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# Multi-target-directed coumarin derivatives: hAChE and BACE1 inhibitors as potential anti-Alzheimer compounds

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**Abstract**—The complex etiology of Alzheimer's disease (AD) prompts scientists to develop multifunctional compounds to combat causes and symptoms of such neurodegeneration. To this aim we designed, synthesized, and tested a series of compounds by introducing halophenylalkylamidic functions on the scaffold of AP2238, which is a dual binding site acetylcholinesterase inhibitor. The inhibitory activity was successfully extended to the beta-site amyloid precursor protein cleavage enzyme, leading to the discovery of a potent inhibitor of this enzyme (**3**) and affording multifunctional compounds (**2**, **6**, **8**) for the treatment of AD.


Alzheimer's disease (AD), a progressive, degenerative disorder of the brain, is believed to be the most common cause of dementia among the elderly. AD is associated with a loss of the presynaptic markers of the cholinergic system in the brain areas related to memory and learning,[1] and is characterized by the presence of amyloid deposits and neurofibrillary tangles in the brain of afflicted individuals. The disease appears to have a heterogeneous etiology, but the deposition of beta-amyloid (Aβ) peptides (amyloid plaques) in the brain is hypothesized to be the root cause of neuronal cell death in AD patients and to play a key role in the progression of the disease.[2,3]

A very attractive approach to lowering Aβ is to inhibit β-secretase, generally referred to as BACE1 (beta-site amyloid precursor protein [APP] cleavage enzyme), a transmembrane aspartyl protease responsible for N-terminal cleavage of the APP leading to the production of the Aβ peptide.[4] Thus, BACE1 has become a target[5] of significant interest for industrial and academic investigation and there is important research underway in the development of new inhibitors for this enzyme.[6,7]

On therapeutic fronts, BACE1 inhibitors need to be less peptidic or nonpeptidic in character to achieve good oral bioavailability and, more important, CNS penetration.

Up to now most of the drugs approved for AD treatment are AChE inhibitors, which are able to enhance cholinergic neurotransmission by increasing acetylcholine (ACh) availability in the synaptic cleft. Considering the mechanism of action of these drugs, they are not expected to interfere with the neurodegenerative cascade of the disease, but only to temporarily mitigate some of the symptoms. However, some studies have shown that they can be effective over a longer period.[8] Moreover, AChE plays an important role in Aβ deposition: besides its catalytic function, AChE also acts as a promoter of Aβ fibril formation, this effect being independent from its normal hydrolyzing activity and associated with the peripheral anionic site (PAS) of the enzyme.[9]

Due to the complexity of AD and the involvement of different enzymes in its progression, the modulation of a single protein might not be sufficient to produce the desired efficacy. In the light of this, researchers are now turning to the design of compounds able to simultaneously hit different targets.[10,11] In a previous work[12] we designed and synthesized a dual binding site AChE inhibitor, AP2238 (Fig. 1), which is able to simultaneously contact both the central and the peripheral anionic sites and which showed an $IC_{50}$ of 44.5 nM against hAChE activity. From a further SAR study[13] a

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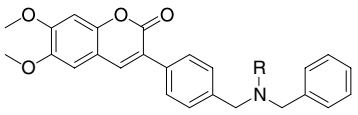



**Figure 1.**

new compound emerged, **1** (AP2243, Fig. 1), with an ethyl instead of a methyl group on the basic nitrogen, showing an improved $IC_{50}$ (18.3 nM).

In this letter, we report new amidic nonpeptidic derivatives in which the structure of **1** (for AChE activity) was maintained, whereas the methoxy groups of the coumarin moiety were alternately substituted by an amidic chain to extend the activity to BACE1. Therefore, we introduced halophenylalkylamidic functions in positions 6 or 7 of the coumarin moiety, choosing a dihalophenyl acid because this moiety emerged as a leitmotif in different BACE1 inhibitors reported in the literature.[14,15] A new series of potential multi-target compounds (**2**–**9**) for AD was synthesized, whose structures are reported in Table 1.

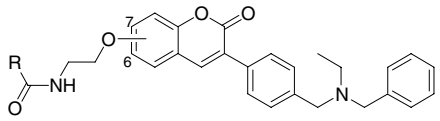
According to Scheme 1, compounds **2**–**5** were synthesized starting from 3-{4-[(benzylethylamino) methyl]-phenyl}-6-methoxychromen-2-one, and compounds **6**–**9** from 3-{4-[(benzylethylamino)methyl]phenyl}-7-methoxychromen-2-one, which were treated with 48% HBr to afford the hydroxy derivatives **10** and **11**, respectively. The subsequent substitution with 2-Boc-aminoethyl bromide gave the intermediates **12** and **13**, respectively. Removal of the protecting group (Boc) by means of $CF_3COOH$ released the primary amino functions (**14** and **15**). The final amidic derivatives (**2**–**9**) were formed by reaction with DCC activated (3,5-difluorophenyl)acetic acid, 3,5-difluorocinnamic acid, 3,4-difluorohydrocinnamic acid or 3,4-dichlorocinnamic acid, via a parallel synthesis procedure: in two series of four distinct reactors, (3,5-difluorophenyl)acetic acid, 3,5-difluorocinnamic acid, 3,4-difluorohydrocinnamic acid, and 3,4-dichlorocinnamic acid (1.3 equiv) were dissolved in $CH_2Cl_2$ under $N_2$ atmosphere. Then, DCC (1.4 equiv) was added to each reactor. Amino derivatives **14** (in the first series) or **15** (in the second series) (1.0 equiv) were added at 0 °C, and each mixture was stirred at room temperature for 2 h under $N_2$. The DCU was filtered off from each solution. Purification of each crude product by flash chromatography yielded the corresponding amides **2**–**9** (Tabel 1).

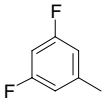
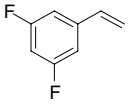
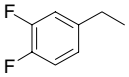
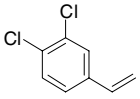
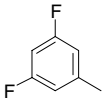
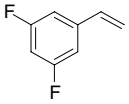
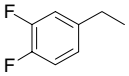
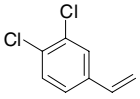
The inhibitory activities of the newly synthesized compounds were studied against hAChE, using the method of Ellman[16] to determine the rate of hydrolysis of acetylthiocholine, and against BACE1, using a spectrofluorometric method.[17]

The results reported in Table 1 for all tested compounds showed a decrease in activity toward hAChE with respect to the reference compound **1**. Compounds **2**, **6**, and **8**, 10-fold less potent than **1**, still maintained a fairly good activity, comparable to the monomethoxycoumarins reported in a previous paper.[13] The considerable decrease in activity shown for compound **5** ($IC_{50}$ = 267 μM), with a *trans*-3,4-dichlorocinnamic substituent, could indicate that molecules carrying bulky groups are not allowed to penetrate into the hAChE gorge to establish a proper interaction, because the substituent in that position might detrimentally interact with hAChE PAS residues. Regarding the activity toward BACE1, the data reported in Table 1 show that all compounds were potent inhibitors, but activity seemed not to be influenced either by the position of the substituent on the coumarin nucleus, or by the different halogen on the phenyl ring. This portion of the molecule was crucial for efficient BACE1 inhibition, as confirmed by the lower potency of the reference compound **1**, which lacked this group and showed inhibition of 42.33% at 210 nM. Compound **3** was the most active of the series, with an $IC_{50}$ of 99 nM.

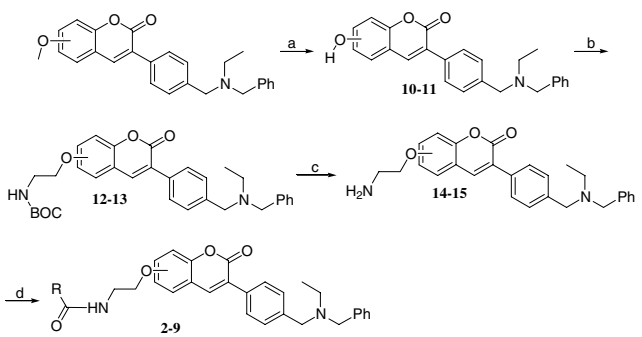
While the binding mode of these derivatives at hAChE gorge resembles that of the parent compound,[12] docking outcomes concerning an inhibitor-BACE1 complex are here reported. Docking simulations were carried out using GOLD[18] and Dock[19] suites and the outcomes grouped together and clusterized with AClAP[20,21] according to the so-called 'holistic approach'.[21] Docking calculations revealed that among all the investigated compounds (see Supplementary data) a similar trend in the binding mode was observed. In the light of this, the binding mode of the most potent BACE1 inhibitor, **3**, is here discussed. The flexibility of the substituent in position 6 on the coumarin moiety led to heterogeneous docking poses. Nevertheless, the availability of the clustering method was very helpful during the analysis of docking solutions. Eight hundred docking poses for compound **3** were obtained as described in Supplementary data. The total number of clusters obtained was 74 but, according to the Chauvenet criterion implemented in AClAP,[20,21] only two clusters were significantly populated. The ascertainment of the ligand–protein complexes, supported also by analysis of the docking solution for the other molecules of the series, led us to consider the binding mode proposed in Figure 1 as plausible. Here, the representative pose of the most populated cluster is shown after the Dock6.1 Amber rescoring.[18] It can be seen that the protonated nitrogen of the ligand is able to reach the acid environment formed by the catalytic diad Asp32 and Asp228; its position inside the active site resembles that of protonated nitrogens of classical hydroxyethylamine inhibitors. The *N*-benzylethyl moiety seems particularly suitable for simultaneously fitting the S1 and S1′ pockets. In Figure 2, the *N*-benzyl group interacts with Tyr198 and Ile226, whereas the *N*-ethyl portion lies on the aromatic ring of Tyr71. In the present docking study, this interaction pattern has also been observed in an inverted fashion having the *N*-benzyl substituent T-shape interacting with Tyr71 and Phe118 and the *N*-ethyl moiety lying on the S1′ pocket. The coumarin core with its 3-phenyl substituent is embedded into the S2 pocket of the enzyme, with the carbonylic group being able to H-bond the side chain

**Table 1.** Structures of studied compounds and their hAChE and BACE1 inhibitory activities, expressed as $IC_{50}$



| Compound | Position | R | $IC_{50} \pm SEM$[a] (μM) | |
|---|---|---|---|---|
| | | | hAChE | BACE1 |
| **1** | — | — | 0.018 ± 0.003 | ~0.238[b] |
| **2** | 6 |  | 0.551 ± 0.049 | 0.149 ± 0.002 |
| **3** | 6 |  | 7.16 ± 0.87 | 0.099 ± 0.005 |
| **4** | 6 |  | 2.77 ± 0.25 | 0.116 ± 0.001 |
| **5** | 6 |  | 267 ± 103 | 0.133 ± 0.004 |
| **6** | 7 |  | 0.181 ± 0.010 | 0.150 ± 0.007 |
| **7** | 7 |  | 4.57 ± 0.39 | 0.121 ± 0.002 |
| **8** | 7 |  | 0.327 ± 0.034 | 0.114 ± 0.007 |
| **9** | 7 |  | 4.23 ± 0.49 | 0.151 ± 0.001 |

[a] Human recombinant AChE and BACE1 were used. $IC_{50}$ values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are means of two independent measurements, each performed in triplicate (SEM, standard error of the mean).
[b] 42.33% inhibition at 201.66 nM.




**Scheme 1.** [a]Synthesis of the studied compounds. [a]Reagents: (a) HBr; (b) 2-(Boc-amino)ethyl bromide; (c) $CF_3COOH$; (d) DCC, (3,5-difluorophenyl)acetic acid, 3,5-difluorocinnamic acid, 3,4-difluorodihydrocinnamic acid or 3,4-dichlorocinnamic acid.

of Arg235. The presence of a substituent in positions 6 or 7 on the coumarin nucleus seems to equally contribute to the relative binding affinity of this series of molecules. This might be due to both the flexibility of the bridging aminoethoxy segment and to the relative structural permittivity of the enzyme portion. The orientation of the 6-substituent might be stabilized by the interaction of the amidic nitrogen with the backbone of the 1OS loop, Gly11. Moreover, the 3,5-difluorophenyl portion might be harbored into a peripheral cationic spot formed by residues Lys9, Arg307, Lys321, Pro160, and Val309.

In conclusion, the introduction of an halophenylalkylamidic function on the scaffold of **1** allowed us to obtain potent BACE1 inhibitors. In particular, compound **3** was the best of the series, showing an $IC_{50}$ of 99 nM. Due to

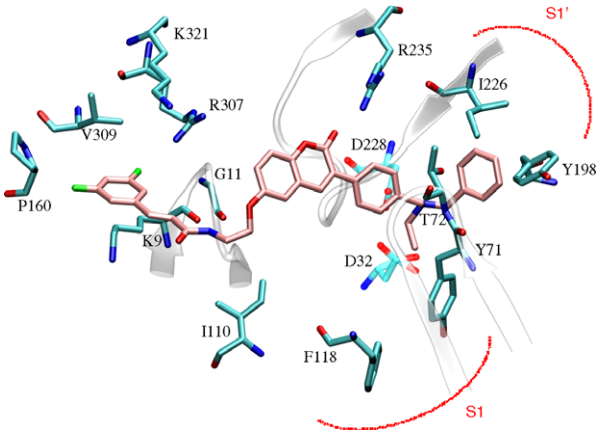



**Figure 2.** Proposed binding mode for compound **3** (carbon atoms in rose) docked at the active site (cyan) of BACE1. S1 and S1′ enzyme subsites are sketched by dotted red lines. Amino acids are indicated as single-letter code.

the multifactorial nature of AD, molecules that modulate the activity of a single protein target are unable to significantly modify the progression of the disease. Following this new paradigm, here we have reported the first dual AChE/BACE1 inhibitors. Promising hits proved to be compounds **2**, **6**, and **8**, which turned out to inhibit both enzymes with sub-micromolar affinity, as required for a good multi-target-directed drug candidate.

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## Supplementary data

Full experimental procedures for the synthesis, computational studies, and biological evaluation of the studied compounds. Supplementary data associated with this article can be found, in the online version, at doi:-doi:10.1016/j.bmcl.2007.09.100.

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