Discovery of ((4*R*,5*S*)-5-Amino-4-(2,4,5-trifluorophenyl)cyclohex-1-enyl)-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8*H*)-yl)methanone (ABT-341), a Highly Potent, Selective, Orally Efficacious, and Safe Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes

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Abstract: Dipeptidyl peptidase IV (DPP4) deactivates glucose-regulating hormones such as GLP-1 and GIP, thus, DPP4 inhibition has become a useful therapy for type 2 diabetes. Optimization of the high-throughput screening lead **6** led to the discovery of **25** (ABT-341), a highly potent, selective, and orally bioavailable DPP4 inhibitor. When dosed orally, **25** dose-dependently reduced glucose excursion in ZDF rats. Amide **25** is safe in a battery of in vitro and in vivo tests and may represent a new therapeutic agent for the treatment of type 2 diabetes.

Dipeptidyl peptidase IV (DPP4,[a] also known as CD26) is a 110-kDa serine protease that is ubiquitously distributed in the body. It modulates biological activities of a number of peptides by cleaving two amino acid residues from the N-terminus.[1] Two of the most prominent endogenous substrates of DPP4 are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP, also known as gastric inhibitory polypeptide), both of which play important roles in regulating blood glucose levels.[2] DPP4 knock-out mice not only showed reduced degradation of both GLP-1 and GIP, but were also healthy without any major immune phenotype (despite the putative importance of DPP4 for immune function), showed improved glucose tolerance, and were resistant to body weight gain on a high-fat diet.[3] DPP4-deficient Fisher rats also showed similar phenotypes.[4] Furthermore, chronic treatment with DPP4 inhibitors preserved islet function in diabetic mice and improved β-cell survival and islet cell neogenesis in streptozotocin-induced diabetic rats,[5] which suggest that DPP4 inhibitors might be able to induce production of new β-cells in type 2 diabetics and thus prevent the progression of the disease. More importantly, several clinical trials in humans show that small-molecule DPP4 inhibitors are well-tolerated, lower blood glucose and/or HbA_{1c} levels, and increase glucose tolerance.[6] All the above evidence suggests that DPP4 inhibitors have the potential to be novel, safe, and useful antidiabetic agents.[7]

Chart 1. Structures of Selected DPP4 Inhibitors in Clinical Trials

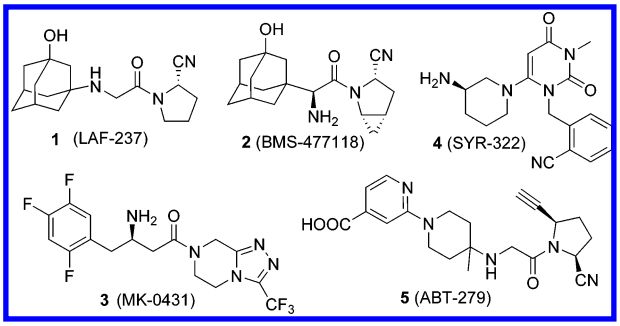
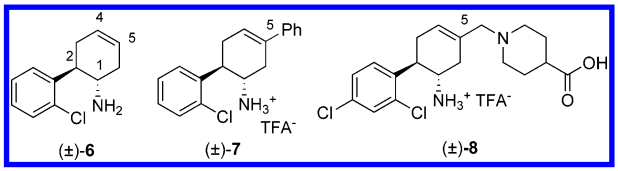


Chart 2



Intensive research efforts have resulted in the discovery of a number of potent DPP4 inhibitors.[7] Pioneering work led to the identification of first-generation DPP4 inhibitors such as **1**[8] and **2**[9] (Chart 1). They are potent but may lack optimal selectivity[10] against other related peptidases such as DPP8[11] and DPP9.[12] Because inhibition of DPP8/9 was linked to toxicity in animal studies,[13] efforts were made to identify second-generation inhibitors that are *selective.* Another study demonstrated that high levels of GLP-1 should be maintained for 24 h for optimal glycemic control.[14] These results provided impetus for us and others to identify potential third-generation DPP4 inhibitors that are not only potent and selective, but also possess pharmacokinetic profiles that provide ≥90% DPP4 inhibition for ≥24 h for maximal efficacy, particularly in more severe diabetic patients (e.g., HbA_{1c} > 9%).[15] Preclinical studies will give indications as to whether this goal is achieved by known DPP4 inhibitors **3**,[16] **4**,[17] **5**,[18] or our new compound, although a definitive answer will await clinical trials in humans.

In this work, cyclohexene-constrained phenethylamine **6** (Chart 2) was identified by high-throughput screening of the Abbott compound collection and subsequently confirmed as a weak DPP4 inhibitor with a K_i of 0.82 μM, as assayed with human DPP4 using Gly-Pro-7-amido-methylcoumarin (Gly-Pro-AMC) as the substrate.[19] After initial SAR exploration revealed that (1) 2,4-dichlorophenyl increases potency over 2-chlorophenyl; (2) the most fruitful position on the cyclohexene ring to modify is C5, as evidenced by compound **7** (K_i < 0.14 μM), acid **8** was identified as a lead with a K_i of 0.045 μM.

The general synthesis of cyclohexene-constrained phenethylamines is outlined in Scheme 1. Thermal Diels−Alder reaction between nitrostyrenes **9** and 2-trimethylsiloxy-1,3-butadiene gave racemic ketones **10** after acidic workup. Vilsmeier bromoformylation of ketones **10** furnished bromoaldehydes **11** in modest yields.[20] After the aldehydes were reduced to alcohols **12**, the bromine atom was removed to afford alcohols **13**. The alcohol group was transformed to a mesylate group, which was replaced with amines. Reduction of the nitro group and subsequent reverse-phase HPLC purification afforded amines **8** or **14** as TFA salts. Alternatively, the nitro group of **12b** was reduced with zinc, and the resulting amino group was protected with a Boc group to afford **15**, which was then debrominated

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[a] Abbreviations: DPP4, dipeptidyl peptidase IV; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide; OGTT, oral glucose tolerance test; HbA1c, glycosylated (or glycated) hemoglobin A.

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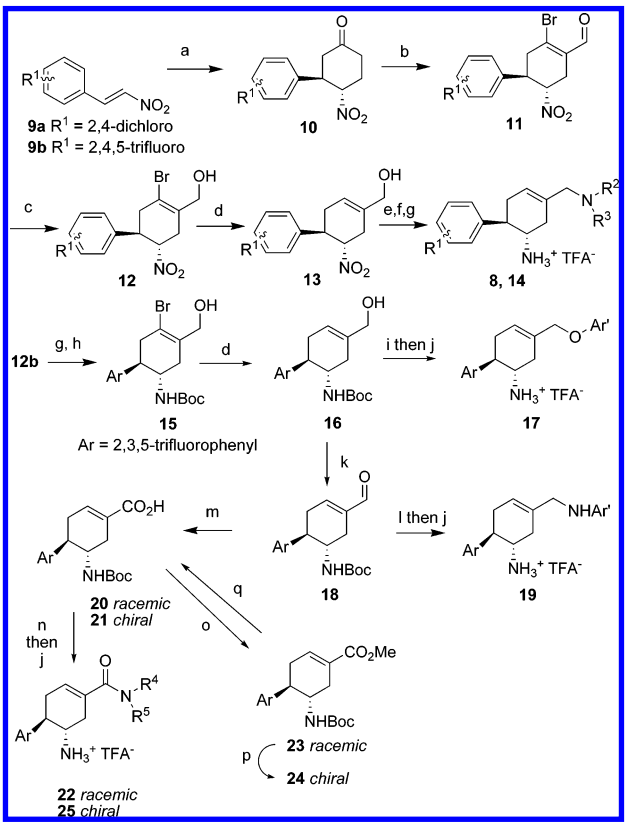
Scheme 1[a]



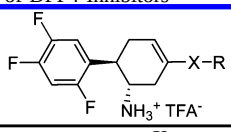
[a] Reagents and conditions: (a) trimethylsiloxy-1,3-butadiene, toluene, 120 °C, then TFA; (b) PBr_3, DMF/CH_2Cl_2, 0 °C to rt; (c) $NaBH_4$ or $NaBH(OAc)_3$, EtOH/CH_2Cl_2; (d) HCO_2H, *n*-Bu_3N, cat. $Pd(PPh_3)_2Cl_2$, DMF, 80 °C; (e) MsCl, CH_2Cl_2, TEA, 0 °C; (f) R^2R^3NH, CH_2Cl_2, TEA; (g) Zn, HOAc/MeOH, reflux; then HPLC; (h) $(Boc)_2O$, THF; (i) Ar′OH, DBAD, PPh_3, toluene, 80 °C; (j) TFA, CH_2Cl_2, rt; (k) Dess−Martin periodinane, CH_2Cl_2; (l) $NaBH_3CN$, Ar′NH_2; (m) $NaClO_2$, isoprene, NaH_2PO_4 buffer, DMSO; (n) R^4R^5NH, TBTU, TEA, DMF, rt; (o) $TMSCHN_2$, MeOH; (p) chiral prep. HPLC; (q) NaOH, THF/H_2O, rt.

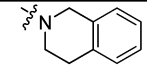
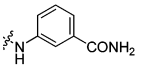
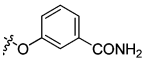
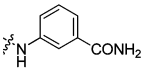
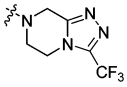
to alcohol **16**. Mitsunobu reaction between **16** and a phenol, then subsequent removal of the Boc group, yielded ether **17**. Alcohol **16** was oxidized to aldehyde **18**, which underwent reductive amination and subsequent removal of the Boc group to give **19**. Aldehyde **18** was further oxidized to acid **20**, which was coupled with an amine, followed by removal of the Boc group to provide amide **22**.

To make optically pure inhibitors, acid **20** was transformed to racemic ester **23**, which was resolved by chiral HPLC to give optically pure ester **24**. Ester **24** was hydrolyzed under basic conditions to give chiral acid **21**, which was converted to optically pure final product **25** in a similar manner to that described for amide **22**.

Table 1 summarizes the SAR of the DPP4 inhibition and selectivity over DPP8 and DPP9. After dichlorophenyl was switched to a less hydrophobic, equipotent trifluorophenyl at the P1 position, bicyclic amine **14** (K_i = 12 nM) was identified and showed significantly improved potency over acid **8**. Moving the phenyl ring closer to the cyclohexene ring and introducing an amidophenyl group resulted in **19** with a single-digit K_i of 6 nM. Analog **17**, with an ether linker, was also very potent, with a K_i of 3.6 nM. However, all of these analogs had limited selectivity over DPP8 and DPP9 (e.g., amine **14** was only 6-fold selective over DPP9). Replacement of the flexible linkers with a more rigid amide significantly improved the selectivity. Thus, amide **22** had a K_i of 4.7 nM against DPP4 and was 1800-fold and 3000-fold selective over DPP8 and DPP9, respectively.

Table 1. SAR Summary of DPP4 Inhibitors[a]



#	X	R	K_i DPP4 (nM)	K_i DPP8 (nM)	K_i DPP9 (nM)
14[b]	CH_2		12	310	73
19[b]	CH_2		6.0	230	500
17[b]	CH_2		3.6	560	430
22[b]	CO		4.7	8,800	14,000
25[c]	CO		1.3 1.6[d]	>30,000	4,000

[a] Values reported are the mean of at least two runs using human DPP4. Potencies were unchanged when rat DPP4 was used. [b] Racemic. [c] Chiral. [d] K_i in 10% of human serum.

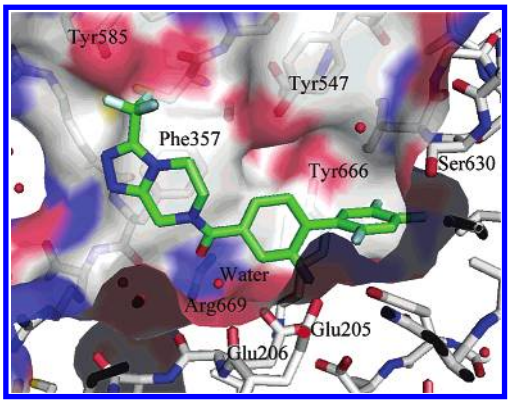


Figure 1. X-ray crystal structure of **25**/huDPP4 complex. Color-coding: all nitrogen atoms are in blue, all oxygen atoms in red and fluorine atoms in cyan. Carbon atoms of **25** are in green, while the carbon atoms of the protein are in gray. The protein surface is shown with atom coloring.

Finally, introduction of a known bicyclic amine[16] provided **25** (A-916165), which showed excellent potency (K_i = 1.3 nM) and selectivity, and the potency was maintained even in the presence of 10% human serum (K_i = 1.6 nM). Neither was there a change in potency when rat DPP4 was used.

The structure of the amide **25**/huDPP4 complex was solved by X-ray crystallography. As shown in Figure 1, the trifluorophenyl occupies the hydrophobic S1 pocket.[21] The amino group on the cyclohexene ring is in close proximity to the side chains of Glu205 and Glu206 for an electrostatic interaction. The carbonyl oxygen of **25** is oriented toward a water molecule positioned for a bridging hydrogen-bonding interaction with the side chain of Arg669. A favorable hydrophobic interaction of the heterocycle with the side chain of Phe357 is observed.

Amide **25** showed superior pharmacokinetic (PK) profiles in all three species tested (Table 2). Amide **25** is characterized by having good exposure across the species, good oral half-lives (5.4 to 6.7 h), large volumes of distribution (7.19 to 3.20 L/kg), and excellent oral bioavailabilities (54 to 104%). Based on the PK in these three species, it is predicted that a 150 mg dose of

Table 2. Selected PK Parameters of Amide **25** in Rat, Dog, and Monkey[a]

		AUC (ng·hr/g)	C_{max} (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	Vss (L/kg)	F (%)
rat	iv	2920			5.3	7.19	
	po	1948	195	4.7	5.4		67
dog	iv	6019			7.0	4.03	
	po	6285	718	0.58	6.7		104
monkey	iv	3557			4.3	3.20	
	po	1905	345	2.7	6.3		54

[a] Dosed at 5 mg/kg in rats ($n = 6$) and 2.5 mg/kg in dogs and monkeys ($n = 6$). AUC, area under curve; C_{max}, max. concentration; T_{max}, time when max. concentration was achieved; $t_{1/2}$, terminal half-life; *V*ss, steady-state volume of distribution; *F*, oral bioavaibility.

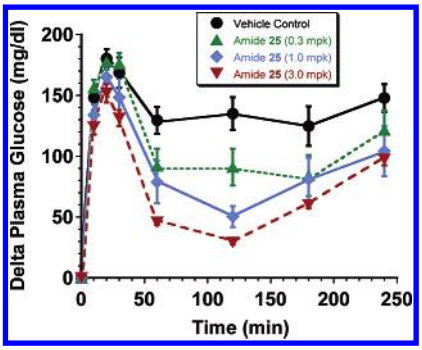


Figure 2. Effects of amide **25** on glucose excursion in female ZDF rats ($n = 10$/group).

amide **25** once a day will provide ≥90% DPP4 inhibition for 24 h in humans.[22]

Next, we profiled amide **25** in our efficacy model. Briefly, 10-week old, female Zucker diabetic fatty (ZDF) rats were dosed with either vehicle or amide **25** orally after an overnight fast. Four hours later ($t = 0$), the rats were allowed free access to a highly palatable, macronutrient balanced food source during the next 4 h. This model is analogous to oral glucose tolerance tests (OGTT) used clinically to routinely evaluate glycemic control, except that the "challenge" is a liquid mixed meal composed of fats, proteins, and carbohydrates, thus a more realistic representation of the nutritional makeup of normal food. Plasma glucose levels were measured at six time points over the course of 4 h, and the change of plasma glucose levels from the baseline is shown in Figure 2. Amide **25** caused a dose-dependent reduction in glucose excursion. The reduction as measured by area under curve (AUC) was 23, 37, and 51% at 0.3, 1.0, and 3.0 mpk, respectively. Consistent with the mechanism of action, active GLP-1 levels (measured at $t = 10$ min) increased by 151%, 163% and 291% at 0.3, 1.0 and 3.0 mpk, respectively. Glucagon levels (measured at $t = 30$ min) decreased by 36, 46, and 61% at 0.3, 1.0, and 3.0 mpk, respectively. The plasma concentration of amide **25** reached 120 ng/mL ($t = 0$), providing 98% ($t = 0$) and 96% ($t = 240$ min) inhibition of DPP4 at 3 mpk.

Besides being efficacious in vivo, amide **25** also possesses an excellent safety profile. It showed no inhibition of major liver metabolic enzymes such as CYP3A4, CYP2D6, and CYP2C9 ($IC_{50} > 30$ μM). It was negative in both mini-Ames and clastogenicity tests[23] (up to 2000 μg/well). It exhibited no hERG binding (dofetilide $K_i > 50$ μM, $IC_{50} > 300$ μM in patch clamp using HEK 293 cells). When amide **25** was tested against a panel of 74 receptor-binding/ion channel assays (Cerep screening) at a concentration of 10 μM,[24] it did not display control-specific binding by 50% or greater in any of the 74 assays. When administered intravenously using an anesthetized, comprehensively instrumented dog model,[25] amide **25** exhibited a benign cardiovascular profile at plasma concentrations vastly higher than those required to achieve efficacy in vivo. As such, amide **25** produced no physiologically relevant effects on mean, systolic, or diastolic arterial pressure, heart rate, cardiac output, pulmonary arterial pressure, pulmonary vascular resistance, or systemic vascular resistance at the highest concentration tested (23.9 ± 1.3 μg/mL) and produced only a small reduction in ventricular contractility at the same concentration. Also, consistent with the absence of hERG binding, amide **25** produced no increase in the QT-interval corrected for changes in heart rate.[22] Amide **25** caused no toxicological effects in a 5-day study in rats when dosed up to 1000 mg/kg/day. Taken together, the combination of superb potency and pharmacokinetic profiles offers the potential for amide **25** to reach maximal efficacy achievable by the mechanism of DPP4 inhibition (as limited by the GLP-1 and GIP secretory capacity). Based on its combined profile of excellent potency, selectivity, efficacy, and safety, amide **25** was selected as a drug development candidate (ABT-341).

In summary, aided with structural information, we were able to quickly optimize the high-throughput screening lead **5**, leading to the discovery of amide **25**, a highly potent, selective, orally efficacious, and potentially third-generation DPP4 inhibitor. When dosed orally, amide **25** dose-dependently reduced glucose excursion, increased active GLP-1 levels, and decreased glucagon levels in ZDF rats. Amide **25** is safe in a battery of in vitro and in vivo safety tests and may represent a new therapeutic agent for the treatment of type 2 diabetes.

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

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