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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4777–4779

Synthesis and dipeptidyl peptidase inhibition of *N*-(4-substituted-2,4-diaminobutanoyl)piperidines

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Received 24 May 2006; revised 27 June 2006; accepted 28 June 2006
Available online 17 July 2006

Abstract—In this paper, we report the synthesis of diastereomerically pure *N*-(4-substituted-2,4-diaminobutanoyl)piperidines. These compounds were prepared to investigate the influence of the 4-substitution on the dipeptidyl peptidase II (DPP II) activity and selectivity of the parent *N*-(2,4-diaminobutanoyl)piperidine. The (4*S*)-methyl compound showed subnanomolar inhibition, comparable with the parent compound. The (4*R*)-methyl group or bigger substituents decreased the activity.

Proline selective serine-type dipeptidyl peptidases cleave off dipeptides from the amino terminus of peptides or proteins with preferentially proline at the penultimate position. Representative examples are dipeptidyl peptidase II, IV, 8 and 9 (DPP II, DPP IV, DPP8 and DPP9) and fibroblast-activation protein α (FAPα). DPP IV is by far the best studied member among these enzymes, and is currently a well-validated target for the treatment of type 2 diabetes. Hence, these peptidases are often referred to as DPP IV activity- and/or structure-homologues (DASH) proteins. DPP II (EC 3.4.14.2) is less well studied. It is a 58-kDa glycoprotein, active as a homodimer formed with a leucine zipper motif and is identical to quiescent cell proline peptidase (QPP, DPP7). It is widely found in the human body and targeted to intracellular vesicles but the natural substrates and physiological functions are largely unknown. No sequence homology has been found between DPP II and DPP IV. It shows, in contrast with the other DPPs, an optimum at acidic pH but it cleaves, like DPP IV, N-terminal dipeptides from oligopeptides with Ala or Pro at the penultimate position.[1,2] The kinetics of DPP II mediated hydrolysis of chromogenic and fluorogenic dipeptide derivatives have been characterized.[3]

The development of potent and selective inhibitors is a very important item in research programmes on DPP II. It is of utmost importance in the unraveling of the biochemical and physiological functions of the enzyme and in the disclosure of potential therapeutic properties of its inhibition.

In a series of previous papers we reported a systematic search for DPP II inhibitors.[4,5] The optimal P1 group appeared to be a piperidine unit and L-2,4-diaminobutyric acid gave the best results as P2 building block. Subnanomolar inhibitors were obtained when the core structure, 2,4-diaminobutanoylpiperidine, was N^4-substituted with a benzyl group such as in UAMC00039 (N^4-(4-chlorobenzyl)-2,4-diaminobutanoylpiperidine). This compound is not only a highly potent inhibitor (K_i of 0.082 ± 0.048 nM) of DPP II but shows a high selectivity towards DPP IV and DPP 8 (SI > 10000). UAMC00039 showed no toxicity and good oral bioavailability.[6]

We further investigated the importance of the diaminobutanoyl chain, more in particular the influence of 4-substituents and prepared a set of 4-alkyl-derivatives (**1**–**7**). These substitutions may indeed influence the flexibility and lipophilicity of the compound and interfere in the interaction with the active site of the enzyme. As well (4*R*)- as (4*S*)-compounds were prepared.

The 4-substituted 2,4-diaminobutyric acids were prepared using the corresponding dehydroaminoacids as

Keywords: 2,4-Diaminoalkanoylpiperidines; Dipeptidyl peptidases; DPP II; Inhibitors.
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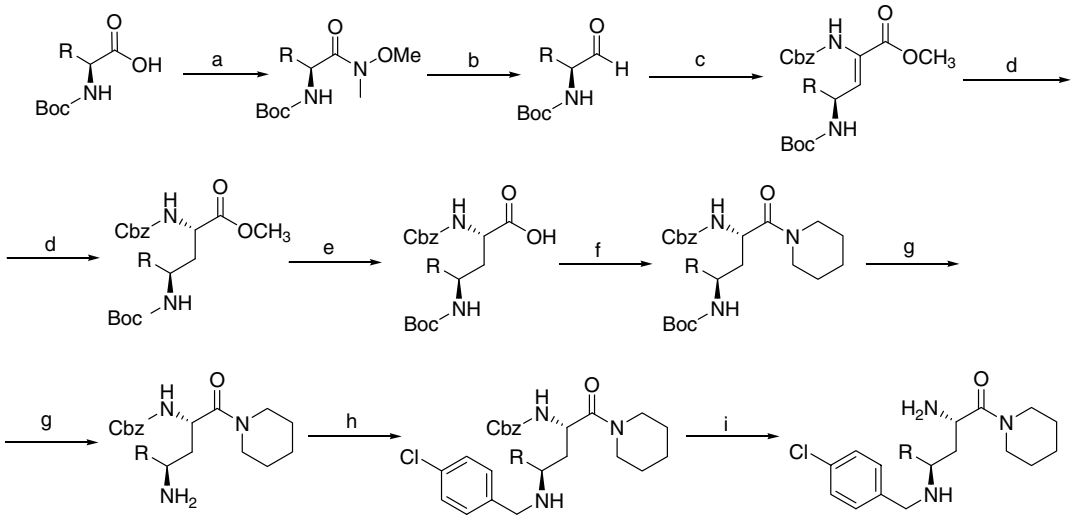
doi:10.1016/j.bmcl.2006.06.082



Scheme 1. Reagents: R=Me, Et, *sec*-But, *iso*-Pro, Bz. (a) *N*,*O*-dimethylhydroxylamine hydrochloride, TBTU, TEA, DMF; (b) $LiAlH_4$, THF; (c) methyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphoryl)-acetate, DBU, DCM; (d) H_2, *R*,*R* [Rh(COD)DIPAMP]$^+$BF4$^-$, EtOH, 3 Bar; (e) 75% KOH/MeOH; (f) piperidine, TBTU, TEA, DMF; (g) TFA/DCM (1:1); (h) *p*-chlorobenzaldehyde, $NaCNBH_3$, AcOH, MeOH; (i) 33% HBr/AcOH.

intermediates (Scheme 1; (2*S*,4*S*) shown). The latter are common starting materials for the synthesis of unnatural amino acids[7] and were prepared through a Horner–Wadsworth–Emmons reaction.[8] This method was already used for the synthesis of protected 2,4-diaminobutyric acid.[9] Another literature described method used a diketopiperazine-derived dehydroamino acid for the stereoselective synthesis of substituted 2,4-diamino acids.[10] We used the commercially available methyl 2-benzyloxycarbonylamino-2-dimethoxyphosphoryl acetate and the corresponding (*S*)- or (*R*)-Boc-protected aminoaldehydes as starting compounds. The latter were prepared from the corresponding amino acids by a lithium aluminium hydride reduction of their *N*-methoxy-*N*-methylamides. Stereoselective hydrogenation of the α,β-unsaturated amino acids with an optically active homogeneous catalyst was used. As (2*R*)-diaminobutyric acid amides were found to be poorly active DPP II inhibitors, we focused on the (2*S*)-configuration and used *R*, *R* [Rh(COD)DIPAMP]$^+$BF4$^-$ as a catalyst[11] and obtained (2*S*)-compounds with a % de of about 80. The piperidyl amides were obtained by TBTU coupling after hydrolysis of the methyl ester and the final *N*-benzyl compounds were prepared by reductive amination with *p*-chlorobenzaldehyde and sodium cyanoborohydride after Boc-deprotection with trifluoroacetic acid. At this stage the (2*S*)-compounds were purified until 100% de by chromatography and finally Z-deprotected by hydrobromic acid/acetic acid.

A set of compounds were prepared with methyl-, ethyl-, isopropyl-, *sec*-butyl and benzyl substituents (Table 1). All compounds were evaluated as described for their ability to inhibit DPP II, DPP IV and DPP 8 and their IC_{50}s compared with reference compound UAMC00039.[5] Results are summarized in Table 1.

Following conclusions can be drawn from the results obtained:

- The (4*S*)-methyl-(2*S*)-compound (**1**) shows about the same activity as the reference compound. The corresponding (2*S*,4*R*)-compound (**2**) is about 100 times less active. The (4*S*)-methyl group does not hinder the inhibitor–enzyme interaction and keeps the inhibitor in its active conformation.
- Larger substituents at the 4-position decrease the activity: the (4*S*)-ethyl-(2*S*)-compound (**3**) and the (4*S*)-benzyl-(2*S*)-compound (**6**) are about 5 times less active. Isopropyl- and *sec*-butyl substitutions (com-

Table 1. Biological data

Entry	R[1]	Stereochemistry	DPP II[a]	DPP IV[a]	DPP 8[a]
1	CH_3	2*S*,4*S*	0.00062 ± 0.00003	325 ± 16	148 ± 16
2	CH_3	2*S*,4*R*	0.057 ± 0.004	>250	65 ± 2
3	C_2H_5	2*S*,4*S*	0.0034 ± 0.0002	>100	>100
4[b]	*i*-Pr	2*S*,4*R*	0.019 ± 0.001	>125	>100
5	*s*-Bu	2*S*,4*R*	0.084 ± 0.006	234 ± 31	>100
6	Bn	2*S*,4*S*	0.0038 ± 0.0002	>125	>100
7	Bn	2*S*,4*R*	0.0465 ± 0.0133	>125	>500
UAMC00039[5]	H	2*S*	0.00022 ± 0.00002	196 ± 8	142 ± 17

[a] IC_{50} (μM); the errors shown are the errors on the fit.
[b] (4*R*) of compounds **4**, **5** corresponds to (4*S*) of compounds **1**, **3**, **6**.

pounds **4**, **5**) afford lower inhibitory activities. The lower activity for (4*R*) compounds is confirmed by benzyl compound **7**.
- The selectivity of *N*-(2,4-diaminobutanoyl)piperidines towards DPP II is confirmed: each compound shows a selectivity index of >1000 over DPP IV and DPP 8.

These results will be used in the investigation of the active site of DPP II and its interaction with inhibitors.

Acknowledgments

This work was supported by the Fund of Scientific Research, Flanders. P. Van der Veken and M.-B. Maes are fellows of the Fund of Scientific Research, Flanders. The authors are grateful to W. Bollaert and N. Lamoen for technical assistance.

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