Discovery of 2-[4-{{2-(2*S*,5*R*)-2-Cyano-5-ethynyl-1-pyrrolidinyl]-2-oxoethyl]amino]-4-methyl-1-piperidinyl]-4-pyridinecarboxylic Acid (ABT-279): A Very Potent, Selective, Effective, and Well-Tolerated Inhibitor of Dipeptidyl Peptidase-IV, Useful for the Treatment of Diabetes

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Dipeptidyl peptidase-IV (DPP-IV) inhibitors are poised to be the next major drug class for the treatment of type 2 diabetes. Structure−activity studies of substitutions at the C5 position of the 2-cyanopyrrolidide warhead led to the discovery of potent inhibitors of DPP-IV that lack activity against DPP8 and DPP9. Further modification led to an extremely potent ($K_{iDPP-IV}$ = 1.0 nM) and selective (K_{iDPP8} > 30 μM; K_{iDPP9} > 30 μM) clinical candidate, ABT-279, that is orally available, efficacious, and remarkably safe in preclinical safety studies.

Diabetes is a major health problem with over 150 million people diagnosed with type 2 diabetes worldwide.[1] It is estimated that only 12% of diagnosed type 2 diabetics in the U.S. achieve adequate glycemic control. Therefore, new therapies with novel mechanisms of action and improved tolerability are urgently needed to more effectively treat this disease. Glucagon-like peptide-1 (GLP-1[a]) is a gut hormone released from L cells in the small intestine and proximal colon in response to the ingestion of nutrients and enhances the glucose-dependent secretion of insulin from pancreatic β-cells (incretin effect).[2] In type 2 diabetic patients, continuous infusion of GLP-1 decreases both fasted and postprandial blood glucose levels, improves β-cell function, and ultimately reduces hemoglobin A1c (HbA1c) concentrations.[3] Discontinuation of GLP-1 infusion in type 2 diabetics leads to the rapid reversion to hyperglycemia because GLP-1 activity is rapidly terminated by the action of the enzyme, dipeptidyl peptidase IV (DPP-IV), which cleaves the N-terminal dipeptide (His-Ala) of GLP-1.[4] Inhibition of DPP-IV activity is, therefore, a logical strategy to amplify the activity of endogenous GLP-1 and other incretins. DPP-IV inhibitors are clinically proven to effectively reduce HbA1c in diabetics and are expected to be the next major new class of oral antidiabetic agents.[5] GLP-1 infusion studies in uncontrolled diabetics have suggested that maximum glycemic control is achieved with 24 h infusion of GLP-1,[6] and our preclinical studies have further demonstrated that maximal glycemic control is achieved with >90% DPP-IV inhibition. In this context, we sought to identify DPP-IV inhibitors that possess a combination of potency and pharmacokinetic profile predicted to produce >90% DPP-IV inhibition for 24 h in man. Another criteria for DPP-IV inhibitor development are selectivity against the closely related enzymes, DPP8 and DPP9. Lack of DPP8/DPP9 selectivity has recently emerged as a potential safety hurdle in the assessment of potential DPP-IV clinical candidates. Specifically, inhibitors of DPP8/DPP9 have been reported to cause profound toxicities in preclinical species.[7] Consequently, our objective was to identify DPP-IV inhibitors that have essentially no DPP8 or DPP9 inhibition at pharmacologically relevant exposures and have the potential to distinguish themselves from earlier clinical candidates in development. Herein we report the synthesis and pharmacologic profiles of a series of 5-alkynyl-2-cyanopyrrolidine compounds. These studies led to the discovery of **42** (ABT-279), a DPP-IV inhibitor with excellent potency ($K_{iDPP-IV}$ = 1.0 nM) and selectivity (K_{iDPP8} > 30 μM; K_{iDPP9} > 30 μM, K_{iDPP7} > 30 μM, K_{iPOP} > 30 μM, $K_{iFAP-\alpha}$ > 30 μM) that is orally available, efficacious, and exceptionally well-tolerated in preclinical safety pharmacology and toxicology studies.

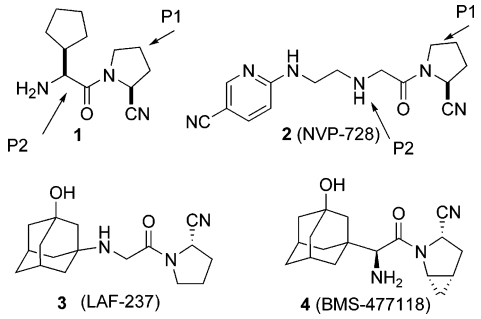

Figure 1. Cyanopyrrolidine DPP-IV inhibitors.

Our initial medicinal chemistry efforts began with the 2-cyanopyrrolidines, illustrated by compounds **1** and **2**, previously shown to be potent inhibitors of DPP-IV.[8] Recently, another two 2-cyanopyrrolidine compounds **3** and **4** have been disclosed that have advanced through Ph III clinical trials[9] (Figure 1). Compounds of this type interact with the enzyme by formation of an imidate with Ser 630 in the S1 pocket of the enzyme and interaction of the primary or secondary amine function of the P2 portion of the molecule with Glu 205/Glu

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[a] Abbreviations: GLP-1, glucagon-like peptide; HbA1c, hemoglobin A1c; DPP, dipeptidyl peptidase; POP, prolyloligo peptidase; FAP, fibroblast activating protein; ZDF, Zucker diabetic fatty; OGTT, oral glucose tolerance test.

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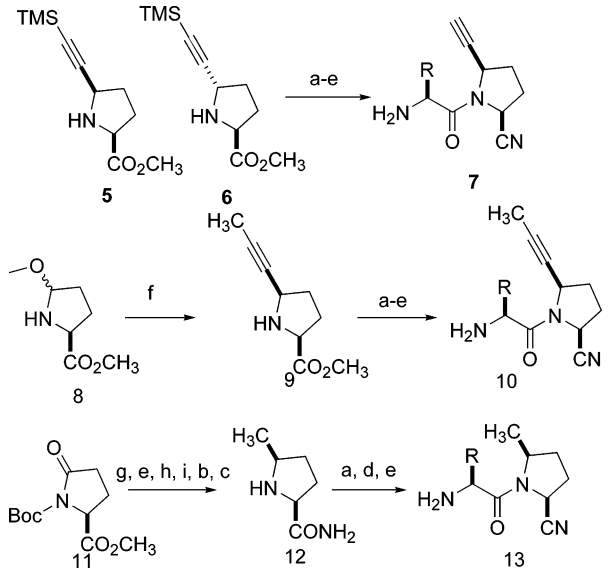
Scheme 1[a]



[a] Reagents: (a) N-Boc amino acid, EDAC, DMAP; (b) LiOH, THF; (c) isobutyl chloroformate, add NH_3 in dioxane; (d) $POCl_3$, imidazole, pyridine; (e) TFA, CH_2Cl_2 or HCl in ether; (f) trimethylsilylpropyne, $AlCl_3$, $SnCl_4$,CH_2Cl_2; (g) MeMgBr; (h) 50 psi H_2, cat. Pd/C; (i) Boc_2O, cat. DMAP.

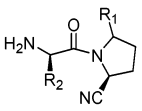
206. At the outset of our work, little was known about the effect of additional substitutions on the P1 pyrrolidine ring. However, we had anticipated that introduction of key substituents at position 4 or 5 of the pyrrolidine ring may lead to compounds with superior selectivity profiles when evaluated against closely related proteases. Ultimately, this premise proved to be correct.

Our initial thoughts on substitution were guided by homology models of DPP-IV based on the published structure of POP, which indicated only small substitutions at the C4 and C5 positions would be tolerated and substitution at C3 were precluded by the size of the S1 pocket. Systematic investigation of small substitutions at the C5 position of the pyrrolidine ring led to the discovery of 5-alkynyl-2-cyano pyrrolidines as potent DPP-IV inhibitors that have exquisite selectivity against the closely related enzymes DPP8 and DPP9.[10]

The synthesis of the dipeptides containing C5-substituted alkynyl pyrrolidines utilized chemistry described by Moeller et al. to prepare intermediates **5** and **6**.[11] Standard peptide-coupling conditions were used to prepare the N-Boc-protected dipeptides. Installation of the nitrile function was accomplished by ester hydrolysis, preparation of the primary amide by a mixed anhydride protocol and dehydration with $POCl_3$ and imidazole in pyridine. The N-Boc group was either removed with TFA or HCl to provide inhibitor **7** (Scheme 1). To prepare the C5 propyne derivative, the previously described hemiaminal was treated with trimethylsilylpropyne and a mixture of aluminum trichloride and tin(IV) chloride. For preparation of the C5 methyl compound, N-Boc-protected pyroglutamic acid methyl ester **8** was treated with methylmagnesium bromide, the Boc group removed and reductive amination accomplished stereoselectively with hydrogen and palladium on carbon. The C5 ethylene compound **18** was prepared by partial reduction of the N-Boc-protected dipeptide with Lindlar's catalyst and subsequent removal of the Boc group with HCl.

All compounds were tested *in vitro* for DPP-IV inhibitory activity using affinity-purified human DPP-IV isolated from Caco-2 cells.[12] SAR comparison of the substitutions at the C5 position reveals that DPP-IV inhibitor potency is critically related to the size and stereochemistry of the C5 substituent.

Table 1. Substitutions on the C5 Position of 2-cyanopyrrolidine with a Primary Amine P2[a]



compd	R_1 (C5 Stereochemistry)	R_2	DPP-IV K_i (nM)
1	-H	c-C_5H_9	1
14	-≡-H (R)	i-Bu	8
15	-CH_3 (R)	i-Bu	49
16	-≡-H (R)	c-C_5H_9	8
17	-CH_3 (R)	c-C_5H_9	97
18	-$CH{=}CH_2$ (R)	c-C_5H_9	3,800
19	-≡-CH_3 (R)	c-C_5H_9	24
20	-≡-H (R)	c-C_6H_{11}	96
21	-≡-H (S)	c-C_6H_{11}	>30,000
22	Ph (S)	sec-Bu	>30,000

[a] All K_i values are the average of at least two runs.

The C5-unsubstituted compound **1** exhibited a potency of 1 nM (Table 1), while terminal alkyne substitution afforded an inhibitor **16** that was only slightly less potent. However, substitution of the larger methyl group **17** causes a significant decrease in DPP-IV inhibitory potency to 97 nM. Even more pronounced, the potency of the ethylene-substituted inhibitor **18** fell to nearly 4 μM. The propynyl substitution was tolerated, however, the analogue containing a phenyl group at C5 was found to be completely inactive. A comparison of inhibitors **20** and **21** illustrates the difference between R and S stereochemistry at the C5 position. Taken together, the SAR is consistent with a relatively small S1 pocket in the DPP−IV enzyme. Indeed, subsequent X-ray crystal structures of compounds **16** and **19** bound in the active site of the enzyme revealed that Tyr 548 is extremely close to the C5 position of the pyrrolidine ring thus restricting the size of tolerated substitutions (Figure 2).[13]

Although we were successful in identifying a substitution at the C5 position that maintained potency, compounds such as **16** did not have the required solution stability to warrant further evaluation.[14] We, therefore, began to investigate the effect of having an *N*-alkylglycine in the P2 portion of the inhibitor, knowing that inhibitors such as **2** had inherently better solution stability than those of type **1**.

To synthesize the *N*-alkylglycine P2, C5-substituted P1 inhibitors, the previously prepared amines were acylated with chloroacetyl chloride, and the ester function was converted to the nitrile, as previously described. The α-chloroamides were then treated with a variety of primary amines (Scheme 2).

The *in vitro* potencies for a small selection of the compounds prepared are illustrated in Table 2. In general, only the terminal alkyne substituent at C5 allowed for *N*-alkyl glycine substituted P2 analogues that had potencies less than 100 nM. In comparing compound **28** with compounds **33** and **34**, there was a significant loss in potency observed with the larger groups at C5. Of note, however, was the observation that analogues **31** and **32** had

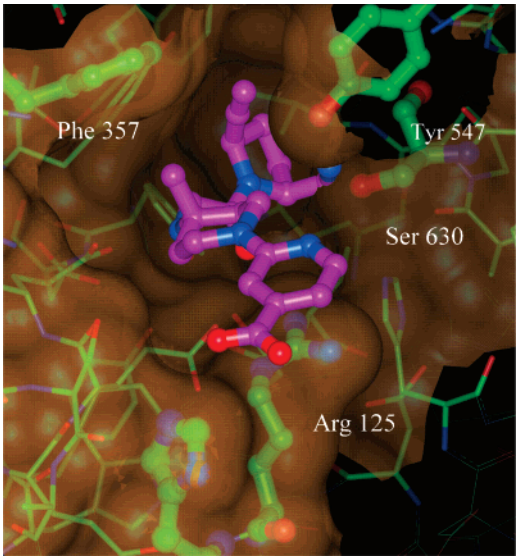
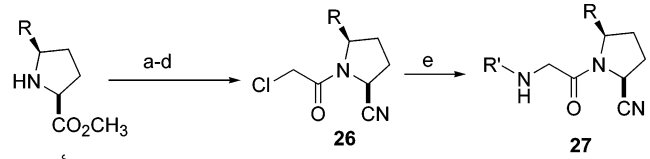


Figure 2. X-ray structure of **42** in active site of DPP-IV (protein data bank code: 2I03).

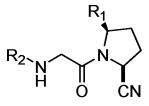
Scheme 2[a]

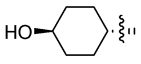
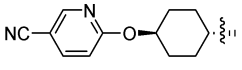


R = -C≡CH **23**
-C≡C-CH3 **24**
—CH3 **25**

[a] Reagents: (a) chloroacetylchoride, Et_3N; (b) LiOH, THF; (c) isobutylchloroformate, NMM, add 0.5 M NH_3 in dioxane; (d) $POCl_3$, imidazole, pyridine; (e) R′NH_2, CH_3CN.

Table 2. *N*-Alkylglycine Substitution in P2 with C5 Substitution in P1[a]

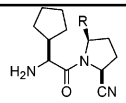
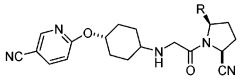
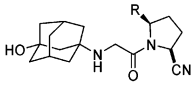
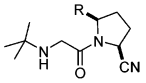
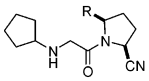


compd	R_1	R_2	DPP-IV K_i(nM)
28	-C≡CH	c-C_5H_9	22
29	-C≡CH	c-C_6H_{11}	79
30	-C≡CH		90
31	-C≡CH		5
32	-C≡CH	t-Bu	25
33	-CH3	c-C_5H_9	811
34	-C≡C-CH3	c-C_5H_9	959
35	-C≡CH		62

[a] All K_i values are the average of at least two runs.

excellent inhibitory potencies against DPP-IV and had significantly longer solution stabilities than those compounds that contained a primary amine in P2. Compound **32**, with the quaternary carbon α to the amine, was stable to heating at 37 °C in phosphate buffer for greater than 48 h.

Table 3. Selectivity of C5 Alkynl vs *des*-Alkynl Cyanopyrrolidines for DPP-IV vs DPP 8 and DPP9[a]

structure	compd	DPP-IV K_i(nM)	DPP8 K_i(nM)	DPP9 K_i(nM)
	**1** R =H	1	4	3
	16 R = -C≡CH	8	17,700	>30,000
	**37** R =H	4	110	10
	31 R = -C≡CH	5	>30,000	23,000
	**3** R =H	4	1342	68
	35 R = -C≡CH	62	>30,000	>30,000
	**38** R =H	10	12,360	3,300
	32 R = -C≡CH	25	>30,000	>30,000
	**39** R =H	11	1,218	293
	28 R = -C≡CH	22	>30,000	>30,000

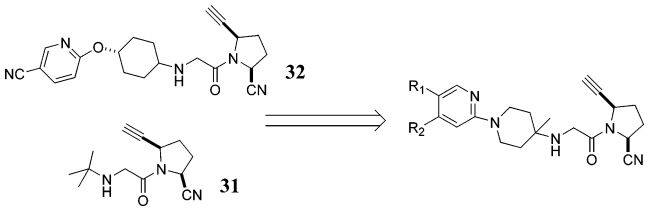
[a] All K_i values are the average of at least two runs.

We also examined the in vitro selectivity of the alkynylcyanopyrrolidines versus their *des*-C5-alkynyl counterparts. Table 3 illustrates the in vitro potencies against DPP-IV, DPP8, and DPP9.[15] In all cases examined, there was a dramatic improvement in inhibitor selectivity against DPP8 and DPP9 due to introduction of the alkyne group at C5 of the P1 cyanopyrrolidine ring. This magnitude of DPP8/9 selectivity was not observed with other C5/C4 substituents on the cyanopyrrolidine ring such as the C5/C4 cyclopropyl modification in compound **4** ($Ki_{DPP-IV} = 0.6$ nM; $Ki_{DPP8} = 130 \pm 12$ nM, $Ki_{DPP9} = 71 \pm 4$ nM).

To further optimize the potency of this series, we chose to focus on merging the chemical stability seen with analogues such as **32** with the high potency seen with more extended analogues such as **31**. To eliminate the need for a chiral center, we focused on substituted piperidine analogues such as compound **40** that had good, but not optimal, potency in our DPP-IV assay. Examination of compounds related to **40** bound in the active site of DPP-IV revealed that Arg 125 and His126 would be in close proximity to the pyridine ring of **40** and postulated that proper substitution with a carboxylate residue on the pyridine ring would impart enhanced binding through a salt bridge between the acid and the protonated form of the histidine. Gratifyingly, compounds **41** and **42** gave enhanced potencies of approximately 1 nM while retaining exquisite selectivity against DPP8, DPP9, DPP7, prolyloligo peptidase (POP) and fibroblast activating protein-α (FAP-α; Table 4).[16,17]

Indeed, an X-ray crystal structure of **42** bound in the active site of human DPP-IV revealed several critical interactions necessary for potent inhibition (Figure 2). Specifically, the pyridine moiety of **42** stacks with Arg 125 of DPP-IV, and His126 forms a salt bridge to the carboxylate of **42**. Another interesting feature of the X-ray structure was the narrow tunnel

Table 4. *N*-Arylated Piperidine DPP-IV Inhibitors



	DPP-IV K_i (nM)	DPP 8 K_i (nM)	DPP 9 K_i (nM)	DPP 7 K_i (nM)	POP K_i (nM)	FAP-α K_i (nM)
40 R1 = −CN R2 = H	3	>30 000	>30 000	>30 000	>30 000	>30 000
41 R1 = $-CO_2H$ R2 = H	1.3	>30 000	>30 000	>30 000	>30 000	>30 000
42 ABT-279 R_1 = H R_2 = CO_2H	1.0	>30 000	>30 000	>30 000	>30 000	>30 000

[a] All K_i values are the average of at least two runs.

Table 5. Selected PK Parameters for **42**[a]

Intravenous Dose					
species (dose)	$T_{1/2}$ (hr)	V_{ss} (L/kg)	V_β (L/Kg)	AUC (μg-hr/mL)	CLp (L/hr-kg)
rat (5 mg/kg)	6.8	2.2	6.7	7.6	0.68
monkey (2.5 mg/kg)	1.7	0.3	1.3	5.2	0.51
dog (2.5 mg/kg)	6.4	1.5	8.5	2.8	0.91

Oral Dose					
species (dose)	$T_{1/2}$ (hr)	C_{max} (μg/mL)	T_{max} (hr)	AUC (μg-hr/mL)	F (%)
rat (5 mg/kg)	4.7	0.37	4.7	2.1	28.0
monkey (2.5 mg/kg)	2.0	0.14	2.7	0.58	11.2
dog (2.5 mg/kg)	5.3	0.3	0.8	0.97	35.2

[a] V_{ss} is the volume at steady-state concentration, V_β is the volume of the beta phase, AUC is the area under the curve, CLp is the plasma clearance, C_{max} is the maximal concentration, T_{max} is the time at maximal concentration, and F is the oral bioavailability. The PK values are averages of at least six rats.

formed by Tyr547 and Phe357 of DPP-IV that accommodates the ethynyl group of **42**.

The pharmacokinetics of **42** were subsequently investigated in the Sprague−Dawley rat, beagle dog, and cynomolgous monkey (Table 5). Compound **42** was characterized by moderate clearance across species with oral half-lives of 2 (monkey) to 5.3 h (dogs).

Compound **42** was efficacious in a Zucker diabetic fatty (ZDF) rat model of impaired glucose tolerance[18] (Figure 3). Briefly, 10-week old female ZDF rats were differentially dosed with either vehicle or **42** after an overnight fast. Four hours later (t = 0), the rats were allowed 4-hour free access to a highly palatable, macronutrient balanced food source (Ensure). This model is analogous to an oral glucose tolerance test (OGTT) used clinically to evaluate glycemic control, except that the "challenge" is a liquid mixed meal. At 4 h post-dose, **42** (1 mg/kg) produced plasma drug levels of 18 ng/mL and caused an 87% inhibition of plasma DPP-IV activity (data not shown). This level of DPP-IV inhibition resulted in a 4-fold increase in circulating active GLP-1 levels (t = 10 min: **42** vs vehicle; 29 pM vs 7 pM) leading to a 60% increase in insulin (t = 10 min: **42** vs vehicle; 39 ng/mL vs 24 ng/mL) and a 50% reduction in glucagon levels (t = 30 min: **42** vs vehicle; 42 pg/dL vs 93 pg/dL), culminating in a 50% reduction in postprandial glucose excursion ($AUC_{glucose\ (0-240\ min)}$; **42** vs vehicle; 12 470 mg/dL·min vs 22 860 mg/dL·min; Figure 3).

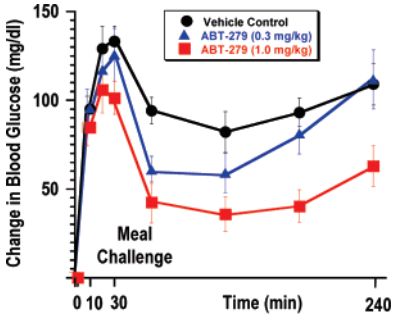


Figure 3. Compound **42** improves glucose tolerance in the female ZDF rat. Compound **42** was orally dosed 4 h prior to the rats being allowed to free feed for 4 h on a mixed meal. Data are expressed as mean ± SEM (n = 10/group).

Compound **42** exhibited an excellent preclinical safety profile. It showed no inhibition of major liver metabolic enzymes such as CYP3A4, CYP2D6, and CYP2C9 (IC_{50} > 30 μM). Compound **42** lacked detectable binding to the hERG channel (K_i > 45.1 μM) and showed a remarkably benign hemodynamic and electrocardiographic profile when administered intravenously to either anesthetized rats or dogs at concentrations up to 28 μg/mL; that is, 900× the effective plasma concentration (30 ng/mL) determined in efficacy studies. It is negative in the miniAmes mutagenicity test up to 2 mg/well and negative in murine *in vivo* clastogenicity test at concentrations up to 2000 mg/kg. In 4-week studies in rats and dogs, the "no observed adverse effect level" was greater than 1000 mg/kg/day (rat AUC_{0-24h} = 39 μg·hr/mL; dog AUC_{0-24h} = 36 μg·hr/mL). Based on its combined profile of excellent potency, selectivity, efficacy, and *in vivo* safety, **42** was selected as a candidate for clinical evaluation in humans.

Experimental Section

The synthesis of compound **42** is indicated below (the last two steps). For more information (procedures for all intermediates and final compounds), see Supporting Information.

(2*S*,5*R*)-5-Ethynyl-1-{*N*-(4-methyl-1-(4-carboxy-pyridin-2-yl)-piperidin-4-yl)glycyl}pyrrolidine-2-carbonitrile (42). To a stirred solution of (2*S*,5*R*)-1-(chloroacetyl)-5-ethynylpyrrolidine-2-carbonitrile (0.058 g, 0.30 mmol) in dioxane (3.0 mL) and water (1.0 mL) at room temperature was added 4-amino-4-methyl-3,4,5,6-tetrahydro-2*H*-(1,2′)bipyridinyl-4′-carboxylic acid *tert*-butyl ester (0.170 g, 0.58 mmol). The reaction mixture was stirred at room temperature for 48 h, concentrated under reduced pressure and purified by flash chromatography with 5% methanol in dichloromethane. The product was mixed with TFA in dichloromethane (1:1, 6 mL), and after 2 h, the volatiles were removed under reduced pressure. The residue was triturated with diethyl ether to provide the titled compound as the TFA salt. MS (CI) m/z 396 $(M + 1)^+$; ^{1}H NMR (300 MHz, methanol-d_4) δ ppm 8.17 (d, 1H), 7.67 (s,-1H), 7.31 (dd, 1H), 4.84 (m, 2H), 4.34−4.15 (m, 4H), 3.41−3.35 (m, 2H), 3.20 (m, 1H), 2.52−2.24 (m, 5H), 2.07−2.00 (m, 4H), 1.59 (s, 3H). Anal. ($C_{21}H_{25}N_5O_3$·2.3 TFA) C, H, N.

Supporting Information Available: Experimental procedures including characterization data for new compounds and biological experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) The K_i for rat DPP-IV is 1.0 nM.

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