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Synthesis and DP-IV inhibition of cyano-pyrazoline derivatives as potent anti-diabetic agents

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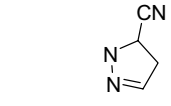
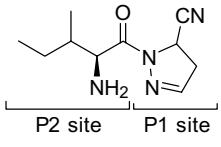
Abstract—A new series of cyano-pyrazoline derivatives with a secondary amine at P-2 site was synthesized through achiral and chiral synthetic methods and evaluated for their ability to inhibit dipeptidyl peptidase IV (DP-IV). Compound **5i** revealed good in vivo efficacy (ED_{50}: 4.1 mg/kg; in vivo DP-IV inhibition). Also chiral derivative (**11b**) having (*S*)-configuration of compound **5i** was found to be more potent.

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]



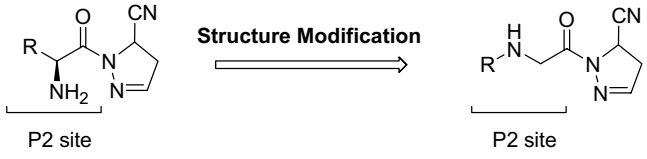
Cyano-pyrazoline skeleton



1a
WO02083128

In the course of the search for DP-IV inhibitor through high throughput screening (HTS) using chemical library of Korea Chemical Bank, cyano-pyrazoline skeleton was discovered as a hit toward DP-IV inhibitor.

Recently, compound **1a** with cyano-pyrazoline moiety was reported by WO02083128[6] as a DP-IV inhibitor. Since under neutral and basic aqueous conditions the P-2 site primary amine of the compound **1a** can nucleophilically attack the carbon of the nitrile to form an inactive cyclic amidine,[7] the modification of the amino acid part of the compound **1a** would be an effective approach to increase in vivo stability.



We now report the synthesis and biological evaluation of cyano-pyrazoline derivatives with the secondary amine at P-2 site as DP-IV inhibitors.

2. Chemistry

The general pathway outlined in Scheme 1 yielded the desired compounds. Acrylonitrile (**2**) was treated with trimethylsilyldiazomethane in toluene–hexane at room

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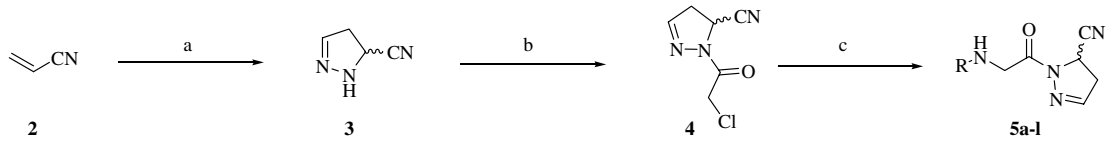
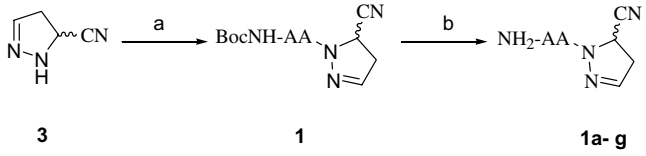


Scheme 1. Reagents and conditions: (a) (i) Me_3SiCHN_2, toluene–hexane, rt; (ii) trifluoroacetic acid, CH_2Cl_2; (b) chloroacetyl chloride, CH_2Cl_2, 0 °C; (c) RNH_2, K_2CO_3, KI, THF, rt.

temperature, followed by desilyation using trifluoroacetic acid to afford the cyano-pyrazoline (**3**), which was acylated by chloroacetyl chloride to afford **4** in 45% yield from **2**. Nucleophilic substitution of compound **4** by diverse primary amines was performed in the presence of K_2CO_3, and KI in THF to produce the desired secondary amine-substituted cyano-pyrazoline derivatives in 40–90% (**5a**–**l**).

Also, chiral cyano-pyrazoline derivatives were synthesized as shown in Scheme 2. Chiral 2,10-camphorsultam derived dipolarphile (**6**) was reacted with trimethylsilyl diazomethane to form the pyrazoline (**7**) by Carreira's procedure,[8] which showed 9:1 diastereoselectivity and was readily separated by chromatography on silica gel. Aminolysis of the sultam moiety produced a chiral 3,4-dihydro-2*H*-pyrazole-3-carboxylic acid amide (**8**) in 60% yield, followed by acylation using chloroacetyl chloride, and dehydration with $POCl_3$ to afford the chiral precursor (**10**) in 56% yield from **8**. Coupling of **10** with primary amines furnished chiral cyano-pyrazoline derivatives (**11b**) in 70% yield.

Compound **1a** and its derivatives were also prepared for comparison to our compounds as shown in Scheme 3. Compound **3** reacted with *N*-Boc-protected amino acid activated ester such as *p*-nitrophenyl or *N*-hydroxy succinimide to give the coupling product (**12**) in 10–30% yields, which was deprotected by trifluoroacetic acid to afford cyano-pyrazoline derivatives with primary amine at P-2 position (**1a**–**g**).

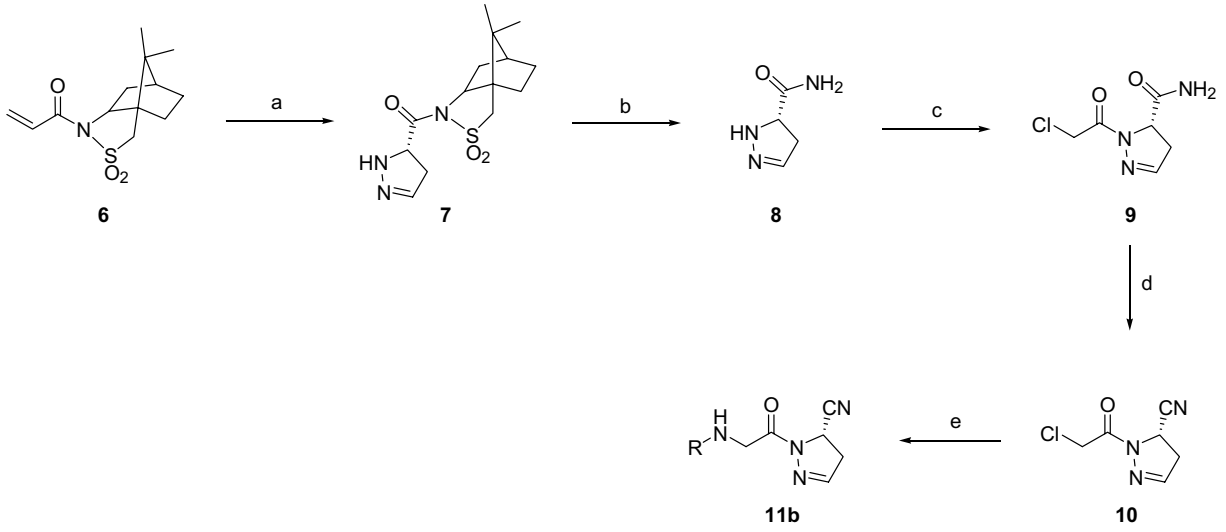


Scheme 3. Reagents and conditions: (a) *N*-*t*-Boc-L-AA *p*-nitrophenyl ester, TEA, CH_2Cl_2, rt or reflux; (b) TFA, CH_2Cl_2, rt.

3. Results

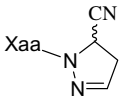
DP-IV enzyme assay was carried out using rat plasma, caco-2, and porcine kidney by measuring 7-amino-4-trifluoromethylcoumarin (AFC) liberated from Ala-Pro-AFC in the presence or absence of a test compound.[9,10] Rat plasma preparation (20 μL) was incubated with Ala-Pro-AFC (40 μM) at room temperature, 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). NVP-728 was used as a reference compound.[7]

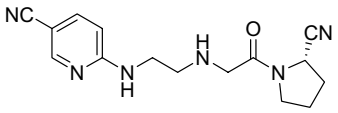
Because compound **1a** reported by WO02083128 did not show any biological activity, compound **1a** and its derivatives (**1b**–**g**) were synthesized and evaluated biological activities in our hand as shown in Table 1. Iso-



Scheme 2. Reagents and conditions: (a) (i) Me_3SiCHN_2, toluene–hexane, rt; (ii) trifluoroacetic acid, CH_2Cl_2; (b) ammonia 2 M methanol solution; (c) chloroacetyl chloride, CH_2Cl_2, 0 °C; (d) $POCl_3$, pyridine, rt; (e) RNH_2, K_2CO_3, KI, THF, rt.

Table 1. Inhibitory activity of cyano-pyrazoline derivatives with primary amine at P-2 position against DP-IV



Compound	Xaa	IC_{50}, μM[a] (rat plasma)	IC_{50}, μM
1a	Isoleucine	0.22	0.13 (caco-2) 0.08 (Porcine)
1b	Alanine	1.2	
1c	Valine	1.9	
1d	Leucine	4.3	
1e	*tert*-Butylglycine	2.1	
1f	Cbz-lycine	0.66	
1g	Cyclohexylglycine	2.3	
NVP-728		0.06[b,7]	

[a] IC_{50} values were determined by curve analysis software (GRAPHPAD PRISM).
[b] Lit. 0.022 μM.

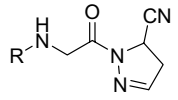
leucine derivative (**1a**) was the best compound of this series in vitro with an IC_{50} value of 0.22, 0.13, and 0.08 μM (rat plasma, caco-2, and porcine kidney, respectively). Alanine and valine analogues (**1b** and **1c**) exhibited IC_{50} values in the range of 1.2–1.9 μM. Other compounds (**1d**–**g**) showed micro or submicromolar inhibitory activities (0.66–4.3 μM).

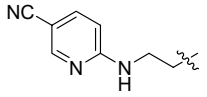
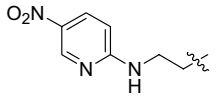
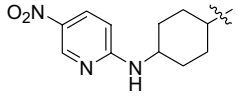
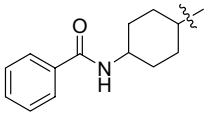
For finding out the candidate of showing good in vivo stability and efficacy, we have prepared and evaluated a new series of cyano-pyrazoline derivatives with secondary amine at P-2. In vitro activities were summarized in Table 2. A *t*-butyl substituent exhibited a weak inhibitory activity against DP-IV (**5a**). Five- to seven-membered cyclic moieties resulted in better than *t*-butyl group with the range of 1.3–2.6 μM (**5b**–**d**). Bi- or tricyclic-substituents showed similar activities to cyclic ones (**5e**–**h**). Heteroarylamino ethyl groups were found to be more potent than alkyl groups (**5i**–**k**). Compound **5i** having the same P2 structure with NVP-728 was the most active in this series with an IC_{50} value of 0.8, 0.41, and 0.96 μM in rat plasma, caco-2, and porcine kidney, respectively.

As a proof of concept, the compounds were evaluated in vivo for their ability to reduce DP-IV activity in normal C57BL/6J mouse as shown in Table 3. Although 2-(2-amino-3-methylpentanoyl)-3,4-dihydro-2*H*-pyrazole-3-carbonitrile (**1a**) showed most active in vitro inhibitory activity, 6-{2-[2-(5-cyano-4,5-dihydropyrazol-1-yl)-2-oxoethylamino]ethylamino}nicotinonitrile (**5i**) was found to be more potent in vivo (4-fold better, ED_{50}: 4.1 mg/kg), thus these data suggest that the cyano-pyrazoline derivative with secondary amine has better in vivo efficacy than that of primary amine at P-2 position.

From this interesting result, we turned our attention to obtaining optically pure 6-{2-[2-(5-cyano-4,5-dihydropyrazol-1-yl)-2-oxoethylamino]ethylamino}nicotinonitrile (**11a** and **11b**). Both chiral isomers (*R* and *S*) of 6-{2-[2-(5-cyano-4,5-dihydropyrazol-1-yl)-2-oxoethylamino]ethylamino}nicotinonitrile (**11a** and **11b**) synthesized using a chiral auxiliary evaluated the biological data as shown in Table 4. The (*R*) configuration com-

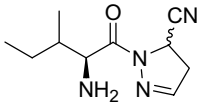
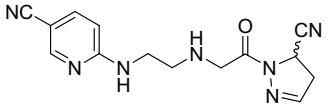
Table 2. Inhibitory activity of cyano-pyrazoline derivatives with secondary amine at P-2 position against DP-IV



Compound	R	IC_{50}, μM[a] (rat plasma)	IC_{50}, μM
5a	*tert*-Butyl	24.4	
5b	Cyclopentyl	1.3	
5c	Cyclohexyl	2.8	
5d	Cyclooctyl	2.6	
5e	Bicyclo[2.2.1]hept-2-yl	2.1	
5f	Adamantan-1-yl	1.5	
5g	Adamantan-2-yl	3.2	
5h	3-Hydroxyadamantan-1-yl	6.4	
5i		0.8	0.41 (caco-2) 0.96 (Porcine)
5j		1.2	
5k		1.2	
5l		4.4	
	NVP-728	0.06[7]	

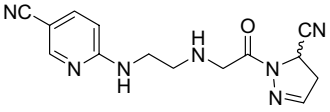
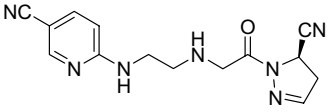
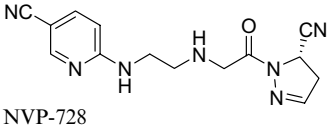
[a] IC_{50} values were determined by curve analysis software (GRAPHPAD PRISM).

Table 3. DP-IV inhibition of selected compounds in vitro and in vivo

Compound		IC_{50}, μM (rat plasma)	ED_{50} (sc, 1 h)[a,b]
1a		0.22	16.1 mg/kg
5i		0.8	4.1 mg/kg

[a] ED_{50} values were determined by curve analysis software (GRAPHPAD PRISM).
[b] $n = 6$.

Table 4. Inhibitory activity of chiral cyano-pyrazoline derivatives against DP-IV

Compound		IC_{50}, μM[a] (rat plasma)	IC_{50}, μM
5i		0.8	0.41 (caco-2) 0.96 (Porcine)
11a[b]		5.20	2.62 (caco-2) 6.82 (Porcine)
11b[c]		0.36	0.20 (caco-2) 0.44 (Porcine)
	NVP-728	0.06[7]	

[a] IC_{50} values were determined by curve analysis software (GRAPHPAD PRISM).
[b] 95% Ee determined by HPLC with chiral column (Chiralcel OD).
[c] 94% Ee.

pound (**11a**) had a diminished inhibitory activity in comparison to the racemic compound (**5i**). (*S*)-6-{2-[2-(5-Cyano-4,5-dihydropyrazol-1-yl)-2-oxoethylamino]-ethylamino}nicotinonitrile (**11b**) exhibited 2-fold more potent with an IC_{50} value of 0.36, 0.20, and 0.44 μM (rat plasma, caco-2, and porcine kidney, respectively), also inhibited DP-IV activity in *ob*/*ob* mouse (30 min; 87%, 60 min; 84% inhibition, postdose, 50 mg/kg dose, $n = 7$). Further investigation of the pharmacological profile of this isomer is in progress.

In conclusion, a new series of cyano-pyrazoline derivatives with secondary amine at P-2 position was synthesized through achiral and chiral synthetic methods and evaluated for their ability to inhibit dipeptidyl peptidase IV (DP-IV). *N*-Alkyl derivative at P-2 position showed moderate in vitro activities. Heteroaryls (**5i**–**k**) exhibited better biological activities. Among them, compound **5i** revealed good in vivo efficacy (ED_{50}: 4.1 mg/kg: in vivo DP-IV inhibition). Also chiral compound (**11b**) having (*S*)-configuration of compound **5i** was found to be more potent.

Acknowledgements

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