Pyrazolidine Derivatives with Heteroaryl Urea as Dipeptidyl Peptidase IV Inhibitors

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In the continuation of efforts to modify the structure of our novel DP-IV inhibitors, a series of pyrazolidine derivatives with heteroaryl urea was synthesized and evaluated for their ability to inhibit dipeptidyl peptidase IV (DP-IV).

Key words dipeptidyl peptidase IV (DP-IV); pyrazolidine; anti-diabetic agent

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—4)] which is an important stimulator of insulin secretion.[5)] Inhibition of DP-IV increases the level of circulating GLP-1 and thus increases insulin secretion,[6,7)] which could ameliorate hyperglycemia in type 2 diabetes. Consequently, DP-IV inhibition has been proposed as a new treatment of type 2 diabetes. On the basis of animal studies and initial clinical trials, it appears that DP-IV inhibitors are capable of significantly lowering glucose concentration, as well as reducing HbA_{1c} levels, with good tolerability and minimal risk of hypoglycemia. Small molecule inhibitors of DP-IV have been reported in the literatures and progressed into clinical trials with positive results.[8—14)]

In a previous paper,[15,16)] we have described the synthesis and biological evaluation of a new series of pyrazolidine derivatives (Fig. 1). Among them, compound **A** was the most active *in vitro*, exhibited IC_{50} value of 1.56 μM (K_i=850 nM). However this compound (**A**) has a labile nitrophenyl moiety, which could result in an undesirable pharmacokinetic profile.

So, the structural modification of nitrophenyl group to heteroaryl group would be an effective approach to find out drug like compound.

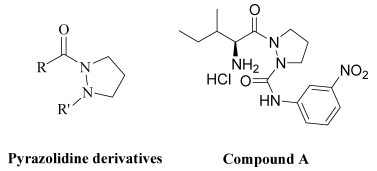
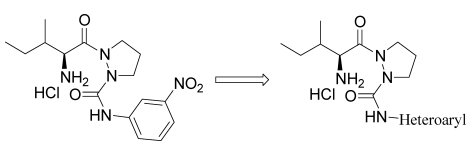


Fig. 1

As a part of our ongoing program on the synthesis and evaluation of novel DP-IV inhibitors, present report focuses on optimization of urea part of our pyrazolidine derivatives with diverse heteroaryl compounds.



Chemistry

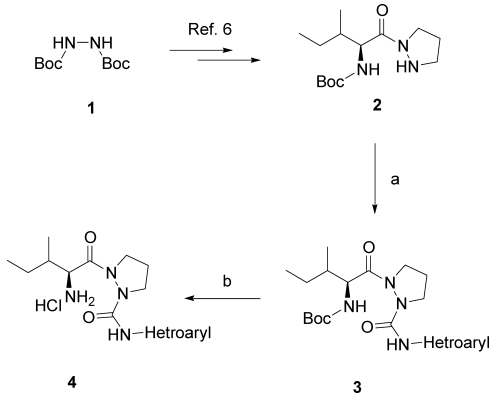
Compounds selected for biological evaluation were prepared as described in Chart 1.

Compound **2**, as a key intermediate, was prepared by our previous paper.[15,16)] Compound **2** reacted with several heteroaryl amines in the presence of phosgene at room temperature to afford heteroaryl urea derivatives (**3**), followed by deprotection using 4 M HCl in dioxane to produce the desired pyrazolidine derivatives with heteroaryl urea (**4**).

Results and Discussion

DP-IV enzyme assay was carried out using rat plasma by measuring 7-amino-4-trifuoromethylcoumarin (AFC) liberated from Ala-Pro-AFC in the presence or absence of a test compound.[17,18)] P32/98 was used as a reference compound.[18)]

First, various pyrazolidine derivatives with 6-membered heteroaryl urea were evaluated the biological activities as shown in Table 1. 4-Pyridyl (**4a**) and 3-pyridyl (**4b**) substituents were inactive. Pyridin-3-ylmethyl derivative (**4c**) also showed no inhibitory activity. However, 2-pyridyl (**4d**) substituent was modestly potent with an IC_{50} of 14.3 μM. This suggests that amine at 2-position of heteroaryl moiety played a role in the DP-IV inhibitory activity. So, we further modified the heteroaryl moiety with amine at 2-position.



(a) Heteroarylamine, phosgene in toluene, CH_2Cl_2, room temperature, 3—12 h; (b) 4 M HCl, dioxane–EtOAc, room temperature, 12 h.

Chart 1

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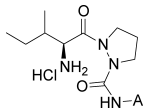
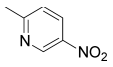
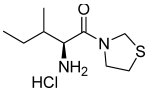
Table 1. Inhibitory Activities of Pyrazolidine Derivatives with 6-Membered Heteroaryl Substituents against DP-IV



Compound	A	IC_{50}, μM[a]
4a		(7.5%)[b]
4b		(15.4%)
4c		(0.9%)
4d		14.3
4e		6.2
4f		7.3
4g		(−13.5%)
4h		4.1%
4i		39.2%
4j		−1.6%
4k		4.7
4l		2.3
4m		10.1
P32/98		2.7[c]

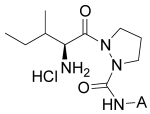
a) IC_{50} values were determined by curve analysis software (GraphPad Prism). *b*) % inhibition at 20 μM is shown in parenthesis. *c*) Lit. 2.8 μM[19]

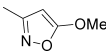
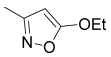
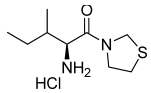
5-Substituted-2-pyridyl derivatives (**4e**, **4f**) showed more potent activities (6.2 and 7.3 μM, respectively), whereas the 3-substituted-2-pyridyl groups (**4g**) resulted in loss of inhibitory activity. Pyrimidines (**4h**, **4i**) and isoquinoline (**4j**) derivatives were not active. Whereas, pyrazine derivatives (**4k**—**m**) exhibited enhanced potency, **4l** (IC_{50}=2.3 μM) was nearly 7 times more potent against DP-IV than the 2-pyridyl (**4d**) substituent (IC_{50}=14.3 μM).

To find out more potent compounds, we turned our attention on replacing urea group with 5-membered heteroaryl derivatives as shown in Table 2. Thiazole substituent (**4n**) showed weak *in vitro* activity (IC_{50}=20.3 μM). Whereas, isooxazole derivatives (**4o**—**s**) exhibited enhanced potency. Among them, **4p** is the most active in this series with an IC_{50} value of 2.2 μM, and more potent than reference P32/98 *in vitro*.

We have evaluated the ability to reduce DP-IV activity *in vivo* in normal C57BL/6J mouse. Compound **4p** showed 2-fold enhanced *in vivo* DP-IV inhibition (ED_{50}=42 mg/kg, s.c.) compared with the previous compound **A** (ED_{50}= 80 mg/kg).

Table 2. Inhibitory Activities of Pyrazolidine Derivatives with 5-Membered Heteroaryl Substituents against DP-IV



Compound	A	IC_{50}, μM[a]
4n		20.3
4o		7.7
4p		2.2
4q		9.2
4r		12
4s		14.4
P32/98		2.7[b]

a) IC_{50} values were determined by curve analysis software (GraphPad Prism). *b*) Lit. 2.8 μM.[19]

Further biological data including parmacokinetic profile, kinetics, selectivity, and *in vivo* efficacy are described in the following paper.

Conclusion

As continued effort on modifing the structure of our DP-IV inhibitor, a series of pyrazolidine derivatives with heteroaryl urea was synthesized and evaluated for their ability to inhibit dipeptidyl peptidase IV (DP-IV). Compound **4p** was the most active in this series, showed a potent inhibitory activity and *in vivo* efficacy.

Experimental

General NMR spectra were recorded on FT-NMR Varian GEMINI (200 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra were recorded on a Shimadzu QD5050 spectrograph.

General Procedure for Synthesis of Compound 3 To a mixture of **2**, amine (1 eq), and pyridine (3 eq) in CH_2Cl_2 was added phosgene (1.5 eq) and stirred at room temperature for 3—24 h. The reaction mixture was diluted with brine, extracted with ethyl acetate. The organic layer was dried with $MgSO_4$, concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give the desired compound. (**3p**) ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 10.17 (s, 1H), 8.99 (s, 1H), 6.61 (s, 1H), 5.01—4.95 (m, 1H), 4.30—4.23 (m, 3H), 3.40—3.30 (m, 2H), 2.26 (s, 3H), 2.00—1.90 (m, 2H), 1.60—1.50 (m, 2H), 1.33—1.07 (m, 10H), 0.85—0.75 (m, 6H). MS (relative intensity) 409 (M^+, 5).

General Procedure for Synthesis of Compound 4 To a solution of compound **3** in ethyl acetate was 4 M HCl in dioxane (10 eq) and stirred at ambient temperature for 3—12 h. The reaction mixture concentrated *in vacuo* and crystallized from ether/hexane to give the final compound. (**4p**) ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 10.4 (s, 1H), 8.18—8.08 (m, 3H), 6.53 (s, 1H), 3.99—3.83 (m, 3H), 3.25—3.05 (m, 2H), 2.20—2.00 (m, 4H), 1.92—1.82 (m, 2H), 1.40—1.30 (m, 1H), 0.94—0.85 (m, 6H).

Determination of Inhibitory Activity against DP-IV DP-IV enzyme

assay was carried out using rat plasma by measuring 7-amino-4-trifuoromethylcoumarin (AFC) liberated from Ala-Pro-AFC in the presence or absence of a test compound.[17,18] 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%. 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).

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