

Mylan Pharmaceuticals Inc.,
Wockhardt Bio AG, Teva Pharmaceuticals USA, Inc.,
Aurobindo Pharma U.S.A. Inc., and Sun Pharmaceutical
Industries, Ltd., Sun Pharma Global FZE and
Amneal Pharmaceuticals LLC,
Petitioners

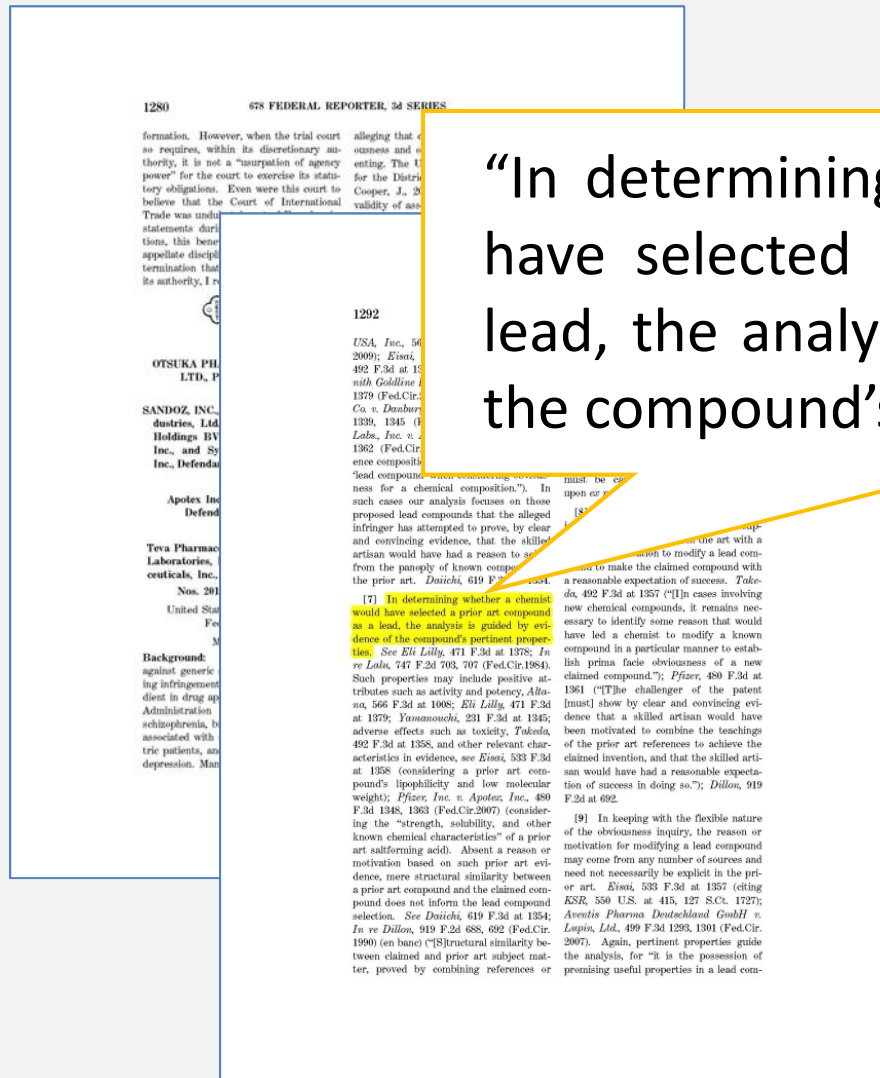
v.

AstraZeneca AB,
Patent Owner

IPR2015-01340
US RE44,186 E

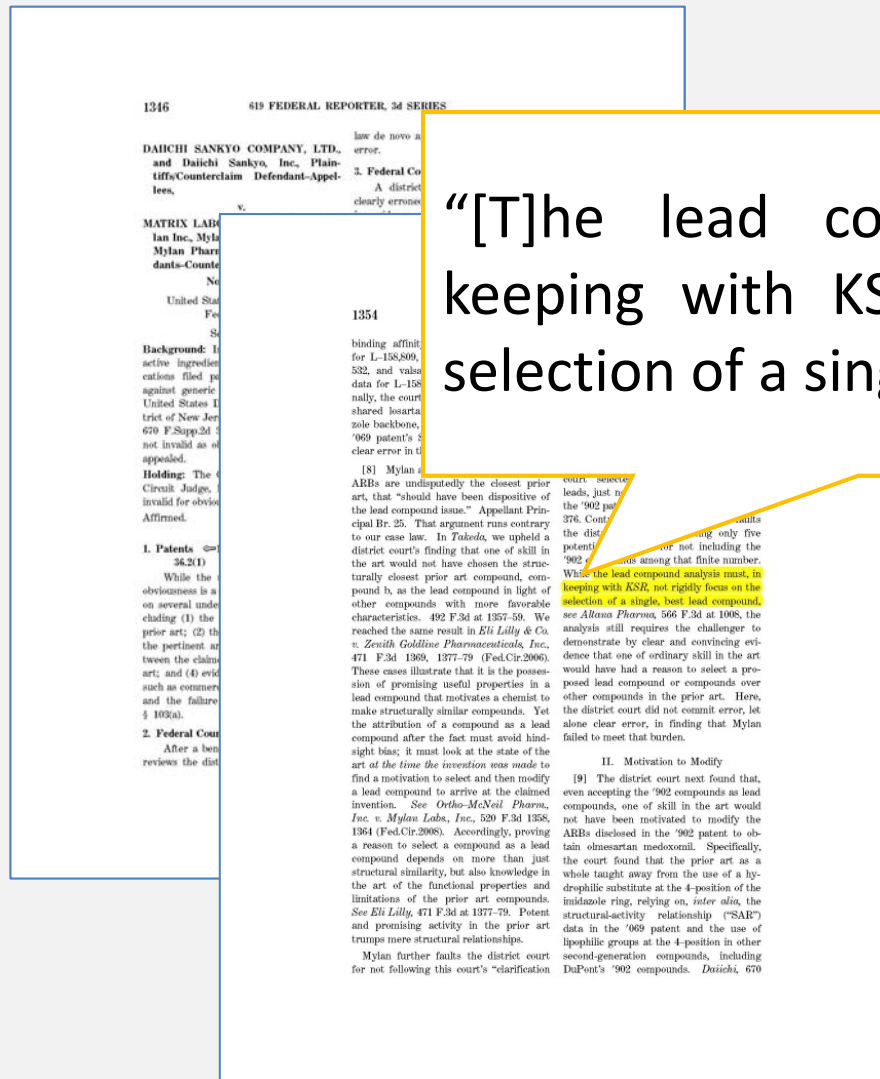
January 25, 2017

Otsuka v. Sandoz, 678 F.3d 1280, 1292 (Fed. Cir. 2012)



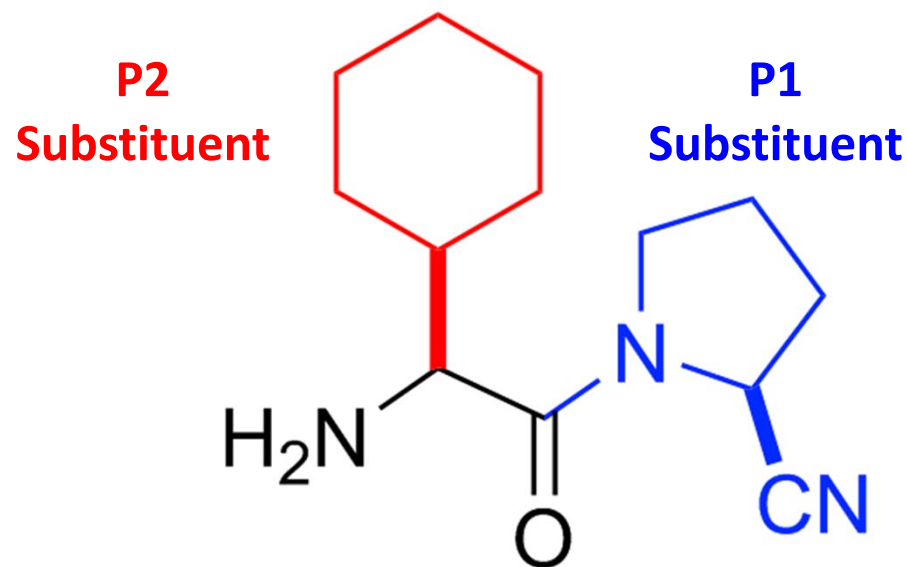
"In determining whether a chemist would have selected a prior art compound as a lead, the analysis is guided by evidence of the compound's pertinent properties."

Daiichi Sankyo v. Matrix Labs., 616 F.3d 1346, 1354 (Fed. Cir. 2010)



“[T]he lead compound analysis must, in keeping with KSR, not rigidly focus on the selection of a single, best lead compound . . .”


Ashworth 25 is a Pertinent Lead Compound



Ashworth 25

- ✓ **Potency:** $K_i < 2 \text{ nM}$
- ✓ **Solution Stability:** $t_{1/2} > 48 \text{ hours}$

Ashworth 25 Has Good Potency and Stability

 Pergamon
Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 16, pp. 1163-1166, 1996
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 0960-894X/96 \$12.00 + 0.00
 PII: S0960-894X(96)00150-4

2-CYANOPYRROLIDIDES AS POTENT, STABLE INHIBITORS OF DIPEPTIDYL PEPTIDASE IV

Doreen M. Ashworth, M.A.

Ferring Research Institute
 S016

1166 D. M. ASHWORTH *et al.*

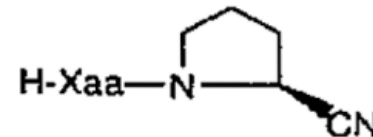
Table II. Dipeptide amines: Potency versus human DP-IV and stability in aqueous solution (pH 7.4).

Compound N ^o	Xaa	K _i (nM) ¹³	t _{1/2} (h) ¹⁹
24	Cpg	1.1 ± 0.2	48
25	Chg	1.4 ± 0.5	>48
26	Ile	2.2 ± 0.5	48
27	Tbg	3.8 ± 0.8	>48
28	Lys(Z)	5.2 ± 1.0	24
29	Pro	22.0 ± 4.0	7.5

These compounds were found to be non-toxic in T cell assays up to 72h and inhibitor 26 had no toxicity when injected into mice (up to 10mg/kg). Compound 25 inhibited proliferation and cytokine release in human T lymphocytes (e.g. proliferation and cytokine release).

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 6) Shilling, F.; Jung, D.; Rasmussen, D.; Ulfert, A.; Buhling, K.; Jankov, O.; Rasmussen, D.; Neukirch, W.; Hübner, B. *Biochemistry Today* 1994, 15, 110.
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 19) The stability of the inhibitors in buffered, aqueous solutions was determined by incubating a solution of the inhibitor (10 μM) in buffered, aqueous solution (pH 7.4) at 37°C. The concentration of the inhibitor was determined by HPLC-MS.

(Received in Belgium 21 February 1996; accepted 13 March 1996)



Compound N ^o	Xaa	K _i (nM) ¹³	t _{1/2} (h) ¹⁹
24	Cpg	1.1 ± 0.2	48
25	Chg	1.4 ± 0.5	>48
26	Ile	2.2 ± 0.5	48
27	Tbg	3.8 ± 0.8	>48
28	Lys(Z)	5.2 ± 1.0	24
29	Pro	22.0 ± 4.0	7.5

Measuring Potency by K_i and IC_{50} Values

Biochemical Pharmacology, Vol. 22, pp. 3099-3108, Pergamon Press, 1973. Printed in Great Britain.

RELATIONSHIP BETWEEN THE INHIBITION CONSTANT (K_i) AND THE CONCENTRATION OF INHIBITOR WHICH CAUSES 50 PER CENT INHIBITION (I_{50}) OF AN ENZYMATIC REACTION*

YUNG-CHI CHENG and WILLIAM H. PRUSOFF

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. 06510, U.S.A.

(Received 15 March 1973; accepted 27 April 1973)

Abstract—A theoretical analysis has been made of the relationship between the inhibition constant (K_i) of a substance and the (I_{50}) value which expresses the concentration of inhibitor required to produce 50 per cent inhibition of an enzymic reaction at a specific substrate concentration. A comparison has been made of the relationships between K_i and I_{50} for monosubstrate reactions when noncompetitive or uncompetitive inhibition kinetics apply, as well as for bisubstrate reactions under conditions of competitive, noncompetitive and uncompetitive inhibition kinetics. Precautions are indicated against the indiscriminate use of I_{50} values in agreement with the author's previous work. The analysis described shows K_i does not equal I_{50} when competitive inhibition kinetics apply; however, K_i is equal to I_{50} under conditions of either noncompetitive or uncompetitive kinetics.

MANY DRUGS are believed to exert their biological effect as a consequence of competitive inhibition. One approach to the understanding of the mechanism of action of drugs has been to study the effect of drug concentration on the activity of an isolated enzyme. Several approaches have been used.

One approach is to measure the concentration of inhibitor producing 50 per cent inhibition such as I_{50} (concentration of inhibitor producing 50 per cent inhibition), $(I/S)_{50}$ (concentration of inhibitor relative to substrate concentration at 50 per cent inhibition), and K_i (the dissociation constant of the enzyme-inhibitor complex, or the reciprocal of the binding affinity of the inhibitor to the enzyme).

Although the relationship between the inhibition constant (K_i) and I_{50} of a competitive inhibitor is well known, a comparison of such values for noncompetitive or uncompetitive reactions when the inhibition is competitive. An understanding of the theoretical basis for the relationship between the experimental I_{50} and K_i values is essential. Although what is known is well known, those who are less familiar with the effect of drugs on enzymes should be aware of the following.

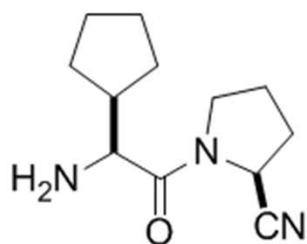
* This research was supported by the National Institutes of Health.

Smaller K_i and IC_{50} values represent greater potency

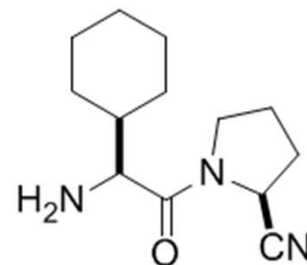
IC_{50} is the “concentration of inhibitor producing 50 per cent inhibition[.]”

K_i is the “dissociation constant of the enzyme-inhibitor complex, or the reciprocal of the binding affinity of the inhibitor to the enzyme[.]”

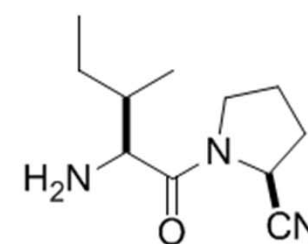
Ashworth I Table II Compounds



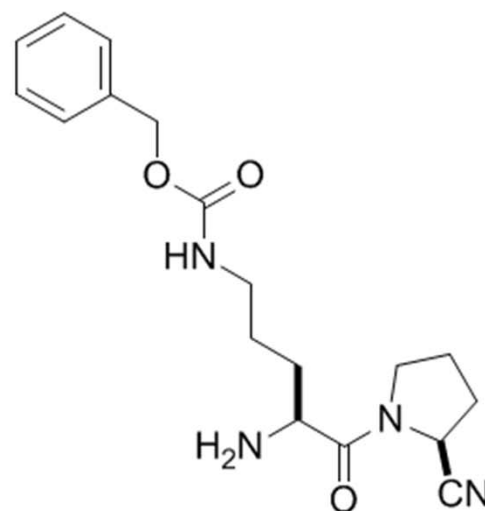
**Cpg Analogue
(Cyclopropylglycine)
Compound 24**



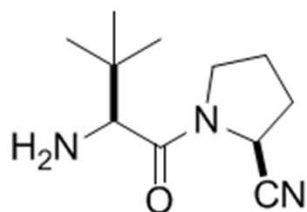
**Chg Analogue
(Cyclohexylglycine)
Compound 25**



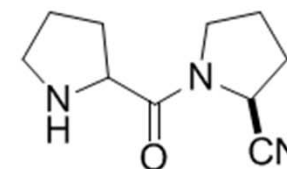
**Ile Analogue
(Isoleucine)
Compound 26**



**Lys(Z) Analogue
(Carboxybenzyl-lysine)
Compound 28**



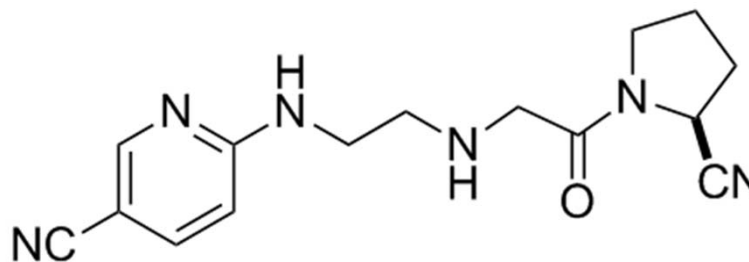
**Tbg Analogue
(Tert-butylglycine)
Compound 27**



**Pro Analogue
(Proline)
Compound 29**

Cyanopyrrolidines Were of Interest

NVP-DPP728, containing a cyanopyrrolidine, entered clinical trials in humans.



NVP-DPP728

UNITED
BEFOR

Case No. IPR2015-01340
Patent RE44,186

E. The most promising DPP-4 inhibitors were in the clinic

Of the various reported DPP-4 inhibitors in the prior art, only two had entered the clinic for evaluation in humans: NVP-DPP728 and P32-98. Ex. 2056, ¶¶88, 143; Ex. 2057, ¶¶40-41. Because of the available data and ongoing clinical trials, these two DPP-4 inhibitors were recognized as the most promising compounds at the time. Ex. 2056, ¶¶154-159.

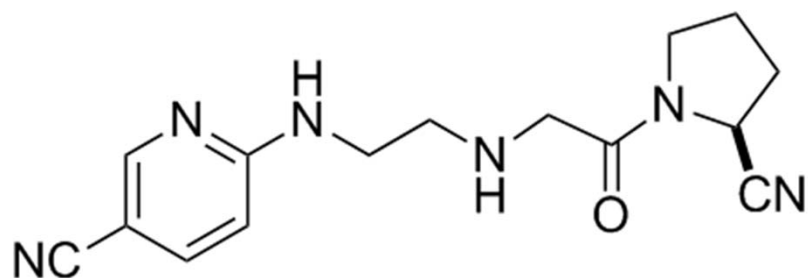
1. Novartis's first clinical trial candidate NVP-DPP728

By the time of the invention, Novartis had selected NVP-DPP728 as a clinical candidate, and it was reported to be safe and effective in initial studies in humans. Ex. 2056, ¶88. Specifically, in a phase 1 clinical trial, NVP-DPP728 increased prandial active GLP-1 levels and reduced prandial glucose excursion without causing low blood sugar ("hypoglycemia") or causing serious adverse events after a single dose of 100 mg in healthy volunteers. Ex. 2056, ¶¶12, 2; Ex. 2056, ¶88; Ex. 2057, ¶41. These data "support[ed] the... glucose-lowering potential of NVP-DPP728 for the treatment... indicated to a person of ordinary skill in the art ("POSA") appeared safe and effective in initial studies in humans. Ex. 2056, ¶156.

After the time of invention, Novartis discontinued NVP-DPP728... was found to have a short half-life *in vivo* and progressed another...

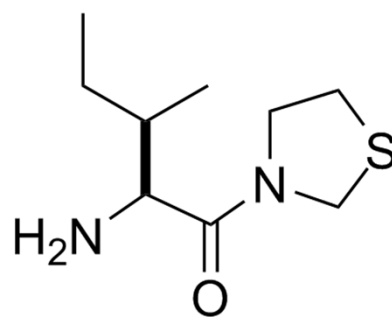
"By the time of the invention, Novartis had selected NVP-DPP728 as a clinical candidate, and it was reported to be safe and effective in initial studies in humans."

Ashworth 25 More Potent than Other Clinical Candidates



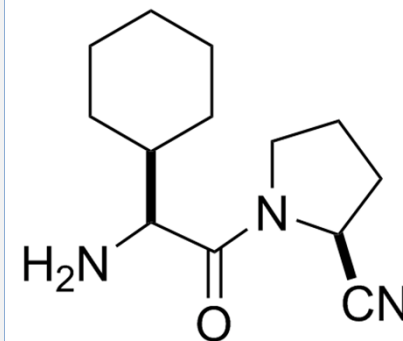
NVP-DPP728

$K_i = 11 \text{ nM}$



P32/98

$IC_{50} = 2800 \text{ nM}$



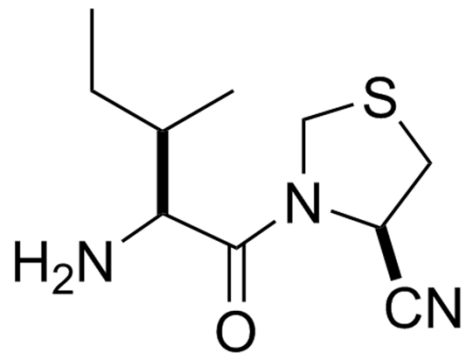
Ashworth 25

$K_i = 1.4 \text{ nM}$

Dr. Weber concedes that “compound 25 of Ashworth-I [is] more potent than the clinical candidates NVP-DPP728 and P32/98[.]”

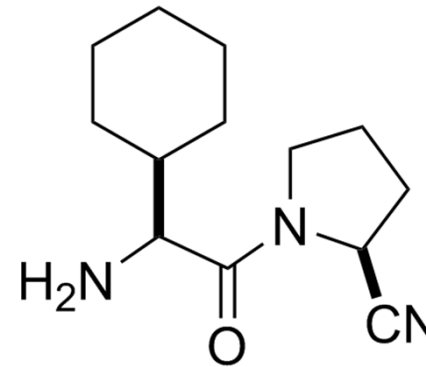
Source: EX1074 (Second Rotella Decl.), ¶11; EX2016 (Hughes) at 11600; EX1007 (Ashworth I) at Table 2, 1166; EX2078 (Schon) at 308; EX2056 (Weber Decl.), ¶172 .

Superior Stability of Ashworth 25



Ashworth II compound 3

$t_{1/2} = 27 \text{ h}$



Ashworth I compound 25

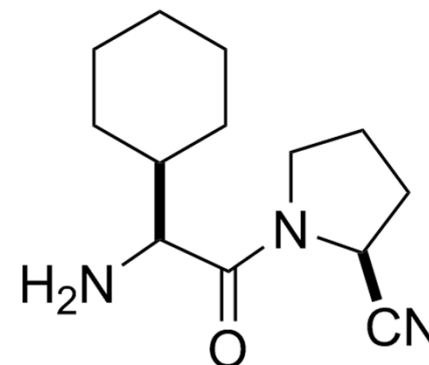
$t_{1/2} = >48 \text{ h}$

Ashworth 25 has greater *in vitro* stability (longer half-life, $t_{1/2}$) than compound 3 from Ashworth II.

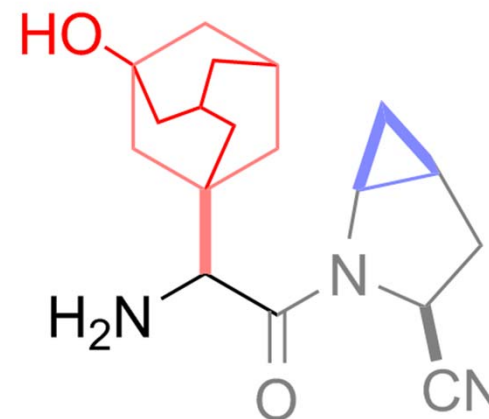
Summary of Structural Differences

- **Cyclopropanation of the pyrrolidine ring**
 - EX1007: Ashworth I
 - EX1010: Hanessian
- **Replace cyclohexyl ring with hydroxyadamantyl**
 - EX1007: Ashworth I
 - EX1008: Villhauer WO 98
 - EX1009: Raag

Ashworth 25

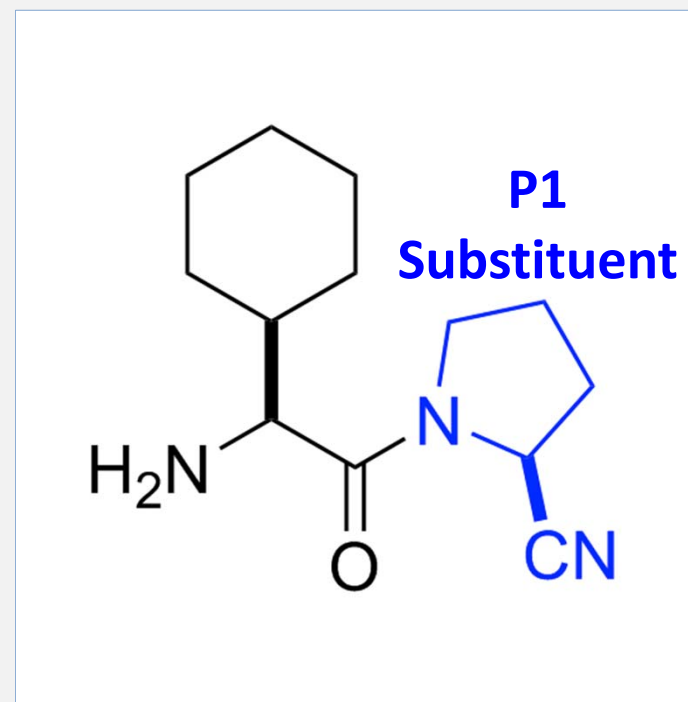


Saxagliptin



Petitioner's Motivation to Modify P1

- **Cyclopropanation**
 - flattens and rigidifies pyrrolidine ring
 - modulates cyano position
- **Optimizes interaction with DPP-4 enzyme to improve activity and stability**



Cyclopropanation Modulates Proline Conformation

“The structures and conformations of **6** and **8** in the solid state were unambiguously confirmed by single-crystal X-ray analysis. Table 1 lists selected torsion angles for compounds **6** and **8**, where **considerable ‘flattening’ of the pyrrolidine ring** is observed relative to *N*-Boc-L-proline [.]”

oides in length. In this “evolution” was done on this region 30% mutagenesis, and four more rounds of *in vitro* selection followed before the second population was cloned. From the sequence, a consensus region was discovered. Certainly then, this work is a pioneering article of how the concept involved than that presented in summary, a novel design to take advantage of that has multiple copies of condensed the many copies of than two days. This was a showed that the new method of copies of individual a consistently selecting the best per selection round, and off sequence.

Since only the original try the screenings, the technique *in vitro* selection of modified could not undergo this process should significantly increase method and is the direction

COMMU

described¹¹¹, phorene¹¹² the consequence variation and of pairs each system. We describe diastereomeric glycosidic acid, action of inter other congeners.

Treatment

hexamethylphosphoramide (HMPA) (10 mL) and ethyl acetate (10 mL) were added to the reaction mixture. The mixture was stirred for 2 h at room temperature. The mixture was then extracted with ethyl acetate (10 mL) and the organic phase was dried over anhydrous sodium sulfate. The solvent was removed by evaporation under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 1/1 v/v) to give compound 10 (1.5 g, 40% yield).

Characterization

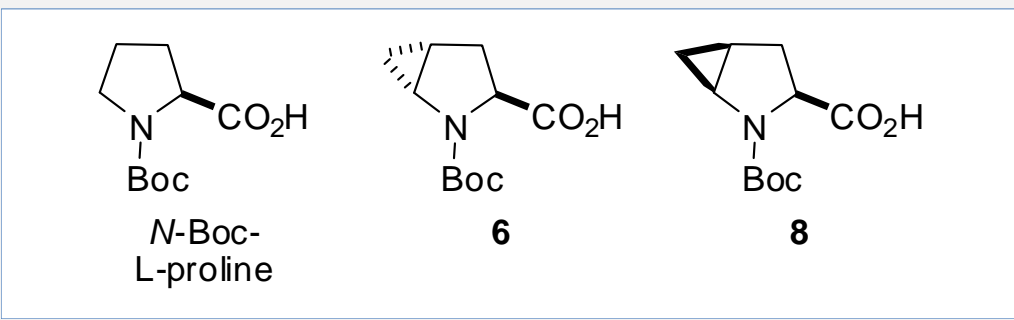
mp: 105–106 °C (lit.¹¹¹ 105–106 °C). ¹H NMR (CDCl₃, δ): 7.2–7.4 (m, 2H, aromatic), 6.8–7.0 (m, 2H, aromatic), 5.8–6.0 (m, 2H, aromatic), 4.5–5.0 (m, 2H, aromatic), 3.5–4.0 (m, 2H, aromatic), 2.0–2.5 (m, 2H, aromatic), 1.0–1.5 (m, 2H, aromatic). IR (KBr): 3400 (broad, OH), 1700 (C=O), 1600 (C=C), 1500 (C=C), 1400 (C=C), 1300 (C=C), 1200 (C=C), 1100 (C=C), 1000 (C=C), 900 (C=C), 800 (C=C), 700 (C=C), 600 (C=C), 500 (C=C). MS (ESI): m/z 200 (M⁺), 220 (M⁺), 240 (M⁺).

Crystallographic Data

Crystal size: 0.5 × 0.5 × 0.5 mm³. Wavelength: Cu Kα (1.5418 Å). Temperature: 100 K. Space group: P2₁. Unit cell dimensions: a = 10.112(4) Å, b = 10.112(4) Å, c = 10.112(4) Å. Volume: 1011.2(4) Å³. Z: 4. Density: 1.213 g/cm³. μ: 0.089 mm⁻¹. R_{int}: 0.028. R₁: 0.028. wR₂: 0.032. S: 1.0. Extinction: none. Goodness-of-fit on I²: 1.000. ORTEP-3 for Windows. Data collection: Bruker AXS. Refinement: Bruker AXS. Software: Bruker AXS.

Keywords: aptamers • conformation • nucleic acids • polymers

© 1997 John Wiley & Sons, Ltd. *J. Chem. Soc. Chem. Commun.* 1997, 1135–1136. DOI: 10.1039/9700001135



Cyclopropanation Confers Conformational Rigidity

Page 1

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

---oO---

ASTRAZENECA AB, Case Number
Plaintiff, 14-664-GMS

vs

AUROBINDO PHARMA
AUROBINDO PHARMA
Defendant

VIDEOTAPED DEPOSITION

Reported by:
THOMAS J. FRASIK
RPR, CSR No. 6961
Job No: 2314618

Pages: 1 - 201

800-567-8658

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1 one would take. The first two had been covered fairly
2 extensively in the prior art, and so that left the
3 third, and that is to fuse an additional ring to the
4 mono-ring, the monocycling system.
5 Q. And where in the prior art do you find a
6 suggestion to fuse an additional ring to a pyrrolidine
7 ring of a DPP4 inhibitor?
8 A. Hanesian illustrated a method to
9 cyclopropanate the pyrrolidine—sorry, the pyrrolidine
10 ring system. That molecule was not studied as a DPP4
11 inhibitor, but it is obvious to one skilled in the art
12 of how one could convert that molecule into a DPP4
13 inhibitor or a DPP4 inhibitor fragment. And I'm
14 referring specifically to a Hanesian 1997 paper. And
15 as I mentioned, in fact, that was one of the papers that
16 was provided to me by my colleagues at Bristol Myers
17 when I started the project.
18 Q. Are you aware of anybody, other than your
19 colleagues at Bristol Myers, who had connected the
20 Hanesian cyclopropanation reaction to an Ashworth-type
21 DPP4 inhibitor before they did?
22 MS. STEINER: Objection to form.
23 THE WITNESS: I would have no way of knowing
24 that at the time I was there.
25 BY MR. LIPSEY:

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1 Q. But you, in the research that you've done for
2 this case, you haven't found any publication connecting
3 cyclopropanation to the modification of an Ashworth-type
4 DPP4 inhibitor other than the publications from your
5 colleagues at BMS, correct?
6 MS. STEINER: Objection to form.
7 THE WITNESS: To the best of my recollection,
8 yes.
9 BY MR. LIPSEY:
10 Q. Okay. And just to be clear, fusing a ring to
11 that pyrrolidine ring does involve replacing two of the
12 hydrogen atoms with something else; correct?
13 A. It does.
14 Q. Okay.
15 A. The difference is that when you fuse a ring,
16 when you fuse two rings together, especially when you
17 have a small ring but it's a difference in degree, that
18 introduces increased rigidity to the structure and can
19 alter the orientation in space of substituents attached
20 to what was previously the monocycling system.
21 That's just a consequence of molecular structure.
22 And in the process of that modification
23 of orientation in space, you may observe effects on
24 potency, you may observe effects on solution stability,
25 you may observe other effects on other properties that

Page 120

1 one might measure in connection with a drug
2 project.
3 Q. And those effects might be either positive
4 negative, correct?
5 A. They might be, yes.
6 Q. Now, let's take a peak at the 1997 Hanesian
7 publication to which you've referred. Let me
8 what's previously been marked for identification
9 Plaintiff's Deposition Exhibit 33.
10 (Previously marked Exhibit 33)
11 was shown to the witness
12 and is inserted hereto.)
13 BY MR. LIPSEY:
14 Q. Is that the 1997 Hanesian publication
15 you referred?
16 A. Yes, it is.
17 Q. And that's one of the publications you
18 about which you were at BMS, correct?
19 A. That's correct.
20 Q. And in the title here, Dr. Hanesian refers
21 to fusing of the pyrrolidine ring. Is that the—what
22 your view, a person of ordinary skill in the art
23 view as desirable in a DPP4 inhibitor?
24 A. That could be concluded. It's simply a
25 structural effect that they observed.

Page 121

1 Q. Well, what I'm trying to get to is a
2 important—is it important to your opinion that
3 cyclopropanation of Hanesian was said to be the
4 pyrrolidine ring?
5 A. Yes. And as I pointed out, since there
6 substituents on that ring, that flattening or flattening
7 that second ring will adjust the orientation in
8 of groups attached to the ring, and that adjustment
9 could prove to be beneficial.
10 Q. Now, there are other ways of cyclopropanating
11 could make it that pyrrolidine ring
12 flatten it, is that right?
13 A. There are other ways.
14 Q. Okay.
15 double bond.
16 yes.
17

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Page 31 of 92 800-567-8658

Q. Okay. And just to be clear, fusing a ring to that pyrrolidine ring does involve replacing two of the hydrogen atoms with something else; correct?

A. It does.

Q. Okay.

A. The difference is that when you fuse a ring, when you fuse two rings together, especially when you have a small ring but it's a difference in degree, that introduces increased rigidity to the structure and can alter the orientation in space of substituents attached to what was previously the monocycling structure. That's just a consequence of molecular structure.

Increase in Cyanopyrrolidine Stability Was Predictable

1 UNITED STATES DISTRICT COURT
2 FOR THE DISTRICT OF DELAWARE
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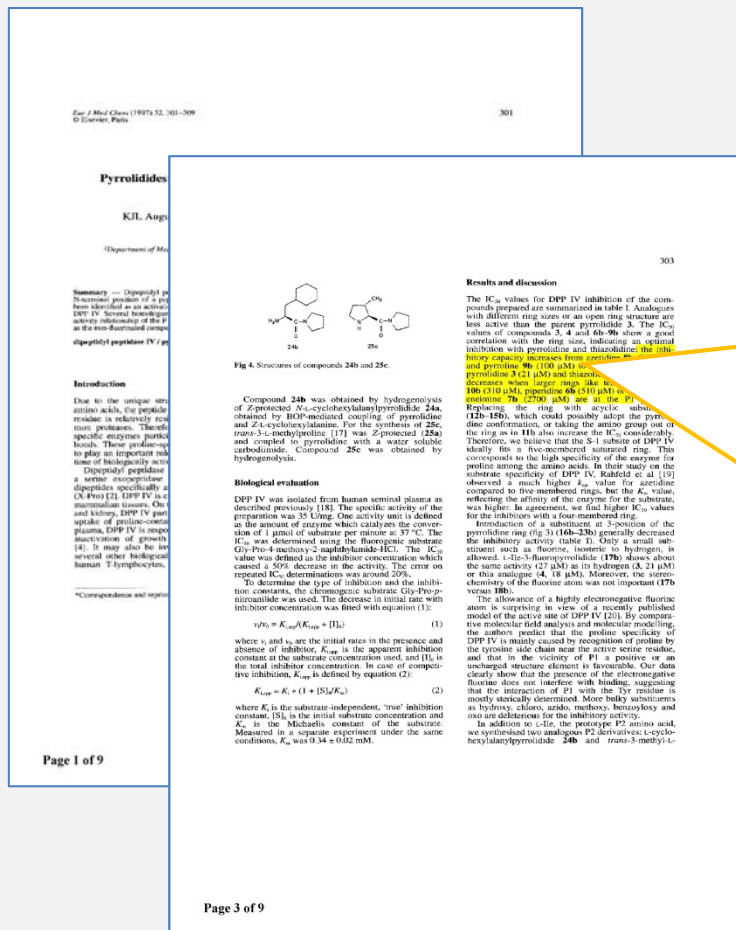
36 (Pages 138 - 141)

973-410-4040

And, in fact, if you turn to page 2591 and look at table 2 -- I'll save you, but you can certainly read this for yourself -- but these are theoretical calculations that predict that the stability of the 4, 5 methano -- sorry -- the 4, 5 cyclopropane CIS is going to be more stable compared to the simple cyanopyrrolidine.

Source: EX2174 (Rotella Depo. Trans.), 139:22-140:3; EX2002 (Magnin) at 2591.

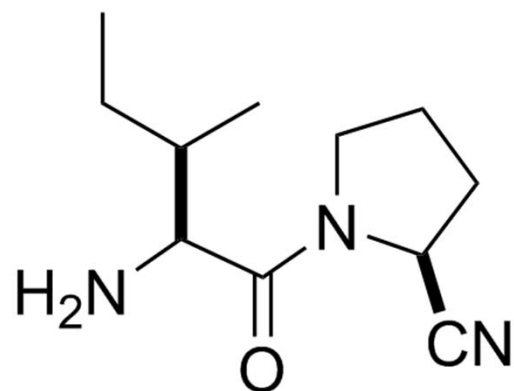
Patent Owner Cites Augustyns 1997



“[T]he inhibitory capacity increases from azetidine **8b** (270 μM) and pyrroline **9b** (100 μM) to the optimal five-rings pyrrolidine **3** (21 μM) and thiazolidine **4** (18 μM), and decreases when larger rings like tetrahydropyridine **10b** (310 μM), piperidine **6b** (510 μM) or hexamethyleneimine **7b** (2700 μM) are at the P1 position.”

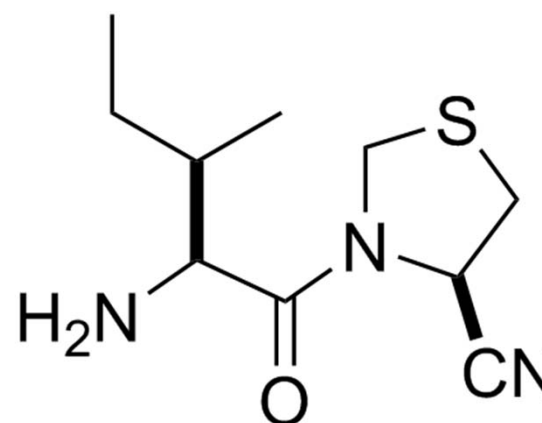
Patent Owner: “Increasing the pyrrolidine ring size to a 6- or 7-membered ring . . . was not well tolerated.”

Small Changes to P1 Ring Size Were Tolerated



Ashworth II compound 5

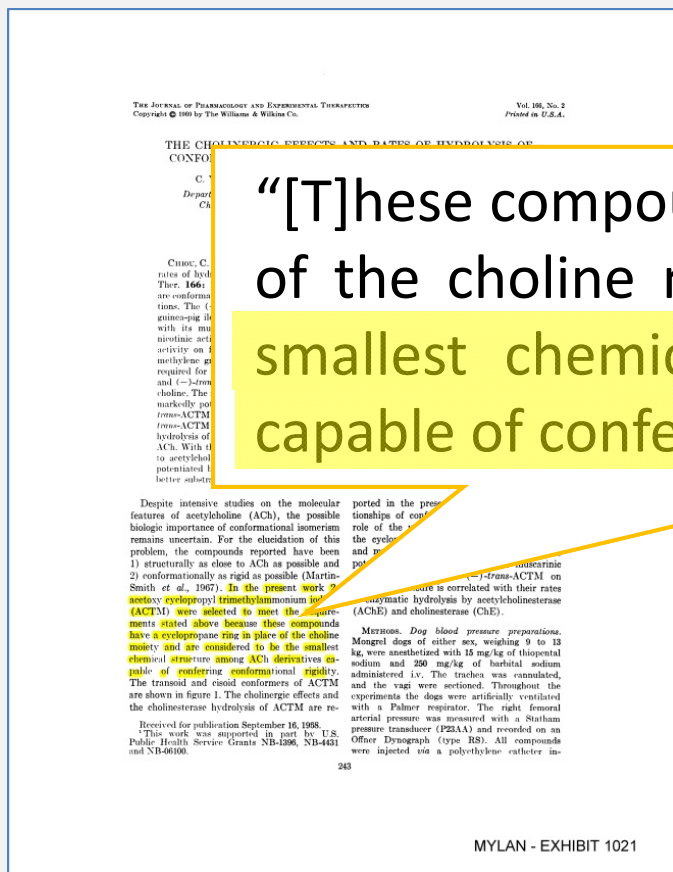
$K_i = 2.2 \text{ nM}$



Ashworth II compound 3

$K_i = 0.41 \text{ nM}$

Cyclopropanation Has Minimal Effect on Ring Size



"[T]hese compounds have a cyclopropane ring in place of the choline moiety and are considered to be the smallest chemical structure among Ach derivatives capable of conferring conformational rigidity."

Cyclopropanation Fits with 5-Membered Ring Preference

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Pyrrolidines: synthesis and structure-activity relationship as inhibitors of dipeptidyl peptidase IV

K.H. Augustyns¹, A.M. Lambrechts¹, M. Borloo¹, I. De Meester¹, I. Vedeckikova¹, G. Vanhooft¹, D. Hendriks¹, S. Scharpe¹, A. Haemers^{1*}

Summary
 N-acyl-pyrrolidines were synthesized from N-acyl-pyrrolidines. The activity of DPP IV was measured in the presence of dipeptidyl peptidase IV.

Introduction
 Due to the amino acid residue in the active site of DPP IV, the enzyme is highly specific for dipeptides. The to play an important role in the regulation of a wide range of biological processes (N-FoxO) [1].

Results and discussion
 The K_{i50} values for DPP IV inhibition of the compounds prepared are summarized in table 1. Analogues with different ring sizes or an open ring structure are less active than the parent pyrrolidine 3. The K_{i50} values of compounds 3, 4 and 6b-9b show a good correlation with the ring size, indicating an optimal inhibition with pyrrolidine and thiazolidine; the inhibitory capacity increases from acetyl-L-tyrosine 9b (270 μ M) and proline 9b (100 μ M) to the optimal five-rings proline 3 (21 μ M) and thiazolidine 4 (14 μ M), and decreases when larger rings like tetrahydropyridine 10b (10 μ M), piperidine 6b (510 μ M) or hexahydroindole 7b (2700 μ M) are at the P1 position. Replacing the ring with acyclic substances (12b-15b), which could possibly adjust the pyrrolidine conformation, or taking the amino group out of the ring as in 11b also increase the K_{i50} considerably. Therefore, we believe that the S-1 subsite of DPP IV ideally fits a five-membered saturated ring, corresponding to the high specificity of the enzyme for proline among the amino acids. In their study on the substrate specificity of DPP IV, Raftfield et al [19] observed a much higher K_{i50} value for acetyl-L-tyrosine compared to five-membered rings, but the K_{i50} value was higher. In agreement, we find higher K_{i50} values for the inhibitors with a four-membered ring.

Introduction of a substituent at 3-position of the pyrrolidine ring (6b-3) (10b-20b) generally decreased the inhibitory activity (table 1). Only a small substituent such as fluorine, isomer to hydrogen, is allowed. L-1c-3-fluoropyrrolidine (17b) shows about the same activity (27 μ M) as its hydrogen (3, 21 μ M) or this analogue (4, 18 μ M). Moreover, the stereochemistry of the fluorine atom was not important (17b versus 18b).

The absence of a highly electronegative fluorine atom is surprising in view of a recently published model of the active site of DPP IV [20]. By comparative molecular field analysis and molecular modeling, the authors predict that the proline specificity of DPP IV is mainly caused by recognition of proline by the tyrosine side chain near the active serine residue, and that in the vicinity of P1 a positive or an uncharged structure element is favourable. Our data clearly show that the presence of the electronegative fluorine does not interfere with binding, suggesting that the interaction of P1 with the Tyr residue is mostly sterically determined. More bulky substituents as hydroxy, chloro, azido, methyl, cyano, nitro and oxo are deleterious for the inhibitory activity.

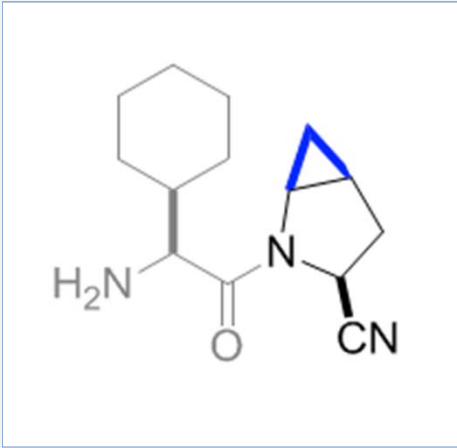
In addition to *cis*-2, the prolylase P2 active site, we synthesized two analogous P2 derivatives: L-cyclohexylalanine-pyrrolidine 24b and *trans*-3-methyl-L-

Fig 4. Structures of compounds 24b and 25c.

Compound 24b was obtained by hydrogenolysis of Z-protected N-(1-cyclohexylalanyl)pyrrolidine 24a, obtained by HOP-mediated coupling of pyrrolidine and Z-L-cyclohexylalanine. For the synthesis of 25c, *trans*-3-methylpyrrolidine (17) was Z-protected (24a) and coupled to pyrrolidine with a water soluble carbodiimide. Compound 25c was obtained by hydrogenolysis.

A POSA “would understand that cyclopropanation preserves the saturated five-membered ring, while also providing controlled modifications to the pyrrolidine conformation, as taught by Hanessian.”

“Therefore, we believe that the S-1 subsite of DPP IV ideally fits a five-membered saturated ring.”



Methods For Cyclopropanating Pyrrolidine were Known

COMMUNICATIONS

oxides in length. In vitro "evolution" was done on this region at 30% mutagenesis, and four more rounds of *in vitro* selection followed before the most successful population was cloned. From these sequences, a consensus region was discovered. Certainly through this work is a pioneering achievement in the field, it is an example of how the conventional protocol is significantly more involved than this procedure.

In summary, a novel approach to take advantage of the multiple copies condensed the early stages of these two days. This work showed that the new in vitro selection of the DNA library (H) is more efficient than the conventional method, and that the use of a multiple copy condensed the early stages of these two days. This work showed that the new in vitro selection of the DNA library (H) is more efficient than the conventional method, and that the use of a multiple copy condensed the early stages of these two days.

COMMUNICATIONS

described^{12,13} the 4,5-methanopyrrolidines are relatively unexplored.¹⁴ Furthermore, structural investigations that study the consequences of introducing strain and its effects on the configuration and stability of amide linkages are not available to compare such systems to their pyrrolidine counterparts.

We describe herein highly stereoselective syntheses of the diastereomeric 4,5-methano-1-pyrrolidines and 5,6-methano-1-pyrrolidines acids by a novel intramolecular cyclopropanation reaction of imino amides and the extension of the methodology to other congeners.¹⁴⁻¹⁶

Treatment of the readily available lactam 1^{17,18} with lithium hexamethylantimonate (LHMMS) and Me₃SnCl₂ gave the *N*-alkylated products 2 (R₁ = -15.3, $\epsilon = 0.43$ in CHCl₃) and 3 (R₁ = -16.0, $\epsilon = 1.23$ in CHCl₃) in 63% and 25% yields, respectively (Scheme 1). The *syn*-isomer 3 could be easily obtained

	1	2	3
δ (N)	-17	-14.4	-14.4
δ (C ₄)	-20	+4.8	+15.3
δ (C ₅)	-20	-2.6	-11.4
δ (C ₆)	-1.9	-0.7	-1.9
δ (N-CO ₂ R)	+1.8	+4.1	+3.6
δ (C ₁ -C ₂)	-0.8	-0.8	-0.8
area deviation of final states	0.00	-0.8	0.00
ϵ	0.00	0.43	1.23

Scheme 1. TBDPS = *t*-BuPh₂Si, TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl, CSA = camphor-10-sulfonic acid.

Scheme 2.

by treatment of the enolate from 2 with the proton source 2,6-di-*tert*-butylphenol.^{19,20} Cisomerization of the hemiaminal from 2 and treatment with TFA led to the (4*R*,5*R*)-methano-1-pyrrolidine derivative 4 (δ _N = -69.3, $\epsilon = 1.81$ in CHCl₃), which was smoothly deprotected to 5, and the latter oxidized to give the crystalline (4*R*,5*R*)-methano-*N*-Boc-1-pyrrolidine in excellent overall yield.

Similar treatment of the *syn*-isomer 3 gave the diastereomeric crystalline acid 8 via its methylaminal derivative 7. The structures and conformations of 6 and 8 in the solid state were unambiguously confirmed by single-crystal X-ray analysis. Table 1 lists selected torsion angles for compounds 6 and 8, where considerable "battering" of the pyrrolidine ring is observed relative to *N*-Boc-1-pyrrolidine²¹ particularly in the case of 6. The flattening of the pyrrolidine ring in 6 is also manifested in the room-temperature value of 0.093 Å for the C₄ and N atoms from the plane defined by C₁, C₂, C₃, and N (0.015 Å in 8). The largest deviation of 0.014 Å in the case of *N*-Boc-pyrrolidine was found for C₂ and C₃ atoms in the plane C₁, N, C₂, C₃; in this case C₄ was distinctly above the plane (0.521 Å). This differs substantially

with aldiminium chloride, followed by trifluoroacetic acid (TFA), led to the (5*S*)-1-(2-propenyl)-4,5-methano-1-pyrrolidine derivative 11 (δ _N = -27.0, $\epsilon = 0.57$ in CHCl₃) on migration of the double bond. Compounds 10 and 11 represent unusually functionalized precursors to constrained α -methanopyrrolidines.

The versatility and generality of the intramolecular carbocyclization reaction with appended triphenylsilyloxy groups via taciturn initiation ions can be demonstrated in the synthesis of bicyclic pyrrolidine congeners (Scheme 3). These compounds are related to the antihepatocarcinogen congerin.²² A highly stereoselective alkylation of the enolate from 1 gave 12 (δ _N = -45.0, $\epsilon = 1.0$ in CHCl₃), which was subjected to a photoinduced intramolecular alkylation²³ to give 13 (δ _N = -23.6,

Scheme 1. TBDPS = *t*-BuPh₂Si, TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl, CSA = camphor-10-sulfonic acid.

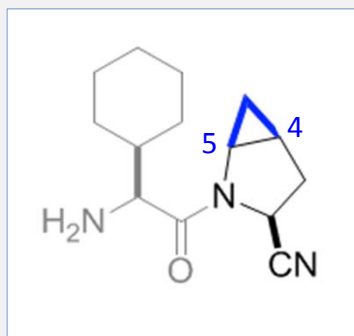
Scheme 2.

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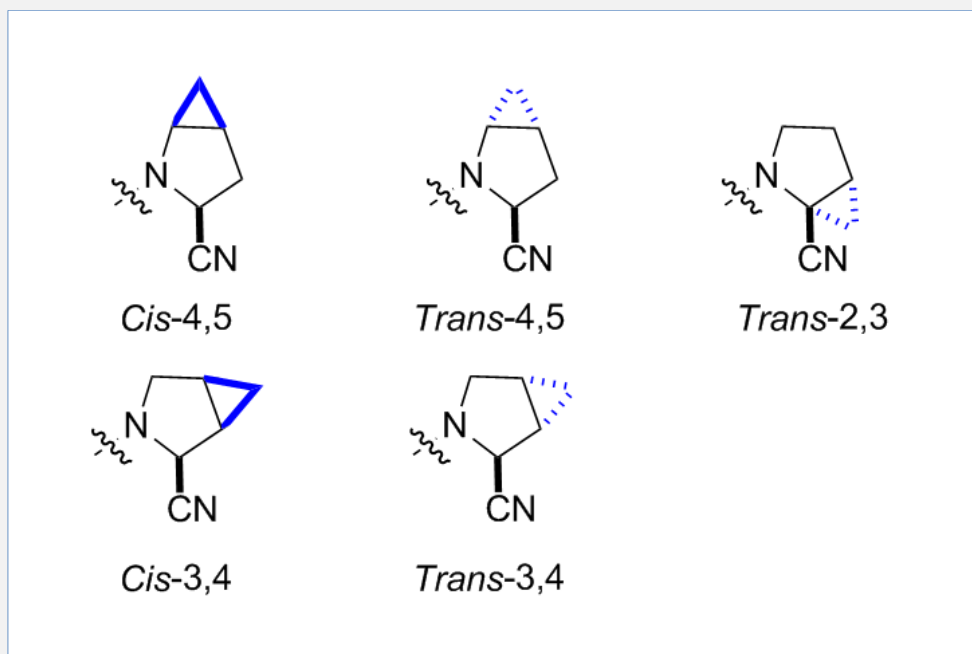
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Hanessian teaches synthesis of *cis*-4,5 and *trans*-4,5 cyclopropyl cyanopyrrolidine.

Limited Number of Positions to Cyclopropanate Pyrrolidine



Only 5 possible ways to cyclopropanate pyrrolidine ring (including *cis* and *trans* isomers).

Magnin Confirms Ease of Evaluating Each Option

J. Med. Chem. 2004, 47, 2587–2598

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 α -Aminoacyl-L-*cis*-4,5-methanoprolineitrile-Based Inhibitors

David R. Magnin,¹ Jeffrey A. Roth,^{1*} Richard B. Siskin,² David J. Augeri,^{1,3} Yanting Huang¹

2588 *Journal of Medicinal Chemistry*, 2004, Vol. 47, No. 18

Department of Solid State P.O. Box 24

Received July 14, 2004

Abstract

Introduction

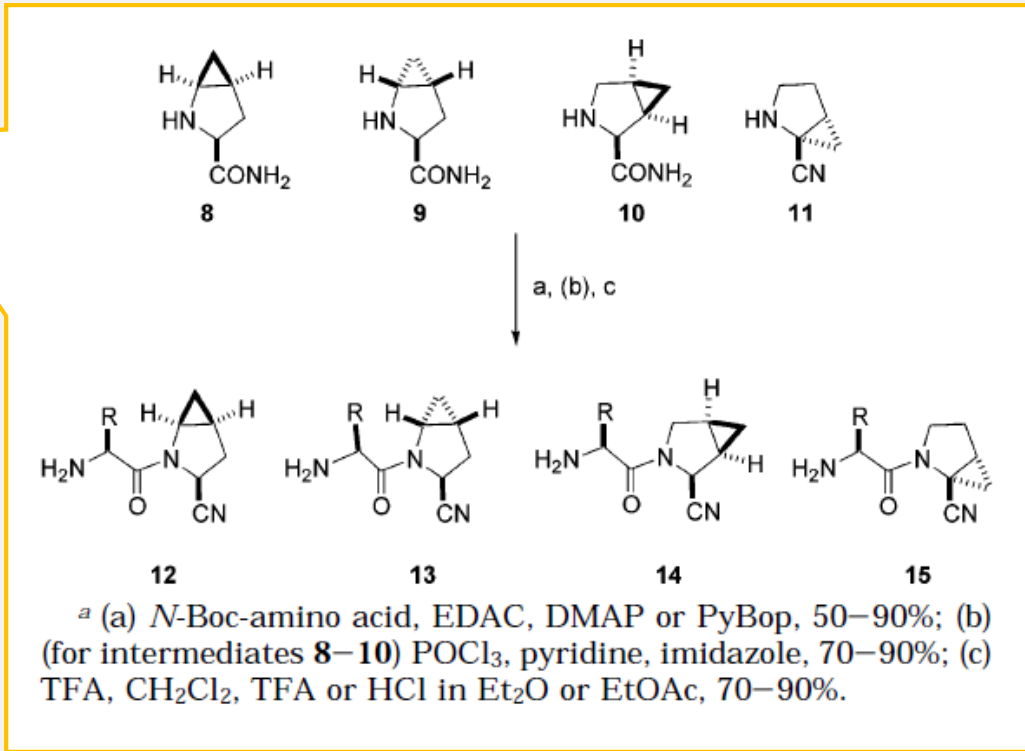
With the type 2 diabetes epidemic, current resistance supplies with glucose drug development interest in hormones known to insulin resistance. 2 diabetes, insulin resistance, and insulin response to insulin.

Chemistry

Dipeptides (12–15) composed of N-terminal isoleucine appended to cyclopropylprolineamides or nitriles derived from L-proline (8–11) were targeted to probe the potential for this type of inhibitor scaffold. Isoleucine was selected as the N-terminal residue because it was the most potent natural amino acid reported in the 2-cyanopyrrolidine series.¹⁹ These inhibitors were expected to provide a dependable inhibitory benchmark for the differing methanoproline structures. The synthetic routes used to prepare the dipeptides derived from L-*cis* and L-*trans*-4,5-methanoproline,^{19,20} L-*cis*-3,4-methanoproline,¹⁹ and L-2,3-methanoproline²⁰ are shown in Scheme 1.

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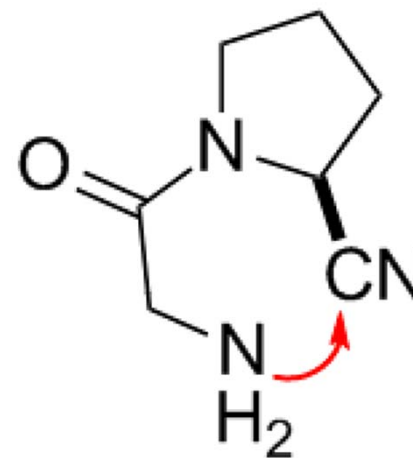
Page 2 of 12



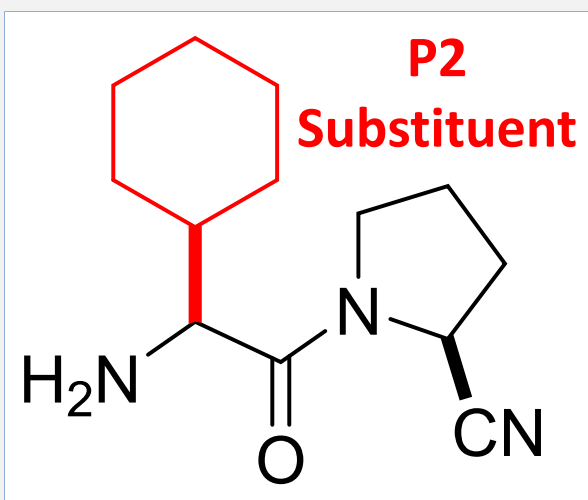
Magnin confirms the straightforward task of screening cyclopropanation derivatives at each of the available cyanopyrrolidine positions.

Motivation to Modify P2

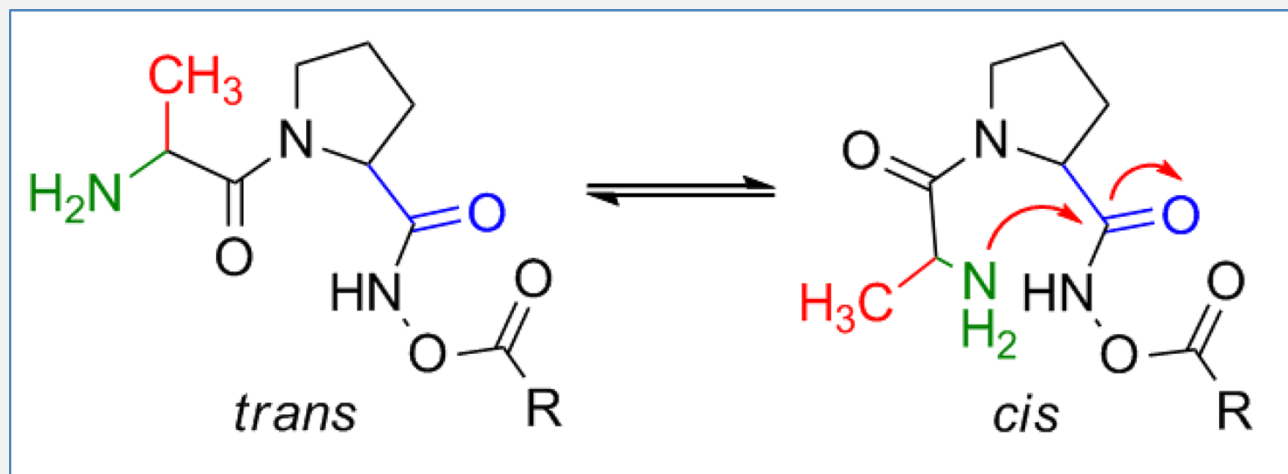
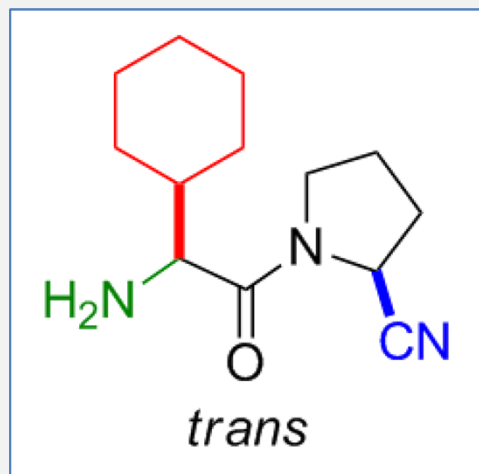
- **Sterically bulky P2 substituents**
 - improve stability
 - improve potency
 - favor *trans* confirmation and reduce cyclization



Cyclization




Intramolecular Cyclization Favored in *Cis*-Conformation



Intramolecular cyclization is minimized by favoring *trans*-conformation, instead of *cis*-conformation.

Bulky P2 Groups Improve Stability


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 0969-594X/96/001165-02

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Table II. Dipeptide analogs: Potency versus aqueous solution (pH 7.4).

Compound N°	Xaa
24	Cpg
25	Chg
26	Ile
27	Tbg
28	Lys(Z)
29	Pro

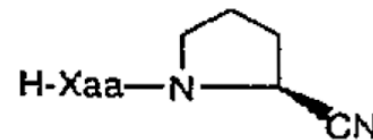
These compounds were found to be stable in aqueous solution (pH 7.4) for up to 48 h.

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- 10) Domsch, H.-J., Fischer, G., Barth, A. and Schwenen, R. L., *J. Org. Chem.* 1988, 54, 2890.
- 11) All compounds were tested in vitro against the human DRY-epitope from MMS, Copenhagen, Denmark. Inhibition was measured using the fluorogenic substrate, H-Ala-Pro-APC at these concentrations per inhibitor. A typical assay (total volume 0.4 ml) contained sodium HEPES 50.3 mM, DTTA 1.01 mM, BSA 1.5 mg mL⁻¹, pH 7.3, DQ-TV 25 µg mL⁻¹, substrate (in 10 and acetate pH 4.0). The reaction was started by the addition of substrate and readings were every 30 sec for 7.5 min, excitation at 395 nm, emission 490 nm. K_i values were determined using Dixon plots.
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- 17) The stability of the inhibitor in buffered, aqueous solution (100 mM Tris, pH 7.0) was monitored by reverse-phase HPLC.

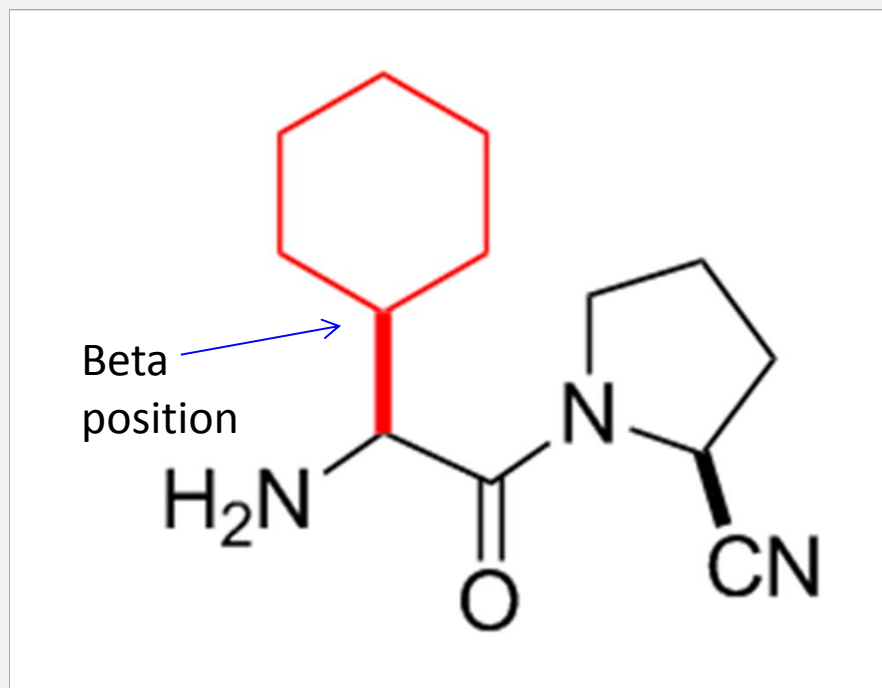
(Received in Belgium 21 February 1996; accepted 19 April 1996)

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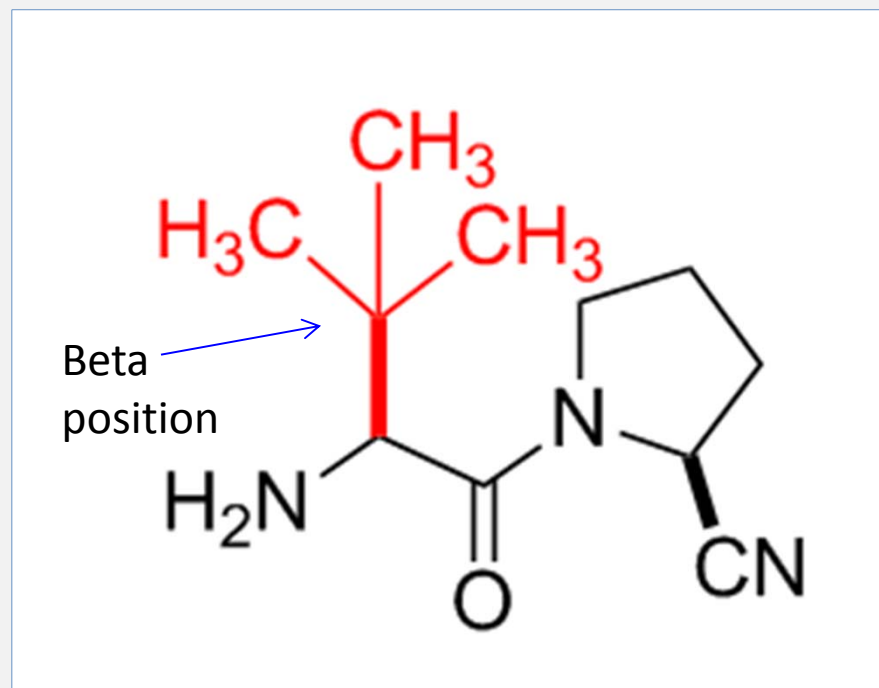
Compound N°	Xaa	K _i (nM) ¹³	t _{1/2} (h) ¹⁹
24	Cpg	1.1 ± 0.2	48
25	Chg	1.4 ± 0.5	>48
26	Ile	2.2 ± 0.5	48
27	Tbg	3.8 ± 0.8	>48
28	Lys(Z)	5.2 ± 1.0	24
29	Pro	22.0 ± 4.0	7.5

Steric Bulk Localized at the Beta Position



Ashworth I Compound 25

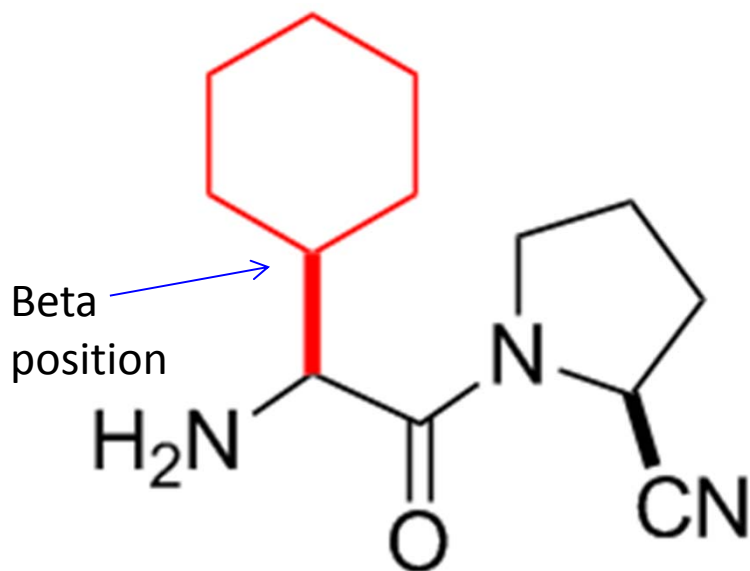
$t_{1/2} = >48$ h
 $K_i = 1.4 \pm 0.5$ nm



Ashworth I Compound 27

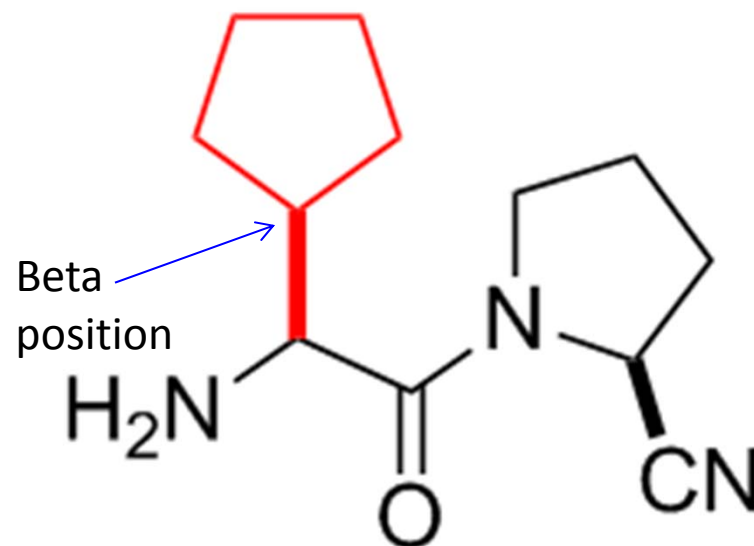
$t_{1/2} = >48$ h
 $K_i = 3.8 \pm 0.8$ nm

Steric Bulk Localized at the Beta Position



Ashworth I Compound 25

$t_{1/2} = >48$ h
 $K_i = 1.4 \pm 0.5$ nm



Ashworth I Compound 24

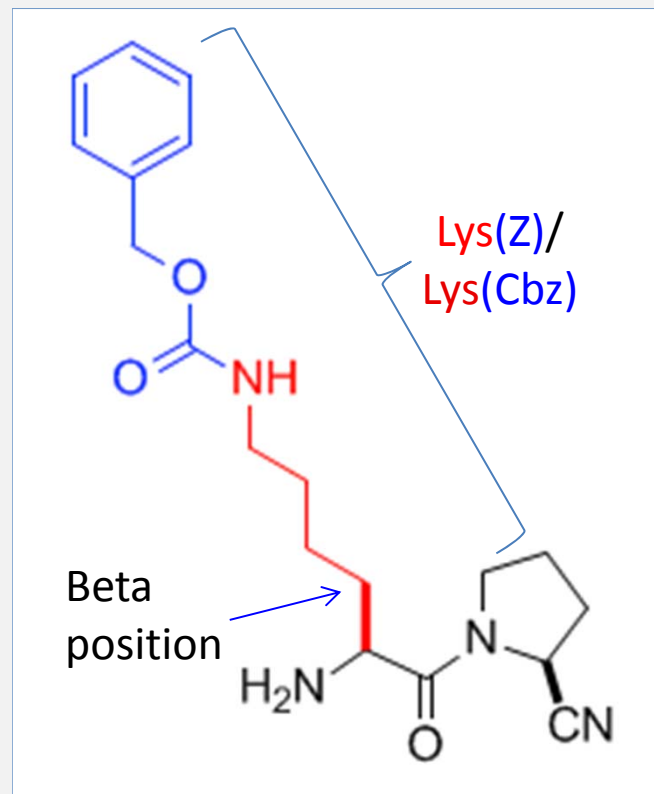
$t_{1/2} = 48$ h
 $K_i = 1.1 \pm 0.2$ nm

More Atoms Alone \neq Steric Bulk

Lys(Z) group is longer with more atoms.

Lys(Z) is less sterically bulky compared to a cyclohexyl group, with a smaller footprint at the β -position.

Lys(Z) has decreased stability compared to a cyclohexyl substituent.



Ashworth I Compound 28

$t_{1/2} = 24$ h

Use of Adamantyl at P2 was Known in DPP-4 Inhibitors

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: C07D 207/00, 401/00, C07K 5/00 A2 (11) International Publication Number: WO 98/19998 (43) International Publication Date: 14 May 1998 (14.05.98)

(21) International Application Number: PCT/EP97/06125 (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GR, HU, IL, IN, JP, KR, KZ, LC, LK, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TH, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO phases (GL, KI, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IR, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, GQ, MR, NE, SN, TD, TG).

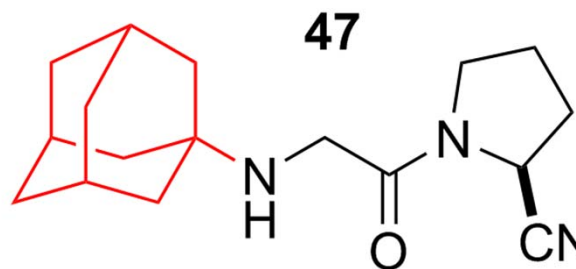
(22) International Filing Date: 5 November 1997 (05.11.97) (71) Applicant (for all designated States except US): NOVARTIS AG (CH/CH); Schwabstrasse 215, CH-4058 Basel (CH).

(72) Inventor(s) and (75) Inventor/Applicant (for US only): VILLHAUER, Edwin, Bernol [US/US]; 20 Dorothy Drive, Morristown, NJ 07960 (US).

(74) Agent: ROTIL, Bernhard, M.; Novartis AG, Patent- und Markenabteilung, Lichtstrasse 35, CH-4002 Basel (CH).

(54) Title: N-SUBSTITUTED 2-CYANOPYRROLIDINES

(57) Abstract



Example No.	R	Form	Analogous to Ex. No.
34	[S,S]-(1-hydroxymethyl)propyl	ch	1 ⁽¹⁾
35	[2-(2-hydroxymethyl)phenyl]thio]phenylmethyl	ch	1 ⁽²⁾
36	2-(2-methoxyphenyl)ethyl	ch	1
37	5-hydroxypentyl	ch	1
38	cyclobutyl	ch	1 off
39	2-(2,4-dichlorophenyl)ethyl	ch	1
40	1-(S)-(+)-hydroxymethyl-3-methylbutyl	ch	1 ⁽³⁾
41	[1R*,2S*]-2-hydroxy-2-phenylethyl	ch	
42	2-(2-fluorophenyl)ethyl	ch	
43	cyclopropyl	ch	
44	[1S(1S,2S,3S,5R)]-2,6,6-trimethylbicyclo[3.1.1]hept-3-yl	ch	1 ⁽⁴⁾
45	(2-phenoxy)ethyl	ch	1
46	2-(3,5-dimethoxyphenyl)ethyl	ch	1
47	1-adamantyl	ch	1
48	1,1,3,3-tetramethylbutyl	ch	1
49	2-adamantyl	ch	1
50	1,1-dimethylpropyl	ch	1
51	benzyl	ch	1
52	1,1-dimethylethyl	ch	1
53	(2-adamantyl)methyl	ch	1
54	2-phenylethyl	ch	1
55	pentyl	ch	1
56	butyl	ch	1

47

1-adamantyl

49

2-adamantyl

53

(2-adamantyl)methyl

white solid; m.p. 290-292°; ¹³C-NMR: 121.60 (ppm)

white fluffy solid; m.p. 68-70°; ¹³C-NMR: 121.55 (ppm)

off-white solid; m.p. 122-124°; ¹³C-NMR: 121.69 (ppm)

white fluffy solid; m.p. 62-64°; ¹³C-NMR: 121.53 (ppm)

white solid; m.p. 58-60°; ¹³C-NMR: 121.38 (ppm)

white solid; m.p. 226-228°; ¹³C-NMR: 121.56 (ppm)

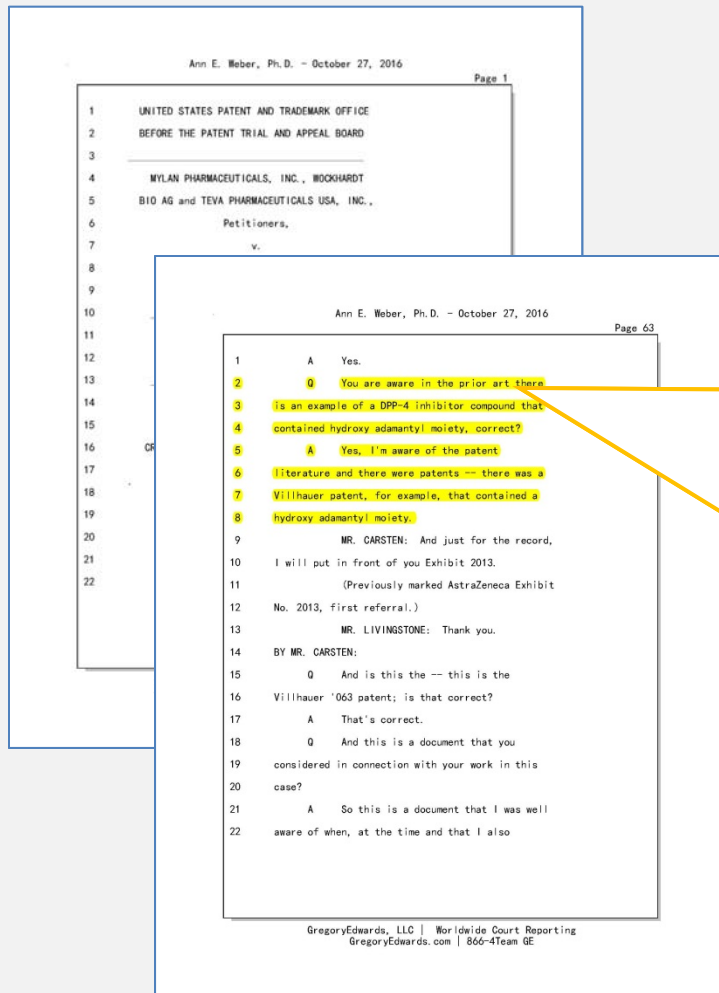
white solid; m.p. 158-160°; ¹³C-NMR: 121.53 (ppm)

white solid; m.p. 275-280° (dec.); ¹³C-NMR: 121.52 (ppm)

white solid; m.p. 176-178°; ¹³C-NMR: 121.67 (ppm)

white solid; m.p. 180-182°; ¹³C-NMR: 121.53 (ppm)

Hydroxyadamantyl Was Known From Villhauer 2000



Q You are aware in the prior art there is an example of a DPP-4 inhibitor compound that contained hydroxy adamantyl moiety, correct?

A Yes, I'm aware of the patent literature and there were patents -- there was a Villhauer patent, for example, that contained a hydroxy adamantyl moiety.

Source: EX1073 (Weber Depo. Trans.), 63:2-8; EX1074 (Second Rotella Decl.), ¶133; EX2013 (Villhauer 2000), 7:15-27.

Hydroxyadamantyl at P2 was Known in DPP-4 Inhibitors

US00166063A

United States Patent [19] **Patent Number:** **6,166,063**
Villhauer [43] **Date of Patent:** **Dec. 26, 2000**

[54] **N-SUBSTITUTED GLYCYL-L-2-CYANOPYRROLIDINES, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN INHIBITING DIPEPTIDYL PEPTIDASE-IV**

[75] **Inventor:** **Edwin Bernard Villhauer, Morrisown, N.J.**

[73] **Assignee:** **Novartis AG, Basel, Switzerland**

[21] **Appl. No.:** **09/488,224**

[22] **Filed:** **Dec. 9, 1999**

Related U.S. Application

[63] **Continuation of application No. 09/209-048/048**

[51] **Int. Cl.:** **A61K 31/07(2006)**

[51] **U.S. Cl.:** **314.423**

[56] **Field of Search**

[56] **References Cited**

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6,166,063

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inhibition, such compositions in unit dosage form and such compositions comprising a pharmaceutically acceptable carrier.

The compounds of formula I (including those of each of the subtypes thereof and each of the examples) may be administered in enantiomerically pure form (e.g., as >98%, preferably >99%) or together with the B enantiomer, e.g., in racemic form. The above dosage ranges are based on the compounds of formula I (excluding the amount of the B enantiomer).

The following examples show representative compounds encompassed by this invention and their synthesis. However, it should be clearly understood that they are for purposes of illustration only.

EXAMPLE 1

Pyridinone, 1-[(3-hydroxy-1-adamantyl)amino]acetyl-2-cyano-, (S)

A. 1-Aminoadamantane-3-ol

Slight modifications to the synthesis found in Klein, Farm. Zs. (1980), 20(7), 910-15, may be used.

To a rapidly stirred, clear and colorless, ice-water chilled mixture of concentrated sulfuric acid (210 mL, 3.943 mmol) and 65% nitric acid (21.0 mL, 217.0 mmol) is added 21.0 g (112.0 mmol) of 1-adamantylamine HCl (99%), in small portions, over 30 minutes. Upon subsequent hydrochloride addition, slight bubbling occurs and the reaction is slightly exothermic. This bubbling, yellow solution is stirred at ice-water temperature for about 2 hours and then at room temperature for 30 hours. This clear, light yellow reaction is then poured into about 100 g of ice and the resulting solution is clear green-blue.

The solution is placed in an ice-water bath and allowed to stir for 30 minutes. Approximately 550 g of 89% pure KOH (6.74 mol) is then added in small portions over 45 minutes. During this addition, the reaction is exothermic, reaching 80° C. and producing copious amounts of brown NO₂ gas. By the end of the addition, the reaction is thick with white solids (both product and salts). The resulting white paste is then poured into a beaker furnished with a pad and washed with 1.2 L of CH₂Cl₂. The CH₂Cl₂ layer is then extracted from the water layer and dried over Na₂SO₄. The solution is then filtered and concentrated (vacuum) to provide 1-aminoadamantane-3-ol as a white solid.

Alternatively, the reaction may be carried out using n-butane as solvent instead of methylene chloride.

Alternatively, 1-aminoadamantane-3-ol can be prepared e.g. as follows: A 2-L, 4-necked, round-bottomed flask is thoroughly flushed with nitrogen. The flask is charged under nitrogen with 420 mL of conc. sulfuric acid (98%). The contents are cooled to 8° C., then slowly (slightly exothermic, 1-10° C. gas evolution) 100.0 g (1.0 mmol) of 1-aminoadamantane hydrochloride are added into the mixture in 8 portions at 9-10° C. over 20 min (minutes), then the binary contents are stirred at 9-10° C. for 20 min to obtain a homogeneous mixture. 72 mL of conc. (concentrated) nitric acid (70%) are added (very exothermic) dropwise into the mixture maintaining inner temperature at 14-15° C. with efficient cooling (at this scale 20 min. needed for this addition). The mixture

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is stirred at 14-15° C. for 20 min, the temperature is allowed to raise to 25° C. over 1 h (hour) (15-20° C. for 30 min, and 20-25° C. for 30 min), then the contents are stirred at 24-25° C. for 5 h (external cooling is needed). 1.7 L of water are charged into a 5-L, 4-necked flask, the water is cooled to 10° C., then the reaction mixture is slowly poured (very exothermic, some NO₂ gas evolution) over 25 min, maintaining the inner temperature below 35° C. to give a blue-green homogeneous solution. The original 2-L flask (slightly exothermic) is rinsed once with 0.3 L of water and the water wash is poured into the 5-L flask. Slowly 900 mL of 50% sodium hydroxide aqueous solution are added (very exothermic, some NO₂ gas evolution) into the 5-L flask over 30 min, at 65-70° C. to bring the pH of the mixture to 13. 100 mL of 1-butanol and 200 mL of toluene are added (free exothermic) under vigorous stirring and allow the mixture to reach 90° C. The bottom aqueous layer is separated for proper disposal. The organic layer is once washed with 100 mL of saturated sodium chloride solution. The saturated sodium chloride wash is saved for disposal. The organic layer is concentrated to 60-65% of the original volume.

The solids are filtered.

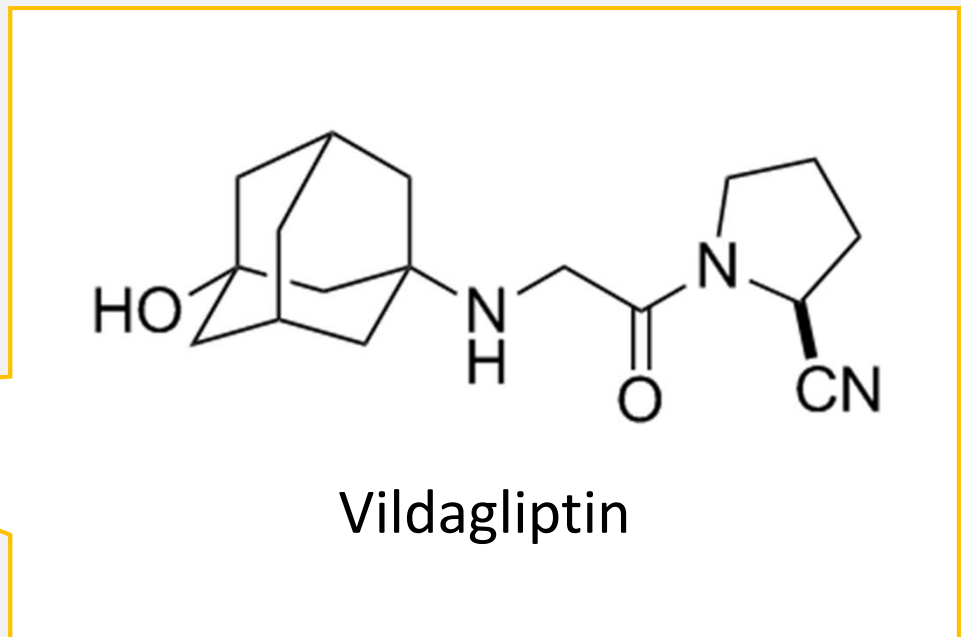
Butcher dried, then once with 80 mL of heptane. The solids are dried at 100° C. in a vacuum oven. The final cake is dried at 100° C. for 10 h to afford 1-aminoadamantane-3-ol as a white solid.

B. 1-Chloroacetyl-2-cyanopyridinone

To a mechanically stirred solution of 20.0 g (180.0 mmol) of dichloroacetyl chloride and 97 g (69.70 mmol) of potassium carbonate in 150 mL of acetonitrile is added a solution of 1-prolineamide 20.0 g (180.0 mmol) in 500 mL of tetrahydrofuran in a dipropyl ether over 45 minutes. This reaction is then mechanically stirred for an additional two hours at room temperature. The reaction is then filtered to remove potassium salts and the filtrate is dried over Na₂SO₄. The Na₂SO₄ is then removed via filtration and to this solution filtrate is added trifluoroacetic anhydride (25.0 mL, 0.180 mmol) in one portion. The reaction is then magnetically stirred for 1 hour at room temperature and the resulting clear yellow-orange solution is concentrated via rotary evaporation. The excess trifluoroacetic anhydride is removed by adding ethyl acetate to the concentrated oil and recovering via rotary evaporation. This removing operation is performed three times. The resulting oil is partitioned between ethyl acetate and water. The product is then extracted into the ethyl acetate and the aqueous layer is then washed twice with ethyl acetate. The combined organic layers are then washed successively with water and being dried over magnesium sulfate, filtered and concentrated to obtain 1-chloroacetyl-2-cyanopyridinone as a yellow solid.

Alternatively, the reaction may be carried out by using, as a mixture, e.g. 2-ethylhexanoic acid sodium hydroxide.

To a heterogeneous solution of the title A compound (1-aminoadamantane-3-ol) (5.00 g, 34.7 mmol) in CH₂Cl₂ (60.0 mL) is added 9.6 g (69 mmol) of K₂CO₃. This heterogeneous mixture is then cooled in an ice-water bath and a solution of 30.0 g (1.0 mmol) of the title B compound (1-chloroacetyl-2-cyanopyridinone) dissolved in 23.0 mL of CH₂Cl₂ is added dropwise over a period of 30 minutes. The resulting mixture is stirred for 2 hours at 0° C. and at room temperature for 6-days. The reaction is then concentrated to



Patent Owner Cites Mentlein 1993

Eur. J. Biochem. 214, 829–835 (1993)
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Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum

Rolf MENTLEIN¹, Baptist GALLWITZ² and Wolfgang F. SCHMIDT²

¹ Anatomisches Institut und
² Abteilung Allgemeine Innere Medizin

(Received February 9/April 16, 1993)

Peptides of the sequence similarity might be in part potent removing dipeptides hormone-releasing factor peptide (GLP-1) and tropin [GLP-1(7–36)] hydrolysed to their octapeptide. VIP with term, the hydrolysis of GIP values of 4–34 μM at purified peptidase w/ concentrations. When ment as with the pat in the case of GIP degradation products peptidase IV, 1 mM production of these metabolites of GIP for the biological act known to be inactive peptidase-IV action inactivation and their

Dipeptidyl-peptidase IV (DPP) aminopeptidase removing dipeptide and synthetic peptide substrates p nine are the penultimate N-ter (1988, for review). Small peptides with proline in this position are those with alanine (Heins et al., human serum, as an ectoenzyme endothelial cells, at kidney brush

Correspondence to R. Mentlein, U Institut, Othovassestrasse 60–66, D-5 Fax: +49 431 8801557.

Abbreviations: DPP IV, dipeptidyl inhibitory polypeptide or glucose-dep tide; GLP-1(7–36)amide, glucagon-insulinotropin or preproglucagon(78– like peptide-2 or preproglucagon(1– mosic-releasing, factor/hormone; PHM, peptide histidine methionine; PACAP, pituitary adenylylate-cycl Enzyme, Dipeptidyl peptidase IV

Page 1 of 7

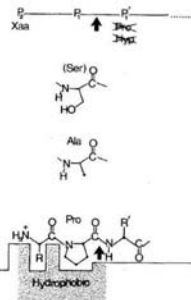


Fig. 4. Schematic representation of the substrate-binding and substrate-cleaving (arrow) sites of DPP IV. Proline and alanine fit in the hydrophobic P₁-substrate-binding pocket, whereas serine appears to be too hydrophilic to yield appreciable binding. In the P₁ position bulky amino acids with an obligate free amino group are preferred. Peptides with Pro or Hyp in the P₁ position are not cleaved by DPP IV. Preferential amino acids for the P₁ position are not known.

as further degradation product could be identified after derivatization with 4-dimethylaminobenzoyl-sulphonyl-chloride (see Experimental Procedures) by identical retention time and co-chromatography with a derivatized, synthetic His-Ala standard. Again, in the presence of L-lys-pyrrolidide (1 mM) and dipiprin A (0.1 mM), the generation of the des-His-Ala-fragment was abolished (<5%). Thus, as concluded from specific inhibition and generation of His-Ala and the des-His-Ala-peptide GLP-1(7–36)amide is cleaved by human serum mainly by action of DPP IV.

In sera of healthy males we measured a mean activity of $55 \pm 12 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ ($n = 6$) with the chromogenic substrate 0.5 mM Gly-Pro-4-nitranilide for DPP IV. No significant differences were found for the peptidase activities in preprandial sera ($n = 3$). In a serum with an activity of $50 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ for Gly-Pro-4-nitranilide, we estimated degradation rates of about $0.3 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ for Tyr-Ala liberation from 20 μM GIP and $0.4 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ for His-Ala liberation from 20 μM GLP-1(7–36)amide.

DISCUSSION

Members of the VIP/glucagon peptide family with N-terminal penultimate alanine are good substrates for DPP IV. GRP(1–29)amide or GRP(1–44)amide as analyzed here and by Bongers et al. (1992), GIP, GLP-1(7–36)amide and PHM are cleaved to their des-Tyr-Ala or des-His-Ala derivatives by the highly purified human enzyme. In contrast, VIP with N-terminal His-Ser was not significantly degraded. This fits well with the known, preferential specificity of DPP IV for

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penultimate proline or alanine residues (Fig. 4). Almost no other naturally occurring amino acid is accepted in this position. Replacement of penultimate Ala in a GRP(1–29)amide derivative by hydrophilic Ser or Gly resulted in dipeptidyl-peptidase-IV substrates of far lower k_m and higher k_c values (Bongers et al., 1992). In contrast, substrates with synthetic hydrophobic derivatives of the proline ring (oxa- or thia derivatives) or short, unbranched hydrophobic (alkyl) derivatives in the P₁ position are good substrates for DPP IV (Rahfeld et al., 1991b; Schetkowsky, 1991). This indicates a hydrophobic substrate (P₁) recognition site for DPP IV where Ser is less well (or not) bound than Ala or Pro (Fig. 4). Moreover, a bulky N-terminal amino acid with free amino group (P₂ position) as with Tyr or His in the peptides investigated here is optimal for

fects of the C-terminus the relatively 1–29–42 residue PHM as comp generic substrate GIP release endocrine K c stimulates issu ence of elevati have clearly required for cal

on longer coco cation of GIP spect to its ma

Cleavage products are numerous or specific, numerous clearly show that dipeptidyl peptidase IV is the main degradation and, considering the above findings, inactivation enzyme for GIP in human serum. The enzyme should be still more active on this peptide hormone at other sites, e.g. endothelial cells of blood vessels, hepatocytes, kidney brush-border membranes (podocytes of the glomerular basement membrane and proximal tubule cells), lymphocytes, chief cells of gastric glands, or epithelial cells of the intestine, where it is found in high concentrations as an ectoenzyme of the plasma membranes (Lojida, 1979; Hartel et al., 1988; Gosrau, 1979; McCaughan et al., 1990; Mentlein et al., 1984). Active hydrolysis by DPP IV might therefore explain why GIP(3–42) has been isolated as a second component (relative yield about 20–30%) beside intact GIP from porcine intestine and has been found as a contaminant of natural GIP preparations (Jörvall et al., 1981; Schmidt et al., 1987).

GLP-1(7–36)amide is a product of the tissue-specific post-translational processing of the glucagon precursor. It is released postprandially from intestinal endocrine L cells and stimulates insulin secretion. Gallwitz et al. (1990) have shown that the C-terminal fragment of the peptide is important for receptor binding of the hormone, but is not sufficient to transduce a biological action as does the intact peptide (raise in cyclic AMP levels in rat insulinoma RIN5F cells). It appears that as in the case of glucagon (Jinson et al., 1989), of GIP (Schmidt et al., 1986, 1987) and of other members of the VIP/glucagon peptide family (Christophe et al., 1989; Robberecht et al., 1992) also for GLP-1(7–36)amide an intact N-terminus is needed for signal transduction and biological action. Provided this, action of DPP IV inactivates GLP-1(7–36)amide.

“Preferential amino acids for the P₁ position are not known.”

Mentlein: Bulky Groups are Preferred at P2

Eur. J. Biochem. 214, 829–835 (1993)
© FEBS 1993

Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum

Rolf MENTLEIN¹, Baptist GALLWITZ² and Wolfgang

¹ Anatomisches Institut und
² Abteilung Allgemeine Innere Medizin der Universität

(Received February 9/April 16, 1993) – EJB 93 0215/3

Peptides of the glucagon/vasoactive sequence similarity at their N-termini might be in part potential targets for removing dipeptides only from peptide hormone-releasing factor(1–29)amide, glucagon-like peptide (GLP) with terminal Tyr, tropin (GLP-1(7–36)amide) and peptide hydrolysed to their des-Xaa-Ala derivative. VIP with terminal His-Ser was the hydrolysis of GLP-1(7–36)amide values of 4–34 μM and V_{max} values of purified peptidase which should allow concentrations. When human serum was mixed with the purified dipeptidyl-peptidase IV, 1 mM Lys-pyrrolidide production of these fragments by secret metabolism of GLP and GLP-1(7–36) for the biological activity of the ment known to be inactive to release insulin peptidase-IV action inactivates these inactivation and their determination by

Dipeptidyl-peptidase IV (DPP IV) is a highly aminopeptidase removing dipeptides from bioactive and synthetic peptide substrates provided that proline are the penultimate N-terminal residues (1988, for review). Small peptides or chromogenic with proline in this position are far better hydrolysed than those with alanine (Heins et al., 1988). DPP IV human serum, as an ectoenzyme on the surface of endothelial cells, at kidney brush-border membrane

Correspondence to R. Mentlein, Universität Kiel, An Institut, Olshausenstrasse 60–66, D-24118 Kiel, Germany. Fax: +49 431 8801557.

Abbreviations: DPP IV, dipeptidyl-peptidase IV; GIP, gastric inhibitory polypeptide or glucose-dependent insulinotropic peptide; GLP-1(7–36)amide, glucagon-like peptide-1(7–36) amide; proglucagon(78–107)amide; GLP-2, like peptide-2 or proglucagon(126–159); GRF, growth hormone-releasing factor(1–29)amide; PHM, peptide histidine methionine; VIP, vasoactive intestinal peptide; PACAP, pituitary adenylylate-cyclase-stimulating polypeptide. Dipeptidyl-peptidase IV (EC 3.4.14.5).

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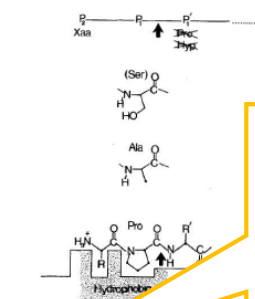


Fig. 4. Schematic representation of the substrate-binding sites of DPP IV. Proline has to fit in the hydrophobic, P₁-substrate-binding pocket, whereas serine appears to be too hydrophilic to yield appreciable binding. In the P₂-position bulky amino acids with an obligate free amino group are preferred. Peptides with Pro or Hyp in the P₁ position are not cleaved by DPP IV. Preferential amino acids for the P₁ position are not known.

as further degradation product could be identified after derivatization with 4-dimethylaminoazobenzene-sulphonyl-chloride (see Experimental Procedures) by identical retention time and co-chromatography with a derivatized, synthetic His-Ala standard. Again, in the presence of Lys-pyrrolidide (1 mM) and diprotin A (0.1 mM), the generation of the des-His-Ala-fragment was abolished (<5%). Thus, as concluded from specific inhibition and generation of His-Ala and the des-His-Ala-peptide GLP-1(7–36)amide is cleaved by human serum mainly by action of DPP IV.

In sera of healthy males we measured a mean activity of $55 \pm 12 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ ($n = 6$) with the chromogenic substrate 0.5 mM Gly-Pro-4-nitranilide for DPP IV. No significant differences were found for the peptidase activities in preprandial and postprandial sera ($n = 3$). In a serum with an activity of $50 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ for Gly-Pro-4-nitranilide, we estimated degradation rates of about $0.3 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ for Tyr-Ala liberation from 20 μM GIP and $0.4 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{l}^{-1}$ for His-Ala liberation from 20 μM GLP-1(7–36)amide.

DISCUSSION

Members of the VIP/glucagon peptide family with N-terminal penultimate alanine are good substrates for DPP IV. GRF(1–29)amide or GRF(1–44)amide as analyzed here and by Bongers et al. (1992), GIP, GLP-1(7–36)amide and PHM are cleaved to their des-Tyr-Ala or des-His-Ala derivatives by the highly purified human enzyme. In contrast, VIP with N-terminal His-Ser was not significantly degraded. This fits well with the known, preferential specificity of DPP IV for

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penultimate proline or alanine residues (Fig. 4). Almost no other naturally occurring amino acid is accepted in this position. Replacement of penultimate Ala in a GRF(1–29)amide derivative by hydrophilic Ser or Gly resulted in dipeptidyl-peptidase-IV substrates of far lower k_m and higher K_m values (Bongers et al., 1992). In contrast, substrates with synthetic hydrophobic derivatives of the proline ring (oxa- or thia de-

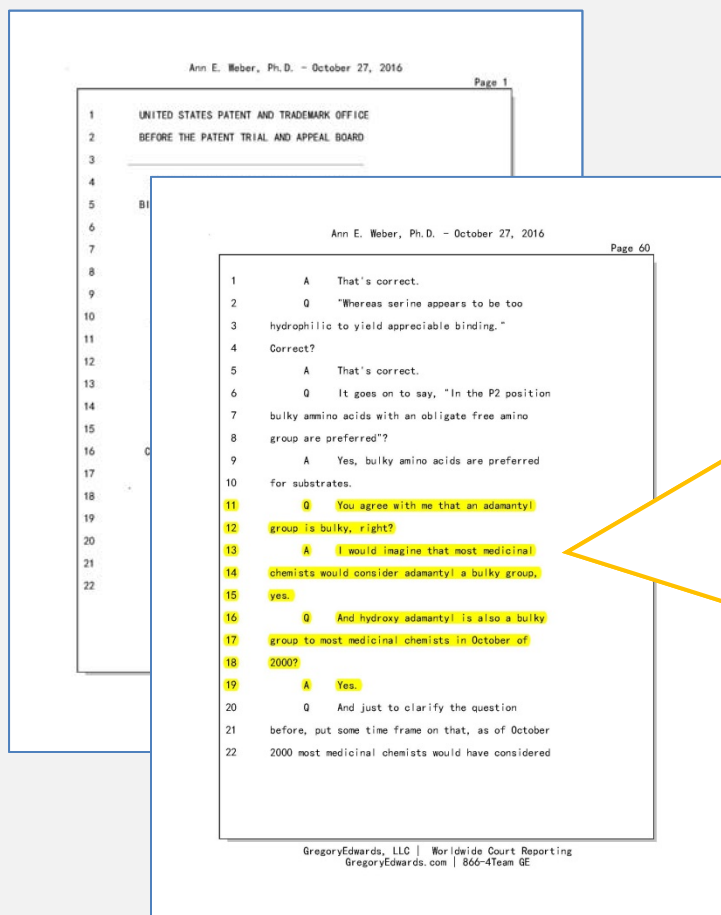
“In the P₂ position bulky amino acids with an obligate free amino group are preferred.”

cal effect) of GIP. Pure des-Tyr-Ala-GIP (3–42) unlike intact GIP did not increase insulin secretion in the presence of 16.7 mM glucose from rat pancreatic islets at physiological or higher concentrations even up to 250 nM. Therefore, truncation of GIP by DPP IV results in its inactivation with respect to its major physiological, the insulinotropic, action.

Cleavage products and influence of specific inhibitors clearly show that dipeptidyl-peptidase IV is the main degradation and, considering the above findings, inactivation enzyme for GIP in human serum. The enzyme should be still more active on this peptide hormone at other sites, e.g. endothelial cells of blood vessels, hepatocytes, kidney brush-border membranes (podocytes of the glomerular basement membrane and proximal tubule cells), lymphocytes, chief cells of gastric glands, or epithelial cells of the intestine, where it is found in high concentrations as an ectoenzyme of the plasma membranes (Lojda, 1979; Hartel et al., 1988; Gosrau, 1979; McCaughan et al., 1990; Mentlein et al., 1984). Active hydrolysis by DPP IV might therefore explain why GIP(3–42) has been isolated as a second component (relative yield about 20–30%) beside intact GIP from porcine intestine and has been found as a contaminant of natural GIP preparations (Jernvall et al., 1981; Schmidt et al., 1987).

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Hydroxyadamantyl is a Bulky Group



Q You agree with me that an adamantyl group is bulky, right?

A I would imagine that most medicinal chemists would consider adamantyl a bulky group, yes.

Q And hydroxy adamantyl is also a bulky group to most medicinal chemists in October of 2000?

A Yes.

Metabolites Guide Modification of Drug Candidates

“Metabolite identification in drug discovery provides early information that can lead to structural changes in the current lead compound, improving such pharmacokinetic parameters as oral bioavailability, half-life ($t_{1/2}$), or C_{max} .”

REVIEWS

HPLC-API/MS/ tool for integrat metabolism int discovery proc

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on the analysis of 40 ul even on 10-20 ul of plate are necessary. Because systems, it is possible are subjected to scall ba at is closed with the inle time points (typical a small volume of blood mass data, three rats c vental bleeding in rats an

- Fewer rats are need on a compound.
- Smaller amounts of dosing.
- There is less variabi
- The entire set of pharmacokinetic parameters (C_{max} , area under curve, $t_{1/2}$) are available for each animal.

Figure 2 shows data obtained from (a) a serial bleeding study and (b) a multi-rat study for the same compound dosed orally at 30 mg/kg. It can be seen that there is a better correlation between the individual data and the mean data for the serial bleeding study than for the multi-rat study.

Metabolite identification

Previously, metabolite identification was reserved for compounds in the development phase. One reason for this was that the standard method for metabolite identification relied on radiolabeled drug. As shown in Figure 3, the process of synthesizing the drug and collecting, purifying, and analyzing the metabolites typically takes 2-4 months. This time frame is not acceptable by current drug discovery standards in the drug discovery phase. HPLC-API/MS/MS can be used for metabolite identification. As shown in Figure 5, once you have metabolite identification can be completed in 1 week in many cases. 1 day is sufficient to obtain useful information on the plasma metabolites of a drug does experimental animal. A more extensive look at other tissues (liver, kidney or other tissues (e.g. brain, heart) or 2 weeks. In either case, significant amount of information on the metabolism of a compound can be obtained in a relatively short period of time.

Metabolite identification in drug discovery provides early information that can lead to structural changes in the current lead compound, improving such pharmacokinetic parameters as oral bioavailability, half-life ($t_{1/2}$), or C_{max} . Often

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DDT Vol. 2, No. 12 December 1997

Metabolites Guide Modification of Drug Candidates

“Early metabolite identification can provide information on how to improve the metabolic stability of the lead structure. In this way, future lead compounds might be a metabolite identified from the previous lead drug or an analogue of the previous drug designed to block the major route of metabolism.”

REVIEWS

HPLC-API/MS/ tool for integrat metabolism int discovery proc

Walter A. Korfmacher, Kathleen John Vande Kassel, No. Debas

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REVIEWS

on the analysis of 40 μ l even on 10–20 μ l of plasma are necessary⁶. Because, quite rarely, it is possible are subjected to serial bleed rat is dosed with the test multiple time points (typical a small volume of blood mean drug, these rats of serial bleeding in rats are

- Fewer rats are needed on a compound.
- Smaller amounts of dosing.
- There is less variability.
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Metabolite identification in drug discovery provides early information that can lead to structural changes in the current lead compound, improving such pharmacokinetic parameters as oral bioavailability, half-life (t_{1/2}), or C_{max}. Often

these parameters stability of the stability, it is y metabolized. progress a lead compo ing that can be placed in the drug metabo via often linked and, in the past, co producing a pharmacokinetic profile of can prov on how to improve the metabolic stability and structure. In this way, future lead compounds can be a metabolite identified from the previous lead drug or an analog of the previous drug designed to block the major route of metabolism. In either case, metabolite information in early drug discovery may lead to a much faster progression from the early lead drug to the final candidate drug.

DOI: 10.1002/jbm.b.10001

Adamantane is Metabolized at Tertiary Carbons

“Adamantane is the only substrate we have investigated, in this study, that is metabolized to a single product despite having a relatively high active site mobility. The single product can be attributed to the existence of only two types of unique carbon atoms in adamantane, together with the greater reactivity of tertiary versus secondary carbons.”

2674 *Biochemistry* 1991, 30

Crystal Structures of Cytochrome P-450 Thiocamphor, and Adamantane: Fact Hydroxylat

Reetta Raag¹ and Thor

Center for Advanced Research in Biotechnology of the Maryland B Grove, 9600 Gudebilly Drive, Rockville, Maryland 20850, and the 1 Maryland College Park, Md

Received September 26, 1990; Revised Mar

ABSTRACT: X-ray crystal structures have been determined substrates camphane, adamantane, and thiocamphor. Unlike bonds to Tyr96 and is metabolized to a single product, a hydrogen bond to the enzyme and all are hydroxylated substrate-enzyme hydrogen bond allows substrate greater regioselectivity of metabolism as well as the inability of heme iron. Tyr96 camphor-P-450_{cam} 687-700). The 9000 in the presence of th understanding the role orientation in the a thiocamphor in quite primarily hydroxyl positioned near Tyr9 camphor-P-450_{cam} identified in the act from substrate hydro molecule has also be the active site in th

The cytochrome P-450 exp many different types of oxid hormone biarylthiol, fatty a tion of foreign compounds [Gonzalez, 1987; Anders, 19 P-450s generally oxidize and facilitating their excretion. O in the cytoplasm as "activated of which are mutagens and 1973; Saito & Omura, 1973; Ito, 1980; Wolf, 1986). sticity of this superfamily, as types of reactions, there is me relationship of P-450s. UG compounds to selectively inh neering novel P-450s to fac environmental contaminants. The best characterized P- a crystal structure is know dioxylase P-450_{cam} (Gunn)

¹Supported in part by NIH G "Crystallographic modification h Protein Data Bank under the file P-450_{cam}-adamantane. ©1991, by cytochrome P-450_{cam}-thiocamphor *Correspondence should be ad University of Maryland.

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2678 *Biochemistry*, V

Table III: Various Substrat

substr	mol wt	hydrogen bond to Y96	no. of iron ligand	radius, Å	P ₄₅₀ /Fe ²⁺	high-spin %	regioselect of substr type
camphor	152	1	6	1.95	1.35	100	1
thiocamphor	152	1	6	1.95	1.35	100	1
adamantane	192	0	6	1.95	1.35	100	2

substr temp factor (Å²)
substr hydroxyl group hydroxyl "efficiency"
L6-substr dist

Le-iron dist	NA	NA	1.95 Å	1.35 Å	2.35 Å (30%)
L6 occupancy	NA	NA	1.00	0.90	
L6 temp factor	NA	NA	14.3 Å ²	19.6 Å ²	
oxygen occupancy	1.00	1.00	0.90	0.91	
cation temp factor	12.1 Å ²	10.0 Å ²	15.5 Å ²	14.2 Å ²	

¹Pauline et al. (1983, 1987). ²Rang and Poulos (1988). ³Fisher and Sigar (1983). ⁴White et al. and Sigar (1983). ⁵Carbon numbering for each substrate begins with C-1 at the top of the six-membered ring. ⁶Numbering proceeds counterclockwise such that the substrate carbon is C-2 and C-3 is in the lower secondary carbon in some substances and a tertiary carbon in others.

the distal ligand is located between the heme iron and the substrate sulfur atom. These two neighbors could conceivably interact via the distal ligand and increase electron density at this location, which could be reflected in an anomalously high ligand occupancy.

Although initial occupancy estimates for the two thiocamphor orientations were successful in eliminating substrate-associated difference electron density, we decided to explore other occupancy combinations because of the discrepancy between the refined occupancy of the distal ligand (0.80) and the estimated occupancy of the thiocamphor orientation (0.30), which would be sterically compatible with the presence of the ligand. After calculating and examining maps based on occupancy combinations ranging from 0.30/0.70 to 0.80/0.20 in increments of 0.10, we concluded that the relative occupancies of thiocamphor orientations 1 and 2 (parts A and B of Figure 4, respectively) are probably around 65% and 35%, respectively, with an error of roughly 10%.

DISCUSSION

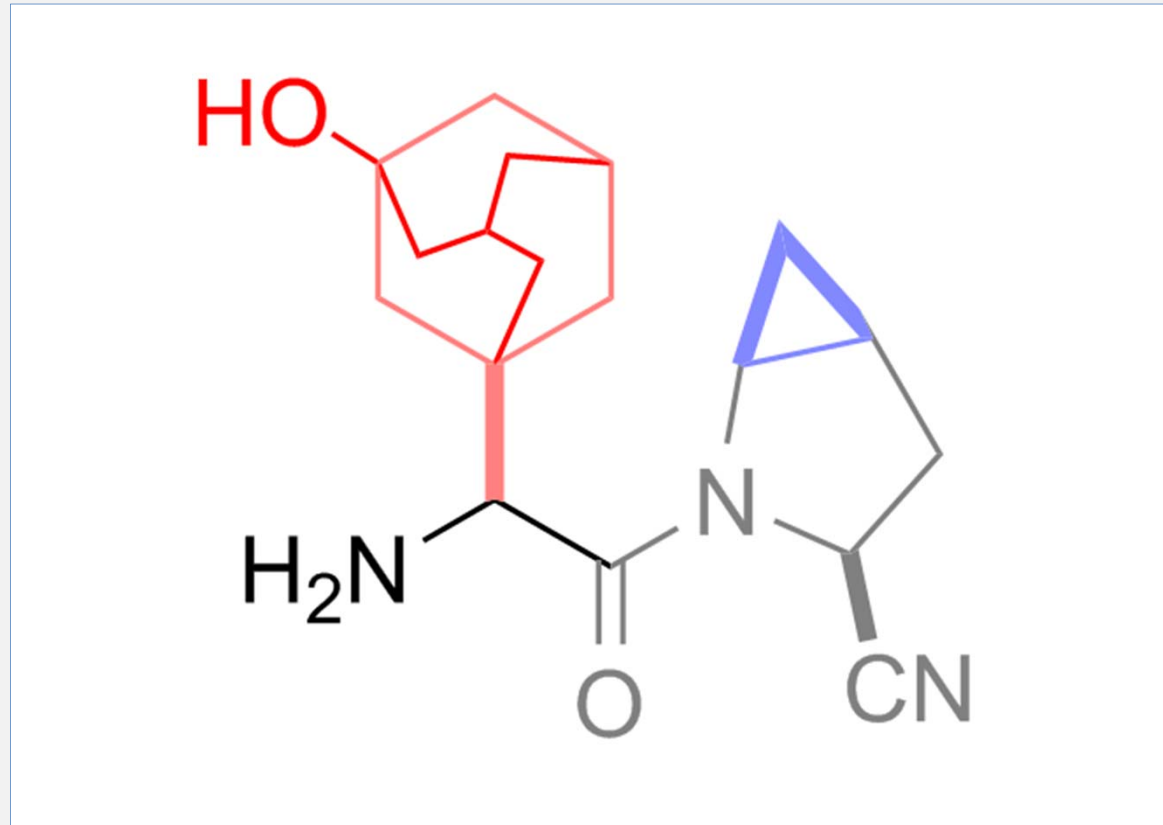
Substrate Hydroxylation Profiles

Camphane. Although camphane is incapable of hydrogen bonding with Tyr96, its similarity to camphor in overall shape and size causes it to be bound in a nearly identical position in the P-450_{cam} active site. The methyl groups of camphane and camphor interact with the same active site features. As with camphor, the 5-carbon atom of camphane is the nearest to the heme iron atom, explaining the observed preference (90% of products) for 5-exo hydroxylation of this substrate (Aikins & Sigar, 1988). That 10% of the products are 6-oxo hydroxylated (Aikins & Sigar, 1988) can be attributed to the enhanced mobility of camphane in the P-450_{cam} active site (Table III).

Hydroxylation profiles and crystallographic data on non-camphor- and camphane-P-450_{cam} complexes, in comparison with camphor complexes, demonstrate that two features, a hydrogen bond to the enzyme and complementary van der Waals interaction of a substrate, tallographic ter regioselectivity of a substrate. Adamantane is metabolized to a single product de actively high active site mobility. The single product can be attributed to the existence of only two types of unique carbon atoms in adamantane, together with the greater reactivity of tertiary versus secondary carbons (White et al., 1984).

Thiocamphor. Thiocamphor binds to P-450_{cam} in two orientations, both of which are different from that preferred by camphor and both of which have sulfur as the substrate atom nearest to iron. A priori, the proximity of the sulfur atom to the heme suggests that the thiocamphor hydroxylation mechanism might involve an initial single electron transfer from sulfur to heme instead of, or in competition with, initial hydrogen abstraction, as is thought to occur with camphor (Ortiz de Montellano, 1986). However, the major products of thiocamphor metabolism are 5- and 6-oxo hydroxylated, and these substrate atoms are among the farthest substrate atoms from the active oxygen location in our thiocamphor-P-450_{cam} model. Modeling of thiocamphor in the orientation preferred by camphor (with full occupancy) resulted in difference electron density maps strongly suggesting that such a model was incorrect. Nevertheless, the products of thiocamphor hydroxylation imply that this substrate is only metabolized when it adopts a camphor-like orientation in the active site. These data lead us to conclude that the conformers seen in the crystal structure are nonproductive and that the camphor-like conformer is fractionally occupied and crystallographically unobservable. Although thiocamphor appears to make a snug van der Waals fit with P-450_{cam}, it may be possible for it to occasionally rotate within the active site to yield a camphor-like complex, as suggested by molecular dynamics simulations of the Tyr96Phe mutant-camphor complex

Hydroxylated Adamantane Can Impede Metabolism



“Blocking metabolism at the 3-position would result in greater metabolic stability.”

Hydroxyadamantyl at P2 was Known in DPP-4 Inhibitors

United States Patent [19] Patent Number: 6,166,063
 Villhauer [45] Date of Patent: Dec. 26, 2000

US00166063A

[54] N-SUBSTITUTED GLYCYL-2-CYANOPYRROLIDINES, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN INHIBITING DIPEPTIDYL PEPTIDASE-IV

[75] Inventor: Edwin Bernard Villhauer, Morrisown, N.J.

[73] Assignee: Novartis AG, Basel, Switzerland

[21] Appl. No.: 09/488,224

[22] Filed: Dec. 9, 1999

Related U.S. Application 1

[65] Continuation of application No. 09/209,488 filed

[51] Int. Cl.⁷: A61K 31/07(2006)

[51] U.S. Cl.: 314.423.5

[56] Field of Search

[50] References Cited

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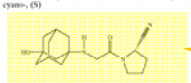
inhibition, such compositions in unit dosage form and such compositions comprising a pharmaceutically acceptable carrier.

The compounds of formula I (including those of each of the subtypes thereof and each of the examples) may be administered in enantiomerically pure form (e.g., >98% enantiomerically pure) or together with the B enantiomer, e.g., in racemic form. The above dosage ranges are based on the compounds of formula I (excluding the amount of the B enantiomer).

The following examples show representative compounds encompassed by this invention and their synthesis. However, it should be clearly understood that they are for purposes of illustration only.

EXAMPLE 1

Pyrimidine, 1-[(3-hydroxy-1-adamantylamino)acetyl]-2-cyano-, (S)



A. 1-Aminoadamantane-3-ol

Slight modifications to the synthesis found in Klein, Farm. Zs. (1980), 20(7), 910-15, may be used.

To a rapidly stirred, clear and colorless, ice-water chilled mixture of concentrated sulfuric acid (210 mL, 3.943 mmol) and 65% nitric acid (21.0 mL, 217.0 mmol) is added 21.0 g (112.0 mmol) of 1-adamantylamine HCl (99%), in small portions over 30 minutes. Upon subsequent hydrochloride addition, slight bubbling occurs and the reaction is slightly exothermic. This bubbling, yellow solution is stirred at ice-water temperature for about 2 hours and then at room temperature for 30 hours. This clear, light yellow reaction is then poured into about 100 g of ice and the resulting solution is clear green-blue.

The solution is placed in an ice-water bath and allowed to stir for 30 minutes. Approximately 550 g of 89% pure KOH (6.74 mol) is then added in small portions over 45 minutes. During this addition, the reaction is exothermic, reaching 80° C. and producing copious amounts of brown NO₂ gas. By the end of the addition, the reaction is thick with white solids (both product and salts). The resulting white paste is then poured into a beaker furnished with a pad and washed with 1.2 L of CH₂Cl₂. The CH₂Cl₂ layer is then extracted from the water layer and dried over Na₂SO₄. The solution is then filtered and concentrated (vacuum) to provide 1-aminoadamantane-3-ol as a white solid.

Alternatively, the reaction may be carried out using n-butane as solvent instead of methylene chloride.

Alternatively, 1-aminoadamantane-3-ol can be prepared e.g. as follows: A 2-L, 4-necked, round-bottomed flask is thoroughly flushed with nitrogen. The flask is charged under nitrogen with 420 mL of conc. sulfuric acid (98%). The content are cooled to 8° C., then slowly (slightly exothermic) a 10% (w/v) solution of 100.0 g (1.000 mol) of 1-aminoadamantane hydrochloride are added into the mixture in 8 portions at 9-10° C. over 20 min (minutes), then the binary contents are stirred at 9-10° C. for 20 min to obtain a homogeneous mixture. 72 mL of conc. (concentrated) nitric acid (70%) are added (very exothermic) dropwise into the mixture maintaining inner temperature at 14-15° C. with efficient cooling (at this scale 20 min. needed for this addition). The mixture is stirred at 14-15° C. for 20 min, the temperature is allowed to raise to 25° C. over 1 h (hour) (15-20° C. for 30 min, and 20-25° C. for 30 min), then the contents are stirred at 24-25° C. for 5 h (external cooling is needed). 1.7 L of water are charged into a 5 L, 4-necked flask, the water is cooled to 10° C., then the reaction mixture is slowly poured (very exothermic, some NO₂ gas evolution) over 25 min, maintaining the inner temperature below 35° C. to give a blue-green homogeneous solution. The original 2-L flask (slightly exothermic) is rinsed once with 0.3 L of water and the water wash is poured into the 5-L flask. Slowly 900 mL of 50% sodium hydroxide aqueous solution are added (very exothermic, some NO₂ gas evolution) into the 5-L flask over 30 min, at 65-70° C. to bring the pH of the mixture to 13. 100 mL of 1-butanol and 200 mL of toluene are added (exothermic) under vigorous stirring and allow the mixture to reach 90° C. The bottom aqueous layer is separated for proper disposal. The organic layer is once washed with 100 mL of saturated sodium chloride solution. The saturated sodium chloride wash is saved for disposal. The organic layer is concentrated at 40-45° C. over 6-8 h (hours) (40-45° C. for 2 h, 40-45° C. for 4 h, 40-45° C. for 2 h).

The solids are filtered.

Butanol dried, then once with 80 mL of heptane. The filtrate is dried at 40-45° C. in a rotary evaporator. The filtrate is dried at 40-45° C. for 10 h to afford 1-aminoadamantane-3-ol as a white solid.

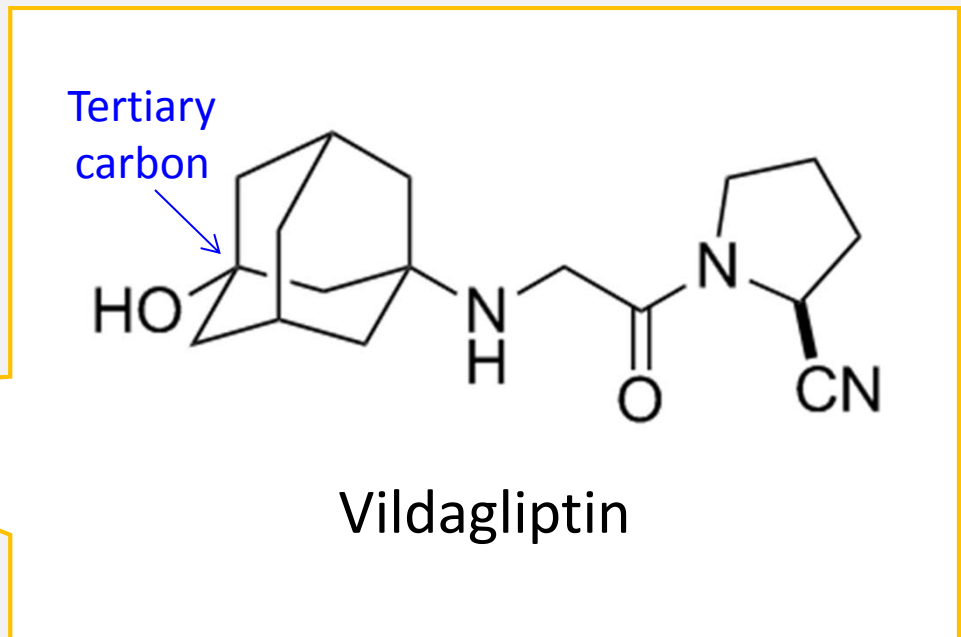
B. 1-Chloroacetyl-2-cyanopyrimidine

To a mechanically stirred solution of 20.0 g (180.0 mmol) of dichloroacetyl chloride and 97 g (69.70 mmol) of potassium carbonate in 150 mL of acetonitrile is added a solution of 1-prolineamide 20.0 g (180.0 mmol) in 500 mL of tetrahydrofuran in a dipropyl ether over 45 minutes. This reaction is then mechanically stirred for an additional two hours at room temperature. The reaction is then filtered to remove potassium salts and the filtrate is dried over Na₂SO₄. The Na₂SO₄ is then removed via filtration and to this solution filtrate is added trifluoroacetic anhydride (25.0 mL, 0.180 mmol) in one portion. The reaction is then magnetically stirred for 1 hour at room temperature and the resulting clear yellow-orange solution is concentrated via rotovap. The excess trifluoroacetic anhydride is removed by adding ethyl acetate to the concentrated oil and nonconcentrating via rotovap. This removing operation is performed three times. The resulting oil is partitioned between ethyl acetate and water. The product is then extracted into the ethyl acetate and the aqueous layer is then washed twice with ethyl acetate. The combined organic layers are then washed successively with water and being dried over magnesium sulfate, filtered and concentrated to obtain 1-chloroacetyl-2-cyanopyrimidine as a yellow solid.

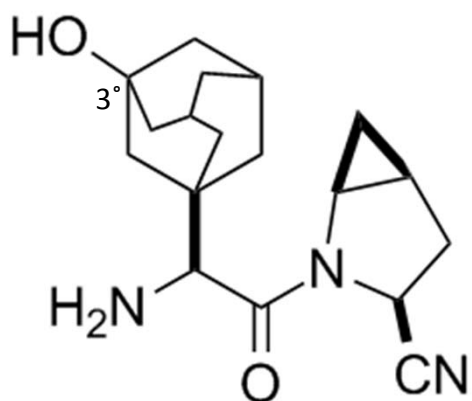
Alternatively, the reaction may be carried out by using, as a mixture, e.g. 2-ethyl-hexanoic acid sodium hydride, C. Pyrimidine, 1-[(3-hydroxy-1-adamantylamino)acetyl]-2-cyano-, (S)

To a heterogeneous solution of the title A compound (1.000 mol) and HCl gas evolution) 100.0 g (1.000 mol) in CH₂Cl₂ (60.0 mL) is added 9.6 g (69.9 mmol) of K₂CO₃. This heterogeneous mixture is then cooled in an ice-water bath and a solution of 30.0 (1.0 mmol) of the title B compound (1-chloroacetyl-2-cyanopyrimidine) dissolved in 25.0 mL of CH₂Cl₂ is added dropwise over a period of 30 minutes. The resulting mixture is stirred for 2 hours at 0° C. and at room temperature for 6-days. The reaction is then concentrated at

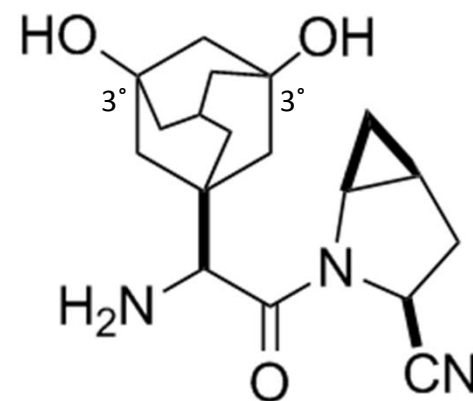
Page 5 of 12



Saxagliptin is also Metabolized at a 3° Carbon



Saxagliptin



Saxagliptin Metabolite M2

M2 metabolite predictably results from a second oxidation at one of only two remaining tertiary adamantyl carbons.

Summary of Structural Modifications

- Cyclopropanate the pyrrolidine ring

- Smallest possible fusion confers conformational rigidity and modulate position of cyano group

Source: EX1007 (Ashworth I) at 1163

EX1010 (Hanessian) at 1882

- Replace cyclohexyl ring with hydroxyadamantyl

- Sterically bulky substituent favors *trans* conformation to maintain or improve stability and prevent intramolecular cyclization; potential to improve potency

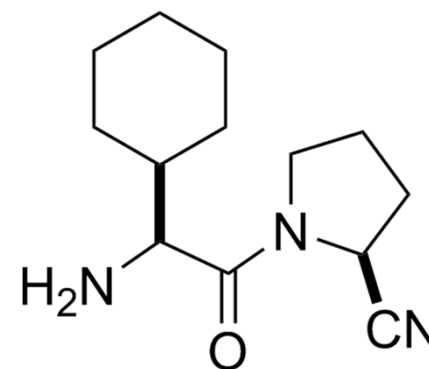
Source: EX1007 (Ashworth I) at 1163

EX1008 (Villhauer) at 13

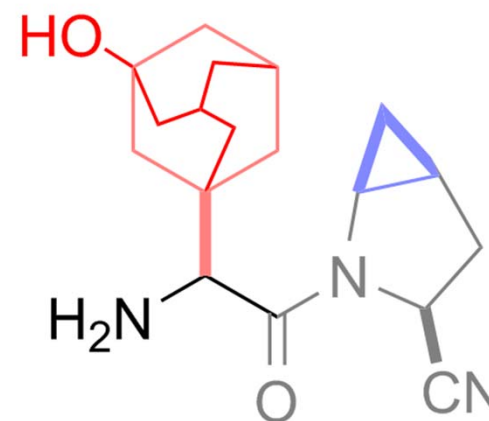
- Routine evaluation of metabolites

Source: EX1009 (Raag)

Ashworth 25



Saxagliptin



Secondary Considerations Don't Overcome *Prima Facie* Case

- Did not meet a long-felt need
- No commercial success
- No evidence of failure of others
- No unexpected results

Metformin is Still the Most Preferred Anti-Diabetic Drug



“Metformin has the strongest evidence base and demonstrated long-term safety as pharmacological therapy for diabetes prevention.”

“In my opinion, there is no drug (including saxagliptin) that stands out above the others as the second best choice alternative to metformin.”

Metformin is Essential Medicine for Type 2 Diabetes

WHO Model List
of
Essential Medicines

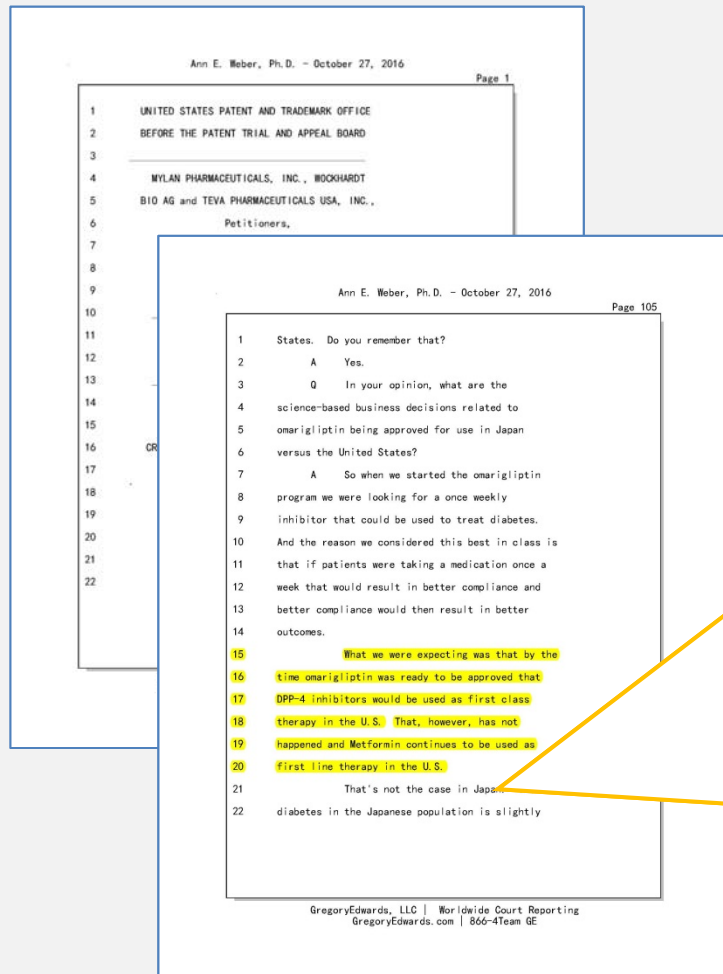
The World Health Organization DOES NOT list saxagliptin as an essential medicine in the treatment of Type 2 Diabetes.

18.5 Insulins and other medicines used for diabetes

□ gliclazide*	Solid oral dosage form: (controlled-release tablets) 30 mg; 60 mg; 80 mg. * glibenclamide not suitable above 60 years.
glucagon	Injection: 1 mg/ mL.
insulin injection (soluble)	Injection: 40 IU/ mL in 10- mL vial; 100 IU/ mL in 10- mL vial.
intermediate-acting insulin	Injection: 40 IU/ mL in 10- mL vial; 100 IU/ mL in 10- mL vial (as compound insulin zinc suspension or isophane insulin).
metformin	Tablet: 500 mg (hydrochloride).
Complementary List [c]	
metformin	Tablet: 500 mg (hydrochloride).

This is a reprint
<http://www.who.int>

Metformin is Still the Most Preferred Anti-Diabetic Drug



“What we were expecting was that by the time omarigliptin was ready to be approved that DPP-4 inhibitors would be used as first class therapy in the U.S. That, however, has not happened and Metformin continues to be used as first line therapy in the U.S.”

Source: EX1073 (Weber Depo. Trans.), 105:15-20;
EX1041 (Tanenberg Decl.), ¶¶20-21; Pet. Reply at 23.

Vildagliptin was Invented Before Saxagliptin

United States Patent [19] [11] Patent Number: **6,166,063**
Villhauer [45] Date of Patent: **Dec. 26, 2000**

[54] **N-SUBSTITUTED GLYCYL-D-CYANOPIPERIDINES, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THE INHIBITING Dipeptidyl PE**

[75] Inventor: **Edwin Bernard Villhauer**

[73] Assignee: **Novartis AG, Basel, S**

[21] Appl. No.: **00488,224**

[22] Filed: **Dec. 9, 1999**

Related U.S. Application 1

[63] Continuation of application No. 09/209,488, abandoned.

[51] Int. Cl.⁷: **A61K 31/07**

[52] U.S. Cl.: **A14423.3**

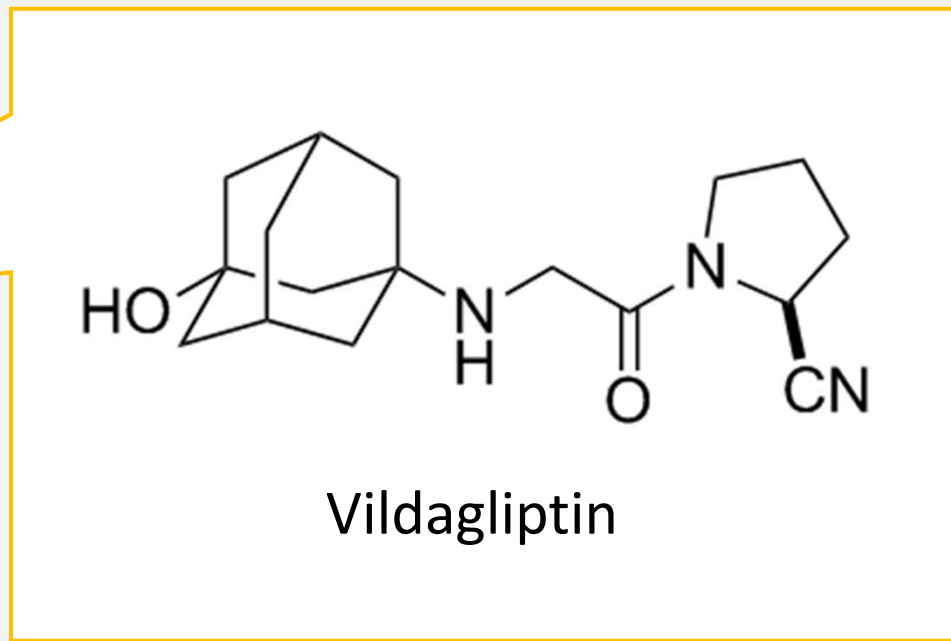
[53] Field of Search:

[56] **References Cited**

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 FOREIGN PATENT DOCUMENTS
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 1531 09 12/1982 Germany
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 W09112063 03/1991 WIPO
 W09116339 03/1991 WIPO
 W09116259 03/1991 WIPO
 W09112499 03/1991 WIPO
 W09113389 03/1991 WIPO
 W09120109 11/1991 WIPO
 W09120691 11/1991 WIPO
 W09120438 12/1991 WIPO
 W09119098 03/1991 WIPO
 W09108001 8/1999 WIPO

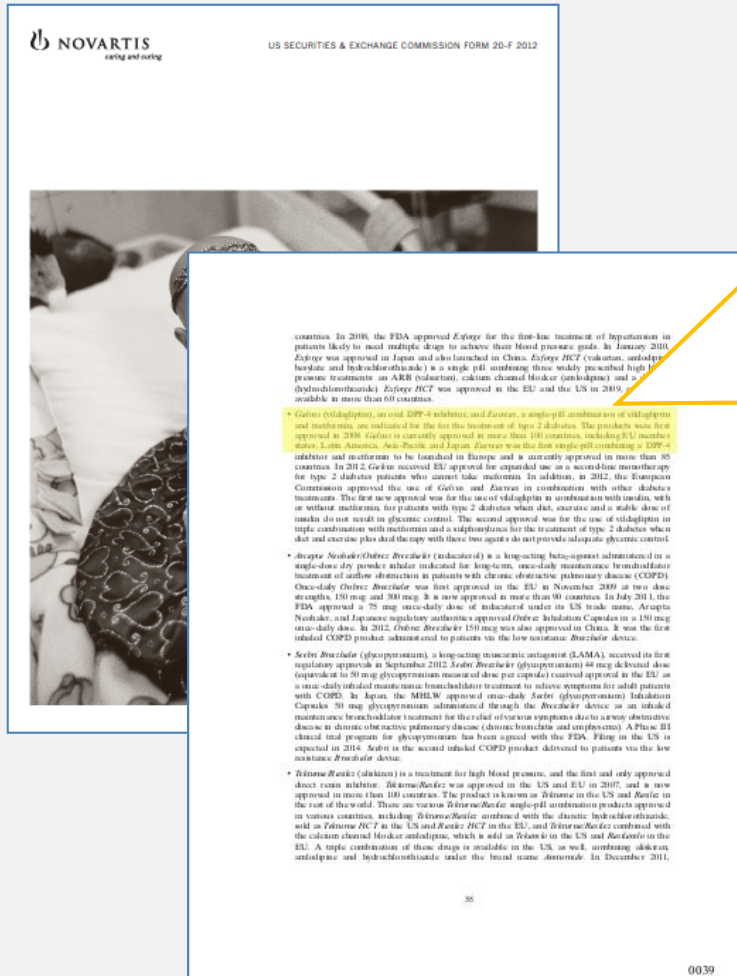
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 Li, et al. Journal of Neuroscience, vol. 6 (1996).

Page 1 of 12



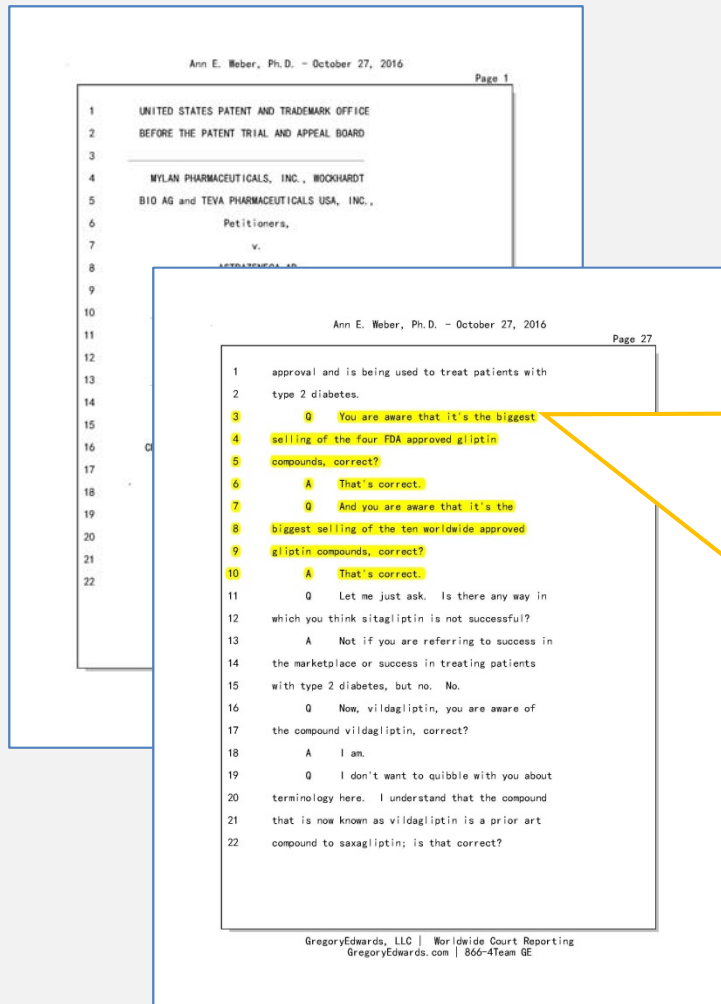
Source: EX2013 (Villhauer 2000), 7:15-27; Pet. Reply at 14.

Vildagliptin Received Regulatory Approval Before Saxagliptin



“Galvus (vildagliptin) , an oral DPP-4 inhibitor, and Eucreas, a single-pill combination of vildagliptin and metformin, are indicated for the treatment of type 2 diabetes. The products were first approved in 2008. Galvus is currently approved in more than 100 countries[.]”

Sitagliptin is the Highest Selling Gliptin



Q You are aware that it's the biggest selling of the four FDA approved gliptin compounds, correct?

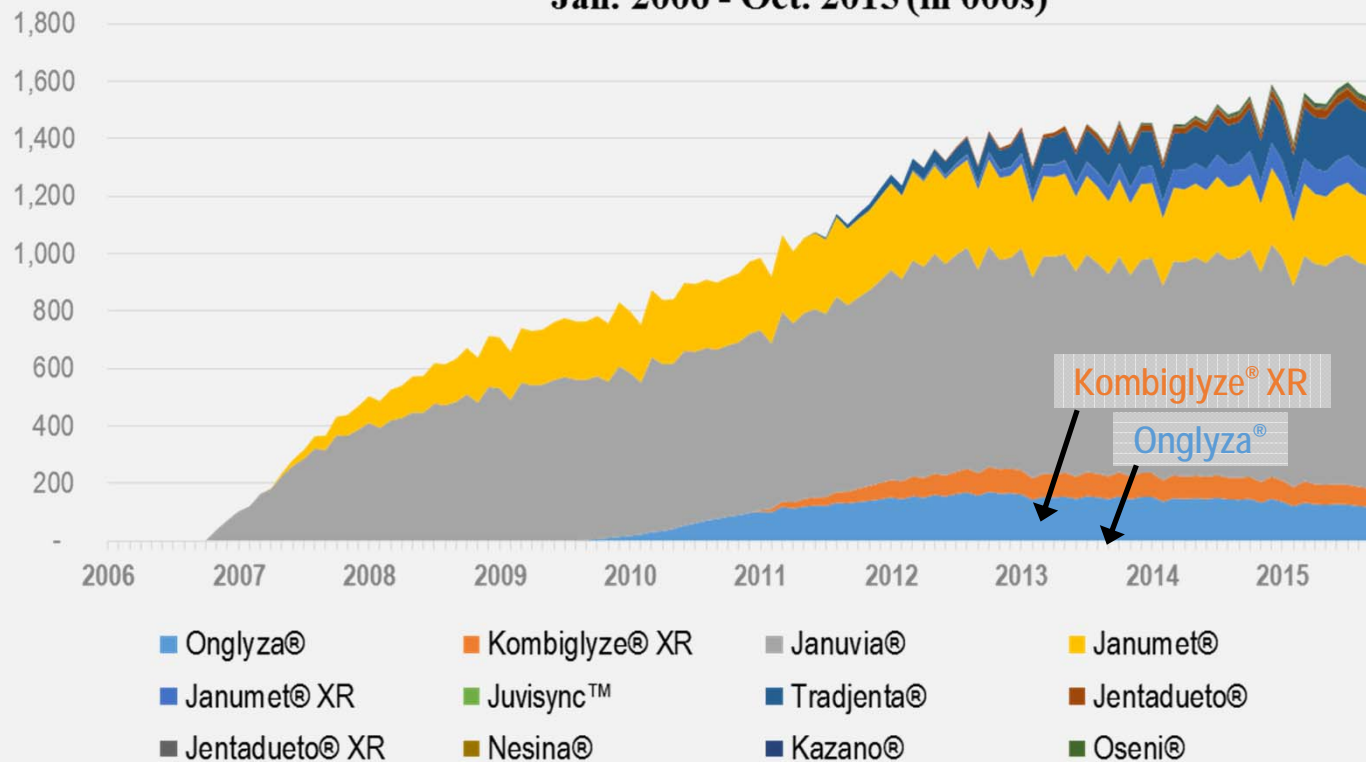
A That's correct.

Q And you are aware that it's the biggest selling of the ten worldwide approved gliptin compounds, correct?

A That's correct.

Saxagliptin Under-Performs Sitagliptin (Januvia®)

DPP-4 Inhibitor Total Prescriptions
Jan. 2006 - Oct. 2015 (in 000s)



Saxagliptin products failed to capture a substantial share of the U.S. DPP-4 inhibitor market.

Source: EX1060B (McDuff Decl.), ¶120; EX1062B (McDuff Attachments) at B-3; EX1035, ¶136; EX2117 (IMS Health Audit); EX1029 (Meyer Depo. Trans.), 422:4-13.

Large and Increasing Rebates for Saxagliptin

Onglyza®							
	2009	2010	2011	2012	2013	2014	2015
Net Sales Adjustments as a % of Gross Sales	11%	32%	33%	37%	35%	54%	66%

Kombiglyze® XR						
	2010	2011	2012	2013	2014	2015
Net Sales Adjustments as a % of Gross Sales	26%	33%	37%	38%	53%	57%

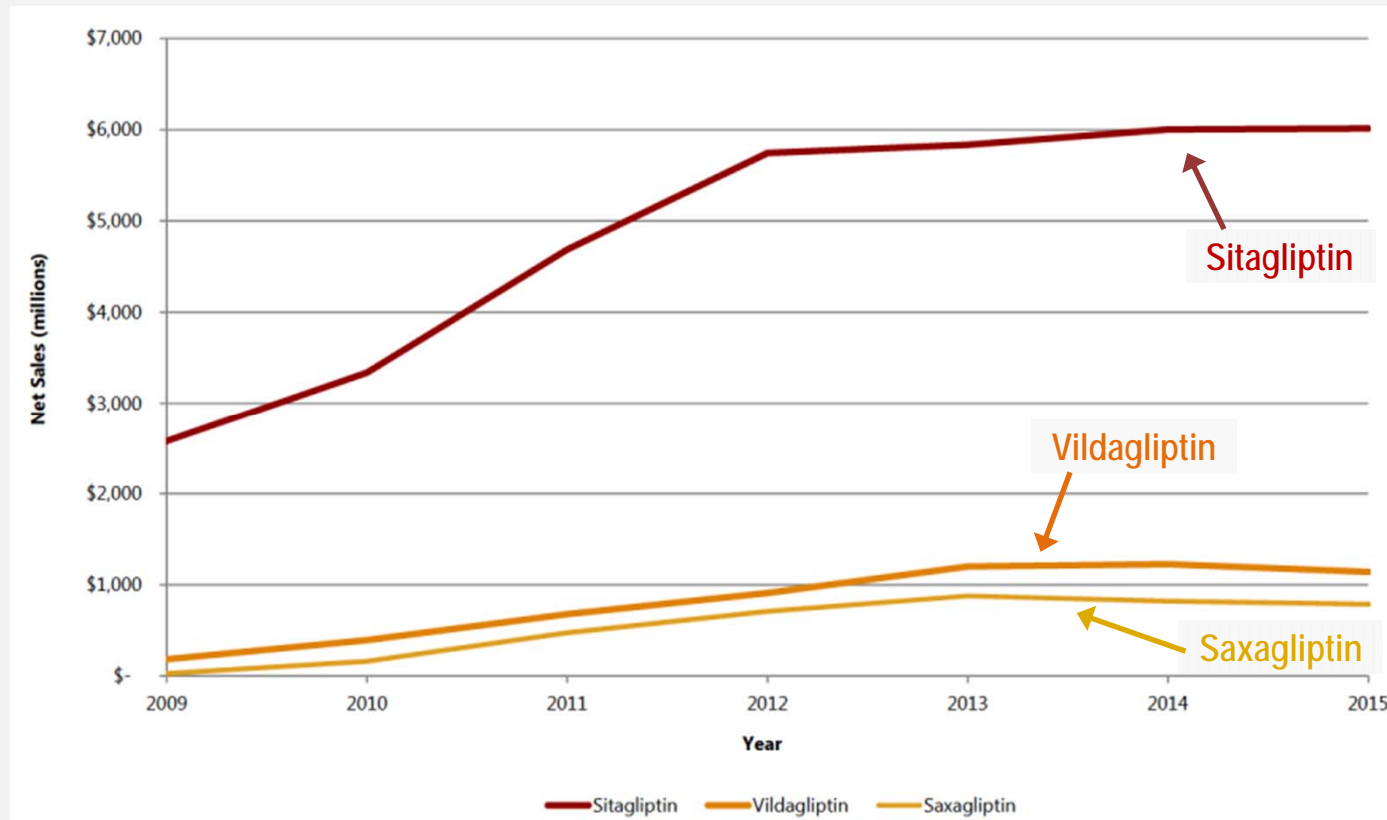
Net sales adjustments are a large percentage of gross sales and have increased continuously and significantly from launch through 2015.

Large Marketing Expenditure on Saxagliptin

Product	Measure	2009 (Jul - Dec)	2010	2011	2012	2013	2014	2015 (Jan - Jul)	Total (2009-2015)	Total (2011-2015)
Promotional Spending (millions)	Onglyza	\$ 44.1	\$ 128.6	\$ 85.5	\$ 65.8	\$ 55.0	\$ 35.7	\$ 14.6	\$ 429.3	\$ 256.7
	Kombiglyze XR	\$ -	\$ 0.5	\$ 54.1	\$ 38.2	\$ 33.1	\$ 11.1	\$ 5.0	\$ 141.9	\$ 141.4
	Total	\$ 44.1	\$ 129.1	\$ 139.5	\$ 104.0	\$ 88.1	\$ 46.8	\$ 19.6	\$ 571.3	\$ 398.1
US Sales (millions)	Onglyza	\$ 13.3	\$ 150.5	\$ 361.1	\$ 481.8	\$ 524.8	\$ 579.0	\$ 338.5	\$ 2,449.0	\$ 2,285.2
	Kombiglyze XR	\$ -	\$ 0.3	\$ 73.0	\$ 200.0	\$ 253.1	\$ 288.1	\$ 180.0	\$ 994.5	\$ 994.2
	Total gross sales	\$ 13.3	\$ 150.8	\$ 434.1	\$ 681.8	\$ 778.0	\$ 867.1	\$ 518.5	\$ 3,443.5	\$ 3,279.4
	Total net sales	\$ 22.0	\$ 119.0	\$ 339.0	\$ 516.0	\$ 591.0	\$ 481.0	\$ 420.0	\$ 2,488.0	\$ 2,347.0
Marketing Share Sales	Onglyza	330.7%	85.5%	23.7%	13.7%	10.5%	6.2%	4.3%	17.5%	11.2%
	Kombiglyze XR	n/a	163.3%	74.1%	19.1%	13.1%	3.8%	2.8%	14.3%	14.2%
	Total gross sales	330.7%	85.6%	32.1%	15.3%	11.3%	5.4%	3.8%	16.6%	12.1%
	Total net sales	200.4%	108.5%	41.2%	20.2%	14.9%	9.7%	4.7%	23.0%	17.0%

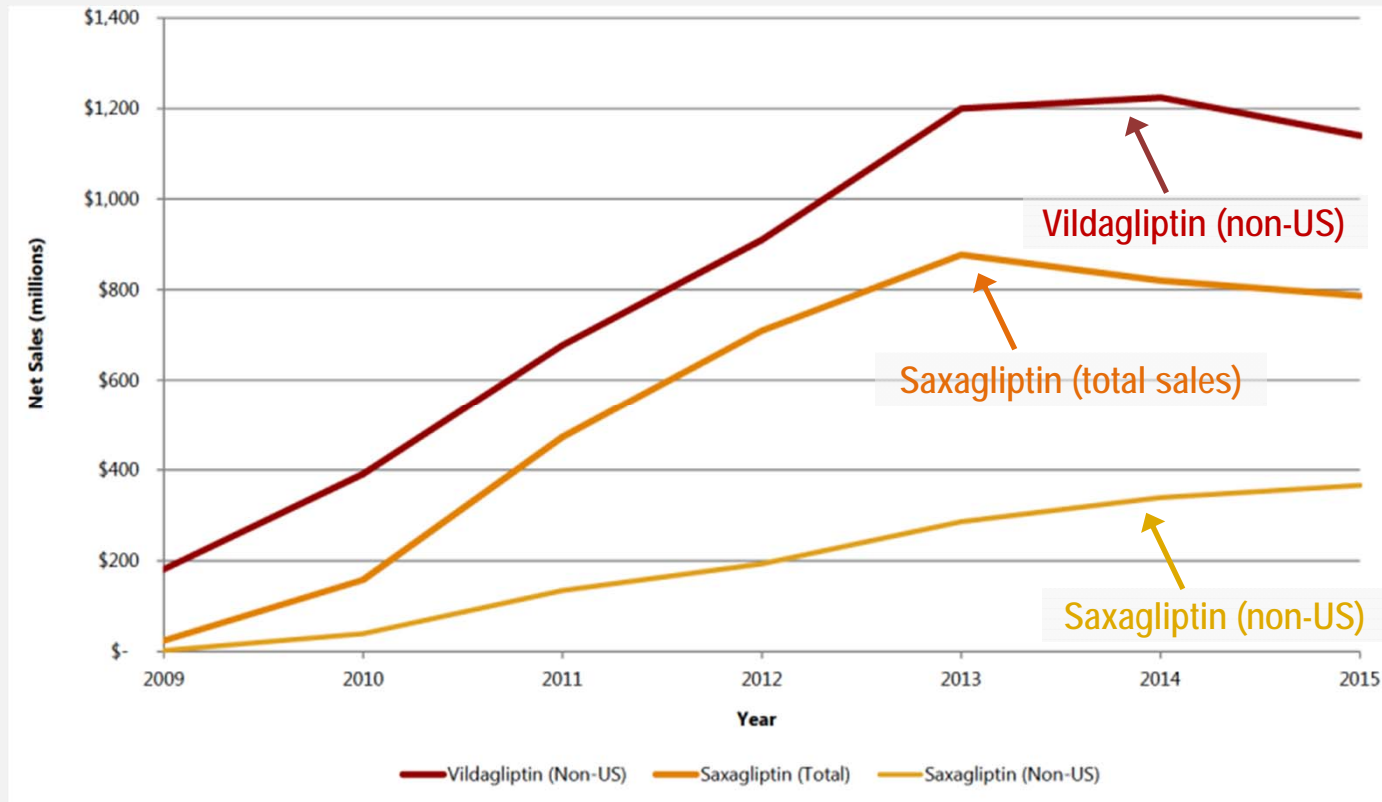
\$571 million in promotional expenditures on Onglyza and Kombiglyze XR in the U.S. from 2009 to 2015, which is 23.0% of total U.S. sales over the same time period.

Saxagliptin Also Underperforms Vildagliptin (Galvus)



Worldwide sales of vildagliptin beat worldwide sales of saxagliptin.

Saxagliptin Also Underperforms Vildagliptin (Galvus)



Even without U.S. sales, worldwide sales of vildagliptin beat worldwide sales of saxagliptin.

They Call Vildagliptin a “Failure”

Ann E. Weber, Ph.D. - October 27, 2016 Page 1

1 UNITED STATES PATENT AND TRADEMARK OFFICE
2 BEFORE THE PATENT TRIAL AND APPEAL BOARD
3
4 MYLAN PHARMACEUTICALS, INC., BOOHHARDT
5 BIO AG and
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Ann E. Weber, Ph.D. - October 27, 2016 Page 23

1 was never advanced to any clinical trials,
2 correct?
3 A I'm not aware of any clinical trials
4 that were conducted
5 Q And this
6 approved, correct?
7 A That's c
8 Q So under
9 and we will get to s
10 but this compound w
11 failure of other
12 this case, correct?
13 A So this
14 that it was not FDA
15 standard I used for
16 would be a failure.
17 Q So just
18 applied for your ase
19 was FDA approval, co
20 A That is
21 Q And was
22 were asked to apply

Ann E. Weber, Ph.D. - October 27, 2016 Page 28

1 A That is correct.
2 Q Now, I recognize that at the time,
3 October 2000, vildagliptin was not known commonly
4 as vildagliptin but rather the structure of the
5 compound itself was known at that point, correct?
6 A That is correct.
7 Q Okay.
8 Vildagliptin, under your standard in
9 this case, is a failure, correct?
10 A That is correct.
11 Q Now, vildagliptin is approved in
12 Europe for treatment of type 2 diabetes as a DPP-4
13 inhibitor, correct?
14 A Vildagliptin is approved in Europe.
15 Q Okay.
16 Going back to the patent, you were
17 talking about Exhibit 1001. We were talking about
18 the compounds listed under claim 8. We had just
19 finished discussing the first compound under
20 claim 8. I would like to ask the same series of
21 questions with regard to the second compound.
22 So with respect to the second

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GregoryEdwards.com | 866-4Team GE

Q So under your analysis in this case, and we will get to some of this a little later, but this compound would be a failure, exhibit a failure of others in connection with your work in this case, correct?

A So this compound by virtue of the fact that it was not FDA approved, and that was the standard I used for failure of others, yes, this would be a failure.

Q Okay.

Vildagliptin, under your standard in this case, is a failure, correct?

A That is correct.

Best-in-class Glyptin “a Failure”

Ann E. Weber, Ph.D. - October 27, 2016 Page 1

1 UNITED STATES PATENT AND TRADEMARK OFFICE
2 BEFORE THE PATENT TRIAL AND APPEAL BOARD

3

4 MYLAN PHARMACEUTICALS, INC., BODKHARDT
5 BIO AG and TEVA PHARMACEUTICALS USA, INC.,
6 Petitioners,
7 v.
8 ASTRAZENECA AB,
9 Patent Owner.

Ann E. Weber, Ph.D. - October 27, 2016 Page 100

15 1 omarigliptin?
16 2 A We use that to describe omarigliptin.
17 3 Let me just take a step back. At the time when we
18 4 were discovering or working on the omarigliptin
19 5 program our goal was to identify a best in class
20 6 inhibitor.
21 7 Q Now, the omarigliptin, I think you
22 8 just said something along the lines of the program
9 to determine or discover omarigliptin. It's the
10 same program that had initially determined
11 sitagliptin, correct?
12 A If you mean by the same program, if
13 you're talking about DPP-4 inhibition as a general
14 program, yes, it is also a DPP-4 inhibitor.
15 Q And omarigliptin is not approved in
16 the United States, correct?
17 A That is correct.
18 Q And so under your analysis in this
19 case omarigliptin would be a failure?
20 A That is correct.
21 Q Now, you're aware that there are lots
22 of reasons why a company might elect not to pursue

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GregoryEdwards.com | 866-4Team GE

Dr. Weber's own “best in class of the gliptins” omarigliptin qualifies as an FDA-approval “failure.”

Q And omarigliptin is not approved in the United States, correct?

A That is correct.

Q And so under your analysis in this case omarigliptin would be a failure?

A That is correct.

FDA-Approved DPP-4 Inhibitors are Interchangeable

169

1 IN THE UNITED STATES DISTRICT COURT
2 IN AND FOR THE DISTRICT OF DELAWARE
3
4 ASTRAZENECA AB,) Civil Action
5)
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1 possibilities of events.
2 Q. Now, Doctor, do
3 interchangeable?
4 A. Relatively.
5 Q. So if a patient we
6 wanted to switch them to
7 Side effects?
8 A. Correct. If a pat
9 one DPP-4 inhibitor and
10 asked to go back on it,
11 again. I might use a di
12
13
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25

Q. Now, Doctor, do you view DPP-4 inhibitors as interchangeable?

A. Relatively.

Q. But generally side effect profile, as you testified earlier, are the same for the class; is that correct?

A. Correct.

Q. Now, you said that the side effect profile for DPP-4 inhibitors, as you testified earlier, are the same for the class; is that correct?

A. They have a favorable side effect profile; is that correct?

Q. And do you agree that DPP-4 inhibitors, as you testified earlier, are clinically effective?

A. I'm sorry. You could rephrase that?

FDA-Approved DPP-4 Inhibitors are Interchangeable

169

1 IN THE UNITED STATES DISTRICT COURT
2 IN AND FOR THE DISTRICT OF DELAWARE

3
4 Lenhard - cross

5 1 Q. You testified earlier that saxagliptin can be a
6 first-
7 2 A.
8 3 Q.
9 4 A. class
10 5 A.
11 6 A. have s
12 7 Q. could
13 8 Q.
14 9 Q. could
15 10 A.
16 11 Q.
17 12 saxagl
18 13 favora
19 14 effica
20 15 A.
21 16 Q.
22 17 class
23 18 A.
24 19 Q.
25 20 A.
21 22 Q.
22 23 any of
23 24 A.
24 25 tabula

1 Q. Is there one that you
2 others?
3 A. Not that I am
4 Q. It's your opinion that the
5 difference in the side effect pr
6 inhibitors on the market. Is that correct?
7 A. Correct.
8 Q. Now, are you aware, Doctor
9 required that a warning be added
10 A. Yes.
11 Q. Do you know what the cost
12 A. There
13
14 A. Okay.
15 Q. So this was an FDA warning
16 Is that correct?
17 A. Yes, it is.
18 Q. You see in the first parag
19 says, A U.S. food and drug Admin
20 review has found that type 2 dia
21 saxagliptin and alogliptin may i
22 disease, particularly in patient
23 kidney disease."

Q. It's your opinion that there is no meaningful difference in the side effect profile of the DPP-4 inhibitors on the market. Is that correct?

A. Correct.

Q. Now, Doctor, you also testified earlier that saxagliptin demonstrated unexpected results because of its favorable side effect profile of once-daily dosing and efficacy. Correct?

A. That's correct.

Q. That would apply to the other members of the DPP-4 class that have been FDA-approved. Correct?

A. That would.

FDA Requires Safety Warning on Saxagliptin Product Label

“Saxagliptin and alogliptin may increase the risk of heart failure, particularly in patients who already have heart or kidney disease. . . . As a result, we are adding new warnings to the drug labels about this safety issue.”

Sitagliptin does not have a heart failure warning.

FDA U.S. Food and Drug Administration
Drug Safety Communication

FDA Drug Safety Communication: FDA adds warnings about heart failure risk to labels of type 2 diabetes medicines containing saxagliptin and alogliptin

This is an update to the FDA Drug Safety Communication: FDA adds warnings about heart failure risk to labels of type 2 diabetes medicines containing saxagliptin (marketed as Onglyza and Kombi) and alogliptin (marketed as Nesina) on February 11, 2014.

Safety Announcement

[4-5-2016] A U.S. Food and Drug Administration (FDA) Drug Safety Communication (DSC) has found that saxagliptin and alogliptin may increase the risk of heart failure, particularly in patients who already have heart or kidney disease. Heart failure can result in the heart not being able to pump enough blood to meet the body's needs. As a result, we are adding new warnings to the drug labels about this safety issue.

Saxagliptin and alogliptin are part of the class of dipeptidyl peptidase-4 (DPP-4) inhibitor drugs, which are used with diet and exercise to lower blood sugar in adults with type 2 diabetes. Untreated, type 2 diabetes can lead to serious health problems, including blindness, nerve and kidney damage, and heart disease (see List of saxagliptin- and alogliptin-containing Medicines).

Patients taking these medicines should contact their health care professionals right away if they develop signs and symptoms of heart failure such as:

- Unusual shortness of breath during daily activities
- Trouble breathing when lying down
- Tiredness, weakness, or fatigue
- Weight gain with swelling in the ankles, feet, legs, or stomach

Patients should not stop taking their medicine without first talking to their health care professionals.

Health care professionals should consider discontinuing the medicine in patients who develop heart failure and monitor their diabetes control. If a patient's blood sugar level is not well-controlled with their current treatment, other diabetes medicines may be required.

JOINT TRIAL EXHIBIT
JTX-146
114-cv-464-GMS

MYLAN - EXHIBIT 1032
Mylan et al. v. AstraZeneca
IPR2015-01340
JTX-146 Page 1 of 4

LEONARD
DEPOSITION
EXHIBIT
2/11/14

Source: EX1032 (FDA Drug Safety Communication);
EX1041 (Tanenberg Decl.), ¶26; EX1060 (McDuff Decl.), ¶35.

Vildagliptin Also Has a Once-Daily Combination Form

4.2 Posology and method of administration

Posology

Adults

When used as monotherapy, in combination with metformin, in combination with thiazolidinedione, in combination with metformin and a sulphonylurea, or in combination with insulin (with or without metformin), the recommended daily dose of vildagliptin is 100 mg, administered as one dose of 50 mg in the morning and one dose of 50 mg in the evening.

When used in dual combination with a sulphonylurea, the recommended dose of vildagliptin is 50 mg once daily administered in the morning. In this patient population, vildagliptin 100 mg daily is more effective than vildagliptin 50 mg once daily.

When used in combination with a sulphonylurea, a lower dose of the sulphonylurea may be considered to reduce the risk of hypoglycaemia.

Doses higher than 100 mg are not recommended.

If a dose of Galvus is missed, it should be taken as soon as the patient remembers. A double dose should not be taken on the same day.

The safety and efficacy of vildagliptin as triple oral therapy in combination with metformin and a thiazolidinedione have not been established.

Additional information on special populations

Elderly (≥ 65 years)

No dose adjustments are necessary in elderly patients (see also sections 5.1 and 5.2).

Renal impairment

No dose adjustment is required in patients with mild renal impairment (creatinine clearance ≥ 50 ml/min). In patients with moderate or severe renal impairment or with end-stage renal disease (ESRD), the recommended dose of Galvus is 50 mg once daily (see also sections 4.4, 5.1 and 5.2).

Hepatic impairment

Galvus should not be used in patients with hepatic impairment, including patients with pre-treatment alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 3x the upper limit of normal (ULN) (see also sections 4.4 and 5.2).

Paediatric population

Galvus is not recommended for use in children and adolescents (< 18 years). The safety and efficacy of Galvus in children and adolescents (< 18 years) have not been established. No data are available (see also section 5.1).

Method of administration

Oral use

Galvus can be administered with or without a meal (see also section 5.2).

4.3 Contraindications

Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.

“When used in dual combination with a sulphonylurea, the recommended dose of vildagliptin is 50 mg once daily administered in the morning.”

SUMMARY

Page 1 of 36

Page 3 of 36

3

Additional Slides

Patent Owner's Cyanopyrrolidine Arguments

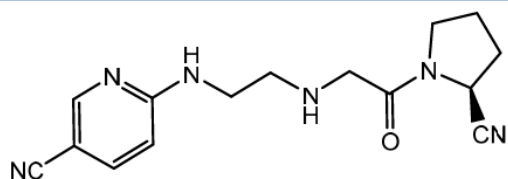


Figure 3: NVP-DPP728

1. NVP-DPP728 and P32/98 were more plausible leads

Case No. IPR2015-01340
Patent RE44,186
compound, vildagliptin, into the clinic. Ex. 2056, ¶¶146, 252; Ex. 2098, 4138.
Vildagliptin, described in the prior art U.S. Patent No. 6,166,063 (Ex. 2013), also had the stabilizing *N*-linkage but ultimately failed to obtain FDA approval. It is approved in Europe but only for administration twice-daily and with a requirement for liver toxicity screening. Ex. 2056, ¶248; Ex. 2057, ¶¶67-70; Ex. 2050, 3-4. The structure of vildagliptin is shown in **Figure 5** below. See Ex. 2013, 5.

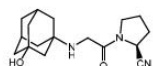


Figure 5: Vildagliptin

2. Merck's first clinical trial candidate P32/98

When Merck began medicinal chemistry on DPP-4 inhibitors, it performed a real-world lead compound analysis. Ex. 2056, ¶¶116-118. Merck scientists were concerned by the presence of a cyano group in the P1 position of Ashworth-I-type compounds because of the potential for cyclization and for toxic cyanide release should amide bond cleavage occur *in vivo*. Ex. 2056, ¶¶116-117; **Figure 6** below.

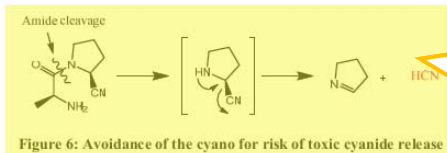


Figure 6: Avoidance of the cyano for risk of toxic cyanide release

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“But there were additional reasons to dismiss Compound 25 as a lead. For instance, the cyano group introduced the concern of toxic cyanide release *in vivo* (Ex. 2056, ¶162), leading Merck to dismiss cyanopyrrolidine compounds[.]”

Amide cleavage

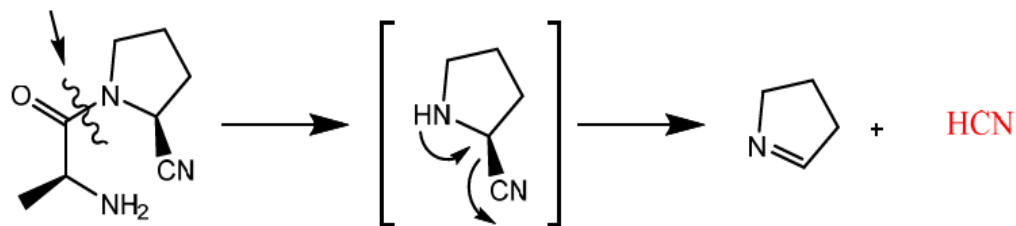


Figure 6: Avoidance of the cyano for risk of toxic cyanide release

Cyanopyrrolidines Do Not Release HCN

Ann E. Weber, Ph.D. - October 27, 2016 Page 1

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1 Q So let me make sure I understand. So
2 you do not criticize Dr. Rotella's sele
3 Ashworth
4 release; is
5 A So
6 cyanide release I wa
7 program at Merck, and rea
8 elected not to have a nitrile
9 Q And that is a concern
10 doesn't have anything to do with your
11 about Dr. Rotella's selection of compou
12 Exhibit 1007; is that your testimony?
13 A So there was no information
14 was no information. This was a hypothe
15 concern. And so my selection of a lead
16 was really based on the data that was k
17 prior art, which included clinical data
18 and NVP-DPP728 and very limited data on
19 hundreds, probably thousands of other m
20 that have been reported in the literatu
21 which compound 25 was one.
22 Q Well, if you turn to Page 6

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Q So let me make sure I understand. So you do not criticize Dr. Rotella's selection of Ashworth compound 25 for fear of toxic cyanide release; is that your testimony?

A So when I was talking about the toxic cyanide release I was actually describing our program at Merck, and really explaining why we had elected not to have a nitrile in the molecule.

Source: EX1073 (Weber Cross-Examination), 52:1-8.

Cyanopyrrolidines Do Not Release HCN

Ann E. Weber, Ph.D. - October 27, 2016 Page 1

1 UNITED STATES PATENT AND TRADEMARK OFFICE
2 BEFORE THE PATENT TRIAL AND APPEAL BOARD

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1 Q So let me make sure I
2 you do not criticize Dr. Rotella's
3 Ashworth compound 25 for fear of t
4 release; is that your testimony?
5
6 A So when I was talking
7 cyanide release I was actually des
8 program at Merck, and really expla
9 elected not to have a nitrile in t
10
11 Q And that is a concern
12 doesn't have anything to do with y
13 about Dr. Rotella's selection of c
14 Exhibit 1007; is that your testimo
15
16 A So t
17 was no information.
18 concern. And so my se
19 was really based on the e
20 prior art, which included o
21 and NVP-DPP728 and very limite
22 hundreds, probably thousands of o
23 that have been reported in the lit
24 which compound 25 was one.
25
26 Q Well, if you turn to P

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Q And that is a concern that -- that doesn't have anything to do with your criticisms about Dr. Rotella's selection of compound 25 from Exhibit 1007; is that your testimony?

A So there was no information -- there was no information. This was a hypothetical concern.

Ashworth I Table I Explores Optimal N-Terminal Residues

 Pergamon
Biorganic & Medicinal Chemistry Letters, Vol. 6, No. 10, pp. 1163-1166, 1996
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PII: S0960-894X(96)00190-4

2-CYANOPYRROLIDIDES OF
1165

Doreen M. Ashworth, Butrus
Ferring Research Institute
S016 7NP, Fax

Abstract: A novel series of dipeptide analogues, incorporating a non-proteinogenic amino acid, were synthesized and evaluated for their inhibitory activity versus human DP-IV and half-life in vivo.

Dipeptidyl peptidase IV (DP-IV) is a membrane-bound enzyme that cleaves dipeptides from the N-terminus of polypeptides (Y-Pro).¹ DP-IV is widely distributed in various tissues, including kidney, liver, intestinal epithelium and activation marker, CD26. Recent evidence² and of a main population of B cells.³

Our interest in DP-IV was based on its role as an inhibitor or antibodies of the enzyme in immunomodulators.^{4,5}

Substrates and inhibitors of DP-IV are of interest as protease inhibitors (e.g. C-terminal chloromethylketones) are inherently useful in the study of the enzyme.

H-Ala

The most potent DP-IV inhibitor (K_i=3nM). However, these boronic

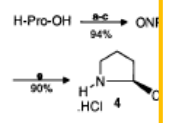
2-Cyanopyrrolidides

To establish an optimal N-terminal residue, we prepared a series of amino acid pyrrolidides.⁶ These compounds were prepared by reaction of the O-succinimide, (ONSu), ester of the required Boc protected amino acid with a slight excess of pyrrolidine in dichloromethane. Subsequent acid catalysed deprotection (4N HCl/dioxane) afforded the inhibitor as its hydrochloride salt. As expected, from the substrate specificity of DP-IV, only (S)-amino acids showed any activity and, as can be seen in Table I, lipophilic amino acids gave more potent compounds. The most potent α-amino acid derivatives were the most potent compounds with the non-proteinogenic amino acid, giving the most active pyrrolidide (compound 5 possessing a K_i value of 64 nM).

We then applied these findings to a series of 2-cyanopyrrolidides.

required a large scale synthesis of 2-cyanopyrrolidides. Compound 4 was prepared from Boc-Pro-NH₂ using a dehydrating agent, but the usual acidic conditions required to deprotect the Boc group from 2-cyanopyrrolidide. Employment of the *o*-nonyl succinimide (ONSu) and a very mild deprotection to be used in the final step. The use of a large volume of diethyl ether afforded the hydrochloride salt.

Scheme I. Preparation of dipeptide nitriles.



Reagents: a. ONPS-Cl, 2N NaOH. b. HONSu, Water soluble carbodiimide. c. conc. NH₄OH, dioxane. d. imidazole (2 equiv.), POCl₃ (4 equiv.), pyridine. e. 4N HCl/dioxane (3 equiv.), diethyl ether. f. Boc-Xaa-OH, pyBop, NEt₃, CH₂Cl₂. g. Trifluoroacetic acid.

The series of dipeptide nitriles described in Table II were prepared via a pyBop¹⁸ mediated coupling of 4 with the required Boc protected amino acid, followed by deprotection with TFA (Scheme I).

We were gratified to find that these compounds were potent inhibitors of DP-IV. The S.A.R. for the N-terminal residue developed in the pyrrolidide series correlated well for the dipeptide nitrile series and the most potent compounds 24, 25, 26 and 27 possessed activity comparable to the boroprolines, 1 and 2. Stability studies¹⁹ revealed excellent half-lives (t_{1/2}) in aqueous solution (pH 7.4) at room temperature (Table II) with several examples having t_{1/2} greater than 48h. Further work on optimisation of the pyrrolidine ring will be reported shortly.]

“To establish an optimal N-terminal residue, we prepared a series of amino acid pyrrolidides[.]”

Bulky Cyclohexyl at P2 Was Most Potent

Pergamon
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2-CYANOPYRROLIDIDES 1165

To establish an optimal *N*-terminal residue, we prepared a series of dipeptide nitriles. Compounds were prepared by reaction of the *O*-succinimide, acid with a slight excess of pyrrolidine in dichloromethane (CH₂Cl₂/dioxane) afforded the inhibitor hydrochloride salt. In this series, only (S)-amino acid derivatives were active. β-branching and bulky side chains gave more potent compounds. In particular, β-branching and bulky side chains gave more potent compounds. In particular, β-branching and bulky side chains gave more potent compounds. In particular, β-branching and bulky side chains gave more potent compounds.

Abstract: A series of dipeptide nitriles were prepared by reaction of the *O*-succinimide, acid with a slight excess of pyrrolidine in dichloromethane (CH₂Cl₂/dioxane) afforded the inhibitor hydrochloride salt. In this series, only (S)-amino acid derivatives were active. β-branching and bulky side chains gave more potent compounds. In particular, β-branching and bulky side chains gave more potent compounds. In particular, β-branching and bulky side chains gave more potent compounds.

Scheme 1. Preparation of dipeptide nitriles.

Reagents: a. ONPS-Cl, 2N NaOH, b. HONSu, Water soluble d. imidazole (2 equiv.), POCl₃ (4 equiv.), pyridine f. Boc-Xaa-OH, pyBop, NEt₃, CH₂Cl₂, g. Trifluoroacetic acid

The series of dipeptide nitriles described in Table II with the required Boc protected amino acid, followed by deprotection. We were gratified to find that these compounds with the *N*-terminal residue developed in the pyrrolidide series correlated with the required Boc protected amino acid, followed by deprotection. We were gratified to find that these compounds with the *N*-terminal residue developed in the pyrrolidide series correlated with the required Boc protected amino acid, followed by deprotection. We were gratified to find that these compounds with the *N*-terminal residue developed in the pyrrolidide series correlated with the required Boc protected amino acid, followed by deprotection.

“In particular, β-branched α-amino acid derivatives were the most potent compounds with the non-proteinogenic amino acid, (S)-cyclohexylglycine providing the most active pyrrolidide (compound 5 possessing a *K_i* value of 64 nM). We then applied these findings to a series of 2-cyanopyrrolidides.”

Ashworth I Table I SAR Applied to Table II

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PII: S0960-894X(96)00190-4

2-CYANOPYRROLIDIDES 1165

Doreen M
Ferring

Abstract: A number of dipeptide nitriles were synthesized and their inhibitory activity was determined in a competitive binding assay using a human DP-IV (K_i=3nM). However, the most potent compounds were found to be dipeptide nitriles with the required Boc protected amino acid residues. We were gratified to find that these N-terminal residue developed in the pyrrolidide series correlated well for the dipeptide nitrile series and the most potent compounds 24, 25, 26 and 27 possessed activity comparable to the boroprolines, 1 and 2.

To establish an optimal *N*-terminal residue, we prepared a series of dipeptide nitriles with the required Boc protected amino acid residues. We were gratified to find that these N-terminal residue developed in the pyrrolidide series correlated well for the dipeptide nitrile series and the most potent compounds 24, 25, 26 and 27 possessed activity comparable to the boroprolines, 1 and 2.

We then applied these findings to a series of 2-cyanopyrrolidide required a large scale synthesis of 2-cyanopyrrolidine 4 (Scheme I), prepared from Boc-Pro-NH₂ using a dehydrating mixture of phosphorus pentachloride and pyridine but the usual acidic conditions required to remove the Boc protecting group were very mild deprotection to be used in the final step. Adding three equivalent volumes of diethyl ether afforded the hydrochloride salt 4 as an off-white solid.

Scheme I. Preparation of dipeptide nitriles.

Reagents: a. ONPS-Cl, 2N NaOH. b. HONSu, Water soluble. c. imidazole (2 equiv.), POCl₃ (4 equiv.), pyridine. d. Boc-Xaa-OH, pyBop, NEt₃, CH₂Cl₂. e. H₂O, Et₃N. f. Boc-Xaa-OH, pyBop, NEt₃, CH₂Cl₂. g. H₂O, Et₃N.

“The series of dipeptide nitriles described in Table II were preparedWe were gratified to find that these compounds were potent inhibitors of DP-IV. The S.A.R. for the N-terminal residue developed in the pyrrolidide series correlated well for the dipeptide nitrile series and the most potent compounds 24, 25, 26 and 27 possessed activity comparable to the boroprolines, 1 and 2.”

Ashworth I Finds Excellent Stability Greater Than 48 Hours

Pergamon
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2-Cyanopyrrolidides 1165

To establish an optimal *N*-terminal residue, we prepared a series of amino acid pyrrolidides.⁶ These compounds were prepared by reaction of the *O*-succinimide, (ONSu), ester of the required Boc protected amino acid with a slight excess of pyrrolidine in dichloromethane. Subsequent acid-catalysed deprotection (4N HCl/dioxane) afforded the inhibitor as its hydrochloride salt. As expected, from DP-IV, only (S)-amino acid derivatives showed any activity and, as can be seen, β-branched α-amino acid derivatives gave more potent compounds. In particular, β-branched α-amino acid derivatives with the non-proteinogenic amino acid, (S)-cyclohexylglycine provided compound 5 possessing a *K_i* value of 64 nM.

We then applied these findings to a series of 2-cyanopyrrolidides. The procedure required a large scale synthesis of 2-cyanopyrrolidine 4 (Scheme I). *N*-Boc-2-cyanopyrrolidine was prepared from Boc-Pro-NH₂ using a dehydrating mixture of phosphorous oxychloride but the usual acidic conditions required to remove the Boc protecting group were very mild deprotection to be used in the final step. Adding three equivalents of 4 volume of diethyl ether afforded the hydrochloride salt 4 as an off-white precipitate.

Scheme I. Preparation of dipeptide nitriles.

Reagents: a. ONPS-Cl, 2N NaOH. b. HONSu, Water soluble carbodiimide. c. conc. NH₄OH, dioxane. d. imidazole (2 equiv.), POCl₃ (4 equiv.), pyridine. e. 4N HCl/dioxane (3 equiv.), diethyl ether. f. Boc-Xaa-OH, pyBop, NEt₃, CH₂Cl₂. g. Trifluoroacetic acid.

The series of dipeptide nitriles described in Table II were prepared via a pyBop¹⁸ method with the required Boc protected amino acid, followed by deprotection with TFA (Scheme I).

We were gratified to find that these compounds were potent inhibitors. The activity of the *N*-terminal residue developed in the pyrrolidide series correlated well for the most potent compounds 24, 25, 26 and 27 possessed activity comparable to the corresponding dipeptides, 1 and 2. Stability studies¹⁹ revealed excellent half-lives (*t_{1/2}*) in aqueous solution (pH 7.4) at room temperature (Table II) with several examples having *t_{1/2}* greater than 48h. Further work on optimisation of the pyrrolidine ring will be reported shortly.

“Stability studies revealed excellent half-lives ($t_{1/2}$) in aqueous solution (pH 7.4) at room temperature (Table II) with several examples having ($t_{1/2}$) greater than 48h.”

Ashworth Promises Further Investigation at P1

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2-Cyanopyrrolidines 1165

To establish an optimal *N*-terminal residue, we prepared a series of amino acid pyrrolidides.⁴ These compounds were prepared by reaction of the *O*-succinimide, (ONSu), ester of the required Boc protected amino acid with a slight excess of pyrrolidine in dichloromethane. Subsequent acid catalysed deprotection (4N HCl/dioxane) afforded the inhibitor as its hydrochloride salt. As expected, from the substrate specificity of DP-IV, only (S)-amino acid derivatives showed any activity and, as can be seen in Table I, lipophilic amino acids gave more potent compounds. In particular, β -branched α -amino acid derivatives were the most potent compounds with the non-proteinogenic amino acid, (S)-cyclohexylglycine providing the most active pyrrolidide (compound 5 possessing a K_i value of 64 nM).

We then applied these findings to a series of 2-cyanopyrrolidides. The preparation of these compounds required a large scale synthesis of 2-cyanopyrrolidine 4 (Scheme I). *N*-Boc-2-cyanopyrrolidine was readily prepared from Boc-Pro-NH₂ using a dehydrating mixture of phosphorus pentachloride, pyridine and imidazole but the usual acidic conditions required to remove the Boc group destroyed the nitrile group. However, very mild deprotection to be used in the final step. Addition of a large volume of diethyl ether afforded the hydrochloride salt 4.

Scheme I. Preparation of dipeptide nitriles.

Reagents: a. ONPS-Cl, 2N NaOH, b. HONSu, Water soluble carbodiimide, c. compound 1, d. imidazole (2 equiv.), POCl₃ (4 equiv.), pyridine, e. 4N HCl/dioxane, f. Boc-Xaa-OH, pyBop, NEt₃, CH₂Cl₂, g. Trifluoroacetic acid

The series of dipeptide nitriles described in Table II were prepared by the mediated coupling of 4 with the required Boc protected amino acid, followed by deprotection (Scheme I).

We were gratified to find that these compounds were potent inhibitors of DP-IV. The S.A.R. for the *N*-terminal residue developed in the pyrrolidide series correlated well for the dipeptide nitrile series and the most potent compounds 24, 25, 26 and 27 possessed activity comparable to the boroprolines, 1 and 2. Stability studies¹⁶ revealed excellent half-lives ($t_{1/2}$) in aqueous solution (pH 7.4) at room temperature (Table II) with several examples having $t_{1/2}$ greater than 48h. Further work on optimisation of the pyrrolidine ring will be reported shortly.

“Further work on optimization of the pyrrolidine ring will be reported shortly.”

Ashworth II Supports Selection of Compound 25

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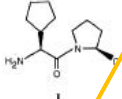
4-CYANTHIAZOLIDIDES AS VERY POTENT, STABLE INHIBITORS OF DIPEPTIDYL PEPTIDASE IV

Doreen M. Ashworth, Butras Atrash, Graham R. Baker, Andrew J. Baxter, Paul D. Jones, D. Michael Jones and Michael Szelke

Ferring Research Institute, Chilworth Research Centre, Chilworth, Southampton, SO16 7NP.

Abstract: A series of stable, very potent inhibitors of dipeptidyl peptidase IV has been developed. A number of dipeptide analogues, incorporating a 4-cyanothiazolidide, were found to have K_i values of 1 nM versus human DP-IV and half-lives of between 5 and 27h in aqueous solution (pH 7.4).
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The serine protease dipeptidyl peptidase IV (DP-IV, EC 3.4.14.5)^{1,2} which is identified as an activation marker CD26 has been the subject of intense scrutiny because it was recently shown that antibodies of this enzyme can inhibit T cell proliferation.^{3,4} However, the physiological role of this enzyme in the immune system and the molecular events mediated by this enzyme are only partly established. It was necessary to develop potent, stable inhibitors of DP-IV to help elucidate the role of this enzyme and to investigate their therapeutic use in a number of disease states such as Alzheimer's disease (GVHD), cancer or AIDS.



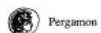
We recently reported a series of aminoacyl-2-cyanothiazolidides^{6,7} which possess K_i values of less than 5 nM versus human DP-IV⁶ and half-lives ($t_{1/2}$) of greater than 48h in aqueous solution (pH 7.4).⁷ This series of inhibitors is exemplified by 1 which has a K_i value of 1.1 nM versus human DP-IV and a half-life of 48h in aqueous buffer (pH 7.4).

* Fax +44 (1703)766253; e-mail pdj@fering.demon.co.uk

Page 1 of 4
2745
AstraZeneca Exhibit 2001
Mylan v. AstraZeneca
IPR2015-01340

“We recently reported a series of aminoacyl-2-cyanopyrrolidides^{6,7} which possess K_i values of less than 5 nM versus human DP-IV and half-lives ($t_{1/2}$) of greater than 48h in aqueous solution (pH 7.4).”

Ashworth II Seeks Even Greater Potency



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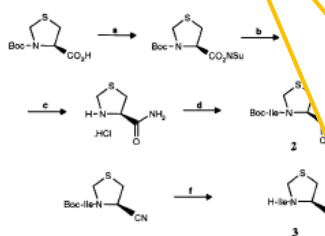
4-CYA

2746

D. M. ASHWORTH *et al.*

In a quest to improve the potency of this class of inhibitors, we investigated replacing the pyrrolidine ring with other nitrogen heterocycles. We chose isoleucine (Ile) as a standard *N*-terminal residue as it was the most potent natural amino acid in the 2-cyanopyrrolidine series.⁷ The preparation of **3** (Scheme 1) illustrates the general route to the series of cyano compounds described in Table I.

Scheme 1. Preparation of 3-isoleucyl-4-cyanothiazolidide



Reagents and Yields: a. *N*-hydroxysuccinimide (HONSu), water solub; b. conc. NH_4OH , dioxane, 96%. c. 4N HCl /dioxane, 99%. d. Boc-Ile-O; e. POCl_3 , imidazole, pyridine, 53%. f. Trifluoroacetic acid, 75%.

A pyBop¹⁰ mediated coupling of 4-amidothiazolidide with Boc pro dipeptide mimic **2** in modest yield. Dehydration of the primary amide function catalysed deprotection yielded the trifluoroacetate salt of **3**.¹¹ From a rang heteroatoms in 5- or 6-membered rings, we were pleased to find that the 4-cyanothiazolidide analogue **3** was approximately 5-fold more active than the 2-cyanopyrrolidine inhibitor **5**⁷ (Table I). However, this increase in activity was accompanied by a slight decrease in stability.

Having established 4-cyanothiazolidide as an optimum C-terminal residue, we prepared further analogues with the best *N*-terminal α -amino acids from the pyrrolidine series.⁷ These compounds were prepared as described in Scheme 1 but Boc-Ile-OH, in step d, was replaced with the required Boc-Xaa-OH. A number of analogues were prepared with sub-nanomolar activity against DP-IV and good stability in aqueous buffer (pH 7.4). (Table II)

“In a quest to improve the potency of this class of inhibitors, we investigated replacing the pyrrolidide ring with other nitrogen heterocycles.”

Abstract:
number of dipepti
1 nM versus huma
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The serine
activation marker
antibodies of this
immune system an
was necessary to
and to investigate
disease (GVHD).

We recent
nM versus human
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Page 1 of 4

Page 2 of 4

Ashworth II Supports Selection of Compound 25

Pergamon
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2746 D. M. ASHWORTH *et al.*

In a quest to improve the potency of this class of inhibitors, we investigate with other nitrogen heterocycles. We chose isoleucine (Ile) as a standard *N*-terminal natural amino acid in the 2-cyanopyrrolidine series.⁷ The preparation of a general route to the series of cyano compounds described in Table I.

Abstract:
number of dipeptidyl aminopeptidase (DAP-IV) activity in human plasma (1 nM versus human plasma) was necessary to investigate the role of this enzyme in the disease (GVHD).

The serine protease activation marker antibodies of this immune system are necessary to investigate the disease (GVHD).

We recently reported that the 4-cyanothiazolidide analogue 3 was approximately 5-fold more active than the 2-cyanopyrrolidide inhibitor 57 (Table I). However, this increase in activity was accompanied by a slight decrease in stability.

Scheme 1. Preparation of 3-isoleucyl-4-cyanothiazolidide.

Reagents and Yields: a. *N*-hydroxysuccinimide (HONSu), 96%; b. conc. NH₄OH, dioxane, 96%; c. 4N HCl/dioxane, 99%; d. Boc-Ile-OH, 53%; e. POCl₃, imidazole, pyridine, 53%; f. Trifluoroacetic acid.

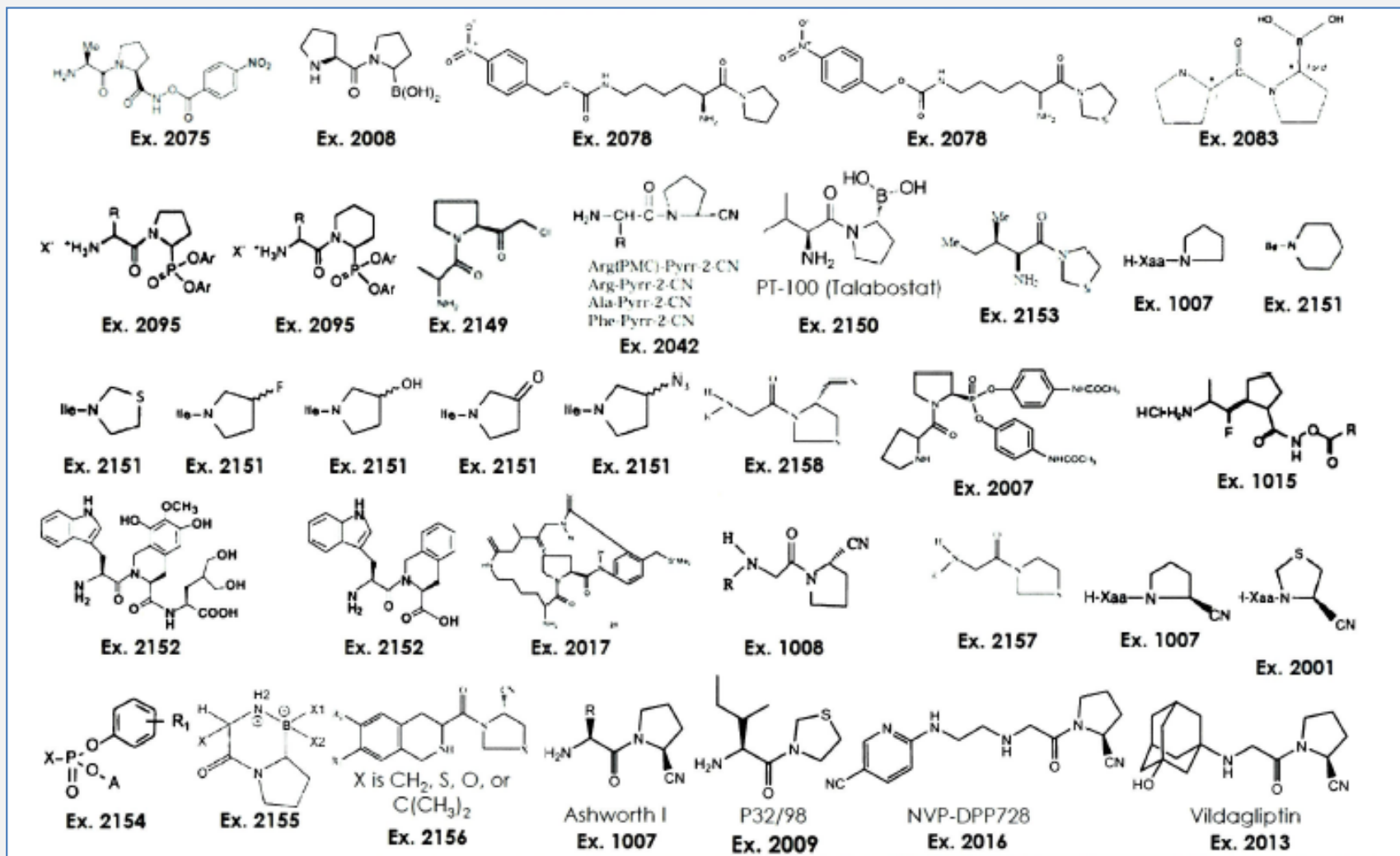
A pyBop¹⁰ mediated coupling of 4-amidothiazolidide dipeptide mimic **2** in modest yield. Dehydration of the catalysed deprotection yielded the trifluoroacetate. heteroatoms in 5- or 6-membered rings, we were pleased to find that the 4-cyanothiazolidide analogue **3** was approximately 5-fold more active than the 2-cyanopyrrolidide inhibitor **57** (Table I). However, this increase in activity was accompanied by a slight decrease in stability.

Having established 4-cyanothiazolidide as an optimum C-terminal residue, we prepared with the best *N*-terminal α-amino acids from the pyrrolidine series.⁷ These analogues were prepared with sub-nanomolar activity against DP-IV and good (7.4), (Table II)

Page 1 of 4
Page 2 of 4

“From a range of compounds with various heteroatoms in 5- or 6-membered rings, we were pleased to find that the 4-cyanothiazolidide analogue **3** was approximately 5-fold more active than the 2-cyanopyrrolidide inhibitor **57** (Table I). However, this increase in activity was accompanied by a slight decrease in stability.”

Many Structures Successfully Inhibit DPP-4



Source: EX2246 (AstraZeneca Demonstrative Exhibit).

In Vivo Toxicity Testing Not Required

Page 1

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

---oOo---

ASTRAZENECA AB, Case Number
Plaintiff, 14-664-GMS
vs (Consolidated)
AUROBINDO PHARMA LTD.
AUROBINDO PHARMA LTD.

VIDEOTAPE

Reported by:
THOMAS J. FRASER
RPR, CSR No. 6
Job No: 23146
Pages: 1 - 20

800-567-8658

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Page 54

1 A. That's correct.
2 Q. And in order to get our nomenclature straight,
3 can we refer to that as being N-linked?
4 A. That's fine.
5 Q. Okay. It appears from your CV and, in fact,
6 from the various honors that you've won and societies
7 that you belong to, that you've spent a career in
8 medicinal chemistry, is that fair?
9 A. Yes, it is.
10 Q. Okay. And let's see if there's some things
11 about the realities of medicinal chemistry that we can
12 agree on before we head off into what we don't agree on.
13 Do you agree that it is often necessary to
14 synthesize a relatively large number of molecules in
15 order to find one that has desirable in vivo activity?
16 MS. STEINER: Objection to form.
17 THE WITNESS: I would not agree with that
18 because it depends on what your starting point is, and
19 it depends on what information you know prior to
20 beginning those sorts of efforts.
21 BY MR. LIPSLEY:
22 Q. What would you say the usual experience is in
23 that regard?
24 A. I would say that there is no usual experience,
25 consider, because the starting points that one chooses

Page 55

1 for initiation of a drug discovery program come from
2 multiple sources and from multiple avenues, and
3 depending on what is known or can be discovered about
4 those molecules dictates the path or -- not dictates, it
5 contributes to the information that you need to obtain
6 for the path forward. And so there are a number
7 of -- as I said, there are a number of avenues for
8 identifying starting points.
9 Q. Well, once you identify a starting point, what
10 in general is the next step?
11 MS. STEINER: Objection to form.
12 THE WITNESS: One key step is to understand the
13 properties of the molecule that you've identified, and
14 those properties are dependent on the specifics
15 of the program.
16 BY MR. LIPSLEY:
17 Q. And one of those properties would be being
18 active against the desired target, correct?
19 A. In a general sense, yes.
20 Q. And another of those properties might be
21 demonstrating a desirable level of potency in its
22 activity towards the desired target, correct?
23 A. Yes.
24 Q. And not every molecule that gets synthesized
25 in a drug discovery program is either active or

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1 sufficiently potent, is that fair?
2 A. That is fair, yes, we can agree on that.
3 Q. Okay. And once one has a molecule
4 that appears to be active and sufficiently potent
5 against the target, the logical next step would be to
6 test the molecule for activity in an animal model, is that fair?
7 MS. STEINER: Objection to form.
8 THE WITNESS: Yes.
9 BY MR. LIPSLEY:
10 Q. Okay. And would you agree with
11 demonstrated activity in a human patient
12 is a good thing for a putative drug development
13 program?
14 A. Yes.
15 Q. Okay. And would you agree with
16 molecules that are active in vitro are active
17 models of the disease being studied?
18 BY MR. LIPSLEY:
19 Q. Sure. Do you agree that not all
20 are active in vitro are active in animal
21 models of the disease being studied?
22 MS. STEINER: Same objection.
23 THE WITNESS: Yes.
24 BY MR. LIPSLEY:
25 Q. And do you agree that not all
26 are active in animal models of the disease
27 are active in human beings with the disease?
28 MS. STEINER: Objection to form.
29 THE WITNESS: Yes.

15 (Pages 54 - 57)

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Q. Okay. And once one has a new molecule that appears to be active and sufficiently potent for the target, the logical next step would be to -- or a logical next step would be to test the molecule for activity in an animal model. Is that fair?

MS. STEINER: Objection to form. Foundation.
THE WITNESS: No.

Dr. Rotella's Prior Art Search

Page 1

1 UNITED STATES DISTRICT COURT
2 FOR THE DISTRICT OF DELAWARE
3 ---oOo---

4 ASTRAZENECA AB, Case Number

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Page 18 Page 20

1 Q. Absolutely.
2 A. And that's the only reason. And I take
3 issue, so.
4 Q. Okay.
5 MS. STEINER: Counsel, based on Dr. Rotella's
6 medical condition, if we could request that we try to
7 take breaks, even short breaks, every hour or hour and
8 a half or so, that would be great.
9 MR. LIPSEY: That's fine with me. I tend to

1 Q. Okay. And when were you first contacted in
2 connection with the possibility of providing expert
3 testimony in this case?
4 A. At some point in 2015. I don't recall the
5 exact date.
6 Q. Early, mid, late?
7 A. I don't recall.
8 Q. Okay. And who contacted you?
9 A. Katherine Hooper.

Page 22 Page 24

1 THE WITNESS: I was asked by counsel to provide
2 opinions based on -- or provide opinions on obviousness
3 related to the discovery, synthesis, identification and
4 discovery of saxagliptin. And I carried out a
5 literature search for documents that might be
6 rel[evant] -- that might be relevant. In some cases,
7 I could -- I could get those references easily.
8 In other cases, I could not.

1 Chemistry Letters from 1996.
2 Q. And that's the one that starts at page 1163?
3 A. Yes.
4 Q. Okay.
5 A. Chung and Prusoff, Biochemical Pharmacology,
6 starting on page 3099.
7 Q. Okay.
8 A. The next paper in the list, starting
9 on page 3100.
10 Q. Okay. I believe you're on page 1881.
11 Q. Okay.
12 A. Korfman.
13 That's all.
14 Q. How about the patent?
15 Yes -- what of those were you
16 commencement of the formulation.
17 A. Oh, yeah. I believe the patent
18 1999, I believe that is the Villanar patent.
19 aware of that one. I don't remember what the
20 patent refers to, the other WO application there.
21 Q. Okay. Any others?
22 A. No.
23 Q. Had you seen either the reissue patent,
24 RE 44,88, which is Defendant's Deposition Exhibit 18,
25 or the original patent, 6,395,767, prior to the

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“Q. What I'm trying to get at is how you came to know of the existence of the materials that you principally rely upon in your report.”

“THE WITNESS: I was asked by counsel to provide...opinions on obviousness related to the discovery[,] synthesis[,] identification[,] and discovery of saxagliptin. And I carried out a literature search for documents that might be rel[evant].... In some cases, I could -- I could get those references easily; in other cases, I could not.”

Dr. Rotella's Prior Art Search

Page 1

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

Page 22

1 THE WITNESS: I was asked by counsel to provide
2 opinions based on -- or provide opinions on obviousness
3 related to the discovery synthesis identification and
4 discovery of saxagliptin. And I carried out a
5 literature search for documents that might be
6 rel -- that might be relevant. In some cases,
7 I could -- I could get those references easily,
8 in other cases, I could not.
9 BY MR. LIPSEY:
10 Q. And how did you go about doing the literature
11 search?
12 A. I used SciFinder to investigate what was known
13 in the field, based on priority dates provided to me by
14 counsel.
15 Q. Did you have preexisting knowledge of any of
16 the literature that you were looking for?
17 MS. STEINER: Objection to form.
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Page 24

1 Chemistry Letters from 1996
2 Q. And that's the one that starts at page 1123?
3 A. Yes.
4 Q. Okay.
5 A. Cheng and Prusoff, Biochemical Pharmacology,
6 starting on page 3099.
7 Q. Okay.
8 A. Chiou, the next paper in the list, starting
9 on page 243.
10 Hanessian, from 1997, starting on page 1881.
11 Q. Okay.
12 K. Korfmacher, Korbhand, Lin, Lipinsky. And
13 that's all.
14 Q. How about the patent literature at the top, did
15 you -- what of those were you familiar with prior to the
16 commencement of the formation of your opinion?
17 A. Oh, yeah. I believe the patent application.

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1 Q. Okay.
2 A. Now --
3 Q. Okay.
4 A. I investigate. The Chiu paper and the Cheng and
5 Prusoff paper, I was aware of from my days in grad
6 school.
7 Q. Okay. Just to give us a frame of reference, to
8 get some dates straight, I'd like the reporter to mark
9 for identification as Plaintiff's Deposition Exhibit 59
10 a copy of what appears to be the CV of Dr. Rotella.
11 (Deposition Exhibit 59 was marked
12 for identification.)
13 BY MR. LIPSEY:
14 Q. Can you identify Plaintiff's Deposition
15 Exhibit 59, please?
16 A. This is the CV that I provided to Wilson
17 Sosen.
18 Q. And was it complete and accurate to the best of
19 your knowledge as of the time your expert report was
20 submitted in January of 2014?
21 A. Yes.
22 Q. Are there any additions, corrections or
23 modifications to it to make it current today?
24 A. There has been some change in my current
25 research funding, but other than that, no.

Page 28

1 A. Prior to the DPP4 program, I worked on the
2 discovery of PDE5 inhibitors.
3 Q. Like Viagra?
4 A. Yes.

Page 29

1 in BMS?
2 A. No. I left the company.
3 Q. OK, okay. I should have figured that out, I'm
4 sorry.
5 And what were your responsibilities as a
6 principal research scientist on the DPP4 project?
7 A. My primary responsibilities were to conceive
8 of and synthesize new DPP4 inhibitors. I worked with
9 other members of the team and supervised two research
10 assistants who worked with me on that project.
11 Q. And who were they?
12 A. Yehang Zhu, Y-e-h-e-n-g-Z-h-u, and Zhong Sun,
13 Z-h-o-n-g S-u-n.
14 Q. And were they your assistants throughout your
15 tenure on the DPP4 project?
16 A. Yes.
17 Q. I gather that at the time you joined the
18 project, saxagliptin had already been discovered, is
19 that right?
20 MS. STEINER: Objection to form.
21 BY MR. LIPSEY:
22 Q. Well, let me ask an open-ended question to make
23 your lawyer happy.
24 What was the status of saxagliptin development,
25 if any, at the time you joined the project?

Page 27

1 Q. And what is there new in your current
2 research funding?
3 A. Unfortunately, nothing. Some of those things
4 have -- some of those have ended.
5 Q. Okay. I guess working chronologically forward
6 in your experience, which actually ends on the second
7 page of Plaintiff's Deposition Exhibit 59, was there any
8 experience you had in any of your prior employment
9 relating to DPP4 or its inhibition prior to the time you
10 joined Bristol Myers Squibb?
11 MS. STEINER: Objection to form.
12 THE WITNESS: No.
13 BY MR. LIPSEY:
14 Q. Okay. Now, it indicates here that you joined
15 Bristol Myers Squibb in 1997, is that right?
16 A. That's correct.
17 Q. And that you left in 2003, is that right?
18 A. That's correct.
19 Q. Okay. During what part of that time did you
20 work on matters relating to DPP4?
21 A. I worked on the DPP4 program for approximately
22 one year. I can't remember more precisely than that.
23 So approximately 2002 to 2003.
24 Q. And what projects had you been working on at
25 BMS prior to joining the DPP4 program?

8 (Pages 26 - 29)

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"[C]an you identify the publications of which you had preexisting knowledge prior to the commencement of the formulation of your opinions?"

A. Ashworth[I]..., Cheng,... Chiou,... Hanessian, Korfmacher,... the Villhauer patent...."

"Q. Were there other materials you were provided when you began working on this program at BMS beyond those two, if you recall?"

A. Well, I'm remarkably certain that all of the ones I identified were part of the literature that we were provided with as a part of working on that program.... I apologize. The Chiu paper and the Cheng and Prusoff paper, I was aware of from my days in graduate school."

Dr. Rotella's Prior Art Search

Page 1

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

---oOo---

ASTRAZENECA AB, Case Number
Plaintiff, 14-664-GMS
vs (Consolidated)
AUROBINDO PHARMA LTD. and
AUROBINDO PHARMA INC. INC

Page 18

1 BY MR. LIPSEY: 2 Q. Okay. Now, I don't want to put words in your
3 mouth, but is it fair to say that the thrust of your
4 expert opinion in this case is that the starting point
5 for drug development of a DPP4 inhibitor would have been
6 the compound of the Ashworth publication that you
7 identified in your report?
8 A. And can I ask if you're referring to the point
9 in time when -- that's an issue here, or are we talking
10 about the current day?
11 Q. No, no, no. At the time that was the subject
12 of your report, before the invention of saxagliptin was
13 made.
14 A. Okay. So to be clear, you're talking about
15 the late 1990s? Because my report was written in
16 and so I want to make certain that we have our date
17 references because -- the reason I say that is because
18 drug discovery has evolved in the period of time
19 Q. Well, which period of time were you referencing
20 in your report?
21 A. I was using the priority dates as provided to me
22 by counsel.
23 Q. Okay. Well, let's use that priority date.
24 And perhaps let's take a look at Defendant's Deposition
25 Exhibit 18, which is the '186 Patent in suit here. And

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1 BY MR. LIPSEY: 2 Q. Which is a copy of the 1996 Ashworth
3 publication starting at page 1163 that we've been
4 calling the Ashworth One publication.
5 Is Plaintiff's Deposition Exhibit 34 the
6 Ashworth One publication which was the starting point
7 for the analysis in your report?
8 A. This was one of the pieces of information that
9 I used, yes.
10 Q. Now, at the time of the filing of the
11 application for the patent in suit, Defendant's
12 Deposition Exhibit 18, February 16, 2001, to your
13 knowledge, had there been a report of the testing of any
14 of the compounds described in Ashworth One, Plaintiff's
15 Deposition Exhibit 34, in humans?

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1 you can see on the face of it there that the application
2 leading to the original patent was filed on February 16,
3 2001.
4 A. Yes. Can we use that date, or is there
5 another date you'd rather use?
6 A. That's fine with me.
7 Q. Okay. Let's use that date.
8 So is it fair to say that as of February 16,
9 2001, the thrust of your opinions in this case is that
10 a person seeking to develop a DPP4 inhibitor would have
11 started with the molecules of what we've been calling
12 the Ashworth One publication?
13 A. That is one potential starting point. There
14 are others.
15 Q. Is that the starting point upon which you based
16 your opinion?
17 A. That is, yes.
18 Q. Okay. I guess we've been talking about it for
19 a while and maybe we better get a copy of it. Ed likes
20 to show you a document that has previously been marked
21 for identification as Plaintiff Deposition Exhibit 34.
22 (Previously marked Exhibit 34
23 was shown to the witness
24 and is annexed hereto.)

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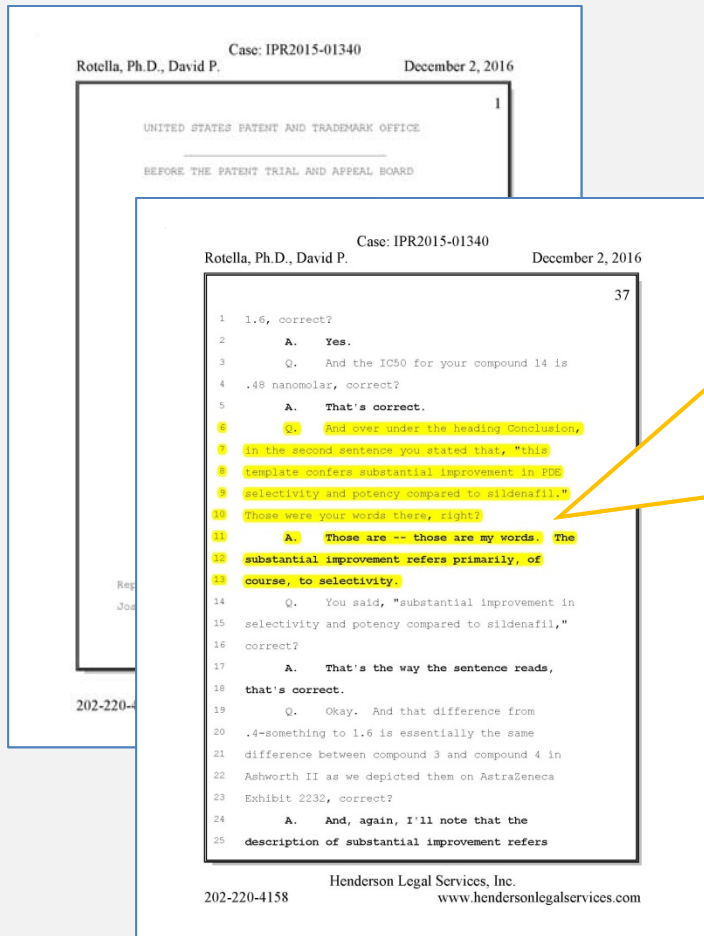
“Q. No, no, no. At the time that was the subject of your report, before the invention of saxagliptin was made.

A. Okay. So to be clear, you're talking about the late 1990s? Because my report was written in 2016, and so I want to make certain that we have our date references because -- the reason I say that is because drug discovery has evolved in the period of time.

Q. Well, which period of time were you referencing in your report?

A. I was using the priority dates as provided to me by counsel.”

Dr. Rotella



“Q. And over under the heading Conclusion, in the second sentence you stated that, ‘this template confers substantial improvement in PDE selectivity and potency compared to sildenafil.’ Those were your words there, right?”

A. Those are – those are my words. The substantial improvement refers primarily, of course, to selectivity.”

CERTIFICATE OF SERVICE

On 23 January 2017, Mylan served a copy of its demonstrative exhibits on counsel for Patent Owner AstraZeneca AB at the following electronic service addresses:

Charles E. Lipsey	charles.lipsey@finnegan.com
Eric E. Grondahl	egrondahl@mccarter.com
John D. Livingstone	john.livingstone@finnegan.com
Anthony A. Hartmann	anthony.hartmann@finnegan.com
M. David Weingarten	david.weingarten@finnegan.com
Nicole A. Conlon	nicole.conlon@finnegan.com
Daniel M. Silver	dsilver@mccarter.com
Kassandra M. Officer	kassandra.officer@finnegan.com

on Wockhardt at the following service electronic addresses:

Frederick R. Ball	FRBall@duanemorris.com
Patrick C. Gallagher	PCGallagher@duanemorris.com

on Teva at the following service electronic addresses:

Iain A. McIntyre	IMcIntyre@carlsoncaspers.com
Gary J. Speier	GSpeier@carlsoncaspers.com

on Aurobindo at the following service electronic addresses:

Sailesh K. Patel SPatel@schiffhardin.com

Joel Wallace JWallace@schiffhardin.com

George Yu GYu@schiffhardin.com

and on Sun/Amneal at the following service electronic addresses:

Samuel S. Park spark@winston.com

Andrew R. Sommer asommer@winston.com

Respectfully submitted,

Dated: 23 January 2017

By: /Richard Torczon /
Richard Torczon, Backup Counsel
Reg. No. 34,448