Propidium-Based Polyamine Ligands as Potent Inhibitors of Acetylcholinesterase and Acetylcholinesterase-Induced Amyloid-β Aggregation

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Abstract: Heterodimers **4** and **5** were effective inhibitors of acetylcholinesterase (AChE) activity and AChE-induced amyloid-β (Aβ) aggregation. The peculiar biological profile of **4** can be relevant in studying the molecular basis underlying the nonclassical action of AChE and in addressing the question whether AChE inhibitors can affect the neurotoxic cascade leading to Alzheimer's disease. Compound **4** emerged as the most potent heterodimer so far available to inhibit AChE-induced Aβ aggregation.

Alzheimer's disease (AD) is characterized by selective neuronal loss in cholinergic population and by massive deposits of aggregated proteins to form, as peculiar lesions, the intracellular neurofibrillary tangles and the extracellular senile plaques. In the last two decades, basic research has tried to bridge the gap between these pathological hallmarks and rational pharmacological treatments, through the formulation of the so-called "cholinergic",[1] "tau",[2] and "amyloid"[3] hypotheses. Notwithstanding, these hypotheses are not lone and not mutually exclusive, and the cholinergic pharmacotherapy has been the subject of criticism; until 2002, the only marketed drugs for AD were the acetylcholinesterase inhibitors (AChEIs) tacrine (**1**), donepezil, rivastigmine, and galantamine.

Recently, following mounting evidence that acetylcholinesterase (AChE) may be involved in functions distinct from the catalytic activity and defined as "nonclassical", a renewed interest in the search of AChEIs has occurred.[4] For example, AChE colocalizes with amyloid-β peptide (Aβ) in neuritic plaques where an abnormal expression has been detected.[5] There is also evidence that AChE can enhance the rate of formation of Aβ fibrils, forming with them stable complexes.[6] It has been speculated that the site responsible for this aggregation-promoting action is localized close to the entrance of the gorge, perhaps overlapping the peripheral anionic site (PAS). In fact, inhibition of the active site did not influence this action, whereas occupancy of the PAS by the specific inhibitor propidium (**2**) blocked it.[7] Crystallographic[8] and computational[9] studies of the AChE/**2** complex identified the amino acid residues that form contacts with allosteric inhibitors, helping to elucidate the nature of interactions that participate in nucleation of amyloid fibrils and in other noncatalytic functions.

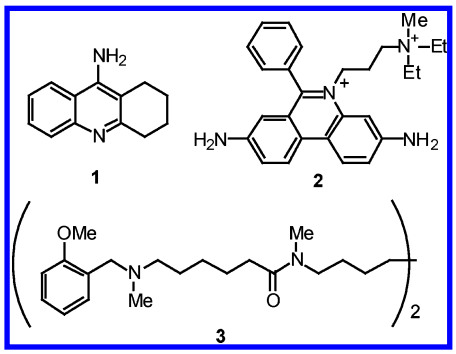

Figure 1. Chemical structures of AChEIs referred to in this study.

Considering the nonclassical AChE functions, their relationships to AD hallmarks, and the putative role of PAS in all these functions, dual-site inhibitors may acquire paramount importance for AD treatment.[10] Moreover, bivalency is a successful approach in medicinal chemistry for improving drug potency and selectivity.[11] Following this rationale, different bivalent ligands were obtained by linking through a spacer two moieties able to contact both acylation and peripheral sites of AChE. The pharmacophoric units resemble the features of well-known inhibitors, such as **1**,[12−14] galantamine,[15] linked by alkylene tethers, whose prominent role was the reduction of the entropy loss, although in some other instances the spacers provide additional sites of interaction.[16−18]

The aim of our study was to design a new series of dual binding site inhibitors based on a polyamine backbone, exploiting the universal template approach, a design strategy proposed by our group[19] and successfully applied in the development of the AChEI caproctamine (**3**).[19] We planned to synthesize new bivalent ligands in which the connecting unit does not act only as spacer, but plays a role in the target recognition process, being able to establish its own interactions with the enzyme. This idea was supported by the consideration that the AChE gorge is lined with several conserved aromatic residues,[20] capable, in principle, to form cation−π interaction with the basic polyamine counterpart.[16] As proof of principle, we chose a triamine backbone to link the structural motif of **2**, as a scaffold for contacting the peripheral site, to the tetrahydroaminoacridine system of **1**, or to the *N*-methyl-2-methoxybenzylamino group of **3** for fishing the active site, affording novel heterobivalent polyamine ligands **4** and **5**. In particular, the choice of the tether for heterodimers **4** and **5** was based on the observation that tacrine dimers bear in their structure, like **4**, secondary amino functions,[21] whereas the polyamine backbone of caproctamine contains, like that of **5**, tertiary amino groups.[19] However, a detailed account for the effect on the biological activity of different tethers, linking the two terminal aromatic moieties of heterodimers related to **4** and **5**, will be reported in due course.

Heterodimers **4** and **5** were prepared by alkylation of **7** and **12**, respectively, with bromide **8**,[22] followed by

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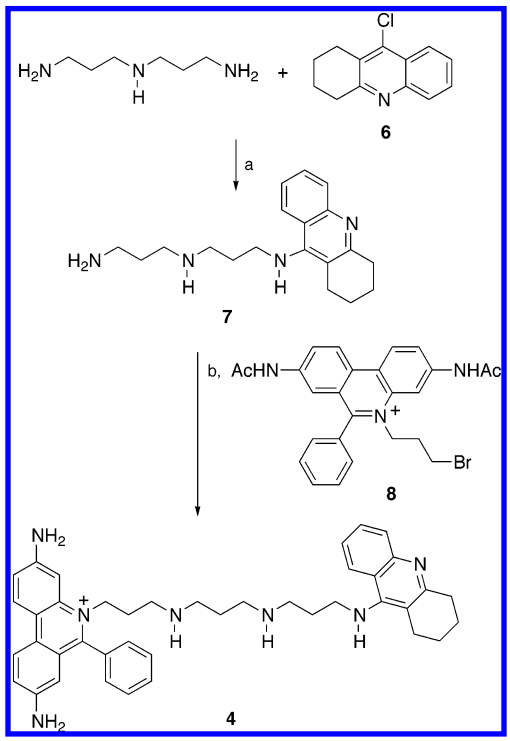
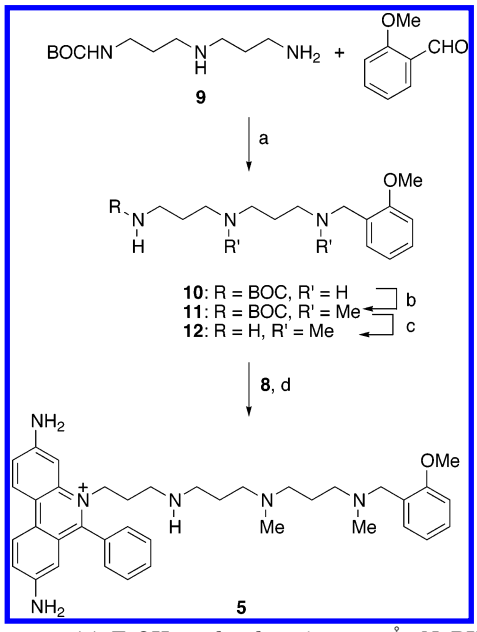
Scheme 1[a]



[a] Reagents: (a) 1-pentanol, KI, reflux; (b) MeOH, reflux, then MeOH, 12 N HCl, reflux.

Scheme 2[a]



[a] Reagents: (a) EtOH, molecular sieves 3 Å, $NaBH_4$, room temperature; (b) HCHO, CH_3COOH, $NaBH_3CN$, EtOH, room temperature; (c) CF_3COOH, room temperature; (d) MeOH, reflux, then MeOH, 12 N HCl, reflux.

acidic hydrolysis of protecting groups. Intermediate **7** was obtained from **6**[23] and N^1-(3-aminopropyl)propane-1,3-diamine with negligible amount of polyalkylation products, due to the low reactivity of **6** (Scheme 1), whereas **12** was synthesized from **9**, which, upon reductive amination with 2-methoxybenzaldehyde to yield **10**, was sequentially methylated (**11**) and deprotected at the terminal amino group (Scheme 2).

The biological profile of **4** and **5** against human recombinant AChE (catalytic and Aβ proaggregating

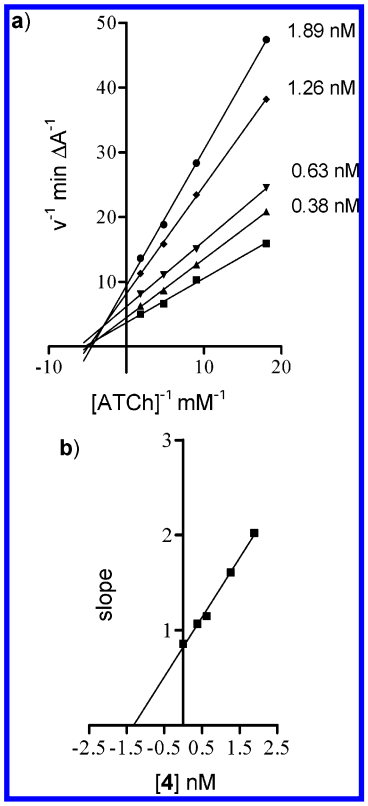


Figure 2. Steady-state inhibition by **4** of AChE hydrolysis of acetylthiocholine (ATCh) (a). Reciprocal plots of initial velocity and substrate concentration (20−550 μM) are reported. Lines were derived from a weighted least-squares analysis of data points. Estimation of K_i value for **4**, from slope replot vs inhibitor concentration (b).

Table 1. In Vitro Biological Data of Compounds **1**−**5**

no.	K_i [a] (nM) AChE	IC_{50} (nM) AChE	IC_{50} (nM) BChE	IC_{50} (μM) Aβ aggregation
1	151 ± 16[b]	424 ± 21	45.8 ± 3.0	na[c]
2	7140 ± 620	32300 ± 2200	13200 ± 400	12.6 ± 2.0
3	104 ± 16[b]	170 ± 2.0	11600 ± 300	na[c]
4	1.49 ± 0.52	1.55 ± 0.16	63.7 ± 2.8	13.8 ± 1.8
5	894 ± 81	952 ± 34	2190 ± 60	17.7 ± 1.6

[a] Estimates of the competitive inhibition constants (K_i) were obtained from replots of the slopes of the graphs of Lineweaver−Burk vs inhibitor concentration. [b] Data from ref 20. [c] na, not active; no significant inhibition up to 100 μM concentration.

actions) and butyrylcholinesterase (BChE) was assessed in comparison with reference compounds **1**−**3**. The inhibitory potency against AChE and isolated serum BChE was evaluated using the method of Ellman.[24] The graphical analysis of steady-state AChE inhibition data for **4** is shown in Figure 2, whereas estimates of competitive inhibition constants K_i and IC_{50} values are reported in Table 1. The ability of **4** and **5** to inhibit the proaggregating action of AChE toward Aβ was assessed through a thioflavin T-based fluorometric assay reported earlier.[7,25]

As postulated and as seen in other series, heterodimerization resulted in a remarkable increase in AChE potency; compound **4** was nearly 20 000-fold more potent than **2** and 300-fold more potent than **1**, consistent with the simultaneous binding to both active and peripheral sites. The same trend was not shown by compound **5**, which displayed a 34-fold increase in potency compared to **2**, whereas it was 5-fold less potent than **3**. In agreement with the IC_{50}, the K_i values showed that **4**

was the most potent among the investigated compounds. In this respect, we should note that in using the same triamine spacer, the distance between the two anchoring points, the phenantridinium nitrogen atom and, in the two cases, the endocyclic nitrogen atom of **1** and the benzylamine of **3**, is not the same. Probably, in the case of **4** the tether length is approaching or is close to the optimum distance, and, consequently, the ligand can easily achieve the dual binding, whereas the optimum tether length for a propidium/*N*-methyl-2-methoxybenzylamine heterodimer may not have been realized in **5**. Alternatively, the substantial difference in the inhibitory AChE activity of **4** and **5** might simply reflect that the tetrahydroacrydine moiety of **4** is a more potent catalytic site ligand than the *N*-methyl-2-methoxybenzylamine function of **5**. Work to define the proper polyamine spacer between the two sites is in progress.

An analysis of the Lineweaver−Burk reciprocal plots of **4** and **5** revealed that there are both an increasing slope and an increasing intercept with higher inhibitor concentrations. The inhibitory behavior of **4** and **5**, as illustrated in Figure 2 for **4**, is strictly similar to that displayed by some reported bis-tetrahydroaminoacridine inhibitors of AChE.[24] Therefore, from the kinetic profile, we derived that compound **4** and **5** cause a mixed type of inhibition.

Furthermore, the most striking result of the present investigation was that both our inhibitors markedly prevented the proaggregating effect of AChE toward Aβ in the fluorometric assay. In particular, **4** and **5** were found to inhibit AChE-induced Aβ aggregation with IC_{50} values comparable to those shown by **2**, a specific PAS ligand and the most effective in this action so far available. On the contrary, **1** and **3** did not show any inhibitory activity (Table 1), which parallels their limited ability to interact with the peripheral site of the enzyme. Moreover, a 100 μM concentration of **4** and **5** inhibits AChE-induced Aβ aggregation by 95% and 79%, respectively, a percentage which is severalfold higher than that of all the other inhibitors ever tested[25] and even significantly higher than that of AP2238, an inhibitor purposely designed to bind both sites of the enzyme (35% inhibition).[26]

The relevant biological profile displayed by **4** and **5** further supports the design strategy carried out and validates the idea that dual inhibitors can modulate AChE catalytic and noncatalytic function. To our knowledge, **4** can be considered a unique AChE inhibitor, combining a potent Aβ antiaggregating action with an elevated AChE inhibitory potency. This feature can be relevant in studying the molecular basis underlying the nonclassical actions of AChE and in addressing the question whether AChEI can affect the neurotoxic cascade leading to AD and, consequently, disease progression.

In conclusion, we have discovered a new dual-binding AChE inhibitor, which, due to an outstanding profile against AChE-induced amyloid aggregation and a potent inhibitory activity, might represent a valuable lead for developing effective drugs to cure AD.

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Supporting Information Available: Full experimental procedures and characterization data for reported compounds. This material is available free of charge via Internet at http://pubs.acs.org.

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