Edited by
János Fischer and C. Robin Ganellin

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The Editors

Prof. Dr. János Fischer
Richter Plc
Gyömröi ut 30
1103 Budapest
Hungary

Prof. Dr. C. Robin Ganellin
University College London
Department of Chemistry
20 Gordon Street
London WC1H OAJ
United Kingdom

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5
Dipeptidyl Peptidase IV Inhibitors for the Treatment of Type 2 Diabetes

Jens-Uwe Peters and Patrizio Mattei

5.1
Introduction

The symptoms of diabetes mellitus, a metabolic disorder characterized by hyperglycemia (abnormally high blood glucose) due to inadequate insulin levels, have been described since antiquity. The introduction of insulin replacement therapy for diabetes in 1922 was a major feat in the history of medicine and was awarded with the Nobel Prize in medicine in the following year. Later in the 1920s, the first oral antidiabetic drugs (OADs) were introduced. Although they were imperfect and later withdrawn, they led to the recognition that two types of diabetics exist – the juvenile type, requiring insulin therapy, and the late-onset type, which also benefits from OAD treatment [1, 2]. The late-onset form, today known as type 2 diabetes, accounts for more than 90% of all diabetic patients and affects about 4% of the world population [3].

The treatment of type 2 diabetes aims to normalize blood glucose levels by diet, exercise, and medication, and is monitored by measuring glycosylated hemoglobin (HbA_{1c}) as a long-term marker of elevated blood glucose. The amount of HbA_{1c} reflects the average glucose level over the last 120 days (the life span of red blood cells) and should be maintained below 7% [4]. Each percentage reduction in HbA_{1c} leads to a 21% reduction of the risk for any diabetes-related end point [5]. Poorly controlled, chronic hyperglycemia causes microvascular damage, which affects organs with delicate capillary systems such as the eyes and kidneys, and can lead to blindness and renal failure. In addition, hyperglycemia leads to atherosclerosis of larger vessels, which increases the risk of myocardial infarction and stroke. An important complication resulting from micro- and macroangiopathy are lesions of the lower limbs ("diabetic foot") that may ultimately require amputation. Unfortunately, the majority of diabetic patients do not reach recommended HbA_{1c} levels and are therefore at risk of developing these disabling comorbidities. Furthermore, the prevalence of type 2 diabetes has increased over recent years, mainly due to higher life expectancies and an increasing prevalence of obesity [3]. Several classes of OADs have been introduced into clinical practice since the 1950s and are widely prescribed. However, they all come along with side effects such as hypoglycemia, weight gain, or gastrointestinal

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problems. Moreover, they often fail to achieve sustained glycemic control. Thus, there is a critical unmet need for OADs with novel modes of action.

In the late 1980s, several research groups could show that the peptidic hormone GLP-1 (glucagon-like peptide 1), which is secreted by the L-cells of the intestinal epithelium in response to food ingestion, is a potent stimulator of glucose-dependent insulin release. This finding raised hopes that exogenous GLP-1 might be used to stimulate the impaired insulin secretion in type 2 diabetic patients. Disappointingly, single subcutaneous injections of GLP-1 were ineffective in normalizing blood glucose [6]. A few years later, it was discovered that DPP-IV (dipeptidyl peptidase IV), a serine protease first isolated in 1966, rapidly cleaves and inactivates GLP-1 [7]. Several research groups recognized the implications of this finding:

- Inhibition of DPP-IV should prevent the rapid degradation of GLP-1 and should thus increase circulating GLP-1 levels.
- Increased GLP-1 levels should enhance glucose-dependent insulin secretion, leading to lower blood glucose levels.
- Consequently, DPP-IV inhibitors should have an antidiabetic effect.

The glucose-lowering/antidiabetic effect of DPP-IV inhibitors was soon demonstrated in animals and humans and triggered enormous research activities throughout the pharmaceutical industry in the first decade of the new millennium [8].

5.2
In Vitro Assays and Animal Models for the Assessment of DPP-IV Inhibitors

The discovery of DPP-IV inhibitors was facilitated by the availability of robust and high-throughput *in vitro* assays, which often rely on a simple chromogenic or fluorogenic readout. For instance, DPP-IV cleaves Ala-Pro-AFC, a peptidyl derivative of 7-amino-4-trifluoromethylcoumarin (AFC), and the green fluorescence of the cleavage product, AFC, can be distinguished from the violet-blue fluorescence of the substrate (Figure 5.1). The cleavage of Ala-Pro-AFC serves as a measure of DPP-IV activity in an *in vitro* assay, in which the candidate inhibitor is evaluated by its ability to suppress the formation of fluorescent AFC. Furthermore, animal models with high relevance to the human disease state were available. For instance, the oral glucose tolerance test (OGTT) in diabetic rats measures the glucose excursion, or the insulin response, after an oral ingestion of a standardized amount of glucose, and is equivalent to the OGTT used in the diagnosis of diabetes in humans. The efficaciousness of DPP-IV inhibitors can be evaluated in such an animal model by their ability to reduce the glucose excursion after their administration prior to the glucose challenge.

5.3
Substrate-Based DPP-IV Inhibitors

Speculations about the relevance of DPP-IV in the processing of bioactive peptides, and its potential role in diseases such as cancer and AIDS, might have provided much

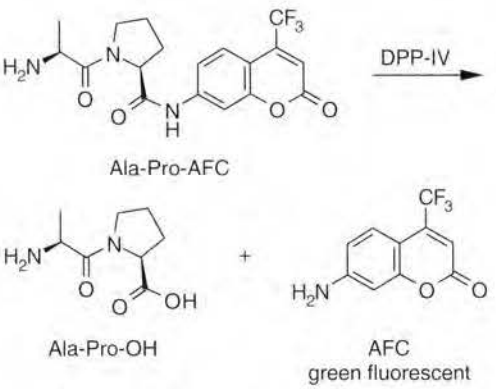


Figure 5.1 DPP-IV liberates AFC from its dipeptidyl derivative, Ala-Pro-AFC. The green fluorescence of the product is used as a readout in a DPP-IV inhibition assay.

of the impetus for DPP-IV inhibitor research in the 1980s [9]. At this time, the ACE inhibitor success story had just proven that substrate-based design is a viable approach to drug discovery, and it seems natural that this concept was also pursued in DPP-IV research. DPP-IV is an endopeptidase that releases dipeptides from the N-terminus of a wide variety of peptidic hormones, with a preference for proline at the penultimate position. This proline preference is pronounced in small substrates (such as Ala-Pro-AFC, Figure 5.1), even if the larger peptide GLP-1 (30 amino acids) is cleaved after an alanine (Figure 5.2).

In the early 1990s, several academic research groups disclosed dipeptide-like DPP-IV inhibitors, in which a pyrrolidine or a thiazolidine replaces the proline, and an attached amino acid with a free amino group mimics the N-terminus of a substrate peptide (Figure 5.3). The scissile peptide bond was either omitted, as in the prototypical DPP-IV inhibitor P32/98 [10], or replaced by a functional group designed to mimic the proteolytic transition state or to covalently bind to the enzyme's active site serine. For instance, prolineboronic acids such as **1** have been designed as transition-state analogues and are reversible, slow-binding inhibitors with activities in the low nanomolar range [11]. Phosphonates such as **2** are irreversible inhibitors, which form stable esters with DPP-IV's catalytically active serine. However, these early types of serine-interacting inhibitors did initially not provide clear advantages over the noncovalent inhibitors, as they were too unstable, too unselective, or did not show a substantially improved activity. Nevertheless, the boronic acid dutogliptin, a DPP-IV inhibitor discovered by Phenomix, has

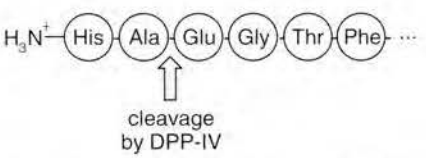
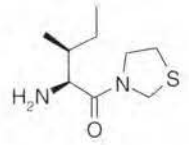
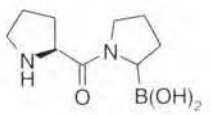


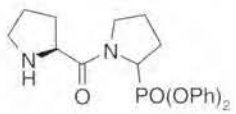
Figure 5.2 DPP-IV cleaves GLP-1 at the penultimate position from the N-terminus.



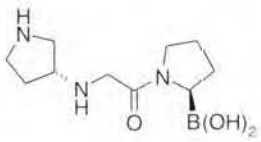
P32/98
K_i = 123 nM
Demuth et al., 1991



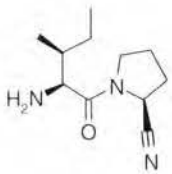
1
IC_{50} = 20 nM
Flentke et al., 1991



2
irreversible
Powers et al., 1995



Dutogliptin
IC_{50} = 25 nM
Phenomix, 2005



3
K_i = 2.2 nM
Ferring, 1996

Figure 5.3 Early substrate-based DPP-IV inhibitors and dutogliptin.

apparently overcome these limitations and entered phase 3 clinical development in 2008 [12, 13].

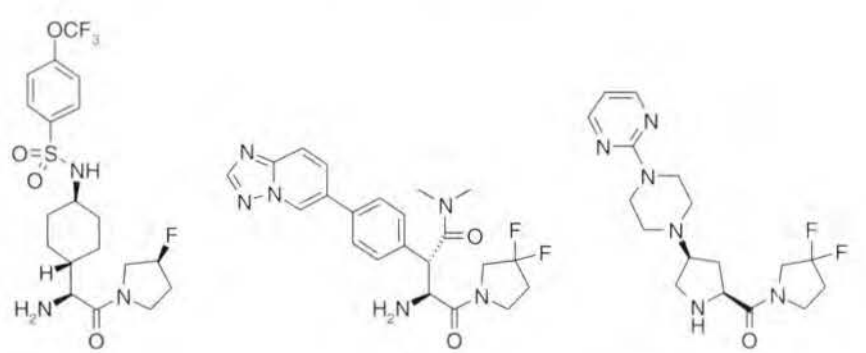
In 1994, a publication demonstrated that nitriles could be used as serine-interacting motifs in inhibitors of prolyl endopeptidase (PEP), a serine protease related to DPP-IV [14]. So far, nitriles had only been known to be cysteine protease inhibitors, but were regarded unreactive to typical serine proteases. This surprising finding prompted Sherwin Wilk's research group at the City University of New York, and researchers working with Paul D. Jenkins at Ferring Pharmaceuticals, to introduce nitriles into their substrate-based DPP-IV inhibitors [15–17]. These new cyanopyrrolidine-type DPP-IV inhibitors, for example, **3** (Figure 5.3), turned out to have an approximately 100-fold improved inhibitory potency, and additionally both a good selectivity profile and an acceptable chemical stability.

Up to this point, the role of DPP-IV in glucose homeostasis was not fully recognized. Rolf Mentlein *et al.* from the University of Kiel had already demonstrated in 1993 that GLP-1 is a substrate of DPP-IV *in vitro* [7], but this did not necessarily mean that DPP-IV would be the main metabolic enzyme of GLP-1 *in vivo*. Actually, **3** was proposed as a potential immunomodulator, as DPP-IV is identical to CD26, a component of the T-cell receptor complex. In 1995, Jens Holst and coworkers from the University of Copenhagen concluded from their studies that DPP-IV is responsible, at least in part, for the observed rapid degradation of GLP-1 in humans and proposed that inhibition of DPP-IV could be a useful adjunct in the management of type 2 diabetes [19]. Shortly thereafter, a collaborating team of scientists working with Hans-Ulrich Demuth from the University of Halle and Christopher H.S. McIntosh and Ray A. Pederson from the University of British Columbia patented DPP-IV

inhibition as a method to lower blood glucose [20]. The patent application was disclosed in 1997 and demonstrated that DPP-IV inhibition with P32/98 did indeed improve glucose tolerance in rats. Demuth, who had spent most of his academic career working on DPP-IV, would later start the biotech company, Probiodrug, to exploit this invention and to bring P32/98 into the clinic. The improvement in glucose tolerance by P32/98 was then reproduced in human healthy volunteers and diabetic patients. P32/98 and the epimeric allo-isoleucyl-thiazolidide were licensed to Merck in late 2000. However, development of both compounds was discontinued in February 2001, after Merck had identified unacceptable toxicity profiles for both compounds. Later, insufficient selectivity over the related dipeptidases DPP-8 and/or DPP-9 was postulated to be the reason for the observed toxicities [21]. At that time, Merck had already identified fluoropyrrolidine **4** (Figure 5.4) as a potential development compound. Because the rationale for subtype selectivity was compelling, **4** was rejected on the basis of a selectivity of only 50-fold over DPP-8 and DPP-9, and medicinal chemistry focused on HTS-based DPP-IV inhibitors, which culminated in the discovery of sitagliptin (see Section 5.4). Further exploration of the substrate analogue series provided **5**, with a selectivity of >10 000-fold over DPP-8/9 [22]. This compound was brought forward as a backup for sitagliptin [23].

Another potent and selective difluoropyrrolidine derivative, PF-00734200, has been discovered by Pfizer. This compound was reported to be in phase 2 clinical studies in September 2008 [24, 25].

During this time, the cyanopyrrolidines originally discovered by Sherwin Wilk and the group at Ferring had become the most popular class of DPP-IV inhibitors, as judged by the number of patent applications [18]. While the SAR around the cyanopyrrolidine ring was rather limited, a wide variety of attached amino acids with lipophilic or polar, negatively or positively charged, side chains were tolerated, which provided ample room for proprietary structures.



4
IC_{50} = 35 nM
Merck, 2004

5
K_i = 8.8 nM
Merck, 2004

PF-00734200
IC_{50} = 13 nM
Pfizer, 2005

Figure 5.4 Pyrrolidides without a serine-interacting motif.

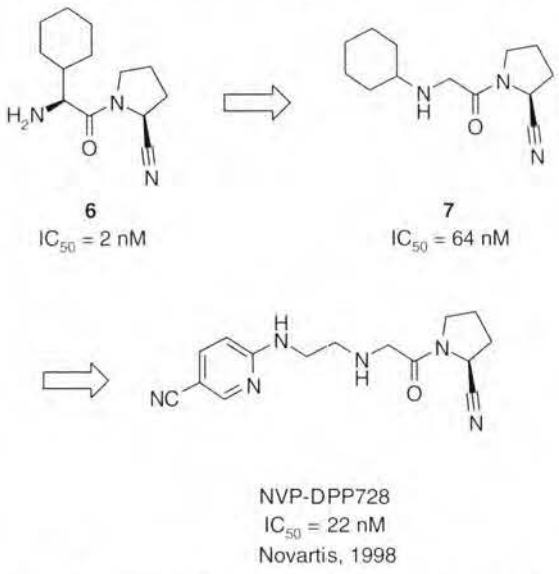


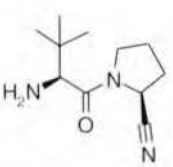
Figure 5.5 Scaffold change leading to *N*-alkylglycine DPP-IV inhibitors; NVP-DPP728 was efficacious in a proof-of-concept trial.

An important extension of this SAR was made already in 1996 by scientists at Novartis. Edwin B. Villhauer, a chemist with a long-standing interest in diabetes, was looking for a new project when Jens Holst's paper was published in 1995. Within a few days, he and his colleagues had a DPP-IV project running. Cells that happened to express DPP-IV were just available and provided an *in vitro* assay. A paper from 1988, describing a DPP-IV substrate with sarcosine (*N*-methylglycine) as an N-terminal amino acid [26], caught Villhauer's attention and led him to explore *N*-alkylglycine cyanopyrrolidines, in which the side chain of the pyrrolidine-attached amino acid is, formally, shifted to the nitrogen atom (e.g., **6** → **7**, Figure 5.5) [27]. The novel *N*-alkylglycine cyanopyrrolidines were amenable to resin-based chemistry, which was a very popular technology in those years, enabling the preparation of 1300 diverse compounds within 7 months. Only a few inhibitors with low nanomolar activities were identified in this campaign, one of them carrying a (5-nitro-pyridin-2-yl)-aminoethyl substituent. Replacement of the nitro functionality by a nitrile then led to NVP-DPP728 (Figure 5.5) with an improved selectivity over DPP-II and PPCE (postproline cleaving enzyme), which were then standard enzymes in DPP-IV selectivity studies. Within only 9 months, the Novartis project team had identified a development compound. Clinical trials with NVP-DPP728 began in 1998. A first phase 2 trial based on the then widely held paradigm that any type 2 diabetes patient treated with a DPP-IV inhibitor should experience an immediate benefit, gave disappointing results and almost stopped the project. A detailed data analysis suggested that patients with a certain level of pancreatic beta cell activity might benefit over a longer time frame. A second trial designed with the hindsight from this analysis was a huge success: after 4 weeks of treatment, NVP-DPP728 reduced

postmeal glucose excursion, fasting glucose, and 24 h mean glucose. For the first time, it was shown that chronic DPP-IV inhibition in diabetic patients was safe and also led to a reduction in HbA_{1c} levels [28].

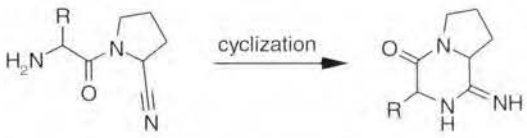
NVP-DPP728's relatively short half-life of 0.85 h was initially not seen as a disadvantage. On the contrary, the many possible physiological roles of DPP-IV made it desirable for a proof-of-concept compound that any potential adverse effects would abate quickly after a discontinuation of administration. DPP-IV cleaves, at least *in vitro*, not only GLP-1 but also several peptidic hormones, neurotransmitters, and chemokines. Of particular concern was initially the fact that DPP-IV is identical to CD26, a surface protein on activated T-cells, which mediates stimulatory signals; fortunately, it was found that NVP-DPP728 had no immunosuppressant effect. (Later on it was shown that the enzymatic activity of DPP-IV is not required for T-cell function.) It might have been envisioned that NVP-DPP728 could be a short-acting, meal-dependently administered drug to reduce postprandial glucose excursion. Such a treatment would allow an intermittent recovery of DPP-IV activity, and the normal regulation of other potential DPP-IV substrates, thus minimizing side effects. However, a team of Novo Nordisk researchers, collaborating with the Miami School of Medicine, demonstrated in 2001 that a 24 h infusion of GLP-1 over 7 days gave a much better outcome for diabetic patients than a 16 h infusion, indicating that a 24-h blockade of DPP-IV was needed to maximize the therapeutic effect [29]. In 2002, Ferring researchers published their results with the long-acting DPP-IV inhibitor FE 999011 (Figure 5.6), which clearly showed that full inhibition of DPP-IV over 24 h gave the best results in animal models of diabetes [30]. In the following years, most companies therefore focused on inhibitors with high metabolic stability, and today all clinically proven inhibitors show >50% plasma DPP-IV inhibition over 24 h.

Apart from the demonstrated clinical efficacy and the facile synthetic access, there might be yet another reason why the *N*-alkylglycine inhibitors became very popular throughout the industry in the following years: it was generally perceived that they had a superior chemical stability. As already mentioned, cyanopyrrolidine DPP-IV inhibitors, and other substrate-based inhibitors with an electrophilic serine-interacting motif, are chemically unstable in solution. This solution instability is due to an intramolecular reaction between the amino function and the electrophilic motif, as depicted in Scheme 5.1. The short solution half-life typically of a few hours was



FE 999011
K_i = 3.8 nM
Ferring, 1996

Figure 5.6 Studies with FE 999011 showed that sustained inhibition of DPP-IV leads to best results in animal models of diabetes.



Scheme 5.1 The limited solution stability of cyanopyrrolidine DPP-IV inhibitors is due to an intramolecular reaction between the mandatory amino and cyano functionalities.

causing problems for formulation and was made responsible for the short *in vivo* half-life of some compounds.

To overcome this limitation, many research groups explored *N*-alkylglycines with sterically hindered amines, which would undergo cyclization less readily. Early on, Novartis scientists had identified an adamantyl derivative **8** (Figure 5.7), which was one of the most potent inhibitors discovered in their program. Also, the primary metabolites of this compound were found to be highly active. Already in 1998, Villhauer synthesized one of the putative metabolites, LAF-237, which turned out to have an excellent solution stability, potent inhibitory activity, and good selectivity over related enzymes [31]. The improved pharmacokinetic profile and longer lasting pharmacodynamic effect of LAF-237 led to a replacement of Novartis' front-runner NVP-DPP728. LAF-237 was later named vildagliptin, in reference to Villhauer, its inventor [32]. Vildagliptin has been, after sitagliptin, the second compound to obtain market approval in the European Union and other countries. In the United States, Novartis has paused its efforts to seek regulatory approval after the FDA had requested additional data to address concerns about the tolerability in patients with renal impairment and skin lesions in nonhuman primates [33] (although no skin

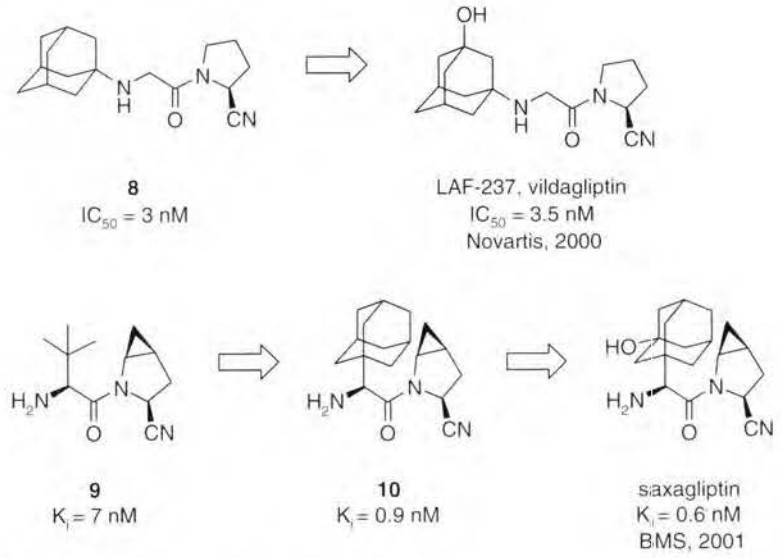


Figure 5.7 Discovery of vildagliptin and saxagliptin.

Table 5.1 Chemical stabilities of primary amine inhibitors.

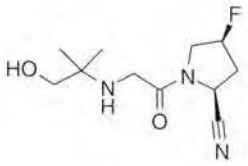
Compound	Half-life[a)]
3 (Figure 5.3)	5 h
FE 999011 (Figure 5.6)	27 h
9 (Figure 5.7)	42 h

a) In aqueous buffer at pH 7.2; 39.5 °C.

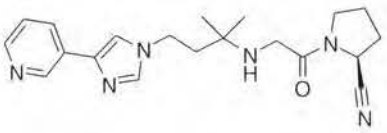
lesions have been observed in humans during clinical trials [67]). Vildagliptin is only moderately selective over DPP-8 and DPP-9. Following the highly publicized Merck study on the potential toxicities associated with DPP-8/9 inhibition [21], Novartis undertook long-term rodent toxicity studies with vildagliptin at exposures that are high enough for complete inhibition of DPP-IV, DPP-8, and DPP-9. As vildagliptin did not display any of the toxicities observed with P32/98 and structurally related molecules, the toxicity of the compounds studied by Merck is more likely the result of unidentified off-target effects that are independent of DPP-8/9, and the relevance of isoform selectivity remains unclear [34].

Researchers at Bristol-Myers Squibb found that converting a tertiary (**3**, Figure 5.3) to a quaternary alpha-carbon (FE 999011, Figure 5.6) improves the solution half-life by fivefold (Table 5.1). The long-lasting pharmacodynamic effect of FE 999011 might, at least in part, be attributed to this improved solution stability. Also, the introduction of a methylene bridge into the cyanopyrrolidine ring leads to steric bulk that similarly improves the chemical stability (compare FE 999011 and **9**, Table 5.1). Molecular modeling demonstrated that these effects are, in both cases, due to intramolecular van der Waals interactions. These interactions disfavor a *cis* conformation of the amide, which is a prerequisite for cyclization, and thereby increase stability [35]. These findings led the Bristol-Myers Squibb scientists, in striking analogy to the efforts at Novartis, to **10** with an adamantyl substituent. This compound showed an excellent plasma-DPP-IV inhibition after oral dosing in rats, despite a low bioavailability (2%). This seemed to indicate that **10** is converted into an active metabolite *in vivo*, which prompted the synthesis of a hydroxy analogue as a putative metabolite. Quite similar to the vildagliptin story, it was found that this metabolite, later named saxagliptin (Figure 5.7), was highly potent and had an excellent solution stability [36]. This high solution stability, together with a relatively high distribution volume, makes saxagliptin a long-acting DPP-IV inhibitor. Bristol-Myers Squibb and AstraZeneca have shared the clinical development and filed a New Drug Application in 2008 [37].

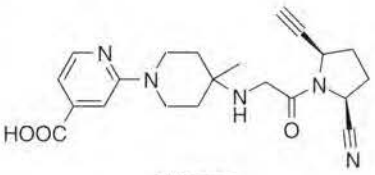
Other companies also came up quickly with *N*-alkylglycines with a wide variety of quaternary N-substituents. TS-021, **11**, and ABT-279 (Figure 5.8) are examples of *N*-alkylglycines that were evaluated in clinical trials. Taisho scientists identified TS-021, which had a much higher solution stability than a previously explored primary amine and an alkylglycine analogue without a quaternary N-substituent [38]. This improved stability translated into markedly higher plasma concentrations in rats, as measured 6 h after oral administration. An oral dose of TS-021 of 0.3 mg/kg in rats almost completely inhibited plasma DPP-IV activity for 120 min and exhibited a significant



TS-021
IC_{50} = 4.6 nM
Taisho, 2002



11
IC_{50} = 20 nM
Roche, 2003



ABT-279
K_i = 1.0 nM
Abbott, 2004

Figure 5.8 Various *N*-alkylglycine compounds in clinical development.

antihyperglycemic effect. The compound underwent phase I clinical studies in 2004, and was licensed to Eli Lilly in 2005; however, no further development was reported. Roche's clinical compound, **11**, was well tolerated in healthy volunteers up to doses of 2 g. In a multiple-dose study, the oral administration of 400 mg of **11** twice daily achieved >50% inhibition of plasma DPP-IV activity over the 12 h dose interval [39]. ABT-279 features a 5-ethynyl substituent on the cyanopyrrolidine ring, which had been demonstrated to improve selectivity over DPP-8/9 [40]. Indeed, the compound has an excellent selectivity over these enzymes as well as related peptidases and other safety-relevant targets. In healthy volunteers, ABT-279 was well tolerated up to doses of 1 g.

A primary amine inhibitor with a bulky side-chain, GSK-23A (Figure 5.9), was discovered at GlaxoSmithKline [41]. A combination of steric and electronic effects

GSK-23A
K_i = 53 nM
GSK, 2003

denagliptin
K_i = 22 nM
GSK, 2003

Figure 5.9 GSK-23A and denagliptin.

might be responsible for a reduced nucleophilicity of the free amine function, which leads to an extraordinarily long half-life of 1733 h in aqueous buffer at pH 7.2 and 37 °C. Denagliptin, another compound from the same company, was developed up to phase 3, but was finally put on hold in 2006 due to unfavorable data from preclinical long-term toxicology experiments [42, 43].

Today, we can look back on more than two decades of research on substrate-based DPP-IV inhibitors. These dipeptide-like compounds provided the first tools to elucidate the function of DPP-IV *in vivo*. Especially, P32/98 and NVP-DPP728 have played a pivotal role in establishing DPP-IV's role in glucose homeostasis and in establishing DPP-IV as a therapeutic target for type 2 diabetes. The exciting results obtained with these and other compounds triggered a race in the pharmaceutical industry toward DPP-IV inhibitors as a novel class of antidiabetic medicines, and many companies embarked on fast-follower projects with similar substrate-based compounds. This research culminated in the discovery of vildagliptin, which has obtained market approval in several countries, and other advanced compounds undergoing clinical development. However, other important classes of DPP-IV inhibitors have also emerged more recently, as will be shown in the next sections.

5.4 Sitagliptin and Analogues

Sitagliptin has been the first DPP-IV inhibitor to be approved as a treatment for type 2 diabetes. Launched by Merck in 2006, the annual sales for 2008 have already exceeded US$ 1000 million. The medicinal chemistry team led by Ann E. Weber started in 1999 and initially focused on substrate analogue inhibitors (see Section 5.3). After the identification of unwanted off-target activity as possible reason for multiorgan toxicity, the objective became to achieve a high (>1000-fold) selectivity over related proline peptidases, especially DPP-8 and DPP-9 [23, 44, 45]. The link between activity at DPP-8/9 and toxicity remains a matter of debate, but the goal per se has successfully guided the team toward the discovery of sitagliptin.

A high-throughput screening of the Merck sample library was performed in parallel with the medicinal chemistry work on substrate analogues. The screening produced only very few hits, among which the legacy compounds **12** and **13** (Figure 5.10) were followed up. At that time, no structural information of DPP-IV was available, and it was (wrongly) assumed that the pyrrolidine subunit of **13** might reside in the S1 substrate specificity pocket. As a consequence, the pyrrolidine was replaced with a thiazolidine, in analogy with substrate analogues such as P32/98. The truncated molecule **14**, with a much reduced molecular weight, was roughly equipotent to **13** but left little room for structural variations. The trifluorophenyl derivative, **15**, had a respectable potency but poor pharmacokinetic properties and an insufficient selectivity over DPP-8 [46].

In the meantime, the weakly active HTS hit **12** was combined with the 3-amino-4-phenylbutyryl side chain of **13**. The resulting hybrid molecule **16** was more than 100-fold more potent. A fluorine substituent at C(2) (**17**) led to an additional fourfold

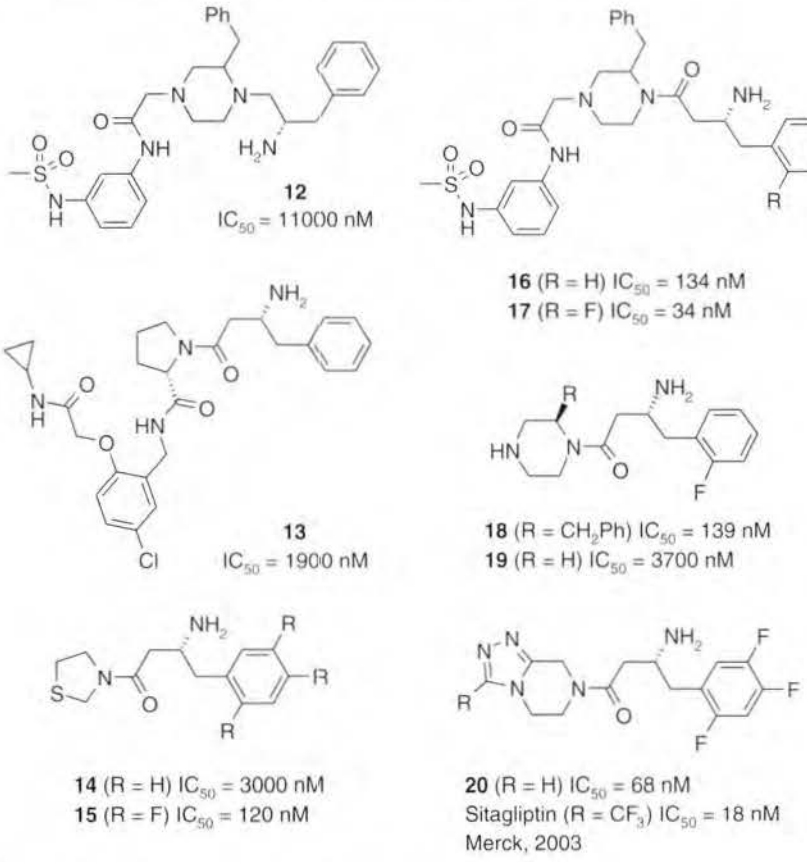


Figure 5.10 Evolution of sitagliptin from screening hits 12 and 13.

potency improvement. By removing the decoration of the piperazine, **18** and **19** were obtained. Molecule **18** was reasonably potent and selective but displayed a poor pharmacokinetic profile, which was attributed to the metabolic instability of the piperazine ring [47]. Unsubstituted piperazine **19** was only marginally active but had a low molecular weight and set the stage for further refinement.

Incorporation of the 2,4,5-trifluoro substitution pattern on the phenethylamine and replacement of the piperazine by a triazolopiperazine led to a significant improvement in potency. The poor bioavailability of **20** was improved to excellent values by installation of a trifluoromethyl group in the triazole ring, resulting in sitagliptin [48].

Interestingly, triazolopiperazines (systematic name: 5,6,7,8-tetrahydro-1,2,4-triazolo[4,3-*a*]pyrazine) have only recently found widespread use. The parent compound was first disclosed by Merck, as late as 2001, as an intermediate for $GABA_A$ ligands as cognition enhancers [49] and soon became a fashionable building block in various Merck projects [50, 51]. Since the public disclosure of sitagliptin as development

compound in 2004, the trifluoromethyl-substituted triazolopiperazine has become a frequently used amine subunit across the medicinal chemistry community.

Sitagliptin was discovered in the absence of biostructural information. However, as soon as Merck had determined the cocrystal structure of sitagliptin within DPP-IV, the rational design of sitagliptin analogues became feasible. The cocrystal structure shows that the trifluorophenyl group occupies the S1 pocket of the enzyme; this pocket is a central recognition motif and normally accommodates the penultimate amino acid of the substrate (Figure 5.11). The fluorine atoms at C(4) and C(5) optimally fit the hydrophobic niche in the back of the S1 pocket, whereas the fluorine at the *ortho* position makes a favorable electrostatic interaction with the side chains of Asn710 and Arg125 [52]. Like the class of substrate-based inhibitors, which use a pyrrolidine or thiazolidine derivative to fill the S1 pocket, this class has a rather limited SAR around the trifluorophenyl group. Accordingly, a number of sitagliptin analogues have been made, which use the 2,4,5-trifluorophenethylamine subunit for selective recognition of DPP-IV but differ in the remaining part of the molecule for additional interactions with the target and refinement of the pharmacokinetic properties. For instance, Merck has designed the cyclic analogue **21** (Figure 5.12), in which the butyryl moiety of sitagliptin is replaced by a cyclohexane. Like sitagliptin, **21** is potent and selective over DPP-8/9 but has improved pharmacokinetic properties, with lower clearance and longer half-lives across species [53].

Researchers at Abbott have adapted the major fragments of the Merck inhibitors sitagliptin and **21** to create their own DPP-IV inhibitor, ABT-341. This compound is a potent DPP-IV inhibitor, is selective over DPP-8/9, and has excellent pharmacokinetic properties, comparable to **21** [54]. Despite the similarity to sitagliptin, the binding mode of ABT-341 is different from that of sitagliptin, in that the triazolopyrazinecarbonyl subunit occupies a different part of the binding pocket and induces some conformational change at the target [55]. The compound was selected as

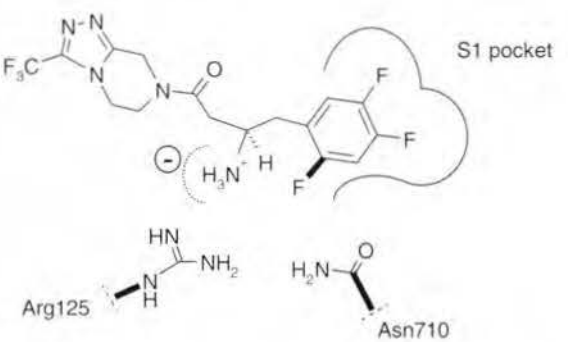
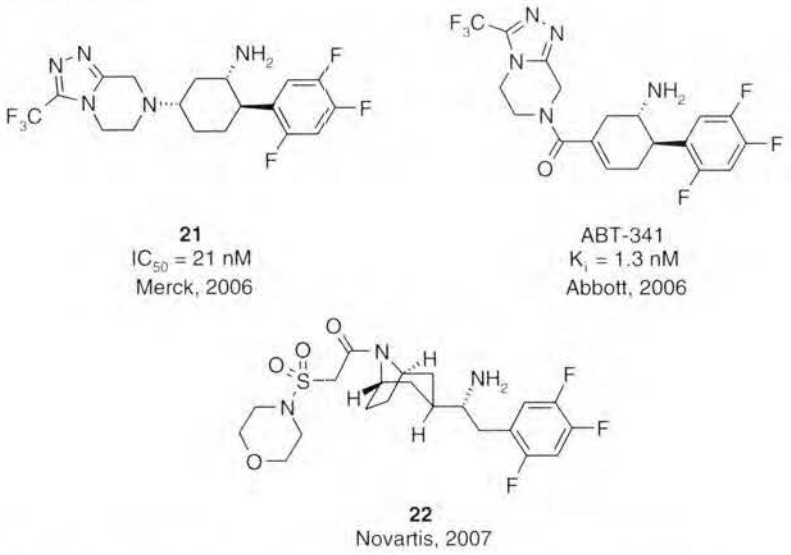


Figure 5.11 Schematic illustration of key interactions of sitagliptin with DPP-IV: the trifluorophenyl substituent resides in the lipophilic S1 pocket. The *ortho*-F makes favorable electrostatic interactions with Arg125 and Asn710. The protonated amine binds to a negatively charged surface of the protein (comprised of Glu205, Glu206, and Tyr662, not shown for clarity).



21
IC_{50} = 21 nM
Merck, 2006

ABT-341
K_i = 1.3 nM
Abbott, 2006

22
Novartis, 2007

Figure 5.12 Sitagliptin analogues with a 2,4,5-trifluorophenethylamine motif.

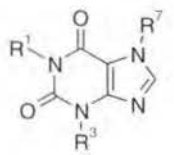
development candidate, but no clinical development has been reported as of December 2008.

Several years after the discovery of vildagliptin, Novartis has also embarked on a DPP-IV follow-on project, using sitagliptin as seed structure. As a late entrant to the phenethylamine class, compound **22**, with a bicyclic subunit, has been identified as a potent DPP-IV inhibitor [56].

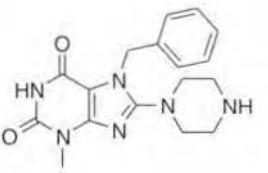
5.5
Xanthines and Analogues

The natural products theophylline, theobromine, and caffeine are known as xanthine alkaloids. They are among the oldest drugs, mainly exhibiting vasodilatory and stimulating effects, which can be rationalized through their actions as (nonselective) phosphodiesterase inhibitors and adenosine receptor antagonists [57]. Owing to their rich pharmacology and chemical tractability, xanthine derivatives are well represented in corporate screening libraries. After DPP-IV had emerged as an attractive target for type 2 diabetes, several companies performed a high-throughput screening to identify novel classes DPP-IV inhibitors.

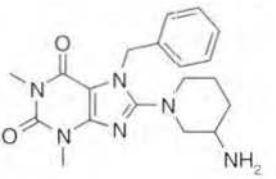
Compound **23** (Figure 5.13) is a commercially available "lead-like" xanthine derivative that inhibits DPP-IV in the low micromolar range. As a consequence, **23** has been discovered as a screening hit by a number of research teams. For instance, Merck invested some limited resources on substituent alterations of **23** with little success but then focused on more promising activities (see preceding sections) [44]. On the other hand, Novo Nordisk and Boehringer Ingelheim have identified



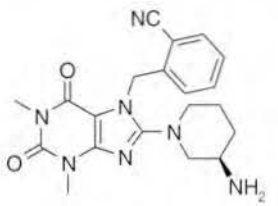
Xanthine $R^1 = R^3 = R^7 = H$
Theophylline $R^1 = R^3 = Me$, $R^7 = H$
Theobromine $R^1 = H$, $R^3 = R^7 = Me$
Caffeine $R^1 = R^3 = R^7 = Me$



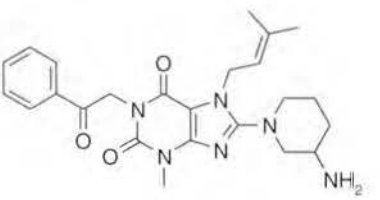
23
IC_{50} = 3900 nM



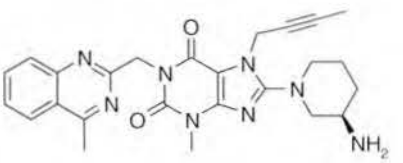
24
IC_{50} = 82 nM
Boehringer Ingelheim, 2002



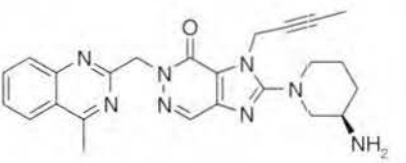
25
Novo Nordisk, 2003



26
IC_{50} = 6 nM



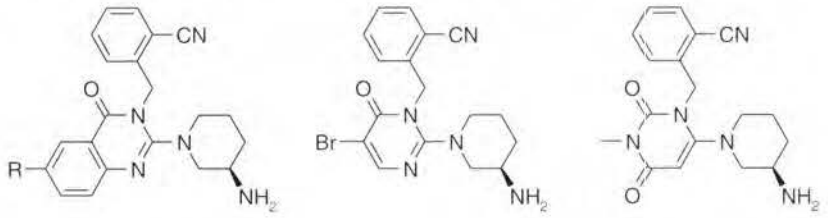
Linagliptin
IC_{50} = 1 nM
Boehringer Ingelheim, 2004



27
IC_{50} = 1 nM
Boehringer Ingelheim, 2004

Figure 5.13 Linagliptin and other DPP-IV inhibitors originating from a commercially available screening compound, 23.

a 3-aminopiperidine subunit to be a superior replacement for the piperazine moiety (compounds **24** and **25**) and filed patent applications, which overlap to a significant degree [58]. Boehringer Ingelheim has best succeeded in elaborating the xanthine series: modification of the substituents at N(1) and N(7) led to **26**, which was very potent on DPP-IV but had unacceptable off-target activities at the hERG channel and the muscarinic receptor M_1. Replacement of the substituent at N(7) by a 2-butynyl group and installation of a quinazolylmethyl substituent in lieu of the phenacyl group gave linagliptin, in which the hERG interaction was greatly reduced and the selectivity over the M_1 receptor was increased to 300-fold [59]. Comparative preclinical *in vivo* characterization with vildagliptin, saxagliptin, sitagliptin, and alogliptin shows that linagliptin has a superior potency and longer duration of action [60]. Linagliptin has entered phase 3 clinical trials in 2008. The X-ray crystal structure of linagliptin within DPP-IV reveals that the 2-butynyl group resides in



28 R = H IC_{50} = 13 nM
29 R = F IC_{50} = 4 nM

30
IC_{50} = 5 nM

Alogliptin
IC_{50} = 7 nM
Syrrx/Takeda, 2005

Figure 5.14 Structural insight led to a successful core replacement of xanthine 25, and finally to alogliptin.

the S1 pocket. The 4-methylquinazolinone group stacks on top of a tryptophan residue of the protein (Trp629); this π–π interaction [61] is not exploited by other classes of DPP-IV inhibitors and contributes to the very high affinity of linagliptin. The main binding contribution of the xanthine moiety comes from another π–π interaction, a stacking of the central uracil ring with a tyrosine side chain (Tyr547). Comparable aromatic–aromatic interactions can also be affected by a wide variety of other heterocycles [58]. For instance, Boehringer Ingelheim has reported analogue **27**, in which the xanthine core has been replaced by an imidazopyridazinone. This compound is equipotent to linagliptin but has a superior selectivity over M_1 (>1000-fold) and a different pharmacokinetic profile [62].

Researchers at Syrrx (now Takeda San Diego) have performed a remarkable scaffold hopping exercise, which provided interesting new classes of patentable DPP-IV inhibitors. Supported by high-throughput structural biology and molecular modeling as the company's core expertise, they started from seed structures such as Novo Nordisk's xanthine derivative **25** (Figure 5.14). In **25**, the cyanobenzyl substituent fills the cavity of the S1 pocket. The cyano group does not engage in a covalent interaction with the enzyme (in contrast to the cyano group in the cyanopyrrolidine series) but makes a favorable electrostatic interaction with the side chains of Asn710 and Arg125, similar to that of the *ortho*-fluorine of sitagliptin [52]. In search for central scaffolds that could take advantage of the π–π interaction with Tyr547 like the xanthine core of **25**, they identified 4-quinazolinone as a suitable heterocyclic replacement. Indeed, compound **28** was very potent. Pharmacokinetic shortcomings were amended by introducing a fluorine at the metabolically vulnerable position of the quinazolinone. Compound **29** had attractive pharmacological and pharmacokinetic properties but showed unacceptable levels of CYP3A4 and hERG inhibition. To minimize the interaction at these off-targets, more polar heterocycles were explored as quinazolinone replacements. Pyrimidinone **30** and the analogous uracil compound, later named alogliptin, retained the potency, and greatly improved the selectivity over the off-targets. Alogliptin, which is the least lipophilic in this series, showed the most favorable pharmacological profile and no evident safety issues [63, 64]. Alogliptin has progressed through clinical development very rapidly, and a New Drug Application has been filed in December 2007.

5.6
Pharmacological Comparison of DPP-IV Inhibitors

DPP-IV is a chemically very tractable target, and several DPP-IV inhibitors have progressed into clinical trials as medicines to treat type 2 diabetes. In this highly competitive field, the structural diversity is remarkable, with a primary or secondary amino group as the sole recurring motif. Nevertheless, a comparison of phase 3 clinical data at therapeutic doses shows that vildagliptin, sitagliptin, and alogliptin (as representative compounds from each structural class) have similar clinical efficacies. Thus, the average reduction of glycosylated hemoglobin (HbA_{1c}) is 0.5–0.8% after 24 or 26 weeks of treatment at therapeutic doses (Table 5.2). It should be noted that the magnitude of the HbA_{1c} reduction depends on the severity of the disease. For instance, vildagliptin (50 mg twice a day) achieves an HbA_{1c} reduction of 0.6% from a baseline-HbA_{1c} of $\leq$8% but a reduction of 1.6% from a baseline of $\geq$10% (similar patterns for HbA_{1c} changes are reported for other classes of OADs).

While the determination of meaningful changes in HbA_{1c} requires long-term treatment of diabetic patients and a correct estimation of the therapeutic dose, DPP-IV inhibition has the benefit of offering an instant-readout biomarker that can forecast the efficacy of the drug in an exploratory setting: DPP-IV activity can be easily determined in blood plasma by measuring the turnover rate of a peptidic substrate using UV spectroscopy. Thus, the notion that sustained inhibition of DPP-IV activity leads to a maximal therapeutic effect [29, 30] has been exploited by Merck in designing phase 1 clinical studies. In healthy volunteers, near-maximal ($\geq$80%) DPP-IV inhibition was achieved at daily doses of $\geq$100 mg (Figure 5.15). The dose of 100 mg/day was confirmed in phase 2 studies to be therapeutically adequate in type 2 diabetic patients and later taken on to phase 3. The successful implementation of a simple pharmacodynamic readout as biomarker enabled Merck to progress sitagliptin from entry into human to phase 3 in only 2.1 years [65].

For vildagliptin, the DPP-IV inhibition after administration of 50 mg is greater than 80% over 12 h but reduced to about 20% after 24 h [66]. Accordingly, the recommended dosing regimen for vildagliptin in the majority of settings is 50 mg

Table 5.2 HbA_{1c} changes after chronic administration of DPP-IV inhibitors (phase 3 data).

	Vildagliptin [67]	Sitagliptin [68]	Alogliptin [74]
Number of subjects	90	229	131
Duration of treatment	24 weeks	24 weeks	26 weeks
Dose	50 mg	100 mg	25 mg
Dosing regimen	Twice daily	Once daily	Once daily
HbA_{1c} baseline	8.6%	8.0%	7.90%
Mean change from baseline HbA_{1c}	−0.8%	−0.6%	−0.59%
HbA_{1c} change from placebo	−0.5%[a)]	−0.8%[b)]	−0.57%[c)]

a) 95% confidence interval: (−0.8; −0.1); $p < 0.05$ compared to placebo.
b) 95% confidence interval: (−1.0; −0.6); $p < 0.001$ compared to placebo.
c) $p < 0.001$ compared to placebo.

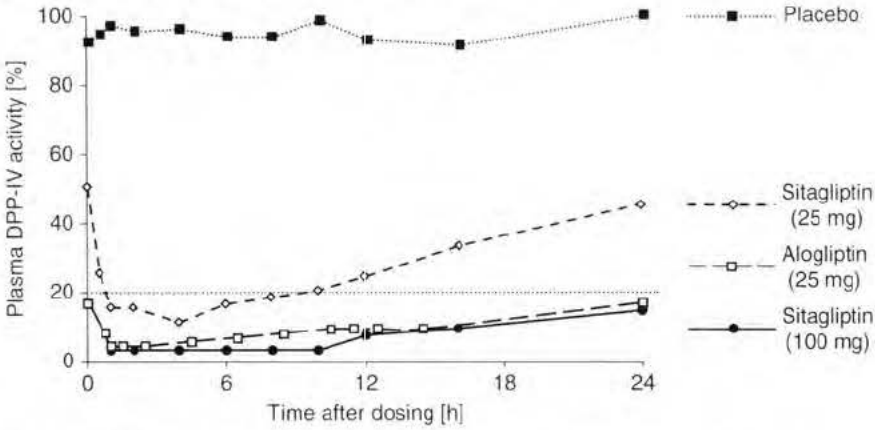


Figure 5.15 Time course of inhibition of plasma DPP-IV activity after administration of placebo [75], and multiple daily oral doses of sitagliptin (after 10 days, healthy volunteers) [75], and alogliptin (after 14 days, type 2 diabetic patients) [69]. Adapted with permission from Excerpta Medica, Inc.: Clinical Therapeutics, copyright 2006, 2008.

twice a day [67]. Alogliptin achieves near-maximal DPP-IV inhibition over 24 h already at much lower doses – a 25 mg dose has approximately the same effect as a 100 mg dose of sitagliptin (Figure 5.15).

DPP-IV inhibitors are typically hydrophilic compounds that are rapidly absorbed. Otherwise, the pharmacokinetic properties of the individual DPP-IV inhibitors are quite distinct (Table 5.3): sitagliptin has a relatively low clearance and a large volume of distribution. This translates into a long terminal half-life. Protein binding is low. Sitagliptin is predominantly excreted unchanged through the kidneys, with limited metabolic contribution through CYP3A4 and CYP2C8. Accordingly, patients with renal impairment should use lower doses [68].

In comparison, vildagliptin has a higher clearance and lower volume of distribution, which is reflected in a relatively short half-life. Protein binding is very low. CYP-dependent metabolism does not occur. The major elimination pathway is hydrolytic

Table 5.3 Pharmacokinetic data of DPP-IV inhibitors.

	Sitagliptin [68]	Vildagliptin [67]
Dose	100 mg	50 mg
t_{max}	1–4 h	1.7–2.5 h
Clearance	350 ml/min	680 ml/min
Volume of distribution	198 l	71 l
Half-life	12.4 h	2 h (intravenous), 3 h (oral)
Bioavailability	87%	85%
Protein binding	38%	9.3%
Renal excretion of parent	79%	23%

metabolism at the cyano group, followed by renal excretion of the inactive metabolite; renal excretion of parent drug accounts only for a minor fraction. Vildagliptin is not recommended for renally impaired patients due to insufficient data. Additional safety concerns are related to elevated levels of liver aminotransferases and skin lesions; therefore, monitoring for liver function and skin disorders is recommended [67].

For the less advanced DPP-IV inhibitors, only limited pharmacokinetic information is available. Alogliptin has pharmacokinetic properties similar to sitagliptin, with an apparent half-life of about 20 h and mainly renal excretion of unmetabolized drug [69].

Saxagliptin is also renally excreted, as parent and active metabolite, both of which have apparent half-lives of about 3 and 5 h, respectively [70]. The conversion of saxagliptin to its active metabolite is mediated by CYP3A4/5, a clear difference from its close structural analogue, vildagliptin [71].

Finally, linagliptin has a completely different pharmacokinetic profile in that renal excretion is only a minor elimination route. The compound is largely bound to plasma proteins, has a very long apparent terminal half-life of about 3 days, and has a bioavailability of 30% [72].

Taken together, DPP-IV inhibitors achieve an average HbA_{1c} reduction of 0.5–0.8% after 6 months, independent of the structural class. Inhibition of DPP-IV activity is a relevant biomarker for antihyperglycemic efficacy, and near-maximal inhibition over 24 h is required for an optimal effect. Besides, the individual compounds differ significantly in their mode of metabolism and excretion, which may be an important consideration for the individual patient.

5.7 Concluding Remarks

DPP-IV inhibitors represent only one of the many classes of drugs to treat patients with type 2 diabetes. The main goal of management of type 2 diabetes is to achieve glycemic levels as close to the nondiabetic range (HbA_{1c} at 4–6%) as practicable, in order to reduce the risk of late-stage complications. A consensus algorithm of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) released in 2008 calls for a therapeutic intervention in cases where HbA_{1c} exceeds 7%. In principle, most patients diagnosed with type 2 diabetes would massively benefit from weight loss and increased physical activity, but only a minority is willing and able to adhere to lifestyle changes in the long term. Therefore, medical management is the common practice, with metformin as first-line treatment. In cases where the HbA_{1c} goal of 7% is not met with metformin alone, either insulin or a sulfonylurea should be added. Alternatively, when hypoglycemia (as frequent side effect of insulin and sulfonylureas) is particularly undesirable, pioglitazone or a GLP-1 agonist can be used as an add-on to metformin. Other approved classes of drugs including DPP-IV inhibitors are not within the list of preferred agents, in part due to their limited clinical data [73].

Sitagliptin, launched in 2006, is often used in combination with metformin. Its rapid rise in popularity is due to the favorable safety profile (no hypoglycemia, no weight gain, and no gastrointestinal side effects). The absence of competition from other DPP-IV inhibitors has also contributed to a highly successful start for this drug. Vildagliptin has been approved in several countries, and other DPP-IV inhibitors are expected to be introduced in the near future. They all lower HbA_{1c} to a similar extent but have quite diverse pharmacokinetic properties. The result of ongoing studies, with focus on long-term benefits and safety, will determine the future role of DPP-IV inhibitors among the options to treat type 2 diabetes.

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Patrizio Mattei

F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, 4070 Basel, Switzerland

Patrizio Mattei was born in Thalwil, Switzerland, in 1968. He studied chemistry at the Eidgenössische Technische Hochschule in Zürich, where he also carried out his doctoral work under the supervision of Professor François Diederich. After a postdoctoral fellowship in Professor Donald Hilvert's group at the Scripps Research Institute in La Jolla, California, he joined F. Hoffmann-La Roche Ltd. as a medicinal chemist in 1999. He is coinventor of more than 40 patents and patent applications, 5 of which are related to DPP-IV inhibitors.

Jens-Uwe Peters

F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, 4070 Basel, Switzerland

Jens-Uwe Peters studied chemistry at the University of Göttingen, where he worked with Professor A. de Meijere, and conducted his doctoral research at the Technical University of Berlin under the guidance of Professor S. Blechert. After 1 year of postdoctoral studies in the group of Professor J. Rebek at the Scripps Research Institute, La Jolla, he joined the Medicinal Chemistry group at F. Hoffmann-La Roche, Ltd. in 1999. Since then, he has been contributing to Roche's DPP-IV project and to numerous other drug discovery projects.