Inhibitors of Dipeptidyl Peptidase 4

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1. INTRODUCTION

Dipeptidyl peptidase 4 (EC 3.4.14.5, DPP-IV, DPP4, CD26) is a ubiquitous serine protease that modulates the biological activities of numerous peptides, including glucagon-like peptide-1 (GLP-1). GLP-1 plays an important role in the control of post-prandial glucose levels by potentiating glucose-stimulated insulin release and inhibiting the release of glucagon. Other actions of GLP-1 include delaying gastric emptying, inducing satiety and increasing *beta* cell mass. GLP-1 has shown efficacy in diabetics, but suffers from a very short physiological half-life ($t_{1/2}$ ~2 min) due to DPP4-mediated cleavage of the active peptide (7-36 amide or 7-37) to an inactive form (9-36 amide or 9-37). Intense research in the pharmaceutical industry aims to discover and develop stable GLP-1 analogs, exogenous agonists of the GLP-1 receptor or small-molecule inhibitors of DPP4. This research has been buoyed recently by positive clinical trial data on GLP-1 analogs and DPP4 inhibitors. The field of DPP4 inhibition has been reviewed extensively [1–12]. This review attempts to provide an update to the previous ARMC article on DPP4 inhibitors [13] covering the primary literature from 2001 through the end of March 2005. It is not the intent of the authors to provide another review of the pharmacology of DPP4, but to concentrate on the medicinal chemistry in the field.

1.1. Function of DPP4

DPP4 functions as a serine protease and cleaves the amino-terminal dipeptide from oligopeptides with a proline or alanine at the penultimate position. Peptides with residues other than Pro or Ala at the penultimate position may also be low-affinity substrates for DPP4. In contrast, DPP4 is not selective with respect to the N-terminal

residue [14] and shows little discrimination of various prime-side residues [15,16]. A number of biologically important peptides are substrates for DPP4 *in vitro* [17,18].

1.2. Structure of DPP4

DPP4 is a 110-kDa glycoprotein expressed on the cell surface and widely distributed throughout the body. Cleavage of the extracellular portion of DPP4 from the 22-residue transmembrane section results in a soluble, circulating form of approximately 100 kDa. Functional DPP4 is a homodimer, although an active heterodimer with fibroblast activation protein has been observed [19]. The consensus sequence for DPP4 is G-W-S-Y-G and the catalytic triad is made up of Ser630, Asp708 and His740. It has been shown that the glycosylation state of the enzyme is not important for enzyme activity, dimerization, and adenosine deaminase binding [20].

Several groups have reported crystal structures of human DPP4 [15,21–24], and one group has reported the structure of porcine DPP4 [25]. These structures show the dimeric nature of the enzyme and reveal that the catalytic site is located in a cavity between the α/β hydrolase domain and an eight-bladed propeller domain. Also revealed is the oxyanion hole, which is composed of the backbone NH of Tyr631 and the OH of Tyr47. A co-complex of DPP4 and the inhibitor Val-pyrrolidide demonstrates that two glutamates in the active site play an important role in substrate binding by forming a salt bridge with the N-terminus of a peptide substrate. The pyrrolidine of the inhibitor effectively fills a hydrophobic pocket that will only accommodate small residues. This pocket engenders DPP4's selectivity for proline at P1. This work also revealed that two openings in the enzyme may provide access to and egress from the catalytic site for some substrates and products [21]. The importance of Tyr547 in the stabilization of the intermediate oxyanion was confirmed through site-directed mutagenesis [26]. Most authors agree that peptides enter the larger side opening to access the active site [15]. It has been postulated that the dipeptide product is expelled through the narrow β-propeller opening [21,24]. The co-complex of DPP4 and a compound related to NVP-DPP728 [23] confirms that cyanopyrrolidine inhibitors form an imidate with the active site serine, consistent with a model proposed earlier [27]. Two groups have observed the trapping of tetrahedral intermediates in co-complexes of peptides with DPP4 [15,24].

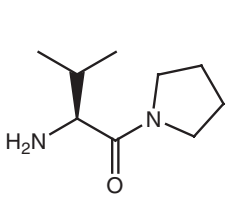
1.3. Therapeutic significance

Relative to wild-type controls, DPP4-deficient mice are resistant to the development of obesity and hyperinsulinemia when fed a high-fat diet [28]. DPP4 knockout mice also show elevated GLP-1 levels and improved metabolic control. Relative to DPP4 positive controls, DPP4-deficient Fischer rats show improved glucose tolerance following an oral glucose challenge due to enhanced insulin release mediated by high levels of active GLP-1 [29,30]. In these studies, the authors note that fasting and post-challenge glucose levels in both strains are similar, supporting previous assertions that hypoglycemia is unlikely during treatment with DPP4 inhibitors.

The use of GLP-1 and its analogs in the treatment of diabetes has been reviewed recently [31,32]. It has been shown that DPP4 inhibition prevents the degradation of endogenous GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) in dogs, thereby preserving the insulinotropic effects of these peptides [33]. In the same study, it was noted that total incretin secretion was reduced, suggesting that feedback mechanisms restrict the secretion of incretins when levels of active peptide are elevated. It has been demonstrated that agonism of the GLP-1 receptor results in growth and differentiation of pancreatic islet *beta* cells [34–36]. If realized in humans, such an effect may result in preservation or restoration of β-cell function in diabetics. In human clinical trials, infusion of GLP-1 led to such beneficial effects as decreases in post-prandial glucose excursions, increases in post-prandial insulin, reductions in HbA_{1c}, weight loss, enhanced insulin sensitivity and improved β-cell function [37,38]. Administration of the GLP-1 analogs exendin-4, CJC-1131 and NN2211 resulted in similar beneficial effects [31,32]. Notably, DPP4 inhibition has been shown to augment the insulin secretion effects of not only GLP-1 and GIP, but also pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide (GRP) [39].

2. PRECLINICAL DPP4 INHIBITORS

Early DPP4 inhibitors closely mimicked DPP4 substrates, as exemplified by valine-pyrrolidide (Val-Pyr, **1**), P32/98 (**2**) and FE 999011 (**3**). A large body of data has been reported for these compounds and provided early biological validation for the use of DPP4 inhibitors as an approach to the treatment of diabetes.



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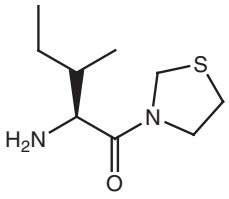

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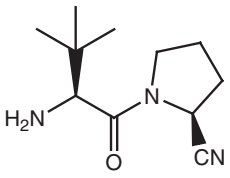

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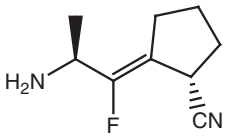
Treatment of six-week-old *db*/*db* mice with Val-Pyr resulted in increased endogenous GLP-1 levels, potentiated insulin secretion and improved glucose tolerance; however, while the effects on GLP-1 and insulin were maintained in mice at 23 weeks of age, the improved glucose control was lost [40]. Studies in rats demonstrated that combining Val-Pyr with metformin leads to reduced food intake and body weight gain, improved glucose tolerance and increases in active plasma GLP-1 and that these effects are absent or less significant when using either drug as monotherapy [41,42]. In related work, treatment of rats with metformin or pioglitazone resulted in reduced serum DPP4 activity. Since the authors found that these agents are not inhibitors of DPP4 *in vitro*, they suggested that the effect resulted from reduced DPP4 secretion [43].

Double incretin receptor knockout (DIRKO) mice are genetically altered to lack both the GLP-1 receptor and the GIP receptor. A study in these animals with

Val-Pyr and a structurally unrelated inhibitor, SYR106124, showed that while these inhibitors provide improved glucose tolerance and increased insulin levels in wild-type and single incretin receptor knock out mice, these effects were lost in the DIRKO mice. This result points to the essential nature of the incretin receptors in the actions of DPP4 inhibitors [44].

While inhibitors such as **4** ($K_i = 6.03\,\mu M$) and **5** ($IC_{50} = 12\,\mu M$) are related to the cyanopyrrolidine DPP4 inhibitors through the use of the fluoroolefin amide isostere, these compounds are only weak inhibitors of the enzyme [45–47].



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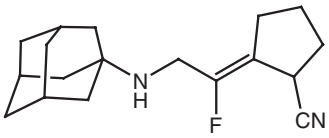

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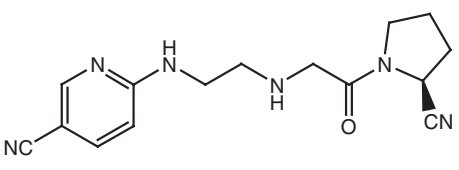
Several recent papers have examined the effects of long-term treatment with P32/98 (**2**) in rodent models of diabetes. A three-month treatment regimen provided sustained improvements in glucose tolerance, increased β-cell responsiveness and improved peripheral insulin sensitivity in Zucker *fa/fa* rats [48,49]. The same investigators have shown that 7 weeks of treatment with **2** enhances β-cell survival and islet neogenesis in a streptozotocin-induced diabetes model [50]. A study designed to compare the effects of **2** with those of rosiglitazone and to the effects of the combination of the two agents found that the DPP4 inhibitor provided improved glucose tolerance in both prediabetic and diabetic animals. While rosiglitazone resulted in increased body weight, **2** was body-weight neutral. However, neither agent was very effective at improving the diabetic condition of older ZDF rats [51]. Studies have shown that the metabolism of **2** is dominated by oxidation of the sulfur atom and glucuronidation of the primary amine [52].

In rodent models of diabetes, chronic treatment with FE 999011 (**3**) provided improved glucose tolerance, postponed the progression to hyperglycemia by 21 days, reduced hypertrigylyceridemia and prevented a rise in circulating free fatty acids [53].

Rodent studies using NVP-DPP728 (**6**, $IC_{50} = 7\,nM$) [54] and the structurally related K579 (**7**, $IC_{50} = 5\,nM$) have demonstrated similar pharmacological effects as those seen with the inhibitors discussed above. In a comparative study, **7** appeared to provide better control of DPP4 activity and glucose excursions than did **6** [55]. Combination of **7** with glibenclamide further enhanced the glucose control without significant hypoglycemia [56].



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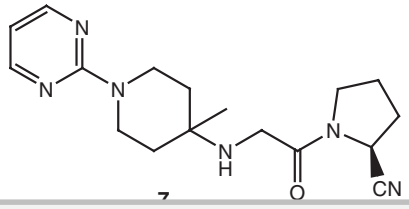

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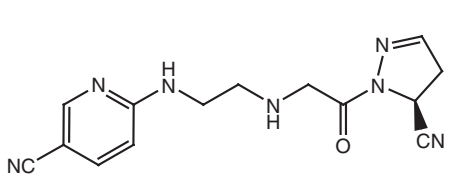
The 2-CN pyrrolidine present in **6** can be substituted by a cyanopyrazoline, but this results in a less potent compound (**8**, $IC_{50} = 360\,nM$) [57]. A pyrazolidine heterocycle has also been examined (**9**, $IC_{50} = 1.56\,\mu M$) [58].



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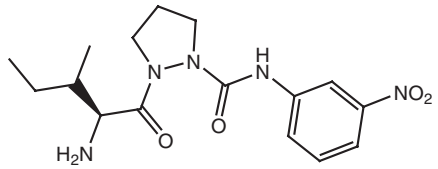

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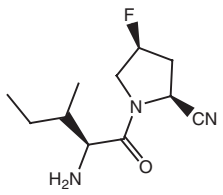
Several groups have examined substituted pyrrolidines in an effort to improve potency or stability of the inhibitors. Attempted incorporation of hydroxy or methoxy substituents at various positions on the ring led to reduced potency, but fluorination at the 4-position gave increased potency as in compound **10** ($IC_{50} = 0.6\,nM$). This compound also displayed increased plasma drug concentrations relative to the unsubstituted inhibitor [59]. In an examination of pyrrolidines cyclopropanated at either the 3,4 or 4,5 positions, it was found that while introduction of the cyclopropane on the face of the pyrrolidine *trans* to the cyano group led to compounds with micromolar IC_{50}s, the *cis*-3,4-methano and *cis*-4,5-methano moieties were well tolerated. One goal of this work was to reduce the intramolecular amine-nitrile cyclization that plagues many cyanopyrrolidine DPP4 inhibitors. Bulky substituents on the amino acid and the cyclopropane moiety provided impressive improvements in solution stability. Compound **11** ($IC_{50} = 1.5\,nM$) has a half-life of 5 hours, while compound **12** ($K_i = 8\,nM$) has one of 27 hours and compound **13** ($K_i = 7\,nM$), 42 hours. Compound **13** reduced glucose excursions following an oral glucose tolerance test (OGTT) in Zucker *fa/fa* rats [60].



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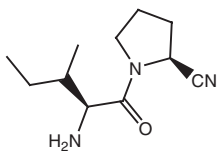

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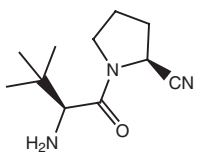

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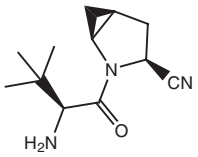

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Ketopyrrolidines and ketoazetidines, which replace the cyano group with a heteroaryl ketone, have also been examined as DPP4 inhibitors. Heteroaryl ketones have been used extensively as reversible serine protease inhibitors and act by providing an electrophilic carbonyl that can form a tetrahedral species with the active site serine. An examination of rings from four to six atoms revealed that only the piperidine derivatives were not inhibitors of the enzyme. 2-Thiazolyl and 2-benzothiazolyl substituents provided sufficient activation of the carbonyl to give low nanomolar inhibitors such as **14** ($IC_{50} = 30$–$42\,nM$). These compounds suffer from an internal cyclization followed by oxidation to give dihydroketopyrazines such as **15** [61].

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