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Non-competitive and selective dipeptidyl peptidase IV inhibitors with phenethylphenylphthalimide skeleton derived from thalidomide-related α-glucosidase inhibitors and liver X receptor antagonists

Kazunori Motoshima, Kazuyuki Sugita, Yuichi Hashimoto *, Minoru Ishikawa

Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

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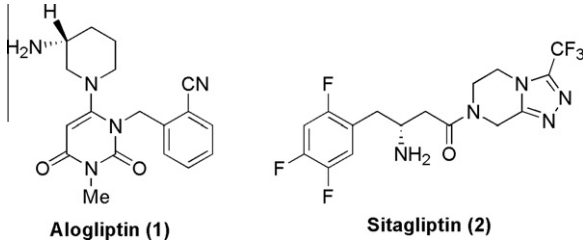
ABSTRACT

Novel dipeptidyl peptidase IV (DPP-IV) inhibitors with a phenethylphenylphthalimide skeleton were prepared based on α-glucosidase inhibitors and liver X receptor (LXR) antagonists derived from thalidomide. Representative compounds showed non-competitive inhibition of DPP-IV and **28a** exhibited 10-fold selectivity for DPP-IV over DPP-8. Compound **28a** is the first non-competitive, selective DPP-IV inhibitor.

Dipeptidyl peptidase IV (DPP-IV; E.C. 3.4.14.5) is a 220–240 kDa homodimeric type II transmembrane glycoprotein catalyzing the cleavage of Xaa-Pro or Xaa-Ala dipeptides preferentially from the N-terminus of polypeptides (where Xaa is any amino acid except for Pro).[1–4] DPP-IV is identical with the CD26 T-cell activating antigen found in almost all human organs and tissues.[5] It is anchored to the plasma membrane of endothelia of almost all organs examined, and is also found in a soluble form in body fluids, such as blood plasma and cerebrospinal fluid.[6] In vivo, this enzyme cleaves various polypeptides, including chemokines and peptide hormones.[4,7] Glucagon-like peptide-1 (GLP-1), an insulin-releasing hormone, is also cleaved and inactivated by DPP-IV.[8] GLP-1 is secreted in response to ingestion of food and stimulates insulin secretion.[9] It has been suggested that potentiation and extension of the action of GLP-1 by DPP-IV inhibition would stimulate insulin secretion only after a meal,[10] and DPP-IV inhibitors have therefore come to be seen as a potential new type of antidiabetic agent free of side effects such as hypoglycemia. Some DPP-IV inhibitors such as alogliptin (**1**) and sitagliptin (**2**) are already on the market (Fig. 1).[11] Almost all of the currently known inhibitors show competitive inhibition.[12,13]

We have been engaged in the creation of bioactive compounds based on the multi-template approach utilizing thalidomide (**3**).[14–19] The concept underlying the multi-template approach is



Alogliptin (1) Sitagliptin (2)

Figure 1. Chemical structures of currently available DPP-IV inhibitors (**1** and **2**).

that the number of three-dimensional spatial structures (fold structures) of human proteins is only approximately 1000, which is much smaller than the number of human proteins, estimated to be 50,000–70,000.[20–22] Therefore, ignoring physical/chemical interactions, a template/scaffold structure which is spatially complementary to one fold structure might serve as a multi-template for structural development of ligands that would interact specifically with 50–70 or more different human proteins. We have focused on thalidomide (**3**) as a candidate multi-template structure. Thalidomide (**3**) is a hypnotic/sedative drug, which was launched in the 1950's, but was withdrawn from the market in the 1960's because of its severe teratogenicity. In spite of this, thalidomide (**3**) has been established to be useful for the treatment of Hansen's disease and multiple myeloma. Additionally, many reports have appeared on its therapeutic potential for the treatment of a range of diseases,

* Corresponding author.
E-mail address: hashimot@iam.u-tokyo.ac.jp (Y. Hashimoto).

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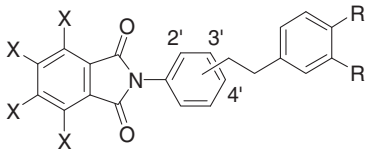
including cancers, rheumatoid arthritis and diabetes.[14–19,23] We have developed many biological response modifiers, including tumor necrosis factor-α (TNF-α) production regulators,[24,25] nitric oxide synthase (NOS) inhibitors,[26,27] cyclooxygenase (COX) inhibitors,[28–30],liver X receptor (LXR) antagonists,[31–34] α-glucosidase inhibitors[34–37] and glycogen phosphorylase inhibitors,[38] by employing the multi-template approach. During these studies, we also discovered novel DPP-IV inhibitors, including PPS-33 (**4**) and 5APP-33 (**5**) (Fig. 2).[39] However, these compounds possess only weak inhibitory activity (**4**: IC_{50} = 57.8 μM, **5**: IC_{50} = 65.9 μM), and they also show LXR-antagonistic and α-glucosidase-inhibitory activity, as well as DPP-IV-inhibitory activity.[33] Recently, we have developed various phenethylphenylphthalimide derivatives, including an α-glucosidase inhibitor (**6**), an LXR antagonist (**7**) and an LXRα-selective antagonist (**8**) (Fig. 2).[34] The above considerations led us to speculate that phenethylphenylphthalimide derivatives might possess DPP-IV-inhibitory activity. Thus, we carried out a screening assay to search for novel DPP-IV inhibitors among our derivatives.

A classical DPP-IV inhibitor, diprotin A (**9**) (Fig. 2),[40] was adopted as a positive control; the extent of inhibition by **9** at 10 μM was 14% under our experimental conditions. In the initial screening of phenethylphenylphthalimide derivatives reported previously,[34] compound **22** showed the most potent DPP-IV-inhibitory activity (Table 1). Among dimethoxy and dihydroxyl derivatives, dihydroxyl derivatives seem to be more potent than dimethoxy derivatives [**12** > **11**, **15** > **14**, **18** > **17**, **21** > **20**, **24** > **23**, **26** > **6**].

Next, we tried to improve the inhibitory activity of compound **22**. Introduction of two methoxy (**23**) or hydroxyl groups (**24**) into compound **22** resulted in decreased DPP-IV-inhibitory activity (Table 1). Based on the above structure–activity information, analogs of **22** which possess electron-withdrawing groups were designed as candidates for more potent DPP-IV inhibitors. An analog of **22** bearing a methyl group was also designed, for comparison with the analog bearing the CF_3 group.

These analogs were synthesized as shown in Scheme 1. Various benzyl halides were reacted with triphenylphosphine to generate triphenylphosphonium salts **30a–d**. Diphenylethene derivatives **31a–d** were prepared as *E*/*Z* mixtures by Wittig reaction of 3-nitrobenzaldehyde with **30a–d**. After reduction of the nitro group and olefin moiety of compounds **31a–d**, phenethylphenylphthalimides **27a–d** were obtained by condensation with tetrachlorophthalic anhydride.

Table 1
DPP-IV-inhibitory activity of phenethylphenylphthalimides (**6**,**9**–**26**)



Compound	X	Position	R	Inhibition ratio at 10 μM (%)
9	—	—	—	14
10	H	2′	H	0
11	H	2′	OMe	0
12	H	2′	OH	4
13	H	3′	H	2
14	H	3′	OMe	0
15	H	3′	OH	7
16	H	4′	H	6
17	H	4′	OMe	0
18	H	4′	OH	7
19	Cl	2′	H	7
20	Cl	2′	OMe	2
21	Cl	2′	OH	12
22	Cl	3′	H	26
23	Cl	3′	OMe	0
24	Cl	3′	OH	5
25	Cl	4′	H	12
6	Cl	4′	OMe	3
26	Cl	4′	OH	11

Introduction of a CF_3 group at the 4″ position of **22** afforded compound **27b**, in which exhibited increased DPP-IV-inhibitory activity (Table 2). On the other hand, the potency of analogs substituted with fluorine, that is, compounds **27a** and **27c**, or a methyl group, that is, compound **27d**, was similar to that of **22** (Table 2). Hence, there does not seem to be a clear relationship between electronic character of substituents (electron-donating or -withdrawing) and DPP-IV-inhibitory activity.

Subsequently, we designed 2″- and 3″-CF_3 analogs in order to examine the effect of the position of the CF_3 group on DPP-IV-inhibitory activity. The analogs were synthesized as shown in Scheme 2, using a route nearly identical to that in Scheme 1.

Surprisingly, a positional shift of the CF_3 group of **27b** from the 4″-position to the 3″- (**28a**) or 2″-position (**28b**) resulted in a dras-

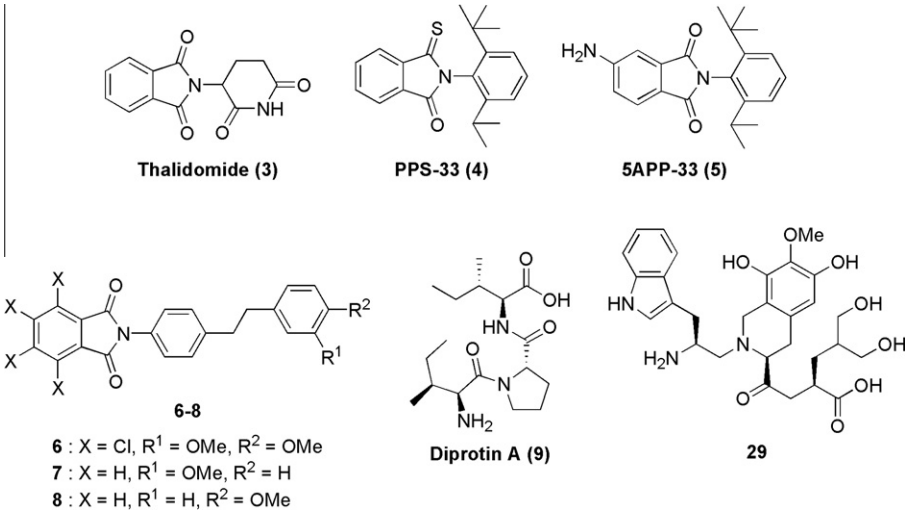
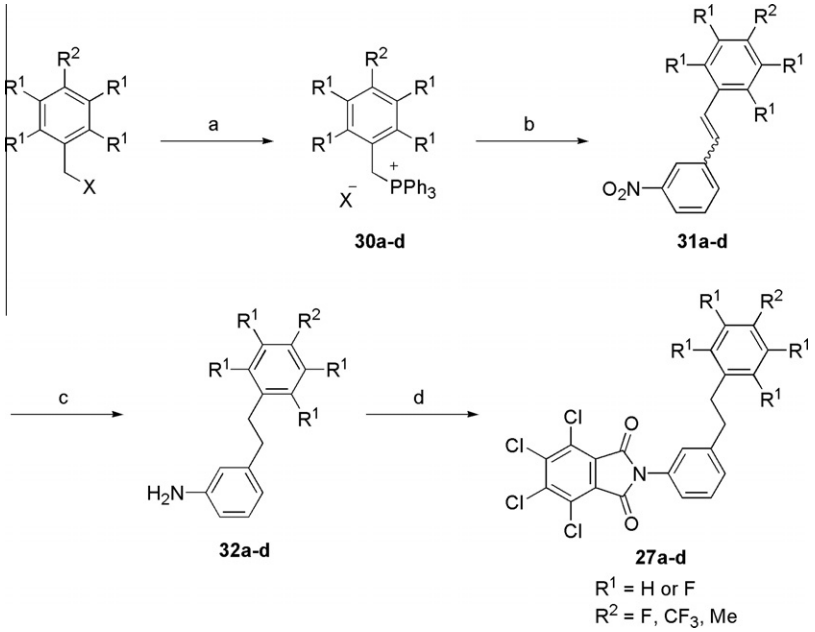
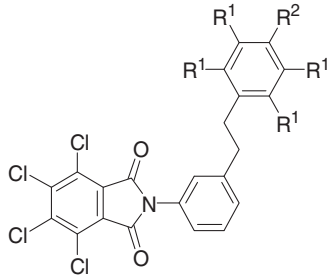


Figure 2. Chemical structures of thalidomide (**3**), PPS-33 (**4**), 5APP-33 (**5**), α-glucosidase inhibitor (**6**), LXR antagonist (**7**), LXRα-selective antagonist (**8**), diprotin A (**9**) and non-competitive DPP-IV inhibitor (**29**).



Scheme 1. Synthesis of phenethylphenylphthalimide analogs with electron-withdrawing substituents **27a–d**. Reagents and conditions: (a) triphenylphosphine, toluene, reflux; (b) K_2CO_3, 18-crown-6, CH_2Cl_2, reflux; (c) H_2, 10% Pd/C, EtOAc, rt; (d) tetrachlorophthalic anhydride, neat, 160 °C.

Table 2
DPP-IV-inhibitory activity of phenethylphenylphthalimides with electron-withdrawing groups (**27a–d**)



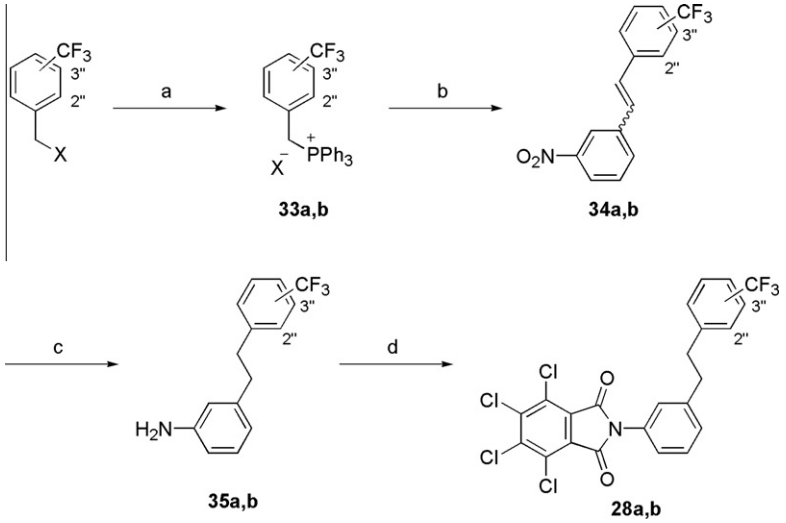
Compound	R^1	R^2	Inhibition ratio at 10 μM (%)
9	—	—	14
22	H	H	26
27a	H	F	20
27b	H	CF_3	38
27c	F	F	19
27d	H	Me	25

tic increase of DPP-IV-inhibitory activity; **28a** and **28b** showed IC_{50} values of 3.1 μM and 4.0 μM, respectively (Table 3).

As mentioned above, almost all currently known DPP-IV inhibitors, including alogliptin (**1**) and sitagliptin (**2**), show competitive inhibition[12,13] and share similar pharmacophores, that is, they possess cyano and/or free amino groups. In terms of chemical structure, compounds **28a** and **28b** are quite different from the known competitive DPP-IV inhibitors. In particular, these phenethylphenylphthalimide derivatives do not possess a cyano or free amino group, unlike the known inhibitors. Therefore, we speculated that the mode of DPP-IV inhibition elicited by **28a** and **28b** might be different from that of the known competitive inhibitors. Indeed, Lineweaver–Burk plot analysis showed that **28a** and **28b** are non-competitive inhibitors (Fig. 3 and data not shown, respectively).

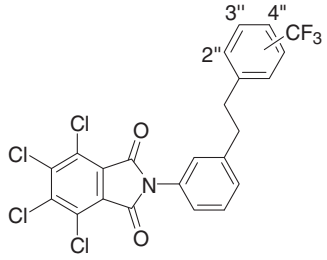
There exist many types of dipeptidylpeptidases with similar substrate selectivity. Among them, DPP-8, which is a cytoplasmic aminopeptidase like DPP-IV,[41] possesses an almost identical active-site amino acid sequence to DPP-4.[41,42] The administration of selective DPP-8 inhibitors to animals results in severe toxic reactions, including alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies and increased mortality.[43] Hence, DPP-IV selectivity over DPP-8 would be an advantage to eliminate side effects. Alogliptin (**1**) and sitagliptin (**2**) show very weak DPP-8-inhibitory activity. That is to say, they are DPP-IV-selective inhibitors.[12,13] Interestingly, although the amino acid sequences of DPP-IV and DPP-8 in the active sites are almost identical, as mentioned above, these proteins show only 51% amino acid sequence homology overall.[41] Therefore, we speculated that non-competitive DPP-IV inhibitors might show selectivity between the two enzymes. As far as we know, compound **29** (Fig. 2) is the only non-competitive DPP-IV inhibitor reported so far, but its activity against DPP-8 was not evaluated.[44] Our phenethylphenylphthalimide derivatives show non-competitive DPP-IV inhibition (vide supra), so we next evaluated their DPP-8-inhibitory activity. We found that compound **28a** possessed 10-fold selectivity for DPP-IV over DPP-8 (Table 4). Compound **28b** also showed three-fold selectivity for DPP-IV. On the other hand, compounds **22** and **27b** showed no selectivity for DPP-IV over DPP-8. These results suggest that the binding site for phenethylphenylphthalimide derivatives in DPP-IV might be similar to that in DPP-8. But, since a positional shift of the CF_3 group in phenethylphenylphthalimide derivatives affected the selectivity for DPP-IV over DPP-8, we speculated that the CF_3 group might interact with some hydrophobic amino acids in DPP-IV and/or DPP-8, and therefore differences in hydrophobic amino acid residues between DPP-IV and DPP-8 might be important for selectivity.

In conclusion, we discovered novel DPP-IV inhibitors among our α-glucosidase inhibitors/LXR antagonists derived from thalidomide (**3**). Structural development led to compound **28a**, which is the first non-competitive and selective DPP-IV inhibitor. Further investigation of the structure–activity relationships aiming at the development of superior DPP-IV inhibitors is in progress.



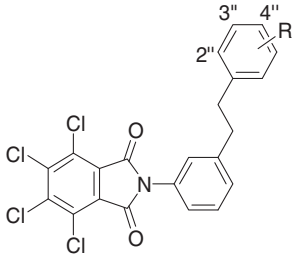
Scheme 2. Synthesis of phenethylphenylphthalimide analogs with CF_3 groups, **28a**, **b**. Reagents and conditions: (a) triphenylphosphine, toluene, reflux; (b) K_2CO_3, 18-crown-6, CH_2Cl_2, reflux; (c) H_2, 10% Pd/C, EtOAc, rt; (d) tetrachlorophthalic anhydride, neat, 160 °C.

Table 3
DPP-IV-inhibitory activity of phenethylphenylphthalimides with CF_3 groups (**28a**,**b**)



Compound	Position	Inhibition ratio at 10 μM (IC_{50} (μM))
5	—	14%
27b	4″	38%
28a	3″	87% (3.1 μM)
28b	2″	86% (4.0 μM)

Table 4
DPP-IV- and DPP-8-inhibitory activities of phenethylphenylphthalimides (**9**, **22**, **27b**, **28a** and **28b**)



Compound	Position	R	Inhibition ratio at 10 mM (IC_{50} (μM))	
			DPP-IV	DPP-8
9	—	—	14%	10%
22	—	H	26%	15% (21 μM)
27b	4″	CF_3	38%	21% (21 μM)
28a	3″	CF_3	87% (3.1 μM)	23% (>30 μM)
28b	2″	CF_3	86% (4.0 μM)	43% (11 μM)

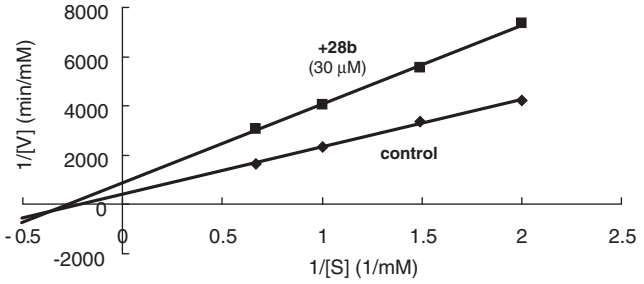


Figure 3. Lineweaver–Burk plot analysis of the inhibition of DPP-IV by **28b**.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.026.

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