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.

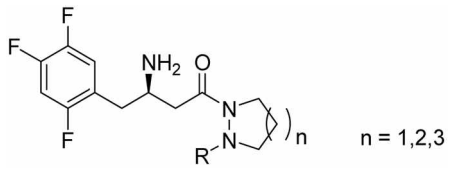
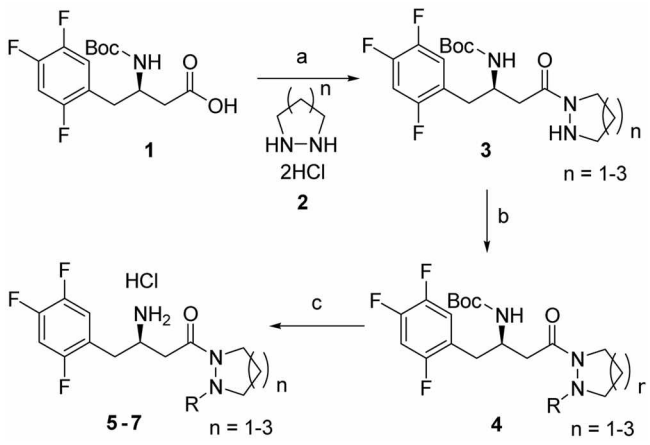


Figure 1. β-aminoacyl-containing cyclic hydrazide derivatives.

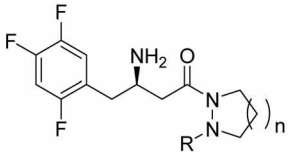
A series of β-aminoacyl-containing cyclic hydrazine derivatives was synthesized by using the route shown in Scheme 1. The detailed synthetic explanation was described in our previous publication.[8]

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

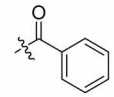
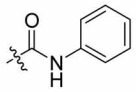
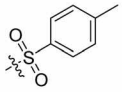
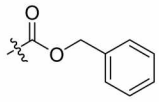
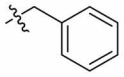


Scheme 1. Reagents and conditions: (a) compound 2, triethylamine, EDCI, CH_2Cl_2, room temperature; (b) electrophiles, CH_2Cl_2, triethylamine, room temperature; (c) HCl, dioxane, room temperature.

Table 1. Inhibitory activity of β-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 β-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

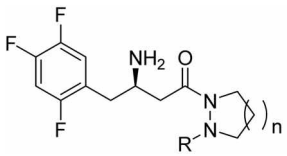
General. All reported yields are isolated yields after column chromatography or crystallization. ^{1}H-NMR spectra were obtained on FT-NMR Varian GEMINI-200FT or Bruker AVANCE-300 with TMS as internal reference. MS spectra were obtained on a Shimadzu QP5050 spectrograph.

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 μL, 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) δ 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

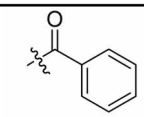
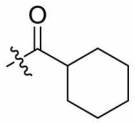
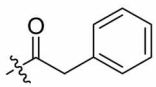
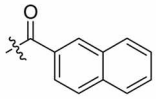
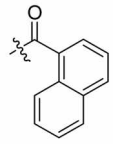
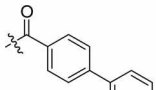
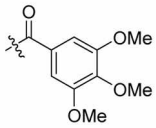
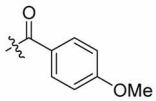
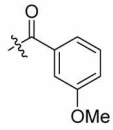
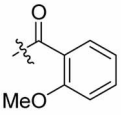
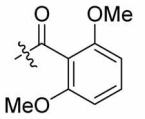
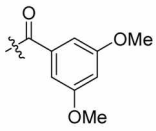
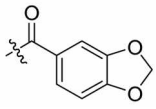
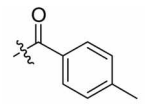
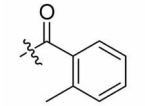
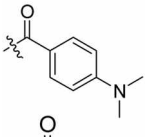
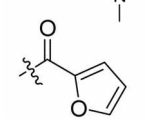
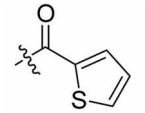
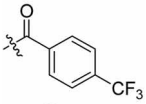
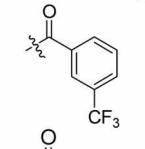
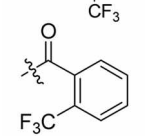
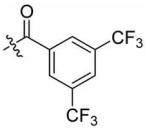
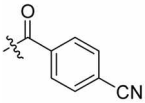
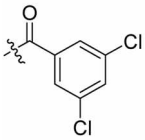
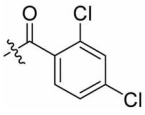
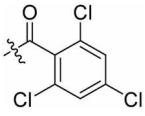
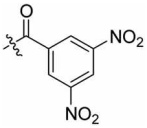
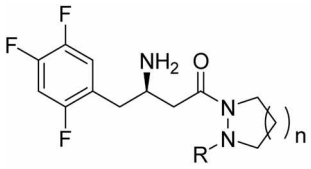
R	Compd (n = 1)	n = 1 % inh. at 100 nM	IC_{50}, nM[a]	Compd (n = 2)	n = 2 % inh. at 100 nM	IC_{50}, nM[a]	Compd (n = 3)	n = 3 % inh. at 100 nM	IC_{50}, nM[a]
	**5-2**	14.32		**6-2**	67.97	74.40	**7-2**	51.12	85.72
	**5-7**	ND		**6-7**	2.21		**7-7**	36.82	
	**5-8**	ND		**6-8**	2.11		**7-8**	14.75	
	**5-9**	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

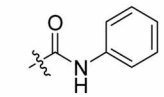
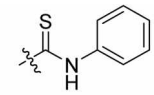
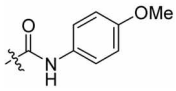
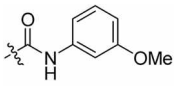
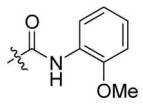
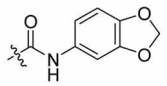
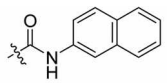
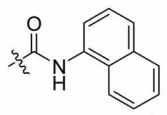
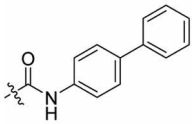
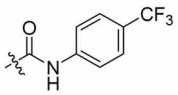
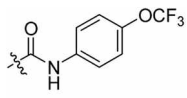
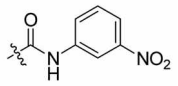
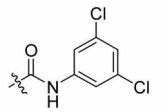
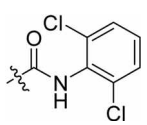
Table 2. Continued

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



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) δ 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 μL of Caco-2 cell lysate was suspended in Tris-HCl (pH 7.5), and then 40 μM 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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