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Rational design and synthesis of potent and long-lasting glutamic acid-based dipeptidyl peptidase IV inhibitors

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ABSTRACT

A series of (2*S*)-cyanopyrrolidines with glutamic acid derivatives at the P2 site have been prepared and evaluated as inhibitors of dipeptidyl peptidase IV (DPP-IV). The structure–activity relationships (SAR) led to the discovery of potent 3-substituted glutamic acid analogues, providing enhanced chemical stability and excellent selectivity over the closely related enzymes, DPP8, DPP-II and FAP. Compound **13f** exhibited the ability to both significantly decrease the glucose excursion and inhibit plasma DPP-IV activity.

Dipeptidyl peptidase IV (DPP-IV, also known as CD26) (EC 3.4.14.5) is a prolyl dipeptidase involved in the in vivo degradation of two insulin-sensing hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), by cleaving at the peptide bond of the penultimate position.[1,2] Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by intestinal L-cells in response to food intake.[3] The active form of GLP-1 is a 30-amino acid peptide, which stimulates insulin release, inhibits glucagons release, and slows gastric emptying, each a benefit in the control of glucose homeostasis in patients with type II diabetes.[4–7] Thus inhibition of DPP-IV extends the half-life of endogenously secreted GLP-1, which in turn enhances insulin secretion and improves the glucose tolerance. DPP-IV inhibitors offer several potential advantages over existing therapies including decreased risk of hypoglycemia, potential for weight loss, and the potential for regeneration and differentiation of pancreatic β-cells.[8] Therefore, DPP-IV has become a validated target for the treatment of type II diabetes, and several inhibitors of DPP-IV are currently undergoing late-stage clinical trials; the first DPP-IV inhibitor, sitagliptin **1** (Januvia, Merck) was approved in October 2006 (Fig. 1),[9,10] vildagliptin **2** (Glavus, Novartis) was approved for use in Europe in September 2007.[11]

With few exceptions, most DPP-IV inhibitors resemble the P2–P1 dipeptidyl substrate cleavage product. As shown in Figure 1, vildagliptin **2** is a covalent inhibitor. Often this electrophilic cyano-

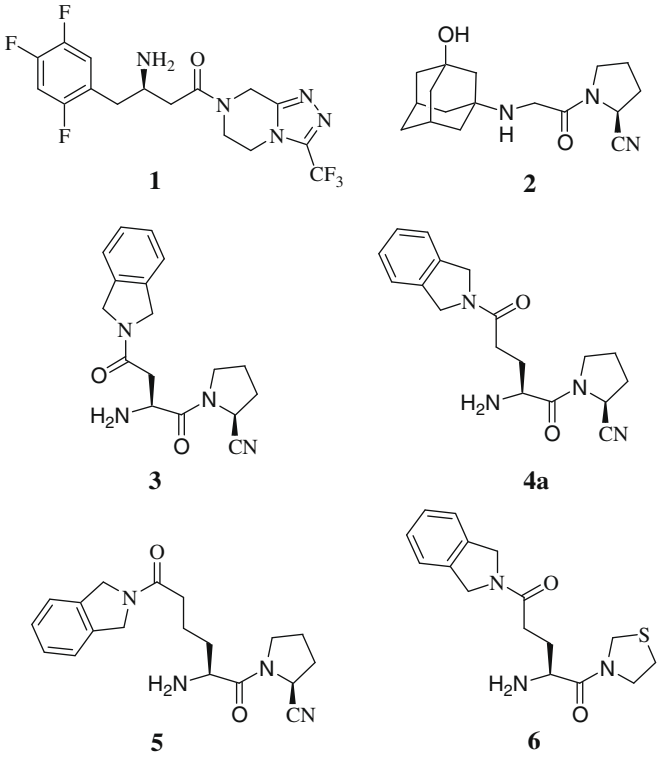

Figure 1. Inhibitors of DPP-IV.

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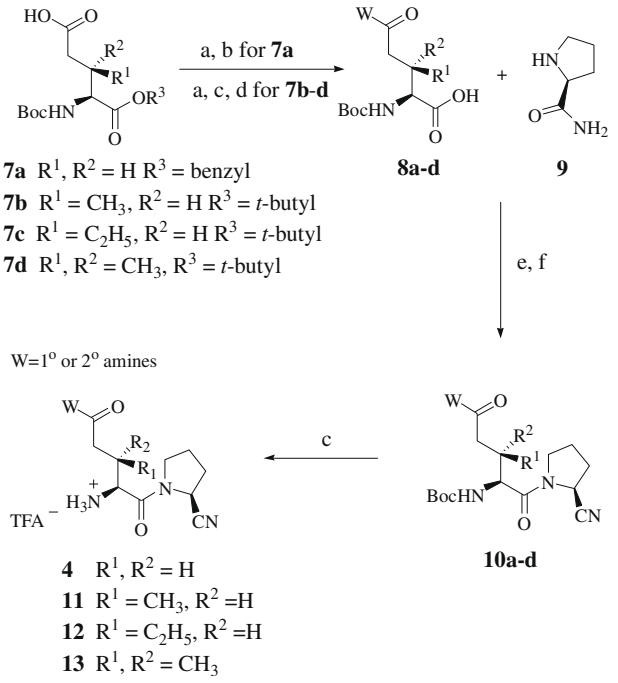
pyrrolidine is able to bind covalently with the serine 630 in the S1 pocket of DPP-IV, whereas the non-covalent inhibitor sitagliptin **1** depend on non-covalent protein–ligand interactions and the substituted phenyl group occupies very well the hydrophobic S1 pocket of the enzyme.[9] Previous report from our laboratories described asparatic acid derivatives as potent DPP-IV inhibitors (**3**, Fig. 1),[12a] but this class of inhibitors lacks selectivity toward DPP8 (Table 1). This selectivity is an important criterion for their further development as antidiabetic agents, since in vivo studies indicate that selective inhibition of DPP8/9 may be associated with profound toxicities.[13] Extensive SAR studies by replacement of aspartic acid with glutamic acid at the P2 site, glutamic acid derivative **4a** is 150-fold more potent in DPP-IV inhibition and 800-fold more selective toward DPP8 than aspartic acid derivative **3**.[12b,12c] In comparison with **4a**, lengthening by one carbon (**5**) results in fivefold lost in DPP-IV potency, and suffers from poor selectivity over DPP8, DPP-II,[14] and FAP (Fibroblast activation protein).[15] The cyanopyrrolidine derivatives **3–5** are potent DPP-IV inhibitors, but the electrophilic nitrile group makes these compound instable due to reaction with the primary amine of the P2 amino acid.[16] Related compound thiazolidine **6** lacking an electrophile possess much greater chemical stability, but this compound shows a drastic reduction in DPP-IV inhibition (IC_{50} = 1.6 μM).

The data shown in Table 1 compare the DPP-IV potency and selectivity of compounds **3–6** to two diabetes drugs **1** and **2**, we selected compound **4a** as a lead compound for further modification in an effort to improve intrinsic selectivity and stability of compound **4a**. Augeri et al. reported that increasing the degree of branching at the β-position of the alkylglycine substituent increases solution stability.[17] Based on the result, we decided to add alkyl substituent in the β-position of the P2 site glutamic acid (Scheme 1, compounds **11–13**) and investigated the effects of methyl, ethyl and dimethyl substituents on stability and selectivity compared to unsubstituted **4**. This study describes here has led to the discovery of the potent, selective and stable DPP-IV inhibitor **13f** and its analogues. This selective **13f** demonstrated in vivo efficacy comparable to that of DPP-IV inhibitor **2**.

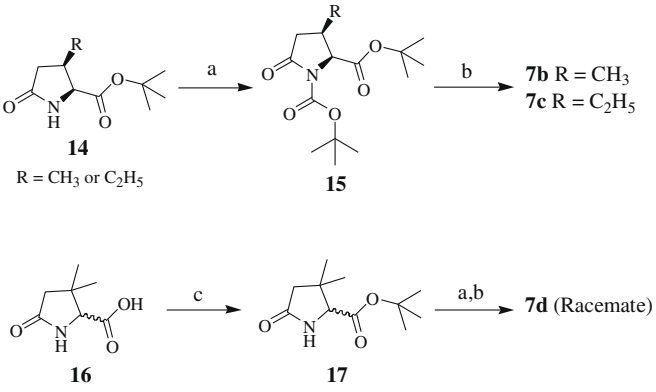
A series of (2*S*)-cyanopyrrolidines with glutamic acid derivatives at the P2 site, compounds **4** and **11–13**, was prepared as described in Scheme 1. Inhibitors **4** and **11–13** were synthesized from **7a–d**, respectively. Boc-L-glutamic acid 1-benzyl ester **7a** is commercially available. The preparation of Boc-protected (3*R*)-3-methyl and -ethyl 1-*tert*-butyl ester **7b–c** and 3,3-dimethyl 1-*tert*-butyl ester **7d** (racemate) will be discussed in Scheme 2. EDC coupling of **7a–d** with primary or secondary amines was followed by hydrogenation to give **8a** or by deprotection of the *tert*-butylcarbamate and *tert*-butyl ester and reprotection of the free amines to give acids **8b–d**. Compounds **8a–d** were then coupled with L-prolinamide **9** followed by dehydration of the amides to provide **10a–d**. (*2S*)-Glutamic acid derivative **10d** (less polar fraction) and another diastereomer of **10d** (more polar fraction) can be separated by chromatography. Removal of the BOC protecting group of **10a–d** with trifluoroacetic acid yielded the desired glutamic acid derivatives **4** and **11–13**, respectively. The assignment of configuration at the α-carbon of 3,3-dimethyl glutamic acid was based on the fact that compound **13a** derived from corresponding **10d** showed at least 10-fold more potent than another diastereomer of **13a** as DPP-IV inhibitor. The result permitted the assignment of (*2S*) configuration of 3,3-dimethyl glutamic acid to the more active compound **13**.

Table 1
Inhibition of DPP-IV, DPP8, DPP-II and FAP by compounds **1–6**

Compd	IC_{50} (μM)			
	DPP-IV	DPP8	DPP-II	FAP
1	0.030	>20	>20	>20
2	0.056	14	>20	>20
3	0.298	0.22	>20	>20
4a	0.002	1.2	15	1.2
5	0.010	0.36	2.6	0.054
6	1.6	>20	>20	12



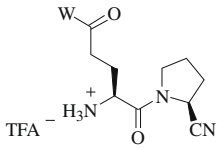
Scheme 1. Synthesis of glutamic acid derivatives: Reagents: (a) EDC, HOBt, 1,4-dioxane/CH_2Cl_2, various amines; (b) H_2, 5% Pd/C, MeOH; (c) TFA, CH_2Cl_2; (d) $(Boc)_2O$, $NaHCO_3$, H_2O/1,4-dioxane; (e) EDC, HOBt, 1,4-dioxane/CH_2Cl_2; (f) imidazole, $POCl_3$, pyridine.



Scheme 2. Synthesis of **7b–d**: Reagents: (a) $(Boc)_2O$, 4-DMAP, CH_2Cl_2; (b) $NaOH_{(aq)}$, THF; (c) DMF-DBA, toluene, 80 °C.

As shown in Scheme 2, Boc-protected **7b–c** and **7d** were prepared from cyclic (*2S,3R*)-3-methyl or ethyl pyroglutamate **14**[18a] and racemic cyclic 3,3-dimethyl pyroglutamic acid **16**,[18b] respectively. Compounds **14** and **16** were synthesized according to the literature procedures.[18] The treatment of **14** with $(Boc)_2O$ in the presence of 4-(dimethylamino)pyridine (4-DMAP) in dichloromethane gave **15**, which selectively hydrolyzed the *N*-Boc protected amide bond using NaOH (1.5 equiv) in THF/H_2O to furnish **7b–c**. Racemic acid **16** was effected by treatment with *N,N*-dimethylformamide di-*tert*-butyl acetal to provide *tert*-butyl ester **17**. Compound **17** can be transformed to 3,3-dimethyl 1-*tert*-butyl ester **7d** (racemate) by two-step reaction as **14** was treated.

Table 2
Inhibition of DPP-IV, DPP8, DPP-II and FAP by compound **4**



4

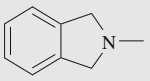
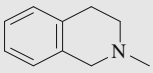
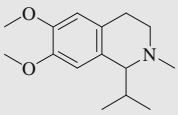
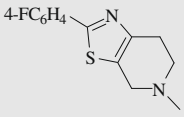
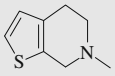
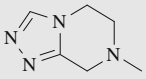
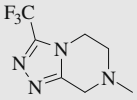
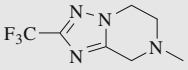
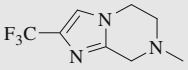
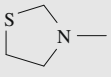
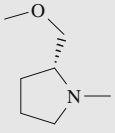
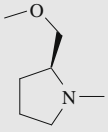
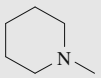
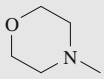
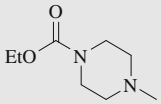
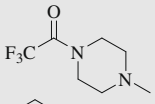
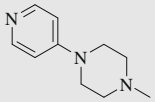
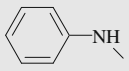
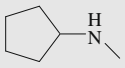
Compd	W	IC_{50} (μM)[a]				SI[c]
		DPP-IV	DPP8	DPP-II	FAP	
4a		0.002	1.2	8.4	1.2	600
4b		0.005	0.83	10	8.8	166
4c[b]		0.035	1.2	4.5	6.1	34
4d		0.040	1.0	12	2.3	25
4e		0.006	2.5	12	7.5	417
4f		0.007	7.2	>20	>20	1028
4g		0.005	5.7	4.5	>20	1140
4h		0.009	11	>20	>20	1222
4i		0.006	7.9	12	14	1316
4j		0.007	5.3	>20	11	757
4k		0.010	3.9	>20	8.3	390
4l		0.022	5.5	>20	21	250
4m		0.007	12	>20	20	1714

Table 2 (*continued*)

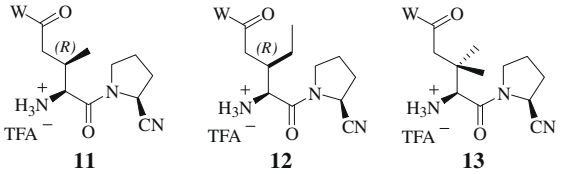
Compd	W	IC_{50} (μM)[a]				SI[c]
		DPP-IV	DPP8	DPP-II	FAP	
4n		0.015	2.7	>20	>20	180
4o		0.017	4.9	>20	>20	288
4p		0.031	2.5	>20	3.1	81
4q		0.008	3.9	>20	19	488
4r		0.019	0.98	17	0.47	52
4s		0.037	1.5	ND	ND	41
4t		0.011	5.4	>20	>20	491

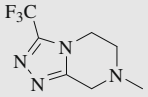
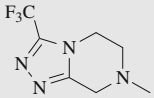
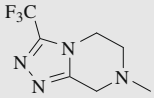
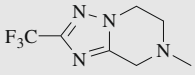
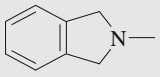
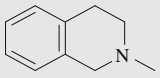
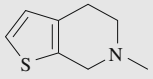
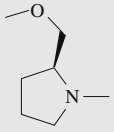
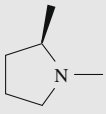
[a] Means of at least three experiments; standard deviations are ±40%; see Ref. 21.
[b] The first carbon position of isoquinoline is racemate.
[c] Selectivity index (SI) = DPP8 IC_{50}/ DPP-IV IC_{50}.

Generally, this class of inhibitors shown in Table 2 and Table 3 exhibited potent DPP-IV inhibition (IC_{50} < 40 nM), therefore improvement of selectivity against the off-target enzymes, in particular DPP-8 and DPP-9 was of great concern. Since inhibition of DPP8 and/or DPP9 is associated with significant toxicity in preclinical species.[13,19] A selectivity (DPP-IV/DPP8) index of >1000 is selected for the development candidates. Firstly, the effects of bicyclic group on the glutamic acid were investigated. To improve the selectivity and to maintain the potency toward DPP-IV, we replaced the isoindoline of lead compound **4a** at 5-position of glutamic acid with isoquinoline, generating isoquinoline analogues **4b** and **4c** (Table 2). Both compounds **4b–c** neither improved DPP-IV potency nor enhanced selectivity toward DPP8, DPP-II and FAP. Having failed to improve both the potency and selectivity of the lead compound **4a**, we set out to investigate the effect of varying heterobicyclic group. Since Gao et al. reported that heteroatoms or polar substituents at the P2 site would help to increase the DPP8 IC_{50}/ DPP-IV IC_{50} ratio, namely, to provide more selective DPP-IV inhibitors.[20] Nevertheless, 4,5,6,7-tetrahydro-thiazolo[5,4-*c*]pyridine **4d** and 4,5,6,7-tetrahydro-thieno[2,3-*c*]pyridine **4e** did not improved potency and selectivity. Compound **4d** showed 18-fold decrease in DPP-IV inhibition compared to lead **4a**. Further modification was carried out with more polar heterobicyclic building blocks, such as triazolopyrazine derivatives **4f–h** and imidazopyrazine **4i**, led to at least a 2.5-fold decrease in DPP-IV inhibitory potency compared to lead compound **4a**. In contrast, all the four compounds were more selective than **4a** for DPP-IV over DPP8 and FAP; the selectivity index (SI) of these heterobicyclic analogues (**4f–i**) for DPP-IV versus DPP8 inhibition is >1000-fold compared with **4a** which SI is 600-fold, and these compounds dramatically improved selectivity with respect to FAP (IC_{50} > 14 μM).

Next, we investigated the effects of varying monocyclic derivatives, such as five-member ring **4j–m** and six-member ring **4n–r**.

Table 3
Inhibition of DPP-IV, DPP8, DPP-II and FAP by compounds **11–13**



Compd	W	IC_{50} (μM)[a]				SI[b]
		DPP-IV	DPP8	DPP-II	FAP	
4a		0.002	1.2	15	1.2	600
11a		0.008	2.0	>20	7.1	250
12a		0.031	17	>20	>20	548
13a		0.012	10	>20	>20	833
13b		0.031	6.2	>20	>20	200
13c		0.012	1.9	>20	>20	158
13d		0.016	1.0	>20	>20	63
13e		0.025	1.2	>20	>20	48
13f		0.014	15	>20	>20	1071
13g		0.013	11	>20	>20	846

[a] Means of at least three experiments; standard deviations are ±40%; see Ref. 21.
[b] Selectivity index (SI) = DPP8 IC_{50}/ DPP-IV IC_{50}.

This approach did not result in improvement in potency but in selectivity (Table 2). Pyrrolidine **4j** exhibited a similar activity as thiazolidine **4k**, but **4j** was more selective than **4k** for DPP-IV over DPP8. Addition of a methoxymethyl group at the 2-position of pyrrolidine provided diasteromers **4l** and **4m**. The chirality at C2 (**4m** vs **4l**) has threefold effect on DPP-IV inhibition in favor of the (*S*) configuration, and *S*-diasteromer **4m** showed significantly better selectivity toward DPP8 (1700-fold) compared to *R*-diasteromer **4l** (250-fold). This methoxymethyl analogue **4m** represents the most selective DPP-IV inhibitor among the 5-substituted glutamic acid series of inhibitors. In comparison with five-member ring **4m**, six-member ring system, such as piperidine **4n** and morpholine **4o** showed a twofold decrease in potency (DPP-IV IC_{50} = 15–17 nM, Table 2), and had reduced selectivity over DPP8. As for piperazine derivatives, ethyl carbamate **4p** was a less potent inhibitor of DPP-IV in this series; its selectivity over DPP8 and FAP was relatively low. Replacement of carbamate in **4p** with trifluoro-acetamide **4q** was equipotent to five-member ring **4m**; however, **4q** displayed unacceptable DPP8 activity (IC_{50} = 3.9 μM). Interestingly, 1-pyridin-4-yl-piperazine **4r** inhibited DPP8 and FAP at submicromolar level. Further modification was carried out at bringing the nitrogen out of the ring system and developing the aniline derivative **4s** and cyclopentylamine derivative **4t**. Aniline substituent was detrimental to DPP-IV inhibition; cyclopentylamine substituent provided no improvement in selectivity over DPP8.

Among these compounds shown in Table 2, compounds **4f**, **4h** and **4m** were potent inhibitors for DPP-IV, with IC_{50} values of <10 nM; >1000-fold selectivity over DPP-IV, DPP-II and FAP. But these compounds (free base forms) are inherently unstable ($t_{1/2}$ < 24 h) because the amino group intramolecularly cyclizes with the nitrile thus forming a cyclic amidine or diketopiperazine.[16,17] For this reason, further development of a series of compounds with alkyl substituent at the β-position of the P2 site glutamic acid was continued. In this Letter, we just report (*R*) configuration at C3 of glutamic acid because (*3S*) configuration gave less selective than (*3R*) configuration (data not shown).

As shown in Table 3, the methyl group at C3 with (*R*) configuration (**11a**) resulted in a slight decrease in both potency and selectivity over DPP8 compared to unsubstituted **4g**. Ethyl analogue **12a** exhibited a sixfold decrease in DPP-IV potency, leading no improvement in selectivity index between DPP-IV and DPP8 relative to **4g**. For this reason, methyl or ethyl analogue was not further optimized. Increasing the bulk of the branching by introduction of a dimethyl group gave compounds **13a–g**. Compared with monoalkyl analogues **11a** and **12a**, compound **13a** still maintained good potency (IC_{50} = 12 nM) and showed 1000-fold selectivity over DPP8. Apparently, dimethyl group is well tolerated at the C3 position; further optimization of a series of compounds with 3,3-dimethyl group was continued. Next, moving the nitrogen from the 2 to 3 position in the triazolopiperazine of **13a** (analogue **13b)** appeared unfavorable for DPP-IV inhibition (IC_{50} = 31 nM). The same general trend for dramatically decreased selectivity over DPP8 when the triazolopiperazine bicycle (**13a**, 833-fold) was replaced by less polar bicycles, such as isoindoline (**13c**, 158-fold), isoquinoline (**13d**, 63-fold) and thiazolo[5,4-*c*]pyridine (**13e**, 48-fold). In comparison with **13a**, both analogues **13c-d** maintained potency, but **13e** gave a twofold decrease in potency. Monocyclic pyrrolidines with methoxymethyl group and methyl group, **13f** and **13g**, remained potency against DPP-IV with >800-fold selectivity versus DPP8. Except for monomethyl analogue **11a**, all the compounds with alkyl substituent at the β-position of the P2 site glutamic acid shown in Table 3 were inactive toward DPP-II and FAP (IC_{50} > 20 μM).

Compound **13f** is a potent and selective DPP-IV inhibitor with a high stability in aqueous solution ($t_{1/2}$ > 7 days in phosphate buffer solution, pH ~7.4), and thus this compound was chosen for more extensive in vivo studies. In an oral glucose tolerance test (OGTT),[21] compounds were administered by oral route at 3 mpk to C57BL/6j mice 30 min before glucose administration (3 g/kg), and then blood samples drawn and analyzed for glucose levels. The glucose AUC was determined from 0 to 120 min, OGTT data on **2** and **13f** are summarized in Figure 2. Both **2** and **13f** significantly suppressed the glucose excursion observed after glucose challenge. For the

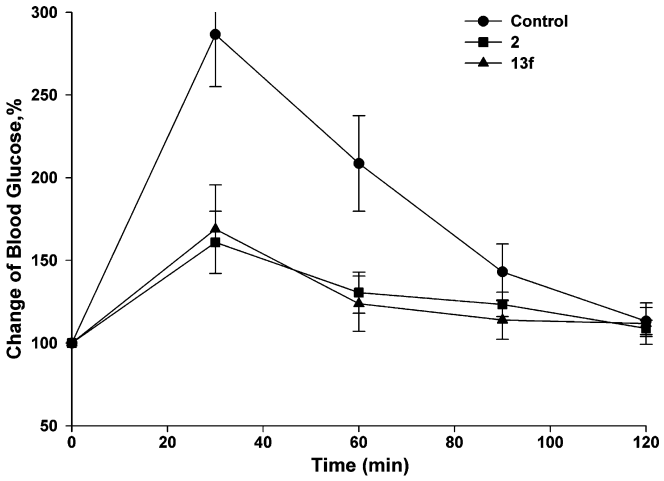


Figure 2. Effects of **2** and **13f** on glucose excursion in C57BL/6j mice (3 mpk po, $n = 6$), control is water.

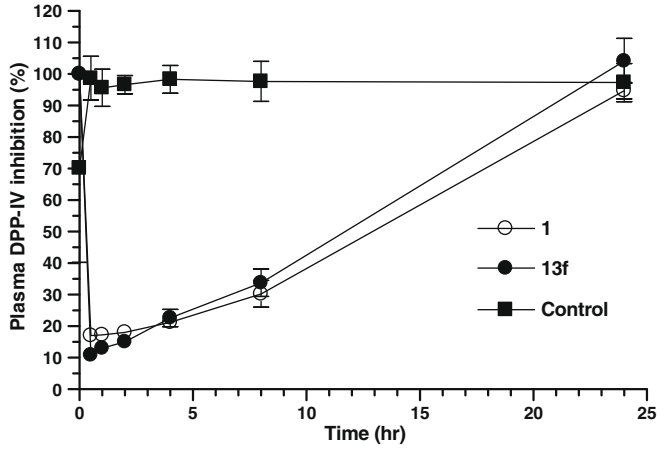


Figure 3. DPP-IV inhibition in rats for **2** ($n = 5$) and **13f** ($n = 4$) at 3 mpk po versus control (water, $n = 3$).

plasma DPP-IV inhibition assay,[21] the compounds were administered to Wistar rats by oral route at 3 mpk, blood samples were collected and analyzed for plasma DPP-IV activity (Fig. 3). A maximum inhibition of plasma DPP-IV activity was observed approximately 30 min after the oral dosing of compounds **2** or **13f**, the inhibition was >80% from 30 min to 4 h and >60% at 8 h. The compound **13f** showed similar inhibition profile of plasma DPP-IV activity as **2**. The in vivo effects shown in Figures 2 and 3 demonstrate the ability of **13f** to both significantly decrease the glucose excursion and inhibit plasma DPP-IV activity, and these pharmacological profiles of **13f** are comparable to those of compound **2**, a DPP-IV inhibitor, which is a diabetes drug for the European market.

In summary, we have identified a novel series of glutamic acid derivatives as potent and selective DPP-IV inhibitors. Notable among these is compound **13f** having 3,3-dimethyl substituents in the β-position of the P2 site glutamic acid. This compound is an $IC_{50} = 14$ nM DPP-IV inhibitors with an excellent selectivity profile over DPP8 ($IC_{50} = 14$ μM), FAP ($IC_{50} > 20$ μM) and DPP-II ($IC_{50} > 20$ μM). The inhibitor also exhibited excellent aqueous stability. The in vivo effects of compound **13f**, including inhibition of plasma DPP-IV activity and suppression of blood glucose elevation, were also demonstrated. The result of these pharmacological studies indicates that **13f** is a potent, selective, and orally available inhibitor of DPP-IV.

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