Publications

"To me, science is an expression of the human spirit, which reaches every sphere of human culture. It gives an aim and meaning to existence as well as a knowledge, understanding, love, and admiration for the world. It gives a deeper meaning to morality and another dimension to esthetics." - Isidor Isaac Rabi

8. Publication

List of Publications from the PhD work

1. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Samadhan Kshirsagar, Mukul Jain, Mubeen Shaikh, Kalapatapu V. V. M. Sairam. “Long-acting peptidomimetics based DPP-IV inhibitors”*Bioorg. Med. Chem. Lett.***2012**, *22*, 3516-3521.

2. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Kiran Shah, Shruti Bhardwaj, Kishan Patel, Kaushil Patel, Rajendra Chopade, Mubeen Shaikh, Kalapatapu V. V. M. Sairam and Mukul Jain. “Design of Peptidomimetics Based DPP-IV Inhibitors, Devoid of CYP liabilities”*Letters in Drug Design & Discovery***2012**, *9*, 867-873.

3. **Pradip Jadav**, Rajesh Bahekar, Shailesh R. Shah, Dipam Patel, Amit Joharapurkar, Mukul Jain, Kalapatapu V. V. M. Sairam and Praveen Kumar Singh. “Design, Synthesis and Biological Evaluation of Novel Aminomethyl-piperidones based DPP-IV Inhibitors”*Bioorg. Med. Chem. Lett.***2014**, *24*, 1918-1922.

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Long-acting peptidomimetics based DPP-IV inhibitors ☆

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ABSTRACT

Pyrrolidine based peptidomimetics are reported as potent and selective DPP-IV inhibitors for the treatment of T2DM. Compounds **16c** and **16d** showed excellent in vitro potency and selectivity towards DPP-IV and the lead compound **16c** showed sustained antihyperglycemic effects, along with improved pharmacokinetic profile.

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease, which selectively cleaves first two amino acids of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1) thereby makes it inactive.[1,2] Inhibition of DPP-IV activity elevates endogenous GIP, GLP-1 and insulin levels thereby improve glucose excursion. Thus, DPP-IV inhibitors hold great potential for the treatment of type 2 diabetes mellitus (T2DM). Structurally, DPP-IV enzyme resembles with several other protease, so while designing new class of DPP-IV inhibitors, it is essential to consider selectivity of DPP-IV inhibitors over other serine protease, especially DPP-2, DPP-8 and DPP-9.[3]

DPP-IV inhibitors are classified as peptidomimetics (α- and β-series) and non-peptidomimetics. In α-series, pyrrolidine derivatives have been widely explored and depending upon nature of substituents (Z; at the C_2 position of pyrrolidine ring), it is divided into; irreversible (Z = diphenyl-phosphonate ester or *O*-acylhydroxamic acid) and reversible (Z = boronic acid/nitrile) inhibitors.[4] The NVP-DPP728, Vildagliptin, Saxagliptin and Denagliptin represents advanced molecules in cyanopyrrolidine series.[5] The β-series, such as Sitagliptin and the non-peptidomimetic DPP-IV inhibitors, including Alogliptin were developed through high-throughput screening (HTS).[6,7] The DPP-IV enzyme has three binding pockets/sites (S_1, S_2 and S_3).[8] The S_1 pocket consists of catalytic triad (Ser_{630}, Asn_{710} and His_{740}) and the S_2 pocket involves key interactions with Glu_{205} and Glu_{206} dyad. The S_3 pocket (Ser_{209}, Arg_{358} and Phe_{357}) of DPP-IV differs a lot from other protease and it govern selectivity against DPP-8 and 9.[9]

In T2DM patients, gliptins (Vildagliptin, Sitagliptin, Alogliptin and Saxagliptin; Fig. 1) do not lower post-prandial glucose to greater extent as monotherapy, but they are more effective in combination.[10] Gliptins demonstrated good oral bioavailability but due to the rapid clearance, most of them exhibit shorter half-life, thereby require repeated dosing. Thus, attempts are still underway to develop long acting DPP-IV inhibitors, which could potentially provide sustained antidiabetic effect by reducing the post-prandial glucose excursion and HbA1c (>1%), to anticipate cost and dosing frequency. One of the approaches to develop long acting DPP-IV inhibitor could be design of a cyanopyrrolidines based slow-binding inhibitors. The cyanopyrrolidines forms a reversible covalent enzyme-inhibitor complex in which inhibitor bind and dissociates slowly (two-step slow-binding inhibition). Consequently the enzyme catalytic activity can be inhibited even after the free drug has been cleared from the circulation, thereby cyanopyrrolidines inhibits DPP-IV activity for longer duration despite their short half-lives (Vildagliptin and Saxagliptin $t_{1/2}$: ~2–4 h).[11] The NVP-DPP728 represents first slow-binding cyanopyrrolidine-based DPP-IV inhibitor and it showed good antidiabetic activity in clinical trials, despite its short half-life ($t_{1/2}$ ~0.85 h).[12] Recently RBx-0597, has been reported as a potent, selective and slow-binding DPP-IV inhibitor.[13]

Considering clinical implication of long acting DPP-IV inhibitors and to overcome the limitations of existing gliptins, we report

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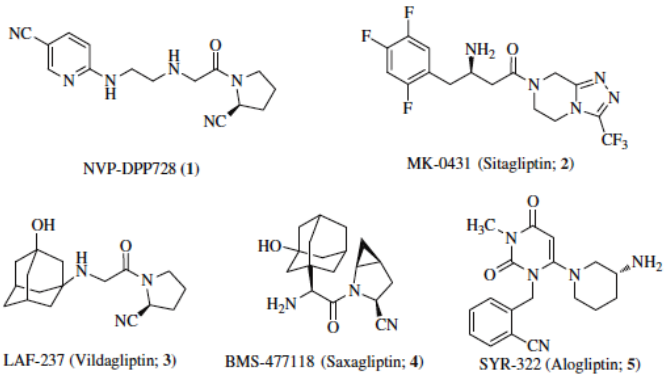


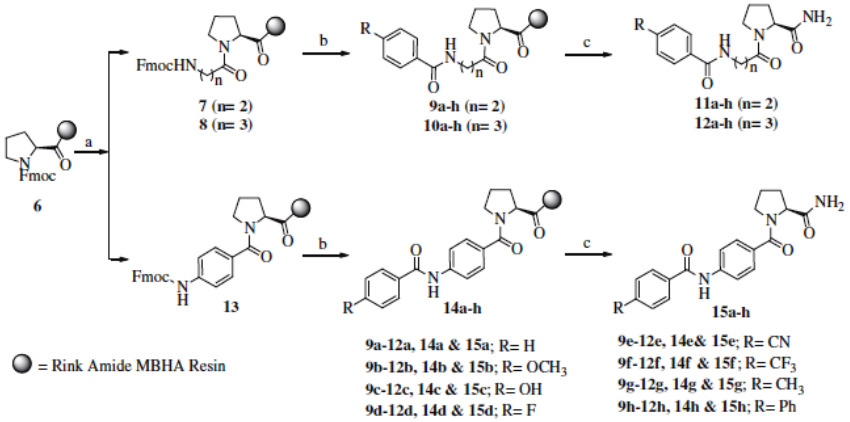
Figure 1. Structurally diverse small molecule based-DPP-IV inhibitors.

design, synthesis and biological evaluation of pyrrolidine containing peptidomimetic based DPP-IV Inhibitors. The peptidomimetics (**11a–h**, **12a–h**, **15a–h**, **16a–d** and **17a,b**) consist of substituted-pyrrolidine ring attached to sterically hinder aromatic acid, with suitable linkers/spacers.

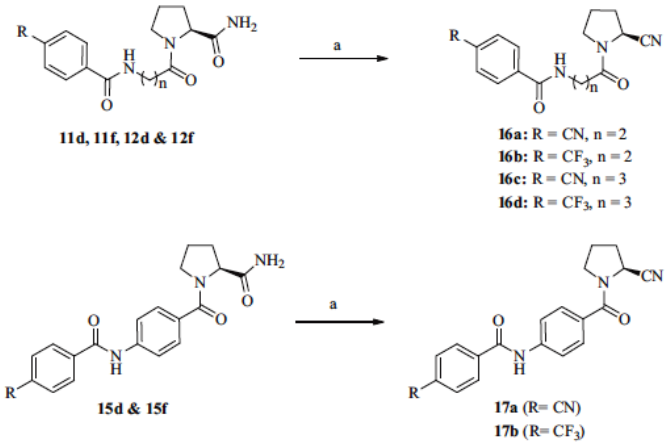
Synthesis (Schemes 1 and 2) of peptidomimetics (**11a–h**, **12a–h**, **15a–h**, **16a–d** and **17a,b**) was carried out, using Fmoc-based solid phase peptide synthesis (SPPS) approach, starting from commercially available Rink-amide MBHA resin, preloaded with Fmoc-protected proline (**6**).[14] Deprotection of **6** with piperidine (20% DMF) and 1,3-diisopropycarbodiimide (DIC) coupling with Fmoc-protected β-Ala (β-alanine), GABA (γ-amino butanoic acid) or PABA (*para*-amino benzoic acid) provided the resin-bound Fmoc-protected dipeptides (**7**, **8** or **13**). Deprotection of **7**, **8** and **13** with piperidine (20% DMF) and DIC coupling with substituted benzoic acids gives resin-bound tripeptides (**9a–h**, **10a–h** and **14a–h**). Trifluroacetic acid (TFA) mediated cleavage of resin-bound peptides (**9a–h**, **10a–h** and **14a–h**) gives pyrrolidinecarboxamides (**11a–h**, **12a–h** and **15a–h**). Trifluroacetic anhydride (TFAA) mediated dehydration of pyrrolidinecarboxamides (**11d**, **11f**, **12d**, **12f**, **15d** and **15f**) afforded title compounds as pyrrolidinecarbonitriles (**16a–d** and **17a,b**).[15] All the test compounds obtained were purified by preparative HPLC (yield 70–85%; HPLC purity >99%) and characterized by various spectroscopic technique (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined within 0.04% of theoretical values (see Supplementary data for analytical and spectral data).

The in vitro DPP-IV inhibitory activity was determined in order to establish the structure–activity relationship (SAR).[16] Two series of the peptidomimetics (**11a–h**, **12a–h**, **15a–h**, **16a–d** and **17a,b**) were prepared (Tables 1 and 2). In the first series (pyrrolidinecarboxamides), 24 compounds (**11a–h**, **12a–h**, **15a–h**) were prepared by linking proline with substituted benzoic acids, using suitable spacers (set-1: β-Ala (**11a–h**); set-2: GABA (**12a–h**) and set-3: PABA (**15a–h**)). In the second series (pyrrolidinecarbonitriles), six compounds (**16a–d** and **17a,b**) were prepared by replacing pyrrolidinecarboxamides with pyrrolidinecarbonitriles. All the test compounds showed varying degrees of DPP-IV inhibitory activity (IC_{50}) depending on the nature of the substituents.

Within the first series (**11a–h**, **12a–h**, **15a–h**), the set-1 (**11a–h**) containing β-alanine spacer attached to *para*-substituted benzamides, showed diverse DPP-IV inhibitory activity depending on the nature of substituents at the *para*-position. Compounds with electron donating groups (**11b**: –OMe and **11c**: –OH) showed weak

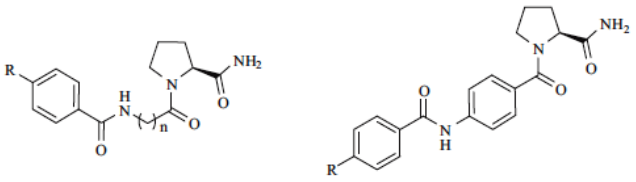


Scheme 1. Synthesis of compounds **11a–h**, **12a–h** and **15a–h**. [a]Reagents and conditions: (a) (1) 20% piperidine in DMF; (2) Fmoc–NH–$(CH_2)_n$–COOH or Fmoc–PABA, HOBt, DIC, DMF, N_2; (b) (1) 20% piperidine in DMF; (2) substituted benzoic acids, HOBt, DIC, DMF, N_2; (c) TFA/H_2O/triisopropylsilane (95:2.5:2.5), 3 h.



Scheme 2. Synthesis of compounds **16a–d** and **17a,b**. [a]Reagents and conditions: (a) TFAA, CH_2Cl_2, 25 °C, 6 h.

Table 1
In vitro DPP-IV inhibitory activity*



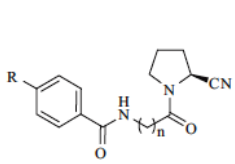
S. No.	R	DPP-IV inhibition**	S. No.	R	DPP-IV inhibition**
11a	–*H*	320 ± 29	**12e**	–*CN*	26 ± 3.1
11b	–OCH_3	890 ± 21	**12f**	–CF_3	19 ± 2.3
11c	–*OH*	863 ± 18	**12g**	–CH_3	694 ± 14
11d	–*F*	93 ± 9.3	**12h**	–*Ph*	104 ± 16
11e	–*CN*	31 ± 2.5	**15a**	–*H*	311 ± 24
11f	–CF_3	28 ± 1.7	**15b**	–OCH_3	879 ± 22
11g	–CH_3	715 ± 27	**15c**	–*OH*	863 ± 13
11h	–*Ph*	107 ± 19	**15d**	–*F*	100 ± 8.5
12a	–*H*	298 ± 19	**15e**	–*CN*	34 ± 7.6
12b	–OCH_3	869 ± 43	**15f**	–CF_3	31 ± 8.3
12c	–*OH*	843 ± 26	**15g**	–CH_3	723 ± 21
12d	–*F*	74 ± 11	**15h**	–*Ph*	116 ± 13

* DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ± SD (n = 3).

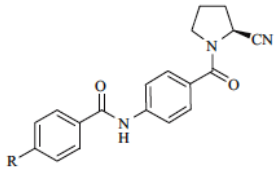
inhibitory activity relative to that of un-substituted (**11a**: –H), whereas compounds with electron withdrawing groups (**11d**; **11e** and **11f**) showed good DPP-IV inhibitory activity. Among the **11d**, **11e** and **11f** tested, **11e** and **11f** showed improved DPP-IV inhibitory activity, which could be due to increase in the electronegativity at *para*-position of benzamide. Aliphatic substitution at *para*-position (**11g**; –CH_3) showed weak inhibitory activity, while aromatic substitution at *para*-position (**11h**; –Ph) showed moderate DPP-IV inhibitory activity. The second and third set of compounds (**12a–h**, **15a–h**) comprising GABA and PABA spacers attached to *para*-substituted benzamides, showed similar trend in DPP-IV inhibitory activity as observed with first set of compounds, with respect to nature of *para*-substituents.

The first series was specifically designed to understand the role of spacer and effect of *para*-substituents on benzamide. The SAR study of first series reveals that the DPP-IV inhibitory activity of test compounds drastically varies with *para*-substituents, whereas alteration in spacers (aliphatic with two/three carbon chain-length versus aromatic) do not exhibit significant change. In general, neutral effect of spacers on inhibitory activity might be due to the flexibility in S_2 pocket and stapled orientation of Glu-dyad. Substituents on *para*-position of benzamide altered inhibitory activity to greater extent because in S_3 pocket, *para*-substituents play crucial role for its interaction with Ser_{209}, Arg_{358} and Phe_{357}. From first series, altogether in three different sets, **11e**, **11f**, **12e**, **12f**, **15e** and **15f** (*para*-nitrile/trifluoromethyl benzamide) were

Table 2
In vitro DPP-IV inhibitory activity and selectivity*



16a-b (n= 2); 16c-d (n= 3)



17a-b

S. No.	R	DPP-IV**	DPP2$	DPP8$	DPP9$
16a	–CN	10.3 ± 1.9	–	–	–
16b	$-CF_3$	13.2 ± 2.3	–	–	–
16c	–CN	2.3 ± 0.9	>25,000	>15,000	>15,000
16d	$-CF_3$	3.8 ± 0.5	>25,000	>15,000	>15,000
17a	–CN	11.6 ± 1.6	–	–	–
17b	$-CF_3$	14.3 ± 2.5	–	–	–
NVP-DPP728#		7.2 ± 1.3	>25,000	>15,000	>15,000
Vildagliptin#		3.2 ± 0.5	–	–	–

* DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ± SD ($n = 3$).
$ DPP2, DPP8 and DPP9 inhibitory activity represented as fold-selectivity wrt DPP-IV inhibitory activity.
# Reported literature values for NVP-DPP728 and Vildagliptin are 7 ± 1.7 and 2.7 ± 0.1 respectively. (Ref. 5b,c).

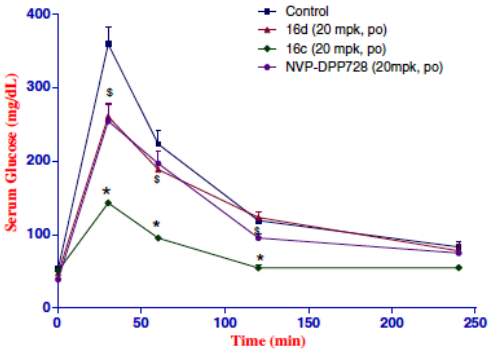
identified as primary lead compounds. Further to study effect of nitrile group on pyrrolidine ring system, a second series (**16a–d** and **17a,b**) was prepared by replacing pyrrolidinecarboxamides of first series lead compounds with pyrrolidinecarbonitriles.

As depicted in Table 2, all the six compounds (**16a–d** and **17a,b**) from second series showed potent inhibitory activity and was found to be comparable with standard compounds (NVP-DPP728 and Vildagliptin).[5] Compared to first series (pyrrolidinecarboxamides), significant improvement in the inhibitory activity was observed with second series (pyrrolidinecarbonitriles) of compounds (**16a–d** and **17a,b**), which could be due to the favorable interactions of pyrrolidinecarbonitriles with the key residues of S_1 pocket. Among six compounds tested (second series), **16c** and **16d** were found to be equipotent as Vildagliptin.

The in vitro selectivity over serine protease (Table 2), especially DPP-2, DPP-8 and DPP-9 was evaluated for most potent compounds (**16c** and **16d**).[16] Compounds **16c** and **16d** showed >25000-fold selectivity over DPP-2 and >15000-fold selectivity over DPP-8 and DPP-9, which was found to be comparable with reference standard compounds (NVP-DDP728). Among all the compounds tested, **16c** and **16d** were found most potent and selective, hence subjected for pharmacodynamic (PD) as well as pharmacokinetic (PK) profiling.

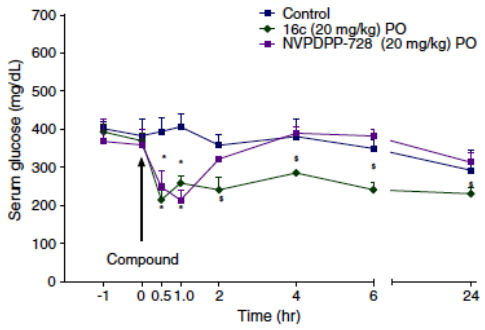
The in vivo antidiabetic activity of **16c**, **16d** and NVP-DPP728 (@ 20 mg/kg, po) was evaluated in male C57BL/6 J mice, using IPGTT (intraperitoneal glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose up to 240 min; mg/dL) is reported (Fig. 2).[17] Compound **16c** showed good oral antidiabetic activity (% decrease in AUC glucose 54.9 ± 3.86), whereas **16d** and NVP-DPP728 (positive control) showed moderate activity upon oral administration (% decrease in AUC glucose 17.4 ± 5.35 and 21.5 ± 6.1, respectively). In C57 mice (IPGTT protocol), it was interesting to observe that **16c** showed suppression in the blood glucose at all the time points (30, 60, 120 and 240 min) compared to vehicle control, while **16d** and NVP-DPP728 showed blood glucose reduction only at 30 and 60 min.

Further to understand the duration of action and effect of test compounds on post-prandial glucose excursion, single dose (@



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 2. In vivo antidiabetic activity of **16c**, **16d** and NVP-DPP728 in C57 mice (OGTT).



*P<0.05, $P<0.01, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 3. In vivo antidiabetic activity of **16c** and NVP-DPP728 in db/db mice.

20 mg/kg, po) antidiabetic activity of **16c** and NVP-DPP728 was evaluated in fed-db/db mice (hyperglycemic animals) for 24 h (Fig. 3). Under fed condition, compared to vehicle control, NVP-DPP728 and **16c** showed good antidiabetic activity (% decrease in AUC glucose 31.4 ± 8.7 and 33.5 ± 7.4, respectively) up to 2 h. However, **16c** showed sustained suppression in serum glucose levels for >8 h (% decrease in AUC glucose, 14.9 ± 6.3 for NVP-DPP728 and 30.8 ± 6.2 for **16c**, after 8 h).

A comparative single dose (20 mg/kg iv or po) PK profile of **16c**, **16d** and NVP-DPP728 was evaluated in male C57BL/6J mice ($n = 6$) and the various PK parameters such as T_{max}, $T_{1/2}$, C_{max}, AUC and %F were recorded (Table 3).[18] In PK study, all the test compounds showed rapid T_{max}, good C_{max} and oral bioavailability (%F ~63–72%). Compound **16c** showed higher AUC (>twofold compared to **16d** and NVP-DPP728 **1**) and extended half-life ($T_{1/2}$: >7 h compared to **16d** and NVP-DPP728). Compound **16c** showed extended half-life and higher AUC, which could be due to its low clearance compared to **16d** (elimination rate constant (kel; h^{-1}), 0.11 ± 0.03 for **16c** and 0.82 ± 0.18 for **16d**). Thus improved pharmacokinetic profile of compound **16c** justifies its potent and sustained antidiabetic activity in C57 and db/db mice.

The molecular docking analysis of **16c** and NVP-DPP728 was carried out using extra precision (XP) Glide docking method (Fig. 4).[9,19] The crystal structure of the DPP-IV enzyme (PDB ID: 2I03) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. For docking study, the ligands were minimized by applying an OPLS-AA forcefield, using ligprep module of Schrödinger.[19]

The overlay of binding poses of **16c** (Turquoise) and NVP-DPP728 (Rose) in the DPP-IV active site is shown in Fig. 4. As observed with NVP-DPP728, **16c** docks very well into all the three sites (G-scores −11.85 (9/9) and −10.16 (7/9) for **16c** and NVP-DPP728 respectively). Both NVP-DPP728 and **16c** showed covalent interaction of cyanopyrrolidine–CN with OH-group of side-chain of Ser_{630} (S_1 pocket) and H-bonding of amide-NH backbone with C=O groups of side-chains of Glu_{205} and Glu_{206} dyad (S_2 pocket), which supports excellent in vitro DPP-IV selectivity of **16c** over other protease. Especially, incorporation of GABA linkage (spacer) and *para*-nitrile benzamide in **16c** allows it to adopt new confirmation, which favors strong H-bonding of benzamide with the NH of guanidine side-chain of Arg_{358} and aromatic π–π stacking of *para*-nitrile phenyl ring with Phe_{357} in S_3 pocket (Fig. 1; Supplementary data). These additional interactions of **16c** in S_3 pocket justify its 3-fold potent DPP-IV inhibitory activity (in vitro) over NVP-DPP728.

Kinetic study of DPP-IV inhibition by radiolabeled NVP-DPP728 established its slow-binding inhibition mechanism and nitrile functionality play crucial role in the formation of the high-affinity complex, via imidate intermediate.[12] Due to unavailability of radiolabeled **16c**, inhibitory kinetic of **16c** was not evaluated. However, docking studies supports involvement of key interactions of **16c** with all the three pockets, which apparently establish slow-binding kinetic of **16c** as cyanopyrrolidine class effect.

In summary, we report SPPS approach to discover peptidomimetic based cyanopyrrolidine derivatives as potent, selective and long acting DPP-IV inhibitors for an effective treatment of T2DM. The lead compound **16c** showed sustained suppression of pre-

Table 3
Pharmacokinetic study parameters[a] of **16c**, **16d** and NVP-DPP728

Compd	T_{max} (h)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (0-∞) h μg/ml	F^* (%)
16c	0.29 ± 0.11	7.1 ± 0.83	7.99 ± 0.33	14.3 ± 1.13	72.5
16d	0.28 ± 0.10	5.9 ± 0.88	0.99 ± 0.14	6.89 ± 1.21	63.1
NVP-DPP728	0.32 ± 0.08	6.2 ± 0.91	0.88 ± 0.11	6.49 ± 1.11	65

[a] In male C57BL/6 J mice ($n = 6$), compounds were administered orally (po) at 20 mg/kg dose and plasma concentration was analyzed by LC–MS, values indicate Mean ± SD.
[*] Oral bioavailability (%F) was calculated wrt to iv AUC (**16c**: 11.02 ± 0.11; **16d**: 10.92 ± 0.12 & NVP-DPP728: 9.98 ± 0.09 h μg/ml) administered at 20 mg/kg dose, iv.

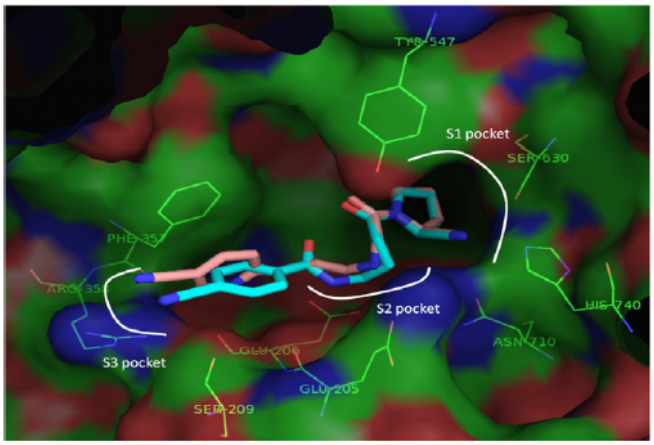


Figure 4. Key interactions of compound **16c** and NVP-DPP728 with active sites of DPP-IV enzyme. Binding pose of compound **16c** (Turquoise) and **NVP-DPP728** (Rose) in the DPP-IV active site is indicated (Surface view: Green), wherein both compounds interact closely with key residues of site S1, S2 and S3.

and post-prandial blood glucose levels (in vivo), which correlates with its extended PK profile.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.078.

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18. Briefly, for single dose PK study, test compounds were administered orally/iv on a body weight basis (20 mg/kg) to overnight fasted male C57BL/6 J mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24 and 30 h post-dose after compounds administration. Approximately 0.3 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at −70 °C and the concentrations of compounds in plasma were determined by the LC–MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C_{18} (2.0 × 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters were calculated using a non-compartmental model of WinNonlin software version 5.2.1.
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Design of Peptidomimetic Based DPP-IV Inhibitors, Devoid of CYP Liabilities#

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Abstract: A peptidomimetic based cyanopyrrolidine derivatives are reported as potent and selective DPP-IV inhibitors. Some of the test compounds (**10l** and **10m**) showed excellent potency and selectivity towards DPP-IV over various serine proteases, without CYP inhibition.

Keywords: CYP, cyanopyrrolidine, DPP-IV inhibitors, Selectivity, Peptidomimetic.

INTRODUCTION

Obesity and diabetes are emerging as the global epidemic of the 21st century and becoming major health problems worldwide.[1] Type 2 diabetes mellitus (T2DM) is characterized by elevated levels of blood glucose, resulting from impaired insulin secretion and/ or insulin resistance.[2] Currently diabetic patients are treated with various antihyperglycemic agents; however, due to the progressive nature of the disease, most of the available antihyperglycemic agents loose sustained glycemic control over a period of time.[3-4] Also, adverse events associated with the existing antihyperglycemic agents raise safety concerns.

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease, [5] which selectively cleaves the N-terminal dipeptide from the penultimate position of Glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagon-Like Peptide (GLP-1) thus makes them inactive.[6-7] Inhibition of DPP-IV activity extend the duration of action of endogenous GLP-1, thereby stimulating insulin secretion, inhibiting glucagon release and slowing gastric emptying.[8-9] Because of these multiple benefits of GLP-1 mediated glucose homeostasis, orally bioavailable DPP-IV inhibitors has been developed as promising therapeutic agents for the treatment of T2DM.

DPP-IV inhibitors offer a number of potential advantages over existing diabetes therapies, including a lowered risk of hypoglycemia and weight gain. Consequently, various DPP-IV inhibitors such as Vildagliptin (NVP-LAF237, **1**), Saxagliptin (BMS-477118, **2**) and Denagliptin (GW-823093, **3**), including Sitagliptin are in clinic for the effective treatment of T2DM Fig. (**1**) [10-14].

DPP-IV enzyme exhibits close structural analogy with several other serine proteases. So while designing new class of DPP-IV inhibitors, it is essential to consider selectivity of DPP-IV inhibitors over other serine protease, especially DPP-2, DPP-8 and DPP-9.[15-16] The X-ray crystal study of various inhibitors in complex with DPP-IV represents three major binding pockets/ sites as S_1, S_2 and S_3. The S_1 pocket consists of catalytic triad (Ser_{630}, Asn_{710} and His_{740}), S_2 pocket consists of Glu dyad (Glu_{205} and Glu_{206}), while S_3 pocket involves interactions with Ser_{209}, Arg_{358} and Phe_{357}.[17-18] Low nanomolar potency can be achieved by optimizing favorable interactions with S_1 and S_2 pockets. The S_3 pocket of DPP-IV differs a lot from DPP-8/9 and the precise interactions with Phe_{357} govern selectivity against DPP-8 and 9 [19-20].

Though several DPP-IV inhibitors are in the market, attempts are still underway to develop potent and selective DPP-IV inhibitors devoid of side effects associated with existing DPP-IV inhibitors.[21] Recently, we disclosed a series of cyanopyrrolidine based peptidomimetics as potent, selective and long acting DPP-IV inhibitors (Compound **4**, Fig. (**1**). [22] However, upon secondary profiling of compound **4**, CYP3A4 and CYP2D6 inhibitions (IC_{50}: 1.1 and 1.9 μM respectively) were observed, which halted its further preclinical development.

Cytochrome P450 (CYP450) enzymes are predominantly expressed in the liver and are essential for the detoxification and the metabolism of drugs. In addition to antidiabetic drugs diabetic patients are treated with a number of other drugs, including anti-hypertensive and lipid-lowering agents. Notably, more than 50% of these drugs are metabolized by CYP3A4 or CYP2D6 enzymes. Drugs can inhibit (decrease), induce (increase) CYP metabolism or may act as a substrate

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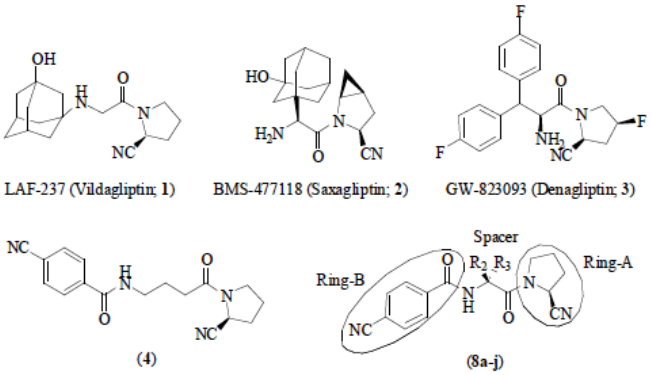


Scheme 1. Structurally diverse small molecule based-DPP-IV inhibitors.

for CYP enzymes. Inhibition of CYP metabolism will likely increase the affected drug's systemic concentrations, whereas induction of metabolism often reduces systemic concentrations [23].

CYP3A4 or CYP2D6 inhibition and induction is clinically relevant to diabetic patients, especially when treated with antidiabetic agents such as Sulfonylureas, Metformin and Meglitanides. For example, Sulfonylureas are known substrates of CYP. Thus inducers and inhibitors of CYP can affect the metabolism of Sulfonylureas. Similarly, Repaglinide is metabolized by the CYP3A4 and a serious drug-drug interactions (DDI) may occur when it is co-administered with CYP inhibitor, such as Gemfibrozil (triglyceride lowering agent), as it increases eightfold exposure of Repaglinide. Pioglitazone is a substrate for CYP3A4 and can be affected by inhibitors (Verapamil, Diltiazem) or inducers (Carbamazepine, Rifampin) of CYP3A4. Thus, CYP inhibition/ induction can have significant consequences on other antidiabetic drugs that are metabolized by these enzymes, which may result in DDI and idiosyncratic drug toxicity (IDT) [24, 25].

In general, affinity of a molecule for CYP can be attenuated by increasing / decreasing the carbon chain length.[26] So to overcome CYP liabilities, amino-alkyl spacer (-$(CH_2)_3$-; 3C) of compound **4** was specifically reduced from 3C to 2C (-$(CH_2)_2$-) and 1C (-CH_2-) and the resulting molecules were examined for CYP inhibitions. Compound **4** with 2C amino-alkyl spacer (i.e. compound **16a** reported in our previous publication,[22] with DPP-IV inhibitory IC_{50}: 10.3 nM) showed weaker CYP3A4 and CYP2D6 inhibitions (IC_{50}: 9.3 and 10.1 μM respectively), while compound **8a** Table **1** with 1C amino-alkyl spacer showed no CYP3A4 and CYP2D6 inhibitions up to 100 μM. However, **8a** showed weak DPP-IV inhibitory activity (IC_{50}: 722 nM). Thus reduction of amino-alkyl spacer attenuates CYP inhibitions but led to a significant drop in DPP-IV inhibitory activity.

Further to improve DPP-IV inhibitory activity of **8a**, two series (**8b-j** and **10a-m**) of structurally constrained cyanopyrrolidine containing peptidomimetic based DPP-IV inhibitors were designed. In the first series suitable modifications were carried out on 1C amino-alkyl spacer of **8a** and altogether nine compounds (**8b-j**) were prepared by linking ring A with ring B, using various α-substituted amino acids spacers. In the second series, thirteen compounds (**10a-m**) were prepared by modifying the best compound obtained from first series, specifically by carrying out suitable changes over ring-A and -B.

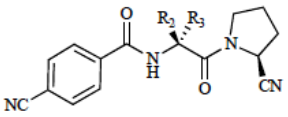
CHEMISTRY

Synthesis (Scheme **1**) of peptidomimetics (**8a-j** and **10a-m**) was carried out using Fmoc-based Solid Phase Peptide Synthesis (SPPS) approach, starting from commercially available Rink-amide MBHA resin, preloaded with Fmoc-protected prolines (**5a-b**).[27] Deprotection of **5a-b** with piperidine (20% DMF) and 1,3-diisopropycarbodiimide (DIC) coupling with Fmoc-protected amino acids provided the resin-bound Fmoc-protected dipeptides (**6a-k**). Deprotection of **6a-k** with piperidine (20% DMF) and DIC coupling with substituted benzoic acids gives resin-bound tripeptides, which upon Trifluroacetic acid (TFA) mediated cleavage gives pyrrolidinecarboxamides (**7a-j** and **9a-m**). Trifluroacetic anhydride (TFAA) mediated dehydration of pyrrolidinecarboxamides (**7a-j** and **9a-m**) afforded title compounds as pyrrolidinecarbonitriles (**8a-j** and **10a-m**).[28] All the test compounds obtained were purified by preparative HPLC (yield 70-85%; HPLC purity >97%) and characterized by various spectroscopic technique (^{13}C NMR, ^{1}H NMR and ESI MS). Elemental analyses were determined and the results were within ±0.04% of theoretical values (see supplementary information for analytical and spectral data).

In Vitro DPP-IV INHIBITION STUDY

The *in vitro* DPP-IV inhibitory activity was determined using fluorescence-based enzymatic assay. The Gly-Pro-AMC was used as a substrate. The substrate was incubated with DPP-IV enzymes in the presence of various concentrations of test compounds. Activity (fluorescence) was measured using Spectra Max fluorometer (Molecular

Table 1. *In vitro* **DPP-IV inhibitory activity***



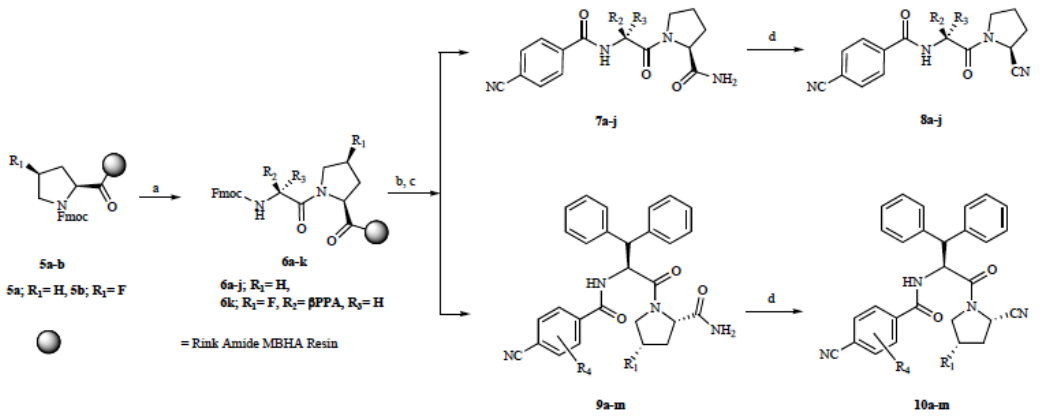
8a-j

S. No	R_2	R_3	Amino acids[$]	DPP-IV inhibition**
8a	H	-H	Gly	722 ± 3.4
8b	$-CH(CH_3)_2$	-H	Val	74 ± 2.4
8c	$-CH(CH_3)(C_2H_5)$	-H	Ile	39 ± 1.2
8d	$-CH_3$	$-CH_3$	Aib	157 ± 3.3
8e	cyclohexyl	-H	Chg	97 ± 2.7
8f	-Ph	-H	Phg	463 ± 3.8
8g	-Bz	-H	Phe	239 ± 1.9
8h	2-F Bz	-H	2-F Phe	197 ± 3.6
8i	2-F Bz	$-CH_3$	α-Me-2-F Phe	137 ± 4.9
8j	$-CH(Ph)_2$	-H	βPPA	**27 ± 1.6**
1	--	--	--	3.2 ± 0.5
4	--	--	--	2.3 ± 0.9

*DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software

** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3)

[$] R_2, R_3 together represents amino acids with absolute (S) stereo configuration.



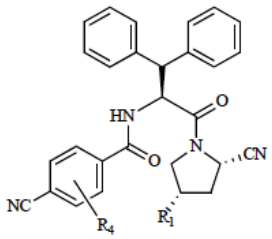
Scheme 1: Synthesis of compounds **8a-j** and **10a-m**

[a] Reagents and conditions: (a) 1. 20% Piperidine in DMF; 2. Fmoc-NH-(CHR_2R_3)-COOH, DIC, DMF, N_2; (b) 1. 20% Piperidine in DMF; 2. Substituted benzoic acids, HOBt, DIC, DMF, N_2; (c) TFA: H_2O: Triisopropylsilane (95:2.5:2.5), 3h; (d) TFAA, CH_2Cl_2, 25 °C, 6h.

Devices, Sunnyvale CA) by exciting at 380 nm and emission at 460 nm. The IC_{50} values were determined for test compounds using Graph Pad prism software.[29]

As shown in Table **1-2**, two series of peptidomimetics (**8a-j** and **10a-m**) were prepared and depending on the nature of substitutions, different degree of DPP-IV inhibitory activity was observed. In the first series, upon linking

Table 2. ***In vitro*** **DPP-IV inhibitory activity***



10a-m

S. No	R_1	R_4	DPP-IV inhibition**	DPP2[$]	DPP8[$]	DPP9[$]
10a	-H	2-CH_3	34 ± 2.9	---	---	---
10b	-H	2-F	22 ± 1.7	---	---	---
10c	-H	3-CH_3	18 ± 1.3	---	---	---
10d	-H	3-F	9.6 ± 0.6	>25,000	>15,000	>15,000
10e	-H	2,5-di-CH_3	31 ± 2.4	---	---	---
10f	-H	2,5-di-F	19 ± 0.7	---	---	---
10g	-H	3-OH	28 ± 2.7	---	---	---
10h	-H	3-OCH_3	23 ± 1.9	---	---	---
10i	-H	3-Cl	11 ± 0.8	>25,000	>15,000	>15,000
10j	-H	3-CN	17 ± 1.3	---	---	---
10k	-H	3-CF_3	14 ± 2.1	---	---	---
10l	-F	3-Cl	**4.2 ± 0.7**	>25,000	>15,000	>15,000
10m	-F	3-F	**2.7 ± 0.3**	>25,000	>15,000	>15,000
3***	--	--	19 ± 3.2			
4	--	--	2.3 ± 0.9	>25,000	>15,000	>15,000

*DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ±SD (n = 3).
[$] DPP2, DPP8 and DPP9 inhibitory activity represented as fold-selectivity wrt DPP-IV inhibitory activity.
*** Reported literature value for Denagliptin 22 nM (Ref: 12)

cyanopyrrolidine (ring A) with *para*-cyanobenzoic acid (ring B), using α-substituted amino acid spacers (Val; **8b**, Ile; **8c** or cyclohexyl glycine (Chg); **8e**), compounds **8b**, **8c** and **8e** showed moderate DPP-IV inhibitory activities. When amino-isobutyric acid (Aib); **8d** or α-methyl-2-fluoro phenyl alanine (α-Me-2-F Phe); **8i**, were introduced as spacer, the resulting compounds however showed weak *in vitro* activities. The compounds **8f**, **8g** and **8h** containing phenyl glycine (Phg), phenyl alanine (Phe) and 2-fluoro phenyl alanine (2-F-Phe) respectively as spacers were also found to be the least potent. However compound **8j** with β-phenyl phenyl alanine (β-PPA) showed the highest DPP-IV inhibitory activity (IC_{50}: 27 nM) within the series.

The first series was specifically designed as analogs of **8a**, to understand the role of α-substituents on 1C amino-alkyl spacer so as to get the low nM DPP-IV inhibitory activity. The SAR study of first series reveals that the DPP-IV inhibitory activity of test compounds drastically varies with the nature of α-substituents and among various substituents screened, β-PPA was found to be favorable. It appears that the DPP-IV enzyme accepts changes in limited steric bulk at S_2 binding pocket, which might be due to the stapled orientation of Glu-dyad in S_2 pocket.

Compound **8j** was identified as primary hit from the first series. Further to improve DPP-IV inhibitory activity of **8j**, second series (**10a-m**) was designed, specifically by carrying out suitable changes over ring-A and -B of **8j** and in second series, five sets of compounds were prepared Table **2**. Substitutions were carried out in set-1 (**10a** and **10b**) on 2nd position, in set-2 (**10c** and **10d**) on 3rd position and in set-3

(**10e** and **10f**) on 2nd and 5th positions of cyano-benzamide (ring-A) , either with electron withdrawing (EW) or electron donating (ED) groups. In set-4 (**10g-10k**), substitutions were carried out specifically on 3rd position of cyano-benzamide (ring-A). Finally, based upon the literature presidencies (favorable substitution of 4F- pyrrolidine in Denagliptin), set-5 (**10l** and **10m**) was prepared by substituting 4th position of cyano-pyrrolidine (ring-B) with fluoro group, to improve the DPP-IV inhibitory activity [30, 31].

All the test compounds from the second series showed significant DPP-IV inhibitory activities. Set-1 and 2 showed improved but similar DPP-IV inhibitory activities, irrespective of electron withdrawing (EW) or electron donating (ED) nature of the substituents. Compare to Set-1 and 3, Set-2 showed very good DPP-IV inhibitory activities.

Based on these results, further changes were made only at 3rd position of cyano-benzamide, as set-4 (**10g-10k**). In set-4, compounds **10j and 10k** with EW groups at *meta* position of cyano-benzamide showed higher DPP-IV inhibitory activities than compounds **10g and 10h**, with ED groups. Among all the compounds tested from second series, halo substituted compounds (**10d** and **10i**) showed excellent DPP-IV inhibitory activities (IC_{50}: 9.6 and 11 nM respectively). The 4-fluoropyrrolidine-carbonitrile derivatives (**10l** and **10m**, set-5) of **10d** and **10i** showed further improvement in DPP-IV inhibitory activities (IC_{50}: 4.2 and 2.7 nM respectively, similar to compound **4**), which could be due to the favorable interactions of 4-fluoro pyrrolidine-carbonitrile with the key residues of S_1 pocket.

In Vitro DPP-IV SELECTIVITY AND CYP INHIBITION STUDIES

The *in vitro* selectivity over serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for most potent compounds **10d**, **10i**, **10l** and **10m** (fold-selectivity listed in Table **2**. [29] All the test compounds showed >25000-fold selectivity over DPP-2 and >15000-fold selectivity over DPP-8 and DPP-9, which was found to be comparable with reference standard compound **4**. Among all the compounds tested, **10l** and **10m** were found to be most potent and selective. To assess the CYP liabilities of these peptidomimetics, **10l** and **10m** were subjected for CYP3A4 and CYP2D6 inhibition studies. For CYP3A and CYP2D6 inhibition studies, Human liver microsomes (0.2 mg/ml), Testosterone (50 µM) / Dextromethorphan (5 µM) respectively, as probe substrates for CYP3A and CYP2D6 were incubated with different concentrations of test compounds at 37°C for 10 min., enzyme activity (% of control) was determined by HPLC-MS/MS and IC_{50} values were calculated. Both the test compounds were found to be devoid of CYP3A4 and CYP2D6 inhibition up to 100 µM concentrations [32].

MOLECULAR MODELING

The molecular docking analysis of **8a**, **10m** and Denagliptin was carried out using extra precision (XP) Glide docking method, to understand their critical interactions with all the three binding sites (S_1, S_2 and S_3) of DPP-IV enzyme (Fig. **2**; binding poses overlay of **8a** (Turquoise), **10m**

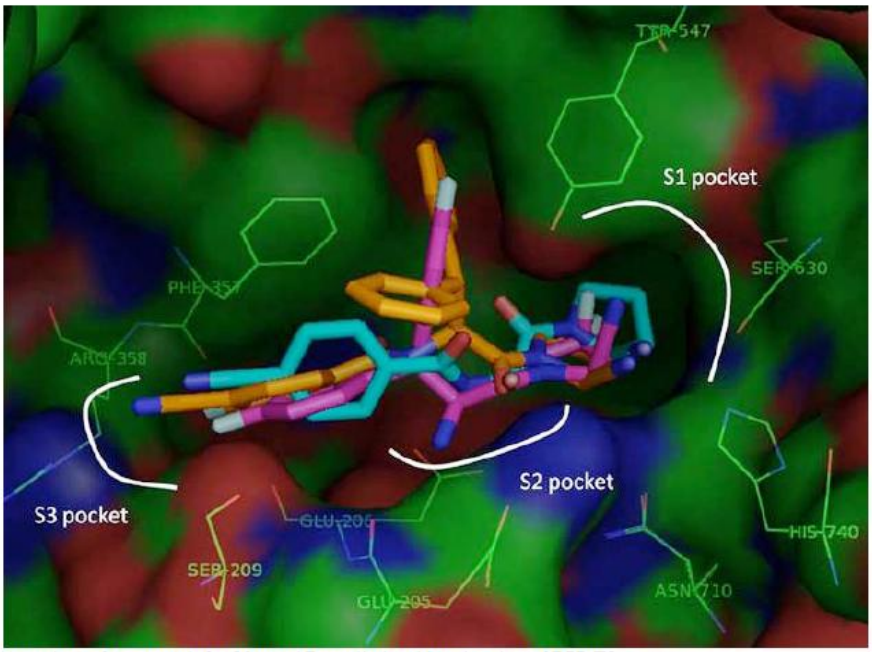


Fig. (2). Key interactions of compounds **8a**, **10m** and Denagliptin with active sites of DPP-IV enzyme.

Binding pose of compound 8a (Turquoise), 10m (Brown) and Denagliptin (Rose) in the DPP-IV active site is indicated (Surface view: Green), wherein compounds 10m and Denagliptin interacts closely with key residues of site S_1, S_2 and S_3.

(Brown) and Denagliptin (Rose)).[19-20, 33-34] The crystal structure of the DPP-IV enzyme (PDB ID: 2ajl) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. After protein structure was prepared, the bound ligand of receptor was defined as grid binding box. For docking study, the ligands were minimized by applying an OPLS-AA force field, using ligprep module of Schrödinger.

The results of docking studies illustrate that all the three compounds interact closely with the key residues of S_1 pocket (as per literature presidencies, cyanopyrrolidine-CN may form covalent bond with OH-group of side-chain of Ser_{630}). In S_2 pocket, benzamide-NH of **10m** and α-amino group of Denagliptin forms H-bonding with C=O groups of side-chains of Glu_{205} and Glu_{206} dyad, while benzamide-NH of **8a** flip away from the Glu dyad. Compound **10m** interact closely in S_3 pocket (aromatic–CN forms H-bonding with the NH of guanidine side-chain of Arg_{358}), while **8a** interact weakly with the key residues of S_2 and S_3 pockets, which may justify its weak *in vitro* DPP-IV inhibitory activity.

Molecule **10m** in our docking studies has shown CH•••π, OH•••π and π•••π interactions. Diphenylmethane in **10m** has all the three aromatic interactions. One of the phenyl ring makes a CH•••π with Phe 357 and Tyr 547. At the same time the same Phe 357 and hydroxyl of Tyr 547 forms a π•••π and OH•••π stabilizing interactions with diphenylmethane. In addition to the above-mentioned aromatic interactions, we have also noticed that fluoro and cyano substituted pyrrolidine forms two CH•••π with Tyr 662 and 666. All the CH•••π interactions are with a range of 2.4 to 2.6 Å, while OH•••π is 2.5 Å and π•••π is 4.4 Å. This π•••π interaction between Phe 357 and diphenylmethane has a parallel alignment of aromatic rings and this might have resulted from the inductive effect of functionalites of respective phenyl rings.

Incorporation of β-PPA linkage (spacer) in **10m** allows it to adopt new confirmation, which may favors covalent interaction of cyanopyrrolidine ring with Ser_{630} (S_1 pocket, covalent interaction of cyanopyrrolidine ring as reported for cyanopyrrolidine derivatives), strong H-bonding of back-bone benzamide-NH with Glu dyad (S_2 pocket) and *para*-nitrile benzamide with Arg_{358}, including aromatic π- π stacking of benzamide with Phe_{357} in S_3 pocket. As observed with Denagliptin, **10m** docks very well into all the three sites (S_1, S_2 and S_3) of DPP-IV crystal structure and these favorable interactions of **10m** across all the three sites of DPP-IV enzyme support its potent *in vitro* DPP-IV inhibitory activity and excellent selectivity over other protease.

CONCLUSION

In summary, we have reported a SPPS approach to discover peptidomimetic based cyanopyrrolidines derivatives as potent and selective inhibitors of DPP-IV and devoid of CYP liabilities. Some of these novel peptidomimetics showed excellent *in vitro* potency and selectivity over other serine proteases, due to their favorable orientations across all the three binding sites.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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DISCLOSURE

The authors declare that there is no conflict of interest that would prejudice the impartiality of the research reported herein.

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Design, synthesis and biological evaluation of novel aminomethyl-piperidones based DPP-IV inhibitors ☆

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ABSTRACT

A series of novel aminomethyl-piperidones were designed and evaluated as potential DPP-IV inhibitors. Optimized analogue **12v** ((4*S*,5*S*)-5-(aminomethyl)-1-(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)-4-(2,5-difluorophenyl)piperidin-2-one) showed excellent in vitro potency and selectivity for DPP-IV over other serine proteases. The lead compound **12v** showed potent and long acting antihyperglycemic effects (in vivo), along with improved pharmacokinetic profile.

The prevalence of Type 2 diabetes mellitus (T2DM) is rapidly increasing (~371 million diabetic patients worldwide) and there is a great need for new drug classes to ameliorate hyperglycemia, while addressing additional accompanying elements of the metabolic syndrome.[1] Dipeptidyl peptidase-IV (DPP-IV) is a serine protease,[2] responsible for the inactivation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), both of which enhance insulin secretion in a glucose-dependent manner.[3] DPP-IV inhibitors are a new class of oral medications, which have been in use for 7 years, as a second-line therapy. DPP-IV inhibitors are generally well tolerated, safe (low risk of hypoglycemia) and weight neutral. There are currently eight gliptins approved worldwide with several more on the way.[4]

In recent years, a large variety of scaffolds being discovered as a next generation DPP-IV inhibitors, particular efforts being made to develop the long acting DPP-IV inhibitors.[4,5] Two drugs (Omarigliptin and Trelagliptin) are currently under development for once-weekly dosing to improve patients compliance.[4] Structurally, DPP-IV enzyme resembles with several other protease, so while designing new class of DPP-IV inhibitors, it is essential to consider selectivity of DPP-IV inhibitors over other serine protease, especially DPP-2, DPP-8 and DPP-9.[6]

Structurally distinct and rigid analogs of Sitagliptin (**1**), such as *N*-aryl aminopiperidine (**2**), aminopiperidine-fused imidazoles (**3**) and tetrahydropyran (**4**) derivatives were identified as a novel class of DPP-IV inhibitors (Fig. 1).[7–10] These newly discovered DPP-IV inhibitors exhibit potent DPP-IV inhibitory activity, good off-target selectivity and improved pharmacokinetic profiles. Earlier, we reported peptidomimetics based long acting DPP-IV inhibitors.[11] In continuation to our ongoing research on DPP-IV inhibitors, we report herein design, synthesis and biological evaluation of novel aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**) based DPP-IV inhibitors. Title compounds are designed based on the piperidone skeleton and we anticipated that the aminomethyl and the amide groups of the piperidone ring might contribute for improved pharmacokinetic and pharmacodynamic effects, along with the potent and selective DPP-IV inhibitory activity.

As depicted in Scheme 1, synthesis of the aminomethyl-piperidones based DPP-IV inhibitors (**12a–v**, **13a–e** and **14a–e**) commenced with a Horner–Wadsworth–Emmons reaction of aldehydes (**5a–c**), followed by Michael addition, to get diester (**6a–c**). Reduction of nitrile group of **6a–c** by hydrogenation, using Adam's catalyst, followed by cyclization and ester regeneration by trimethylsilyldiazomethane yielded piperidone-carboxylate (**7a–c**), with >85% *trans* selectivity.[12] *Trans* racemic mixture [(3*R*,4*S*) and (3*S*,4*R*)] of (**7a–c**) were isolated in pure form by

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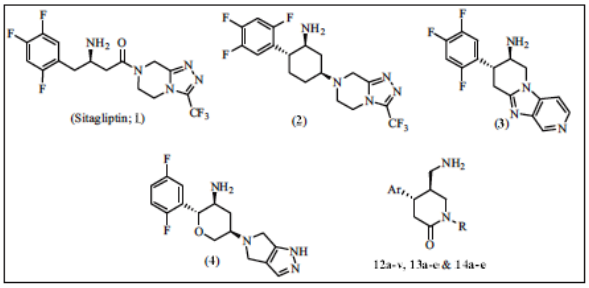
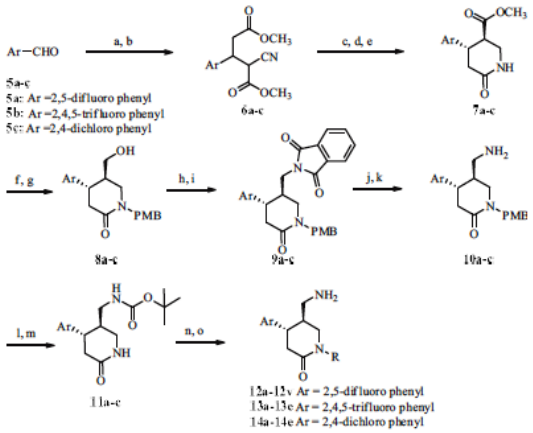


Figure 1. Structurally diverse small molecule based-DPP-IV inhibitors.



Scheme 1. Synthesis of compounds **12a–v**, **13a–e** and **14a–e**. Reagents and conditions: (a) $(Et_2O)_2POCH_2COOMe$, Na_2CO_3, EtOH; (b) $NCCH_2COOMe$, NaOMe, MeOH; (c) H_2, PtO_2, HCl, MeOH; (d) K_2CO_3, toluene/MeOH; (e) Me_3SiCHN_2, Et2O/MeOH; (f) PMB-Br, NaHMDS, THF/DMF(4:1), −78 °C; (g) $LiAlH_4$, THF, 0 °C; (h) CH_3SO_2Cl, NEt_3, DCM, 0 °C; (i) potassium phthalimide, DMF, 90 °C; (j) NH_2–NH_2, EtOH, 25 °C; (k) chiral resolution: D-tartaric acid, MeOH; (l) Boc_2O, NEt_3, THF/H_2O(3:2), 25 °C; (m) CAN, CH_3CN/H_2O(3:1), 25 °C; (n) R–X, CuI, *N,N*-dimethylethylenediamine, toluene, reflux or R–X, NaH, DMF, 0 °C to 25 °C; (o) concd HCl/EtOAc(1:3), −50 °C, 2 h, 0 °C, 1 h.

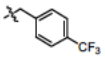
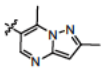
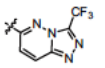
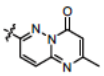
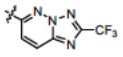
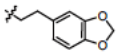
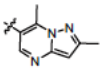
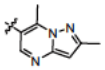
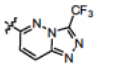
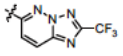
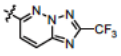
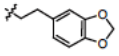
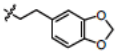
removing corresponding *cis* racemic mixture [(3*R*,4*R*) and (3*S*,4*S*)], by column chromatography (mobile phase: 0–3% methanol in DCM, using 100–200 mesh silicagel). Amide –*NH* protection of *trans* racemic **7a–c** with *para*-methoxy benzyl (PMB) group and reduction of ester with lithium aluminium hydride (LiAlH4) yielded *trans* racemic alcohol (**8a–c**). Subsequently, **8a–c** were converted to a good leaving group (methanesulfonate derivatives), which upon treatment with potassium phthalimide via Gabriel synthesis type reaction lead to the formation of *trans* racemic phthalimido-piperidones (**9a–c**).

Hydrazinolysis of phthalimido group of **9a-c** lead to the formation of *trans* racemic aminopiperidones (**10a–c**). *trans* racemic **10a–c** was subjected for chiral resolution (**10a–c** was added to a solution of D-tartaric acid (1.1 equiv D-tartaric acid, dissolved in 100 ml methanol) and the mixture was stirred for 15 h at 25 °C, solid precipitated was filtered off, washed with methanol (200 ml) and dried to get enantiomerically pure (4*S*,5*S*) desired piperidones (**10a–c**) as a tartrate salt, with >97% ee (chiral HPLC analysis conditions: CHIRALCEL OD-H column, using mobile phase as n-hexane and 0.1% diethyl amine in EtOH (98:02)). Further, protection of primary amine of **10a–c** with Boc-group and subsequent oxidative removal of PMB group gave Boc-aminopiperidones (**11a–c**). Various haloheterocycles/halo-aromatics of the interest were coupled with **11a–c**, by Goldberg reaction[13] or by nucleophilic substitution, followed by Boc-deprotection to get the chiral pure (4*S*,5*S*) aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**).[14] All the test compounds obtained were purified by preparative HPLC (yield 70–85%; HPLC purity >97% and chiral purity >97%ee) and characterized by various spectroscopic techniques (^{13}C NMR, ^1H NMR and ESI MS). Elemental analyses were determined within 0.04% of theoretical values (see Supplementary data for analytical and spectral data).

The in vitro DPP-IV inhibitory activity was determined in order to establish the structure-activity relationship (SAR).[15] Three sets of the aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**) were prepared (Table 1). In the first set (Ar = 2,5-difluoro phenyl), 22 compounds (**12a–v**) were prepared by coupling 2,5-difluoro phenyl-aminopiperidone (**11a**) with various halo-heterocycles/halo-aromatics. In the second set (Ar = 2,4,5-trifluoro phenyl), 5 compounds (**13a–e**) were prepared by replacing 2,5-difluoro phenyl with 2,4,5-trifluoro phenyl, while in third set (Ar = 2,4-dichloro phenyl), 5 compounds (**14a–e**) were prepared by replacing 2,5-difluoro phenyl with 2,4-dichloro phenyl. All the test compounds showed varying degrees of DPP-IV inhibitory activity (IC_{50}), depending on the nature of the substituents.

Within the first set (**12a–v**), test compounds showed diverse DPP-IV inhibitory activity depending on the nature of substituents on piperidone ring system. Compounds with electron withdrawing groups (**12b**: –CN, **12c**: –F and **12d**: $-CF_3$) at *para*-position of phenyl ring system showed improved DPP-IV inhibitory activity, compared to unsubstituted derivative (R = –Ph; **12a**). Compounds with electron donating groups (**12e**: –OMe and **12f**: $-SO_2$–Me) at *para*-position of phenyl ring showed further improvement in in vitro DPP-IV inhibitory activity. Replacement of phenyl ring system with 3-pyridyl (**12g**) and further substitutions with electron donating (**12h**) and withdrawing (**12i** and **12j**) groups at *para*-position showed moderate DPP-IV inhibitory activity. Replacement of phenyl ring system with quinoline (**12m**), triazolo[4,3-*a*]pyrazine (**12n**), 2-methyl-pyrimido[1,2-*b*]pyridazinone (**12o**), benzyl (**12k**) and further substitutions with electron withdrawing (**12l**) groups at *para*-position showed moderate DPP-IV inhibitory activity. Substitutions with ethylbenzene (**12p**), ethylpyridine (**12q**), dimethylpyrazolo[1,5-*a*]-pyrimidine (**12r**), 3-methyl-triazolo [4,3-*b*]pyridazine (**12s**), 3-trifluoromethyl-triazolo[4,3-*b*]pyridazine (**12t**) and 2-trifluoromethyl-triazolo[1,5-*b*]pyridazine (**12u**) showed good DPP-IV inhibitory activity, while **12v** (methylenedioxy phenethyl) showed superior DPP-IV inhibitory activity (IC_{50}: 8.5 ± 0.4 nM), compared to Sitagliptin (IC_{50}: 18±2.4 nM).

Table 1
In vitro DPP-IV inhibitory activity of aminomethyl-piperidones (**12a–v**, **13a–e** and **14a–e**).*

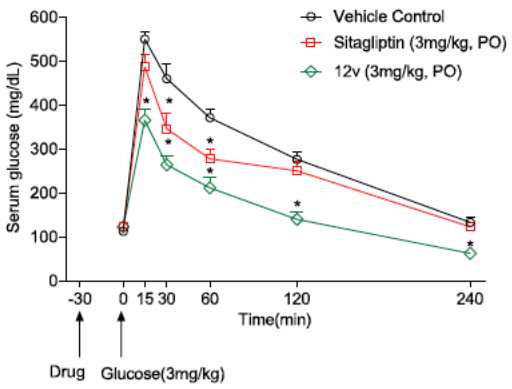
Compd	R	IC_{50}** (nM)	Compd	R	IC_{50}** (nM)	Compd	R	IC_{50}** (nM)
12a		1436 ± 12.3	**12l**		910 ± 3.1	**13a**		157 ± 4.1
12b		378 ± 1.4	**12m**		1034 ± 21.2	**13b**		119 ± 1.0
12c		382 ± 4.5	**12n**		1023 ± 3.1	**13c**		125 ± 2.7
12d		342 ± 3.3	**12o**		997 ± 13.5	**13d**		111 ± 2.1
12e		217 ± 8.6	**12p**		119 ± 4.2	**13e**		19 ± 5.1
12f		193 ± 8.4	**12q**		84 ± 2.6	**14a**		197 ± 4.2
12g		1388 ± 5.9	**12r**		77.6 ± 1.2	**14b**		148 ± 3.7
12h		452 ± 3.7	**12s**		122 ± 3.2	**14c**		134 ± 7.3
12i		443 ± 5.3	**12t**		79 ± 0.2	**14d**		137 ± 9.6
12j		404 ± 7.7	**12u**		74 ± 0.9	**14e**		43 ± 3.2
12k		885 ± 11.2	**12v**		**8.5 ± 0.4**	**Sitagliptin**	–	**18 ± 2.4**

Bold IC_{50} values of 12v and sitagliptin represents most potent compounds in Table 1.
* DPP-IV inhibitory activity determined by fluorescence-based assay; fluorescence measured using Spectra Max fluorometer (Molecular Devices, CA) by exciting at 380 nm and emission at 460 nm. IC_{50} determined using Graph Pad prism software.
** DPP-IV inhibitory activity represented as IC_{50} (nM), expressed as the mean ± SD (n = 3).

In the second set (**13a–e**, Ar = 2,4,5-trifluoro phenyl), all the five compounds showed good activity, but compared to 2,5-difluoro phenyl series (Set-1 analogs, **12q**, **12r**, **12t**, **12u** and **12v**), in vitro DPP-IV inhibition were found to be bit weaker, while in set three (**14a–e**, Ar = 2,4-dichloro phenyl), in vitro DPP-IV inhibition were found to be slight weaker than Set-1 and Set-2 corresponding analogs. Thus the nature and position of halogen atom on aromatic ring system contributed significantly towards in vitro DPP-IV inhibition.

The in vitro selectivity over serine protease, especially DPP-2, DPP-8 and DPP-9 was evaluated for **12V** and it showed >5000-fold selectivity over DPP-2 and >10,000-fold selectivity over DPP-8 and DPP-9.[15] To assess the CYP liabilities, **12v** was subjected for CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition studies (@ 1, 10 and 100 μM concentrations) and the test compound **12v** was found to be devoid of CYP1A2, CYP2C8, CYP2C9, CYP2D6, CYP2C19 and CYP3A4 inhibition up to 100 μM concentrations.[16]

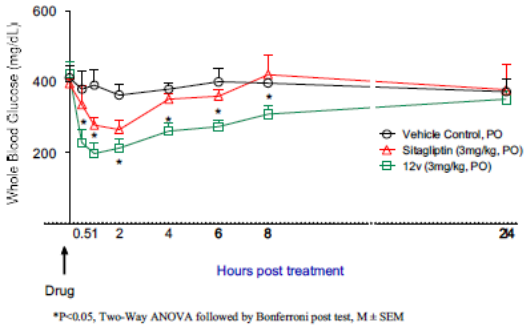
Detailed pharmacodynamic (PD) as well as pharmacokinetic (PK) profiling of **12v** was carried out. The in vivo antidiabetic activity of **12v** and Sitagliptin (@ 3 mg/kg, p.o.) was evaluated in male C57BL/6J mice, using OGTT (oral glucose tolerance test) protocol and changes in serum glucose levels (AUC glucose up to 240 min; mg/dL) was estimated (Fig. 2).[17] Compound **12v** showed good oral antidiabetic activity (% decrease in AUC glucose 38.9 ± 5.20), which



*P<0.05, Two-Way ANOVA followed by Bonferroni posttest, Mean ± SEM

Figure 2. In vivo antidiabetic activity of **12v** and Sitagliptin in C57 mice.

was found to be better than Sitagliptin (% decrease in AUC glucose 17.9 ± 4.58). In C57 mice, it was interesting to observe that **12v** showed suppression in the blood glucose at all the time points



*P<0.05, Two-Way ANOVA followed by Bonferroni post test, M ± SEM

Figure 3. In vivo antidiabetic activity of **12v** and Sitagliptin in db/db mice.

(15, 30, 60, 120 and 240 min) compared to vehicle control, while Sitagliptin showed blood glucose reduction only at 30 and 60 min.

Further to understand the duration of action and effect of **12v** on post-prandial glucose excursion, single dose (@ 3 mg/kg, po) antidiabetic activity of **12v** and Sitagliptin was evaluated in fed-db/db mice (hyperglycemic animals) for 24 h (Fig. 3). Under fed condition, compared to vehicle control, **12v** and Sitagliptin showed good antidiabetic activity (% decrease in AUC glucose 38.29 ± 12.13 and 20.80 ± 11.06, respectively) up to 2 h. However, **12v** showed prolonged suppression of serum glucose levels (% decrease in AUC glucose 20.62 ± 7.05 for **12v** and 1.48 ± 11.84 for Sitagliptin, up to 24 h).

A comparative single dose (3 mg/kg iv or p.o.) PK profile of **12v** and Sitagliptin was evaluated in male C57BL/6J mice ($n = 6$) and the various PK parameters (T_{max}, $T_{1/2}$, C_{max}, AUC and %F) were recorded (Table 2).[18] In PK study, **12v** showed rapid T_{max}, higher AUC (~twofold compared to Sitagliptin), extended half-life ($T_{1/2}$: >8 h) compared to Sitagliptin and good oral bioavailability (%F: 79.5%). Compound **12v** showed extended half-life and higher AUC, which could be due to its low clearance compared to Sitagliptin (elimination rate constant (kel; h^{-1}), 0.12 ± 0.02 for **12v** and 0.84 ± 0.14 for Sitagliptin). Thus improved pharmacokinetic profile of compound **12v** justifies its potent and prolonged antidiabetic activity in C57 and db/db mice.

Interestingly, various gliptins, currently used in the clinic (Sitagliptin, Vildagliptin, Saxagliptin, Alogliptin and Linagliptin), exhibit short half-life thereby requires once or twice daily drug administration.[19] Further to regulate the pre- and post-prandial blood glucose and thereby to control HbA1c, several long-acting DPP-IV inhibitors (Omarigliptin and Trelagliptin) are under developments, as once-weekly drugs.[4] Their clinical efficacy and side effects profile appear to be comparable with other gliptins in the class, however, their infrequent dosing creates a niche and promotes patients compliance.[4] In this context, overall pre-clinical profile of **12v** demonstrated added advantages over currently practiced gliptins and appears to serve as long-acting DPP-IV inhibitors.

The molecular docking analysis of **12v** and Sitagliptin, in the binding pocket of DPP-IV was carried out using extra precision (XP) Glide docking method (Fig. 4).[20] The X-ray structure of the DPP-IV enzyme (PDB ID: 2OQI) was obtained from the protein data bank and the protein structure was prepared using protein preparation wizard module of Schrödinger. For docking study, the ligands were geometrically optimized and prepared by using ligprep module of Schrödinger.[18] The overlay of binding poses of **12v** (Orange) and Sitagliptin (Maroon) in the DPP-IV active site is shown in Figure 4. As observed with Sitagliptin, **12v** docks very well into all the three sites (G-scores −11.81 (9/9) and −10.99 (9/9) for **12v** and Sitagliptin respectively). Although, G-score of **12v** and Sitagliptin are comparable, however, in vitro, DPP-IV IC_{50} of **12v** is half of that of Sitagliptin, which could be due to favorable interactions of **12v**, in all the three binding pockets. Di-fluorophenyl ring of **12v** occupies S_1 pocket. In S_2 pocket, aminomethyl groups of piperidone ring forms H-bonding with the side-chains of Glu_{205} and Glu_{206} dyad, while methylenedioxy phenyl ring of **12v** accommodates very well in S_3 pocket, which together supports excellent in vitro DPP-IV activity and selectivity of **12v** over other protease.

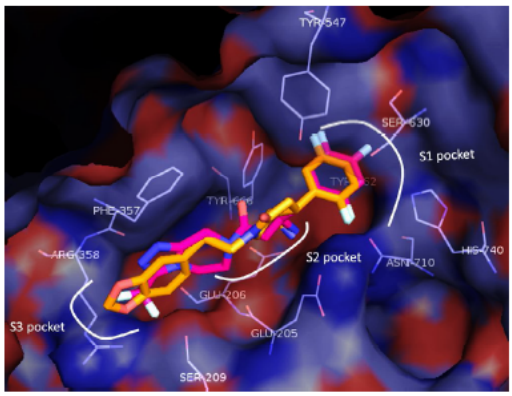


Figure 4. Key interactions of compound **12v** and Sitagliptin with active sites of DPP-IV enzyme. Binding pose of compound **12v** (Orange) and Sitagliptin (Maroon) in the DPP-IV active site is indicated (Surface view: Blue), wherein both compounds interact closely with key residues of site S_1, S_2 and S_3.

In summary, we report discovery of novel aminomethyl-piperidone derivatives as potent, selective and long acting DPP-IV inhibitors for the treatment of T2DM. The lead compound **12v** ((4*S*,5*S*)-5-(aminomethyl)-1-(2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)-4-(2,5-difluorophenyl)-piperidin-2-one) showed prolonged suppression of pre-and post-prandial blood glucose levels (in vivo), which correlates with its extended PK profile.

Acknowledgment

We are grateful to the management of Zydus Group for encouragement and support.

Table 2
Pharmacokinetic study parameters[a] of **12v** and Sitagliptin

Compd	T_{max} (h)	C_{max} (μg/ml)	$T_{1/2}$ (h)	AUC (0-∞) h μg/ml	%F[b]
12v	0.28 ± 0.12	0.42 ± 0.03	8.99 ± 0.31	1.01 ± 0.09	79.5
Sitagliptin	0.22 ± 0.10	0.31 ± 0.01	1.56 ± 0.11	0.56 ± 0.02	75.7

[a] In male C57BL/6J mice ($n = 6$), compounds were administered orally (p.o.) at 3 mg/kg dose and plasma concentration was analyzed by LC–MS, values indicate mean ± SD.
[b] Oral bioavailability (%F) was calculated wrt to iv AUC (**12v**: 1.27 ± 0.08 and Sitagliptin: 0.74 ± 0.09 h μg/ml) administered at 3 mg/kg dose, iv.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.009.

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17. (a) Chen, D.; Wang, M. *Diabetes Obes. Metab.* **2005**, *7*, 307; (b) Kim, J. G.; Baggio, L. L.; Bridon, D. P.; Castaigne, J. P.; Robitaille, M. F.; Jette, L.; Benquet, C.; Drucker, D. J. *Diabetes* **2003**, *52*, 751; (c) Study was conducted in male C57BL/6J (using OGTT protocol) or without glucose load, in db/db mice (age 8–12 weeks). All animal experiments were conducted according to the internationally valid guidelines following approval by the 'Zydus Research Center Animal Ethical Committee'. Two days prior to the study, the animals were randomized and divided into 2 groups ($n = 6$), based upon their fed glucose levels. Animals were left for 2 days under acclimatization and maintained on a standard diet. On the day of experiment, food was withdrawn from all the cages, water was given ad-libitum and were kept for overnight fasting. Briefly, in OGTT protocol (C57 mice) overnight fasted mice were dosed orally (p.o.) with the test compounds (3 mg/kg), 0.5 h prior to the oral glucose load, while in db/db mice, fed mice were dosed orally (p.o.) with the test compounds (3 mg/kg) and the blood samples were collected at various time points. Blood samples were centrifuged and the separated serum was immediately subjected for the glucose estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, California). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (Ver 4.0) was used to plot an area under the curve (0–240 min AUC). The AUC obtained from graphs were analyzed for two-way ANOVA, followed by Bonferroni post test, using Graph Pad prism software.
18. Briefly, for single dose PK study, test compounds were administered orally/iv on a body weight basis (3 mg/kg) to overnight fasted male C57BL/6 J mice. Serial blood samples were collected in microcentrifuge tubes containing EDTA at pre-dose, 0.15, 0.3, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 36 and 48 h post-dose after compounds administration. Approximately 0.2 ml of blood was collected at each time point and centrifuged at 4 °C. The obtained plasma was frozen, stored at −70 °C and the concentrations of compounds in plasma were determined by the LC–MS/MS (Shimadzu LC10AD, USA), using YMC hydrosphere C18 (2.0 × 50 mm, 3 μm) column (YMC Inc., USA). The pharmacokinetic parameters, such as T_{max}, $t_{1/2}$, C_{max}, AUC and %F were calculated using a non-compartmental model of WinNonlin software version 5.2.1.
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