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Discovery, SAR, and X-ray structure of novel biaryl-based dipeptidyl peptidase IV inhibitors

Lei Qiao, Christian A. Baumann, Carl S. Crysler, Nisha S. Ninan, Marta C. Abad, John C. Spurlino, Renee L. DesJarlais, Jukka Kervinen, Mike P. Neeper, Shariff S. Bayoumy, Robyn Williams, Ingrid C. Deckman, Malini Dasgupta, Rolanda L. Reed, Norman D. Huebert, Bruce E. Tomczuk and Kevin J. Moriarty*

Johnson & Johnson Pharmaceutical Research and Development, 665 Stockton Drive, Exton, PA 19341, USA

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Abstract—The discovery, SAR, and X-ray crystal structure of novel biarylaminoacyl-(*S*)-2-cyano-pyrrolidines and biarylaminoacyl-thiazolidines as potent inhibitors of dipeptidyl peptidase IV (DPP IV) are reported.

Glucagon-like peptide-1 (GLP-1) stimulates glucose-dependent insulin secretion, inhibits hepatic glucose production, lowers blood glucose levels, and promotes the growth and differentiation of β-cells. GLP-1 may also play a role in suppressing appetite in humans and in modulating peripheral glucose uptake.[1] The hormone's activity is rapidly abolished by the N-terminal cleavage of the peptide by the aminopeptidase dipeptidyl peptidase IV (DPP IV, CD26, EC 3.4.14.5), a sequence-specific non-classical serine protease.[2] Both mice and rats genetically deficient in DPP IV have shown enhanced insulin secretion and accelerated clearance of blood glucose partly due to increased levels of active GLP-1.[3]

Since Type 2 diabetes is a disease characterized by elevated blood glucose levels and a relative insufficiency of insulin, a therapeutic agent that extends the duration of GLP-1 action may aid in controlling glucose homeostasis, by enhancing β-cell glucose-stimulated insulin release and promoting insulin gene expression and its biosynthesis.[4] In addition, elevation of GLP-1 levels may increase β-cell proliferation and survival. Thus, DPP IV inhibition has the potential to be a promising new therapy for elevating plasma GLP-1 levels and the treatment of Type 2 diabetes.[5]

Most of the small molecule inhibitors of DPP IV reported in the literature have a 1° or 2° amine at the P2 site with the P1 site occupied by a proline mimetic (Fig. 1).[6] The most active inhibitors incorporate an electrophilic group (e.g., $P(O)(OPh)_2$, CO–NH–O–COR′,

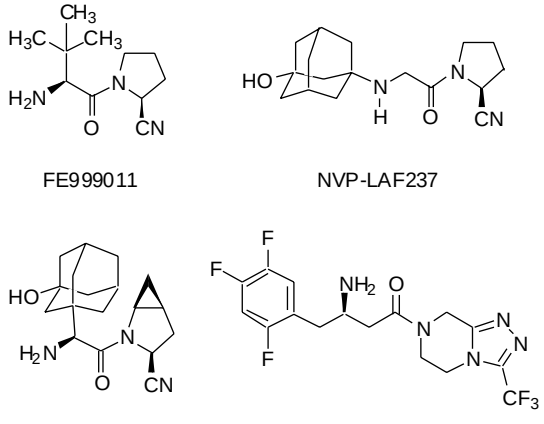

Figure 1. Selected inhibitors of DPP IV.

Keywords: Dipeptidyl peptidase IV Inhibitors; SAR; X-ray structure.
* Corresponding author. Tel.: +1 610 458 5264x6592; fax: +1 610 458 8249; e-mail: kmoriart@prdus.jnj.com

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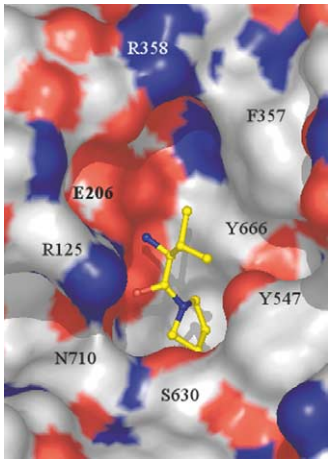
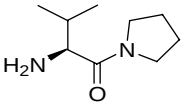
$B(OH)_2$, or CN) as a replacement for the normally cleaved P1–P1′ substrate amide on the proline at P1 and providing a reactive center to interact with the DPP IV active site serine residue. Low nanomolar inhibition and chemical stability adequate for oral administration have been obtained with the nitrile containing inhibitors.[6c–e,7] Recently inhibitors lacking an electrophilic group with good activity have also appeared in the literature.[6f,8]

Our goal was to identify non-nitrile inhibitors with good potency and ADME characteristics. Optimizing the P2 interactions could allow for the removal of the electrophilic group and still retain acceptable characteristics. In this paper, we report the preparation of a series of P2 optimized nitrile containing biaryl DPP IV inhibitors. The SAR from this series was used to identify non-nitrile inhibitors with low nanomolar potency and good pharmacological properties. In addition, the X-ray crystal structure of DPP IV with inhibitor 1-[2-(*S*)-amino-3-biphenyl-4-yl-propionyl]-pyrrolidine-2-(*S*)-carbonitrile bound has been determined, revealing the nature of the covalent interaction of the nitrile group with the active-site serine.

Analysis of the published crystal structure[9] of human DPP IV with valine pyrrolidide bound in the active site (Fig. 2, PDB ID:1N1M) revealed a linear hydrophobic pocket at the S2 subsite formed by the side chains of Phe357 and Arg358. We envisioned that filling this pocket with appropriate groups would lead to more potent inhibitors and potentially allow for the removal of the nitrile moiety at P1.[10]

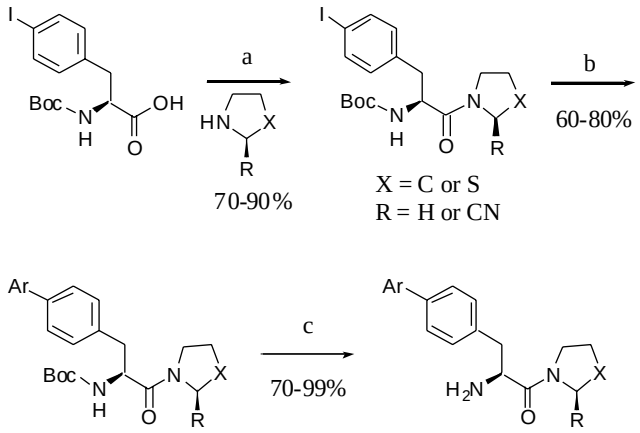
The general synthetic route to the biaryl-based inhibitors is shown in Scheme 1. The commercially available





Ki = 2μM

Figure 2. Space filling picture of DPP IV in complex with valine pyrrolidide. PDB ID is 1N1M. Inhibitor is shown in yellow with nitrogen colored blue and oxygen colored red.

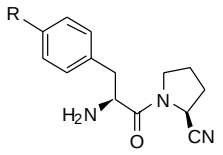


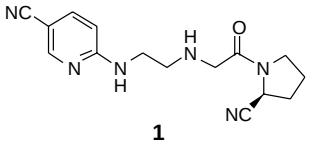
Scheme 1. Synthesis of biaryl-based DPP IV inhibitors. Reagents: (a) EDCI, HOBt, DIEA, CH_2Cl_2; (b) $ArB(OH)_2$, $Pd(OAc)_2$, Na_2CO_3; (c) TFA, CH_2Cl_2, thioanisole.

Boc-L-4-iodophenylalanine was coupled to the proline mimetic using EDCI and HOBt in CH_2Cl_2 to give the 4-iodophenylalanine derivatives in 70–90% yields. The iodo-compounds were then converted to the biaryls using a $Pd(OAc)_2$ catalyzed Suzuki reaction[11] (60–80%). Removal of the Boc-protecting group with trifluoroacetic acid in the presence of thioanisole gave final products in 70–99% yields.

A survey of the S2 site was undertaken using the 2-(*S*)-cyano-pyrrolidine at the S1 as our initial probe with the intention of extrapolating the findings of this study to non-nitrile analogs (Table 1). Entries **2**–**5** indicated that non-polar groups are favored at the 4-position of phenylalanine moiety, giving potencies similar to the reference NVP-DPP728 (entry **1**).[12] Placement of a carboxylic acid moiety at the 4-position of the phenylalanine (entry **4**) resulted in a 7-fold loss of potency compared to the known[7b] compound entry **2**. However, addition of a phenyl ring at the 4-position (entry **5**) resulted in a 5-fold improvement in activity. Substitution on the terminal phenyl ring was then explored.[13] Substitution with bulky or electron-donating groups, for example, 4-methyl or 3,4-dimethoxy groups (entries **6** and **7**), gave less active compounds compared with the unsubstituted entry **5**. However, when fluorine was placed to the 4-position (entry **8**), a 4-fold improvement in activity was observed. This increase in activity was attributed to a better fit in the targeted lipophilic pocket and a stronger edge-to-face π–π interaction between the middle ring of the biphenyl group and the Phe357. Moving the fluorine to the 2-position of the terminal phenyl ring (entry **9**) was also tolerated. Di-substitution with fluorine at the 2- and 4-positions (entry **10**) had an additive effect to give a compound with further increased activity. And lastly replacement of the terminal phenyl ring with a furan (entry **11**) gave a compound with activities comparable to that of the known[10a] 4-iodophenyl-alaninyl-(*S*)-2-cyano-pyrrolidine (entry **3**).

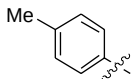
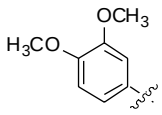
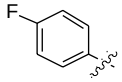
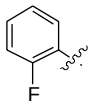
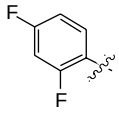
Table 1. K_i values of the 4-substituted phenylalanine cyano-(*S*)-pyrrolidine derivatives[a]





1

K_i (μM): 0.027

Compound	R	K_i (μM)
2		0.063
3		0.034
4		0.470
5		0.013
6		0.020
7		0.026
8		0.0031
9		0.0053
10		0.0022
11		0.036

[a] Potency was assessed by a kinetic method monitored at 405 nm similar to that of Hughes et al.[12] IC_{50} was determined from the slope of regression lines of modified Dixon plots of uninhibited velocity/inhibited velocity versus inhibitor concentration. K_i was calculated using substrate concentration [S], substrate K_m, and IC_{50} with $K_i = IC_{50} \times (1/(1 + [S]/K_m))$.[14] Compounds were assayed in duplicate. The assay coefficient of variation was 10.1% ($N = 5$).

A crystal structure of compound **5** covalently bound to the active site of the enzyme was determined (Figs. 3A and B).[15] The structural information validated the inhibitor design and binding hypothesis. The inhibitor is covalently bound to active site Ser630 through the nitrile at P1. The pyrrolidine ring fits into the hydrophobic S1 pocket formed by the side chains of Arg125, Tyr547, Ser630, Tyr662, and Tyr666. The main chain of the P_2-residue interacts with the enzyme at two anchor sites. Glu206 forms a salt bridge with the free amino terminus of the P2 residue.[9] The Nδ2

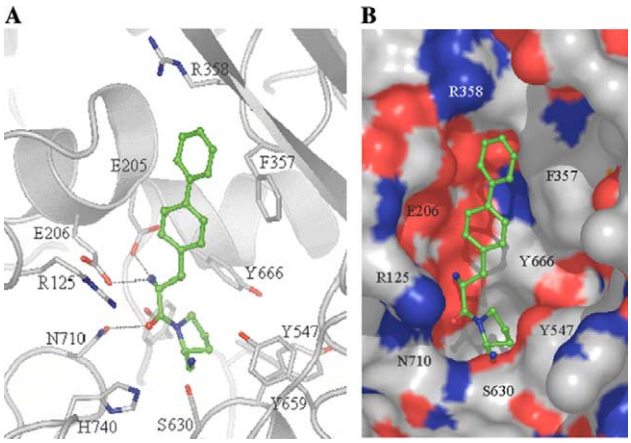
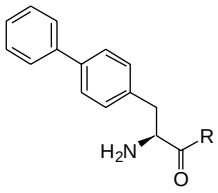


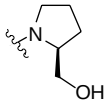
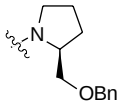
Figure 3. X-ray structure of DPP IV in complex with compound **5**. PDB code is 2AJL. (A) Active site residues are depicted in gray. Inhibitor is in green and atoms are colored by element with nitrogen blue and oxygen red. (B) Space filling picture showing the ligand-binding cavity.

of Asn710 stabilizes the P2 carbonyl oxygen atom. The biphenyl extends into the lipophilic pocket formed by the side chains of Phe357 and methylene groups of the Arg358 side chain. The surface representation clearly shows the biphenyl group extending into the lipophilic pocket (Fig. 3B). The middle ring of the biphenyl group is situated in the pocket in such a way as to allow for an edge to face π–π interaction with the Phe357.

Using the information gleaned from the crystal structure and the SAR generated from our study of the 4-substituted phenylalanine cyano-(*S*)-pyrrolidines, we looked to extend our results to non-nitrile inhibitors. The nitrile group forms a reversible covalent bond with the active site serine and yields potent inhibitors. However, under neutral and basic aqueous conditions, the P2 site amine could potentially undergo a nucleophilic attack of the carbon of the nitrile to form an inactive cyclic amidine.[16] For this reason it was of interest to identify non-nitrile containing and reversible inhibitors, which may offer an advantage over existing inhibitors. A quick survey of the proline mimics was done using 4-phenyl-phenylalanine as P2 moiety (Table 2). Replacement of the (*S*)-cyano-pyrrolidine with a thiazolidine ring gave compound **12** with sufficient potency (360 nM) to build on. Exchange of the terminal phenyl ring with a pyridyl ring (Table 3, Entry **18**) had little effect on the activity. Placement of a carboxylic acid to the 4-position of the biaryl gave compound **19** with only slightly increased activity. However,sss addition of a carboxylic acid at the 3-position increased the potency by 2-fold (entry **20**), indicating a potential interaction of the acid moiety with R358. Addition of a cyano- or fluorine atom to the 4-position of the biphenyl (entry **21**, **22**) also resulted in a 2-fold increase in potency. Interestingly parallel to the SAR of the (*S*)-cyano-pyrrolidine series, disubstitution with fluorine at the 2- and 4-positions (entry **23**) again demonstrated an additive

Table 2. K_i values of 4-phenylphenylalaninyl proline mimetics



Compound	R	K_i (μM)
5		0.013
12		0.36
13		1.16
14		>12.8
15		>12.8
16		>12.8

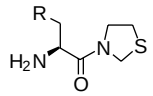
effect and increased the potency by about 4-fold to 96 nM and gave us our most potent inhibitor of this series to date.

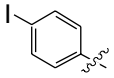
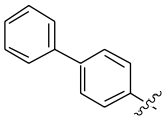
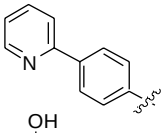
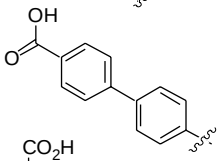
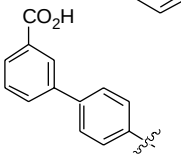
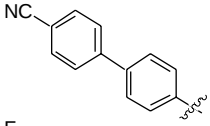
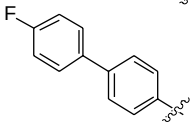
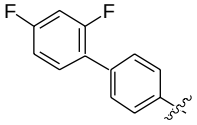
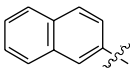
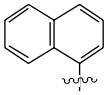
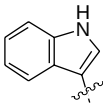
The size requirements of the S2 pocket were also probed. Replacement of the linear biphenyl group with naphthalen-2-yl group resulted in a huge loss of activity (Table 3, entry **24**). A naphthalen-1-yl or a 1*H*-indol-3-yl group in place of the biaryl was detrimental to activity (entry **25**, **26**).

After the discovery that the biaryl thiazolidines were potent inhibitors of DPP IV, a study was conducted to investigate Caco-2 permeability and microsome stability of the thiazolidine analogs compared to the corresponding nitrile derivatives. Five compounds were selected and the results are shown in Table 4. In most cases, changing from (*S*)-cyano-pyrrolidine to the thiazolidine improved the Caco-2 permeability while maintaining good human liver microsome stability.

In summary, a series of novel biaryl inhibitors of DPP IV was identified using a structure-based approach. The K_i of the biaryl (*S*)-cyano-pyrrolidines reached a lower single digit nanomolar range, and a preliminary

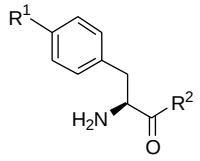
Table 3. K_i value of aminoacylthiazolidines

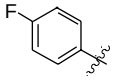
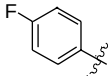
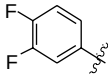


Compound	R	K_i (μM)
17		0.98
12		0.36
18		0.355
19		0.31
20		0.166
21		0.16
22		0.17
23		0.096
24		8.9
25		>12.8
26		>12.8

SAR was established. The SAR was then successfully transferred to non-cyano containing biaryl thiazolidines, and double-digit nanomolar K_i as well as improved ADME characteristics were achieved. A crystal structure of human DPP IV in complex with one of our inhibitors was obtained, and the structural information is expected to facilitate future lead optimization efforts.

Table 4. Caco-2 permeability and human microsome stability study of selected compounds



Compound	R^1	R^2	Caco-2[a]		HLM[b]
			A → B	B → A	
5			2.66	3.82	52.3
12			4.56	1.5	62
8			7.21	4.45	6.7
22			16.81	5.61	78.5
10			4.93	4.93	73.9

[a] P_{app}, apparent permeability ($\times 10^{-6}$ cm/s) at 10 μM compound concentration.
[b] % remaining after 10 min of incubation at 1 μM compound concentration.

References and notes

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15. (a) PDB code is 2AJL; (b) DPP IV Purification. The cDNA fragment encoding the soluble region Ser39-Pro766 of human DPP IV was inserted into the baculovirus vector pDEST20 (Invitrogen) and expressed in Hi5 insect cells. Active DPP IV was purified from the concentrated cell media on a Q-Sepharose (0–2 M NaCl gradient in 10 mM Tris–HCl, pH 8.0) followed by phenyl-Sepharose 6FF (1.5–0 M $(NH_4)_2SO_4$ gradient in 20 mM Tris–HCl, pH 8.0). Purified DPP IV (0.5 mg/mL) was dialyzed against 20 mM Tris–HCl, pH 8.0, 25 mM NaCl, flash-frozen in liquid nitrogen, and stored at −80 °C for enzyme assays. For crystallographic purposes, DPP IV was concentrated to 18 mg/mL by filtration (Ultracel-4, 30000 MWCO, Millipore) after dialysis. (c) DPP IV crystallization, data collection, molecular replacement, and structure refinement. DPP IV in complex with compound **5** was crystallized by micro seeding from a solution containing 25% polyethylene glycol 4000 and 0.1 M Tris at pH 7.5. The crystals were transferred to a cryoprotectant solution containing the crystallization solution and 10% glycerol. X-ray diffraction data to a resolution of 2.5 Å were collected on a Bruker AXS Proteum 6000 detector. Diffraction data were indexed, integrated, and scaled using the Proteum Processing Program suite from Bruker AXS. The DPP IV crystals belong to the $P2_1$ space group, with unit cell parameters $a = 65.28$, $b = 126.88$, $c = 110.83$ Å, and $\beta = 99.4°$. The structure was determined and refined by molecular replacement with CNX (Brunger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J., Nilges, M.; Pannu, N. S.; Read, R. J., Rice, L. M.; Simonson, T.; Warren, G. L. Acta Crystallogr. **1998**, *D54*, 905) using the structure of DPP IV with valine-pyrrolidide as a search model (PDB ID: 1NIM). All model building was done using O (Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. Acta Crystallogr. **1991**, *A47*, 110). The final structure was refined to an *R*-factor of 22.6.
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