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(3*R*)-4-[(3*R*)-3-Amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(2,2,2-trifluoroethyl)-1,4-diazepan-2-one, a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes

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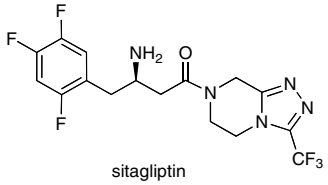
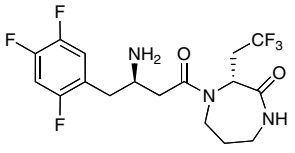
Abstract—Replacement of the triazolopiperazine ring of sitagliptin (DPP-4 IC_{50} = 18 nM) with 3-(2,2,2-trifluoroethyl)-1,4-diazepan-2-one gave dipeptidyl peptidase IV (DPP-4) inhibitor **1** which is potent (DPP-4 IC_{50} = 2.6 nM), selective, and efficacious in an oral glucose tolerance test in mice. It was selected for extensive preclinical development as a potential back-up candidate to sitagliptin.

The incretin hormone glucagon-like peptide 1 (GLP-1) stimulates insulin biosynthesis and secretion, and inhibits glucagon release in a glucose-dependent manner.[1,2] GLP-1 and its analogs have been the subject of intense research related to the treatment of type 2 diabetes.[3,4] However, active GLP-1 (GLP-1[7–36]amide) is rapidly degraded in vivo through the action of dipeptidyl peptidase IV (DPP-4), a serine protease which cleaves a dipeptide from the N-terminus to give the inactive GLP-1[9–36]amide.[5,6] Small-molecule inhibitors[7,8] of DPP-4 have been shown to prolong the beneficial effects of this incretin hormone, as well as stabilize other incretin hormones such as glucose-dependent insulinotropic polypeptide (GIP).[9] Indeed, improved glucose tolerance in diabetic patients was achieved in human clinical trials with several small-molecule DPP-4 inhibitors,[10] including sitagliptin.[10f] In this paper, we describe the synthesis, SAR, and biological evaluation of (3*R*)-4-[(3*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(2,2,2-trifluoroethyl)-1,4-diazepan-2-one (**1**), which was chosen for extensive pre-clinical studies as a potential back-up to sitagliptin.



sitagliptin



1

The DPP-4 inhibitors **5** reported in this manuscript were prepared from β-amino acid intermediates **2**[10f,11] and substituted heterocyclic amines **3** using standard peptide coupling conditions followed by deprotection as shown in Scheme 1.

The imidazolone (n = 0) and piperazinone (n = 1) heterocycles **3** are commercially available. The diazepanones (n = 2) are prepared as shown in Scheme 2. The hydrochloride salt of amino ester **6** is condensed with acrylonitrile **7** and the amino group of the product formed is protected as its *tert*-butoxylcarbonyl (Boc)

Keywords: DPP-IV; Diabetes; Diazepanone.
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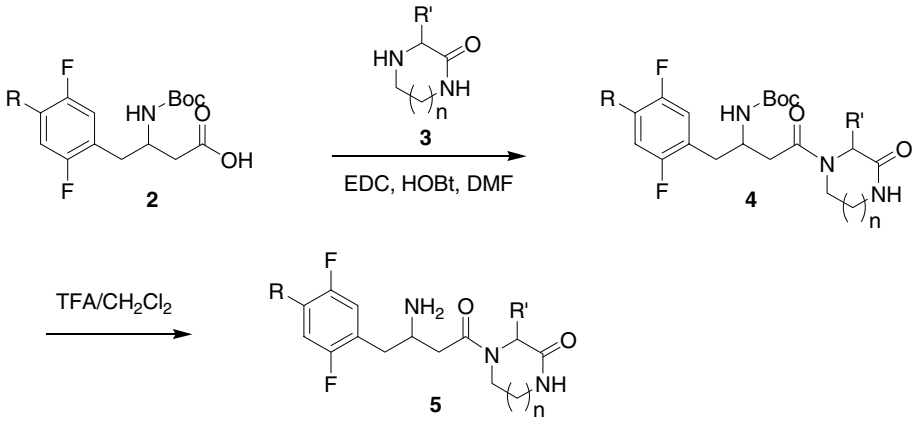
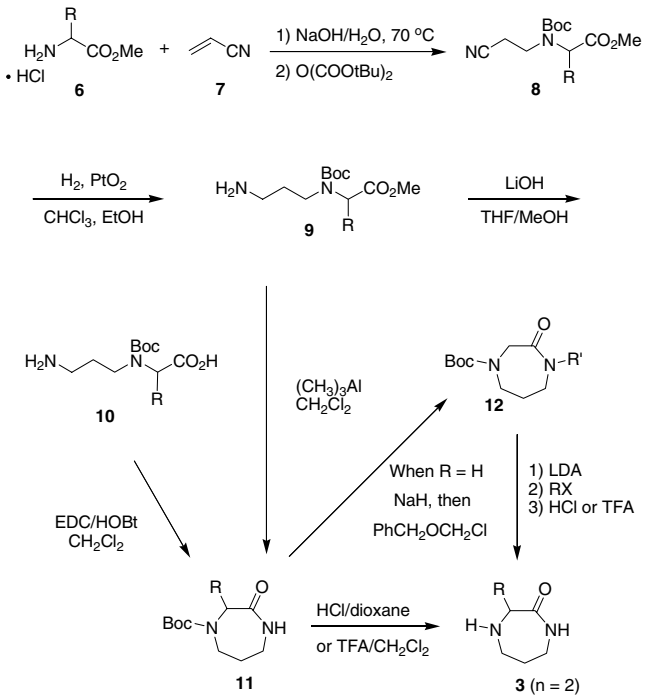


Scheme 1.



Scheme 2. Synthesis of hexahydrodiazepinones.

derivative to provide **8**, which is reduced to primary amine **9**. Amino ester **9** was hydrolyzed to acid **10** and cyclized using amino acid coupling reagents such as EDC to provide intermediate **11**. Alternatively, direct cyclization of **9** to *N*-protected hexahydrodiazepinone **11** was also conducted using trimethylaluminum. Deprotection of Boc derivative **11** by treatment with hydrogen chloride in dioxane or trifluoroacetic acid in dichloromethane provided diazepanone **3** ($n = 2$). Protected diazepanone **12** (R′ = BOM), made from **11**, wherein R is hydrogen, was alkylated using bases such as lithium diisopropyl amide followed by treatment with various alkyl halides. Deprotection gave **3** ($n = 2$).

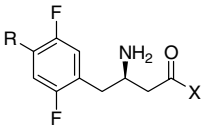
Compounds **1**, **13**–**26** were evaluated in vitro for their inhibition of hDPP-4 activity.[12] The inhibitors were also tested against DASH family members, including DPP8,[13] DPP9,[14] and fibroblast activation protein (FAP, also called seprase),[15] PEP, and other proline specific enzymes[16] with DPP-4-like activity, including quiescent cell proline dipeptidase (QPP, also known as DPP-II),[12,17] APP and prolidase. Activity against PEP, FAP, APP, and prolidase was typically $\geqslant 100$ μM and is thus not reported. DPP8 and DPP9 activity was similar, thus only DPP8 data are reported. Selectivity over the latter two enzymes was of particular concern since safety studies using a DPP8/9 selective inhibitor suggest that inhibition of DPP8 and/or DPP9 may be associated with profound toxicity in preclinical species.[18] The relevance of this toxicity to humans has yet to be determined.

DPP-4 inhibitory activities of compounds **1**, **13**–**26**[19] are listed in Tables 1–4. As shown in Table 1, the difluorophenyl analog **13** of sitagliptin has an IC_{50} of 27 nM in the DPP-4 assay.[10f] Introduction of a methyl group gave **14**, which is a more potent ($IC_{50} = 7.2$ nM) DPP-4 inhibitor.[20] Replacement of the triazolopiperazine of **14** with various ring size cyclic amides, that is, imidazolone, piperazolone, and diazepanone gave analogs **15**, **16**, and **17** with IC_{50}s of 160, 89, and 14 nM, respectively. Similar to what was seen with sitagliptin, the trifluorophenyl diazepanone **18** was more potent than the difluorophenyl analog **17**.

Since diazepanone **18** is the most potent compound in this series and has slightly better activity than sitagliptin, we investigated the activities of the four possible stereoisomers of this compound. As shown in Table 2, the decreasing order of DPP-4 inhibitory activity is: **18** (*R*,*R*) > **19** (*R*,*S*) > **20** (*S*,*S*) > **21** (*S*,*R*).

The most potent (*R*,*R*) isomer **18** was then elaborated further by modifying the methyl adjacent to the carbonyl group of the diazepanone ring. As shown in Table 3, the unsubstituted analog **22** showed greatly decreased DPP-4 inhibitory potency. Aryl-alkyl substituents give compounds such as **24**, **25**, and **26**, which are subnanomolar inhibitors of DPP-4 and display high selectivity against QPP, DPP8, and the other proline peptidases tested. However, these compounds have poor oral bioavailability (data not shown). While ethyl analog **23** is less active than the methyl derivative, the trifluoroethyl

Table 1. Activities of imidazolone, piperazone, and diazepanone DPP-4 inhibitors



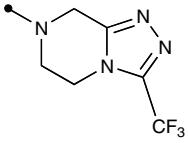
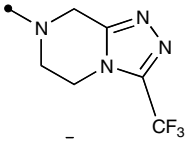
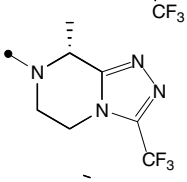
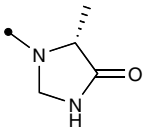
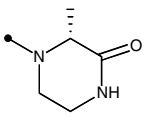
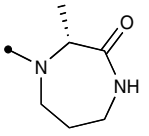
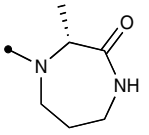
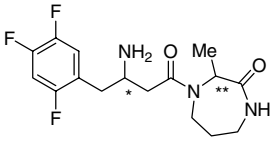
Compound	R	X	DPP-4 IC_{50} (nM)
Sitagliptin	F		18
13	H		27
14	H		7.2
15	H		160
16	H		89
17	H		14
18	F		6.6

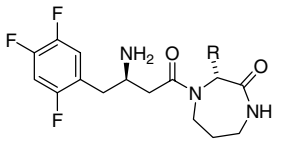
Table 2. Inhibitory properties of diazepanone isomers



Compound	Stereochemistry*	Stereochemistry**	DPP-4 IC_{50} (nM)
18	*R*	*R*	6.6
19	*R*	*S*	150
20	*S*	*S*	2,300
21	*S*	*R*	67,000

substituted analog **1** has increased activity (IC_{50} = 2.6 nM) and >10,000-fold selectivity over QPP and DPP8.

Table 3. Inhibitory properties of substituted diazepanone analogs



Compound	R	IC_{50} (nM)		
		DPP-4	QPP	DPP8
22	H	140	>100,000	82,000
18	Me	6.6	59,000	46,000
23	Et	16	47,000	13,000
1	CH_2CF_3	2.6	59,000	28,000
24	CH_2(Ph)	0.91	17,000	21,000
25	CH_2(2-pyridyl)	0.49	89,000	53,000
26	CH_2(2-pyrazolyl)	0.29	75,000	80,000

Table 4. Mean pharmacokinetic parameters of **1** and **18** in nonclinical species

Parameter	Rat		Dog	
	1	**18**	**1**	**18**
Dose iv/po (mg/kg)	1/2	1/2	1/2	1/2
Cl_p (mL/min/kg)	94	88	18	9
$T_{1/2}$ (h)	2	1	9	4.3
F_{oral} (%)	36	26	95	100
NAUC (μM h/(mg/kg))	0.2	0.15	2.2	8.5

Cl_p, plasma clearance; F_{oral}, oral bioavailability; nAUC, dose-normalized area under the plasma concentration versus time curve. Pharmacokinetic parameters were obtained following a iv (1 mg/kg) or po (2 mg/kg) dose of **1** and **18** (amorphous trifluoroacetic acid salt in rats, hydrochloride salt in dogs) in water.

The pharmacokinetic properties of the two most promising analogs **1** and **18** were then determined in male Sprague–Dawley rats and beagle dogs (1 mg/kg iv, 2 mg/kg po). As shown in Table 4, both compounds exhibited high plasma clearance and moderate oral bioavailability in rats. In dogs, however, plasma clearance was moderate and oral bioavailability was very good. In both species, the terminal half-life of trifluoroethyl analog **1** was longer than methyl analog **18**, and thus compound **1** was chosen for more extensive study.

Trifluoroethyl analog **1** is a competitive, reversible inhibitor of DPP-4 (IC_{50} = 2.6 nM), twice as active as **18**. It is highly selective over all proteases tested (IC_{50}s > 23 μM), including DPP8 and DPP9, and a panel of >170 enzyme and receptor assays at Panlabs (>10 μM). In an oral glucose tolerance test (OGTT)[10f,11] in lean mice, **1** significantly reduced blood glucose excursion in a dose-dependent manner from 0.1 mg/kg (16% reduction) to 3 mg/kg (46% reduction). At the 3 mg/kg dosage, plasma DPP-4 activity was inhibited by >80%, the targeted inhibition associated with maximal efficacy, with a corresponding plasma concentration of 266 nM. In DPP-4-deficient mice, no inhibition of glucose excursion was observed following administration of **1** (10 mg/kg po), indicating that the reduction observed in wild type mice is a mechanism-based effect.

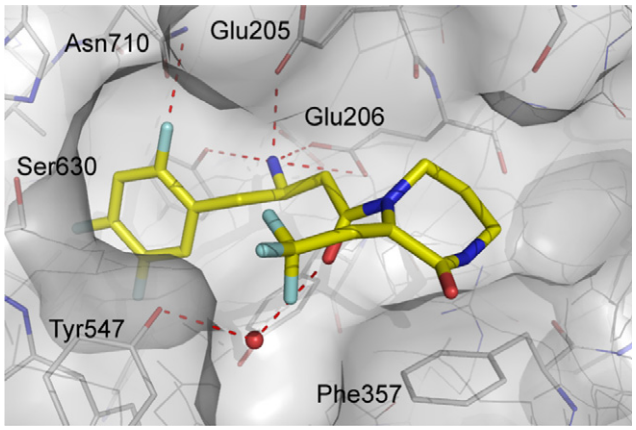


Figure 1. X-ray structure of compound **1** bound to active site of DPP-4.

Figure 1 shows compound **1** bound to the active site of DPP-4 as presented in the crystal structure. The basic amine group hydrogen bonds with the side chains of Glu205 and Glu206. The 2-F atom of the P1 fragment is within hydrogen bonding distance from the side chain of Asn710 and the amide carbonyl interacts with the side-chain hydroxyl of Tyr547 through a water molecule. The 7-membered ring group fills the hydrophobic area above the side chain of Phe357. Comparison of the structure of **1** and **18** (not shown) indicates that the methyl group points toward a hydrophobic pocket formed by the side chains of Phe357, Tyr547, and Tyr666, and that the larger trifluoroethyl substituent provides a better complementarity to the binding site, thus increasing the compound potency. The crystal structure was solved at 2.35 Å resolution. Coordinates for the structure of DPP-4 in complex with **1** and **18** have been deposited with the Protein Data Bank, accession codes 2IIT and 2IIV, respectively.

In summary, a novel series of potent diazepanone-based DPP-4 inhibitors have been discovered. Based on its DPP-4 potency, selectivity, in vivo efficacy, and pharmacokinetic profile, (3*R*)-4-[(3*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoyl]-3-(2,2,2-trifluoroethyl)-1,4-diazepan-2-one (**1**) was chosen for preclinical evaluation as a potential back-up to sitagliptin for the treatment of type 2 diabetes mellitus.

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References and notes

1. (a) Holst, J. J. *Gastroenterology* **1994**, *107*, 1048; (b) Orsakov, C. *Diabetologia* **1992**, *35*, 701.
2. Drucker, D. J. *Diabetes* **1998**, *47*, 159.
3. Ahren, B. E. *Drug Discovery Today* **2004**, *1*, 207.
4. Deacon, C. F. *Diabetes* **2004**, *53*, 2181.
5. Kieffer, T. J.; McIntosh, C. H. S.; Pederson, T. A. *Endocrinology* **1995**, *136*, 3585.
6. Deacon, C. F.; Nauck, M. A.; Toft-Nielson, M.; Pridal, L.; Willms, B.; Holst, J. J. *Diabetes* **1995**, *44*, 1126.
7. Weber, A. E. *J. Med. Chem.* **2004**, *46*, 4135.
8. Deacon, C. F.; Ahren, B.; Holst, J. J. *Expert Opin. Investig. Drugs* **2004**, *13*, 1091.
9. (a) Gautier, J. F.; Fetita, S.; Sobngwi, E.; Salaun-Martin, C. *Diabetes Metab.* **2005**, *31*, 233; (b) Ahren, B.; Hughes, T. E. *Endocrinology* **2005**, *146*, 2055.
10. (a) Ahren, B.; Simonsson, E.; Larsson, H.; Landin-Olsson, M.; Torgeirsson, H.; Jansson, P.-A.; Sandqvist, M.; Bavenholm, P.; Efendic, S.; Eriksson, J. W.; Dickinson, S.; Holmes, D. *Diabetes Care* **2002**, *25*, 869; (b) Ahren, B.; Gomis, R.; Standl, E.; Mills, D.; Schweizer, A. *Diabetes Care* **2004**, *27*, 2874; (c) Ristic, S.; Byiers, S.; Foley, J.; Holmes, D. *Diabetes Obes. Metab.* **2005**, *7*, 692; (d) Matthew, C.; Daniel, J. D. *Diabetes Care* **2006**, *29*, 435; (e) Raz, I.; Hanefeld, M.; Xu, L.; Caria, C.; Williams-Herman, D.; Khatami, H. *Diabetologia* **2006,** doi:10.1007/s00125-006-0416-z; (f) Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, K.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R.; Wu, J.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. *J. Med. Chem.* **2005**, *48*, 141.
11. Brockunier, L.; He, J.; Beconi, M.; Colwell, L. F., Jr.; Habulihaz, B.; He, H.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R.; Teffera, Y.; Wu, J. K.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4763.
12. Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T.; Thornberry, N. A. *Biochem. J.* **2003**, *371*, 525.
13. Abbot, C. A.; Yu, D. M.; Woollatt, E.; Sutherland, G. R.; McCaughan, G. W.; Gorrell, M. D. Eur. *J. Biochem.* **2000**, *267*, 6140.
14. Olsen, C.; Wagtmann, N. *Gene* **2002**, *299*, 185.
15. Scallan, M. J.; Raj, B. K. M.; Calvo, B.; Garin-Chesa, P.; Sanz-Moncasi, M. P.; Healey, J. H.; Old, L. J.; Rettig, W. *J. Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5657.
16. (a) Sedo, A.; Malik, R. *Biochim. Biophys. Acta* **2001**, *1550*, 107; (b) Rosenblum, J. S.; Kozarich, J. W. *Curr. Opin. Chem. Biol.* **2003**, *7*, 496.
17. McDonald, J. K.; Leibach, F. H.; Grindeland, R. E.; Ellis, S. *J. Biol. Chem.* **1968**, *243*, 4143.
18. Lankas, G. R.; Leiting, B.; Sinha Roy, R.; Eiermann, G. J.; Beconi, M. G.; Biftu, T.; Chan, C.-C.; Edmondson, S.; Feeney, W. P.; He, H.; Ippolito, D. E.; Kim, D.; Lyons, K. A.; Ok, H. O.; Patel, R. A.; Petrov, A. N.; Pryor, K. A.; Qian, X.; Reigle, L.; Woods, A.; Wu, J.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
19. All new compounds were characterized by ^{1}H NMR and LC–MS prior to submission for biological evaluation.
20. Linda Brockunier, unpublished.