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Lipase-Catalysed Synthesis of New Acetylcholinesterase Inhibitors: *N*-Benzylpiperidine Aminoacid Derivatives

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Abstract—New acetylcholinesterase inhibitors were synthetized via a lipase-mediated regioselective amidation using *Candida antarctica* lipase B as a biocatalyst in the key step. The new compounds have two different structural fragments: a *N*-benzylpiperidine moiety to anchor the enzyme active site and a dicarboxylic aminoacid to act as a biological carrier. Some analogues of *N*-benzylpiperazine were also synthesised and studied but they did not display AChE inhibitor activity. A preliminary structure–activity relationship study was performed employing some computational techniques as similarity indices and electrostatic potential maps.

Introduction

The cholinergic hypothesis postulates the deficit of cholinergic functions in the brain as one of the main causes of memory impairments in Alzheimer's disease (AD) patients.[1] As a consequence, potentiation of central cholinergic action has become an effective approach for the palliative treatment of mild to moderate cases of AD.[2] One of the strategies to enhance cholinergic neurotransmission is the inhibition of acetylcholinesterase (AChE),[3] the enzyme responsible for the metabolic breakdown of acetylcholine (ACh). In fact, this mechanism is the one that allowed the development of drugs presently approved for the treatment of AD (i.e. tacrine, donepezil, Fig. 1).[4] However, there are many other different mechanisms acting on the neurodegenerative process. Many research efforts are currently focused on developing hybrid-compounds with dual mechanism of action that potentiate the cognitive effect and decrease side effects.[5]

Continuing with our work on AChE inhibitors,[6] here we report the synthesis, biological evaluation and structure–activity relationship of *N*-benzylpiperidine amino acid derivatives (Fig. 2; X = CH, R′ = H). For comparative purposes, some piperazine analogues (X = N, R′ = H, Cl, NO_2 and Ph) were also synthetized and studied. The new designed compounds have two different structural fragments, coupled via regioselective lipase-catalysed amidation: the *N*-benzylpiperidine moiety present in donepezil[7] to anchor in the AChE active site and a dicarboxylic amino acid which acts as a biological carrier. Two groups of compounds have been studied: the α-amides of *S*-glutamic[8] (structure **I**) and the ω-amides of *S*-α-aminoadipic ($n = 2$) and *R,S*-α-aminopimelic ($n = 3$) acid derivatives (structure **II**). Acetyl and carbobenzoxy (Cbz) were the *N*-protecting groups used. The dicarboxylic amino acid derivatives were selected because they have a free functional group in their structure, which could be used for subsequent transformations in hybrid compounds affording a dual biological activity, i.e. by linkage with an m_1 agonist.

Results and Discussion

Synthesis

Candida antarctica lipase B (CAL from here on) catalysed amidation of diesters of *N*-protected-*S*-glutamic acid regioselectively affords α-monoamides.[9] Amidation with 4-(2-aminoethyl)-*N*-benzylpiperidine (obtained by reduction of the corresponding nitrile)[10] as nucleophile yielded **1** and **2** (Scheme 1). Piperazine analogues **4**–**7** were obtained by using commercially available 1-(2-aminoethyl)piperazine as the nucleophile of a doubly regioselective, acyl donor and nucleophile, amidation followed by the *N*-alkylation of the α-monoamide **3**

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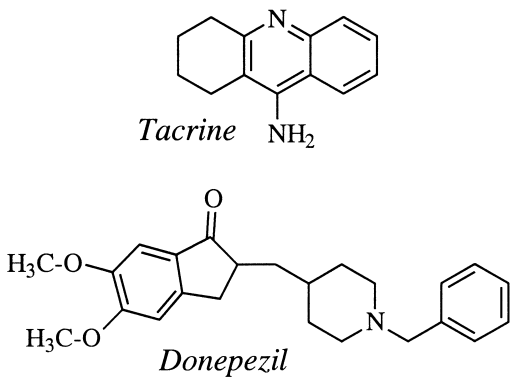


Figure 1.

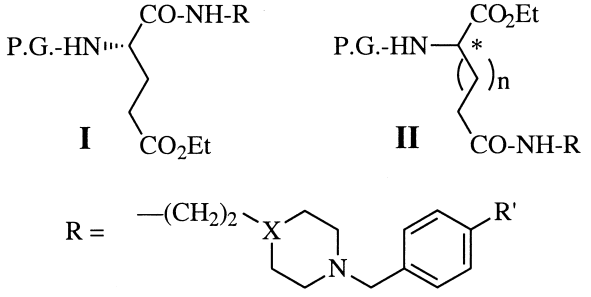


Figure 2.

with a mixture of the selected benzyl halide and triethylamine (TEA).

The enzymatic amidation takes regioselectively place on the ω-ester group when the side chain is longer than that of the glutamic acid.[11] Thus, when the reactions, showed in Scheme 1, were applied to *S*-α-aminoadipic and *R,S*-α-aminopimelic derivatives, the corresponding δ- and ϵ-monoamides were regioselectively obtained: *N*-benzylpiperidines **8**, **9**, **12** and **13** and, via *N*-benzylation of **10** and **14**, *N*-benzylpiperazines **11** and **15** (Fig. 3).

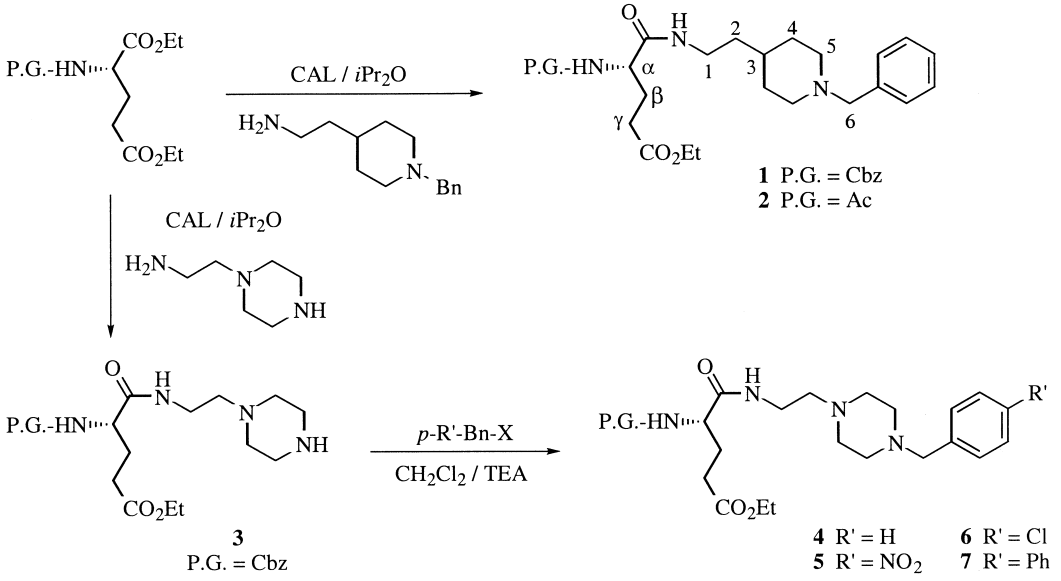
Structural elucidation

The structures of all new compounds were elucidated according to their analytical and spectroscopic data (^{1}H and ^{13}C NMR) which are collected in the experimental section and in Tables 1, 2 and 3.

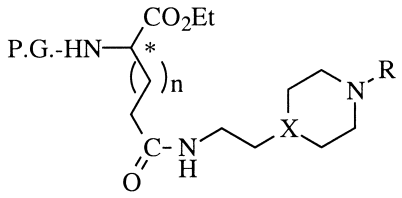
The monoamidation position was determined using the chemical shift displacement rule previously determined by our group.[12] It was observed that amide substitution at position α produced an upfield shift of the α-CH proton of about 0.2 ppm, while no appreciable change was observed for the γ-CH_2 resonances (Fig. 4). In contrast, the γ-monoamide derivatives show their γ-CH_2 protons more shielded than the corresponding diester ($\Delta\delta \approx 0.2$ ppm). Differences in the ^{13}C NMR spectra of the regioisomeric monoamide derivatives were also found, as are depicted in Figure 4.

Concerning the new synthetized glutamic derivatives (compounds **1**–**7**), higher chemical shift differences in the α-CH than in ω-CH_2 were observed (Table 1), pointing to a regioselective α-amidation. In addition, the monoamidation site was confirmed unequivocally for compounds **1** and **3** by bidimensional experiments such as HMQC for one bond correlation or HMBC sequences for long-distance couplings. Thus, α-methylene protons ($\delta = 4.21$ and 4.18 ppm respectively) correlated exclusively with the α-CO ($\delta = 171.1$ and 170.9 ppm), while ω-CO ($\delta = 173.4$ and 173.3 ppm) correlated with the methylenic protons of the ethyl group ($\delta = 4.12$ and 4.07 ppm). On the other hand, in aminoadipic and aminopimelic ester derivatives (compounds **8**–**15**), higher chemical shift differences, both in ^{1}H and ^{13}C, on the side chain methylene moiety were observed (Table 1) which evidence a regioselective monoamidation on the side chain.

Additionally, derivatives **3**, **10** and **14**, in which 4-(2-aminoethyl)-piperazine was used as nucleophile in the



Scheme 1.



S-α-aminoadipic acid derivatives (n = 2)

8: P.G. = Cbz, X = CH, R = Bn

9: P.G. = Ac, X = CH, R = Bn

10: P.G. = Cbz, X = N, R = H

11: P.G. = Cbz, X = N, R = Bn

R,S-α-aminopimelic acid derivatives (n = 3)

12: P.G. = Cbz, X = CH, R = Bn

13: P.G. = Ac, X = CH, R = Bn

14: P.G. = Cbz, X = N, R = H

15: P.G. = Cbz, X = N, R = Bn

Figure 3.

Table 1. Measured differences in the chemical shifts between lipase-catalyzed synthetised monoamide derivatives and the corresponding diester and inhibitory activity on human erythrocytes isolated AChE of the monoamide aminoacids derivatives **1**–**15**

	^{1}H NMR (δ, ppm)[a]		^{13}C NMR (δ, ppm)[a]		AChE inhb. IC_{50} [b]
Compound	α-CH	ω-CH_2	α-CH	ω-CH_2	(μM)
1	0.21	0.02	−0.8	−0.2	7.1 ± 0.4
2	0.15	−0.04	−1.3	−0.2	83 ± 5
3	0.18	−0.03	−1.1	−0.4	> 100
4	—	—	—	—	86 ± 2
5	—	—	—	—	> 100
6	—	—	—	—	> 100
7	—	—	—	—	> 100
8	−0.06	0.16	−0.5	−2.8	0.20 ± 0.02
9	−0.05	0.15	0.2	−2.1	0.52 ± 0.01
10	−0.02	0.14	−0.1	−2.1	> 100
11	—	—	—	—	2.5 ± 0.4
12	−0.04	0.14	0.0	−2.4	0.60 ± 0.02
13	0.01	0.13	0.0	−2.3	1.7 ± 0.3
14	0.01	0.15	0.0	−1.7	> 100
15	—	—	—	—	3.6 ± 0.02

[a] $\Delta\delta = \delta_{diester} - \delta_{amide}$.

[b] All values are expressed as mean ± standard error of the mean of at least four experiments. IC_{50}: 50% inhibitory concentration of acetylcholinesterase activity (μM). References values: tacrine, IC_{50} = 0.13 ± 0.02, donepezil,[10] IC_{50} = 0.04 ± 0.01.

enzymatic reaction, showed a broad triplet at δ≈6.5 ppm interchangeable with deuterium dioxide, which indicates a regioselective amidation on the primary versus secondary starting material amine group.

Biological results

The in vitro inhibition of AChE for the new synthetized aminoacid monoamide derivatives **1–15** was determined by the method of Ellman et al.[13] using tacrine as reference. The results are presented in Table 1. They show that some of the new compounds (nos. **8**, **9** and **12**) inhibit the enzyme with IC_{50} values comprised between 0.2 and 0.6 μM.

Some preliminary structure–activity relationships could be derived. Firstly, all the compounds with a *N*-benzyl moiety showed inhibition of AChE. However, substitution on its aromatic ring leads to completely inactive compounds (nos. **5**–**7**). The *N*-benzylpiperidine derivatives inhibit more efficiently the AChE than the *N*-benzylpiperazine ones (compounds **1**–**2**, **8**–**9**, **12**–**13** versus **4**, **11** and **15**). Lipophilicity in the *N*-blocking group of the aminoacid slightly increases the enzymatic inhibition since compounds with the benzyloxycarbonyl group were more active than the ones with the acetyl group (compounds **1**, **8**, **12** versus **2**, **9**, **13** respectively). One possible explanation for this result could be that the gorge leading to the active site of AChE is lined with aromatic residues,[14] therefore, hydrophobic interactions could stabilize compounds with a benzyloxycarbonyl moiety through the aromatic channel. Finally, concerning the aminoacid, the best results were obtained for the aminoadipic and aminopimelic derivatives which in all cases were more potent than the glutamic ones (compounds **8**–**15** versus **1**–**7**). This fact could be related to the monoamidation position more than to the length of the aminoacid side chain.

It is generally thought that non-covalent forces dominate the receptor drug interactions and these forces can be described in terms of steric and electrostatic effects. In an attempt to relate these effects with the biological activity found in this work, some molecular modeling studies have been additionally performed.

Similarity indices measures are one of the most recent techniques succesfully employed for this purpose.[15] Here, compounds **8**–**11** have been selected for this study. Molecular similarity indices were calculated using donepezil as reference. Due to the quiral centre present in donepezil both enantiomers were taken into account. The best correlation was found with (*S*)-isomer. Similarity indices for shape and electrostatic potential alone did not reveal any correspondence with biological activity, however, the combined index, based on an equal contribution of shape and charge, showed a good correlation with enzymatic inhibition finding similarity around 35% for inhibitors **8**, **9** and **11,** in contrast to the lower similarity found (24%) for the inactive derivative **10.** These results could explain the indispensable requirement of the *N*-benzyl moiety for AChE inhibition.

Moreover, the comparative study of the electrostatic potential surfaces of the most active compound, the γ-monoamide **8**, and (*S*)-donepezil showed a common pattern for the electrostatic distribution (Fig. 5), with the three previously established pharmacophoric areas of charge in common.[16] These data could explain the

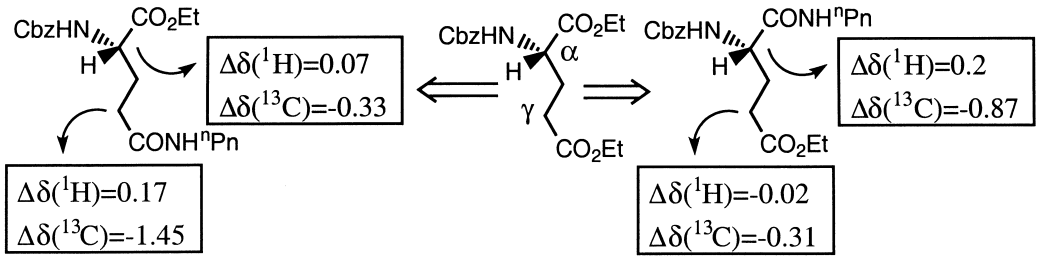


Figure 4.

good AChE inhibition found in the *N*-benzylpiperidine aminoacid ω-derivatives here synthetized.

The synthetic methodology here employed together with the theoretical models proposed will be used for further development of ω-monoamide aminoacid derivatives as AChE inhibitors. The free α-ethoxycarbonyl group will be used for subsequent transformation in hybrid compounds, i.e. by linkage with an m_1 agonist or a radical scavenger, searching a dual biological activity which could improve the therapeutic profile of anti-Alzheimer's drugs.

Experimental

Chemical procedures

Melting points were determined with a Reichert–Jung Thermovar apparatus and are uncorrected. Flash column chromatography was carried out at medium pressure using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–240 mesh ASTM) with the indicated solvent as eluent. ^{1}H NMR spectra were obtained on Varian XL-300 and Gemini-200 spectrometers working at 300 and 200 MHz respectively. Typical spectral parameters were: spectral width 10 ppm, pulse width 9 μs (57°), data size 32 K. ^{13}C NMR experiments were carried out on the Varian Gemini-200 spectrometer operating at 50 MHz. The acquisition parameters were: spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 μs (57°), data size 32 K. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si. H and C atoms are numbered as in Scheme 1. Analytical HPLC was performed on a Beckman chromatograph using a DeltaPak C_{18} column (3.9×150 mm, 5 μm), using different rates of acetonitrile (CH_3CN)-H_2O (with 0.5 mL/L of tifluoroacetic acid) as eluent and an UV detector. Elemental analyses were performed by the analytical department at C.N.Q.O. (CSIC) and the results obtained were within ±0.4% of the theoretical values. CAL was the Novo Nordisk's immobilized preparation Novozym 435 and was used as received. Diethyl *N*-protected dicarboxylic amino acids were obtained from the commercial free amino acids following standard procedures.[17]

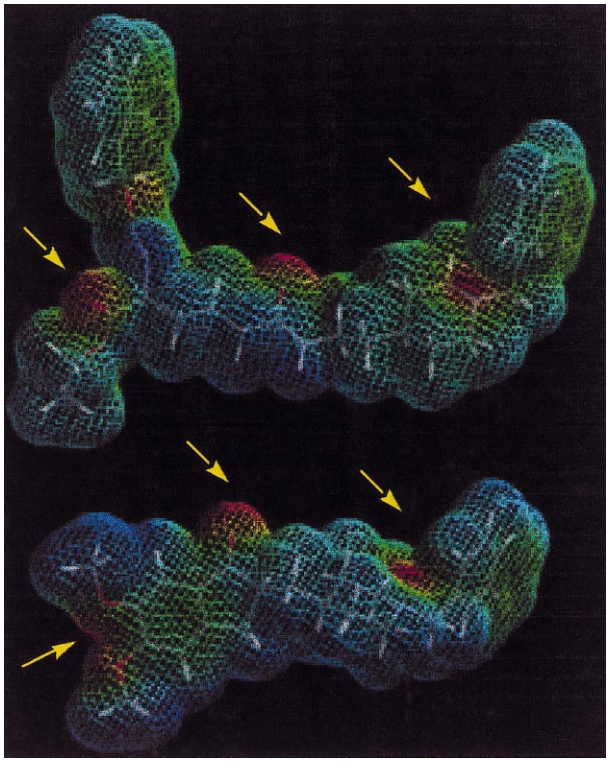


Figure 5. Electrostatic potential maps of inhibitor **8** and (*S*)-donepezil showing the pharmacophoric charge areas in common.

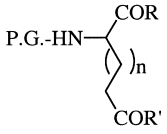
General method for enzymatic synthesis

To a solution of the corresponding *N*-blocked aminoacid diethyl esther (20 mM) and amine (50 mM) in diisopropylether, CAL (50 mg/mL) and 4 Å molecular sieves (50 mg/mL) were added. The reaction mixture was stirred in an orbital shaker at 60 °C. After that time, the enzyme and molecular sieves were filtered off and washed with methanol. The combined organic solution was evaporated to dryness. In the case of piperazine derivatives, **3**, **10** and **14**, the residue was dissolved in dichloromethane (60 mL) and washed with water (3×25 mL). The organic phase was dryed ($MgSO_4$), the solvent was eliminated under reduced pressure and the residue was used in the next step without further purification. In piperidine derivatives, the residue was purified by silica gel column chromatography using EtOAc:MeOH as eluent.

N-Benzylpiperidine derivatives

These derivatives were obtained when *N*-benzyl-4-(2-aminomethyl)piperidine was the amine used as nucleophile in the enzymatic amidations, following the general procedure.

Table 2. Significant ^{1}H NMR data of monoamide derivatives **1**–**15** (200 MHz, $CDCl_3$ δ ppm)



No.	*n*	P.G.	R	R′	α	β-CH_2	γ-CH_2	δ-CH_2	ε-CH_2	α-NH	R	R′
											3.19 (1-CH_2, NHPd)	
1	1	Cbz	NHPd[a]	OEt	4.18	1.84–2.12	2.27–2.48	—	—	5.88	6.53 (NH, NHPd) 3.43 (CH_2, NBn) 3.26 (1-CH_2, NHPd)	1.19 (CH_3, Et) 4.07 (CH_2, Et)
2	1	Ac	NHPd	OEt	4.41	1.88–2.55	2.30–2.57	—	—	6.52	6.45 (NH, NHPd) 3.47 (CH_2, NBn)	1.26 (CH_3, Et) 4.14 (CH_2, Et)
3	1	Cbz	NHPz[b]	OEt	4.21	1.89–2.17	2.33–2.54	—	—	5.67	3.33 (1-CH_2, NHPz) 6.67 (CH, NHPz) 3.31 (1-CH_2, NHPz)	1.24 (CH_3, Et) 4.12 (CH_2, Et)
4	1	Cbz	NHPz	OEt	4.18	1.90–2.32	2.33–2.45	—	—	5.65	6.61 (NH, NHPz) 3.47 (CH_2, NBn) 3.31 (1-CH_2, NHPz)	1.23 (CH_3, Et) 4.11 (CH_2, Et)
5	1	Cbz	NHPz	OEt	4.18	1.90–2.20	2.30–2.57	—	—	5.65	6.56 (NH, NHPz) 3.55 (CH_2, NBn) 3.26 (1-CH_2, NHPz)	1.23 (CH_3, Et) 4.11 (CH_2, Et)
6	1	Cbz	NHPz	OEt	4.15	1.82–2.10	2.28–2.44	—	—	5.64	6.57 (NH, NHPz) 3.37 (CH_2, NBn) 3.30 (1-CH_2, NHPz)	1.18 (CH_3, Et) 4.05 (CH_2, Et)
7	1	Cbz	NHPz	OEt	4.19	1.87–2.14	2.32–2.54	—	—	5.63	6.60 (NH, NHPz) 3.50 (CH_2, NBn) 3.22 (1-CH_2, NHPd)	1.21 (CH_3, Et) 4.09 (CH_2, Et)
8	2	Cbz	OEt	NHPd	4.35	1.58–1.97	1.58 1.77	2.11–2.19	— —	5.35	1.23 (CH_3, Et) 4.11 (CH_2, Et) 3.26 (1-CH_2, NHPd)	5.55 (NH, NHPd) 3.45 (CH_2, NBn)
9	2	Ac	OEt	NHPd	4.57	1.62–1.87	1.62–1.87	2.10–2.32	—	6.22	1.28 (CH_3, Et) 4.20 (CH_2, Et)	5.56 (NH, NHPd) 3.49 (CH_2, NBn)
10	2	Cbz	OEt	NHPz	4.31	1.62–1.97	1.62–1.73	2.18	—	5.55	1.25 (CH_3, Et) 4.17 (CH_2, Et)	3.29 (1-CH_2, NHPz) 6.09 (NH, NHPz) 3.44 (1-CH_2, NHPz)
11	2	Cbz	OEt	NHPz	4.27	1.59–1.88	1.59–1.69	2.06–2.22	—	5.46	1.20 (CH_3, Et) 4.12 (CH_2, Et)	5.99 (NH, NHPz) 3.55 (CH_2, NBn) 3.22 (1-CH_2, NHPd)
12	3	Cbz	OEt	NHPd	4.31	1.52–2.00	1.52–1.72	1.38	2.10	5.33	1.24 (CH_3, Et) 4.16 (CH_2, Et)	5.42 (NH, NHPd) 3.46 (CH_2, NBn) 3.19 (1-CH_2, NHPd)
13	3	Ac	OEt	NHPd	4.51	1.50–1.86	1.50–1.86	1.18–1.49	2.10	6.31	1.22 (CH_3, Et) 4.13 (CH_2, Et)	5.77 (NH, NHPd) 3.50 (CH_2, NBn)
14	3	Cbz	OEt	NHPz	4.26	1.54–1.84	1.54–1.63	1.31	2.10	5.42	1.20 (CH_3, Et) 4.12 (CH_2, Et)	3.25 (1-CH_2, NHPz) 6.06 (NH, NHPz) 3.27 (1-CH_2, NHPz)
15	3	Cbz	OEt	NHPz	4.29	1.53–1.90	1.53–1.90	1.34	2.11	5.42	1.22 (CH_3, Et) 4.14 (CH_2, Et)	6.07 (NH, NHPz) 3.39 (CH_2, NBn)

[a]Pd = 2-(4-benzylpiperidinyl)ethyl.
[b]Pz = 2-(1-piperazinyl)ethyl.

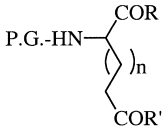
1-(*N*-Benzylpiperidin-4-yl-2-ethylamido)-*N*-benzyloxycarbonyl-*S*-glutamic acid 5-ethyl ester (1). Reaction time, 5 h. Chromatographic conditions: HPLC, CH_3CN:H_2O 40:60, λ = 215 nm; column, EtOAc:MeOH 60:1. Yield, 61%. Colorless solid mp 107–108 °C, $[\alpha]_D$: −3.7° (*c* = 1, $CHCl_3$). Anal. calcd for $C_{29}H_{39}N_3O_5$: C, 68.34; H, 7.71; N, 8.25. Found: C, 68.38; H, 7.85; N, 8.30.

1-(*N*-Benzylpiperidin-4-yl-2-ethylamido)-*N*-acetyl-*S*-glutamic acid 5-ethyl ester (2). Reaction time, 14 h. Chromatographic conditions: HPLC, CH_3CN:H_2O 20:80, λ = 200 nm; column, EtOAc:MeOH 15:1. Yield, 68%. Colorless solid mp 99–100 °C, $[\alpha]_D$: −9.4° (*c* = 1, $CHCl_3$). Anal. calcd for $C_{23}H_{35}N_3O_4$: C, 66.16; H, 8.45; N, 10.06. Found: C, 66.27; H, 8.28; N, 9.94.

6-(*N*-Benzylpiperidin-4-yl-2-ethylamido)-*N*-benzyloxycarbonyl-*S*-α-amino-adipic acid 1-ethyl ester (8). Reaction time, 9 h. Chromatographic conditions: HPLC, CH_3CN:H_2O 50:50, λ = 215 nm; column, EtOAc:MeOH 30:1. Yield, 70%. Colorless solid mp 85–86 °C, $[\alpha]_D$: +2.4° (*c* = 1, $CHCl_3$). Anal. calcd for $C_{30}H_{41}N_3O_5$: C, 68.81; H, 7.89; N, 8.02. Found: C, 69.02; H, 7.84; N, 7.96.

6-(*N*-Benzylpiperidin-4-yl-2-ethylamido)-*N*-acetyl-*S*-α-aminoadipic acid 1-ethyl ester (9). Reaction time, 7 h.

Table 3. Significant ^{13}C NMR data of monoamide derivatives **1**–**15** (50 MHz, $CDCl_3$, δ ppm)



No.	*n*	P.G.	R	R′	α-CH	β-CH_2	γ-CH_2	δ-CH_2	ε-CH_2	R	R′
										37.20 (1-CH_2, NHPd)	
1	1	Cbz	NHPd[a]	OEt	54.10	28.00	30.30	—	—	35.90 (2-CH_2, NHPd)	14.00 (CH_3, Et)
										63.30 (CH_2, NBn)	60.60 (CH_2, Et)
										36.00 (1-CH_2, NHPd)	
2	1	Ac	NHPd	OEt	52.40	27.90	30.50	—	—	37.20 (2-CH_2, NHPd)	14.10 (CH_3, Et)
										63.40 (CH_2, NBn)	60.70 (CH_2, Et)
3	1	Cbz	NHPz[b]	OEt	54.40	28.30	30.50	—	—	35.90 (1-CH_2, NHPz)	14.20 (CH_3, Et)
										56.70 (2-CH_2, NHPz)	60.80 (CH_2, Et)
										36.10 (1-CH_2, NHPz)	
4	1	Cbz	NHPz	OEt	54.20	28.20	30.30	—	—	56.20 (2-CH_2, NHPz)	14.20 (CH_3, Et)
										62.90 (CH_2, NBn)	60.70 (CH_2, Et)
										36.10 (1-CH_2, NHPz)	
5	1	Cbz	NHPz	OEt	54.30	28.30	30.40	—	—	56.20 (2-CH_2, NHPz)	14.20 (CH_3, Et)
										62.00 (CH_2, NBn)	60.70 (CH_2, Et)
										36.00 (1-CH_2, NHPz)	
6	1	Cbz	NHPz	OEt	54.20	28.30	30.30	—	—	56.20 (2-CH_2, NHPz)	14.20 (CH_3, Et)
										62.10 (CH_2, NBn)	60.70 (CH_2, Et)
										36.00 (1-CH_2, NHPz)	
7	1	Cbz	NHPz	OEt	54.20	28.30	30.40	—	—	56.20 (20CH_2, NHPz)	14.20 (CH_3, Et)
										62.60 (CH_2, NBn)	60.70 (CH_2, Et)
											37.10 (1-CH_2, NHPd)
8	2	Cbz	OEt	NHPd	54.10	32.90	22.10	36.40	—	14.80 (CH_3, Et)	38.00 (2-CH_2, NHPd)
										62.20 (CH_2, Et)	64.10 (CH_2, NBn)
											36.30 (1-CH_2, NHPd)
9	2	Ac	OEt	NHPd	51.60	31.90	21.40	35.50	—	14.20 (CH_3, Et)	37.20 (2-CH_2, NHPd)
										61.60 (CH_2, Et)	63.50 (CH_2, NBn)
10	2	Cbz	OEt	NHPz	53.70	32.00	21.50	35.70	—	14.20 (CH_3, Et)	35.60 (1-CH_2, NHPz)
										61.50 (CH_2, Et)	56.10 (2-CH_2, NHPz)
											35.80 (1-CH_2, NHPz)
11	2	Cbz	OEt	NHPz	53.60	31.90	21.40	35.60	—	14.20 (CH_3, Et)	56.50 (2-CH_2, NHPz)
										61.50 (CH_2, Et)	62.90 (CH_2, NBn)
											36.30 (1-CH_2, NHPd)
12	3	Cbz	OEt	NHPd	53.70	32.40	24.80	25.10	36.30	14.10 (CH_3, Et)	37.20 (2-CH_2, NHPd)
										61.50 (CH_2, Et)	63.40 (CH_2, NBn)
											36.10 (1-CH_2, NHPd)
13	3	Ac	OEt	NHPd	52.00	32.00	24.80	25.00	36.10	14.10 (CH_3, Et)	37.00 (2-CH_2, NHPd)
										61.30 (CH_2, Et)	63.00 (CH_2, NBn)
14	3	Cbz	OEt	NHPz	53.70	32.30	24.80	25.10	35.60	14.10 (CH_3, Et)	36.20 (1-CH_2, NHPz)
										61.40 (CH_2, Et)	57.10 (2-CH_2, NHPz)
											35.80 (1-CH_2, NHPz)
15	3	Cbz	OEt	NHPz	53.80	32.40	24.80	25.10	36.20	14.10 (CH_3, Et)	56.50 (2-CH_2, NHPz)
										61.40 (CH_2, Et)	62.90 (CH_2, NBn)

[a]Pd = 2-(4-benzylpiperidinyl)ethyl.
[b]Pz = 2-(1-piperazinyl)ethyl.

Chromatographic conditions: HPLC, CH_3CN:H_2O 20:80, λ = 200 nm; column, EtOAc:MeOH 15:1. Yield, 73%. Colorless solid mp 110–111 °C, $[\alpha]_D$: +2.8° (c = 1, $CHCl_3$). Anal. calcd for $C_{24}H_{37}N_3O_4$: C, 66.79; H, 8.64; N, 9.74. Found: C, 66.79; H, 8.53; N, 9.57.

7-(*N*-Benzylpiperidin-4-yl-2-ethylamido)-*N*-benzyloxycarbonyl-*R*,*S*-α-amino-pimelic acid 1-ethyl ester (12). Reaction time, 7 h. Chromatographic conditions: HPLC, CH_3CN:H_2O 50:50, λ = 215 nm; column, EtOAc:MeOH 15:1. Yield, 72%. Colorless syrup. Anal. calcd for $C_{31}H_{43}N_3O_5$: C, 69.25; H, 8.06; N, 7.81. Found: C, 69.08; H, 8.12; N, 7.74.

7-(*N*-Benzylpiperidin-4-yl-2-ethylamido)-*N*-acetyl-*R*,*S*-α-aminopimelic acid 1-ethyl ester (13). Reaction time, 6 h. Chromatographic conditions: HPLC, CH_3CN:H_2O 25:75, λ = 200 nm; column, EtOAc:MeOH 20:1. Yield, 71%. Colorless syrup. Anal. calcd for $C_{25}H_{39}N_3O_4$: C, 67.39; H, 8.82; N, 9.43. Found: C, 67.40; H, 8.89; N, 9.35.

***N*-Benzylpiperazine derivatives.** These compounds were obtained in a two step process: following the general procedure, the enzymatic amidation of the diesters using 2-(1-piperazinyl)ethylamine as nucleophile and the subsequent *N*-benzylation of the *N*-free piperazine intermediates **3**, **10** and **14** obtained.

1-(Piperazinyl-2-ethylamido)-*N*-benzyloxycarbonyl-*S*-glutamic acid 5-ethyl ester (3). Reaction time, 5 h. Chromatographic conditions: HPLC, $CH_3CN:H_2O$ 40:60, $\lambda = 215$ nm. Yield, 83%. Colorless syrup, $[\alpha]_D$: $+3.8°$ (c = 1, $CHCl_3$). Anal. calcd for $C_{21}H_{32}N_4O_5$: C 59.98; H, 7.67; N, 13.32. Found: C, 60.18; H, 7.52; N, 13.60.

6-(Piperazinyl-2-ethylamido)-*N*-benzyloxycarbonyl-*S*-α-aminoadipic acid 1-ethyl ester (10). Reaction time, 5 h. Chromatographic conditions: HPLC, $CH_3CN:H_2O$ 50:50, $\lambda = 215$ nm. Yield, 75%. Colorless syrup, $[\alpha]_D$: $+3.2°$ ($c = 1$, $CHCl_3$). Anal. calcd for $C_{22}H_{34}N_4O_5$: C, 60.81; H, 7.89; N, 12.89. Found: C, 61.11; H, 7.97; N, 12.93.

6-(Piperazinyl-2-ethylamido)-*N*-benzyloxycarbonyl-*R*,*S*-α-aminopimelic acid 1-ethyl ester (14). Reaction time, 6 h. Chromatographic conditions: HPLC, $CH_3CN:H_2O$ 50:50, $\lambda = 215$ nm. Yield, 81%. Colorless syrup. Anal. calcd for $C_{23}H_{36}N_4O_5$: C, 61.59; H, 8.09; N, 12.49. Found: C, 62.70; H, 7.78; N, 12.53.

***N*-Benzylation general procedure.** A solution of the corresponding benzylhalide (0.25 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise to a stirred ice-cooled solution of the monoamide **3**, **10** or **14** (0.23 mmol) and triethylamine (0.24 mmol) in anhydrous CH_2Cl_2 (15 mL). The mixture was stirred 12 h (TLC, $CHCl_3$:acetone 1:1) at room temperature and then washed with HCL 0.1 N (3×10 mL) and water. The organic solution was dried ($MgSO_4$), evaporated and the remaining residue eluted on a silicagel column.

1-(*N*-Benzylpiperazinyl-2-ethylamido)-*N*-benzyloxycarbonyl-*S*-glutamic acid 5-ethyl ester (4). Yield 60%. Eluent EtOAc:MeOH 20:1. Colorless solid mp 100–101 °C, $[\alpha]_D$: $+2.8°$ ($c = 1$, $CHCl_3$). Anal. calcd for $C_{28}H_{38}N_4O_5$: C, 65.86; H, 7.50; N, 10.97. Found: C, 65.98; H, 7.72; N, 10.92.

1-[*N*-(4-Nitrophenylmethyl)piperazinyl-2-ethylamido]-*N*-benzyloxycarbonyl-*S*-glutamic acid 5-ethyl ester (5). Yield 91%. Eluent, EtOAc:MeOH 15:1. Reddish syrup, $[\alpha]_D$: $+2.1°$ ($c = 1$, $CHCl_3$). Anal. calcd for $C_{28}H_{37}N_5O_7$: C, 60.53; H, 6.71; N, 12.60. Found: C, 60.86; H, 6.75; N, 12.45.

1-[*N*-(4-Chlorophenylmethyl)piperazinyl-2-ethylamido]-*N*-benzyloxy-carbonyl-*S*-glutamic acid 5-ethyl ester (6). Yield 58%. Eluent, EtOAc:MeOH 20:1. Colorless solid mp 93–94 °C, $[\alpha]_D$: $+3.0°$ ($c = 1$, $CHCl_3$). Anal. calcd for $C_{28}H_{37}N_4ClO_5$: C, 61.70; H, 6.84; N, 10.28. Found: C, 61.93; H, 6.75; N, 10.08.

1-[*N*-(4-Biphenylylmethyl)piperazinyl-2-ethylamido]-*N*-benzyloxycarbonyl-*S*-glutamic acid 5-ethyl ester (7). Yield 44%. Eluent EtOAc:MeOH 30:1. Colorless solid mp 122–123 °C, $[\alpha]_D$: $+3.7°$ ($c = 1$, $CHCl_3$). Anal. calcd for $C_{34}H_{42}N_4O_5$: C, 69.60; H, 7.22; N, 9.55. Found: C, 69.35; H, 7.23; N, 9.31.

6-(*N*-Benzylpiperazinyl-2-ethylamido)-*N*-benzyloxycarbonyl-*S*-α-amino-adipic acid 1-ethyl ester (11). Yield 78%. Eluent EtOAc:MeOH 20:1. Colorless solid mp 80–81 °C, $[\alpha]_D$: $+4.4°$ ($c = 1$, $CHCl_3$). Anal. calcd for $C_{29}H_{40}N_4O_5$: C, 66.39; H, 7.68; N, 10.68. Found: C, 66.24; H, 7.51; N, 10.52.

7-(*N*-Benzylpiperazinyl-2-ethylamido)-*N*-benzyloxycarbonyl-*R*,*S*-α-amino-pimelic acid 1-ethyl ester (15). Yield 61%. Eluent EtOAc:MeOH 20:1. Colorless syrup. Anal. calcd for $C_{30}H_{42}N_4O_5$: C, 66.89; H, 7.86; N, 10.40. Found: C, 67.02; H, 7.89; N, 10.29.

Modeling methods

Semiempirical methods. All compounds were built in SPARTAN.[18] Each molecule were fully optimised using the semi-empirical method AM1.[19] Partial atomic charges required for calculation of electrostatic potential were evaluated by fitting point to electrostatic potential.

Similarity calculations. Molecular alignment and molecular similarity indices were carried out using TSAR package.[20] Molecular alignment were carried out using a function based on a mixture of shape, electrostatic charge and lipophilicity. The combined property (cp) for atom i is calculated as follows:

$$cp(i) = 1.0w_S + q_i w_Q + l_i w_L$$

where w_S, w_Q and w_L are user-defined weights (shape, charge and lipophilicity) and q_i and l_i are the partial charge and lipophilicity for atom i respectively. The best results were obtained when $w_S = 1$, $w_Q = 0$, $w_L = 0$. Molecular similarity indices were calculated without optimisation of previous alignment.

Biological methods

The method of Ellman et al.[12] was followed. The assay solution consisted of 0.1 M phosphate buffer pH 8, 200 μM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellan's reagent), 0.02 unit/mL AChE (Sigma Chemical Co., from human erythrocytes), and 400 μM acethylthiocholine iodide as the substrate of the enzymatic reaction. The compounds tested were added to the assay solution and pre incubated with the enzyme for 10 min at 30 °C. After that period, the substrate was added. The absorbance changes at 412 nm were recorded for 5 min with a Perkin–Elmer 550 SE UV/VIS spectrometer, the reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated. The IC_{50} is defined as the concentration of each compound that reduces a 50% the enzymatic activity with respect to that without inhibitors.

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