma. Compounds were measured in 100 mM phosphate buffer pH 8.0 at 30 °C, using acetylthiocholine and butyrylthiocholine (0.4 mM) as substrates, respectively. In both cases, 5,5′-dithiobis(2-nitrobenzoic)acid (DTNB, Ellman's reagent, 0.2 mM) was used and the values of IC_{50} were calculated by UV spectroscopy, from the absorbance changes at 412 nm.[62] Experiments were performed in triplicate.

Oxygen Radical Absorbance Capacity Assay. The ORAC-FL method of Ou et al.[66] partially modified by Dávalos et al.[67] was followed, using a Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters. The equipment was controlled by Fluorostar Galaxy software (version 4.11-0) for fluorescence measurement. 2,2′-Azobis-(amidinopropane) dihydrochloride (AAPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), and fluorescein (FL) were purchased from Sigma-Aldrich. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. Antioxidant (20 μL) and FL (120 μL; 70 mM, final concentration) solutions were placed in a black 96-well microplate (96F untreat, Nunc). The mixture was preincubated for 15 min at 37 °C, and then AAPH solution (60 μL, 12 mM, final concentration) was added rapidly using a multichannel pipet. The microplate was immediately placed in the reader and the fluorescence recorded every minute for 80 min. The microplate was automatically shaken prior to each reading. Samples were measured at eight different concentrations (0.1−1 μM). A blank (FL + AAPH in phosphate buffer) instead of the sample solution and eight calibration solutions using trolox (1−8 μM) were also carried out in each assay. All the reaction mixtures were prepared in duplicate, and at least three independent assays were performed for each sample. Raw data were exported from the Fluostar Galaxy Software to an Excel sheet for further calculations. Antioxidant curves (fluorescence vs time) were first normalized to the curve of the blank corresponding to the same assay, and the area under the fluorescence decay curve (AUC) was calculated. The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. Regression equations between net AUC and antioxidant concentration were calculated for all the samples. ORAC-FL values were expressed as trolox equivalents by using the standard curve calculated for each assay, where the ORAC-FL value of trolox was taken as 1.

In Vitro Blood−Brain Barrier Penetration Assay. Prediction of the brain penetration was evaluated using a parallel artificial membrane permeation assay (PAMPA), in a manner similar to that described previously.[70] Commercial drugs, phosphate buffer saline solution at pH 7.4 (PBS), and dodecane were purchased from Sigma, Aldrich, Across, and Fluka. Millex filter units (PVDF membrane, diameter 25 mm, pore size 0.45 μM) were acquired from Millipore. The porcine brain lipid (PBL) was obtained from Avanti Polar Lipids. The donor microplate was a 96-well filter plate (PVDF membrane, pore size 0.45 μM), and the acceptor microplate was an indented 96-well plate, both from Millipore. The acceptor 96-well microplate was filled with 180 μL of PBS:ethanol (70:30), and the filter surface of the donor microplate was impregnated with 4 μL of porcine brain lipid (PBL) in dodecane (20 mg mL^{-1}). Compounds were dissolved in PBS:ethanol (70:30) at 1 mg mL^{-1}, filtered through a Millex filter, and then added to the donor wells (180 μL). The donor filter plate was carefully put on the acceptor plate to form a sandwich, which was left undisturbed for 120 min at 25 °C. After incubation, the donor plate was carefully removed and the concentration of compounds in the acceptor wells was determined by UV spectroscopy. Every sample was analyzed at five wavelengths, in four wells and at least in three independent runs, and the results are given as the mean ± standard deviation. In each experiment, 20 quality control standards of known BBB permeability were included to validate the analysis set.

BACE-1 Inhibition Assay. BACE-1 full protein (His·Tag, Human Recombinant, NSO cells) was purchased from Calbiochem (PF 125), and rhodamine derivative substrate which contains the peptide quencher sequence RhoRVNLDAEFK (Panvera peptide) was acquired from Invitrogen (Milan, Italy). Sodium acetate and DMSO were obtained from common commercial suppliers. Purified water from Mili-RX system (Millipore, Milford, MA) was used to prepare buffers and standard solutions. Spectrofluorometric analyses were carried out on Tecan Safire spectrofluorometer (working at 544 and 590 nm as excitation and emission wavelengths) using black with clear bottom microtiter plates (Corning 3711, 384 wells). Stock solutions of tested compounds were prepared at 10 mM in DMSO and diluted with 100 mM sodium acetate buffer (pH 4) with 0.001% Triton X-100. For each reaction, 10 μL of BACE-1 enzyme (18.8 nM, final concentration) was incubated with 5 μL of the tested compound for 60 min. Then, the reaction was started by addition of 1 μL of FRET peptide substrate (Panvera peptide 0.25 μM, final concentration). The final volume in each reaction is 20 μL. The mixture was incubated at 28 °C for 60 min. To stop the reaction, 20 μL of BACE-1 stock solution (sodium acetate, 2.5 M) was added to each well. The fluorometric assay was followed by reading the increase of the fluorescence signal at 590 nm with the time. The DMSO concentration in the final reaction volume was maintained at 5% (v/v) to guarantee no significant loss of enzymatic activity. The fluorescence intensities, with and without inhibitor were compared, and the percent inhibition due to the presence of tested compounds was calculated. The background signal, measured in control wells containing all the reagents except BACE-1, was subtracted from each reaction mixture. The inhibition (%) due to the presence of eight increasing concentrations of test compounds was calculated by the following expression: $100 - (\mathrm{IF_i}/\mathrm{IF_o} \times 100)$ where $\mathrm{IF_i}$ and $\mathrm{IF_o}$ are the fluorescence intensities obtained for BACE-1 in the presence and in the absence of inhibitor, respectively. The inhibition curves were obtained by plotting the percent inhibition or activity (%) versus the logarithm of concentration of the inhibitor. The regression parameters were determined, and the IC$_{50}$ value was extrapolated (GraphPad Prism 4.0, GraphPah Software Inc., dose−response inhibition, log [I] vs normalized response). To demonstrate the reliability of this assay, the peptidomimetic inhibitor OM99-2 (β-secreatase inhibitor, Calbiochem, Merck; Nottingham, UK) was serially diluted into the reaction wells, and its IC$_{50}$ value was calculated (IC$_{50}$ = 0.033 μM), being in agreement with the published data.[78−80]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Elemental analysis results of new tacrine−4-oxo-4*H*-chromene hybrids **3**−**30**; experimental details for PAMPA-BBB and for human BACE-1 inhibition assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

[†] This paper comprises a part of M.I.F.-B's Ph.D thesis.

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■ ABBREVIATIONS USED

Aβ, β-amyloid peptide; ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; BBB, blood−brain barrier; BuChE, butyrylcholinesterase; CAS, catalytic active site; ChEs, cholinesterases; CNS, central nervous system; hAChE, human AChE; hBuChE, human BuChE; ORAC-FL, oxygen-radical absorbance capacity by fluorescence; PAMPA-BBB, parallel artificial membrane permeation assay for the blood−brain barrier permeation; PAS, peripheral anionic site; PVDF, polyvinylidene fluoride; ROS, reactive oxygen species; SD, standard deviation

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