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An example of designed multiple ligands spanning protein classes: Dual MCH-1R antagonists/DPPIV inhibitors

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ABSTRACT

A ligand-based approach to identify potential starting points for a dual MCH-1R antagonist/DPPIV inhibitor medicinal chemistry program was undertaken. Potential ligand pairs were identified by analysis of MCH-1R and DPPIV in vitro data. A highly targeted synthetic effort lead to the discovery of pyridone **11**, a dual MCH-1R antagonist/DPPIV inhibitor with selectivity over DPP8 and DPP9.

Type II diabetes, characterised by high blood glucose in the context of insulin resistance, is due to a number of lifestyle and genetic factors. Risk factors include poor diet, lack of physical activity, obesity and paradoxically, prenatal under-nutrition. Obese individuals with diabetes have poorer control of blood glucose levels; conversely, intentional weight loss is associated with reduced mortality among individuals with diabetes.[1] The majority of current drug treatments are either weight neutral (Metformin, Acarbose, DPPIV inhibitors) or cause some weight gain (insulin, Thiazolidinediones). An exception is GLP-1 analogues such as Exanatide, however, they require administration by subcutaneous injection.[2] Thus there remains an unmet medical need for oral anti-diabetics that give weight loss.

Oral dosing of MCH-1R antagonists is well documented to give significant weight loss in rodent models. To date, few programs have advanced far in the clinic, and validation in man remains elusive.[3] DPPIV inhibitors have been on the market for over 5 years, and been shown to be well tolerated and weight neutral. The combination of a MCH-1R antagonist and a DPPIV inhibitor would require co-formulation of two active ingredients, with the inherent problems of achieving compatibility in the physicochemical, pharmacokinetic and pharmacodynamic properties of the two active ingredients. However, a single chemical entity combining MCH-1R antagonism and DPPIV inhibition could circumvent such issues, and may provide an oral anti-diabetic agent that gives significant weight loss.

The term 'designed multiple ligand' (DML) was first proposed by Morphy et al. as a generic phrase to describe compounds rationally designed to modulate multiple targets relevant to a disease.[4] Examples of ligands designed to have activity toward unrelated proteins (and within typical oral drug-like space) are rare. Ostensibly, the disparity between the binding sites of unrelated proteins may seem too great a technical challenge to identify drug-like ligands; nevertheless, examples have been reported.[5] The undertaking of such a seemingly daunting technical challenge is driven by the potential for multi-target drugs to achieve greater clinical efficacy over exquisitely selective drugs.[6,7]

A number of strategies have been proposed to identify chemical starting points for DMLs. Linking of pharmacophores from selective ligands, via a long chain is a common method exemplified. However, unless the individual pharmacophores are small or highly overlapped, the resulting ligand will have molecular properties outside typical drug-like space.[8] Throughout medicinal chemistry, common structural motifs in ligands reoccur. In designing a dual ligand for a peptidase (DPPIV) and a Class A GPCR (MCH-1R) we undertook a ligand-based approach to identify starting points for a medicinal chemistry program. Figure 1 shows the structures of FDA approved DPPIV inhibitors, and some literature MCH-1R antagonists. Despite a lack of obvious structural similarity between the ligands shown, we believed that a number of features of these two targets may permit the design of dual ligands with drug-like properties:

- Ligands for both targets display rich structural diversity, and considerable SAR information is available from the literature.

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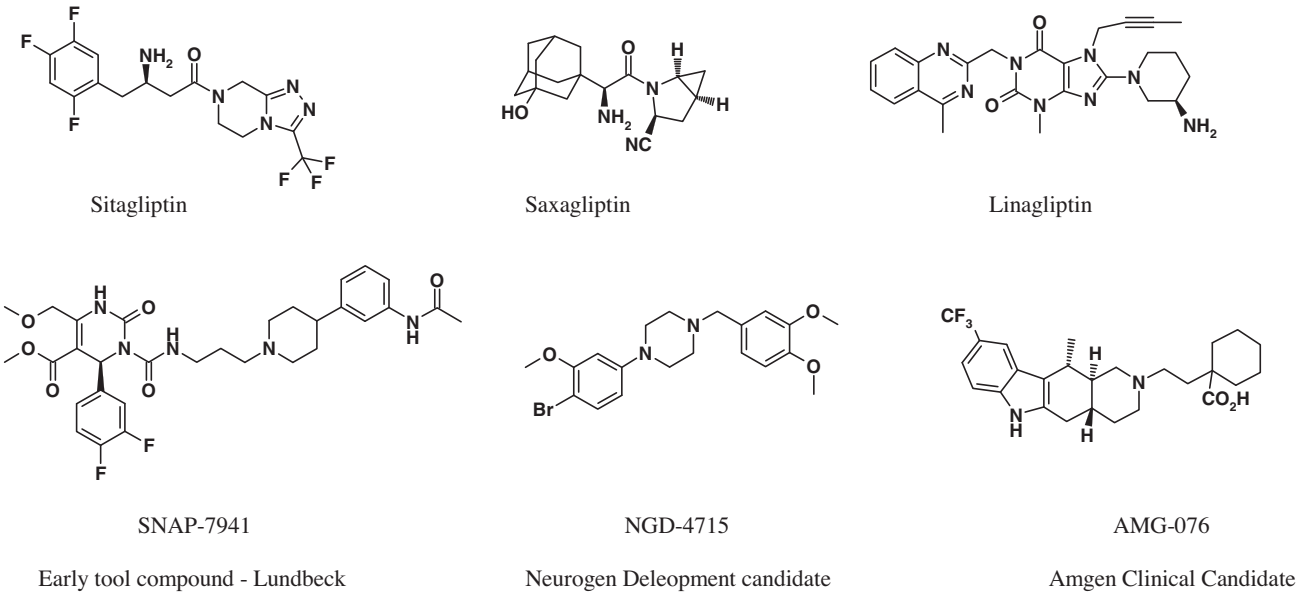


Figure 1. Structures of FDA approved DPPIV inhibitors ('gliptins') and some literature MCH-1H antagonists.

- Many potent DPPIV inhibitors are reported which possess low molecular weight (and hence high ligand efficiency).
- Extensive X-ray crystallography data exists for inhibitors bound to DPPIV which could aid in the design process.
- X-ray crystallography has shown that DPPIV possesses a large solvent exposed cavity proximal to the active site. This has the potential to accommodate a significant part of the pharmacophore of a MCH-1R ligand.

Ligand data was obtained for these targets from two sources. Nexus is an in-house database containing data abstracted from the medicinal chemistry literature; and in vitro data was extracted from patents using proprietary techniques. In vitro data was collated for ~1500 MCH-1R antagonists, and ~2500 DPPIV inhibitors. Analysis identified a number of cases where MCH-1R and DPPIV ligands shared common structural motifs. Two examples of this, **1** & **2**, and **2** & **3** are shown in Figure 2.

All three molecules comprise a common core containing a pyrolidine ring substituted on nitrogen with a heterocycle. MCH-1R antagonist **1** (K_i 6 nM)[9] has an amino group at the 3-position of the pyrolidine, and likewise DPPIV inhibitor **2** (K_i 20 nM).[10] On the other hand, **2** has an additional 2,4,5-trifluorophenyl ring at the 4-position, that fills the S1 pocket in DPPIV and is critical to activity. Incorporation of the appropriately substituted phenyl ring at the 4-position of the pyrolidine ring in **1** would furnish the potential dual MCH-1R antagonist/DPPIV inhibitor **4**.[11] An indication for the tolerance of a phenyl ring at this location in MCH-1R ligands is gained from antagonist **3** (binding 25 nM)[12] although it does lack the amino group present in **1** and **2**. Docking of **4** into a DPPIV crystal structure suggested there was sufficient room to accommodate the ligand.

The plethora of MCH-1R ligand data in the public domain afforded the opportunity to explore many ideas for potential dual ligands. MCH-1R antagonists believed to adopt a similar binding mode to **1** and **3** were identified; from these, potential dual ligands were constructed virtually in a similar manner to **4**. To provide compounds with drug-like properties, and to maximise the potential for blood brain barrier penetration, limits on physiochemical properties were set at PSA $\leqslant$ 80 Å^2, Log$D \leqslant$ 3.5 and molecular weight $\leqslant$ 500.[13] Out of 26 cores examined, only six met the criteria for synthesis.[14] By investing considerable effort in the design phase, and selecting only the highest quality ideas, the resource intensive activities of synthesis and testing were minimised.[15]

Synthesis of the appropriate electrophiles was conducted as outlined in Scheme 1. Alkylation of 5-hydroxy indan-1-one, followed by ring expansion with sodium azide afforded the isoquinolin-1-one, with subsequent copper-mediated coupling with 2-fluoro-5-iodo pyridine affording the final building block for **8**. Building blocks for **4**, **6** and **9** were prepared using analogous copper-mediated coupling; the precursors being commercially available or their synthesis outlined in the literature.[16] Amides **5** and **7** were prepared starting from alkylation of the appropriate phenol, followed by hydrolysis and amide coupling under standard conditions. Finally, compounds **4** to **9** were prepared via S_NAr reaction of the electrophiles with the corresponding protected di-substituted pyrolidine as outlined in Scheme 2.[17]

Compounds were screened for functional activity in CHO cells stably expressing human MCH-1R receptor. Results are shown in Table 1 for inhibition of agonist (h-MCH) response at 5 μM. For the majority of analogues there was significant inhibition of agonist response, and compounds with >40% inhibition underwent full IC_{50} determination. Pleasingly, pyridone **4** showed excellent functional MCH-1R antagonism, IC_{50} 0.14 μM, whilst analogues **5** and **8** showed micromolar levels of activity. Pyridone **4** also showed good DPPIV inhibition, IC_{50} 1.65 μM, although possessed no significant selectivity over DPP8 and DPP9 (Table 2).

Encouraged by the generation of a dual MCH-1R antagonist/DPPIV inhibitor, the effect of varying the substituent filling the S1 pocket in DPPIV was probed. These were prepared in a similar fashion to Scheme 2. Pyridone **10**, the 2,4,5-trifluorophenyl analogue of **4**, showed comparable levels of functional MCH-1R antagonism, DPPIV inhibition and selectivity.[18] Ring expansion of pyrolidine **2** to the corresponding piperidine derivative is reported to yield a more potent DPPIV inhibitor (K_i 6 nM).[19] Thus the equivalent piperidine analogue **11** was prepared. Gratifyingly, **11** showed submicromolar MCH-1R antagonism/DPPIV inhibition (IC_{50} 0.44 μM/0.35 μM, respectively) and selectivity over DPP8 and DPP9.[20] The requirement of the S1 substituent for DPPIV inhi-

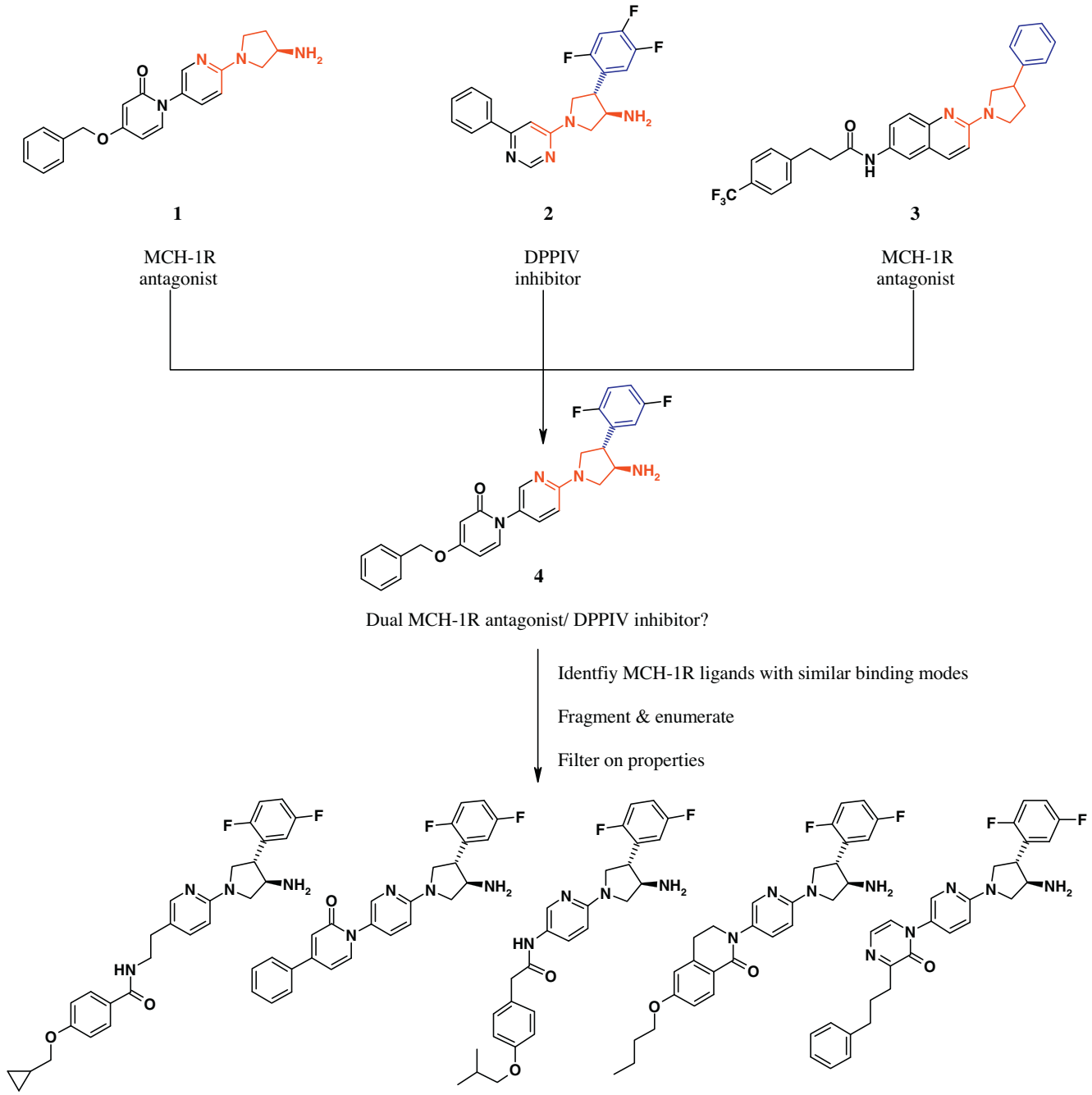


Figure 2. Outline of a ligand-based approach to identify potential dual MCH-1R antagonists/DPPIV inhibitors.

Table 1
Molecular properties and MCH-1R antagonism data for the initial set of analogues

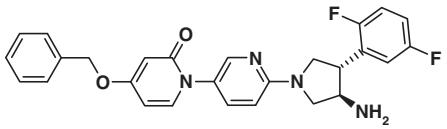
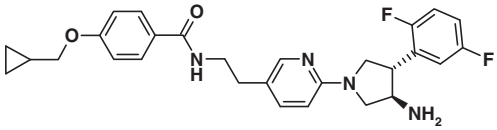
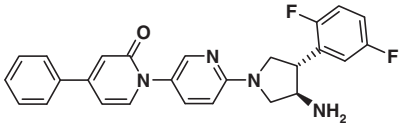
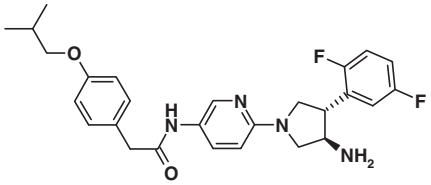
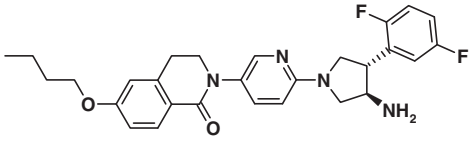
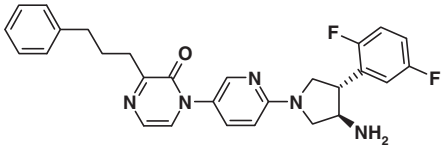
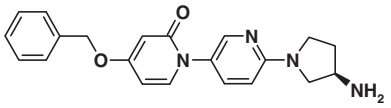
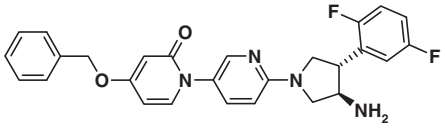
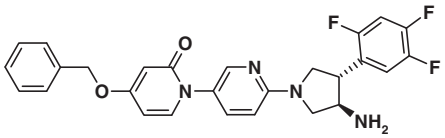
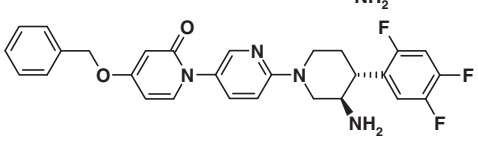
Compd	Structure[a]	%Inhibition at 5 μM[b]	MCH-1R IC_{50}[c] (μM)	PSA (Å^2)	Log$D$[d]
4		104	0.14	72	1.9
5		43	3.8	80	2.8

Table 1 (*continued*)

Compd	Structure[a]	%Inhibition at 5 μM[b]	MCH-1R IC_{50}[c] (μM)	PSA (Å^2)	Log *D*[d]
6		39	ND	62	2.4
7		35	ND	80	3.5
8		44	4.0	72	3.1
9		52	>3.0	75	2.4

[a] All compounds were >95% pure by LCMS, and characterised by NMR and MS.
[b] %Inhibition of control agonist response at 5 μM in CHO cells stably expressing human MCH-1R (conducted at Cerep).
[c] *N* = 2.
[d] ACD Log *D* at pH 7.4.

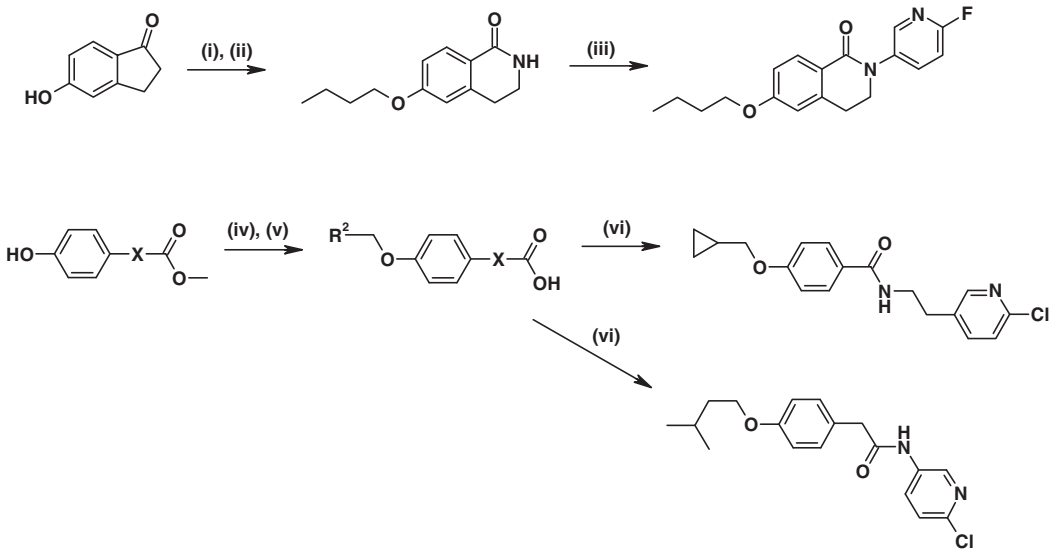
Table 2
In vitro data for some pyridone derivatives

Compd	Structure	IC_{50} (μM)					Log *D*[c]
		MCH-1R	DPPIV[a]	DPP8[a]	DPP9[a]	hERG[b]	
1		0.02	>100	>100	>100	ND	-0.4
4		0.14	1.65	2.26	3.28	8.8	1.9
10		0.11	1.1	2.6	2.2	6.1	2.1
11		0.44	0.35	>50	>50	ND	2.2

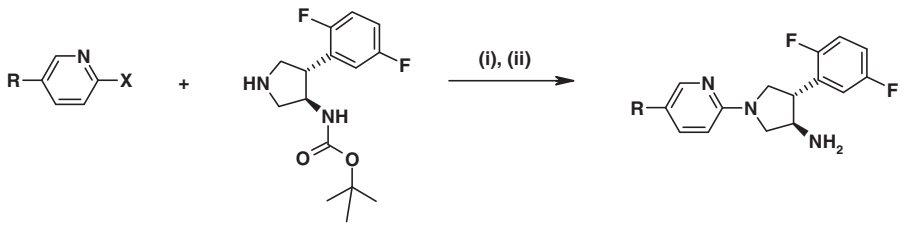
[a] For assay details see Ref. [5a]; *N* = 2.
[b] Performed at Essen Bioscience.
[c] ACD Log *D* at pH 7.4; ND = not determined.

bition is underscored by pyrolidine **1**, synthesised as a control, which showed MCH-1R IC_{50} 0.02 μM, but IC_{50} >100 μM for DPPIV, DPP8 and DPP9.

Several pharmaceutical companies have invested heavily in the identification of viable MCH-1R antagonists, the main hurdle preventing successful development of such compounds being cardio-



Scheme 1. Reagents and conditions: (i) Butylbromide, potassium carbonate, DMF, 85%; (ii) sodium azide, TFA, 80 °C, 12 h, 82%; (iii) DMF, 2-fluoro-5-iodo pyridine, potassium carbonate, *trans*-N,N-dimethylcyclohexadiamine, 110 °C, 18 h, 35%; (iv) Alkyl bromide, potassium carbonate, DMF; (v) sodium hydroxide, methanol; (vi) Amine, HBTU, triethylamine, DMF



Scheme 2. Reagents and conditions: (i) Diisopropylethylamine, DMSO, 150 °C, microwave, or potassium carbonate, DMF, 110 °C, microwave; (ii) TFA, DCM, room temperature.

vascular safety, in particular as a result of hERG inhibition. The hERG data obtained for compounds **4** and **10** (IC_{50} 8.8 μM and 6.1 μM, respectively) suggest this chemotype represents a reasonable starting point for lead optimisation.

In summary, the desire to produce a new anti-diabetic with weight loss in a single molecular entity drove an effort to design a ligand with dual activity at two unrelated protein targets. A ligand-based approach was developed to identify starting points for a dual MCH-1R antagonist/DPPIV inhibitor medicinal chemistry program, with properties designed to maximise the potential for CNS exposure. Through the highly targeted synthesis of only a handful of molecules, potent dual MCH-1R antagonists/DPPIV inhibitors were identified, with one compound having selectivity over DPP8 and DPP9.

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20. 1-(2-Pyridyl)-3-amino-4-(2,4,5-trifluorophenyl)piperidine, the analogue of **11** lacking the benzyloxypyridone tail, is a reported DPPIV inhibitor (Ref. 17, compound **14**, IC_{50} = 0.058 μM). Although it is not strictly correct to directly compare this value with that for analogue **11**, the data does suggest that the benzyloxypyridone tail may reduce DPPIV potency. This could be due to the requirement for positioning the terminal lipophilic aryl ring within the solvent exposed cavity of the DPPIV protein.