

Dipeptidyl Peptidase-IV Inhibitors: An Evolving Treatment for Type 2 Diabetes from the Incretin Concept

Yau-Jan Chyan¹ and Lee-Ming Chuang^{2,3,*}

¹Development Center for Biotechnology, Taipei, Taiwan; ²Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; ³Graduate Institute of Clinical Medicine, National Taiwan University Medical College, Taipei, Taiwan

Received: September 1, 2006; Accepted: November 24, 2006; Revised: November 27, 2006

Abstract: Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1) are the 2 major incretin hormones released after meals to enhance glucose-stimulated insulin secretion. In patients with type 2 diabetes, a loss of activity of GIP for insulinotropic function and a reduced secretion of GLP-1 exist in response to oral glucose while GLP-1 action is preserved. GLP-1 is therefore an attractive avenue for treating type 2 diabetes. Due to the short circulating half-life of GLP-1, which is degraded by dipeptidyl peptidase IV (DPP-IV), 2 approaches have been undertaken. One is to develop long-acting GLP-1 analogs, such as exendin-4 that is resistant to degradation. Here we review another approach for developing DPP-IV inhibitors. This group of potential drugs covers several major chemical classes and their derivatives, such as amino acid amide, carbocyclic, alkylamine, and heterocyclic compounds. More than 100 patents have been issued for DPP-IV inhibitors to be used either as a monotherapy or in combination with other antidiabetic agents for the treatment of type 2 diabetes, as well as metabolic syndrome, osteoporosis, and arthritis. Structure-based drug design is currently under intensive investigation for future development of more selective therapeutic agents.

Keywords: Dipeptidyl peptidase IV, DPP-IV inhibitors, GLP-1, type 2 diabetes.

INTRODUCTION

Diabetes mellitus, a chronic disease, is one of the main threats to human health in the 21st century. The total number of people with diabetes worldwide was estimated at between 151 million and 171 million in the year 2000. The disease is projected to increase to 221 million in 2010 and 366 million by 2030 [1]. While type 1 diabetes is characterized by β -cell failure, partly due to autoimmune destruction [2], type 2 diabetes arises as a result of β -cell failure combined with concomitant insulin resistance [3]. Patients with insulin resistance do not develop hyperglycemia until their β -cells are unable to meet the demands for insulin; thus, enhancement of insulin secretion from the islet β cell is a practical target for treatment of patients with type 2 diabetes. Current insulin secretagogues, including sulfonylureas and glitinides, frequently exhibit a secondary failure and may cause hypoglycemia in patients with type 2 diabetes [4]; hence, much interest has been shown in the identification of newer agents that enhance insulin secretion in a sustained glucose-dependent manner in patients with type 2 diabetes.

THE INCRETIN CONCEPT

Because orally ingested glucose leads to a far greater insulin response than intravenous glucose with similar postprandial plasma glucose excursions, the phenomenon has been termed the "incretin effect" [5, 6]. Up to two thirds of insulin normally secreted in relation to meal intake is

thought to be due to the insulinotropic actions of the so-called incretin hormones [7]. Studies have revealed that secretion of incretin hormones is diminished in type 2 diabetes [8].

TWO MAJOR INCRETIN HORMONES

Two gastrointestinal hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are the 2 major incretin hormones identified to date [9, 10]. GLP-1 is a product of the glucagon gene [11], which is mapped on the human chromosome 2q36-q37. It is expressed in pancreatic β -cells and the L-cells located predominantly in the ileum and colon, although GLP-1-producing L-cells have also been identified more proximally in the duodenum and jejunum [12]. The protein product of the glucagon gene in pancreatic β -cells is glucagon while the expression in the L-cells is the release of the C-terminal products GLP-1 and GLP-2, which show approximately a 50% sequence homology with glucagon [13]. On the contrary, GIP (also known as gastric inhibitory polypeptide) is a 42-amino acid hormone produced from a different gene on chromosome 17q21.3-q22.

INCRETIN DEFECTS AND THE THERAPEUTIC POTENTIAL OF GLP-1 IN TYPE 2 DIABETES MELLITUS

Interestingly, patients with type 2 diabetes are characterized by 2 defects related to incretin effect: 1) the secretion of GLP-1 is decreased and 2) the insulinotropic effect of GIP is reduced [14]. In addition, GLP-1 may represent a more attractive treatment option for type 2 diabetes because of its multiple effects, including the simulation of satiety in the

*Address correspondence to this author at the Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan; Tel: +886-2-23123456; Ext. 5038; Fax: +886-2-23938859; E-mail: leeming@ha.mc.ntu.edu.tw

central nervous system by crossing the blood-brain barrier. Therefore, the development of GLP-1 therapeutics, instead of a GIP supplement, is now being considered for type 2 diabetes [15,16].

GLP-1 stimulates glucose-dependent insulin secretion [17-19] and insulin gene expression [20], inhibits glucagon secretion [21, 22], and delays gastric emptying [23]. *In vitro* and *in vivo* data showed that GLP-1 increases β -cell mass by stimulating islet cell neogenesis and inhibiting apoptosis of islets [24-26]. GLP-1 infusion in conscious dogs with advanced dilated cardiomyopathy showed dramatic improvements in left ventricular function and systemic hemodynamics through its insulinomimetic effect on myocardial glucose uptake [27]. The physiological functions and its beneficial effects on pathophysiological conditions of GLP-1 are summarized in Table 1.

Table 1. Physiological Functions of GLP-1

<ul style="list-style-type: none"> • Stimulates insulin secretion, glucose-dependently • Increases β-cell mass in animal models • Decreases glucagon secretion, glucose-dependently • Delays gastric emptying, decreases food intake and body weight • Improves insulin sensitivity; enhances glucose disposal • Has beneficial cardiovascular effects • Has beneficial CNS effects in animal models
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GLP-1 METABOLISM

GLP-1 is derived from a larger proglucagon precursor that encodes related proglucagon derived peptides, including glucagon, GLP-2, oxyntomodulin, and glicentin [28]. GLP-1 secretion from the distal gut is controlled by both neural and endocrine signals initiated by nutrient entry in the proximal GI tract, as well as by subsequent direct contact of open-type L-cells with digested nutrients. Ingestion of a mixed meal or a meal enriched with specific fats and complex carbohydrates is particularly effective in stimulating GIP and GLP-1 release in human subjects [29, 30]. Although the vagal nerve, via M1 muscarinic receptors, along with several neuroendocrine peptides contribute to the regulation of GLP-1 release in rodents [31, 32], the factors responsible for rapid nutrient-stimulated GLP-1 release in human subjects are largely unknown.

GLP-1(7-37) is a naturally occurring peptide hormone; however, GLP-1(7-36) amide is even more abundant under physiological conditions. The 2 forms of GLP-1 secreted after meal ingestion, GLP-1(7-37) and GLP-1(7-36) amide, differ by a single amino acid. Both peptides are equipotent and exhibit identical plasma half-lives and biologic activities acting through the same receptor [33,34]. The majority (80%) of circulating active GLP-1 is GLP-1(7-36) amide [35]. Compared with GLP-1(7-37), GLP-1(1-37) has a 100-fold lower affinity for the GLP-1 receptor [36].

Native GLP-1 is degraded rapidly with a very short plasma half-life for either GLP-1(7-37) (6.1 ± 0.8 min) or GLP-1(7-36) amide (5.3 ± 0.4 min) after intravenous injection

in healthy subjects. GLP-1 is heavily degraded by the serum enzyme dipeptidyl peptidase (DPP)-IV, leading to GLP-1(9-37) or GLP-1(9-36) amide, which may function as weak competitive antagonists at the receptor level. Evidence suggests that the membrane-bound ectopeptidases are involved in the postsecretory metabolism of GLP-1. However, the physiological relevance of this pathway remains to be elucidated [37].

The clinical development of GLP-1 and its long-acting degradation-resistant GLP-1 analogs are beyond the scope of this present review.

DIPEPTIDYL PEPTIDASE IV (DPP-IV; EC 3.4.14.5)

DPP-IV/CD26 is a cell-surface protease belonging to the prolyl oligopeptidase family. DPP-IV was first reported in 1966 as glycyl-prolyl-naphthylamidase [38] and later named dipeptidyl peptidase IV, as recommended by the Enzyme Commission. Under the enzyme classification list, within the same *clans* (SC), *families* (S9), and *subfamilies* (B), some enzymes are apparent other than DPP-IV, such as DPP 9, DPP 8, DPP 6 (DPPX), fibroblast activation protein (FAR), and so forth (<http://www.chem.qmw.ac.uk/iubmb/enzyme/index.html>) with different degrees of amino acid identity and, therefore, enzymatic specificity. Recently, the crystal structure of DPP-IV has been solved [39, 40]. In the natural states, DPP-IV monomers associate to form homodimers [41]. DPP-IV also forms heterodimers with FAR on the cell surface of fibroblasts and melanocytes [42, 43].

DPP-IV is identical to adenosine deaminase (ADA) complexing protein-2 and to the T-cell activation antigen CD26. Sequence analyses reveal that ADA-binding protein is identical to CD26, a T-cell activation molecule and a 110-kD glycoprotein that is also present on epithelial cells of various tissues, including liver, kidney, and intestine [44, 45]. This enzyme cleaves a dipeptide from the N-terminus of the protein, resulting in inactivation of incretin hormones. In fact, metabolites, such as GIP 3-42 and GLP-1 9-36 amide, can act as antagonists at their respective receptors [46, 47]. The DPP-IV enzyme occurs both in soluble form in circulation and membrane-bound form on the endothelial surfaces, so that the conversion can occur intravascularly as well as upon organ and tissue passage [48].

To establish the role for DPP-IV/CD26 in physiologic glucose homeostasis, a knockout mice deficient for CD26 was generated. Targeted inactivation of the CD26 gene yielded healthy mice that had normal blood glucose levels in a fasted state. However, a reduced glycemic excursion was demonstrated after a glucose challenge. Levels of glucose-stimulated circulating GLP-1 and insulin were increased in CD26^{-/-} mice. More interestingly, a pharmacologic inhibitor of DPP-IV improved glucose tolerance in the wild-type mice, but not in CD26^{-/-} mice [49]. Further, studies indicated that DPP-IV plays a role in body weight control and insulin sensitivity in mice. When mice were fed a high-fat diet, the wild-type mice displayed accelerated weight gain and hyperinsulinemia, but the mice lacking the DPP-IV gene were refractory to obesity and hyperinsulinemia [50]. Using pair-feeding and indirect calorimetry techniques, both reduced food intake and increased energy expenditure were

found and accounted for resistance to obesity in the DPP IV-null mice. Moreover, ablation of the DPP-IV gene was also associated with protection against streptozotocin-induced loss of β -cell mass. Similarly, in Fischer DPP-IV mutant rats with a naturally occurring mutation in the DPP-IV gene, data also suggested that glycemic excursion after glucose loading was reduced with an increase in circulating GLP-1 and insulin levels [51]. These findings not only provide an *in vivo* role of DPP-IV in the physiologic regulation of glucose homeostasis but also provide a basis for therapeutic intervention with DPP-IV inhibitors in treating metabolic disorders related to diabetes and obesity.

CLINICAL DEVELOPMENT OF DPP-IV INHIBITORS

DPP-IV/CD26 exerts its biological effects via 2 distinct mechanisms of action. First, it binds ADA and, when activated, conveys intracellular signals independent of its enzymatic function via dimerization and activation of intracellular signaling pathways. The signaling properties of membrane-associated CD26 have been most extensively characterized in T-cells. Second, when functioning as an enzyme, DPP-IV rapidly inactivates the insulinotropic hormone GLP-1. Thus, inhibition of DPP-IV by DPP-IV inhibitors enhances the hormone activity of GLP-1 and other bioactive peptides (e.g., GIP, pituitary adenylyl cyclase-activating polypeptide-38 [PACAP38] and gastrin-releasing peptide [GRP]), thereby stimulating the release of insulin and reducing the secretion of glucagon. Both effects contribute to regulation of elevated blood glucose levels in

type 2 diabetic patients. The major advantage of DPP-IV inhibitors is its ability to achieve sustainable reductions in hemoglobin A1c with an orally administered, well-tolerated agent. In addition, available data from clinical phase II studies suggest that long-term treatment with DPP-IV inhibitors was not associated with weight gain [52-54]; however, this was not observed in animal studies [55,56]. Preclinical and clinical profiles obtained thus far for DPP-IV inhibitors are very promising and of potential therapeutic value. Increase of biological activities of GLP-1 by DPP-IV inhibitors could improve hyperglycemia control via facilitating insulin functions in gastric and blood, and provide better control of glucose influx and utilization in peripheral tissues (Fig. 1). The outstanding benefits of DPP-IV class compounds are 1) orally active, 2) glucose dependency, 3) glucose competence, 4) inhibition of glucagons secretion, and 4) effect on food intake and satiety.

DPP-IV INHIBITORS UNDER DEVELOPMENT FOR TREATMENT OF DIABETES

Currently, worldwide sales of drugs for type 2 diabetes exceed \$12 billion (≈9.9 billion) per year. The increased interest of the pharmaceutical industry in DPP-IV inhibitors reflects their market attractivity and patent application. There are over 100 patents filed with several different chemical structures, including amino acid amide, carbocyclic, alkylamine, and heterocyclic (mainly pyrrolidine, pyridine, and xanthine derivatives). With available favorable clinical and preclinical studies, DPP-IV inhibitors have become a research area of intense focus with a number of

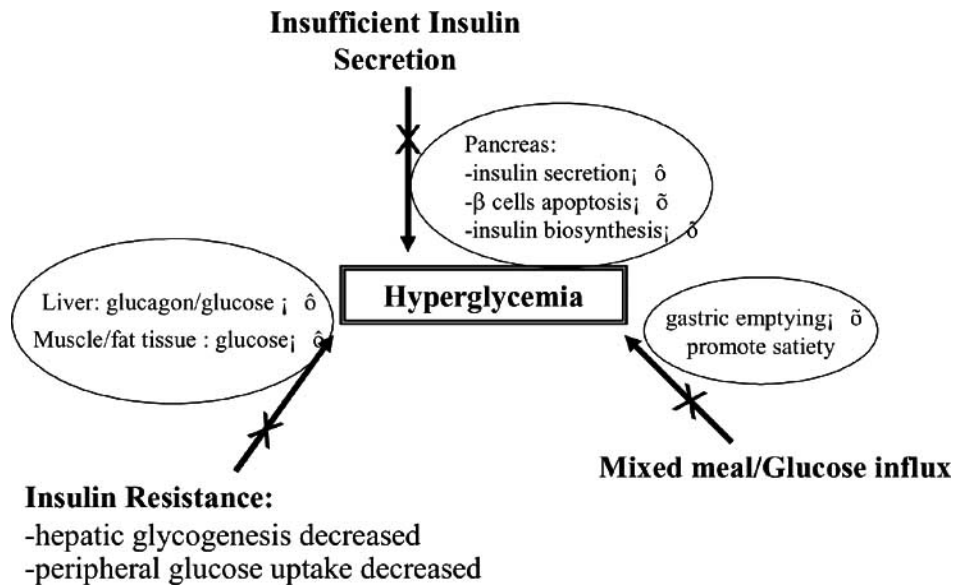


Fig. (1). Roles of DPP-IV inhibitors under hyperglycemia.

Legend: Hyperglycemia is caused by excess food consumption, reduced insulin secretion, and increased insulin resistance with the impaired peripheral glucose uptake. All these aspects involved in deregulation of glucose homeostatic in type 2 diabetes could be corrected (marked with X) by GLP-1, whose bioactivities were eliminated by the protease activity of DPP4. With DPP4 inhibitors to prolong GLP-1 half life in serum, the normoglycemia could be achieved by slowing gastric emptying and promote satiety, or enhancing insulin secretion/biosynthesis and prevent β cell death, and also improving the peripheral glucose utilization and correcting the insulin resistance (as shown in circles).

pharmaceutical companies involved in the development of what is hoped is a new diabetes therapy that will affect approximately 8-10% of the adult western population.

Up to date there are more than 20 different DPP-IV inhibitors developed for various therapeutic interests - mainly type 2 (non-insulin-dependent) diabetes. Although, a number of DPP-IV inhibitors have been described, all have limitations relating to potency, stability, or toxicity. Accordingly, a great need exists for novel DPP-IV inhibitors which are useful in treating conditions mediated by DPP-IV inhibition and which do not suffer from the abovementioned limitations. Among currently developed oral DPP-IV inhibitors, vildagliptin (LAF237), sitagliptin (MK-0431) and saxagliptin (BMS-477118) from Novartis, Merck & Co., and BMS, respectively, are in advanced phase III development and are competing to be "first in class" of a new oral treatment modality for type 2 diabetes. The most advanced DPP-IV inhibitor-related compounds are described below (see Table 2).

Vildagliptin (LAF237)

Novartis has been developing a series of pyrrolidine-based compounds with DPP-IV inhibitor activities, including valine-pyrrolidine and N-substitute 2-cyanopyrrolidine (DPP728 and LAF237). Structure-activity relationship (SAR) studies have revealed structure-related stability with the LAF237 compound. Also, studies of specificity of LAF237 revealed that the respective IC₅₀ for DPP-IV was in the lower nM range. One other related enzyme found to be inhibited by LAF237 was DPP8 with an IC₅₀ of 9.0 ± 0.1 mM; the activities of DPP-II, prololigopeptidase, aminopeptidase P, and aminopeptidase N were not significantly affected by vildagliptin [57,58].

In a phase IIb study, 42 patients were given oral LAF237 plus metformin once daily while 29 patients received placebo plus metformin. HbA_{1c} levels were measured over the course of 52 weeks. In patients treated with LAF237 plus metformin, HbA_{1c} levels decreased significantly, and those levels were maintained up to 52 weeks. In the patients who continued on metformin alone, glycemia control deteriorated over time. At the end of study, an average difference of 1.1% in HbA_{1c} between the 2 groups (p<0.0001) was shown. Glucose levels measured after 8 to 12 hours of fasting and 1 to 2 hours after eating a meal were also reduced in patients taking metformin plus LAF237 versus continued therapy with metformin alone. Also LAF237 was found to be well tolerated in these studies [54, 59]. Currently, phase IIb/III trial results demonstrate strong efficacy in lowering HbA_{1c} levels and excellent tolerability without weight gain, which is consistent with earlier studies. These results also indicate the ability of LAF237 to improve and sustain pancreatic islet cell function and insulin sensitivity, along with better glycemia control over a 1-year period [54, 60]. With such strong positive phase IIb/III trial results, the first regulatory submission of LAF237 is expected in 2006.

Saxagliptin (BMS-477118)

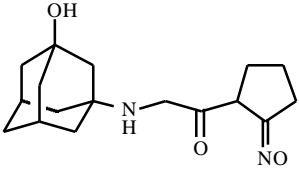
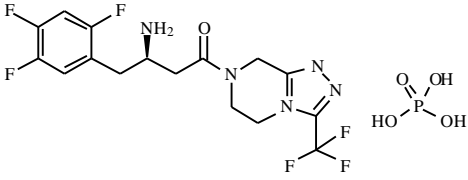
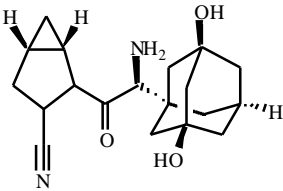
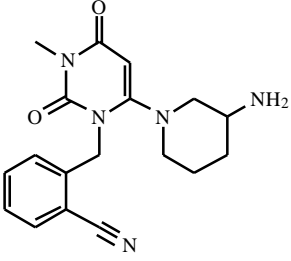
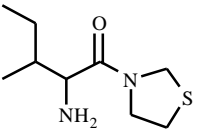
Bristol-Myers Squibb (BMS) is developing saxagliptin (BMS-477118; pyrrolidine-based structure), an oral DPP-IV inhibitor, for a potential once-daily treatment of type 2 diabetes. In early structure-activity relationships (SAR)

studies, BMS has investigated a series of 3,4- and 4,5-disubstituted methanoproline nitrile dipeptide mimetics and found that compounds with the highest degree of beta-branching had the greatest stability. The stable compounds have demonstrated some anti-diabetes activities by suppressing postprandial glucose elevation [61-63]. Several patents have been filed for BMS-477118 including the specific process for preparation of intermediates involved in the production of saxagliptin, including the enzymatic process allowing for a faster procedure and the isolation of crystalline forms without the use of ammonium hydroxide [64-66]. In addition, saxagliptin has been shown not to inhibit T-cell activity *in vivo* [61]. In some clinical studies, saxagliptin exhibited several beneficial effects: for example, 10 mg qd patients showed glucose and HbA_{1c} lowering, and good toleration of the drug. Phase III trials (both as a monotherapy and in combination with other oral agents) were initiated during late 2005 with results expected soon. According to BMS, the US patent covering composition of matter for saxagliptin will expire in 2021.

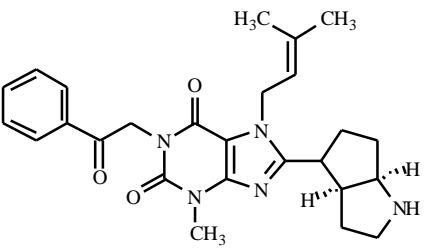
MK-431

In collaboration with Banyu Pharmaceutical Co. Ltd. (Merck's Japanese subsidiary) and Ono Pharmaceutical Co Ltd., Merck is developing sitagliptin (Januvia), a DPP-IV inhibitor, for potential once-daily treatment of type 2 diabetes. The original discovery of substituted piperazines as novel DPP-IV inhibitors was achieved through SAR studies leading to the incorporation of multiple fluorine atoms and modification of the piperazine ring to yield potent and selective compounds related to MK-431. MK-431 is a beta-amino acid-based compound, which is the monophosphate salt of (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine. Key factors in optimizing the affinity and pharmacokinetic properties of the structurally novel piperazine family included the proper location of two or more fluorine atoms onto the phenyl ring of the compounds, and substitution of the metabolically labile piperazine moiety with more metabolically robust heterocyclic analogs [67-70], which not only improved the affinity and bioactivities of derived compounds, but also dramatically improved the bioavailability about 20 folds in rats [69,70]. Optimization of the fluorine substitution pattern of the phenyl ring of the beta-amino group, from 3,4-di-F to 2,5-di-F and 2,4,5-tri-F, led to further affinity improvements and the discovery of MK-431, which was selected for development [69]. Attempted deletion of the trifluoromethyl group of the MK-431 structure led to a drastic loss of bioavailability in rats (from 76 to 3%) and a 4-fold decrease in DPP-IV inhibitory potency, demonstrating the importance of this group for the pharmacokinetic profile and potency of the compound. Intriguingly, the 2-fluorophenyl analog was also more selective for DPP-IV versus a related enzyme, QPP (quiescent cell proline peptidase, also called DPP-II). This is of importance because QPP is thought to be significant in the physiology of immune function. Selectivity against this enzyme is, therefore, an appropriate goal for compound optimization [71]. Throughout its studies, MK-431 was also highly selective for DPP-IV versus other proteases, including aminopeptidase P, prolidase, prolendopeptidase (also called prololigopeptidase or post-proline cleaving

Table 2. Summary for DPP-IV Inhibitors in the Advanced Developmental Stages

Name	Structure	Company (originator)	Actions	status	Related Patents
LAF237	 <p>Cyanopyrrolidine derivatives</p>	Novartis	Glucagen-like peptide 1 metabolism modulator, Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	Pre-registration	WO9819998 WO0034241 US000746249 US6110949 EP0937040
Sitagliptin/ MK0431 (Januvia)	 <p>Piperazines derivatives</p>	Merck & Co Inc	Glucagen-like peptide 1 metabolism modulator, Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	FDA approved	WO-05072530 WO-05030127 WO-05020920 WO-04085661 WO-03004498 WO-05097733 WO-06033848
Saxagliptin (BMS-477118)	 <p>1-cis-4,5-methanoproline nitrile</p>	Bristol-Myers Squibb Co	Insulin sensitizer, Dipeptidyl peptidase IV inhibitor	Phase III	WO-00168603 WO-04052850 WO-05094323 WO-05106011 WO-05108594 WO-05115982 WO-05117841 WO-06020664
SYR-322	 <p>Pyrimidine derivatives</p>	Takeda San Diego Inc	Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	Phase III	WO-05095381 US20050070706 US20050070531
PHX1149	*	Phenomix Corp	Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	Phase II	
GRC-8200	tricyclic derivatives*	Glenmark Pharm. Ltd.	Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	Phase II	WO-05033099
Ile-Thiazolidide	 <p>amino acid cyclic amide derivative</p>	Probiodrug AG	Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	Phase II	US-7084120 US6949515

(Table 2) Contd....

Name	Structure	Company (originator)	Actions	status	Related Patents
SSR-162369	 <p style="text-align: center;">biocyclic 8-pyrrolidinoxanthine</p>	SANOFI-AVENTIS DEUTSCHLAN ND GMBH	Hypoglycemic agent, Dipeptidyl peptidase IV inhibitor	Phase I	WO06015701 WO06015700 WO06015699 WO06015691

* Information not available (database search from Delphion, covered up to July 2006).

enzyme), QPP (IC_{50} values $> 10 \mu M$), and the more closely related enzymes, FAPalpha (fibroblast activation protein-alpha, also called seprase; IC_{50} value $> 100 \mu M$), DPP 8 (IC_{50} value = $48 \mu M$) and DPP 9 (IC_{50} value $> 100 \mu M$) [69]. This was an important finding, as inhibition of the closely related DPP 8 and DPP 9 enzymes has been associated with severe toxicity in animal studies. The toxic effects of DPP-IV inhibitors identified thus far in preclinical studies have included alopecia, thrombocytopenia, anemia, enlarged spleen, multiple histological pathologies, and animal mortality shown in dogs [72]. In addition, the observation of skin lesions in monkeys administered DPP-IV inhibitors led to a decision by the US Food and Drug Administration to require testing of all DPP-IV inhibitors in monkeys. Data from these studies should clarify whether skin lesions are due to DPP-IV inhibition itself or to some other mechanism of action, for example the inhibition of related enzymes, such as DPP-8 or DPP-9. MK-431 was reported to be safe and well tolerated in phase IIb trials and to lower blood glucose levels effectively [73,74]. It is anticipated that dosing frequency will be once daily. Concurrently, Merck is currently developing MK-431A, a combination of sitagliptin + metformin (qv), for the treatment of type 2 diabetes; a New Drug Application (NDA) file is slated for 2007.

SYR322

Takeda San Diego (formerly Syrrx) has been developing a series of small molecules with moiety of heterocyclic structures (pyrimidine and 1,2-thiazine/piperidine) as orally-available DPP-IV inhibitor, for the potential treatment of diabetes. In 2006, a global phase III trial began. The company is also investigating additional DPP-IV inhibitors as part of this program and several Investigational New Drug applications were filed in the United States. Initially, Takeda San Diego cooperated with PPD, the pharmaceutical services provider, to jointly develop and commercialize Syrrx-designed human DPP-IV inhibitors for the treatment of type 2 diabetes and other diseases in 2003. Among highly potent SYR-series compounds, SYR-111791 and SYR-11792 had IC_{50} values of 70 and 16 nM, respectively; SYR-110085A, dosed at 3 mg/kg, has been shown to lower plasma DPP-IV activity in beagle dogs [75]. Another candidate, SYR322, has been shown to lower plasma DPP-IV concentration in

monkeys. In this study, the half-life of SYR322 was 5.7 h with a bioavailability of 100%. SYR322 was also found to reduce glucose excursion following an oral glucose tolerance test in C57BL/6 mice [76]. In early 2004, two different patentable Syrrx-designed chemical classes as DPP-IV inhibitors, SYR619 and SYR322, have been filed for the Investigational New Drugs. These compounds were considered to be variants from nearly all other DPP-IV inhibitors, which were based on the almost ubiquitous 2-pyrrolonitrile chemotype [77]. In January 2006, a global phase III trial of SYR322 began. The use of DPP-IV inhibitors for treating diabetes with secondary sulfonylurea failure was claimed [78]. Takeda's approach, as claimed in this application, provides a highly distinct chemotype that may provide some distinct advantages in comparison with the other DPP-IV inhibitors that are in advanced developmental stage such as vildagliptin.

PHX-1149

Phenomix is developing PHX-1149, the lead from a series of orally active DPP-IV inhibitors, including PHX-1004, for the potential treatment of type 2 diabetes. At the Metabolic Diseases World Summit in San Francisco, CA in 2005, PHX1149 was announced to have low protein binding, high aqueous solubility and a low V_d , suggestive of extravascular localization, where DPP-IV is located, also minimizing off-target toxicity. Pharmacokinetic and pharmacodynamics studies in dog and monkey supported once-daily dosing regimes in humans. Also, 9 mg/kg doses in dogs and monkeys produce over 50% and 80% DPP-IV inhibition after 24 hours, respectively. The half-life of PHX-1149 was 6.3 hours, time to C_{max} was 0.8 hour, C_{max} concentration was 555 ng/ml and AUC concentration was 2030 hour \cdot ng/ml, with excellent selectivity over other DPP-IV-related proteases [79]. A 4-week phase II trial of PHX1149 was initiated early 2006 and the primary endpoint was measurement of post-meal glucose levels.

GRC8200

Glenmark Pharmaceuticals has exploited several different patentable chemical moiety derivatives as DPP-IV inhibitors, including acetamide, carbazoylamine, pyrrolidine and cyclophenyl. GRC8200 and GRC-8116, tetrahydro-carbazole

and closely related tricyclic derivatives with DPP-IV inhibitor action, are now in phase II clinical trial. Initial preclinical studies demonstrated both compounds with excellent IC_{50} values of 1.61 and 6.27 nM, respectively, against a human recombinant DPP-IV enzyme assay. Furthermore, these 2 compounds had specificities over 10,000-fold and over 600-fold, respectively, over DPP-II, PPCE and the other proteases tested [80,81]. In 2005, Glenmark patented the process for the preparation of tetrahydro-carbazole and closely related tricyclic derivatives of DPP-IV inhibitors [82]. Results from the initial phase I clinical trial showed that GRC8200 was well tolerated at all dose levels with a linear pharmacokinetic profile, supporting once-daily dosing. Phase II trial begins in 2006.

SSR-162369

In February 2006, Sanofi-Aventis reported that the DPP-IV inhibitor SSR-162369 was in phase I trials. However, this compound is probably a structural analog of vildagliptin, which has demonstrated some specificity problems related to DPP-IV inhibition. Recently, Sanofi-Aventis has applied a patent related to novel bicyclic 8-pyrrolidinoxanthine derivatives [83]. This application is one of four concurrent filings from Sanofi-Aventis (84-86), substantially augmenting the company's patent filings in the area. Furthermore, the compounds claimed in these applications are xanthine derivatives and bear greater resemblance to the bicyclic heterocycle sitagliptin. However, no suitable advantage was given and no compounds were specifically claimed, for example, 8-(*cis*-hexahydropyrrolo[3,2-*b*]pyrrol-1-yl)-3-methyl-7-(3-methyl-butyl-2-enyl)-1-(2-oxo-2-phenylethyl)-3,7-dihydropurin-2,6-dione, which had an IC_{50} value of 18 nM. In addition, in using these compounds as hypoglycemic agents for the treatment of type 2 diabetes, they were found to be useful for the treatment of psychiatric disorders (e.g., depression, anxiety, schizophrenia, and circadian rhythm disorders), immune disorders, drug abuse, cancer, arthritis, osteoarthritis, osteoporosis, sleep disorders, sleep apnea, male and female sexual dysfunction, inflammation, skin disease (e.g., acne and psoriasis), pigmentation disorders, steroid metabolism disorders, mycosis and neurodegenerative disorders (e.g., multiple sclerosis and Alzheimer's disease) and as weight-loss promoters.

GSK-23A

GlaxoSmithKline (GSK) has investigated a series of 2-cyano-4-fluoro-1-thiovalylpyrrolidine inhibitors of DPP-IV as hypoglycemia agents. Among other similar bioactive compounds, the preliminary studies indicated that GSK-23A, when administered to ob/ob mice at an oral dose of 10 mg/kg twice daily for 8 weeks and then with a subsequent 4-hour fasting, the GLP-1 level was double that of the vehicle control. Triglyceride levels also decreased and there was no change in insulin and cholesterol levels observed. Dogs dosed with 1 mg/kg GSK-23A showed good inhibition of DPP-IV after 24 hours. The compound had an oral bioavailability of 32% and 80% in rats and dogs, respectively. [87]. Recently, GSK has developed one compound displayed a K_i value of 53 nM and when dosed orally, 84% DPP-IV inhibition was achieved in rats (1 mg/kg at 6 hours), and > 12 hours above the IC_{50} value at 0.2 mg/kg in dogs. The compound had a half-life of 2.3 and 3.9 hours in rats and

dogs, respectively [88]. A total of three patents from GSK, all published in January 2003, claim pyrrolidine and thiazolidine and fluoropyrrolidine as DPP-IV inhibitors. Now these compounds are still at the discovery phase.

COMPOUNDS ON THE PIPELINE AND NEWCOMERS

There are a number of DPP-IV inhibitors being studied, with names such as vildagliptin, saxagliptin and sitagliptin. These agents basically inhibit 90% of the action of DPP-IV and increase the life of GLP-1 in the body by about 3 times. These agents have been tested alone and in combination with metformin, and in general, are able to reduce HbA1c by about 1% over 12 weeks. The weight reduction is not as noticeable as with the GLP-1 analogues. These medications were safe and tolerable in clinical trials. Another advanced DPP-IV inhibitor, not previously mentioned, is isoleucine thiazolidide (Ile-thiazolidide, P32/98) by Probiobdrug, which is in phase II study. P32/98 is a competitive transition-state substrate analog inhibitor of DPP-IV, with a K_i of 130 nM [89]. When given orally in humans, P32/98 exhibited a dose-dependent decrease of plasma DPP-IV and a concomitant increase of active GLP-1 [90], suggesting efficient intestinal absorption. Researchers at the Technical University of Munich have recently shown that the oral activity of P32/98 results from its ability to bind at the intestinal PEPT1 transporter thereby allowing transport across the gut wall [91]. Such technology opens the way for the rational design of peptide-linked drugs, like several DPP-IV inhibitors, with improved intestinal absorption. In addition, harnessing peptide transporters to allow the improved uptake and targeting of therapeutics can be extended to other drug classes and indications.

Furthermore, a number of biotechnology companies have also announced active DPP-IV inhibitor compounds. Borhringer Ingelheim Corp. has investigated xanthine-based DPP-IV inhibitors suitable for once-daily use in glucose level control [92]. Recently, Abbott Laboratories have also developed acyl thiazolidide-based DPP-IV inhibitors which exhibited selectivity over related serine proteases DPP7, DPP8, POP and FAP- with promising oral pharmacokinetic profiles ($t_{1/2} = 0.5h$, $F = 57.6\%$, $V_{ss} = 1.38 L/Kg$, $CL_p = 1.94 L/H/Kg$) in preclinical studies [93]. With these promising results, Abbott has filed 2 utility patents, WO0502376 and WO0402682, for exploiting these compounds as the potential drugs for various indications including diabetes. Pfizer has been investigating a series of DPP-IV inhibitors for the potential treatment of type 2 diabetes mellitus. In early 2003, a fluorinated cyclic amide inhibitor (CP-867534-01) was revealed as one of the compounds in this series [94]. However, due to the structure-related phenomenon of sloughing of the colonic epithelium, which manifested itself as the passage of bloody, mucoid stools within 2 hrs of dosing, CP-867534-01 was discontinued in the early discovery phase. Later, DP-893 (a novel sulfonamide derivative) displayed an effective IC_{50} value against the human recombinant form of DPP-IV and a half-life of about 5 hrs and 2 hrs in dogs and rats, respectively [95].

Recently, the structure activities relationship studies of DPP-IV were key tools for the optimization step for the kind of drugs involved in multidiscipline biological systems. In

addition to its role in glucose homeostasis, DPP-IV has been implicated in immune disorders, HIV-1 infection and tumor progression due to the similarity of DPP-IV-related gene family. Therefore, a key question for DPP-IV as a potential target for the type 2 diabetes therapeutics fields is the specificity of inhibitors. Studies have examined the two most advanced DPP-IV inhibitor drug candidates, LAF237 and MK431, in respect to structure-related selectivity. DPP-IV inhibitors with N-substituted glycine in the P2 site, such as LAF-237 or DPP-728 [96], or 2-cyanopyrrolidine with P2-site 4-substituted glutamic acid derivatives [97] or with an aromatic moiety involving in hydrophobic interaction with the side chain of Phe357, such as α -amino acid MK-431 [98], provided excellent selectivity over DPP8. Inhibition of DPP8 by an isoindoline containing compound was found to give rise to profound toxicity in rats, including anemia, multiple histologic pathologies and mortality [72]. Minding these results, selective inhibition of DPP-IV may be required for an acceptable safety and tolerability level of future antihyperglycemic agents of this type. On the other hand, inhibitors of the related enzyme PEP (EC 3.4.21.26) have potential as drugs to treat neurodegenerative disorders [98].

CURRENT & FUTURE DEVELOPMENTS

The therapeutic potential of inhibitors of post-proline cleaving enzymes like DPP-IV has been the focus of recent pharmaceutical research. Three DPP-IV inhibitors are in advanced clinical development: saxagliptin (Bristol-Myers Squibb Co.), which is in phase III clinical trials, and vildagliptin (Novartis) and sitagliptin (Merck & Co Inc.), both of which have had New Drug Applications filed. Regulatory filings of the first class DPP-IV inhibitors are to be expected in 2006. Available data show differences in duration of action and anticipated dosing frequency, whereas data to compare clinical efficacy and safety is not available presently. The DPP-IV inhibitors are evaluated being as monotherapy or in combination with other antidiabetic drugs, e.g. metformin, thiazolidinediones, and/or PPAR agonists.

Although human trial results in type 2 diabetes with DPP-IV inhibitors look promising, the lack of selectivity, i.e. inhibition of the structurally related enzymes DPP-8 and DPP-9, has been a potential concern. Based on the crystal structure resolved, it is expected to develop certain therapeutic agents such as small peptide via binding to the catalytic binding site as a "substrate-selective" DPP-IV inhibitor [96, 99-104]. Recently, one exiting finding is to show the activity of such DPP-IV inhibitors is due in part to their specific absorption by the small intestinal di- and tri-peptide uptake transporter, PEPT1 [91], thus establishing a system for optimizing the orally active peptidomimetic drugs such as amino acid-based DPP-IV inhibitors.

CONCLUDING REMARK

In general, the safety profiles of most DPP-IV inhibitors are very promising but additional studies are certainly needed to obtain a thorough insight in the *in vivo* effects of DPP-IV inhibition. The beneficial effects of DPP-IV inhibitors on treatment for type 2 diabetes not only offer advantages over the current therapies but also provide more therapeutic applications beyond the treatment for diabetes

due to the biological diversity of DPP-IV. So far, there is no sufficient data available to make sweeping generalization about the long-term effects of various DPP-4 inhibitors on T-cell signaling and immune functions *in vivo*.

REFERENCES

- [1] Zimmet P, Alberti KGMM, Shaw J. Global and societal implications of the diabetes epidemics. *Nature* 2001; 414: 782-87.
- [2] Atkinson M, Maclaren N. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med* 1994; 331, 1428-1436.
- [3] DeFronzo RA, Bonadonna RC, Ferannini E. Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15 1992; 318-68.
- [4] Lebovitz HE. Insulin secretagogues: old and new. *Diabetes Rev* 1999; 7: 139-153.
- [5] Erick H, Stimmler L, Hlad CJ, Arai Y. Plasma insulin responses to oral and intravenous glucose administration. *J Clin Endocrinol Metab* 1964; 24:1076-82.
- [6] McIntyre N, Holdsworth CD, Turner DS. Intestinal factors in the control of insulin secretion. *J Clin Endocrinol Metab* 1965; 25:1317-24.
- [7] Nauck MA, Homberger E, Siegel EG, *et al.* Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986; 63: 492-98.
- [8] Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986; 29: 46-52.
- [9] Creutzfeldt W. The entero-insular axis in type 2 diabetes- incretins as therapeutic agents. *Exp Clin Endocrinol Diabetes* 2001; 109 Suppl 2: S288-303.
- [10] Fehmann HC, Goke R, Goke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev* 1995; 16:390-410.
- [11] Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human proglucagon gene. *Nature* 1983; 304:368 -71.
- [12] Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem* 1986; 261:11880 -89.
- [13] Orskov C, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology* 1986; 119:1467-75.
- [14] Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab* 2004; 287:E199-206.
- [15] Holst JJ, Orskov C. The incretin approach for diabetes treatment: modulation of islet hormone release by GLP-1 agonism. *Diabetes*. 2004; 53 Suppl 3:S197-204.
- [16] Holst JJ. Gastric inhibitory polypeptide analogues: do they have a therapeutic role in diabetes mellitus similar to that of glucagon-like Peptide-1? *BioDrugs* 2002; 16:175-81.
- [17] Mojsov S, Weir GC, Habener JF. Insulinotropin: Glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 1987; 79:616-19.
- [18] Kreymann B, Ghatei MA, Williams G, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987; 2: 1300-1304.
- [19] Orskov C, Holst JJ, Nielsen OV. Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology* 1988; 123:2009-13.
- [20] Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA* 1987; 84: 3434-38.
- [21] Matsuyama T, Komatsu R, Namba M, Watanabe N, Itoh H, Tarui S. Glucagonlike peptide-1 (7-36 amide): a potent glucagonostatic and insulinotropic hormone. *Diabetes Res Clin Pract* 1988; 5:281-84.
- [22] Weir GC, Mojsov S, Hendrick GK, Habener JF. Glucagon-like peptide 1(7-37) actions on endocrine pancreas. *Diabetes* 1989; 38:338-42.

- [23] Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 1993; 38: 665-73.
- [24] Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 2003; 278:471-78.
- [25] Drucker DJ. Glucagon-like peptides: regulators of cell proliferation, differentiation and apoptosis. *Mol Endocrinol* 2003; 17: 161-71.
- [26] Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF. Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology* 2002; 143:3152-61.
- [27] Nikolaidis LA, Elahi D, Hentosz T, Doverspike A, Huerbin R, Zourelis L, Stolarski C, Shen YT, Shannon RP. Recombinant Glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. *Circulation* 2004;110:955-61.
- [28] Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human proglucagon gene. *Nature* 1983; 304:368-71.
- [29] Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagonlike peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 1995; 56:117-26.
- [30] Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1(7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol* 1993; 138:159-66.
- [31] Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrientinduced glucagon-like peptide-1 secretion. *Endocrinology* 1999; 140:1687-94.
- [32] Anini Y, Hansotia T, Brubaker PL. Muscarinic receptors control postprandial release of glucagon-like peptide-1: *in vivo* and *in vitro* studies in rats. *Endocrinology* 2002; 143:2420-26.
- [33] Orskov C, Wettergren A, Holst JJ. Biological effects and metabolic rates of glucagon-like peptide 1(7-36)amide and glucagon-like peptide 1(7-37) in healthy subjects are indistinguishable. *Diabetes* 1993; 42:658-61.
- [34] Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7.36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1993; 36:741-44.
- [35] Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine extended glucagon-like peptide 1 in humans. *Diabetes* 1994; 43:535-39.
- [36] Dillon JS, Tanizawa Y, Wheeler MB, Leng XH, Ligon BB, Rabin DU, Yoo-Warren H, Permutt MA, Boyd AE 3rd. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. *Endocrinology*. 1993;133:1907-10.
- [37] Hupésodmann K, McGregor GP, Bridenbaugh R, *et al.* Characterization of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36)amide and comparison of the substrate specificity of the enzyme for other glucagons-like peptides. *Regul Pept* 1995; 58:149-56.
- [38] Hopsu-Havu VK, Glenner GG. A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta-naphthylamide. *Histochemie* 1966; 7:197-201.
- [39] Bocskei Z, Fuxreiter M, Naray-Szabo G, Szabo E, Polgar L. Crystallization and preliminary X-ray analysis of porcine muscle prolyl oligopeptidase. *Acta Crystallogr D Biol Crystallogr*. 1998; 54(Pt 6 Pt 2):1414-15.
- [40] Fulop V, Bocskei Z, Polgar L. Prolyl oligopeptidase: an unusual beta-propeller domain regulates proteolysis. *Cell* 1998; 94:161-70.
- [41] Lambeir AM, Diaz Pereira JF, Chacon P, *et al.* A prediction of DPP IV/CD26 domain structure from a physico-chemical investigation of dipeptidyl peptidase IV (CD26) from human seminal plasma. *Biochim Biophys Acta*. 1997; 1340: 215-26.
- [42] Wesley UV, Albino AP, Tiwari S, Houghton AN. A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. *J Exp Med*. 1999; 190:311-22.
- [43] Ghersi G, Dong H, Goldstein LA, *et al.* Regulation of fibroblast migration on collagenous matrix by a cell surface peptidase complex. *J Biol Chem* 2002; 277:29231-41.
- [44] Kameoka J, Tanaka T, Nojima Y, Schlossman S. F, Morimoto C. Direct association of adenosine deaminase with a T cell activation antigen, CD26. *Science* 1993; 261: 466-69.
- [45] Morrison ME, Vijayasarithi S, Engelstein D, Albino AP, Houghton AN. A marker for neoplastic progression of human melanocytes is a cell surface ectopeptidase. *J. Exp Med* 1993; 117: 1135-43.
- [46] Gault VA, Parker JC, Harriott P, Flatt PR, O'Harte FP. Evidence that the major degradation product of glucose-dependent insulinotropic polypeptide, GIP(3-42), is a GIP receptor antagonist *in vivo*. *J Endocrinol* 2002; 175:525-33.
- [47] Knudsen LB, Pridal L. Glucagon-like peptide-1-(9-36) amide is a major metabolite of glucagon-like peptide-1-(7-36) amide after *in vivo* administration to dogs, and it acts as an antagonist on the pancreatic receptor. *Eur J Pharmacol* 1996; 318:429-35.
- [48] Mentlein R. Dipeptidyl-peptidase IV (CD26)—role in the inactivation of regulatory peptides. *Regul Pept* 1996; 85:9-24.
- [49] Marguet D, Baggio L, Kobayashi T, *et al.* Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc Nat Acad Sci USA* 2000; 97: 6874-79.
- [50] Conarello SL, Li Z, Ronan J, *et al.* Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Nat Acad Sci USA* 2003; 100: 6825-30.
- [51] Nagakura T, Yasuda N, Yamazaki K, *et al.* Improved glucose tolerance via enhanced glucose-dependent insulin secretion in dipeptidyl peptidase IV deficient Fischer rats. *Biochem Biophys Res Commun* 2001; 284:501-506.
- [52] Weber A. Dipeptidyl peptidase IV inhibitors for the treatment of diabetes. *J Med Chem* 2004; 47:4135-41.
- [53] Barlocco D. LAF-237. *Curr Opin Invest Drugs* 2004;5:1094-100.
- [54] Ahrén B, Gomis R, Standl E, Mills D, Schweizer A. Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. *Diabetes Care* 2004; 27:2874-80.
- [55] Balkan B, Kwasnik L, Miserendino R, Holst JJ, Li X. Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7.36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. *Diabetologia* 1999; 42:1324-31.
- [56] Pauly RP, Demuth HU, Rosche F, *et al.* Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor Iletinazolidide. *Metabolism* 1999; 48:385-89.
- [57] Deacon CF, Hughes TE, Holst JJ. Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. *Diabetes* 1998; 47: 764-69.
- [58] Brandt I, Joossens J, Chen X, Maes M-B, Schärpe S, Meester de I, Lambeir A-M. Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by Vildagliptin ((2S)-{[(3-hydroxyadamantan-1-yl) amino]acetyl}-pyrrolidine-2-carbonitrile) *Biochem Pharmacol* 2005; 70:134-43.
- [59] Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase 4 reduces glycemia, sustains insulin levels, and reduces glucagons levels in type 2 diabetes. *J Clin Endocrinol Metab* 2004; 89:2078-84.
- [60] Mari A, Sallas WM, He YL, Watson C, Ligueros-Saylan M, Folet JE. LAF237 is a DPP-4 inhibitor that improves model-assessed β -cell function in drug-naïve patients with type 2 diabetes. *EASD conference* (2004).
- [61] Hamann LG, Augeri DJ, Betebenner DA, *et al.* Design, synthesis, and pharmacology of BMS-477118: a long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type II diabetes. *228th ACS Meeting* (2004) Philadelphia, USA.
- [62] Magnin DR, Robl JA, Sulsky RB, *et al.* Synthesis of novel potent dipeptidyl peptidase IV inhibitors with enhanced chemical stability: interplay between the N-terminal amino acid alkyl side chain and the cyclopropyl group of alpha-aminoacyl-L-cis-4,5- methanopropinonitrile-based inhibitors. *J Med Chem* 2004; 47:2587-98.
- [63] Augeri DJ, Robl JA, Betebenner DA, *et al.* Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2005; 48:5025-37.
- [64] Patel, R.N., Hanson, R.L., Gill, I., Brzozowski,D.B., Skonezny, P.M., Politino, M., Chen, J.G., Moris-Varas, F., White, B.J.: US20050256019A1 (2005) .
- [65] Politino, M., Cadin, M.M., Skonezny, P.M., Chen, J.G.: WO05106011 (2005).

- [66] Patel, R.N., Hanson, R.L., Gill, I., Brzozowski, D.B., Skonezny, P.M., Politino, M., Chen, J.G., Moris-Varas, F., White, B.J.: WO05108594 (2005).
- [67] Ashton WT, Dong H, Sisco RM, *et al.* Diastereoselective synthesis and configuration-dependent activity of (3-substituted-cycloalkyl) glycine pyrrolidides and thiazolidides as dipeptidyl peptidase IV inhibitors. *226th ACS Meeting* (2003).
- [68] Mastracchio A, Edmondson SD, Parmee ER, *et al.* Heterocycle linked cyclohexylglycine derivatives as novel dipeptidyl peptidase-IV inhibitors. *226th ACS Meeting* (2003).
- [69] Kim D, Wang L, Beconi M, *et al.* (2R)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: A potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2005; 48: 141-51.
- [70] Villhauer EB, Brinkman JA, Naderi GB, *et al.* 1-[[[(3-hydroxy-1-adamantyl)amino] acetyl]-2-cyano-(S)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* 2003; 46:2774-89.
- [71] Xu J, Ok HO, Gonzalez EJ, *et al.* Discovery of potent and selective beta-homophenylalanine based dipeptidyl peptidase IV inhibitors. *Bioorg Med Chem Lett* 2004; 14:4759-62.
- [72] Lankas GR, Leiting B, Roy RS, *et al.* Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9. *Diabetes* 2005; 54:2988-94.
- [73] Scott R, Herman G, Zhao P, Chen X, Wu M, Stein P. Twelve-week efficacy and tolerability of MK-0431, a dipeptidyl IV (DPP-IV) inhibitor, in the treatment of type 2 diabetes (T2D). *65th ADA Meeting*. (2005), 41-OR.
- [74] Brazg R, Thomas K, Zhao P, Xu L, Chen X, Stein P. Effect of adding MK-0431 to on-going metformin therapy in type 2 diabetic patients who have inadequate glycaemic control on metformin. *65th ADA Meeting* (2005). 11-OR.
- [75] Gwaltney II SL, Aertgeerts K, Feng J, *et al.* Design and synthesis of potent, selective, and orally efficacious DPP4 inhibitors accelerated by high-throughput structural biology *231st ACS Meeting* (2006).
- [76] Christopher RJ, Davenport JM, Gwaltney S, *et al.* Pharmacokinetic and pharmacodynamic profiles of SYR-322, a novel inhibitor of dipeptidyl peptidase-IV, in rats, dogs, and monkeys. *Diabetes* 2006; 55:6 suppl 1(452 P).
- [77] Feng, J., Gwaltney, S.L., Stafford, J.A., Zhang, Z.: WO05095381 (2005).
- [78] Asakawa, T.: WO05032590 (2005).
- [79] Li X, Winn DT, Farrell AMO, *et al.* Preclinical development of PHX1149, an orally available, potent and selective DPP4 (Dipeptidyl Peptidase) inhibitor. *Diabetes* 2006; 55:6 suppl 1(538 P).
- [80] Narayanan S. Novel orally active dipeptidyl peptidase IV inhibitors exhibiting potent activity in animal models of diabetes. *4th International Metabolic Diseases Drug Discovery World Summit* (2005) California USA.
- [81] Vakkalanka SKVS, Anupindi R, Varanasi KVS, *et al.* GRC 8200: A long acting DPP-IV inhibitor exhibiting excellent PK-PD correlation in rat, dog and monkey. *Diabetes* 2005 54:6 Suppl 1(Abs 1529-P).
- [82] Gopalan, B., Joshi, H.V., Shah, D.M., Ghorpade, S.R.: WO05033099 (2005).
- [83] Schoenafinger, K., Jaehne, G., Defossa, E., Schwink, L., Wagner, H., Buning, C., Tschank, G., Werner, U.: WO06015701 (2006).
- [84] Schoenafinger, K., Jaehne, G., Defossa, E., Schwink, L., Wagner, H., Buning, C., Tschank, G., Werner, U.: WO06015700, (2006).
- [85] Schoenafinger, K., Jaehne, G., Defossa, E., Schwink, L., Wagner, H., Buning, C., Tschank, G., Werner, U.: WO06015699 (2006).
- [86] Schoenafinger, K., Jaehne, G., Defossa, E., Billen, G., Wagner, H., Buning, C., Tschank, G., Werner, U.: WO06015691 (2006).
- [87] Haffner CD, McDougald DL, Reister SM, *et al.* Synthesis and biological evaluation of potent, selective, orally active 4-fluoro-2-cyanopyrrolidine inhibitors of DPP-IV. *228th ACS Meeting* (2004) Philadelphia, USA.
- [88] Haffner CD, McDougald DL, Reister SM, *et al.* a 2-Cyano-4-fluoro-1- thiovalylpyrrolidine analogues as potent inhibitors of DPP-IV. *Bioorg Med Chem Lett* 2005; 15:5257-61.
- [89] Schön E, Born I, Demuth HU, *et al.* Dipeptidyl peptidase IV in the immune system. Effects of specific enzyme inhibitors on activity of dipeptidyl peptidase IV and proliferation of human lymphocytes. *Biol Chem Hoppe Seyler* 1991; 372:305-11.
- [90] Demuth HU, McIntosh CH, Pederson RA. Type 2 diabetes-therapy with dipeptidyl peptidase IV inhibitors. *Biochim Biophys Acta* 2005; 1751: 33-44.
- [91] Hoffmann T, Glund K, McIntosh CH, *et al.* In: Mizutani S, Turner AJ, Nomura S, Ino K. Eds, DPP-IV inhibition as treatment of type II diabetes, in *Cell-Surface Aminopeptidases: Basic and Clinical Aspects*. San Diego, Elsevier Science. 2001; 381-87.
- [92] Tadayyon M, Thomas L, Besenfelder U, *et al.* Pharmacodynamic and pharmacokinetic characteristics of novel long-acting, xanthine-based DPP-IV inhibitors in the rat and Rhesus monkey. *Diabetes* 2005; 54:6 Suppl 1(Abs 565-P).
- [93] Shuai Q, Patel J, Zanze I, *et al.* Acyl thiazolidides-novel potent DPP-IV inhibitors. *230th ACS Meeting* (2005) Washington DC, USA.
- [94] Parker JC, Hulin B, Vanvolkenburg MA, *et al.* A novel fluorinated cyclic amide inhibitor of dipeptidyl peptidase IV improves glucose tolerance in murine models of diabetes mellitus. *Diabetes* 2003; 52:Suppl 6(Abs 16-LB).
- [95] Parker JC, Wright WS, Olson TV, *et al.* A novel inhibitor of dipeptidyl peptidase IV improves glucose tolerance in models of diabetes mellitus. *Diabetes* 2005; 54:6 Suppl 1(Abs 450-P).
- [96] Oefner C, D'Arcy A, Mac Sweeney A, Pierau S, Gardiner R, Dale GE. High-resolution structure of human apo dipeptidyl peptidase IV/CD26 and its complex with 1-[[2-[(5-iodopyridin-2-yl)amino]ethyl]amino]-acetyl]-2-cyano-(S)-pyrrolidine. *Acta Crystallogr D Biol Crystallogr* 2003; 59:1206-12.
- [97] Lu IL, Lee SJ, Tsu H, *et al.* Glutamic acid analogues as potent dipeptidyl peptidase IV and 8 inhibitors. *Bioorg Med Chem Lett* 2005; 5:3271-75.
- [98] Rosenblum JS, Kozarich JW. Prolyl peptidases: a serine protease subfamily with high potential for drug discovery. *Curr Opin Chem Biol* 2003; 7:496-504.
- [99] Hiramatsu H, Yamamoto A, Kyono K, *et al.* The crystal structure of human dipeptidyl peptidase IV (DPP IV) complex with diprotin A. *Biol Chem* 2004; 385:561-64.
- [100] Aertgeerts K, Ye S, Tennant MG, *et al.* Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation. *Protein Sci* 2004; 13:412-21.
- [101] Rasmussen HB, Branner S, Wiberg FC, Wagtman N. Crystal structure of human dipeptidyl peptidase IV/CD 26 in complex with a substrate analog. *Nat Struct Biol* 2003; 10:19-25.
- [102] Engel M, Hoffmann T, Wagner L, *et al.* The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism. *Proc Natl Acad Sci USA* 2003; 100:5063-68.
- [103] Brandt I, Joossens J, Chen X, Maes M-B, Scharpe S, De Meester I, Lambier A-M. Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by Vildagliptin ((2S)-[[[3-hydroxyadamantan-1-yl) amino]acetyl]-pyrrolidine-2- carbonitrile). *Biochem Pharmacol* 2005; 70:134-43.
- [104] Nordhoff S, Cerezo-Gálvez S, Feurer A, *et al.* The reversed binding of α -phenethylamine inhibitors of DPP-IV: X-ray structures and properties of novel fragment and elaborated inhibitors. *Bioorg Med Chem Lett* 2006; 16:1744-48.