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Optimization of 1,4-diazepan-2-one containing dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes

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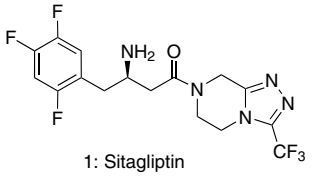
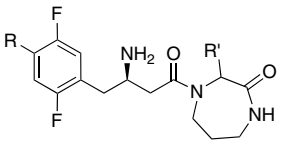
Abstract—Following the discovery of *N*-acyl-1,4-diazepan-2-one as a novel pharmacophore for potent and selective DPP-4 inhibitors, optimization of this new lead with different substitution on the seven-membered ring resulted in several highly potent and selective, orally bioavailable, and efficacious DPP-4 inhibitors, such as 3*R*-methyl-1-cyclopropyl-1,4-diazepan-2-one derivative **9i** (DPP-4 IC_{50} = 8.0 nM) and 3*R*,6*R*-dimethyl-1,4-diazepan-2-one derivative **14a** (DPP-4 IC_{50} = 9.7 nM).

Glucose-dependent insulin secretion (GDIS) is of fundamental importance in blood sugar regulation and is the object of intense research and development in both academia's and pharmaceutical industry's search for novel treatments of type 2 diabetes. The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) both play important roles in GDIS,[1,2] and therapeutic use of GLP-1 and GIP as potential antidiabetic agents has been studied.[3,4] Both hormones are deactivated rapidly in vivo through the action of dipeptidyl peptidase IV (DPP-4), a serine exopeptidase which cleaves a dipeptide from the N-terminus.[5,6] Thus, GLP-1 must be administered via continuous infusion in order to have sustained efficacy.[7,8] Development of DPP-4-resistant GLP-1 analogs, such as exenatide,[9] proved to be an effective way to circumvent this problem. Small-molecule inhibitors of DPP-4, on the other hand, have been shown to prolong the beneficial effects of endogenous GLP-1,[10,11] as well as to stabilize GIP.[12] Human clinical trials of several small-molecule DPP-4 inhibitors, including sitagliptin[13] and vildagliptin,[14] have shown improved glucose tolerance in diabetic patients.

Sitagliptin (**1**) is a β-alanine-based DPP-4 inhibitor containing a triazolopiperazine moiety. Continued SAR studies on **1** led to the discovery of 1,4-diazepan-2-one derivatives (**2a**–**d**).[15] In this paper, we report further optimization of this new lead.



1: Sitagliptin



2a: R = H, R' = Me
2b: R = F, R' = Me
2c: R = F, R' = H
2d: R = F, R' = CH_2CF_3

First we extended the SAR to 1-alkylated derivatives, probing the space that became vacant after removal of the triazolo group in **1**. Thus, intermediate **3**[15] was alkylated under the typical NaH/R–X/DMF conditions (Scheme 1). Partial epimerization at C2 was observed and resulting diastereomers were separated after the coupling step with **8**.[16] Alternatively, bulky alkyl groups, such as *tert*-butyl, that do not undergo nucleophilic substitution under these conditions, were introduced via a route outlined in Scheme 1. The

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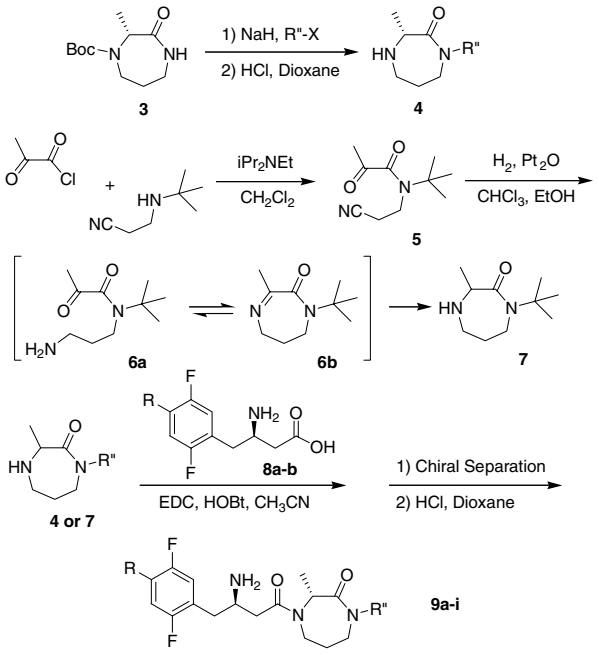


Scheme 1.

commercially available adduct of *tert*-butylamine to acrylonitrile was acylated with pyruvic acid via its acid chloride to form precursor **5**. In one pot under a hydrogen balloon in the presence of platinum oxide as catalyst, the nitrile group of **5** was reduced to primary amine **6a**, which underwent intramolecular condensation with the keto group to form cyclic imine **6b**. This equilibrium was driven to completion by the further reduction of **6b** under the reaction conditions to the desired 1-*tert*-butyl 1,4-diazepan-2-one **7**. The carbodiimide-mediated amide bond formation with intermediates **8a** and **8b** (**8a**: R = H, **8b**: R = F), followed by chiral LC separation and subsequent deprotection, yielded 1-alkyl 1,4-diazepan-2-one derivatives **9a**–**j**.[17]

These compounds were evaluated in vitro for their inhibition of DPP-4 activity[18] and selectivity against DASH family members.[19] Among them, selectivity against DPP-8 and DPP-9 was of particular concern since safety studies using a selective DPP-8/9 dual inhibitor suggest that inhibition of DPP-8 and/or DPP-9 is associated with profound toxicity in preclinical species.[20] DPP-8 and DPP-9 activities are similar, thus only DPP8 data are reported.[20] A selectivity (DPP-4/DPP-8) ratio of >1000 is targeted for the development candidates. Also reported are inhibition data against quiescent cell proline dipeptidase (QPP, also known as DPP-7).[18,21] All new compounds were tested inactive (IC_{50} > 50 μM) against fibroblast activation protein.

As shown in Table 1, most 1-alkylated compounds (**9a**–**j**) have very similar DPP-4 inhibition to their parent NH compound **2a** or **2b**, even the one with a fairly large substituent (**9g**, IC_{50} = 10.5 nM), indicating a sizable hydrophobic pocket in that part of the DPP-4 active site. This is consistent with the observation that polar groups, such as a carboxylic acid group, reduce potency (**9c**, IC_{50} = 69.9 nM). Since many of these compounds were highly potent and select in our initial screening and counter-screening, pharmacokinetic properties became increasingly important in selecting candidates for further evaluation.

The pharmacokinetic properties of several representative compounds in male Sprague–Dawley rats (1 mg/kg iv, 2 mg/kg po) are summarized in Table 2. Consistent with earlier observations, this class of compounds usually exhibits high plasma clearance and moderate oral bioavailability in rats.[15] In this case, trifluorophenyl analogs have better PK parameters than the corresponding difluorophenyl analogs, e.g., **9i** versus **9d**. Compared to our early lead **2d**,[15] cyclopropyl analog **9i** has improved oral bioavailability, half-life, and exposure. Also observed in these PK studies was the 1-dealkylated metabolite (**2a** or**2b**), which itself is a potent DPP-4 inhibitor. In the case of **9e** (1-methyl), the in vivo conversion is as high as 17%. A cyclopropyl group (**9d** and **9i**) significantly reduced this conversion to about 3%.

In an effort to further suppress this in vivo dealkylation possibly through acyl iminium ion intermediates, a proline derivative **12** was made and evaluated (Scheme 2). Although compound **12** is a potent DPP-4 inhibitor (IC_{50} = 23.9 nM), no overall improvement in drug exposure was observed in PK studies on this compound in rat (Table 2). The result indicated, however, that small substituents in the bottom half of the diazepanone ring may be tolerated.

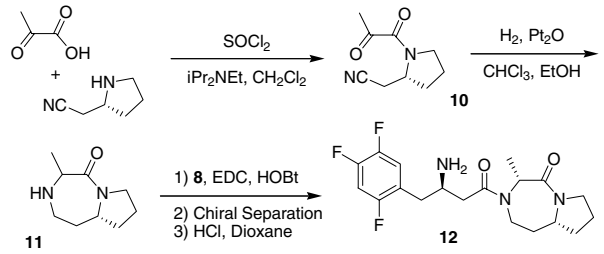
Table 1. Activities of 1-substituted diazepanone (**9**) DPP-4 inhibitors

Compound	R	R″	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)
2a	H	H	11.5	>100,000	58,000
9a	H	Et	20.7	>100,000	38,000
9b	H	CH_2CO_2Me	11.6	>100,000	29,000
9c	H	CH_2CO_2H	69.9	>100,000	>100,000
9d	H	*c*Pr	9.0	>100,000	45,000
2b	F	H	6.6	59,000	46,000
9e	F	Me	6.6	>100,000	21,000
9f	F	CH_2CH_2OH	15.1	78,000	53,000
9g	F	CH_2CH_2OBn	10.5	17,000	15,000
9h	F	CH_2CF_3	19.2	56,000	42,000
9i	F	*c*Pr	8.0	46,000	22,000
9j	F	*t*Bu	9.6	35,000	55,000

Table 2. Mean pharmacokinetic parameters of selected 1,4-diazepanone analogs in rats

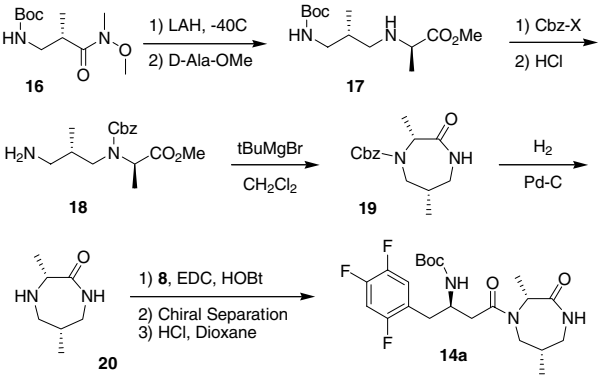
Compound	Pharmacokinetic parameter				
	Cl_p (mL/min/kg)	$t_{1/2}$ (h)	F_{oral} (%)	C_{max} (μM)	Conversion to **2a** or **2b**
2d	94	2	36	0.09	n/a
9a	152	0.8	7.4	0.03	n/a
9d	132	1.6	19	0.05	3.4%
9e	113	1.3	29	0.13	17%
9h	82	1.7	23	0.08	n/a
9i	**51**	**4.3**	**45**	**0.57**	**3%**
12	152	1.4	21	0.04	n/a
15f	97	2.0	8.1	0.01	n/a

Cl_p, plasma clearance; F_{oral}, oral bioavailability; C_{max}, peak plasma concentration after indicated oral dosing. Pharmacokinetic parameters were obtained following a iv (1 mg/kg) or po (2 mg/kg) dose (amorphous trifluoroacetic acid salt) in water. n/a, data not available.



Scheme 2.

Based on the route outlined in Scheme 3, we were able to synthesize the 5-, 6-, and 7-methyl analogs **13**, **14**, and **15**, respectively. Reductive animation of D-alanine methyl ester with desired aldehydes, readily obtained from corresponding Weinreb amides, forms the first nitrogen link between the top and bottom pieces. After protecting group manipulation, cyclization to diazepanone was accomplished by treatment with *tert*-butylmagnesium bromide.[15] From previous studies, we have learned that the (*R*) configuration at C2 is required for DPP-4 inhibitory activity.[15] As a result of the second methylation, there is a pair of diastereomers to be evaluated. In the case of the 5-methyl analogs, the diastereomers were separated, but their absolute configurations have yet to be determined. For 6- and 7-methyl analogs, enantiomerically pure starting materials were used, and therefore, the configurations of the final products are known.

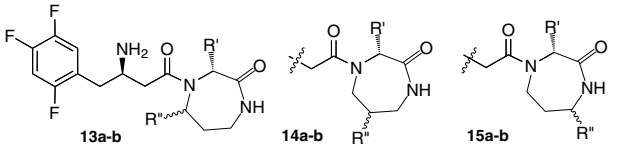


Scheme 3.

As shown in Table 3, the chirality at C5 (**13a** vs **13b**) has little effect on DPP-4 inhibition (IC_{50} ratio of 1:1.4). The ratio increases to moderate at C6 (**14a**:**14b** = 1:3.4) in favor of the (*S*) configuration and to quite large at C7 (**15a**:**15b** = 1:26) in favor of the (*R*) configuration. The change in preferred configuration is rather a result of nomenclature than the actual spatial orientation of the methyl group. It is clear that substitution at C5 (**13a** and **13b** vs **2c**) is not readily tolerated by the enzyme resulting in a 5-fold decrease in potency. On the other hand, C6 (**14a** and **14b**, IC_{50} = 9.7 and 33 nM, respectively) and C7 (**15a**, *R*-diastereomer, IC_{50} = 15.1 nM) substitutions appeared to be well-tolerated, and both potencies and selectivities remain excellent, comparable to **2b**.

Next, we modified **14a**–**14b** by replacing the 2-methyl group with other alkyl groups identified in our previous studies, such as ethyl and trifluoroethyl groups.[15] In this case, however, such replacements do not result in improvement in potency as we had observed previously (Table 4). Although both diastereomeric methyl groups are well-tolerated at the C6 position, the 6,6-dimethyl analog (**14g**, IC_{50} = 142 nM) is not. It is much less active than either one of the monomethyl analog **14a** or **14b**.

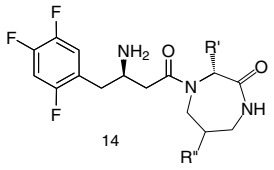
Table 3. Activities of 5-, 6-, 7-methyl diazepanone DPP-4 inhibitors



Compound	R′	R″	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)
2c	H	H	142	>100,000	42,000
13a	H	Me[a]	765	44,000	>100,000
13b	H	Me[a]	1050	>100,000	>100,000
2b	Me	H	6.6	59,000	46,000
14a	Me	(*S*)-Me	9.7	38,000	21,000
14b	Me	(*R*)-Me	33.0	71,000	93,000
15a	Me	(*R*)-Me	15.1	72,000	38,000
15b	Me	(*S*)-Me	397	73,000	70,000

[a] Diastereomeric methyl groups. Absolute configurations have yet to be determined.

Table 4. Activities of 6-methyl diazepanone **14** DPP-4 inhibitors

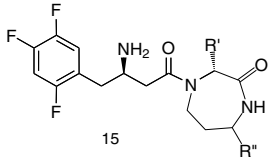


Compound	R′	R″	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)
14a	Me	(*S*)-Me	9.7	38,000	21,000
14b	Me	(*R*)-Me	33.1	71,000	93,000
14c	Et	(*S*)-Me	37.3	61,000	41,000
14d	Et	(*R*)-Me	60.3	90,000	>100,000
14e	CH_2CF_3	(*S*)-Me	24.6	31,000	16,000
14f	CH_2CF_3	(*R*)-Me	143	42,000	89,000
14g	Me	di-Me	142	17,000	26,000

Similar modifications were done on C7 methylated diazepanone **15a** (Table 5, only active diastereomers are shown). Benzyl analogs in this series (**15d** and **15f**, IC_{50} = 1.49 and 2.77 nM, respectively) have superior potencies compared to the parent 2-methyl analog **15a**, consistent with our observation from previous SAR studies on **2a**–**c**.[15] However, they suffer from reduced selectivity. Although pyridomethyl analog **15e** has comparable potency and selectivity, it has an unacceptable pharmacokinetic profile (Table 2). Replacement of the 7-methyl group with other substituents (**15g**–**i**) resulted in no improvement in terms of potency and selectivity, indicating limited space in this region of the enzyme active site.

Thus, the two most promising compounds **14a** and **15a** were chosen for extensive pharmacokinetic screening, and the data are summarized in Table 6. Across the species in rat, dog, and monkey, **14a** and **15a** exhibited good to excellent pharmacokinetic profiles. Similar to the 1-alkylated analogs **9** in Table 2, these two compounds also showed high plasma clearance in rats. This appears to be common among β-alanine-based DPP-4 inhibitors, and addition of a methyl group at C6 or C7 does provide compounds with improved oral bioavailability and drug exposure.[15] In dogs, plasma clearance is much reduced, resulting in excellent pharmacokinetic parameters including longer half-lives and good drug exposure. Plasma clearance in monkeys is relatively high for both compounds **14a** and **15a** (39, and 42 mL/min/mg, respectively), but oral bioavailability (59% and 71%, respectively) remains very good. Although predicting pharmacokinetic parameters in human is very difficult, based on clinical studies with sitagliptin,[22] good drug exposure and long half-lives suitable for once-a-day dosing can be expected for both compounds.

Table 5. Activities of 7-substituted diazepanone **15** DPP-4 inhibitors



Compound	R′	R″	DPP-4 IC_{50} (nM)	QPP IC_{50} (nM)	DPP-8 IC_{50} (nM)
15a	Me	(*R*)-Me	15.1	38,000	21,000
15c	CH_2CF_3	(R)-Me	34.3	42,000	12,000
15d	2-MeBn	(*R*)-Me	1.49	6,800	19,000
15e	2-F-Bn	(*R*)-Me	2.77	18,000	5,300
15f	2-PyMe	(*R*)-Me	11.0	27,000	34,000
15g	(*R*)-Me	CF_3[a]	139	>100,000	76,000
15h	(*R*)-Me	(*R*)-Et	29.6	49,000	20,000
15i	(*R*)-Me	2-F–Bn[a]	126	8,700	19,000

[a] Presumed (*R*) configuration based on DPP-4 activies.

Table 6. Mean pharmacokinetic parameters of **14a** and **15a** in rat, dog, and monkey

Pharmacokinetic parameter	Rat		Dog		Monkey	
	14a	**15a**	**14a**	**15a**	**14a**	**15a**
Cl_p (mL/min/kg)	92.8	87.0	8.1	8.5	39.4	42.3
$T_{1/2}$ (h)	1.5	1.5	8.1	7.2	4.4	4.7
F_{oral} (%)	49	64	77	88	59	71
C_{max} (μM)	0.14	0.17	1.21	1.29	0.29	0.43

Cl_p, plasma clearance; F_{oral}, oral bioavailability; C_{max}, peak plasma concentration after indicated oral dosing. Pharmacokinetic parameters were obtained following a iv (1 mg/kg) or po (2 mg/kg) dose in water.

In pharmacodynamic studies, oral glucose tolerance tests (OGTT) in lean male mice were conducted to determine the efficacy of these DPP-4 inhibitors. Compounds **9i**, **14a**, and **15a** or water (vehicle) were administered 60 min prior to an oral dextrose challenge (5 g/kg). Control animals received water only. The glucose AUC was determined from 0 to 120 min. Percent inhibition values for each treatment were generated from the AUC data normalized to the water-challenged controls, and percent inhibition of DPP-4 in plasma was measured at each dose level.

OGTT data on **9i**, **14a**, and **15a** are summarized in Table 7. For **14a**, significantly reduced blood glucose excursions were observed in a dose-dependent manner from 0.03 mg/kg (27% inhibition), 0.1 mg/kg (46% inhibition), to 0.3 mg/kg (49% inhibition). Because of the highly potent inhibition of **14a** against mouse DPP-4 (IC_{50} = 1.0 nM), the fully efficacious level was reached at a low dose of 0.1 mg/kg. In a separate OGTT of **14a**, plasma DPP-4 inhibition, compound concentration, and active GLP-1 level were measured 20 min after dextrose challenge. At a dose of 0.1 mg/kg, the corresponding

Table 7. Oral glucose tolerance test of **9i**, **14a**, and **15a** in lean mice

Compound	Glucose AUC % vehicle at indicated dose (mg/kg)				
	0.03	0.1	0.3	1.0	3.0
9i	n/a	n/a	28	40	43
14a	27	46	49	52	50
15a	n/a	19	34	52	55

C57BL/6N male lean mice were used for the test. n/a, data not available.

plasma concentration of **14a** reached 4.4 nM, and an uncorrected 72% inhibition of plasma DPP-4 activity was observed,[23] resulting in about a 3-fold increase in active GLP-1 levels, and full efficacy in glucose reduction. Because **9i** (mouse DPP-4 IC_{50} = 18 nM) and **15a** (mouse DPP-4 IC_{50} = 26 nM) are much less potent than **14a** against mouse DPP-4, higher doses (3 mg/kg for **9i** and 1 mg/kg for **15a**, respectively) have to be used to reach maximum efficacy based on at least 80% inhibition of plasma DPP-4. Nonetheless, all three compounds showed good efficacy in glucose reduction, comparable to that of sitagliptin.[16] Good correlations among plasma concentration, DPP-4 inhibition, GLP-1 elevation (data not shown), and glucose AUC reduction strongly suggest that the observed enhancement in glucose tolerance is indeed mechanism based and mediated at least in part by the GLP-1.

In summary, following the discovery of the novel β-alanine-derived 1,4-diazepanone-containing DPP-4 inhibitors, we have explored different substitution patterns of the diazepanone ring through continued SAR studies. Representative compounds, such as 1-substituted **9i**, 6-substituted **14a**, and 7-substituted **15a**, all have good potency against DPP-4, and good selectivity against QPP, DPP-8, and other closely related peptidases. They have good to excellent pharmacokinetic properties across species from rat and dog to monkey. And they have shown good efficacy after oral dosing in rodents at dose levels correlated well to their intrinsic potency. Optimization of this promising lead continues as we try to explore specific interactions in the enzyme active site identified by structural biology, and with input from computer modeling.

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23. It should be noted that % inhibition as determined using the in vitro assay under estimates the % inhibition achieved in vivo, as compound **14a** is a competitive, rapidly reversible inhibitor and assay of plasma DPP-4 activity requires (1) dilution of plasma which results in a dilution of the total inhibitor, and (2) presence of substrate that competes with inhibitor for binding to the enzyme.