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The design of potent and selective inhibitors of DPP-4: Optimization of ADME properties by amide replacements

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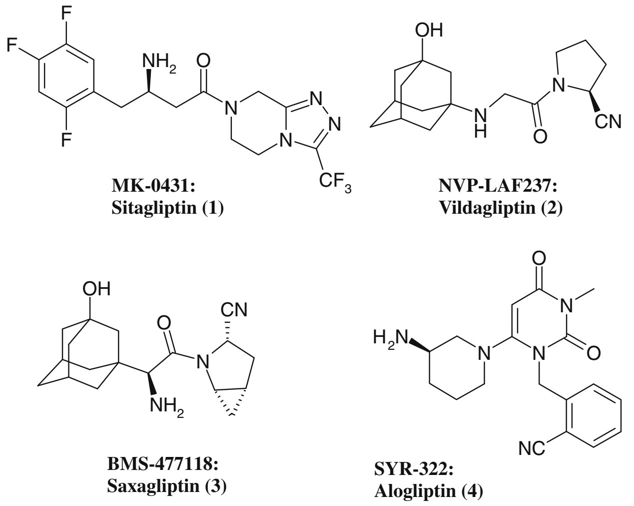
ABSTRACT

For a series of β-homophenylalanine based inhibitors of dipeptidyl peptidase IV ADME properties were improved by the incorporation of amide replacements. These efforts led to a novel series of potent and selective inhibitors of DPP-4 that exhibit an attractive pharmacokinetic profile and show excellent efficacy in an animal model of diabetes.

The inhibition of the serine protease dipeptidyl peptidase IV (DPP-4) has emerged as a major field of diabetes research.[1] The tremendous interest results from the recognition that orally active DPP-4 inhibitors have the potential to improve blood glucose and hemoglobin A_{1c} levels with low risk of hypoglycemia and other side effects associated with the current treatments for diabetes. DPP-4 is an ubiquitously expressed member of the prolyl oligopeptidase family that preferentially cleaves Xaa-Pro or Xaa-Ala N-terminal dipeptides from its substrates.[2] The incretin hormone glucagon-like peptide-1 (GLP-1) that is centrally involved in glucose homeostasis, insulin secretion, and proliferation of pancreatic β-cells serves as a natural substrate for DPP-4.[3] Inhibition of DPP-4 reduces its rapid degradation and sustains the action of the intact hormone.[4] To date, sitagliptin (**1**),[5] vildagliptin (**2**),[6] and saxagliptin (**3**)[7] have obtained approval as first representatives of this class of novel antidiabetic agents, closely followed by others such as alogliptin (**4**)[8] (Fig. 1).

In a previous Letter, we have described a series of β-homophenylalanine based pyrrolidin-2-ylmethyl-amides (**5**).[9] Early examples were found to be potent and selective inhibitors of DPP-4. However, low Caco-2 permeability and a high efflux ratio raised concerns regarding pharmacokinetic properties suitable for oral



MK-0431: Sitagliptin (1)

NVP-LAF237: Vildagliptin (2)

BMS-477118: Saxagliptin (3)

SYR-322: Alogliptin (4)

Figure 1. Selected DPP-4 inhibitors.

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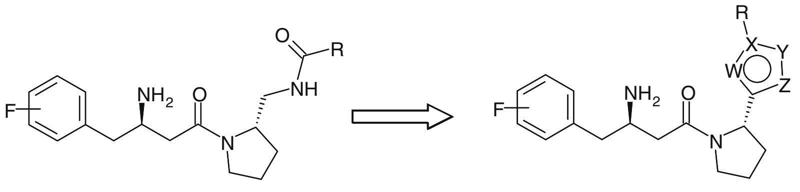


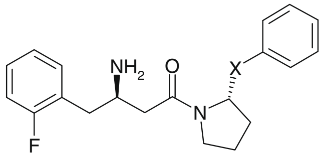
5

6-12

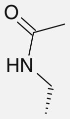
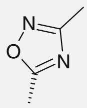
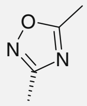
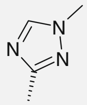
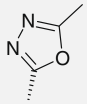
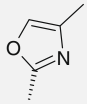
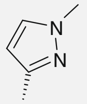
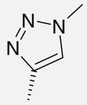
W, X, Y, Z = C, N, O

Figure 2. Heteroaryl replacements of amides.

Table 1
Heterocyclic replacements of linker moiety



5a-12a

Compd	X	DPP-4 IC_{50} (μM)	HLM	RLM
			(% Metabolized after 1 h)	
5a		0.090	16	76
6a		0.031	38	89
7a		0.090	24	81
8a		0.062	1	18
9a		0.120	16	33
10a		0.076	38	85
11a		0.180	32	82
12a		0.130	13	36

administration. As discussed previously,[10] we hypothesized that a large hydration sphere carried by the adjacent amides might account for this issue. In our search for structural modifications to overcome this while maintaining favorable properties, we envisaged to replace the side chain amide with a bioisosteric 1,2,4-oxadiazole and related heterocycles (**6**–**12**, Fig. 2).

Initially, we synthesized a range of simple phenyl substituted five-membered heterocyclic derivatives (**6a**–**12a**) that were readily accessible by methods described in the literature to evaluate the general influence of the various heterocyclic linkers on DPP-4 inhibition (Table 1) and essential in vitro ADME properties.[11]

1,2,4-Oxadiazoles were synthesized starting from a suitable nitrile that was converted into an amidoxime and subsequently cyclized with the corresponding carboxylic acid to furnish intermediates **16** or **19** (Scheme 1). For 1,2,4-triazole synthesis cyanopyrrolidine **17** was first transformed into the corresponding imidate ester. Reaction with phenylhydrazine followed by cyclization with triethyl orthoformate yielded intermediate **21** (Scheme 1).[12]

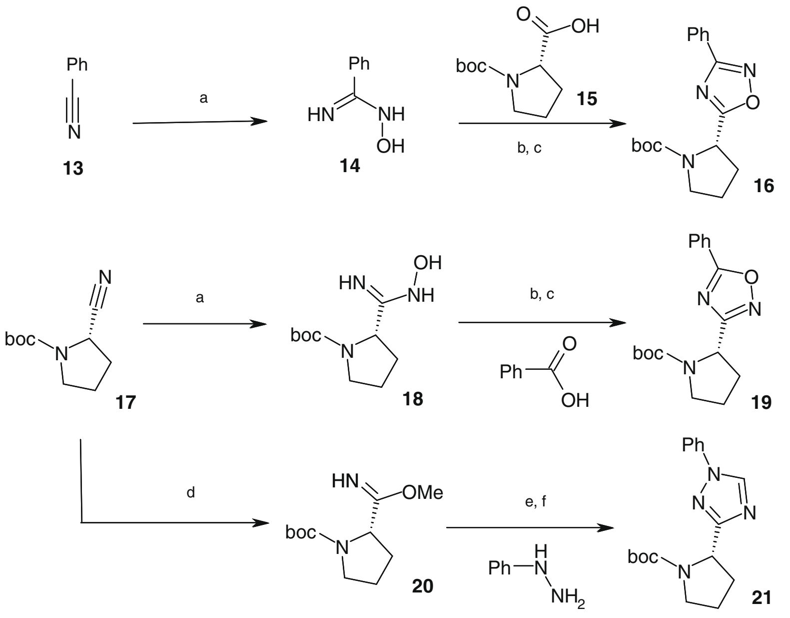
The 1,3,4-oxadiazole intermediate **24** was obtained by coupling the hydrazide **22** to Boc-protected (*S*)-proline (**15**) and subsequent cyclization employing Burgess reagent (Scheme 2).[13] (*S*)-Proline also served as starting material for the synthesis of oxazole **27** via amide coupling with (2)-amino-2-phenylethanol (**26**) and subsequent cyclization and oxidation.[14] Additionally, the pyrazole **30** was synthesized from (*S*)-proline (**15**) via transformation to the TMS alkyne (**29**) and reaction with phenylhydrazine (Scheme 2).[15] Finally, a 1,2,3-triazole intermediate **33** was synthesized by addition of phenylazide to alkyne **32** (Scheme 3).[16]

Intermediates (**16**, **19**, **21**, **24**, **27**, **30, and 33**) were deprotected and coupled with the β-amino acid moiety (**34**) under standard conditions to yield after final deprotection the target compounds (**6**–**12**, Scheme 4).

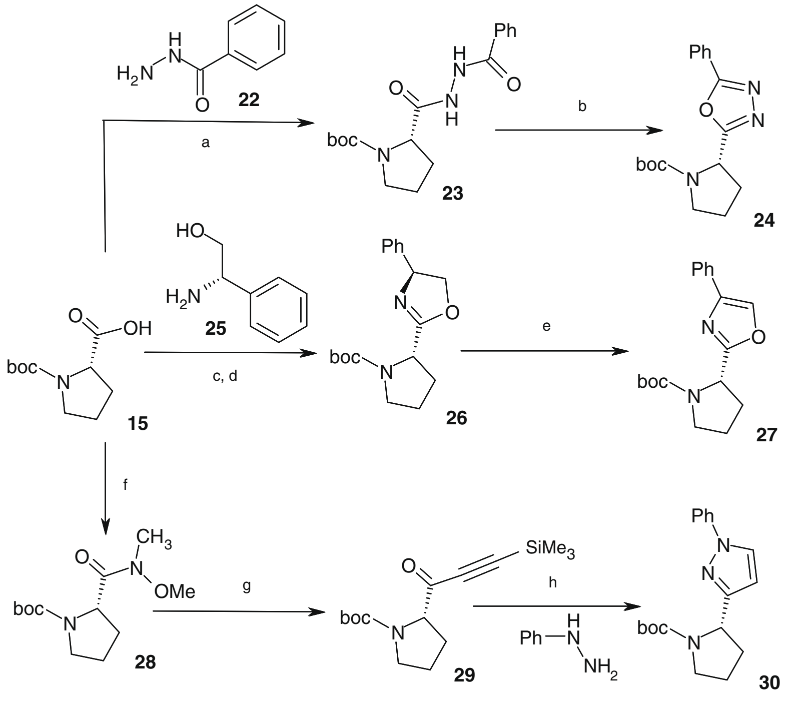
Gratifyingly, oxadiazoles and related five-membered heterocycles (**6a**–**12a**) were shown to be potent inhibitors of DPP-4, within the same range as the corresponding amide **5a**.[17] As it turned out, the orientation of the heteroatoms plays an important role for potency. For instance, isomers **7a** and **9a** lost 3–4-fold potency compared to the most potent representative oxadiazole **6a**. Also the triazoles **8a** and **12a** were found to be somewhat less potent.

Since we aimed for inhibitors that exhibit a long plasma half-life an initial screen for metabolic stability was performed. Not unexpectedly, differences for the various heterocycles were noticed. Triazoles **8a** (clog *P*: 2.04)[18] and **12a** and 1,3,4-oxadiazole **9a** (clog *P*: 1.38) were found to be more stable than oxazole **10a** (clog *P*: 2.76) and pyrazole **11a** (clog *P*: 3.03). 1,2,4-Oxadiazoles **6a** and **7a** which are comprising an NO-bond exhibited lower stability than 1,3,4-oxadiazole **9a**. In conclusion to these results for DPP-4 inhibition and metabolic stability, oxadiazoles of types **6** and **7** and triazoles of type **8** were regarded as most promising and used for further optimization in conjunction with the more potent trifluorophenyl β-amino acid moiety (Tables 2 and 3). The selection of substituents was especially guided by the intention to obtain compounds exhibiting long plasma half-lives. Based on earlier experience we focused on possibly metabolically robust moieties and aimed for reduced lipophilicity.[10]

Overall, oxadiazoles of the type **6** showed highly potent DPP-4 inhibition with IC_{50} values well below 10 nM. Type **7** appeared again about threefold less potent with IC_{50} values in the range of



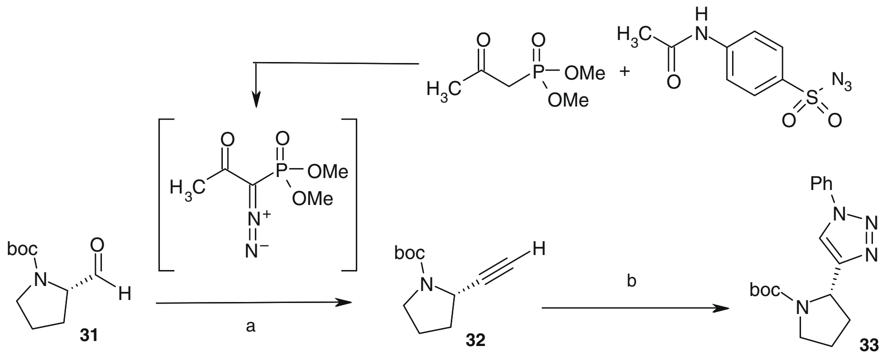
Scheme 1. Synthesis of intermediates **16**, **19**, and **21**. Reagents: (a) NH_2OH, K_2CO_3, MeOH; (b) DIC, DCM; (c) Pyridine, reflux; (d) Na, MeOH, then HCl in 1,4-dioxane; (e) NEt_3, MeOH; (f) $HC(OEt)_3$, pyridine, reflux.



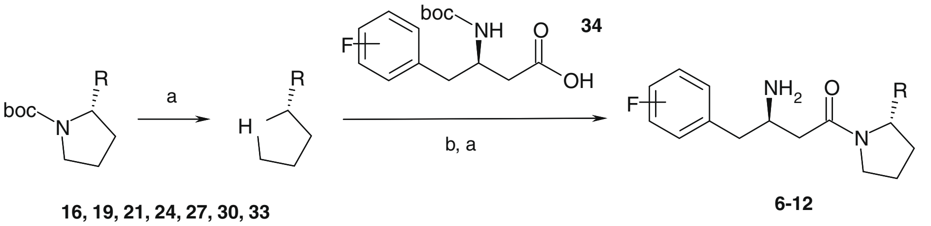
Scheme 2. Synthesis of intermediates **24**, **27**, and **30**. Reagents: (a) HBTU, HOBt, NEt_3, DMF; (b) Burgess reagent, DCM, reflux; (c) EDC, HOBt, NEt_3, DMF; (d) deoxofluor, DCM, −20 °C; (e) NiO_2, benzene, 150 °C, μW; (f) HNMe(OMe), HATU, DIPEA, DMF; (g) Me_3SiCCH, EtMgBr, THF; (h) Na_2CO_3, EtOH, H_2O, 65 °C.

10–20 nM, compound **7g** being an exception to the latter trend. Alkyl, heterocyclic and heteroaryl substituents are equally tolerated. Selectivities against related enzymes were found to be excellent for both types.[19] Unfortunately triazoles **8b** and **8c** turned out to be somewhat less potent than aimed for (IC_{50} <20 nM), a result which focused further efforts on oxadiazoles in general.

Subsequently Caco-2 bidirectional transport and metabolic stability were investigated.[20] As shown in Table 4 for selected examples, Caco-2 permeability (A–B, B–A) varies strongly with the substituent on the oxadiazole. In general, the more lipophilic representatives **6c** (*c*log *P*: 2.14→log *D*: 1.85) or **7c** show good A–B permeability and an excellent efflux ratio, however, suffer from low to moderate stability towards liver microsomes, in particular from rat. On the other hand, the less lipophilic representative **6h** exhibits low A–B permeability and a poor efflux ratio, however, provides high stability towards microsomes. A reasonable balance of properties is provided by compounds **6m** and **7f**. In comparison

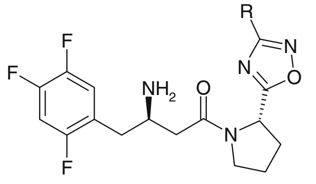


Scheme 3. Synthesis of intermediate **33**. Reagents and conditions: (a) K_2CO_3, CH_3CN, then aldehyde in MeOH, rt; (b) phenylazide, DMF, reflux, (mixture of isomers: 2.8:1 in favor of the desired substitution pattern).

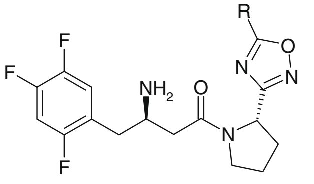


Scheme 4. Synthesis of inhibitors **6–12**. Reagents: (a) TFA, DCM; (b) **34**, EDC, HOBt, Et_3N, DMF.

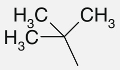
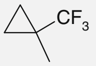
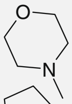
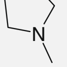
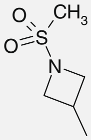
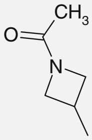
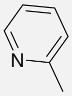
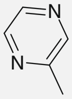
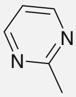
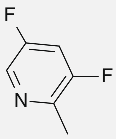
Table 2
SAR and selectivity on two isomeric oxadiazoles



6b-6m



7b-7g

Compd	R	DPP-4	DPP-7	DPP-8	DPP-9	POP
		IC₅₀ (μM)				
6b		0.007	3.8	26.0	17.0	57.0
6c		0.002	7.0	52.0	24.0	39.0
6d		0.003	4.0	37.0	11.0	>100
6e		0.002	2.8	8.2	7.4	61.0
6f		0.004	5.6	21.0	4.8	>100
6g		0.004	3.2	27.0	6.6	>100
6h		0.001	3.8	13.0	8.2	43.0
6i		0.005	17.0	18.0	9.8	65.0
6j		0.010	0.886	2.5	0.91	15.0
6k		0.002	2.3	4.5	1.6	>100
6l		0.004	2.2	34.0	5.1	48.0
6m		0.003	2.0	10.0	3.9	>100
7b		0.017	11.0	23.0	12.0	>100

(continued on next page)

Table 2 (*continued*)

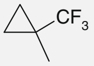
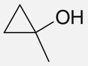
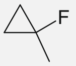
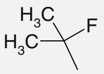
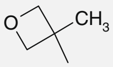
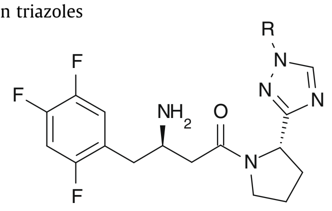
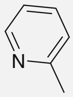
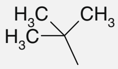
Compd	R	DPP-4	DPP-7	DPP-8	DPP-9	POP
		IC$_{50}$ (μM)				
7c		0.011	10.0	26.0	17.0	33.0
7d		0.017	23.0	35.0	19.0	45.0
7e		0.022	14.0	41.0	14.0	72.0
7f		0.016	16.0	41.0	27.0	>100
7g		0.002	38.0	72.0	27.0	>100

Table 3
SAR and selectivity on triazoles



8b, 8c

Compd	R	DPP-4	DPP-7	DPP-8	DPP-9	POP
		IC$_{50}$ (μM)				
8b		0.028	nd	nd	nd	nd
8c		0.032	36.0	25.0	36.0	>100

nd = not determined.

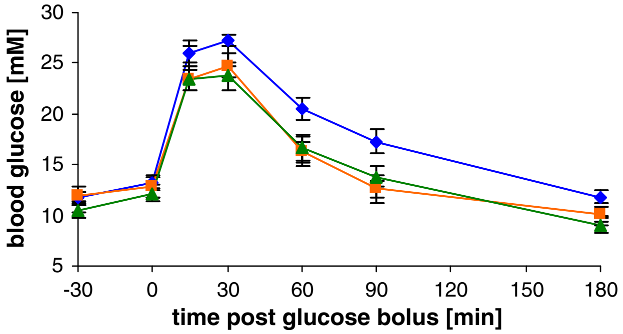


Figure 3. Oral glucose tolerance test (OGTT) with **6m** (green) and **7f** (orange) compared to vehicle (blue) in *ob*/*ob* mice.

to corresponding initial amides (e.g., **5b**, R = cyclopropyl: B–A/A–B 84 vs **6b**; and **5c**, R = CF(CH$_3$)$_2$: B–A/A–B 33 vs **7f**)[10] a marked improvement of the efflux ratio was observed. These results nicely demonstrate the successful implementation of our strategy to reduce the hydration sphere.

Selected compounds were investigated for their pharmacokinetic properties in male Wistar rats (Table 5). Oral bioavailability and C_{max} vary dramatically for the representatives within the series (e.g., **6c** vs **7f**). Compound **6c** is readily metabolized as already indicated by the microsomal stability values, exhibiting low C_{max} and oral bioavailability and a short half-life. Compound **7f** is well absorbed and shows excellent bioavailability and a high C_{max}, however, a short half-life.[21] Compared with amide **5c** oral bioavailability was improved from $F = 24\%$ to $F = 100\%$ for **7f**.[10] An attractive, balanced profile for oral bioavailability, half-life and exposure was observed for compound **6m**.

Efficacy in an animal model of diabetes was shown by improving oral glucose tolerance in *ob*/*ob* mice.[22] Data for the compounds **6m** and **7f** are shown in Figure 3. Oral administration of 3 mg/kg of the compound 30 min prior to a glucose challenge of 1 g/kg significantly reduced peak blood glucose excursion.

In summary, we have successfully implemented structural modifications in a series of β-homophenylalanine based DPP-4 inhibitors in order to improve absorption across membranes while potency in DPP-4 inhibition and selectivity towards related enzymes was maintained. Representatives **6m** and **7f** of this novel chemical series exhibit attractive pharmacokinetic profiles and show excellent efficacy in an animal model of diabetes. Further

Table 4
In vitro ADME parameters of selected compounds

Compd	Caco-2, $P_{app} \times 10^{-6}$cm/s		Caco-2 efflux ratio (B–A/A–B)	Microsomal stability $t_{1/2}$ (min)		Log *D*
	A–B	B–A		Human	Rat	
6b	21	52	2.5	>120	71	1.61
6c	20	49	2.4	80	8	1.85
6h	0.15	28	140	>120	>120	0.96
6m	3.0	73	24	>120	>120	1.32
7c	23	49	2.1	>120	60	2.16
7f	14	62	4.3	>120	102	1.46

Table 5
Pharmacokinetic profiles in rats for selected compounds

Compd	$t_{1/2}$ (h)	Cl (L/h/kg)	AUC$_{norm}$ (μM h)	Vz (L/kg)	$C_{max_{norm}}$ (μM)	*F* (%)
6c	1.6	7.2	0.004	20.5	0.002	3
6m	2.4	6.0	0.60	17.7	0.10	56
7c	2.3	6.9	0.09	17.5	0.02	43
7f	1.0	1.9	0.77	2.9	0.37	100

Pharmacokinetic parameters were obtained following an iv (1 mg/kg) or a po (3 mg/kg) dose.

evaluation is focused on preclinical safety and more comprehensive efficacy profiling. Results will be reported in due course.

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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.2009.09.078.

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20. Details on the ADME assays can be found in Ref. 10.
21. Compounds **6c**, **6m**, and **7c** exhibit low to moderate ppb (rat), **7f** shows very low ppb.
22. Compound (3 mg/kg) or vehicle was administered po to *ob*/*ob* mice 30 min prior to an oral glucose challenge of 1 g/kg. Time points for blood glucose measurement were −30 (shortly prior to the administration of test item); 0, 15, 30, 60, 90, and 180 min after glucose application. Data are expressed as mean ± SEM (*n* = 12/group). The blood glucose lowering effect of MK-0431 is comparable to **6m** and **7f** in this experiment. % Inhibition of plasma DPP-4 activity in an assay containing 80% mouse plasma at 60 and 180 min was found to be 79 ± 5 and 64 ± 7 for **6m** and 70 ± 3 and 56 ± 5 for **7f**.