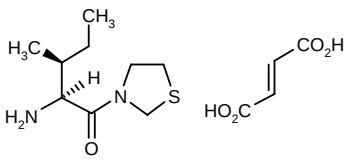
Drugs of the Future 2001, 26(9): 859-864

CCC: 0377-8282/2001

P32/98

Antidiabetic
Dipeptidyl-Peptidase IV Inhibitor

2(*S*)-Amino-3(*S*)-methyl-1-(3-thiazolidinyl)pentan-1-one fumarate
3-(L-Isoleucyl)thiazolidine fumarate
3-[2(*S*)-Amino-3(*S*)-methylpentanoyl]thiazolidine fumarate



$C_9H_{18}N_2OS.C_4H_4O_4$ Mol wt: 318.3918

CAS: 251572-70-0
CAS: 251571-89-8 (as monoacetate)
CAS: 136259-20-6 (as free base)
CAS: 140233-53-0 (as hydrochloride)
CAS: 251572-86-8 (as hemifumarate)

EN: 277261

Synthesis

The condensation of *N*-(*tert*-butoxycarbonyl)-L-isoleucine (I) with thiazolidine (II) by means of isobutyl chloroformate and *N*-ethylmorpholine (NEM) in THF gives 3-[*N*-(*tert*-butoxycarbonyl)-L-isoleucyl]thiazolidine (III), which is deprotected with HCl and thioanisole in acetic acid (1, 2). Scheme 1.

Introduction

Diabetes mellitus is a group of several diseases characterized by chronic hyperglycemia with disturbances in fat, carbohydrate and protein metabolism due to abnormal insulin secretion and/or action. Diabetes can be classified into two major subtypes. Type 1 diabetes, also known as juvenile-onset diabetes (previously referred to as insulin-dependent diabetes mellitus or IDDM), is due to the autoimmune destruction of pancreatic β cells resulting in the inability of the pancreas to produce insulin. Type 1 diabetes can be caused by autoimmune, genetic and/or environmental factors and accounts for 5-10% of all reported cases in the Western world. It usually develops before the age of 40 with most cases presenting before the age of 20. In contrast, type 2 diabetes or adult-onset diabetes (previously referred to as non-insulin-dependent diabetes mellitus or NIDDM) accounts for over 90% of the reported diabetic cases reported in the Western world. In general, individuals suffering from type 2 diabetes produce sufficient amounts of insulin although their bodies cannot use it effectively. Genetic predisposition and environmental factors contribute to its development and risk factors include obesity, physical inactivity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance and race/ethnicity. There are other types of diabetes that are much less frequent and they include gestational diabetes, drug-induced diabetes and diabetes secondary to illness or infection (3).

A total of 15.7 million Americans have been reported by the U.S. Centers for Disease Control and Prevention to have diabetes and 13.4 million have impaired glucose tolerance including insulin resistance syndrome. The World Health Organization reported that there were 154.4 million diabetics worldwide in the year 2000. There is no indication that prevalence of the disease is stabilizing, and the World Health Organization predicts that there will be approximately 300 million individuals with diabetes by the year 2005 (3).

Several drugs are available for the treatment of diabetes mellitus. These include various insulin formulations (*e.g.*, very short-acting, short-acting, intermediate-acting, long-acting and biphasic insulins) in addition to biguanides, sulfonylureas, α-glucosidase inhibitors, insulin sensitizers and insulin secretagogues. However, the search for new agents is ongoing. Those agents which enhance insulin secretion and are available or under development for the treatment of type 2 diabetes are shown in Table I (3).

One novel class of antidiabetic agents are inhibitors of the regulatory enzyme, dipeptidyl peptidase (DPPIV; CD26; EC 3.4.14.5) that is involved in signal transduction processes occurring during the immune responses leading to development of type 2 diabetes. DPPIV was first

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Scheme 1: Synthesis of P32/98

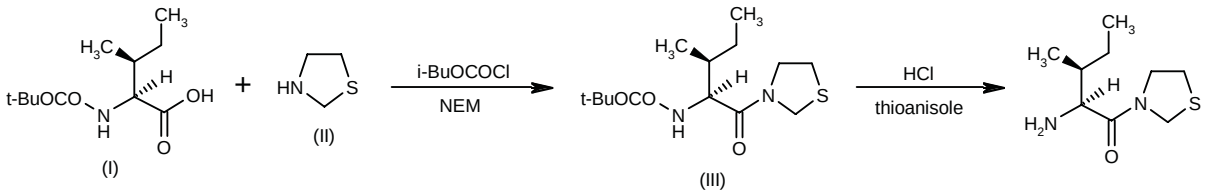
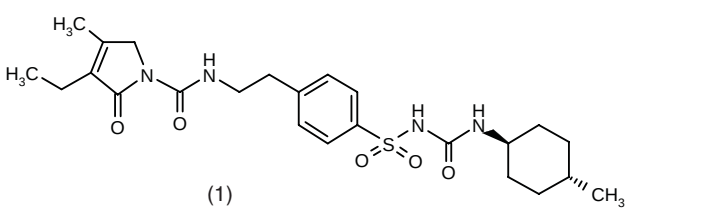


Table I: Drugs that enhance insulin secretion for type 2 diabetes (Prous Science Drug R&D Backgrounders database).

Drug Name	Company	Description	Status
1. Glimepiride (*Amaryl*)	Aventis	Sulfonylurea	Launched-1995
2. Repaglinide (*NovoNorm*, *Prandin*)	Boehringer Ingelheim/Novo Nordisk	Postprandial glucose regulator	Launched-1998
3. Nateglinide (*Fastic*, *Starlix*)	Ajinomoto/Novartis/Merck KGaA	Postprandial glucose regulator	Launched-1999
4. Mitiglinide calcium hydrate	Kissei/Servier	Postprandial glucose regulator	Phase III
5. AC-2993+	Amylin	GLP-1 analogue	Phase II
6. Betatropin	Restoragen	GLP-1	Phase II
7. LAF-237+	Novartis	DPP IV inhibitor	Phase II
8. NN-2211	Novo Nordisk	GLP-1 derivative	Phase II
9. NVP-DPP-728	Novartis	DPP IV inhibitor	Phase II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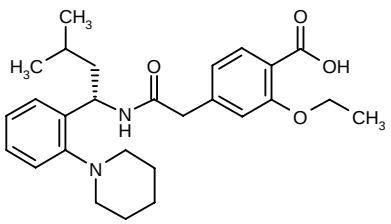
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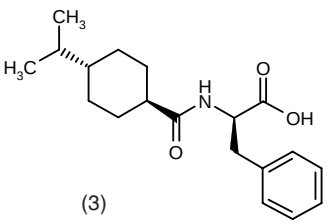
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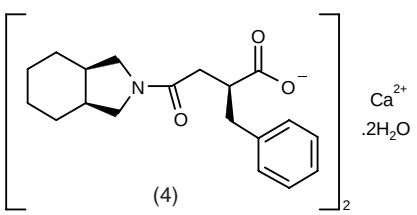
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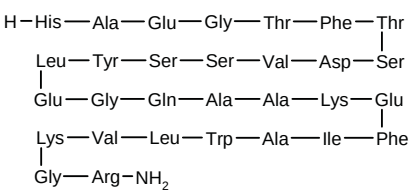

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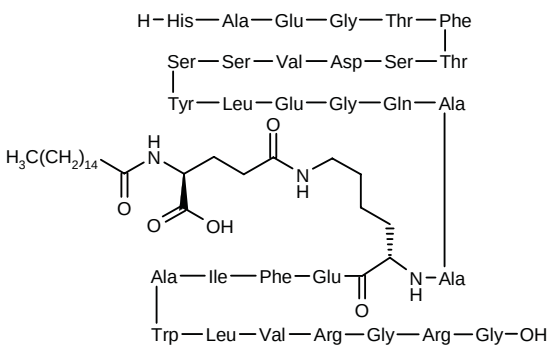

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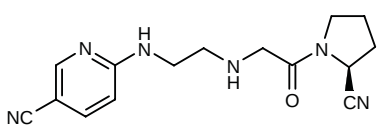
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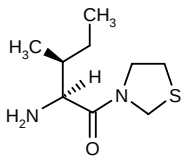
Table I (Cont.): Drugs that enhance insulin secretion for type 2 diabetes (Prous Science Drug R&D Backgrounders database).

Drug Name	Company	Description	Status
10. P32/98	Probiodrug/Merck & Co.	DPP IV inhibitor	Phase II
11. JTT-608	Japan Tobacco	Postprandial glucose regulator	Phase II
12. BIM-51077+	Biomeasure/Beaufour-Ipsen	GLP-1 analogue	Preclinical
13. Ro-27-4375+	Roche	Glucokinase activator	Preclinical
14. Ro-28-1675+	Roche	Glucokinase activator	Preclinical
15. SDZ-272-070	Novartis	DPP IV inhibitor	Preclinical
16. Aib(8,35)hGLP-1(7-37)NH_2	SCRAS	GLP-1 analogue	Biological Testing
17. AZM-134+	Alizyme	Low-molecular-weight GLP-1 mimetic	Biological Testing
18. MITO-2915+	MitoKor	Modulator of mitochondrial metabolism	Biological Testing



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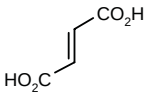

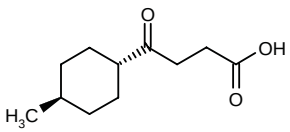

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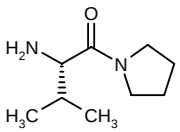

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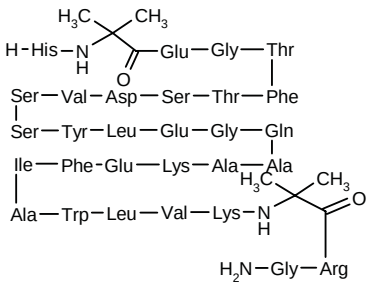

(16)

+Structure not yet detected

described in 1967 and is present in many human tissues (4-6). It is a serine protease existing as a dimer that specifically cleaves oligopeptides after the penultimate proline or alanine residue in the amino terminus. Circulating DPPIV rapidly inactivates 2 incretins: glucose-dependent insulinotropic polypeptide (GIP_{1-42}) and the truncated form of glucagon-like peptide-1 ($GLP\text{-}1_{7-36}$). These hormones are released from the gut in response to ingested nutrients. When blood glucose levels are elevated, incretins act on the pancreas to potentiate glucose-induced insulin secretion. Hydrolysis of GIP_{1-42} and $GLP\text{-}1_{7-36}$ by DPPIV yields the amino-terminally truncated forms of the incretins, GIP_{3-42} and $GLP\text{-}1_{9-36}$, which are not insulinotropic (3, 7, 8). Thus, it was speculated that suppression of DPPIV would result in enhancement of GIP and GLP-1 activity leading to improvement in glucose tolerance. As a result, DPPIV has become a particularly attractive target for antidiabetic therapy. DPPIV inhibitors have a significant advantage over GLP-1 and its analogues in that they are smaller molecules and therefore are potentially orally available. One such agent, P32/98 (Ile-thiazolidide), a highly specific, reversible, competitive, transition-state analogue inhibitor of DPPIV (K_i = 0.126 μM for DPPIV isolated from pig kidney; IC_{50} = 2.8 ± 0.2 μmol/l for human lymphocyte DPPIV) (1, 9, 10) has been selected for further development as an agent that has the potential to improve glucose tolerance and therefore be advantageous in the management of type 2 diabetes.

Pharmacological Actions

The efficacy of P32/98 in inhibiting DPPIV and improving glucose tolerance has been demonstrated in a number of preclinical *in vivo* studies conducted in rats.

A study using overnight fasted Wistar rats administered [^{125}I]-$GLP\text{-}1_{7-36}$ (about 1 mCi/pmol i.v. to achieve circulating levels of 50-100 pmol/l), showed that P32/98 (1.5 μmol loading dose followed by 0.75 μmol/min infusion for 30 min i.v. starting 20 min before [^{125}I]-$GLP\text{-}1_{7-36}$) treatment inhibited endogenous DPPIV by about 70%, resulting in the presence of 90% of the intact labeled hormone at 5 min after injection as compared to only 13.4% observed at 2 min in controls. When rats were administered intraduodenal (i.d.) glucose (1 g/kg bolus at time 0; 50% dextrose) to induce endogenous incretin release, P32/98 treatment suppressed plasma DPPIV activity maximally by 71.4 ± 2.2% at 30 min, with marked inhibition of 59.9 ± 14.4% still observed at 90 min. Moreover, the circulating half-life of intact $GLP\text{-}1_{7-36}$ released in response to i.d. glucose in treated animals was increased. Although both P32/98-treated and untreated animals displayed similar peak insulin levels (477 ± 153 pmol/l and 433.2 ± 87.6 pmol/l, respectively) in response to i.d. glucose, peaks were reached earlier in treated animals (20 *vs.* 30 min) and plasma glucose levels starting at 45 min were significantly lower as compared to controls (11).

Other *in vivo* studies using male Wistar rats demonstrated that administration of P32/98 (10-100 μmol/300 g in a 40% glucose solution p.o.) improved glucose tolerance. P32/98 significantly and dose-dependently decreased plasma DPPIV-activity (55 ± 3 and 88 ± 2% with 10 and 100 μmol/300 g, respectively, *vs.* 14 ± 3% in controls); P32/98 doses of 2.5 and 7.5 μmol/300 g also decreased DPPIV activity but inhibition did not reach significance (34 ± 4 and 42 ± 6%) . P32/98 at a dose of 5 μmol/300 g was found to induce significantly earlier (10 *vs.* 13 ± 4 min) and higher peak (32 ± 10 *vs.* 16 ± 2 ng/l)

plasma insulin levels in addition to lower glucose AUC_{0-60} min values as compared to controls; treatment with this dose also slightly reduced C-peptide AUC values (168 ± 36 *vs.* 228 ± 46 nmol·min/l). When P32/98 (2.5 μmol/300 g in NaCl p.o.) was administered prior (0-60 min) to oral glucose loads (2 g/kg), a significant improvement in glucose AUC values (mmol·min/l) was seen at 10 (90 ± 21), 20 (77 ± 16) and 40 (71 ± 20) min as compared to 0 (135 ± 16), 5 (108 ± 13) and 60 (107 ± 29) min, thus indicating improved glucose tolerance with treatment (12).

P32/98 (25 mg/kg p.o.) was shown to improve glucose tolerance in studies using diabetic Zucker fatty rats when treatment was initiated at 9 weeks of age and continued until 18 weeks of age. Treatment was associated with an increase in circulating plasma insulin concentrations. When P32/98 treatment was delayed until animals were 12 or 15 weeks and diabetes was more advanced, improvements in glucose tolerance continued to be observed (13).

Further efficacy was demonstrated for chronic P32/98 dosing in studies using diabetic Zucker fatty rats. In a study using 20-week old animals, oral administration of P32/98 (4.43 or 21.61 mg/kg) or a P32/98 prodrug (7.63 mg/kg) for 21 days, resulted in a decrease in morning blood glucose levels after only 7 days of treatment as compared to untreated fatty controls (4.7 ± 0.3 , 5.4 ± 0.7 and 5.6 ± 0.2 mmol/l for the two P32/98 doses and prodrug, respectively, *vs.* 6.8 ± 0.7 and 5 ± 0.1 mmol/l in fatty and lean controls, respectively); day-night blood glucose profiles also decreased in treated animals. In addition, HbA1c levels on day 21 were reduced with P32/98 treatment. Treatment did not alter the weights of the small and large bowel which were larger in fatty rats as compared to lean rats (14).

Chronic, 21-day treatment of diabetic (hyperglycemic and hyperinsulinemic) obese Zucker rats with P32/98 (21.61 mg/kg p.o. once daily at 17:00 h) was shown to improve the metabolic control of diabetes. A study comparing the effects of P32/98 with glibenclamide (1 mg/kg p.o. once daily at 17:00 h) reported that by day 5, while blood glucose levels were elevated to 10 mM in animals treated with glibenclamide, P32/98-treated animals displayed significantly normalized levels (5.2 ± 0.3 mM). Examination of 24-h profiles revealed that glycemia peaks were significantly less in P32/98-treated animals (8.3 ± 0.5 mM) as compared to the control (12.2 ± 1.3 mM) and glibenclamide (14.8 ± 1.5 mM) groups. P32/98 treatment also resulted in an earlier increase in peak insulin levels and total daily plasma insulin levels were markedly lower as compared to control and glibenclamide-treated animals, indicating improved insulin sensitivity. Both P32/98 and glibenclamide treatment caused significant reductions in HbA1c from day 0 to 14 (from 1.55 ± 0.10 to 1.04 ±0.06% for P32/98; from 1.55 ± 0.06 to 1.28 ± 0.15% for glibenclamide) as compared to controls (from 1.63 ± 0.06 to 1.7 ± 0.24%); the reduction in HbA1c levels in both treatment groups was maintained for 7 days after the discontinuation of treatment (15).

The improvements in glucose tolerance, insulin sensitivity and hyperinsulinemia observed with P32/98 treatment (10 mg/kg b.i.d. p.o.) in fatty Zucker rats were found to be sustained with long-term administration (100 days). Treatment resulted in significant reductions in fasting (5.8 ± 0.2 *vs.* 7.8 ± 0.2 mM) and peak (12 ± 0.7 *vs.* 20.4 ± 2.4 mM) blood glucose levels as compared to controls with no changes in plasma insulin levels. Results from examination of 24-h profiles showed that treatment decreased blood glucose excursion to 67.5% of the controls which was concomitant with a lower plasma insulin profile (about 79% of controls). No alterations in caloric intake were observed with treatment (16).

Clinical Studies

The safety and tolerability of P32/98 were demonstrated in a randomized, double-blind, placebo-controlled study conducted in 36 healthy male volunteers who received 2 single ascending doses (7.5, 15, 30, 60, 120 and 240 mg p.o.) of the agent 10 min prior to a standard oral glucose tolerance test (OGTT). P32/98 was well tolerated. An improvement in glucose tolerance of more than 50% was observed in subjects treated with the highest dose. Moreover, drug-dependent reductions in plasma DPPIV activity and concomitant increases in bioactive GLP-1 plasma levels were also observed (17) (Box 1).

The safety and efficacy of P32/98 were also demonstrated in a single-dose, open trial conducted in 24

Box 1: P32/98 in the modulation of diabetes mellitus (17) [Prous Science Integrity database].

Design	Randomized, double-blind, placebo-controlled clinical study
Population	Healthy volunteers (n = 36); patients with diabetes (n = 24)
Treatments	P32/98, 15 mg p.o. s.d. → 30 mg p.o. s.d. → 60 mg p.o. s.d. → 120 mg p.o. s.d. → 240 mg p.o. s.d. → 480 mg p.o. s.d (n = 18) Placebo (n = 18) P32/98, 60 mg p.o. s.d. (n = 24)
Results	Glucose tolerance in healthy volunteers, change @ 15 min: P480 (>50%) Glucose tolerance in diabetics, change @ 15 min: P60 (33%)
Conclusions	P32/98 improved glucose tolerance in patients with diabetes and in healthy volunteers

Box 2: P32/98 inhibitors in type 2 diabetes (18) [Prous Science Integrity database].

Design	Open, crossover clinical study
Population	Patients with type 2 diabetes receiving P32/98 prior to an OGTT (75 g) after overnight fasting (n = 24)
Treatments	P32/98, 60 mg p.o. s.d. Placebo
Results	Acute glucose tolerance improvement in patients already on metformin (%): P32/98 (7) on diet (%): P32/98 (22) on acarbose or glybenclamide (%): P32/98 (33) Patients with elevated insulin response of 150% (%): P32/98 (100)
Conclusions	P32/98 improved postprandial blood glucose tolerance in type 2 diabetes

patients with type 2 diabetes. Patients were first administered an OGTT following a 12-h washout of previous medications and an overnight fast. Seven days later and following another 12-h washout period from previous medication and overnight fast, patients received one P32/98 tablet (60 mg p.o.) and were subjected to an OGTT 15 min later. Treatment was well tolerated. Although those patients previously on regular metformin treatment were the least effective responders, displaying only a 7% improvement in glucose tolerance, they exhibited the highest insulin and proinsulin secretion levels during P32/98 treatment. In contrast, those patients previously on a controlled diet or regular acarbose or glibenclamide displayed improvements in glucose tolerance between 22-33% after single-dose P32/98. A 5.8-fold increase in blood bioactive GLP-1(7-36) and an increase in insulin response of 150% were also seen in all patients (17, 18) (Box 2).

P32/98 is currently undergoing phase II trials for the treatment of type 2 diabetes (19).

Manufacturer

Probiodrug GmbH (DE); licensed to Merck & Co., Inc. (US).

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