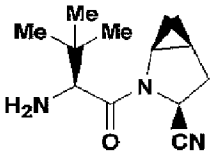
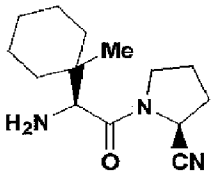
DP4 Chemistry Significant Events **March 30 ,2000**

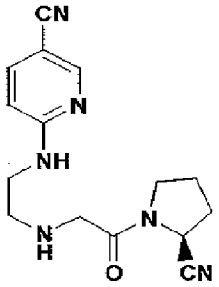
Executive Summary: Pharmacokinetic evaluation in cynos of our most potent and proprietary DP4 inhibitor, BMS-405295, showed this compound to have good oral systemic bioavailability (79%) with a half-life of 3.6 h. This compound had also previously demonstrated good PK in the rat (F = 77%, $t_{1/2}$ = 2.8 h). Novartis' clinical candidate DPP 728 (BMS-428245) was recently synthesized and found to exhibit in vitro activity (Ki = 7 nM) comparable to our current leads. Variation of the N-terminal amino acid of BMS-405295 resulted several compounds with good in vitro potency, including BMS-429636. In vivo testing of these newer analogs, as well as the Novartis compound, are planned.



BMS-405295
DP4 Ki = 6 nM
ED_{50} = 3.3 mg/kg, po

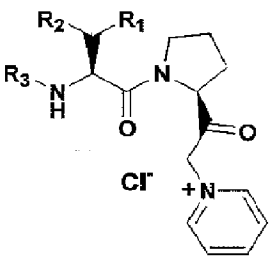


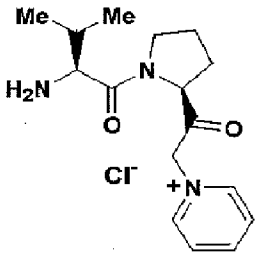
BMS-429636
DP4 Ki = 4 nM



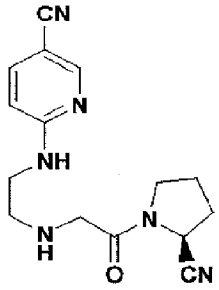
DPP 728
(BMS-428245)
DP4 Ki = 7 nM

Competitive Update: Probiodrug has recently disclosed (patent application WO 99672798) dipeptide based DP4 inhibitors containing an activated alpha-pyridyl ketone. These compounds presumably irreversibly inactivate the enzyme via displacement of the pyridyl functionality by the active site serine, forming a covalent intermediate. The specified compound caused an increase in time to DP4 mediated degradation of substrate from 1 minute to 100 minutes at a concentration of 2.8 μM

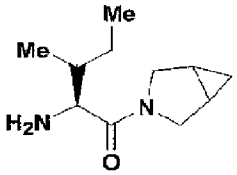




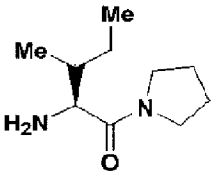
Monthly Summary The goal of the program is to discover small molecule inhibitors of dipeptidyl peptidase IV (DP4) for use in the treatment and prevention of diabetes. Inhibition of DP4 should prevent the degradation of GLP-1 and potentiate its action in vivo. Two new chemotypes were recently explored. In agreement with reported values, Novartis' clinical candidate DPP 728 (BMS-428245) was found to be a 7 nM inhibitor of DP4 in vitro. In vivo testing of this compound is scheduled. A unique bicyclic pyrrolidide BMS-428073, a hybrid between BMS-378738 and the Probiodrug compound BMS-326430, was found to be poorly active against DP4. On the basis of this data, we believe that incorporation of an alpha-cyano functionality in the ring structure would not sufficiently boost its activity to desired levels.



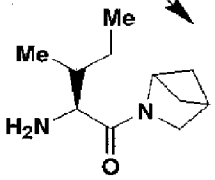
DPP 728
(BMS-428245)
DP4 Ki = 7 nM



BMS-378738
DP4 Ki = 1400nM

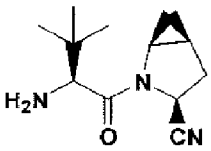


BMS-326430
DP4 Ki = 440 nM



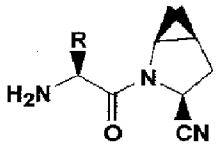
BMS-428245
DP4 Ki = 5300 nM

Pharmacokinetic evaluation in cynos of our most potent and proprietary DP4 inhibitor, BMS-405295, showed this compound to have good oral systemic bioavailability (79%) with a half-life of 3.6 h. This compound had also previously demonstrated good PK in the rat (F = 77%, $t_{1/2}$ = 2.8 h). With the discovery of BMS-405295, a substantial amount of effort has been directed towards the incorporation of highly beta-branched amino acids at the N-terminus of our methano substituted cyano pyrrolidides. Some of the recent variations are shown below. The cyclohexyl/methyl derivative BMS-429636 was only slightly more potent than BMS-405295. Introduction of more highly branched and entropically disordered substituents led to a gradual reduction in inhibitory activity. Thus, although nearly isostructural with BMS-429636, the diethylmethyl and the triethyl derivatives BMS-429806 and BMS-429880 were 4 to 8 fold less active respectively. Based on this observation, future derivatives will incorporate greater conformational restriction in the alkyl group at the N-terminus.



BMS-405295
DP4 Ki = 6 nM
ED_{50} = 3.3 mg/kg, po

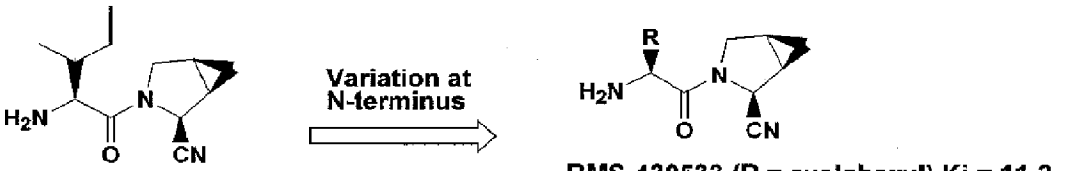
Variation at
N-terminus



BMS #	R	DP4 Ki (nM)
405295		6.2
429636		4.5
429769		6.2
429787		10.2
429806		19.9

429880		38.2
430148		37.8

The first SAR analysis at the N-terminus of the related 3,4-methano cyano pyrrolidide series has been performed. Unlike the 4,5-methano series, limited variation of the alkyl group in the 3,4 series demonstrated essentially no differences with respect to inhibitory activity. This unexpected finding suggests a slight divergence of SAR between the two methano-substituted chemotypes and limits the predictability of activity within these series. Other variations are planned.



BMS-383680
DP4 Ki = 15 nM

BMS-430533 (R = cyclohexyl) Ki = 11.2
BMS-430516 (R = t-butyl) Ki = 11.6 nM
BMS-430526 (R = isopropyl) Ki = 12.1 nM

In vivo studies: Metabolic Diseases Biology has successfully developed a GLP-1(7-36) specific N-terminal directed antibody, enabling the quantification of intact GLP-1 at pM levels in plasma. Application of this technique to DP4 inhibitor treated rats is reported below. Normal fasted rats were dosed with BMS-420597 (Ki = 2 nM, plasma DP4 ED50 = 3.8 mg/kg) at 30 mg/kg p.o., followed 30 min later by glucose challenge and 20 min later with plasma sampling. This resulted in strong inhibition of plasma DP4 activity to an average of 12% of control, and a 270% increase ($p = 0.107$) in endogenous intact GLP-1(7-36) over basal values (Table below). These results, coupled with other recent studies, confirm the correlation between DP4 inhibition and GLP-1 potentiation in vivo and enable a good functional read-out on the activity of our compounds in vivo.

Effect of BMS-420597 on plasma DP4 actvity and endogenous GLP-1(7-36) levels 20 min after glucose challenge. Drug or vehicle given p.o. 30 minutes before oral glucose challenge.

	plasma DP4 act. (units)	**plasma GLP-1(7-36)**
vehicle	32.1 ± 2.5	5.5 ± 1.0
BMS-420597	3.7 ± 0.2	15.0 ± 5.6*
(30 mg/kg)	*(12% of control)*	*(270% of control)*

* $p = 0.107$; n = 4 rats per group