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1-((3*S*,4*S*)-4-Amino-1-(4-substituted-1,3,5-triazin-2-yl) pyrrolidin-3-yl)-5,5-difluoropiperidin-2-one inhibitors of DPP-4 for the treatment of type 2 diabetes

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ABSTRACT

A 3-amino-4-substituted pyrrolidine series of dipeptidyl peptidase IV (DPP-4) inhibitors was rapidly developed into a candidate series by identification of a polar valerolactam replacement for the lipophilic 2,4,5-trifluorophenyl pharmacophore. The addition of a *gem*-difluoro substituent to the lactam improved overall DPP-4 inhibition and an efficient asymmetric route to 3,4-diaminopyrrolidines was developed. Advanced profiling of a subset of analogs identified **5o** with an acceptable human DPP-4 inhibition profile based on a rat PK/PD model and a projected human dose that was suitable for clinical development.

Type 2 diabetes mellitus (TTDM) is a chronic disorder characterized by hyperglycemia coupled with a gradual decline in insulin sensitivity and insulin secretion. An increasing incidence of obesity has, in part, fueled the rise in new TTDM diagnoses and continues to make the treatment of TTDM a critical global health care issue.[1] Present pharmaceutical therapy often fails to provide adequate long-term glycemic control and many patients are unable to achieve their targeted plasma glucose levels through available regimens. The incretin hormones glucagon-like peptide-1 (GLP-1) and gastrointestinal inhibitory peptide (GIP) are released post-prandially from the L-cells of the intestine and stimulate the release of insulin from pancreatic β-cells.[2] However, these incretins are rapidly degraded in vivo by peptidases. Dipeptidyl peptidase IV (DPP-4) is a widely distributed serine protease that specifically cleaves N-terminal dipeptides from polypeptides with proline or alanine at the penultimate position. In vivo administration of DPP-4 inhibitors to patients results in higher circulating concentrations of endogenous GLP-1 and subsequent decrease in plasma glucose. Long term treatment with a DPP-4 inhibitor leads to a reduction in circulating HbA1c (glycosylated hemoglobin). It has been suggested that DPP-4 inhibition may also offer the potential to improve the insulin producing function of the pancreas through either β-cell preservation or regeneration.[3] From a safety standpoint, DPP-4 inhibition offers a significantly reduced risk of hypoglycemia because the endogenous insulin secretagogue (GLP-1) is generated only in response to a glucose stimulus. Therefore, DPP-4 inhibition has emerged as a promising new treatment of type 2 diabetes.[4]

Several DPP-4 inhibitors are now approved drugs, including first in class inhibitor sitagliptin **1**, as well as vildagliptin **2** and saxagliptin **3**. Several other candidates such as gosogliptin **4**[5] and others[6] have been reported in advanced clinical trials (Fig. 1). We were interested in finding additional novel small molecular weight inhibitors of DPP-4 that lacked the 2-cyanopyrrolidide as a back-up to our clinical candidate **4**. This account describes the discovery of a pyrrolidine series of DPP-4 inhibitors **5** and the tactics used to manage pre-clinical safety risks to identify a clinical candidate.

Based on emerging clinical data and PK/PD modeling, the laboratory objectives for the next generation effort was to provide ⩾80% inhibition of DPP-4 for at least 8 h in humans with a once-a-day oral therapy. Candidate compounds would demonstrate >500-fold selectivity against DPP-2 and DPP-8.[7] The successful candidate would also demonstrate a wide safety margin in in vitro safety assays: a therapeutic index versus the hERG patch-clamp assay for QTc prolongation (hERG IC_{50}/DPP-4 K_i ⩾ 300), a clean profile in the in vitro genetic toxicity assays (Ames and micronucleus), and minimal activity in a broad panel of receptors and enzymes. In addi-

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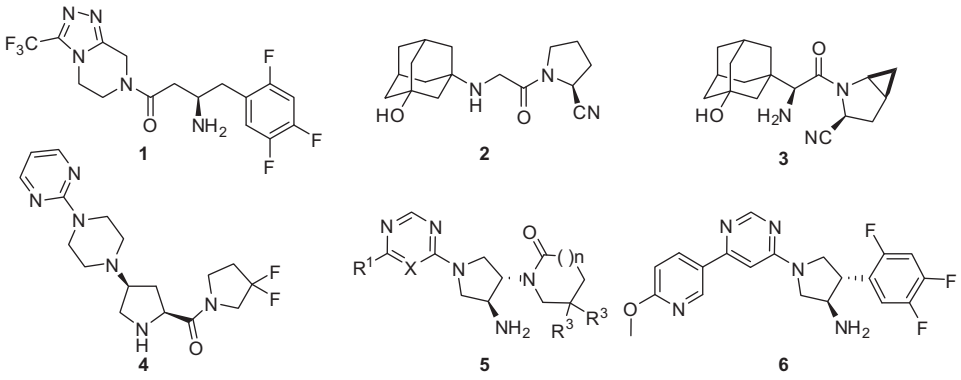


Figure 1. Approved DPP-4 inhibitors and advanced clinical candidates.

tion, physicochemical property space consistent with reducing in vivo toxicological outcomes (c log P <3, TPSA >75) was targeted.[8]

The 2,4,5-trifluorophenyl moiety has been established as a proline amide replacement in the S1 pocket of DPP-4 inhibitors.[9] Earlier efforts from Pfizer laboratories identified potent, isoform-selective DPP-4 inhibitors containing the 3-aminopyrrolidine core (e.g., **6**) as a suitable scaffold to explore substituents in the S2 pocket.[10] However, compounds like **6** were more lipophilic (Δc log P >1.2) than typical DPP-4 inhibitors (Fig. 1), which led to moderate to high intrinsic clearance in human microsomes. Therefore, we sought a suitable polar replacement for the 2,4,5-trifluorophenyl moiety that would result in a more efficient use of the lipophilicity contained in the pharmacophore. Superposition of crystal structures **4** and **6** showed that the carbonyl of pyrrolidine amide of **4** and the 2-fluoro of **6** occupied the same space (Fig. 2). We reasoned that incorporation of an N-linked lactam would offer H-bonding potential through the carbonyl (to Asn710) and the ability to lower log P. Recent literature examples have also shown that a lactam moiety can function as a proline amide surrogate.[11] Thus, we focused on optimization of more polar S1 pocket binding groups.

An initial synthetic route to the requisite (3*S*,4*S*)-diaminopyrrolidine core was fashioned from work reported by Still.[12] The commercially available unnatural tartaric acid **7** was readily converted into the bis-azide **8**. Mono-reduction under Staüdinger conditions and reaction of the amine with an appropriate γ- or δ-chloro acid followed by cyclization generated the azido butyro- or valerolactam, **9** or **10**, respectively. Reduction of the azide and protecting group manipulation afforded the pyrrolidine templates **11** or **12** for analog preparation.

A comparison of a small series of five- and six-membered lactam analogs to the corresponding 2,4,5-trifluorophenyl compounds showed that the more polar lactams were effective pharmacophores for this template (Table 1). The butyrolactams (**5b**, **5e**, **5h**) were less potent inhibitors of DPP-4 than the aryl congeners (**5a**, **5d**, **5g**) but were significantly less lipophilic (Δc log P −2.0). Addition of a methylene unit provided valerolactams (**5c**, **5f**, **5i**) that showed improved DPP-4 inhibition. In this case, the 10-fold gain in potency in going from butyrolactam to valerolactam corresponded to the gain in binding energy from a small, optimally-buried lipophilic group.[13] The reduction in c log P offered by the lactam moieties brought a significant decrease in intrinsic clearance in human liver microsomes.

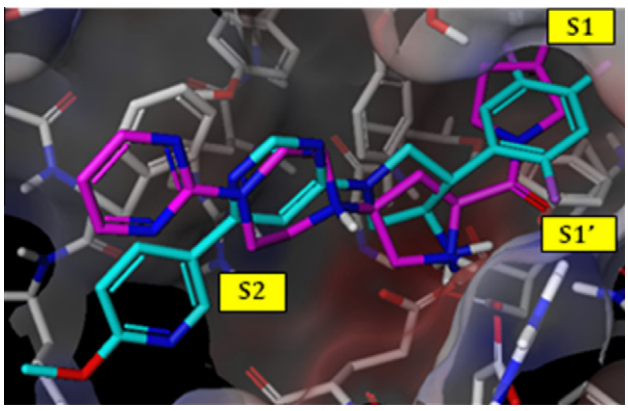


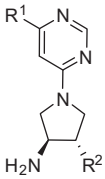
Figure 2. Superposition of compounds **4** (magenta) and **6** (cyan).

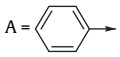
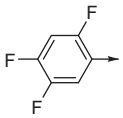
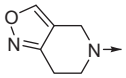
The co-crystal structure of compound **5c** with human DPP-4 confirmed the expected binding mode in the active site. The primary amine makes hydrogen bonds to Tyr662, Glu205, and Glu206. The valerolactam occupies the S1 pocket (Tyr662, Tyr666, Val656, Val711, Tyr631, Trp659) and the lactam carbonyl make a hydrogen bond with Asn710. The phenyl extends into a pocket comprised of Phe357, Arg358, Glu206, and Ser209. The pyrimidine ring is involved in a π-stacking interaction with Phe357 (Fig. 3).[14]

Using the valerolactam template **12**, a range of substituents (heterocycles, amide, sulfonamides; data not shown) was rapidly probed using parallel chemistry. The 2,4-pyrimidine or triazine heterocycles offered the best balance of ease of synthesis and flexibility to explore a range of substituents. In general, analogs made with these heterocycles resulted in high selectivity over the DPP-2 isoform (IC_{50} >30 μM) regardless of the substitution pattern. However, for pyridinyl **5j** or phenyl **5k** analogs, the DPP-4 inhibition and selectivity over DPP-8 remained sub-optimal. As demonstrated by analogs in Table 1, amine substituents off of the pyrimidine are suitable replacements for aryl moieties and make more efficient use of the lipophilicity. Incorporation of 3,3-difluoropyrrolidine as an aryl isostere provided analogs **5l** and **5m** that achieved modest potency gains and higher selectivity over DPP-8 (Table 2).

The rigidity of the S1 pocket appears to leave little room for additional substitution on the lactam moiety but subtle changes of substituents in the S1 binding pocket can have a profound impact on potency. Superposition of the crystal structure of **5c** with multiple crystal structures of DPP-4 inhibitors with fluorine atoms occupying the S1 pocket (sitagliptin, gosogliptin, **6**) showed some unoccupied space, suggesting that additional interactions could be achieved. Thus, incorporation of a *gem*-difluoro substitution at C5 of the valerolactam moiety gave **5n**, a compound that retained selectivity over DPP-8 and improved inhibition of DPP-4 by about threefold (Table 2). Further refinement of the heterocycle **5o** or substituent **5p** led to additional analogs meeting many of our objectives. Advanced screening of selected compounds in routine in vitro safety assays (hERG patch-clamp, micronucleus and Ames assays) did not uncover any issues with this series.

Table 1
DPP-4 inhibition and ADME properties of N1-substituted 3-aminopyrrolidines **5**



Compd	R^1	R^2	DPP-4 IC_{50}[a] (nM)	*c* log *P*	TPSA	HLM CL_{int}[b] (μL/min/mg)
5a		A = 	13	3.47	55	85
5b		B = 	488	1.43	75	<7.0
5c		C = 	47	1.99	88	<7.2
5d		A	1.6	1.36	84	56
5e		B	153	−0.68	105	<7.0
5f		C	12	−0.12	105	<7.5
5g		A	19	2.76	67	38
5h		B	1400	0.72	87	<7.0
5i		C	74	1.28	87	<7.0

[a] Values are means of three experiments.
[b] Values show the intrinsic clearance of a compound as measured by the disappearance of the parent compound in cultured human liver microsomes.

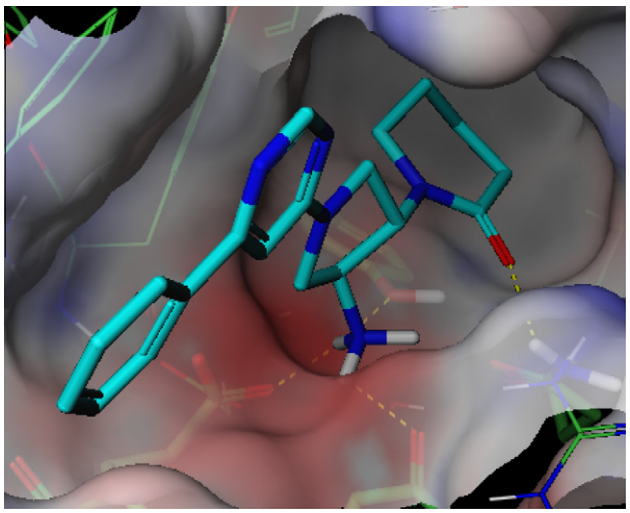


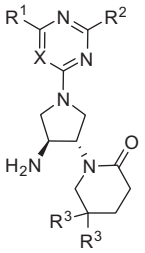
Figure 3. Binding interactions in the active site. Compound **5c** co-crystallized with human DPP-4.

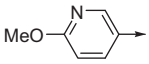
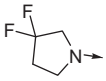
Operating in higher probability safety space (*c* log *P* <3, TPSA > 75) not only brings in vivo safety benefits but also increases probability of obtaining compounds with higher solubility and lower intrinsic clearance.[15] We found that a careful balance of intrinsic permeability and ionization state is needed to maximize the fraction of the dose absorbed. In general, compounds in Table 2 had moderate permeability when assessed in an RRCK (low efflux MDCK) assay. However, 3,3-difluoropyrrolidinopyrimidine analogs **5l**, **5n**, and **5p** have a second basic center (pK_{a2} ~7.6) that effectively renders them partially dibasic at physiological pH. The triazine analogs **5m** and **5o** have a pK_{a2} that is three log units lower and Δc log *P* −0.8 than the corresponding pyrimidines.[16] In order to test whether these properties would have an impact on bioavailability, **5l–5o** were selected for pharmacokinetic (PK) profiling (Table 3). Compounds **5m** and **5o** exhibited moderate clearance, moderate volume of distribution, moderate half-life and good bioavailability, while **5l** and **5n** were characterized by higher clearance and shorter half-life. Based on the desirable PK properties in rat, **5o** was advanced to PK profiling in dog, where moderate clearance, moderate volume of distribution, moderate half-life and good bioavailability were again noted. Of significance, a twofold increase in bioavailability for triazines versus pyrimidine (cf. **5l** vs **5m**, **5n** vs **5o**), which was consistent with log *P* mediated clearance reduction, was observed. An ex vivo measurement of DPP-4 inhibition from these rat PK studies (5 mg/kg, po) indicated that **5l–5m** were capable of inhibiting DPP-4 at the IC_{50} level for at least 16 h.[17]

Compound **5o** was assessed in an advanced battery of in vitro assays (Table 4). Of note was the high selectivity over closely related serine proteases, P450 enzymes and a wide panel of enzymes, receptors and ion channels. On the basis of desirable drug-like properties, excellent PK properties and expected PD effect in rodents, a human dose projection from modeling was conducted for **5o**. The human dose for **5o** was predicted by PK–PD simulation utilizing the projected human pharmacokinetic parameters and the associated DPP-4 inhibition.[18] Modeling suggested that a 100 mg dose of **5o** would provide >80% inhibition of DPP-4 for 20 h in humans.

With a suitable compound in hand, improvements to the synthetic route for the diaminopyrrolidine core were undertaken (Scheme 2). Specifically, the azide intermediates in the initial synthetic route are generally not acceptable in a development process route.[19] Compound **13**, synthesized as described in Scheme 1, was treated with Burgess reagent to affect cyclization to sulfamate

Table 2
DPP-4 inhibition and membrane permeability of N-substituted (3*S*,4*S*)-3-amino-4-(valerolactam-*N*-yl)pyrrolidines



Compd	R^1	R^2	R^3	X	DPP-4 IC_{50}[a] (nM)	DPP-8 IC_{50} (nM)	*c* log *P*	TPSA	pK_{a2}[b]	RRCK[c] P_{app} AB (10^{-6} cm/s)
5j		H	H	CH	39	11,400	1.43	98	3.03	2.7
5k		H	H	N	59	26,000	1.11	88	2.6	35.7
5l		H	H	CH	26	>30,000	1.20	79	7.58	1.8
5m		H	H	N	37	>30,000	0.39	92	4.26	3.8
5n		H	F	CH	8.4	>30,000	1.48	79	7.58	3.4
5o		H	F	N	23 ± 9	>30,000	0.67	92	4.26	5.0
5p		CH_3	F	CH	13	>30,000	1.98	79	7.59	3.9

[a] Values are means of three experiments.
[b] Calculated pK_a from ACD version 9.0.
[c] RRCK permeability assessment is conducted with low efflux Madin-Darby canine kidney (MDCK) cells, where the cells are double-FACS sorted to isolate the cells that showed no P-gp/MDR functional expression.

Table 3
Pharmacokinetic profiles of selected DPP-4 inhibitors[a]

Compd	Species	CL_p[b] (ml/min/kg)	V_{dss}[c] (L/kg)	$t_{1/2}$ (h)	*F* (%)	f_u[d]
5l	Rat	60.1	2.3	0.78	21	0.75
5m	Rat	36.8	4.7	5.0	52	0.86
5n	Rat	84	5.7	2.4	50	0.82
5o	Rat	46	8.4	3.6	90	0.83[e]
	Dog	7.2	1.9	5.9	150	

[a] Rats ($n = 2$) were dosed with 1 mg/kg (iv) and 5 mg/kg (po) in saline and 5% methyl cellulose, respectively.
[b] Clearance of compound from plasma.
[c] Volume of distribution calculated from the iv portion of the experiment.
[d] The unbound fraction of drug was measured using fresh plasma.
[e] The value measured in human plasma was 0.83.

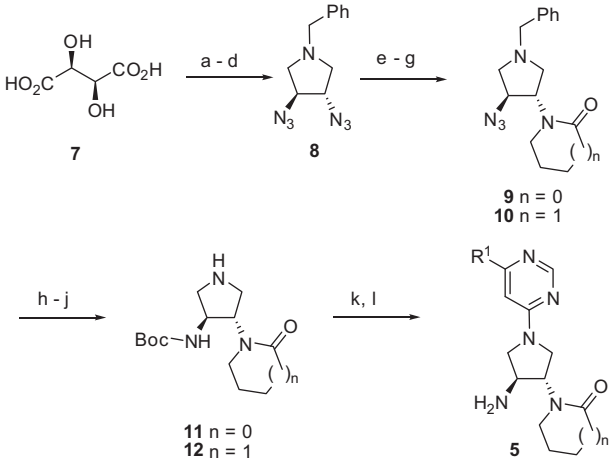
Table 4
Pharmacological and ADME properties of **5o**

		5o[a]
Human Plasma DPP-4 K_i[b]		14.4 ± 1.6 nM (4)
Rat Plasma DPP-4 K_i[b]		2.1 ± 0.3 nM (5)
Selectivity[c]		>1000×
DPP-2	>30,000 nM	
DPP-3	>30,000 nM	
DPP-9	>30,000 nM	
FAP	>30,000 nM	
POP	>30,000 nM	
Projected Human CL[d]		2.9 mL/min/kg
Projected Human V_d[e]		1.5 L/kg
Projected *F*%[f]		80
CEREP broad receptor and enzyme profile (68 assays)		IC_{50} >10 μM
CYP1A2, 2C9, 2D6, 3A4		IC_{50} >15 μM

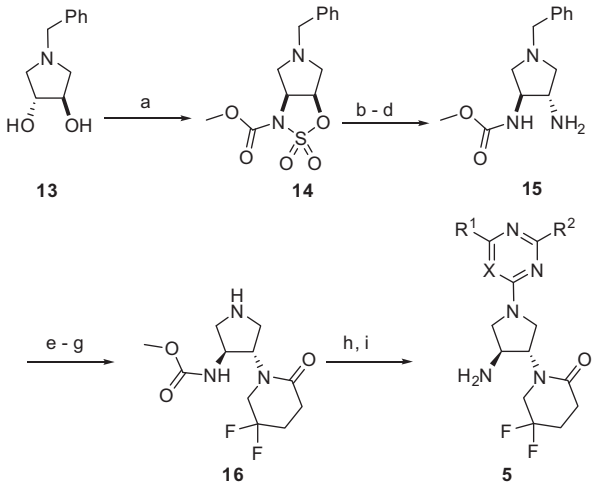
[a] Values are means of at least three experiments.
[b] Data are means ± SEM (*n*). K_i values were calculated using the Cheng–Prusoff equation.
[c] DPP-2, DPP-3, DPP-9, FAP, POP.[9]
[d] Predicted by dog single species scaling and scaling of human in vitro microsome data.
[e] Projected by dog–human proportionality.
[f] Projected value based on pre-clinical *F*%.

14.[20] Opening of sulfamate **14** with potassium phthalimide and subsequent loss of sulfinic acid provided a differentiated diamine substrate that was treated with hydrazine hydrate to afford aminocarbamate **15**. Acylation of the amine with 5-chloro-4,4-difluoropentanoic acid[21] followed by base-induced cyclization and benzyl deprotection provided key pyrrolidine intermediate **16**. Displacement of the appropriately substituted chloro-heterocycle and acidic deprotection of the methyl carbamate afforded the desired target **5o**.

In conclusion, a 3-amino-4-substituted pyrrolidine series of DPP-4 inhibitors was rapidly developed into a candidate series. A more polar valerolactam replacement for the lipophilic 2,4,5-trifluorophenyl pharmacophore was identified and the addition of a *gem*-difluoro substituent to the lactam improved overall DPP-4



Scheme 1. Chiral pool route to (3*S*,4*S*) 3-amino-4-(lactam-*N*-yl) pyrrolidine. Reagent and conditions: (a) $BnNH_2$, $PhCH_3$; (b) Red-Al, THF, 0 °C; (c) MsCl, Et_3N, CH_2Cl_2; (d) NaN_3, DMF, 100 °C; (e) PPh_3, THF/H_2O, heat; (f) $Cl(CH_2)_3(CH_2)_n–CO_2H$. propanephosphonic acid cyclic anhydride, Et_3N, EtOAc, heat; (g) NaH, DMF; (h) PPh_3, THF/H_2O, heat; (i) Boc_2O, THF; (j) H_2 (75 psi), 10% Pd/C, AcOH, EtOAc/EtOH, 50 °C; (k) Het-Cl, *t*-BuOH, heat; (l) TFA, CH_2Cl_2; NaOH, H_2O.



Scheme 2. The second generation asymmetric route to (3*S*,4*S*) 3-amino-4-(lactam-*N*-yl) pyrrolidine Reagent and conditions: (a) Burgess reagent, dioxane, heat; (b) K-phthalimide, CH_3CN, 80 °C; (c) 2 N HCl; (d) $N_2H_4{\cdot}H_2O$, EtOH, heat; (e) $HO_2C(CH_2)_3CF_2CH_2Cl$, propanephosphonic acid cyclic anhydride, Et_3N, EtOAc, heat; (f) NaH, THF/DMF; (g) H_2 (75 psi), $Pd(OH)_2$/C, EtOH/AcOH, 50 °C; h) Het-Cl, DIPEA, *t*-BuOH, heat; (i) PPA, heat.

inhibition. The PK properties were enhanced by lowering the basicity and lipophilicity of the core heterocycle. Advanced profiling in a rat PK/PD model and subsequent human projections identified **5o** as having an acceptable human DPP-4 inhibition profile with a projected dose of 100 mg/q.d. that was suitable for a drug development candidate. A non-azide, asymmetric route to the candidate compound was also discovered.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.055. These data include MOL files and InChiKeys of the most important compounds described in this article.

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