1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(*S*)-pyrrolidinecarbonitrile: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties

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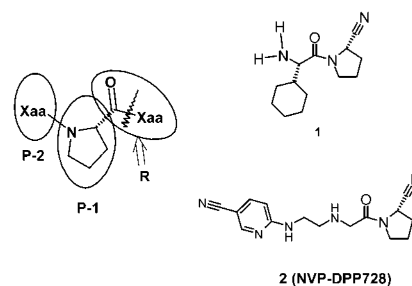
Received March 26, 2002

Abstract: Dipeptidyl peptidase IV (DPP-IV) inhibition has the potential to become a valuable therapy for type 2 diabetes. We report the first use of solid-phase synthesis in the discovery of a new DPP-IV inhibitor class and a solution-phase synthesis that is practical up to the multikilogram scale. One compound, NVP-DPP728 (**2**), is profiled as a potent, selective, and short-acting DPP-IV inhibitor that has excellent oral bioavailability and potent antihyperglycemic activity.

Introduction. Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5) is a ubiquitous yet highly specific serine protease that cleaves N-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position.[1] The biological activities of many circulating regulatory peptides are altered or abolished by the action of DPP-IV in vitro.[2] However, in part because of the multiplicity of enzymes exhibiting DPP-IV-like activity,[3] the in vivo role of DPP-IV in mediating the cleavage and determining the action of most substrates has yet to be established. One exception is with the incretin known as glucagon-like peptide-1 (GLP-1), the most potent insulinotropic hormone known.[4] Numerous studies with DPP-IV[5] and DPP-IV inhibitors[6−9] support a principal role of DPP-IV in the inactivation of GLP-1 in vivo. More importantly, the contribution of DPP-IV catalytic activity to blood glucose control through GLP-1 inactivation has recently been confirmed.[10] Because of multiple benefits of GLP-1 augmentation, DPP-IV inhibition has been recognized as a mechanistic approach of potential value in the treatment of type 2 diabetes.[11] By extending the duration of action of GLP-1, one would stimulate insulin secretion, inhibit glucagon release,[12] and slow gastric emptying;[13] each a benefit in the control of glucose homeostasis. DPP-IV inhibition, through the preservation of active GLP-1 levels, has the potential to slow or even prevent the progression of type 2 diabetes by stimulating insulin gene expression and biosynthesis, increasing the expression of the β-cell's glucose-sensing mechanism and promoting genes involved in the differentiation (neogenesis) of β-cells.[14] GLP-1 may play a role in acutely suppressing appetite in humans[15] and may play a role in mediating peripheral glucose uptake.[16] Since the blood glucose lowering effects of GLP-1 are dependent on elevated blood glucose and abate as glucose levels return to normal, the incidence of hypoglycemia during treatment with a DPP-IV inhibitor is expected to be very low.[17]

Chart 1



With few exceptions,[18−20] DPP-IV inhibitors resemble the P2−P1 dipeptidyl substrate cleavage product, where the P-1 site contains a proline mimic.[21] A straightforward replacement of the normally cleaved P-1 substrate amide (R in Chart 1) with an electrophile provides both irreversible (R = P(O)(OPh)$_2$, CO−NH−O−COR′) and reversible (R = B(OH)$_2$, H, CN) inhibitors.[22] Low nanomolar inhibition and chemical stability adequate for oral administration are obtained only with nitrile replacement of a substrate P-1 site amide (X$_{aa}$-(2*S*)-cyanopyrrolidines[23−25] and X$_{aa}$-(4*R*)-cyanothiazolidines[26]). Cyclohexylglycine-(2*S*)-cyanopyrrolidine **1** is one of the more potent, selective, and stable representatives of this nitrile class (K_i of 1.4 nM, >1000-fold selectivity over closely related peptidases, and $t_{1/2}$ stability of >48 h at pH 7.4).[24]

Until recently, a constant in DPP-IV inhibitor design had been an L-amino acid with a protonatable N-terminal primary amine in the P-2 site. Noticing that *N*-methylglycine was recognized in the substrate P-2 site,[21a] we were curious to investigate whether structurally more complicated N-substituted glycines would be tolerated at the P-2 site. We were gratified to find that a number of diverse P-2 site N-substituted glycines provided potent inhibition when combined with a (2*S*)-cyanopyrrolidide in the P-1 site (**8** in Scheme 1).[27] Intensive evaluation of this class[28] has led to the selection of the slow binding inhibitor **2** (NVP-DPP728)[29] as a clinical development candidate for type 2 diabetes. Herein, a tandem resin−solution parallel synthesis that led to the discovery of the P-2 site of **2** is described along with a solution-based method for multigram synthesis. Additionally, we report on the pharmacologic profile of this selective DPP-IV inhibitor, which exhibits excellent potency and oral bioavailability.

Chemistry. The preparation of a library of N-substituted 2-(*S*)-pyrrolidinecarbonitriles **8** has been carried out in a tandem five-step solid-phase and three step solution-phase sequence starting from commercially available Fmoc-protected Rink amide AM resin **4** as

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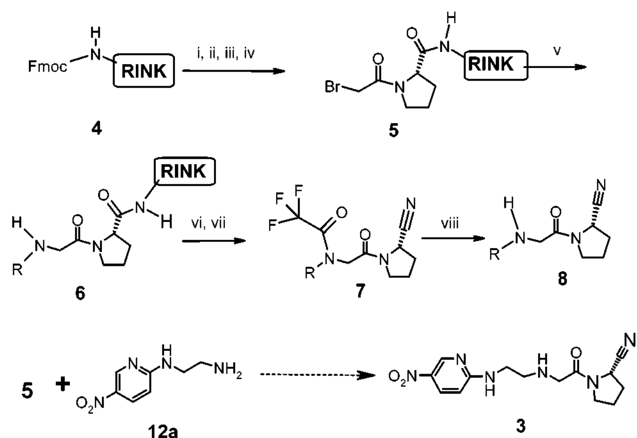
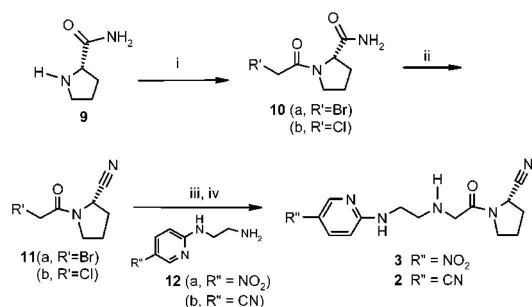
10.1021/jm025522z CCC: $22.00 © 2002 American Chemical Society
Published on Web 05/09/2002

Scheme 1[a]



[a] Reagents: (i) 20% piperidine/DMF; (ii) Fmoc-proline, DIC, DMF; (iii) 20% piperidine/DMF; (iv) $BrCH_2COOH$, DIC, DMF; (v) RNH_2, DMSO; (vi) 95% TFA/H_2O; (vii) TFAA, THF; (viii) NH_3/MeOH.

Scheme 2[a]



[a] Reagents: (i) $BrCH_2COBr$, Et_3N, CH_2Cl_2, and DMAP for **10a** while $ClCH_2COCl$, K_2CO_3, and THF for **10b**; (ii) **10a** for **11a** and **10b** for **11b**, TFAA, CH_2Cl_2; (iii) **11a**, THF, **12a** for **3** and **11b**, THF, **12b** for **2**; (iv) excess HCl/THF for di-HCl of **2** and di-HCl if **3** and 1 equiv of ethanolic HCl for mono-HCl of **2**.

described in Scheme 1.[30] Successive deprotection of **4** with piperidine, 1,3-diisopropylcarbodiimide (DIC) coupling with Fmoc-protected proline, deprotection with piperidine, and finally DIC coupling with bromoacetic acid provided the resin-bound α-bromoacetyl prolinamide **5**. Analogous to Zuckermann's synthesis for solid-phase peptoid libraries,[31] **5** was treated with a diverse array of over 200 primary aliphatic amines to provide a library of discreet, resin-bound N-substituted glycine-2-(*S*)-pyrrolidinecarboxamides (**6**). Trifluoroacetic acid (TFA) resin cleavage of **6** followed by amide dehydration with trifluoroacetic anhydride (TFAA) afforded N-substituted *N*-trifluoroacetylated-2-(*S*)-pyrrolidinecarbonitriles **7**. Deacetylation of **7** with ammonia in methanol provided the product library of N-substituted 2-(*S*)-pyrrolidinecarbonitriles (**8**) in a 1 to 1 mixture with trifluoroacetamide.[32] The commercially available 2-(2-aminoethylamino)-5-nitropyridine (**12a**) provided resin-derived **3** (IC_{50} of 20 ± 3 nM in the DPP-IV Caco-2 assay) as one of the few low nanomolar DPP-IV inhibitors from this library effort. Compound **3** served as our starting point for the structure–activity relationship (SAR) effort that led to the title compound **2**.[28] A solution-based preparation of **2** and **3** had been carried out in three steps beginning with L-prolinamide (**9**) as shown in Scheme 2. Coupling of **9** with either bromoacetyl bromide or chloroacetyl chloride provided **10a** or **10b**, respectively.

Table 1. DPP-IV Inhibition and Selectivity Assays[a]

	Caco-2[b]	rat plasma[b]	human plasma[b]	PPCE[c]	DPP-II[d]
1	2.0 ± 0.3	2.8 ± 0.2	3.2 ± 0.19	41 000 ± 14 000	102 000 ± 20 000
2	22.0 ± 2.0	6.0 ± 1.0	7.0 ± 1.7	190 000 ± 46 000	110 000 ± 5800
3	8.0 ± 3.0	17 ± 0.3	8.7 ± 0.8	16 000 ± 1200	12 000 ± 580

[a] Values are IC_{50} (nM) expressed as the mean ± SD of three independent determinations. Procedures are described in Supporting Information. [b] Primary DPP-IV assays. [c] Extract from human erythrocytes. [d] Extract from bovine kidney homogenate.

Amide dehydration of **10a** and **10b** with trifluoroacetic anhydride produced **11a** and **11b** as solids that were stable for months at room temperature. Coupling of bromide **11a** with an excess of **12a** provided **3**, which was isolated as the dihydrochloride salt. The coupling of commercially available 5-cyano-2-chloropyridine with excess ethylenediamine provided **12b**. Reaction of **11b** with excess **12b** provided **2**, which was isolated as either the mono- or the dihydrochloride salt. The monohydrochloride **2** possessed a solubility of >100 mg/mL in distilled water and crystallized as a hemihydrate trans-amide rotomer with (*S*) chirality, as evidenced by the X-ray crystallographic analysis.[33] In solution, **2** was a mixture of cis- and trans-amide rotomers according to NMR. With minor modifications, the present solution synthesis has provided **2** on the 100 kg scale.

Results and Discussion. Compounds **1**–**3** were evaluated in vitro for their inhibition of DPP-IV extracted from Caco2 cells as well as from rat and human plasma (Table 1). Since under neutral and basic aqueous conditions the P-2 site amine can nucleophilically attack the carbon of the pyrrolidide-nitrile to form an inactive cyclic amidine,[28] the stability of **2** was examined under assay conditions. Under the assay conditions employed, this intramolecular cyclization was slow ($t_{1/2} > 2$ days), resulting in less than 1% of **2** converting during the time frame of the experiment. As shown in Table 1, compound **2** potently inhibited both human and rat plasma DPP-IV and human epithelial cell-surface DPP-IV (IC_{50} = 7, 6, and 22 nM, respectively). Also, **2** was highly selective for DPP-IV over closely related peptidases, post-proline-cleaving enzyme (PPCE) and DPP-II[34] (Table 1). In addition, the in vitro specificity of **2** was profiled in over 100 receptor and enzyme assays and no significant binding was observed (10 μM).

In vivo evaluation of **2** in rat[9] and human[35] has supported the connection between DPP-IV inhibition and an improvement in oral glucose tolerance through an increase in active GLP-1 levels. We also found that **2** rapidly and effectively improved the metabolic profile in nonhuman primates. Oral administration of **2** (1 μmol/kg) significantly reduced plasma glucose levels (38% reduction in the 0–90 min glucose AUC, $p < 0.05$) in cynomologus monkey compared to the control during an oral glucose tolerance test (OGTT) (Figure 1). Additionally, peak glucose levels are significantly reduced in treated animals compared to control (98 ± 4 vs 88 ± 3 mg/dL, $p < 0.05$). When administered 30 min before an OGTT study, **2** maximally inhibited plasma DPP-IV activity (89%) 25 min postdose and provided a ≥70% DPP-IV inhibition throughout the study.

Pharmacokinetic evaluation of **2** was performed in male Sprague–Dawley rats and male cynomolgus mon-

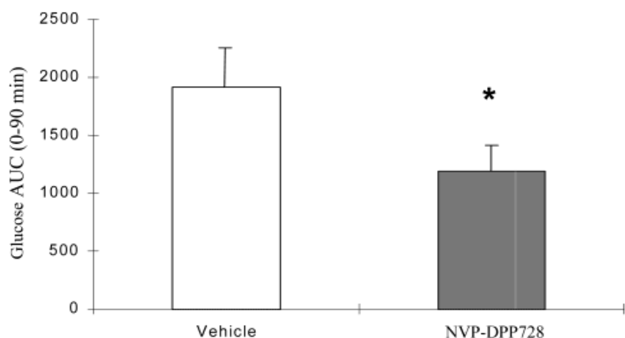


Figure 1. Incremental area under the glucose curve (from 0 to 90 min) during oral glucose tolerance tests (OGTTs) performed in seven anesthetized cynomolgus monkeys following oral administration of vehicle or NVP-DPP728 (**2**) (1 μmol/kg, po), mean ± SEM, (*) $p < 0.05$. Vehicle is 0.5% carboxymethylcellulose in 0.2% Tween 80. Experimental procedure is detailed in Supporting Information.

keys. After an oral dose of 10 μmol/kg, C_{max} was 3.65 μM in rat and 7.56 μM in monkey. Absolute bioavailability was high in both rat and monkey at ≥74%. The steady-state volumes of distribution are similar in rat (670 mL/kg) and monkey (841 mL/kg), suggesting that **2** is distributed principally in the body fluids. Clearance of **2** from plasma is moderate at about 28 ± 1.5 mL min^{-1} kg^{-1} in the rat and 21.9 ± 3.1 mL min^{-1} kg^{-1} in the monkey. After an oral dose in monkey of 1 μmol/kg, **2** provided a half-life of 0.85 h and inhibited plasma DPP-IV activity by >50% for 4 h. A 100 mg oral dose of **2** in humans provided a similar half-live of 0.85 h, a >80% inhibition of plasma DPP-IV activity for ~4 h, a significant increase in active GLP-1 levels, and an improvement in metabolic control.[35] As a reversible DPP-IV inhibitor possessing a relatively short half-life, **2** might most effectively be taken with a meal when GLP-1 secretion is at its maximal rate.

In summary, we report here the use of combined solid-phase and solution-phase chemistry to discover a potent, selective, and short-duration DPP-IV inhibitor that also has excellent oral bioavailability. The favorable pharmacokinetic profile for NVP-DPP728 (**2**) led to the selection of this compound for further study in a clinical setting for type 2 diabetes.[35] Profiling of this new class of DPP-IV inhibitors is under study and will be reported in due course.

Acknowledgment. The authors thank Dr. Phillip E. Fanwick from Purdue University for conducting the X-ray crystallographic analysis of the monohydrochloride of **2**. The authors thank Prasad Kapa and Eric M. Loeser for crystalline monohydrochloride of **2**, Börk Balkan, Elina Dunn, Elizabeth D. Graham, Brenda H. Hamilton, Lori A. Kwasnik, Xue Li, Robert H. Tullman, Michele Valentin, Kathy Ramos, and R. Eric Walter for their assistance in the in vivo analysis of **2**, and Robert C. Anderson and Philip A. Bell for helpful discussions.

Supporting Information Available: Experimental procedures including characterization data for all compounds, biological methods, ORTEP drawing, and atomic coordinate information for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM025522Z