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A comparative study of the binding modes of recently launched dipeptidyl peptidase IV inhibitors in the active site

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ABSTRACT

In recent years, various dipeptidyl peptidase IV (DPP-4) inhibitors have been released as therapeutic drugs for type 2 diabetes in many countries. In spite of their diverse chemical structures, no comparative studies of their binding modes in the active site of DPP-4 have been disclosed. We determined the co-crystal structure of vildagliptin with DPP-4 by X-ray crystallography and compared the binding modes of six launched inhibitors in DPP-4. The inhibitors were categorized into three classes on the basis of their binding subsites: (i) vildagliptin and saxagliptin (Class 1) form interactions with the core S_1 and S_2 subsites and a covalent bond with Ser630 in the catalytic triad; (ii) alogliptin and linagliptin (Class 2) form interactions with the S'_1 and/or S'_2 subsites in addition to the S_1 and S_2 subsites; and (iii) sitagliptin and teneligliptin (Class 3) form interactions with the S_1, S_2 and S_2 extensive subsites. The present study revealed that the additional interactions with the S'_1, S'_2 or S_2 extensive subsite may increase DPP-4 inhibition beyond the level afforded by the fundamental interactions with the S_1 and S_2 subsites and are more effective than forming a covalent bond with Ser630.

1. Introduction

Dipeptidyl peptidase IV (DPP-4, EC 3.4.14.5) inhibitors are a new class of oral anti-hyperglycemic agents for the treatment of type 2 diabetes. The glucose lowering effect of DPP-4 inhibitors is mediated by suppressing the degradation of the incretin hormone glucagon-like peptide-1 and stimulating insulin secretion in response to increased blood glucose levels [1]. Prescriptions for recently launched DPP-4 inhibitors for type 2 diabetes have been expanding because of their high effectiveness and safety.

Among the recently marketed DPP-4 inhibitors (Table 1), vildagliptin [2], saxagliptin [3] and teneligliptin [4] are peptide mimetic compounds, which have been discovered by replacing segments of peptide-based substrates [5]. In contrast, sitagliptin [6], alogliptin [7] and linagliptin [8] are non-peptide mimetic compounds, which have been discovered by optimization of the initial lead compounds identified by random screening [5]. Therefore, their chemical structures are diverse, suggesting that each of their binding modes in DPP-4 would be unique.

DPP-4 is a highly specific serine protease that recognizes an amino acid sequence having proline or alanine at the N-terminal penultimate (P_1) position and inactivates or generates biologically active peptides [9]. The amino acid sequence and three-dimensional structure of DPP-4 are well known [10,11]. The structure comprises a β-propeller domain and a catalytic domain, which together embrace an internal cavity housing the active center. This cavity is connected to the bulk solvent by a "propeller opening" and a "side opening" [12]. The conventional hypothesis suggests that substrates and inhibitors enter or leave the active site via the side opening [12,13].

While some comparative studies on the pharmacological effects of DPP-4 inhibitors have been reported [14], there have been no reports comparing their binding modes in DPP-4. X-ray co-crystal structures of five inhibitors, sitagliptin [6], saxagliptin [15], alogliptin [16], linagliptin [8] and teneligliptin [4], with DPP-4 were determined by each originator except vildagliptin. Because these inhibitors have diverse chemical structures, a comparative study of their binding modes in DPP-4 is of considerable interest. Although it is well known that all DPP-4 inhibitors bind to the S_1 and S_2 subsites in common, it has not been systematically understood whether other subsites exist and whether each inhibitor binds to these in a distinct manner. In this study, we determined the co-crystal structure of vildagliptin with DPP-4, analyzed those

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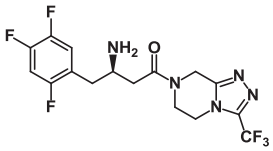
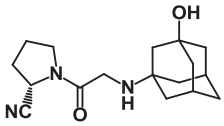
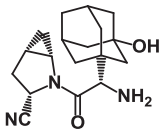
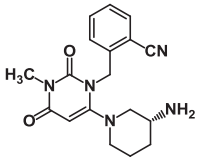
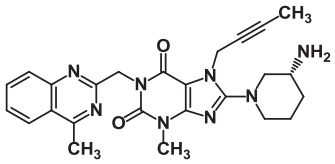
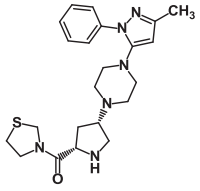
Table 1
Recently launched DPP-4 inhibitors.

Compound name	Chemical structure	Release year	Originator
Sitagliptin		2006	Merck & co.
Vildagliptin		2007	Novartis
Saxagliptin		2009	Astrazeneca and bristol-myers squibb
Alogliptin		2010	Takeda
Linagliptin		2011	Boehringer ingelheim
Teneligliptin		2012	Mitsubishi tanabe pharma

Data were collected in Thomson Reuters Integrity on February 4, 2013.

of the six inhibitors in parallel and studied the relationships between their binding interactions with DPP-4 and their inhibitory activity.

2. Materials and methods

2.1. Synthesis of vildagliptin

Vildagliptin was prepared according to the method described by Villhauer et al. [2].

2.2. X-ray crystallographic studies

The protein of human DPP-4 (33-766) secreted from insect cells was purified and crystallized according to the method reported by Hiramatsu et al. [17] The protein–inhibitor complex was obtained by soaking a preformed DPP-4 crystal in the presence of vildagliptin and preserving it in liquid nitrogen for data collection at 100 K. X-ray diffraction data were collected at the High Energy Accelerator Research Organization (KEK) beam line BL5 and processed using the program HKL2000 [18]. The structure of the DPP-4–inhibitor complex was solved by molecular replacement with the program PHASER [19], utilizing the previously determined coordinates of DPP-4 with the Protein Data Bank (PDB) accession code 3VJK. Data collection and model refinement statistics are summarized in Table 2.

2.3. Comparison of X-ray complex structures

The co-crystal structures of five inhibitors with human DPP-4 have been reported [PDB: 1X70 (sitagliptin), 3BJM (saxagliptin), 3G0B (alogliptin), 2RGU (linagliptin), and 3VJK (teneligliptin)]. They were superimposed on the co-crystal structure of a substrate peptide, diprotin A with DPP-4 (PDB: 1NU8) to analyze the binding subsites. The molecular modeling software Molecular Operating Environment version 2011.10 (Chemical Computing Group, Inc., Montreal, Canada) was used for analysis and graphical visualization of the X-ray co-crystal structures.

The contact area between the inhibitor and DPP-4 was calculated using the molecular modeling software Discovery Studio version 3.5 (Accelrys, Inc., San Diego, USA). For each co-crystal structure, the molecular surface area of the inhibitor, and its solvent-exposed surface area in DPP-4 were calculated. The difference between these areas was defined as the contact area.

3. Results and discussion

3.1. X-ray co-crystal structures of six inhibitors with DPP-4

3.1.1. Definition of subsites in the active site of DPP-4

In the active site of a protease, subsites are generally defined by the binding site of the substrate peptide [20]. The amino acids in the substrate peptide are numbered from the point of cleavage

Table 2
Data collection and refinement statistics.

	Vildagliptin
PDB entry code	3W2T
Crystal	
Space group	$P2_12_12_1$
Unit cell parameters: *a* (Å)	118.22
b (Å)	126.24
c (Å)	138.09
Data	
Resolution (Å)	50.00–2.36 (2.44–2.36)
Unique reflections	82418 (7691)
Redundancy	5.0 (4.6)
Completeness (%)	97.1 (91.7)
R_{merge}[a]	0.080 (0.234)
I/σ (I)	16.3 (6.96)
Refinement	
Resolution (Å)	30.00–2.36 (2.42–2.36)
Unique reflections	78,227 (5407)
Completeness (%)	97.3 (92.1)
Data in the test set	4103 (265)
R-work	0.180 (0.206)
R-free	0.231 (0.287)
R.m.s.d. bond lengths (Å)	0.011
R.m.s.d. bond angles (°)	1.319
Ramachandran plot	
Favored regions (%)	96.1
Allowed regions (%)	3.9
No. of non-H atoms/average B ($Å^2$)	
Protein	12,228/32.7
Ligand	44/23.7
Water	1027/34.7

Values in parentheses are for the highest-resolution shell.
[a] $R_{merge} = \sum|(I - \langle I\rangle)|/\sum(I)$, where I is the observed intensity.

(P_2, P_1, P'_1, P'_2 ...), and the protein subsites occupied by the respective amino acids are also numbered in the same fashion (S_2, S_1, S'_1, S'_2...). In the case of DPP-4, the N-terminus of the substrate peptide is recognized by Glu205 and Glu206, and Ser630 cleaves at the N-terminus penultimate position (P_1). Although, in principle, no subsites are defined after S_2 in DPP-4, our recent study has shown that not the substrates but the inhibitors can bind well beyond the S_2 subsite to increase their inhibitory activity [4,21]. We therefore defined the site beyond S_2 as the S_2 extensive subsite, which is composed of Val207, Ser209, Phe357 and Arg358.

3.1.2. Binding mode of vildagliptin

The co-crystal structure of vildagliptin with DPP-4 is shown in Fig. 1(A). The cyanopyrrolidine binds to the S_1 subsite, with the nitrile forming a covalent imidate adduct with the hydroxyl of Ser630 in the catalytic triad. The imidate nitrogen forms a hydrogen bond with the side-chain hydroxyl of Tyr547. The remaining part including the adamantane binds to the S_2 subsite, where the carbonyl group forms a hydrogen bond with Asn710 and the amino group forms salt bridges with Glu205 and Glu206. The hydroxyl group on the adamantyl moiety forms hydrogen bonds with His126 and Ser209 via the water molecules.

3.1.3. Categorization of the six inhibitors on the basis of their binding subsites

The co-crystal structures of the six inhibitors with DPP-4 superimposed on that of the substrate peptide (diprotin A, Ile-Pro-Ile) are shown in Fig. 1(B)–(H). We categorized the six inhibitors into three classes on the basis of their binding subsites. (i) Vildagliptin and saxagliptin have the most basic binding modes, binding to only the S_1 and S_2 subsites (Class 1). (ii) Alogliptin and linagliptin bind to the S_1, S_2 and S'_1 subsites. Moreover, only linagliptin additionally binds to the S'_2 subsite (Class 2). (iii) Sitagliptin and teneligliptin bind to the S_1, S_2 and S_2 extensive subsites (Class 3). Fig. 2 shows the concept of this categorization.

3.2. Relationship between the inhibitory activity and the binding mode of each class

We focus on the characteristic binding interactions with DPP-4 because other details have been described in previous studies [4,6,8,15,16]. It is well known that all the DPP-4 inhibitors form salt bridges with Glu205 and Glu206 in the S_2 subsite, which have vital roles in the inhibitory activity. The potency of the six DPP-4 inhibitors is shown in Table 3 [22].

3.2.1. Class 1: vildagliptin and saxagliptin

Because vildagliptin and saxagliptin were designed as peptide mimetics, they overlap with the P_1 and P_2 residues of the substrate peptide. As described above, their cyanopyrrolidine moieties bind to the S_1 subsite, forming a covalent bond between the nitrile group and Ser630, and their hydroxy adamantyl groups bind to the S_2 subsite. While they bind in almost the same mode, one of the reasons why saxagliptin has 5-fold higher activity than vildagliptin is attributed to the cyclopropanated cyanopyrrolidine of saxagliptin. Although it was originally intended to enhance the chemical stability of the cyanopyrrolidine [3], introduction of the cyclopropane moiety afforded an additional hydrophobic interaction with the side chain of Tyr666 in the S_1 subsite. Moreover, the direct hydrogen bond between the hydroxyl group of saxagliptin and the side chain of Tyr547 may also contribute to its higher potency.

3.2.2. Class 2: alogliptin and linagliptin

The chemical structures of alogliptin and linagliptin are far different from those of the substrate peptides. The cyanobenzyl group of alogliptin and the butynyl group of linagliptin bind to the S_1 subsite. Their uracil rings form π–π interactions with Tyr547, which undergoes a conformational change in the S'_1 subsite. One of the reasons why linagliptin has 8-fold higher activity than alogliptin may be because only linagliptin binds to the S'_2 subsite. The phenyl component of the quinazoline substituent forms a π–π interaction with Trp629 located in the S'_2 subsite [23]. Eckhardt et al. reported that the introduction of the quinazoline moiety improved its potency 88-fold [8].

3.2.3. Class 3: sitagliptin and teneligliptin

The trifluorophenyl moiety of sitagliptin and the thiazoline moiety of teneligliptin bind to the S_1 subsite. The triazolopyrazine moiety and trifluoromethyl substituent of sitagliptin and the (1-phenylpyrazol-5-yl) piperazine moiety, referred to here as the "anchor lock domain," of teneligliptin bind to the S_2 extensive subsite. Although both inhibitors appear to bind to the subsites in the same manner, teneligliptin has 5-fold higher activity. We suggest three potential reasons for the difference. The first reason may be related to their chemical structures. Because teneligliptin consists of a considerably rigid "J-shaped" structure formed by five rings, four of which are directly connected, the loss in entropy is small upon binding to DPP-4. The second reason may be related to the binding interactions with the S_2 subsite. The carbonyl group of teneligliptin, derived from the peptide mimetics, forms a hydrogen bond with the side chain of Asn710. The third reason may be related to the binding to the S_2 extensive subsite. As shown in Fig. 3, for teneligliptin, introduction of the "anchor lock domain", which binds to the S_2 extensive subsite, increased the activity by 1500-fold over the corresponding fragment that binds to S_1 and S_2 only

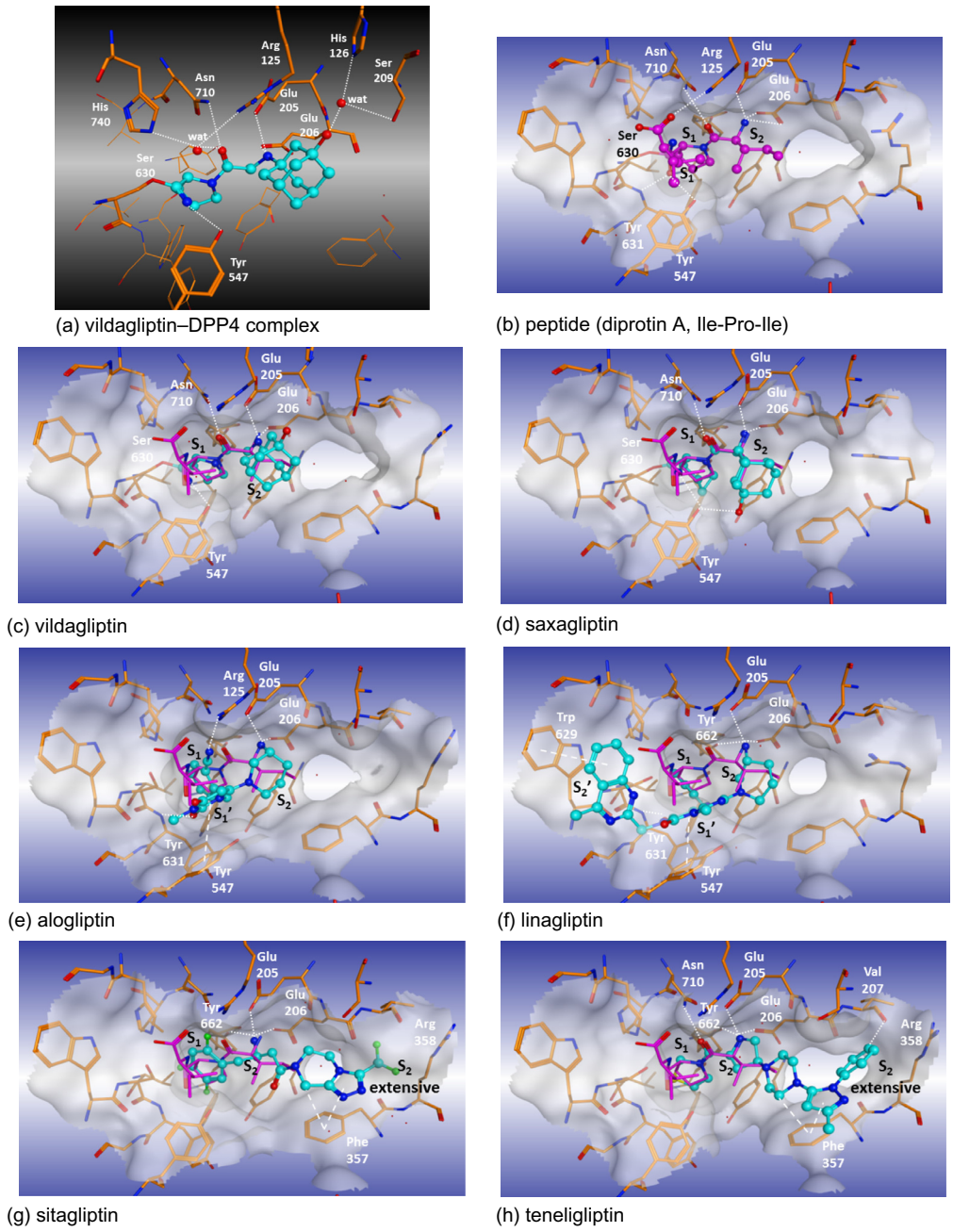


Fig. 1. Binding modes of each inhibitor in the active site of DPP-4. (A) Co-crystal structure of vildagliptin (cyan) bound to DPP-4 (orange) (PDB: 3W2T). (B) Co-crystal structure of the substrate peptide, diproin A (magenta) bound to DPP-4 (orange). (C)–(H) Co-crystal structures of each inhibitor (cyan) bound to DPP-4 (orange) superimposed on the substrate peptide (magenta). The active site of DPP-4 is shown as a gray-colored surface. Blue, red, yellow and green colors indicate nitrogen, oxygen, sulfur and fluorine atoms respectively, and others indicate carbon atoms. Interactions between inhibitors and water molecules are not shown in (B)–(H). PDB codes are noted in Section 2.

[4,24]. On the other hand, for sitagliptin, previous studies revealed that the introduction of the substituent binding to the S_2 extensive subsite increased the activity by 7-fold [6,25]. To investigate the reason for the difference in increased activity, we applied the estimation method (see Section 2) to the calculation of contact areas in the S_2 extensive subsite. The results showed that teneligliptin has a contact area of 0.92 nm^2 (total contact area, 2.08 nm^2), while sitagliptin has a contact area of 0.71 nm^2 (total contact area, 1.90 nm^2). This result indicates that teneligliptin may bind more tightly to the S_2 extensive subsite as a result of stronger hydrophobic interactions mediated by the "anchor lock domain". Binding of the anchor lock domain may relate to the residence time of the inhibitor in DPP-4 and the long in vivo duration of action.

3.3. Particularity of the S_2 extensive subsite

As mentioned above, the S_2 extensive subsite, which is not involved in substrate binding, contributes to increase the inhibitory activity for some DPP-4 inhibitors, but the particularity of the S_2 extensive subsite has not been well known. In other related prolyl peptidases, including DPP-8, DPP-9 and fibroblast activation

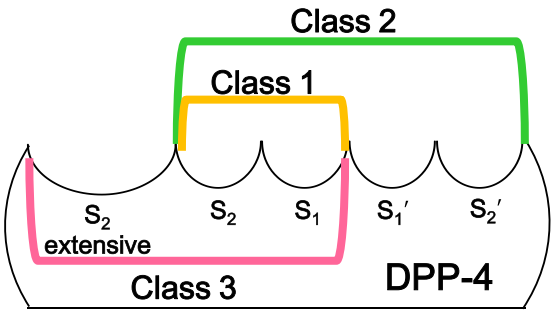


Fig. 2. The concept of three classes on the basis of the inhibitor's binding subsites.

Table 3
The DPP-4 inhibitory activity [22].

Compound	DPP-4 inhibition, IC_{50} (nmol/L)
Vildagliptin	29.2
Saxagliptin	6.3
Alogliptin	4.9
Linagliptin	0.6
Sitagliptin	10.3
Teneligliptin	1.9

protein (FAP, PDB: 1Z68), the S_2 extensive subsite cannot be clearly defined. As a result of our comparison of the corresponding amino acid sequences and three-dimensional structures [26,27] of these proteins, it was found that inhibitors cannot have sufficient hydrophobic interactions with the region beyond S_2 in DPP-8 and DPP-9, and FAP does not have the subsite where the inhibitors can bind beyond S_2. Binding to the S_2 extensive subsite, DPP-4 inhibitors can increase not only their inhibitory activity but also their selectivity against other related prolyl peptidases.

In conclusion, we comparatively present X-ray co-crystal structures of six inhibitors with DPP-4 and categorized them into three classes on the basis of their binding subsites. As a result of the comparative study of the three classes, it is suggested that DPP-4 inhibition tended to increase with an increase in the number of binding subsites. Furthermore, the additional interactions with the S_1', S_2' or S_2 extensive subsite may increase DPP-4 inhibition beyond the level afforded by the fundamental interactions with the S_1 and S_2 subsites and are more effective than forming a covalent bond with Ser630.

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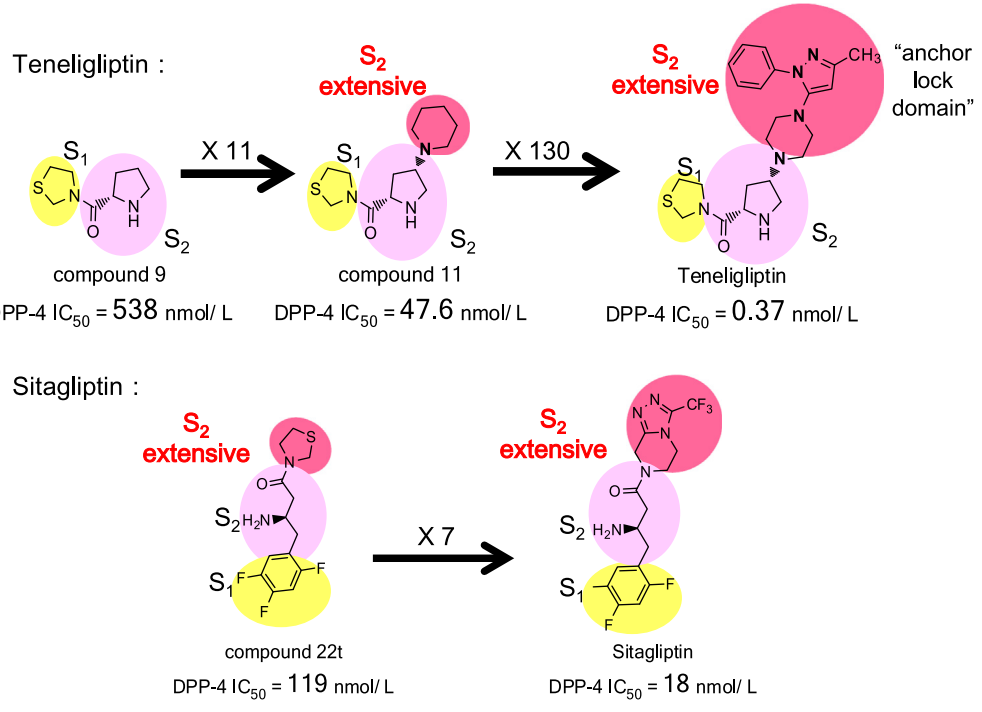

Fig. 3. The effect of binding to the S_2 extensive subsite. The activity of compounds **9** and **11** is obtained from [24] and that of teneligliptin is obtained from [4]. They were assayed in the same system. The activity of compound **22t** and sitagliptin is obtained from [25] and [6], respectively.

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