Dipeptidyl Peptidase IV Inhibitors for the Treatment of Diabetes

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Received December 24, 2003

Introduction

Ingestion of food results in the release of peptide hormones in the gut, termed incretins, that regulate insulin in a glucose-dependent manner.[1] When blood glucose levels are high, the incretin hormone glucagon-like peptide 1 (GLP-1[7−36] amide or GLP-1) stimulates insulin secretion and biosynthesis and inhibits glucagon release. In addition, it serves as an "ileal brake", slowing gastric emptying and reducing appetite. GLP-1 also appears to regulate the growth and differentiation of the insulin-producing β cells in pancreatic islets in rodents. Thus, GLP-1 therapy for the treatment of type 2 diabetes is an area of active research.[2]

GLP-1 is rapidly degraded in vivo through the action of dipeptidyl peptidase IV (DPP-IV), which cleaves the N-terminal two amino acids to give the inactive GLP-1[9−36] amide (Figure 1).[3] Thus, GLP-1 must be administered via chronic infusion in order to achieve sustained elevated plasma levels. DPP-IV resistant GLP-1 analogues represent one means to circumvent this issue, but like GLP-1, these are peptides that must be administered parenterally. Orally bioavailable, small-molecule agonists of the GLP-1 receptor have yet to be reported, though several patents claim low molecular weight GLP-1 agonists and potentiators.[4] Inhibition of DPP-IV, which leads to an increase in circulating levels of endogenous GLP-1, is an alternative approach that appears highly amenable to drug discovery.[5]

DPP-IV Substrates

A cell surface serine protease, DPP-IV[6] is ubiquitously expressed, with the highest levels found in the kidney and the lower levels in liver, pancreas, placenta, thymus, spleen, epithelial cells, vascular endothelium, and lymphoid and myeloid cells. A soluble form is shed into the circulation. Substrate specificity studies point to DPP-IV's strong preference for cleavage of peptides containing a proline residue in P_1,[7] though interestingly GLP-1 and related glucagon family members contain alanine at this position. A wide range of substituents are allowed at P_2, and there is also little preference for specific residues on the prime side except that proline and hydroxyproline are disfavored at P_1'.

While a large number of peptides are cleaved by DPP-IV in vitro,[8] very few have been shown to be endogenous substrates based on the following stringent criteria: (i) cleavage occurs in vitro at the penultimate residue; (ii) cleavage products are observed in vivo but are absent in the presence of a selective inhibitor or in DPP-IV$^{-/-}$ mice; (iii) cleavage is the major route of clearance of the peptide. GLP-1 meets these criteria, as does the incretin hormone glucose-dependent insulinotropic polypeptide (GIP, also known as gastric inhibitory peptide).[9] GIP, which is secreted in the proximal gut in response to food, stimulates insulin secretion in a glucose-dependent manner and is believed to account for approximately half the incretin response in healthy humans.[9c] Unlike GLP-1, the insulinotropic effects of GIP are reduced in type 2 diabetics, and this may contribute to the reduced incretin effects in these patients.

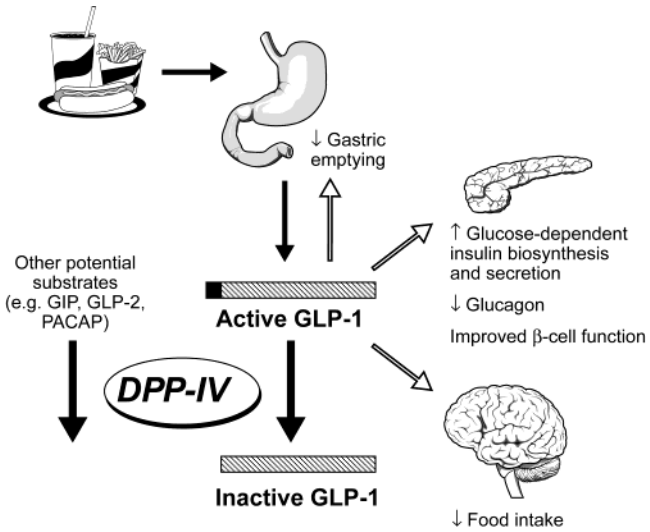

Figure 1. DPP-IV regulates glucose homeostasis via inactivation of GLP-1 and other incretin hormones.

DPP-IV inhibitors evoke decreases in glucose excursion following an oral glucose challenge. Recent studies demonstrate efficacy of inhibitors in mice lacking one or both of the receptors for GLP-1 and GIP.[10] Clearly there are other substrates in addition to these incretins that contribute to the biological activity of DPP-IV inhibitors. One potential candidate is pituitary adenylate cyclase-activating polypeptide (PACAP), a pancreatic neuropeptide that regulates lipid and carbohydrate metabolism. Intravenous administration of this peptide to mice results in rapid cleavage at the penultimate residue. The DPP-IV cleavage product is absent in DPP-IV$^{-/-}$ mice, suggesting a potential role for the enzyme in in vivo processing of PACAP.[11]

DPP-IV Inhibitor SAR

In light of DPP-IV's substrate specificity, it is not surprising that α-aminoacylpyrrolidine derivatives have been widely explored as DPP-IV inhibitors. The most potent of these contain an electrophile at the 2-position of the pyrrolidine ring (Figure 2), which forms an adduct with the active site serine. Irreversible inhibitors con-

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10.1021/jm030628v CCC: $27.50
Published on Web 07/20/2004

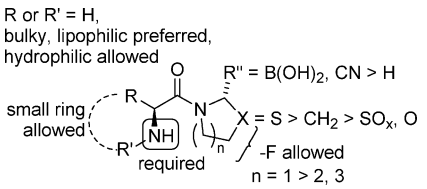


Figure 2. SAR of reversible α-aminoacylproline-derived DPP-IV inhibitors.

taining diphenylphosphonate esters[12] and *O*-acylhydroxamic acids[13] have been identified. A boronic acid moiety provides highly potent inhibitors that are slowly reversible,[14] but the most extensively studied agents are those containing a nitrile at this position. Replacement of the pyrrolidine with thiazolidine gives derivatives with increased potency; however, larger rings (e.g., piperidine, homopiperidine) or those containing other heteroatoms (e.g., oxazolidine) are less potent.[15] With the exception of fluorine, substituents on the pyrrolidine ring are not well-tolerated. In the thiazolidine series, oxidation of the sulfur to sulfoxide or sulfone leads to a decrease in activity.

A basic amine at P_2 is strictly required for inhibition. Consistent with the substrate specificity studies, a wide range of side chains at P_2 are tolerated, including bulky, lipophilic groups, and those containing polar functionality. Branching at this position is preferred, and of the simple amino acid substituents, isoleucyl, and cyclopentylglycyl, and cyclohexylglycyl provide the most potent inhibitors.[16]

Peptides containing sarcosine at P_2 are also substrates for DPP-IV, and this knowledge led to the exploration of N-substituted glycine derivatives as DPP-IV inhibitors.[17] Like their α-substituted amino acid counterparts, these inhibitors tolerate both straight-chain and cyclic substituents at this position, with polar and lipophilic side chains including the very bulky adamantyl group. Two derivatives from this class have been studied in the clinic: DPP728 (**1a**) and LAF237 (**2**, IC_{50} = 22 and 3.5 nM, respectively; Chart 1).

Substituents on nitrogen appear to fill the same S_2 site as those on the α-carbon. Indeed *N*,α-bis-substituted analogues show greatly decreased potency.[17c] Small rings bridging carbon and nitrogen are tolerated, including proline at P_2. A series of tetrahydroisoquinoline-3-carbonylcyanopyrrolidine derivatives are also reported to have good DPP-IV inhibitory activity (e.g., **3**; IC_{50} = 4 nM).[18]

Because of the presence of the required basic amine, electrophile-containing inhibitors generally exhibit a high degree of solution instability. This may contribute in part to the relatively short half-life of these derivatives in vivo. Product-like inhibitors, those lacking an electrophile, have also been developed. While more stable, they typically are much less potent than the corresponding nitriles. One of these, *threo*-isoleucylthiazolidide or P32/98 (**4**, K_i = 126 nM),[19] was advanced to clinical trials. Cyclohexylglycylpyrrolidide (**5**, K_i = 64 nM) is among the most potent DPP-IV inhibitors lacking an electrophilic serine trap.[16] Recently, derivatives with substitution at the 4-position of the cyclohexyl ring were reported to have increased potency. The 4-(2,2,2-trifluoroethyl)sulfonamidophenylsulfonylamino derivative **6** has an IC_{50} of 2.6 nM and is >1000-fold selective over the related prolyl peptidase QPP (quiescent cell proline dipeptidase).[20]

The amide bond is not strictly required for potency, and inhibitors such as **7** containing a fluoroolefin amide bond replacement have been reported.[21] A number of heterocyclic structures devoid of peptide-like character have also been shown to inhibit DPP-IV. These include xanthine derivatives such as **8** (IC_{50} = 5 nM)[22] and isoquinoline[23] and isoquinolone[24] derivatives **9** and **10** (IC_{50} = 320 and 250 nM, respectively).

The X-ray crystal structure of DPP-IV bound to an inhibitor has recently been solved by several laboratories.[25] The enzyme is a homodimer. Each subunit comprises an α/β-hydrolase domain and an eight-bladed β-propeller domain. A large cavity, roughly 30−45 Å wide, is located between the two domains, and inhibitors bind to a small pocket in this cavity, with key residues from both domains making up the binding site. The basic amine forms a salt bridge with Glu205 and, in some cases, Glu206 from the β-propeller domain. Arg125, also from that domain, stabilizes the amide carbonyl moiety. The proline binding pocket is formed by a group of hydrophobic, primarily aromatic residues from the α/β-hydrolase domain, leaving little room to accommodate larger substituents at that position. The active site serine, Ser630, forms an imidate with the nitrile as predicted. The S_2 site is bounded by Ser209, Phe357, and Arg358. This pocket readily accommodates the 5-iodopyrid-2-ylaminoethyl side chain of glycine derivative **1b** and the 4-iodobenzyl side chain of phenylalanine derivative **11**. Thus, the crystal structures provide ready explanations for the structural requirements of the basic amine and pyrrolidine residues, the increased potency of the nitrile-containing analogues, and the less stringent requirements for moieties at P_2. It remains to be seen how this information will be used to design the next generation of DPP-IV inhibitors.

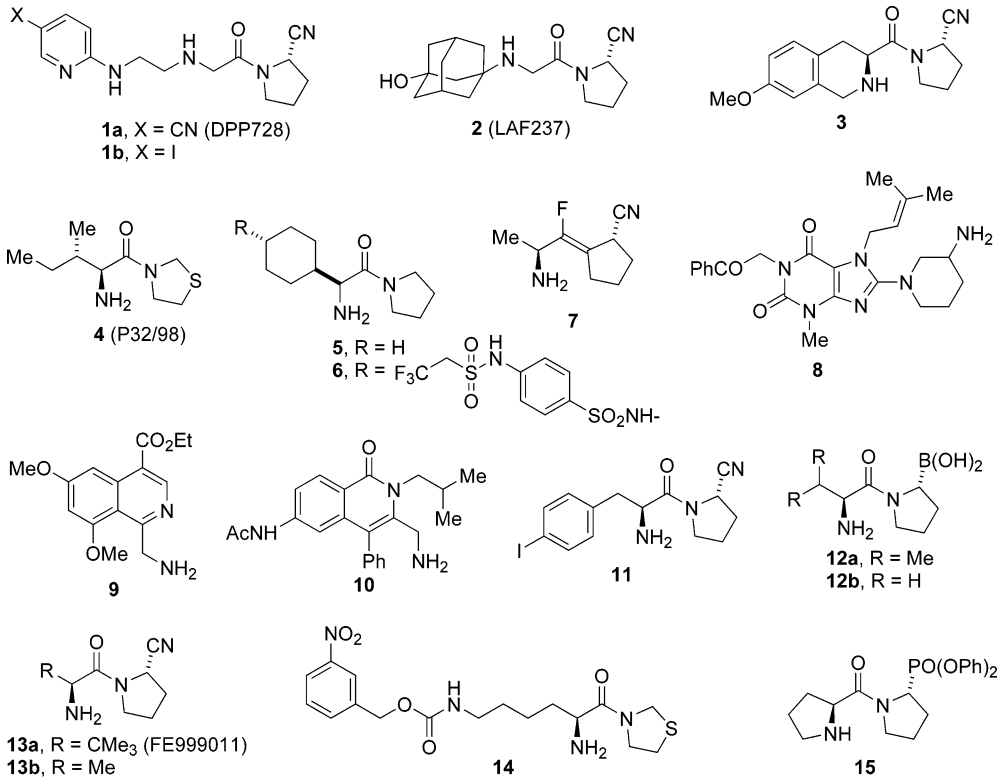
Related Enzymes

In the current inhibitor designs, selectivity over a wide range of proteases is generally possible. The stringent requirement for a basic amine provides inhibitors with selectivity over proline endopeptidases, while the preference for pyrrolidine at P_1 provides selectivity over other aminopeptidases.[17a] However, inhibitors that are thought to be specific for DPP-IV may in fact inhibit other enzymes in the "DPP-IV activity and/or structural homologue" (DASH) family.[26] Several family members have only recently been described, and thus, selectivity data are not generally available.

Fibroblast activation protein α (FAP-α, also known as seprase) shares the highest sequence homology to DPP-IV. This enzyme, which is found in tissue remodeling sites and tumors but not in healthy adult tissue, may be important in wound healing and cancer invasion. Recently the DPP-IV inhibitor Val-boro-Pro (**12a**) was shown to inhibit both DPP-IV and FAP.[27] This compound's ability to stimulate regeneration of neutrophils following cyclophosphamide treatment, originally attributed to DPP-IV inhibition, was noted in both wild-type and DPP-IV-deficient mice, suggesting that another prolyl peptidase such as FAP-α might be responsible for the observed biological activity.

Two other closely related DPP-IV-like proteins, DPP8[28] and DPP9,[29] are soluble, cytoplasmic enzymes that are

Chart 1. Structures of DPP-IV Inhibitors



ubiquitously expressed. DPP8 is up-regulated on activated T-cells, while high levels of DPP9 are found in skeletal muscle, heart, and liver. The latter enzyme was originally reported to lack peptidase activity, but that has recently been refuted.[29b] The biological function of both proteins is currently unknown, as is the degree to which "selective" DPP-IV compounds inhibit them.

Quiescent cell proline dipeptidase (QPP), renamed DPP7, appears to be identical to DPP II[30] and is located in intracellular vesicles. This enzyme shares sequence homology to prolyl carboxypeptidase but has DPP-IV-like, prolyl aminodipeptidase activity. Val-boro-Pro (**12a**), which inhibits DPP-IV with a K_i of 2 nM, is a 125 nM inhibitor of QPP.[31] Treatment of peripheral blood monocytes with this inhibitor induces apoptosis in quiescent but not activated lymphocytes. This effect is seen in T-cells lacking DPP-IV and has thus been attributed to the compound's ability to inhibit QPP in these cells. Selective inhibitors of QPP have been reported and may be useful in determining the biological role of this enzyme.[32]

Selectivity over these related enzymes may prove to be important for identifying safe and well tolerated inhibitors. In addition, caution must be used in interpreting studies with DPP-IV inhibitors because it is clear that in some cases, biological effects have been incorrectly attributed to DPP-IV inhibition.

Preclinical Proof of Concept Studies

There is a growing body of evidence to suggest that inhibition of DPP-IV will have therapeutic effects in treating diabetes. DPP-IV$^{-/-}$ mice show decreased blood glucose levels accompanied by an increase in insulin following an oral glucose challenge.[33] In addition, Fischer F344/DuCrj rats, which have a natural point mutation in DPP-IV affecting trafficking of the enzyme, have greatly reduced plasma DPP-IV activity and show improved glucose tolerance.[34]

Acute inhibition of DPP-IV by small-molecule inhibitors leads to increases in plasma GLP-1 levels and decreases in glucose excursion following an oral glucose challenge in both normal mice and rats and in animal models of diabetes and impaired glucose tolerance, including diet-induced obese (DIO) mice[35] and Zucker fatty rats.[36] An increase in insulin precedes the decrease in blood glucose, suggesting that the mechanism of glucose lowering is increased insulin secretion. The indirect effect of these compounds is also supported by the observation that DPP-IV inhibitors have no effect on glucose-stimulated insulin secretion in isolated islets.[35] In db/db mice, DPP-IV inhibition reduces glucose excursion in young animals but not in older animals with impaired β-cell function and pronounced insulin resistance.[37] Data from this acute study thus suggest that DPP-IV inhibitors may not prove to be efficacious in advanced diabetics but rather in patients with early stages of the disease.

A number of chronic animal studies provide support for the use of DPP-IV inhibitors in the long-term treatment of diabetes. Chronic administration of DPP-IV inhibitor isoleucylthiazolidide (**4**) to VDF Zucker rats, a model characterized by mild hyperglycemia, hyperinsulinemia, and insulin resistance, resulted in a decrease in the 24 h glucose profile and a progressive decrease in both fasting and peak blood glucose levels.[38] Following 12 weeks of treatment, an increase in glucose uptake in soleus muscle was evident as was an increase in the rate of insulin secretion in perfused pancreases from treated animals. The first-phase insulin response, which was absent in controls, was restored in the treated animals. Euglycemic−hyperinsulinemic clamp

studies showed an increase in glucose disposal and a decrease in hepatic glucose output.[39]

Chronic studies in Zucker diabetic fatty (ZDF) rats, which become overtly diabetic at about 8 weeks of age, suggest that DPP-IV inhibition may delay the development of disease.[40] Treatment of 6 week old animals with the potent DPP-IV inhibitor FE 999011 (**13a**, $IC_{50} = 7$ nM) delayed the onset of hyperglycemia from day 8 in vehicle-treated animals to day 15 in rats dosed with 10 mg/kg FE 999011 QD. In ZDF rats dosed with 10 mg/kg b.i.d., the onset was delayed to day 24, suggesting that near-complete, 24 h inhibition of DPP-IV is necessary to obtain maximal efficacy. Free fatty acids and triglycerides were maintained below levels considered toxic to β-cells in the b.i.d.-treated animals; thus, preservation of islet function is a possible mechanism for the delayed onset of diabetes.

There is additional evidence to suggest that chronic DPP-IV inhibition may preserve or restore islet function. In isolated islets from DIO mice treated with DPP728 (**1a**), an increase in insulin response at medium glucose concentrations was noted while maximal glucose-stimulated insulin secretion was not effected.[41] This was accompanied by an increase in GLUT-2, a β-cell glucose transporter. While there was no effect on body weight in the treated animals, islet size was normal; thus, DPP-IV inhibition appears to counteract the increase in islet size that is typically seen in animals fed a high-fat diet.

In a recent report,[42] Wistar rats were treated chronically with isoleucylthiazolidide (**4**) beginning 1 week before or 1 week after administration of streptazotocin (STZ), a toxin that destroys pancreatic β-cells. In the early treatment group, postprandial glucose levels were less than both the late treatment and control STZ-treated groups and plasma insulin levels were higher. The early treatment group showed an increase in glucose-stimulated insulin secretion in perfused pancreas studies and an increase in β-cell number, indicating a cytoprotective effect of DPP-IV inhibition. After 6 weeks, the late treatment group also showed a progressive decrease in glucose and an increase in insulin. Both early and late treatment groups had increases in the smallest size subset of islets relative to STZ-treated controls, with near-normal β-cell fractions, suggesting β-cell regeneration or islet neogenesis.

Taken together, data from preclinical studies indicate that treatment with a DPP-IV inhibitor may provide improved efficacy in the early stages of diabetes and may delay progression of the disease. The potential for preservation and regeneration of β-cells suggests a role for DPP-IV inhibition even in the late stages of diabetes.

Clinical Studies

Preliminary clinical results have been disclosed on three DPP-IV inhibitors: isoleucylthiazolidide (**4**), DPP728 (**1a**), and LAF237 (**2**). Isoleucylthiazolidide was reported to be safe and well-tolerated in normal volunteers at doses up to 240 mg.[43] DPP-IV was inhibited in a dose-dependent manner. In an open label study in diabetic patients a decrease in glucose excursion following an OGTT was seen at a dose of 60 mg.[44] Increases in both active GLP-1 and GIP were noted.

Patients with mild diabetes were treated for 4 weeks with DPP728.[45] Because of the short half-life of this compound in humans ($t_{1/2} = 50$ min), the total daily dose of 300 mg was divided (150 mg b.i.d. and 100 mg t.i.d.). Both dosing regimens led to decreases in fasting and prandial glucose and mean 24 h glucose relative to placebo. While the drug was generally well-tolerated, transient pruritus localized to the palms was noted in treated subjects, perhaps due to potentiation of an unknown bioactive peptide such as substance P.

Development of DPP728 has been discontinued in favor of LAF237, which has a profile suitable for once daily dosing. Following administration to diabetic patients at a dose of 100 mg qd for 4 weeks, decreases in fasting glucose, postprandial glucose, and postprandial glucagon levels were seen.[46]

While these initial clinical studies appear promising, the long-term safety, efficacy, and durability of DPP-IV inhibitor treatment remain to be established.

Additional Opportunities and Potential Pitfalls

With its mechanism of glucose-dependent insulin biosynthesis and secretion, DPP-IV inhibition provides the potential opportunity for excellent synergy with existing diabetes treatments. Combination therapy with insulin sensitizing agents such as PPARγ agonists and agents that control hepatic glucose output such as biguanides may prove to be particularly effective. The former combination has been explored in obese Zucker rats.[47] Following a 10 day treatment, a synergistic effect was observed with DPP-IV inhibitor LAF237 (**2**) and PPARγ agonist pioglitazone, in particular, on the increase in rate of glucose disposal.

Additional opportunities may exist in the treatment of diseases beyond diabetes. When fed a high-fat diet, DPP-IV$^{-/-}$ mice and DPP-IV-deficient Fischer F344/DuCrj rats are resistant to weight gain, suggesting a role for DPP-IV inhibition in the treatment of obesity.[48] Food intake in both the DPP-IV$^{-/-}$ mice and Fischer rats is reduced. Pair-fed wild-type mice weigh more than the DPP-IV$^{-/-}$ animals; thus, a metabolic component may exist. In animal studies with DPP-IV inhibitors, however, weight loss is typically minimal or not seen at all. While it is unclear how these results will translate into clinical findings, DPP-IV inhibitors are not likely to cause the weight gain that is often associated with current diabetes medications.

Potentiation of endogenous substrates beyond GLP-1 and GIP may provide further therapeutic opportunities. For example, GLP-2, an intestinal growth factor released in the gut in response to nutrient ingestion, is inactivated by DPP-IV in vivo in rats.[49] Thus, inhibition of DPP-IV may prove to be useful in the treatment of intestinal injury and disease. This indication remains to be fully explored. DPP-IV is also thought to regulate endomorphin-2, a tetrapeptide (Tyr-Pro-Phe-Phe-NH_2) with high affinity for the μ opioid receptor.[50] The icv administration of DPP-IV inhibitors alanylpyrrolidine-2-nitrile[50a] (**13b**) and DiprotinA[50b] (Ile-Pro-Ile) evoked dose-dependent potentiation of endomorphin-2 induced analgesia in the mouse paw withdrawal and tail flick models, respectively.

There are a variety of other substrates that DPP-IV cleaves in vitro.[8] These include chemokines such as RANTES, eotaxin, IP-10, and SDF-1α, neuropeptides such as substance P, β-casomorphin, NPY, and PYY,

and growth factors such as GRH. In many cases, the DPP-IV cleavage product is inactive or has altered receptor specificity. Additional studies are needed to determine whether these are in vivo substrates and, if so, whether DPP-IV inhibition will lead to desired therapeutic benefits in other diseases or will result in potentially toxic side effects.

DPP-IV has a number of proposed functions in addition to its role in metabolic control.[6] It binds adenosine deaminase, an enzyme important in the normal development and function of the immune system, and likely modulates local concentrations of adenosine. It also has a binding site for the extracellular matrix proteins collagen and fibronection, though its role as an adhesion molecule remains unclear. DPP-IV is identical to the cell surface marker CD26 and serves as a costimulatory molecule in T-cell activation. While DPP-IV inhibitors have been shown to inhibit T-cell activation in vitro, concentrations required for this activity are well above their reported K_i values; thus, a role for enzymatic activity in this function appears unlikely.

Because of the potential role of DPP-IV in the immune system, inhibitors have been studied in a number of immunological models. DPP-IV inhibitors Ala-boro-Pro (**12b**) and Lys(Z-NO_2) thiazolidide (**14**) as well as two natural product DPP-IV inhibitors have been shown to inhibit hind paw swelling in collagen- and alkyldiamine-induced models of arthritis in rats.[51] The irreversible inhibitor Pro-Pro-diphenyl phosphonate (**15**) prolonged allograph survival in a rat cardiac transplant model.[52] With the discovery of additional enzymes possessing DPP-IV-like activity, these results remain to be confirmed with more selective DPP-IV inhibitors.

Conclusions

Inhibition of DPP-IV is an attractive new approach to the treatment of type 2 diabetes. Because DPP-IV inhibitors stimulate insulin secretion in a glucose-dependent fashion, the potential for hypoglycemic side effects is minimal. The lack of weight gain, and potential for weight loss, with DPP-IV inhibitor treatment provides another potential benefit to diabetics, the vast majority of whom are obese. Finally, recent data suggesting restorative effects on pancreatic islets provide hope that DPP-IV inhibitors will slow or perhaps reverse the course of disease. The promise of this treatment remains to be realized as potent and selective inhibitors progress through clinical studies.

Biography

Ann E. Weber received her B.S. degree in Chemistry from the University of Notre Dame and Ph.D. degree in Organic Synthesis from Harvard University, working in the laboratories of Professor David Evans. She joined Merck & Co. in 1987. Currently she is a Senior Director in the Department of Medicinal Chemistry, focusing primarily on the discovery of novel chemotherapeutics for the treatment of metabolic disorders.

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JM030628V