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Diprolyl nitriles as potent dipeptidyl peptidase IV inhibitors

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Abstract—Dipeptidyl peptidase IV (DPP4) is a multifunctional type II transmembrane serine peptidase which regulates various physiological processes, most notably plasma glucose homeostasis by cleaving peptide hormones glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide. Inhibition of DPP4 is a potentially valuable therapy for type 2 diabetes. Synthesis and structure–activity relationships of a series of substituted diprolyl nitriles are described, leading to the identification of compound **1** with a measured DPP4 K_i of 3.6 nM.

Dipeptidyl peptidase IV (DPP4, EC 3.4.14.5; CD) is a highly specific and multifunctional type II transmembrane serine peptidase which is widely distributed in mammalian tissues and found abundantly in the kidney, liver, and intestinal epithelium.[1] DPP4 effects dipeptide cleavage from the N-terminus of peptide substrates with either proline or alanine at the penultimate position.[2] Among the peptidic substrates cleaved by DPP4 are several incretin hormones of physiological relevance to the regulation of metabolic processes. These incretins include glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are released from the gastrointestinal tract in response to nutrient ingestion, and which function to enhance glucose-stimulated insulin secretion through activation of signaling pathways via their respective receptors. However, GLP-1 and GIP are deactivated by N-terminal cleavage by DPP4. Hence, blocking GLP-1 and GIP degradation via inhibition of DPP4 activity represents a potentially valuable means of treatment of type 2 diabetes.[3] Several DPP4 inhibitors have advanced into clinic trials, including P32/98,[4] NVP-DPP728,[5a,b] NVP-LAF237,[5c] and MK-0431[6] (Fig. 1). Clinical results with P32/98 helped to provide proof of principle for the application of DPP4 inhibitors as glucose lowering agents.[7] Additionally, 4-week placebo-controlled clinical studies of NVP-DPP728 and NVP-LAF237, where significant reductions in glycosylated hemoglobin (HbA1c), fasting glucose and maximum prandial peak

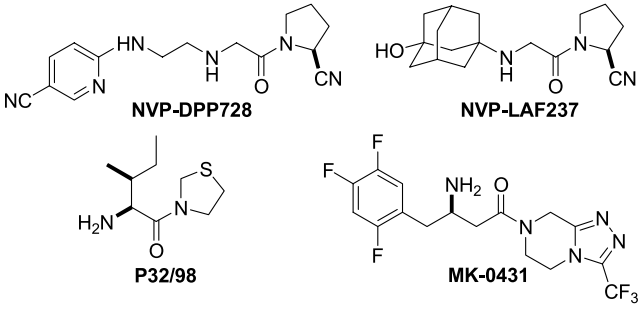

Figure 1. Selected clinically studied DPP4 inhibitors.

Keywords: Diprolyl nitriles; Dipeptidyl peptidase IV.
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glucose excursions were observed in diabetic patients, lend further support to the utility of DPP4 inhibition in the treatment of type 2 diabetes.[8]

Small molecule inhibitors of DPP4 have been extensively studied for nearly two decades.[9] The majority of inhibitors reported to date share common structural features and resemble the N-terminal dipeptide residues of the enzymatic substrates: a penultimate N-terminal proline mimic joined to an additional amino acid or similar surrogate in the P2 position through an acyl linkage. A common structural motif in DPP4 inhibitor design has been the presence of an L-amino acid with a protonatable N-terminal primary amine or the equivalent N-substituted glycine occupying the P2 position.

Initial reports by Ashworth et al.[10] demonstrated that simple prolyl–prolyl nitriles were effective inhibitors of DPP4. No further structure–activity relationships (SARs) in this series were reported, suggesting an opportunity for further investigation with cyclic L-amino acids in the P2 position.[11] We were pleased to find that 4-cis-substituted L-prolines in the P2 position provided potent inhibition when linked to our previously disclosed P1 group 4,5-methano-pyrrolidine.[12] Herein, we report the synthesis and structure–activity relationships of cyclic amino acid P2 position DPP4 inhibitors leading to the discovery of compound **1** (Fig. 2).

The general synthetic route employed for the preparation of compounds **6–18** involved a three-step procedure (Scheme 1). Boc- or Fmoc-protected amino acids **2**[13] were coupled to *cis*-4,5-methano-L-prolineamide **3**,[12] followed by dehydration of the resulting amides to the corresponding nitriles **4** and deprotection of the prolyl nitrogen protecting group gave inhibitors **6–18**. The preparation of (*S*)-4-amino-(*S*)-prolyl-3,4-methanoproline nitriles **19**, **20**, and **1** involved further functionalization (Scheme 2). Fmoc intermediate **4** (for the synthesis of compound **18**) was deprotected using piperidine to give amine **5**. Respective acylation, sulfonylation, or alkylation of this common intermediate, followed by subsequent Boc group deprotection, provided compounds **19**, **20**, and **1** in good yields.

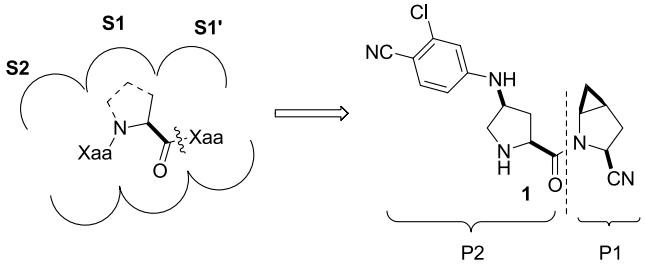
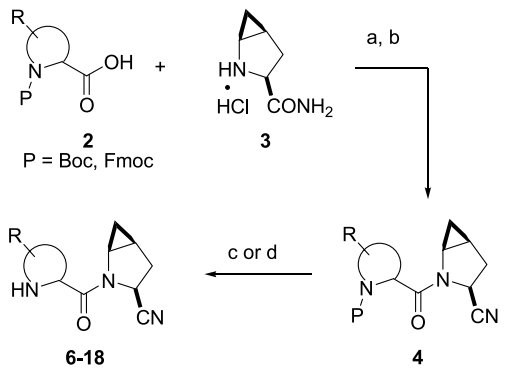


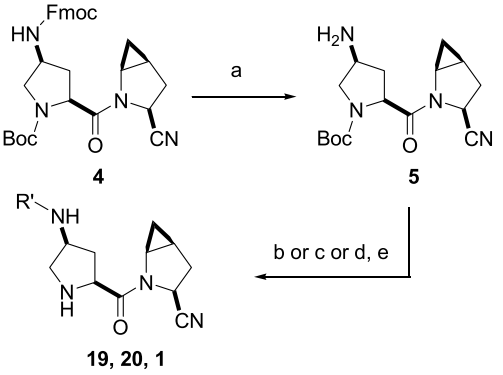
Figure 2. Cyclic P2 position analysis leading to inhibitor **1**.



Scheme 1. Reagents: (a) EDC, HOBT, *i*-Pr_2EtN, CH_2Cl_2; (b) $POCl_3$, pyridine; (c) TFA, CH_2Cl_2; (d) piperidine, CH_2Cl_2.

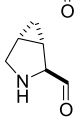
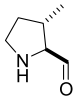
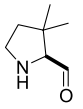
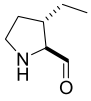
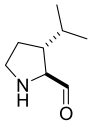
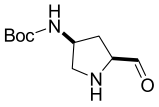
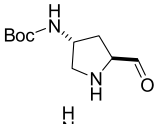
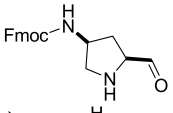
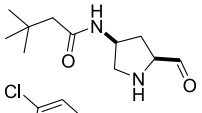
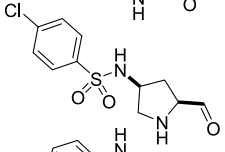
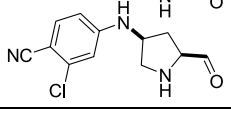
As an initial approach to develop more potent inhibitors of DPP4, the effect of ring size of cyclic amino acids was surveyed (compounds **6–9**, Table 1). The results demonstrated exquisite sensitivity to this parameter, and suggested that a five-membered ring in the P2 position was greatly favored over either a four-membered or six-membered ring (compare piperidinyl analogue **6** and azetidinyl analogue **9** with prolyl analogue **7**). Moreover, the L-prolyl analogue **7** (K_i = 305 nM) was more potent than the corresponding D-prolyl analogue **8**, demonstrating that the L-cyclic amino acid is preferred in the S2 pocket of the enzyme, following the same trend observed for acyclic amino acids at this site.[9] Interestingly, methano fused prolyl analogues **10** and **11** showed very different activities, with the 3,4-methano fused prolyl **10** (K_i = 52 nM) exhibiting much greater potency than the weakly active 4,5-methano fused prolyl analogue **11**. This finding suggested that the 3- and 4-positions on the proline ring are very sensitive to structural changes and might be worthy of further exploration to improve activity.

We began by examining the effects of proline ring substitutions, probing a series of 3-position analogues **12–15** for DPP4 activity. Compound **12** (*trans*-methyl at the 3-position) showed a slight improvement in activity over the unadorned proline P2 compound **7**, and geminal dimethyl substitution at this position (**13**) further enhanced activity. The *trans*-ethyl analogue **14** exhibited an equal potency relative to proline analogue **7**, while the *trans*-isopropyl analogue **15** was 2-fold less potent. This inverse correlation of potency with substituent size perhaps reflects the strict limits of steric accommodation in this region of the binding pocket.



Scheme 2. Reagents: (a) Piperidine, CH_2Cl_2; (b) *t*-butylacetyl chloride, CH_2Cl_2; (c) *p*-chlorophenylsulfonyl chloride, CH_2Cl_2; (d) 2-chloro-4-flouro-benzonitrile, *i*-Pr_2EtN; (e) TFA, CH_2Cl_2.

Table 1. DPP4 inhibition for compounds **1** and **6–20**

Compound	P2 unit	DPP4 K_i, nM
6		>10,000
7		305 ± 21
8		>10,000
9		>10,000
10		52 ± 5
11		>10,000
12		126 ± 14
13		37 ± 4
14		290 ± 23
15		695 ± 36
16		140 ± 17
17		492 ± 61
18		57 ± 16
19		133 ± 5
20		39 ± 1
1		3.6 ± 0.3

We then switched our emphasis to the examination of 4-amino substitution of the P2 L-proline. We first attempted to identify the favored stereochemistry by using a Boc-protected amino group as a probe. The cis analogue **16** was found to be 3-fold more potent than trans analogue **17**, demonstrating that a cis substituent is favored at the 4-position of proline, and that reasonably bulky groups could be tolerated. Replacement of the Boc-amino group with the isosteric *t*-butylacetamido group (**19**) did not affect activity. However, the larger Fmoc-amino substituent (**18**) enhanced activity, implying that aromatic substituents may be preferred. Targeted exploration of aryl moieties which had proven effective at enhancing binding interactions in the N-substituted glycine P2 series reported by Villhauer et al.[5c] led us to explore the *para*-chlorophenylsulfonamido (**20**) and 3-chloro-4-cyanophenylamino (**1**) analogues. Compound **20** provided approximately 8-fold improvement relative to the unsubstituted proline analogue **7.** More strikingly, compound **1**, with a K_i of 3.6 nM, exhibited nearly a 100-fold increase in potency. This series of compounds had comparable potency with other 4,5-methano-pyrrolidine DPP4 inhibitors. However, these compounds showed shorter half lives in solution stability study, exemplified by compound **1** ($t_{1/2}$ = 15 h at 37 °C and 10 μM in pH 7.0 buffer).[12]

The results shown here, that 3-, and in particular, 4-substituted proline P2 groups exhibit potent DPP4 inhibitory activity, are further understood by modeling these inhibitors in the DPP4 active site.[14] One potential binding mode for the L-prolyl-proline compound **7** in the DPP4 active site shows the cyanopyrrolidine portion of the ligand partially obscured by the protein surface (Fig. 3). The model suggests that substituents at the 4-position have the best opportunity to fill the S2 pocket. While both cis and trans substituents appear feasible, the size and nature of trans substituents may be more limited due to their proximity to the base of the S2 pocket. The prolyl substituents have the potential to favorably interact with residues that line the S2 pocket, including Arg125, Ser209, Phe357, Arg358, Tyr547, and Tyr666. These residues are primarily but not exclusively hydrophobic with strong aromatic character,

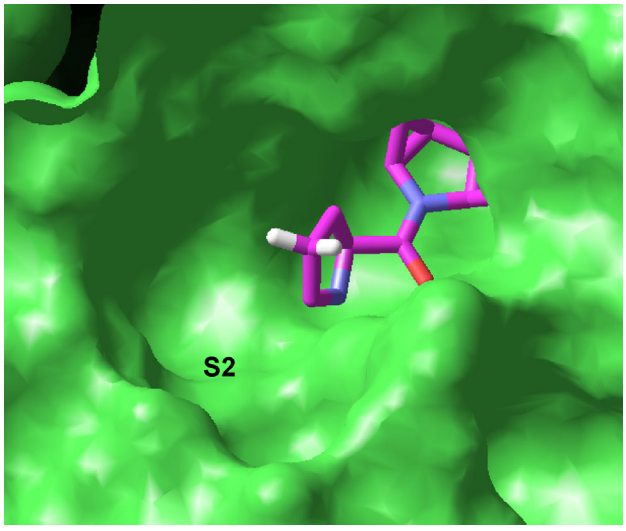


Figure 3. Compound **7** modeled in the DPP4 active site; the S2 pocket is labeled.

indicating that complementary hydrophobic and/or aromatic substitutions on the proline should be preferred.

We have synthesized and studied the SAR of a series of cyclic P2 amino acid 4,5-methanoprolinenitrile DPP4 inhibitors. The results demonstrate that the inhibitory activity is greatly enhanced in the five-membered ring cyclic amino acid (proline) relative to either the four-membered or six-membered ring analogues, and that the L-cyclic amino acid is the preferred stereoisomer to bind in the S2 pocket of the enzyme. Most importantly, the 4-position of the P2 proline can comfortably accommodate cis substituents, and aromatic groups in this region significantly enhance DPP4 inhibitory potency. From this work, compound **1** has been identified as a highly potent inhibitor of DPP4.

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13. For the synthesis of compounds **2** leading to inhibitors **14** and **15**, see Tiba, O.; Overberger, C. G. *J. Polym. Sci. Polym. Chem.* **1987**, *25*, 3437.
14. The binding model shown was obtained by sampling conformations of L-prolyl-proline in the gas phase using the Monte Carlo tool in Macromodel v. 8.1031 (Schrodinger, Inc.) and minimizing representative structures in AMPAC v. 6.55 (Semichem, Inc.) with the SAM1 Hamiltonian. Two low energy conformations were obtained, distinguished by rotation of the P2 pyrrolidine. The structures were overlaid on the bound ligand of pdb structure 1N1M (Ref. 15) based on the common acylpyrrolidine substructure; one conformation was discarded due to van der Waals clashes with the protein in the S2 pocket; the remaining conformation (depicted in Fig. 3) places the charged amino group close to that of the 1N1M ligand. The figure was created with Maestro v. 7.0.110 (Schrodinger, Inc.) using a surface probe radius of 1.8 Å. The protein surface is colored green; carbons in the ligand are colored magenta, oxygens are red, and nitrogens are blue. The hydrogens on C4 are shown explicitly in white; all other hydrogens are hidden.
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