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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 IC_{50} value of 1.56 μM and ED_{50} value of 80 mg/kg (in vivo DP-IV inhibition; po).

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 position[6] (Fig. 1).

This compound (Fig. 1) showed a good in vitro and in vivo DP-IV inhibitory activity, but it contained an electrophilic nitrile. This prompted us to synthesize a new series of pyrazolidine derivatives lacking an electrophile.

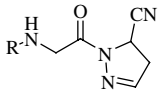
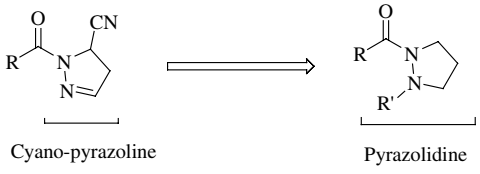


Figure 1.



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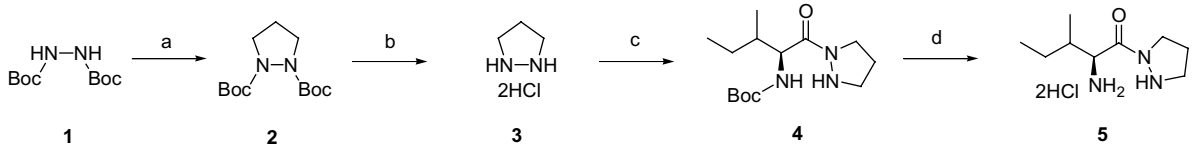
Di-*tert*-butyldihydrazodiformate (**1**) was treated with 1,3-dibromopropane in the presence of Et_4NBr in toluene under reflux, followed by deprotection of Boc using 4 M HCl to afford the pyrazolidine 2HCl (**3**) in 87% yield from **1** by Boros's procedure.[7] Compound **3** was acylated by Boc–isoleucine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

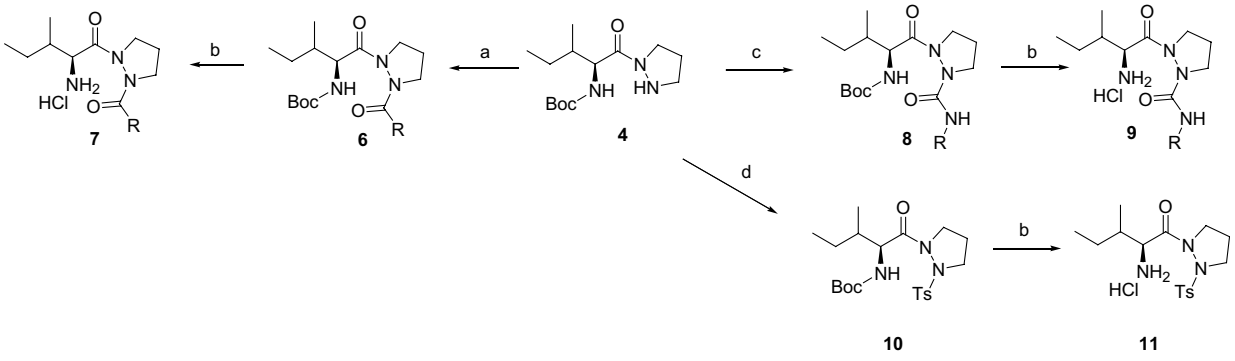
Boc–isoleucine pyrazolidide (**4**) reacted with diverse electrophiles to form the corresponding N-substituted isoleucine pyrazolidides as shown in Scheme 2. Compound **4** was acylated by activated formyl, acetyl and benzoyl group, followed by deprotection using HCl to produce the desired **7a**–**c**. 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 H_2 produced the monosubstituted pyrazolidine (**16**), which upon treatment with benzyl bromide



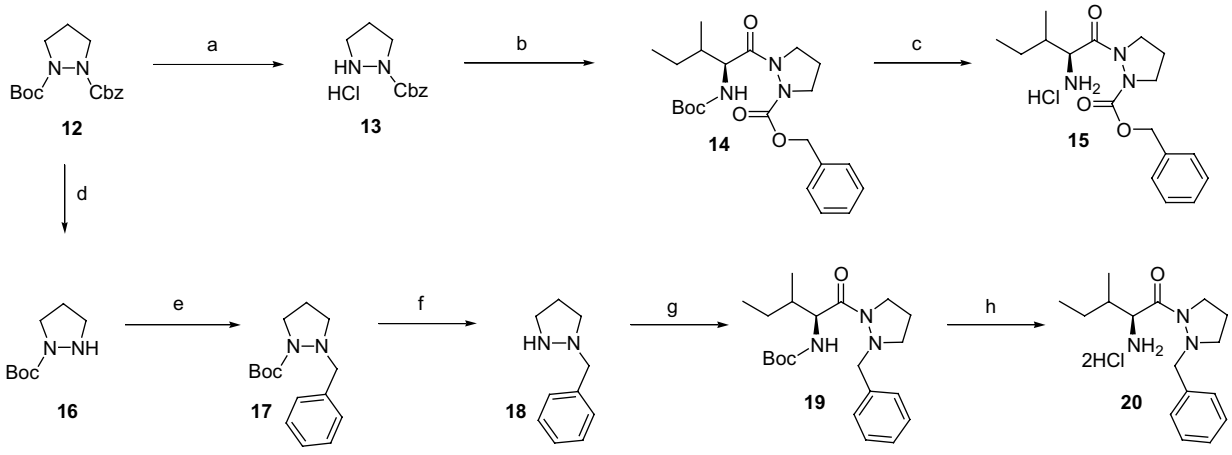
1 2 3 4 5

Scheme 1. Reagents and conditions: (a) 50% NaOH, dibromopropane, Et_4NBr, toluene, reflux, 5 h, 87%; (b) 4 M HCl, dioxane, 100%; (c) Boc–isoleucine, EDCI, TEA, CH_2Cl_2, 78%; (d) 4 M HCl, dioxane–EtOAc, rt, 12 h, 87%.



7 6 4 8 9 10 11

Scheme 2. Reagents and conditions: (a) HCOOH, EDCI, CH_2Cl_2, 0 °C–rt, 45%; Ac_2O, TEA, CH_2Cl_2, 90%; BzCl, TEA, CH_2Cl_2, 67%; (b) 4 M HCl, dioxane–EtOAc, rt, 12 h, 70–95%; (c) RNCO, CH_2Cl_2; rt, 12 h, 70–80%; (d) TsCl, Py, CH_2Cl_2, rt, 15 h, 33%.



12 13 14 15 16 17 18 19 20

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, H_2, MeOH, rt, 93%; (e) benzyl bromide, K_2CO_3, 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–pyrazolidide (**20**).

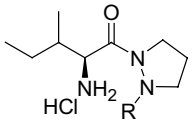
3. Results

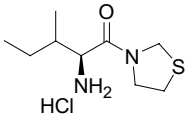
DP-IV enzyme assay was carried out using rat plasma by measuring 7-amino-4-trifluoromethylcoumarin (AFC) liberated from Ala-Pro-AFC in the presence or absence of a test compound.[9,10] Rat plasma preparation (20 μL) was incubated with Ala-Pro-AFC (40 μM) at room temperature, pH 7.8 for 1 h in the presence or absence of test compounds (20 μM). 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 isoleucine–pyrazolidide (**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 IC_{50} of 15 μM. 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 IC_{50} of 23.3 μM. Benzyl group (**20**) was not active.

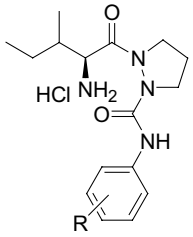
Table 1. Inhibitory activities of pyrazolidine derivatives against DP-IV



Compd	R	IC_{50},[a] μM
5	H	na
7a	CHO	na
7b	CH_3CO	na
7c	C_6H_5CO	na
9a	EtNHCO	na
9b	$C_6H_5CH_2NHCO$–	na
9c	C_6H_5NHCO	15.0
11	Tosyl	na
15	$C_6H_5CH_2OCO$	23.3
20	$C_6H_5CH_2$	na
P32/98		2.7[b]

[a] IC_{50} values were determined by curve analysis software (GraphPad Prism).
[b] Lit. 2.8 μM.

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



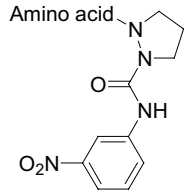
Compd	R	IC_{50},[a] μM
9d	4-CF_3	25.0
9e	2-CF_3	na
9f	4-CN	9.20
9g	3-CN	4.04
9h	4-NO_2	7.06
9i	3-NO_2	1.56
9j	2-NO_2	na
9k	3-Cl-4-NO_2	4.71
9l	3-F-4-NO_2	2.27
9m	4-CO_2Et	3.90
9n	3-CO_2Et	8.80
9o	2-CO_2Et	na
9p	3,4-Dichloro	5.60
P32/98		2.7

[a] IC_{50} 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* CF_3–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 IC_{50} value of 4.04 μM. Phenyl derivatives having NO_2 (**9h**,**i**) except for NO_2 at *ortho* position (**9j**) were found to be more potent. Compound **9i** having NO_2 at *meta* position was the most active in this series with an IC_{50} value of 1.56 μM, 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

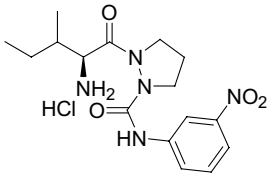
Table 3. Inhibitory activities of amino acid derivatives against DP-IV



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

[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

Compd	Structure	IC_{50}, μM (rat plasma)	ED_{50} (po, 1 h)[a,b]
9i		1.56	80 mg/kg

[a] ED_{50} 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.

Acknowledgements

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