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Seco-prolinenitrile inhibitors of dipeptidyl peptidase IV define minimal pharmacophore requirements at P1

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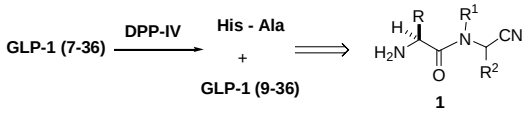
Abstract—A series of seco-prolinenitrile-containing dipeptides were synthesized and assayed as inhibitors of the N-terminal sequence-specific serine protease dipeptidyl peptidase IV, a promising new target for treatment of type 2 diabetes. The inhibitors described herein assess the minimum structural requirements at P1 for this enzyme, resulting in the identification of inhibitors with low nM potency.

The incretin hormone glucagon-like peptide-1 (GLP-1) is released from the gastrointestinal tract in response to nutrient ingestion and is known to function as a mediator of glucose stimulated insulin secretion.[1] Several clinical studies have shown that administration of this peptide or its analogues results in antihyperglycemic action in subjects with type 2 diabetes.[2] Additional studies have demonstrated a β-cell protective effect through increased stimulation of GLP-1 receptors.[3] Consequently, approaches to the treatment of type 2 diabetes based on the GLP-1 axis have attracted much focus from the scientific community. Although GLP-1 is secreted as GLP-1 (7–36) amide from the small and large intestines in response to dietary signals, it is rapidly truncated to GLP-1 (9–36) amide by cleavage of the N-terminal dipeptide residues by dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5), a sequence-specific serine protease which catalyzes the cleavage of dipeptides from the N-terminus of proteins with the sequence H-X-Pro-Y or H-X-Ala-Y (where X, Y = any amino acid, Y ≠ Pro).[4] Inhibition of DPP-IV has been shown to be effective at potentiating circulating levels of GLP-1 (7–36) and therefore offers a new oral therapeutic approach for the treatment of type 2 diabetes.[5] A number of DPP-IV inhibitors have recently advanced to late phase clinical trials[6] and are showing robust antidiabetic effects.[7]

The P1-derived sequence specificity of the DPP-IV suggests two entry points for inhibitor design from a peptide cleavage product formation perspective (Scheme 1): dipeptides derived from either proline (**1**, where R^1 and R^2 form a 5-membered ring) or alanine (R^1 = H, R^2 = Me) occupying the P1 position. Although numerous examples of proline- or proline mimetic-derived P1-containing dipeptidic inhibitors have been described,[8] there have been no reported examples of inhibitors with alanine-based P1 units. Moreover, we envisioned that the alanine fragment of **1** could be



Scheme 1. Cleavage of GLP-1 (7–36) to GLP-(9–36): design of product development inhibitors **1**.

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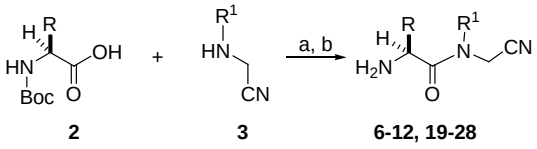
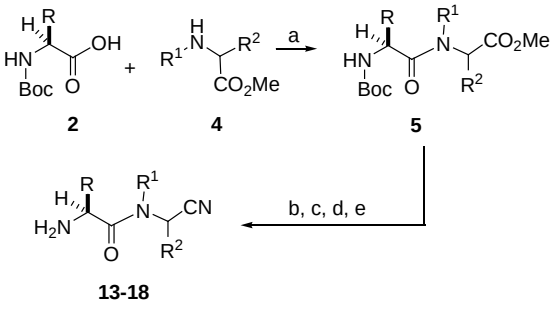
alternatively represented as a substituted glycine fragment, thus providing compounds **1**, where R^1 and R^2 could be independently varied. Herein we describe the in vitro structure–activity relationships around dipeptidic DPP-IV inhibitors **1** which incorporate highly branched N-terminal (P2) amino acids previously shown from our earlier studies to be preferred moieties.[9]



Scheme 2. Preparation of glycinenitrile dipeptidic DPP-IV inhibitors: Reagents: (a) EDAC, CH_2Cl_2, HOAt, NEt_3, 60–90%; (b) CH_2Cl_2, TFA or HCl, 50–90%.



Scheme 3. Preparation of alaninenitrile dipeptidic nitrile DPP-IV inhibitors: Reagents and conditions: (a) EDAC, CH_2Cl_2, HOAt, NEt_3, 60–90%; (b) NaOH, MeOH/H_2O, 50 °C; (c) NMO, *i*-BuOCOCl, NH_3, −15 °C; (d) TFAA, CH_2Cl_2; (e) CH_2Cl_2, TFA or HCl, 50–90%.

Our initial exploration of structure–activity relationships within this seco-proline inhibitor scaffold was addressed through a parallel array format. Coupling of Boc-protected amino acids **2** with amino-nitriles **3** under standard conditions (EDAC, HOAT, and NEt_3) gave, after extraction and solvent removal, essentially pure products (Scheme 2). Further purification was generally accomplished by simple filtration through silica gel cartridges (EtOAc/hexane). Subsequent acid promoted deprotection of the Boc group gave inhibitors **1** as their TFA or HCl salts.

Alanine- and *gem*-dimethylglycine P1-containing inhibitors were prepared by coupling of the appropriate Boc-protected N-terminal amino acids **2** with commercially available amino acid methyl esters **4**, followed by saponification, primary amide formation, dehydration to the corresponding nitrile, and Boc group removal (Scheme 3). All the compounds obtained in each series gave appropriate ^1H and ^{13}C NMR and positive ion MS data.[10]

All compounds were tested in vitro against purified human DPP-IV using the substrate H-Ala-Pro-*p*NA, measuring production of *p*-nitroaniline at 405 nm over 15 min[6c] (Table 1).

The prototype compound in this series (compound **6**), bearing a simple *N*-methylglycinenitrile P1 unit and L-valine at P2, demonstrated modest inhibitory potency

Table 1. Inhibition constants for seco-proline-based DPP-IV inhibitors

Compound	R	R^1	R^2	K_i[a] (nM)
6	*i*-Pr	Me	H	260 ± 83
7	*t*-Bu	Me	H	507 ± 186
8	*t*-Bu	Et	H	182 ± 11
9	*t*-Bu	*n*-Bu	H	>10,000
10	*t*-Bu	Allyl	H	212 ± 42
11	*t*-Bu	Cyclopropyl	H	1311 ± 556
12	*t*-Bu	Cyclobutyl	H	140 ± 48
13[b]	*t*-Bu	Me	Me	513 ± 308
14	*t*-Bu	Me	Me	>10,000
15	*t*-Bu	H	Me	4422 ± 350
16	*t*-Bu	H	di-Me	>10,000
17	Ad-1-yl[c]	H	Me	399 ± 85
18	Ad-1-yl	H	di-Me	>10,000
19	Ad-1-yl	H	H	>10,000
20	Ad-1-yl	Me	H	74 ± 13
21	Ad-1-yl	Et	H	27 ± 3
22	Ad-1-yl	*n*-Pr	H	673 ± 220
23	Ad-1-yl	Allyl	H	37 ± 3
24	Ad-1-yl	Cyclopropyl	H	188 ± 39
25	Ad-1-yl	Bn	H	>10,000
26	3-OH-Ad-1-yl	Me	H	18 ± 2
27	3-OH-Ad-1-yl	Et	H	3 ± 0.6
28	3,5-di-OH-Ad-1-yl	Me	H	23 ± 5

[a] Values represent means ± SEM of three experiments.
[b] Inhibitors **13**, **15**, and **17** have the L-Ala-derived stereochemistry at R_2, inhibitor **14** is derived from Ala having the D-configuration.
[c] Ad, adamantyl.

(260 nM) against DPP-IV (Table 1). This represents essentially a two log diminishment in potency versus typical cyanoprolinenitrile inhibitors bearing similar P2 moieties.[9] Evaluation of a variety of small alkyl substitutions on the glycinenitrile amino functionality fixing P2 as *tert*-Leu failed to yield significant gains in potency, though it did establish rather strict size limitations for this group. For example, potency drops off precipitously for the *N*-(*n*-butyl) compound **9** compared with the smaller ethyl- or allyl-substituted analogues **8** and **10**, respectively.

As DPP-IV specificity requirements readily accommodate alanine in the P1 position, it was not unanticipated that the corresponding alaninenitrile inhibitor **13** was equipotent to the glycine-based analogue **7**. Likewise, as previously demonstrated in proline-based P1-containing systems, the importance of stereochemical configuration at this center was critical for activity, with the D-alanine derived analogue **14** exhibiting greatly reduced inhibitory potency, having a K_i of >10,000 nM. An unoccupied valence on the glycine or alanine nitrogen was highly disfavored, as evidenced by the diminished activity of compounds **15**–**19**, regardless of other structural features.

As previously demonstrated in our 4,5-methanoprolinenitrile series,[6c] installation of an adamantylglycine P2 unit led to significant enhancements in potency (compounds **20**–**25**). Again, strict size limitations on the N-substituent (R^1) were observed, most strikingly evident in the effect of subtle steric differences between *n*-Pr (**22**, 673 nM) and allyl (**23**, 37 nM) analogues. Further enhancement in potency could be achieved through mono- or di-hydroxylation of the adamantyl skeleton (**26**–**28**). This latter effect may be due, in part, to hydroxyl group H-bonding interactions with Tyr547, which is known to function as a stabilizing component of the oxyanion hole formed during substrate hydrolysis.[2] Analogue **27**, bearing an *N*-ethylglycinenitrile P1 group, was the most potent (K_i = 3 nM) compound observed in this seco-proline series of DPP-IV inhibitors.

Compound **26** was evaluated for chemical stability under conditions where intramolecular cyclization reactions were known to be favored, pH 8.5 and 39 °C. The $t_{1/2}$ was found to be approximately 10 h.[11] Although compounds of the present series provide no improvement in chemical stability compared with the corresponding proline-based inhibitors, potent in vivo antihyperglycemic activity was demonstrated for compound **26** in an oral glucose tolerance test (oGTT) in Zucker$^{fa/fa}$ rats.[12]

This study of P1 seco-prolinenitriles demonstrates the first examples of open-chain P1-based inhibitors of DPP-IV and shows that potent inhibitory activity can be achieved when a highly branched amino acid is incorporated at the P2 (N-terminal) position. This characterization of minimal P1 structural requirements, in conjunction with the data available from structural biology approaches[13] and mechanistic studies[14], is expected to aid in the design and development of new and more effective DPP-IV inhibitors for the treatment of type II diabetes.

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