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

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Abstract

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

Keywords: Alzheimer's disease, $A\beta$ -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 (A β) is the major component of extracellular seniles plaques found in Alzheimer's brain patients; this soluble A β has been found to be neurotoxic in vitro [1]. Cleavages of amyloid peptide precursor protein (APP) by both β and γ -secretases release A β 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 A β peptide production through γ -secretase inhibition. For example LY-411,575 (see Figure 1) which is a potent A β 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 A β -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 $(R^1, R^2 \text{ and } R^3)$ 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 A β -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-(5bromopentyl)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-Difluoromandelyl)-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 K₂CO₃ (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, CH₂Cl₂/MeOH, 95:5) to give the desired compound 2a as a white solid.

2a. (Yield) 13%, R_f 0.86 (CH₂Cl₂/MeOH 95:5). ¹H NMR (CDCl₃, 250 MHz) & 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). ¹³C NMR (CDCl₃, 62.9 MHz)



Scheme 1. Reagents and conditions: (i) *N*-benzyl piperidone, pyrrolidine, MeOH, reflux; (ii) HCl.H₂N-OH, pyridine, EtOH, reflux; (iii) DIBAH, CH₂Cl₂, 0°C; (iv) H₂, 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 , $35^{\circ}C$, 24h; (ii) $Br(CH_2)_4$ -phthalimide, CH_3CN , K_2CO_3 , reflux, 12h; (iii) H_2NNH_2 , 24h; (iv) RCO_2H , BOP, DIEA, CH_2Cl_2 , rt, 18h.

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 C₂₃H₃₀N₂O₃: C 72.22, H 7.91, N 7.32. Found: C 75.36, H 7.55, N 7.07%.

2b. (Yield) 13%, $R_f 0.86$ (CH₂Cl₂/MeOH 95:5). ¹H NMR (CDCl₃, 250 MHz) δ : 1.61–1.79 (m 2H), 1.90–2.08 (m, 8H), 2.11–2.88 (m, 6H), 3.31 (t, $\tilde{J} = 5.4$ Hz, 2H), 3.72 (s, 3H), 4.98 (m, $\tilde{J} = 4.6$ Hz, 1H), 6.17 (d, $\tilde{J} = 2.8$ Hz, 1H), 6.23 (dd, $\tilde{J} = 2.9$, 8.7 Hz, 1H), 6.78 (d, $\tilde{J} = 8.7$ Hz, 1H), 7.21–7.36 (m, 5H). ¹³C NMR (CDCl₃, 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 C₂₃H₃₀N₂O₃: C 72.22, H 7.91, N 7.32. Found: C 75.52, H 7.65, N 7.15%. **2c.** (Yield) 12%, ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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 C₂₇H₃₃N₃O₄: 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, CH₂Cl₂/MeOH, 95:5) to give quantitatively the desired compound **2d** (33 mg) as a yellow oil. R_f 0.67 (CH₂Cl₂/MeOH, 95:5). ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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 C₁₉H₃₁N₃O₂: 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 3,5difluorophenylacetyl alanine acid (0.11 g, 0.46 mmol) was dissolved in freshly distilled CH₂Cl₂ (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 $(80 \,\mu\text{L}, 0.46 \,\text{mmol})$ was added dropwise. The reaction mixture was stirred for 1 h at room temperature and then cooled once again to 0°C. A CH₂Cl₂ 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 H₂O (10 mL), brine (10 mL), 5% aqueous NaHCO₃ (2 × 10 mL), and brine (10 mL), was dried over anhydrous MgSO₄, 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 (CH₂Cl₂/MeOH, 95: 5). ¹H NMR (CDCl₃, 250 MHz) δ : 1.32 (d, $\mathcal{F} = 6.6$ 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). ¹³C NMR (CDCl₃, 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, $\tilde{\gamma} = 25.5$ Hz, 1C), 112.0, 112.4, 118.7, $121.2 (d, \mathcal{J} = 24.8 \text{ Hz}, 2\text{C}), 124.4, 138.4 (t, \mathcal{J} = 9.7 \text{ Hz},$ 1C), 142.2, 143.4, 163.2 (dd, $f = 246.8 \,\text{Hz}, 2C$), 168.5, 170.2. ESI-MS m/z [M + H]⁺ = 444. Anal. Calcd. for C₂₄H₂₇F₂N₃O₃: C 65.00, H 6.14, N 9.47. Found: C 65.37, H 6.22, N 9.13%.

3b. (Yield) 38%, $R_f 0.47$ (CH₂Cl₂/MeOH, 95:5). ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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, f = 25.5 Hz, 1C), 109.4, 109.9, 118.8, 121.3 (d, f = 24.8 Hz, 2C), 124.4, 138.3 (t, f = 9.7 Hz, 1C), 142.2, 143.4, 163.2 (dd, f = 246.8 Hz, 2C), 170.3, 170.8. ESI-MS *m*/*z* [M + H]⁺ = 460. Anal. Calcd. for C₂₄H₂₇F₂N₃O₄: 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). ¹H NMR (CDCl₃, 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 (CH₂Cl₂/MeOH 95:5). ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃, 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 C₂₂H₃₂N₂O₃S₂: 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 A β 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 A β peptide production with IC₅₀ values ranging between 0.2 and 0.6µM (Table I).

Since A β -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 A β peptide production, were found β -secretase inhibitors at least up to 100 μ M. These results suggest that the observed inhibition of A β -peptide production, by analogues 2**a** and 2**b** 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 $A\beta$ -peptide inhibitory activity of the resulting derivatives. Inhibitors **2a** and **2b**, substituted on the

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

Compounds	A β 40 IC ₅₀ (μ M) ^{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 diffuoro 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 3hydroxy-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 A β -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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