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Synthesis and biological activity of *N*-substituted spiro[benzoxazepine-piperidine] Aβ-peptide production inhibitors

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Abstract
Synthesis and biological evaluation of an spiro[benzoxazepine-piperidine] class of Aβ-peptide production inhibitors for treatment of Alzheimer's disease are described.

Keywords: *Alzheimer's disease, Aβ-peptide, spiro[benzoxazepine-piperidine], γ-secretase, inhibition*

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory loss and cognitive decline. The amyloid peptide (Aβ) is the major component of extracellular seniles plaques found in Alzheimer's brain patients; this soluble Aβ has been found to be neurotoxic in vitro [1]. Cleavages of amyloid peptide precursor protein (APP) by both β- and γ-secretases release Aβ peptide fragments. These cleavage events are thought to play a key role in the neurodegenerative pathways responsible for AD pathology. Inhibition of proteases responsible for the unfavourable cleavage of APP has been reported to be an attractive point of intervention which could alter pathophysiology of the disease rather than act as palliative treatment.

From a literature search, it was observed that N-[N-(3,5-difluorophenylacetyl-L-alanyl)]-S-phenylglycine *tert*-butyl ester known as DAPT [2,3] (see Figure 1) and some related analogues in which the motifs 3,5-difluorophenylacetyl-L-alanyl or 3,5-difluoromandelyl-L-alanyl are coupled to bulky heterocyclic moieties [4–7] are among the most potent inhibitors of Aβ peptide production through γ-secretase inhibition. For example LY-411,575 (see Figure 1) which is a potent Aβ peptide inhibitor in vitro and in vivo, includes in its structure the 3,5-difluoromandelyl-L-alanyl motif linked to a benzodiazepine moiety [8]. From a synthetic point of view, this promising series of derivatives are rather complex molecules since they present three chiral centers, and appeared to present several potential liabilities that may limit their use for the treatment of AD.

Materials and methods

Herein we report the synthesis and the cellular inhibitory activity of new 4,5-dihydro-3H-spiro[1,5-benzoxazepine-2,4′-piperidine] substituted at the position R^2 by a simple 3-hydroxy-3-phenyl propyl moiety on the production of Aβ-peptide (general structure shown on Figure 2).

The bulky heterocyclic moiety 4,5-dihydro-3H-spiro[1,5-benzoxazepine-2,4′-piperidine] was selected because:

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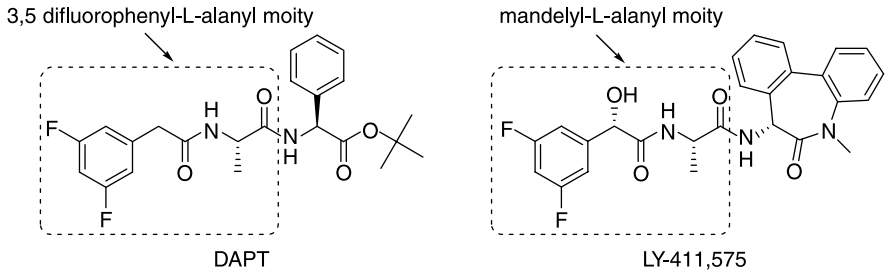


Figure 1. Structure of DAPT and LY-411, 575.

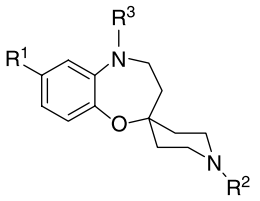


Figure 2. General structure of spiro[benzoxazepine-piperidine].

– it has been observed that bioactive drugs incorporating in their structure a piperidinyl ring linked to an other cycle through a spiranyl carbon, can adopt specific conformations in which the nitrogen electron doublet can have different orientations [9,10].
– this scaffold presents different positions on which substituents (R^1, R^2 and R^3) could be introduced.

Introduction of lipoic acid substituent (compound **3d**) was selected because it has been recently reported [11] that lipoic acid, an universal antioxidant [12,13], could also protect neurons against cytotoxicity induced by Aβ-peptide and stabilize cognitive functions in patients with AD [14].

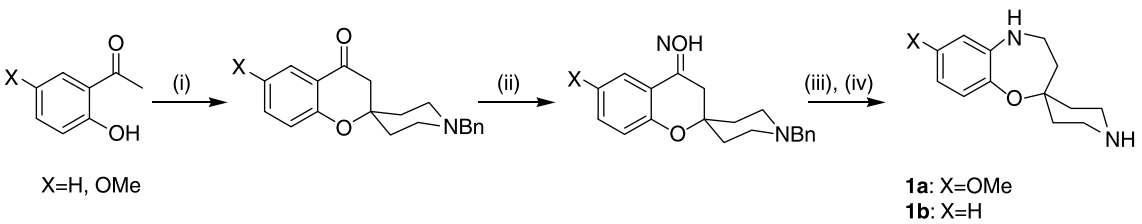
The spiro[benzoxazepine-piperidine] scaffold (see Scheme 1) has been synthesized according to reported procedure [15].

The keys intermediates **1a**, **1b** were subsequently functionalized with various halide derivatives or carboxylic acids (Scheme 2). Compound **1a** was *N*-alkylated [16] with different alkyl halides such as (S)-(−)-3-chloro-1-phenyl-1-propanol, or (R)-(+)-3-chloro-1-phenyl-1-propanol and *N*-(5-bromopentyl)phthalimide. This reaction performed in the presence of potassium carbonate in acetonitrile as solvent gives respectively compounds **2a**, **2b**, **and 2c**. After removal of the phthalimide protecting group of **2c** by hydrazine monohydrate in ethanol [17], compound **2d** was obtained. Acylation of the secondary amine **1a** or **1b** with acids: *N*-(3,5-Difluorophenylacetyl)-L-alanine, *N*-(3,5-Difluoro-mandelyl)-L-alanine [18], DAPT derivative [5] and (+/−)-α-Lipoic acid [19], in the presence of BOP and diisopropylethylamine afford the desired analogues **3a**, **3b**, **3c** and **3d**.

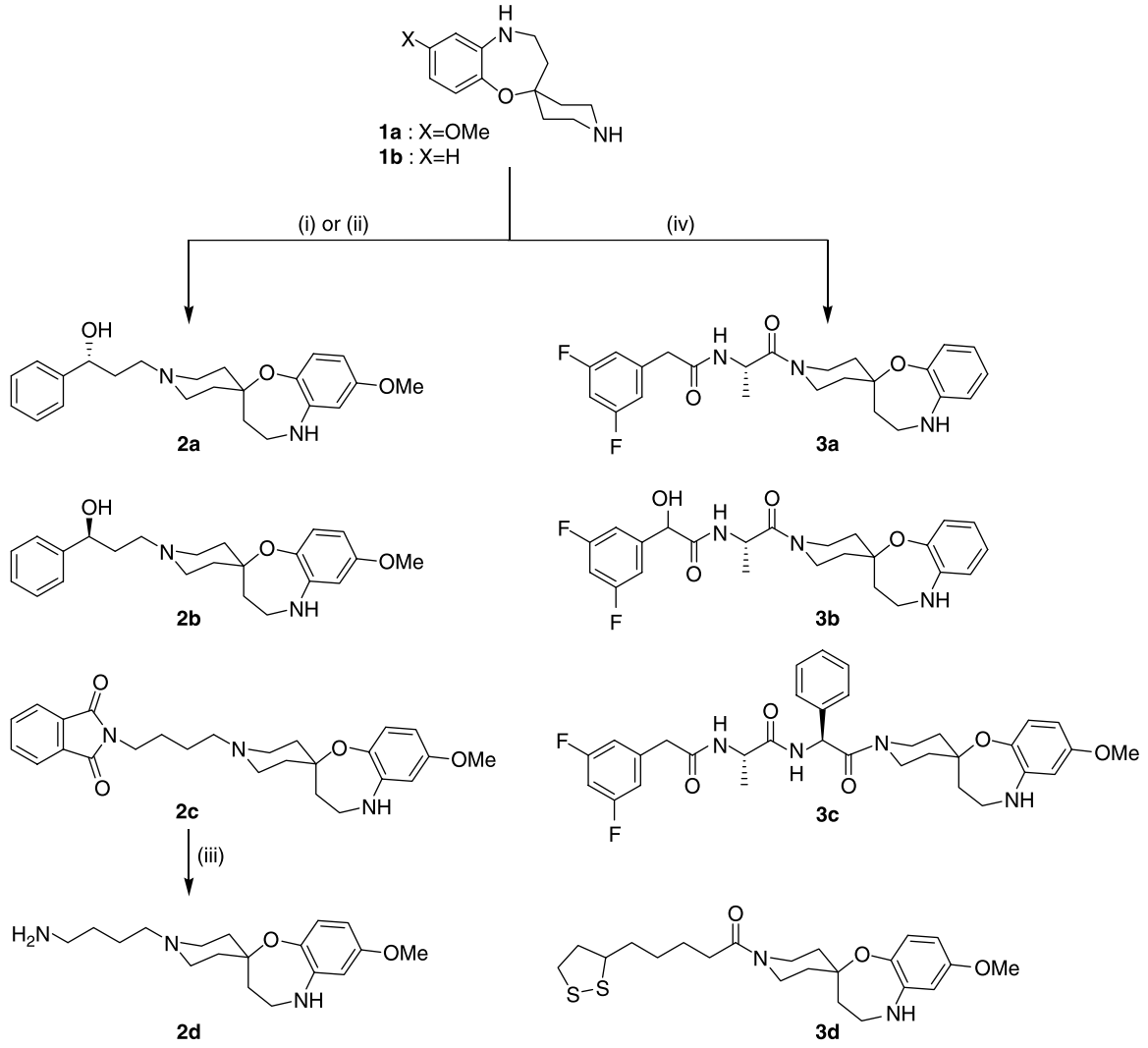
Synthesis

*General procedure for compound **2a**–**2c***. To a stirred solution of **1a** (0.16 g, 0.65 mmol) in *i*-PrOH (5 mL) was added K_2CO_3 (0.18 g, 1.29 mmol). A solution of the (R)-(−)-3-chloro-1-phenyl-1- propanol (0.11 g, 0.65 mmol) in acetonitrile (3 mL) was added slowly, and the reaction mixture was stirred at room temperature for 18 h. After concentration under reduced pressure, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5) to give the desired compound **2a** as a white solid.

2a. (Yield) 13%, R_f 0.86 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.61–1.79 (m 2H), 1.90–2.08 (m, 8H), 2.11–2.88 (m, 6H), 3.31 (t, J = 5.4 Hz, 2H), 3.72 (s, 3H), 4.98 (m, J = 4.6 Hz, 1H), 6.17 (d, J = 2.8 Hz, 1H), 6.23 (dd, J = 2.9, 8.7 Hz, 1H), 6.78 (d, J = 8.7 Hz, 1H), 7.21–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz)



Scheme 1. Reagents and conditions: (i) *N*-benzyl piperidone, pyrrolidine, MeOH, reflux; (ii) HCl.H_2N-OH, pyridine, EtOH, reflux; (iii) DIBAH, CH_2Cl_2, 0°C; (iv) H_2, $Pd(OH)_2$ (10%), MeOH, rt, 80%.



Scheme 2. Reagents and conditions: (i) $ClCH_2CH_2CH(Ph)OH$ (*S*) or (*R*), CH_3CN, K_2CO_3, 35°C, 24 h; (ii) $Br(CH_2)_4$-phthalimide, CH_3CN, K_2CO_3, reflux, 12 h; (iii) H_2NNH_2, 24 h; (iv) RCO_2H, BOP, DIEA, CH_2Cl_2, rt, 18 h.

δ: 33.6, 34.4, 34.5, 40.7, 48.7, 50.1, 55.3, 56.8, 74.7, 75.7, 77.2, 103.9, 104.2, 124.6, 125.5 (2C), 126.9, 128.2 (2C), 137.2, 143.1, 144.7, 156.3. ESI-MS *m/z* $[M + H]^+ = 383$. Anal. Calcd. for $C_{23}H_{30}N_2O_3$: C 72.22, H 7.91, N 7.32. Found: C 75.36, H 7.55, N 7.07%.

2b. (Yield) 13%, R_f 0.86 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$, 250 MHz) *δ*: 1.61–1.79 (m 2H), 1.90–2.08 (m, 8H), 2.11–2.88 (m, 6H), 3.31 (t, $\mathcal{J} = 5.4$ Hz, 2H), 3.72 (s, 3H), 4.98 (m, $\mathcal{J} = 4.6$ Hz, 1H), 6.17 (d, $\mathcal{J} = 2.8$ Hz, 1H), 6.23 (dd, $\mathcal{J} = 2.9$, 8.7 Hz, 1H), 6.78 (d, $\mathcal{J} = 8.7$ Hz, 1H), 7.21–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) *δ*: 33.6, 34.4, 34.5, 40.7, 48.7, 50.1, 55.3, 56.8, 74.7, 75.7, 77.2, 103.9, 104.2, 124.6, 125.5 (2C), 126.9, 128.2 (2C), 137.2, 143.1, 144.7, 156.3. ESI-MS *m/z* $[M + H]^+ = 383$. Anal. Calcd. for $C_{23}H_{30}N_2O_3$: C 72.22, H 7.91, N 7.32. Found: C 75.52, H 7.65, N 7.15%.

2c. (Yield) 12%, ^{1}H NMR ($CDCl_3$, 250 MHz) *δ*: 1.22–1.51 (m, 2H), 1.55–1.75 (m, 6H), 1.88 (t, $\mathcal{J} = 5.4$ Hz, 2H), 1.92–2.03 (m, 2H), 2.36–2.49 (m, 4H), 2.63–2.71 (m, 2H), 3.28 (t, $\mathcal{J} = 5.4$ Hz, 2H), 3.65–3.68 (m, 2H), 3.71 (s, 3H), 6.16 (d, $\mathcal{J} = 3.0$ Hz, 1H), 6.24 (dd, $\mathcal{J} = 2.9$, 5.7 Hz, 1H), 6.82 (d, $\mathcal{J} = 8.7$ Hz, 1H), 7.68–7.72 (m, 2H), 7.81–7.87 (m, 2H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) *δ*: 24.9, 26.4, 28.5, 32.4, 34.7, 38.0, 40.8 (2C), 41.8, 49.4 (2C), 55.3, 58.5, 76.1, 103.9, 104.1, 123.2 (2C), 124.8, 132.1, 133.8 (2C), 137.6, 143.2, 156.1, 168.4 (2C).ESI-MS *m/z* $[M + H]^+ = 464$. Anal. Calcd. for $C_{27}H_{33}N_3O_4$: C 72.22, H 7.91, N 7.32. Found: C 75.52, H 7.65, N 7.15%.

2d. To a stirred solution of **2c** (50 mg, 0.10 mmol) in EtOH (5 mL) was added anhydrous hydrazine (0.15 mmol). The reaction mixture was stirred at reflux for 2 h. and then allowed to cool to room temperature. After concentration under reduced pressure, the residue was purified by column

chromatography (silica gel, CH_2Cl_2/MeOH, 95:5) to give quantitatively the desired compound **2d** (33 mg) as a yellow oil. R_f 0.67 (CH_2Cl_2/MeOH, 95:5). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.22–1.51 (m, 2H), 1.55–1.75 (m, 6H), 1.88 (t, $\mathcal{J}$ = 5.4 Hz, 2H), 1.92–2.03 (m, 2H), 2.33–2.47 (m, 4H), 2.61–2.75 (m, 4H), 3.28 (t, $\mathcal{J}$ = 5.4 Hz, 2H), 3.71 (s, 3H), 6.16 (d, $\mathcal{J}$ = 3.0 Hz, 1H), 6.24 (dd, $\mathcal{J}$ = 2.9, 5.7 Hz, 1H), 6.84 (d, $\mathcal{J}$ = 8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 24.9, 26.9, 26.7, 30.3, 32.8, 40.8 (2C), 41.7, 49.5 (2C), 55.3, 58.9, 76.1, 103.9, 104.1, 124.8, 137.6, 143.2, 156.1. ESI-MS *m/z* $[M + H]^+ = 334$. Anal. Calcd. for $C_{19}H_{31}N_3O_2$: C 68.43, H 9.37, N 12.60. Found: C 68.85, H 9.34, N 12.27%.

*General procedure for compound **3a**–**3d***. The 3,5-difluorophenylacetyl alanine acid (0.11 g, 0.46 mmol) was dissolved in freshly distilled CH_2Cl_2 (5 mL) in the presence of BOP reagent (0.24 g, 0.55 mmol). The reaction mixture was cooled to 0°C and then DIEA (80 μL, 0.46 mmol) was added dropwise. The reaction mixture was stirred for 1 h at room temperature and then cooled once again to 0°C. A CH_2Cl_2 solution of the benzoxazepine piperidine derivative **1a** (0.30 g, 0.46 mmol) and DIEA (240 μL, 1.38 mmol) was added dropwise. The solution was allowed to warm and stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (20 mL). The organic layer was washed successively by using H_2O (10 mL), brine (10 mL), 5% aqueous $NaHCO_3$ (2 × 10 mL), and brine (10 mL), was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH, 95:5) to give the title compound **3a** (76 mg, yield: 37%) as a white solid. R_f 0.48 (CH_2Cl_2/MeOH, 95: 5). ^{1}H NMR ($CDCl_3$,250 MHz) δ: 1.32 (d, $\mathcal{J}$ = 6.6 Hz, 3H), 1.38–1.46 (m, 2H), 1.88–1.92 (m, 2H), 2.05–2.16 (m, 2H), 3.05–3.19 (m, 1H), 3.28–3.33 (m, 2H), 3.51 (s, 2H), 3.59–3.64 (m, 2H), 4.25–4.42 (brs, 1H), 4.85–4.90 (m, 1H), 6.63–6.92 (m, 7H), 8.05 (brs, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ:18.5, 34.4, 35.6, 38.4, 40.7, 41.3, 41.6, 43.0, 45.4, 73.3, 102.7 (t, $\mathcal{J}$ = 25.5 Hz, 1C), 112.0, 112.4, 118.7, 121.2 (d, $\mathcal{J}$ = 24.8 Hz, 2C), 124.4, 138.4 (t, $\mathcal{J}$ = 9.7 Hz, 1C), 142.2, 143.4, 163.2 (dd, $\mathcal{J}$ = 246.8 Hz, 2C), 168.5, 170.2. ESI-MS *m/z* $[M + H]^+ = 444$. Anal. Calcd. for $C_{24}H_{27}F_2N_3O_3$: C 65.00, H 6.14, N 9.47. Found: C 65.37, H 6.22, N 9.13%.

3b. (Yield) 38%,. R_f0.47 (CH_2Cl_2/MeOH, 95:5). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.25–1.60 (m, 5H), 1.88–1.93 (m, 2H), 2.04–2.12 (m, 2H), 3.07–3.19 (m, 2H), 3.25–3.35 (m, 2H), 3.56–3.81 (m, 3H), 4.31–4.42 (m, 1H), 4.83–4.89 (m, 1H), 5.04 (brs, 1H), 6.64–7.41 (m, 7H), 7.95 (brs, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 18.5, 29.7, 34.4, 35.6, 38.4, 40.7, 41.6, 45.2, 72.9, 76.32, 103.5 (t, $\mathcal{J}$ = 25.5 Hz, 1C), 109.4, 109.9, 118.8, 121.3 (d, $\mathcal{J}$ = 24.8 Hz, 2C), 124.4, 138.3 (t, $\mathcal{J}$ = 9.7 Hz, 1C), 142.2, 143.4, 163.2 (dd, $\mathcal{J}$ = 246.8 Hz, 2C), 170.3, 170.8. ESI-MS *m/z* $[M + H]^+ = 460$. Anal. Calcd. for $C_{24}H_{27}F_2N_3O_4$: C 62.73, H 5.92, N 9.15. Found: C 62.45, H 6.08, N 9.12%.

3c. (Yield) 45%, R_f 0.71 (EtOAc). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.30 (d, $\mathcal{J}$ = 6.8 Hz, 3H), 1.58–1.70 (m, 2H), 1.80–1.97 (m, 4H), 2.48–2.71 (m, 4H), 3.21 (t, $\mathcal{J}$ = 5.4 Hz, 2H), 3.61–3.69 (m, 5H), 4.40–4.49 (m, 1H), 5.75 (s, 1H), 6.13–6.42 (m, 3H), 6.74–6.78 (m, 1H), 6.89–7.08 (m, 2H), 7.29–7.50 (m, 5H). ESI-MS *m/z* $[M + H]^+ = 607$. Anal. Calcd. for $C_{33}H_{36}F_2N_4O_5$: C 65.33, H 5.98, N 9.24. Found: C 65.17, H 5.63, N 9.51%.

3d. (Yield) 52%, R_f 0.51 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.39–1.50 (m, 4H), 1.57–1.73 (m, 6H), 1.84–2.05 (m, 3H), 2.29–2.49 (m, 5H), 2.97–3.18 (m, 2H), 3.26–3.31 (m, 2H), 3.48–3.58 (m, 3H), 3.69 (s, 3H), 6.16 (d, $\mathcal{J}$ = 2.8 Hz, 1H), 6.22 (dd, $\mathcal{J}$ = 2.9, 5.7 Hz, 1H), 6.78 (d, $\mathcal{J}$ = 8.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ: 25.1, 29.2, 33.0, 34.3, 34.7, 35.7, 37.7, 38.5, 40.3, 40.6, 41.5, 41.7, 55.4, 56.5, 76.4, 104.0, 104.3, 124.5, 137.3, 143.0, 156.4, 171.2. ESI-MS *m/z* $[M + H]^+ = 437$. Anal. Calcd. for $C_{22}H_{32}N_2O_3S_2$: C 60.52, H 7.39, N 6.42. Found: C 60.37, H 7.45, N 6.01%.

Results and discussion

The new synthesized compounds were first assayed for their inhibitory properties on Aβ peptide production using a conventional cellular model [20]. Inhibitory potencies for each compound was determined using: HEK 293 cells over expressing APP, according to known literature procedure [20–22]. Among the tested compounds, **2a** and **2b** were the most potent to inhibit Aβ peptide production with IC_{50} values ranging between 0.2 and 0.6μM (Table I).

Since Aβ-peptide production can result from cleavages of the amyloid peptide precursor protein (APP) by both β- and γ-secretases, compounds were also assayed as β-secretase inhibitors, using a fluorescence resonance energy transfer (FRET) assay [18]. In this enzymatic assay, no one of the compounds found active on Aβ peptide production, were found β-secretase inhibitors at least up to 100μM. These results suggest that the observed inhibition of Aβ-peptide production, by analogues **2a** and **2b** did not result from β-secretase inhibition but more likely from γ-secretase inhibition.

These results, suggest that substitution on the nitrogen atom of the piperidine ring greatly influences Aβ-peptide inhibitory activity of the resulting derivatives. Inhibitors **2a** and **2b**, substituted on the

Table I. Inhibitory activities of spiro[benzoxazepine-piperidine] derivatives.

Compounds	Aβ40 IC_{50} (μM)[a, b]
2a	0.2 ± 0.3
2b	0.5 ± 0.1
2c	Inactive (100 μM)
2d	Inactive (100 μM)
3a	Inactive (100 μM)
3b	4.7 ± 0.2
3c	Inactive (100 μM)
3d	6 ± 0.7

[a] Potency determination for the ability of compounds to reduce Ab40 production from HEK293 cells; [b] using a cell viability standard assay [23], the whole compounds did not elicit any cell cytotoxicity at least below 1 mM concentrations.

nitrogen atom of the piperidinyl ring, by a chiral moiety 3-hydroxy-3-phenyl-propyl group appeared equipotent to their corresponding **3a** and **3b** derivatives which are substituted by a difluoro phenylacetyl-L-alanyl moiety. It should be also underlined that both enantiomers **2a** and **2b** are equipotent in our assay. In contrast analogues bearing at the N-4 position of the piperazine ring other substituents such as N-acyl-ω-aminobutyl **2d** or N-acyl-lipoyl **3d** were denied of any inhibitory activity.

Conclusions

Based on the molecular structure simplicity and relatively high potency of analogues **2a** and **2b**, it could be of interest to investigate the effects of this 3-hydroxy-3-phenyl-propyl substituent on other positions of the 4,5-dihydro-3H-spiro[1,5-benzoxazepine-2,4′-piperidine] scaffold. These preliminary results are of interest since these new analogues bearing a heterocyclic moiety linked to a simple side chain containing only one chiral center represent new cellular Aβ-peptide inhibitor prototype, which structure can be optimized. Additional in vitro screening assays such as cell free and NICD (Notch intracellular domain) assays are needed in order to evaluate their potential for treatment of AD [24].

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