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Synthesis and evaluation of pyrazolidine derivatives as dipeptidyl peptidase IV (DP-IV) inhibitors

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Abstract—A new series of pyrazolidine derivatives was synthesized and evaluated for their ability to inhibit dipeptidyl peptidase IV (DP-IV). Compound **9i** was the most active in this series, exhibited IC₅₀ value of 1.56 μ M and ED₅₀ value of 80 mg/kg (in vivo DP-IV inhibition; po).

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1. Introduction

The serine peptidase dipeptidyl peptidase IV (DP-IV) modulates the biological activity of several peptide hormones, chemokines and neuropeptides by specifically cleaving after a proline or alanine at amino acid position 2 from the N-terminus.¹ DP-IV cleaves and inactivates glucagon-like peptide 1 (GLP-1),² which is an important stimulator of insulin secretion.³ Inhibition of DP-IV increases the level of circulating GLP-1 and thus increases insulin secretion,⁴ which could ameliorate hyperglycemia in type 2 diabetes. Consequently, DP-IV inhibition has been proposed as a new treatment of type 2 diabetes. Small molecule inhibitors of DP-IV have been reported in the literatures and progressed into clinical trials with positive results.⁵

Recently, we have reported the synthesis and biological evaluation of a new series of cyano-pyrazoline derivatives with the secondary amine at P-2 position⁶ (Fig. 1).

This compound (Fig. 1) showed a good in vitro and in vivo DP-IV inhibitory activity, but it contained an elec-

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Figure 1.

trophilic nitrile. This prompted us to synthesize a new series of pyrazolidine derivatives lacking an electrophile.



The structural modification of cyano-pyrazoline to pyrazolidine would be an effective approach to increase compound stability. We now report the synthesis and biological evaluation of pyrazolidine derivatives as DP-IV inhibitors.

2. Chemistry

A series of pyrazolidine derivatives were synthesized according to the synthetic Schemes 1–3.

Keywords: Dipeptidyl peptidase IV; DP-IV; Pyrazolidine; Anti-diabetic agent.

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Di-*tert*-butyldihydrazodiformate (1) was treated with 1,3-dibromopropane in the presence of Et_4NBr in toluene under reflux, followed by deprotection of Boc using 4 M HCl to afford the pyrazolidine 2HCl (3) in 87% yield from 1 by Boros's procedure.⁷ Compound 3 was acylated by Boc–isoleucine and EDCI to produce key intermediate 4 (Boc–isoleucine–pyrazolidide) in 78% yield. Compound 4 was deprotected by HCl to afford the desired isoleucine–pyrazolidide (5).

Boc-isoleucine pyrazolidide (4) reacted with diverse electrophiles to form the corresponding N-substituted isoleucine pyrazolidides as shown in Scheme 2. Compound 4 was acylated by activated formyl, acetyl and benzoyl group, followed by deprotection using HCl to produce the desired 7a-c. Also, 4 was coupled with ethyl, benzyl and phenyl isocyanate to afford 9a-c. Introduction of tosyl group at NH of Boc–isoleucine– pyrazolidide (4) was achieved using TsCl as an electrophile to give the corresponding compound (11).

In order to cover possible substituents at NH of isoleucine-pyrazolidide (4), benzyloxycarbonyl and benzyl group were also prepared as shown in Scheme 3. Compound 12^7 was deprotected by HCl to produce 13, followed by acylation using N-Boc-isoleucine and EDCI, deprotection of NHBoc, and then converted to the final compound 15. Hydrogenation of 12 with Pd/C under H₂ produced the monosubstituted pyrazolidine (16), which upon treatment with benzyl bromide



Scheme 1. Reagents and conditions: (a) 50% NaOH, dibromopropane, Et₄NBr, toluene, reflux, 5 h, 87%; (b) 4 M HCl, dioxane, 100%; (c) Boc-isoleucine, EDCI, TEA, CH₂Cl₂, 78%; (d) 4 M HCl, dioxane–EtOAc, rt, 12 h, 87%.



Scheme 2. Reagents and conditions: (a) HCOOH, EDCI, CH₂Cl₂, 0 °C-rt, 45%; Ac₂O, TEA, CH₂Cl₂, 90%; BzCl, TEA, CH₂Cl₂, 67%; (b) 4 M HCl, dioxane–EtOAc, rt, 12 h, 70–95%; (c) RNCO, CH₂Cl₂; rt, 12 h, 70–80%; (d) TsCl, Py, CH₂Cl₂, rt, 15 h, 33%.



Scheme 3. Reagents and conditions: (a) 4 M HCl, dioxane, rt, 80%; (b) Boc–isoleucine, EDCI, CH_2Cl_2 , rt, 40%; (c) 4 M HCl, dioxane–EtOAc, rt, 93%; (d) Pd/C, H₂, MeOH, rt, 93%; (e) benzyl bromide, K₂CO₃, acetone, reflux, 52%; (f) trifluoroacetic acid, CH_2Cl_2 , 77%; (g) Boc–isoleucine, TEA, EDCI, CH_2Cl_2 , rt, 30%; (h) 4 M HCl, dioxane–EtOAc, rt, 92%.

afforded **17**. Compound **17** was deprotected, acylated and deprotected to give the benzylated isoleucine–pyr-azolidide (**20**).

3. Results

DP-IV enzyme assay was carried out using rat plasma by measuring 7-amino-4-trifluoromethylcoumarin (AFC) liberated from Ala-Pro-AFC in the presence or absence of a test compound.^{9,10} Rat plasma preparation (20 μ L) was incubated with Ala-Pro-AFC (40 μ M) at room temperature, pH 7.8 for 1 h in the presence or absence of test compounds (20 μ M). Test compounds were dissolved in DMSO. DMSO concentration in the assay mixture was 5%, which did not affect enzyme activity. After 1 h incubation, the fluorescence of AFC released by the reaction was measured at 360 nm (excitation wavelength) and at 485 nm (emission wavelength). **P32/98** was used as a reference compound.⁸

Various isoleucine-pyrazolidide derivatives were evaluated the biological activities as shown in Tables 1 and 2. N-Boc-isoleucine-pyrazolidide (4) and isoleucinepyrazolidide (5) were inactive. Also, amide substituents such as formyl (7a), acetyl (7b) and benzoyl (7c) showed no inhibitory activity. Ethyl (9a) and benzyl urea (9b) were inactive, but phenyl substituent (9c) was modestly potent with an IC₅₀ of 15 μ M. So, we further modified this phenyl urea structure by adoption of diverse substituents as shown in Table 2.

Meanwhile, tosyl (11) derivative resulted in loss of inhibitory activity. Carbamate compound (15) was also moderately active with IC₅₀ of 23.3 μ M. Benzyl group (20) was not active.

Table 1. Inhibitory activities of pyrazolidine derivatives against DP-IV



 a IC_{50} values were determined by curve analysis software (GraphPad Prism). b Lit. 2.8 $\mu M.$

Table 2. Inhibitory activities of urea derivatives against DP-IV



	R	
Compd	R	IC ₅₀ , ^a μM
9d	4-CF ₃	25.0
9e	2-CF ₃	na
9f	4-CN	9.20
9g	3-CN	4.04
9h	4-NO ₂	7.06
9i	3-NO ₂	1.56
9j	2-NO ₂	na
9k	3-Cl-4-NO ₂	4.71
91	3-F-4-NO ₂	2.27
9m	4-CO ₂ Et	3.90
9n	3-CO ₂ Et	8.80
90	2-CO ₂ Et	na
9р	3,4-Dichloro	5.60
P32/98		2.7

 a IC₅₀ values were determined by curve analysis software (GraphPad Prism).

In Table 2, the effects of the substituents (R) on phenyl urea group were investigated. *para* or *ortho* CF₃-phenyl substituents (9d and 9e) were less active than phenyl (9b) or not active. CN-phenyl groups (9f and 9g) showed better in vitro activities than 9c, particularly, CN-3-phenyl (9g) exhibited IC₅₀ value of 4.04 μ M. Phenyl derivatives having NO₂ (9h,i) except for NO₂ at *ortho* position (9j) were found to be more potent. Compound 9i having NO₂ at *meta* position was the most active in this series with an IC₅₀ value of 1.56 μ M, and more potent than reference P32/98 in vitro.

From these results, we turned our attention to replacing the amino acid group of compound **9i** with other various amino acid as shown in Table 3. Although valine and

Table 3. Inhibitory activities of amino acid derivatives against DP-IV

	Amino acid N O = NH $O_2N = $	
Compd	Amino acid	IC ₅₀ , ^a μM
9i	Isoleucine	1.56
21	Valine	2.1
22	Leucine	20.7
23	Proline	na
24	Cyclohexylglycine	4.6
P32/98		2.7

 $^{\rm a}$ IC_{50} values were determined by curve analysis software (GraphPad Prism).

Table 4. DP-IV inhibition of selected compound in vitro and in vivo



 a ED₅₀ values were determined by curve analysis software (GraphPad Prism).

 $^{b}n = 6.$

cyclohexylglycine derivatives (21 and 24) exhibited good in vitro activities, isoleucine 9i was still the most active in vitro.

As a proof of concept, these compounds were evaluated in vivo for their ability to reduce DP-IV activity in normal C57BL/6J mice. As shown in Table 4, compound 9i, which was the most active in vitro showed in vivo efficacy with an ED_{50} value of 80 mg/kg, (po at 1 h postdose).

In conclusion, a new series of pyrazolidine derivatives was synthesized and evaluated for their ability to inhibit dipeptidyl peptidase IV (DP-IV). Compound **9i** was the most active in this series, showed a potent inhibitory activity and in vivo efficacy. Further studies aimed at improving efficacy are in progress and will be reported in due course.

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