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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1744–1748

The reversed binding of β-phenethylamine inhibitors of DPP-IV: X-ray structures and properties of novel fragment and elaborated inhibitors

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Received 6 May 2005; revised 29 November 2005; accepted 30 November 2005
Available online 11 January 2006

Abstract—The co-crystal structure of β-phenethylamine fragment inhibitor **5** bound to DPP-IV revealed that the phenyl ring occupied the proline pocket of the enzyme. This finding provided the basis for a general hypothesis of a reverse binding mode for β-phenethylamine-based DPP-IV inhibitors. Novel inhibitor design concepts that obviate substrate-like structure–activity relationships (SAR) were thereby enabled, and novel, potent inhibitors were discovered.

Diabetes mellitus is a major worldwide health concern with an anticipated global prevalence of 220 million cases by 2010.[1a] Type 2 diabetes accounts for >90% of cases in industrialized nations.[1b] In the USA, some 10% of the population is believed to be diabetic.[1a] The discovery of small-molecule inhibitors of the enzyme dipeptidyl peptidase-IV (DPP-IV) is a major field of endeavour for the development of new drugs to treat type 2 diabetes.[1] DPP-IV is a member of the prolyl oligopeptidase family of serine proteases that cleaves dipeptides from peptide substrates having proline (or alanine) in the penultimate position.[2] DPP-IV is responsible for the inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). These hormones are centrally involved in insulin release and utilization following the ingestion of a meal. GLP-1-based therapy is currently a very persuasive approach to treating type 2 diabetes.[3] GLP-1 itself has remarkable efficacy in humans when given intravenously, lowering blood glucose and modulating insulin levels in a glucose-dependent fashion with the concurrent low risk of hypoglycaemia. Inhibiting DPP-IV reduces its rapid ($t_{1/2}$ < 1 min) degradation of GLP-1, increasing circulating levels of the active hormone in vivo and prolonging its beneficial effects.

In contrast to other serine protease targets, for example thrombin, where the discovery of structural novelty has been limited in scope and evolved over decades, the evolution of small-molecule DPP-IV inhibitors has been explosive, and a remarkable diversity of structures has appeared in the primary and patent literature in the past 12–18 months.[4] The intense interest is due to the fact that DPP-IV is a clinically validated target. While there is no marketed drug as yet, several companies have announced successful proof-of-concept clinical trials with structurally diverse, oral DPP-IV inhibitors (Fig. 1): ProBioDrug (P32/98, **1**),[5] Novartis (NVP-DPP728, **2**,[6a,b] Vildagliptin: NVP-LAF237, **3**[6c]), and most recently Merck (MK-0431, **4**[7]). P32/98 is a substrate-derived non-covalent inhibitor, with an unusually low molecular weight and modest potency in vitro (DPP-IV IC_{50} 140 nM, human; in-house data); NVP-DPP728 and NVP-LAF237, whose cyanopyrrolidine ring has a substrate-like function, are potent, reversible covalent inhibitors in which the cyano group is the active-site serine trap; and Merck has pursued non-covalent inhibitors based on literature compounds and screening leads, culminating in the potent and selective

Keywords: Dipeptidyl peptidase-IV; DPP-IV; Type 2 diabetes; Glucagon-like peptide-1; GLP-1.
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doi:10.1016/j.bmcl.2005.11.103

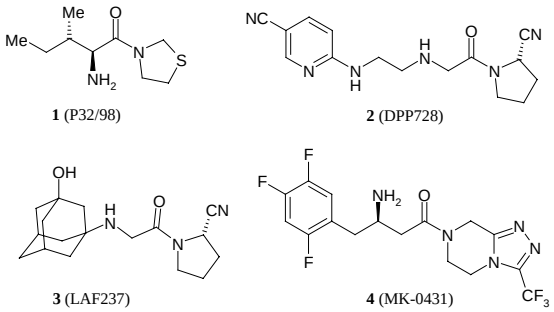


Figure 1. Clinical DPP-IV inhibitors.

β-phenethylamine derivative MK-0431. Early concerns that inhibition of DPP-IV, which has multiple substrates, would lead to general toxicity are gradually receding as a confluence of potent, selective compounds, animal data and positive clinical data emerge.[8]

Across the spectrum of existing DPP-IV inhibitors, and by analogy to substrates, a proline or pyrrolidine ring or analogue is commonly found that has been shown by X-ray crystallography to bind to DPP-IV in a substrate-like mode in the proline-binding pocket—a pocket that has restricted volume and therefore has severe structure–activity relationship (SAR) constraints.[9]

Our own endeavours to produce novel, clinically relevant non-covalent inhibitors of DPP-IV were triggered and inspired by the discovery of an unexpected 'reverse' binding mode for β-phenethylamine-based inhibitors, as revealed by X-ray crystallography.[10] These findings are the subject of this communication from our laboratories.

In-house screening of our fragment collection uncovered the β-phenethylamines **5** and (racemic) **6** (Fig. 2) as weak inhibitors of DPP-IV (**5**, DPP-IV IC_{50} 33 μM, human; IC_{50} 30 μM, porcine; **6**, DPP-IV IC_{50} 33 μM, human; IC_{50} 46 μM, porcine). The binding mode for **5** was established by X-ray crystallography using porcine enzyme and revealed that the phenyl ring was located in the hydrophobic proline pocket (S1) lined by Y662, V656, Y666 and V711. The (protonated) amino group was anchored by a multiple hydrogen-bonding network to E205, E206 and Y662, and the cyclopentyl ring was found to occupy a similar position to the isopropyl group of the α-amino acid-derived inhibitor Val-Pyr (Fig. 3a).

Subsequent to an α-amino acid-derived series of inhibitors, which, by analogy with Val-Pyr presumably bind to DPP-IV in a substrate-like fashion, Merck disclosed the screening hit **7** (DPP-IV IC_{50} 1.9 μM) and derivatives,[11] that contained a β-phenethylamino group. If such compounds, despite their resemblance to substrate-like inhibitors, bound by analogy to **5**—with the phenyl ring, and not the proline ring, in the proline pocket—hypotheses for novel inhibitor designs would emerge. In particular: (i) the proline ring SAR would not be restricted by the limited dimensions of the proline-binding pocket; and (ii) proline side-chain modifications would not be constrained to a substrate-like SAR.

The tetrahydroisoquinoline (TIC) derivative **8** was chosen to probe the hypothesis that proline could be replaced by a much larger ring that could not be accommodated in the DPP-IV proline-binding pocket, and the proline transposed amide **9** (cf. **7**) was chosen to probe the hypothesis that side-chain SAR on the proline ring would not necessarily require a substrate-like amide. The incorporation of an *ortho*-fluoro atom was expected to boost activity as it was anticipated this would bind into the lipophilic proline-binding pocket.

The synthesis of **8** is shown in Scheme 1. Commercial Boc-protected (*S*)-2-carboxytetrahydroisoquinoline **10a** was converted to the primary amide **10b** using standard amide coupling conditions of EDC and HOBt. Deprotection followed by coupling with the Boc-β-amino acid **12**, followed by further deprotection produced **8**.[12] Following a similar strategy, compound **9** was synthesized as outlined in Scheme 2. Commercial Boc-protected (*S*)-2-aminomethylpyrrolidine **13a** was coupled with benzoic acid to give **13b**, the Boc group was removed un-

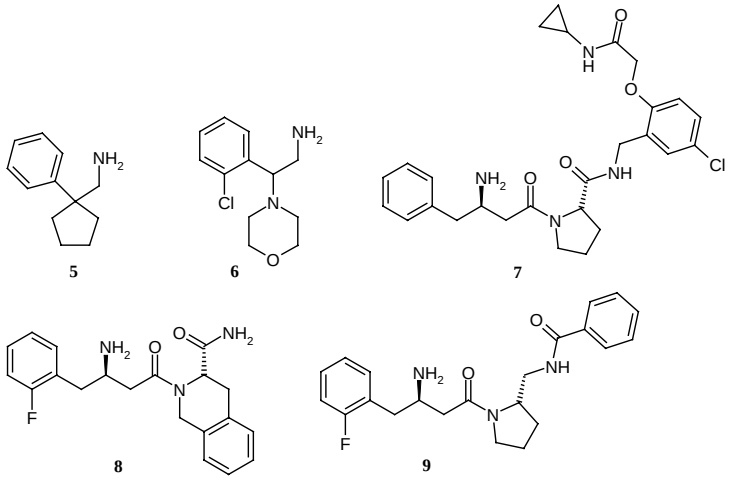


Figure 2. β-Phenethylamine DPP-IV inhibitors.

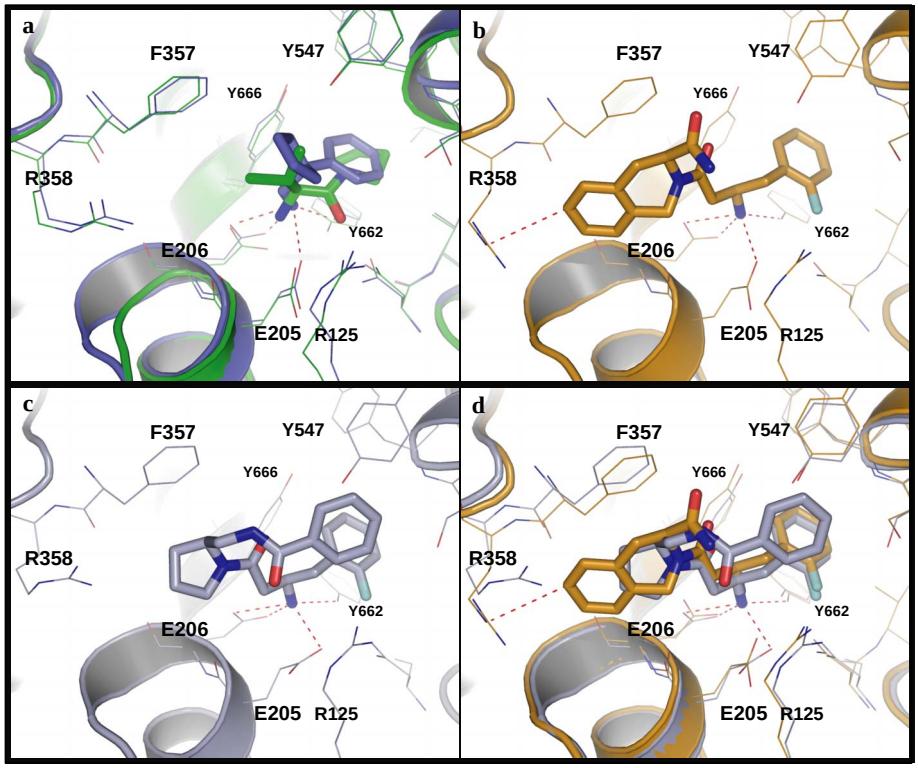
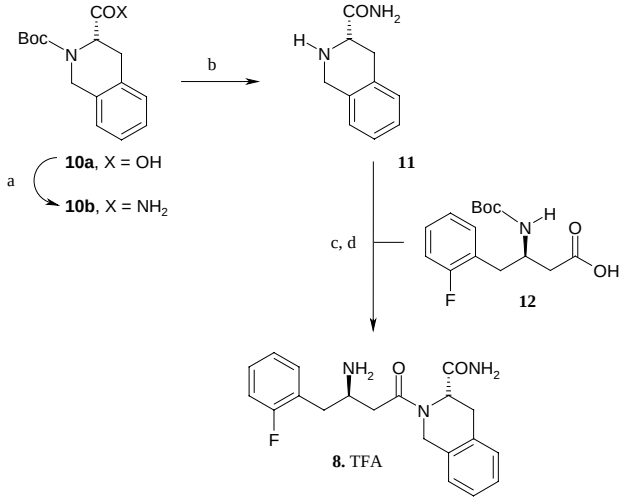
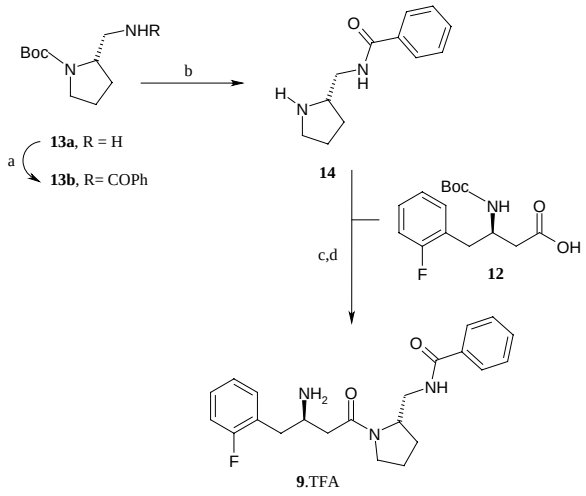


Figure 3. (a–d) Crystal structures of DPP-IV leads: (a) in-house screening hit **5** (blue) aligned with Val-Pyr (green, taken from PDB entry 1n1m[9b]); (b) reversed binding mode of TIC compound (orange) **8**; (c) reversed amide **9** (grey) proved the non-substrate-like binding mode of the proline ring; (d) overlay of **8** and **9**. Figures were generated using PYMOL version 0.98 (Delano Scientific, www.pymol.org).



Scheme 1. Synthesis of inhibitor **8**. Reagents: (a) NH_3/dioxane, EDC, HOBt, DIEA, DMF; (b) TFA, DCM; (c) EDC, HOBt, DIEA, DMF; (d) TFA, DCM.



Scheme 2. Synthesis of inhibitor **9**. Reagents: (a) Benzoic acid, EDC, HOBt, DIEA, DMF; (b) TFA, DCM; (c) EDC, HOBt, DIEA, DMF; (d) TFA, DCM.

der standard conditions, and the pyrrolidine moiety coupled with acid **12** and deprotected to give **9**.[12]

Gratifyingly, **8** and **9** proved to be potent inhibitors of human DPP-IV[13] (**8**: DPP-IV IC_{50} 23 nM; **9**: DPP-IV IC_{50} 90 nM) and porcine (**8**: DPP-IV: 60% inhibition at 100 nM; **9**: DPP-IV IC_{50} 410 nM). X-ray analysis of **8** bound to porcine DPP-IV revealed the reverse binding mode and analogy with **5** (Fig. 3b). Thus, the fluorophenyl ring occupies the proline-binding pocket, the cationic amino group was anchored to the glutamates and the tyrosine, and the TIC ring was located distal to the proline pocket with lipophilic contacts to F357 and a close cation–π interaction to R358. Likewise, X-ray analysis of **9** bound to human DPP-IV (Fig. 3c) revealed the reverse binding mode where the proline of **9** was distal to S1. The phenyl ring of the transposed side-chain amide

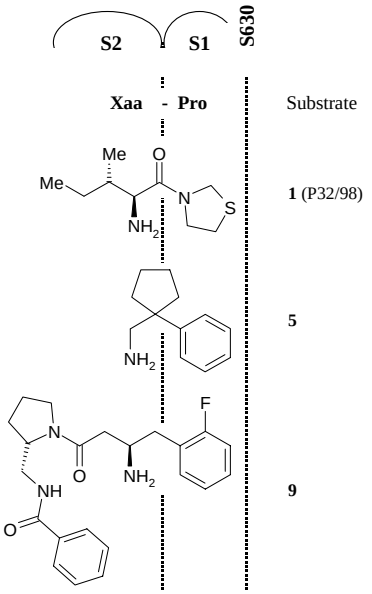


Figure 4. Illustration of the reversed binding mode by means of compounds **5** and **9**, which have the five-membered ring distal to the S1 site, contrary to the substrate-like inhibitor **1** (see also Figs. 3a and c).

had contacts with Y547 and R125 of the protease. Overall, **9** had a very similar binding mode to **8**. The TIC ring of **8** protrudes into the enzyme well beyond the proline ring of **9** (Fig. 3d) and this observation provided the basis for a thorough exploration of non-proline rings and bio-isosteres. Figure 4 further illustrates the new binding mode of our compound series compared to substrate-like inhibitors such as **1**.[11c]

The findings presented here provided a foundation and stimulus for novel DPP-IV inhibitor design, and the rapid evolution of potent, selective, orally bioavailable compounds. These results will be reported in due course.

Acknowledgments

The authors gratefully acknowledge Proteros Biostructures GmbH, Am Klopferspitz 19, 82152, Martinsried, Germany, for co-crystallization of our inhibitors and obtaining X-ray data, Sabine Bostel for synthesizing target molecules **8** and **9**, and Barbara Hoffmann, Carmen Fischer and Ute Schmitt for technical assistance.

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12. All final compounds were characterized by ^{1}H NMR and LC–MS (Reprosil-Pur ODS3, 5 μm, 1 × 60 mm column, linear gradient from 5% to 95% acetonitrile in water with 0.1% TFA over 5 min, flow rate 250 μl/min). *Compound* ***8***. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.68–3.30 (m, 6H), 3.74 (m, 1H), 4.37–4.69 (m, 2H), 4.93 (m, 1H), 6.86 (br s, 1H), 7.05–7.49 (m, 9H), 7.86 (br s, 3H). LC–MS rt 1.97 min, *m*/*z* 356 $(M+H)^+$. *Compound* ***9***. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 1.75–1.95 (m, 4H), 2.50–3.37 (m, 8H), 3.69–3.80 (m, 1H), 3.91 (m, 0.3H), 4.18 (m, 0.7H), 7.12–7.19 (m, 2H), 7.28–7.33 (m, 5H), 7.37–7.52 (m, 2H), 7.92 (br s, 3H), 8.40 (m, 0.7H), 8.63 (m, 0.3H). LC–MS rt 2.02 min, *m*/*z* 384 $(M+H)^+$.
13. Inhibition of DPP-IV peptidase activity was monitored with a continuous fluorimetric assay. Compounds (stock solutions are prepared with DMSO) are preincubated with 50 pM DPP-IV employing a buffer containing 10 mM Hepes, 150 mM NaCl and 0.005% Tween 20 (pH 7.4). The reaction is started by the addition of 16 μM Gly-Pro-AMC and the fluorescence of liberated AMC is detected for 10 min at 25 °C with a fluorescence reader using an excitation wavelength of 370 nm and an emission wavelength of 450 nm. The final concentration of DMSO is 1%. To assess the inhibitory potential of the compounds, IC_{50} values were determined from at least two independent measurements carried out as triplicates. Soluble human DPP-IV lacking the transmembrane anchor (Gly31-Pro766) was expressed in a recombinant *Pichia methanolica* strain as Pre-Pro-alpha-mating fusion. The secreted product (rhuDPP-IV-Gly31-Pro766) was purified from fermentation broth (>90% purity) and deglycosylated.