Drugs of the Future 2005, 30(4): 337-343

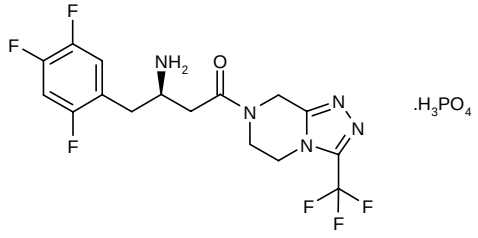
CCC: 0377-8282/2005
DOI: 10.1358/dof.2005.030.04.898484

MK-0431

Agent for Type 2 Diabetes
Dipeptidyl-Peptidase IV (CD26) Inhibitor

MK-431
ONO-5435

3(*R*)-Amino-1-[3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphate



$C_{16}H_{15}F_6N_5O.H_3O_4P$
Mol wt: 505.3102
CAS: 654671-78-0
CAS: 486460-32-6 (as free base)
EN: 333966

Abstract

The incretin hormone glucagon-like peptide-1 (GLP-1, GLP-1[7-36]amide) plays a crucial role in the regulation of insulin by acting on the pancreas to potentiate glucose-induced insulin secretion. GLP-1 also beneficially slows gastric emptying, reduces appetite and restores β-cell function, and has been the subject of research efforts to develop agents for the treatment of type 2 diabetes. However, GLP-1 has an extremely short half-life and is not suitable for therapeutic use. It is rapidly hydrolyzed by the circulating enzyme dipeptidyl-peptidase IV (DPP-IV), which cleaves the molecule at the *N*-terminal, giving rise to the inactive truncated fragment GLP-1(9-36)amide. On the other hand, administration of a DPP-IV inhibitor could enhance the half-life of GLP-1 and could therefore produce the same pleiotropic effects as exogenously administered GLP-1 or GLP-1 analogues. Thus, one of the newer targets for the treatment of diabetes is the serine protease DPP-IV. MK-0431 (Ono-5435) is a novel, potent, orally active β-amino acid-derived DPP-IV inhibitor that has exhibited good pharmacokinetics in mice, rats, dogs and monkeys and was chosen for further development as a treatment for type 2 diabetes. It has been shown to be effective in insulin-resistant mice and mice with diet-induced obesity, and was safe and effective in patients with type 2 diabetes. The agent has reached phase III development as a treatment for this condition.

Synthesis

MK-0431 can be obtained by several ways:

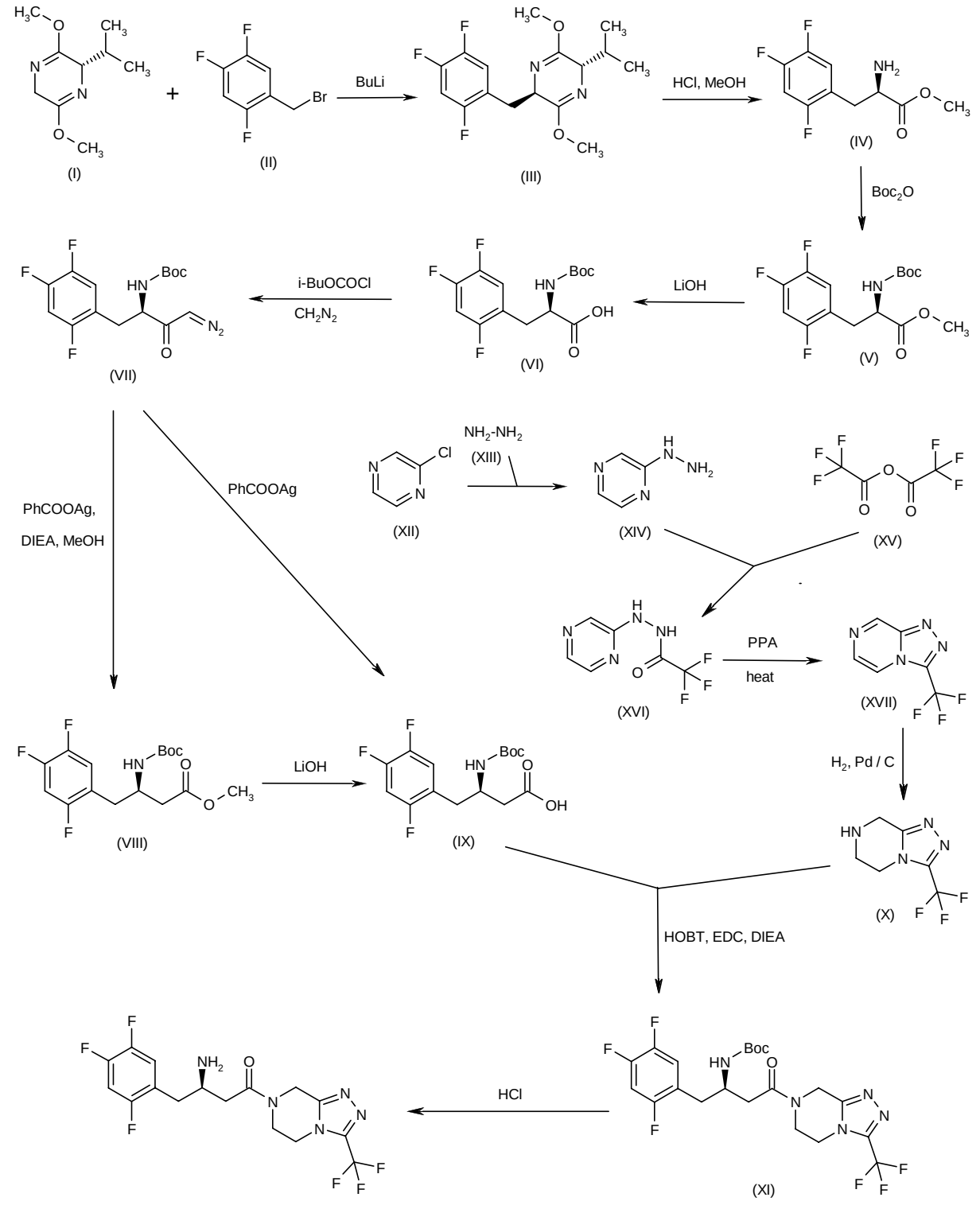
1) Condensation of 2(*S*)-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (I) with 2,4,5-trifluorobenzyl bromide (II) by means of BuLi in THF gives the adduct (III), which is hydrolyzed with HCl in methanol to yield 2(*R*)-amino-3-(2,4,5-trifluorophenyl)propionic acid methyl ester (IV). Protection of the amine group of compound (IV) by reaction with Boc_2O and TEA in dichloromethane affords the *N*-protected α-amino ester (V), which is hydrolyzed by means of LiOH in THF/water to provide the corresponding *N*-protected α-amino acid (VI). Reaction of amino acid (VI) with isobutyl chloroformate and diazomethane affords the diazoketone (VII), which is treated with silver benzoate and DIEA in MeOH to provide the *N*-protected β-amino ester (VIII). Hydrolysis of ester (VIII) with LiOH in THF/water gives the corresponding *N*-protected β-amino acid (IX), which can also be obtained directly by reaction of diazoketone (VII) with silver benzoate in dioxane/water. Condensation of amino acid (IX) with 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazine (X) by means of HOBT, EDC and DIEA in DMF yields the adduct (XI), which is finally deprotected by means of HCl in methanol and treated with phosphoric acid (1, 2). Scheme 1.

The intermediate 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazine (X) can be obtained as follows: Reaction of 2-chloropyrazine (XII) with hydrazine (XIII) in refluxing ethanol gives 2-hydrazinopyrazine (XIV), which is acylated with trifluoroacetic anhydride (XV) to yield the hydrazide (XVI). Cyclization of hydrazide (XVI) by means of PPA at 140 °C affords 3-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]pyrazine (XVII), which is finally hydrogenated with H_2 over Pd/C in ethanol (1, 2). Scheme 1.

2) Reaction of ethyl trifluoroacetate (XVIII) with hydrazine (XIII) in acetonitrile/water gives the corresponding hydrazide (XIX), which is condensed with

L.A. Sorbera, J. Castañer. Prous Science, P.O. Box 540, 08080, Barcelona, Spain.

Scheme 1: Synthesis of MK-0431



chloroacetyl chloride (XX) by means of NaOH in water to yield the bishydrazide (XXI). Cyclization of compound (XXI) by means of $POCl_3$ in hot acetonitrile affords the oxadiazole (XXII), which is treated with ethylenediamine (XXIII) in methanol to provide *N*-[(*Z*)-piperazin-2-ylidene]-trifluoroacetohydrazide (XXIV). Cyclization of hydrazide (XXIV) in refluxing methanol gives 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo[4,3-*a*]pyrazine (X), which by coupling with the adduct (XXV) – obtained by reaction of 2-(2,4,5-trifluorophenyl)acetic acid (XXVI) with Meldrum's acid (XXVII) by means of pivaloyl chloride and DIEA in dimethylacetamide – provides the 3-oxobutyramide (XXVIII). Reaction of butyramide (XXVIII) with 2(*S*)-phenylglycinamide (XXIX) in isopropanol/AcOH provides the enamine (XXX), which is enantioselectively reduced with of H_2 over PtO_2 in THF/methanol to give the desired (*R*)-enantiomer (XXXI). Finally, the chiral auxiliary of (XXXI) is removed by transfer hydrogenolysis with HCOOH over $Pd(OH)_2$/C in hot THF/methanol/water (3). Scheme 2.

3) Alternatively, reaction of the 3-oxobutyramide (XXVIII) with ammonium acetate and ammonium hydroxide in refluxing methanol/water provides the enamine (XXXII), which is finally submitted to an enantioselective hydrogenation with H_2 over chloro(1,5-cyclooctadiene)-rhodium(I) dimer and (*R*,*S*)-*t*-Bu Josiphos as chiral catalyst (4, 5). Scheme 2.

4) Condensation of 2-(2,4,5-trifluorophenyl)acetic acid (XXVI) with Meldrum's acid (XXVII) by means of oxalyl chloride in DMF/dichloromethane gives the adduct (XXV), which is treated with refluxing MeOH to yield 3-oxo-4-(2,4,5-trifluorophenyl)butyric acid methyl ester (XXXIII). Enantioselective reduction of ester (XXXIII) with H_2 over the chiral catalyst (*S*)-BINAP-$RuCl_2$ in methanol affords 3(*S*)-hydroxy-4-(2,4,5-trifluorophenyl)butyric acid methyl ester (XXXIV), which is hydrolyzed with LiOH in THF/water to provide the corresponding free carboxylic acid (XXXV). Reaction of acid (XXXV) with *O*-benzylhydroxylamine (XXXVI) and EDC in aqueous HCl gives the benzyl butyrohydroxamic ester (XXXVII), which is cyclized by means of DIAD and PPh_3 in toluene to yield 1-benzyloxy-4(*R*)-(2,4,5-trifuorobenzyl)azetidin-2-one (XXXVIII). Cleavage of the azetidine ring by means of LiOH in THF provides 3(*R*)-(benzyloxyamino)-4-(2,4,5-trifluorophenyl)butyric acid (XXXIX), which is condensed with 3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-*a*]pyrazine (X) by means of EDC and NMM in DMF/water to yield the adduct (XL). Finally, this compound is debenzylated by hydrogenation with H_2 over Pd/C in methanol (6). Scheme 3.

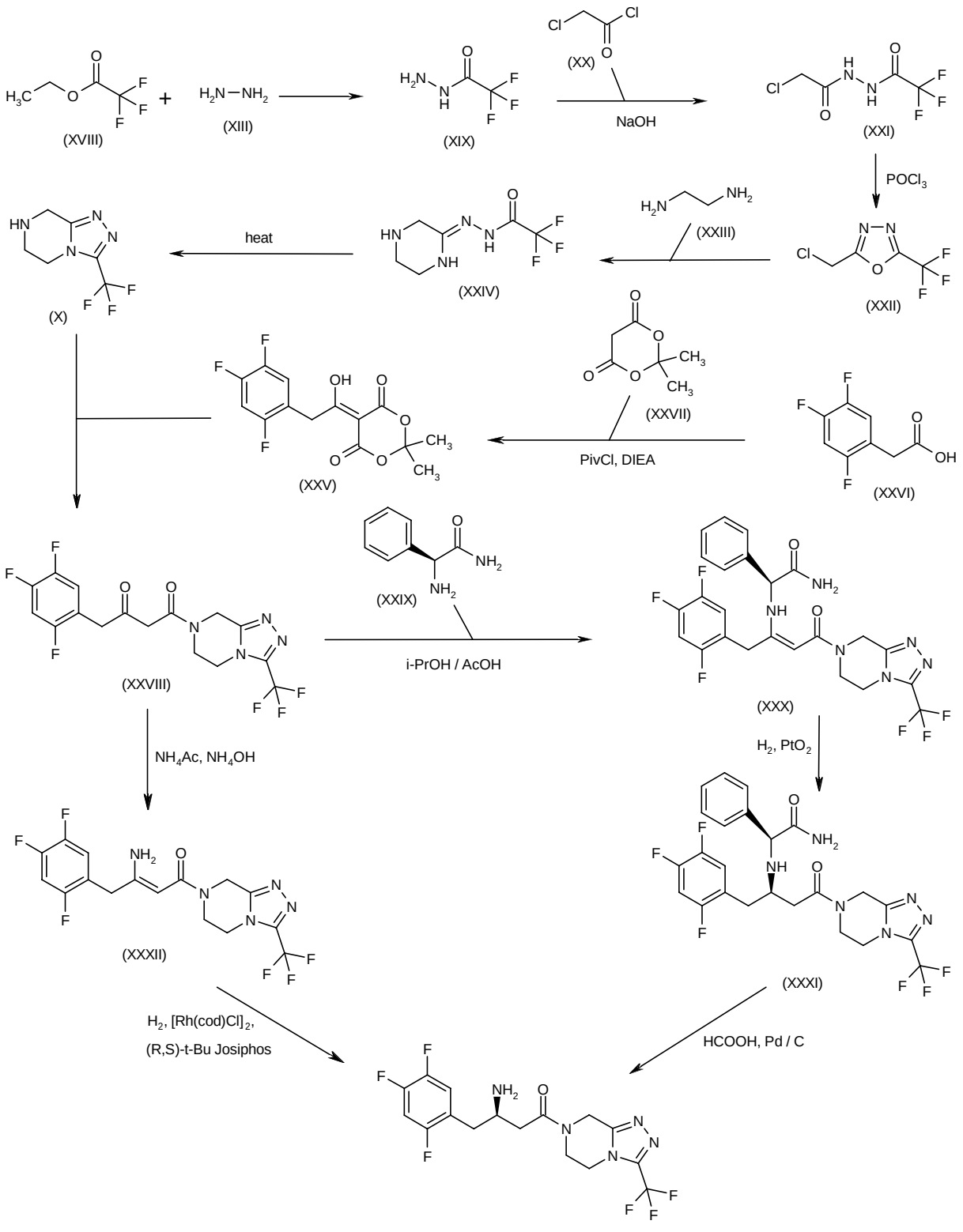
Introduction

Diabetes is a multifactorial disease that is classified as chronic hyperglycemia due to defects in insulin secretion, action or both, which results in abnormalities in carbohydrate, fat and protein metabolism. The World Health Organization (WHO) reported in 2000 a worldwide prevalence of 154.4 million subjects with diabetes and predicts that by the year 2025 there will be nearly 300 million diabetics. The American Diabetes Association estimates that there are a total of 18.2 million Americans suffering from diabetes, of whom two-thirds are diagnosed and one-third are not. Over 90% of the diabetic patient population in the Western world has been diagnosed with type 2 diabetes (non-insulin-dependent diabetes, or adult-onset diabetes). Type 2 diabetes occurs when the body is unable to efficiently use the insulin it produces and glucose levels in the blood become abnormally elevated. This hyperglycemia contributes to numerous acute or chronic complications, such as atherosclerosis, heart disease, stroke, hypertension, end-stage renal disease and blindness, among others. Type 2 diabetes is strongly favored by genetic disposition. However, environmental factors also contribute significantly to its development. The risk factors for type 2 diabetes include age, obesity, physical inactivity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance and race/ethnicity (7-10).

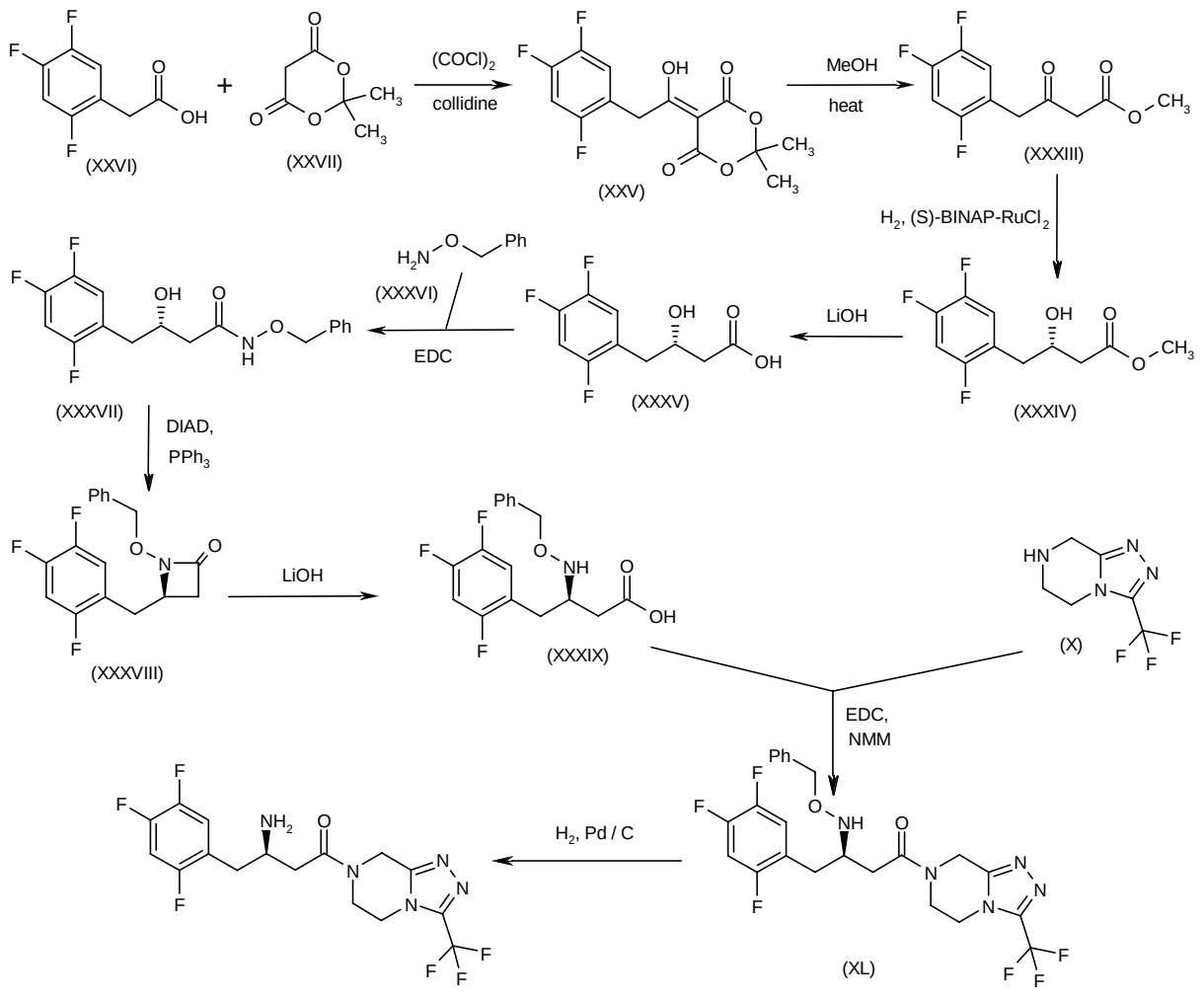
There are several therapeutic options for the treatment of type 2 diabetes, which can include lifestyle modification with diet and exercise, as well as drug therapy. The incretin hormone glucagon-like peptide-1 (GLP-1, GLP-1[7-36]amide) has been the subject of research efforts to develop agents for the treatment of type 2 diabetes. Hormonal regulation of insulin secretion in response to glucose involves pancreatic and gastrointestinal hormones. Gut-derived insulin-releasing incretins such as gastric-inhibitory polypeptide (GIP) and GLP-1 play a crucial role in the regulation of insulin by acting on the pancreas to potentiate glucose-induced insulin secretion. Because GLP-1 regulates insulin in a strictly glucose-dependent manner, there is very little risk of hypoglycemia. GLP-1 has been shown to slow gastric emptying and reduce appetite, and recently, it was demonstrated in rodent models to positively regulate islet cell mass by inducing replication of islet cells and promoting neogenesis of islet cells from pancreatic ductal cells. In addition, GLP-1 attenuates apoptosis in isolated human islets and cultured β-cells. Due to these beneficial actions, GLP-1 was proposed to be a potential agent for the treatment of diabetes. However, GLP-1 is not suitable for therapeutic use due to its extremely short half-life. GLP-1 is only active if administered as a continuous i.v. infusion because it is rapidly hydrolyzed by the circulating enzyme dipeptidyl-peptidase IV (DPP-IV), which cleaves the molecule at the *N*-terminal, giving rise to the inactive truncated fragment GLP-1(9-36)amide. Moreover, GLP-1(9-36) has been shown to act as a GLP-1 receptor antagonist, blocking the effects of the intact hormone. On the other hand, administration of a DPP-IV inhibitor could enhance the half-life of GLP-1 and therefore produce the same pleiotropic effects as exogenously administered GLP-1 or GLP-1 analogues (11-18).

Thus, one of the newer targets for the treatment of diabetes is the serine protease DPP-IV. MK-0431 (Ono-5435) is a novel, potent, orally active β-amino acid-

Scheme 2: Synthesis of MK-0431



Scheme 3: Synthesis of MK-0431



derived DPP-IV inhibitor that exhibits good pharmacokinetics in mice, rats, dogs and monkeys and was chosen for further development as a treatment for type 2 diabetes (1).

Pharmacological Actions

MK-0431 is the phosphate salt of a triazolopiperazine-based compound that was found to be a potent and highly selective inhibitor of DPP-IV (K_i = 9 nM; IC_{50} = 18 nM *vs.* > 100,000 nM for DPP-9 and QPP [quiescent cell proline dipeptidase or DPP-II] and 48,000 nM for DPP-8) (1, 19, 20).

Results from oral glucose tolerance tests (OGTTs) conducted in lean mice given a single oral dose of MK-0431 (0.1-3 mg/kg given 1 h prior to an oral dextrose challenge) revealed that the agent effectively and dose-dependently improved glucose tolerance (23% and 55% reduction in glucose excursions at 0.1 and 3 mg/kg, respectively). Following administration of a dose of 3 mg/kg, C_{max} levels were about 600 nM, with circulating GLP-1 increased 2-3-fold and plasma DPP-IV activity inhibited 84-96%. The increase in GLP-1 plasma levels was similar to that observed when DPP-IV-deficient mice were challenged with glucose. Acute administration of MK-0431 (0.3, 3 and 30 mg/kg p.o.) effectively lowered blood glucose in mice with diet-induced obesity who were hyperglycemic and hyperinsulinemic and showed impaired glucose tolerance in OGTTs, which is consistent with insulin resistance observed in type 2 diabetes. Almost complete normalization in glucose excursions was observed with an oral dose of 3 mg/kg, which corresponded to a plasma C_{max} value of about 700 nM (1, 20, 21).

Pharmacokinetics

The free base exhibited excellent i.v. (1 mg/kg) and oral (2 mg/kg) pharmacokinetics in mice, rats, dogs and monkeys. The oral bioavailability of the compound was 61%, 76%, 100% and 68%, respectively. C_{max} and AUC values obtained following oral dosing in rats, dogs and monkeys were 0.33, 2.2 and 0.33 μM, respectively, and 0.52, 8.3 and 1 μM·h/mg/kg, respectively; $t_{1/2}$ and clearance values were 1.7, 4.9 and 3.7 h, respectively, and 60, 6 and 28 ml/min/kg, respectively (1, 19, 20).

Clinical Studies

A randomized, placebo-controlled, crossover study in 56 patients with type 2 diabetes on diet and exercise therapy examined the safety, tolerability and efficacy of single oral doses of MK-0431 (25 or 200 mg) separated by a 7-day washout period. The agent was well tolerated. Significant reductions in glycemic excursions as compared to placebo were observed in OGTTs performed after an overnight fast and 2 h postdosing, such that significant 22% and 26% reductions in incremental glucose AUC values were observed with the doses of 25 and 200 mg, respectively. Each dose was associated with a significant increase in active GLP-1 levels of about 2-fold and an increase in the active to total GLP-1 ratio after an OGTT as compared to placebo. In addition, treatment with doses of 25 and 200 mg of MK-0431 was also associated with significant increases in plasma insulin AUC (22% and 23%, respectively) and plasma C-peptide AUC (13% and 21%, respectively), as well as significant reductions in plasma glucagon AUC values (8% and 14%, respectively) as compared to placebo. Results indicate the potential efficacy of the agent in the treatment of type 2 diabetes (22).

MK-0431 has advanced to phase III clinical trials for the treatment of type 2 diabetes, with regulatory submissions planned for 2006. The compound was recently licensed to Ono Pharmaceutical (Ono-5435) for Japan, where it is in phase II clinical evaluation and will be developed and marketed in collaboration with Merck's subsidiary Banyu (23, 24).

Sources

Merck & Co., Inc. (US); Banyu Pharmaceutical Co., Ltd. (JP); Ono Pharmaceutical Co., Ltd. (JP).

References

1. Kim, D., Wang, L., Beconi, M. et al. *(2R)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: A potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes.* J Med Chem 2005, 48: 141-51.

2. Edmondson, S.D., Fisher, M.H., Kim, D., MacCoss, M., Parmee, E.R., Weber, A.E., Xu, J. (Merck & Co., Inc.). *β-Amino tetrahydroimidazo[1,2-a]pyrazines and tetrahydrotrioazole-[4,3-a]pyrazines as dipeptidyl peptidase inhibitors for the treatment or prevention of diabetes.* EP 1412357, JP 2004536115, US 2003100563, US 6699871, WO 0304498.

3. Dreher, S.D., Ikemoto, N., Njolito, E., Rivera, N.R., Tellers, D.M., Xiao, Y. (Merck & Co., Inc.). *Process to chiral β-amino acid derivatives.* WO 0485661.

4. Xiao, Y., Armstrong, J.D. III, Krska, S.W., Njolito, E., Rivera, N.R., Sun, Y., Rosner, T. (Merck & Co., Inc.). *Process for the preparation of chiral beta amino acid derivatives by asymmetric hydrogenation.* WO 0485378.

5. Cypes, S.H., Chen, A.M., Ferlita, R.R., Hansen, K., Lee, I., Vydra, V.K., Wenslow, R.M. Jr. (Merck & Co., Inc.). *Phosphoric acid salt of a dipeptidyl peptidase-IV inhibitor.* US 2005032804, WO 0503135.

6. Angelaud, R., Armstrong, J.D. III, Askin, D., Balsells, J., Hansen, K., Lee, J., Maligres, P.E., Rivera, N.R., Xiao, Y., Zhong, Y.-L. (Merck & Co., Inc.). *Process and intermediates for the preparation of beta-amino acid amide dipeptidyl peptidase-IV inhibitors.* WO 0487650.

7. Prous Science Drug R&D Backgrounders: *Diabetes (online publication).* Updated April 28, 2005.

8. Saltiel, A.R. *Series introduction: The molecular and physiological basis of insulin resistance: Emerging implications for metabolic and cardiovascular diseases.* J Clin Invest 2000, 106: 163-4.

9. Narayan, K.M., Boyle, J.P., Thompson, T.J., Sorensen, S.W., Williamson, D.F. *Lifetime risk for diabetes mellitus in the United States.* JAMA - J Am Med Assoc 2003, 290: 1884-90.

10. American Diabetes Association. *Diagnosis and classification of diabetes mellitus.* Diabetes Care 2004, 27: S5-10.

11. Ruilope, L.M. *Recent developments in the treatment of type 2 diabetes mellitus.* Cardiovasc Drugs Ther 2003, 17: 151-8.

12. Orskov, C. *Glucagon-like peptide-1, a new hormone of the entero-insular axis.* Diabetologia 1992, 35: 701-11.

13. Zander, M., Madsbad, S., Madsen, J.L., Holst, J.J. *Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: A parallel-group study.* Lancet 2002, 359: 824-30.

14. Deacon, C.F., Holst, J.J., Carr, R.D. *Glucagon-like peptide-1: A basis for new approaches to the management of diabetes.* Drugs Today 1999, 35: 159-70.

15. Perfetti, R., Hui, H. *The role of GLP-1 in the life and death of pancreatic beta cells.* Horm Metab Res 2004, 36: 804-10.

16. Holz, G.G., Chepurny, O.G. *Glucagon-like peptide-1 synthetic analogs: New therapeutic agents for use in the treatment of diabetes mellitus.* Curr Med Chem 2003, 10: 2471-8.

17. Wiedeman, P.E., Trevillyan, J.M. *Dipeptidyl peptidase IV inhibitors for the treatment of impaired glucose tolerance and type 2 diabetes.* Curr Opin Investig Drugs 2003, 4: 412-20.

18. Ahren, B., Schmitz, O. *GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes.* Horm Metab Res 2004, 36: 867-76.

19. Kim, D., Wang, L., Kowalchick, J. et al. *MK-0431: A potent, orally active DP-IV inhibitor for the treatment of type 2 diabetes.* 228th ACS Natl Meet (Aug 22-26, Philadelphia) 2004, Abst MEDI 208.

20. Thornberry, N.A., Eiermann, G., Kim, D. et al. *Dipeptidyl peptidase IV (DP-IV) inhibition for the treatment of type 2 diabetes: Potential importance of selective inhibition and discovery of MK-0431.* 40th Annu Meet Eur Assoc Study Diabetes (Sept 5-9, Munich) 2004, Abst 113.

21. Weber, A.E., Kim, D., Beconi, M. et al. *MK-0431 is a potent, selective, dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes.* 64th Annu Meet Sci Sess Am Diabetes Assoc (June 4-8, Orlando) 2004, Abst 633-P.

22. Herman, G.A., Zhao, P.-L., Dietrich, B. et al. *The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics.* 64th Annu Meet Sci Sess Am Diabetes Assoc (June 4-8, Orlando) 2004, Abst 353-OR.

23. *Merck 2004 Annual Business Briefing.* Merck & Co., Inc. Web Site December 14, 2004.

24. *Ono and Merck in product licensing arrangements.* Ono Pharmaceutical Press Release Nov 10, 2004.