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(3R,4S)-4-(2,4,5-Trifluorophenyl)-pyrrolidin-3-ylamine inhibitors of dipeptidyl peptidase IV: Synthesis, in vitro, in vivo, and X-ray crystallographic characterization

Stephen W. Wright,* Mark J. Ammirati, Kim M. Andrews, Anne M. Brodeur, Dennis E. Danley, Shawn D. Doran, Jay S. Lillquist, Shenping Liu, Lester D. McClure, R. Kirk McPherson, Thanh V. Olson, Stephen J. Orena, Janice C. Parker, Benjamin N. Rocke, Walter C. Soeller, Carolyn B. Soglia, Judith L. Treadway, Maria A. VanVolkenburg, Zhengrong Zhao and Eric D. Cox

Pfizer Global Research and Development, Cardiovascular and Metabolic Diseases, MS 8220-3141, Eastern Point Road, Groton, CT 06340, USA

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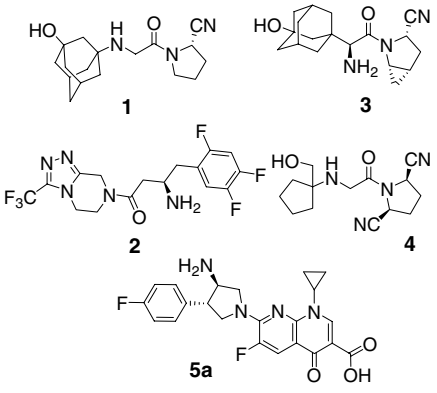
Abstract—A series of pyrrolidine based inhibitors of dipeptidyl peptidase IV were developed from a high throughput screening hit for the treatment of type 2 diabetes. Potency, selectivity, and pharmacokinetic properties were optimized resulting in the identification of a pre-clinical candidate for further profiling.

Type 2 diabetes is a severe and increasingly prevalent disease.[1] Diabetics may suffer debilitating cardiovascular, eye, kidney, and nerve damage and are at risk of premature handicap and death due to these and other diabetic complications, which are the result of glucose toxicity caused by their hyperglycemia. A progressive reduction in insulin sensitivity and insulin secretion are hallmarks of the disease, which eventually result in failure of the pancreatic islet cells and dependence on exogenous insulin. The incretin hormone glucagon-like peptide 1 (GLP-1) is a potent stimulator of endogenous insulin release. GLP-1 has beneficial effects on islet β-cell function and insulin sensitivity without induction of hypoglycemia.[2] Studies in rodents have indicated that GLP-1 may stop or reverse the loss of β-cell function.[3] Unfortunately, GLP-1 is rapidly degraded in vivo by the serine protease dipeptidyl peptidase IV (DPP4); therefore inhibition of DPP4 has emerged as a promising approach for the treatment of Type 2 diabetes.[4] This has been substantiated by the results of clinical trials of several inhibitors,[5] including vildagliptin **1** (LAF-237),[6] sitagliptin **2** (MK-0431),[7] and saxagliptin **3** (BMS-477118).[8]

We have previously reported our studies with the inhibitor **4** based on a *cis*-2,5-dicyanopyrrolidine template.[9] The quinolone **5a**, previously prepared as part of an antibacterial program, was identified in a high throughput screen of our corporate compound collection as an inhibitor of DPP4 (IC_{50} = 140 nM). We were intrigued by the unusual structure of **5a** and sought to determine whether it could be parlayed into a novel series of DPP4 inhibitors. This paper reports our discovery of a series of 3-amino-4-phenyl pyrrolidine inhibitors of DPP4.[10]



Keywords: DPPIV inhibitors; Diabetes; Structure-based design.
* Corresponding author. Tel. +1 860 441 5831; fax: +1 860 715 4483; e-mail: stephen.w.wright@pfizer.com

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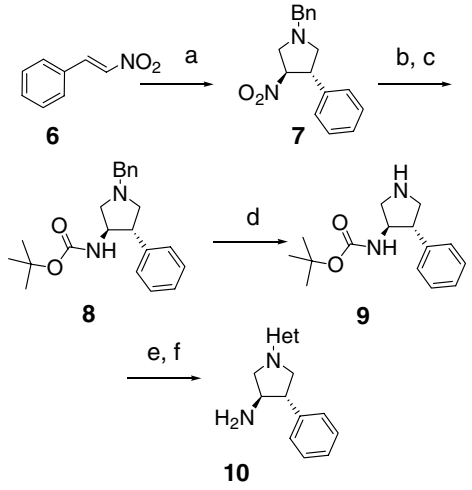
Substructure searching and screening quickly revealed key SAR information (Table 1): the *trans*-3,4-disubstituted pyrrolidine was more potent than the *cis* isomer, and enzyme inhibitory potency was critically dependent upon the substitution pattern of the phenyl ring.

We hypothesized that the *trans*-3-amino-4-phenyl pyrrolidine was the key pharmacophore, and docking experiments provided further support for this hypothesis. Recognizing that the quinolone fragment of **5** was undesirable,[11] we screened a variety of heterocycles as potential replacements for the quinolone fragment using the *trans*-3-amino-4-phenyl pyrrolidine scaffold. These compounds were prepared as racemates using the route shown in Scheme 1.

Nitrostyrene **6** was treated with the azomethine ylide generated from *N*-benzyl-*N*-(methoxy)methyl-*N*-trimethylsilylmethylamine to provide pyrrolidine (±)**7**.[12] Reduction of (±)**7** with hydrogen in the presence of Raney nickel was followed by protection with BOC anhydride to give (±)**8**. Subsequent debenzylation with hydrogen and Pd/C in acetic acid afforded (±)**9**, which was coupled with heteroaryl chlorides using parallel synthesis to provide (±)**10a**–**10m**. This effort identified the 6-phenylpyrimidine (±)**10a** as the best replacement for the quinolone, albeit with some loss of potency (Table 2).[13] Pharmacokinetic evaluation of (±)**10a** in the rat showed a correlation between clearance predicted from in vitro microsomal screens and in vivo clearance, and acceptable bioavailability (32%), suggesting that derivatives of (±)**10a** could be found that had acceptable pharmacokinetic properties.

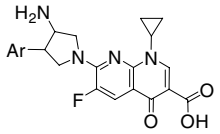


Scheme 1. Reagents and conditions: (a) *N*-benzyl-*N*-(methoxy)methyl-*N*-trimethylsilylmethylamine, TFA, CH_2Cl_2, 0 °C; (b) H_2, RaNi, MeOH–NH_3, 25 °C; (c) Boc_2O, Et_3N, THF, 25 °C; (d) H_2, Pd/C, EtOH, AcOH, 50 °C; (e) chloroheterocycle, *i*-Pr_2NEt, *t*-BuOH, 110 °C (microwave); (f) HCl, 1,4-dioxane, 25 °C.

Further improvement in potency was realized by the introduction of a 2,4,5-trifluorophenyl substitution pattern on the phenyl ring attached to the pyrrolidine 4-position.[14] The necessary 2,4,5-trifluorophenyl template ((±)**12**, Scheme 2) was prepared from **11** by the same sequence of reactions presented in Scheme 1.

Further SAR development was focused on the phenyl ring attached to the pyrimidine ring as shown in Scheme 3. Suzuki coupling of an appropriately substituted boronic acid with 4,6-dichloropyrimidine afforded the

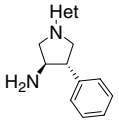
Table 1. Aryl substitution, pyrrolidine stereochemistry, and DPP4 enzyme inhibition assay results for compounds **5a**–**5k**



Compound	Ar	3-NH_2-4-Ar stereochemistry	DPP4 IC_{50}, nM[a]
5a	4-FC_6H_4	*trans*	140
5b	2-$CH_3OC_6H_4$	*cis*	2000
5c	2-$CH_3OC_6H_4$	*trans*	200
5d	C_6H_5	*trans*	150
5e	4-$H_2NC_6H_4$	*trans*	>3000
5f	4-*i*PrC_6H_4	*trans*	>3000
5g	4-$MeO_2CC_6H_4$	*trans*	>3000
5h	4-$CH_3OC_6H_4$	*trans*	>3000
5i	3,4-$Cl_2C_6H_4$	*trans*	1000
5j	4-Me_2NC6H_4	*trans*	>3000
5k	4-HOC_6H_4	*trans*	>3000

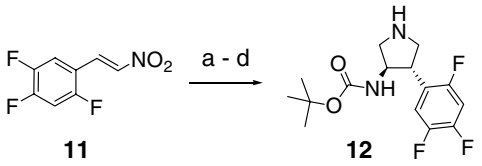
[a] Recombinant wild type human enzyme. Means of at least three experiments; standard deviations are ±15%. An IC_{50} of >3000 indicates that no curve was noted in the dose–response up to 3 μM; see Ref. 9.

Table 2. Heterocycle and DPP4 enzyme inhibition assay results for compounds **10a**–**10m**

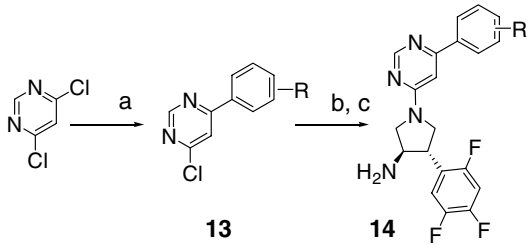


Compound	Heterocycle	DPP4 IC_{50}, nM[a]
10a	6-Phenyl-pyrimidin-4-yl	790
10b	6-(3-Cyano)phenyl-pyrimidin-4-yl	1400
10c	6-(4-Methoxy)phenyl-pyrimidin-4-yl	2300
10d	6-(3-Chloro)phenyl-pyrimidin-4-yl	2900
10e	(3-Cyano)pyridin-2-yl	>3000
10f	6-(2-Chloro)phenyl-pyrimidin-4-yl	>3000
10g	6-Chloro-2-phenyl-pyrimidin-4-yl	>3000
10h	9-Methyl-9H-purin-6-yl	>3000
10i	6-(4-Hydroxy)phenyl-pyrimidin-4-yl	>3000
10j	6-(4-Chloro)phenyl-pyrimidin-4-yl	>3000
10k	(5-Acetyl)pyridin-2-yl	>3000
10l	1-Quinoxalin-2-yl	>3000
10m	1-Quinolin-2-yl	>3000

[a] Recombinant wild type human enzyme. Means of at least three experiments; standard deviations are ±15%. An IC_{50} of >3000 indicates that no curve was noted in the dose–response up to 3 μM; see Ref. 9.



Scheme 2. Reagents and conditions: (a) *N*-benzyl-*N*-(methoxy)methyl-*N*-trimethylsilylmethylamine, TFA, CH_2Cl_2, 0 °C; (b) H_2, RaNi, MeOH–NH_3, 25 °C; (c) Boc_2O, Et_3N, THF, 25 °C; (d) H_2, Pd/C, EtOH, AcOH, 50 °C.



Scheme 3. Reagents and conditions: (a) Boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 90 °C; (b) (±)**12**, *i*-Pr_2Net, *t*-BuOH, 110 °C; (c) HCl, 1,4-dioxane, 25 °C.

4-chloro-6-(substituted)phenyl pyrimidines **13**, which were aminated with (±)**12** and deprotected with HCl/dioxane.

This effort revealed that potency was optimal with a substituent *meta*- to the biaryl bond, and further effort was directed toward increasing the diversity of *meta*- substituents and replacement of the *meta*- substituted benzene ring by a 3-pyridyl group (Table 3).

Compounds **14a**, **14j**, and **15b** emerged as compounds of interest based on potency and in vitro microsomal stability. These compounds were prepared as single enantiomers from the Boc-protected pyrrolidine enantiomer (+)**12**, which was obtained by classical resolution of the racemate (±)**12** using (S)-naproxen (See Table 4).[15]

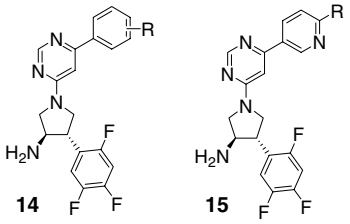
Compound (+)**15b** was selected for pharmacokinetic study in the rat and dog (Table 5) [16] based on its potency, selectivity,[17] in vivo activity,[18] and lack of CYP inhibition.

The co-crystal structure of (+)**15b** confirmed the expected binding mode (Fig. 1). The primary amine makes hydrogen bonds to Y662, E205, and E206. The trifluorophenyl ring occupies the S1 pocket (Y662, Y666, V656, V711, Y631, and W659). The pyridine extends into a pocket comprised of F357, R358, E206, and S209, and the methoxy group forms a hydrogen bond to R358. The pyrimidine ring is involved in a pi-stacking interaction with F357.

In conclusion, a high throughput screening hit identified a *trans*-3-amino-4-phenyl pyrrolidine as a template for a novel series of DPP4 inhibitors. By use of structure-based design and parallel synthesis, the series was advanced with improvements in potency, ADME properties, and selectivity versus other DPP isoforms. Compound (+)**15b** was identified as a compound for further pre-clinical profiling due to its PK/PD profile in the rat.

X-Ray crystal structures have been deposited in the RCSB protein data bank with code 2QJR.

Table 3. Aryl substitution and DPP4 enzyme inhibition assay results for compounds **14a**–**14w**, **15a**, and **15b**



Compound	R	DPP4 IC_{50}, nM[a]
14a	H[b]	21
14b	2-Cl	260
14c	2-CN	93
14d	2-$COCH_3$	230
14e	2-F	57
14f	2-OMe	180
14g	3-Cl	190
14h	3-CN	37
14i	3-$COCH_3$	19
14j	3-F	51
14k	3-OMe	47
14l	4-Cl	470
14m	4-CN	130
14n	4-$COCH_3$	160
14o	4-F	51
14p	4-OMe	120
14q	3-CF_3	71
14r	3-OCF_3	1300
14s	3-CH_2OH	32
14t	3-CO_2H[b]	5.0
14u	3-$NHCOCH_3$	14
14v	3-NO_2	120
14w	3-OH	70
15a	H	31
15b	OMe	37

[a] Recombinant wild type human enzyme. Means of at least three experiments; standard deviations are ±15%. An IC_{50} of >3000 indicates that no curve was noted in the dose–response up to 3 μM; see Ref. 9.
[b] See also Ref. 10.

Table 4. DPP4, DPP2, DPP8 enzyme inhibition assay results and predicted human extraction ratio for compounds **14a**, **14j**, and **15b**

Compound	DPP4 IC_{50}, nM[a]	DPP2 IC_{50}, μM[a]	DPP8 IC_{50}, μM[a]	Predicted human CL[b]
(+)14a	13	18	3.0	0.81
(+)14j	14	15	1.6	<0.31
(+)15b	6.4	19	3.2	<0.31

[a] Recombinant wild type human enzyme. Means of at least three experiments; standard deviations are ±15%; see Ref. 9.
[b] Predicted hepatic clearance (CLh) from human liver microsomal lability assay divided by human liver blood flow (20 mL/min/kg).

Table 5. Pharmacokinetic parameters for compound **15b** in rat and dog

Species	Unbound fraction (%)	CL, mL/min/kg	Vss, L/kg	$t_{1/2}$, h (i.v.)	$t_{1/2}$, h (p.o.)	F%
Rat	6	4.4	0.67	2.8	3.3	57
Dog	17	4.8	1.9	5.1	nd[a]	nd[a]
Human	6	1.3[b]	2.0[b]			50[b]

[a] Not determined.
[b] Projected values based on rat single-species allometric scaling (see Ref. 19).

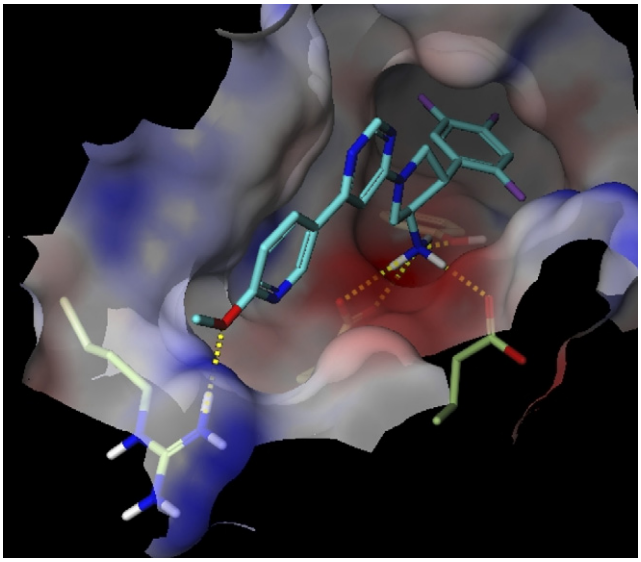


Figure 1. Compound (+)**15b** co-crystallized with human DPP4 showing hydrogen bond from Arg 358 to methoxypyridine.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.081.

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13. Analogs incorporating amides, amines, sulfonamides, and urea substituents placed on the pyrrolidine ring nitrogen atom were also evaluated; in general these were less potent as DPP4 inhibitors.
14. The application disclosing MK-0431 published concurrently with our efforts to increase potency by appropriate substitution of this phenyl ring; for example, a 2,5-difluorophenyl substitution improved potency to 61 nM from 790 nM for **10a**. We obtained internal co-crystal structures using compounds similar to **2** and our pyrrolidine inhibitors and found a similar aryl binding mode in these structures. See Edmondson, D. D.; Parmee, E.; Weber, A. E.; Xu, J. Patent WO 03/000180, 2003; *Chem. Abstr.* **2003**, *138*, 73536.
15. The absolute configuration of (+)**12** ($[a]_D$ = 61° (c = 1.5, EtOH) was based upon agreement with protein crystal structures as well as identity to a sample of (+)**12** prepared by diastereoselective azomethine ylide cycloaddition to (S)-4-phenyl-3-[(E)-3-(2,4,5-trifluoro-phenyl)-acryloyl]-oxazolidin-2- one; see U.S. Patent 5,618,949, 1997; *Chem. Abstr.* **1997**, *126*, 330549.
16. All procedures involving animals were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee. Oral pharmacokinetic evaluations were dosed in 0.5% methylcellulose formulation at 5 mg kg^{-1} in the rat and in sterile saline solutions at 1 mg kg^{-1} in the dog. Intravenous evaluations were conducted at 1 mg kg^{-1} in sterile saline. Pharmacodynamic determinations were made by assay of DPP4 activity in plasma samples taken from the PK study; see reference 7 for plasma DPP4

activity assay. PK and PD data for (+)**15b** are presented in the Supporting information.
17. Compound (+)**15b** had $IC_{50} > 30\ \mu M$ against DPP3, DPP9, FAP, and POP.
18. Compound (+)**15b** was dosed in fasted, diabetic KK/H1J mice and the response of the mice in an OGTT was measured; see Ref. 9. See Supporting information for in vivo glucose lowering data for (+)**15b**.
19. $CL_{predicted} = (BW_{human}/BW_{rat})^{0.75} * (CL_{animal} * BW_{animal}/Fu_{animal}) * (Fu_{human}/BW_{human})$, where BW, body weight, Fu, fraction unbound, CL, i.v. clearance, and 0.75, allometric scaling factor.