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Piperidinyl-2-phenethylamino inhibitors of DPP-IV for the treatment of Type 2 diabetes

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ABSTRACT

A highly ligand efficient lead molecule was rapidly developed into a DPP-IV selective candidate series using focused small library synthesis. A significant hurdle for series advancement was genetic safety since some agents in this series impaired chromosome division that was detected using the in vitro micronucleus assay. A recently developed high-throughput imaging-based in vitro micronucleus assay enabled the identification of chemical space with a low probability of micronucleus activity. Advanced profiling of a subset within this space identified a compound with a clean safety profile, an acceptable human DPP-IV inhibition profile based on the rat PK/PD model and a projected human dose that was suitable for clinical development.

An increasing incidence of obesity has, in part, fueled the rise in new Type 2 Diabetes Mellitus (TTDM) diagnoses and continues to make the treatment of TTDM a critical global health care issue.[1] Present pharmaceutical therapy fails to provide adequate glycemic control and many patients are unable to achieve their targeted plasma glucose levels through available regimens. Inhibition of the serine protease dipeptidyl peptidase-IV (DPP-IV) has recently emerged as an effective biological target for the treatment of TTDM.[2] Two of the targets for DPP-IV are the incretin hormones glucagon-like peptide-1 (GLP1) and gastrointestinal inhibitory peptide (GIP), bioactive molecules that are secreted by the gut in response to food intake.[3] Binding of GLP1 to the pancreas and pancreatic β-cells leads to insulin secretion and results in enhanced glucose disposal. GLP1 has also been reported to provide benefits to the pancreas through improved beta cell function and possible regeneration.[4] From a safety standpoint, DPP-IV inhibition offers a significantly reduced risk of hypoglycemia because the endogenous insulin secretagogue (GLP1) is generated only in response to a glucose stimulus. A first in class DPP-IV inhibitor Januvia® (MK-0431, **1**) has been approved and other agents are in review at the FDA [Galvus™ (LAF-237, **2**); Alogliptin (SYR-322, **3**)] (Fig. 1). We were interested in finding additional novel small molecular weight inhibitors of DPP-IV that lacked the 2-cyanopyrrolidide as a back-up to our clinical candidate, PF-00734200 (**4**). This account describes the discovery of a novel piperidinyl-2-phenethylamino series of DPP-IV inhibitors and the tactics used to manage pre-clinical safety risks to identify promising clinical candidates.

The laboratory objective of the back-up effort was a once-a-day oral therapy that would provide ⩾80% inhibition of DPP-IV for at least 8 h in humans. Compounds would have demonstrated >100-fold selectivity against DPP2 and DPP8 and have rat PK properties that would translate into acceptable human PK parameters. The successful candidate must also have no QTc prolongation risk, as judged by a 300x therapeutic index against hERG (hERG IC_{50}/DPP-IV $K_i \geqslant 300$) ($K_i = IC_{50}/1.66$) and a clean profile in the in vitro genetic toxicity assays (Bioluminescent Salmonella Assay and in vitro micronucleus assay).[5]

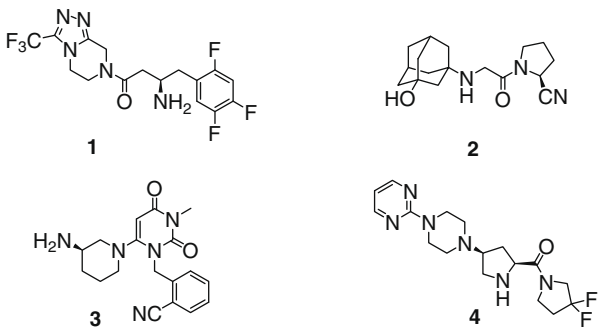

Figure 1. Advanced DPP-IV inhibitor clinical candidates.

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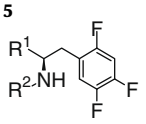
Table 1
DPP-IV inhibition of C1-substituted 1-amino-2-(2,4,5-trifluoro)phenylethanes, **5**



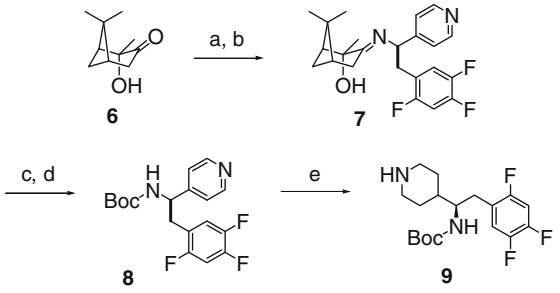
5

Compounds	R^1	R^2	DPP-IV inhibition IC_{50}, μM[a]	Ligand efficiency (LE)[b]
5a	H	H	>30	
5b	*i*-Pr	H	1.93	0.52
5c	Cyclohexyl	H	3.58	0.41
5d	Cyclohexyl-CH_2–	H	>30	<0.32
5e	*t*-Bu	H	25.9	0.39
5f	–$(CH_2)_4$–		>30	<0.39
5g	4-CH_3O_2CPh–	H	3.25	0.34
5h	1-(*R*)-4-CH_3O_2CPh–	H	1.42	0.36
5i	1-(*S*)-4-CH_3O_2CPh–	H	>30	<0.28
5j	1-(*R*)-4-Pyridyl	H	1.97	0.43
5k	1-(*R*)-4-Piperidinyl	H	0.272	0.50
5l	*N*-Boc-piperidin-4-yl	H	0.27	0.36

[a] Values are means of three experiments.
[b] Ligand efficiency is measured from the following equation: LE = $-1.4 \log K_i$/# of heavy atoms. K_i was determined from the IC_{50} values using the Cheng–Prussof equation: $K_i = IC_{50}/(1 + [S]/K_m)$ where [S] = 50 μM and K_m = 57 μM.

The 2,4,5-trifluorophenethylamino substructure was an established proline amide replacement in the S1 pocket and structural information suggested that the S2 pocket was sufficiently large and flexible enough to accommodate a variety of drug-like motifs.[6] As part of a lead-hopping effort around the pharmacophore substructure, a series of simple phenethylamine derivatives were made and screened. While the un-substituted parent structure **5a** was an inefficient inhibitor of DPP-IV action, simple alkyl (**5b and c**) produced highly ligand efficient molecules (Table 1). A synthetic strategy using accessible chemical space was employed to probe the area directly outside of the S1 pocket and enabled the discovery of a viable, novel lead series. Substitution near the primary amine disrupted binding to the critical glutamate residues on the floor of S1 pocket: quaternary substitution alpha to the phenethylamine chain (**5e**), secondary nitrogen in the form of a saturated heterocycle (**5f**) or the extension of the alkyl substituent from the main backbone (**5d**) was not tolerated. However, non-polar, racemic aryl substitution **5g** on the ethyl chain was tolerated and asymmetric HPLC separation of the 4-methylbenzoate analogs established that the preferred amino stereochemistry was (*R*). The S2 pocket also tolerated basic polarity (4-pyridyl (**5j**) and 4-piperidinyl (**5k**)) and the *N*-Boc analog (**5l**) provided an opportunity to effectively explore this region of the binding site using a variety of functional groups that could modulate the physical chemical properties of the core.

An efficient route to the library-enabled template **9** was devised using the asymmetric alkylation method of Shioiri (Scheme 1).[7] The commercially available 4-aminomethylpyridine was reacted with (*S*)-hydroxypinanone **6** to form the corresponding imine and diastereoselective alkylation with 2,4,5-trifluorobenzyl chloride proceeded in good yield to afford **7** with excellent diastereoselectivity (>98% de). Removal of the imine through *trans*-oximation provided the primary amine intermediate that was readily converted into the *N*-Boc derivative **8**. Hydrogenation of the pyridine heterocycle (Pt_2O, AcOH) afforded the key piperidine intermediate **9** that could be recrystallized from EtOH to afford the *N*-Boc piperidine in good yield.



6 **7** **8** **9**

Scheme 1. Asymmetric route to (*R*)-1-(piperidin-4-yl)-1-amino-2-(2,4,5-trifluoro)phenylethane. Reagents and conditions: (a) 4-aminomethylpyridine, $BF_3{\cdot}Et_2O$, $PhCH_3$; (b) *n*-BuLi, THF, <−20 °C then 2,4,5-F_3BnCl; (c) NH_2OH, EtOH, heat; (d) Boc_2O, 1,4-dioxane; (e) H_2, Pt_2O, EtOH, AcOH.

Having identified a suitable template, medium speed synthesis methods were used to produce a diverse set of analogs to probe the DPP selectivity SAR, generate benchmark data for in silico ADME modeling and to survey the behavior in the secondary safety screens. The 2-(2,4,5-trifluoro)phenethylamino moiety effectively delivered the required selectivity over the DPP8 isoform (IC_{50} = 5–30 μM) regardless of the substitution elsewhere in the molecule. Selectivity over the DPP2 isoform could be achieved by modulating the potency against DPP-IV and DPP2 in tandem: piperidine-1-yl substituents with rotational flexibility such as amides, sulfonamides and alkyl chains improved DPP-IV inhibition and generally decreased the activity against DPP2. The DPP-IV IC_{50} values in conjunction with the in vitro ADME screening data could be used to filter compounds for rat PK/PD experiments. Criteria used for selection were: DPP-IV IC_{50} <100 nM; clearance in rat and human microsomes of <19 or <5 mg/mL/min, respectively, and Apical→Basal (AB) apparent permeability >10 cm^{-1}/s in MDCK cells with negligible efflux (BA/AB <3); single point dofetilide inhibition <20% at 10 μM compound. In addition, safety data was generated on a subset of analogs that indicated an unacceptable level of hERG inhibition and an increase incidence of micronuclei (aneugenic activity) (3/4) in the in vitro micronucleus assay.[8] At this stage, an assessment of this series suggested that compounds with an acceptable selectivity profile and appropriate PK properties could be identified (Table 2).

Because not all of the compounds in this series were positive in the micronucleus assay, the activity was not believed to be specific to the pharmacophore. However, the usual low-throughput assay had insufficient capacity to handle the volume of samples needed to de-risk this chemical series in a timely manner; the microscopic scoring of the micronuclei is a laborious and time-consuming process. A recently developed image analysis algorithm for micronuclei scoring and implementation of automated microplate technologies enabled the testing up to forty compounds at a time.[9] The ability