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PSEUDOPEPTIDE INHIBITORS OF AMINOPEPTIDASES CONTAINING THE Ψ[CH(CN)NH] SURROGATE AS A TRANSITION-STATE MIMIC

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Abstract: The introduction of the Ψ[CH(CN)NH] peptide bond surrogate, as a mimic of the tetrahedral transition-state involved in peptidase action, into analogues of H-Phe-Leu-OH and H-Phe-Leu-Pro-OMe led to AP-B and AP-M inhibitors, which were 10-fold less active than bestatin against AP-B and 10-fold more active against AP-M. The transormation of this peptide bond surrogate into the analogue Ψ[CH($CONH_2$)NH] afforded inactive compounds.

Aminopeptidase B (AP-B, EC 3.4.11.6) and aminopeptidase M (AP-M, EC 3.4.11.2) are two membrane bound APs which play a key role in processes of inflamation, immunity, oncogenesis, metastasis, virus infection and pain[1-6]. These enzymes are inhibited by the natural dipeptide bestatin [[*N*-(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine = (2*S*,3*R*)-AHPBA-Leu][7], and the analogue tetrapeptide probestin [(2*S*,3*R*)-AHPBA-Leu-Pro-Pro][8]. Bestatin, has been approved for clinical use as immunomodifier and antitumor agent[9], activities that have been related with the mentioned APs inhibitory properties[10]. The structural similarity between the sp^3 geometry of the *C*-2 hydroxy group of the AHPBA residue and the probable tetrahedral intermediate in the amide bond hydrolysis by APs has been the basis to propose that bestatin may function as a transition-state analogue inhibitor[11].

Replacement of the scissible peptide bond with suitable transition-state mimics has proved to be an useful starting point for the search of metabolically stable peptidase inhibitors[12]. As a result of our recent studies[13-15] on the cyanomethylaneamino group [CH(CN)NH] as a new type of peptide bond surrogate, it was demostrated by semiempirical quantum mechanic calculations that this peptidase-resisting group could be a good mimic of the tetrahedral transition-state involved in peptidase action[13]. These results and our current interest in APs inhibitors[16] focused our attention on the incorporation of the cyanomethyleneamino group into bestatin and probestin analogues.

The present paper describes the AP-B and AP-M inhibition by the Ψ[CH(CN)NH][17] pseudodi- and pseudotripeptides **1a-1d** and **1e**. In order to support our hypothesis concerning the utility of the cyanomethyleneamino peptide bond surrogate for the search of enzyme inhibitors, pseudopeptides **2a** and **2e** in which this surrogate has been transformed into a non suitable transition-state mimic, such as the [CH($CONH_2$)NH] group, have also been prepared and included in the AP inhibition assays.

H-PheΨ[CH(CN)NH]Xaa-OMe

1

a : Xaa = Leu **d** : Xaa = Phe

b : Xaa = Val **e** : Xaa = Leu-Pro

c : Xaa = Lys(Z)

H-PheΨ[CH($CONH_2$)NH]Xaa-OMe

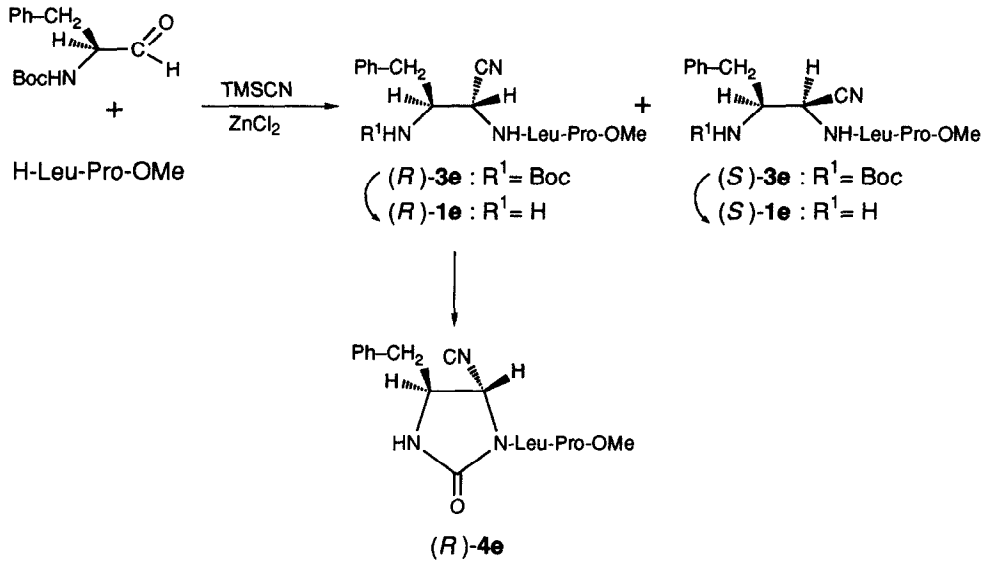
2

a : Xaa = Leu

e : Xaa = Leu-Pro

In a similar way to that described for the synthesis of pseudodipeptides **1a-d**[14], pseudotripeptides **1e** were prepared following the general procedure reported for the synthesis of Ψ[CH(CN)NH] pseudopeptides in solution[14]. Thus, reaction of *N*-Boc-phenylalaninal with Leu-Pro-OMe and trimethylsilyl cyanide (TMSCN), in the presence of $ZnCl_2$ as catalyst, gave a 3:1 mixture of the *N*-protected pseudotripeptides (*R*)- and (*S*)-**3e**. Chromatographic resolution of these two epimers, and subsequent Boc removal with HCl methanolic solution provided the corresponding deprotected analogues **1e** (scheme 1)[18]. The assigment of the absolute configuration at the new chiral center in (*R*)- and (*S*)-**1e** was carried out on the basis of the $J_{4,5}$ value in the ^{1}H-NMR spectrum of the 2-oxoimidazolidine (*R*)-**4e**, obtained from (*R*)-**1e** by reaction with bis(trichloromethyl)carbonate[14].

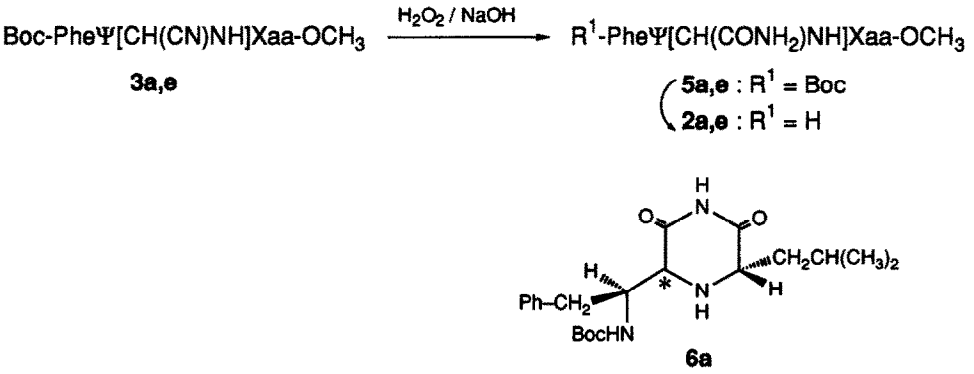
Scheme 1



Oxidative cyano-hydratation in the *N*-Boc-protected cyanomethyleneamino pseudotripeptides (*R*)- and (*S*)-**3e** with basic hydrogen peroxide under phase-transfer conditions, using *n*-tetrabutylammonium hydrogen sulphate as catalyst [19,20], gave the corresponding carbamoylmethyleneamino derivatives (*R*)- and (*S*)-**5e**, respectively (Scheme 2). Boc-Removal with TFA afforded the corresponding deprotected pseudotripeptides **2e**

as ditrifluoroacetates. However, in the application of this methodology to the (*R,S*)-epimeric mixture of *N*-Boc cyanomethyleneamino pseudodipeptides **3a**, which could not be separated, only the (*S*)-epimer of the carbamoylmethyleneamino derivative (*S*)-**5a** could be isolated from the reaction mixture, along with both (*R*)- and (*S*)-epimers of the 2,6-dioxopiperazine **6a**[20]. These compounds resulted from the easy cyclization of the intermediates 4-carbamoyl esters **5a** in the basic medium required for the cyano-hydratation[21]. This cyclization in (*R*)-**5a** was so fast that it could not be isolated in this reaction, and therefore it was not possible to assay this compound as AP inhibitor.

Scheme 2



The inhibitory potencies of the *N*-Boc-protected and unprotected Ψ[CH(CN)NH] pseudopeptides **3a-e** and **1a-e** and of the carbamoylmethyleneamino derivatives **2a** and **2e** against AP-B (associated with the surface of murine L cells)[1] and AP-M[22] are listed in table 1. For comparative purposes the dipeptide H-Phe-Leu-OH, the tripeptide H-Phe-Leu-Pro-OMe and bestatin were also included in the AP-assays. All the *N*-deprotected cyanomethyleneamino pseudopeptides **1**, except for **1d**, showed IC_{50} values in the 10^{-5} and 10^{-6} M range against AP-B and AP-M, respectively. In comparison with bestatin, these compounds were approximately 10-fold less active against AP-B, but 10-fold more active against AP-M, and, therefore, more selective for this last AP. These results are more significant when compared with those of the peptidic models H-Phe-Leu-OH and H-Phe-Leu-Pro-OMe, which were inactive at concentrations below 10^{-3}.

Although the number of assayed cyanomethyleneamino pseudopeptides **1a-e** is not enough to draw structure-activity conclusions, the 10-100 fold decrease in the activity of the (*R*)- and (*S*)-**1d** derivatives with respect to compounds **1a-c** indicated, as in the case of bestatin[23], the preference for aliphatic residues in position P'_1(Xaa). In contrast to the increase in the AP-inhibitory potency observed when bestatin is compared with probestin[8], and in the case of other probestin analogues[16c], the *C*-terminal extension of the pseudodipeptides **1a** with a Pro residue had not a significant effect on the activity of these compounds. Also, unlike bestatin, the stereochemistry of the cyanomethyleneamino group in these pseudopeptides [compare (*R*)-**1e** and (*S*)-**1e**] does not seem to influence significantly the activity. As in the case of many AP inhibitors[4,23], .

the *N*-Boc protected pseudopeptides **3a-e** were inactive, probably due to the preferential binding of APs to *N*-deprotected peptides.

Table 1: Inhibitory potency of compounds **1a-e, 2a,e** and **3a-e** on AP-B[a] and AP-M[b]

Compound	AP-B[c] $IC_{50}(\mu M)$	AP-M[c] $IC_{50}(\mu M)$
(*R*,*S*)-**1a**	50	2.4
(*R*,*S*)-**1b**	36	3.8
(*R*,*S*)-**1c**	44	5.0
(*R*)-**1d**	221	85
(*S*)-**1d**	293	146
(*R*)-**1e**	28	2.9
(*S*)-**1e**	14	3.4
(*S*)-**2a**	>1000	>1000
(*R*)-**2e**	>1000	783
(*S*)-**2e**	>1000	>1000
(*R*,*S*)-**3a**	>1000	>1000
(*R*,*S*)-**3b**	>1000	>1000
(*R*,*S*)-**3c**	>1000	>1000
(*R*)-**3d**	>1000	>1000
(*S*)-**3d**	>1000	>1000
(*R*)-**3e**	>1000	>1000
(*R*)-**3e**	>1000	>1000
H-Phe-Leu-OH	>1000	>1000
H-Phe-Leu-Pro-OMe	824	793
Bestatin	6	19.4

[a] Murine L cells surface-associate AP-B activities were determined following the Aoyagi method[1], using L-lysine-2-naphthylamide hydrochloride as substrate (0.5 mM, Km = 0.9×10^{-4} M). [b] AP-M Assays were carried out as previously described[22], using L-leucine-2-naphthylamide hydrochloride as substrate (0.5 mM, Km = 0.6×10^{-4} M). [c] Values are the mean of 4-5 experiments with different concentrations of inhibitor. S.E. were less than 10% of the mean.

Finally, the transformation of the active cyanomethyleneamino pseudopeptides **1a** and **1e** into their corresponding carbamoylmethyleneamino derivatives **2a** and **2e** led to inactive compounds. This loss of activity could be explained in terms of the differences in steric and electronic properties between the [$CH(CONH_2)NH$]

group and the tetrahedral transition-state intermediate involved in peptide bond hydrolysis, deduced from the semiempirical quantum mechanic calculations[24] indicated in fig 1.

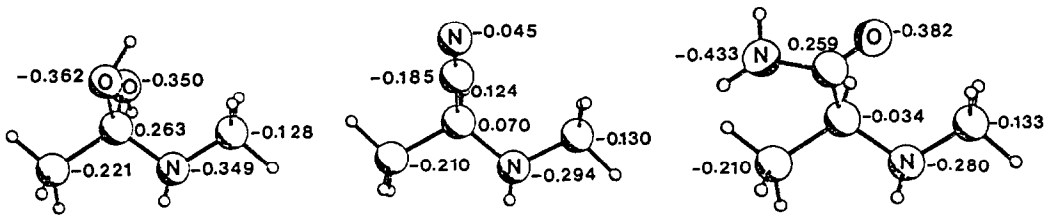


Figure 1: Optimized geometry and atomic charges for the tetrahedral transition-state, the [CH(CN)NH] and the [$CH(CONH_2)NH$] groups[24].

In conclusion, the AP-inhibition results reported here support the hypothesis concerning the suitability of the cyanomethyleneamino group as a good mimic of the tetrahedral transition-state, and the interest of Ψ[CH(CN)NH] pseudopeptides in the search of metabolically stable peptidase inhibitors

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