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Synthesis and biological evaluation of homopiperazine derivatives with β-aminoacyl group as dipeptidyl peptidase IV inhibitors

Jin Hee Ahn [a,*], Woul Seong Park [b], Mi Ae Jun [a], Mi Sik Shin [a], Seung Kyu Kang [a], Ki Young Kim [a], Sang Dal Rhee [a], Myung Ae Bae [a], Kwang Rok Kim [a], Sung Gyu Kim [c], Sun Young Kim [c], Sang Kwon Sohn [c], Nam Sook Kang [a], Jie Oh Lee [d], Duck Hyung Lee [b], Hyae Gyeong Cheon [a], Sung Soo Kim [a,*]

[a] Drug Discovery Division, Korea Research Institute of Chemical Technology, Yuseong-Gu, Daejeon 305-600, Republic of Korea
[b] Department of Chemistry, Sogang University, Seoul 121-742, Republic of Korea
[c] Namyang Research Center, Yungjin Pharm. Co., Ltd, Musong-dong, Kyungki-do, Republic of Korea
[d] Korea Advanced Institute of Science and Technology, Yuseong-gu, Daejeon 305-701, Republic of Korea

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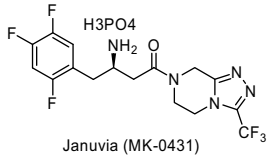
ABSTRACT

Compounds with homopiperazine skeleton are designed to find a potent DPP-IV inhibitor without inhibiting CYP. Thus a series of β-aminoacyl-containing homopiperazine derivatives was synthesized and evaluated. Compounds with acid moiety were found to be potent inhibitors of DPP-IV without inhibiting CYP 3A4. More specifically, compound **7m** showed nanomolar activity with no inhibition towards five subtypes of CYPs, was considered as a prototype for further derivatization. Based on its X-ray co-crystal structure with human DPP-IV, we identified compounds **7s** and **7t** which showed good in vitro activity, no CYP inhibition, and good selectivity.

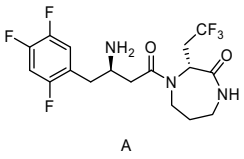
A non-insulin dependent diabetes mellitus (NIDDM) is characterized by chronic hyperglycemia, and belongs to a group of metabolic disorders with multiple etiologies. It is very common and may result from insulin resistance, inadequate secretion of insulin, hepatic glucose overproduction or glucose intolerance.[1]

Glucagon like peptide-1 (GLP-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 sustained insulin secretion, which regulate an elevated glucose level. It also retards gastric emptying, induction of satiety and stimulation, regeneration and differentiation of islet β-cells.[3] A 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 N-terminus position. 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.

In recent past, many reports on use of small molecules as inhibitors of DPP-IV are available in the literauture.[7] Merck described a series of structurally novel β-amino amide derivatives, and among them MK-0431 (sitagliptin) was launched into the market in 2006.[8] Also, Merck has developed 1,4-diazepine-2-one derivative (A) as a potential back-up candidate.[9]

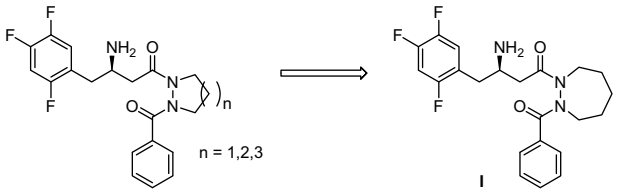


Januvia (MK-0431)



A

In our earlier report, we have described the synthesis and biological evaluation of a series of β-aminoacyl group containing cyclic hydrazine derivatives (Fig. 1).[10]



I

Figure 1. β-Aminoacyl-containing cyclic hydrazine derivatives.

* Corresponding authors. Tel.: +82 42 860 7076; fax: +82 42 860 7160.
E-mail address: jhahn@krict.re.kr (J.H. Ahn).

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Table 1
In vitro activity against CYP.

CYP subtype	Activity at 10 μM (%)
1A2	91.5
2C9	71.5
2C19	90.3
2D6	51.0
3A4	5.4

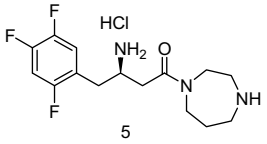
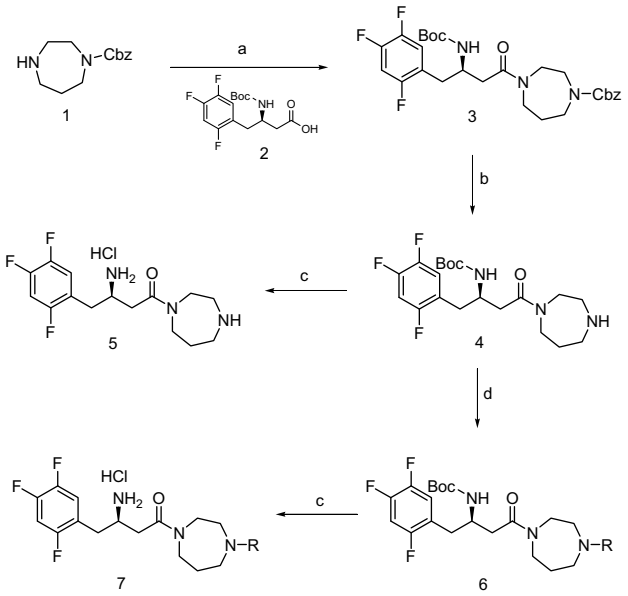


Figure 2. Homopiperazine derivative 5.



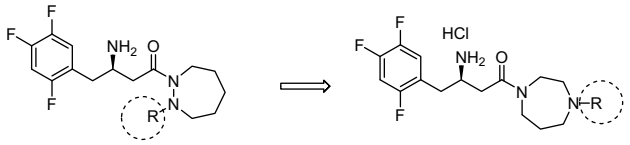
Scheme 1. Reagents and conditions: (a) (*R*)-3-*BocNH*-4-(2,4,5-trifluorophenyl)butanoic acid (2), EDCI, Et_3N, CH_2Cl_2, room temperature, 12 h; (b) 10% Pd/C, H_2 balloon, MeOH, room temperature, 12 h; (c) 4 M HCl, ethyl acetate, room temperature, 12 h; (d) electrophile, CH_2Cl_2, TEA, room temperature.

Among the cyclic hydrazine derivatives, compound **I** showed good potency, selectivity and in vivo efficacy. In course of time, we have further evaluated toxicity related test with this compound (**I**), and found to inhibit CYP 3A4 subtype as shown in Table 1.

Cytochrome P450 (CYP) enzymes play a major role in metabolizing drug molecules. Many lead candidate molecules in pharmaceutical development fail due to inhibition of one or more isozymic forms of CYP enzymes. CYP 3A4, is one of the most important cytochrome P450s present in human liver,[11] has ability to metabolize >50% of administered therapeutic agents. It accounts for the large number of documented drug–drug interactions associated with CYP 3A4 inhibition.[12] To overcome CYP inhibition (particularly CYP 3A4), we have synthesized over 100 compounds based on cyclic hydrazine, however all the compounds found to show CYP 3A4 inhibition.

In our recent findings, we identified homopiperazine derivative **5**, which showed moderate DPP-IV inhibition (IC_{50} 1.44 μM) and no CYP 3A4 inhibition (93.3% at 10 μM) (Fig. 2).

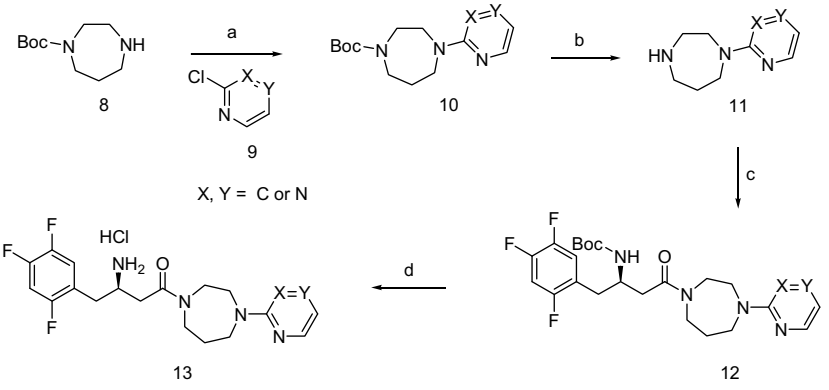
These observations prompted us to turn our attention from cyclic hydrazine to homopiperazine skeleton.



We now wish to report here the synthesis and biological evaluation of a series of β-aminoacyl-containing homopiperazine derivatives as DPP-IV inhibitors. The sequence of reaction steps involved in synthesis is shown in Schemes 1 and 2. *Cbz*-protected homopiperazine (**1**) was reacted with β-amino acid (**2**) in presence of EDCI to provide the coupled product (**3**). It is on reduction with 10% palladium on carbon in hydrogen atmosphere to provide a key intermediate **4**, followed by *boc*-deprotection using 4 M HCl to result corresponding HCl salt (**5**). The compound **4** is also derivatized with diverse electrophiles to obtain compounds **6**, followed by deprotection to give compound **7**.

Similarly, *Boc*-protected homopiperazine (**8**) was reacted with heteroaryl chloride to give the coupled compound **10**, followed by *boc*-deprotection to give product **11**. The compounds **11** were further coupled with β-amino acid to give **12**, and on deprotection with HCl to afford compounds **13**.

The β-aminoacyl containing homopiperazine derivatives (**5, 7** and **13**) were evaluated in vitro for DPP-IV inhibition, and the results were summarized in Table 2. MK-0431 and compound **I** were used as reference.



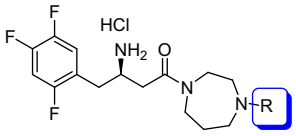
Scheme 2. Reagents and conditions: (a) **9**, ethylene glycol, 130 °C, 2 h; (b) 4 M HCl, ethyl acetate, room temperature, 12 h; (c) (*R*)-3-*BocNH*-4-(2,4,5-trifluorophenyl)butanoic acid (2), EDCI, Et_3N, CH_2Cl_2, room temperature, 12 h; (d) 4 M HCl, ethyl acetate, room temperature, 12 h.

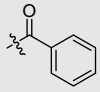
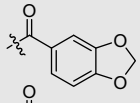
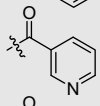
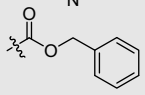
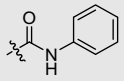
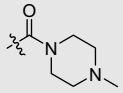
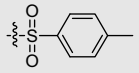
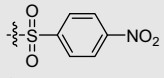
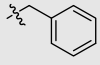
The activity data of basic verses substituted homopiperazine derivatives is compared. *N*-Aroyl substituted homopiperazines such as **7a**, **7c** and **7d** showed nearly 3-fold more potent than that of the basic homopiperazine **5** (IC_{50} 1440 nM), however activity of acetyl derivative **7b** is diminished. Carbamate **7e** (IC_{50} 490 nM), heteroaryl **13a** and **13b** exhibited equal potency with that of aroyl derivatives. Urea derivatives such as **7f** and **7g** demonstrated weaker activities. *N*-Aralkyl substituted compound **7k** (IC_{50} 387 nM) showed better in vitro inhibitory activity than that of alkyl **7l** (IC_{50} 6400 nM). Tosyl derivative **7h** (IC_{50} 350 nM) found to show highest activity, and is a potential candidate for further derivatization.

Based on the above data, we chose three representative compounds (**7a**, **7h** and **13b**) for CYP re-evaluation. Unfortunately, all the compounds found to show CYP 3A4 inhibition (Table 3).

Our recent experience[13] with acid moiety in a molecule promoted good DPP-IV inhibitory activity with no CYP 3A4 inhibition. Thus, we have synthesized number of compounds having acid or acid equivalent group, and evaluated. Fortunately, acid derivatives (**7m–7o**) showed no CYP 3A4 inhibition at 10 μM concentration as

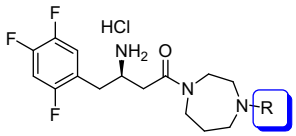
Table 2
In vitro DPP-IV inhibitory activity of homopiperazine derivatives with β-aminoacyl group.



Compound	R	IC_{50}[a] (nM)
5	H	1440
7a		510
7b		3400
7c		490
7d		410
7e		490
7f		1392
7g		1500
7h		350
7i		3100
7j		405
7k		387
7l		6400
13a		597
13b		423
MK-0431		87
I		70

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

Table 3
In vitro CYP 3A4 activity of homopiperazine derivatives with β-aminoacyl group.



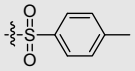
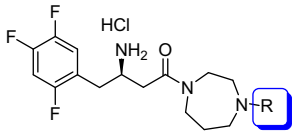
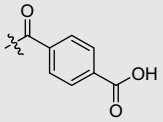
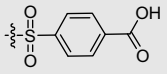
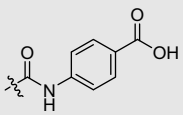
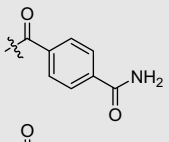
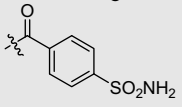
Compound	R	CYP 3A4 activity at 10 μM (%)
7a		2.1
7h		1.2
13b		16.9
MK-0431		89.0

Table 4
In vitro DPP-IV and CYP 3A4 activity of homopiperazine derivatives with β-aminoacyl group.



Entry	R	IC_{50}[a] (nM)	CYP 3A4 activity at 10 μM (%)
7m		223	74.6
7n		239	83.3
7o		788	83.4
7p		180	5.7
7q		288	36.6
MK-0431		87	86.1

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

shown in Table 4. Amide and sulfonamide derivatives (**7p** and **7q**) still inhibited CYP 3A4.

Compound **7m** being not shown CYP 3A4 inhibition, was further screened with other CYP subtypes and found to show no inhibition (Table 5).

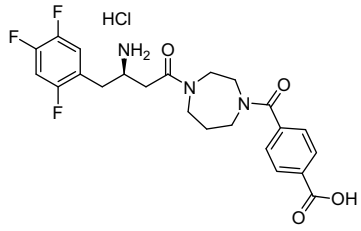
Therefore, we determined the crystal structure of human DPP-IV complexed with **7m**. As can be seen by viewing the binding site region in the complex (Fig. 3), the β-aminoacyl group has a similar conformation as that of MK-0431.[8] Also, the carbonyl moiety in amino acyl group showed water-bridged hydrogen bonding with Y547. Whereas, carboxy or benzoyl part showed no significant interaction, therefore we further derivatized.

We have derivatized homopiperazine skeleton with acid moiety, and evaluated the inhibitory activities. The results are summarized in Table 6. Substituents in *meta* position (**7s** and **7t**) exhibited better activity than *para* (**7m** and **7r**) with IC_{50} value of 50–67 nM, as well as no CYP 3A4 inhibition.

Compounds **7s** and **7t** were further investigated for their selectivity towards a variety of DPP-IV related peptidases, and found to show good selectivity against DPP-2 and DPP-8 (Table 7).

In conclusion, this investigation has led to the synthesis and biological evaluation of a series of β-aminoacyl-containing homopiperazine derivatives. Several homopiperazine derivatives with acid moiety were found to be potent inhibitors of DPP-IV with no CYP 3A4 inhibition. The compound **7m** showed submicromolar activity with no CYP inhibition towards five subtypes, and is a prototype for further derivatization. Based on the results, we identified **7s** and **7t**, which showed good in vitro activity, no CYP inhibition, and good selectivity. Further studies are underway to optimize this compound class for the treatment of diabetes.

Table 5
In vitro activity against five CYP subtypes.



CYP subtype	Activity at 10 μM (%)
1A2	97.3
2C9	103.2
2C19	96.0
2D6	89.1
3A4	74.6

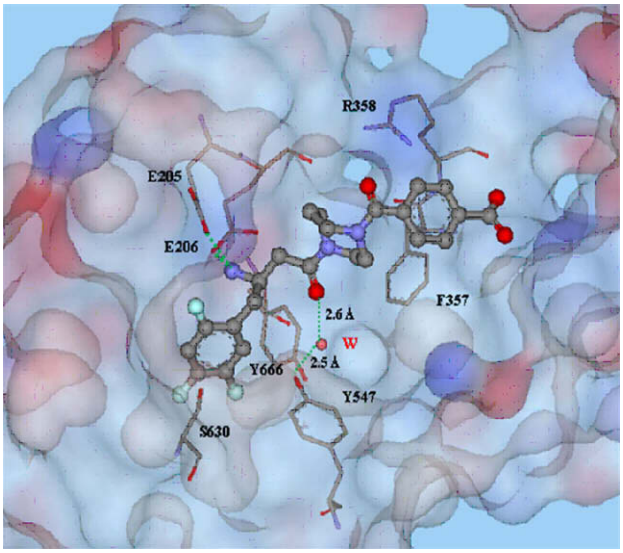
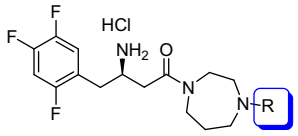
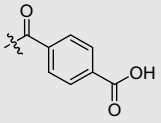
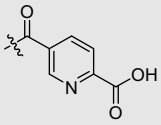
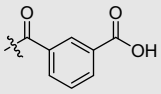
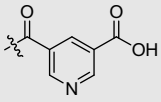
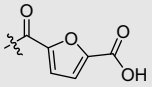


Figure 3. X-ray co-crystal structure of **7m** with DPP-IV (pdb code 3EIO).

Table 6
In vitro DPP-IV inhibitory activity and CYP 3A4 activity of homopiperazine derivatives with β-aminoacyl group.



Entry	R	IC_{50}[a] (nM)	CYP 3A4 activity at 10 μM (%)
7m		223	74.6
7r		150	55.5
7s		50	66.1
7t		67	74.0
7u		260	100
MK-0431		87	86.1

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

Table 7
Selectivity of compounds **7s** and **7t** towards DPP-IV related enzymes.

Compound	DPP-IV IC_{50} [a](nM)	DPP-2 IC_{50} [a](nM)	DPP-8 IC_{50} [a](nM)
7s	50	46,770	4265
7t	67	99,434	18,197

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

Acknowledgments

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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.2008.10.076.

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