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Novel N-substituted 4-hydrazino piperidine derivative as a dipeptidyl peptidase IV inhibitor

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ABSTRACT

A novel class of N-substituted 4-hydrazino piperidine derivatives were designed, synthesized and evaluated for DPP IV inhibition. The SAR studies on the N-substituted piperidine led to the discovery of compound **22e** as a potent DPP IV inhibitor (IC_{50} 88 nM), which is highly selective over other peptidases. In vivo efficacy indicates that compound **22e** stimulates insulin release in response to glucose load and improves glucose tolerance in n5-STZ and Zucker Diabetic Fatty (ZDF) rats.

Type-2 diabetes mellitus (T2DM) is characterized by variable degrees of insulin resistance and pancreatic beta-cell dysfunction, which leads to hyperglycemia.[1] With the progressive nature of T2DM, development of drugs that retard or prevent the transition from the pre-diabetic state of impaired glucose tolerance to overt diabetes have therapeutic potential in attenuating the rapid rise in the disease prevalence. Furthermore, with the currently existing forms of therapy, insulin secretory capacity is ultimately lost in later stages of diabetes.

Glucagon-like peptide-1 (GLP-1) based therapy is a new paradigm in the management of T2DM. GLP-1 was shown to retain insulinotropic action without risk of hypoglycemia when given to type-2 diabetic patient.[2] GLP-1, produced by L-cell in distal small bowel, stimulates glucose dependent insulin secretion. However, its effects are short-lived as a result of rapid inactivation by Dipeptidyl Peptidase IV (DPP IV). Thus to prolong the beneficial effects of GLP-1, research has been focused on inhibition of DPP IV enzyme.

DPP IV is an abundantly distributed serine protease located on endothelial cells of the blood vessels throughout the body and circulates as soluble enzyme. It cleaves peptides containing proline or alanine at penultimate position from the amino termini of substrate proteins. There are several other DPPs like DPP II, DPP VI, DPP VII, DPP VIII, and DPP IX, hence the high selectivity of inhibitor would be an advantage to eliminate side effects associated with other DPPs.[3]

For the treatment of T2DM, several DPP IV inhibitors are progressing through preclinical and clinical trials.[4] Some of the selected reversible DPP IV inhibitors have been reported.[5] The crystal structure of DPP IV enzyme is known in the literature.[6] These information can be effectively utilized to search for new classes of DPP IV inhibitors. We have employed these structural informations to design and synthesize a series of novel substituted 4-hydrazino piperidine derivatives which were evaluated for DPP IV inhibitory activity. Herein, we report the results of our study in relation to the synthesis, in vitro DPP IV inhibitory activity, and SAR of new piperidine analogs. The selectivity and in vivo efficacy profile of one of the potent derivative **22e** has also been discussed.

Novel compounds belonging to N-substituted 4-hydrazino piperidine series were synthesized by coupling reaction of *R*(−)3-(2-chloro-acetyl)-thiazolidine-4-carbonitrile (**1**), its isomer (**2**) or (*S*)1-(2-chloro-acetyl)-pyrrolidine-2-carbonitrile (**3**) with compound (**8**) as described in Scheme 1.

The key intermediate **1** was synthesized starting from *R*(−)thiazolidine-4-carboxylic acid following literature method.[7] The synthesis of another key intermediate **8** is described in Scheme 1.

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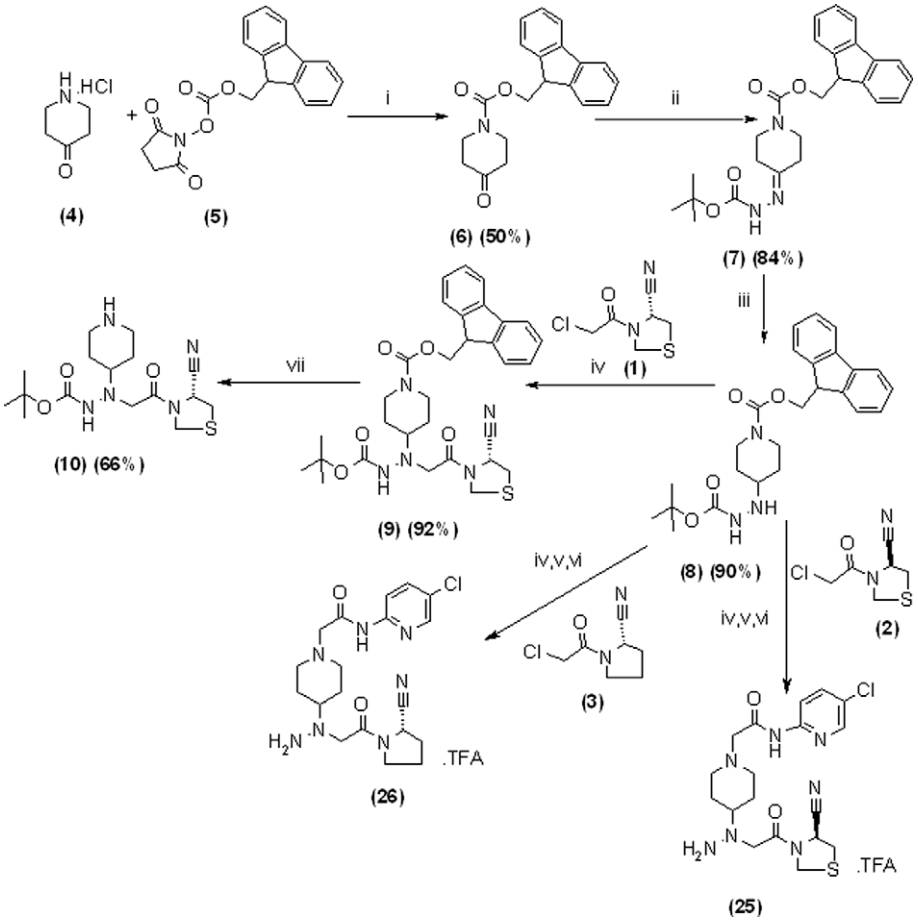


Scheme 1. Synthesis of compounds **10**, **25** and **26**. Reagents and conditions: (i) Na_2CO_3, CH_2Cl_2–water, 0 °C–rt, 3 h; (ii) *t*-butyl carbazate, CH_2Cl_2 or IPA, 80–85 °C, 3 h; (iii) $NaBH_4$, AcOH, CH_2Cl_2, 0–5 °C, 30 min; (iv) **1** or **2** or **3**, K_2CO_3, KI, EtOAc, 65–70 °C, 18 h; (v) (a) morpholine, CH_2Cl_2, 0 °C–rt, 30 min; (b) **19v**, acetone, 50–60 °C, 8 h; (vi) TFA, CH_2Cl_2, 20–25 °C, 30 min; (vii) morpholine, CH_2Cl_2, 0 °C–rt, 30 min.

The N-protection of 4-piperidone hydrochloride (**4**) with Fmoc-succinimide (**5**) resulted in **6**. Reaction of **6** with *t*-butylcarbazate in CH_2Cl_2 or IPA afforded imine **7**. Reduction of **7** with $NaBH_4$ in the presence of AcOH in CH_2Cl_2 yielded N-protected hydrazino piperidine (**8**). The N-alkylation reaction of compound **8** with **1** in the presence of K_2CO_3 and KI in ethyl acetate afforded **9**. The removal of Fmoc -group from **9** was achieved using morpholine in CH_2Cl_2 to furnish compound **10**.

Deprotection of Boc-group in the presence of TFA yielded 3-[2-(*N*-piperidin-4-yl-hydrazino)-acetyl]-thiazolidine-4-carbonitrile (**11**) (Scheme 2). Installing different functionalities on nitrogen of piperidine ring in **10** led to *N*-acyl, sulfonamides and N-alkylated compounds (**13a–d**, **16a–h** and **18a–h**) as delineated in Scheme 2. Reaction of **10** with various substituted sulfonyl chlorides, acid chlorides and carbamoyl chlorides in the presence of TEA in THF afforded compounds (**12a–d**), (**15a–f**) and (**15g–h**), respectively.

Further N-benzylation or N-alkylation of **10** with substituted benzyl chlorides or substituted alkyl chlorides in the presence of KI and K_2CO_3 yielded their respective Boc protected derivatives (**17a–h**). The Boc deprotection of (**12a–d**, **15a–h** and **17a–h**) were carried out in TFA to afford (**13a–d**, **16a–h** and **18a–h**) as TFA salts. The ethyl hydrazine derivative (**14**) was prepared by the treatment of compound (**13c**) with acetaldehyde in MeOH followed by reduction with $NaBH_4$ (Scheme 2).

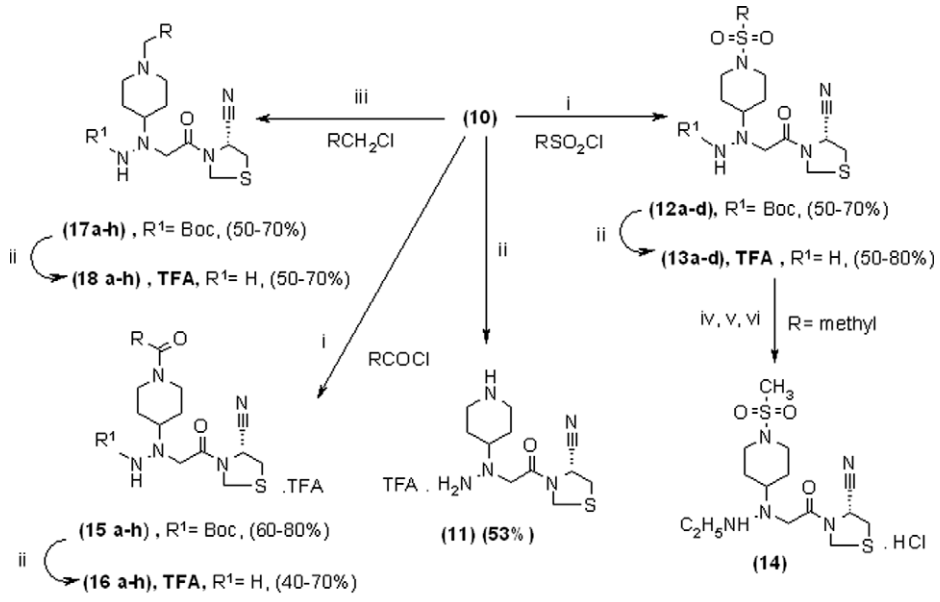
Synthesis of **21a** and various N-substituted acetamide derivatives (**21b–v**) are shown in Scheme 3. The reaction of **10** with chloroacetamide (**19a**) or various N-substituted chloroacetamides (**19b–v**) in the presence of TEA in acetone yielded compounds **20a–v**.

The N-substituted chloroacetamide derivatives (**19b–v**) were prepared by reacting respective primary amine with chloroacetyl chloride in the presence of TEA in THF. Deprotection of Boc-group in compounds **20a–v** using TFA afforded respective products **21a–v** as TFA salts. The hydrochloride salt of compounds **21f**, **21o**, **21t**, **21u** and **21v** were prepared by deprotection of Boc protected compounds **20f**, **20o**, **20t**, **20u** and **20v** with MeCN–HCl in MeCN, which resulted to compounds **22a–e**. The ethyl hydrazine derivative (**23**) was prepared by the treatment of compound **22e** with acetaldehyde in MeOH followed by reduction with $NaBH_4$, further hydrochloride salt was prepared using MeCN–HCl (Scheme 3).

Similarly the compound (**24**) was prepared from **22e** by first, neutralizing with NH_4OH and further reacting with methyl chloroformate in DMF in the presence of K_2CO_3 and then further converted to its hydrochloride salt in MeCN–HCl. Spectroscopic data for all compounds were in accordance with their established structures.

All synthesized compounds were tested in vitro for their DPP IV enzyme inhibitory activity via spectrophotometric method,[8] where Gly-Pro-*p*-nitroanilide was used as the substrate for the DPP IV enzyme from porcine kidney and *p*-nitroanilide, cleaved from the substrate and measured at 385 nm. IC_{50} was determined to compare the DPP IV inhibitory activity of synthesized compounds.

This work is focused on development of a series around compound **11** (IC_{50} 12076 nM). Introduction of aromatic or aliphatic

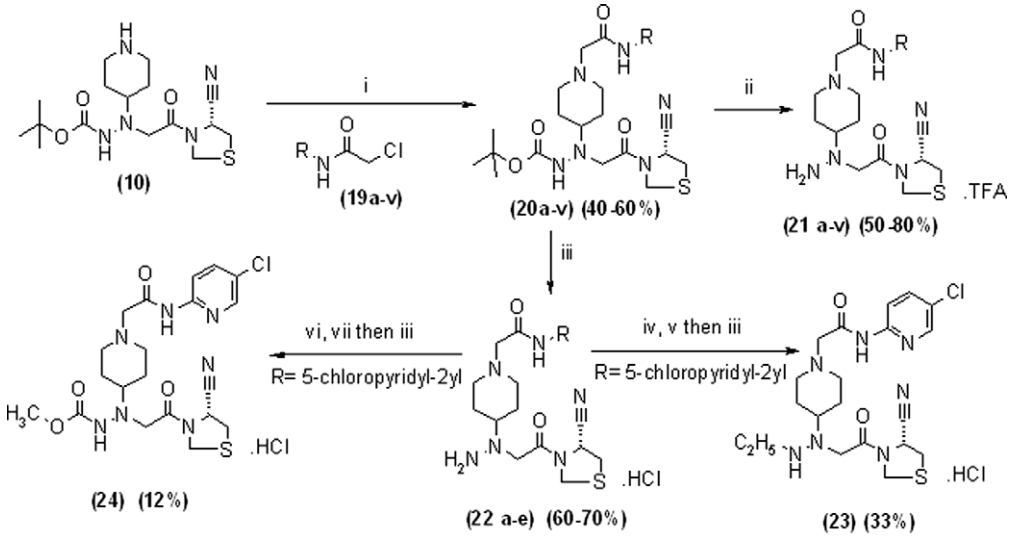


15a, **16a**: R= 4-methylcyclohexyl
15b, **16b**: R= methyl
15c, **16c**: R= 2,3-dichlorophenyl
15d, **16d**: R= t-butyl
15e, **16e**: R= (pyridin-3-yl)methyl
15f, **16f**: R= (morpholin-4-yl)methyl
15g, **16g**: R= N-methylethanamino
15h, **16h**: R= morpholin-4-yl

17a, **18a**: R= 4-fluorophenyl
17b, **18b**: R= 4-nitrophenyl
17c, **18c**: R= phenyl
17d, **18d**: R= 4-cyanophenyl
17e, **18e**: R= pyridin-3-yl
17f, **18f**: R= (phenoxy)methyl
17g, **18g**: R= (2-cyclopropylamino-2-oxo)ethyl
17h, **18h**: R= 2-cyanobiphenyl-4-yl

12a, **13a**: R= 4-methoxyphenyl
12b, **13b**: R= 4-chlorophenyl
12c, **13c**: R= methyl
12d, **13d**: R= 4-methylphenyl

Scheme 2. Synthesis of compounds **11**, **13a–d**, **14**, **16a–h** and **18a–h**. Reagents and conditions: (i) RSO_2Cl or RCOCl, or substituted carbamoyl chloride, TEA, THF, rt, 2–8 h; (ii) TFA, CH_2Cl_2, 20–25 °C, 30 min; (iii) RCH_2Cl, K_2CO_3, KI, THF, 25–80 °C, 8–10 h; (iv) CH_3CHO, MeOH, rt, 1 h; (v) $NaBH_4$, MeOH, rt, 1 h; (vi) MeCN·HCl, MeCN, rt, 1 h.



19a, **20a**, **21a**: R= H
19b, **20b**, **21b**: R= (2-methoxy)ethyl
19c, **20c**, **21c**: R=(3-isopropoxy)propyl
19d, **20d**, **21d**: R= adamantan-1-yl
19e, **20e**, **21e**: R= cyclohexyl
19f, **20f**, **21f**, **22a**: R= cyclopropyl
19g, **20g**, **21g**: R= (2-(thiophen-2-yl))ethyl
19h, **20h**, **21h**: R= 2-fluorobenzyl
19i, **20i**, **21i**: R= 4-chlorophenyl
19j, **20j**, **21j**: R= 3-chloro-4-fluorophenyl
19k, **20k**, **21k**: R= 4-trifluoromethylphenyl

19l, **20l**, **21l**: R= 3,4-methelenedioxyphenyl
19m, **20m**, **21m**: R= 3,4-ethelenedioxyphenyl
19n, **20n**, **21n**: R= 4-cyanophenyl
19o, **20o**, **21o**, **22d**: R= 4-aminosulfonylphenyl
19p, **20p**, **21p**: R= 5-bromothiazol-2-yl
19q, **20q**, **21q**: R= 4,5-dimethylthiazol-2-yl
19r, **20r**, **21r**: R= benzothiazol-2-yl
19s, **20s**, **21s**: R= pyrimidin-2-yl
19t, **20t**, **21t**, **22b**: R= 5-cyanopyridin-2-yl
19u, **20u**, **21u**, **22c**: R= 2-chloropyridin-3-yl
19v, **20v**, **21v**, **22e**: R= 5-chloropyridin-2-yl

Scheme 3. Synthesis of compounds **21a–v**, **22a–e**, **23** and **24**. Reagents and conditions: (i) TEA, acetone, 50–60 °C, 8–20 h; (ii) TFA, CH_2Cl_2, 20–25 °C, 30 min; (iii) MeCN·HCl, MeCN, 20–25 °C, 1 h; (iv) CH_3CHO, MeOH, rt, 1 h; (v) $NaBH_4$, MeOH, rt, 1 h; (vi) NH_4OH, EtOAc; (vii) CH_3OCOCl, K_2CO_3, DMF, rt, 3 h.

sulfonyl represented by **13a–d** or aromatic or aliphatic carbonyl group exemplified by **16a–d** at nitrogen of piperidine ring of **11** displayed improvement in inhibitory activity (IC_{50} 1449–6677 nM). The introduction of cyclic urea moiety at nitrogen of piperidine in compound **11** results in improvement in activity (**16h**, IC_{50} 790 nM) as compared to open chain urea derivative **16g**. Installing substituted benzyl group in **18a–d** or pyridine-3-yl-methyl in **18e** at secondary amino group of piperidine retains inhibitory activity in sub-micromolar range. The compound with 4-nitrobenzyl group **18b** and 4-cyanobenzyl group **18d** exhibit better activity (IC_{50} 698 nM and 710 nM, respectively) as compared to unsubstituted benzyl **18c** and 4-flourobenzyl derivative **18a**. This indicates that the electron withdrawing substituents on phenyl ring are more suited for bioactivity (Table 1).

Further incorporation of aminocarbonylmethyl group in **11** produced **21a** which showed better activity (IC_{50} 817 nM). The development of trends of SAR around **21a** and its analogue **21b–v** are shown in Table 1.

Compounds having different substitutions on nitrogen atom of acetamido group like substituted phenyl **21i–o**, heteroaryl such as pyridine **21t–v**, pyrimidine **21s**, thaizole **21p–q**, benzothiazolyl **21r**, and substituted alkyl **21b–c**, cycloalkyl **21e–f**, tricycloalkyl **21d**, benzyl **21h**, thiophene-ethyl **21g** were prepared to assess the SAR. The 4-aminosulfonylphenyl **21o** group was found to be the most potent in various substituted phenyl analogs. Among heteroaryls, compounds with pyridine ring were found to be more potent as compared to other heteroaryl ring. Various substitutions on pyridine ring were well tolerated irrespective of their position on ring as in case of **21t–v**.

The substituent *N*-cyclopropyl-acetamido in **21f** showed good inhibitory activity (IC_{50} 236 nM) whereas introduction of *N*-cyclopropylamino-carbonylmethyl (**18g**) lowers the activity by about sixfold, which suggest that an increase in alkyl chain length lead to the reduction in inhibitory activity.

To investigate the effect of thiazolidine ring, the thaizolidine derivatives were synthesized. They were found to be more potent than their corresponding pyrrolidine derivative as observed in case of **21v** (IC_{50} 120 nM) and compound **26** (IC_{50} 3700 nM). Further to confirm the effect of stereochemistry of cyano group present on thiazolidine ring, the *R*(−) isomer **21v** was found ~34 times more potent than its *S*-isomer (**25**, IC_{50} 4048 nM).

Among all compounds, **22e** was found to be the most potent compound with IC_{50} 88 nM. However, introduction of small alkyl group on free nitrogen of hydrazine group in compound **22e** like *N*-ethyl analog (**23**, IC_{50} 490 nM) and methoxycarbonyl analog (**24**), leads to the drastic reduction in inhibitory potency (IC_{50} 38450 nM) as compared to compound **22e**.

Table 1
DPP IV inhibitory effect of N-substituted piperidine derivatives

Compd	IC_{50} (nM)	Compd	IC_{50} (nM)	Compd	IC_{50} (nM)
11	12,076	**18d**	710	**21m**	222
13a	6677	**18e**	4767	**21n**	200
13b	1449	**18f**	638	**21o**	98
13c	1459	**18g**	1760	**21p**	226
13d	2701	**18h**	1022	**21q**	185
14	3755	**21a**	817	**21r**	134
16a	866	**21b**	1027	**21s**	143
16b	1534	**21c**	468	**21t**	110
16c	1793	**21d**	523	**21u**	119
16d	4149	**21e**	506	**21v**	120
16e	667	**21f**	236	**22a**[a]	217
16f	830	**21g**	415	**22b**[a]	130
16g	1228	**21h**	510	**22c**[a]	124
16h	790	**21i**	258	**22d**[a]	90
18a	2228	**21j**	359	**22e**[a]	88
18b	698	**21k**	308	**23**[a]	490
18c	1631	**21l**	240	**24**[a]	38,450

[a] Hydrochloride salt.

Table 2
Selectivity of representative compound **22e**

IC_{50} (nM)						
DPP IV	PEP	DPP II	Trypsin	Elastase	DPP VIII	DPP IX
88	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^5$	$>10^5$

PEP: prolyl endopeptidase.

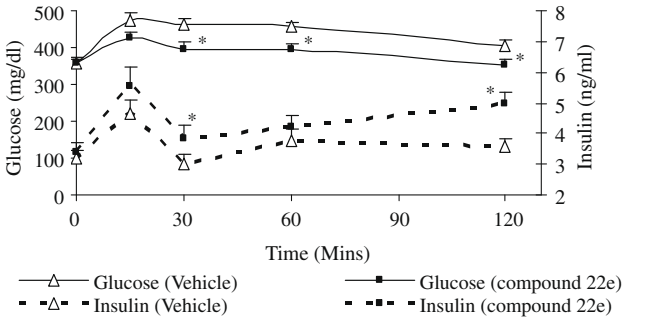


Figure 1. Effect of 6 weeks treatment of compound **22e** (44 mg/kg; $n = 14$) on plasma glucose and insulin levels during OGTT in ZDF rats.

The isozyme selectivity of **22e** was investigated and it was found to be highly selective against other DPPs like PEP, DPP II, Trypsin, Elastase, DPP VIII, and DPP IX (Table 2).

Compound **22e** was further evaluated for its in vivo efficacy in n5-STZ and ZDF rats. To correlate in vitro DPP IV inhibitory activity to in vivo scenario, plasma DPP IV inhibition (% of basal) was monitored over 12 h after a single oral administration (22 mg/kg) of **22e** in n5-STZ rats. It produces maximum plasma DPP IV inhibition within 30 min with significant decrease in the activity observed till 4 h (% inhibition with respect to vehicle 39.81 ± 3.71%, $p < 0.05$ vs vehicle).

In single dose study, compound **22e** decreased glucose excursion and the AUC-glucose during oral glucose tolerance test (OGTT) in n5-STZ rat and was able to stimulate insulin response in a situation where insulin secretory function is failing, as compared to that of control. We also evaluated the long term treatment effect of **22e**, after oral administration, in ZDF rats which become insulin resistant and hyperinsulinemic, when they are five to six weeks old. Treatment was initiated at the age of 6 weeks. OGTT performed at the end of 6 weeks of treatment showed that compound **22e** reduced the glucose excursions and increase insulin release during OGTT in ZDF rats (Fig. 1, $^*p < 0.05$ vs vehicle).

Thus, compound **22e**, has a capacity to augment insulin release in response to glucose load and thus improve glycemic control through its inhibitory effect on DPP IV enzyme.

In conclusion, we have found that *N*-(5-chloropyridin-2-yl) substitution at acetamido group of compound **21a** showed better activity among all compounds. The 4-cyanothiazolidine has potential to elicit better inhibitory activity (**21v** IC_{50} 120 nM) as compared to 4-cyanopyrrolidine containing compound **26** with IC_{50} 3700 nM. Free amino of hydrazine moiety is essential for better activity as it was found that small substitutions on free amino group of hydrazine leads to reduction in inhibition activity (**23**–**24**). These results suggest that further modification or substitution on pyridine ring may yield potent compounds with better in vitro and in vivo efficacy.

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Supplementary data

Supplementary data (experimental procedures and results for in vitro and in vivo DPP IV inhibition assay, experimental procedures of in vivo single dose and long term studies and spectroscopic data of compounds **21v**, **22e** and **26**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.058.

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