# SAR Study of β-Aminoacyl-Containing Cyclic Hydrazide Derivatives as DPP-IV Inhibitors

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In continuation of our efforts to further derivatize dipeptidyl peptidase IV (DPP-IV) inhibitors, a series of β-aminoacyl-containing 5-, 6- and 7- membered cyclic hydrazide derivatives was synthesized. All the compounds were evaluated for their ability to inhibit DPP-IV, and an optimum structural unit on basic skeleton is identified to show good *in vitro* activity.

**Key Words :** Dipeptidyl peptidase IV, Diabetes, Cyclic hydrazide

## Introduction

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]

GLP-1[2] is released from L cells of the small intestine in response to digestion of food, and plays an important role in secretion of insulin. Increased activity of GLP-1 will lead to sustained insulin secretion, which normalize an elevated glucose level. It also retards gastric emptying, induction of satiety and stimulation, regeneration & 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, several reports on use of small molecules as inhibitors of DPP-IV is available in literauture.[7]

In our previous paper,[8] we have described the synthesis and biological evaluation of β-aminoacyl-containing cyclic hydrazine derivatives with only 6 examples. In continuation of our efforts, we have further derivatized the core compounds with diversified substituents, in order to find a potential candidate as DPP-IV inhibitor. We now wish to report here the detailed SAR study of β-aminoacyl-containing cyclic hydrazide derivatives as DPP-IV inhibitors.

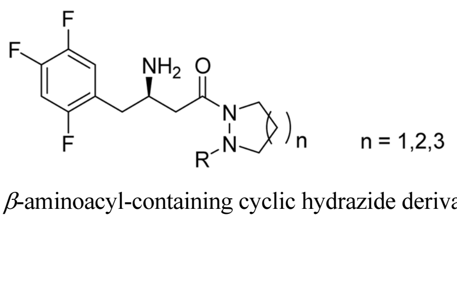
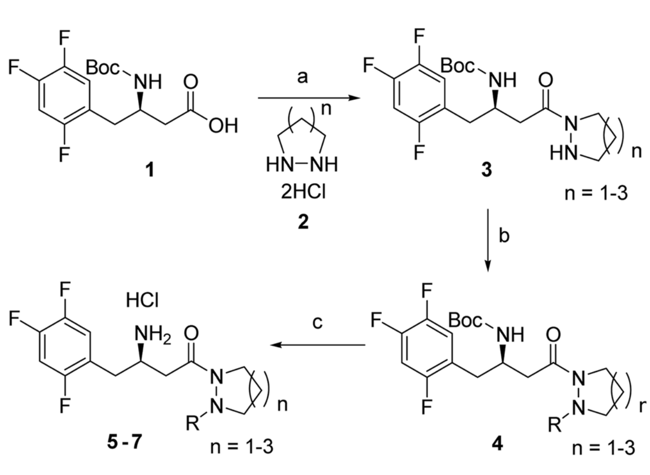



**Figure 1**. β-aminoacyl-containing cyclic hydrazide derivatives.

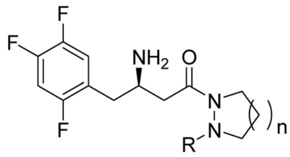
A series of β-aminoacyl-containing cyclic hydrazine derivatives was synthesized by using the route shown in Scheme 1. The detailed synthetic explanation was described in our previous publication.[8]

5-, 6- and 7-Membered cyclic hydrazide derivatives with β-aminoacyl group were evaluated *in vitro* for their inhibitions against DPP-IV. MK-0431 was used as a reference compound. Compounds which showed more than 50% inhibition of DPP-IV at 100 nM, were considered as promising and the $IC_{50}$ values of the compounds were determined. The data are compared with ring size and also various functionalities such as acyl, benzoyl, urea, sulfonyl, carbamate, and alkyl groups. Basic compounds (R = H, **5-1**, **6-1** and **7-1**) couldn't reach 50% inhibition at 100 nM, however benzoyl substituents promoted activity. More particularly 6- and 7- membered benzoyl hydrazides (**6-2** and **7-2**) showed good *in vitro* inhibitory activities with $IC_{50}$ values

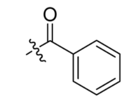
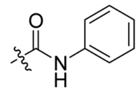
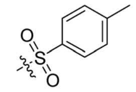
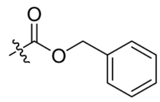
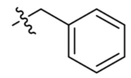



**Scheme 1**. Reagents and conditions: (a) compound 2, triethylamine, EDCI, $CH_2Cl_2$, room temperature; (b) electrophiles, $CH_2Cl_2$, triethylamine, room temperature; (c) HCl, dioxane, room temperature.

**Table 1**. Inhibitory activity of $\beta$-aminoacyl-containing cyclic hydrazide derivatives against DPP-IV




n = 1,2,3

| R | Compd (n = 1) | % inh. at 100 nM | IC$_{50}$, nM[a] | Compd (n = 2) | % inh. at 100 nM | IC$_{50}$, nM[a] | Compd (n = 3) | % inh. at 100 nM | IC$_{50}$, nM[a] |
|---|---|---|---|---|---|---|---|---|---|
| **H** | **5-1** | 3.94 | | **6-1** | 22.10 | | **7-1** | 20.14 | |
|  | **5-2** | 14.32 | | **6-2** | 67.97 | 74.40 | **7-2** | 51.12 | 85.72 |
|  | **5-3** | 11.51 | | **6-3** | 5.65 | | **7-3** | 51.71 | 95.07 |
|  | **5-4** | 14.68 | | **6-4** | ND[b] | | **7-4** | 16.25 | |
|  | **5-5** | ND[b] | | **6-5** | 20.64 | | **7-5** | 41.95 | |
|  | **5-6** | ND[b] | | **6-6** | ND[b] | | **7-6** | 1.59 | |
| MK-0431 | | | | IC$_{50}$ = 65.42 nM | | | | | |

[a]IC$_{50}$ values were determined from direct regression curve analysis. [b]not determined.

of 74.40 nM and 85.72 nM respectively. In case of urea, 7-membered hydrazide displayed a good activity with 95.07 nM. All other substituents such as sulfonyl, carbamate and aralkyl groups showed weak activities.

The benzoyl derivatives (**6-2** and **7-2**) being demonstrated good activity further derivatized with various substituted benzoyl derivatives and evaluated. Some compounds (**7-11**, **7-13**, **7-14**, **7-15**, **7-17**, **7-18** and **7-22**) showed good activities and compound (**7-18**) is found to be most active with an IC$_{50}$ value 32.80 nM. The details are tabulated in Table 2.

Urea based substituent also being active, it is further derivatized with various substituents and evaluated. Compound 7-39 showed better activity than other urea based derivatives, and the details of activity data is tabulated in Table 3.

From the SAR data, we have chosen compound **7-18** to evaluate *in vivo* for their ability to reduce DPP-IV activity in normal C57BL/6J mice. Oral administration of compounds **7-18**, at 10 mg/kg dose, resulted in *ca* 70% inhibition of plasma DPP-IV activity after 2 h.

## Conclusion

Diverse $\beta$-aminoacyl-containing 5-, 6- and 7-membered cyclic hydrazide derivatives were synthesized and evaluated for their ability to inhibit dipeptidyl peptidase IV (DPP-IV). Among them, **7-18** emerged as the most active compound with an IC$_{50}$ value of 32.8 nM, and evaluated for its *in vivo* DPP-IV inhibitory activity.

## Experimental

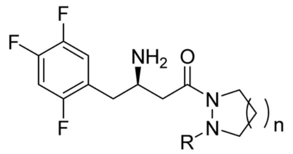
**General.** All reported yields are isolated yields after column chromatography or crystallization. $^1$H-NMR spectra were obtained on FT-NMR Varian GEMINI-200FT or Bruker AVANCE-300 with TMS as internal reference. MS spectra were obtained on a Shimadzu QP5050 spectrograph.

**Synthetic Procedure for Representative Compound 7-18:** A mixture of (R)-tert-butyl 4-(1,2-diazepan-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate (30 mg, 0.072 mmol), Benzo[1,3]dioxole-5-carbonyl chloride (20 mg, 0.108 mmol), and triethylamine (20 $\mu$L, 0.144 mmol) in $CH_2Cl_2$ (2 mL) was stirred for 1 h at room temperature. The reaction mixture was diluted with brine and $CH_2Cl_2$. The organic layer was separated, dried and evaporated. The residue was purified by silica gel column chromatography to give (R)-*tert*-butyl 4-(2-(benzo[d][1,3]dioxole-5-carbonyl)-1,2-diazepan-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate (36 mg, 89%) as an oil.

$^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.00-6.77 (m, 5H), 6.02 (s, 2H), 5.65-5.10 (br., s, 1H), 4.22-4.09 (m, 1H), 3.22-2.48 (m, 7H), 1.91-1.42 (m, 7H), 1.35 (s, 9H); MS m/z 563 ($M^+$).

To a solution of (R)-*tert*-butyl 4-(2-(benzo[d][1,3]dioxole-5-carbonyl)-1,2-diazepan-1-yl)-4-oxo-1-(2,4,5-trifluorophenyl)butan-2-ylcarbamate (50 mg, 0.089 mmol) in EtOAc (2 mL), was added 4 M-HCl/1,4-dioxane (0.5 mL) and the mixture was stirred for 12 h at room temperature. The solvents were evaporated, and the residue was crystallized with ether to give (R)-3-amino-1-(2-(benzo[d][1,3]dioxole-

**Table 2**. Inhibitory activity of *β*-aminoacyl-containing cyclic hydrazide derivatives with *N*-acyl substituents against DPP-IV




n = 1,2,3

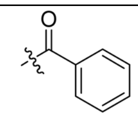
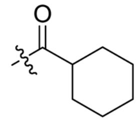
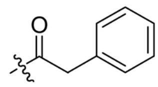
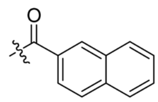
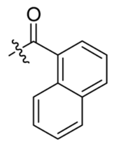
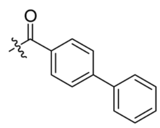
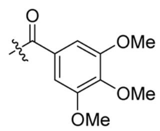
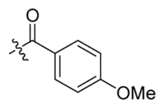
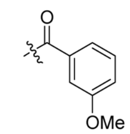
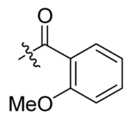
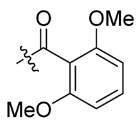
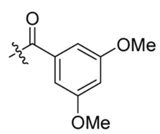
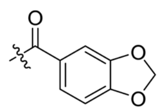
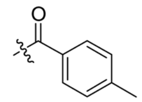
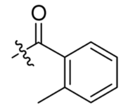
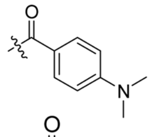
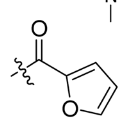
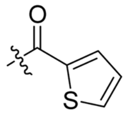
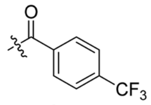
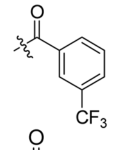
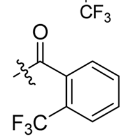
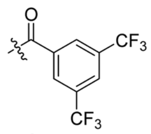
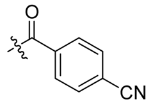
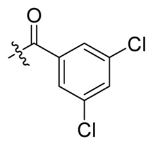
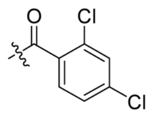
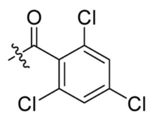
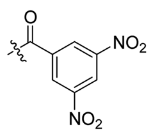
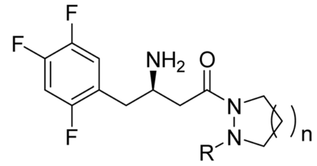
| R | Compd (n = 1) | n = 1 % inh. at 100 nM | IC$_{50}$, nM[a] | Compd (n = 2) | n = 2 % inh. at 100 nM | IC$_{50}$, nM[a] | Compd (n = 3) | n = 3 % inh. at 100 nM | IC$_{50}$, nM[a] |
|---|---|---|---|---|---|---|---|---|---|
|  | **5-2** | 14.32 | | **6-2** | 67.97 | 74.40 | **7-2** | 51.12 | 85.72 |
|  | **5-7** | ND | | **6-7** | 2.21 | | **7-7** | 36.82 | |
|  | **5-8** | ND | | **6-8** | 2.11 | | **7-8** | 14.75 | |
|  | **5-9** | ND | | **6-9** | 4.47 | | **7-9** | 28.87 | |
|  | **5-10** | ND | | **6-10** | 23.74 | | **7-10** | 33.83 | |
|  | **5-11** | ND | | **6-11** | 15.46 | | **7-11** | 55.41 | 85.04 |
|  | **5-12** | ND | | **6-12** | 0.76 | | **7-12** | 9.54 | |
|  | **5-13** | 15.32 | | **6-13** | 34.53 | | **7-13** | 75.08 | 35.42 |
|  | **5-14** | ND | | **6-14** | 35.15 | | **7-14** | 55.68 | 83.07 |
|  | **5-15** | ND | | **6-15** | 35.14 | | **7-15** | 57.14 | 82.10 |
|  | **5-16** | ND | | **6-16** | 8.10 | | **7-16** | 2.36 | |
|  | **5-17** | ND | | **6-17** | 38.87 | | **7-17** | 68.42 | 41.04 |
|  | **5-18** | 16.08 | | **6-18** | 27.53 | | **7-18** | 74.07 | 32.80 |

**Table 2**. Continued

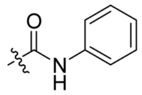
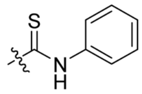
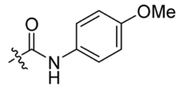
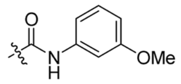
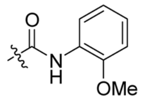
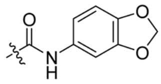
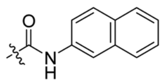
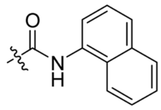
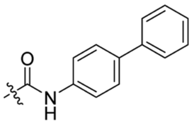
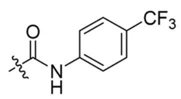
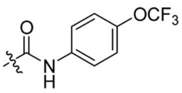
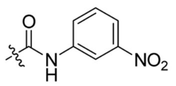
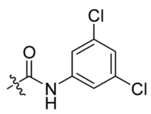
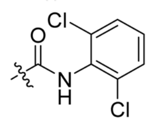
| R | Compd (n = 1) | n = 1 % inh. at 100 nM | $IC_{50}$, nM[a] | Compd (n = 2) | n = 2 % inh. at 100 nM | $IC_{50}$, nM[a] | Compd (n = 3) | n = 3 % inh. at 100 nM | $IC_{50}$, nM[a] |
|---|---|---|---|---|---|---|---|---|---|
|  | **5-19** | ND | | **6-19** | 20.01 | | **7-19** | 21.75 | |
|  | **5-20** | ND | | **6-20** | 1.78 | | **7-20** | 21.69 | |
|  | **5-21** | ND | | **6-21** | 30.33 | | **7-21** | 36.58 | |
|  | **5-22** | ND | | **6-22** | 20.19 | | **7-22** | 60.59 | 69.60 |
|  | **5-23** | ND | | **6-23** | 36.87 | | **7-23** | 36.57 | |
|  | **5-24** | ND | | **6-24** | 38.82 | | **7-24** | 45.72 | |
|  | **5-25** | ND | | **6-25** | 11.56 | | **7-25** | 43.22 | |
|  | **5-26** | ND | | **6-26** | 1.47 | | **7-26** | 22.04 | |
|  | **5-27** | ND | | **6-27** | 0.37 | | **7-27** | 3.77 | |
|  | **5-28** | ND | | **6-28** | 0.43 | | **7-28** | 28.89 | |
|  | **5-29** | ND | | **6-29** | 5.21 | | **7-29** | 41.40 | |
|  | **5-30** | ND | | **6-30** | 35.47 | | **7-30** | 30.19 | |
|  | **5-31** | ND | | **6-31** | 13.94 | | **7-31** | 8.10 | |
|  | **5-32** | ND | | **6-32** | 23.68 | | **7-32** | 14.45 | |
| MK-0431 | | | | | $IC_{50}$ = 65.42 nM | | | | |

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

**Table 3**. Inhibitory activity of *β*-aminoacyl-containing cyclic hydrazide derivatives with urea substituents against DPP-IV



n = 1,2,3

| R | Compd (n = 1) | % inh. at 100 nM | $IC_{50}$, nM[a] | Compd (n = 2) | % inh. at 100 nM | $IC_{50}$, nM[a] | Compd (n = 3) | % inh. at 100 nM | $IC_{50}$, nM[a] |
|---|---|---|---|---|---|---|---|---|---|
|  | **5-3** | 11.51 | | **6-3** | 5.65 | | **7-3** | 51.71 | 95.07 |
|  | **5-33** | ND | | **6-33** | 11.88 | | **7-33** | 7.70 | |
|  | **5-34** | ND | | **6-34** | 18.27 | | **7-34** | 34.96 | |
|  | **5-35** | ND | | **6-35** | 19.62 | | **7-35** | 52.82 | 93.20 |
|  | **5-36** | ND | | **6-36** | 25.12 | | **7-36** | 10.77 | |
|  | **5-37** | ND | | **6-37** | 7.14 | | **7-37** | 49.82 | |
|  | **5-38** | ND | | **6-38** | 15.16 | | **7-38** | 42.59 | |
|  | **5-39** | ND | | **6-39** | 18.73 | | **7-39** | 66.19 | 47.90 |
|  | **5-40** | ND | | **6-40** | 13.08 | | **7-40** | 38.04 | |
|  | **5-41** | ND | | **6-41** | 18.44 | | **7-41** | 28.58 | |
|  | **5-42** | ND | | **6-42** | 20.77 | | **7-42** | 19.20 | |
|  | **5-43** | ND | | **6-43** | 21.05 | | **7-43** | 43.87 | |
|  | **5-44** | ND | | **6-44** | 5.68 | | **7-44** | 26.99 | |
|  | **5-45** | ND | | **6-45** | 9.35 | | **7-45** | 7.58 | |
| MK-0431 | | | | $IC_{50}$ = 65.42 nM | | | | | |

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

5-carbonyl)-1,2-diazepan-1-yl)-4-(2,4,5-trifluorophenyl)-butan-1-one hydrochloride (40 mg, 90%) as a solid.

$^1$H NMR (DMSO-d$_6$, 500 MHz) $\delta$ 8.23 (s, 1H), 8.14 (s, 1H), 7.57-7.51 (m, 2H), 7.13-7.10 (m, 1H), 7.04-7.03 (m, 1H), 6.98-6.77 (m, 1H), 6.12 (s, 2H), 4.00-3.90 (m, 1H), 3.88-3.86 (m, 1H), 3.75-3.72 (m, 2H), 3.17-3.07 (m, 1H), 3.03-2.88 (m, 3H), 2.81-2.73 (m, 1H), 1.78-1.47 (m, 6H).

**Determination of Inhibitory Activity against DPP-IV.** 10 $\mu$L of Caco-2 cell lysate was suspended in Tris-HCl (pH 7.5), and then 40 $\mu$M Ala-Pro-AFC (ICN Biomedicals, Inc) was added. After treatment of compounds, the mixture was incubated for 60 min at 24 °C. AFC as a indicator of DPP-IV activity was detected at 405/510 nm (Ex/Em) by Fluorometer, Synergy HT (Biotek). IC$_{50}$ was calculated by Prism 4.0 software (GarphPad Software, Inc).

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