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Discovery of β-aminoacyl containing thiazolidine derivatives as potent and selective dipeptidyl peptidase IV inhibitors

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ABSTRACT

A series of β-aminoacyl containing thiazolidine derivatives was synthesized and evaluated for their ability to inhibit DPP-IV. Several thiazolidine derivatives with an acid moiety were found to be potent DPP-IV inhibitors. Among them, compound **2da** is the most active in this series with an IC_{50} value of 1 nM, and it showed excellent selectivity over DPP-IV related enzymes including DPP-2, DPP-8, and DPP-9. Compound **2da** is chemically and metabolically stable, and showed no CYP inhibition, hERG binding or cytotoxicity. Compound **2db**, an ester prodrug of **2da**, showed good in vivo DPP-IV inhibition after oral administration in rat and dog models.

Glucagon like peptide-1 (GLP-1)[1,2] is released from L cells of the small intestine in response to the digestion of food, and plays an important role in secretion of insulin. Increased activity of GLP-1 will lead to increase insulin secretion, which regulates an elevated glucose level. It also retards gastric emptying, induces of satiety and stimulates, regenerates, and differentiates islet β-cells.[3] Dipeptidyl peptidase IV (DPP-IV), a serine protease present in many tissues and body fluids, exist either with membrane bound or soluble enzyme. It degrades GLP-1 (GLP-1[7–36] amide) into inactive GLP [9–36] amide[4,5] at the N-terminus. Inhibition of DPP-IV increases the concentration of GLP-1, as a result increases insulin secretion,[6] which can ameliorate hyperglycemia in type 2 diabetes.

Many reports on the use of small molecules as DPP-IV inhibitor are available in the literature.[7] Among them, Merck described a series of structurally novel β-amino amide derivatives, of which MK-0431 (Sitagliptin) was approved for the treatment of type II diabetes in 2006.[8]

Merck researchers also identified a proline derived homophenylalanine derivative (**1**, Fig. 1), which is a potent and highly selective DPP-IV inhibitor, but exhibited poor bioavailability.[9]

Meanwhile, we have identified several series of β-aminoacyl derivatives.[10,11] as DPP-IV inhibitors (Fig. 2). However more active and less toxic compounds are still in great need.

Based on above mentioned compounds, we identified β-aminoacyl containing thiazolidine derivatives (**2**, Fig. 3), and now wish to report the synthesis and biological evaluation of β-aminoacyl-containing thiazolidine derivatives as DPP-IV inhibitors.

The general and key compound's syntheses are outlined in Schemes 1 and 2. As shown in Scheme 1, commercially available (*R*)-3-(*tert*-butoxycarbonyl amino)-4-(2,4,5-trifluorophenyl)butanoic acid **3** was coupled with racemic ethyl thiazolidine-2-carboxylate in presence of EDCI to provide the amide **4**, which was treated with LiOH to provide the corresponding acid **5**. The compound **5** was amidated with diverse amines to yield amide derivatives, which were deprotected by 4 M HCl to give compound **2**.

The chiral key compounds **2da** and **2db** were synthesized as shown in Scheme 2. Racemic ethyl thiazolidine-2-carboxylate was converted to optically active **6** (>99% ee) by crystallization induced dynamic resolution using L-tartaric acid.[12] (*S*)-Ethyl thiazolidine-2-carboxylate **6** treated with (*R*)-3-BocNH-4-(2,4,5-trifluorophenyl)butanoic acid in the presence of EDCI and DMAP to give the corresponding amide, which was hydrolyzed with LiOH to afford **7**. To complete the syntheses, 4-bromobenzonitrile reacted with D-valine **8** in the presence of copper iodide and K_3PO_4 in dimethylacetamide to afford compound **9**, which was reduced by nickel (II) chloride and sodium borohydride to give the corresponding amine which was protected with Boc_2O for purification and deprotected with 4 M HCl to give compound **10**. The compound **10** was then coupled with compound **7** to afford compound **11**, which was hydrolyzed by LiOH and deprotected with

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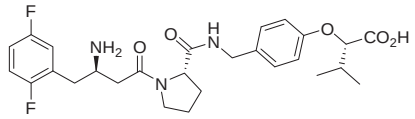


Figure 1. Merck's compound **1**.

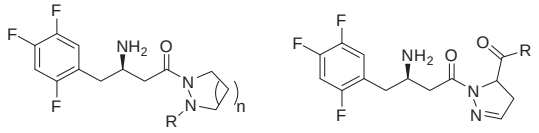
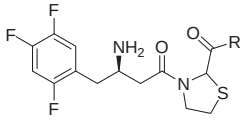
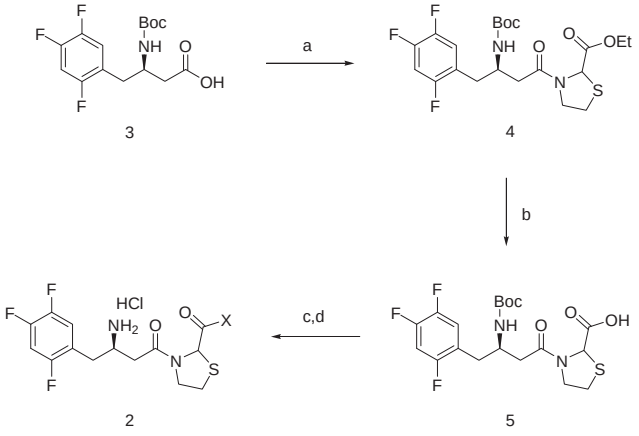


Figure 2. β-Aminoacyl derivatives discovered by the authors.



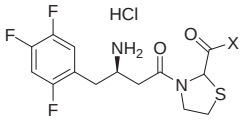
(2)

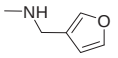
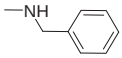
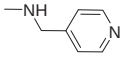
Figure 3. β-Aminoacyl-containing thiazolidine derivative.



Scheme 1. Reagents and conditions: (a) ethyl thiazolidine-2-carboxylate, EDCI, Et_3N, CH_2Cl_2, room temperature, 12 h; (b) LiOH, THF, H_2O, room temperature, 12 h; (c) amines, EDCI, Et_3N, CH_2Cl_2, room temperature, 12 h; (d) 4 M HCl, ethyl acetate, room temperature, 12 h.

Table 1
In vitro DPP-IV inhibition activity of β-aminoacyl thiazolidine derivatives



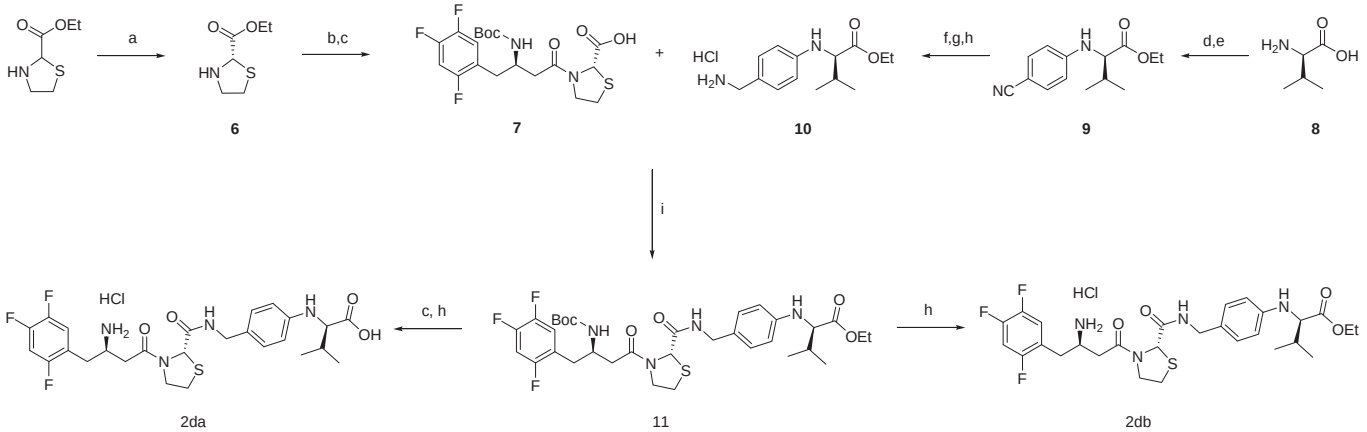
Compound	X	IC_{50}[a] (nM)
2a	OH	601
2b	OMe	204
2c	$NHCH_2CO_2H$	212
2d	$NHCH_2CO_2Et$	101
2e	$N(CH_3)_2$	1700
2f		807
2g		680
2h		88
2i		77
2j		357
MK-0431		20

[a] IC_{50} values were determined from direct regression curve analysis.

4 M HCl to give the final **2da**. Similarly, compound **11** was directly deprotected with 4 M HCl to afford final ester **2db**.

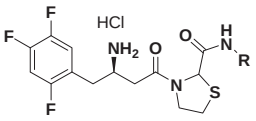
The β-aminoacyl containing thiazolidine derivatives were evaluated in vitro for DPP-IV inhibition, and the results are summarized in Table 1 through **5**. Sitagliptin (MK-0431) was used as a reference compound. β-Aminoacyl thiazolidine-2-carboxylic acid **2a** showed moderate inhibition activity with an IC_{50} value of 601 nM. Acid derivatives such as ester (**2b**) and amide (**2c** and **2d**) were 3–6-fold more potent than that of **2a**. However activities of N,N-disubstituted amides (**2e**, **2f**, and **2g**) were diminished. Arylalkyl amide derivatives (**2h** and **2i**) exhibited good in vitro activities with IC_{50} values in the range of 77–88 nM.

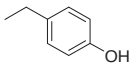
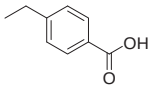
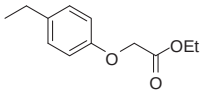
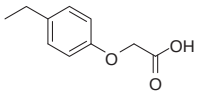
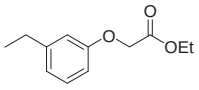
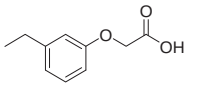
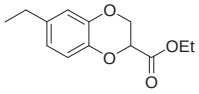
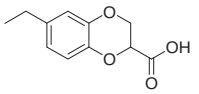
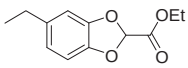
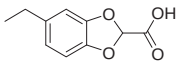
Based on the above data, we derivatized the benzyl group of **2i** with diverse substituents and their inhibitory activities are summarized in Table 2. Hydroxy (**2k**) or acid (**2l**) substituents showed moderate inhibitory activities with IC_{50} values in the range of 229–328 nM. *para*-Phenoxyacetic acid and ester derivatives (**2m** and **2n**) were active with IC_{50} values in the range of 41–51 nM.



Scheme 2. Reagents and conditions: (a) (i) L-tartaric acid, ethanol, diethyl ether, room temperature to 40 °C, 10 days; (ii) 10% sodium bicarbonate, diethyl ether, H_2O, 10 °C, 0.5 h; (b) (*R*)-3-BocNH-4-(2,4,5-trifluorophenyl)butanoic acid, room temperature, EDCI, DMAP, Et_3N, CH_2Cl_2, room temperature, 12 h; (c) LiOH, THF, MeOH, H_2O, room temperature, 3 h; (d) 4-bromobenzonitrile, K_3PO_4, CuI, dimethylacetamide, 90 °C, 48 h; (e) ethyl iodide, K_2CO_3, acetone, reflux, 2 h; (f) nickel (II) chloride, $NaBH_4$, ethanol, room temperature, 0.5 h; (g) Boc_2O, $NaHCO_3$, room temperature, 3 h; (h) 4 M HCl, dioxane, CH_2Cl_2, room temperature, 12 h; (i) EDCI, Et_3N, CH_2Cl_2, room temperature, 10 h.

Table 2
In vitro DPP-IV inhibition activity of β-aminoacyl thiazolidine derivatives



Compound	R	IC_{50}[a] (nM)
2k		328
2l		229
2m		41
2n		51
2o		95
2p		102
2q		112
2r		192
2s		64
2t		64
MK-0431		20

[a] IC_{50} values were determined from direct regression curve analysis.

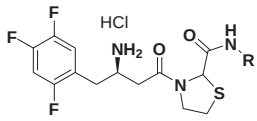
However, *meta* substituents (**2o** and **2p**) and *meta–para* disubstituents (**2q**–**2t**) exhibited weaker activities than that of *para* substituents.

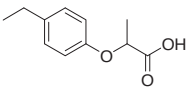
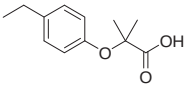
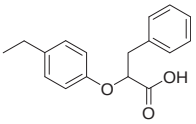
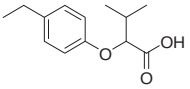
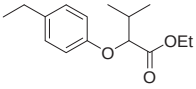
We further modified *para*-phenoxyacetic acid derivatives with substituents at the α-position of the acetic acid moiety. As shown in Table 3, α-methyl derivative **2u** was two fold less potent than unsubstituted compound **2n**. α,α-Dimethyl derivative **2v** was similar to unsubstituted compound **2n** with an IC_{50} value of 56 nM. A benzyl substituent at the α-position showed improved inhibitory activity (IC_{50} = 28 nM). Isopropyl substituent **2x** was the most active in this series with an IC_{50} value of 6 nM. However, **2x** exhibited a very weak in vivo DPP-IV inhibition after oral administration, which is a similar result to Merck's compound **1**.[9] Therefore, we further looked for a potent compound with amino acid substituted benzyl derivatives.

β-Aminoacyl containing thiazolidine derivatives with an amino acid moiety were evaluated for their inhibitory potency, and the results are summarized in Table 4. *N*-Methyl valine substituent **2aa** and dimethylglycine substituent **2ba** exhibited weaker activity than **2x**. Fortunately, valine derivative **2ca** showed a good in vitro potency with an IC_{50} value of 3 nM.

Compound **2ca** is a mixture of four stereoisomers when made from racemic thiazolidine and valine starting material. As shown in Table 5, each isomer and its ethyl ester were evaluated for their

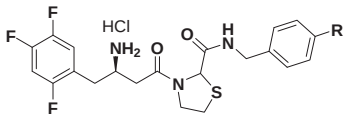
Table 3
In vitro DPP-IV inhibition activity of β-aminoacyl thiazolidine derivatives

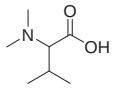
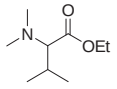
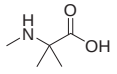
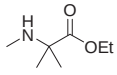
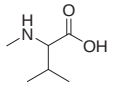
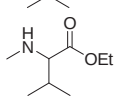


Entry	R	IC_{50}[a] (nM)
2u		216
2v		56
2w		28
2x		6
2y		77
MK-0431		20

[a] IC_{50} values were determined from direct regression curve analysis.

Table 4
In vitro DPP-IV inhibition activity of β-aminoacyl thiazolidine derivatives

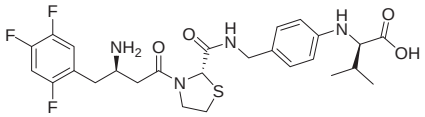
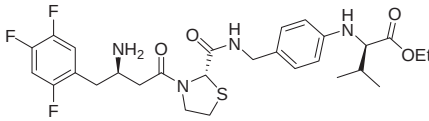
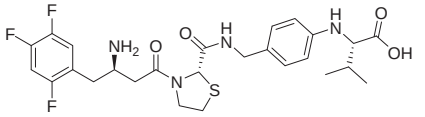
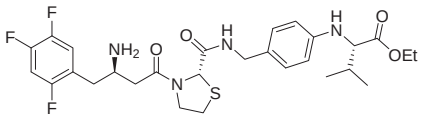
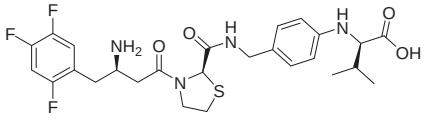
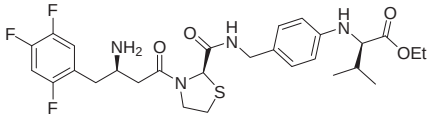
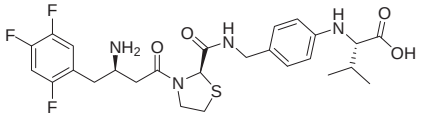
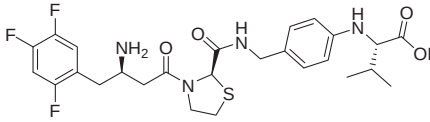


Entry	R	IC_{50}[a] (nM)
2aa		80
2ab		80
2ba		13
2bb		68
2ca		3
2cb		50
MK-0431		20

[a] IC_{50} values were determined from direct regression curve analysis.

inhibitory activity. The chirality of the 2-position of thiazolidine is important [**2da** (1 nM) vs **2fa** (10 nM) and **2ea** (1.2 nM) vs **2ga** (13 nM)], but that of the valine isomer (D or L valine) does not affect the in vitro activity [**2da** (1 nM) vs **2ea** (1.2 nM)]. Compound **2da** was the most active in this series with an IC_{50} value of 1 nM. Therefore, we further evaluated its selectivity, stability, and in vivo activity.

Table 5
In vitro DPP-IV inhibition activity of β-aminoacyl thiazolidine derivatives

Entry	R	IC_{50}[a] (nM)
2da		1
2db		17
2ea		1.2
2eb		25
2fa		10
2fb		175
2ga		13
2gb		309
MK-0431		20

[a] IC_{50} values were determined from direct regression curve analysis.

Table 6
Selectivity of compound **2da** towards DPP-IV related enzymes

DPP-IV related enzyme	IC_{50} (μM)[a]	Fold
DPP-IV	0.001	
DPP-2	>100	100,000
DPP-8	21	21,000
DPP-9	13	13,000
APN	>100	100,000
POP	>100	100,000
Trypsin	>100	100,000
Elastase	>100	100,000

[a] IC_{50} values were determined from direct regression curve analysis.

Compound **2da** was investigated for its selectivity towards a variety of DPP-IV related peptidases, and found to be selective against DPP-IV related isozymes (Table 6).

Compound **2da** is metabolically stable in human and rat liver microsomes, with 90% of the parent compound remaining after 1 h incubation. Also, **2da** is chemically stable in various pH conditions with at least 90% of the parent compound remaining after 1 day at room temperature as shown in Table 7.

As shown in Table 8, compound **2da** was examined in a CYP inhibition assay with 5 CYP subtypes (3A4, 1A2, 2C9, 2C19, and

Table 7
Stability of compound **2da**

	Remaining%	Condition
Human liver microsome	>90	1 h incubation
Rat liver microsome	>90	1 h incubation
Buffer solution pH 1,3,5,7,9	>90	24 h incubation

Table 8
CYP, hERG, and cytotoxicity study of compound **2da**

Assay	Results
CYP	3A4: 3.5% inhibition at 10 μM 1A2: 2.9% inhibition at 10 μM 2C9: 26.8% inhibition at 10 μM 2C19: 46.9% inhibition at 10 μM 2D6: 12.2% inhibition at 10 μM
hERG binding assay	EC_{50} = 408 μM
Cytotoxicity	HepG2 cells: no cytotoxicity at 100 μM WI-38 cells: no cytotoxicity at 100 μM

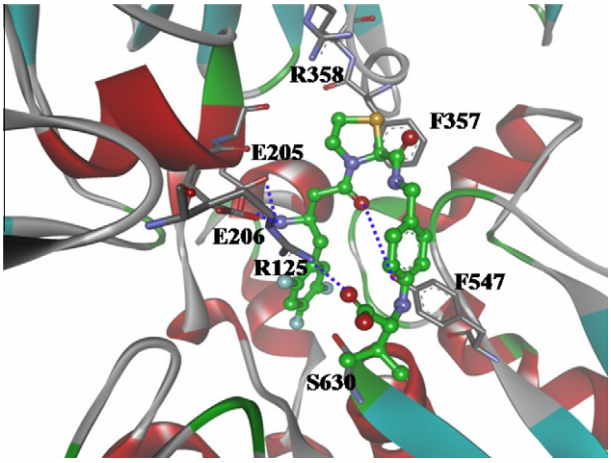


Figure 4. Binding mode and X-ray crystal structure of compound **2da** with DPP-IV. PDB deposition number 3Q8W.

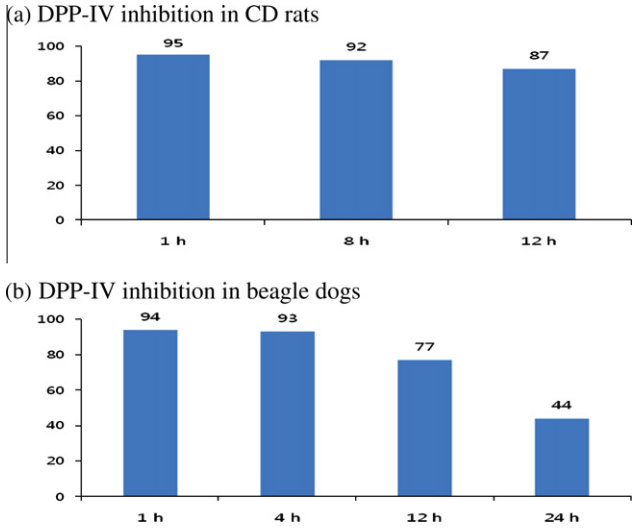


Figure 5. In vivo DPP-IV inhibition of compound **2db** in (a) CD rats (n = 3) and (b) beagle dogs (n = 3). Compound was orally administered in a single dose of 10 mg/kg (CD rats) and 3 mg/kg (beagle dogs).

2D6), and exhibited less than 50% inhibition toward 5 subtypes at 10 μM. Compound **2da** showed no hERG binding with an EC_{50} value of 408 μM. Compound **2da** is not cytotoxic up to 100 μM in a cell cytotoxicity assay with two cell lines (HepG2 and WI-38 cells).

We investigated a binding mode of compound **2da** with molecular modeling and X-ray co-crystallization. Calculations for docking were carried out using LigandFit[13] interfaced with Accelrys DiscoveryStudio 2.5, using our previous complex (pdb entry; 2OLE) as a reference structure. We set the O atoms of carboxylic group in side chain of E205 and E206 as interaction sites with default parameters in LigandFit. As can be seen by viewing the binding site region in Figure 4, the β-aminoacyl group has a similar conformation to that of MK-0431,[8] and the carbonyl moiety in amino acyl group is possible to form the water-bridged hydrogen bonding with Y547, as shown in our previous literature.[10] Also, carboxylic moiety forms the strong ionic interaction with guanidium group in R125 residue, inducing the most favorable activity. The X-ray co-crystal structure of human DPP-IV complexed with **2da** showed the same result with modeling study.

In order to evaluate in vivo efficacy in rat and dog model, we used compound **2db** as a prodrug, which is very rapidly converted to **2da** in plasma, to increase absorption after oral administration. The compound **2db** showed good in vivo DPP-IV inhibitions in rat and dog model as shown in Figure 5.

Oral administration of compound **2db** at 10 mpk in CD rats resulted in ca 90% inhibition of plasma DPP-IV activity up to 12 h. Also, **2db** exhibited ~80% DPP-IV inhibition at 3 mg/kg after 12 h in beagle dogs.

In conclusion, we have identified a series of β-aminoacyl containing thiazolidine derivatives as DPP-IV inhibitors. Several thiazolidine derivatives with acid moiety were found to be potent DPP-IV inhibitors. Among them, the compound **2da** is the most active in this series with an IC_{50} value of 1 nM. The compound **2da** showed excellent selectivity toward DPP-IV related enzymes including DPP-2, DPP-8, and DPP-9. The compound **2da** is chemically and metabolically stable, also showed no CYP inhibition, hERG binding, and cytotoxicity. From the modeling and co-crystal structure of **2da** with DPP-IV, **2da** bound the active pocket of DPP-IV and showed additional binding of side chain of **2da**. The in vivo efficacy of **2db** as a prodrug of **2da** showed good DPP-IV inhibition after oral administration in rat and dog model.

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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.2011.01.041.

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