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Synthesis and Biological Evaluation of Novel Tacrine Derivatives and Tacrine–Coumarin Hybrids as Cholinesterase Inhibitors

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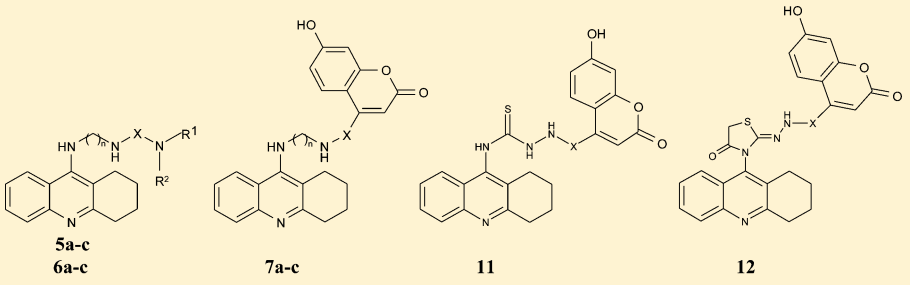
Supporting Information



5, 6: X = $CO(CH_2)_2$, n = 2-4
5: NR^1R^2 = Methylcyclohexylamine, 6: NR^1R^2 = Ethylcyclohexylamine
7: X = $COCH_2$, n = 2-4; 11, 12: X = $COCH_2$

ABSTRACT: A series of novel tacrine derivatives and tacrine–coumarin heterodimers were designed, synthesized, and biologically evaluated for their potential inhibitory effect on both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Of these compounds, tacrine–coumarin heterodimer **7c** and tacrine derivative **6b** were found to be the most potent inhibitors of human AChE (*h*AChE), demonstrating IC_{50} values of 0.0154 and 0.0263 μM. Ligands **6b**, **6c**, and **7c** exhibited the highest levels of inhibitory activity against human BuChE (*h*BuChE), demonstrating IC_{50} values that range from 0.228 to 0.328 μM. Docking studies were performed in order to predict the binding modes of compounds **6b** and **7c** with *h*AChE/*h*BuChE.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that results in the progressive and irreversible loss of higher brain functions, including memory, cognition, and reason.[1−3] The pathological hallmarks of AD include widespread neuronal degeneration, neuritic plaques containing β-amyloid (Aβ), and τ-rich neurofibrillary tangles (NFT).[4] Currently, most therapeutic treatments for AD are drugs that aim to inhibit enzyme acetylcholinesterase (AChE), thereby increasing acetylcholine (ACh) concentration in cholinergic synaptic clefts.[5] The only two classes of drugs currently available for AD treatment are the group of acetylcholinesterase inhibitors (AChEIs) (tacrine (Figure 1), donepezil, rivastigmine, galantamine) and the *N*-methyl-D-aspartate receptor (NMDAR) antagonist memantine, although it is important to note that the inhibition of the catalytic activity of AChE is not the only mechanism that is responsible for the neuroprotective effect of AChEIs.[5] The effectiveness of ChE inhibitors in AD treatment is limited by their ability to penetrate through the blood–brain barrier (BBB).[6] The important factors, which may influence passive penetration into the central nervous system (CNS), are the structure, lipophilicity, molecular weight, and the presence of charge in AChEIs.[6] The penetration into CNS is typically confirmed by their therapeutic effect (improved cognitive and memory functions, improved behavioral deficits) or by their potency to inhibit cholinesterase in the brain.[7] Therefore, all drugs that were used successfully in the therapy of AD should be considered as CNS+ targeted.[6] In recent years, major research efforts have been devoted to the development of compounds that are able to bind simultaneously to both the peripheral anionic site (PAS) and the catalytic anionic site (CAS) of the enzyme. The neuroprotective effect of the compounds that have resulted from these studies is based on their ability to react with the peripheral site and to block the interaction of AChE with Aβ (β-amyloid peptide).[8,9] Compounds that demonstrate a dual binding affinity with

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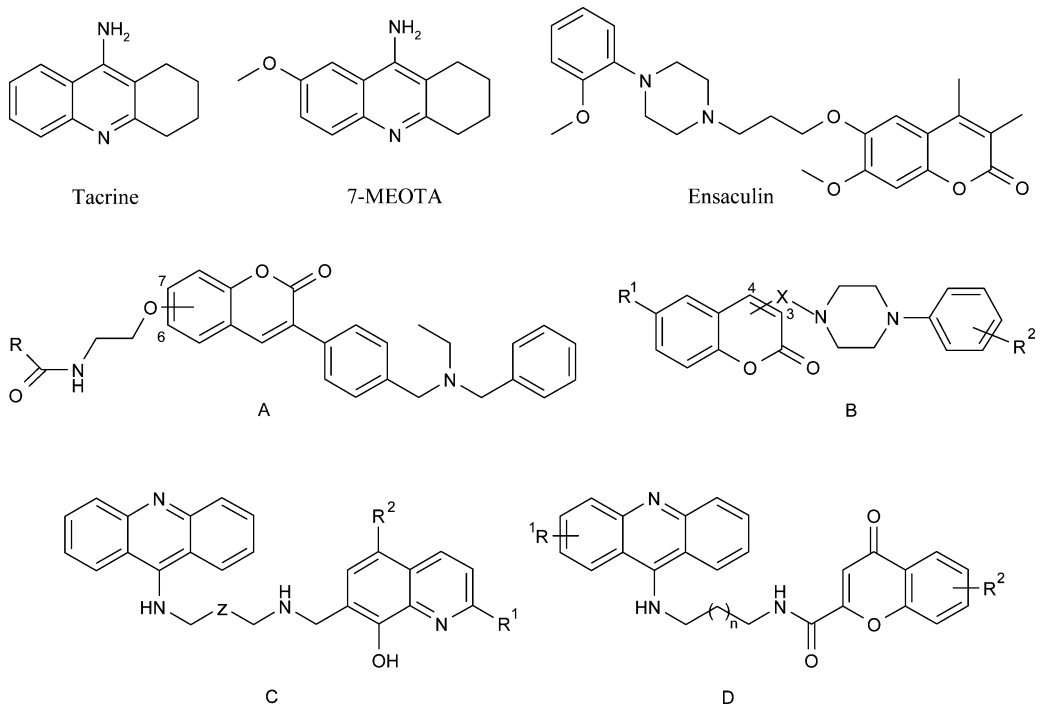

Figure 1. Chemical structures of tacrine, 7-MEOTA, ensaculine, coumarin derivatives **A**, **B**, tacrine−quinoline heterodimers **C**, and tacrine−4-oxo-4*H*-chromene hybrids **D**.

AChE have also been used in the development of a new type of therapeutic agent that prevents Aβ aggregation.[10,11]

While there is evidence of stable or slowly falling levels of AChE activity in the brains of patients with AD, slight increases in BuChE activity have also been reported. Both enzymes would therefore appear to be suitable targets for the development of ChE inhibitors in the treatment of AD.[12] These two enzymes differ in terms of substrate specificity and kinetics and also show varying levels of activity in different regions of the brain.[13a−c] The two molecules are also structurally different; the three aromatic residues present in the PAS of AChE are absent from the PAS of BuChE. One result of this is that typical PAS ligands exhibit a weaker affinity toward BuChE than to AChE.[14]

Tacrine (1,2,3,4-tetrahydroacridine, Figure 1) is noncompetitive reversible acetyl/butyrylcholinesterase inhibitor that selectively binds to the catalytic side of AChE. The main issue associated with the use of tacrine was the significant side effects, in particular the hepatotoxicity and cholinergic effects upon the gastrointestinal tract.[15,16] Tacrine is not effective in all cases of AD because its metabolization to different hydroxy metabolites depends on individual activities of the cytochrome P450 isozyme family;[17] some of these are pharmacologically active but are also slightly toxic. In order to find compounds with reduced side effects, a series of THA derivatives and analogues was synthesized. 7-MEOTA (9-amino-7-methoxy-1,2,3,4-tetrahydroacridine, Figure 1) was found to be a potent and less toxic ChEI that is free of the serious side effects related to tacrine.[15]

More importantly, tacrine actually prevents the aggegation and deposition of Aβ-amyloid plaques, an ability that is related to its interaction with the PAS of AChE.[18,19] The development of tacrine based dimers and hybrids with improved pharmacological properties has been the focus of a great deal of research in recent years.[20−24] Coumarin derivatives have been reported to possess a wide variety of biological effects such as antioxidant,[25,26] antimicrobial,[27] antifugal,[28] anticoagulant,[29] anti-inflammatory,[30−32] anticancer,[33] antitubercular[34] activities. Recent studies have also shown that coumarin analogues exhibit potent AChE activity, a finding that has led to these compounds being seen as potential drugs in the treatment of AD.[35−37] Many compounds containing a coumarin scaffold, such as ensaculin (Figure 1), have been successfully used as AChEIs.[38,39]

Zhou et al. have designed and synthesized a series of ensaculin analogues with phenylpiperazine functional groups[38] at position 3, 4, or 6 (Figure 1, coumarin derivatives **B**). The analogues substituted at position 3 and/or position 4 of the coumarin ring demonstrated higher levels of anti-AChEI activity than 6-substituted coumarins (IC_{50} of 6.7−9.3 and 4.5−7.9 μM). Piazzi et al. had earlier synthesized a series of coumarin hybrids with halophenylalkylamidic functional groups at position 6 or 7 of the coumarin moiety (Figure 1, coumarin derivatives **A**) and reported their high potential as multipotent anti-AD drug candidates. These compounds were found to exhibit considerable acetylcholinesterase inhibitory activity (IC_{50} = 0.181−0.551 μM) and BACE-1 inhibitory activity (IC_{50} = 0.099−0.151 μM).[35]

Fernández-Bachiller et al. recently designed and synthesized a series of novel multifunctional compounds with antioxidant and metal-binding properties which also demonstrated an inhibitory effect on Aβ aggregation and the dual inhibition of AChE and BuChE[40,41] (Figure 1). These tacrine−8-hydroxyquinoline (Figure 1, derivatives **C**) and tacrine−4-oxo-4*H*-chromene hybrids (Figure 1, derivatives **D**) showed excellent inhibitory activity against *h*AChE and *h*BuChE and β-secretase 1 (BACE-1) at both nano and picomolar concentrations.

On the basis of previous work published by Rodriguez-Franco's group in the field of AD,[40,41] Spuch et al. investigated the neurotoxic effects of a new tacrine−melatonin hybrid, *N*-(2-

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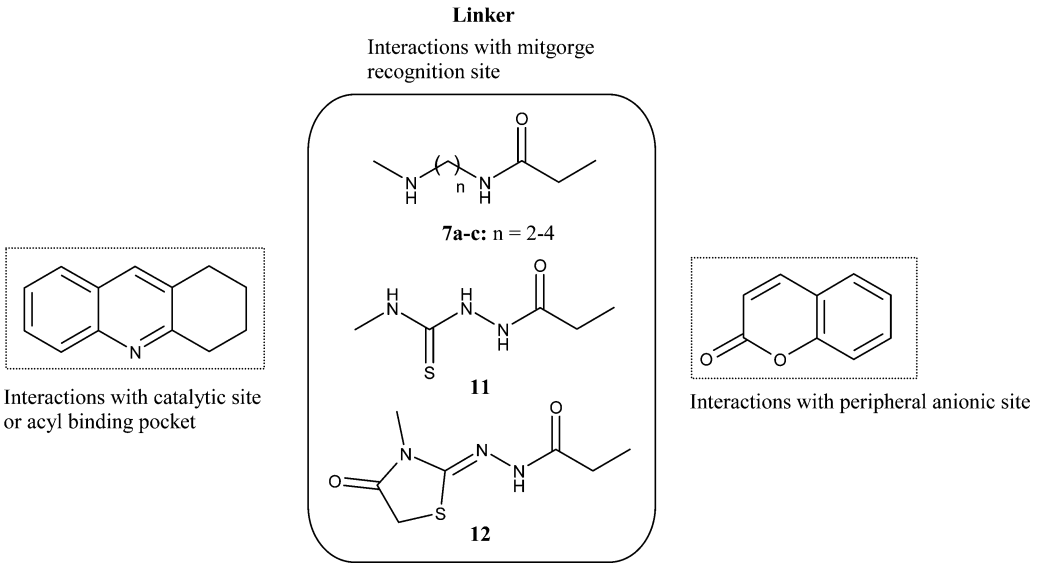
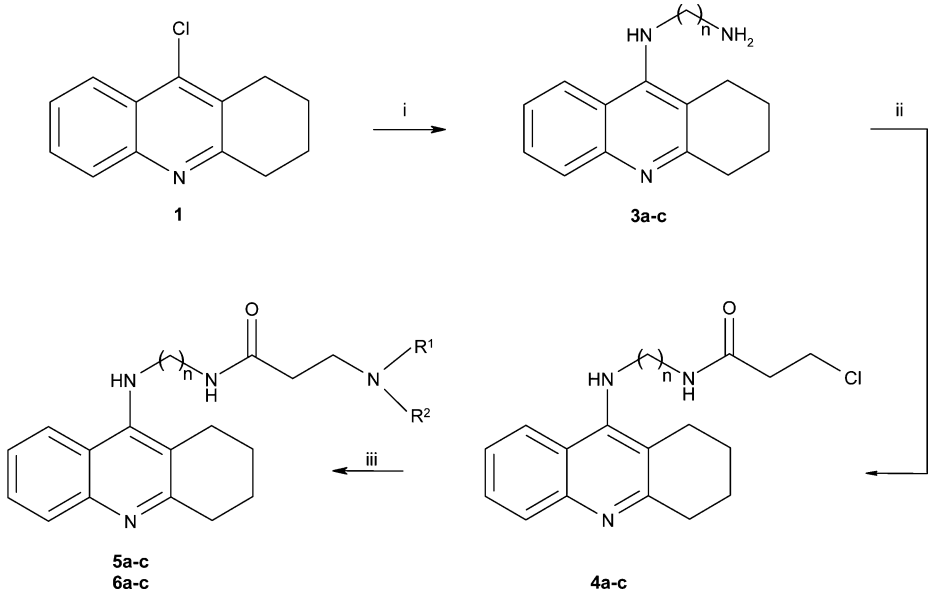


Figure 2. Design strategy for heterodimers **7**, **11**, and **12**.

Scheme 1. Synthesis of Tacrine Derivatives 4a–c, 5a–c, 6a–c[a]



[a]Reagents and conditions: (i) diaminoalkane (n = 2–4), phenol, reflux, 2 h; (ii) CPC, Et_3N, CH_2Cl_2, 0 °C, 2 h; (iii) secondary amine, DIPEA, CH_3CN, rt, 4 h.

(1*H*-indol-3-yl)ethyl)-7-(1,2,3,4-tetrahydroacridin-9-ylamino)-heptanamide.[42] This new hybrid is a potent inhibitor of *h*AChE and shows a high oxygen radical absorbance capacity which has been proven to cause a reduction in Aβ deposits.[42] Antequera et al.[43] investigated the modulatin effect of a multifunctional tacrine–8-hydroxyquinoline hybrid (IQM-622) with evidence of cholinergic, antioxidant, coopper-complexing, and neuroprotective properties. The authors found that the IQM-622 hybrid controls pathological processes at the cellular and neuronal level and holds considerable potential for the treatment of AD-associated brain damage.

As a continuation of our previous research,[44,45] this study describes the synthesis, docking studies, and biological evaluation of a series of novel tacrine derivatives and tacrine–coumarin hybrids. The aim of the research presented in this study was to combine tacrine with the coumarin scaffold in order to capitalize on the ChE inhibitory qualities of the former at the CAS, and the aromatic characteristics of the latter with AChE at the PAS. On the basis of our knowledge of the well-known structure of AChE, we decided to connect the tacrine and coumarin fragments using alkylenediamine tethers of different lengths, thiosemicarbazides tethers, and linkers with thiazolidinone heterocycle (Figure 2). Such linkers could be lodged in the narrow enzymatic cavity, thereby allowing simultaneous interaction between the heteroaromatic fragments and both the CAS and PAS of AChE.

RESULTS AND DISCUSSION

Synthesis of Tacrine Derivatives and Tacrine–Coumarin Hybrids. Two starting compounds, 9-chloro-1,2,3,4-tetrahydroacridine[46] (**1**) and (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid (**2**), were employed in the synthesis

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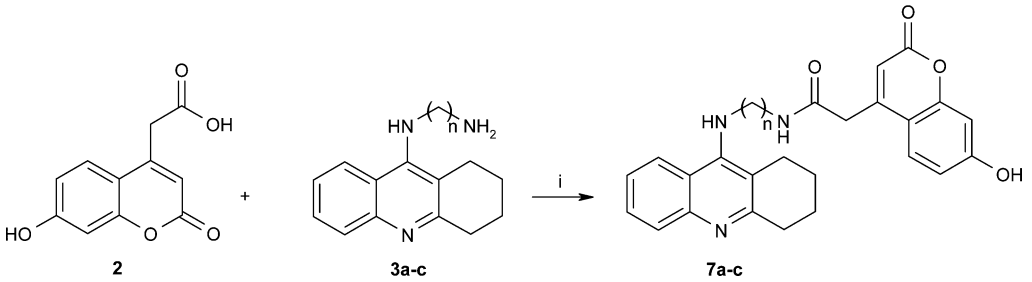
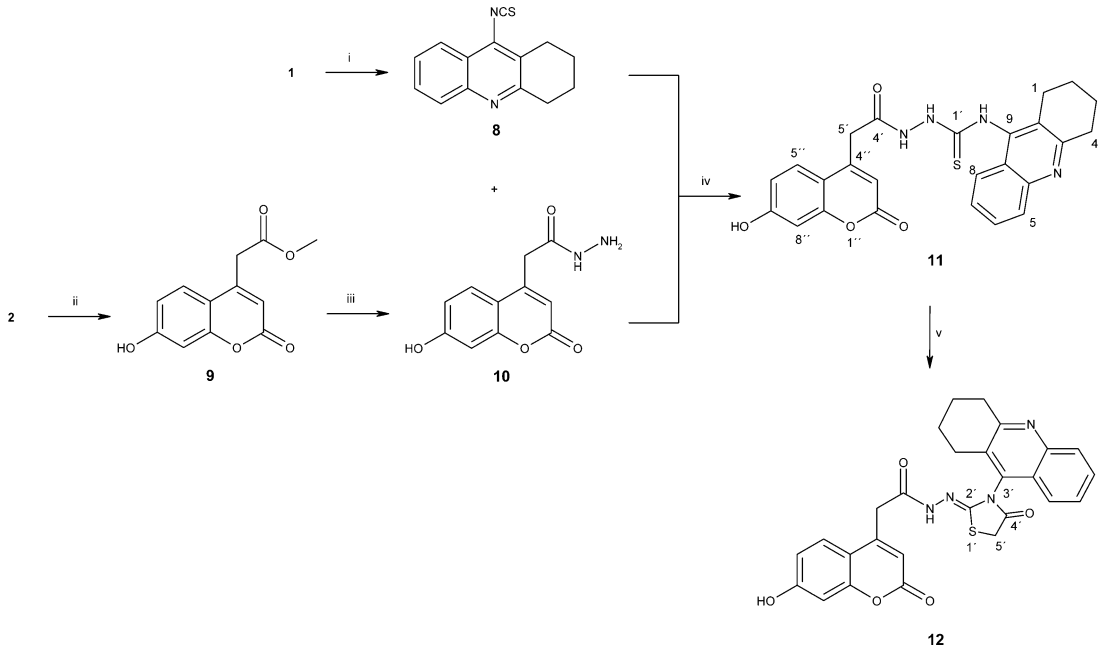
Scheme 2. Synthesis of Tacrine−Coumarin Heterodimers 7a−c[a]



[a]Reagents and conditions: (i) (n = 2−4), CDI, CH_2Cl_2, rt, 24 h.

Scheme 3. Synthesis of Tacrine−Coumarin Hybrids 11 and 12[a]



[a]Reagents and conditions: (i) AgSCN, toluene, 125−130 °C, 6 h; (ii) CH_3COCl, methanol, 2 h; (iii) NH_2−NH_2·H_2O, dry ethanol, reflux, 24 h; (iv) CH_3CH_2OH, rt, 24 h; (v) $BrCH_2COOCH_3$, TEA, $CHCl_3$, 25 h, rt.

of desired derivatives, the synthetic route of which is outlined in Schemes 1−3. The reaction of 9-chloro-1,2,3,4-tetrahydroacridine (**1**) with ethylene-, propane-, and butane-1,4-diamine, respectively, in phenol[47] produced three distinct intermediate compounds, *N*-(1,2,3,4-tetrahydroacridin-9-yl)alkanediamines **3a−c**. These derivatives were then reacted with 3-chloropropionyl chloride (CPC)[48,49] to produce the halogenated intermediates chloropropionamides **4a−c** (Scheme 1). A further reaction of chloropropionamides **4a−c** with secondary amines (methylcyclohexylamine/ethylcyclohexylamine) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in CH_3CN produced the target compounds: derivatives **5a−c** and **6a−c** (Scheme 1).

(7-Hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid (**2**) was prepared by condensing resorcinol with citric acid in the presence of concentrated sulfuric acid following a previously published procedure.[50] The reaction between (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid (**2**) and synthons **3a−c**, which had been obtained in the earlier process, in the presence of 1,1′-carbonyldiimidazole (CDI) in CH_2Cl_2 for 24 h resulted in the synthesis of the target compounds: novel tacrine−coumarin heterodimers **7a−c** with extended alkyl linkers (Scheme 2).

In order to synthesize tacrine−coumarin hybrids **11** and **12**, (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid methyl ester (**9**) and (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid hydrazide (**10**) were first prepared according to a previously published method.[51]

The reaction of hydrazide **10** with 9-isothiocyanato-1,2,3,4-tetrahydroacridine (**8**) in ethanol at room temperature produced *N*-[2-(1,2,3,4-tetrahydroacridin-9-yl)-2-[2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetyl]-1-hydrazinecarbothioamide (**11**). The cyclization of thiosemicarbazide **11** with methyl bromoacetate in $CHCl_3$ in the presence of TEA resulted in thiazolidinone derivative **12** at a 70% yield in the form of a yellow powder (Scheme 3).

Inhibition of Human AChE and BuChE. The extent of inhibition was expressed as the chemical concentration at which 50% of enzyme activity was inhibited (IC_{50}). The IC_{50} values of compounds **5a−c**, **6a−c**, **7a−c**, **11**, and **12** were determined against human erythrocytal AChE (*h*AChE, EC 3.1.1.7) and human plasmatic butyrylcholinesterase (*h*BuChE, E.C. 3.1.1.8) using the method of Ellman et al.[52] The IC_{50} values and

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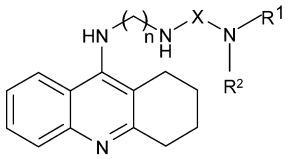
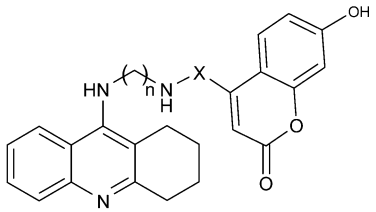
Table 1. In Vitro hAChE and *h*BuChE Inhibitory Activity and Selectivity Index of Tacrine Derivatives, 5a–c, 6a–c, and Tacrine–Coumarin Heterodimers 7a–c, 11, and 12



5a-c, 6a-c



7a-c

compd	X	R^1/R^2	*n*	IC_{50} ± SD [a] (μM) *h*AChE	IC_{50} ± SD [b] (μM) *h*BuChE	SI [c]
5a	$CO(CH_2)_2$	Me/Cy	2	0.58 ± 0.26	2.03 ± 0.14	3.50
5b	$CO(CH_2)_2$	Me/Cy	3	0.398 ± 0.40	2.38 ± 0.29	5.98
5c	$CO(CH_2)_2$	Me/Cy	4	0.104 ± 0.09	2.23 ± 0.04	21.40
6a	$CO(CH_2)_2$	Et/Cy	2	0.233 ± 0.19	0.765 ± 0.82	3.28
6b	$CO(CH_2)_2$	Et/Cy	3	0.0263 ± 0.22	0.267 ± 0.34	10.15
6c	$CO(CH_2)_2$	Et/Cy	4	0.0942 ± 0.11	0.228 ± 0.11	2.42
7a	$COCH_2$		2	2.91 ± 0.20	12.6 ± 0.11	4.33
7b	$COCH_2$		3	0.277 ± 0.27	0.827 ± 0.77	2.99
7c	$COCH_2$		4	0.0154 ± 0.17	0.328 ± 0.21	21.30
11				8.17 ± 1.63	1.42 ± 0.24	0.174
12				15.7 ± 3.14	5.62 ± 0.97	0.358
tacrine				0.5 ± 0.1	0.023 ± 0.004	0,046
7-MEOTA				15 ± 2.9	21 ± 3.4	1,4

[a] IC_{50}: 50% inhibitory concentration (mean ± SD of three experiments) of AChE. [b] IC_{50}: 50% inhibitory concentration (mean ± SD of three experiments) of BuChE. [c] Selectivity index for AChE is defined as IC_{50}(*h*BuChE)/IC_{50}(*h*AChE).

selectivity indices (SI) of tacrine derivatives **5a–c** and **6a–c**, tacrine–coumarin heterodimers **7a–c**, **11**, **12** and the control compounds of tacrine and 7-MEOTA[53] are summarized in Table 1. The synthesized derivatives demonstrated inhibitory activity against both *h*AChE and *h*BuChE with IC_{50} values ranging from micromolar to submicromolar concentrations. The results listed in Table 1 clearly show that the variation of the alkyl chain length influences both the anti-AChE and the anti-BuChE activities of the compounds.

The IC_{50} values of the compounds suggest that the inhibitory effect of tacrine derivatives **5a–c**, **6a–c** and tacrine–coumarin heterodimers **7a–c** was far stronger on AChE than on BuChE. Among these compounds, tacrine–coumarin heterodimer **7c** displayed excellent inhibition of *h*AChE; the IC_{50} value of 0.0154 μM is approximately 32 times higher than that of tacrine and 974 times higher than that of 7-MEOTA. The most potent inhibitor of *h*BuChE, tacrine derivative **6c**, showed an IC_{50} value of 0.228 μM; however, while this value is approximately 92 times higher than 7-MEOTA, it is still around 10 times less effective than tacrine itself. Compounds **5c**, **6c**, and **7c** with four methylene groups between the tacrine and amide group, and compound **6b** with three methylene groups were the best inhibitors of AChE in their series. Derivatives **6a–c** and **7b,c** demonstrated a similar trend in the inhibition of BuChE. From these results it can be seen that the length of the alkyl spacer and the amine moiety at the end of the chain have some influence on the levels of inhibition. When the methyl group (Me) in cyclohexylamine (**5a–c**) was replaced with an ethyl group (Et) (**6a–c**), an increase in anti-AChE and anti-BuChE activity was observed. When the alkyl chain was replaced with a thiosemicarbazide linker **11** or thiazolidinone moiety **12**, the AChE inhibitory activity dramatically decreased (IC_{50} = 8.17, 15.7 μM).

Tacrine derivatives **5a–c** and **6a–c** were found to be between 3 and 530 times more potent than thiosemicarbazide **11** and between 6 and 1000 times more potent than tacrine–coumarin heterodimer **12**. The best selectivity for *h*AChE was demonstrated by compounds **5c** and **7c** (SI = 21.40 and 21.30) and for *h*BuChE by compound **11** (SI = 0.174). The inhibition type was determined for selected ligands **6b**, **6c**, **7c**, **11**, and **12** using the Lineweaver–Burk plot. The Lineweaver–Burk plot revealed that ligands **6b**, **6c**, **7c** noncompetitively inhibited *h*AChE, and IC_{50} values of 0.0263, 0.0942, 0.00154 μM, respectively, were recorded (Table 1). Tacrine–coumarin heterodimers **11**, **12** were competitive inhibitors, showing IC_{50} values of 8.17 and 15.7 μM.

The dissociation constants K_{i1} of selected ligands, describing the stability of the enzyme–inhibitor complex, are listed in Table 2.

The K_{i1} value of 0.0041 μM for ligand **7c** indicates that this compound demonstrates the strongest affinity toward *h*AChE, a value that is 55 times higher than that of tacrine and 510 times higher than that of 7-MEOTA.

Table 2. Dissociation Constants K_{i1} and K_{i2} of Some Selected Ligands and Reference Compounds

compd	K_{i1} [a] (μM)	K_{i2} [b] (μM)
6b	0.0804	0.0789
6c	0.199	0.328
7c	0.0041	0.0234
11	1.94	92.8
12	2.36	65.3
tacrine	0.225	0.101
7-MEOTA	2.09	6.34

[a] K_{i1}: dissociation constant for AChE–inhibitor complex. [b] K_{i2}: dissociation constant for AChE–inhibitor–substrate complex.

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Molecular Modeling. In order to reveal the possible intermolecular interactions behind the inhibitory activities of novel tacrine derivatives, a molecular modeling study was carried out using docking programs AUTODOCK 4.2 and DOCK 6.5. The results of the modeling are shown in Figures 2−5.

The first stage of the process was the localization of ligand binding poses using Autodock. After a visual inspection of the poses with the highest negative binding energy, the structures that fill the catalytic cavity of the enzyme were chosen as the inputs for Amber rescoring. This led to the next stage, the redocking linked with Amber force field molecular dynamics. The main advantage of Amber rescoring is that the positions of both the ligand and the active site of the enzyme can be flexible, allowing small structural rearrangements to reproduce the so-called "induced fit".

Docking simulations were performed with the compounds that had been found to be the most active against *h*AChE and *h*BuChE, derivatives **6b** and **7c** (for more details see Experimental Section).

In order to allow sufficient space sampling for each derivative, all possible ligand structural combinations were built into the simulation. *R*/*S* configurations on atoms of nitrogen were combined with s-cis and s-trans conformations on the amide −NH−CO− bond. The result of these molecular combinations was a set of eight distinct structures for derivate **6b** and four different structures for derivative **7c** (see Supporting Information data, Figure S01). All of these structures were used as input geometries for docking simulations in order to reveal all of the possible interactions between the ligand and the active site of the enzyme. The protonation level of the ligands in physiological pH was also studied using the Marvin software pack. [http://www.chemaxon.com]. Autodock results were visually evaluated, and the top pose of the most negative cluster with a proper catalytic cavity orientation was selected for rescoring in AMBER molecular mechanics force field using DOCK software. Results were summarized according to the binding ability of the ligands.

The pose of derivative **6b** with the lowest binding energy toward *h*AChE is depicted in Figure 3. Plausible $\pi-\pi$ binding interactions might be found in the CAS of the enzyme between the tacrine core and the aromatic residue of Trp86. More complex stabilization might result from the hydrogen bonds between the ligand spacer and Tyr337, Tyr124 (see Supporting Information data, Figure S02). The existence of weak binding interactions might also be proposed between the endocyclic tacrine nitrogen and the amino acids of the inner part of the catalytic cavity of the enzyme. The docking run of derivative **7c** also proposed plausible $\pi-\pi$ stacking intermolecular interactions within the Trp86−tacrine−Tyr337 and Trp286−coumarine core (Figure 4).

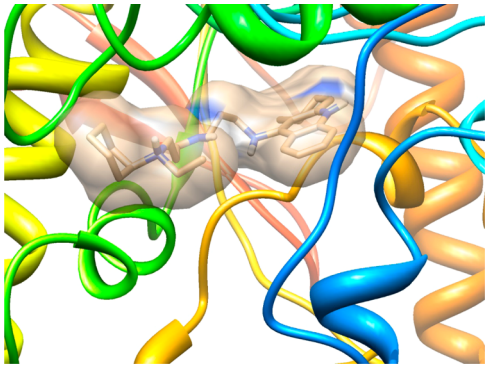

Figure 3. Top-score docking pose of derivative **6b** with molecular surface depicting putative structural orientation in the active-site gorge of the *h*AChE in a ribbon style.[41]

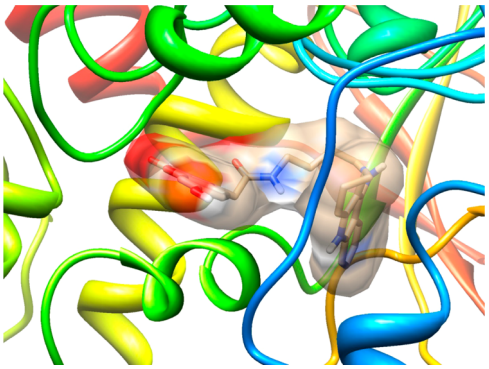

Figure 4. Top-score docking pose of derivative **7c** with molecular surface depicting putative structural orientation in the active-site gorge of the *h*AChE in a ribbon style.[41]

As is depicted in Figure 5, the ternary inhibition complex of compound **6b** with *h*BuChE shows evidence of direct interaction with the catalytic triad via His438. Additional hydrogen bonds formed between the amino acids of the catalytic cavity and derivative **6b** are depicted in Figure S04 (see Supporting Information). The binding of compound **7c** within the catalytic site of *h*BuChE is proposed in the ligand's folded state locked by the intramolecular interaction of the coumarine carbonyl oxygen and tacrine endocyclic nitrogen (Figure 6). It is possible to suggest that derivative **7c** could interact with enzyme via the hydrogen bond with carbonyl group and also through the histidine core of His438 (see Supporting Information, Figure S05).

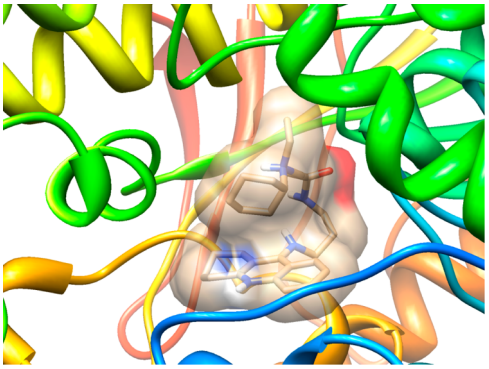

Figure 5. Top-score docking pose of derivative **6b** with molecular surface depicting putative structural orientation in the active-site gorge of the *h*BuChE in a ribbon style.[41]

CONCLUSION

This study describes the synthesis and biological evaluation of a series of new tacrine derivatives (**5a−c**, **6a−c**) and tacrine−coumarin heterodimers (**7a−c**, **11**, **12**). Some of the studied compounds demonstrated higher levels of inhibition of AChE and BuChE in comparison to those of the control compounds

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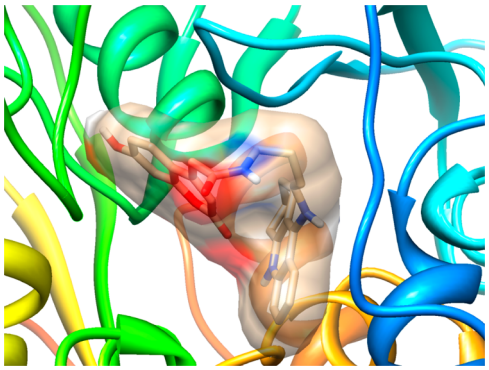

Figure 6. Top-score docking pose of derivative **7c** with molecular surface depicting putative structural orientation in the active-site gorge of the *h*BuChE in a ribbon style.[41]

of tacrine **1** and 7-MEOTA. The most potent inhibitors of *h*AChE were compounds **7c** and **6b** which showed IC_{50} values of 0.0154 and 0.0263 μM, while compounds **6c**, **6b**, and **7c** demonstrated the highest efficiency against *h*BuChE with a range of IC_{50} values from 0.228 to 0.328 μM. The highest selectivity indices were found for compounds **5c** and **7c** (SI = 21.40, 21.30) and **11** (SI = 0.174). From the results, it can be concluded that the length of the alkyl spacer and the amine moiety at the end of the chain have a considerable effect on the inhibitory effect of the compounds. There was a clear decrease in inhibitory activity when the alkyl chain was replaced with a thiosemicarbazide linker or thiazolidinone moiety. Molecular modeling studies confirmed that these hybrids target both the CAS and PAS of AChE.

EXPERIMENTAL SECTION

Chemistry. General Methods. All solvents, chemicals, and reagents were obtained commercially and used without purification. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Mercury Plus NMR spectrometer using $CDCl_3$ or DMSO-d_6 as solvents with tetramethylsilane as an internal standard. Chemical shifts, δ, are given in parts per million (ppm), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling constants, *J*, are expressed in hertz (Hz). Thin-layer chromatography was performed on Macherey-Nagel Alugram Sil G/UV254 plates, and spots were visualized with UV light. Chromatographic separations were performed on silica gel 60 (0.063−0.040 mm, Merck) column chromatography. Melting points were recorded on a Boetius hot-plate apparatus and are uncorrected. Yields refer to isolated pure products and were not maximized. CHN analysis was performed on a CHN analyzer PerkinElmer 2400.

General Procedure for the Synthesis of *N*1-[*n*-(1,2,3,4-Tetrahydroacridin-9-ylamino)alkyl]-3-chloropropanamides 4a−c. A solution of corresponding *N*1-(1,2,3,4-tetrahydroacridin-9-yl)diamine (**3**, 0.74 mM) in anhydrous CH_2Cl_2 (4 mL) and Et_3N (0.74 mM) was added dropwise to a solution of chloropropionyl chloride (0.07 mL, 0.74 mM) in anhydrous CH_2Cl_2 (2 mL) over 40 min at −5 °C. The mixture was stirred for 30 min at 0 °C and for 1 h at room temperature. After completion of the reaction, the residue was washed with 5% aqueous Na_2CO_3 solution (2 mL). The organic layer was separated and dried over Na_2SO_4. Removal of the solvents produced a residue which was purified using column chromatography, eluent CH_2Cl_2−MeOH (4:1).

***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)ethyl]-3-chloropropanamide (4a).** Compound **3a** was treated with chloropropionyl chloride according to a commonly used procedure to give the desired product **4a** in the form of a yellow solid (42%). Mp 87−92 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.74−1.90 (m, 4H, 2 × CH_2, H-2,3), 2.56 (t, 2H, CH_2, H-5′, *J* = 6.2 Hz), 2.62−2.74 (m, 2H, CH_2, H-1), 2.95−3.07 (m, 2H, CH_2, H-4), 3.47 (t, 2H, CH_2, H-2′, *J* = 5.6 Hz), 3.75 (t, 2H, CH_2, H-6′, *J* = 6.0 Hz), 3.97 (t, 2H, CH_2, H-1′, *J* = 5.6 Hz), 7.55 (dd, 1H, CH, H-7, *J* = 7.0, 8.4 Hz), 7.85 (dd, 1H, CH, H-6, *J* = 6.8, 8.4 Hz), 7.95 (d, 1H, CH, H-5, *J* = 8.4 Hz), 8.46 (d, 1H, CH, H-8, *J* = 8.8 Hz). ^{13}C NMR (DMSO-d_6) δ 20.2, 21.4 (C-2,3), 23.6 (C-1), 28.3 (C-4), 37.9 (C-5′), 39.8 (C-2′), 40.8 (C-6′), 47.8 (C-1′), 112.3 (C-9a), 115.3 (C-8a), 119.0 (C-5), 125.0 (C-7), 125.2 (C-8), 132.5 (C-6), 139.9 (C-10a), 151.4 (C-9), 155.2 (C-4a), 170.3 (C-4′). Anal. Calcd for $C_{18}H_{22}N_3O$ (331.85): C, 65.15; H, 6.68; N, 12.66. Found: C, 65.12; H, 6.63; N, 12.64.

***N*1-[3-(1,2,3,4-Tetrahydroacridin-9-ylamino)propyl]-3-chloropropanamide (4b).** Compound **3b** was treated with chloropropionyl chloride according to a commonly used procedure to give the desired product **4b** in the form of a yellow oil (47%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.75−1.93 (m, 6H, 3 × CH_2, H-2,3,2′), 2.57 (t, 2H, CH_2, H-6′, *J* = 6.4 Hz), 2.65−2.74 (m, 2H, CH_2, H-1), 2.96−3.03 (m, 2H, CH_2, H-4), 3.13−3.24 (m, 2H, CH_2, H-3′), 3.72−3.88 (m, 2H, 2 × CH_2, H-1′,7′), 7.53 (ddd, 1H, CH, H-7, *J* = 1.2, 6.8, 8.4 Hz), 7.81 (dd, 1H, CH, H-6, *J* = 6.8, 8.4 Hz), 7.89 (dd, 1H, CH, H-5, *J* = 1.2, 8.8 Hz), 8.36 (d, 1H, CH, H-8, *J* = 8.8 Hz). ^{13}C NMR (DMSO-d_6) δ 20.5, 21.6 (C-2,3), 24.0 (C-1), 28.8 (C-4), 30.0 (C-2′), 35.6 (C-3′), 38.7 (C-6′), 41.0 (C-7′), 44.6 (C-1′), 112.0 (C-9a), 116.2 (C-8a), 124.5 (C-8), 124.7 (C-7), 120.6 (C-5), 131.7 (C-6), 139.3 (C-10a), 151.8 (C-9), 154.7 (C-4a), 169.3 (C-5′). Anal. Calcd for $C_{19}H_{24}N_3O$ (345.88): C, 65.98; H, 6.99; N, 12.15. Found: C, 65.95; H, 6.96; N, 12.12.

***N*1-[4-(1,2,3,4-Tetrahydroacridin-9-ylamino)butyl]-3-chloropropanamide (4c).** Compound **3c** was treated with chloropropionyl chloride according to a commonly used procedure to give the desired product **4c** in the form of a yellow oil (45%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41−1.52 (m, 2H, CH_2, H-3′), 1.62−1.72 (m, 2H, CH_2, H-2′), 1.79−1.88 (m, 4H, 2 × CH_2, H-2,3), 2.50−2.60 (m, 2H, CH_2, H-7′), 2.63−2.72 (m, 2H, CH_2, H-1), 2.96−3.02 (m, 2H, CH_2, H-4), 3.03−3.11 (m, 2H, CH_2, H-4′), 3.62−3.84 (m, 4H, 2 × CH_2, H-1′,8′), 7.49 (dd, 1H, CH, H-7, *J* = 7.0, 8.2 Hz), 7.74 (dd, 1H, CH, H-6, *J* = 6.8, 8.4 Hz), 7.85 (d, 1H, CH, H-5, *J* = 8.4 Hz), 8.31 (d, 1H, CH, H-8, *J* = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ 20.8, 21.7 (C-2,3), 24.1 (C-1), 26.1 (C-3′), 27.3 (C-2′), 29.6 (C-4), 37.9 (C-4′), 38.1 (C-7′), 40.9 (C-8′), 46.9 (C-1′), 112.5 (C-9a), 116.9 (C-8a), 122.0 (C-5), 124.2 (C-8), 124.3 (C-7), 130.8 (C-6), 140.2 (C-10a), 151.8 (C-9), 154.7 (C-4a), 169.6 (C-6′). Anal. Calcd for $C_{20}H_{26}N_3O$ (359.90): C, 66.75; H, 7.28; N, 11.68. Found: C, 66.73; H, 7.25; N, 11.63.

General Procedure for the Synthesis of *N*1-[*n*-(1,2,3,4-Tetrahydroacridin-9-ylamino)alkyl]-3-[cyclohexyl(methyl)-amino]propanamides 5a−c. A mixture of corresponding *N*1-[*n*-(1,2,3,4-tetrahydroacridin-9-ylamino)alkyl]-3-chloropropanamide (**4**, 0.17 mM), *N*-methylcyclohexylamine (0.022 ml, 0.17 mM), and *N*,*N*-diisopropylethylamine (0.04 mL, 0.231 mM) was stirred under N_2 in acetonitrile (2 mL) for 4 h at room temperature. The solvent was removed under reduced pressure. CH_2Cl_2 (1 mL) and water (1 mL) were added to the crude product. The water layer was extracted with CH_2Cl_2 (3 × 1 mL). The combined organic extracts were dried over $MgSO_4$. The residue was purified using column chromatography, eluent CH_2Cl_2−MeOH (4:1).

***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)ethyl]-3-[cyclohexyl(methyl)amino]propanamide (5a).** Compound **4a** was treated with methylcyclohexylamine according to a commonly used procedure to give the desired product **5a** in the form of a yellow oil (77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16−1.36 (m, 10H, 5 × CH_2, H-9′,10′,11′,12′,13′), 1.87−1.97 (m, 4H, 2 × CH_2, H-2,3), 2.30 (s, 3H, CH_3, H-1″), 2.40−2.50 (m, 1H, CH, H-8′), 2.62 (t, 2H, CH_2, H-5′, *J* = 6.4 Hz), 2.69−2.78 (m, 2H, CH_2, H-1), 3.03−3.10 (m, 2H, CH_2, H-4), 3.59 (t, 2H, CH_2, H-2′, *J* = 5.6 Hz), 3.62−3.72 (m, 2H, CH_2, H-1′), 3.82 (t, 2H, CH_2, H-6′, *J* = 6.4 Hz), 7.36 (ddd, 1H, CH, H-7, *J* = 1.2, 7.2, 8.4 Hz), 7.55 (ddd, 1H, CH, H-6, *J* = 1.2, 6.8, 8.0 Hz), 7.89−7.96 (m, 2H, 2 × CH, H-5,8). ^{13}C NMR ($CDCl_3$) δ 22.7, 23.0 (C-2,3), 25.1 (C-1), 24.8 (C-10′,11′,12′) 29.7 (C-9′,13′), 33.9 (C-4), 37.8 (C-1″), 39.6 (C-5′), 40.1 (C-6′), 40.8 (C-2′), 49.1 (C-1′), 58.5 (C-8′), 116.1 (C-9a), 120.3 (C-8a), 122.8 (C-8), 124.0 (C-7),

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127.3 (C-5), 128.5 (C-6), 147.0 (C-10a), 152.2 (C-9), 158.8 (C-4a), 169.3 (C-4′). Anal. Calcd for $C_{25}H_{36}N_4O$ (408.59): C, 73.49; H, 8.88; N, 13.71. Found: C, 73.50; H, 8.85; N, 13.68.

***N*1-[3-(1,2,3,4-Tetrahydroacridin-9-ylamino)propyl]-3-[cyclohexyl(methyl)amino]propanamide (5b).** Compound **4b** was treated with methylcyclohexylamine according to a commonly used procedure to give the desired product **5b** in the form of a yellow oil (59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10−1.35 (m, 10H, 5 × CH_2, H-10′,11′,12′,13′,14′), 1.80−2.03 (m, 9H, 3 × CH_2, CH_3, H-2,3,1″,2′), 2.38−2.52 (m, 1H, CH, H-9′), 2.69 (t, 2H, CH_2, H-6′, J = 6.0 Hz), 2.72−2.82 (m, 2H, CH_2, H-1), 2.98−3.10 (m, 2H, CH_2, H-4), 3.40−3.50 (m, 2H, CH_2, H-3′), 3.55−3.65 (m, 2H, CH_2, H-1′), 3.82 (t, 2H, CH_2, H-7′, J = 6.0 Hz), 7.35 (dd, 1H, CH, H-7, J = 7.2, 8.0 Hz), 7.52 (dd, 1H, CH, H-6, J = 7.2, 8.0 Hz), 7.91 (d, 1H, CH, H-5, J = 8.0 Hz), 8.07 (d, 1H, CH, H-8, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$) δ 22.4, 22.9 (C-2,3), 24.9, 25.0 (C-1,11′,13′), 25.9 (C-12′), 29.7 (C-10′,14′), 31.2 (C-2′), 33.9 (C-4), 36.5 (C-3′), 37.8 (C-1″), 39.4 (C-6′), 40.5 (C-7′), 44.9 (C-1′), 58.5 (C-9′), 115.6 (C-9a), 119.7 (C-8a), 123.0 (C-8), 124.1 (C-7), 126.9 (C-5), 129.0 (C-6), 145.7 (C-10a), 151.7 (C-9), 157.1 (C-4a), 170.6 (C-5′). Anal. Calcd for $C_{26}H_{38}N_4O$ (422.62): C, 73.89; H, 9.06; N, 13.26. Found: C, 73.87; H, 9.03; N, 13.24.

***N*1-[4-(1,2,3,4-Tetrahydroacridin-9-ylamino)butyl]-3-[cyclohexyl(methyl)amino]propanamide (5c).** Compound **4c** was treated with methylcyclohexylamine according to a general procedure to give the desired product **5c** as yellow oil (50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18−1.38 (m, 10H, 5 × CH_2, H-11′,12′,13′,14′,15′), 1.60−1.68 (m, 2H, CH_2, H-3′), 1.68−1.80 (m, 2H, CH_2, H-2′), 1.82−1.95 (m, 4H, 2 × CH_2, H-2,3), 2.30 (s, 3H, CH_3, H-1″), 2.47−2.50 (m, 1H, CH, H-10′), 2.62 (t, 2H, CH_2, H-7′, J = 6.8 Hz), 2.63−2.72 (m, 2H, CH_2, H-1), 3.00−3.08 (m, 2H, CH_2, H-4), 3.26−3.37 (m, 2H, CH_2, H-4′), 3.53 (t, 2H, CH_2, H-1′, J = 6.8 Hz), 3.80 (t, 2H, CH_2, H-8′, J = 6.4 Hz), 7.34 (dd, 1H, CH, H-7, J = 7.2, 8.0 Hz), 7.53 (dd, 1H, CH, H-6, J = 7.2, 8.4 Hz), 7.92 (d, 1H, CH, H-5, J = 8.0 Hz), 7.96 (d, 1H, CH, H-8, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ 22.7, 22.9 (C-2,3), 24.8 (C-1,2′), 25.1 (C-12′, 14′), 27.1 (C-3′), 29.2 (C-13′), 31.3 (C-11′,15′), 33.4 (C-4), 37.8 (C-1″), 39.2 (C-4′), 39.6 (C-7′), 40.4 (C-8′), 48.8 (C-1′), 58.5 (C-10′), 115.8 (C-9a), 119.9 (C-8a), 122.9 (C-8), 123.9 (C-7), 127.8 (C-5), 128.8 (C-6), 146.5 (C-10a), 151.1 (C-9), 157.8 (C-4a), 169.7 (C-6′). Anal. Calcd for $C_{27}H_{40}N_4O$ (436.65): C, 74.27; H, 9.23; N, 12.83. Found: C, 74.24; H, 9.21; N, 12.80.

General Procedure for the Synthesis of *N*1-[*n*-(1,2,3,4-Tetrahydroacridin-9-ylamino)alkyl]-3-[cyclohexyl(ethyl)-amino]propanamides 6a−c. A mixture of corresponding *N*1-[*n*-(1,2,3,4-tetrahydroacridin-9-ylamino)alkyl]-3-chloropropanamide (**4**, 0.12 mM), *N*-ethylcyclohexylamine (0.018 mL, 0.12 mM), and *N*,*N*-diisopropylethylamine (0.027 ml, 0.155 mM) was stirred under N_2 in acetonitrile (2 mL) for 4 h at room temperature. The solvent was removed under reduced pressure. CH_2Cl_2 (1 mL) and water were added to the crude product. The water layer was treated with CH_2Cl_2 (3 × 1 mL). The organic layer was dried over $MgSO_4$. A residue was purified using column chromatography, eluent CH_2Cl_2−MeOH (4:1).

***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)ethyl]-3-[cyclohexyl(ethyl)amino]propanamide (6a).** Compound **4a** was treated with ethylcyclohexylamine according to a commonly used procedure to give the desired product **6a** in the form of a yellow oil (62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82−0.98 (m, 3H, CH_3, H-2″), 1.10−1.43 (m, 10H, 5 × CH_2, H-9′,10′,11′,12′,13′), 1.83−1.97 (m, 4H, 2 × CH_2, H-2,3), 2.42−2.60 (m, 1H, CH, H-8′), 2.63−2.72 (m, 2H, CH_2, H-1), 2.75 (t, 2H, CH_2, H-5′, J = 6.2 Hz), 2.80−2.88 (m, 2H, CH_2, H-1″), 2.97−3.13 (m, 1H, CH, H-4), 3.70−3.78 (m, 2H, CH_2, H-2′), 3.79−3.93 (m, 4H, 2 × CH_2, H-1′,6′), 7.26 (dd, 1H, CH, H-7, J = 7.2, 8.0 Hz), 7.47 (dd, 1H, CH, H-6, J = 7.2, 8.0 Hz), 7.96 (d, 1H, CH, H-5, J = 8.4 Hz), 8.02 (d, 1H, CH, H-8, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$) δ 15.1 (C-2″), 22.5,23.1 (C-2,3), 25.0 (C-1), 25.6 (C-10′,11′,12′), 29.7 (C-9′,13′), 33.8 (C-4), 39.2 (C-5′), 40.2 (C-2′), 40.3 (C-6′), 41.0 (C-1″), 49.6 (C-1′), 56.7 (C-8′), 116.3 (C-9a), 120.4 (C-8a), 123.1 (C-8), 124.3 (C-7), 128.2 (C-5), 128.8 (C-6), 147.0 (C-10a), 151.0 (C-9), 158.8 (C-4a), 169.5 (C-4′). Anal. Calcd for $C_{26}H_{38}N_4O$ (422.62): C, 73.89; H, 9.06; N, 13.26. Found: C, 73.86; H, 9.01; N, 13.23.

***N*1-[3-(1,2,3,4-Tetrahydroacridin-9-ylamino)propyl]-3-[cyclohexyl(ethyl)amino]propanamide (6b).** Compound **4b** was treated with ethylcyclohexylamine according to a commonly used procedure to give the desired product **6b** in the form of a yellow oil (50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08−1.33 (m, 13H, 5 × CH_2, CH_3, H-10′,11′,12′,13′,14′,2″), 1.78−1.85 (m, 2H, CH_2, H-2′), 1.87−1.96 (m, 4H, 2 × CH_2, H-2,3), 2.44−2.55 (m, 1H, CH, H-9′), 2.63−2.81 (m, 6H, 3 × CH_2, H-1,6′,1″), 3.01−3.12 (m, 2H, CH_2, H-4), 3.41−3.50 (m, 2H, CH_2, H-3′), 3.50−3.57 (m, 2H, CH_2, H-1′), 3.83 (t, 2H, CH_2, H-7′, J = 6.4 Hz), 7.34 (ddd, 1H, CH, H-7, J = 1.2, 7.2, 8.4 Hz), 7.53 (ddd, 1H, CH, H-6, J = 1.2, 7.2, 8.4 Hz), 7.90 (d, 1H, CH, H-5, J = 8.4 Hz), 8.03 (d, 1H, CH, H-8, J = 8.8 Hz). ^{13}C NMR ($CDCl_3$) δ 15.1 (C-2″), 22.7, 23.1 (C-2,3), 25.1 (C-1,11′,13′), 26.1 (C-12′), 29.7 (C-10′,14′), 31.3 (C-2′), 33.7 (C-4), 36.7 (C-3′), 39.6 (C-6′), 40.3 (C-7′), 41.0 (C-1″), 45.2 (C-1′), 56.8 (C-9′), 116.4 (C-9a), 120.3 (C-8a), 122.7 (C-8), 123.9 (C-7), 128.2 (C-5), 128.5 (C-6), 146.8 (C-10a), 150.9 (C-9), 158.2 (C-4a), 170.4 (C-5″). Anal. Calcd for $C_{27}H_{40}N_4O$ (436.65): C, 74.27; H, 9.23; N, 12.83. Found: C, 74.26; H, 9.19; N, 12.79.

***N*1-[4-(1,2,3,4-Tetrahydroacridin-9-ylamino)butyl]-3-[cyclohexyl(ethyl)amino]propanamide (6c).** Compound **4c** was treated with ethylcyclohexylamine according to a commonly used procedure to give the desired product **6c** in the form of a yellow oil (51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07−1.36 (m, 13H, 5 × CH_2, CH_3, H-11′,12′,13′,14′,15′,2″), 1.58−1.80 (m, 4H, 2 × CH_2, H-2′,3′), 1.86−1.98 (m, 4H, 2 × CH_2, H-2,3), 2.43−2.55 (m, 1H, CH, H-10′), 2.59 (t, 2H, CH_2, H-7′, J = 6.4 Hz), 2.65−2.77 (m, 4H, 2 × CH_2, H-1,1″), 3.00−3.07 (m, 2H, CH_2, H-4), 3.29−3.40 (m, 2H, CH_2, H-4′), 3.45−3.52 (m, 2H, CH_2, H-1′), 3.76−3.85 (m, 2H, CH_2, H-8′), 7.34 (ddd, 1H, CH, H-7, J = 1.2, 6.8, 8.0 Hz), 7.53 (ddd, 1H, CH, H-6, J = 1.2, 6.8, 8.4 Hz), 7.90 (d, 1H, CH, H-5, J = 8.0 Hz), 7.98 (d, 1H, CH, H-8, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$) δ 15.2 (C-2″), 22.8, 23.1 (C-2,3), 25.1 (C-1,2′,12′,14′), 26.1 (C-13′), 27.2 (C-3′), 29.7 (C-11′,15′), 34.1 (C-4), 39.2 (C-4′), 39.6 (C-7′), 40.3 (C-8′), 40.9 (C-1″), 48.9 (C-1′), 56.8 (C-10′), 116.4 (C-9a), 120.4 (C-8a), 122.7 (C-8), 123.8 (C-7), 128.3 (C-6), 128.7 (C-5), 147.4 (C-10a), 150.5 (C-9), 158.6 (C-4a), 169.8 (C-6′). Anal. Calcd for $C_{28}H_{42}N_4O$ (450.67): C, 74.62; H, 9.39; N, 12.43. Found: C, 74.59; H, 9.36; N, 12.39.

General Procedure for the Synthesis of *N*1-[*n*-(1,2,3,4-tetrahydroacridin-9-ylamino)alkyl]-2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetamides 7a−c. A mixture of (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid (**2**, 0.153 g, 0.7 mM) in anhydrous CH_2Cl_2 (6 mL) and carbonyldiimidazole (0.124 g, 0.765 mmol) was stirred for 1.5 h at room temperature. A solution of corresponding *N*-(*n*-aminoalkyl)-*N*-(1,2,3,4-tetrahydroacridin-9-yl)amine (**3**, 0.7 mM) in anhydrous CH_2Cl_2 (2 mL) was added. The mixture was stirred for 24 h. Water (6 mL) was added to the mixture. The organic layer was separated, dried over Na_2SO_4, and the solvents were evaporated. The residue was purified using column chromatography, eluent EtAC−MeOH−NH_3OH (6:2:0.2).

***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)ethyl]-2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetamide (7a).** Compound **3a** was treated with (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid according to a commonly used procedure to give the desired product **7a** in the form of a yellow oil (34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80−1.92 (m, 4H, 2 × CH_2, H-2,3), 2.80−2.84 (m, 2H, CH_2, H-1), 3.02−3.08 (m, 2H, CH_2, H-4), 3.45−3.65 (m, 4H, 2 × CH_2, H-1′,2′), 3.73 (s, 2H, CH_2, H-5′), 6.24 (s, 1H, CH, H-3″), 6.75 (s, 1H, CH, H-8″), 6.81 (dd, 1H, CH, H-6″, J = 2.4, 8.8 Hz), 7.44−7.62 (m, 3H, 3 × CH, H-6,7,5″), 7.85 (d, 1H, CH, H-5, J = 8.4 Hz), 7.95 (d, 1H, CH, H-8, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ 22.3 (C-2,3), 24.3 (C-1), 33.2 (C-4), 38.0 (C-5′), 52.1 (C-1′,2′), 102.3 (C-8″), 112.0 (C-3″,4a″), 113.0 (C-6″), 115.7 (C-9a), 119.4 (C-8a), 122.6 (C-8), 124.2 (C-7), 126.6 (C-5″), 128.3 (C-5), 128.7 (C-6), 146.7 (C-10a), 149.5 (C-4″), 150.9 (C-9), 154.9 (C-8a″), 158.2 (C-4a), 160.1 (C-2″), 161.2 (C-7″), 171.7 (C-4′). Anal. Calcd for $C_{26}H_{25}N_3O4$ (443.51): C, 70.41; H, 5.68; N, 9.47. Found: C, 70.39; H, 5.65; N, 9.43.

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***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)propyl]-2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetamide (7b).** Compound **3b** was treated with (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid according to a commonly used procedure to give the desired product **7b** in the form of a yellow oil (40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.77−2.0 (m, 6H, 3 × CH_2, H-2,3,2′), 2.78−2.83 (m, 2H, CH_2, H-1), 3.05−3.10 (m, 2H, CH_2, H-4), 3.49−3.56 (m, 2H, CH_2, H-1′), 3.56−3.67 (m, 2H, CH_2, H-3′), 3.72 (s, 2H, CH_2, H-6′), 6.18 (s, 1H, CH, H-3″), 6.75 (s, 1H, CH, H-8″), 6.83 (dd, 1H, CH, H-6″, J = 2.4, 8.8 Hz), 7.30−7.45 (m, 2H, 2 × CH, H-7,5″), 7.45−7.60 (m, 1H, CH, H-6), 8.0 (d, 1H, CH, H-5, J = 8.4 Hz), 8.00 (d, 1H, CH, H-8, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ 22.2, 22.3 (C-2,3), 24.3 (C-1), 33.5 (C-4), 31.2 (C-2′), 37.9 (C-3′,6′), 47.6 (C-1′), 103.4 (C-8″), 111.5 (C-3″,4a″), 114.1 (C-6″), 115.7 (C-9a), 120.4 (C-8a), 122.8 (C-8), 124.2 (C-7), 126.6 (C-5″), 128.4 (C-5), 129.1 (C-6), 146.9 (C-10a), 150.0 (C-4″), 151.9 (C-9), 155.2 (C-8a″), 157.2 (C-4a), 160.5 (C-2″), 161.5 (C-7″), 171.9 (C-5′). Anal. Calcd for $C_{27}H_{27}N_3O4$ (457.53): C, 70.88; H, 5.95; N, 9.18. Found: C, 70.86; H, 5.92; N, 9.17.

***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-ylamino)butyl]-2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetamide (7c).** Compound **3c** was treated with (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid according to a commonly used procedure to give the desired product **7c** in the form of a yellow oil (33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.66−1.80 (m, 4H, 2 × CH_2, H-2′,3′), 1.80−1.92 (m, 4H, 2 × CH_2, H-2,3), 2.60−2.70 (m, 2H, CH_2, H-1), 2.98−3.06 (m, 2H, CH_2, H-4), 3.38−3.48 (m, 2H, CH_2, H-4′), 3.54−3.62 (m, 2H, CH_2, H-1′), 3.70 (s, 2H, CH_2, H-7′), 6.22 (s, 1H, CH, H-3″), 6.70 (s, 1H, CH, H-8″), 6.83 (d, 1H, CH, H-6″, J = 8.8 Hz), 7.30−7.42 (m, 2H, 2 × CH, H-7,5″), 7.49−7.55 (m, 1H, CH, H-6), 7.84 (d, 1H, CH, H-5, J = 8.4 Hz), 7.95 (d, 1H, CH, H-8, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ 22.5, 22.7 (C-2,3), 24.5 (C-1), 26.8, 28.8 (C-2′,3′), 32.4 (C-4), 37.9 (C-7′), 40.5 (C-4′), 48.7 (C-1′), 103.4 (C-8″), 111.5 (C-4a″), 111.8 (C-3″), 114.3 (C-6″), 115.7 (C-9a), 119.4 (C-8a), 123.2 (C-8), 124.0 (C-7), 126.6 (C-5″), 128.2 (C-5), 128.8 (C-6), 145.6 (C-10a), 149.4 (C-4″), 151.8 (C-9), 155.4 (C-8a″), 157.2 (C-4a), 160.5 (C-2″), 161.9 (C-7″), 171.8 (C-6′). Anal. Calcd for $C_{28}H_{29}N_3O4$ (471.56): C, 71.32; H, 6.20; N, 8.91. Found: C, 71.29; H, 6.18; N, 8.89.

***N*1-[2-(1,2,3,4-Tetrahydroacridin-9-yl)-2-[2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetyl]-1-hydrazinecarbothioamide (11).** 9-Isothiocyanato-1,2,3,4-tetrahydroacridine (**8**, 0.18 g, 0.75 mM) was added to a solution of (7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetic acid hydrazide (**10**, 0.125 g, 0.5 mM) in ethanol (5 mL). The mixture was stirred for 24 h at room temperature. The resulting precipitate was collected by filtration and dried in order to produce compound **11** at a yield of 84%. Mp 170−175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.68−1.94 (m, 4H, 2 × CH_2, H-2,3), 2.73−2.87 (m, 2H, CH_2, H-1), 2.96−3.09 (m, 2H, CH_2, H-4), 3.77 (s, 2H, CH_2, H-5′), 6.26 (s, 1H, CH, H-3″), 6.70 (s, 1H, CH, H-8″), 6.77 (d, 1H, CH, H-6″, J = 2.4, 8.8 Hz), 7.42−7.48 (m, 1H, CH, H-7), 7.58−7.70 (m, 2H, 2 × CH, H-6,5″), 7.76 (d, 1H, CH, H-8, J = 8.4 Hz), 7.90 (d, 1H, CH, H-5, J = 8.4 Hz), 9.33 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 21.9, 22.5 (C-2,3), 24.5 (C-1), 33.4 (C-4), 36.8 (C-5′), 102.3 (C-8″), 111.3 (C-4a″), 112.9 (C-3″), 113.2 (C-6″), 123.5 (C-8), 125.4 (C-8a), 125.6 (C-7), 126.7 (C-9a), 126.8 (C-5″), 128.7 (C-5), 129.2 (C-6), 141.3 (C-9), 146.6 (C-10a), 150.5 (C-4″), 155.1 (C-8a″), 158.5 (C-4a), 160.3 (C-2″), 161.3 (C-7″), 168.2 (C-4′), 181.5 (C-1′). Anal. Calcd for $C_{25}H_{22}N_4O_4S$ (474.54): C, 63.28; H, 4.67; N, 11.81. Found: C, 63.25; H, 4.64; N, 11.78.

***N*′1-[4-Oxo-3-(1,2,3,4-tetrahydroakridin-9-yl)-1,3-thiazolan-2-yliden]-2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)-ethanohydrazide (12).** Methyl bromoacetate (0.024 mL, 0.25 mM) and triethylamine (0.025 mL, 0.25 mM) were added to a solution of *N*1-[2-(1,2,3,4-tetrahydroacridin-9-yl)-2-[2-(7-hydroxy-2-oxo-2*H*-4-chromenyl)acetyl]-1-hydrazinecarbothioamide (**11**, 0.12 g, 0.25 mM) in $CHCl_3$ (2 mL). The mixture was stirred for 25 h at room temperature. The solvent was removed, and the crude solid product was purified using chromatography, eluent EtAc/MeOH/NH_3 (6:2:0.2), to give **12** at a yield of 70%. Mp 118−120 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80−1.92 (m, 4H, 2 × CH_2, H-2,3), 2.62−2.72 (m, 2H, CH_2, H-1), 3.06−3.17 (m, 2H, CH_2, H-4), 4.00 (s, 2H, CH_2, H-9′), 4.24 (s, 2H, CH_2, H-5′), 6.35 (s, 1H, CH, H-3″), 6.45 (dd, 1H, CH, H-6″, J = 2.4, 8.8 Hz), 6.70 (s, 1H, CH, H-8″), 7.50−7.57 (m, 1H, CH, H-7), 7.58−7.68 (m, 2H, 2 × CH, H-6,5″), 7.86 (d, 1H, CH, H-8, J = 8.8 Hz), 7.98 (d, 1H, CH, H-5, J = 8.0 Hz), 10.50 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 21.6, 22.5 (C-2,3), 23.8 (C-1), 31.1 (C-5′), 31.4 (C-4), 36.6 (C-9′), 102.2 (C-8″), 111.1 (C-4a″), 112.2 (C-3″), 112.7 (C-6″), 119.6 (C-9a), 123.3 (C-7), 123.6 (C-8), 124.2 (C-8a), 126.4(C-5), 126.9 (C-6,5″), 131.1 (C-9), 143.0 (C-10a), 150.2 (C-4″), 154.9 (C-8a″), 158.4 (C-4a), 160.2 (C-2″), 161.3 (C-7″), 167.6 (C-2′), 168.4 (C-8′), 171.9 (C-4′). Anal. Calcd for $C_{27}H_{22}N_4O_5S$ (514.56): C, 63.02; H, 4.31; N, 10.89. Found: C, 62.90; H, 4.29; N, 10.86.

Biochemical Studies. Cholinesterase Inhibitory Activities. The AChE and BuChE inhibitory activity of the tested drugs was determined using Ellman's method[52] and is expressed as IC_{50}, meaning the concentration at which cholinesterase activity is reduced by 50%. Human recombinant AChE (*h*AChE; EC 3.1.1.7), human plasmatic BuChE (*h*BuChE; EC 3.1.1.8), 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB), phosphate buffer (PB, pH 7.4), acetylthiocholine (ATC), butylthiocholine (BTC), and tacrine hydrochloride were purchased from Sigma-Aldrich, Praque, Czech Republic. Polystyrene cuvettes (Brand GmbH + Co. KG, Denmark) were used for measuring purposes. All experiments were carried out in 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 7.4. Enzyme solutions were prepared at 2.0 units/mL in 2 mL aliquots. The assay medium (1 mL) consisted of 650 μL of 0.1 M phosphate buffer (pH 7.4), 200 μL of 0.01 M DTNB, 25 μL of enzyme, and 100 μL of 0.01 M substrate (ATC chloride solution). Assay solutions with inhibitor (10^{-3}−10^{-10} M) were preincubated for 5 min. The reaction was initiated by an immediate addition of 100 μL of substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals using a spectrophotometer Helios-Zeta (Thermospectronic, Cambridge, U.K.). Each experiment was carried out in triplicate. The study using in vitro BuChE was carried out using a method similar to that described above. The corresponding selectivity indices of the compounds were also calculated (ratio = [IC_{50}(*h*BuChE)]/[IC_{50}(*h*AChE)]) according to a method optimized to allow the constants K_{i1} and K_{i2} to be determined. Software Origin 6.1 (Northamption, MA, USA) was used for statistical data evaluation.

Docking Simulation. Molecular models of derivatives **6b** and **7c** were created using the building options included in the Marvin 5.1.4 2008 software pack, ChemAxon [http://www.chemaxon.com]. The same software was used to determine the overall protonization of the compounds. Docking simulations were carried out using AUTODOCK, version 4.2. MGL TOOLS 1.4.5 (revision 30) was used to prepare the input files.[54,55]

Molecules of water with other nonenzymatic molecules were removed, and missing hydrogens were added. A united atom representation for ligands and enzymes was used, and Gasteiger partial atomic charges for proteins and ligands were added. For the initial docking simulation, the energy grid was set to the coordinates x = 116.4, y = 104.3, z = −130.6 for *h*AChE (PDB code 1B41) and x = 138.7, y = 116.3, z = 41.0 for *h*BuChE (PDB code 1P01), with an active site with the dimensions of 80 points × 80 points × 80 points and a spacing of 0.375 Å. Flexible ligand docking was performed for the compounds. Autotors was used to define the rotatable bonds in the ligands. Docking runs were performed using the Lamarckian genetic algorithm. Docking began with a population of random ligand conformations in random orientations and at random translations. The results of each docking experiment were derived from 100 different runs which were set to terminate after a maximum of 5 000 000 energy evaluations or 27 000 generations. The population size was set to 500, and the other parameters were used as default. Thus, the ligand pose with the lowest energy was chosen as the space for the construction of the rerun energy grid with the coordinates x = 116.40, y = 108.3, z = −132.9 and with dimensions of 46 points × 46 points × 46 points for *h*AChE (PDB code 1B41). For *h*BuChE (PDB code 1P01), the coordinates were set as x = 136.0, y = 114.3, z = 38.7 and with dimensions of 46 points × 46 points × 46 points. Spacing was set at 0.375 Å for both enzymes. The subsequent redocking run used the

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same parameters as those described above.[56] DOCK Amber rescoring was performed using DOCK, version 6.5.[57] A generalized Born/surface area (GB/SA) continuum model was used for solvation. In order to reduce the computational cost with no penalty to accuracy, the atoms 3 Å distant from the site of the ligand binding were kept frozen with 100 minimization and 3000 MD steps. Other input parameters were set as defaults in the program. The enzymes were modeled in their physiologically active forms with neutral His and deprotonated Glu, which, together with Ser, form the catalytic triad. The charge distribution of the inhibitors was determined using a MOPAC semiempirical modul at the AM1-bcc level, a parm99.dat parameter set for protein atoms, and GAFF atom types for the ligand.[58,59]

■ ASSOCIATED CONTENT

Supporting Information

Structures applied in docking runs; top-score docking poses of derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AD, Alzheimer's disease; NFT, neurofibrillary tangles; AChE, acetylcholinesterase; ACh, acetylcholine; AChEI, acetylcholinesterase inhibitor; NMDAR, *N*-methyl-D-aspartate receptor; PAS, peripheral anionic site; CAS, catalytic anionic site; Aβ, β-amyloid peptide; BuChE, butyrylcholinesterase; *h*AChE, human acetylcholinesterase; *h*BuChE, human butyrylcholinesterase; CPC, chloropropionyl chloride; IC_{50}, 50% inhibitory concentration; SI, selectivity index

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