Molecular Recognition of Ligands in Dipeptidyl Peptidase IV

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Abstract: The serine protease dipeptidyl peptidase IV (DPP-IV) is a clinically validated target for the treatment of type II diabetes and has received considerable interest from the pharmaceutical industry over the last years. Concomitant with a large variety of published small molecule DPP-IV inhibitors almost twenty co-crystal structures have been released to the public as of May 2006. In this review, we discuss the structural characteristics of the DPP-IV binding site and use the available X-ray information together with published structure-activity relationship data to identify the molecular interactions that are most important for tight enzyme-inhibitor binding. Optimized interactions with the two key recognition motifs, *i.e.* the lipophilic S1 pocket and the negatively charged Glu 205/206 pair, result in large gains in binding free energy, which can be further improved by additional favorable contacts to side chains that flank the active site. First examples show that the lessons learned from the X-ray structures can be successfully incorporated into the design of novel DPP-IV inhibitors.

INTRODUCTION

Prolyl peptidases are a relatively small family of enzymes that are able to cleave peptide bonds after a proline residue [1]. One of the best characterized members of this class is the serine protease dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5), which specifically removes N-terminal dipeptides from substrates containing proline, and to some extent alanine, at the penultimate position [2]. An important *in vivo* active substrate of DPP-IV is the incretin glucagon-like peptide 1 (GLP-1) which has a stimulating effect on insulin secretion in a meal-dependent manner [3]. As DPP-IV is responsible for rapid degradation of GLP-1 levels in plasma the concept of DPP-IV inhibition to increase the half-life of this hormone and prolong its beneficial effects has been pursued as a new potential therapeutic approach to the treatment of type 2 diabetes [4-7].

As discussed in this issue and in several excellent reviews [6,7], there has been an explosion of patents and publications in recent years in particular from the pharmaceutical industry. The high interest in DPP-IV is on the one hand because it is a clinically validated target. On the other hand, the DPP-IV inhibitor binding site seems to be highly drugable in the sense that tight, specific binding to the enzyme can be achieved with small molecules without compromising favorable physico-chemical properties that are important for good pharmacokinetics. While no DPP-IV inhibitor is on the market yet, several molecules have progressed to phase II clinical trials and beyond: vildagliptin **1** (Novartis, preregistered) [8], sitagliptin **2** (Merck, preregistered) [9], saxagliptin **3** (Bristol-Myers Squibb, phase III) [10], SYR322 **4** (Takeda, phase III) [11], denagliptin **5** (Glaxo SmithKline, phase II) [12], 815541 (GlaxoSmith Kline/Tanabe, phase II), PSN9301 (Osi-Prosidion, phase II), NVP-DPP728 **6** (Novartis, phase II discontinued) [13], and P3298 **7** (Merck/Probiodrug, phase II discontinued) [14].

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The overview of the advanced inhibitors **1-7** in Fig. (**1**) shows that structurally diverse molecules are able to interact strongly with DPP-IV. For the design of novel DPP-IV inhibitors, it is of interest to identify those protein-ligand interactions that are crucial for achieving tight binding. More generally, a structural understanding why DPP-IV binds so many drug-like molecules might be useful for a better assessment of protein drugability in the future [15]. These questions can be addressed to some extent by X-ray crystallography. The first crystal structure of DPP-IV was published in 2003 by Rasmussen *et al*. [16] revealing its complex with the inhibitor valine-pyrrolidide **8**. As can be seen from Fig. (**2**), the number of structures deposited to the Protein Data Bank (PDB) [17] has increased steadily since then, most of them have been solved as protein-inhibitor complexes [9,18-24]. The abundance of publicly available DPP-IV X-ray information provides a good basis to analyze the molecular recognition processes in this enzyme.

This review focuses on three aspects of DPP-IV inhibitor interactions. Firstly, important structural information of the DPP-IV binding site gleaned from the existing X-ray studies is summarized. Secondly, we highlight the central molecular recognition interactions as they have emerged from analyses of complex crystal structures and chemical probing. Lastly, first attempts to translate the lessons learned from the X-ray structures into the design of novel chemotypes are reviewed.

GENERAL STRUCTURAL ASPECTS

Human DPP-IV is a 766 amino acid transmembrane glycoprotein consisting of a cytoplasmic tail (residues 1-6), a transmembrane region (residues 7-28), and an extracellular part (29-766) [16]. The extracellular region can be further subdivided into two domains: a) the catalytic domain (residues 508-766) which shows an α/β hydrolase fold and contains the catalytic triad Ser630 – Asp708 – His740 and b) an eight-bladed β propeller domain (residues 56-497) which also contributes to the inhibitor binding site [19]. DPP-IV is enzymatically active as a homodimer and this is also the assembly predominantly found in the asymmetric

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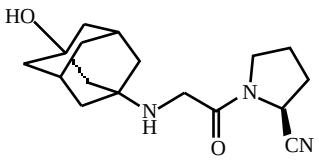
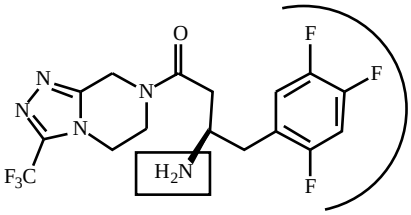
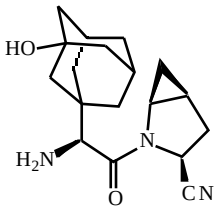


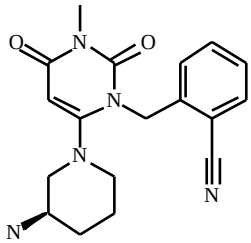
1, Vildagliptin
IC_{50} = 3.5 nM
Novartis



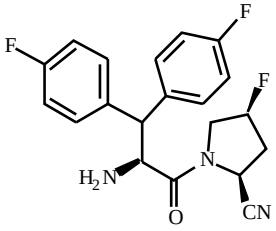
2, Sitagliptin
IC_{50} = 18 nM
Merck



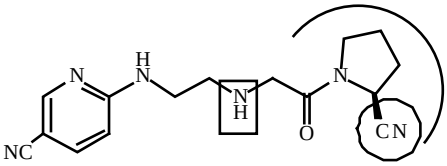
3, Saxagliptin
IC_{50} = 0.5 nM
Bristol-Myers Squibb



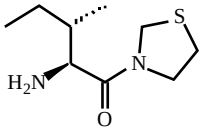
4, SYR-322
IC_{50} = 4 nM
Takeda



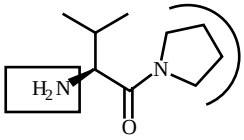
5, Denagliptin
IC_{50} = 22 nM
GlaxoSmithKline



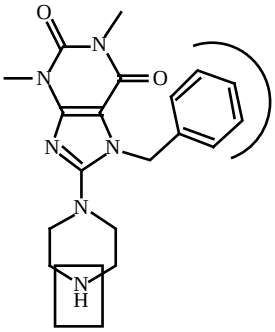
6, NVP-DPP728
IC_{50} = 22 nM
Novartis



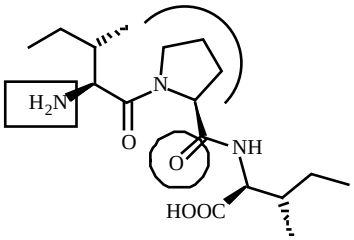
7, P3298
K_i = 123 nM
Merck/Probiodrug



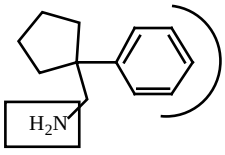
8, Val-pyrrolidide
IC_{50} = 2 μM



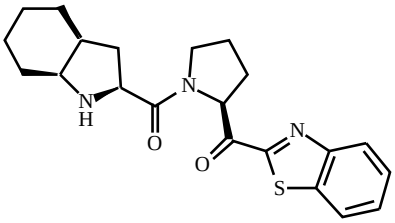
9, BDPX
K_i = 5.4 μM
Novo Nordisk



10, Diprotin A
apparent IC_{50} = 1.1 μM

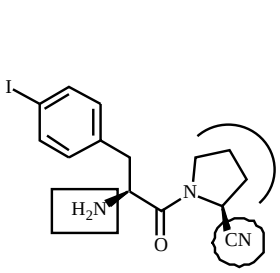


11
IC_{50} = 40 μM
Santhera

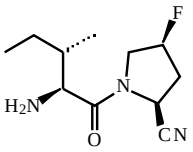


12
IC_{50} = 39 nM
Guilford Pharma

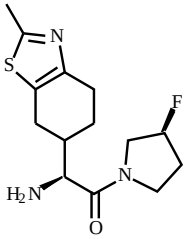
Fig. (1) Contd….



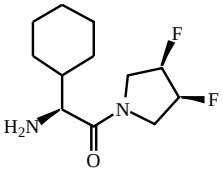
13
K_i = 25 nM



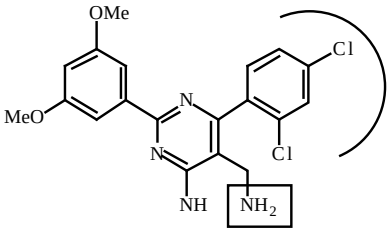
14
IC_{50} = 0.6 nM
Taisho



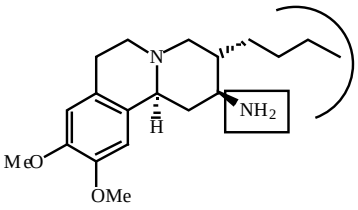
15
IC_{50} = 43 nM
Merck



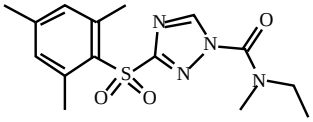
16
K_i = 61 nM
Pfizer



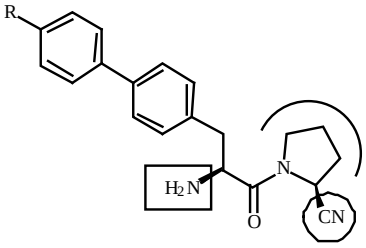
17
IC_{50} = 0.1 nM
Roche



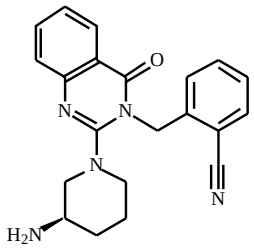
19
IC_{50} = 500 nM
Roche



21
IC_{50} = 0.35 nM
Eisai



22a, R=H
K_i = 13 nM
22b, R=F
K_i = 3 nM
Johnson & Johnson



23
IC_{50} = 10 nM
Takeda

Fig. (1). Selected inhibitors of DPP-IV. Ligands for which crystal structure information is available have underlined numbers. For these ligands, the location of the S1 pocket is indicated by a curved line, the atoms interacting with the Glu 205/206 dyad are in a rectangular box, and the atoms forming a covalent bond to Ser 630 are encircled.

unit of the known X-ray structures. The catalytic site lies in a large cavity between the two extracellular domains and can be accessed through two active site openings. The crystal structure of DPP-IV with a decapeptide suggests that substrates access the catalytic site through a cleft between the two domains [20]. The second opening which is located in the β propeller domain might be used for the dipeptidic product to leave the enzyme [19].

The shape and hydrophobic/hydrogen bonding properties of the inhibitor binding site of DPP-IV are illustrated in Fig. (**3a,b left**) for the bound cyanopyrrolidine **6** and xan-thine derivative **9** [22]. We use color coding to distinguish hydrogen bond acceptor/donor functionalities and aromatic/ non-aromatic hydrophobic surface patches. The binding site is open in the front for solvent access and a considerable number of ligand atoms are in contact with surrounding water. Hydrophobic and hydrophilic patches in the environment of the ligands are roughly equal in size with hydrogen-bond acceptor groups dominating the hydrophilic part. As can be seen from the protein-ligand interactions in Fig. (**3a,b**

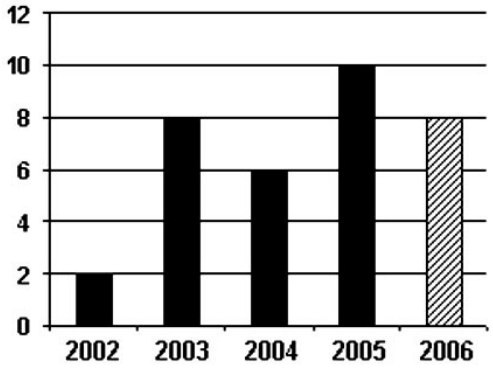


Fig. (2). Annual number of structures deposited to the Protein Data Bank. The grey bar indicates the structures deposited in the first quarter of 2006.

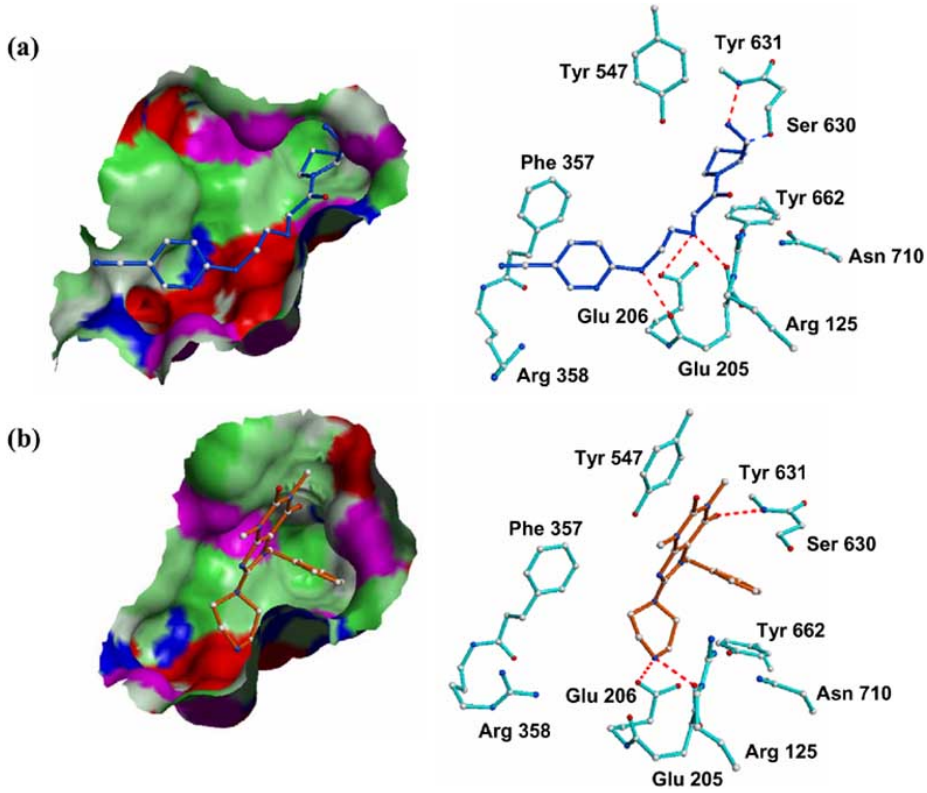


Fig. (3). Illustration of solvent-accessible surface of the DPP-IV binding site (left) as well as of important protein-ligand interactions (right) for two inhibitors. (a) X-ray complex structure of cyanopyrrolidine NVP-DPP728, **6**, with human DPP-IV, (b) crystal structure of xanthine derivative BDPX, **9**, with porcine kidney DPP-IV (PDB-id: 2aj8). Surfaces are colored by hydrophobic and hydrogen bonding (HB) properties: HB acceptor (red), HB donor (blue), HB acceptor/donor (magenta), hydrophobic (grey), aromatic hydrophobic (green). Dashed red lines indicate protein-ligand hydrogen bonds and the dashed blue line shows the covalent linkage between NVP-DPP728 and Ser 630. Residues Tyr 631, Val 656, Trp 659, Tyr 666, and Val 711 lining the S1 pocket in the back are removed for the sake of clarity.

right), this is due to backbone carbonyl groups pointing into the binding site at the bottom of the pocket and the negatively charged side chains of the Glu 205/206 dyad which strongly bind to basic groups such as the secondary amines of the two ligands. Crystal structures with the low-turnover substrate diprotin A (Ile-Pro-Ile, **10**) confirmed that the two carboxylates of the Glu dyad make short hydrogen bonds to the N-terminus of the peptide (d = 2.6Å) providing a strong ligand recognition motif [19,25]. For ligands containing primary amines, the third hydrogen bond is typically made to the hydroxyl group of Tyr 662.

The two binding modes displayed in Fig. (**3**) provide a good illustration of the interactions made by inhibitors to achieve tight binding to DPP-IV. Apart from the ionic interactions with the Glu dyad both ligands fill the proline-specific S1 pocket in the back with hydrophobic fragments. The low micromolar affinity of the small Val-pyrrolidide **8** and of the β-phenethylamine fragment **11** from Santhera Pharmaceuticals [24], shows that substantial binding affinity can be gained by optimized interactions with the S1 and Glu dyad anchor sites. The importance of these two pharmacophores is also underpinned by the fact that almost all inhibitor classes share the presence of a lipophilic moiety in close proximity to a primary or secondary amine.

Additional ligand-protein interactions that are seen in Fig. (**3**) are a covalent bond to the catalytic Ser 630 by a cyano electrophile mimicking the transition state of peptide cleavage, as well as hydrogen bonds to the backbone NH of Tyr 631, which forms the oxyanion hole, and to the backbone carbonyl of Glu 205. The aromatic rings of Phe 357 and Tyr 547 are exposed to the binding site and provide opportunities for additional lipophilic interactions. Each of the two ligands in Fig. (**3**) uses one of these two aromatic residues for π-π stacking. Finally, the polar Arg 125 and Asn 710 side chains are located close to the carbonyl amide linking the P1 fragment with the N-terminus of substrates (termed P2 amide recognition region throughout this text). As observed for the classical serine proteases, few DPP-IV inhibitors explore the C-terminal direction of the binding site (S1'-S2'-…). One noteworthy exception are the ketopyrrolidines **12**, or related ketoazetidines, in which the benzothiazole ring is an optimized substituent of the S1' site [26]. Replacing the benzothiazole moiety with a methyl or phenyl group renders the molecules inactive, indicating that a significant amount of additional binding free energy can be gained on the C-terminal end of the scissile bond. In the next paragraph, we will use the structural information together with examples of published structure-activity relationship (SAR) data to investigate the importance of the different interaction regions in more detail.

The large number of X-ray structures allows an assessment of the flexibility of the DPP-IV binding site, which is of relevance for computational drug design. A superposition of the available complex crystal structures reveals very little movement in the active site (Fig. (**4**)). Notable exceptions are Ser 630 and Tyr 547, which both can adopt two conformations, and especially the flexible side chain of Arg 358 at the end of the binding pocket. The crystallographic temperature factors of atoms of Arg 358 are typically high and the average position of its side chain is strongly influenced by the bound ligand. In contrast, the other arginine residue, Arg 125, shows little movement, presumably due to the stabilizing effect of its salt bridge with Glu 205. The two binding modes in Fig. (**3**) reveal the induced fit effect of the xanthine core which triggers a rotation of the aromatic ring of Tyr 547 from its usual orientation for better π-π interaction.

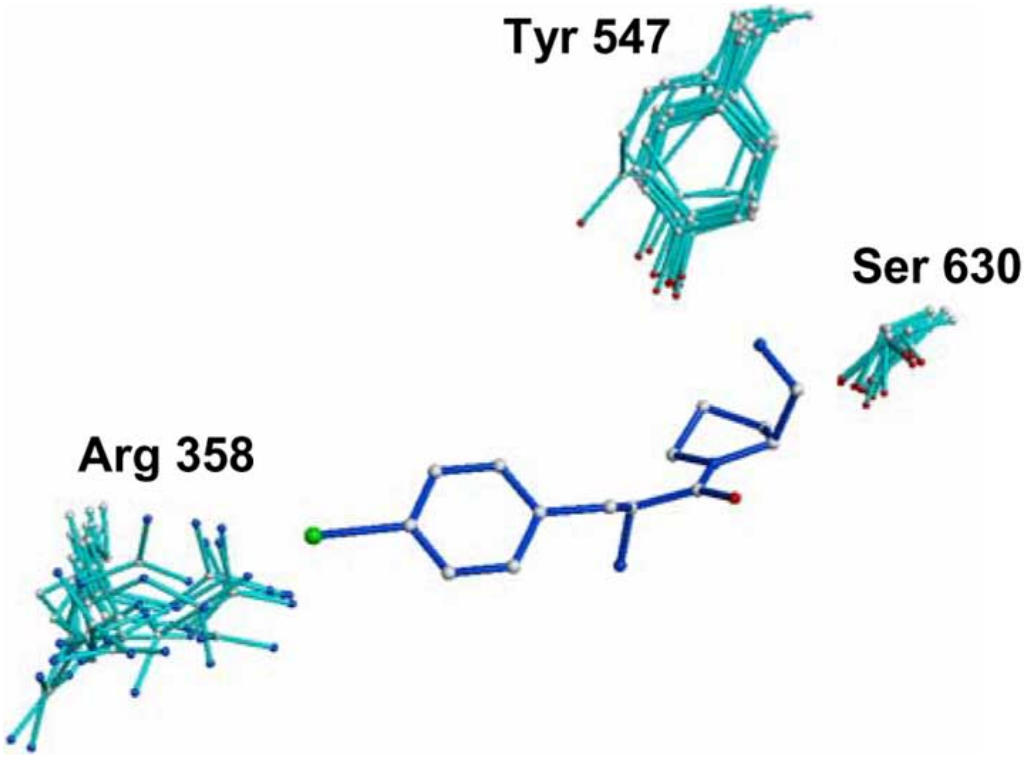


Fig. (4). Illustration of flexibility in the active site of DPP-IV. Shown are the residues for which different conformations are seen in the X-ray complex structures. The cyanopyrrolidine inhibitor **13** is displayed to indicate the positions of the flexible residues relative to a bound ligand (PDB-id: 1orw).

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