Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 22, pp. 2745-2748, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00491-X

4-CYANOTHIAZOLIDIDES AS VERY POTENT, STABLE INHIBITORS OF DIPEPTIDYL PEPTIDASE IV

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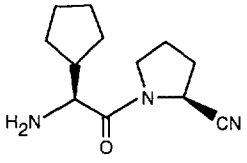
Abstract: A series of stable, very potent inhibitors of dipeptidyl peptidase IV has been developed. A number of dipeptide analogues, incorporating a 4-cyanothiazolidide, were found to have K_i values of less than 1 nM versus human DP-IV and half-lives of between 5 and 27h in aqueous solution (pH 7.4).

The serine protease dipeptidyl peptidase IV (DP-IV, EC 3.4.14.5)[1,2] which is identical to the T cell activation marker CD26 has been the subject of intense scrutiny because it was recently shown that inhibitors or antibodies of this enzyme can inhibit T cell proliferation.[3,4] However, the physiological role of DP-IV in the immune system and the molecular events mediated by this enzyme are only partly established[5] and we felt that it was necessary to develop potent, stable inhibitors of DP-IV to help elucidate the biological role of the enzyme and to investigate their therapeutic use in a number of disease states such as inflammation, graft versus host disease (GVHD), cancer or AIDS.



1

We recently reported a series of aminoacyl-2-cyanopyrrolidides[6,7] which possess K_i values of less than 5 nM versus human DP-IV[8] and half-lives ($t_{½}$) of greater than 48h in aqueous solution (pH 7.4).[9] This series of inhibitors is exemplified by **1** which has a K_i value of 1.1 nM versus human DP-IV and a half-life of 48h in aqueous buffer (pH 7.4).

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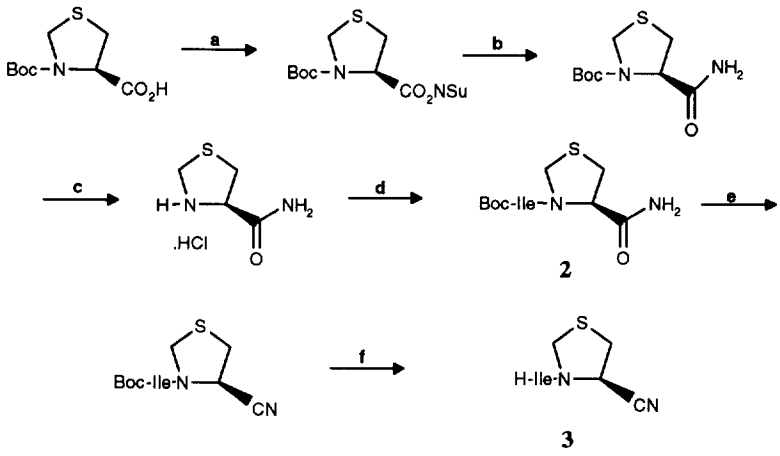
In a quest to improve the potency of this class of inhibitors, we investigated replacing the pyrrolidide ring with other nitrogen heterocycles. We chose isoleucine (Ile) as a standard *N*-terminal residue as it was the most potent natural amino acid in the 2-cyanopyrrolidide series.[7] The preparation of **3** (**Scheme I**) illustrates the general route to the series of cyano compounds described in **Table I.**

Scheme I. *Preparation of 3-isoleucyl-4-cyanothiazolidide.*

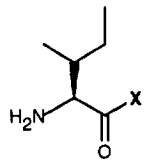


Reagents and Yields: **a.** *N*-hydroxysuccinimide (HONSu), water soluble carbodiimide, CH_2Cl_2. 99%. **b.** conc. NH_4OH, dioxane. 96%. **c.** 4N HCl/dioxane. 99%. **d.** Boc-Ile-OH, PyBop, CH_2Cl_2, NEt_3. 38%. **e.** $POCl_3$, imidazole, pyridine. 53%. **f.** Trifluoroacetic acid. 75%.

A pyBop[10] mediated coupling of 4-amidothiazolidide with Boc protected isoleucine afforded the dipeptide mimic **2** in modest yield. Dehydration of the primary amide function to a nitrile and subsequent acid catalysed deprotection yielded the trifluoroacetate salt of **3**.[11] From a range of compounds with various heteroatoms in 5- or 6-membered rings, we were pleased to find that the 4-cyanothiazolidide analogue **3** was approximately 5-fold more active than the 2-cyanopyrrolidide inhibitor **5**[7] (**Table I**). However, this increase in activity was accompanied by a slight decrease in stability.

Having established 4-cyanothiazolidide as an optimum *C*-terminal residue, we prepared further analogues with the best *N*-terminal α-amino acids from the pyrrolidide series.[7] These compounds were prepared as described in **Scheme I** but Boc-Ile-OH, in step **d**, was replaced with the required Boc-Xaa-OH. A number of analogues were prepared with sub-nanomolar activity against DP-IV and good stability in aqueous buffer (pH 7.4). (**Table II**)

Table I. *Isoleucyl heterocyclic nitriles: Potency versus human DP-IV and stability in aqueous solution.*



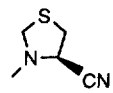
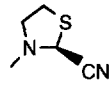
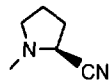
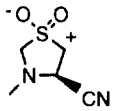
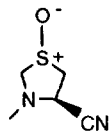
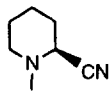
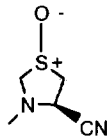
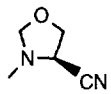
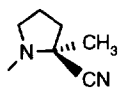
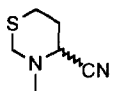
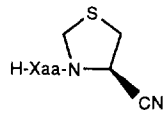
Compound N°	X	K_i (nM)[a]	$t_{1/2}$(h)[b]
3		0.41 ± 0.15	27
4[12]		1.70 ± 0.50	3
5		2.2 ± 0.50	48
6		21.0 ± 5.0	4
7[13]		34.0 ± 7.0	1.25
8		260 ± 50	>48
9[13]		440 ± 200	1.5
10		450 ± 100	>48
11		4,200 ± 900	48
12		6,000 ± 1,500	>48

Table II. *3-Aminoacyl-4-cyanothiazolidides: Potency versus human DP-IV and stability in aqueous solution (pH 7.4).*



Compound N°	Xaa	K_i (nM)[a]	$t_{1/2}$ (h)[a]
3	Ile	0.41 ± 0.15	27
13	Cyclopentylglycine	0.50 ± 0.10	5
14	Cyclohexylglycine	0.80 ± 0.20	16
15	Lys(Cbz)	5.00 ± 1.00	>48

These new, stable, low molecular weight inhibitors should offer the opportunity to study the physiological role of DP-IV and possibly have therapeutic benefits. We are currently exploring the effects of these compounds on lymphocytes (e.g. proliferation and cytokine release) and further details will be reported in due course.

REFERENCES AND NOTES

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2) Hegen, M.; Niedobitek, G.; Clein, C.E.; Stein, H.; Fleischer, B. *J. Immunol.* **1990**, *144*, 2908.
3) Schon, E.; Born, I.; Demuth, H.-U.; Faust, J.; Neubert, K.; Steinmetzer, T.; Barth, A.; Ansorge, S. *Biol. Chem. Hoppe-Seyler* **1991**, *372*, 305.
4) Flentke, G.R.; Munoz, E.; Huber, B.T.; Plaut, A.G.; Kettner, C.A.; Bachovchin, W.W. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 1556.
5) A review has recently been published, discussing the evidence that CD26 has important functions in the immune system. Fleischer, B. *Immunology Today* **1994**, *15*, 180.
6) Patent Cooperation Treaty (PCT) *WO 95/15309* (6 Dec. **1993**).
7) Ashworth, D.M.; Atrash, B.; Baker, G.R.; Baxter, A.J.; Jenkins, P.D.; Jones, D.M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163.
8) All compounds were tested in vitro against pure human DP-IV (purchased from M&E, Copenhagen, Denmark). Inhibition was determined using the fluorogenic substrate, H-Ala-Pro-AFC at three concentrations per inhibitor. A typical assay (total volume 0.4 mL) comprised sodium HEPES 83.3 mM, EDTA 1.67 mM, BSA 1.5 mg mL^{-1}, pH 7.8, DP-IV 25 μU mL^{-1}, inhibitor (in 10 mM acetate pH 4.0). The reaction was started by the addition of substrate and readings taken every 30 sec for 7.5 min, excitation at 395 nm, emission 450nm. K_i values were determined using Dixon plots.
9) The stability of the inhibitors in buffered, aqueous solution (100 mM Tris, pH 7.4) was monitored by reverse-phase HPLC. The inhibitors with half-lives of less than 48h decompose to multiple products which have not been characterised.
10) Martinez, J.; Bali, J.P.; Rodriguez, M.; Castro, B.; Laur, J.; Lignon, M.-F. *J. Med. Chem.* **1988**, *28*, 1874.
11) All compounds were >95% pure by HPLC and were characterised by a combination of ^{1}H NMR, ^{13}C NMR and FAB mass spectrometry.
12) Compound **4** is the active diastereomer of a pair separated by preparative HPLC and was assigned the (S,S) stereochemistry. The (S,R) diastereomer was inactive versus human DP-IV.
13) Compounds **7** and **9** are separated diastereomers (preparative HPLC) whose absolute stereochemistry was not determined.

(*Received in Belgium* 3 *September* 1996; *accepted* 15 *October* 1996)