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Potent non-nitrile dipeptidic dipeptidyl peptidase IV inhibitors

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Abstract—The synthesis and structure–activity relationships of novel dipeptidyl peptidase IV inhibitors replacing the classical cyanopyrrolidine P1 group with other small nitrogen heterocycles are described. A unique potency enhancement was achieved with β-branched natural and unnatural amino acids, particularly adamantylglycines, linked to a (2*S*,3*R*)-2,3-methanopyrrolidine based scaffold.

Dipeptidyl peptidase IV (DPP-IV) is an exopeptidase ubiquitously expressed in mammalian tissues, specifically on epithelial and endothelial cells and lymphocytes, which specifically cleaves dipeptides from the amino terminus of peptide substrates with proline or alanine at the penultimate position.[1] DPP-IV is responsible for the degradation of several important incretin hormones, most notably the gut hormone glucagon-like peptide-1 (GLP-1) which is released post-prandially from the L-cells of the intestine, and acts to potentiate glucose-stimulated insulin secretion resulting in the lowering of plasma glucose.[2] Due to DPP-IV's actions, the circulating half-life of GLP-1 is <90 s. Several DPP-IV inhibitors have reached late stages of clinical development (Fig. 1), including the dipeptidic inhibitors vildagliptin[3] and saxagliptin,[4] and the non-peptidic, structurally novel sitagliptin[5] and alogliptin.[6] Robust antidiabetic efficacy has been demonstrated clinically with DPP-IV inhibitors, and the most advanced compound has recently gained FDA approval for treatment of type 2 diabetes.[7]

Although multiple distinct chemical classes of DPP-IV inhibitors have been disclosed spanning diverse structural types,[8] some of the most potent compounds to date are those containing a proline mimetic cyanopyrrolidine P1 group.[9] This enhanced potency is thought to be due in part to a transient covalent trapping of the active site Ser630 hydroxyl of DPP-IV by the nitrile group, resulting in delayed dissociation kinetics and slow-tight binding of certain inhibitors.[10]

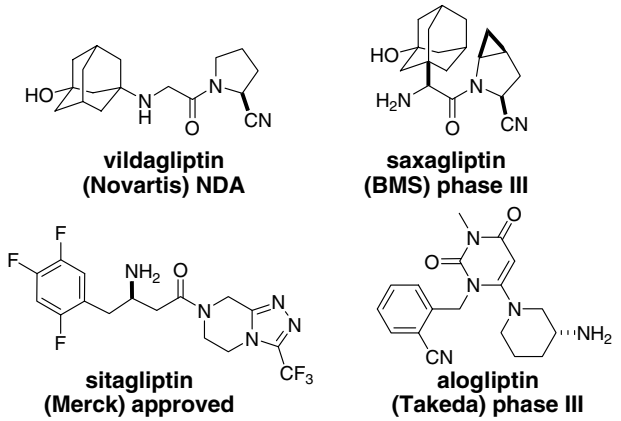

Figure 1. Clinically advanced DPP-IV inhibitors.

Along with this potency enhancement, chemical stability issues had been noted with early generations of nitrile-based inhibitors. While these issues were largely resolved

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with more advanced molecules, we sought to understand whether adequate potency might be achieved in a dipeptidic inhibitor without a serine trap in order to obviate this potential chemical stability issue altogether. These efforts were initially both guided and tempered by two fundamental assumptions derived from extensive internal and external structure–activity relationship (SAR) analysis: (1) that very strict steric constraints exist around the pyrrolidine ring of cyanopyrrolidide-based inhibitors, with only hydrogen,[11] fluoro,[12] acetylene,[13] nitrile,[14] or methano[15] substitution permitted; and (2) that presence of a nitrile moiety on the pyrrolidine ring is critical to achieving potent activity. The overall strategy we pursued involved exploration of both P2 and P1 residues of the dipetide mimics lacking a prolinenitrile moiety.

The presumption that the presence of a nitrile group is critical to achieving potent DPP-IV inhibition in pyrrolidine-derived P1 containing inhibitors is based on known thiazolidine-based inhibitors such as isoleucine thiazolidide (P32/98, K_i = 126 nM)[16] and simple pyrrolidine-based inhibitors such as valine pyrrolidide (K_i = 470 nM)[17] and fluoropyrrolidides,[18] which exhibit considerably weaker (typically >10-fold) potency than their respective cyanopyrrolidine analogues. Furthermore, the des-cyano analogue of NVP-DPP728 was reported to have very weak potency (K_i = 15.6 μM).[19] These inhibitors also lack slow-binding kinetic properties, as an additional consequence of their reduced affinity. Nonetheless, valine pyrrolidide was found to potentiate plasma levels of active GLP-1 and insulin in response to glucose,[20] and isoleucine thiazolidide was shown to improve glucose tolerance in obese Zucker rats.[21] Studies with these DPP-IV inhibitor series showed a preference for β-branched L-amino acids for improved potency. We sought to probe the validity of this assumption regarding a requirement for a nitrile moiety for potent inhibition, and to evaluate whether DPP-IV inhibitors possessing bulkier P2 groups which more fully fill the S2 pocket of the enzyme (proven to result in potency enhancements in the nitrile series, such as with saxagliptin) might experience enhanced potency with simple P1 groups. A partial list of those non-nitrile containing proline surrogates evaluated in this study is shown in Figure 2.

Proline surrogates **1**–**5** are available commercially. Methanopyrrolidines **6**–**8** were prepared as follows. 3,4-Methanopyrrolidine **6** was prepared according to a known procedure (Scheme 1).[22] Thus, ethyl chloroacetate (**9**) and ethyl acrylate (**10**) were reacted in the presence of NaH to give the diethyl cyclopropanedicarboxylate **11**. Saponification with NaOH afforded the corresponding

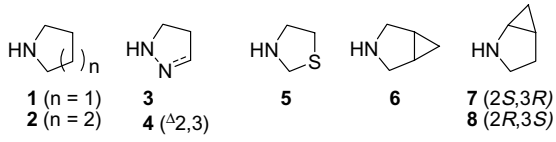
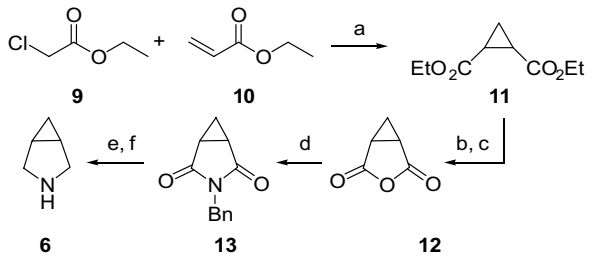


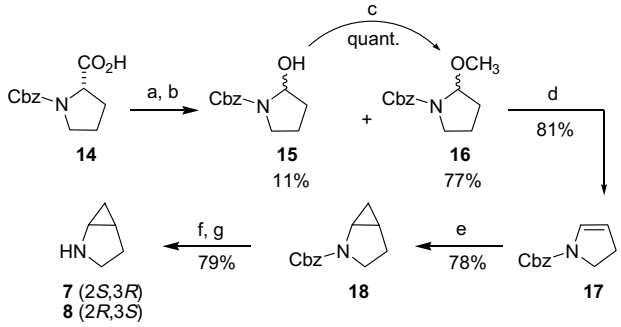
Figure 2. P1 Proline surrogates used in synthesis of non-nitrile DPP-IV inhibitors.



Scheme 1. Reagents and conditions: (a) NaH, toluene, rt, 2.5 h, 35 °C, 1.5 h; (b) NaOH, reflux, 8–10 h; (c) Ac_2O, reflux 40 min; (d) $BnNH_2$, toluene, 180 °C, 1.0 h, 150 °C, 20 h; (e) Red-Al, Et_2O, 0 °C, 70 min, reflux 3 h, then rt, o.n.; (f) i—10% Pd/C, CH_3OH, HOAc, 40 psi, 4 d, ii—4.0 N HCl/dioxane.

diacid which was converted to the methanosuccinic anhydride **12** by heating in Ac_2O. Reaction of **12** with benzylamine in toluene gave the corresponding benzyl azabicyclohexane-2,4-dione **13**. Finally, reduction of the imide with Red-Al followed by catalytic hydrogenation in the presence of 10% Pd/C effected N-debenzylation to give the desired 3,4-methanoproline **8** which was obtained as the HCl salt by filtering the methanolic solution directly into a solution of 4.0 N HCl in dioxane.

The initial syntheses of enantiomeric 2,3-methanopyrrolidines **7** and **8** utilized a stereorandom construction of a racemic 2,3-methanopyrrolidine, which was subsequently coupled to a suitably protected homochiral amino acid prior to resolution at the analogue stage (Scheme 2). Commercially available Cbz-protected L-proline (**14**) was oxidatively decarboxylated by treatment with iodobenzene diacetate and elemental iodine in CH_2Cl_2, followed by stirring in methanol to provide the racemic protected 2-methoxypyrrolidine **16** in 77% yield, along with the corresponding hydroxy product **15** (11%). The hydroxy product could be recycled by quantitative conversion to the desired methoxy compound **16** by treatment with pyridinium *p*-toluene sulfonate (PPTS) in MeOH. Dehydration of methoxy compound **16** was achieved by treatment with Hunig's base and TMSOTf to give protected dihydropyrrole **17** in 81% yield. Standard cyclopropanation conditions



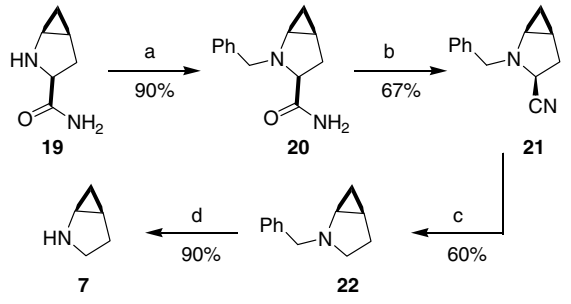
Scheme 2. Reagents and conditions: (a) iodobenzene diacetate, I_2, CH_2Cl_2, rt; (b) MeOH, rt; (c) PPTS, MeOH, rt, 20 h; (d) TMSOTf, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 0 °C; (e) diethylzinc, $ClCH_2I$, Et_2O, 0 °C to rt; (f) H_2, 10% Pd/C, HCl, EtOH; (g) chiral HPLC resolution.

(diethylzinc, chloroiodomethane) to give the methano product **18**, followed by deprotection of the Cbz group under acidic conditions, afforded the racemic 2,3-methanopyrrolidine **7/8** as the corresponding HCl salt in 62% overall yield for the two steps.

In a second generation synthesis, the desired 2*S*,3*R*-stereoisomer **7** could be obtained in optically pure form by a formal deamidation of a key intermediate used in the preparation of saxagliptin (Scheme 3). Beginning with L-4,5-methanoprolinamide (**19**),[15] protection of the proline nitrogen was accomplished using benzyl bromide and Hunig's base in CH_2Cl_2 to give intermediate **20** in 90% yield. Dehydration of the amide to the corresponding nitrile was achieved using TFAA and triethylamine in CH_2Cl_2 to give cyano compound **21** in 67% yield. Reductive removal of the cyano group by treatment with $NaBH_4$ in aqueous ethanol afforded benzyl protected methanopyrrolidine **22** in 60% yield. Removal of the benzyl protecting group was accomplished by treatment with α-chloroethyl acetyl chloride (ACE-Cl) in refluxing CH_2Cl_2 to give the desired (2*S*,3*R*)-2,3-methanopyrrolidine **7** in optically pure form as the HCl salt in 90% yield.

The series of dipeptides in the present study were then prepared via standard peptide coupling (PyBOP/NMO or EDAC/HOBT/DMAP) of the appropriate P1 proline surrogate with the various Boc-protected P2 L-amino acids. Subsequent removal of the Boc-protecting group with TFA in CH_2Cl_2 or HCl in dioxane afforded inhibitors **28**–**40** as their corresponding TFA or HCl salts.[23] All compounds were tested in vitro against purified human DPP-IV under steady state conditions with gly-pro-*p*-nitroanilide as substrate as previously described (Table 1).[4]

We systematically examined the influence of both P1 and P2 moiety contributions to DPP-IV inhibitory potency, beginning with a survey of both natural and unnatural amino acids in the P2 position, while fixing the P1 subunit as the homochiral des-cyano methanopyrrolidine (**7**) corresponding to saxagliptin. As shown previously for nitrile containing inhibitors, P2 amino acids with aryl (**23**–**28**) or polar (**29**–**36**) side-chains failed to exhibit any appreciable DPP-IV inhibition



Scheme 3. Reagents and conditions: (a) benzyl bromide, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt; (b) trifluoroacetic acid anhydride, TEA, CH_2Cl_2, 0 °C; (c) $NaBH_4$, EtOH/H_2O, rt; (d) 1-chloroethyl chloroformate, CH_2Cl_2, reflux.

Table 1. Inhibition constants versus human DPP-IV for compounds **23**–**60**

Compound	P1	P2-Xaa[a]	DPP4 K_i[b] (nM)
Saxagliptin	2*S*-CN-7	3-HO-Ad-Gly	0.6 ± 0.06
23	7	Ph-Gly	>10,000
24	7	Phe	3653 ± 206
25	7	4-Cl-Phe	877 ± 286
26	7	His	>10,000
27	7	Tyr	3007 ± 180
28	7	Trp	>10,000
29	7	Asn	>10,000
30	7	*N*-Ac-Lys	>10,000
31	7	Orn	>10,000
32	7	Ser	>10,000
33	7	*O*-Me-Ser	>10,000
34	7	*O*-*t*-Bu-Ser	>10,000
35	7	homo-Ser	>10,000
36	7	Thr	>10,000
37	7	*n*-Bu-Gly	3257 ± 453
38	7	Leu	>10,000
39	7	Neopentyl-Gly	1010 ± 146
40	7	Val	1065 ± 485
41	7	Ile	530 ± 36
42	7	Allo-Ile	731 ± 76
43	7	*tert*-Leu	356 ± 68
44	8	*tert* -Leu	>10,000
45	3	*tert*-Leu	>10,000
46	7	β,β-di-*i*-Pr-Ala	112 ± 9
47	7	3,3,5,5-tetra-Me-Ch-Gly	152 ± 21
48	7	3-HO-Ad-Gly	10 ± 3
49	7	3,5-di-HO-Ad-Gly	14 ± 7
50	8	3-HO-Ad-Gly	1944 ± 334
51	1	3-HO-Ad-Gly	49 ± 8
52	4	3-HO-Ad-Gly	3311 ± 563
53	5	3-HO-Ad-Gly	28 ± 3
54	(±)-2-Me-1	3-HO-Ad-Gly	>10,000
55	Isoindole	3-HO-Ad-Gly	1420 ± 196
56	Indoline	3-HO-Ad-Gly	>10,000
57	6	3,5-di-HO-Ad-Gly	270 ± 58
58	7	5,7-di-Me-3-HO-Ad-Gly	2.9 ± 0.5
59	2	5,7-di-Me-3-HO-Ad-Gly	607 ± 27
60	7	*N*-(3-HO-Ad)Gly	3081 ± 790

[a] All P2 amino acids bear the natural L-configuration at the α-stereocenter.

[b] All K_i values are mean ± SD of at least triplicate determinations.

(K_is $\geqslant 1$ μM). Also consistent with findings in the analogous nitrile series, a strong potency dependence on β-branching in the P2 side-chain was revealed in comparison of those simple alkyl side-chains with and without β-branching (compare **37**–**39** vs. **40**–**43**). Increasing the steric bulk of the β-branched substituents gave only modest incremental enhancement of potency (**46**, **47**). In all of these cases, it appeared that potency versus the corresponding nitrile series suffered an approximately 20- to 50-fold loss.

Combining our optimized P2 group present in saxagliptin (3-hydroxyadamantylglycine) with the 2*S*,3*R*-methanopyrrolidine **7** gave compound **48**, with potency equivalent to that of some of the most active nitrile-containing inhibitors in the clinic (K_i = 10 nM); similar

potency was observed in the dihydroxy compound **49** (K_i = 14 nM). As was noted for the nitrile-containing series, a strong stereochemical preference was maintained for the methano bridge bearing the 2*S*,3*R*-configuration (compare **43** with **44** and **48** with **50**). The apparently unique potency enhancing properties of this bulky P2 unit are further demonstrated with simple pyrrolidine (**51**) and thiazolidine (**53**) P1 groups, though the 3- to 5-fold diminished potency compared with **48** serve to validate the role of the methano bridge in this precise regio- and stereochemical orientation (compare with **57**) in favorably contributing to DPP-IV binding affinity. It is noteworthy that strict steric constraints exist in the S1 pocket, such that even simple methyl substitution as in **54** essentially destroys all activity. The most potent compound in the series was obtained by packing further bulk into the S2 pocket (**58**, K_i = 2.9 nM), though a change as subtle as opening the bicyclic methanopyrrolidine to a piperidine (**59**) results in a 200-fold drop in potency. Interestingly, methanopyrrolidine analogues with N-linked substitution analogous to vildagliptin (**60**) failed to demonstrate any significant DPP-IV inhibitory activity.

The unique structural features imparted to inhibitors by the hydroxyadamantylglycine P2 group appear to be capable of conferring significant potency to non-nitrile compounds, though limited by the same narrow steric and stereochemical requirements shown for nitrile-containing inhibitors. Interestingly, several of the more potent analogues in this series have retained some slow binding kinetic properties, despite the lack of a nitrile (dissociation rate increases from 4.6×10^{-5}/s for saxagliptin to 2.0×10^{-3}/s for compound **48** at 25 °C, unpublished results). Compound **48** maintains potent and fully efficacious antihyperglycemic effects in rodent models and mirrors the PK and safety profiles of clinical lead compound saxagliptin, yet is incapable of undergoing degradative cyclization. As previously discussed, this inhibitor also shows uniquely potent inhibition relative to other non-cyano compounds, suggesting that the summation of contributions to the binding energy of this compound in the active site is largely dominated by the P2 hydroxyadamantylglycine moiety. These studies have shown the development of chemically more stable and potent DPP-IV inhibitors in the low nM range, specifically compounds **48**, **49** and **58**. Further studies examining in vivo pharmacological effects as well as biochemical and biophysical aspects of the binding interactions for these potent compounds will be the subject of forthcoming disclosures from these laboratories.

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23. Data for compound **48** (HCl salt): HPLC (Phenominex Luna 3 μ C18 4.6 × 150 mm, 95% A to 95% B (A = H_2O + 0.05% TFA, B = CH_3CN + 0.05% TFA, flow rate 1 mL/min, linear gradient over 42 min) retention time 13.37 min (97.9%); Chiral analytical HPLC (Chiralpak AD 10 μ 4.6 × 250 mm, 80% heptane + 20% 1:1 EtOH–MeOH + 0.1% DEA, flow rate 1 mL/min, isocratic) retention time 10.56 min (98.2% ee); LC/MS *m*/*z* 291 $[M+H]^+$; ^1H NMR (D_2O, 400 MHz) δ 4.16 (s, 1H), 3.82 (ddd, 1H, J = 13.2, 10.3, 2.9 Hz), 3.48 (td, 1H, J = 6.2, 2.6 Hz), 2.94 (dt, 1H, J = 13.1, 8.7 Hz), 2.14 (bs, 2H), 1.94–2.05 (m, 1H), 1.88 (ddd, 1H, J = 12.4, 8.4, 3.3 Hz), 1.74 (ddd, 1H, J = 8.8, 11.4, 5.2), 1.3–1.73 (m, 12H), 0.74–0.85 (m, 1H), 0.65–0.71 (td, 1H, J = 5.7, 2.6); ^{13}C NMR (D_2O, 100 MHz) δ 167.3, 69.1, 59.6, 45.3, 45.1, 43.1, 39.7, 38.2, 37.1, 36.8, 36.6, 34.5, 30.2, 30.1, 24.4, 18.9, 12.8; Anal. Calcd for $C_{18}H_{25}N_3O_3 \cdot 1.64\ HCl \cdot 1.33\ H_20$: C, 54.56; H, 8.16; N, 7.49; Cl, 15.57. Found: C, 54.42; H, 7.86; N, 7.35; Cl, 15.57. KF, 6.39.