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# Discovery of DA-1229: A potent, long acting dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes

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# ABSTRACT

A series of  $\beta$ -amino amide containing substituted piperazine-2-one derivatives was synthesized and evaluated as inhibitors of dipeptidyl pepdidase-4 (DPP-4) for the treatment of type 2 diabetes. As results of intensive SAR study of the series, (*R*)-4-[(*R*)-3-amino-4-(2,4,5-trifluorophenyl)-butanoyl]-3-(*t*-butoxymethyl)-piperazin-2-one (DA-1229) displayed potent DPP-4 inhibition pattern in several animal models, was selected for clinical development.

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Type 2 diabetes mellitus is a chronic disorder characterized by hyperglycemia coupled with a gradual decline in insulin sensitivity and insulin secretion. The main goal of the management of type 2 diabetes is to achieve glycemic control as close to the non diabetic range as practicable, in order to reduce the risk of late-stage complications.<sup>1</sup> However, the therapeutic effect provided by existing medications is often not sustainable, since the multi-organ defects responsible for the disease are only insufficiently addressed.<sup>2</sup> The incretin hormone glucagon-like peptide 1 (GLP-1) is a potent stimulator of endogenous insulin release. GLP-1 has beneficial effects on islet  $\beta$ -cell function and insulin sensitivity without induction of hypoglycemia.<sup>3</sup> Further contributing factors are the inhibition of glucagon release from pancreatic  $\alpha$ -cells, reduction of food intake and retardation of gastric emptying, which are mediated by GLP-1.<sup>4</sup> Unfortunately, GLP-1 is rapidly degraded in vivo by the serine protease dipeptidyl peptidase-4 (DPP-4);<sup>5</sup> therefore DPP-4 inhibitors have emerged as a new therapeutic option to treat type 2 diabetes.<sup>6-10</sup> Clinical proof of concept has been established for several DPP-4 clinical candidates, four of which have become marketed drugs. Januvia<sup>™</sup> (sitagliptin), Onglyza<sup>™</sup> (Saxagliptin), Galvus™ (vildagliptin) and Nesina™ (alogliptin) have been approved for the treatment of type 2 diabetes (Fig. 1). Long-term studies with DPP-4 inhibitors in patients are underway in order to confirm the safety and sustainability of these effects, and, in particular, their ability to prevent the progressive loss of  $\beta$ -cell function. On the basis of these background, we discovered various kind of DPP-4 inhibitors including DA-1229. We also disclose that our initial design concept came from  $\beta$ -amino acid thiazolidides<sup>11</sup> and some substituted piperazines.<sup>12</sup>



Figure 1. DPP-4 inhibitors in market.

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Scheme 1. Overall design of β-amino amide containing substituted piperazine-2-one derivatives. Reagents: (a) EDC, HOBt, DIPEA/DMF or MC; (b) HCl in ether/MeOH.

As shown in Scheme 1, the target compound **4** and **5** were prepared from commercial Boc protected  $\beta$ -amino acid **1** and substituted heterocyclic amine **2** using standard peptide coupling conditions followed by deprotection of amine.

The substituted heterocyclic amine **8** was prepared as shown in Scheme 2. Commercial Boc-protected piperazin-2-one (**6**) was coupled with alkyl or aryl halide to give compound **7**, respectively. Deprotection of Boc derivatives **7** by treatment with hydrogen chloride in ether provided compound **8**.

As shown in Table 1, introduction of the simple methyl **4a** resulted in DPP-IV inhibitory activity with an IC<sub>50</sub> value of around 100 nM. Substitution of pyridine **4b** or non aromatic replacement **4e** showed decreased activity compared to methyl substitution. In case of five-membered hetero fusion ring **4d** and bi-phenyl **4c** increased potency by twofold than methyl. We concluded that there were limitations of activity elevation and the essential moiety might be the benzyl **4f** which is possible to make hydrophobic interaction with side chain of Phe357<sup>13,14,16</sup> as shown in Figure 2.



Scheme 2. Reagents: (a) R<sup>1</sup>Br, NaH, or R<sup>1</sup>Br/DMF, Pd(OAc)<sub>2</sub>, DPPF, KO-*t*-Bu, THF; (b) HCl in ether/MeOH.

## Table 1

DPP-4 inhibition activities of various substituted compound 4



Compound	$\mathbb{R}^1$	DPP-4 $IC_{50}^{15}$ (nM)
4a	$\sim$	92.6
4b	N N	311
4c		44.8
4d	N <sup>2</sup> N N	42.7
4e	∕_N_O	116
4f		54.1

So, we tried to study more about benzyl moiety in that position and then various other substituents were introduced.

As shown in Table 2, there was no effect on activity according to position and size of halogens (**4m**, **4n**, **4o**, **4p**;  $\sim$ 30 nM). In the case of nitro group, *ortho*-**4h** is threefold more potent than *para*-**4g**. We



**Figure 2.** Docking structure of **4f** in the active site (from 1X70) of DPP-4. Trifluorophenyl ring is energetically stabilized in the hydrophobic pocket formed by Tyr547, Tyr662 and Tyr666. The amine group forms H-bond with side chain of Glu205, Tyr662. Phenyl ring of **4f** interacts with the side chain of Phe357 and forms edge-phase pi–pi interaction.

## Table 2

DPP-4 inhibition activities of various substituted compound 4



Compound	R <sup>1</sup>	DPP-4 IC <sub>50</sub> (nM)
4g	4-Nitro-benzyl	38.5
4h	2-Nitro-benzyl	13.7
4i	2-Cyano-benzyl	48.9
4j	2-Trifluoromethyl-benzyl	50.7
4k	4-Ethyl-benzyl	38.9
41	4-Methoxy-benzyl	33.9
4m	4-Fluoro-benzyl	33.8
4n	4-Iodo-benzyl	30.8
40	3-Chloro-benzyl	26.7
4p	2-Chloro-benzyl	29.0
4q	2-Nitro-4,5-dimethoxy-benzyl	7.40

Table 3DPP-4 inhibition activities of chiral substituted compound 5



Compound	Х	R <sup>2</sup>	DPP-4 $IC_{50}(nM)$
5a	0	Н	12
5b	0	Methyl	7.4
5c	0	Ethyl	4.3
5d	0	Cyclopentyl	1.7
5e	0	Isopropyl	2.2
5f	0	tert-Butyl	0.9
5g	Ν	Ethylmethyl	17
5h	Ν	Diethyl	11
5i	Ν	$-(CH_2CH_2)_2O$	5.2
5j	S	tert-Butyl	1.3

achieved with an IC<sub>50</sub> value of around 10 nM, so further investigation was conducted with *ortho*-nitro group. Replacement of nitro group to electron-withdrawing cyano **4i** or trifluoromethyl **4j** resulted in 3- to 4-fold decrease of inhibition activity. From these results, we fixed *ortho* position substituent to nitro group and through additional substitution of methoxy to *meta* and *para* **4q**, we got the highest inhibitory activity (7.4 nM) in these series.

On the other side, we tried R' substitution (compound **5**) for more activity enhancing and the results are shown in Table 3. The substituted heterocyclic amine **18** was prepared as shown in Scheme 3.

The N-protected aziridine compound **9** was reacted with various alcohols (X = O) or amines (X = N) or thiol (X = S) to provide compound **10**, followed by deprotection using Pd/C and hydrogen gas gave compound **11** which was condensed with Boc-amino acetaldehyde to provide **12** having a variety of substituents X and R<sup>2</sup>. Compound **12** was protected with Cbz, followed by deprotection of Boc to provide **13** which was conducted using trimethyl-aluminium and deprotected to give **15**. Additionally, compound **5a** was prepared from p-serine methyl ester (**11**; X = O, R = H).

From our preliminary study for proper chirality, in case of polar hydroxyl group 5a, (R)-form was threefold more potent than (S)form and compound 5a overcame CYP inhibition problem. For example, compound 41 showed DPP4 IC<sub>50</sub> of 33.9 nM and CYP3A4 IC<sub>50</sub> of 1075 nM (Selectivity Index CYP3A4 IC<sub>50</sub>/DPP4 IC<sub>50</sub>  $\approx$  31.7). On the other hand, introduction of hydroxylmethyl substituent raised selectivity index more than 1000-fold. So we set the new start point to hydroxy derivatives. The DPP-4 inhibitory activities of hydroxyl derivatives (5b, 5c, 5e, 5d) were directly correlated to the number of alkyl size (the IC<sub>50</sub> value of methyl, ethyl, isopropyl and cyclopentyl were 7.4, 4.3, 2.2 and 1.7 nM, respectively). In this SAR, we found tert-butyl addition to (R)-hydroxy moiety 5f was highly potent with an IC<sub>50</sub> value of around 1 nM. Replacement of oxygen in the hydroxyl linker to nitrogen showed similar activity pattern which were dependent on the bulkiness of direct substituent to nitrogen (5g, 5h, 5i). Finally incorporating sulfur instead of the oxygen of hydroxy resulted in highly potent DPP-4 inhibitory activity (5j)

Representative analog in the series, compound **5f** was selected for evaluation of in vivo efficacy. In Sprague Dawley rats, compound **5f** dose-dependently inhibited plasma DPP-4 activity within 30 min of administration. At 24 h following oral administration of compound **5f** (1, 3, 10 mg/kg), plasma DPP-4 activity was inhibited by 72.5  $\pm$  2.4%, 82.9  $\pm$  1.6% and 87.2  $\pm$  0.6%, respectively (Fig. 3).



**Figure 3.** Inhibition of DPP-4 activity in plasma obtained from SD rats after single oral administration of compound **5f** at different doses. Data were presented as means ± SEM.



Scheme 3. Reagents: (a) R<sup>2</sup>XH, BF<sub>3</sub>OEt<sub>2</sub>/CHCl<sub>3</sub>; (b) Pd/C, H<sub>2</sub>(g)/MeOH; (c) Boc-aminoacetaldehyde, NaBH(OAc)<sub>3</sub>/MC; (d) CbzCl/THF; (e) HCl in ether/MeOH; (f) (CH<sub>3</sub>)<sub>3</sub>Al/MC; (g) Pd/C, H<sub>2</sub>(g)/MeOH.

Table 4



Figure 4. Effects of compound 5f on the change of blood glucose levels in C57BL/6 mice after oral glucose loading. Data were presented as means ± SEM.

#### References

Preliminary pharmacological and kinetic data of compound 5f

CYP450, 3A4/2D6/2C9/2C19/1A2	>50 µM
Human liver microsome $t_{1/2}$	>90 min
$t_{1/2}$ (rat)	6.10 h
CL (rat)	60.5 mL/min/kg
V <sub>ss</sub> (rat)	12.0 L/kg
Foral (rat)	74.8%
hERG, patch clamp $IC_{50}$	79.6 μM

In a separated experiment, compound **5f** was evaluated using oral glucose tolerance test (OGTT) in the C57BL/6J mice.<sup>17</sup> Compound 5f (0.1, 0.3 or 1.0 mg/kg) significantly inhibited the elevation of blood glucose and reduced the glucose AUC in dosagedependent manner achieving maximal efficacy at 1 mg/kg, with 63% inhibition (Fig. 4).

Compound **5f** was submitted to the advanced test including preliminary experiments on pharmacokinetic parameters in rats (Table 4). Additionally, compound 5f was applied for a panel of receptors and enzymes and it showed no known liabilities at test concentrations of 1 µM.18

In conclusion, we have shown that variations of  $\beta$ -amino amide containing the piperazine-2-one scaffold used in the clinical development compound 5f (DA-1229), which retains excellent DPP-4 inhibitory activity, in vivo efficacy and preliminary safety profiles, as well as subtype selectivity.<sup>19</sup> DA-1229 is currently undergoing phase II clinical trials and holds the potential for once-daily treatment of type 2 diabetics.

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