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Novel heterocyclic DPP-4 inhibitors for the treatment of type 2 diabetes

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ABSTRACT

Novel deazaxanthine-based DPP-4 inhibitors have been identified that are potent (IC_{50} <10 nM) and highly selective versus other dipeptidyl peptidases. Their synthesis and SAR are reported, along with initial efforts to improve the PK profile through decoration of the deazaxanthine core. Optimisation of compound **3a** resulted in the identification of compound (*S*)-**4i**, which displayed an improved in vitro and ADME profile. Further enhancements to the PK profile were possible by changing from the deazahypoxanthine to the deazaxanthine template, culminating in compound **12g**, which displayed good ex vivo DPP-4 inhibition and a superior PK profile in rat, suggestive of once daily dosing in man.

An attractive approach to the treatment of type 2 diabetes is based on targeting the gut hormone glucagon-like peptide (GLP-1), which is released into the bloodstream in response to a meal. GLP-1 is anti-diabetic due to its ability to stimulate insulin secretion, inhibit glucagon production and increase β-cell mass.[1] A problem however is that GLP-1 is rapidly inactivated by the enzyme dipeptidyl peptidase 4 (DPP-4).[2] The inhibition of DPP-4 will increase endogenous levels of active GLP-1 by extending its half-life and consequently improve both β-cell insulin secretion and glycaemic control.[3] This concept has been validated by the approval of the covalent inhibitor Vildagliptin (Galvus®, Novartis, Fig. 1)[4] and the non-covalent inhibitor Sitagliptin (Januvia®, Merck)[5] for the treatment of diabetes.

One approach towards identifying novel, highly potent and selective, non-covalent inhibitors of DPP-4 is presented in this Letter. Intensive research in the DPP-4 area has resulted in the identification of numerous 'xanthine-related' non-covalent inhibitors.[6,7] Linagliptin (Tradjenta®, Boehringer-Ingelheim), as the most advanced member of this compound class, showed efficacy in phase III clinical trials and has recently been approved by both the FDA and EMEA.[8,9]

Our initial efforts in this area centred on modifications to the xanthine imidazopyrimidinedione core, which resulted in the identification of the 4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-d]pyrimidine-7-carbonitrile scaffold **3a** (deazahypoxanthine, Fig. 1). It was envisaged that the different structural and electronic nature of the deazahypoxanthine core compared with typical xanthines, could offer advantages in terms of potency/selectivity as well as ADME properties. Compound **3a** showed encouraging activity in the low micromolar range and was our starting point in the search for an improved deaza(hypo)xanthine-based DPP-4 inhibitor.

The synthesis of deazahypoxanthines commenced from compound **1** and involved selective alkylation at N-5 using an appropriate alkyl halide and DIPEA as the base (Scheme 1).[10] The choice of base appeared key to obtaining good regiocontrol of alkylation at N-5 versus N-3 (use of potassium carbonate gave isomeric mixtures at N-5 and N-3 although no O-alkylation was ob-

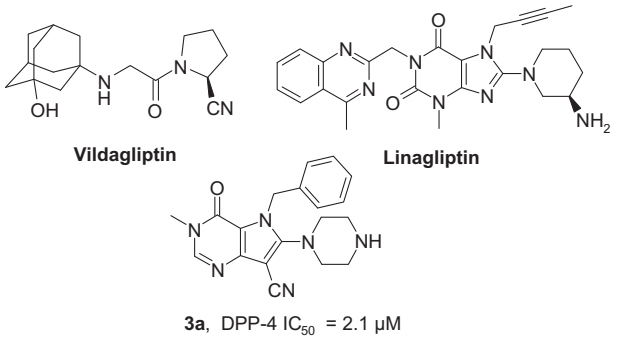

Figure 1. Vildagliptin, Linagliptin and deazahypoxanthine hit, **3a**.

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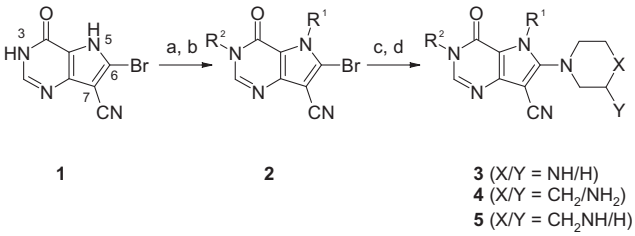


Scheme 1. Synthesis of 7-cyanodeazahypoxanthines. (a) R^1X, DIPEA, DMF, rt, 18 h. (30–69%); (b) R^2X, K_2CO_3, DMF, rt, 1 h, (63–97%); (c), Boc diamine, DMA, μ-wave, 160 °C, 45 min (40–90%); (d) TFA/DCM [1:1], rt, 1 h, (70–95%).

served). A second alkylation at N-3 could then be effected with potassium carbonate to give compound **2**. Typically, the synthesis was concluded by the incorporation of the 6-amino functionality, which was undertaken using a mono Boc-protected diamine under microwave irradiation at elevated temperatures and gave, after Boc removal with TFA/DCM, the desired DPP-4 inhibitor compounds **3**, **4** and **5**.

The initial investigation in the deazahypoxanthine series centred on incorporating structural elements found in known 'xanthine-based' inhibitors at R^1 and R^2 (Table 1).[6a,7] Altering R^2 to a 3-methoxyacetophenone group gave **3b**, which exhibited improved DPP-4 inhibition.[11] Further modifications at R^1 in **3c** and **3d** led to greatly enhanced activity, presumably due to the reduced steric demands of the but-2-ynyl and isoprenyl groups allowing a favourable conformation of the C-6 aminopiperazine. Changing from pipera-

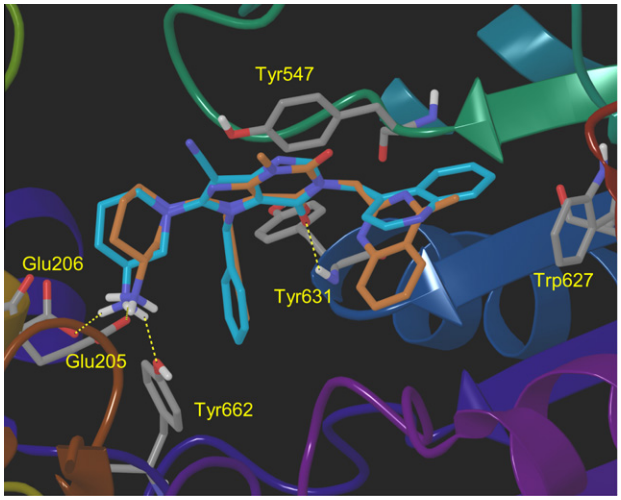
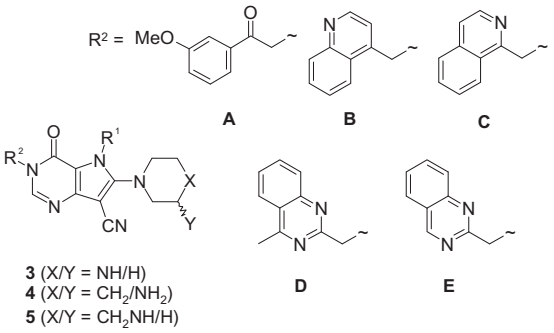


Figure 2. X-ray structure of compound (*S*)-**4c** (cyan carbon atoms 4A5S) bound to DPP-IV (key residues shown with grey carbon atoms). Superimposed is the bound conformation of Linagliptin (orange carbon atoms, PDB entry 2RGU [6]).

Table 1
DPP-4 inhibition of 7-cyanodeazahypoxanthines



Compounds	R^1	R^2	DPP-4 IC_{50} (nM)	Caco-2 (10^6 cm/sec) (A/B)/EFR	CL_{int} (mL/min/kg)[b]
3a	Bn	Me	2100	/	/
3b	Bn	A	400	/	/
3c	$CH_2C{\equiv}CMe$	A	23	/	/
3d	$CH_2CH{=}CMe_2$	A	10	2.6/2.7[a]	180
(*S*)-**4a**	Bn	Me	20	1.8/5[a]	45
(*R*)-**4b**	Bn	A	25	/	/
(*S*)-**4b**	Bn	A	4	0.9/3.3[a]	43
(*S*)-**4c**	Bn	B	17	1.6/4.4[a]	63
(*R*)-**4d**	$CH_2C{\equiv}CMe$	A	60	/	/
(*S*)-**4d**	$CH_2C{\equiv}CMe$	A	15	0.4/13[a]	21
(*S*)-**4e**	$CH_2C{\equiv}CMe$	B	10	1.2/11.8	34
(*S*)-**4f**	$CH_2C{\equiv}CMe$	C	5	0.5/20	28
(*R*)-**4g**	$CH_2CH{=}CMe_2$	A	4	0.9/4.4	8
(*S*)-**4g**	$CH_2CH{=}CMe_2$	A	1	0.5/1.6	11
(*S*)-**4h**	$CH_2CH{=}CMe_2$	B	4	1.7/2.5[a]	46
(*S*)-**4i**	$CH_2CH{=}CMe_2$	C	0.5	6.1/1.8	59
(*S*)-**4j**	$CH_2CH{=}CMe_2$	D	3	1.9/3.7[a]	87
(*S*)-**4k**	$CH_2CH{=}CMe_2$	E	8	/	67
(*S*)-**4l**	$CH_2CH{=}CMe_2$	Me	9	3/4.4[a]	37
Linagliptin	$CH_2C{\equiv}CMe$	D	0.1	0.8/18	/
5a	Bn	C	60	1.2/4.8[a]	65
5b	$CH_2CH{=}CMe_2$	C	7	3.9/1.1[a]	NT
5c	$CH_2C{\equiv}CMe$	C	6	1/5.5[a]	48
5d	$CH_2C{\equiv}CMe$	D	9	1.7/—	NT
5e	$CH_2C{\equiv}CMe$	E	45	0.6/—	NT

[a] Efflux observed.
[b] Rat liver microsome data.

zine to the 3-aminopiperidine substituent was also beneficial; a similar effect has also been observed in other scaffolds[12] with compound (*S*)-**4a** being 100-fold more potent than the piperazine analogue **3a**. An examination of both the (*R*)- and (*S*)-aminopiperidine enantiomers in compounds **4b**, **4d** and **4g** identified a preference for the (*S*)-aminopiperidine over the (*R*) enantiomer in all cases.

A selection of compounds (*S*)-**4e**, (*S*)-**4f** and (*S*)-**4h–k**, bearing heterobicyclic groups at R^2, were prepared. Although potencies did not approach that of Linagliptin, all analogues exhibited acceptable potency in the single nM range.[13] Comparing compounds (*S*)-**4e** and (*S*)-**4f** with (*S*)-**4h** and (*S*)-**4i** indicated a slight preference for the isoprenyl over the but-2-ynyl substituent. The homopiperazine compounds **5b–d** prepared with isoprenyl/but-2-ynyl substitution also gave acceptable potency.

Figure 2 shows the X-ray crystal structure of compound (*S*)-**4c** in complex with human DPP-4. Compound (*S*)-**4c** forms three charge-assisted hydrogen bonds from the primary amine to Glu205, Glu206 and Tyr662. The S1 subsite is occupied by the phenyl ring of the compound's benzyl moiety.

Characteristic of this class of compound is the face-to-face π-stacking interaction formed between the deazahypoxanthine scaffold and the phenol ring of Tyr547, which moves from its position in the apo structure to permit this contact.[6] The carbonyl oxygen of the deazahypoxanthine accepts a hydrogen bond from the backbone NH of Tyr631. Finally, the quinoline ring system makes an edge-to-face π-stacking interaction with the indole ring of Trp627. Figure 2 also overlays the bound conformation of Linagliptin showing the commonalities and differences in binding mode between the two compounds.

Selected ADME properties for deazahypoxanthine compounds are shown in Table 1. In general members of this series showed moderate to high clearance in vitro in rat liver microsomes and low Caco-2 permeability with possible efflux contributing to reduced exposure in vivo (data not shown). In the same assay Linagliptin was found to exhibit very high levels of efflux, similar to related deazahypoxanthine analogues, (*S*)-**4d–f**. A further examination of compound (*S*)-**4a** in an MDR-MDCK cell-line indicated P-gp mediated efflux may account for the poor absorption of many deazahypoxanthine analogues. However, as most compounds profiled also exhibited moderate to high clearance, a combination of these two factors must be responsible for their limited exposure. Compound (*S*)-**4i**, which exhibited acceptable in vitro DPP-4 inhibition and moderate exposure in vivo, was profiled in the rat pharmacodynamic model, measuring ex vivo inhibition of DPP-4, and showed reasonable inhibition (60%) 5 h post dose (3 mg/kg po). Although this compound showed high in vitro clearance (CL_{int} = 59 mL/min/kg), the high primary potency and superior permeability, compared to the majority of other examples, drives the observed efficacy. Compound (*S*)-**4i** showed excellent selectivity versus other proteases (>30,000-fold vs DPP-2, 8 & 9). However, the high CYP3A4 inhibition (IC_{50} = 1.7 μM) and in vitro clearance were deemed unacceptable for compound progression.

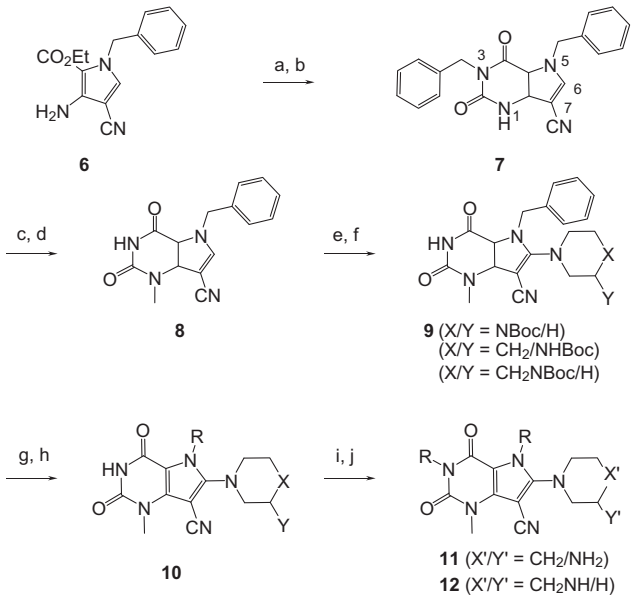
In an effort to improve permeability and potency, an examination of the related 5-Me-7-cyanodeaza xanthine scaffold was undertaken (Scheme 2).

Condensation of the benzyl-protected aminopyrrole **6** with benzyl isocyanate gave an intermediate benzyl urea, which underwent base-promoted cyclisation to give **7**.[14] The template was methylated at N-1 then regioselectively debenzylated at N-3 using BBr_3 to give compound **8**. Incorporation of the amino functionality in position 6 was achieved by bromination and nucleophilic displacement with the appropriate mono Boc-protected diamines under microwave irradiation, at elevated temperatures, to give **9**. Removal of the N-5 benzyl substituent (where necessary) could only be achieved using transfer hydrogenation with ammonium formate/10% Pd/C. Regioselective N-5 alkylation using DIPEA and the appropriate R^1 alkylating agent gave **10**. The final deazaxanthine compounds were obtained after N-3 alkylation using K_2CO_3 base and Boc removal with TFA/DCM to afford **11** and **12**.

To probe the SAR in the deazaxanthine series, several compounds were prepared (Table 2) which included analogues containing the preferred substituents identified earlier (Table 1).

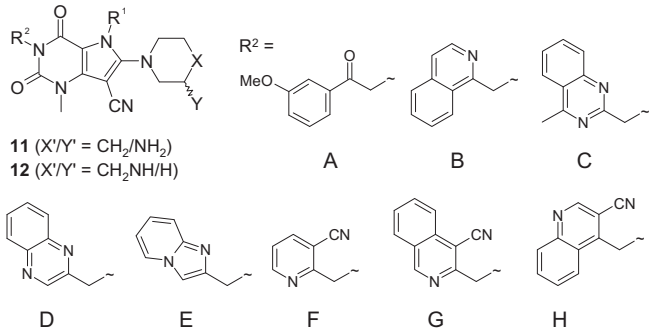
A comparison of deazahypoxanthines (Table 1) with deazaxanthine compounds (Table 2), bearing the (*S*)-aminopiperidine substituent, indicated that an improvement in DPP-4 inhibition was generally observed for deazaxanthine analogues containing identical R^1/R^2 substituents. However, the SAR showed that the compound activity was more closely linked to variation in R^1/R^2 substituents rather than the core scaffold. Interestingly, the but-2-ynyl containing enantiomers (*R*)-**11k** and (*S*)-**11k** did not follow the previously observed SAR and showed a reversal of the generally preferred stereochemistry at the 3-aminopiperidine (the (*R*)-isomer was >10-fold more active against DPP-4 compared with the (*S*)-isomer). A similar trend was also observed for related Linagliptin analogues bearing the but-2-ynyl substituent.[6a] A comparison of (*R*)-**11k** with Linagliptin, having identical $R^1/R^2/R^3$ substituents showed greater DPP-4 inhibition for Linagliptin (Table 2). Although the SAR was found to track well between xanthine-related series, some differences have been observed. For example compound (*S*)-**11j** gave a marked improvement in DPP-4 inhibition compared to (S)-**11k** (60-fold), whereas the corresponding direct xanthine-derived analogue is reported to show comparable activity to Linagliptin.[6a] An examination of the homopiperazine amino substituent indicated that similar activities to the deazahypoxanthine analogues could be obtained.

Molecular modelling was used to design targets (*S*)-**11h**, **12e**, **12f**, **12i** and **12j**, in which a cyano residue was introduced into the R^2 substituent (P1′ substituent) with the aim of forming additional hydrogen bonding interactions with Lys554 at the DPP-4 active site. However, these compounds gave no increase in potency over related *des*-cyano analogues. CYP3A4 inhibition of compounds



Scheme 2. Synthesis of 7-cyanodeazaxanthines. (a) BnNCO, Pyr, rt, 18 h, (56–100%); (b) NaOMe, DMF, 70 °C, 4 h, (89–96%); (c) MeI, K_2CO_3, DMF, rt, 4 h, (87–100%); (d) BBr_3, xylene, reflux, 6 h, (85–100%); (e) Br_2, AcOH, 45 °C, 18 h. (68–90%); (f) Boc diamine, *N*-methyl morpholine, DMA, μ-wave, 160 °C, 20 min, (36–86%); (g) ammonium formate, 10% Pd/C, 75 °C, 30 min, (60–90%); (h) R^1Br, DIPEA, DMF, 60 °C, (25–48%); (i) R^2Br, K_2CO_3, DMF, rt, 18 h, (75–95%); (j) TFA/DCM [1:1], rt, 1 h, (75–93%).

Table 2
DPP-4 inhibition of 7-cyanodeazaxanthines



Compounds	R^1	R^2	DPP-4 IC_{50} (nM)	hERG[a] IC_{50} (μM)
(*S*)-**11a**	Bn	A	1.5	0.1
(*S*)-**11b**	Bn	B	4	2.8
(*S*)-**11c**	Bn	Me	10	4.3
(*S*)-**4h**	$CH_2CH{=}CMe_2$	B	0.5	4.2
(*S*)-**11d**	$CH_2CH{=}CMe_2$	A	0.5	1.6
(*S*)-**11e**	$CH_2CH{=}CMe_2$	B	1	9
(*S*)-**11f**	$CH_2CH{=}CMe_2$	C	2	6.7
(*S*)-**11g**	$CH_2CH{=}CMe_2$	D	9	5
(*S*)-**11h**	$CH_2CH{=}CMe_2$	F	25	16.9
(*S*)-**11i**	$CH_2C{\equiv}CMe$	A	2	1.3
(*S*)-**11j**	$CH_2C{\equiv}CMe$	B	4	28.2
(*R*)-**11k**	$CH_2C{\equiv}CMe$	C	20	21.7
(*S*)-**11k**	$CH_2C{\equiv}CMe$	C	250	>30
Linagliptin	$CH_2C{\equiv}CMe$	C	0.1	>30
12a	$CH_2CH{=}CMe_2$	A	2	1.1
12b	$CH_2CH{=}CMe_2$	B	3	1.9
12c	$CH_2CH{=}CMe_2$	C	2	2
12d	$CH_2CH{=}CMe_2$	E	10	1.8
12e	$CH_2CH{=}CMe_2$	G	1	1.8
12f	$CH_2CH{=}CMe_2$	H	5.5	0.9
12g	$CH_2C{\equiv}CMe$	B	5	3.4 (10)[b]
12h	$CH_2C{\equiv}CMe$	C	7.5	12
12i	$CH_2C{\equiv}CMe$	G	1	12
12j	$CH_2C{\equiv}CMe$	H	2	4.4

[a] Dofetilide binding assay.
[b] Qpatch IC_{50} (μM).

bearing either benzyl or isoprenyl substitution at R^1 was found to be pronounced ($IC_{50} \leqslant 3$ μM). Encouragingly, the potent DPP-4 inhibitors **12g** and **12j** (Table 2), bearing the but-2-ynyl substituent at R^1, gave reduced inhibition of CYP3A4 ($IC_{50} \geqslant 10$ μM). Inhibition of the other CYP isoforms by the deazaxanthine compounds was generally acceptable, with typical values for the IC_{50} of >10 μM. The majority of compounds exhibited moderate to high affinity for the dofetilide-binding site of the hERG channel (Table 2), although **12g** inhibited the hERG channel with moderate potency, having an IC_{50} of 10 μM in the Qpatch assay.

Activity against the related proteases DPP-2/8/9 has been associated with toxicity in animal models;[15] however the majority of the deazaxanthine analogues showed excellent selectivity against these enzymes ($IC_{50} \geqslant 30$ μM). Selectivity against the other closely related endopeptidases, prolyl endopeptidase (PEP) and fibroblast activation protein peptidase (FAP) was also excellent for the majority of compounds ($IC_{50} \geqslant 30$ μM). Notable exceptions were compounds (*S*)-**11f** and **12h**, bearing the 4-methylquinazoline substituent at R^2, which gave a reduced selectivity margin versus FAP (IC_{50} = 1 and 4.7 μM, respectively). This issue was highlighted for Linagliptin, also containing the 4-methylquinazoline substituent at R^2 (89-fold selective vs FAP).[6b] This trend has been observed in other xanthine-based DPP-4 inhibitors, where further elaboration of R^2 has led to FAP inhibitors for the treatment of hyperproliferative diseases.[16]

A summary of key PK data from both the deazaxanthine and deazahypoxanthine series is presented in Table 3. The compounds are grouped as 'matched-pairs' to highlight the improvements achieved by moving to the deazaxanthine template. The data clearly show advantages in terms of exposure leading to better overall bioavailability for the deazaxanthine compounds. Presumably, the combination of reduced efflux and clearance is responsible for their better in vivo profiles. A direct comparison with Linagliptin can be made; in our hands this compound exhibited poor exposure in rat most probably due to excessive efflux.

Compound **12g** in particular, showed rapid absorption (T_{max} = 0.33 h), low clearance and an acceptable bioavailability of 67%. **12g** was further profiled in the rat by measuring ex vivo inhibition of DPP-4, and was found to give extended DPP-4 inhibition of ⩾70% up to eight hours post administration at 3 mg/kg po (Fig. 3).

Compound **12g** showed a favourable selectivity profile (>6000-fold) over the related proteases DPP-2/8/9, PEP and FAP. Another relevant off-target issue identified during the development of Linagliptin was the muscarinic M1 receptor (Linagliptin IC_{50} = 295 nM).[6] A comparison with compounds profiled across both the deazaxanthine and deazahypoxanthine series, which include **12g**, showed IC_{50}s >10 μM at this receptor.

In conclusion, a novel class of DPP4 inhibitor, derived from a xanthine-like scaffold, has been identified.[17] Extensive derivatisation in the deazahypoxanthine series revealed clear SAR which was used to design related deazaxanthine compounds. Deazaxanthine derivatives were found to show improved exposure as exemplified by compound **12g**, which exhibited a favourable selectivity and ADME profile, a promising PK profile in the rat and excellent in vivo activity.

Table 3
Selected PK data—matched pairs analysis

Compounds	C_{max} (nM)	AUC (nM h)	T½ (h)	CL (mL/min/kg)[a]	F%	Caco-2 (10^6 cm/sec) (A/B)/EFR
Linagliptin	21	185	/	34	9	0.8/18[c]
(*S*)-**4a**	143	539	1.4	58	21	1.8/7.2[c]
(*S*)-**11c**	266	2329	4.2	18	33	2.6/4.3[c]
(*S*)-**4b**	46	218	3.5	65	14	0.9/3.3[c]
(*S*)-**11a**	674	4998	4.4	5	24	0.9/1
(*S*)-**4d**	13.7	314	3.7	33	10	0.4/13[c]
(*S*)-**11i**	207	923	ND	21	18	3/1.3
(*S*)-**4h**	156	711	/	59[b]	/	6.1/0.3
(*S*)-**11e**	193	1744	3.3	43	75	2.9/1.2
5b	83	678	1.8	40	25	1.9/2.2
12b	139	1017	3.2	42	34	5.3/0.6
5c	124	574	2	48	/	1/5.5[c]
12g	1061	7308	3.1	10	67	2.4/3.7[c]

ND = not determined.
[a] iv leg run at 1 mg/kg.
[b] Rat liver microsome data (RLM).
[c] Efflux observed.

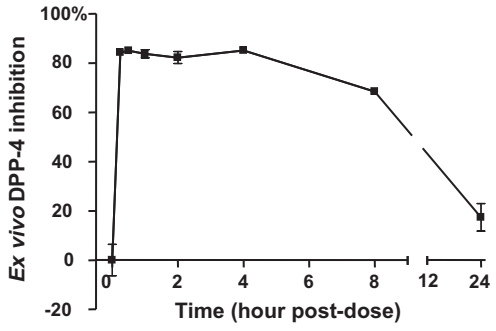


Figure 3. Inhibition of plasma DPP-4 activity after oral administration of compound **12g** (3 mg/kg) in Wistar rats. Data are represented as the mean SEM ($n = 3$).

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References and notes

1. (a) Pospisilik, J. A.; Stafford, S. G.; Demuth, H.-U.; Brownsey, R.; Parkhouse, H.; Finegood, D. T.; McIntosh, C. H. S.; Pederson, R. A. *Diabetes* **2002**, *51*, 943; (b) Drucker, D. J. *Endocrinology* **2003**, *144*, 5145; (c) Murphy, K. G.; Dhillo, W. S.; Bloom, S. R. *Endocrine Rev.* **2006**, *27*, 719.
2. (a) Mentlein, R.; Gallwitz, B.; Schmidt, W. E. *Eur. J. Biochem.* **1993**, *214*, 829; (b) Kieffer, T. J.; McIntosh, C. H. S.; Pederson, R. A. *Endocrinology* **1995**, *136*, 3585.
3. (a) Thorens, B. *Diabetes Metab.* **1995**, *21*, 311; (b) Meier, J. J.; Nauck, M. A.; Schmidt, W. E.; Gallwitz, B. *Regul. Pept.* **2002**, *101*, 1.
4. http://www.fiercebiotech.com/press-releases/galvus-new-treatment-patients-type-2-diabetes-receives-european-approval.
5. Biftu, T.; Scapin, G.; Singha, S.; Fenga, D.; Becker, J. W.; Eiermann, G.; Hea, H.; Lyonsa, K.; Patela, S.; Petrovb, A.; Sinha-Royc, R.; Zhang, B.; Wuc, J.; Zhang, X.; Dossd, G. A.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3384.
6. (a) Eckhardt, M.; Langkopf, E.; Todayyon, M.; Thomas, L.; Nar, H.; Pfrengle, W.; Guth, B.; Lotz, R.; Sieger, P.; Fuchs, H.; Himmelsbach, F. *J. Med. Chem.* **2007**, *50*, 6450; (b) Deacon, C. F.; Holst, J. J. *Expert Opin. Investig. Drugs* **2010**, *19*(1), 133.
7. (a) Matthias, E.; Hauel, N.; Himmelsbach, F.; Langkopf, E.; Nar, H.; Todayyon, M.; Thomas, L.; Guth, B.; Lotz, R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3158; (b) Havale, H.; Pal, M. *Bioorg. Med. Chem.* **2009**, *17*, 1783; Pyrrolopyrimidinones with deviating structure have been reported as DPP-4 inhibitors in Nakahira, H.; Kimura, H.; Hochigai, H. WO2006/068163; (c) Nishio, Y.; Kimura, H.; Tosaki, S.; Sugaru, E.; Sakai, M.; Horiguchi, M.; Masui, Y.; Ono, M.; Nakagawa, T.; Nakahira, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7246.
8. Taskinen, M. R.; Rosenstock, J.; Tamminen, I.; Kubiak, R.; Patel, S.; Dugi, K. A. *Diabetes Obes. Metab.* **2011**, *13*, 65.
9. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm253501.
10. Kataoka, K.; Kosugi, T.; Ishii, T.; Takeuchi, T.; Tsutsumi, T.; Nakano, A.; Unoki, G.; Yamamoto, M.; Sakai, Y. WO2003/070729.
11. Compounds were tested for their inhibitory potency against human DPP-4. Human DPP-4 (amino acids 39–766) with a C-terminal step-tag was expressed using the baculovirus system and purified to >80%, and stored in a 25 mM Tris buffer, pH 9, containing 300 mM NaCl at −80 °C. The substrate was kept at 5 mM stock solution in DMSO at −20 °C and the assay buffer for the DPP-4 reaction was 25 nM Tris/HCl, pH 7.5, containing 140 mM NaCl, 10 mM KCl and 0.05% (w/v) CHAPS.
12. This observed boost in affinity moving from piperazine to aminopiperidine has been rationalised in terms of an additional hydrogen bonding ability and lower energy conformation: Engel, M.; Hoffmann, T.; Manhart, S.; Heiser, U.; Chambre, S.; Huber, R.; Demuth, H.-U.; Bode, W. *J. Mol. Biol.* **2006**, *356*, 768.
13. Linagliptin was prepared according to Himmelsbach, F.; Langkopf, E.; Eckhardt, M.; Mark, M.; Maier, R.; Todayyon, M.. WO2004/018468.
14. Milton, J.; Wren, S.; Wang, S.; Folkes, A.; Chuckowree, I.; Hancox, T; Miller, W.; Sohal, S. WO2004065389.
15. Lankas, G. R.; Leiting, B.; Roy, R. S.; 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. K.; Zaller, D.; Zhang, X.; Zhu, L.; Weber, A. E.; Thornberry, N. A. *Diabetes* **2005**, *54*, 2988.
16. (a) Thomas, L.; Eckhardt, M.; Langkopf, E.; Tadayyon, M.; Himmelsbach, F.; Mark, M. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 175; (b) Himmelsbach, F.; Mark, M.; Tadayyon, M.; Thomas, L. WO2009/024542.
17. Baeschlin, D. K.; Clark, D. E.; Dunsdon, S. J.; Fenton, G.; Fillmore, A.; Harris, N. V.; Higgs, C.; Hurley, C. A.; Krintel, S. L.; MacKenzie, R. E.; Ostermann, N.; Sirockin, F.; Sutton, J. M. WO2007/071738.