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Novel peptidomimetics as BACE-1 inhibitors: Synthesis, molecular modeling, and biological studies

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ABSTRACT

Aiming at identifying new scaffolds for BACE-1 inhibition devoid of the pharmacokinetic drawbacks of peptide-like structures, we investigated a series of novel peptidomimetics based on a 1,4-benzodiazepine (BDZ) core **1a–h** and their seco-analogues **2a–d**. We herein discuss synthesis, molecular modeling and in vitro studies which, starting from **1a**, led to the seco-analogues (*R*)-**2c** and (*S*)-**2d** endowed with BACE-1 inhibition properties in the micromolar range both on the isolated enzyme and in cellular studies. These data can encourage to pursue these analogues as hits for the development of a new series of BACE-1 inhibitors active on whole-cells.

Alzheimer's disease (AD), one of the most prevalent neurodegenerative disorders among the elderly, is pathologically characterized by the extracellular accumulation of amyloid-β (Aβ) plaques and by intracellular neurofibrillary tangles. Considerable evidence indicates a central role of the Aβ peptide and its aggregation in the pathogenesis of AD.[1] High levels of soluble Aβ peptides correlate to cognitive decline in AD[2,3] and the original amyloid cascade hypothesis has evolved to propose that soluble oligomeric Aβ assemblies precede deposition and are the proximal cause of synaptic dysfunction and early impairment in AD.[4] Aβ40 and Aβ42[5] are the two major isoforms of Aβ found in AD brains. Although the Aβ40 is the most abundant isoform, Aβ42 is enriched in AD brains as it progressively accumulates into extracellular senile plaques.[4] The proteolytic enzyme β-secretase (BACE-1) catalyzes the rate-limiting step of the Aβ generation by cleaving the Met671–Asp672 peptide bond of amyloid precursor protein (APP) at the extracellular space.[6,7] Currently available therapies for AD only treat disease symptoms and do not address the underlying disease processes.[8] On the basis of a number of seminal in vitro and in vivo studies the aspartic protease BACE-1 has been recognized as a relevant drug target for the development of AD disease-modifying therapies.[9] BACE-1 potent inhibitors have been produced by academic and industrial research.[10] However very few of them fulfill the requirements for in vivo biological and clinical studies and only recently, orally available highly efficient BACE-1 inhibitors became available.[11] To identify new scaffolds for BACE-1 inhibition to overcome the well-known pharmacokinetic drawbacks of peptide-like structures, we decided to search for novel peptidomimetic compounds based on the C_8/C_9-substituted 1,4-benzodiazepine (BDZ) structural system or on the seco-1,4-BDZ scaffold, represented by the core structures **1** and **2** depicted in Figure 1.

The BDZ system has not been explored to date for developing BACE-1 inhibitors, and the BDZ system is one of the most important privileged pharmacogenic structures for drug discovery endowed with a high degree of druggability. This prompted us to develop compounds **1a–h**. Furthermore, we recently discovered a versatile synthetic protocol for the synthesis of C_8 and C_9 modified

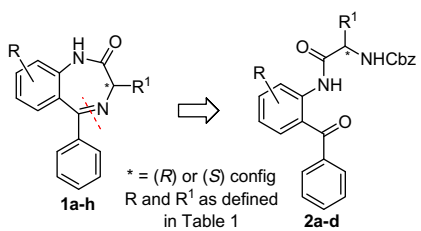

Figure 1. Title compounds: benzodiazepines **1a–h** and seco-analogues **2a–d**.

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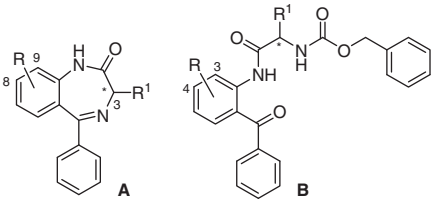
1,4-BDZs which gave us the opportunity to investigate BDZs bearing at these positions protonatable and non-protonatable functions.[12] Thus, we started our research program with the synthesis and evaluation of a series of BDZs and their seco-analogues (**1** and **2**, Fig. 1). In particular the compounds were functionalized with a piperazine group at C_9 and/or with the hydroxyethylamine (HEA) moiety introduced at various positions of the BDZ scaffold of **1** and of the seco-BDZs **2** (see Table 1).

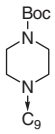
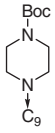
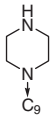
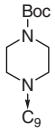
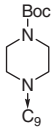
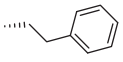
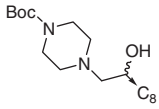
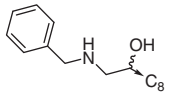
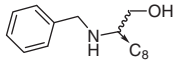
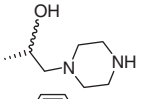
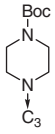
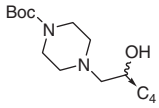
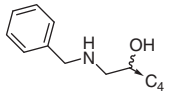
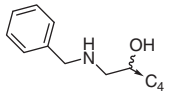
Compounds **1a**,**b** were prepared as described in Scheme 1 following a synthetic procedure previously described for the preparation of analogues **1c**,**d**.[12] The L- or D-Cbz-protected phenylglycines were activated by means of hexachloroacetone (HCA) and triphenylphosphine, and then treated with the piperazino-substituted aminobenzophenone **3**[12] to give compound **2a**. After deprotection and cyclization (*R*)- and (*S*)-**1a** were obtained. Treatment of (*S*)-**1a** with HCl gave the amine (*S*)-**1b**.

The synthesis of the BDZ scaffold necessary for the preparation of the C_8-hydroxyethylamino-BDZs was performed starting from the benzophenone **4**[12] (Scheme 2) which was coupled with Cbz or Fmoc protected phenylglycine and successively exposed to tributylvinyl tin[13] to give **5a**,**b**. These latter compounds were oxidized in the presence of *m*-chloroperbenzoic acid (*m*-CPBA). The epoxide ring **6a** (obtained as a mixture of two diasteroeisomers) was subjected to a nucleophilic ring opening reaction by exposure to *N*-Boc-piperazine. The resulting hydroxyethylamino-derivative **2b** was obtained in good yield. When **6a** was treated with benzylamine in the presence of lithium perchlorate[14] both regioisomers were obtained (**2c** and **2d**). Deprotection of *N*-Cbz **2b** followed by cyclization furnished compound **1e**. Starting from **2c**,**d,** deprotection of the *N*-Cbz under different reaction conditions was unsuccessful, so the corresponding *N*-Fmoc protected derivatives **2e**,**f** were prepared. Deprotection of the Fmoc-group in the presence of diethylamine was achieved and the intermediates were cyclized to **1f**,**g**. Scheme 3 reports the synthesis of **1h**. Starting from the commercially available aminobenzophenone **7**, coupling with L-Cbz-Met followed by a *m*-CPBA oxidation provided the sulfoxide (*S*)**-8**. The sulfoxide was then submitted to a thermal elimination reaction by refluxing in xylene ((*S*)**-9**). The olefin **9** was oxidized to the corresponding epoxide **10** (mixture of two diastereoisomers) which was reacted with *N*-Boc-piperazine. After *N*-Cbz and *N*-Boc deprotection and cyclization, **1h** was obtained.

The BDZs **1a**–**h** and the seco-analogues **2a**–**d**, bearing or not the HEA substructure, were tested against the isolated BACE-1 enzyme in fluorescence-based assays (Table 1). Analytical data for the tested compounds are given as Supplementary Information (SI). HEA fragment is a peptide cleavage transition state mimetic, which found multiple applications in targeting aspartyl proteases.[15] Consequently, to improve solubility, the piperazine moiety, present in **1a**–**d** (**1a**, Log*S* = −4.77, **1b**, Log*S* = −1.83, calculated at pH 7, ACD/Labs V12.0, Toronto, Canada), was combined to the HEA fragment (**1e**,**h**) (**1e**, Log*S* = −4.37, **1h**, Log*S* = 0.44). To evaluate the effect of the piperazine ring we synthesized analogues **1f**,**g** exclusively bearing the HEA moiety. In the BDZs series only **1a**, **1g** and **1h** showed inhibition of BACE-1 in the micromolar range (Table 1). Starting from **1a** we also investigated the stereoselectivity of interaction and we observed that (*R*)-**1a** and (*S*)-**1a** showed a comparable potency. Removal of the carbamoyl moiety from **1a** dramatically reduced potency (**1b**). Moreover, tethering the phenyl ring at C_3 of **1a** by one or two methylenes led to inactive analogues (**1c** and **1d**). The BDZ core structure was also decorated at C_8 or C_3 with HEA functionalities bearing or not the piperazine system (**1e**–**h**). Compounds **1e**,**f** present a substitution at the HEA-carbon atom bearing the OH, while the HEA fragment of **1g**,**h** was substituted on the carbon atom close to the nitrogen. As regard to **1b** and **1f**, when only the piperazine (**1b**) or the HEA group (**1f**) is present, neither

Table 1
BACE-1 inhibition activity of compounds **1a**–**h** and **2a**–**d** as IC_{50} (μM)[a]



Compd	A/B	R	R^1	IC_{50}[b] (μM)
(*R*)-**1a**	A			43
(*S*)-**1a**	A			35
(*S*)-**1b**	A			NA[c]
(*S*)-**1c**	A			NA[c]
(*S*)-**1d**	A			NA[c]
(*R*)-**1e**	A			NA[c]
(*R*)-**1f**	A			NA[c]
(*R*)-**1g**	A			10.9
(*S*)-**1h**	A	—		12.5
(*S*)-**2a**	B			NA[c]
(*R*)-**2b**	B			4.9
(*S*)-**2c**	B			2.4
(*R*)-**2c**	B			2.6